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Córdoba

Departamento de
Química Analítica

EVALUACIÓN DEL POTENCIAL DE SÓLIDOS MONOLÍTICOS MODIFICADOS CON NANOPARTÍCULAS EN TÉCNICAS DE MICROEXTRACCIÓN

EVALUATION OF THE POTENTIAL OF MONOLITHIC SOLIDS MODIFIED WITH NANOPARTICLES IN MICROEXTRACTION TECHNIQUES

TESIS DOCTORAL

Beatriz Mª Fresco Cala

Córdoba, 2018

TITULO: *EVALUACIÓN DEL POTENCIAL DE SÓLIDOS MONOLÍTICOS MODIFICADOS CON NANOPARTÍCULAS EN TÉCNICAS DE MICROEXTRACCIÓN*

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EVALUACIÓN DEL POTENCIAL DE SÓLIDOS MONOLÍTICOS MODIFICADOS CON NANOPARTÍCULAS EN TÉCNICAS DE MICROEXTRACCIÓN

LA DIRECTORA,

Fdo. **Mª Soledad Cárdenas Aranzana**
Catedrática del Departamento de
Química Analítica de la Universidad
de Córdoba

Trabajo presentado para aspirar al Grado de Doctor en Ciencias

LA DOCTORANDA,

Beatriz

Fdo. **Beatriz Mª Fresco Cala**

Mª Soledad Cárdenas Aranzana, Catedrática del Departamento de Química Analítica de la Universidad de Córdoba, en calidad de directora de la Tesis Doctoral presentada por la Licenciada Beatriz Mª Fresco Cala.

CERTIFICA:

Que la Tesis Doctoral "**EVALUACIÓN DEL POTENCIAL DE SÓLIDOS MONOLÍTICOS MODIFICADOS CON NANOPARTÍCULAS EN TÉCNICAS DE MICROEXTRACCIÓN**" ha sido desarrollada en los laboratorios del Departamento de Química Analítica de la Universidad de Córdoba (España}, en el Departamento de Química Analítica de la Universidad de Valencia, y en el Instituto de Química Analítica de la Academia de las Ciencias de República Checa, y que, a mi juicio, reúne todos los requisitos exigidos a este tipo de trabajo.

Que Beatriz Mª Fresco Cala es la primera autora de todos los trabajos científicos presentados en esta Tesis Doctoral. De acuerdo con la normativa de esta Universidad y los acuerdos internos del Grupo de Investigación, el primer autor es el responsable de la realización del trabajo experimental y de la producción del manuscrito. Además, ha participado activamente en las reuniones periódicas con la directora para evaluar y discutir los resultados obtenidos durante la Tesis Doctoral.

Y para que conste y surta los efectos pertinentes, expido el presente certificado en la ciudad de Córdoba, a 5 de junio de 2018.

Mª Soledad Cárdenas Aranzana

Mª Soledad Cárdenas Aranzana, Full Professor of Analytical Chemistry Department of the University of Córdoba, in quality of supervisor of the Doctoral Thesis of Beatriz Mª Fresco Cala.

CERTIFIES THAT:

The Doctoral Thesis entitled "**EVALUATION OF THE POTENTIAL OF MONOLITHIC SOLIDS MODIFIED WITH NANOPARTICLES IN MICROEXTRACTION TECHNIQUES**" has been developed in the laboratories of the Department of Analytical Chemistry of University of Córdoba (Spain), Department of Analytical Chemistry of University of Valencia (Spain), and Institute of Analytical Chemistry of the Czech Academy of Sciences (Czech Republic), and according to our judgement the Thesis fulfils all the requirements of this type of scientific work.

Beatriz Mª Fresco Cala is the first author of all the scientific articles developed during the experimental phase of the Thesis. According to both the University rules and internal agreements in our research group, the first author of a paper is the full responsible for the implementation of the experimental work and also to produce the first draft of the paper. In addition, she has also actively participated in the meetings with the supervisors to check and discuss the progress of the doctoral work.

Córdoba, 5th June 2018.

Mª Soledad Cárdenas Aranzana

TÍTULO DE LA TESIS: EVALUACIÓN DEL POTENCIAL DE SÓLIDOS MONOLÍTICOS MODIFICADOS CON NANOPARTÍCULAS EN TÉCNICAS DE MICROEXTRACCIÓN

DOCTORANDA: Beatriz Fresco Cala

INFORME RAZONADO DE LA DIRECTORA DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

Beatriz Fresco Cala cursó con distinción la Licenciatura en Ciencias Químicas, obteniendo el premio extraordinario de Licenciatura, y los estudios de Máster en Química Fina. La concesión de una beca de iniciación a la investigación en el curso académico 2010-2011 fue el comienzo de su vinculación con el grupo de investigación. Continuó como beneficiaria de una beca de colaboración en el curso académico 2013-2014 y posterior concesión de una beca de formación de profesorado universitario (FPU-2014) para la realización de su tesis doctoral, cuya Memoria se presenta para su defensa bajo la modalidad de doctorado internacional y como compendio de publicaciones.

La tesis doctoral evalúa el potencial de sólidos monolíticos modificados con nanopartículas en técnicas de microextracción. El trabajo experimental ha evolucionado desde la inmovilización de las nanopartículas sobre el monolito formado previamente, hasta la preparación de estos materiales constituidos únicamente con nanomateriales de carbono. Los sólidos sintetizados se han incorporado a distintos formatos de microextracción: capilares, *spin-column*, puntas de pipeta o unidades con agitación integrada.

Además de la familiarización con procedimientos de síntesis, la doctoranda ha tenido la ocasión de formarse en distintas técnicas de caracterización tanto microscópicas como espectroscópicas, así como medidas de porosidad. Se han seleccionado distintos problemas analíticos modelo para la evaluación del potencial de los sólidos-unidades de microextracción preparadas empleando cromatografía de líquidos y de gases para la determinación de los analitos.

Durante estos cuatro años ha realizado dos estancias de investigación. La primera de ellas en la Universidad de Valencia bajo la supervisión del Dr. Herrero-Martínez, y la segunda en el Instituto de Química Analítica de la Academia de las Ciencias de la República Checa en el grupo del Dr. Foret y que le permite cumplir uno de los requisitos necesarios para optar a la mención de doctorado internacional.

Como consecuencia de la investigación realizada, se han publicado (o están en vías de publicación) 13 artículos científicos, 15 comunicaciones a congresos nacionales e internacionales y 1 capítulo de libro.

La doctoranda ha colaborado en la impartición de clases prácticas en el Grado de Química y Ciencia y Tecnología de los Alimentos durante 3 cursos académicos. Ha participado en la dirección de dos TFGs del Grado de Química, lo que le ha permitido iniciarse en las tareas de dirección de investigación en estudiantes en formación. Su participación en actividades de divulgación ha sido notable, tal como se puede acreditar en el anexo correspondiente de esta Memoria.

Además de las competencias inherentes al trabajo experimental, ha demostrado iniciativa en la investigación, proponiendo alternativas a las distintas dificultades que se han ido planteando durante estos cuatro años. Cabe resaltar su constancia en el trabajo diario y compromiso con las responsabilidades asignadas en la supervisión de los estudiantes en formación. Su capacidad para el trabajo en grupo queda reflejada en las colaboraciones en los artículos científicos publicados.

Finalmente, considero que la tesis doctoral cumple con los requisitos de originalidad, innovación y calidad exigibles a este tipo de trabajo.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 5 de junio de 2018

Firma de la directora

Fdo.: Mª Soledad Cárdenas Aranzana

MENCIÓN DOCTORADO INTERNACIONAL

Mediante la defensa de esta Memoria de Tesis Doctoral se pretende optar a la obtención de la Mención de "Doctorado Internacional" habida cuenta de que la doctoranda reúne los requisitos para tal mención (R.D. 99/2011, de 28 de enero):

1. Cuenta con los informes favorables de dos doctores pertenecientes a instituciones de Enseñanza Superior de países distintos a España.

2. Uno de los miembros del tribunal que ha de evaluar la Tesis pertenece a un centro de Enseñanza Superior de otro país distinto a España.

3. Parte de la defensa de la Tesis Doctoral se realizará en una lengua distinta de las lenguas oficiales en España.

4. La doctoranda ha realizado una estancia de tres meses en el Instituto de Química Analítica de la Academia de las Ciencias de la República Checa, gracias a la concesión de una ayuda para estancias en el extranjero del Programa Estatal de Promoción del Talento y su Empleabilidad (Ministerio de Educación, Cultura y Deporte).

INFORME DE FACTORES DE IMPACTO

A continuación, se adjunta una tabla con el factor de impacto y el cuartil del *Journal Citation Reports* en el que se encuentran las publicaciones derivadas de la presente Tesis Doctoral correspondiente al año de su publicación o al último disponible.

Revista	Año	Factor de impacto	Área	Cuartil / Decil
Analytica Chimica Acta	2018	5.123	Química Analítica	D1
Microchimica Acta	2018	5.705	Química Analítica	D1
	2017	5.705		
	2016	4.580		
Talanta	2018	4.244	Química Analítica	D1
Journal of Chromatography A	2016	3.981	Química Analítica	Q1
	2018	3.716		
Nanomaterials (Open Access)	2018	3.504	Multidisciplinar	Q1
RSC Advances (Open Access)	2018	2.936	Multidisciplinar	Q2
Microchemical Journal	2018	2.746	Química Analítica	Q2

Otras publicaciones relacionadas con la Tesis Doctoral.

Revista	**Año**	**Factor de impacto**	**Área**	**Cuartil / Decil**
Microchimica Acta	2014	3.741	Química Analítica	Q1
Molecules (Open Access)	2018	3.098	Química Orgánica	Q2

Agradezco al Ministerio de Educación, Cultura y Deporte la concesión
de la ayuda de Formación del Profesorado Universitario
(FPU13/03896) que ha hecho posible mi dedicación a este trabajo
durante los últimos cuatro años.

Agradezco al Comité Europeo para Relaciones Académicas (EUACC) de Lilly de España por haber reconocido el trabajo desarrollado en la presente Tesis Doctoral otorgándole uno de los Premios Lilly de Investigación para Alumnos de Doctorado 2018

Soy de las que piensan que la Ciencia tiene una gran belleza. Un científico en su laboratorio no es sólo un técnico: es también un niño colocado ante fenómenos naturales que le impresionan como un cuento de hadas.

–Marie Curie–

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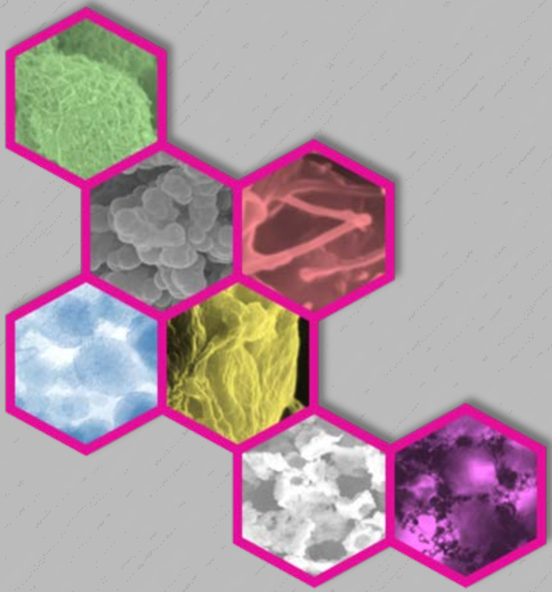
Introducción

Objeto

Aim



La Química Analítica es la ciencia que desarrolla, optimiza y aplica procesos de medida para obtener información sobre la composición y la naturaleza (bio)química de la materia. Sin embargo, las necesidades que tiene nuestra sociedad actualmente, en cuanto a información química se refiere, son muy distintas de los años que nos preceden. Se necesita determinar compuestos presentes en concentraciones cada vez más pequeñas y en muestras más complejas, así como hacerlo de una forma más rápida y eficiente. Por tanto, la Química Analítica debe evolucionar en función de las demandas de una sociedad progresivamente compleja, alcanzando objetivos mucho más ambiciosos que los planteados hasta ahora. De esta manera, el uso de nanomateriales como fases extractantes ha dado paso al desarrollo de sistemas miniaturizados de medida. Las mejores propiedades sorbentes que exhiben estos nanomateriales, asociadas a su pequeño tamaño, frente a los sólidos convencionales son clave a la hora de incrementar tanto la sensibilidad como la selectividad de la etapa instrumental, logrando aislar y preconcentrar los compuestos de interés de la matriz de la muestra con éxito.

Entre el amplio abanico de nanomateriales existente cabe destacar las estructuras derivadas del grafeno, es decir, aquellas basadas en láminas formadas por átomos de carbono unidos entre sí. Los nanotubos y los nanocuernos de carbono pertenecen a esta familia de materiales, los cuales además de las excepcionales propiedades físicas, químicas, magnéticas, térmicas, electrónicas y ópticas ya presentes en el grafeno, exhiben una elevada reactividad química ligada a sus extremos cónicos. Sin embargo, esa misma reactividad puede ir en detrimento de uso como fases sorbentes empaquetadas debido a problemas derivados de agregación y formación de sólidos amorfos macroscópicos. Como solución a dicho problema suelen combinarse o inmovilizarse en soportes, membranas o polímeros, potenciando alguna determinada propiedad, incluyendo una nueva funcionalidad y/o aumentando la superficie de estos. En este contexto, el desarrollo de nuevos materiales híbridos basados en la combinación de sólidos monolíticos, polímeros con una estructura continua pero altamente porosa, y nanopartículas juega un papel transcendental en la búsqueda de nuevas unidades de microextracción.

Con esta premisa, el objetivo principal de la Tesis Doctoral que se presenta en esta Memoria ha sido la evaluación del potencial de los sólidos monolíticos modificados con nanoestructuras de carbono como material sorbente en técnicas de microextracción en fase sólida, culminando con el desarrollo de estructuras monolíticas formadas únicamente por nanopartículas. De acuerdo con este objetivo general surgen diferentes objetivos específicos que se presentan a continuación:

- Estudio bibliográfico como primera toma de contacto con el campo en el que se desarrolla la investigación. Esto se ha materializado en la publicación de una revisión crítica que constituye la ***Introducción*** de esta Tesis Doctoral.

- Estudio de los procesos de inclusión de las nanopartículas en la matriz polimérica. Así, se estudiarán diferentes estrategias de introducción de las nanopartículas, tales como anclaje químico y fotoquímico a la superficie del polímero, así como atrapamiento de las nanopartículas en la estructura monolítica, que son ampliamente discutidas en el ***Bloque I***.

- Evaluación de diferentes formatos de microextracción, tal como se muestra en el ***Bloque II*** de esta Memoria.

- Desarrollo de nuevas estrategias de síntesis que permitan obtener estructuras monolíticas formadas exclusivamente por nanopartículas de carbono. Esta investigación se recoge en el ***Bloque III***.

- Caracterización de los sólidos sintetizados mediante diversas técnicas instrumentales. Esto se ha llevado a cabo a lo largo de la Tesis Doctoral.

- Aplicación de las unidades de extracción desarrolladas a la resolución de problemas medioambientales, clínicos y/o de alimentos, de manera transversal en el conjunto de publicaciones de esta Memoria.
- Utilización de los sólidos sintetizados como fases estacionarias en separaciones cromatográficas, tal como se muestra en el ***Capítulo 2*** de esta Memoria.

Analytical Chemistry is the science that develops, optimizes and applies measurement processes to obtain information about the composition and the (bio) chemical nature of the matter. However, the current needs of our society, in terms of chemical information, are very different from those stated several decades ago. It is necessary to determine compounds at very low concentrations in a great variety of complex matrices, as well as to do it more quickly and efficiently. Therefore, Analytical Chemistry should evolve according to the demands of a progressively complex society reaching much more ambitious objectives than those proposed up to now. In this way, the use of nanomaterials as sorbent phases has given a way to develop miniaturized extraction systems. The enhanced sorbent properties of the nanomaterials, associated with their small size, compared to conventional solids is the reason behind the improvement of both the sensitivity and selectivity of the methods via the isolation and preconcentration of the target compounds from the samples.

Among the wide range of existing nanomaterials, it is worth mentioning the structures derived from graphene, that is, those based on sheets formed by carbon atoms joined together. Carbon nanotubes and nanohorns belong to this family of materials, which besides the exceptional physical, chemical, magnetic, thermal, electronic and optical properties already present in graphene, exhibit a high chemical reactivity associated to their conical shape. However, that same reactivity can be detrimental to use them as packed sorbent phases because of their high tendency to form macroscopic amorphous aggregates of higher sizes. To overcome this problem, they are usually combined or immobilized in supports, membranes or polymers, improving some specific properties of them, including a new functionality and/or increasing their surface. In this context, the development of new hybrid materials based on the combination of monolithic solids, polymers with a continuous and highly porous structure, and nanoparticles plays a transcendental role in the search for new and superior microextraction units.

Based on the foregoing, the primary aim of this Doctoral Thesis has been the evaluation of the potential of monolithic solids modified with carbon nanostructures and their use as a sorbent phase in microextraction techniques, culminating with the development of monolithic structures formed exclusively by nanoparticles. The specific objectives derived from this general one are as follows:

- Bibliographic study of this research field as first step of the doctoral training. This has been materialized in the publication of a critical review that constitutes the ***Introduction*** of this Doctoral Thesis.

- Evaluation of different methodologies for the inclusion of nanoparticles in the monolithic solid, including chemical and photochemical attachment to the porous surface as well as their embedding in the polymeric network. These approaches are studied and widely discussed in ***Block I***.

- Evaluation and development of several microextraction units, as shown in the ***Block II*** of this Report.

- Development of new procedures for the synthesis of monolithic solids formed solely by carbon nanoparticles. Results of these studies are set out in ***Block III***.

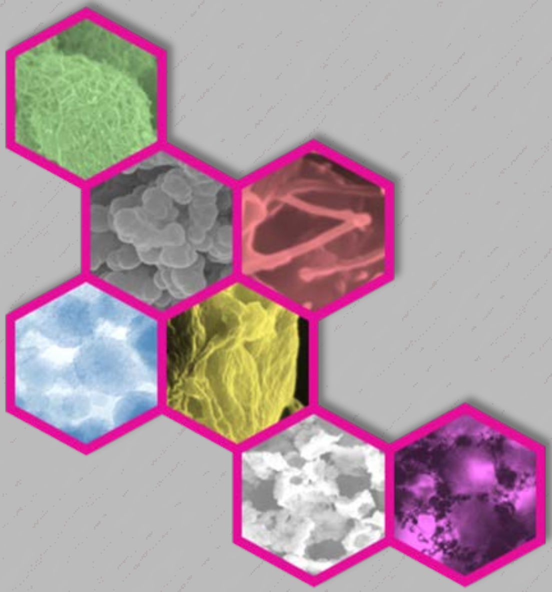
- Characterization of the synthesized solids by means of diverse microscopic and spectroscopic techniques. This has been carried out all along this Doctoral Thesis.

- Practical application of the developed microextraction units to environmental, agrifood and/or biological samples. These applications have been evaluated transversally in each of the chapters.

- Use of the synthesized solids as stationary phases in separation techniques, as shown in the ***Chapter 2*** of this Report.

Introducción

Introduction



Potential of nanoparticle-based hybrid monoliths as sorbents in microextraction techniques

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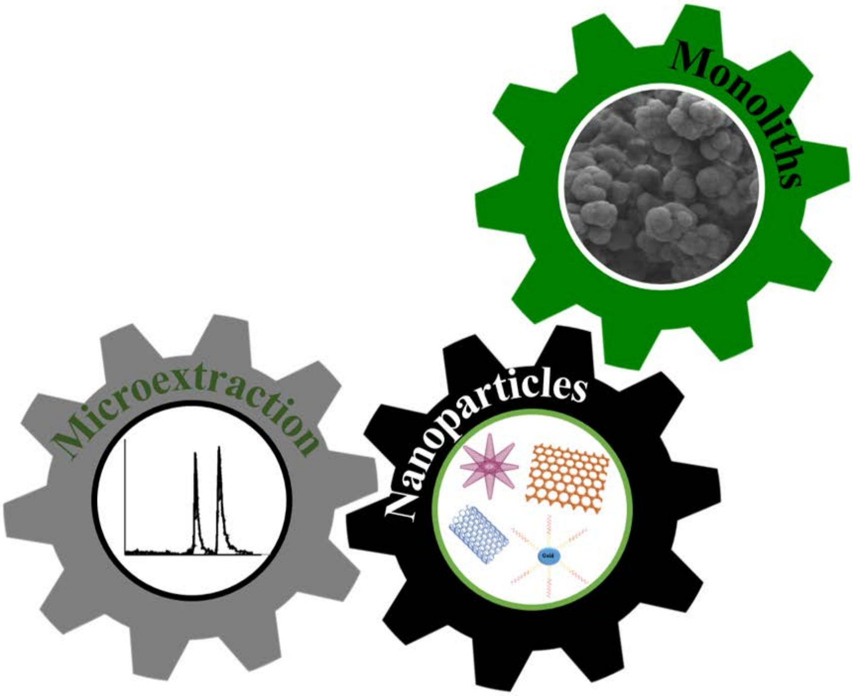

Keywords

Organic monoliths; Silica monoliths; Metallic nanoparticles; Metal oxide nanoparticles; Carbon nanoparticles; Micro-solid phase extraction.

Abstract

This article reviews the synthesis and uses of nanoparticle-based hybrid monoliths in microextraction techniques. Synthesizing monolithic solids containing nanoparticles allows the advantages of the two sorbent phases to be synergistically combined. The resulting hybrid material exhibits outstanding capabilities for isolating the target compounds from the samples. In addition to conventional in-cartridge solid-phase extraction, the monoliths can be easily adapted for use in most of the usual formats of micro-solid phase extraction including capillaries, pipette tips, spin columns, microfluidic chips and stirred units. The most salient uses devised so far are described and critically discussed in this article, which deals with both silica and organic monolithic solids modified with nanostructured materials including carbonaceous and metal or metal oxide nanoparticles.

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1. Introduction

The growing miniaturization of the different steps of the analytical process has also reached sample treatment [1]. Downsizing equipment allows amounts or volumes of sample considerably smaller than those required by a conventional analytical procedure to be handled. Also, it reduces consumption of reagents and the need to dispose of waste, and enables the analysis of scant (e.g., biological) samples and valuable or unique objects. However, if an extraction technique is to be implemented in miniaturized format, the phase containing the extractant, whether solid or liquid, must be efficient enough to offset the potential disadvantages of the small sample volume or amount used. In response to this problem, new sorbent materials have arisen as alternatives to conventional silica-based or polymeric phases such as those typically used in on-cartridge solid-phase extraction (SPE). So far, micro-solid phase extraction (μ-SPE) has been made possible by monolithic solids, nanoparticles (NPs) and composite materials [2–5], and so has liquid phase microextraction (LPME) by ionic liquids [6] and supramolecular solvents [7]. Despite the salient contributions of LPME to efficient extraction, this technique falls outside the scope of this article.

Analytically, a monolithic solid can be defined as a unique, continuous structure containing large pores that confer it a high permeability [8]. Unlike packed bed materials, monolithic solids contain no voids between particles; as a result, the interaction of analytes with monolithic surfaces is less markedly affected by diffusion phenomena. Monoliths can also be prepared *in situ* by following an uncomplicated, reproducible procedure that usually involves mixing selected monomers, porogens and either an initiator [9, 10] for organic monoliths or using the sol-gel approach [11] to obtain silica monoliths. The synthetic process can be facilitated by using an external source of energy such as heat or ultraviolet (UV) light. Because monolith-based sorbents can be prepared in a vast variety of forms [12, 13], they are compatible with most microextraction formats [14]. Also, they can be directly attached to the inner walls of the support in order to dispense with frits. However, most monoliths have the disadvantage of a smaller specific surface area than other types of µ-SPE sorbents.

A nanomaterial can be defined as a solid at least one of whose dimensions is smaller than 100 nm —a threshold established by convention. The nanometric size of nanomaterials endows them with outstanding electrical, optical, mechanical, magnetic or sorptive properties that set them apart from micro- and macrosolids [15]. The most paradigmatic example of nanomaterials are no doubt carbon nanoparticles (particularly carbon nanotubes and graphene, which are the most widely cited by far [16, 17]). Other types of organic nanomaterials, and also inorganic nanoparticles consisting of metals or metal oxides, have been extensively used in different steps of the analytical process [18]. Efficient analyte isolation and preconcentration with nanomaterials relies heavily on their sorption capacity (specifically, on maintaining a high surface-to-volume ratio throughout the separation process). This requires avoiding aggregation of the nanoparticles since aggregates are amorphous and not nanometrically sized, which dramatically diminishes their sorption capacity.

One elegant way of circumventing the shortcomings the previous two types of sorbent materials while boosting their performance as sorbent phases is by developing effective synthetic pathways to obtain hybrid materials synergistically combining the favorable sorptive properties of monolithic phases (viz., absence of interstitial voids, controlled pore size, mechanical stability, fast mass transfer under dynamic conditions) and nanoparticles (viz., high surface/volume ratios, flexible interaction chemistries and easy functionalization) [18]. A number of nanostructures have in recent years been used in combination with monolithic materials to develop new, hybrid sorbent phases of increased selectivity and sorbent capacity [19, 20]. The exponential growth of literature on nanoparticle-modified monolithic solids is consistent with their brilliant present and anticipates a promising future.

This article reviews the analytical uses of new monolithic solids in the µ-SPE realm. It discusses the synthetic procedures involved, and the preferred microextraction formats. Also, it specifies the types of nanoparticles used in each case and highlights the resulting advantages relative to bare monoliths. Finally, it includes a critical Conclusion section that predicts new uses for these novel materials in the near future.

2. Synthesis of monolithic solids

Monolithic solids can be classified into organic, silica-based and hybrid monoliths depending on the nature of the monomers involved. Each class of monolithic solid has specific advantages and disadvantages concerning sample treatment for microextraction. Because they constitute the subject matter of this article, hybrid monoliths containing nanoparticles are also included in the definition.

2.1. Organic monoliths

Organic polymer monoliths, which were first reported three decades ago, are obtained by polymerization of monomers, crosslinkers, porogen solvents and initiators in appropriate proportions. The monomer is usually an acrylamide, methacrylate or acrylate, or styrene, whereas the crosslinker is most often ethylene dimethacrylate (EDMA). Most monoliths of this type are obtained by using a conventional polymerization technique involving free radical reactions that are initiated by heating or UV radiation [21, 22]. However, alternative polymerization initiation methods based on high-energy radiation such as γ-rays or electron beams [9] afford flexibility in monolith morphology and efficient control of pore size. One key benefit of these methods is that neither an initiator nor the presence of functional groups at chain ends in the monomer is needed. In fact, the polymers can be obtained at any temperature and in almost any type of container including stainless steel tubes. However, high-energy processes require using sophisticated equipment under more stringent safety measures.

2.2. Silica monoliths

Inorganic (silica) monoliths are prepared either by the sol-gel method or from bare silica particles [23]. The sol-gel method (Figure 1) involves mixing an alcohol and an alkoxysilane, which are hydrolyzed in the presence of water under initially acidic conditions [11]. Then, the polymerization rate and phase separation are controlled by increasing the pH. The variables influencing gel formation (e.g., monomer/porogen ratio, reaction temperature, pH) must be carefully examined in order to use the particular values leading to the desired type of pore network in the monolith.

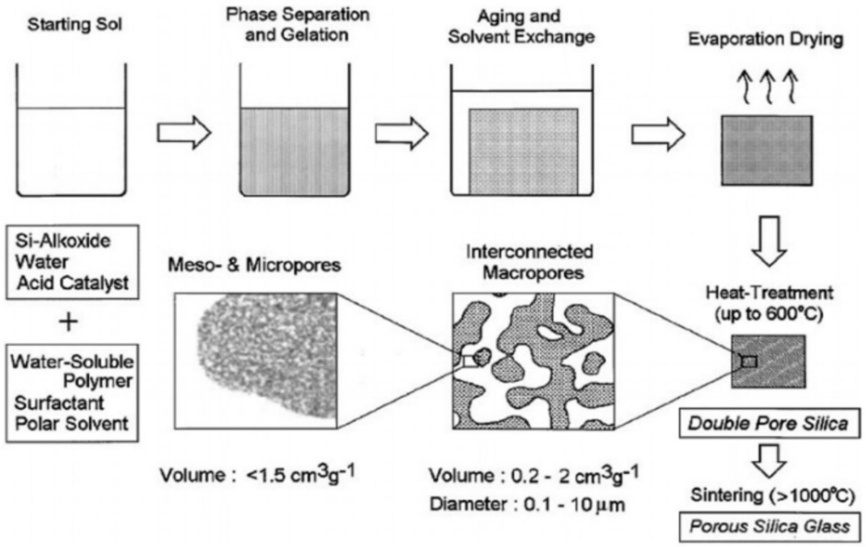

Figure 1. Production of monolithic silica rods and columns. Reprinted from reference 23, with permission.

Silica monoliths provide some substantial advantages over organic polymer monoliths. Thus, they possess greater physical strength and are less prone to swelling under the action of organic solvents than are organic monoliths. Also, macropore and mesopore characteristics in monoliths can be controlled separately through the conditions of the sol-gel preparation procedure [24, 25]. Finally, silica-based monoliths possess a chemically active surface that can be readily derivatized with a variety of functional groups. Above pH 4, however, underivatized silica contains negatively charged silanol groups that are potential sites for strong secondary interactions [26] and may result in irreproducible elution of some analytes (particularly basic compounds). Also, silica monoliths prepared with sol-gel technology solid tend to shrink at the end of the process unless they are covalently attached to the support walls. This shortcoming, however, cannot be completely avoided, especially with monolith diameters exceeding 4.6 mm.

2.3. Hybrid monoliths and nanoparticle-based hybrid monoliths

The scope of monolithic solids in separation techniques can be expanded by using compounds intended to confer the final solid specific functionalities or enhanced sorption capabilities. One attractive example in this context is the preparation and functionalization of silica-based monoliths in a single step by co-condensation of organosilanes such as $R_nSi(OR')_{3-n}$ to obtain a surface with the desired properties [27, 28]. Monoliths synthetized in this way are commonly referred to as “hybrid monoliths”. This type of monolithic sorbent has gained prominence by virtue of its unique characteristics, which include mechanical and pH stability, solvent resistance, easy preparation and tunable polarity [29].

As stated in the Introduction, incorporating nanoparticles into a monolithic structure has opened up a promising but challenging research line. Nanoparticle-containing monoliths have been primarily used for chromatographic and electrophoretic separation [19, 30, 31], and, only recently, for (micro)extraction.

Nanoparticles can essentially be added to a monolithic structure in two different ways, namely:

1. By direct addition, as a dispersion in a porogen solvent, to the reaction mixture and subsequent polymerization. Once the monolithic solid has formed, the nanoparticles are embedded in it.

2. By immobilization on the monolith, which is functionalized before the nanostructures are anchored through electrostatic or covalent interactions on its pores and surface by flowing a nanoparticle dispersion.

Each approach has its own advantages and disadvantages. For example, incorporating the nanoparticles into the reaction mixture allows the resulting hybrid monolith to be reused a number of times with no loss of efficiency thanks to its high stability. It should be noted, however, that not all nanoparticles (NPs) may be available (e.g., those at the surface) for interaction with the analytes. Also, if the NPs are used in excessive amounts, they may aggregate or even sediment, thereby distorting the porous structure of the bare solid and rendering it useless for extraction purposes.

By contrast, incorporating NPs onto a monolith surface requires an additional step to functionalize the monolith after polymerization and before an NP dispersion is flowed through it for electrostatic or covalent attachment to its surface (Figure 2). Incorporating NPs considerably increases the specific surface of the monolith material and leads to an increased extraction efficiency. However, it is not always possible to ensure uniform coating because aggregation and/or sedimentation can eventually block pores and prevent the passage of solvents. Also, small amounts of NPs may detach from the solid and impair reusability of the extractant.

Both approaches have been assessed in terms of performance of the resulting monolithic solids in µ-SPE. Their most salient uses are described in the following sections according to extraction format (see Figure 3).

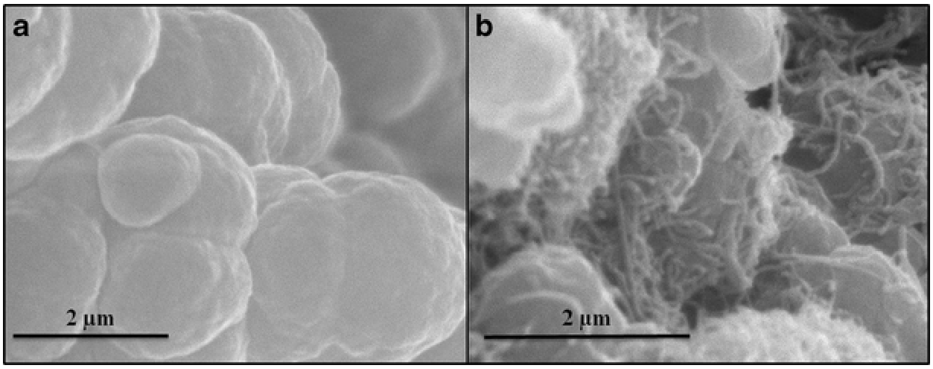

Figure 2. Scanning electron micrographs of **(a)** a poly(BA-co-EGDMA) monolith (×15000) and **(b)** a poly(BA-*co*-EGDMA-c-MWCNTs) monolith (×20000). Reprinted from reference 42, with permission.

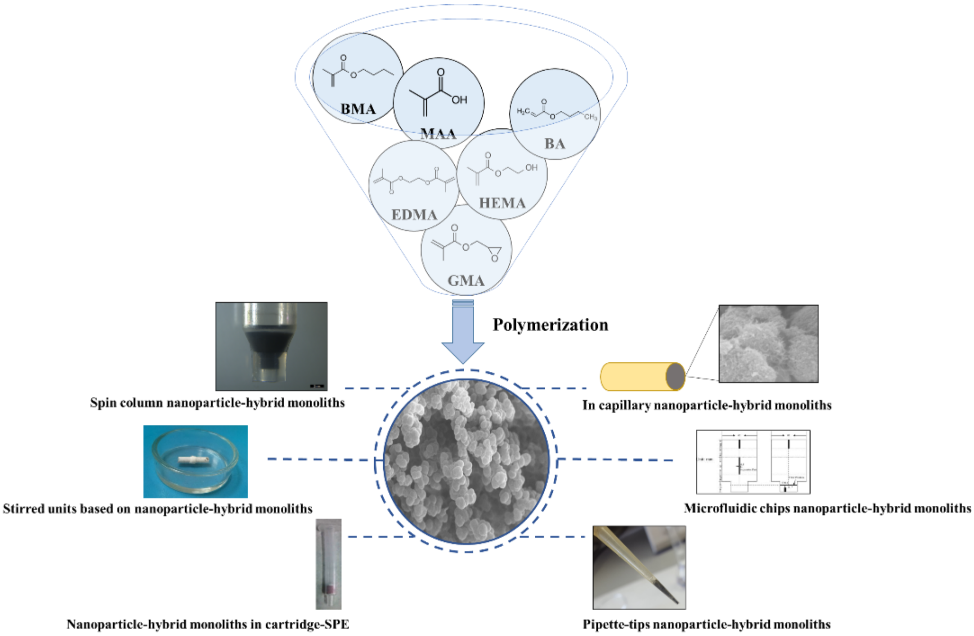

Figure 3. Most widely used monomers for monolith preparation and formats used in microsolid phase extraction. Reprinted from references 73, 76 and 80, with permission.

3. The role of nanoparticles in microextraction

In recent years, a wide variety of carbon NPs including carbon nanotubes (CNTs), graphene and single-walled carbon nanohorns (SWNHs) have been used as sorbents. Worth special note here is the contribution of CNTs to improving separation techniques [17, 32] through their exceptional mechanical, electric/electronic, magnetic and surface properties. However, the high tendency of CNTs to aggregating is a strong constraint for use in dispersive microsolid phase extraction (d-µSPE) or in flow configurations, which usually entail immobilization onto a solid support such as a membrane [33] or polymer [4].

The discovery of new carbon NPs with unique properties in recent years has opened up new avenues for preparing sorbent materials affording isolation and preconcentration of analytes from complex matrices. For example, carbon nanostructured aerogels have been used to extract environmental pollutants from water [34, 35]. Also, conical carbon nanostructures called “single-walled nanohorns” (SWNHs), which contain a horn-shaped tip (cone angle ca. 20°) and a cylindrical nanotube section, have emerged as one of the most promising nanosized sorbent materials by virtue of their rich chemical reactivity, small size and ability to form stable spherical aggregates resembling dahlias through van der Waals interactions between individual horns [36, 37].

Metal and metal oxide nanoparticles such as gold nanoparticles (AuNPs), iron oxide nanoparticles (IONs) and cuprous oxide nanoparticles (CONPs) possess exceptional properties which have prompted their use for many purposes including analytical separations. In fact, these types of NPs have distinct properties including high electrical and thermal conductivity. Also, colloidal gold and silver possess excellent optical properties thanks to their ability to produce surface plasmon resonance (SPR) as a consequence of the collective oscillation of electrons in the conduction band. This phenomenon results in the characteristic red and yellow colour of Au and Ag colloids, respectively, and in substantially enhanced electric fields on their surface. In addition, the high polarizability of metallic NPs results in strong interactions with nearby fluorescent chromophores that either decrease or increase their quantum yield. In any case, one of the most important chemical properties of metal and metal oxide NPs as regards separation is their affinity for some functional groups such as cyano, mercapto or amino [38], which has, for example, enabled the selective recognition and extraction of proteins from biological samples [39].

Magnetic nanoparticles (MNPs) based on iron oxides (Fe_2O_3 and Fe_3O_4) have been widely used for extraction purposes [40–42]. MNPs can be dispersed in the sample and subsequently collected easily with an external magnet thanks to their superparamagnetic properties. However, they result in nonspecific interactions and are unstable at extreme pH values —a problem that can be circumvented by coating the nanoparticles with a polymer layer to increase their chemical stability and boost selectivity in the interactions.

4. In-capillary nanoparticle-hybrid monoliths

Monoliths synthetized inside a capillary have gained increasing popularity thanks to their easy anchoring onto monolith inner walls and their combination affording online coupling for automatic analysis [13]. Fused silica capillaries are usually pretreated with 3-(trimethoxysilyl)propyl methacrylate to facilitate covalent binding of the monolithic phase to the capillary inner wall [43]. Then, the capillary is filled with the polymerization mixture before its two ends are sealed. Unreacted monomers and porogenic solvents remaining after the polymerization reaction must be removed before the resulting unit can be used to isolate and preconcentrate analytes. As with other microextraction formats, nanoparticles have also been incorporated into monolithic capillaries by using either of the two above-described choices to improve their extraction efficiency [44]. In some cases, derivatization of the porous surface of the monolith is required before the nanoparticles can be incorporated. Table 1 lists the selected uses of this type of monolith discussed in this section.

Table 1. Selected examples of in-capillary nanoparticle-hybrid monoliths.

	Monolithic solid	**Nanoparticles**	**Inclusion strategy**	**Analytes**	**Instrumental technique**	**LODs ($\mu g\cdot L^{-1}$)**	**Reference**
Metal and metal oxide nanoparticles	Poly(HEMA-*co*-EDMA)	HANPs	Embedded	Phosphopeptides	MALDI-MS	-	[45]
	Poly(GMA-*co*-EDMA)	AuNPs	On-surface	Captopril	Capillary electrophoresis-UV	-	[46]
	Poly(GMA-*co*-PEGDA)	AuNPs	On-surface	Glycoproteins	MALDI-MS	-	[47]
	Poly(GMA-*co*-EDMA)	ION	On-surface	Phosphopeptides	MALDI-MS	-	[48]
	Poly(NIPAM-*co*-GMA-*co*-EDMA)	γ- Al_2O_3 NPs	On-surface	Sudan dyes	LC-UV	0.13-0.29	[49]
	Poly(NIPAM-*co*-MBA)	γ- Al_2O_3 NPs	Embedded	Synthetic food dyes	LC-UV	9.3-11.5	[50]
	Poly(BMA-*co*-EDMA)	CONPs	On-surface	Polychlorinated biphenyls	GC–ECD	0.011-0.09	[51]
	Poly(MAA-*co*-EDMA)	ZnO NPs	Embedded	Fluoroquinolone antibiotics	LC-MS/MS	0.007-0.034	[52]
	Poly(MAA-*co*-EDMA)	TiO_2 NPs	Embedded	Gd^{+3} and Gd-based contrast agents	ICP-MS	3.6	[53]
	Poly(PEG-*co*-EDMA)	MNPs	Embedded	Steroid hormones	LC-DAD	0.06-0.25	[54]
	Poly(AM-*co*-EDMA)	MNPs	Embedded	Ultraviolet filters	LC-DAD	0.04-0.26	[55]
Carbon nanostructures	Poly(BMA-*co*-EDMA	GN	Embedded	Glucocorticoids	LC-MS	0.13-1.93	[56]
	Poly(GMA-*co*-EDMA)	GO and GN	On-surface	Sarcosine	LC-MS/MS	1.0	[57]
	Poly(BA-*co*-EDMA)	SWNHs, SWCNTs, and MWCNTs	On-surface	Triazine herbicides	GC-MS	0.03-0.1	[58]
	Poly(MAA-*co*-EDMA)	SWCNTs	Embedded	Triazine herbicides	Real time mass spectrometry	0.02-0.14	[59]
	Poly(TEOS)	SWCNTs, MWCNTs, and SWNHs	Embedded	PAHs	GC-MS	0.1-0.3	[60]

NIPAM, N-isopropylacrylamide; MBA, N, N'-methylenebisacrylamide; PEG, polyethylene glicol; AM, 1-allyl-3-methylimidazolium bis[trifluoromethylsulfonyl]imide; TEOS, tetraethoxysilane.

Svec et al. [45] used a poly(2-hidroxyethyl methacrylate-*co*-ethylene dimethacrylate) [poly(HEMA-*co*-EDMA)] monolithic capillary column with embedded commercial hydroxyapatite nanoparticles (HANPs) for protein separation and selective enrichment of phosphopeptides from complex peptide mixtures of ovalbumin, α-casein and β-casein digests. Nanoparticles were incorporated into the monolith by admixing in the polymerization mixture because HANPs have a strong tendency to agglomerate owing to the presence of positively charged calcium groups and negatively charged phosphate groups on NP surfaces. Extracted analytes were released for analysis by matrix-assisted laser desorption/ionization coupled to mass spectrometry (MALDI-MS).

In 2011, Zhang et al. [46] developed a new approach to the preparation of polymer monoliths providing enhanced coverage of pore surfaces with AuNPs for the selective extraction of thiol-containing compounds (particularly captopril traces) from plasma. They prepared monoliths based on poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) [poly(GMA-*co*-EDMA)] inside a fused silica capillary and then modified them chemically with cystamine and immobilized gold NPs onto the surface of monolith pores. AuNPs were synthetized in the laboratory and pumped through the poly(GMA-*co*-EDMA) monolith with a hand syringe until the entire monolithic solid turned reddish. The capillary was then cut into a piece 2 cm long and fitted to a syringe barrel by using the syringe pinhead. The extraction procedure included conditioning, sampling, washing and elution, the eluate being analyzed by capillary electrophoresis with UV detection at 230 nm.

Wu et al. [47] also attached AuNPs to a methacrylate monolithic capillary for enrichment with glycoprotein from human plasma samples. Using poly(ethylene glycol diacrylate) (PEGDA) as a crosslinker containing the gold nanoparticles increased the hydrophilicity, biocompatibility and surface area of the monolithic material, thereby leading to improved selectivity and binding capacity for glycoprotein enrichment.

Metal oxide nanoparticles have also been incorporated into polymer-based monolithic columns to improve their separation efficiency and enrichment capabilities. Thus, Krenkova et al. [48] prepared an ION-coated methacrylate monolithic capillary column for the selective isolation of low-abundant phosphopeptides from complex protein digests and their off-line characterization by MALDI-MS. The monolithic material was synthesized inside a vinylized UV-transparent fused silica capillary and its surface functionalized with quaternary ammonium groups in order to anchor the IONs. The resulting monolithic column exhibited superior performance, and better efficiency and selectivity for phosphoproteomic analysis, than existing sorbents.

Jia et al. used a γ-alumina (γ-Al_2O_3) NP-functionalized porous polymer monolith to enrich Sudan dyes in red wine [49] and synthetic food dyes in soft drinks [50]. For the former purpose, they flushed a γ-Al_2O_3 NP dispersion through the monolithic column to immobilize nanoparticles on its surface; for the latter, they added NPs directly to the reaction mixture containing the monomers and porogen solvents. In both cases, the extraction device included a plastic syringe connected to the monolithic capillary (530 µm i.d.) by a plastic pinhead connector and the microextraction procedure involved the following steps: (1) flushing methanol and NaH_2PO_4 to condition the capillary; (2) injecting the sample; (3) washing the capillary with NaH_2PO_4; and (4) eluting the analytes with methanol for analysis by liquid chromatography (LC).

A novel poly(butyl methacrylate-*co*-ethylene dimethacrylate) [poly(BMA-*co*-EDMA)] monolithic column was prepared in situ and modified with allylamine-β-cyclodextrin (ALA-β-CD) and CONPs [51]. The microextraction unit was used to extract polychlorinated biphenyls from wine samples. A programmable syringe pump was used to flush the solvents through the capillary throughout the extraction process. The extraction efficiency of poly(BMA-*co*-EDMA) and poly(BMA-*co*-EDMA-ALA-β-CD-CONPs) monolithic columns was compared and the modified monolith found to provide greater peak areas. These results can be ascribed to the hydrophobicity of the monolith and ALA-β-CD, the porous structure and the sorbent efficiency of CONPs improving the capabilities of the material and the kinetics of the process, thereby allowing greater amounts of analytes to be retained and sensitivity in the chromatographic analysis to be enhanced as a consequence.

Liu et al. [52] prepared a methacrylic acid (MAA)-based polymer monolith modified with zinc oxide (ZnO) NPs and used it for the simultaneous determination of antibiotics by LC. ZnO NPs were directly added to the polymerization mixture and the extraction device consisted of a 5 mL plastic syringe. The resulting poly(MAA-*co*-EDMA-ZnO) monolithic column exhibited recovery values of 70.7–108.3%, and excellent stability and reproducibility. These authors also examined the sorption capacity of polymer monoliths functionalized with indium oxide nanoparticles. The modified monolithic capillaries were successfully coupled with LC tandem mass spectrometry (MS/MS) for the determination synthetic colorants in food samples.

In another study, a poly(MAA-*co*-EDMA) monolithic column with embedded titanium dioxide (TiO_2) NPs was used to speciate Gd^{+3}/Gd-based contrast agents (Gd-DPTA) in human urine samples [53]. This type of NP possesses excellent sorption properties for compounds containing phosphate groups and dispense with the need for oxidation, reduction or subtraction, which is a critical factor for Gd speciation in clinical analysis. Using the monolithic capillary microextraction unit in online combination with inductively coupled plasma mass spectrometry (ICP-MS) led to the lowest limits of detection (LODs) for Gd reported to date.

The superparamagnetic properties of IONs have been used to improve the extraction efficiency of monolithic polymers and led to the development of a new extraction approach called “magnetism-enhanced monolith-based in-tube solid-phase microextraction” (ME-MB/IT-SPME) [54]. The greatest novelty of this approach is that it uses the principle of diamagnetic repulsion by applying a magnetic field in different directions in the analyte retention and elution steps. For this purpose, the monolithic capillary column is wrapped in a magnetic coil and connected to a high-pressure pump. While the sample solution is being passed through the capillary, a magnetic field is applied in the same direction. For desorption, however, the magnetic field is applied in the opposite direction of the solvent flow. Extraction is done at room temperature in any case. This approach was used to determine six steroid hormones as a proof of concept, and found to boost the extraction capabilities of the solid by effect of the applied magnetic field. All microextraction variables including the magnetic field intensity, retention and elution flow rates, sample volume and desorption solvent were carefully examined. The ensuing methodology was also used for the online analysis of five organic ultraviolet filters in environmental water samples [55]. For this purpose, the ionic liquid 1-allyl-3-methylimidazoliumbis [(trifluoromethyl)sulfonyl]imide (AM) was for the first time used as a functional monomer to prepare MNP-doped monoliths. Under optimal conditions, the hybrid monolith afforded fast, acceptably efficient extraction and allowed the target analytes to be monitored at trace levels. Magnetically based extraction methods provide the added advantages of being inexpensive and environmentally friendly.

Concerning carbon nanoparticles, Tong et al. [56] prepared a porous polymer monolithic column with embedded graphene nanosheets (GNs) which they use to extract and enrich nine glucocorticoids in cosmetics for LC-MS analysis. The variables influencing microextraction performance and monolith capabilities were optimized, the resulting poly(BMA-*co*-EDMA-GN) monolith being characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM) and scanning electron microscopy (SEM). A comparison of poly(BMA-*co*-EDMA) monoliths with and without nanoparticles, and direct LC-MS analysis, in terms of performance revealed a high extraction capacity in the GN-embedded monolith. Also, the synthetic procedure proved quite reproducible and poly(BMA-*co*-

EDMA-GN) stable as regards extraction performance. Graphene was functionalized with poly(vinylpyrrolidinone) (PVP) to ensure good dispersion in the porogens by exploiting the hydrophilic nature of PVP-GNs. Although increasing the proportion of PVP-GNs in the polymerization mixture improved the extraction efficiency, excessive amounts considerably raised backpressure. Tong et al. [57] used a slightly different approach to determine sarcosine by LC-MS/MS. Thus, they prepared poly(GMA-*co*-EDMA) monoliths modified with graphene oxide (GO) and GNs also synthesized inside a capillary.

Our group [58] assessed the performance of three different types of carbon NPs [viz., SWNHs, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs)] as modifiers of a poly(BA-*co*-EDMA) monolith for the extraction of triazine herbicides from water and orange juice. Passing an NP dispersion through the monolithic solid caused the nanoparticles to be immobilized on pore surfaces. The resulting hybrid monolith exhibited better extraction efficiency than the bare monolith. However, the best results were obtained with a poly(BA-*co*-EDMA-MWNTs) monolithic unit. This can be partly ascribed to the fact that the size and number of MWCNT sheets were both greater than those of SWCNTs and SWNHs, thereby favoring π interactions with the analytes. Triazine herbicides have also been preconcentrated from water samples by using a poly(MAA-*co*-EDMA-*co*-SWCNTs) monolith [59]. Oxidized SWCNTs were incorporated into monoliths by adding NPs to the porogen solvent. In this case, the microextraction unit was for the first time coupled with direct, real-time analysis by MS. Unlike offline extraction methods, extraction was completed within a few minutes and with no need for chromatographic separation. The extraction conditions were systematically optimized and the monolith morphology was characterized by SEM.

Until fairly recently, the development of microextraction units based on silica monoliths containing embedded carbon nanostructures was an unexplored area. Our group [60] examined the sorption efficiency of silica monolithic capillaries containing three different types of carbon NPs (viz., carboxylated SWCNTs, carboxylated MWCNTs, and oxidized SWNHs) in the extraction of polycyclic aromatic hydrocarbons (PAHs) from water as a model analytical problem. The nanoparticles were directly included in the polymerization mixture and the sol-gel method was used to prepare the monoliths. Incorporating the nanostructures altered the hydrophilicity of the silica monolith and enabled the adsorption of nonpolar compounds such as PAHs through π–π stacking and hydrophobic interactions.

5. Pipette-tip nanoparticle-hybrid monoliths

Solid-phase extraction can also be miniaturized by packing the sorbent in conventional, disposable polypropylene pipette tips. With these modified units, the microextraction procedure involves repeated aspirating–dispensing cycles that can be performed with a manual or automated micropipetter. Both conventional sorbents and nanostructured solids have been used in this format [61].

Miyazaki et al. [62] used monolithic silica packed in a pipette tip back in 2004. The synthetic procedure is quite straightforward as it only requires preparing the silica monolith in a mold and its further fixing into a 200 µL pipette tip by supersonic adhesion. Various versions of pipette tip-based devices have been subsequently reported [63, 64] some of which such as OMIX (Varian) and MonoTip (GL Sciences) have become commercially available.

Combining monolithic solids and nanoparticles in microextraction units was first proposed by Hsieh et al. [65] for the enrichment of phosphopeptides from a tryptic digest of β-casein prior to MALDI–MS analysis. They prepared a monolithic solid containing TiO_2 NPs by aspirating 1 µL of reaction mixture into a polypropylene pipette tip and inserting a piece of capillary into the tip in order to create a central channel inside the monolithic phase before initiating the photopolymerization reaction. The unit was irradiated with UV light at 365 nm for 20 min and the capillary removed from the pipette tip after polymerization. Unreacted monomer and unbound TiO_2 NPs were removed by washing the monolithic solid with methanol. The authors preferred TiO_2 NPs to TiO_2 microparticles because the former had a larger surface area and dispersed better in the monomer/porogen mixed solution, thus resulting in more uniform distribution on the monolithic solid. The microextraction unit proved highly capable of preconcentrating and isolating mono- and multiphosphorylated peptides from tryptic digest α-casein and β-casein; also, it outperformed commercial ZipTipMC tips in efficiency, selectivity and recovery.

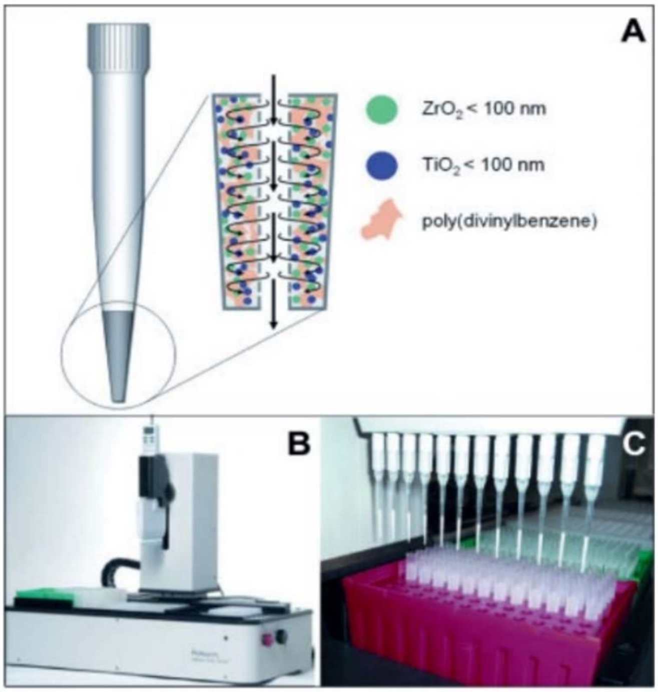

Figure 4. (A) Highly porous hollow monolithic tip structure. **(B)** MEATM Personal Purification and Enrichment System. **(C)** Custom-made TiO_2/ZrO_2-embedded poly(DVB) extraction tips. Reprinted from reference 66, with permission.

Poly(divinylbenzene) [poly(DVB)] monoliths containing embedded TiO_2, zirconium dioxide (ZrO_2) or a mixture of TiO_2 and ZrO_2 nanopowder were evaluated for the selective enrichment of phosphorylated peptides from tryptic digests [66]. The hybrid monolith was synthetized in a 10 µL pipette tip via one-step initiated free-radical polymerization with azobisisobutyronitrile (AIBN) as initiator and 2 µL of polymerization mixture. The tip was placed in an oven at 80 ºC for 2.5 h and, once the polymerization reaction completed, washed with acetonitrile and methanol to remove unreacted components. The best results as regards extraction of phosphopeptides were obtained with a mixture of nanostructured TiO_2 and ZrO_2 powder. As can be seen from Figure 4, the whole extraction–elution procedure was automated by using robotic liquid handling systems, which allowed more than a hundred samples per day to be processed. Based on the results, the monolithic microextraction unit was more selective than commercial phosphorylation-enrichment kits.

Covalent attachment of lectin-modified AuNPs to a poly propylene-encased monolith was first proposed by Alwael et al. [67]. The nanomaterial increased the surface area of the sorbent. The monolith was in situ synthesized in a polypropylene tip modified with grafted chains of EDMA to ensure covalent bonding between the EDMA monolith bed and the polypropylene wall. The differences between the microextraction units prepared with and without modification of the inner surface of the tip are apparent from the SEM images of Figure 5. A guide ring was shipped over the outside of the modified tip to obtain a repeatable bed volume for each monolith. AuNPs were immobilized onto the monolithic surface in two sequential steps, namely: (1) amination of the monolith surface by reacting grafted polymer chains of vinyl azlactone with ethylenediamine; and (2) immobilization of 20 nm citrate-stabilized AuNPs. SEM was used to confirm the presence of AuNPs and their forming a uniform surface coating. The selectivity of the pipette tip device was demonstrated with a complex mixture of non-glycosylated and glycosylated proteins differing in terminal sugar structure. Finally, the effect of the sample matrix was evaluated in terms of recovery of galactosylated glycoproteins in an Escherichia coli cell lysate, and the efficiency and selectivity were validated by LC.

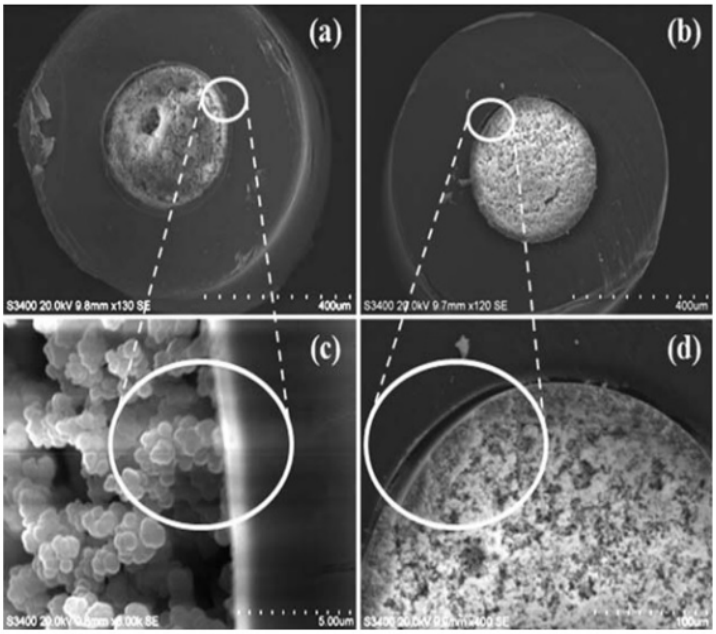

Figure 5. Scanning electron micrographs of a porous polymer monolith formed within a polypropylene pipette tip that was either modified by photografting (**A and C**) or not modified (**B and D**) prior to polymerization. Reprinted from reference 67, with permission.

More recently, Krenkova et al. reported a similar method for phosphopeptide enrichment in which IONs and HANPs were attached to the surface of a poly(HEMA-*co*-EDMA) monolith [68]. First, a colloid containing 20 nm IONs was flushed through the monolith, the color immediately turning reddish (Figure 6). This group has also incorporated HANPs into poly(HEMA-*co*-EDMA) monoliths simply by admixing them in the polymerization mixture and then starting in situ polymerization. A comparison with commercially available TiO_2 pipette tips revealed that NP-modified monoliths were more suitable for extracting phosphorylated peptides from peptide mixtures of α-casein and β-casein digests. Also, both types of NP-modified monoliths were highly stable even if the pH of the retention/elution solution was changed, which facilitated their reuse. A carbon nanotube-modified monolithic pipette tip device was recently developed for extracting antidepressants from human urine by using a fast photopolymerization approach (reaction time 2 h) to prevent MWCNTs sedimentation in the polymerization mixture [69]. Including MWCNTs in the monolithic solid increased its sorption capacity through additional π interactions of aromatic groups in the antidepressants with carbon nanoparticles.

6. Spin-column nanoparticle-hybrid monoliths

In 2008, Namera et al. [70] introduced the use of a monolithic silica spin column for sample extraction. The difference between spin columns and other microextraction devices is that, in the former, the sample solution and solvents are passed through the sorbent phase by centrifugation. For this purpose, the columns must be inserted in a rotor in each of the four steps of the extraction process, namely: conditioning, loading, washing and elution. The rotation speed used has a marked influence on the extraction efficiency that should be carefully examined in each case. Despite this limitation, the errors associated with sample handling in spin columns are considerably small relative to other microextraction formats such as pipette tips [71]. Also, spin columns afford the simultaneous processing of several samples. The operational simplicity, easy synthetic procedure and low elution volume of this type of microextraction device has enabled the successful isolation of analytes from different types of matrices including urine, serum and blood. In most cases, silica-based monolithic solids are preferentially used in this format; as a result, the extraction efficiency is also affected by the pH of the sample solution.

As with other microextraction formats, modifying monoliths with NPs has also been explored. Thus, Alwy et al. [72] developed a new microextraction unit using a monolithic spin column. For this purpose, they immobilized citrate-stabilized Fe_3O_4 NPs approximately 16 nm in size onto commercial silica monolithic spin columns (MonoSpin). The cartridge comprised a silica monolithic disk of bimodal pore morphology containing macropores (2–5 µm) and mesopores (2–50 nm). The nanoparticles were immobilized with the single-layer or layer-by-layer method, which were assessed for performance in terms of the surface density of Fe_3O_4 NPs. SEM images revealed that the layer-by-layer approach provided a thicker, more uniform NP coating. The resulting monolith was used to extract phosphorylated compounds at a 5 µM concentration with recoveries from 80 to 86%.

Recently, our group developed a straightforward, cost-effective, efficient methodology for preparing and attaching organic polymeric monoliths containing oxidized SWNHs to spin columns [73]. The housing polypropylene surface was irradiated with UV light to facilitate covalent functionalization and hence robust attachment of the monolith. This strategy was previously used to anchor methacrylate monoliths to cylindrical tubes [74]; to our knowledge, however, this was the first time an organic monolith was confined in a spin column. Also, including SWNHs in the polymer matrix provided hybrid materials with a large specific surface area and enhanced sorption capabilities. The potential of the resulting solids was demonstrated with the isolation and preconcentration of nonsteroidal anti-inflammatory drugs from biological samples.

7. Microfluidic-chip nanoparticle-hybrid monoliths

The interest in incorporating monolithic solids into microfluidic platforms for extracting and preconcentrating analytes has grown steadily ever since the first instance was reported by Yu et al. in 2001 [75]. The greatest advantage of microfluidic chips is the ability to integrate several analytical processes in a small device. Monolithic stationary phases are a great choice here as they can be formed directly inside microchip channels. Consequently, introducing NPs in monolithic materials can substantially increase their extraction efficiency and provide microchips with additional functionalities. For example, γ-alumina nanoparticles were added to a conventional poly(MAA-*co*-EDMA) monolith in an ultraviolet-transparent polymethyl methacrylate microfluidic chip [76]. The resulting poly(MAA-*co*-EDMA-Al_2O_3) monolith-based solid-phase microextraction unit was coupled to an optical fiber spectrophotometer to determine 2-amino-4-chlorophenol in chlorzoxazone tablets. γ-Al_2O_3 NPs were directly dispersed in the porogens and the polymerization reaction was in situ initiated by UV irradiation. SEM images of poly(MAA-*co*-EDMA) and poly(MAA-*co*-EDMA-Al_2O_3) monoliths revealed that microglobules in the NP-modified monolith were smaller, possibly as a result of the excellent mechanical properties of γ-Al_2O_3 increasing the specific surface area of the solid. Monoliths with and without NPs were compared in terms of extraction capacity and the poly(MAA-*co*-EDMA-Al_2O_3) monolith was found to exhibit an enrichment factor of 17.0 versus only 7.3 for the poly(MAA-*co*-EDMA) monolith. The preparation method thus provided an effective, robust approach to detecting analytes present at low concentrations in real samples.

8. Stirred units based on nanoparticle-hybrid monoliths

The previous sections are concerned with microextraction devices involving a synthetic monolithic solid and flowing solvents through its pores. However, the porous solid can alternatively be directly inserted in the sample provided the sample is properly stirred because efficient extraction with these devices relies heavily on fast mass transfer between the two phases. The sample can be stirred with an external apparatus or simultaneously with extraction in the same device. Integrated extraction/stirring techniques include stir-bar and stir-cake sorptive extraction [77]. Stir-bar extraction involves coating a glass tube having a magnetic core with the monolithic extraction phase, whether polar or nonpolar [78, 79]. Because anchoring a polymer onto the surface of a magnetic stir bar is a time-consuming process, Díaz-Álvarez et al. [80] developed an easy, alternative procedure for preparing imprinted stir bars containing embedded iron II and III oxide NPs. The superparamagnetism of the IONs conferred the hybrid monolith magnetic properties for use as a stir unit. Imprinted stir bars were prepared by bulk polymerization inside glass vial inserts used as molds. The magnetic nanoparticles were directly added and homogeneously dispersed in the polymerization mixture. However, the ION surface had to be previously modified with poly(vinyl alcohol) in order to avoid deterioration and facilitate attachment to the polymeric network through chemical bonding for increased robustness of the resulting material. In any case, the amount of NPs attached never exceeded at 7% because higher proportions had an adverse effect on mechanical stability in the resulting stir bars. The bars were assessed for performance in the extraction of triazines from soil samples, where the synthetic material exhibited a high selectivity. Our group developed another hybrid monolithic unit with integrated stirring that combined the properties of SWNHs and organic polymer monoliths [81]. The synthetic procedure, which involved copolymerization of methacrylate monomers and SWNHs, was easy and reproducible, and provided a stable, indivisible device.

Also, the presence of carbon nanoparticles led to increased porosity and rigidity, and ultimately to a higher sorbent efficiency.

Stir-cake sorptive extraction uses a monolithic cake placed in a customized holder including a protected iron wire [82]. This configuration avoids direct contact of the solid with the sample vessel. Although various organically based monoliths have to date been reported [83–85], no NP-modified monolithic solids appear to have been used in this format.

9. Nanoparticle-hybrid monoliths for in-cartridge solid-phase extraction

Monolithic solids have also been used to pack cartridges for isolating and preconcentrating analytes in a great variety of complex sample matrices [26, 86, 87]. This microextraction format has several advantages including low associated costs, short processing times, low solvent consumption, simplicity and ease of automation that have fostered its use in routine laboratories. However, extraction cartridges typically consist of silica-based material (C8 or C18), which has certain shortcomings such as a low breakthrough volume, the risk of secondary interactions with residual silanol groups, a narrow pH stability range and poor reusability [88, 89]. It is thus highly desirable to develop alternative hybrid materials to overcome these problems, and also to boost sensitivity and selectivity.

Vergara-Barberán et al. [90] reported a novel AuNP-modified monolithic sorbent for protein separation where the nanoparticles were immobilized onto an amino-functionalized monolithic surface. They assessed the extraction efficiency of the combination with proteins as model compounds. For this purpose, the monolithic bulk solid was ground in a mortar and sieved down to less than 100 μm by passage through a steel screen. High recovery values for several samples and acceptable reusability of the sorbent were demonstrated by packing only 50 mg of modified polymer in comparison with traditional C8 or C18 cartridges.

Inspired by this approach, the authors used the previous sorbent to determine small molecules such as azoxystrobin and chlorothalonil in environmental samples [91]. The ensuing method provided high recoveries, wide linear ranges and low detection limits for the two analytes thanks to the good affinity of cyano groups present in the target compounds for gold surfaces.

10. Future trends

Monolithic solids have brought about considerable improvements in both chromatographic and nonchromatographic separation techniques. In fact, they have allowed most of the shortcomings of the packed materials to be circumvented. Also, the recent incorporation of nanoparticles has led to even better performance. The combination of monolithic solids and carbon NPs is especially attractive as it reduces or even completely avoids the typical aggregation of NPs in dispersive µ-SPE or flow-based configurations. Any loss of these nanostructures can detract from their outstanding sorptive properties, and hence from their usefulness and competitiveness. The next few years will witness the consolidation of these hybrid monoliths in different extraction formats and their extension to a broader variety of analytical problems.

Hybrid monoliths built inside a capillary constitute the most flexible configuration among microextraction formats since they afford automated passage of the sample and solvents through the extraction unit and can be online coupled to highly sensitive detectors such as mass spectrometers. Also monolith-housing capillaries provide low detection limits and make the overall analytical process more competitive. Embedding nanoparticles, whether inorganic or carbonaceous, in a hybrid monolith is the most effective way of avoiding interferences or backpressure problems arising from detachment of NPs from the monolithic skeleton. The main drawbacks of this configuration are the high complexity of the synthetic procedure and potential stability problems with the nanoparticles, which tend to deposit onto the capillary lower wall by gravity during the polymerization step.

Hybrid monoliths prepared inside pipette tips or spin columns also have some advantages but mainly simplicity of the synthetic procedure and a low cost of the ingredients. In addition, pipette tips and spin columns are compatible with the equipment usually available in routine laboratories and afford the simultaneous processing of a relatively large number of samples for increased throughput. Because the supports are transparent to UV light, polymerization is completed more rapidly and the risk of NP sedimentation substantially reduced as a result. Both pipette tips and spin columns can be easily reused or disposed of, the latter choice being more effective to avoid cross-contamination in handling biological samples and, in general, complex matrices. However, neither can process large volumes, so the sensitivity can only be increased by repeating the sampling step as many times as needed to obtain an adequate enrichment factor.

Although hybrid monoliths allow a wide variety of analytical problems to be addressed, the uses of carbon NPs have focused on the determination of organic contaminants in environmental and agrifood samples. Noble metal NPs are the most compatible with biochemical analytes such as proteins by virtue of their strong interactions with specific functional groups (e.g., thiol) usually present in such analytes.

Probably, the inclusion of (bio)recognition elements to increase selectivity will also be considered shortly. In fact, although still limited, there has been some research in this direction. Molecularly imprinted polymers and some restricted-access materials provide selective ingredients that are preferably used in core-shell nanoparticles, however. Also, antibodies have clearly been surpassed by aptamers in this context owing to their better stability and compatibility with solvents [92]. The monolith-NP-aptamer combination usually includes AuNPs because they are highly biocompatible and perform very well as intermediaries for efficient anchoring of the aptamer [93–95]. These formats are usually prepared as capillary columns and used as affinity stationary phases combined with LC separation. The selectivity of these hybrid solids has been assessed with α-thrombin [93] and ochratoxin A [94] as analytes. Finally, the synthesis of monolithic solids based on nanoparticles (mainly carbon NPs) alone as in aero and xerogels, and in 3D structures, is one field to be explored in the near future in the (micro)extraction realm.

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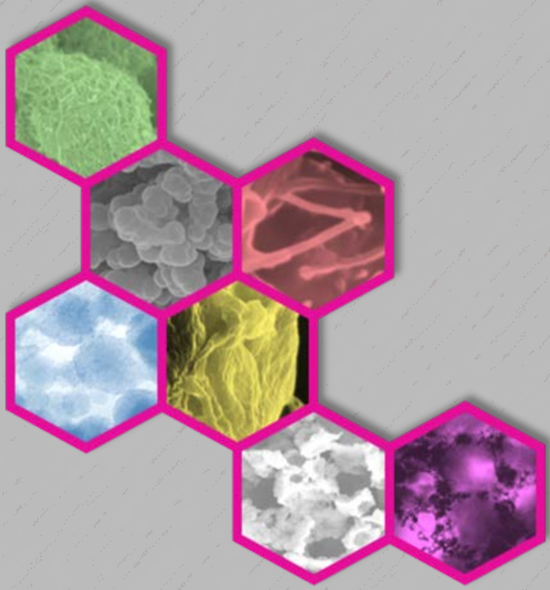
Bloque I

Sólidos Híbridos Basados en la Combinación de Polímeros Monolíticos y Nanopartículas de Carbono Preparados en Capilares

Hybrid Solids Based on the Combination of Monolithic Polymers and Carbon Nanoparticles Prepared in Capillaries



El desarrollo de nuevos sistemas de separación miniaturizados es actualmente uno de los principales desafíos en el campo de la cromatografía ya que gracias al uso de columnas con diámetros más pequeños se consigue reducir el consumo de reactivos y disolventes, así como los tiempos de análisis, y por tanto los costes, manteniendo o incluso mejorando la resolución cromatográfica y la sensibilidad del proceso de medida. En este contexto, las columnas capilares monolíticas han supuesto un punto de inflexión debido a las ventajas que presentan frente a otros tipos de fases estacionarias, entre las que destacan su versatilidad, estabilidad en un amplio rango de pH y su idoneidad para ser preparadas *in situ* en el interior del capilar. Sin embargo, a pesar de que se están realizando grandes esfuerzos para mejorar la instrumentación y metodologías necesarias para el uso de estos sistemas miniaturizados, aún existen una serie de problemas técnicos relacionados con el control y manejo de los conectores, los sistemas de inyección, así como las elevadas presiones alcanzadas en las columnas. Probablemente estos son algunos de los motivos de su limitado uso en el contexto de la microextracción. No obstante, el empleo de columnas capilares monolíticas para la extracción y preconcentración de compuestos de interés puede suponer grandes beneficios entre los que destacan: la obtención de bajos límites de detección y cuantificación a partir de bajos volúmenes de muestra, y la posibilidad de acoplamiento a diversas técnicas de detección lo cual reduce significativamente los errores humanos causados por la manipulación. Por tanto, uno de los retos planteados en esta Tesis Doctoral es la preparación de columnas capilares monolíticas, las cuales se modifican con nanoestructuras de carbono que mejoran sus propiedades sorbentes para su posterior aplicación en microextracción en fase sólida, y en última instancia en cromatografía líquida miniaturizada.

A lo largo de este Bloque de la memoria de la Tesis Doctoral se presentan diversas unidades de microextracción basadas en el uso de sólido monolíticos híbridos sintetizados en el interior de columnas capilares de sílice fundida. Además, se describen diferentes estrategias de inclusión de las nanopartículas de carbono en los sólidos monolíticos, entre las que se encuentran; el anclaje químico (***Capítulo 1***) y fotoquímico (***Capítulo 2***) a la superficie de polímeros de naturaleza orgánica, así como el atrapamiento de las nanopartículas en la matriz polimérica tanto de polímeros orgánicos (***Capítulo 3***) como de base inorgánica, es decir, de sílice (***Capítulo 4***).

Capítulo 1

Improved Microextraction of Selected Triazines Using Polymer Monoliths Modified with Carboxylated Multi-Walled Carbon Nanotubes

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Improved microextraction of selected triazines using polymer monoliths modified with carboxylated multi-walled carbon nanotubes

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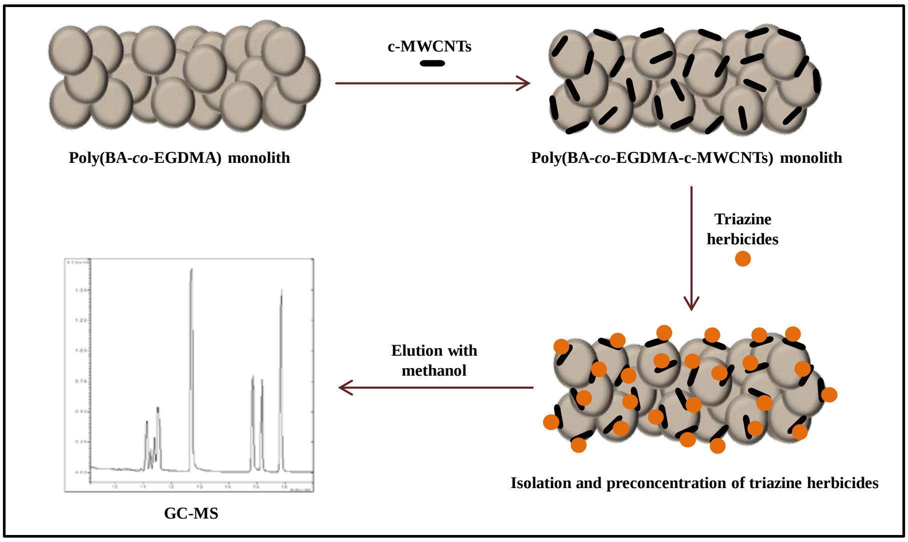


Graphical Abstract

Immobilization of carboxylated multi-walled carbon nanotubes (c-MWCNTs) on the poly(BA-*co*-EGDMA) monolith prepared inside a fused silica capillary results in improved extraction of nine target triazines from water and juice samples.

Abstract

This article reports on the enhancement of the capacity of an acrylate-based monolithic solid sorbent by anchoring carboxylated multi-walled carbon nanotubes (c-MWCNTs) in its pores and on its surface. Monolithic poly(butyl acrylate-*co*-ethyleneglycol dimethacrylate) [poly(BA-*co*-EGDMA)] was synthetized inside a fused silica capillary via free-radical polymerization, and an ethanolic dispersion of c-MWCNTs was passed through the capillary. The resulting poly(BA-*co*-EGDMA-c-MWCNTs) monolith was characterized by scanning electron microscopy to confirm the presence of the c-MWCNTs. The effect of using three different kinds of carbon nanoparticles and the microextraction step were studied using triazine herbicides as model compounds. The use of c-MWCNTs resulted in best performance in terms of extraction enhancement (compared to carboxylated single-walled carbon nanotubes and oxidized single-walled carbon nanohorns). The use of these carbon nanoparticles improved the extraction of triazines in any case when compared to using a bare poly(BA-*co*-EGDMA) monolith. The triazines were then quantified by gas chromatography with mass spectrometric detection. Detection limits ranged from 0.03 to 0.1 $\mu g \cdot L^{-1}$ (except for simazine; 0.6 $\mu g \cdot L^{-1}$), and the precision (relative standard deviation) varied between 3.0 and 11.4 %. The reproducibility between units is <14.3 % (expressed as relative standard deviation) which demonstrates the robustness of the method. The method was applied to analyze an unknown sample of orange juice and gave a value of 0.18 $\mu g \cdot L^{-1}$ for prometryn. Finally, the analysis of spiked samples of water and orange juices yielded recoveries ranging from 81 to 113 % and 75 to 125 %, respectively.

Keywords

Monolithic solid; Carboxylated multi-walled carbon nanotubes; (Micro)Solid phase extraction; Herbicides; Gas chromatography; Triazines; Orange juice.

1. Introduction

Sample preparation has been the focus of intense research in order to improve the isolation and preconcentration steps of the analytical procedures. Current trends in this context involve the simplification and miniaturization of separation techniques in both solid and liquid phase formats. The success of these tendencies depends on the efficiency of the extracting medium [1]. Nanostructured materials can be identified as a turning point on the development of new miniaturized approaches [2], as they are more efficient than silica-based or polymeric sorbents due to their high aspect ratio and chemical nature.

Monoliths are a continuous piece of a highly porous material, allowing solvents to flow through their large pores (>50 nm macropores, 2–50 nm mesopores). The monoliths can be classified in three types: polymer monoliths [3], silica-based monoliths [4] and organic-silica hybrid monoliths [5]. In the chromatographic and electrophoretic context, these materials have some advantages over particle packed columns such as: easy synthesis, mechanical stability and direct linkage of the solid with the inner walls of the support. Besides, they feature tolerance to high flows allowing fast separations of target analytes, much more efficient mass transfer, great diversity in shapes and supports and good synthesis reproducibility. Due to their versatility, sorbent monoliths have been used to improve chromatographic [6–8] and electrophoretic [9, 10] separations. Their potential has also been evaluated in the microextraction context [11–14].

Carbon nanoparticles (CNPs) have been extensively used in microextraction techniques thanks to their outstanding sorbent capacity [15]. This property is usually ascribed to the high surface to volume ratio of the nanomaterials. However, a relevant disadvantage of using CNPs as sorbent, and carbon nanotubes in particular, is their aggregation tendency due to their low solubility in common organic solvents and water. This fact hinders their use in conventional cartridge-SPE formats and also limits their packing in microcolumn inserted in flow configurations because of the high back-pressure generated. Therefore, in order to benefit from their sorbent capacity, CNPs have to be efficiency dispersed or immobilized on a surface/support, such as disk [16], controlled-pore glass [17] or porous-hollow fiber [18] to minimize or avoid the presence of aggregates.

Although there are references dealing with the use of nanoparticles to improve the chromatographic or electrophoretic separations [19–23], the combination of nanoparticles and monolithic solids as extraction phase is scarcely reported [24–26]. In the particular case of multi-walled carbon nanotubes (MWCNTs), they exhibit limited solubility in most of the porogen solvents used for the synthesis of the monolith. Aggregates of MWCNTs are observed even at very low concentrations in the polymerization mixture.

This study evaluates the potential of a poly(butyl acrylate-*co*-ethyleneglycol dimethacrylate) monolithic capillary modified with carboxylated multi-walled carbon nanotubes as a microextraction unit for preconcentration of triazine herbicides (prometon, simazine, atrazine, propazine, terbumeton, secbumeton, simetryn, prometryn and terbutryn) from waters and orange juices. The preparation of the hybrid solid has been deeply studied as well as all the variables affecting the microextraction process. Gas chromatography with mass spectrometric detection was used for analytes identification and quantification.

2. Experimental section

2.1 Reagents, materials and samples

All reagents were of analytical grade or better. Triazines (prometon, simazine, atrazine, propazine, terbumeton, secbumeton, simetryn, prometryn and terbutryn) were purchased from Sigma-Aldrich (Madrid, Spain. http://www.sigmaaldrich.com). Standard solutions of each analyte were prepared in methanol

(Sigma-Aldrich) at a concentration of 1 g·L^{-1} and stored at 4 °C. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in ultrapure Milli-Q water. Methanol was also used for triazines elution.

Uncoated fused-silica capillaries (320 µm i.d., Sigma Aldrich) were used for the preparation of the monolithic extraction unit. Ferrules 1/16″ ID, PEEK tubing 1/16″ and internal union zero volume 1/16″ to 1/16″ (Sigma-Aldrich) were also employed. The reagents used for the synthesis of the monolithic phase, butyl acrylate (BA), ethyleneglycol dimethacrylate (EGDMA), lauroyl peroxide (LPO), 2-propanol (2-PrOH), formamide, 3-(trimethoxysilyl)propyl methacrylate, ethanol, sodium hydroxide, hydrochloric acid, acetone and acetic acid were purchased from Sigma-Aldrich. Carboxylated multi-walled carbon nanotubes (c-MWCNTs, <8 nm o.d., 10–30 µm length, >95 wt% purity, 3.86 wt% functional content) and carboxylated single-walled carbon nanotubes (c-SWCNTs, 1–2 nm o.d., 5–30 µm length, >90 wt% purity, 2.73 wt% functional content) were obtained from Sigma-Aldrich. Single-walled nanohorns were purchased from Carbonium S.r.l (Padua, Italy. http://www.carbonium.it/public/site/index.php). The production of SWNHs was carried out, according to the information reported by the manufacturer, by direct graphite evaporation in Ar flow and the purity obtained was above 90 wt%. SWNHs form stable dahlia-shaped aggregates with an average diameter of 60–80 nm. Individually, the lengths of these SWNHs are in the range of 40 to 50 nm, and the diameter in the cylindrical structure varies between 4 and 5 nm. Table S1 presents the schematic structure as well as the TEM micrographs obtained for the three carbon nanoparticles used in this article. A 7 % (*v/v*) aqueous solution of ethylenediamine (Sigma-Aldrich) was used to immobilize the c-MWCNTs on the monolith. The dispersion of the c-MWCNTs was made in ethanol. In brief, 0.5 mg of c-MWCNTs were weighed, added to a glass vial and ultrasonic-assisted dispersed in 50 mL of ethanol for 30 min.

Tap and river water samples were selected for the determination of the target compounds using the monolithic microextraction unit. Water samples from the Guadalquivir river were collected in amber glass bottles (Sigma-Aldrich) without headspace and stored at 4 °C until analysis. All the aliquots were filtered using a 0.45 µm disposable Nylon filter (Análisis Vínicos, Córdoba, Spain. http://www.analisisvinicos.com) prior to analysis. The water samples were prepared with the analytes at a concentration of 1 $\mu g \cdot L^{-1}$ and then they were left to stand for 24 h prior to the analysis. The oranges and juice samples were purchased from local markets and stored at 4 °C until their use. The squeezed juice was prepared in the laboratory prior to analysis. 1 mL of both orange juices were diluted with Milli-Q water to 5 mL and filtered through a 0.20 µm disposable Nylon filter prior to the analysis.

2.2 Apparatus

SP-400 NanobaumeTM System was used to pump the c-MWCNTs dispersion through the monolithic microextraction unit. For analytes preconcentration and elution, a micro-HPLC pump Jasco 1585 (Jasco Analítica Spain, Madrid, Spain. http://www.jasco-spain.com) was employed. The poly(BA-*co*-EGDMA-c-MWCNTs) microextraction unit was connected to the pump by means of a stainless steel internal union fitted with a PEEK adapter.

Chromatographic analyses were carried out on a gas chromatograph (Varian CP-3800)-mass spectrometer (Varian 1200 MS/MS) working under single quadrupole mode and with an electron multiplier detector. The gas chromatograph was equipped with a fused silica capillary column VF-5 ms (30 m × 0.25 mm i.d.) coated with 5% phenyl-95% dimethylpolysiloxane (film thickness 0.25 µm) (Sigma-Aldrich) to separate the nine analytes.

The GC oven was programmed as follows: the initial temperature, 40 °C, was maintained for 2 min, raised up to 170 °C at 10 °C·min^{-1} and then immediately ramped at 2 ºC·min^{-1} up to 200 ºC. The final temperature, 260 °C, was reached with a ramp of 10 °C·min^{-1} and maintained for 2 min. The injector temperature was 280 °C and the splitless mode was selected. The injection volume, 2 µL of methanol, was measured with a 5 µL microsyringe (Hamilton Co., Nevada, USA). The carrier gas used was helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.0 mL·min^{-1}, and it was regulated by digital pressure controller. The transfer line and ionization source were maintained at 280 ºC and 250 ºC, respectively.

The ionization mode employed in the mass spectrometer was electron impact (EI) with ionization energy of 70 eV. Mass spectra were acquired using the selected ion monitoring mode (SIM), dividing the analysis time in four temporal windows: the first one with m/z 200, 201, 210 and 214 (from 9 to 12.15 min), the second one selecting the m/z 196 (from 12.15 to 13.72 min), the third one with m/z 213 and 241 (from 12.15 to 15.45) and the fourth temporal window selecting m/z 226 (15.45 to 28.25), all of them at 1 scan/s. Chromatograms were acquired and processed using MS Workstation on an AMD Sempro™ Processor computer (https://www.bruker.com) which also controlled the whole system.

A JEOL JSM 6300 scanning electron microscopy (Isaza, Alcobendas, Spain) was also used to obtain the micrographs of the monolithic solid with and without carbon nanoparticles. An ultrasonic bath model 3510 from Branson (Connecticut, USA) was also used in different steps of the procedure. In the preparation of the poly(BA-*co*-EGDMA) monolithic capillary, an oven (Binder, Madrid, Spain) was also needed to maintain the temperature at 70 ºC during the polymerization step.

2.3 Preparation of monolithic solid

The fused-silica capillary was pretreated to favor the covalent binding of the monolithic phase to the capillary inner wall [19]. For this aim, the capillary (1 m in length) was flushed with acetone (5 min) and Milli-Q water (20 min) at a flow rate of 1 mL·min^{-1}. NaOH (0.2 M) was sequentially pumped through the capillary using the micro-HPLC pump for 30 min at a flow rate of 50 µL·min^{-1}. Then, the capillary was rinsed with Milli-Q water (1 mL·min^{-1}, 20 min), and then a 0.2 M HCl stream was passed for 30 min (50 µL·min^{-1}) to protonate the silanol groups previously formed. Next, the acid was removed with Milli-Q water and ethanol (1 mL·min^{-1}, 30 min), followed by a 20% (*v/v*) solution of 3-(trimethoxysilyl)propyl methacrylate in ethanol (adjusted to pH 5 using acetic acid) at a flow rate of 50 µL·min^{-1} (45 min). Finally, the capillary was washed with acetone (1 mL·min^{-1}, 20 min) and dried under a nitrogen stream. The whole pretreatment of fused-silica capillary was performed at room temperature. Pieces of 3 cm were then used to synthesize the monolith.

The polymerization mixture is composed of 20 wt% monomers (25 wt% BA and 75 wt% EGDMA) and 80 wt% porogens (50 wt% 2-PrOH and 50 wt% formamide). As free-radical initiator, 0.3 wt% of LPO (out of the total weight of monomers) was added to the polymerization mixture. This reaction mixture was sonicated for 20 min and purged with nitrogen for 10 min. A piece of the pretreated fused-silica capillary (3 cm in length) was filled with the reactant solution by means of a syringe and then sealed with a septum at both ends. Next, the capillary was introduced into an oven at 70 ºC for 24 h. After completing the polymerization reaction, poly(BA-*co*-EGDMA) monolith was washed with methanol to remove the unreacted monomers and porogenic solvents.

2.4 Immobilization of carboxylated multi-walled carbon nanotubes on the monolith

In order to immobilize the carbon nanoparticles on the monolithic solid, primary amine functional groups were generated on its pores and surface [27]. For this purpose, an ethanolic solution of ethylenediamine (7% (*v/v*)) was pumped through the capillary for 90 min at a flow rate of 50 µL·min^{-1}. Then, the capillary was washed with water to neutral pH for 30 min at a flow rate 0.3 mL·min^{-1}. Next, the poly(BA-*co*-EGDMA) monolith was dried under a nitrogen stream and an ethanolic dispersion of 0.01 g·L^{-1} of the c-MWCNTs was pumped at a flow rate of 0.3 mL·min^{-1} for 5 min under continuous stirring using the set-up represented in Fig. 1A.

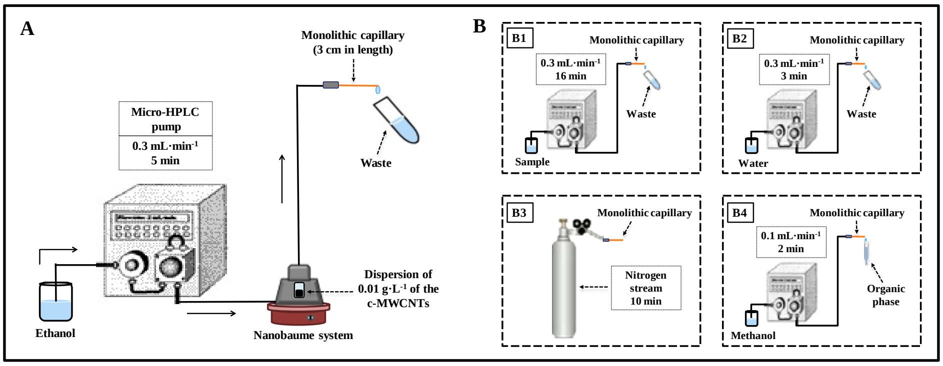


Figure 1. (A) Instrumental set-up used for the immobilization of c- MWCNTs on the monolithic capillary. **(B)** Schematic representation of the microextraction procedure for the extraction of the triazine herbicides from waters and orange juice; ***(B1)*** sampling, ***(B2)*** washing, ***(B3)*** drying, and ***(B4)*** desorption.

Micrographs of the cross-section of monolithic capillary columns (320 µm i.d.) were obtained for the poly(BA-*co*-EGDMA) monolith (Fig. 2A) and the poly(BA-*co*-EGDMA-c-MWCNTs) monolith (Fig. 2B). The micrographs were obtained using scanning electron microscopy. The section of the capillaries was coated with gold to increase the conductivity. The presence of the nanoparticles was corroborated by comparing both micrographs.

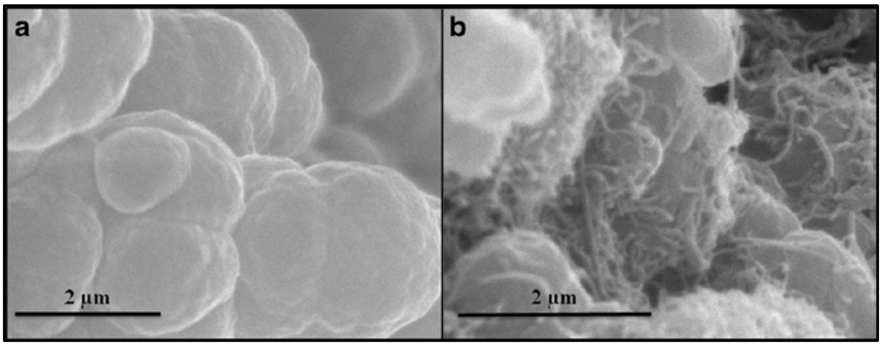


Figure 2. (a, ×15000) Scanning electron microscopy of poly(BA-*co*-EGDMA) and **(b, ×20000)** poly(BA-*co*-EGDMA-c-MWCNTs) monolith.

2.5 Microextraction procedure

The poly(BA-*co*-EGDMA-c-MWCNTs) monolith was used for the isolation and preconcentration of triazine herbicides from waters and juices. The microextraction is schematically depicted in Fig.1b and it is as follows. First, 3 mL of aqueous standards or samples containing the nine target analytes at concentrations within the linear range were passed through the microextraction unit at a flow rate of 0.3 $mL\cdot min^{-1}$ for 10 min, followed by Milli-Q water (3 min). Prior to elution, the aqueous phase remaining in the column was removed by means of a nitrogen stream (10 min). After that, the retained analytes were eluted with 200 μL of methanol at flow rate of 0.1 mL·min^{-1}. An evaporation-redissolution step was included in order to reduce the final volume to 20 μL, thus increasing the method sensitivity. Finally, 2 μL of the organic phase with the extracted analytes were injected into the gas chromatograph/mass spectrometer for their separation and detection. The chromatographic peak areas were used as analytical signals.

Between samples, the poly(BA-*co*-EGDMA-c-MWCNTs) monolith was conditioned with methanol (1.3 mL, 0.1 mL·min^{-1}), dried with a nitrogen stream for 10 min and finally rinsed out with Milli-Q water. Following this procedure, the monolithic capillary can be reused for 20 times without efficiency losses. Longer uses reduce the extraction efficiencies in ca. 30 %.

3. Results and discussion

3.1 Variables affecting to the preparation of the poly(BA-*co*-EGDMA-c-MWCNTs) monolith

The polymerization was carried out in one-step procedure using a 3 cm pretreated silica capillary, mixing monomers, porogens and an initiator at 70 ºC for 24 h. The temperature and reaction time were fixed according to the indications of Viklund et al. [28]. The initial experimental conditions were: 20 wt% monomers (50 wt% BA and 50 wt% EGDMA) and 80 wt% porogens (50 wt% 2-PrOH and 50 wt% formamide). A solution of LPO (0.3 wt% out of the total weight of the monomers) was used as an initiator of the reaction. The poly(BA-*co*-EGDMA) monolithic capillary was evaluated using the determination of triazine herbicides as model compounds. For this purpose, 4.8 mL of a working standard solution containing the nine analytes at a concentration of 1 μg·mL^{-1} was preconcentrated, using methanol as eluent. Three replicate analyses for each monolithic column to evaluate also the repeatability of the different extraction units.

The first variable studied was the monomers/porogens ratio within the following proportions: 20/80% (*w/w*), 40/60% (*w/w*), 60/40% (*w/w*) and 80/20% (*w/w*). The high percentage of monomers resulted in the smaller pores and therefore it led to an increased flow resistance. Thus a 20/80% (*w/w*) ratio was selected for further experiments.

Next, the BA/EGDMA ratio was studied within the following percentages: 75/25% (*w/w*), 60/40% (*w/w*), 40/60% (*w/w*) and 25/75% (*w/w*). When the ratio was 75/25% (*w/w*) the resulting pores were too small, and the high backpressure generated hindered the flow of solvents through the capillary. However, although monoliths prepared with the other ratios exhibited a favourable permeability to flow the solvents, the extraction efficiency of the bare monolithic solid towards the triazines was very low as it shown in the Fig. 3A.

Therefore, the inclusion of carbon nanoparticles in the microextraction unit was deeply studied, including both the type and their amount. For this purpose, commercially available carboxylated carbon nanotubes (c-SWCNTs, c-MWCNTs) and laboratory-oxidized carbon nanohorns (o-SWNHs) were selected taking into consideration their better dispersibility in organic media [29]. Dispersions of each carbon nanoparticle were prepared at a concentration of 1 $mg\cdot L^{-1}$. A volume of 900 µL was flushed through the monolithic capillary column at a flow rate of 0.3 $mL\cdot min^{-1}$ in order to retain the nanoparticles on the microporous material.

When the proportion was 60/40% (*w/w*), the monolithic solid was collapsed under pressure during the procedure of the immobilization of the carbon nanoparticles. While for the ratios 40/60% (*w/w*) and 25/75% (*w/w*) it was possible to anchor the NPs on the monolithic surfaces, obtaining a greater increase when the percentage of BA decreased as regards that of the cross-linker. We attribute this to the highest adsorption on the larges pores when dispersions of the NPs were passed through the monolithic solid. For this reason, the selected ratio was 25/75% (*w/w*) of BA/EGDMA. Fig. 3A exemplified this behavior for c-MWCNTs and terbutryn as model NPs and compound, respectively.

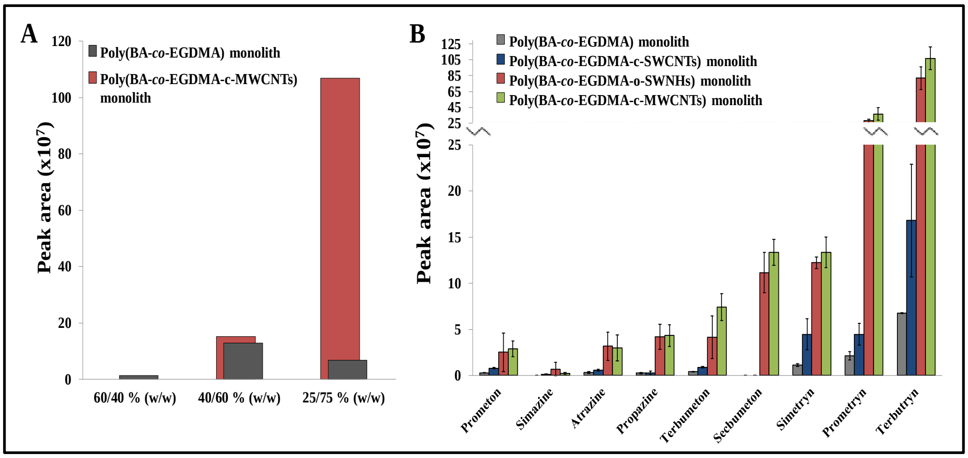


Figure 3. (A) The relative extraction efficiency for terbutryn as model compound, using the bare poly(BA-*co*-EGDMA) monolith and the poly(BA-*co*-EGDMA-c-MWCNTs) monolith as microextraction unit. **(B)** Comparison of the analytical performance of poly(BA-*co*-EGDMA) monolith without nanoparticles and the monolith with c-MWCNTs, o-SWNHs and c-MWCNTs immobilized on its pores for 20/80 % (*w/w*) and 25/75% (*w/w*) proportions of monomers/porogens and monomers/cross-linker ratios, respectively.

Besides, as it is shown in Fig. 3B, the presence of the c-MWCNTs, c-SWCNTs, and o-SWNHs increased the retention of the triazines on the microextraction unit in comparison with the bare monolithic solid. The best results were obtained using c-MWCNTs as modifiers of the bare poly(BA-*co*-EGDMA) monolith. They exhibit the highest sorbent capacity owing to their larger size and number of sheets in comparison with the other carbon nanoparticles (c-SWCNTs and o-SWNHs). Also, the precision of the results (n=3) expressed as standard deviation and reflected in the Fig. 3B as error bars, was better for almost all the analytes. The better performance of o-SWNHs as regards c-SWCNTs can be explained taking into consideration their ability to form stable large aggregates (60–80 nm) which results in higher extraction capacity. From these results, c-MWCNTs were selected.

The influence of the amount of c-MWCNTs immobilized on the monolithic solid surface was tested using ethanolic dispersions of the nanoparticles at concentrations of 0.001, 0.01 and 0.05 g·L^{-1}. Aliquots of 900 µL were passed through the column at a flow rate of 0.3 mL·min^{-1}. The highest concentration generated a backpressure in the system, probably due to the aggregation of the nanoparticles which resulted in pores blockage. The results obtained for the other two concentrations are shown in Fig. 4. As expected, the higher amount of nanoparticles resulted in higher efficiency and therefore, a concentration of 0.01 g·L^{-1} of the c-MWCNTs in ethanol was selected.

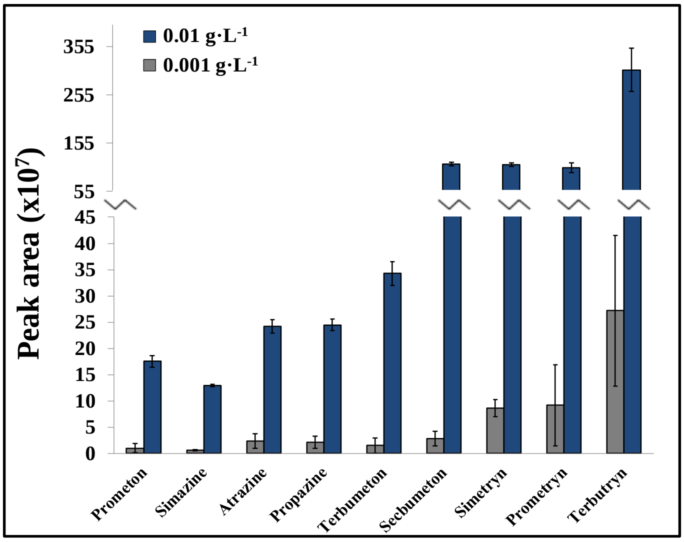


Figure 4. Influence of the concentration of the c-MWCNTs dispersion on the triazines retention.

This dispersion was flushed in an interval from 3 to 25 min at a flow rate of 0.3 mL·min^{-1} (0.9–7.5 mL). The graphic comparison of the results given in Fig. 5, shows that 1.5 mL (5 min) was the best option since higher values resulted in a decrease of the extraction, probably due to bundle formation on the pore and surface of the monolith.

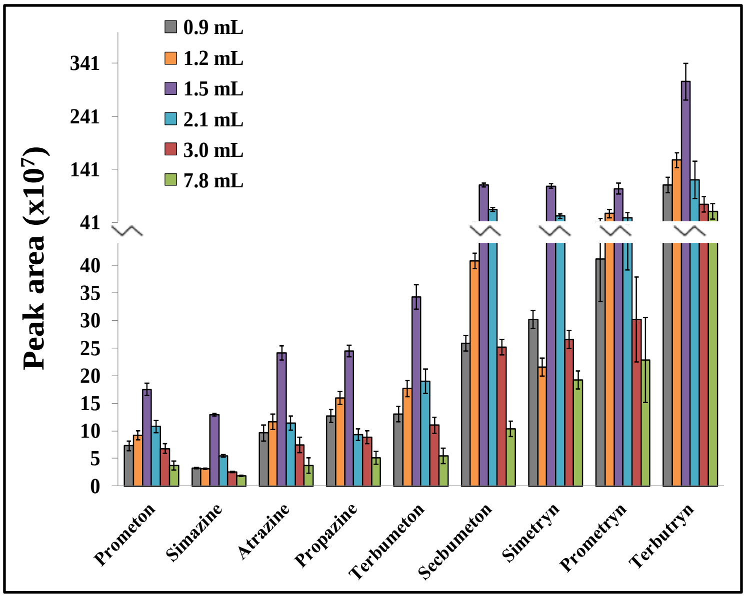


Figure 5. Effect of the volume of the 0.01 g·L^{-1} c-MWCNT dispersion passed through the poly(BA-*co*-EGDMA) monolith at a flow rate of 0.3 mL·min^{-1}.

3.2 Evaluation of the variables affecting to the microextraction process

The variables directly related with the extraction step were studied using aqueous standards containing the selected triazines at a concentration of 1 µg·mL^{-1}. The following parameters were optimized: (a) Sample flow rate; (b) sample volume and; (c) elution volume. Respective data and Figures are given in the *Electronic Supporting Material*.

The following experimental conditions were found to give the best results: (a) a sample flow rate of 0.3 mL·min^{-1}, (b) a sample volume of 3 mL and, (c) an elution volume of 0.2 mL.

3.3 Analytical figures of merit

Once optimized, the monolithic extraction unit was characterized in terms of sensitivity, linearity, and precision. The corresponding calibration graphs were constructed by using aqueous standards containing the nine analytes at concentrations in the range 0.1–1000 µg·L^{-1}. Standards were processed in duplicate using the optimized method, and 2 µL of the organic extract was injected into the GC/MS for analysis. The corresponding equations were obtained by plotting the peak areas of the characteristic m/z fragment ions against the concentration for each target analyte.

The limits of detection (LODs) were calculated as the concentrations giving a signal-to-noise ratio (S/N) of 3. As it can be seen in Table 1, they were in the range 0.03–0.6 µg·L^{-1}. The limits of quantification (LOQs) were calculated as the concentration providing chromatographic peak areas ten times higher than the background noise and varied between 0.1 and 0.4 µg·L^{-1} for all analytes (simazine excepted, 1 µg·L^{-1}).

The precision of the method (intra and inter-day conditions), expressed as relative standard deviation (RSD) was calculated from five individual standards prepared at a concentration of 1 µg·mL^{-1} and it was lower than 11.4 % for all the analytes.

Table 1. Analytical figures of merit of poly(BA-*co*-EGDMA-c-MWCNTs) monolithic microextraction unit to the determination of the target triazines.

				Precision		
Analyte	**m/z**	**LOD ($\mu g \cdot L^{-1}$)**	**LOQ ($\mu g \cdot L^{-1}$)**	**Intra-day RSD (%, n=5)**	**Inter-day RSD (%, n=5)**	**Inter-units RSD (%, n=5)**
Prometon	210	0.10	0.4	9.4	11.4	6.8
Simazine	201	0.60	1	4.2	7.1	7.9
Atrazine	200	0.10	0.4	11.4	9.9	3.9
Propazine	214	0.10	0.4	6.0	5.3	8.2
Terbumeton	210	0.10	0.4	7.2	10.3	8.7
Secbumeton	196	0.03	0.1	3.0	7.4	10.0
Simetryn	213	0.03	0.1	4.1	8.6	9.0
Prometryn	241	0.03	0.1	5.9	8.1	13.8
Terbutryn	226	0.03	0.1	10.5	11.3	14.3

Fig. 6 shows a chromatograph with the different m/z fragment ions obtained after the analysis of a standard with the nine triazines (1 $\mu g \cdot L^{-1}$) following the microextraction procedure. In addition, the reproducibility between extraction units was evaluated. For this purpose, five poly(BA-*co*-EGDMA-c-MWCNTs) monolithic microextraction units were prepared and a standard solution of the nine triazines (1 $\mu g \cdot mL^{-1}$) was analyzed. The results, expressed as RSD, are also given in Table 1 and they were acceptable in all cases.

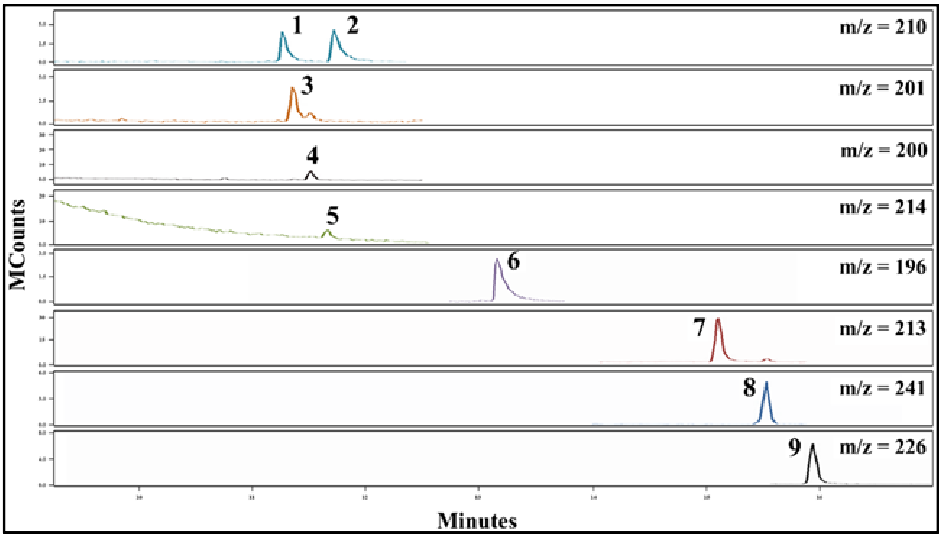


Figure 6. Chromatogram obtained after monolith microextraction of a standard with the target analytes at a concentration of 1 µg·L^{-1}. Peaks: **(*1*)** Prometon, **(*2*)** Terbumeton, **(*3*)** Simazine, **(*4*)** Atrazine, **(*5*)** Propazine, **(*6*)** Secbumeton, **(*7*)** Simetryn, **(*8*)** Prometryn, **(*9*)** Terbutryn.

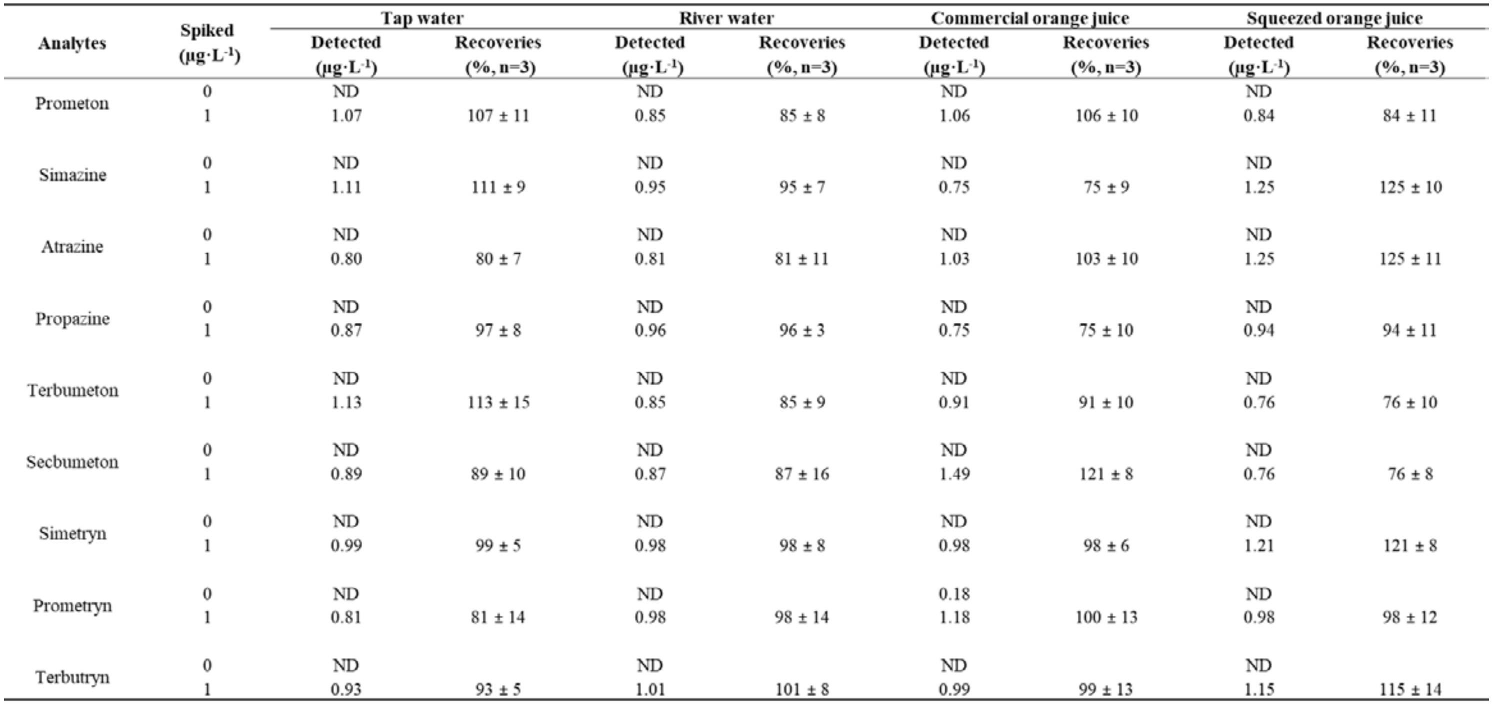
3.4 Analysis of water and orange juice samples

Prior to the analysis of real samples, the identification of potential interferences from the matrix on the quantification of the analytes is a relevant issue, especially when analyzing unknown samples. Therefore, the accuracy of the proposed method was evaluated through a recovery study. As neither certified reference materials (CRMs) nor quality control (QC) samples were available for this specific analytical problem, different blank water and juice samples were fortified with the nine target analytes (prometon, simazine, atrazine, propazine, terbumeton, secbumeton, simetryn, prometryn and terbutryn) at a concentration of 1 µg·L^{-1}, and they were left to stand for 24 h prior to analysis.

The fortified samples were analyzed using the extraction method, and the concentration for each triazine was calculated by interpolating the peak area obtained in the corresponding calibration graph. The recovery values were calculated dividing the concentration found by the concentration added and expressed in percentage. Each sample was analyzed by triplicate; the results obtained are listed in Table 2. As it can be seen, they were acceptable in all instances and they are ranged from 75 to 125 %.

The extraction method was applied to the determination of the target triazines in two water samples (river and tap waters) and two types of orange juice samples (squeezed and commercial). Aliquots of 3 mL of water and juice samples were passed through the poly(BA-*co*-EGDMA-c-MWCNTs) monolith and processed under the optimum conditions. As a result, none of the analytes were found in waters and squeezed orange juices. However, as it can be seen in Table 2 a low content of prometryn was detected in commercial orange juices. Herbicide residues may be present in the juice made from concentrate due to the great consumption on agrochemical for the protection of crops in agriculture and they can be transferred from orange peels to juices.

Table 2. Recovery study for prometon, simazine, atrazine, propazine, terbumeton, secbumeton, simetryn, prometryn and terbutryn spiked to water samples and orange juices analyzed following c-MWCNTs monolithic extraction unit.



Analytes	Spiked (µg·L^{-1})	Tap water		River water		Commercial orange juice		Squeezed orange juice	
		Detected (µg·L^{-1})	Recoveries (%, n=3)	Detected (µg·L^{-1})	Recoveries (%, n=3)	Detected (µg·L^{-1})	Recoveries (%, n=3)	Detected (µg·L^{-1})	Recoveries (%, n=3)
Prometon	0	ND		ND		ND		ND	
	1	1.07	107 ± 11	0.85	85 ± 8	1.06	106 ± 10	0.84	84 ± 11
Simazine	0	ND		ND		ND		ND	
	1	1.11	111 ± 9	0.95	95 ± 7	0.75	75 ± 9	1.25	125 ± 10
Atrazine	0	ND		ND		ND		ND	
	1	0.80	80 ± 7	0.81	81 ± 11	1.03	103 ± 10	1.25	125 ± 11
Propazine	0	ND		ND		ND		ND	
	1	0.87	97 ± 8	0.96	96 ± 3	0.75	75 ± 10	0.94	94 ± 11
Terbumeton	0	ND		ND		ND		ND	
	1	1.13	113 ± 15	0.85	85 ± 9	0.91	91 ± 10	0.76	76 ± 10
Secbumeton	0	ND		ND		ND		ND	
	1	0.89	89 ± 10	0.87	87 ± 16	1.49	121 ± 8	0.76	76 ± 8
Simetryn	0	ND		ND		ND		ND	
	1	0.99	99 ± 5	0.98	98 ± 8	0.98	98 ± 6	1.21	121 ± 8
Prometryn	0	ND		ND		0.18		ND	
	1	0.81	81 ± 14	0.98	98 ± 14	1.18	100 ± 13	0.98	98 ± 12
Terbutryn	0	ND		ND		ND		ND	
	1	0.93	93 ± 5	1.01	101 ± 8	0.99	99 ± 13	1.15	115 ± 14

ND: not detected

4. Conclusions

The use of monolithic solids in the microextraction context has been recently reviewed [14]. Table 3 summarizes the comparison of the present method with other monolithic packings for the extraction and isolation of triazine herbicides from different samples. Most of these extraction units are based on methacrylate monolithic columns, and LODs ranged from 0.18 to 95.0 $\mu g \cdot L^{-1}$. The extraction of efficiency of these porous materials has been enhanced by the incorporation of nanoparticles, and especifically carbonaceous ones, in the monolith. In this regard, carboxylated single-walled carbon nanotubes, have been used to improve the sorption capacity of poly(MAA-*co*-EDMA) monoliths. In this approach, the nanoparticles are added to the polymerization mixture in such a way that they are finally embedded into the solid. This procedure presents as an advantage the higher stability of the hybrid sorbent as the nanoparticles are included in the polymer. However, only those nanoparticles remaining on the pores are available for interaction. In addition, nanoparticles must be soluble in the polymerization mixture (usually the porogenic solvent) to minimize the aggregation of the material. Moreover, avoiding the sedimentation of the nanomaterial during polymerization also has to be taken into account in order to obtain a homogeneous distribution.

Table 3. Comparison of the performance of the poly(BA-*co*-EGDMA-c-MWCNTs) monolith versus other monolithic sorbent described in the literature for the determination of triazine herbicides

Monolithic sorbent	Selected triazines	Packing	Sample	LODs	Precision (RSD, %)	Recovery (%)	Reference
Poly(MAA-*co*-EDMA-SWCNT) monolith	Simazine, atrazine, prometon, ametryn, propazine, and prometryne	Monolithic capillary column	Lake water and orange juice	0.02-0.14 $\mu g \cdot L^{-1}$	3.1-10.9	85.0-106.0	[26]
Poly(MAA-*co*-EGDMA) monolith	Cyanazine, simazine, atrazine, prometon, ametryn, and prometryn	Monolithic capillary column	Cereals	1.1-2.8 $\mu g \cdot kg^{-1}$	1.4-5.5	73.4-107.2	[30]
Poly(MAA-*co*-EDMA) monolith	Atrazine, 2-amino-4-methoxy-6-methyl-1,3,5-triazine, terbutylazine, and ametryn	Monolithic MIP-SPME fiber	Lake water	0.18-0.35 $\mu g \cdot L^{-1}$	5.3-12.0	72.8-113.2	[31]
Poly(MAA-*co*-EGDMA) monolith	Atrazine, simazine, propazine, cyanazine, ametryn, terbutryn, and prometryn	Monolithic MIP-SPME fiber	Tap water, onion and rice	20-88 $\mu g \cdot L^{-1}$	6.5-11.6	87.8-99.6	[32]
Poly(MAA-*co*-EGDMA) monolith	Atrazine, simazine, cyanazine, ametryn, prometryn and terbutryn	Monolithic MIP-SPME fiber	Grape juice, tap water and groundwater.	2.6-42 $\mu g \cdot L^{-1}$	4.4-12.1	82.1-93.5	[33]
Poly(MAA-*co*-EDMA) monolith	Ametryn, prometryn, terbutryn, atrazine, simazine, propazine, and cyanazine	Monolithic MIP-SPME fiber	Tap water, rice, maize and onion	14-95 $\mu g \cdot L^{-1}$	5.2-11.8	85.1-99.8	[34]
Poly(BA-*co*-EGDMA-c-MWCNTs) monolith	Prometon, terbumeton, simazine, atrazine, propazine, secbumeton, simetryn, prometryn, terbutryn	Monolithic capillary column	Tap and river water, and orange juice	0.03-0.1 $\mu g \cdot L^{-1}$	3.0-11.4	75.0-125.0	Present work

LODs limit of detections, RSD relative standard deviation.

Acknowledgments

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Electronic Supplementary Material

1. Evaluation of the variables affecting to the microextraction process

The variables directly related with the extraction step were studied using aqueous standards containing the selected triazines at a concentration of 1 µg·mL^{-1}. The initial experimental conditions for the microextraction process were: 4.8 mL of aqueous standard pumped at 0.3 mL·min^{-1} and an elution with 500 µL of methanol at a flow rate 0.1 mL·min^{-1}.

The first variable studied the sample flow rate. A sample volume of 4.8 mL was pumped at 0.3, 0.4 and 0.5 mL·min^{-1}. As it was expected, the best results in terms of peak area corresponded to 0.3 mL·min^{-1} as it maximized the contact time between the analyte and the sorbent (Fig. S1). Lower flow-rates were not evaluated as they negatively affected to the sample throughput.

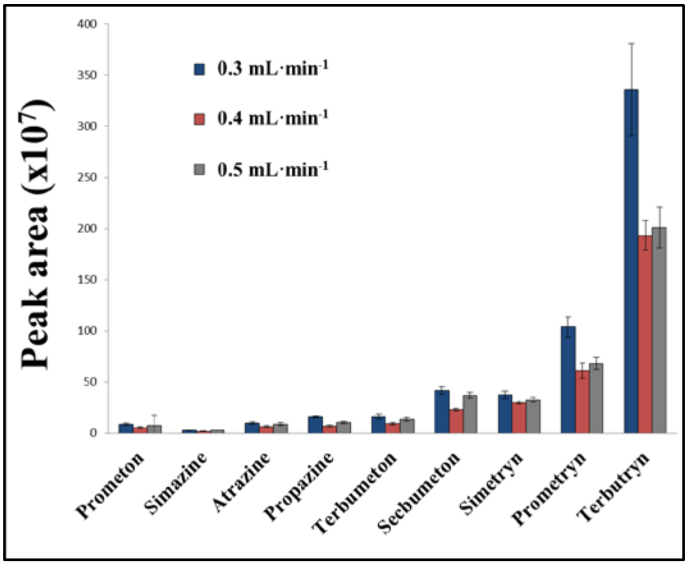


Figure S1. Effect of the sample flow rate on the extraction of target analytes.

The next variable evaluated was the sample volume within the interval 1.5–6 mL. As it can be seen in Fig. S2, the maximum extraction efficiency for the target triazines was achieved for a volume of 3 mL, decreasing over this value due to a breakthrough effect.

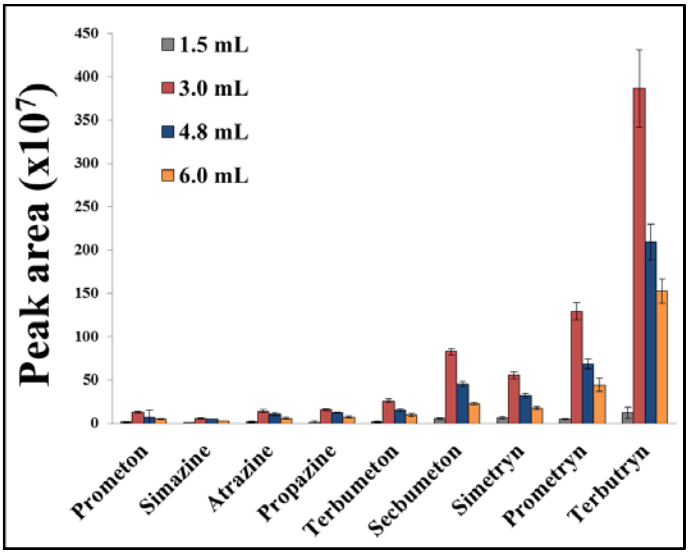
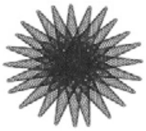
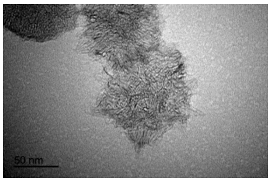
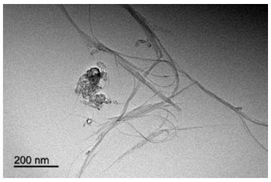
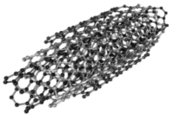
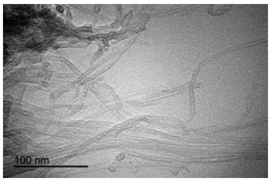


Figure S2. Effect of the sample volume on the extraction of target analytes.

Finally, the elution volume was tested in range of 100–500 µL. The flow rate was maintained at 0.1 mL·min^{-1} and the methanolic extract was collected in a 1.5 mL glass vial. Quantitative elution was obtained for a volume of 200 µL.

Table S1. Schematic representation and transmission electron microscopy (TEM) images of the three carbon nanostructures.

Carbon nanoparticle	Structure	TEM images
SWNH		
SWCNT		
MWCNT		

Capítulo
2

Carbon Nanostructures Incorporated on Methacrylate Monoliths for Separation of Small Molecules by Nano-Liquid Chromatography

Microchemical Journal, 139 (2018) 222-229

Carbon nanostructures incorporated on methacrylate monoliths for separation of small molecules by nano-liquid chromatography

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Highlights

- Comparison of embedded and photografted SWNHs on polymethacrylate monoliths.
- First example of photografted carbon nanostructures on polymeric monolithic columns.
- Effect of the amount of SWNHs and grafting time on the performance of composites.
- Enhanced nano-LC separations of small aromatic molecules.

Keywords

Methacrylate monoliths; Carbon nanohorns; Multi-walled carbon nanotubes; Nano-LC; Photografting; UV initiation.

Abstract

Carbon nanostructures have been incorporated to polymethacrylate monoliths to develop novel stationary phases with enhanced separation performance in nano-liquid chromatography (nano-LC). Two incorporation approaches of these nanomaterials in monoliths were investigated. Single-walled carbon nanohorns (SWNHs) were first incorporated to the polymerization mixture, followed by UV initiation. The resulting stationary phases with embedded SWNHs led to an increase in the separation performance of analytes compared to the parent monolith. Alternatively, a two-step photo-grafting approach was also adopted to attach the carbon nanoparticles onto the polymer monolith surface. The effect of the amount of SWNHs and irradiation time on the performance of the resulting composites was investigated. Moreover, these novel stationary phases were characterized by scanning electron microscopy. The photografted modified with SWNHs led to an enhanced separation performance (up to 6000 plates·m^{-1}) for test analytes. Besides, stationary phases containing oxidized SWNHs and multi-walled carbon nanotubes (MWCNTs) grafted on polymeric monoliths were prepared and a comparison in terms of separation performance was accomplished. The photografted columns with SWNHs also showed satisfactory reproducibility in terms of retention time and efficiency (RSD values below 8.6 and 1.4%, respectively).

1. Introduction

Polymethacrylate-based monolithic columns developed by Svec et al. [1–3] have several good features as easily adjustable polarity, fine control for pore characteristics and high stability under extreme pH conditions (pH 2–12). These stationary phases have proven ideal media for the efficient separation of biopolymers (e.g. proteins) using gradient elution in high-performance liquid chromatography (HPLC) [4, 5] or simply isocratic elution in capillary electrochromatography (CEC) [6–8]. However, these macroporous polymeric monoliths suffer from low surface areas due to the absence of a mesoporous structure and in turn limited retention toward small solutes in HPLC.

In any case, the ideal flow features of these polymer-based monoliths such as their high permeability and through-pores make them suitable supports for the incorporation of nanoparticles, which can be exploited to develop stationary phases that can improve the low surface area (by increasing the surface-to-volume ratio) as well as to afford distinct selectivity toward a wide range of solutes.

In this context, the preparation of polymer-based monolithic columns modified with carbon nanostructures has gained relevance in the field of chromatographic separations. This fact is due to unique properties of carbon nanoparticles, such as their large specific surface and hydrophobic nature, which improves in both selectivity and retention of small molecules. Along this topic, Li et al. [9] have studied the incorporation of pretreated single-walled carbon nanotubes (SWCNTs) into a vinylbenzyl chloride-based monolithic column for micro-LC and CEC. The presence of these nanostructures in the polymeric matrix improved the efficiency and enhanced retention of capillary/nano-LC separations. Also, multi-walled carbon nanotubes (MWCNTs) have been embedded into polymeric matrices containing glycidyl methacrylate (GMA) [10, 11] and benzyl methacrylate [12] as bulk monomers. Other types of carbon nanoparticles less common studied such as single-walled carbon nanohorns (SWNHs) have been recently described. Thus, Zhao et al. [13] have entrapped single-walled nanohorns (SWNHs) into a poly(styrene-*co*-divinylbenzene) monoliths using thermally initiated polymerization to afford capillary columns for CEC.

In all these works, the composites have been usually prepared by thermal polymerization, which involves rather long reaction times (20–24 h). This undoubtedly favours the aggregation and sedimentation phenomena of these carbon nanomaterials in the polymerization mixture, being a drawback for fully displaying their excellent adsorption capabilities. In this sense, the adoption of faster initiating ways, such as UV initiation, should be desirable. Thus, polymerization can be accomplished in short times (15–30 min), giving polymer networks with homogenously embedded carbon nanoparticles. Thus, in a previous work [14], we have demonstrated that this initiating way could be used to prepare polymeric monoliths with embedded carboxy-modified SWCNTs for CEC separation of polyaromatic hydrocarbons and even able to enantioseparate N-derivatives of amino acids. On the other hand, in all these studies, carbon nanomaterials are encapsulated within the polymer matrix, being few of them accessible to interact with analytes, which did not allow to fully exploit the capabilities of these carbon nanomaterials. In this sense, alternatives based on direct attachment to these nanoparticles to pore surface instead to their direct addition to the polymerization mixture should be also explored. Thus, Chambers et al. [10] attached shortened oxidized MWCNTs on the pore surface of a monolith via electrostatic interactions between carboxylate ions present in the surface of nanoparticles and an amine functionalized GMA-based monolith. This strategy led to improvements in both retention and efficiency compared to the embedding approach of MWCNT.

An alternative strategy for functionalization of polymeric-based monoliths involves the use of UV photografting techniques [15–19]. Essentially, these methods involve filling the pores of a monolith with a functional monomer in the presence of a free radical initiator (benzophenone) and an inert solvent, giving as a result the grafting of target monomer directly onto the surface by a process of hydrogen abstraction [20, 21]. In this way, grafted groups onto the monolithic matrix with different functionalities such as anion exchange [22, 23], cation exchange [24, 25], or protein immobilized [18] via grafted reactive functional monomers [26–29] can be produced. Also, photo-grafting methodology has been successfully used for surface functionalization of carbon nanotubes (MWCNTs) [30] and other carbon nanostructures (graphene) [31]. However, to our knowledge, the immobilization of these carbon nanomaterials onto pore surface monolith using UV-photografting has not yet been explored.

Within carbon nanostructures, SWNHs show good adsorbent ability due to their high surface area and its unique conical structure. They have enough large intertubular gaps for permeation of small molecules, which provide them an enhanced adsorption capacity as regards other carbon nanostructures. The rough surface structure of the SWCNH aggregates (with dahlia-like shape) results in weak Van der Waals interactions between aggregates and thus SWCNH has better dispersion in solvents than carbon nanotubes [32]. Moreover, oxidation conditions can be applied to generate opening side-wall nanowindows, which permit the penetration of small molecules and increase their surface specific area. Due to their unique structures and properties, several studies have demonstrated their potential in analytical field, mostly in the context of microextraction techniques [33–35], with scarce applications in chromatographic area [13, 36].

In this work, SWNHs and other carbon nanostructures have been incorporated to methacrylate monoliths for its application in nano-LC. For this purpose, two incorporation strategies were investigated. The carbon nanoparticles were embedded into the monoliths by simply admixing them with the components of the polymerization mixture before UV irradiation or the nanostructures were attached by photografting onto pore surface of monolith. For both approaches, poly(GMA-*co*-EDMA), which is a well-studied material, was chosen as the parent monolith. The incorporation of increasing amounts of the carbon nanoparticles into the monolith and its effect on nano-LC separation of test analytes was investigated. Differences in separation performance between both approaches were discussed. Besides, a comparison in terms of chromatographic performance between photo-grafted SWNHs, oxidized SWNHs and MWCNTs was carried out.

2. Materials and methods

2.1. Reagents, materials and samples

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), 3-(trimethoxysilyl)propyl methacrylate, 1-dodecanol, and cyclohexanol were purchased from Sigma-Aldrich (Madrid, Spain). HPLC-grade methanol (MeOH), acetonitrile (ACN), dimethyl sulfoxide (DMSO) and acetic acid were from VWR (Radnor, Boston, MA, USA). Azobisisobutyronitrile (AIBN), benzophenone (BP), and uracil were from Fluka (Buchs, Switzerland). Multi-walled carbon nanotubes (MWCNTs, N 90% carbon basis, D × L 110–170 nm × 5-9 µm length) were obtained from Sigma-Aldrich. Single-walled nanohorns (SWNHs) were purchased from Carbonium S.r.l (Padua, Italy). SWNHs form stable dahlia-shaped aggregates with an average diameter of 60–80 nm. Individually, the lengths of these SWNHs were in the range of 40 to 50 nm, and the diameter in the cylindrical structure varies between 4 and 5 nm. Laboratory-oxidized carbon nanohorns (o-SWNHs) were prepared following a procedure previously optimized [35]. Briefly, SWNHs were weighed (5 mg) and added to a glass vial, which was further introduced into a microwave oven, being the solid irradiated at 800 W for 10 min. Dispersions of SWNHs, o-SWNHs and MWCNTs were prepared in DMSO at a concentration of 0.5 $mg \cdot mL^{-1}$. Alkyl benzenes from Riedel-de Haën (Seelze, Germany) were used as test solutes. Deionized water was obtained with a Barnstead deionizer (Sybron, Boston, MA, USA). Stock solutions of alkyl benzenes (1 mg·mL^{-1}) were prepared independently in MeOH and kept at 4 °C until their use.

Uncoated fused-silica capillaries 375 µm O.D. × 100 µm I.D. (Polymicro Technologies, Phoenix, AZ, USA) with UV-transparent external coating were used for preparation of monolithic columns.

2.2. Instrumentation

An UV crosslinker (model CL1000) from UVP Inc. (Upland, CA, USA) equipped with UV lamps (5 × 8 W, 254 nm) was used for the preparation and photografting of the monolithic columns. A syringe pump (Model 100, KD Scientific, New Hope, PA, USA) was employed to introduce the reagents into the monolithic capillary columns. An HPLC pump (1100 series, Agilent Technologies, Waldbronn, Germany) was used for washing the prepared monolithic columns. A SP-400 Nanobaume™ system (Western Fluids Engineering, Wildomar, CA, USA) connected to Agilent 1100 HPLC pump was used to pump the SWNH, o-SWNH or MWCNT dispersion through the monolithic capillary columns.

SEM photographs of monolithic materials were taken with a scanning electron microscope (S-4800, Hitachi, Ibaraki, Japan) provided by a field emission gun, and an EMIP 3.0 image data acquisition system. Samples for SEM analysis were previously sputter-coated with Au/Pd for 2 min to avoid charging problems; this treatment was necessary since these materials were not electrically conducting.

A capillary liquid chromatography (cLC) system from Agilent (1200 series, Waldbronn, Germany), constituted by a degasser (Agilent part G1379B), a capillary pump (G1376A) providing a primary flow which was split by an electromagnetic proportional valve (EMPV, G1361-60000), a capillary-flow sensor (maximal flow rate, 20 µL·min^{-1}), a micro-well plate autosampler

(G1377A, 1260 Infinity series) with a micrometering device which provides injection volumes from 0.01 to 8 μL with the installed standard loop capillary, and UV detector equipped with a 5 nL quartz cell (10 mm path length cell, Sunchrom, Germany), was used for the separations. Data acquisition was performed with the ChemStation Software (Rev.A.10.01, Agilent).

2.3. Preparation of polymeric monolithic columns

To ensure covalent attachment of the monoliths to the inner wall of the fused-silica capillaries, surface modification with 3-(trimethoxysilyl)propyl methacrylate was performed [37]. A polymerization mixture containing GMA (20 wt%), EDMA (5 wt%), cyclohexanol (70 wt%), 1-dodecanol (5 wt%), and AIBN as initiator (1 wt% with respect to the monomers) [38] was used for the preparation of monolithic columns. The mixture was sonicated for 10 min, purged with nitrogen for 10 min, and then filled into the silanized capillary up to a length of 10 cm. To initiate the photo-polymerization, columns were placed in the UV crosslinker at 0.9 $J \cdot cm^{-2}$ for 15 min. After UV polymerization and using HPLC pump, the resulting columns were washed for 30 min with MeOH to remove the pore-forming solvents and any possible unreacted monomers.

2.4. Embedding of carbon nanoparticles in methacrylate monoliths

Different contents of the SWNHs (from 0.01 to 0.1 wt%) were dispersed in the polymerization mixture, which was obtained by weighing the appropriate amounts of GMA and EDMA as monomers, a binary mixture of cyclohexanol and 1-dodecanol as porogenic solvents and AIBN as photoinitiator, whose composition is indicated above. To avoid sedimentation processes during capillary filling, the dispersions were stirred throughout the whole time of capillary filling with a syringe pump. Then, the polymerization process was performed as described in Section 2.3.

2.5. Photografting of polymeric monoliths with carbon nanostructures

The post-polymerization functionalization (photografting) of the GMA-based monoliths was performed using a two-step approach as described previously in literature [18,39]. Thus, a 5 wt% solution of BP (as the photo-initiator) in MeOH was pumped through the methacrylate monolithic column (0.06 µL·h^{-1}) for 30 min to completely fill the pores. The capillary was then end-capped with rubber and exposed to UV irradiation for 10 min at 0.9 J·cm^{-2}.

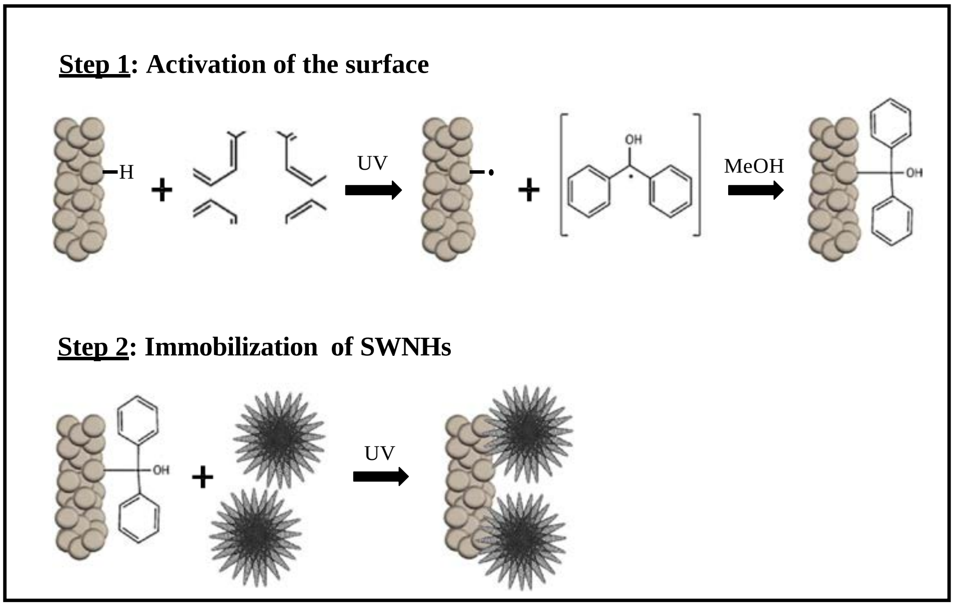

Figure 1. Schematic representation of the two-step photografting procedure to graft SWNHs onto methacrylate monolithic stationary phases.

After the initiator was attached to the surface (see Fig. 1, step 1), the monolith was washed with MeOH. Next, several percentages of carbon nanoparticles (SWNHs, o-SWNHs or MWCNTs) were dispersed in DMSO. The columns were flushed with these dispersions using the nanobaume system. Next, UV irradiation at 0. 9 $J \cdot cm^{-2}$ was performed for various times. After UV grafting (see Fig. 1, step 2), the resulting columns were thoroughly flushed with MeOH to flush out any unreacted chemicals. All solutions were sonicated and purged with nitrogen for 10 min prior to use to remove all oxygen.

3. Results and discussion

3.1. Preparation and characterization of UV-polymerized monoliths with embedded SWNHs

The choice of GMA as relatively polar monomer was done in order to examine better the possible changes in separation performance of polymeric monoliths after incorporation of SWNHs. One of the most common methods to incorporate nanoparticles in organic monolithic materials is by direct addition of them in the polymerization mixture. This method is simple but can lead to sedimentation problems, which would produce an inhomogeneity in the prepared materials. Therefore, it is necessary to uniformly disperse these nanomaterials in the monomer/porogens mixture to prepare a homogeneous polymer monolith in a capillary column format. The conditions to prepare UV-polymerized GMA-based monoliths were adapted from our previous work [38]. The SWNHs were well-dispersed in this mixture, giving rise to a homogenous and stable suspension (up to 0.1 wt%) for at least 6 h. This time was considered quite safe in relation to the 15 min that is required for UV polymerization. In addition, the stirring step during capillary filling, introduced by the authors in a previous work [40], assured a homogenous distribution of the carbon nanomaterials in the polymeric matrix.

Next, several monolithic capillary columns were prepared by incorporating increasing amount of SWNHs to the polymerization mixture (final concentration in the mixtures, 0.01–0.1 wt%). For comparison, a parent monolithic column (without SWNHs) was also prepared. Fig. S1 shows the SEM micrographs of the UV polymerized monoliths obtained both in the absence and in the presence of 0.05 wt% of SWNHs. As observed, the morphological differences between these both monoliths were small. Besides, the presence of SWNHs on the monolith surface was difficult to detect in these SEM pictures, since most of the SWNHs were located inside the polymer globules. Although a few of them can protrude from the globule surfaces, their simple distinction is not easy, due to the structural resemblance between the dahlia-like SWCNHs and the typical cauliflower-like globules of organic monoliths. Fig. 2 shows the chromatograms obtained with a mixture of alkyl benzenes using the parent monolith and monoliths prepared at several percentages of SWNHs. As it can be seen, the addition of SWNHs led to a separation of these solutes compared to the parent monolith (Fig. 2A). For instance, as shown in Table 1, the retention factors (k) changed from 1.8 in the parent monolith to k-values ranged 0.9–7.3 for the columns prepared with 0.01 wt% (Fig. 2B). When the SWNH content was increased from 0.01 to 0.1 wt%, a moderate increase in the retention of all tested compounds was also observed (see Table 1), being the chromatographic performance also modified. Thus, the resolution between propyl and butyl benzene increased from the monolith prepared with 0.01 wt% (Rs=1.0) to that prepared with 0.02 wt% SWNHs (Rs=1.6); however, the resolution decreased for 0.1 wt% SWNHs (Rs=1.0). It could suggest that the SWNH surfaces are at least partially exposed to the mobile phase, and probably they can interact with the alkyl benzenes through hydrophobic (Van der Waals interactions) and π–π interactions between the free π-bonds of sp^2 orbitals of the SWNHs and the aromatic rings of the analytes. However, at higher SWNH content could lead to aggregation and sedimentation phenomena leading to a worst separation performance as shown in the 0.1 wt% column (Fig. 2D and

Table 1). These results are comparable to those obtained for Zhao et al. [13], where the alkyl benzenes were injected into a CEC system showing a small increase in the k-values at lower SWNHs contents (ca. 0.05 wt%).

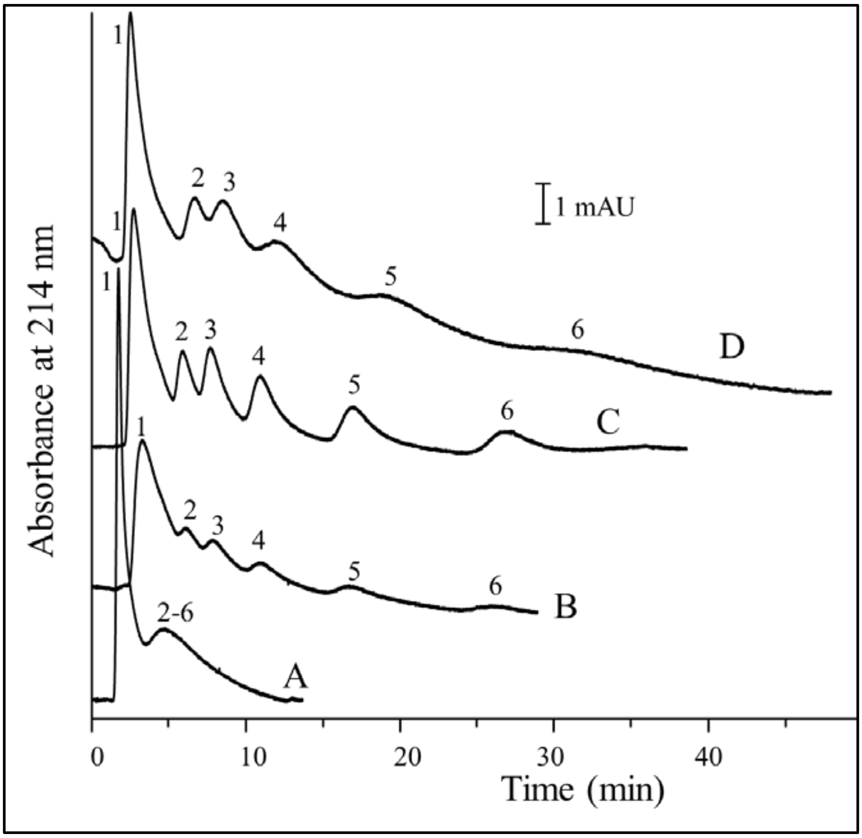

Figure 2. Separations of alkyl benzene test mixture using monoliths prepared in absence **(A)** and in presence of 0.01 **(B)**, 0.02 and **(C)** 0.1 of wt% SWNHs. LC conditions: column, 100 mm × 100 µm i.d.; mobile phase 50:50 (*v/v*) MeOH/water; flow rate 1 µL·min^{-1}. Peak identification: uracil **(1)**, toluene **(2)**, ethylbenzene **(3)**, propylbenzene **(4)**, butylbenzene **(5)**, and pentylbenzene **(6)**.

Regarding the efficiency, hybrid monoliths prepared with a 0.02 wt% of SWNHs showed the best efficiency for alkyl benzenes, reaching up to 4000 plates·m^{-1}. These results are higher to those reported by Zhao et al. [13] for a CEC monolithic column containing entrapped SWNHs (up to 2000 plates·m^{-1}).

Table 1. Retention factor and resolution values at different SWNH contents in embedded poly (GMA-*co*-EDMA) monolithic columns.

SWNH content (wt. %)	**Toluene**	**Ethyl benzene**		**Propyl benzene**		**Butyl benzene**		**Pentyl benzene**	
	k	***k***	***Rs***^{a}	***k***	***Rs***^{a}	***k***	***Rs***^{a}	***k***	***Rs***^{a}
0.00	1.8	1.8	-	1.8	-	1.8	-	1.8	-
0.01	0.9	1.4	<0.5	2.4	0.7	4.2	1.0	7.3	1.7
0.02	1.2	1.9	<0.5	3.2	0.9	5.5	1.6	9.4	2.2
0.10	1.8	2.5	<0.5	4.1	<0.5	7.1	1.0	12.3	1.9

[a] *Resolution (Rs) was calculated between adjacent peaks. LC conditions as in Fig. 2.*

3.2. Preparation and characterization of monolithic columns photo-grafted with SWNHs

An alternative strategy to incorporate SWNH into the monolithic columns involves their direct attachment to the pore surface by photografting. To perform this process, the GMA-based monolith described in the previous section was selected as parent (ungrafted) monolith. Then, a sequential two-step photografting process (see Fig. 1), was assessed using BP as photoinitiator to functionalize the pore surface of the methacrylate polymer. In the first step, BP is excited and abstracts a hydrogen from the monolith surface, generating surface radicals and semipinacol radicals, which combine to form surface photoinitiators. In the second step, the semipinacol groups are released from the surface, allowing the carbon nanostructures to bind to the free radical left at the surface. Consequently, the SWNHs are covalently photo-grafted onto the monolithic surface due to the strong tendency of radicals to react with the unsaturated double bonds of SWNHs [41].

In this work, the conditions used for the first step were taken from literature [22, 39], whereas the concentration of SWNHs and irradiation time were investigated. To perform this second step, the circulation of dispersions of SWNHs through the monolith is required. Thus, several organic solvents such as ACN, MeOH, DMSO, among others were tested, being DMSO able to produce stable dispersions of up to 50 $\mu g \cdot mL^{-1}$ of SWNHs. This solvent has also demonstrated to be an effective solvent for the dispersion of MWCNTs [42, 43].

Monolithic capillary columns were then prepared by conducting photografting experiments containing several concentrations of SWNHs (10–50 $\mu g \cdot mL^{-1}$) in the DMSO dispersion, by keeping constant the irradiation time. For comparison, a parent (ungrafted) monolithic column was also prepared. Fig. 3 shows SEM micrographs of parent monoliths and grafted with SWNHs (10 $\mu g \cdot mL^{-1}$) at irradiation time of 10 min. As it can be seen, before grafting, the flow through pores was well-defined within the parent monolith (Fig. 3A), being the globular structure clearly distinguished. After grafting (Fig. 3B), the through-pore channels were reduced, which was probably due to the microglobules were covered with grafted SWNHs. Although the typical shapes of SWNHs and globules showed a strong resemblance, as we mentioned above, in Fig. 3B, clusters with smaller size (attributed possibly to dahlia-like shape SWCNH aggregates) than globules of parent monolith were clearly evidenced. Moreover, when the concentration of grafted SWNHs was risen, an increase in backpressure (up to 2.1 fold for 50 $\mu g \cdot mL^{-1}$) was produced, which provided an additional evidence of the attachment of the SWNHs to the monolith surface.

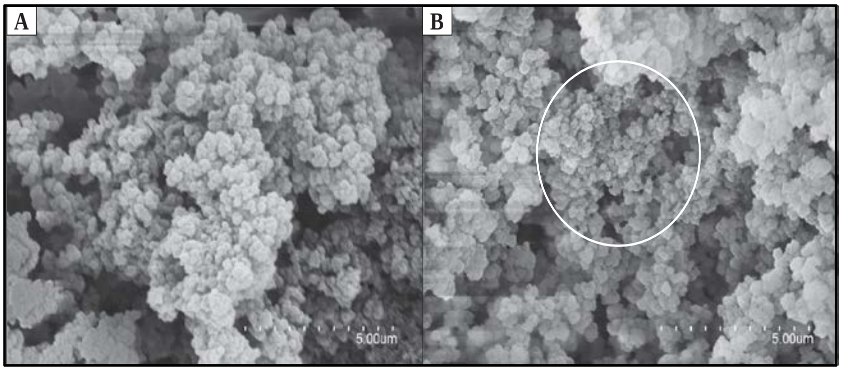

Figure 3. SEM micrographs of parent (ungrafted) monolith **(A)** and column prepared by photografting at 10 µg·mL^{-1} of SWNH **(B)**. Photografting conditions: first step, 5 wt% BP for 10 min; second step, irradiation at selected SWNH content for 10 min.

The chromatographic performance (k- and Rs-values) of the prepared monolithic stationary phases with photografted SWNHs was then evaluated (Table 2). As shown in Fig. 4 (from A to C) and Table 2, when the SWNH concentration was increased in the photografting process (from 10 to 50 µg·mL^{-1}), an increase in k- and resolution values were observed compared to ungrafted monolith (Fig. 2A). For instance, with increasing SWNH content, the k- and Rs values varied from 3.2 to 12.9 and <0.5 to 2.1, respectively. However, the increase in the amount of SWNH (from 20 to 50 µg·mL^{-1}) grafted onto the polymer system caused slight reduction in the k-values. This behavior suggests that the monolith surface is probably saturated with a compact layer of grafted chains, and no more SWNHs, which are still in the solution, could be attached onto the surface. A possible explanation of this saturation could be related with a self-screening effect, wherein the nanostructure begins to absorb the applied polymerization energy, which translates into lower concentration of available energetic free radicals and thus immobilized SWNHs [44, 45].

Table 2. Retention factor and resolution values in different columns prepared with photografting of several carbon nanostructures (SWNHs, o-SWNHs and MWCNTs) onto poly(GMA-*co*-EDMA) monolith.

Carbon nanoparticle type	**Nanoparticle concentration ($mg \cdot L^{-1}$)**	**Photografting time (min)**	**Toluene**	**Ethyl benzene**		**Propyl benzene**		**Butyl benzene**		**Pentyl benzene**	
			k	*k*	Rs^{a}	*k*	Rs^{a}	*k*	Rs^{a}	*k*	Rs^{a}
-	0	0	1.8	1.8	-	1.8	-	1.8	-	1.8	-
SWNHs	10	10	1.2	1.2	<0.5	1.5	<0.5	1.9	<0.5	3.2	<0.5
	20	10	2.2	3.2	1.0	5.0	1.3	8.5	1.6	14.0	1.6
	50	10	1.8	2.8	1.4	4.5	1.6	7.6	1.6	12.9	2.1
o-SWNHs	50	5	1.6	2.2	1.0	3.2	0.9	5.5	1.7	17.8	1.9
	50	20	1.8	2.7	<0.5	4.3	1.0	7.3	1.6	12.1	1.4
	50	10	2.1	3.1	0.8	4.8	1.0	8.2	1.6	13.2	1.8
MWCNTs	50	10	1.7	2.7	<0.5	4.3	<0.5	7.0	1.2	11.3	1.9

In any case, these improvements in both separation and retention result from the incorporation of SWNHs onto the pore surface, and the high affinity of the immobilized nanostructures for aromatic compounds as explained in Section 3.1.

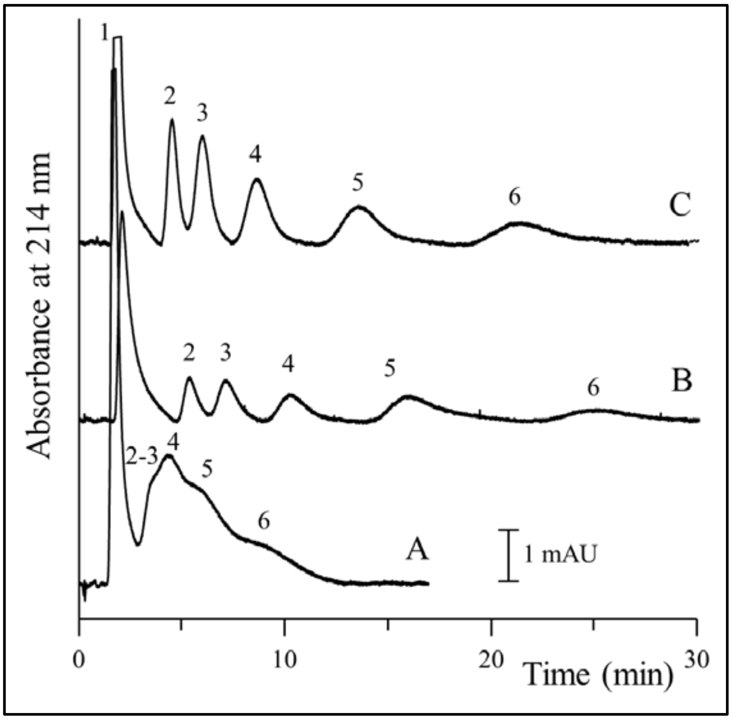

Figure 4. Separation of alkyl benzenes on monoliths photographed with several SWNHs concentrations: 10 **(A)**, 20 **(B)** and 50 **(C)** µg·mL^{-1}. Photografting conditions as in Fig. 4, LC conditions and peak identification as in Fig. 2.

The effect of grafting time was also investigated in monolithic columns prepared with 50 µg·mL^{-1} SWNHs. The morphological characterization of these monoliths revealed that no visible changes were evidenced on the surface along the irradiation time (data not shown), although an increased backpressure in the monolithic column resulted along this experiment. Fig. 5 shows the effect of photografting time on separation performance using alkyl benzenes as test solutes. The results showed that an increase in the irradiation time from 5 (Fig. 5A) to 10 min (Fig. 4C) led to of a progressive increase in retention and resolution (see Table 2). However, extending the grafting time to 20 min (Fig. 5B), a decrease in resolution (particularly for the peaks labelled as 2–4) was clearly evidenced. As mentioned before, this behavior can be explained by a complete saturation of the monolith surface by SWNH layers, which can block the nanohorn internal access and consequently, its interaction with analytes. Higher irradiation times resulted in unfeasibly high column backpressures or complete pore blockage.

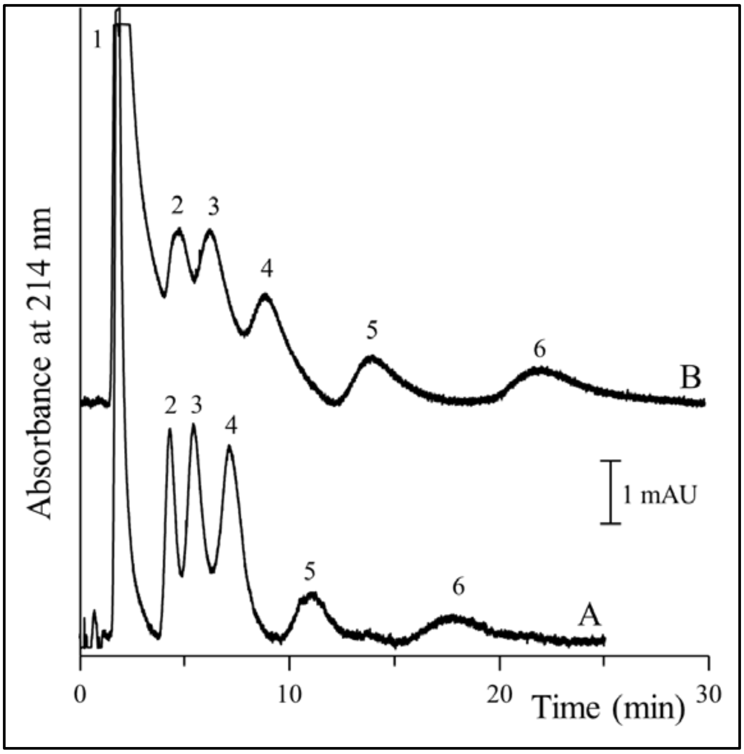

Figure 5. Effect of the grafting time on separation of alkyl benzenes: 5 **(A)** and 20 **(B)** min. Monoliths grafted with 50 µg·mL^{-1} of SWNHs, other photografting conditions are the same as in Fig. 4, LC conditions and peak identification as in Fig. 2.

Within these SWNH-grafted monoliths described here, the column prepared with 50 μg·mL^{-1} of SWNHs for 10 min of grafting time showed the largest resolution and efficiency (up to 6000 plates·m^{-1}) at flow rate of 1 μg·mL^{-1} for alkyl benzenes. Certainly, the separation performance achieved for the tested solutes was higher than those previously obtained by the embedding approach (see Table 1).

Regards to other works reported for monoliths containing other carbon nanostructures and evaluated using capillary/nano-LC, our column efficiencies were comparable with the values found by Aqel et al. (8800–11,100 plates·m^{-1} for ketones at 2 μg·mL^{-1}) [12], although were lower than those described by Chambers et al. [10], that provided up to 30,000 plates·m^{-1} for retained benzene at 0.25 μg·mL^{-1} in monoliths attached with short oxidized MWNTs. These differences in column performance could be related with the slow diffusion of the solutes within the network of dahlia-SWNHs.

A comparison in terms of chromatographic performance with poly (GMA-*co*-EDMA) monoliths photografted with other types of carbon nanoparticles, o-SWNHs and MWCNTs, was then performed (Fig. 6). These columns were prepared using the same grafting conditions as described above (50 μg·mL^{-1} of the carbon nanoparticle and 10 min of irradiation time). SEM micrographs of these grafted stationary phases were also taken. The photografting with o-SWNHs gave similar results to those described above for SWNHs, whereas the treatment with MWCNTs evidenced the presence of this nanostructured material on the globule surface (Fig. S2). These last nanostructures could be distinguished due to the differences in length between the carbon nanostructures as well as the different shape of their corresponding aggregates.

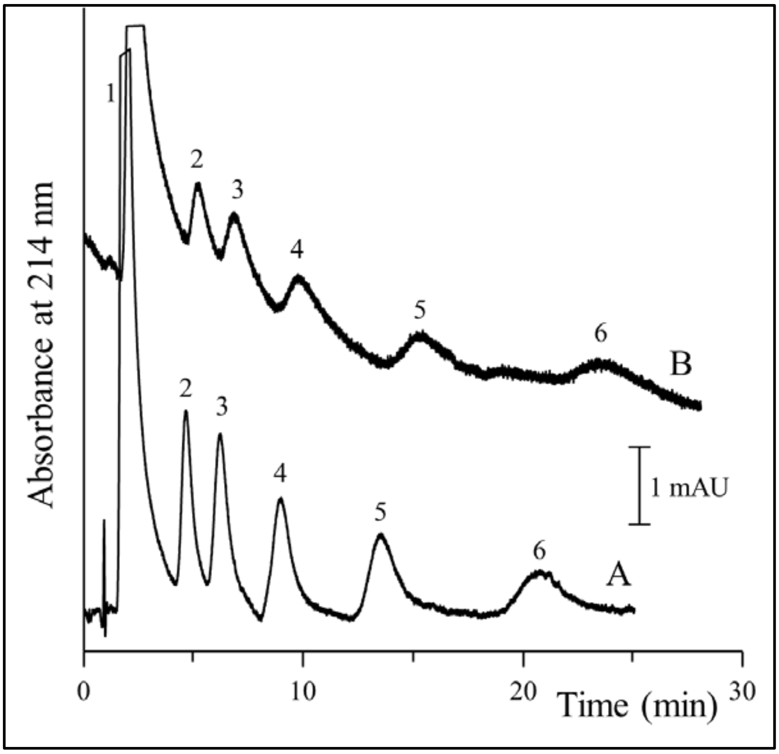

Figure 6. Separation of alkyl benzenes on grafted columns with o-SWNHs **(A)** and MWCNTs **(B)**. Monoliths grafted with 50 µg·mL^{-1} of each carbon nanostructure for 10 min, other photografting conditions are the same as in Fig. 4, LC conditions and peak identification as in Fig. 2.

As shown in Fig. 6, the column prepared with o-SWNHs showed a slightly lower retention than that obtained with a non-oxidized SWNH-grafted monolith (see Table 2 and Figs. 4C and 6A). This behavior can be explained as these o-SWNHs are more hydrophilic than their pristine counterparts, but in any case, they still provide enough hydrophobicity to the column, thus enabling separation (for instance, as shown in Table 2, the Rs values obtained for each peak pair ranged from 0.8 to 1.8). Additionally, these columns provided similar efficiencies values than SWNH-grafted monoliths. On the other hand, the chromatographic features obtained for the MWCNT-grafted (Fig. 6B) monolithic column showed worst separation performance and similar retention factors than those obtained with the SWNHs immobilized monolith (see Table 2). The lower efficiencies and resolution values showed by the columns photografted with MWCNTs could be explained by less available interaction surface since these nanostructures are solid cylinders and the analytes cannot enter inside the structure like in the SWNHs, where one of the sides has an aperture allowing the interaction inside the nanostructure giving higher available surface. These results are in accordance with those reported by Zhao et al. [13] where the use of single-walled carbon nanotubes also showed lower retention factors and worse efficiencies (up to 1000 plates·m^{-1}) and resolution values than the SWNHs tested in the same work.

Finally, the reproducibility of preparation of columns grafted with SWNHs was studied. Thus, the run-to-run repeatability was evaluated from series of three injections of propyl benzene as representative analyte, while the column-to-column reproducibility was estimated with three columns prepared from the same polymerization mixture, polymerized using the same process and grafting conditions matching those used for the preparation of the column indicated in Fig. 4C. Satisfactory relative standard deviation (RSD) values, obtained for retention time and efficiency, for run-to-run were below 4.5 and 0.7, respectively; and for column-to-column, the precisions were below 8.6 and 1.4 %, respectively.

4. Conclusions

In this work, the preparation and characterization of UV-initiated GMA-based monolithic columns with incorporated SWNHs and other nanostructures for nano-LC have been described. Thus, different strategies to introduce these nanomaterials either through embedding into polymeric matrix or by attachment onto pore surface via photografting were tried. Experimental preparation conditions for each approach were optimized in terms of SWNHs concentration and other variables. The prepared stationary phases with these nanoparticles were characterized morphologically by SEM. In both approaches, changes in monolith morphology were difficult to distinguish due to the strong structural resemblance between the SWNHs aggregates and polymer globules.

The chromatographic performance of polymer monoliths containing either embedded or grafted SWNHs was evaluated using alkyl benzenes as test analytes. In general, the incorporation of small amounts of SWNHs in composite columns featured enhanced retention and separation of aromatic solutes compared to the generic monolith. Our results suggest that retention by the SWCNHs incorporated monolithic columns was strengthened based on hydrophobic and π–π stacking properties of SWCNHs. The higher retention seems to be due to the increase of the surface area of the monolith, produced by the presence of carbon nanoparticles onto the surface which adds Van der Waals and π–π interactions.

A comparison in terms of performance was done for both approaches. Under respective optimum conditions, both gave similar behaviour, although the photografting approach gave better separation performance. Additionally, the monoliths obtained by this last strategy exhibited satisfactory reproducibilities in their chromatographic behaviour. However, the separation efficiencies were lower than those achieved by other groups via attachment of short MWCNTs [10]. In any case, our results clearly demonstrate the ability of these carbon nanostructures to significantly affect the separation performance of monolithic columns.

The chromatographic behavior found with composites containing SWNHs was compared with those obtained with other carbon nanostructures such as o-SWNHs and MWCNTs. As a result, SWNHs or its oxidized counterparts immobilized on a monolith surface seem to be more adequate than MWCNTs, since better separation performance of the target analytes was obtained with the formers.

To the authors' knowledge, this work represents the first instance of nano-LC separation of small solutes on grafted polymer monoliths with carbon nanostructures. Given the simplicity of the photografting protocol, we expect this methodology can be extended in the development of novel composite materials will find a broad range of applications.

Acknowledgments

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Supplementary Information

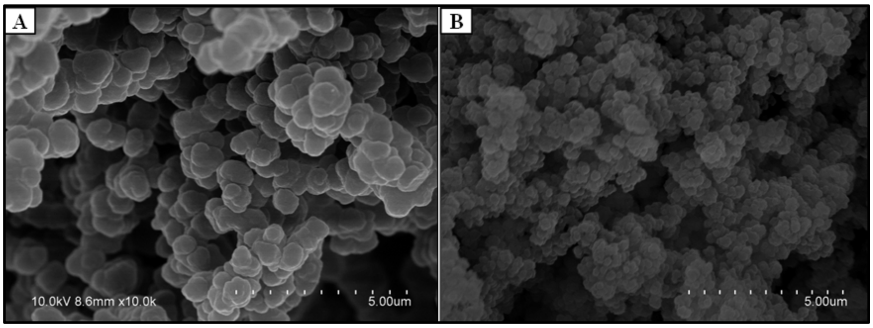

Figure S1. SEM micrographs of monoliths prepared from polymerization mixtures in the absence **(A)** and containing 0.05 wt% **(B)** of SWNHs

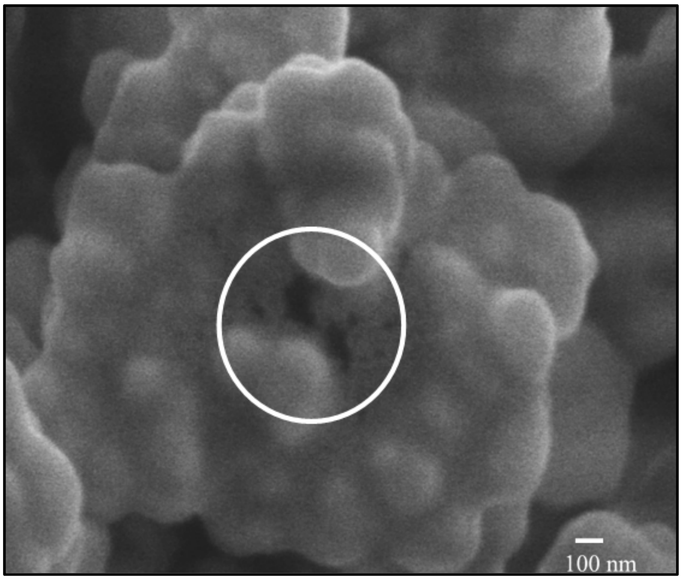

Figure S2. SEM micrograph of column prepared by photografting at 50 µg·mL^{-1} of MWCNTS. Photografting conditions: first step, 5 wt% BP for 10 min; second step, irradiation at selected SWNH content for 10 min.

Capítulo 3

Effect of Carbon Nanohorns in the Radical Polymerization of Methacrylate Monolithic Capillary Columns and Their Application as Extractant Phases

En revisión en Talanta

Effect of carbon nanohorns in the radical polymerization of methacrylate monolithic capillary columns and their application as extractant phases

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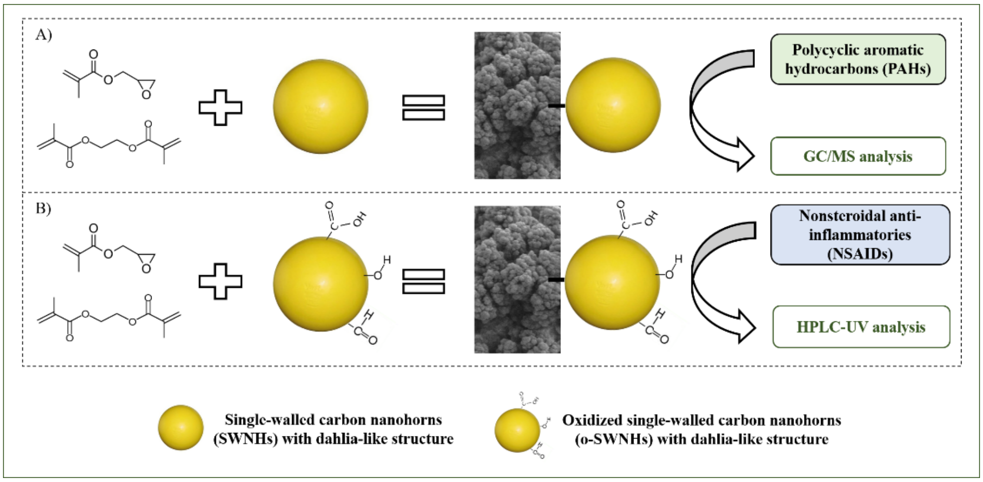

Keywords

Carbon nanoparticles; Conical nanostructures; Hybrid monolith; Microextraction; Polycyclic aromatic hydrocarbons; Nonsteroidal anti-inflammatory drugs.


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Capítulo

4

Preparation and Evaluation of Micro and Meso Porous Silica Monoliths with Embedded Carbon Nanoparticles for the Extraction of Non-Polar Compounds from Waters

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Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters

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Highlights

- A silica-carbon nanoparticles monolith has been synthetized.
- The hybrid sorbent was prepared in a fused silica capillary.
- Carbon nanostructures improve the extraction efficiency of the monolith.
- PAHs have been determined in waters using the proposed microextraction unit.

Keywords

Silica monolith; Solid-phase microextraction; Polycyclic aromatic hydrocarbons; Carbon nanoparticles; Monolithic hybrid solid.

Abstract

A novel hybrid micro and meso porous silica monolith with embedded carbon nanoparticles (Si-CNPs monolith) was prepared inside a fused silica capillary (3 cm in length) and used as a sorbent for solid-phase microextraction. The hybrid monolithic capillary was synthetized by hydrolysis and polycondensation of a mixture of tetraethoxysilane (TEOS), ethanol, and three different carbon nanoparticles such as carboxylated single-walled carbon nanotubes (c-SWCNTs), carboxylated multi-walled carbon nanotubes (c-MWCNTs), and oxidized single-walled carbon nanohorns (o-SWNHs) via a two-step catalytic sol-gel process. Compared with silica monolith without carbon nanoparticles, the developed monolithic capillary column exhibited a higher extraction efficiency towards the analytes which can be ascribed to the presence of the carbon nanoparticles. In this regard, the best performance was achieved for silica monolith with embedded c-MWCNTs. The resulted monolithic capillaries were also characterized by scanning electron microscopy (SEM), elemental analysis and nitrogen intrusion porosimetry. Variables affecting to the preparation of the sorbent phase including three different carbon nanoparticles and extraction parameters were studied in depth using polycyclic aromatic hydrocarbons (PAHs) as target analytes. Gas chromatography-mass spectrometry was selected as instrumental technique. Detection limits range from 0.1 to 0.3 $\mu g \cdot L^{-1}$, and the inter-extraction units precision (expressed as relative standard deviation) is between 5.9 and 14.4 %.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of non-polar organic compounds consisting of two or more benzene rings which are byproducts of petroleum processing or combustion of hydrocarbons. PAHs are of environmental concern due to their significant toxicity and potential carcinogenic properties at relatively low levels [1].In this regard, the concentration of benzo[a]pyrene has been used as indicator of total contamination by PAHs being 0.2 $\mu g \cdot L^{-1}$ the maximum level established by Agency for toxic substances & Disease Registry (ATSDR) in waters [2].

Analytical methods including gas chromatography (GC) [3], gas chromatography coupled with mass spectrometry (GC-MS) [4], high-performance liquid chromatography with fluorescence detection (HPLC-FLD) [5], thin-layer chromatography (TLC) [6], and spectrofluorimetry [7] have traditionally been used to determine PAHs. However, preconcentration and clean-up steps are needed to achieve the required sensitivity and selectivity. In this context, new microextraction techniques have been recently developed based on the simplification and the miniaturization of classical separation techniques in both solid and liquid phase formats. The incorporation of nanostructured materials as well as hybrid sorbents is behind the consolidation of these new approaches particularly due to their high superficial area and variety of interactions [8].

Monolithic solids have gained prominence as new separation material because of their unique properties including fast dynamic transport, simplicity of their preparation, frit-free construction, good loading capacity and low backpressure. To date, both organic polymer and silica-based monoliths have been used in several formats in the extraction context such as micropipette-tips, spin columns, microfluidics chips, and capillary columns [9, 10]. Carbon nanoparticles, especially carbon nanotubes, have received special research attention since their discovery thanks to their unique and outstanding properties. Their excellent properties in terms of extraction efficiency have resulted in their use in different microextraction formats [11]. Hybrid monoliths prepared via sol-gel from silica precursors and organic frameworks including carbon nanoparticles combine the advantages of both [12–14]. The main challenge is to achieve a good and stable dispersion of the carbon nanoparticles in the polymerization mixture in order to obtain a homogeneous solid.

In this article, micro and meso porous silica monoliths with embedded carbon nanoparticles have been successfully synthesized inside a fused silica capillary and evaluated for the determination of non-polar compounds such as PAHs in tap and river water samples. Gas chromatography-mass spectrometry was selected as instrumental technique. All the variables related to the preparation of the monolith as well as those affecting to the microextraction process were evaluated in depth. In addition, the monolithic solid with and without carbon nanoparticles was characterized by elemental analysis to confirm their presence into the silica network and a porosimetry study was also carried out in order to know the size distribution of the pores.

2. Experimental section

2.1. Reagents, materials and samples

The reagents used were of analytical grade or better. Polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene and benzo[a]pyrene) were purchased from Sigma-Aldrich (Madrid, Spain. www.sigmaaldrich.com). Stock standard solutions of each analyte were prepared in methanol (Sigma-Aldrich) at a concentration of 1 $g\cdot L^{-1}$ and stored at 4 ºC. Working solutions were prepared by dilution of the stocks in ultrapure Milli-Q water (Millipore Corp.; Madrid, Spain). In the extraction procedure, methanol from Sigma-Aldrich was employed as eluent.

Uncoated fused-silica capillaries (320 µm i.d., Sigma Aldrich) were used for the preparation of the monolithic extraction unit. Ferrules 1/16” ID, PEEK tubing 1/16” and internal union zero volume 1/16” to 1/16” (Sigma-Aldrich) were also employed.

The reagents needed for the synthesis of the monolithic phase, tetraethyl orthosilicate (TEOS), ethanol, Milli-Q water, 3-(trimethoxysilyl)propyl methacrylate, methanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), acetone and acetic acid were purchased from Sigma-Aldrich.

Carboxylated multi-walled carbon nanotubes (c-MWCNTs, <8 nm o.d., 10–30 µm length, >95 wt% purity, 3.86 wt% functional content) and carboxylated single-walled carbon nanotubes (c-SWCNTs, 1–2 nm o.d., 5–30 µm length, >90 wt% purity, 2.73 wt% functional content) were obtained from Sigma-Aldrich. Single-walled nanohorns were purchased from Carbonium S.r.l (Padua, Italy. http://www.carbonium.it/public/site/index.php). SWNHs form stable dahlia-shaped aggregates with an average diameter of 60–80 nm. Individually, the lengths of these SWNHs are in the range of 40 to 50 nm, and the diameter in the cylindrical structure varies between 4–5 nm.

The dispersions of the carboxylated carbon nanotubes (c-SWCNTs and c-MWCNTs) were made in ethanol. In brief, 5 mg of c-MWCNTs were weighed, added to a glass vial and ultrasonic-assisted dispersed in 50 mL of ethanol for 30 min. On the other hand, carbon nanohorns (SWNHs) were weighed (5 mg) and added to a glass vial, which was further introduced into a microwave oven, being the solid irradiated at 800 W for 10 min. After cooling at room temperature, the oxidized carbon nanohorns (o-SWNHs) were dispersed in 50 mL of ethanol and sonicated for 30 min.

Tap and river water samples were selected for the determination of the target compounds using the monolithic microextraction unit. Water samples from the Guadalquivir river were collected in amber glass bottles without headspace. The spiked samples were prepared by adding the analytes at a concentration of 20 μg·L^{-1}, and then they were left to stand for 24 h until the analysis.

2.2. Chromatographic analysis

Gas chromatographic/mass spectrometric analyses were carried out on a gas chromatograph (Varian CP-3800)-mass spectrometer (Varian 1200 MS/MS) working under single quadrupole mode and with an electron multiplier detector. The gas chromatograph was equipped with a fused silica capillary column VF-5 ms (30 m × 0.25 mm i.d.) coated with 5 % phenyl-95 % dimethylpolysiloxane (film thickness 0.25 μm) (Sigma-Aldrich) to separate the six analytes. System control and data acquisition was achieved with an HP1701CA MS ChemStation software.

The column temperature program was as follows: the initial temperature, 80 ºC, was kept for 2.5 min, raised up to 200 ºC at 25 °C·min^{-1} (maintained for 1 min) and further to 250 ºC at 10 ºC·min^{-1}. Then it was immediately ramped up to 285 ºC at 5 ºC·min^{-1}. The final temperature, 300 ºC, was reached with a ramp of 30 ºC·min^{-1} and maintained for 1 min. The quadrupole mass spectrometer detector was operated in selected ion monitoring mode, recording the following fragment-ions characteristic of each analyte: 128 (from 3.0 to 6.4 min) for naphthalene, 178 (from 6.4 to 10.58 min) for phenanthrene, 202 (from 10.58 to 14.0 min) for fluoranthene and pyrene, 228 (from 14.0 to 17.0 min) for benz[a]anthracene, 252 (from 17.0 to 21.0 min) for benzo[a]pyrene, all of them at 1 scan/s. Electron impact ionization (70 eV) was used for analytes fragmentation. The injector temperature was 270 ºC and the splitless mode was selected. The injection volume, 2 μL of methanol, was measured with a 5 μL microsyringe (Hamilton Co., Nevada, USA). The carrier gas used was helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.0 mL·min^{-1}, and it was regulated by digital pressure controller. The transfer line and ionization source were maintained at 280 ºC and 250 ºC, respectively.

2.3. Preparation of Si-CNPs monolithic solid

It is well known that silica monoliths tend to shrink during the post-heat treatment, even in small devices such as capillaries. However, this shrinkage can be minimized or prevented by a covalent attachment of the monolith to the capillary inner walls. Therefore, the hybrid monolith was synthetized inside a fused silica capillary (3 cm in length) which was previously modified with 7% solution of 3-(trimethoxysilyl)propyl methacrylate in ethanol [15].

A schematic representation of the synthesis procedure is shown in the Fig. 1. The hybrid monolith was prepared by hydrolysis and polycondensation of precursors via a two-step catalytic sol-gel process according to a previously described procedure with some modifications [16]. It should be noticed that the rate of hydrolysis and condensation of the precursors is the most important factor to control the final monolithic structure. The kinetics of each step of the sol-gel process is pH-dependent. Low pH values produce a fast hydrolysis of alkoxysilanes generating hydroxyl groups, while high pH values accelerate the condensation reactions [16]. This pH variation was regulated by adding hydrochloric acid (0.5 M) or ammonium hydroxide (0.5 M) as required In the acid-catalyzed hydrolysis step, 160 µL of the TEOS was mixed with a solution of 200 µL of ethanol containing one of the three carbon nanoparticles studied (0.1 $g \cdot L^{-1}$), 20 µL of Milli-Q water, and 10 µL of HCl (0.5 M) in a 1.5 mL eppendorf vial. The pre-polymerization mixture solution was sonicated at 20 ºC for 4 h, and then 25 µL of ammonium (0.5 M) were added into the solution. After shaking the mixture, the modified capillaries were filled with the homogenous polymerization mixture up to a length of 3 cm, and sealed at both ends with two pieces of rubbers. Next, the capillaries were introduced into an oven at 40 °C for 12 h. After polymerization and using a micro-HPLC pump, the resulting columns were rinsed with ethanol to remove any possible unreacted monomer and the soluble hydrolysis products. Then, the monolithic capillary was flushed with a 5 % (*v/v*) solution of acetic acid in methanol for 10 min (0.1 mL·min^{-1}), followed by Milli-Q water (0.1 mL·min^{-1}, 10 min) in order to conditioning the surface monolith prior to use.

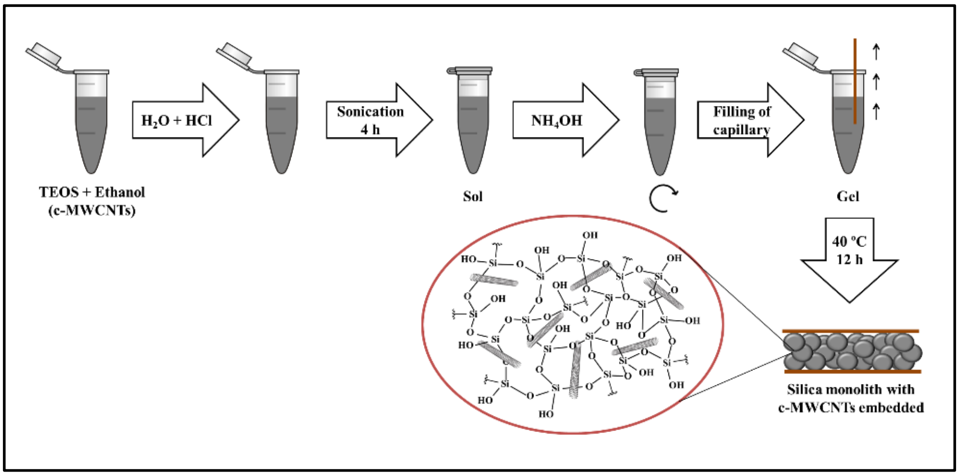

Figure 1. Scheme of the sol-gel process and the filling of the fused silica capillary.

2.4. Characterization

A JEOL JSM 6300 scanning electron microscopy (Isaza, Alcobendas, Spain) was also used to obtain the micrographs of the monolithic solid with and without carbon nanoparticles to evaluate the morphological and structural characteristics of the monolithic sorbent. The monolith was fixed on the stub by a double-sided sticky tape and then coated with gold. Transmission electron microscopy (TEM) images were recorded by use of a JEOL JEM 1400 microscope (Isaza, Alcobendas, Spain) operating at an accelerating voltage of 120 kV. The TEM micrographs were used to characterize the nanoparticles size and morphology.

Elemental analysis was carried out on an elemental analyzer CHNS Eurovector EA 3000. Nitrogen adsorption/desorption experiments were carried out at -196 ºC using a Quantachrome® ASiQwin™-Automated Gas Sorption Data. The specific surface area values were calculated according to the BET (Brunauer-Emmett-Teller) equation. T-plot method was used to determine the micropore surface areas, and the average pore volumes were evaluated from the desorption branches of isotherms based on the BJH (Barrett-Joyner-Halenda) model.

2.5. Analytical procedure

The microextraction procedure followed for the isolation and preconcentration of PAHs from waters comprised the following steps. First, aliquots of 2 mL of aqueous standards or water samples, containing the target analytes, were passed through the polymer monolithic capillary at a flow rate of 0.1 $mL \cdot min^{-1}$ by means of the micro-HPLC pump. The monolith was washed with 500 µL of Milli-Q water (0.1 $mL \cdot min^{-1}$), and then the aqueous phase remaining in the capillary was eliminated by means of a nitrogen stream (10 min). After this step, the analytes were eluted pumping methanol (500 µL, 0.1 $mL \cdot min^{-1}$), and the eluent was collected into a vial, evaporated and the residue was redisolved in 10 µL of methanol. Finally, 2 µL of the organic phase with the extracted analytes were analyzed by GC/MS. The chromatographic peak areas were used as analytical signals. Between extractions, the monolith was sequentially conditioned with a 5 % (*v/v*) solution of acetic acid in methanol and Milli-Q water (10 min in each case) at a flow rate of 0.1 $mL \cdot min^{-1}$. No degradation effects or deformation of the monolithic structure was observed in terms of extraction efficiency with repetitive microextraction cycles. The extraction unit can be used repeatedly owing to its compatibility with organic solvents as well as water [17].

3. Results and discussion

3.1. Variables affecting to the preparation of the monolith

The preparation of the bare monolith was carried out as described in section 2.3. The incorporation of carbon nanoparticles (c-SWCNTs, c-MWCNTs and o-SWNHs) in the monolith was deeply studied. As it was previously mentioned, the homogenous and stable dispersion of the nanoparticles is crucial for this aim. Therefore, dispersions of each carbon nanoparticle were prepared at a concentration of 0.1 g·L^{-1} and several organic solvents (methanol, ethanol and 2-propanol) were studied to efficiently disperse the carbon nanoparticles. Fig. 2 shows the behavior for each carbon nanoparticle in the different organic media.

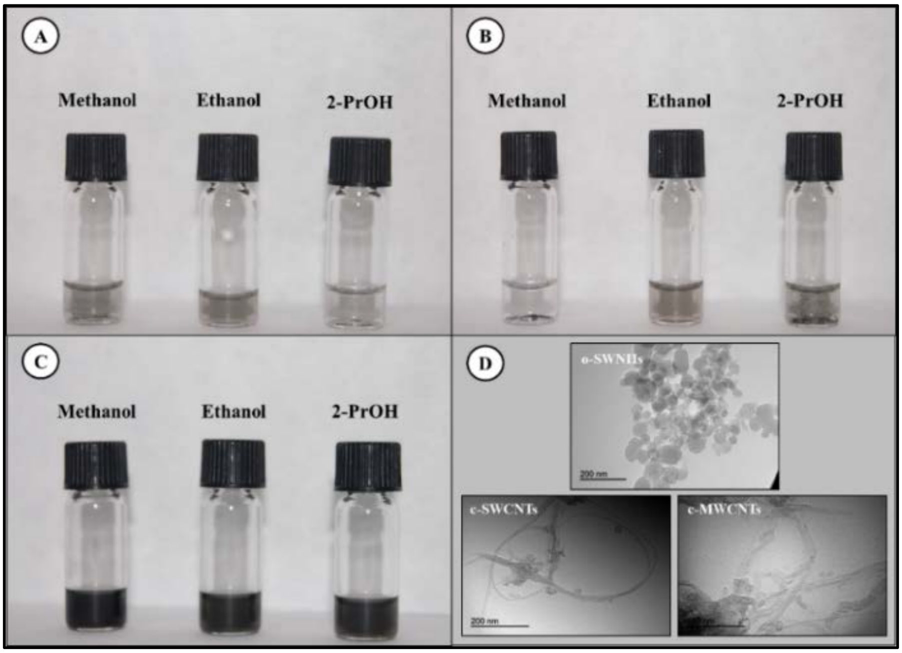

Figure 2. Photographs of the dispersions of c-SWCNTs **(A)**, c-MWCNTs **(B)**, and o-SWNHs **(C)** in methanol, ethanol, and 2-PrOH; and TEM images **(D)** of the three carbon nanostructures.

As it can be seen, ethanol resulted to be the best choice to disperse the carboxylated carbon nanotubes (Fig. 2A and 2B), while good dispersion of o-SWNHs was obtained in the three solvents (Fig. 2C). Therefore, ethanol was selected as optimum solvent for the efficient dispersion of the carbon nanoparticles used in this article. Fig. 2D also presents the TEM micrographs obtained for the dispersions of c-SWCNTs, c-MWCNTs, and o-SWNHs.

Next, the influence of these carbon nanoparticles incorporated to the monolith was evaluated in terms of extraction efficiency using aqueous standards containing benzo[a]pyrene as model compound at a concentration of 1 μg·mL^{-1}. As it can be seen in Fig. 3, the introduction of the c-MWCNTs, c-SWCNTs, and o-SWNHs into the network of silica monoliths improved the extraction of benzo[a]pyrene in comparison with the bare monolith. In fact, the best performance was achieved using c-MWCNTs due to their large specific surface area and number of sheets.

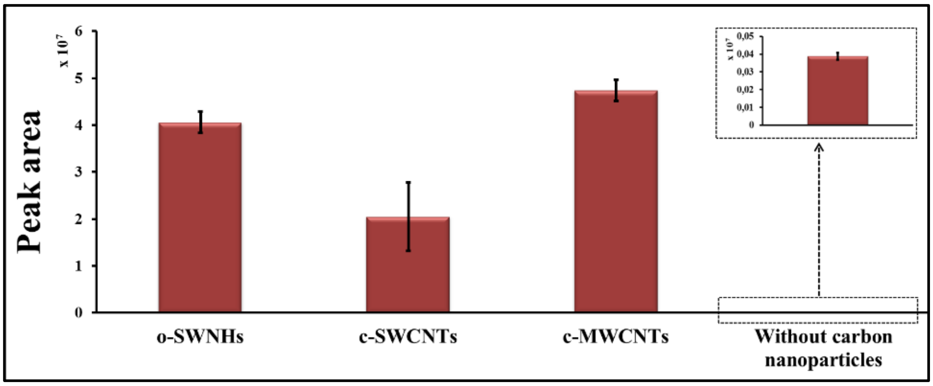

Figure 3. Comparison of the analytical performance of silica monolith without nanoparticles and the monolith containing embedded o-SWNHs, c-SWCNTs and c-MWCNTs.

On the other hand, o-SWNHs present an extensive surface area and horn interstices which provide them with an exceptional adsorption capacity. Besides, the oxidation conditions can produce nanowindows, generating additional interaction sites on the conical and curved ends. All this combined with the formation of stable aggregates improve their extraction capacity explaining the better performance in comparison with c-SWCNTs. Based on the results obtained, this, c-MWNTs were selected for further experiments.

Next, the amount of c-MWCNTs was evaluated at concentrations of 0.01, 0.05 and 0.1 g·L^{-1}. Monolithic solid formed at concentrations of nanoparticles higher than 0.1 g·L^{-1} showed non-homogenous distribution of the c-MWCNTs due to the aggregation of the carbon nanoparticles decreasing the extraction efficiency. As it is shown in Fig. 4, the signals increased with the concentration, and therefore a concentration of 0.1 g·L^{-1} of the c-MWCNTs in ethanol was selected.

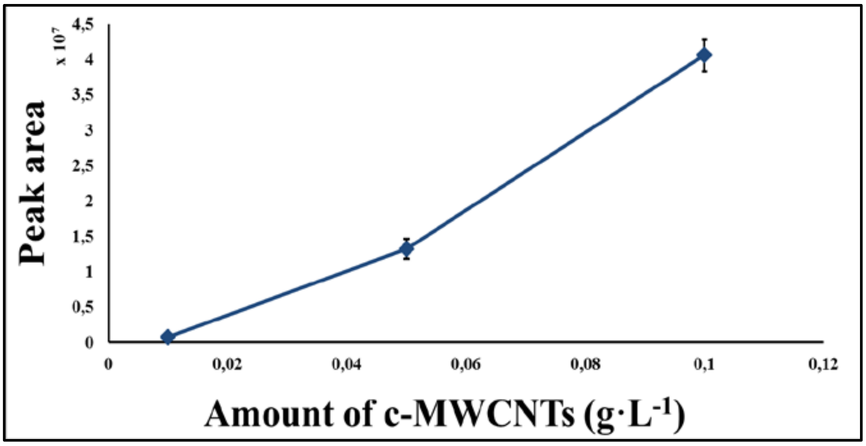

Figure 4. Influence of the concentration of the c-MWCNTs dispersion on the PAHs retention.

3.2. Characterization

Once optimized, the synthetized monoliths were characterized by SEM, elemental analysis, and nitrogen adsorption/desorption measurements.

Micrographs of the monolithic sorbents were obtained for the bare silica, Si-SWNHs, Si-SWCNTs, and Si-MWCNTs monolith. As it can be seen in Fig. 5, the silica monolithic solids exhibit homogeneous and porous networks that consisted of interconnected microglobules. It is worth to mention that the graph of the Si-SWNHs monolith (Fig. 5B) shows that SWNHs form spherical aggregates with dahlia-like structures, while the monolithic solids modified with carbon nanotubes (Fig. 5C and D) maintain practically the same microscopic morphology as it showed for the bare silica monolith (Fig. 5A).

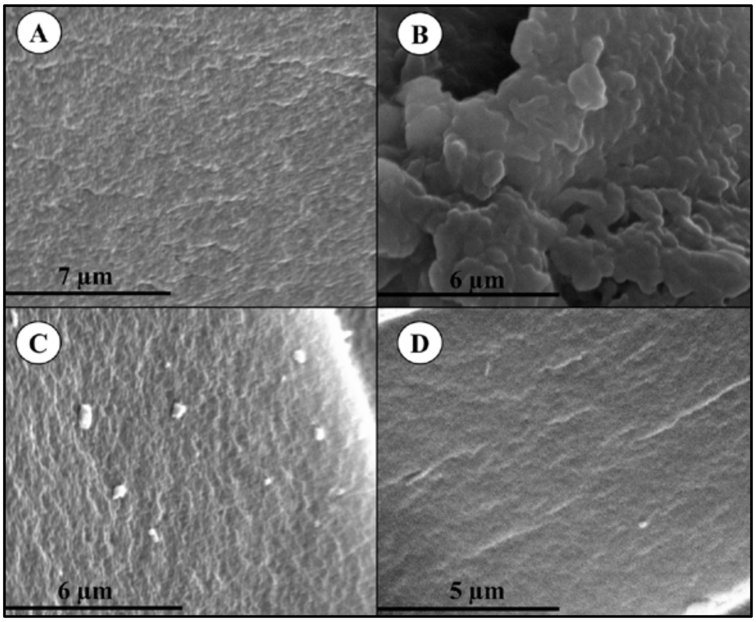

Figure 5. Scanning electron microscopy of silica monolith **(A)**, Si-SWNHs **(B)**, Si-SWCNTs **(C)**, and Si-MWCNTs **(D)**.

The C% of the bare silica, Si-SWNHs, Si-SWNCTs, and Si-MWCNTs monolith was determined by elemental analysis and resulted to be 0.72 %, 4.24 %, 1.94 %, and 2.48 %, respectively. The increase in the proportion of the carbon in the synthetized material demonstrated the successful incorporation of the carbon nanoparticles into the silica network. As expected, the highest percentage was found for the single walled carbon nanohorns due to its size and weight.

Isotherms of nitrogen adsorption/desorption for each type of synthetized monolithic solid are described in Fig. 6. As it can be seen, the isotherms for silica, Si-SWNHs, and Si-SWCNTs monoliths were type I in the BDDT classification [18], while the nitrogen isotherms obtained for the Si-MWCNTs monolith was the type IV, exhibiting H1 hysteresis loops which indicates a mesoporous solid. The BET surface and pore volume values are compiled in Table 1 together with the micropore surface areas.

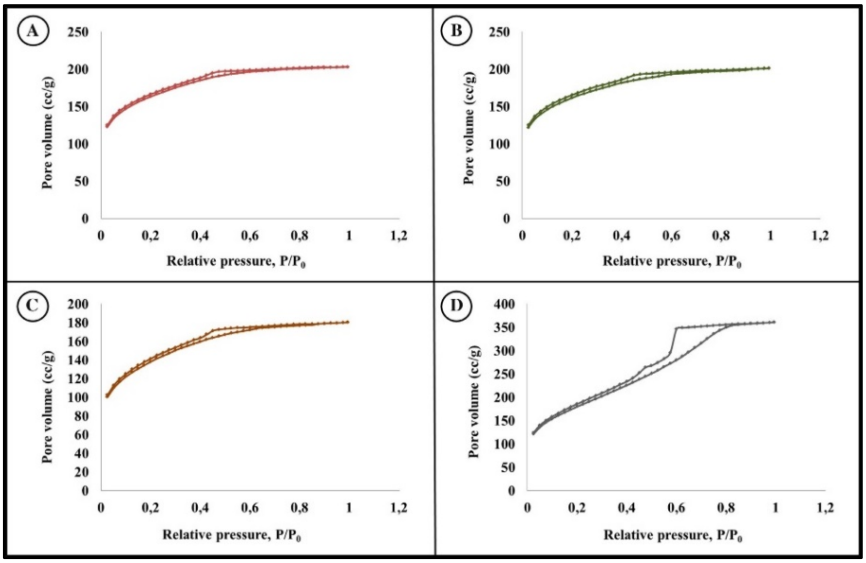

Figure 6. N_2 adsorption and desorption isotherms of silica monolith **(A)**, Si-SWNHs **(B)**, Si-SWCNTs **(C)**, and Si-MWCNTs **(D)**. P: sample pressure; Po: saturation

Table 1. Textural properties of the synthetized monolithic solids.

	Silica monolith	**Si-SWNHs monolith**	**Si-SWCNTs monolith**	**Si-MWCNTs monolith**
Specific surface area, BET ($m^2\cdot g^{-1}$)	594.07	588.25	497.37	646.82
Micropore surface area ($m^2\cdot g^{-1}$)	345.41	352.80	255.52	108.89
Average pore volume ($cc\cdot g^{-1}$)	0.314	0.311	0.279	0.557

3.3. Evaluation of the variables affecting to the microextraction process

As it is a novel extraction procedure, a univariate approach was selected to study the effect of each single variable in the extraction process, using aqueous standards containing benzo[a]pyrene as model compound at a concentration of 1 $\mu g\cdot mL^{-1}$. The initial experimental conditions for the microextraction process were: 3 mL of aqueous standard and an elution volume of 200 µL of methanol. Flow rates used for preconcentration and elution step were fixed at 0.1 $mL\cdot min^{-1}$.

The sample and eluent volumes are critical parameters for the method sensitivity, since they will determine the preconcentration factor. The sample volume was studied within the interval 1–4 mL. The maximum extraction efficiency for the target triazines was achieved for a volume of 2 mL, decreasing over this value due to a breakthrough effect. The volume of methanol required for analytes elution was studied between 100 and 500 µL. The methanolic extract was collected in a 1.5 mL glass vial, and a volume of 500 µL was necessary to quantitatively elute the target analytes which was fixed as optimum. An evaporation–redissolution step was included in order to reduce the final volume to 10 µL, thus increasing the method sensitivity. Finally, 2 µL of the organic phase with the extracted analytes were injected into the gas chromatograph/mass spectrometer for their separation and detection. The chromatographic peak areas were used as analytical signals. Between samples, the monolithic capillary was conditioned with a 5% (v/v) solution of acetic acid in methanol for 10 min. The pH of the solvent is a critical parameter in the conditioning in order to achieve a reproducible elution as underivatized silica exhibit secondary interactions between analytes and negative charged silanol groups at pH >4 [19]. Milli-Q water was sequentially passed through the hybrid monolithic capillary at a flow rate of 0.1 $mL\cdot min^{-1}$ for 10 min.

3.4. Analytical figures of merit and analysis of water samples

The analytical figures of merit of the proposed method are summarized in Table 2. The calibration curves for PAHs was constructed by using working aqueous standards prepared in duplicate at controlled concentrations which were subjected to the optimized extraction procedure, and 2 µL of the organic extract was injected into the GC/MS for analysis. The corresponding equations were obtained by plotting the peak areas of the characteristic m/z fragment ions against the concentration for each target analyte. The method was characterized on the basis of its linearity, sensitivity, and precision.

The corresponding calibration graphs were constructed by extracting in duplicate nine working aqueous standards containing the analytes at concentrations in the range from 0.3 to 1000 $\mu g\cdot L^{-1}$. The sensitivity of the method was evaluated according to the limit of detection (LOD) and quantification (LOQ). LODs, calculated by using a signal-to-noise ratio (S/N) of 3, were 0.1 $\mu g\cdot L^{-1}$ and the LOQs, calculated by using a S/N of 10, were 0.3 $\mu g\cdot L^{-1}$ for all analytes.

Table 2. Analytical figures of merit of Si-MWCNTs monolithic microextraction unit to the determination of the target polycyclic aromatic hydrocarbons.

Analyte	**m/z**	**LOD ($\mu g\cdot L^{-1}$)**	**LOQ ($\mu g\cdot L^{-1}$)**	**Precision**	
				Intra-extraction units RSD (%, n=5)	**Inter-extraction units RSD (%, n=5)**
Naphthalene	128	0.1	0.3	14.1	12.9
Phenanthrene	178	0.1	0.3	10.2	14.4
Fluoranthene	202	0.1	0.3	8.8	10.9
Pyrene	202	0.1	0.3	8.4	7.9
Benz[a] anthracene	228	0.1	0.3	10.8	10.7
Benzo[a] pyrene	252	0.1	0.3	13.3	13.9

LOD limit of detection, LOQ limit of quantification, RSD relative standard deviation

The linearity was maintained at least in the interval 0.3–1000 $\mu g\cdot L^{-1}$ for all analytes. Fig. 7 shows a SIM chromatograph with the different m/z fragments ions obtained after monolithic microextraction procedure of a blank water and a standard sample with the target analytes at 0.1 $\mu g\cdot L^{-1}$. The precision of the method was studied under repeatability and reproducibility experimental conditions. Repeatability (intra-extraction units conditions), expressed as relative standard deviation (RSD) was calculated from five individual standards prepared at a concentration of 1 $\mu g\cdot mL^{-1}$ and it was lower than 14.1 % for all the analytes. The reproducibility between extraction units (inter-extraction units) was also evaluated and the results, expressed as RSD (n=5) ranged from 5.9 to 14.4 %.

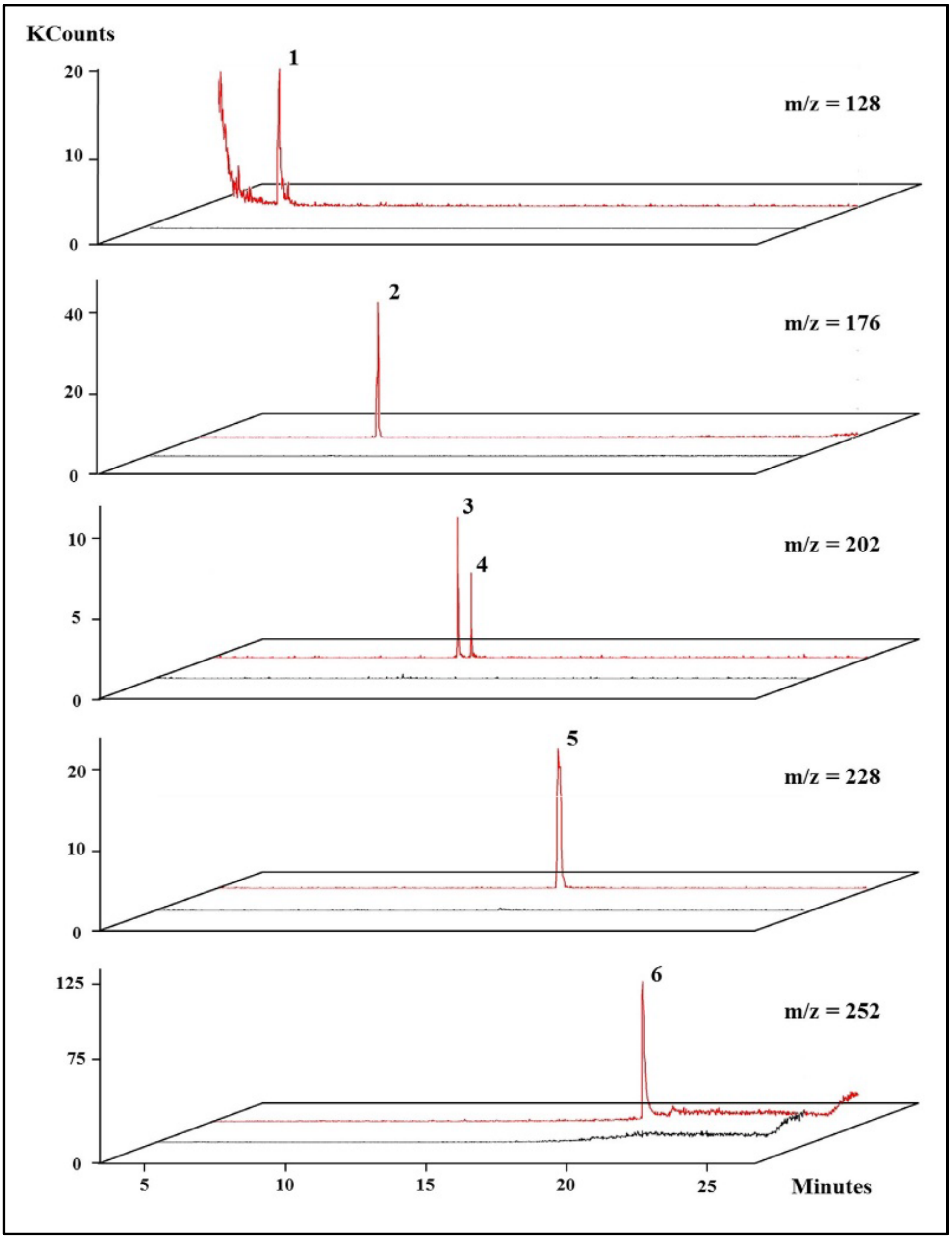

Figure 7. SIM chromatogram obtained after monolithic microextraction procedure of a blank water (green) and a standard sample with the target analytes at 0.1 µg L^{-1} (red). Peaks: **(1)** Naphthalene, **(2)** Phenantrene, **(3)** Fluoranthene, **(4)** Pyrene, **(5)** Benz[a]anthracene, **(6)** Benzo[a]pyrene.

Once optimized and analytically characterized, the proposed method was applied to the determination of the target PAHs in environmental water samples (tap and river). The samples were analyzed in order to find any potential presence of the analytes. Since the analytes were not detected, validation samples were prepared using blank waters samples (tap and river waters) fortified with the six target analytes (naphthalene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene and benzo[a]pyrene) at a concentration of 20 $\mu g \cdot L^{-1}$. Samples were maintained in amber glass bottles without headspace and in the dark for 24 h until analysis. Then, the fortified samples were analyzed using the extraction method, and the concentration for each PAH was calculated by interpolating the peak area obtained in the corresponding calibration graph.

The recovery values were calculated dividing the concentration found by the concentration added, and expressed in percentage. The recovery values (average of three replicates) obtained for each of the fortified samples analyzed are shown in Table 3, and they ranged from 72 to 124 %.

Table 3. Recovery study for naphthalene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene, and benzo[a]pyrene spiked to water samples analyzed following Si-MWCNTs monolithic extraction unit.

Analyte	**Tap water (%, n=3)**	**River water (%, n=3)**
Naphthalene	113 ± 7	105 ± 5
Phenanthrene	85 ± 11	82 ± 14
Fluoranthene	99 ± 9	76 ± 9
Pyrene	101 ± 8	72 ± 8
Benz[a]anthracene	124 ± 5	121 ± 10
Benzo[a]pyrene	114 ± 14	109 ± 14

4. Conclusions

In this study, a novel silica monolith with embedded carbon nanoparticles was synthetized inside a fused silica capillary for the extraction of non-polar compounds from water samples. The hybrid monolithic solid sorbent was prepared by two-step catalytic sol-gel process and the NPs were directly added into the polymerization mixture. The most critical point of the synthesis was to achieve stable dispersions of the NPs due to their tendency to form aggregates over time.

A comparison between the silica monolith without and with carbon nanoparticles was carried out. Hence, the three selected NPs were evaluated as sorbents improving the extraction capacity in any case. Among them, the best results were obtained for the c-MWCNTs being selected as optimum. Variables affecting to the preparation of the monolithic solid as well as the extraction conditions were evaluated by optimizing experimental parameters.

This work demonstrates that the addition of the carbon NPs into silica solid monolithic significantly increases the extraction efficiency for the target analytes due to π–π stacking interaction and hydrophobic effect. In addition, the proposed microextraction method shows favorable analytical features in comparison to the previously reported analytical methods for the determination of PAHs in water samples. By way of example, Table 4 summarizes the main analytical information of the selected references related to the proposed microextraction method, including LODs and LOQs as well as extraction format used. As it can be derived from the data shown in the table, the proposed method presents similar limits of quantification compared with the majority of the other approaches. The lowest LOQs were obtained for [21], were an on-line thermal desorption step of the retained analytes was implemented. This fact dramatically improves the method sensitivity as it avoids the dilution inherent to the chemical elution. Also, the inclusion of the fluorimetic detection resulted in a better sensitivity thanks to the native fluorescence of PAHs. Nevertheless, it should be highlighted that present hybrid sorbent has been optimized for achieve the concentration level established by ATSDR, and therefore, a better sensitivity is not required in water samples.

Acknowledgments

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Table 4. Comparison of the developed Si-CNPs monolith with other monolithic solids reported for the determination of PAHs.

Monolithic sorbent	Selected PAHs	Packing	Sample	LODs	LOQs	Detection	Ref.
Si-CNPs monolith	Naphthalene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene, and benzo[a]pyrene	Monolithic capillary column	Water samples	0.1 µg·L^{-1}	0.3 µg·L^{-1}	GC-MS	This work
Molecularly imprinted sol-gel polymer	Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene	Packed SPE	Water samples	5.2-12.6 ng·L^{-1}	0.10-1.0 µg·L^{-1}	GC-MS	[20]
Poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith	Naphthalene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, benz[a]anthracene, benzo[k], fluoranthene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene	Monolithic capillary column	Water samples	2.8-11.5 ng·L^{-1}	0.012-0.05 µg·L^{-1}	GC-MS	[21]
Graphene monolith	Naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, and benz[a]anthracene	Monolithic fiber	Water samples	4.0-50.0 ng·L^{-1}	0.05-0.5 µg·L^{-1}	GC	[22]
Hybrid organic-inorganic	Biphenyl, fluorene, phenanthrene, and fluoranthene	Monolithic capillary column	Water samples	2.4-8.1 µg·L^{-1}	8.0-27.8 µg·L^{-1}	µ-HPLC-UV	[23]
Poly(vinylpyrrolidone-*co*-divinylbenzene)	Naphthalene, fluorene, and fluoranthene	Stir bar	Water samples	0.045-0.093 µg·L^{-1}	-	GC-MS	[24]
PolyHIPE monolith	Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene	Pipette tips	Water samples	0.004-0.228 ng·L^{-1}	0.1-1.0 µg·L^{-1}	HPLC-FLD	[25]

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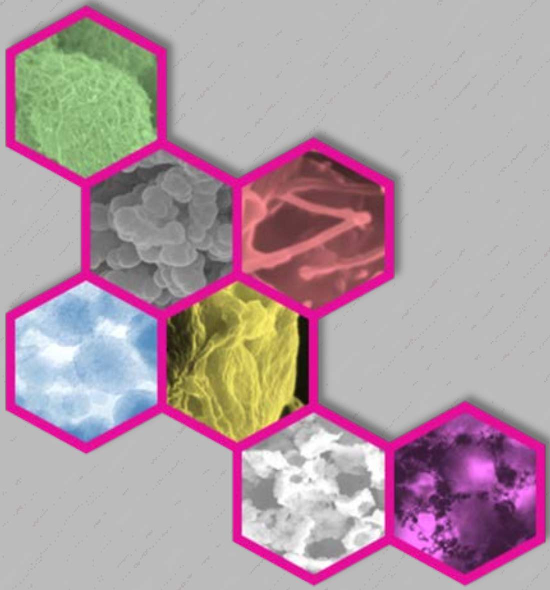
Bloque II

Sólidos Híbridos Basados en la Combinación de Polímeros Monolíticos y Nanopartículas de Carbono Preparados en Otros Formatos de Microextracción

Hybrid Solids Based on the Combination of Monolithic Polymers and Carbon Nanoparticles Prepared in Other Microextraction Formats



La conversión de los monómeros hacia la formación del polímero sólido está fuertemente ligada al volumen/cantidad de mezcla de polimerización. En general, cuando se preparan sólidos monolíticos de tamaño macroscópico se incrementa la contracción durante el proceso de polimerización y/o el tiempo para completar la polimerización aumenta, lo cual puede causar fisuras o huecos y como resultado unidades de microextracción con poca estabilidad mecánica. Esto es un factor clave cuando se sintetizan sólidos monolíticos en dispositivos de microextracción, tales como columnas de centrifugación (*spin-columns)*, puntas de pipeta (*pipette-tips*), o barras de agitación (*stir-bars*).

Una forma habitual para intentar prevenir este tipo de comportamiento es el anclaje químico del sólido a las paredes del soporte. Pero si además se añaden nanopartículas de carbono a la mezcla de reacción, no sólo se mejora la eficiencia de la extracción, sino que se puede también aumentar la velocidad de polimerización y, en consecuencia, la robustez y morfología del sólido final.

Así, en el ***Capítulo 5*** de la memoria de la Tesis Doctoral se presenta la síntesis de un sólido monolítico con nanocuernos de carbono en el interior de un dispositivo de polipropileno (*spin-column*) para la determinación de anti-inflamatorios en muestras de orina. Siguiendo esta línea, en el ***Capítulo 6*** se aborda la preparación de un sólido monolítico híbrido en una punta de pipeta. Esta vez se incorporaron nanotubos de carbono de pared múltiple, y la unidad se optimizó para la separación y preconcentración de antidepresivos de muestras de orina.

Por otro lado, en el ***Capítulo 7*** se describe la fabricación de una unidad de microextracción monolítica con agitación integrada. En este caso, las nanopartículas (nanocuernos de carbono) permitieron obtener un sólido altamente poroso, pero suficientemente rígido para soportar la fricción causada por la agitación magnética durante las etapas de extracción. Finalmente, dicha unidad de extracción se empleó con éxito en la determinación de filtros solares (parabenos) en muestras de origen medioambiental y biológico.

Capítulo 5

Preparation of Porous Methacrylate Monoliths with Oxidized Single-Walled Carbon Nanohorns for the Extraction of Nonsteroidal Anti-Inflammatory Drugs from Urine Samples

Microchimica Acta, 184 (2017) 1863-1871

Preparation of porous methacrylate monoliths with oxidized single-walled carbon nanohorns for the extraction of nonsteroidal anti-inflammatory drugs from urine samples

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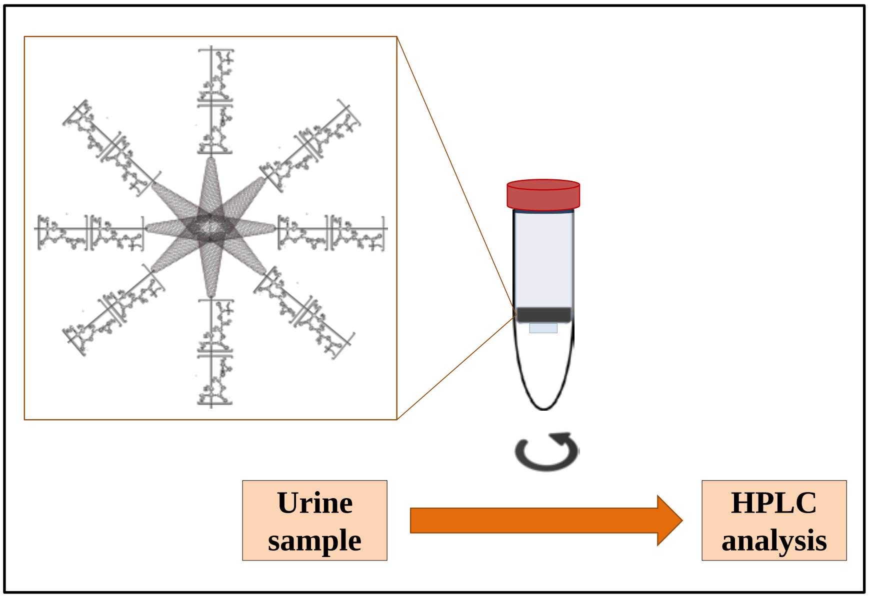

Graphical abstract

A hybrid monolithic solid based on the copolymerization of methacrylate monomers and single-walled carbon nanohorns was covalently anchored and synthetized into a spin column device via photo-polymerization approach. The spin column was used for the extraction of nonsteroidal anti-inflammatory drugs.

Abstract

A copolymer was prepared from glycidyl methacrylate, ethylene glycol dimethacrylate and oxidized single-walled carbon nanohorns via photo-polymerization and used in spin columns for the extraction of nonsteroidal anti-inflammatory drugs (NSAIDs) from human urine samples. All microextraction procedures (loading, washing and elution) can be performed by centrifugation. The hybrid monolithic polymers were characterized by scanning electron microscopy and nitrogen intrusion porosimetry. Following elution with methanol, the NSAIDs (naproxen, fenbufen, flurbiprofen, and ibuprofen) were quantified by reversed-phase HPLC with UV detection. The detection limits varied between 0.1 and 10 $\mu g \cdot L^{-1}$, and the precision (relative standard deviation) ranged from 3.5 to 11.8%. Relative recoveries between 81 and 106% were found when analyzing spiked urine samples.

Keywords

Centrifugal spin columns; Photo-polymerization; (Micro)Solid-phase extraction; High-performance liquid chromatography; Biological samples.

1. Introduction

Since the first synthesis of monolithic polymers at the end of the twentieth century [1] organic monoliths derived from methacrylates, acrylamides, and styrenes have been successfully applied as stationary phases in chromatography [2], and as sorbents in sample treatment techniques [3] but to a lesser extent. Among the microextraction formats where monolithic sorbents have been used, spins columns can be cited as one of the most advantageous. Thus, the spin-column format offers a simple operation procedure, allows a high-throughput sample, requires a low eluate volume and does not involve solvent evaporation, all features in rough agreement with the principles of green analytical chemistry. Monolithic silica spin column was first introduced by Namera and Saito in 2008 [4, 5]. The monolithic solid is packed in the bottom of the column unit without using frits, and then solvents are passed through the sorbent phase by centrifugation. This approach has been used to extract target compounds from biological matrices [6, 7]. While there are already several monolithic silica spin columns used to isolate and preconcentrate target analytes, a potential growth area of interest may be the development of polymer-based monoliths. In this context, Güzel et al. have developed an erbium phosphate doped poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate (poly(GMA-*co*-EDMA)) spin column for selective enrichment of phosphopeptides [8].

To achieve more specific interaction of the analytes with the polymeric networks, nanomaterials including graphene [9], carbon nanotubes [10, 11], and carbon nanohorns [12], have been combined with monolithic material to prepare novel stationary phases or sorbents with enhanced performances. Single-walled carbon nanohorns (SWNHs) were firstly discovered by Iijima et al. in 1999 [13]. Concretely, SWNHs are cone-shaped carbon structures with a single wall, which have natural tendency to form dahlia-shaped aggregates [14]. Their conical structure provides high porosity and large surface area showing a good absorbent capacity for organic compounds [15]. Up to date, their potential and usefulness has widely demonstrated in microextraction techniques [16–19].

In this study, a hybrid monolithic phase based on a combination of methacrylate monomers and oxidized SWNHs (o-SWNHs) has been synthetized into a spin column device employing a UV-polymerization approach. To ensure covalent attachment of the monolith to the inner wall of the polypropylene device, a surface modification was first carried out with grafted chains of EDMA. Then, the optimization of variables affecting to the more convenient preparation of the hybrid monolith in terms of uniformity, rigidity and robustness was addressed. Moreover, the monolith copolymerized with o-SWNHs was characterized by scanning electron microscopy (SEM) and a porosimetry study was also carried out. The spin column was evaluated for the extraction and preconcentration of four nonsteroidal anti-inflammatory drugs (NSAIDs) in urine samples prior to HPLC-UV analysis.

2. Experimental section

2.1. Reagents and materials

All reagents were of analytical grade or better. Nonsteroidal anti-inflammatory drugs (NSAIDs) (naproxen, fenbufen, flurbiprofen, and ibuprofen) were purchased from Sigma-Aldrich (Madrid, Spain. http://www.sigmaaldrich.com). Standard solutions of each analyte were prepared in methanol (Sigma-Aldrich) at a concentration of 1 $g \cdot L^{-1}$ and stored at 4 °C. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in ultrapure Milli-Q water. Methanol was also used for NSAIDs elution.

Single-walled carbon nanohorns (SWNHs) were purchased from Carbonium S.r.l. (Padua, Italy). SWNHs form stable dahlia-shaped aggregates with an average diameter of 60–80 nm. Individually, the lengths of these SWNHs are in a range

40–50 nm, and the diameter in the cylindrical structure varies between 4 and 5 nm. Laboratory-oxidized carbon nanohorns were prepared following a procedure previously optimized [18]. In brief, SWNHs were weighed (5 mg) and added to a glass vial, which was further introduced into a microwave oven, being the solid irradiated at 800 W for 10 min.

Acetonitrile, acetic acid (Scharlab, Barcelona, Spain. http://scharlab.com) and ultrapure Milli-Q water were employed as components of the chromatographic mobile phase. The reagents used for the synthesis of the monolithic phase, glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), cyclohexanol, 1-dodecanol, lauroyl peroxide (LPO), ethanol, acetone, 2,2-dimethoxy-2-phenylacetophenone (DMPA), α,α′-azobisisobutyronitrile (AIBN), and benzophenone (BP) were purchased from Sigma-Aldrich. The spin columns were supplied by Sigma-Aldrich (SigmaPrep™ Spin Columns with Break-Away Tip), which have a maximum volume of 700 µL.

Blank urine samples were collected from healthy adult volunteers and stored in polytetrafluoroethylene flasks at -20 °C until analysis. Prior to the microextraction process, urine samples were diluted to 1:1 with phosphate buffer (pH 2.11, 25 mM). Next, each sample filtered through a disposable nylon filter of 0.45 µm of pore size (Análisis Vínicos, Tomelloso, Spain. http://www.analisisvinicos.com). In order to validate the method, samples collected from individuals treated with NSAIDs, were also employed.

2.2. Instrumentation

The photo-polymerization was carried out using a UV crosslinker (model CL1000) from UVP Inc. (Upland, CA, USA) equipped with UV lamps (5 × 8 W, 254 nm). SEM photographs of monolithic materials were taken with a scanning electron microscope (S-4100, Hitachi, Ibaraki, Japan) provided by a field emission gun and an EMIP 3.0 image data acquisition system (Rontec, Normanton, UK). Samples for SEM analysis were previously sputter-coated with Au/Pd for 2 min to avoid charging problems. This treatment was necessary since these materials are not electrically conducting. Optical microscope SZH (Olympus) was used to obtain the pictures of the hybrid monolithic solid. An ultracentrifuge (Sigma Laborzentrifugen, model 2–15 Osterode am Harz, Germany) was used for the different extraction steps.

Nitrogen adsorption/desorption experiments were carried out at -196 °C using a Quantachrome®ASiQwin™-AutomatedmGas Sorption Data. The specific surface area values were calculated according to the BET (Brunauer-Emmett-Teller) eq. T-plot method was used to determine the micropore surface areas, and the average pore volumes were evaluated from the desorption branches of isotherms based on the BJH (Barrett-Joyner-Halenda) model.

An 1100 Series HPLC chromatograph (Agilent Technologies, Waldbronn, Germany), provided with a quaternary pump, including a thermostatic column compartment and a UV-Vis diode array detector was used. For the optimized procedure, a Kinetex C18 column (Phenomenex, Torrance, US, 2.6 µm, 100 Å, 150 × 4.6 mm) was used. The flow rate was 1 $\mathrm{mL \cdot min^{-1}}$. UV detection wavelengths were set at 220, 230, and 254 nm using 360 nm as reference. Separation was accomplished using two mobile phase solvents: ultrapure Milli-Q water (mobile phase A) and ACN (mobile phase B) both containing 0.1% (*v/v*) of acetic acid. An isocratic gradient (50% B) in 11 min was performed at 25 °C. Prior to use, all mobile phases were degassed with a D-78224 ultrasonic bath (Elma, Germany).

2.3. Modification of spin wall surface and preparation of hybrid monolithic material

To ensure covalent attachment of the monolithic beds to the inner spin column wall for enhanced mechanical stability, the polypropylene wall surface was previously photo-chemically modified with BP and EDMA. For this purpose, the spin column was sequentially washed with ethanol and acetone, and dried under a nitrogen stream. Next, it was filled with 80 μL of a 5% *w/v* methanolic BP solution and irradiated with UV light at 1 J·cm^{-2} for 10 min. BP was removed with methanol followed by a drying step with nitrogen. Then, the spin column was filled with a 15% (*v/v*) methanolic EDMA solution (80 μL) and placed again under UV irradiation for 10 min. Finally, the spin column modified was washed with acetone and dried under a nitrogen stream.

The polymerization mixture is composed of 60 wt% monomers (48 wt% GMA and 12 wt% EDMA), 40 wt% porogens (37 wt% cyclohexanol and 3 wt% 1-dodecanol) and 0.3 wt% of LPO (out of the total weight of monomers) as free-radical initiator. In the next step, o-SWNHs (0.1 wt%) was added to the polymerization mixture. Afterward, the mixture was sonicated for 10 min and purged with nitrogen for an additional 10 min. The spin column previously modified was filled with 70 μL of the polymerization mixture. Polymerization was accomplished by irradiation of the spin column within the UV crosslinker chamber at 1 J·cm^{-2} for 4 h. After polymerization, the spin column was thoroughly washed with methanol and Milli-Q water to remove the pore-forming solvents and any possible unreacted monomers.

2.4. Microextraction procedure

The poly(GMA-*co*-EDMA-*co*-o-SWNHs) monolithic material was used for the extraction of NSAIDs from urine samples. The general scheme of the microextraction procedure is depicted in Fig. 1. Prior to extraction of NSAIDs, the hybrid monolith was preconditioned with 0.1 mL of methanol and 0.1 mL of Milli-Q water by centrifugation at 14000 rpm (18,000 *g*) for 10 min, respectively. Urine samples were diluted to 1:1 with phosphate buffer (pH 2.11) and filtered through a disposable nylon filter (0.45 μm). Then, 0.6 mL of sample, were placed into the pre-conditioned spin column, and it was centrifuged at 18,000 *g* for 55 min. The monolithic phase was then rinsed with Milli-Q water (0.1 mL) by centrifuging (18,000 *g*) for 10 min. Finally, the adsorbed analytes were eluted with 50 μL of methanol (5 min, 18,000 *g*) for further HPLC analysis.

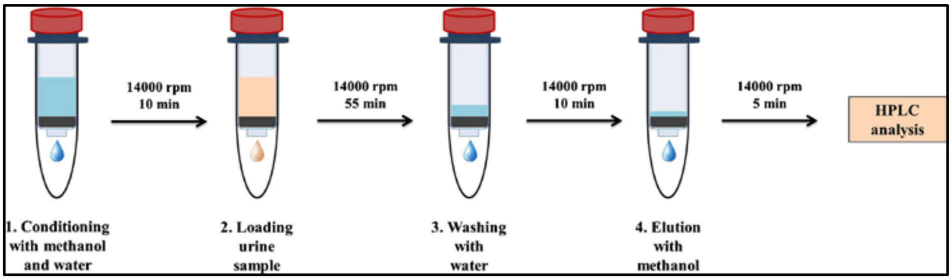

Figure 1. Scheme of the microextraction procedure using the monolithic spin column.

3. Results and discussion

3.1. Surface attachment of monolith to polypropylene spin column

Polypropylene surface modification is required for the successful attachment of the monolithic polymer. This fact prevents both the formation of voids at the monolith-wall interface due to the shrinkage of the monolith during the polymerization process in bulk polymerization as well as the monolith detachment during extraction operation steps. This is especially remarkable in non-treated conical housing materials. Supersonic adhesion is usually used to fix monolithic silica rod into the spin column devices. However, covalent binding of the organic polymer-based monoliths to the spin column inner wall has not yet been reported. In this work, a UV grafting process based on the initial UV immobilization of BP derived free-radical initiator followed by the grafting of a polymer layer (EDMA) with a multiplicity of pendant double bonds from the polypropylene inner surface has been optimized. The influence of the UV irradiation time, at both steps, keeping the irradiation power level fixed, has been studied within the interval 5–60 min. The results showed that 10 min was selected as the time required to reach a covalent attachment of the hybrid monolith to the spin column wall (respective figure is given in the *Supporting Information*).

3.2. Choice of materials

One of the benefits of polymer monolithic materials is their high permeability due to their large through pores. However, these materials exhibit small surface areas due the absence of meso- and nano-porous structures, which can reduce the number of interaction sites required to achieve a sufficient sample loading capacity, an important parameter in sorption-based extraction techniques. Then, the combination of monolithic technology and the specific features of SWNHs is an attractive way of obtaining novel sorbents with enhanced adsorption (retention) performances. In order to demonstrate the improvement in the extraction performance of polymeric monoliths after incorporation SWNHs, monoliths from a relatively polar monomer, glycidyl methacrylate (GMA) were selected.

3.3. Incorporation of o-SWNHs to methacrylate monoliths

Taking into account these considerations, our approach was to incorporate SWNHs in the monolith by direct addition (dispersion) of these carbon nanostructures to the polymerization mixture in order to obtain a hybrid monolith with mechanical stability and large extraction efficiency. To reach this goal, initial polymerization conditions were as follows; 20 wt% monomers (15 wt% GMA and 5 wt% EDMA) and 80 wt% (75 wt% cyclohexanol and 5 wt% 1-dodecanol), in the presence of different free-radical initiators. Furthermore, 0.1 wt% of o-SWNHs was well-dispersed in this mixture by sonication for 10 min, and then purged with nitrogen. Once the mixture was filled into the spin column, it was placed under UV irradiation for 4 h.

Thus, several types of free-radical initiators were tested in terms of the polymerization rate as well as homogeneity of the polymer formed. The behavior of AIBN (1 wt%), DMPA (0.2 wt%) and LPO (0.3 wt% out of the total weight of the monomers), which affects the kinetics of the free-radical polymerization as well as the morphology of the resulting polymer [20, 21], was evaluated. When AIBN or DMPA were used as radical initiator, the o-SWNHs were entrapped into the monolithic network remaining some carbon nanoparticles on the pore surface available to interact with the analytes. However, a copolymerization of monomers (GMA and EDMA) with o-SWNHs was produced using an organic peroxide initiator such as LPO. This may be due to the existence of a competition between monomer and o-SWNHs for the initiator radicals [22]. In this regard, the role of initiator is a key point, AIBN and DMPA undergo a reaction mechanism which leads to the production and subsequent propagation of monomer radicals in comparison with the active o-SWNHs surface generated by LPO. Thus, hybrid monoliths initiated with LPO exhibited the most favourable and homogeneous structure for the extraction performance, and therefore LPO was selected as photo-initiator.

The second variable studied, in order to obtain a rigid and stable monolithic polymer with high extraction capacity, was the monomers/porogens ratio within the following proportions: 20/80 % (*w/w*), 40/60 % (*w/w*), 60/40 % (*w/w*), and 80/ 20 % (*w/w*). The permeability and mechanical stability of the hybrid monolith were controlled by the percentage of porogenic solvents. While monoliths prepared with ratios of 20/80 and 40/60 % (*w/w*) showed a too porous and brittle structure causing their break in the centrifugation step, when the proportion was 80/20 % (*w/w*) the monolithic phase resulted in the smaller pores, hindering the flow of solvents through it. The best morphological characteristics were obtained for a 60/40 % (*w/w*) ratio and therefore it was selected for further experiments.

Next, the amount of o-SWNHs was evaluated in the range from 0.05 to 0.5 wt%. Monolithic polymers formed at high concentrations of nanoparticles showed a more rigid structure avoiding the breakage of the material during the passage of the solvents through it and promoting the reuse of the monolithic solid. By contrast, monoliths were not formed in the absence of o-SWNHs in the polymerization mixture because polymer radicals for methacrylate monomers are not as reactive as the o-SWNHs conical surface due to its reactivity is associated with their areas rich in pentagons and heptagons [23].

When the percentage was 0.5 wt%, the solid resulted in the smaller pores and therefore it led to an increased flow resistance. Thus, an amount of 0.1 wt% of SWNHs was selected as the best compromise for high throughput purposes. Figure 2 shows a picture of this hybrid monolithic polymer into the spin column.

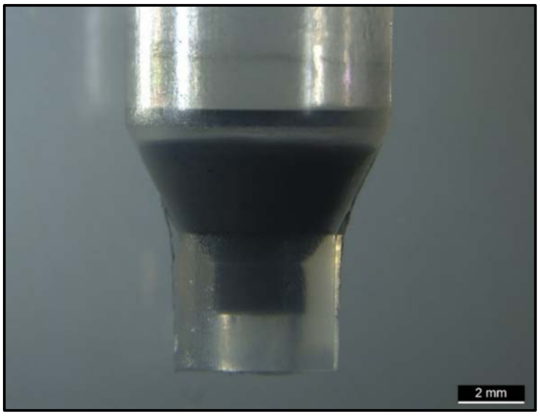

Figure 2. Photograph of the hybrid monolith into the spin column device.

3.4. Characterization of hybrid monolithic material

The monoliths were characterized by SEM, elemental analysis, and nitrogen adsorption/desorption measurements. Figure 3 shows the SEM images of the UV polymerized monoliths obtained in presence of 0.1 wt% of SWNHs at different magnification powers. The macroporous structure with different levels of pore sizes is visible which demonstrated large pore (~ 3 μm) sizes, and consequently, an adequate permeability (Fig. 3a). A larger magnification provided evidence that the addition of SWNHs in the polymeric matrix led to globules with larger surface roughness compared to those found in the typical globular structure of polymeric monoliths [1, 2, 20, 21].

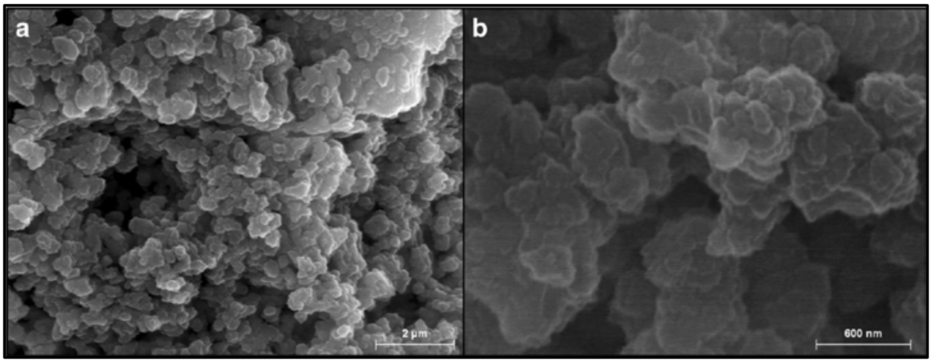

Figure 3. SEM micrographs of hybrid monolithic material at ×1000 **a**, and ×4000 **b** magnification.

Data from nitrogen adsorption-desorption isotherms evidenced that the monolithic solid exhibited an isotherm type IV which is typical of solids with a mainly mesoporous structure. Furthermore, t-plots (using Harkins-Jura correlation) from the adsorption branch of the isotherm, showed the absence of microporosity. The specific surface area of the hybrid solid was determined by the BET method and the pore size distribution by the method of Barrett, Joyner, and Halenda (BJH). The values of surface area, pore diameter, and pore volume, obtained are compiled in Table 1. Thus, the hybrid monoliths containing o-SWNHs showed larger values surface areas (ca. 700 $m^2 \cdot g^{-1}$) compared to the typical organic polymer-based monolith, where these values did not exceed few tens of $m^2 \cdot g^{-1}$. This remarkable increase in surface area will benefit undoubtedly the retention and extraction efficiency, which will be described in detail below.

Table 1. Porous properties of the synthesized hybrid monolithic polymer.

	Specific surface area, BET ($m^2 \cdot g^{-1}$)	**Average pore diameter (Å)**	**Average pore volume ($cm^3 \cdot g^{-1}$)**
Poly(GMA-*co*-EDMA-*co*-o-SWNHs) monolith	696.439	43.32	0.75

3.5. Application to the extraction and preconcentration of NSAIDs from urine samples

The hybrid monolithic sorbent presented in this work was employed for the extraction of the four NSAIDs (naproxen, fenbufen, flurbiprofen, and ibuprofen) from urine samples following the microextraction procedure described previously. The extraction conditions were adapted from previous studies of our research group [24]. Prior to (micro)solid-phase extraction using the poly(GMA-*co*-EDMA-*co*-o-SWNHs) spin column, each sample was diluted to 1:1 with phosphate buffer and filtered through a disposable nylon filter. The pH of the sample was adjusted to 2.11 by using dilute phosphate buffer to maximize the interaction of the analyte with the hybrid monolith via hydrophobic and hydrogen bonds interactions.

The method was validated in terms of sensitivity, linearity, and precision. The corresponding calibration graphs were constructed by extracting in duplicate nine working aqueous standards containing the four analytes at different concentrations (0.5–10,000 $\mu g \cdot L^{-1}$). For all the analytes, a good linearity ($R>0.99$) was observed. The limits of detection (LODs) were calculated by using a signal-to-noise ratio of 3, giving values comprised between 0.1 (naproxen) and 10 (ibuprofen) $\mu g \cdot L^{-1}$ (Table 2). The limits of quantification (LOQs), calculated as the concentration providing chromatographic peak areas ten times higher than the background noise, ranged from 0.5 to 20 μg·L^{-1} (Table 2). The precision of the method (intra and inter-units), expressed as relative standard deviation (RSD) and also given in Table 2, was calculated from three individual standards prepared at a concentration of 50 μg·L^{-1} and it was lower than 11.8% for all the analytes. The enrichment factors for all the analytes were calculated by comparison of the slopes of the calibration graphs before and after the extraction process. They were in the range from 10.4 to 13.2 (see Table 3). The absolute extraction recoveries, which refer to the percentage of total analyte that can be extracted efficiently by the sorbent and finally eluted with methanol, were in the interval of 81–106%.

Table 2. Analytical figures of merit of poly(GMA-*co*-EDMA-*co*-o-SWNHs) monolithic microextraction unit for determination of the target NSAIDs.

				Precision	
Analyte	**λ (nm)**	**LOD (μg·L^{-1})**	**LOQ (μg·L^{-1})**	**Intra- spin column RSD (%, n=3)**	**Inter- spin columns RSD (%, n=3)**
Naproxen	230	0.1	0.5	4.0	3.5
Fenbufen	220	0.5	1	11.8	9.1
Flurbiprofen	254	0.5	1	5.4	8.0
Ibuprofen	220	10	20	5.8	9.8

LOD: limit of detection, LOQ: limit of quantification, RSD: relative standard deviation.

The identification of potential interferences from the matrix on the quantification of the analytes is a relevant issue, especially when analyzing unknown samples. Therefore, the accuracy of the method was evaluated through a recovery study. Different blank urine samples were fortified with the four target analytes (naproxen, fenbufen, flurbiprofen, and ibuprofen) at a concentration of 50 μg·L^{-1}, and they were left to stand for 24 h prior to analysis. Then, the fortified samples were analyzed using the extraction method, and the concentration for each NSAID was calculated by interpolating the peak area obtained in the corresponding calibration graph.

The recovery values were calculated dividing the concentration found by the concentration added, and expressed in percentage. Each sample was analyzed by triplicate; the results obtained are listed in Table 3. As it can be seen, in all instances, excellent recovery values (ranged from 81.3 to 105.6 %) were obtained.

Table 3. Recovery study for the four NSAIDs spiked to urine samples analyzed following o-SWNHs monolithic spin column unit.

Analyte	**R ± SD**	**EF**	**ER (%)**
Naproxen	99 ± 3.0	11.8	98.3
Fenbufen	81 ± 6.1	10.4	86.6
Flurbiprofen	106 ± 4.8	11.5	95.8
Ibuprofen	101 ± 5.6	13.2	110.0

R, relative extraction recovery expressed as percentage. SD, standard deviation. EF, enrichment factor. ER, absolute extraction recovery.

The method was then applied to the determination of the NSAIDs in urine samples (3 h after drug intake). Urine samples, collected from an individual treated with naproxen (550 mg) were analyzed. The extraction was carried out for standards and spiked samples following the procedure detailed above. The presence of naproxen was definitely confirmed by the comparison of its retention time with the spiked standard of the same analyte. The corresponding value was 1.79 mg·L^{-1} which is comparable with pharmacokinetic values for the NSAIDs, and is closely related to half-life values reported in literature [25, 26]. Furthermore, the method allows the quantitative determination of the target analytes without interference of endogenous compounds.

3.6. Comparison with other nanomaterial-based extraction procedures

Table 4 compares the characteristic features for our method with other nanomaterial-based extraction methods reported in the literature for the determination of NSAIDs in biological samples. Regarding absolute extraction recovery values obtained in this study, these were similar to those found in most reported studies with the exception of those given in reference [30], where the recoveries were quite low. Concerning the LODs, most of these studies have been focused on the determination of naproxen, in this sense, our LOD value was similar [31] or better [28] using magnetic SPE. Besides, our method provided the widest linearity range of all the methods reported. Regarding to the preparation of sorbent few simple steps are required, and moreover our protocol simplifies the handling of more samples simultaneously and speeds the preconcentration process of NSAIDs. In particular, this method allowed a sample throughput of 10 samples·h^{-1}, whereas a rate of 1–2 samples·h^{-1} may be achieved with other nanomaterials-based protocols [27, 29].

Table 4. An overview on recently reported nanomaterial-based methods for preconcentration and determination of NSAIDs.

Nanomaterials used	**NSAIDs selected**	**Method applied**	**Sample**	**LOD ($\mu g \cdot L^{-1}$)**	**Linearity range ($\mu g \cdot L^{-1}$)**	**ER (%)**	**Ref.**
Magnetic nanoparticles	Indoprofen, ketoprofen, naproxen, zaltoprofen, flurbiprofen, diclofenac, ibuprofen, meclofenamic acid, mefenamic acid, flufenamic acid and tolfenamic acid	MSPE-HPLC/DAD	Water samples	0.32-1.44	5-800	-	[27]
Magnetic nanoparticles	Naproxen	MSPE-HPLC-UV	Human plasma and urine samples	0.07	0.5-500	99	[28]
Magnetic nanoparticles	Naproxen, indomethacin, diclofenac, and ibuprofen	MSPE-HPLC	Human urine and wastewater samples	2.0-7.0	7.0-200	91.7-96.2	[29]
Polyaniline/hydroxyl multi-walled carbon nanotubes	Ketoprofen, naproxen, and fenbufen	SBSE-HPLC-UV	Environmental water and sediment samples	0.09-0.81	2-1000	46.2-52.9	[30]
Molecularly imprinted polymer-coated magnetic multi-walled carbon nanotubes	Naproxen	MIP-SPE-FL	Human urine samples	2.0	4-40.0	100	[31]
Poly(GMA-*co*-EDMA-*co*-o-SWNHs) monolith	Naproxen, Fenbufen, Flurbiprofen, and ibuprofen	MSCME-HPLC-DAD	Human urine samples	0.1-10	0.1-10000	86.6-110	This work

MSPE, magnetic solid-phase extraction. SBSE, stir bar sorptive extraction. MIP-SPE, molecularly imprinted polymer solid-phase extraction. MSCME, monolithic spin column microextraction. LOD, limit of detection. ER, absolute extraction recovery.

4. Conclusions

A monolithic spin column, based on copolymerization between methacrylate monomers and o-SWNHs, was prepared via in situ photo-polymerization. The potential of this new sorbent was evaluated for the preconcentration of NSAIDs from urine samples. The hybrid monolith was characterized by SEM and nitrogen intrusion porosimetry. A UV strategy for the covalent functionalization of the housing polypropylene surface to achieve a robust attachment of monolith has been carried out, thus improving its mechanical stability, without the need of retaining frits, in comparison with non-anchored monoliths. To our knowledge, this is the first report that employs this modification protocol combined with organic monoliths confined within these microextraction supports. The high adsorption capacity and specific surface area has been attributed to the participation of o-SWNHs as monomer in the polymerization reaction due to their enhanced reactivity associated with their conical shape. The results obtained demonstrated that the hybrid monolithic spin columns can be successfully applied in bioanalysis owing to its unique characteristic including their cost-effective preparation, porosity and chemical stability in a wide pH range. Although the method requires high centrifugation rates in extraction procedure, it provides good analytical features in term of recoveries, excellent linearity and LODs compared to other nanomaterial-based extraction methods, and a high throughput sample preparation.

Acknowledgments

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Supporting Information

1. Surface attachment of monolith to polypropylene spin column

To evaluate the successful covalent attachment of the monolith to the spin column wall, a simple visual inspection and scanning electron microscopy (SEM) micrographs of the support were performed. Then, SEM image of hybrid monolith were carried out in EDMA-modified spin column (Fig. S1). As it can be seen, no significant gap between the inner wall surface of spin column and the polymeric monolith was found, which confirmed that the monolith was tightly attached.

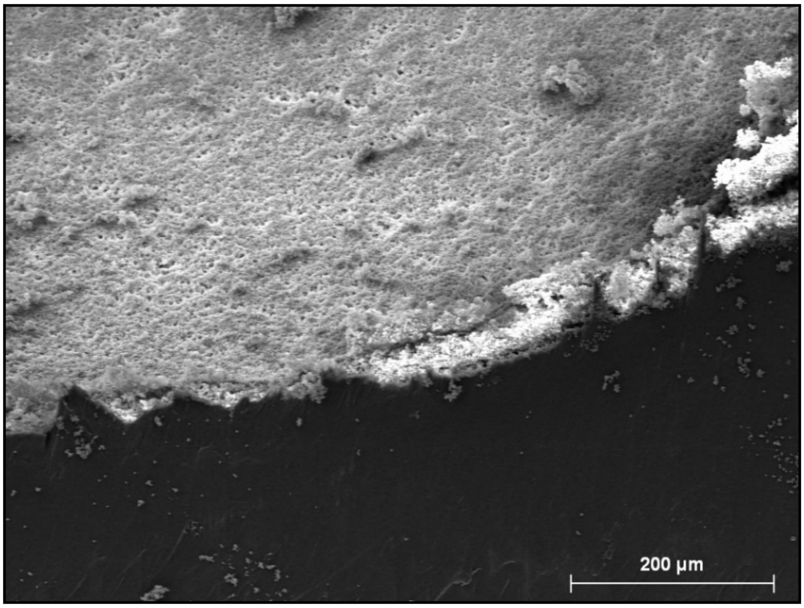

Figure S1. SEM micrograph of hybrid monolithic material into a spin column device.

Capítulo

6

Carbon Nanotube-Modified Monolithic Polymethacrylate Pipette Tips for (Micro)Solid-Phase Extraction of Antidepressants from Urine Samples

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Carbon nanotube-modified monolithic polymethacrylate pipette tips for (micro)solid-phase extraction of antidepressants from urine samples

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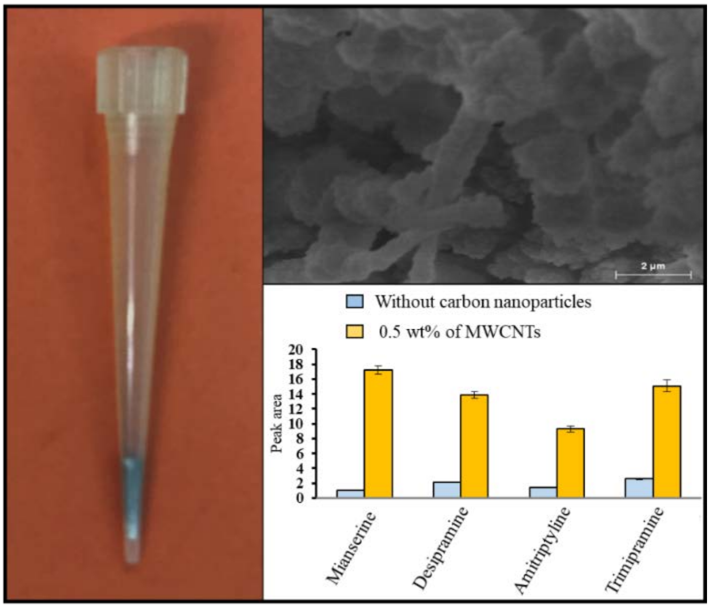

Graphical Abstract

Hybrid polymer monoliths containing multi-walled carbon nanotubes (MWCNTs) were prepared in pipette tips by photo-polymerization approach. The extraction devices were used for the extraction of antidepressants in urine samples.

Abstract

This work evaluates the potential of methacrylate monoliths with multi-walled carbon nanotubes incorporated into the polymeric network for the extraction of antidepressants from human urine. The method is based on a micropipette solid-phase extraction tip containing a hybrid monolithic material covalently attached to the polypropylene housing. A polymer layer made from poly(ethylene dimethacrylate) was bound to the inner surface of a polypropylene tip via UV grafting. The preparation of the monolith and the microextraction steps were optimized in terms of adsorption capacity. Limits of detection ranged from 9 to 15 μg·L^{-1}. The average precision of the method varied between 3 and 5% (intra-tips), and from 4 to 14% (inter-tips). The accuracy of the method was evaluated through a recovery study by using spiked samples.

Keywords

Carbon nanoparticles; Hybrid monolith; Microextraction; Porous polymer; Photografting process; UV attachment; Drugs; Liquid chromatography; Biological samples.

1. Introduction

Tetracyclic antidepressants (TCAs) are commonly used for treatment of psychiatric disorders such as depression and anxiety [1–3]. To minimize the risk of overdose and adverse effects of these drugs, their measurement in biological fluids is mandatory [4]. Several methods have been reported for the extraction of TCAs from human body fluids using conventional sample pretreatment techniques including liquid-liquid extraction [5], and solid-phase extraction (SPE) [6]. However, these approaches present main shortcomings such as large volumes of solvents as well as tedious manual operating procedures. In the last years, new microextraction techniques based on the simplification and the miniaturization of classical separation techniques have been developed [7–9].

Nanostructured materials can be identified as a turning point on the development of these new techniques particularly due to their high superficial area and variety of interactions [10, 11]. In this context, carbon nanotubes have been used as SPE sorbents for the analytical extraction of drugs, pesticides or natural compounds in different media such as biological fluids, drug preparations, environment, plants or animal organs [12, 13]. From an analytical point of view, carbon nanotubes are especially suitable for analytical applications derived from their nanometric size [14]. First, they exhibit interesting chemical properties and a high specific surface when used as a sorbent. In addition, they open up new approaches, providing exceptional possibilities for further miniaturization. Therefore, in order to benefit from their sorbent capacity, carbon nanotubes have to be efficiency embedded into a material or immobilized on a surface/support, such as disk [15], porous-hollow fiber [16], and polymer monoliths [17]. Among these alternatives, the combination of monolithic solids and the specific features of carbon nanotubes is an attractive way of obtaining novel stationary phases with high permeability, different selectivities, and enhanced performances. Wang et al. [18] have studied the incorporation of single-walled carbon nanotubes (SWCNTs) into a poly(MAA-*co*-EDMA) monolithic capillary column for enrichment of six triazine herbicides from water samples. Single- and multi-walled carbon nanotubes (MWCNTs) have been also embedded into polymerization mixtures containing tetraethoxysilane (TEOS) as bulk monomer [19]. However, the main challenge to include the carbon nanoparticles directly in the polymerization mixture is their tendency to aggregation, which can negatively affect to the final monolithic structure. This fact is frequently detected when thermal polymerizations are used. Faster polymerization kinetics and phase separation are achieved under UV initiation, which prevents the sedimentation of the nanoparticles at the bottom of the support [20].

A poly(GMA-*co*-EDMA) monolith with embedded MWCNTs have been prepared into a pipette tip device, and applied to isolate four antidepressants from human urine samples. In particular, MWCNTs were selected as modifiers of the polymer monolith on the basis of their sorbent capacity owing to their larger size and number of sheets compared with the SWCNTs [17, 19]. Moreover, the use of non-oxidized CNTs also avoids possible secondary interactions between analytes and negative charged carboxylic groups in alkaline media. Concerning the preparation of the microextraction unit, it involves two steps. In the first one, the polymer monolith is anchored to the polypropylene surface of the tips by photografting approach, while in the second one, the *in situ* polymerization of the monolithic phase (within the pipette tips) under UV light is developed. To the best of our knowledge, this is the first time that this hybrid monolithic phase (methacrylate monolith with carbon nanotubes) has been evaluated in this microextraction format. The preparation of the hybrid monolith has been studied in depth as well as all the variables related to the microextraction process. High-performance liquid chromatography (HPLC) was used for analytes identification and quantification.

2. Experimental section

2.1. Reagents and materials

All reagents were of analytical grade or better. Antidepressants (mianserine, trimipramine, desipramine, and amitriptyline) were purchased from Sigma-Aldrich (Madrid, Spain. http://www.sigmaaldrich.com). Standard solutions of each analyte were prepared in methanol (Sigma-Aldrich) at a concentration of 1 $g \cdot L^{-1}$ and stored at 4 °C. Working standard solutions were prepared daily by rigorous dilution of the stocks in ultrapure Milli-Q water. Methanol (MeOH) was also used for antidepressants elution.

Acetonitrile (ACN), acetic acid (Scharlab, Barcelona, Spain. http://scharlab.com/) and ultrapure Milli-Q water were employed as components of the chromatographic mobile phase.

The reagents used for the synthesis of the monolithic phase, glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), cyclohexanol, 1-dodecanol, lauroyl peroxide (LPO), were purchased from Sigma-Aldrich. Multi-walled carbon nanotubes (MWCNTs, >90% carbon basis, D×L 110–170 nm × 5–9 μm) were also obtained from Sigma-Aldrich.

Blank urine samples were collected from healthy adult volunteers and stored in polytetrafluoroethylene flasks at -20 °C until analysis. Prior to the microextraction process, each sample was filtered through a disposable nylon filter (0.45 μm of pore size, Análisis Vínicos, Tomelloso, Spain. http://www.analisisvinicos.com). Ionic strength and pH were also adjusted to the optimum values.

2.2. Instrumentation

Photo-polymerization and photo-grafting processes were performed into an UV crosslinker (model CL1000) from UVP Inc. (Upland, CA, USA) equipped with UV lamps (5 × 8 W, 254 nm). SEM micrographs of polymeric materials were obtained with a scanning electron microscope (S-4100, Hitachi, Japan). For these measurements, the materials were previously coated with a very thin-layer of conductive carbon.

A concentrator Plus (Eppendorf AG, Hamburg, Germany) was used in the extraction procedure. A syringe pump (model 100, KD Scientific, New Hope, PA, USA) was used for all washing and elution steps of monoliths within pipette tips.

Chromatographic analyses were carried out using a HP 1100 liquid chromatograph (Agilent, CA, USA) equipped with a single wavelength photometer and a binary high-pressure pump for mobile-phase delivery. Data analysis was performed using HP ChemStation software (Agilent). Chromatographic separation was achieved on a LiChrosorb® C18 column (4.6 × 150 mm; Agilent) under isocratic conditions. The mobile phase consisted of (A) water containing 0.1% (*v/v*) of acetic acid and triethylamine (10 mM), and (B) ACN at a flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$. An isocratic mode (40% B) was performed in 15 min. The injection volume was 10 µL with partial loop with needle overfill injection mode. The separated analytes were determined at 254 nm.

2.3. Modification of the inner surface of polypropylene tips

Prior to preparation of monolithic phase into the tip, the inner surface of the polypropylene tip was modified by photografting with EDMA [21, 22]. In brief, the inner surface of 200 µL polypropylene tip was sequentially washed with methanol and acetone, and dried under a nitrogen stream to remove any impurities of the inner walls. Then, the tip was filled with 50 µL of a deoxygenated 5% (*w/v*) methanolic BP solution and sequentially irradiated with UV light (1 J·cm^{-2}) for 30 min followed by a methanol rinse and drying step with nitrogen. Next, the tip was filled with 50 µL of a 15% (*v/v*) methanolic EDMA solution and irradiated again with UV light for 30 min. Finally, the modified tip was washed thoroughly with acetone and dried under a nitrogen stream before use.

2.4. Preparation of MWCNT-modified monolithic pipette tips

Preparation of hybrid monolithic bed within 200 µL pipette tips was carried out under UV irradiation. MWCNTs were directly added to the polymerization mixture before filling the tips. Details of the procedure are described in *Electronic Supporting Material (ESM)*.

2.5. Microextraction procedure

Extraction of four antidepressants from human urine samples was achieved using the MWCNT-modified monolithic pipette tips. The reagent or sample solutions were put into a syringe and loaded by using a syringe pump. Prior to extraction, the hybrid monolithic tips were sequentially preconditioned with MeOH (200 µL) and Milli-Q water (200 µL) using a syringe pump at a flow rate of 200 µL·h^{-1} for 15 min. Ionic strength and pH of urine samples and standard solutions were previously adjusted at a concentration of 300 g·L^{-1} (NaCl) and 10, respectively. Aliquots of samples (600 µL) containing the four target analytes within the linear range were passed through the tips at a flow rate of 100 µL·h^{-1} for 15 min, followed by Milli-Q water (50 µL) for 5 min. After washing, the analytes were eluted from the monolithic phase with a 5% (*v/v*) solution of acetic acid in MeOH (20 µL) at a flow rate of 100 µL·h^{-1} and collected for further HPLC analysis.

3. Results and discussion

3.1. Surface attachment of monolith to polypropylene pipette tips

A poly(GMA-*co*-EDMA) monolith with embedded multi-walled carbon nanotubes was fabricated in a pipette-tip by UV polymerization process. Pipette tips made from polypropylene were selected as they are UV transparent (thus making feasible all UV-initiated processes) [22–24] and resistant to a wide range of solvents. In order to prevent the formation of voids at the monolith-wall interface and improve their mechanical stability during the microextraction procedure, an EDMA layer was anchored to the inner surface of the polypropylene tips using a sequential two-step photografting approach [25]. In the first step, the benzophenone was grafted to the inner surface, while in the second step, a solution of EDMA was photografted to generate vinyl functionalities to assure the posterior binding of monolith to the polypropylene wall.

The effects of the UV irradiation time in both steps were studied within the interval 5–60 min by keeping the UV irradiation level fixed. The results showed that 30 min was selected as the time required to achieve a covalent attachment of the hybrid monolith to the surface tip wall (see Fig. S1 in *ESM*).

3.2. Preparation of the hybrid monoliths in pipette tips

Then, a poly(GMA-*co*-EDMA) monolith was prepared in the pretreated pipette tip (200 μL) by UV-initiated radical polymerization. The initial composition to prepare polymerization mixtures were as follows: 20 wt% monomers (15 wt% GMA and 5 wt% EDMA) and 80 wt% porogens (75 wt% cyclohexanol and 5 wt% 1-dodecanol), in the presence of 0.3 wt% LPO (out of the total weight of the monomers). Next, 0.1 wt% of MWCNTs were dispersed in this mixture by sonication for 10 min, and then purged with nitrogen. Once the mixture was filled into the pipette tip following the recommendations of reference [22], it was placed under UV light for 2 h. This polymerization time was based on a previous work [21], where other carbon nanostructures were incorporated in hybrid monoliths in spin column devices.

The ratio monomer/porogens can affect the pore size and the permeability of the resulting polymer. For this purpose, several ratios of this variable ranging from 20/80 to 80/20% (*w/w*) were investigated. The results showed that higher percentages of monomers, such as 80/20 and 60/40% (*w/w*), hampered the flow-through of the solvents. However, when the percentage of monomers was lower than 40% (*w/w*), the monolithic structure was not rigid enough. Therefore, 40/60% (*w/w*) ratio was selected as the best compromise to obtain mechanically stable monoliths in the pipette tips.

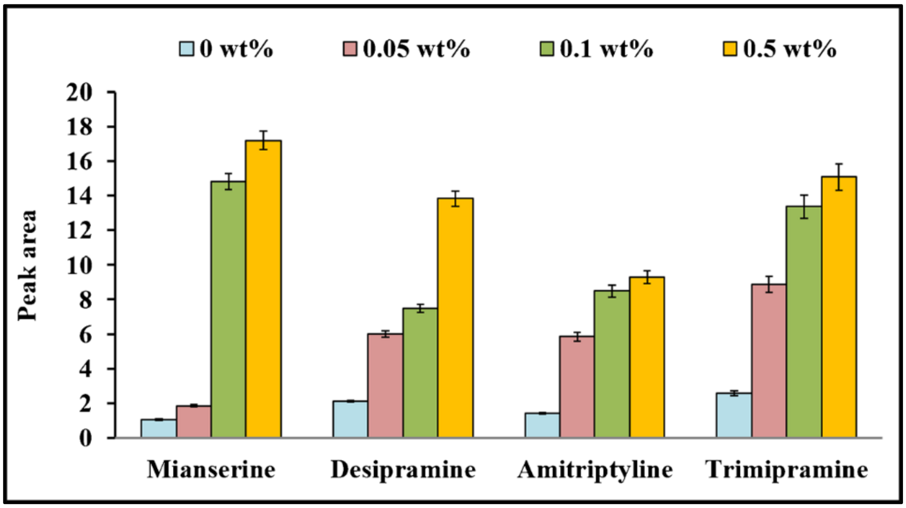

Figure 1. Influence of MWCNTs content on the adsorption capability of hybrid monolith in pipette tip.

Next, the amount of MWCNTs was studied in the range from 0.05 to 0.5 wt% in terms of extraction efficiency using aqueous standards containing the four target drugs (as model compounds) at a concentration of 1000 µg·L^{-1}. The graphic comparison of the results is given in Fig. 1, showing that higher concentrations of carbon nanotubes resulted in an increase of the extraction. This was probably due to the interaction of the aromatic groups of the antidepressants with the protruding MWCNTs from the globules surfaces through π-interactions. Therefore, an amount of 0.5 wt% of the carbon nanotubes in the polymerization mixture was selected. Higher percentages of MWCNTs led to a lack of homogenous distribution of the nanoparticles in the polymeric matrices due to the aggregation tendency of the carbon nanoparticles. Figure 2 shows an image of the selected hybrid monolith into the pipette tip, where a darkening in the color of the generic (or parent) monolith is evidenced.

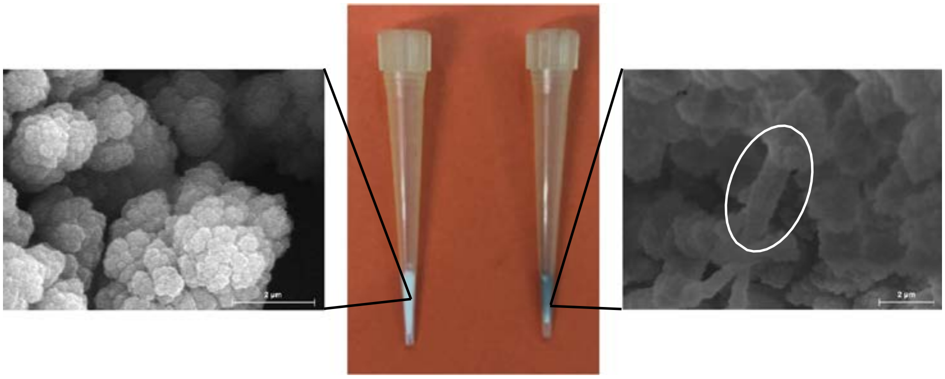

Figure 2. Images of monolithic pipette tips and its corresponding SEM micrographs.

This polymeric material was also characterized by SEM. The presence of some MWCNTs (or nanotubes) was distinguished in either protruding from the globule surfaces or crossing the through pores of monolith (or over two close points of the surface). Raman spectra of pristine MWCNT material and the monolith prepared with these nanostructures were also done (Fig. S2). The characteristic Raman signals of carbon graphite vibrations, G-band (1550 cm^{-1}) and D-band (1320 cm^{-1}), were found in the carbon nanostructures, although this last one was quite weak, suggesting a high crystallinity of the pristine MWCNTs. The former band was also detected in the Raman spectrum of composites, which corroborates the presence of these carbon nanostructures in polymeric monoliths.

3.3. Variables affecting to the microextraction process

The following parameters were optimized: (a) Sample pH value; (b) elution solvent; (c) concentration of salt and (d) sample volume. Respective data and figures are given in the *ESM*. The experimental conditions that gave the best results were: (a) sample pH: 10; (b) elution solvent: MeOH:acetic acid (5 wt%); (c) salt concentration: 300 $g \cdot L^{-1}$, and (d) sample volume: 600 μL.

3.4. Analytical figures of merit and analysis of urine samples

The method was evaluated in terms of linearity, sensitivity, and precision. The corresponding calibration graphs were constructed by using nine working aqueous standards containing the four analytes at different concentrations. For all the analytes, a good linearity ($R>0.99$) was observed in the range 14–1000 $\mu g \cdot L^{-1}$.

The limits of detection (LODs), which were defined using a S/N ratio of three, which are listed in Table 1, varied between 9 and 15 $\mu g \cdot L^{-1}$. The limits of quantification (LOQs), calculated as the concentration providing chromatographic peak areas ten times higher than the background noise, ranged from 14 to 30 µg·L^{-1} (Table 1). The precision of the method (expressed as relative standard deviation, RSD), which was calculated for three individual standards prepared at 100 µg·L^{-1}, varied between 3.1 and 5.1%.

Table 1. Analytical figures of merit of poly(GMA-*co*-EDMA-MWCNTs) monolithic microextraction unit for determination of the target antidepressants at 100 µg·L^{-1}.

Analyte	**LOD (µg·L^{-1})**	**LOQ (µg·L^{-1})**	**Regression equation**[a]	**Precision**	
				Intra-Tips RSD (%, n=3)	**Inter-Tips RSD (%, n=3)**
Mianserine	13	25	Y = (0.0524 ± 0.0018)X – (1.2704 ± 0.3556)	3	7
Desipramine	9	14	Y = (0.1106 ± 0.0040)X – (1.3001 ± 0.5580)	3	4
Amitriptyline	15	30	Y = (0.0943 ± 0.0062)X – (2.2201 ± 0.7597)	4	14
Trimipramine	15	29	Y = (0.0788 ± 0.0028)X – (1.8587 ± 0.5655)	5	10

[a] *Y= Peak area, X = Concentration expressed as µg·L^{-1}.*

The enrichment factors for all the analytes were calculated by comparison of the slopes of the calibration graphs before and after the extraction process (Table 2). They were in the range from 24.0 to 36.0.

Table 2. Recovery study for the four antidepressants spiked to urine samples analyzed following MWCNTs monolithic tip unit.

Analyte	**R ± SD at 200 µg·L^{-1}**	**R ± SD at 1000 µg·L^{-1}**	**EF**
Mianserine	74 ± 5	86 ± 13	24.9
Desipramine	72 ± 9	108 ± 16	28.2
Amitriptyline	85 ± 15	83 ± 15	36.0
Trimipramine	77 ± 16	93 ± 15	24.0

R, recovery expressed as percentage, SD standard deviation, EF enrichment factor.

Once analytically characterized, the method was applied to the determination of the target antidepressants in urine samples from healthy volunteers. Each sample was filtered through a disposable nylon filter (0.45 µm) and the extraction was carried out following the protocol described above. The samples were analyzed in order to find any potential presence of the analytes. Figure 3 illustrates HPLC-UV chromatograms of blank and spiked urine samples at two concentration levels (200 and 1000 µg·L^{-1}). The results showed that any target drug was detected in the non-spiked real samples. Consequently, validation samples were prepared using blank urine samples fortified with the four target analytes at these concentrations levels and processed following the optimized microextraction procedure. The recovery values were calculated by dividing the concentration found by the concentration added, and expressed in percentage. Each sample was analyzed by triplicate; and the results obtained are listed in Table 2. As it can be seen, they were excellent in all instances and ranged from 74 to 108%.

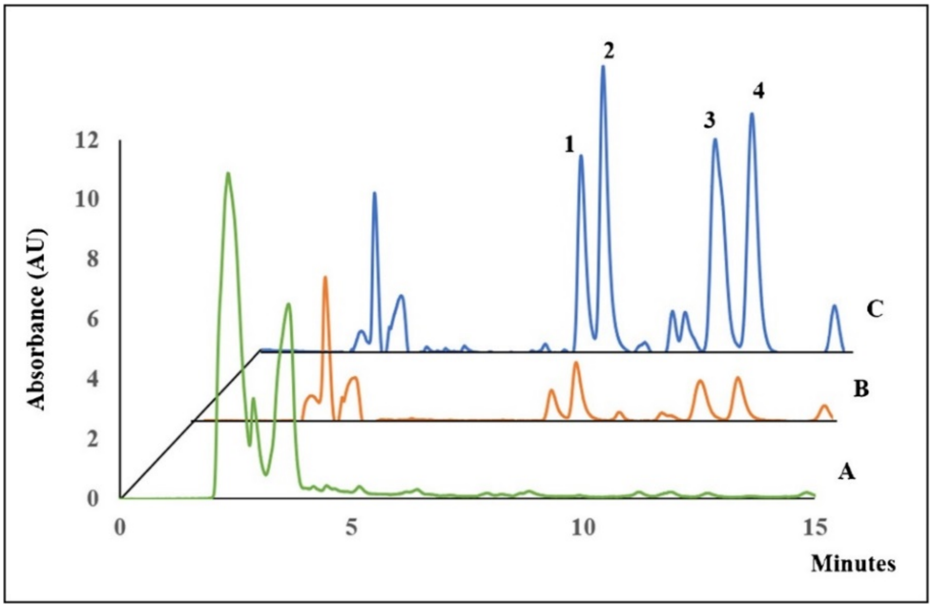

Figure 3. HPLC-UV chromatograms of TCAs obtained after microextraction procedure: **(A)** blank urine, **(B)** spiked urine at 200 μg·L^{-1}, and **(C)** at 1000 μg·L^{-1}. Peaks: **(1)** mianserine, **(2)** desipramine, **(3)** amitriptyline, and **(4)** trimipramine.

A comparative study of our method with other recently reported sample preparation protocols was also done (Table 3). The results revealed that the method using common equipment accessible (such as UV detector) in most analytical laboratories showed good extraction efficiencies, a wide linear range and satisfactory LODs [26–29, 31]. However, the LODs were higher than those obtained using other (nano)materials combined with highly sensitive and sophisticated techniques (GC-MS) [30, 32]. Besides, the preparation of our sorbent (synthesis and incorporation of MWCNTs) is easier and cheaper than other sorbents reported (e.g. SPME fibers), which certainly makes our microextraction procedure economically feasible.

Table 3. An overview on recently reported nanomaterial-based methods for preconcentration of antidepressants in biological fluids.

Material used	**Extraction procedure**	**Coupled technique**	**Matrix**	**Linear range (µg L^{-1})**	**LOD (µg L^{-1})**	**Recovery (%)**	**Reference**
MNPs-CTAB	SPME	HPLC-UV	Urine	10-400	3-5	64-71	[26]
ER-GO	HS-CF-SPME	GC-FID	Water, plasma	0.7-500 (water) 2-500 (plasma)	0.08 (water) 0.30 (plasma)	73-96 (plasma)	[27]
PCL-PPy composite	SPME	IMS	Urine, plasma	0.7-16 (urine) 40-207 (plasma)	0.23 (urine) 0.45 (plasma)	105-120 (urine) 91-101 (plasma)	[28]
PINTP	EC-IT-SPME	HPLC-UV	Water, plasma	80-1000	< 40	83-120	[29]
PDA-Ag-PPy nanocomposite	MEPS	GC-MS	Urine	0.1-100	0.03-0.05	88-104	[30]
Fe_3O_4-$MgSiO_3$	MSPE	HPLC-UV	Urine, serum	10-500	1.7-2.8	75-115 (urine) 72-113 (serum)	[31]
Fe_3O_4-MCM-41	MSPE	GC-MS	Urine, plasma	0.05-500	0.015 (urine) 0.02 (plasma)	86-115	[32]
CNTs incorporated polymer monolith	microSPE	HPLC-UV	Urine	14-1000	8.6-15.2	72-108	Present work

Abbreviations: EC-IT: electrochemically controlled in-tube, ER-GO: electrochemically reduced graphene oxide, GC-FID: gas chromatography flame ionization detector, HS-CF: Headspace cold fiber, IMS: ion mobility spectrometry, MNPs-CTAB: magnetic nanoparticles-cetyltrimethyl ammonium bromide, MEPS: microextraction in packed syringe, MS: mass spectrometry, MSPE: magnetic solid-phase extraction, PINTP: poly(indole-co-thiophene), PCL: poly(ε-caprolactone), PDA-Ag-PPy: polydopamine-silver nanoparticles-polypyrrole, SPME: solid-phase microextraction.

4. Conclusions

This study presents, for the first time, the preparation of a MWCNTs-modified monolithic pipette tips UV-initiated polymerization. The MWCNTs were added to the polymerization mixtures in such a way that some nanotubes protruded from the monolith surface being able to interact with the analytes of interest. The covalent attachment of the hybrid monolith on the inner part of the pipette tip avoids contraction of the extractant phase after synthesis and improves its stability during the extraction process. The microextraction unit was evaluated using the determination of four antidepressants in urine samples as model analytical problem. The analytical figures obtained allow the determination of the analytes in human urines with minimal interference from sample matrix. The presence of the MWCNTs in the monolith results in a better sensibility as the antidepressants since they are easily retained due to the additional π-interactions that are generated during the retention step. Compared with other existing nanomaterial-based methods for samples preparation and analysis of antidepressants, our SPE protocol is simpler and more cost-effective; however, it provided LODs higher than those obtained using more sophisticated techniques such MS detection. In any case, our method constitutes a promising alternative for the preconcentration of antidepressants from biological samples.

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Electronic Supplementary Material

1. Preparation of MWCNT-modified monolithic pipette tips

A polymerization mixture consisted of 40wt% monomers (32 wt% GMA and 8 wt% EDMA), 60 wt% porogens (55 wt% cyclohexanol and 5 wt% 1-dodecanol) and 0.3 wt% of LPO (out of the total weight of monomers) as free-radical initiator was prepared. MWCNTs (0.5 wt%) were added to the polymerization mixture, and afterwards the mixture was sonicated for 10 min and purged with nitrogen for an additional 10 min. The modified polypropylene tip was carefully filled with 10 µL of the mixture following the strategy described by Alwael and co-workers [22]. Then, the tips were placed into the UV crosslinker chamber, irradiated with 1 $J\cdot cm^{-2}$ for 2 h. After polymerization, the resulting hybrid monolith was washed with MeOH and water to remove the porogens and any unreacted monomers.

2. Surface attachment of monolith to polypropylene pipette tip

To evaluate the successful covalent attachment of the monolith to the pipette tip, SEM micrographs of the polymeric material within the pipette tip, without and with wall modification (via photo-grafting approach) were taken (Fig. S1). Clearly, the wall modified tips at photo-grafting times of 30 and 60 min (traces C and D, respectively) showed that the monolith was tightly attached to the polypropylene inner wall. The unmodified tip evidenced a large void (trace A), whereas the tip modified at photo-grafting time of 15 min (trace B) did not assure a firm binding to the wall pipette tip.

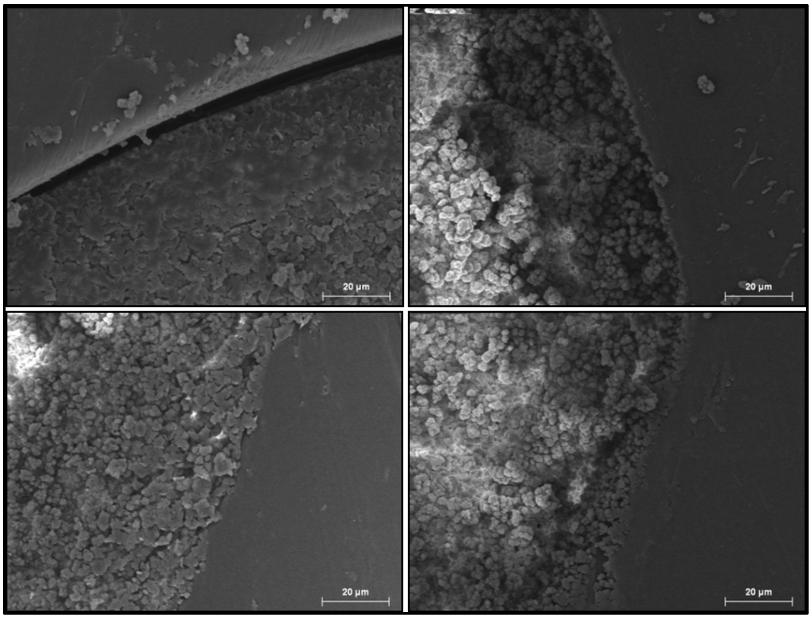

Figure S1. SEM micrographs of hybrid monolithic material within a polypropylene tip, which was unmodified **(A)** and modified by photo-grafting treatment at different times: 15 **(B)**, 30 **(C)** and 60 min **(D)**.

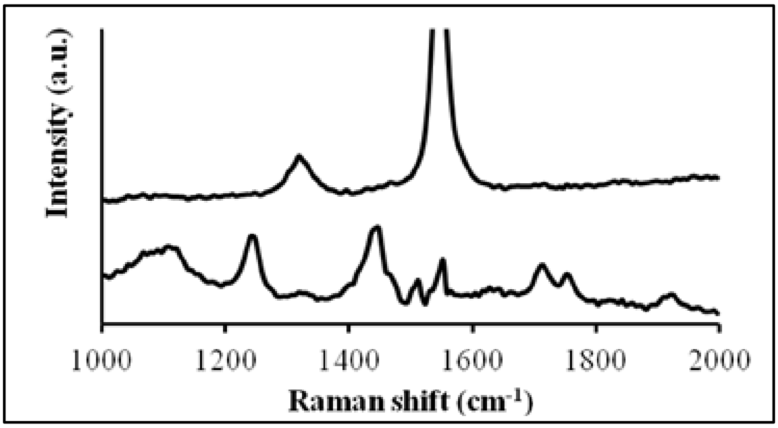

Figure S2. Raman spectra of **(A)** pristine MWCNTs and **(B)** polymeric monolith containing MWCNTs.

3. Variables affecting to the microextraction process

In the order to achieve the best method performance, the variables that influence on the extraction of the analytes were deeply studied: type of eluent, pH, ionic strength and sample volume. Along optimization extraction process, an aqueous standard containing the analytes at a concentration of 1000 µg·L^{-1} was used as test mixture. The initial conditions for the extraction process were: 200 µL of sample (adjusted at pH 9) without NaCl, followed by washing with this solution, and elution with 20 µL of ACN. Separation and quantification of the analytes was carried out by HPLC-UV.

The first variable studied was sample pH since it can affect the analytes' retention since they present ionizable groups. Antidepressants are weak bases with pKa values above 8. Therefore, the sample pH was evaluated within the interval 8–11. The best results were obtained at pH 10 since it ensures that the neutral form of these compounds is predominant and can interact better with hydrophobic surface of the protruding MWCNTs.

The selection of a proper elution solvent is of major concern for the optimization of a SPE process. For this purpose, different organic solvents were tested as eluents such as ACN, ACN:acetic acid (5 wt%), MeOH, and MeOH:acetic acid (5 wt%). As shown in Figure S3A, MeOH:acetic acid (5 wt%) provided the best results, and it was selected as optimum. 20 µL of this mixture was required to achieve a quantitative elution of the analytes.

It is well-known that the addition of salt in an extraction procedure can benefit the efficiency because of the salting-out effect. In this case, the influence of the ionic strength was evaluated in the range from 0 to 300 g·L^{-1} using sodium chloride as model electrolyte (Figure S3B).

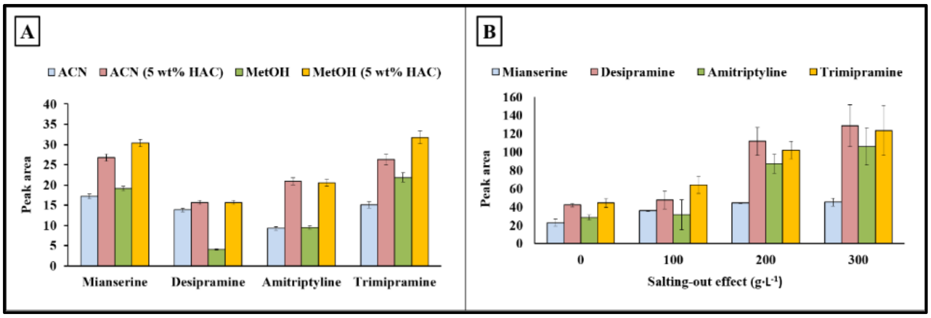

Figure S3. Effect of the type of the **(A)** eluting solvent and **(B)** ionic strength on the retention of TCAs. Conditions: concentrations, 1000 µg·L^{-1} of each analyte; sample volume, 200 µL; eluent volume, 20 µL.

As it can be seen, the peak areas of all analytes reached its high peak area values at a concentration of 300 g·L^{-1} of NaCl. According to the results, a concentration of 300 g·L^{-1} of NaCl was added to the sample.

The next variable evaluated was the sample volume within the interval 200–600 µL (Figure S4). The best results in terms of peak area corresponded to 600 µL, which indicates the remarkable sorbent capacity of the hybrid monolithic phase towards the target antidepressants. Although higher sample volumes would have been used, 600 µL was selected for further analysis as it was enough to achieve the required sensitivity within a reasonable sampling time.

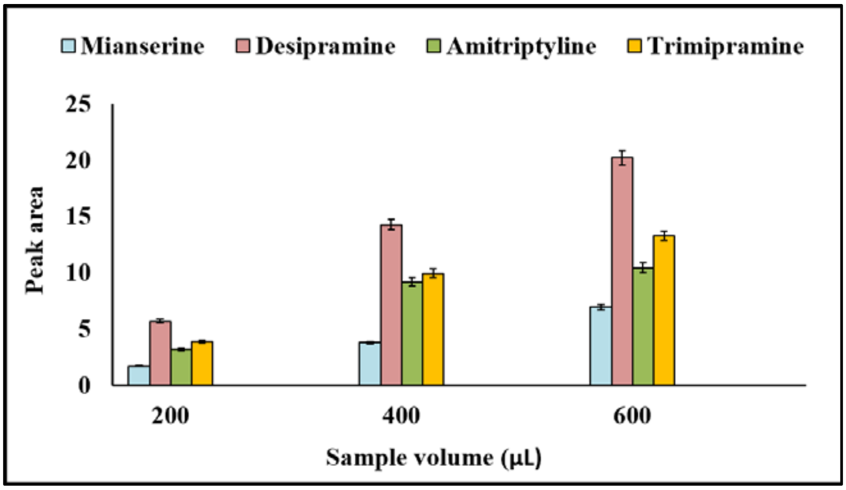

Figure S4. Effect of sample volume on the adsorption capability of TCAs. Conditions: concentrations, 1000 µg·L^{-1} of each analyte in different sample volumes; eluent volume, 20 µL of MeOH:acetic acid (5 wt%) containing 300 g·L^{-1} NaCl.

Capítulo

7

Nanostructured Hybrid Monolith with Integrated Stirring for the Extraction of UV-Filters from Water and Urine Samples

Talanta, 182 (2018) 391-395

Nanostructured hybrid monolith with integrated stirring for the extraction of UV-filters from water and urine samples

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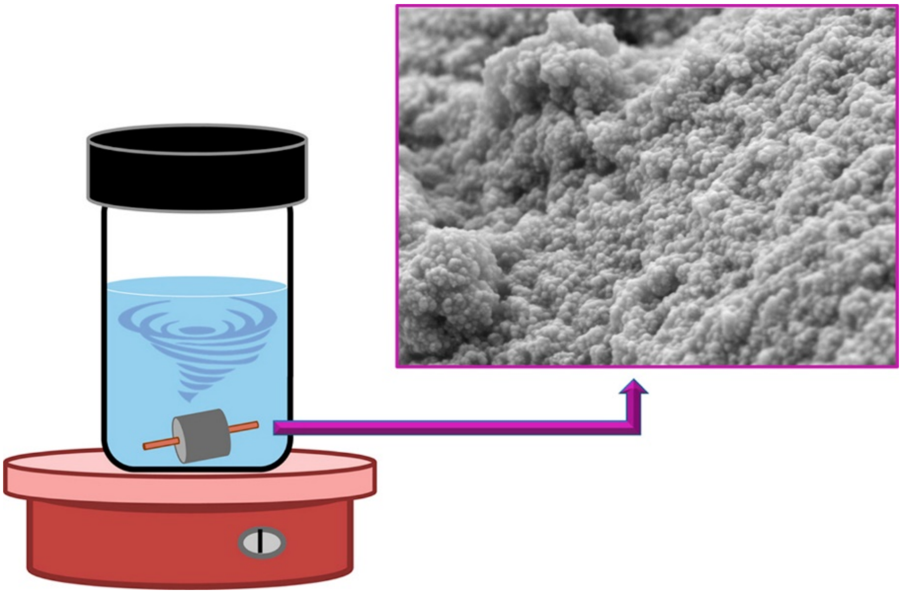

Keywords

Carbon nanohorns; Benzophenones; Stir bar; Monolithic solid; Microextraction.

Highlights

- A hybrid monolith with stirring integrated has been developed.
- The monolithic solid was prepared by copolymerization of methacrylate monomers and o-SWNHs.
- The morphology and porosity as well as the effect of the presence of the SWNHs has been evaluated.
- UV-filters have been determined in water and urine samples using the proposed microextraction unit.

Abstract

This article presents a monolithic extraction unit with integrated stirring using carbon nanohorns and methacrylate-based compounds as monomers. The hybrid monolithic material was prepared by thermal polymerization at 70 °C for 24 h, and was applied for the extraction of UV-filters from waters and human urine samples. To achieve the integrated stirring unit, the monolith was grown over an ironware. Variables dealing with the polymerization mixture composition and the microextraction procedure were studied in depth. The resulting hybrid monolithic polymer was also characterized by scanning electron microscopy (SEM) and nitrogen intrusion porosimetry. The target analytes were quantified by UPLC-DAD, and the limits of detection were between 1 and 10 $\mu g \cdot L^{-1}$. The precision of the method (inter extraction units) expressed as relative standard deviation ranged from 5.4% and 7.9%. Also, relative recoveries values of the analyte spiked to swimming pool water and urine samples varied in the interval 72–124 and 71–114%, respectively.

1. Introduction

Benzophenones (BPs) are a family of compounds which have the ability to absorb and dissipate ultraviolet light, and therefore, are used in the cosmetic industry as UV-filters to protect the skin against solar UV radiation [1]. Thus, these compounds may be absorbed through the skin, accumulate in the body and/or excreted in the urine [2]. In addition, studies have shown that UV-filters may be significantly accumulated in swimming pools through direct contact [3]. However, a maximum concentration has been established for each country legislation due to their dermatological implications [4]. Moreover, they have been also linked to the development of endocrine and reproductive problems. For all these reasons, it is important to develop new methods for the determination of UV-filters both in biological samples and in waters.

Microextraction techniques are processes which depend on thermodynamic and kinetics factors. Therefore, it is crucial to enhance the mass transference during the extraction in order to improve the extraction yield [5]. In such conditions, the developed new extraction modes with integrated stirring in the same device such as stir bar [6], stir cake [7], or stir membrane [8] are good choices as they simplify the extraction to a large extent. Apart from the commercial ones, in most of these cases, the preparation of the microextraction unit involves an additional step for the attachment of the material to the surface of the selected device. As a main drawback, detachment of part of the material due to the friction can occur during the stirring. This is especially relevant when a thin film of the extraction medium is covered the support. However, this problem can be solved using stir cake sorptive extraction modality which possesses an excellent longevity because the sorbent does not come in contact with the vessel wall during stirring [9].

Monolithic materials, which consist of a rigid macroporous structure, are very attractive in this context [7, 10, 11]. They can be easily prepared and their interconnecting pores allow the solvents flow through them, resulting in a good permeability [12]. In addition, to improve the sorbent capacity and the specific surface area of the material as well, nanomaterials such as carbon nanotubes [13, 14], or carbon nanohorns [15, 16], have been incorporate into the polymer networks. In a previous work, our group demonstrated the participation of oxidized single-walled carbon nanohorns (o-SWNHs) as monomer in polymerization reactions when lauroyl peroxide (LPO) is used as free-radical initiator [15]. Thus, a uniform, rigid and robust hybrid monolithic solid is obtained by the copolymerization of organic monomers and carbon nanohorns. The principal difference between carbon nanoparticles entrapped into the monolithic network and copolymerized is that in this case, the most of the nanoparticles are accessible to interact with the analytes.

In this work, we describe the synthesis of a hybrid solid with integrated stirring combining the properties of the carbon nanohorns and organic polymer monoliths. Hybrid monolithic stir bar has been obtained by copolymerization of methacrylate monomers and o-SWNHs using LPO as free-radical initiator. An ironware was introduced directly in the polymerization mixture to integrate the stirring, resulting in a rigid and stable piece of monolithic solid with the ironware inside. The effects of preparation and extraction variables were studied in detail, and the sorbent capacity of the material was evaluated for the extraction of the benzophenones in swimming pool water and human urine samples as model analytical problems.

2. Experimental section

2.1. Chemicals and materials

Benzophenones (benzophenone-2, 4-hydroxybenzophenone, benzophenone-1, benzophenone-8, and benzophenone-3) were obtained from Sigma-Aldrich (Madrid, Spain. http://www.sigmaaldrich.com). Standard solutions of each benzophenone were prepared in methanol (Sigma-Aldrich) at a concentration of 1 $g·L^{-1}$ and stored at 4 °C until their use. Working standard solutions were prepared by dilution of the stock standard solutions in ultrapure Milli-Q water. Methanol was also used for benzophenones elution. Acetonitrile (Scharlab, Barcelona, Spain. http://scharlab.com) and ultrapure Milli-Q water were used as chromatographic mobile phases.

The reagents used for the preparation of the monolithic solid, glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), cyclohexanol, 1-dodecanol, and LPO were purchased from Sigma-Aldrich. Single-walled carbon nanohorns (SWNHs) with dahlia-like structure (40–50 nm in length) were supplied by Carbonium S.r.l. (Padua, Italy. http://www.carbonium.it/public/site/index.php). Laboratory-oxidized carbon nanohorns were prepared following a procedure previously optimized [17].

Blank human urine samples were collected from healthy adult volunteers and stored in polytetrafluoroethylene (PTFE) flasks at -20 °C until analysis. Swimming pool water samples were collected in amber glass bottles without headspace and stored at 4 °C until their analysis.

2.2. Preparation of the hybrid monolithic material

The hybrid monolith was prepared by thermally initiated free-radical polymerization according to previously described conditions with some modifications [15]. Brieftly, the polymerization mixture consists of 60 wt% monomers (48 wt% GMA and 12 wt% EDMA), 40 wt% porogens (37 wt% cyclohexanol and 3 wt% 1-dodecanol) and 0.3 wt% of LPO (out of the total weight of monomers) as free-radical initiator. Moreover, o-SWNHs (0.1 wt%) was also added and the dispersion was homogenized by immersion in an ultrasonic bath (5 min), and purged with nitrogen for 10 min. Subsequently, a cylindrical polypropylene device (5 mm i.d.) containing an ironware was filled with 80 µL of the polymerization mixture. Next, the device was introduced into an oven at 70 °C for 24 h. After polymerization, the monolithic stir was washed with methanol and Milli-Q water to remove the pore-forming solvents and any possible unreacted monomers for 1 h at 700 rpm.

2.3. Microextraction procedure

The extraction procedure (Fig. 1), which is based on stirring adsorption and desorption modes, was as follows: the monolithic stirring extraction unit, previously conditioned with methanol and water, was immersed into 10 mL of the aqueous standard or sample and stirred at 1100 rpm for 150 min. After this step, the monolithic unit was withdrawn from the sample by means of an external magnet and washed with Milli-Q water (500 µL) at 1100 rpm for 5 min. Next, 300 µL of methanol were used to elute the analytes (5 min, 1100 rpm). The extract was evaporated to dryness under a nitrogen stream and the residue is finally redissolved in 150 µL of mobile phase for further UPLC-DAD analysis.

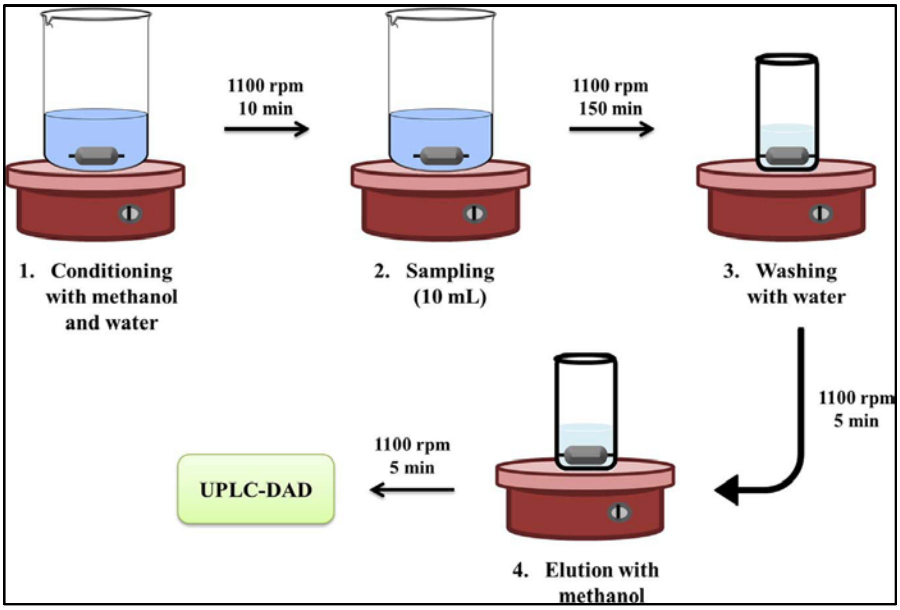

Figure 1. Scheme of the microextraction procedure using the developed monolithic solid.

Once finalized the microextraction procedure, the monolithic stirring extraction unit was sequentially washed with 10 mL of methanol and water. It can be reused at least 250 times without altering its physical properties or the adsorption capacity.

3. Results and discussion

3.1. Preparation and characterization of the monolithic stirring unit

The polymerization mixture is composed of monomers (GMA and EDMA) and porogens (cyclohexanol and 1-dodecanol), in the presence of free-radical initiator (LPO), being the o-SWNHs (0.1 wt%) directly dispersed in this mixture by sonication. The amount of carbon nanohorns as well as their effect in the polymerization was deeply studied in a previous work [15].

Different monomers/porogens ratios were evaluated in order to prepare a hybrid monolith which exhibited sufficient rigidity and mechanical stability to endure the damage produced at high stirring velocities. Another key aspect of the final monolithic solid should be its large extraction efficiency. Thus, monolithic stir bar with monomers/ porogens ratios of 80/20% (*w/w*), 60/40% (*w/w*), 20/80% (*w/w*), and 40/60% (*w/w*) were prepared and tested using BPs as model compounds. Ratios with low percentage of monomers resulted in a great amount of macroporous which tend to a loose network structure causing their breakage during the microextraction process. However, monoliths at 80/20 and 60/40% (*w/w*) ratios exhibited rigid and stable structures. The graphic comparison of the results is given in Fig. 2, showing the highest sorbent capacity for the 60/40% (*w/w*) ratio. This can be explained by the fact that the high percentage of monomers resulted in the smaller pores and therefore it led to a decrease in permeability, and subsequent problems of diffusion of the analytes.

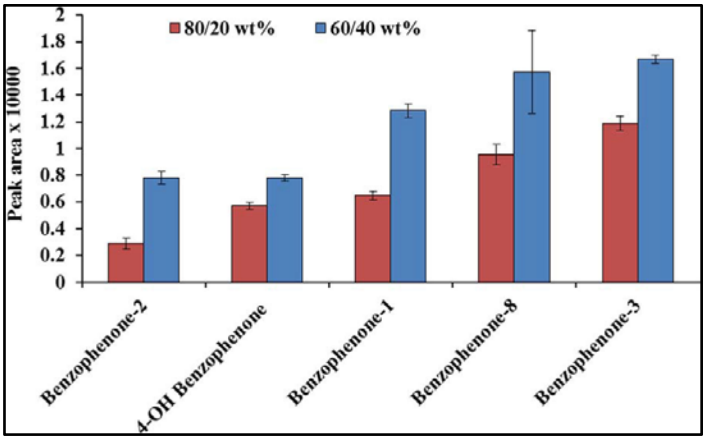

Figure 2. Comparison of extraction performance of hybrid monolithic material with a monomers/porogens proportions of 80/20 and 60/40% (*w/w*).

Fig. 3 shows the SEM images of both monoliths. As expected, significant differences in the morphology were observed between them. Monoliths prepared with 80/20% (*w/w*) (Fig. 3A) showed a dense polymeric network with low permeability, while for monoliths prepared with 60/ 40% (*w/w*) (Fig. 3B) the number of void spaces and size of the through pores as well as the number of globules is increased.

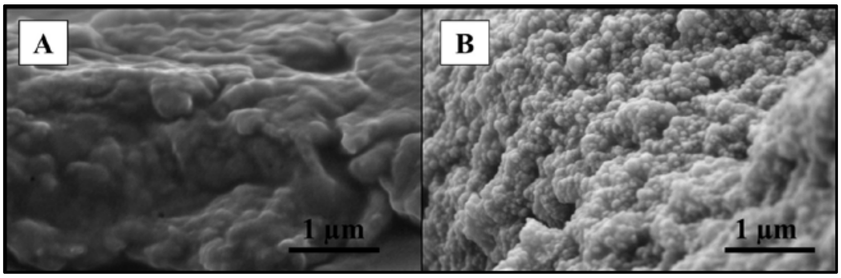

Figure 3. SEM micrographs of hybrid monolithic material with a monomers/porogens proportions of 80/20 **(A)**, and 60/40 **(B)** % (*w/w*).

Nitrogen intrusion porosimetry was performed to assess the porosity of the monoliths. The textural values of monoliths prepared with 80/20 and 60/40% (*w/w*) including specific surface area, pore diameter, and pore volume, are shown in the Table 1. The specific surface area of the hybrid solid was determined by the BET method and the pore size distribution by the method of Barrett, Joyner, and Halenda (BJH). As it can be seen, monoliths with a monomers/porogens ratio of 60/40% present higher specific surface area leading to an extraction performance improvement. In addition, pore diameter and volume are also higher allowing a better mass transfer during the extraction process.

Table 1. Porous properties of the synthetized hybrid monolithic polymers.

Monomers/porogens ratio (%)	Specific surface area, BET ($m^2 \cdot g^{-1}$)	Average pore diameter (Å)	Average pore volume ($cm^3 \cdot g^{-1}$)
80/20	12.39	40.8	0.012
60/40	44.02	164.4	0.18

3.2. Evaluation of the variables affecting to the microextraction process

All the variables involved in the extraction procedure were studied following an univariable methodology in order to isolate the effect of each variable on the new proposed method. The initial experimental conditions for the microextraction process were: 5 mL of aqueous standard stirring at 300 rpm for 15 min, and an elution with 300 µL of methanol at 300 rpm for 5 min.

Sample volume (a), stirring rate (b), and extraction time (c) were studied and optimized given the best results for the following conditions (Discussion and related Figures are shown in the *Electronic supporting material*): (a) sample volume of 10 mL, (b) stirring rate of 1100 rpm and, (c) extraction time of 150 min.

3.3. Analytical performance

The linearity, sensitivity, and precision of the proposed method was deeply investigated. A linear range from 5 to 500 $\mu g \cdot L^{-1}$ was obtained by extracting, in duplicate, nine working aqueous standards of the five analytes (R>0.99 for all them). The limits of detection (LODs), which were calculated by using a signal-to-noise ratio of 3, ranged from 1 to 10 $\mu g \cdot L^{-1}$. The limits of quantification (LOQs), defined as the concentration providing chromatographic peak areas ten times higher than the background noise, ranged from 5 to 20 $\mu g \cdot L^{-1}$ (Table 2). Meanwhile, the precision of the method (intra and inter- stirs, n=3, 50 $\mu g \cdot L^{-1}$), expressed as relative standard deviation (RSD), was lower than 9.13% for all the analytes as it is shown Table 2. The enrichment factors were calculated by comparison of the slopes of the calibration graphs before and after the extraction process. They were in the range from 16 (for 4-hydroxybenzophenone) to 32 (for benzophenone-3).

Once optimized and analytically characterized, the possible matrix effects as well as its applicability in human urine and pool water samples analysis was also evaluated. Firstly, the samples were analyzed in order to find any potential presence of the analytes. Since the analytes were not detected, validation samples were prepared using blank urine and pool water samples fortified with the five target analytes (benzophenone-2, 4-hydroxybenzophenone, benzophenone-1, benzophenone-8, and benzophenone-3) at 25, 200, and 500 $\mu g \cdot L^{-1}$, and they were left to stand for 24 h prior to analysis. Accordingly, the recovery values obtained for each of the fortified samples analyzed are shown in Table 3, and they varied between 71% and 124%, respectively. Thus, high recovery values demonstrated that there are no inferences of the matrix with the analytes. In addition, the detection system based on DAD allows to obtain a higher spectral purity and consequently, to difference the analytes from potentially interfering substances.

Table 2. Analytical figures of merit of the monolithic microextraction unit to the determination of the target BPs.

Analyte	**LOD (μg·L^{-1})**	**LOQ (μg·L^{-1})**	**Precision**	
			Intra-extraction units RSD (%, n=5)	**Inter-extraction units RSD (%, n=5)**
Benzophenone-2	5	10	6.6	5.8
4-Hydroxybenzophenone	10	20	5.7	5.7
Benzophenone-1	5	10	5.6	6.1
Benzophenone-8	1	5	5.4	9.1
Benzophenone-3	5	10	7.9	8.4

LOD limit of detection, LOQ limit of quantification, RSD relative standard deviation.

Table 3. Recovery study for the target BPs using the monolithic extraction unit.

Analyte	**Swimming pool water samples (%, n=3)**			**Human urine samples (%, n=3)**		
	25 µg·L^{-1}	**200 µg·L^{-1}**	**500 µg·L^{-1}**	**25 µg·L^{-1}**	**200 µg·L^{-1}**	**500 µg·L^{-1}**
Benzophenone-2	72 ± 5	78 ± 7	87 ± 10	71 ± 4	89 ± 9	93 ± 10
4-Hydroxybenzophenone	92 ± 5	101 ± 9	101 ± 11	80 ± 7	96 ± 16	107 ± 16
Benzophenone-1	82 ± 4	82 ± 9	101 ± 10	76 ± 5	88 ± 16	114 ± 15
Benzophenone-8	120 ± 6	79 ± 9	101 ± 4	113 ± 9	85 ± 8	105 ± 10
Benzophenone-3	124 ± 5	91 ± 13	102 ± 4	74 ± 4	94 ± 8	101 ± 5

4. Conclusions

In the present work, a hybrid monolith based on the copolymerization of carbon nanohorns and methacrylate monomers with integrated stirring has been developed for the preconcentration and isolation of UV-filters from water and urine samples. The synthesis procedure to obtain the monolithic microextraction unit was easy and reproducible resulting in an indivisible device which can be used several times without efficiency losses. Moreover, variables related to the preparation of the solid as well as variables affecting to the microextraction procedure were deeply studied. The morphological characteristics of the hybrid monolith were also evaluated. Therefore, the presence of the carbon nanohorns in the polymerization mixture contributed to the porosity and rigidity of the final solid as to the improvement of the sorbent capacity because they can interact with the target analytes via hydrogen bonding, π–π stacking, electrostatic forces, van der Waals forces and hydrophobic interactions. Concerning extraction efficiency, the proposed stirring unit presents similar enrichment factors compared with previous monolith-based stir extraction devices such as stir cake sorptive extraction units [18–20] or stir bars [21]. Although the time extraction and the reusability are also similar, as an added benefit of the proposed device, the elution time required for a quantitative elution of the analytes is lower (5 min), whereas a time of 30–60 min is necessary with other approaches. Regarding the sensitivity, the LODs values for our method were comparable even with other nanomaterial-based dispersive microextraction methods [22, 23] reported in the literature for the determination of UV-filters in water samples.

Acknowledgments

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Electronic Supplementary Material

1. Instrumentation

Liquid chromatographic analyses of the target analytes were performed using a Waters-Acquity™ Ultra Performance LC system (Waters Corp., Madrid, Spain) with an Acquity UPLC® BEH C18 column (1.7 µm, 2.1 mm × 100 mm). The mobile phase consisted of (A) water and (B) acetonitrile at a flow rate of 0.4 $mL\cdot min^{-1}$ using an isocratic elution program (45 % of acetonitrile) at 60 ºC. The injection volume was 10 µL with partial loop with needle overfill injection mode. The separated analytes were determined using a PDA eλ (extended wavelength) detector (Waters) at 214 nm. System control was achieved with Empower software.

In the preparation of the monolithic solid, an oven (Binder, Madrid, Spain) was also needed to maintain the temperature at 70 ºC during the polymerization step. An ultrasonic bath model 3510 from Branson (Connecticut, USA) was also used in different steps of the procedure. A VELP Scientifica Magnetic Stirrer-MST was employed for the extraction procedure.

A JEOL JSM 7800F scanning electron microscopy (Isaza, Alcobendas, Spain) was used to obtain the micrographs of the monoliths. Nitrogen adsorption/desorption experiments were carried out at -196 ºC using a Quantachrome®ASiQwin™-Automated Gas Sorption Data. The specific surface area values were calculated according to the BET (Brunauer-Emmett-Teller) equation. T-plot method was used to determine the micropore surface areas, and the average pore volumes were evaluated from the desorption branches of isotherms based on the BJH (Barrett-Joyner-Halenda) model.

2. Evaluation of the variables affecting to the microextraction process

The variables directly related with the extraction step were studied using aqueous standards containing the selected BPs at a concentration of 0.5 µg·mL^{-1}. The initial experimental conditions for the microextraction process were: 5 mL of aqueous standard stirring at 300 rpm for 15 min, and an elution with 300 µL of methanol at 300 rpm for 5 min.

The first variable studied the sample volume within the interval 2–20 mL. As it can be seen in Fig. S1, the maximum extraction efficiency for the target analytes was achieved for a volume of 10 mL remaining almost constant for higher volumes. This effect may be ascribed to a lower stirring capacity of the hybrid monolithic stir bar when higher sample volumes were employed.

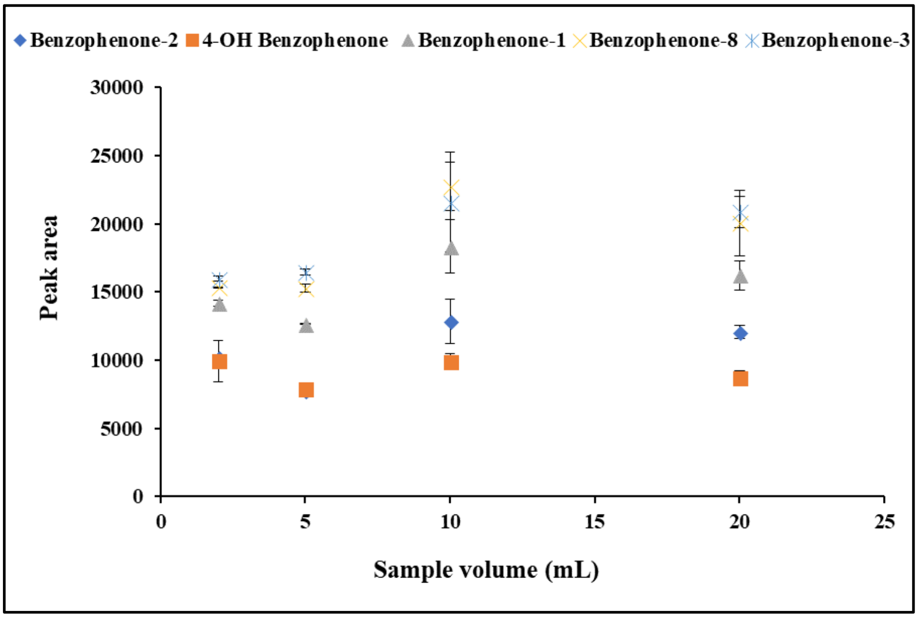


Figure S1. Influence of the sample volume on the BPs retention.

It is well-known that the stirring rate can affect the mass transfer of the analytes during the extraction process. For this reason, six different stirring rates (0, 100, 300, 500, 700 and 1100 rpm) were evaluated. As it can be seen in Fig. S2, the extraction increased with speed obtaining the best results at 1100 rpm for all analytes. The use of stirring rates higher than 1100 rpm induced a decreased in the extraction unit rotation due to the shape and size of the stir bar, and it may also shorten the lifetime of the monolithic stir. For these reasons, a speed of 1100 rpm was chosen for further studies.

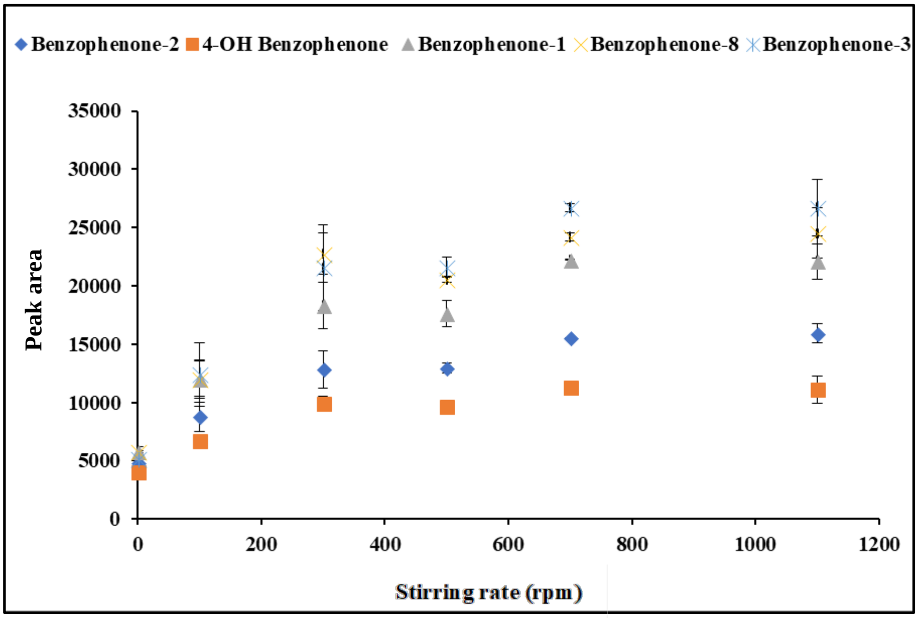


Figure S2. Influence of the stirring rate on the BPs retention.

The influence of the extraction time is also an important variable in the extraction process. This variable was investigated in the range from 10 to 180 min. Results, which are depicted in Fig. S3, indicated that the extraction efficiency markedly increases with the extraction time up to 150 min. As other sorptive techniques, like SPME or SBSE, stir phase sorptive extraction with stirring integrated requires a longer time to reach the partition equilibrium. Consequently, 150 min was selected as extraction time in the following research.

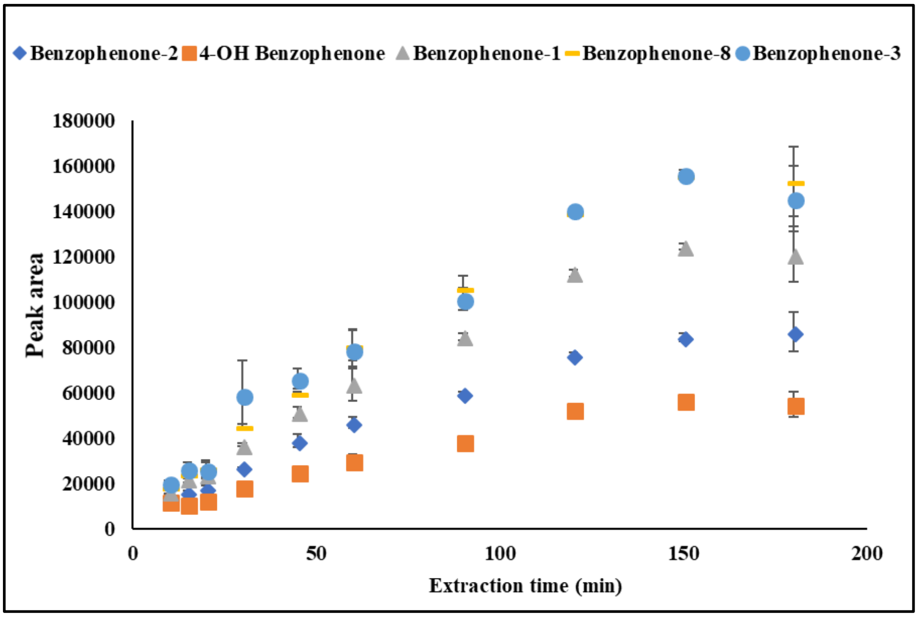


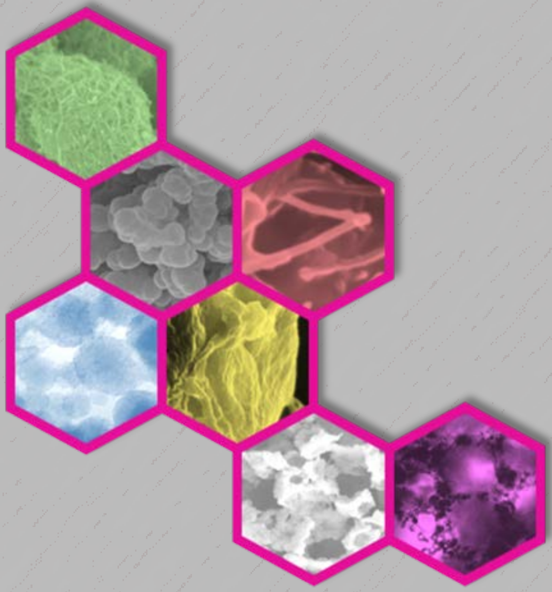
Figure S3. Influence of the extraction time on the BPs retention.

Finally, the elution time was also tested (data not shown). Quantitative elution was obtained for a time of 5 min and 300 µl of methanol. Moreover, in order to enhance the chromatographic analysis, the final extract was evaporated under a N_2 stream and redissolved in a lower volume. This step is possible since no losses by evaporation are observed for the target analytes. For UPLC-DAD analysis the final residue was redissolved in 150 µL of mobile phase allowing the enhancement of the preconcentration factors.

Bloque III

Sólidos Monolíticos Basados en el Uso de Nanopartículas de Carbono Como Monómeros Principales

Monolithic Solids Based on the Use of Carbon Nanoparticles as Main Monomers



El diseño y desarrollo de materiales nanoestructurados permite la creación de nuevos solidos, dispositivos y sistemas con características únicas y distintas de los mismos materiales en la escala macroscópica. De esta manera, al igual que los polímeros convencionales son macromoléculas constituidas por la repetición de unidades monoméricas, los materiales poliméricos nanoestructurados pueden prepararse siguiendo una aproximación conocida como *bottom-up* basada en la unión entre sí de las nanopartículas. El aspecto más relevante de estos sólidos macroscópicos es que está constituido por partículas de tamaño nanométrico, las cuales determinan sus características y propiedades funcionales.

En este Bloque se abordan diferentes estrategias para la síntesis de sólidos monolíticos nanoestructurados y su posterior uso como fases sorbentes en microextracción. En particular se ha evaluado el potencial de los nanocuernos de carbono para actuar como monómeros principales en mezclas de polimerización (***Capítulo 8***), y su capacidad para autoensamblarse sin la necesidad de adicionar componentes extra como entrecruzadores o iniciadores (***Capítulo 9***). Por último, se presenta también una nueva ruta de síntesis de sólidos monolíticos formados por quantum-dots de grafeno (***Capítulo 10***), en la cual simultáneamente se generan las nanopartículas, a partir de sus precursores orgánicos, y se autoensamblan formando un sólido poroso macroscópico.

Capítulo 8

Carbon Nanohorns as a Porous Monolithic Extraction Phase Prepared in Polypropylene Tips by Emulsion

En revisión en Journal of Chromatography A

Carbon nanohorns as a porous monolithic extraction phase prepared in polypropylene tips by emulsion photopolymerization

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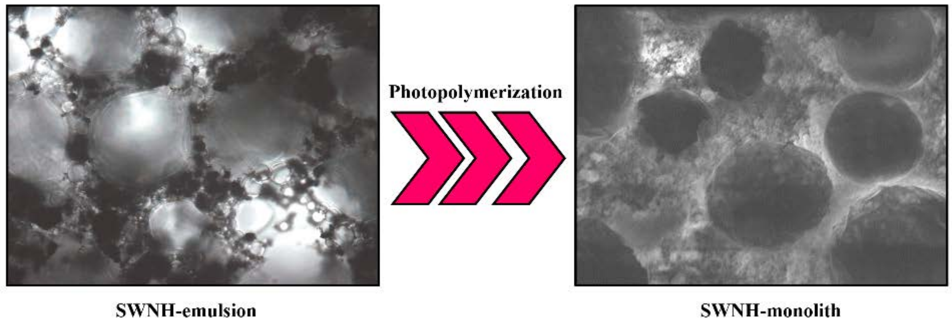

Keywords

SWNH-dahlia structures; Monolithic solids; Pipette tips; Photopolymerization; Photografting; Microextraction; Parabens; Urine.

Capítulo

9

Monolithic Solid Based on Single-Walled Carbon Nanohorns: Preparation, Characterization, and Practical Evaluation as a Sorbent

Nanomaterials, 8 (2018) 370

Monolithic solid based on single-walled carbon nanohorns: preparation, characterization, and practical evaluation as a sorbent

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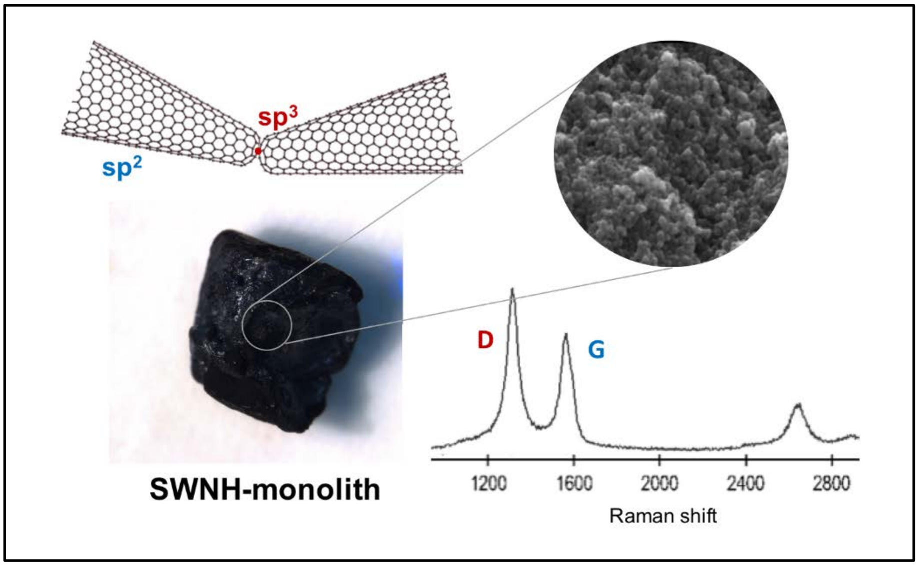


Keywords

Carbon monolith; Carbon nanoparticles; Volatile organic compounds; Headspace microextraction; Single-walled carbon nanohorns; Gas chromatography

Abstract

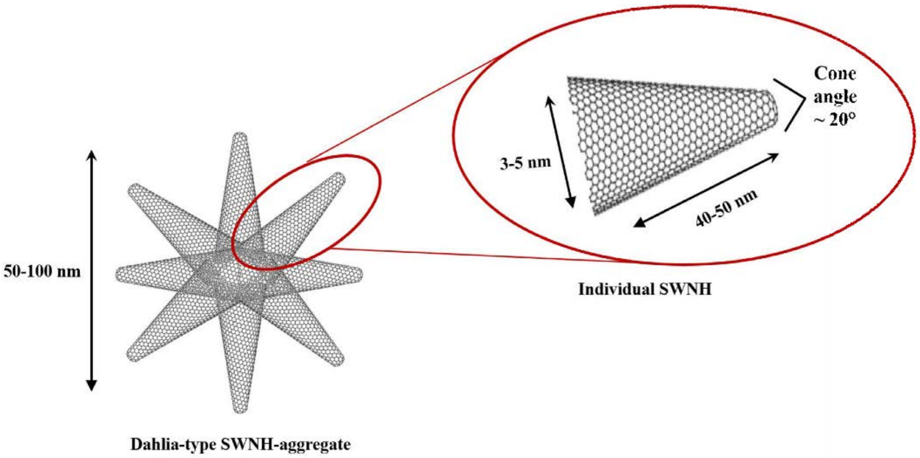
A monolithic solid based solely on single walled carbon nanohorns (SWNHs) was prepared without the need of radical initiators or gelators. The procedure involves the preparation of a wet jelly-like system of pristine SWNHs followed by slow drying (48 h) at 25 °C. As a result, a robust and stable porous network was formed due to the interaction between SWNHs not only via π-π and van der Waals interactions, but also via the formation of carbon bonds similar to those observed within dahlia aggregates. Pristine SWNHs and the SWNH monolith were characterized by several techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal laser scanning microscopy, Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and nitrogen intrusion porosimetry. Taking into account the efficiency of carbon nanoparticles in sorption processes, the potential applicability of the SWNH-monolith in this research field was explored using toluene; m-, p-, and o-xylene; ethylbenzene; and styrene, as target analytes. Detection limits were 0.01 $\mu g \cdot L^{-1}$ in all cases and the inter-day precision was in the interval 7.4–15.7%. The sorbent performance of the nanostructured monolithic solid was evaluated by extracting the selected compounds from different water samples with recovery values between 81.5% and 116.4%.

1. Introduction

The number of publications in the field of nanoscience and nanotechnology has greatly increased in the last years due to the exceptional physical and chemical properties of the nanomatter [1–4]. Within the sample preparation context, nanoparticles have been extensively used to improve the performance of extractant phases [5–7]. In particular, carbon nanostructures have demonstrated a unique sorbent capacity due to their extraordinary surface-volume ratio and the possibility to interact with organic molecules via non-covalent forces. However, most of them are non-porous in nature and possess a high tendency to aggregate when packed as a powder, which requires immobilization on the surface of particles or polymers which are then packed in conventional separation units [8]. These stationary phases present the main problems associated with this type of packing, including the need for frits, bubble formation, and poor mass transfer.

Monolithic solids are porous polymeric networks capable of providing fast mass transport and a high permeability [9]. Many efforts have been devoted to the development of carbon nanoparticle-modified monolithic polymers, which combine the unique and inherent advantages of both materials [10–13]. Moreover, interconnected porous structures formed in their majority or entirety by carbon nanomaterials are highly desirable [14, 15]. These structures can be prepared via chemical vapor deposition (CVD), although a significant amount of residual metal catalyst can be found in the final monolith [16, 17]. In addition, wet chemistry methods such as gelation above a critical concentration have been used to create mesoporous materials [18], mainly based on carbon nanotubes. Nevertheless, in most of the cases, extra-linkers (gelation promoters) are needed, as well as the final addition of a structure stabilizer [19, 20].

Single walled carbon nanohorns (SWNHs) are carbon nanoparticles characterized by a conical structure with a horn-shaped tip (cone angle of approximately 20°), as well as a cylindrical nanotube section (3–5 nm in diameter and 40–50 nm in length) [21, 22]. A feature of SWNHs is their high reactivity, owing to their small size and curvature, which generate an extra negative surface charge, allowing them to react with other radical monomers and initiators [23, 24]. In addition, carbon nanohorns are able to form stable aggregates via van der Waals interactions, as well as single C–C bonds between the individual carbon nanohorns [25]. SWNHs have been described to form different types of spherical aggregates, which have been so-called dahlia-, bud-, and seed-type aggregates [26, 27]. The resulting spherical dahlia-shaped aggregates (Scheme 1) provide them with an extended surface area and an enhanced sorption capacity in comparison with other carbon nanoparticles such as carbon nanotubes [28]. The inherent tendency of SWNHs to aggregate enables the formation of monoliths without the need for further agents, resulting in a cleaner solid without any impurities from the synthesis.



Scheme 1. Scheme of dahlia-type aggregates of single walled carbon nanohorns. Detail of an individual single-walled carbon nanohorns (SWNHs) showing its dimensions and the cone angle.

In this work, we have prepared the first macroscopic SWNH-monolithic solid composed of only dahlia-type aggregates of SWNHs without the assistance of extra components such as dispersants, crosslinkers, or gelation promoters. The gelation is driven by physical interactions including van der Waals forces and π–π stacking. The wet jelly-like system was converted to the corresponding monolithic structure following low rate drying in a polypropylene cell. The solid porous macroscopic structure showed higher stability towards different solvents compared to the monolithic solid composed of only carbon nanotubes. Pristine SWNHs and monoliths were characterized by different techniques, e.g., scanning electron microscopy (SEM), transmission electron microscopy (TEM), nitrogen intrusion porosimetry, Raman spectroscopy, and X-ray photoelectron spectroscopy (XPS), revealing the presence of sp^3 carbons responsible for the long-term stability of the structure. As a proof of concept, its applicability in the microextraction context has been evaluated for the determination of toluene; ethylbenzene; o-, m-, and p-xylene; and styrene (TEXS) in water samples.

2. Materials and methods

2.1. Reagents, materials, and samples

All reagents were of analytical grade or better. TEXS (toluene; ethylbenzene; o-, m-, and p-xylene; and styrene) were acquired from Sigma-Aldrich (Madrid, Spain, http://www.sigmaaldrich.com). Individual stock standard solutions (1 $g \cdot L^{-1}$) were prepared in hexane and stored at 4 °C. Working standard solutions were prepared daily by rigorous dilution of the stocks in ultrapure Mill-Q water.

Single-walled carbon nanohorns (SWNHs, >90 wt % purity, 40–50 nm in length and 4–5 nm in diameter) were purchased from Carbonium S.r.l. (Padua, Italy, http://www.carbonium.it/public/site/index.php). SWNHs were dispersed in chloroform (HPLC gradient grade, Sigma-Aldrich, Madrid, Spain) while n-hexane (Panreac, Madrid, Spain) was selected as the eluent.

Tap and Guadalquivir river water samples were selected for the determination of the target compounds following the proposed SWNH-monolithic (micro)solid-phase extraction approach. River waters were collected in amber glass bottles and maintained without headspace at 4 °C until analysis.

2.2. Apparatus

Chromatographic equipment was a GC Varian CP-3800 coupled to a Varian 1200 MS/MS working under single quadrupole mode and with an electron multiplier detector. The separation of the six analytes was accomplished on a 5% phenyl-95% dimethylpolysiloxane fused silica capillary column (HP-5MS UI, 30 m × 0.25 mm i.d. × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). The temperature program was: 40 °C (2 min) initial, raised up to 60 °C at 30 $°C·min^{-1}$ and then immediately ramped at 5 $°C·min^{-1}$ up to 75 °C. The final temperature, 280 °C (1 min), was reached with a ramp of 40 $°C·min^{-1}$. The injector working in splitless mode was kept at 225 °C. The injection volume of n-hexane, 2 µL, was measured with a 5 µL microsyringe (Hamilton Co., Reno, NV, USA). Helium (6.0 grade, Air Liquide, Seville, Spain) was used as the carrier gas at a flow rate of 1.0 $mL·min^{-1}$. It was regulated by a digital pressure and flow controller. The transfer line and ionization source temperatures were 280 °C and 250 °C, respectively.

The ionization mode in the mass spectrometer was electron impact (EI) with an ionization energy of 70 eV. Mass spectra were acquired using the selected ion monitoring mode (SIM), selecting the m/z 91 at 1 $scan·s^{-1}$. Chromatograms were acquired and processed using the MS Workstation (Varian) on an AMD Sempro™ Processor computer, which also controlled the whole system.

In the preparation of the monolith, a vortex stirrer from IKA® (Staufen, Germany) and an ultrasonic bath (50 W, 40 KHz) form J. P. Selecta (Barcelona, Spain) were used. Besides, an oven (Binder, Madrid, Spain) was also used for drying the monolithic solid at 25 °C.

2.3. Preparation of the monolithic solid based on SWNHs

The macroscopic monoliths were fabricated by crosslinking SWNHs in concentrated suspensions to form wet jelly-like systems. They were prepared by mixing 10 mg of SWNHs and 80 µL of chloroform into a polypropylene device. Next, the mixture was sonicated for 5 min in order to ensure a uniform dispersion of the SWNHs, and then incubated at 25 °C for 48 h. Once formed, the molds were disassembled to obtain the stable macroscopic monolith (Figure 1).

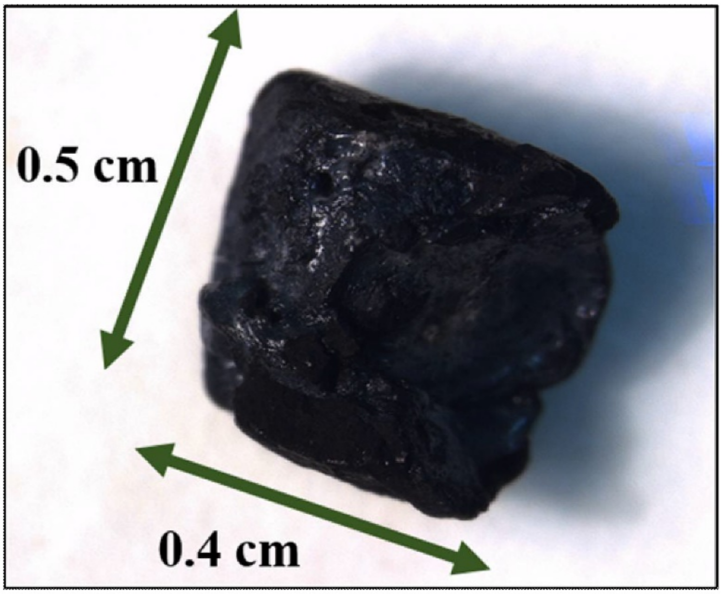


Figure 1. Photograph of the SWNH-monolith.

2.4. Characterization

Several techniques were used with the goal of characterizing the developed monolith. Raman measurements were carried out with a confocal Raman spectrometer (alpha500, Witec GmbH, Ulm, Germany). A frequency doubled Nd:YAG laser at 532 nm (second harmonic generation) was employed for excitation using a laser power of 2.48 mW (measured prior the objective lens). The laser beam was focused on the sample using a 20×/0.4 Zeiss objective. Raman spectra were acquired with an integration time of 2 s, averaging 10 spectra. For the measurements, solid samples of pristine SWNHs and SWNHs dispersed in chloroform and quickly dried with and without sonication treatment, as well as SWNH-monoliths, were deposited on a glass slide. Measurements were performed at different points of the sample and the average value was calculated. The height of the D and G bands, as well as their ratio, were calculated.

Three-dimensional non-invasive assessment of monolithic surface was performed by confocal microscopy (Leica DCM8, L'Hospitalet de Llobregat, Spain). Surface roughness is given by the average roughness value, Ra, which is the arithmetic mean of the departure of the profile from the center line of a line scan of the surface.

X-ray photoelectron spectroscopy (XPS) was measured with a PHI VersaProbe II spectrometer from Physical Electronics (PHI, Chanhassen, MN, USA), equipped with an Al Kα X-ray radiation source (1486.6 eV). The beam diameter was 200 µm.

A JEOL JSM 7800F scanning electron microscope (Isaza, Alcobendas, Spain) was used to obtain the micrographs of the SWNH-monoliths, and a Philips CM-10 (Philips, Amsterdam, Netherlands) transmission electron microscope (TEM) was used to obtain the micrographs of the SWNHs' dahlia-like structure.

Nitrogen adsorption/desorption experiments were carried out at -196 °C using Quantachrome® ASiQwin™-Automated Gas Sorption Data. The specific surface area values were calculated according to the BET (Brunauer-Emmett-Teller) equation. The average pore diameter and volumes were evaluated from the desorption branches of isotherms based on the BJH (Barrett-Joyner-Halenda) model.

3. Results and discussion

3.1. Synthesis of SWNH-monolith

The formation of pure carbon nanohorn monoliths has been deeply studied in this work. The main objective was the fabrication of a monolithic solid based on pristine SWNHs maintaining the unique characteristics and properties related with their shape and size. For that purpose, a given amount of SWNHs was dispersed in an organic solvent (chloroform) and sonicated for 5 min. Above a critical concentration, the mixture formed a slightly sticky system due to van der Waals and π–π interactions. At high concentrations, the distance between the dahlias aggregates is reduced, inducing SWNH interactions. In fact, higher concentrations lead to denser and mesoporous structures, while if the concentration is too low, only a precipitate is formed [19]. Thus, pure SWNH systems were obtained at a concentration of 125 $mg \cdot mL^{-1}$, allowing the complete removal of the solvent by drying at 25 °C without destroying the monolith.

Usually, radical initiators are used for the covalent functionalization of carbon nanostructures [29], as well as for the fabrication of macroscopic stable carbon scaffolds [30], while the use of linkers or initiators helps to obtain interlinked solids with excellent mechanical resilience. Herein, the fabrication of mechanically stable carbon nanohorns without the need for radical initiators has been possible thanks to the enhanced chemistry reactivity of SWNHs and their tendency to form stable aggregates. The stability of the SWNH-monoliths was evaluated by their immersion in different solvents including water, methanol, and hexane (see Figure S1), and via submission to high temperatures, in order to evaluate their practical stability for future applications. In all cases, the monolith exhibited great stability, maintaining its shape and rigidity. Moreover, once the monolith structure was formed, the structures were manipulated with the aid of tweezers for the subsequent microextraction procedure. It has to be pointed out that the solid monolithic structure was not damaged by manual handling with the tweezers during the different steps of the extraction process.

3.2. Microscopic and pore characterization of SWNH-monoliths

As previously mentioned, SWNHs possess a conical structure with a horn-shaped tip and a cylindrical nanotube section (~ 2–5 nm in diameter and 40–50 nm in length). The conical shape of carbon nanohorns is the direct consequence of introducing six pentagons within the network of hexagons of the graphene sheet pentagons. An additional heptagon is also required to counteract the curvature change of one pentagon.

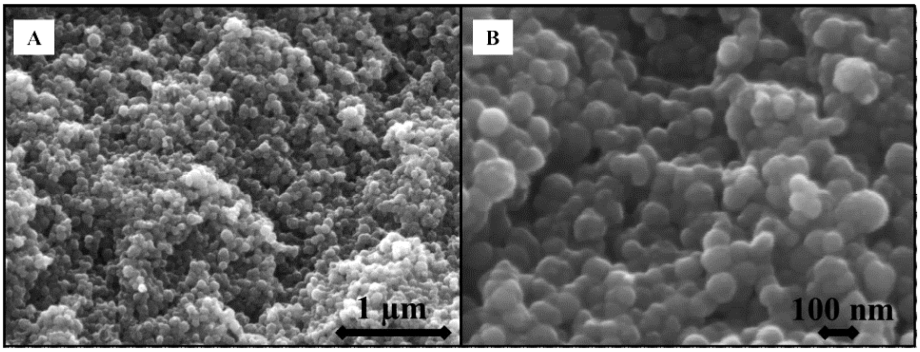


Figure 2. Scanning electron microscopy (SEM) of the SWNH-monolithic solid at 27,000× **(A)**, and 70,000× **(B)** magnification.

SEM images of the SWNH-monoliths were acquired to characterize their morphology and porous structure (Figure 2). From the microphotographs at a 27,000× magnification (Figure 2A), the monolithic solid clearly exhibited interconnected SWNHs which results in a porous architecture. At a larger magnification (70,000×, Figure 2B) of the globules, the image showed that the size of globules varies between 50–100 nm, which is coherent with the diameter of the spherical dahlia-shaped aggregates. This fact was corroborated by the TEM micrographs acquired from pristine SWNH dahlia aggregates (Figure 3), which also showed that the nanoparticles are aggregated into dahlia-type structures of a nearly spherical shape, showing a narrow size distribution (Figure 3A). A magnified TEM micrograph of an SWNH dahlia-aggregate (Figure 3B) showed conical horn-like protrusions in the edge area, corresponding to the individual SWNHs.

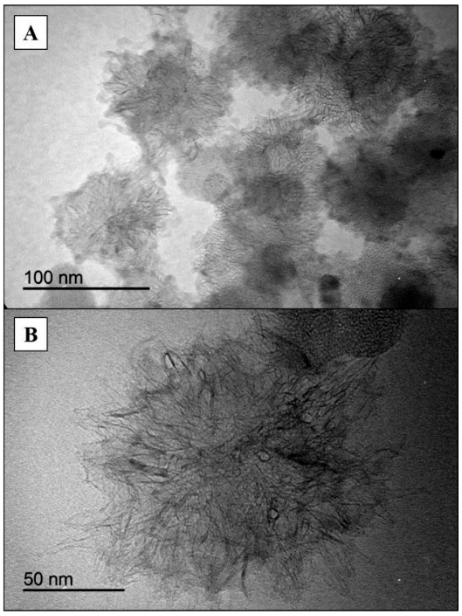


Figure 3. Transmission electron microscopy images of SWNHs at low **(A)** and high **(B)** magnification.

The confocal profile image of the SWNH-monolith is shown in Figure 4. 2D and 3D representations of their surface topographies with a color scale for the z-range calibrated in micrometers (µm) are illustrated. It is possible to distinguish the surface roughness of the macroscopic SWNH-monolithic solid as well as the depth profile along a vertical line with a mean of the absolute values of the profile heights measured from a mean line averaged over the profile (Ra) of 3.685 µm.

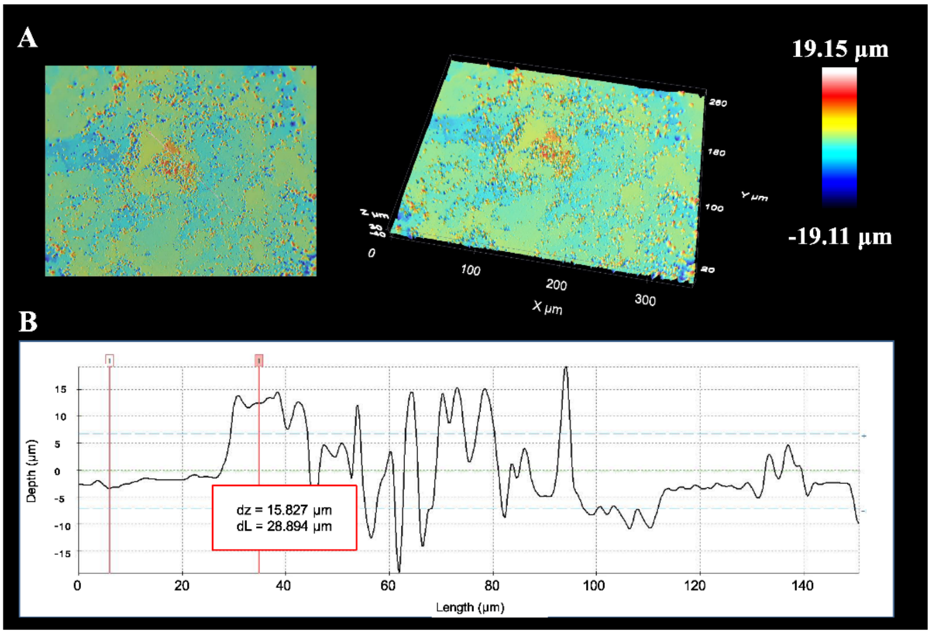


Figure 4. 2D and 3D images of the SWNH-monolith **(A)**, and its depth profile **(B)** in confocal microscopy.

Nitrogen intrusion porosimetry measurements were performed to assess the porosity of SWNH-monoliths. The textural values including the specific surface area, pore diameter, and pore volume, are compiled in Table 1. Data from N_2 adsorption-desorption isotherms evidenced that the solid exhibited an isotherm type IV with a step at a relative pressure around 0.7–0.9 (Figure 5). This is typical of pores with sizes in the high mesopore range.

Table 1. Porous properties of the SWNHs monolithic solid.

Specific surface area, BET ($m^2 \cdot g^{-1}$)	Average pore diameter (Å)	Average pore volume ($cc \cdot g^{-1}$)
154.68	166	0.64

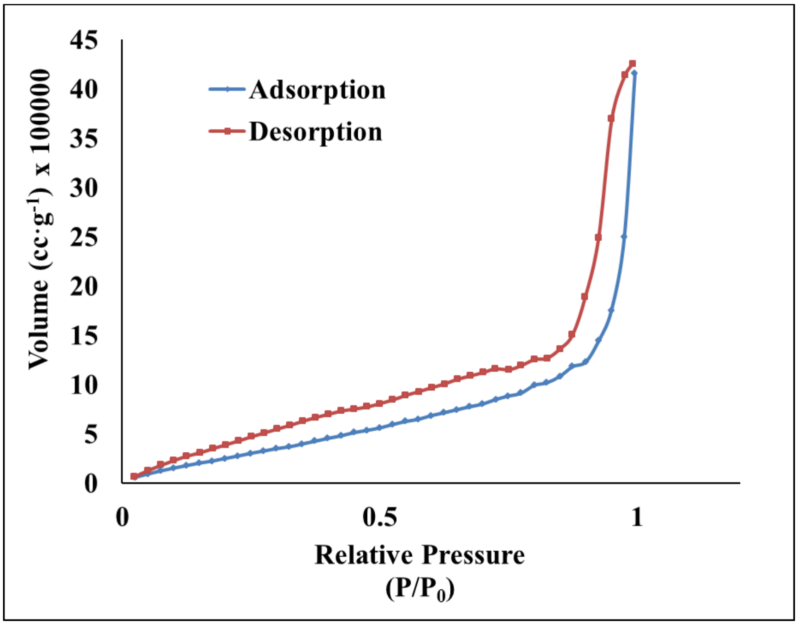


Figure 5. Nitrogen adsorption and desorption isotherms of the SWNH-monolith.

3.3. Spectroscopic characterization of SWNH-monoliths

Moreover, the synthesized SWNH-based monoliths and pristine SWNHs were also characterized by different spectroscopic techniques, i.e., Raman spectroscopy and X-ray photoelectron spectroscopy. Figure 6A shows the Raman spectra of pristine SWNHs (1), that of SWNHs dispersed in chloroform followed by a rapid evaporation of the solvent with (3) and without (2) the previous sonication step, and the SWNH-monolith prepared as previously described (4). As can be seen, the Raman spectrum of the pristine SWNHs at room conditions exhibits the characteristic bands typical of sp^2 defected carbon materials, i.e., the G band (~ 1589 cm^{-1}) associated with the double bonds between the carbon atoms (C=C), which originates from in-plane E2g stretching vibrations in the sp^2 bonded carbon atoms [31], and the D band (~1350 cm^{-1}), assigned to A1g-symmetry modes [32], which is related to the introduction of pentagons and heptagons resulting in the typical conical-shape of SWNHs, as well as with the presence of single-bonding sp^3 carbon atoms (C–C) [26,33].

The spectrum of the SWNH-monolith material is also dominated by these two characteristic bands; nevertheless, an increase in the D feature as regards the G band is observed compared to the pristine material. The intensity ratio of the D and G bands was calculated for the different materials. As shown in Figure 6B, the intensity ratio I_D/I_G increases from 1.04 for pristine SWNHs to 1.46 for the SWNH-monolith. The D band is associated with structure imperfections/defects, thus, the effect of the sonication process on the structure of the SWNHs was evaluated. For this purpose, solid SWNHs were dispersed in chloroform and a rapid evaporation process was carried out without previous sonication of the solid (spectrum 2 in Figure 6A). On the other hand, SWNHs dispersed in chloroform were submitted to the same sonication conditions as those of the monolith, with the drying step taking place in a short time (spectrum 3, Figure 6A). As can be seen in the spectra and deduced from the calculated I_D/I_G values, sonication of the nanomaterial at such conditions does not lead to the introduction of defects within the SWNHs' structure and, thus, to the observed variations in the Raman bands.

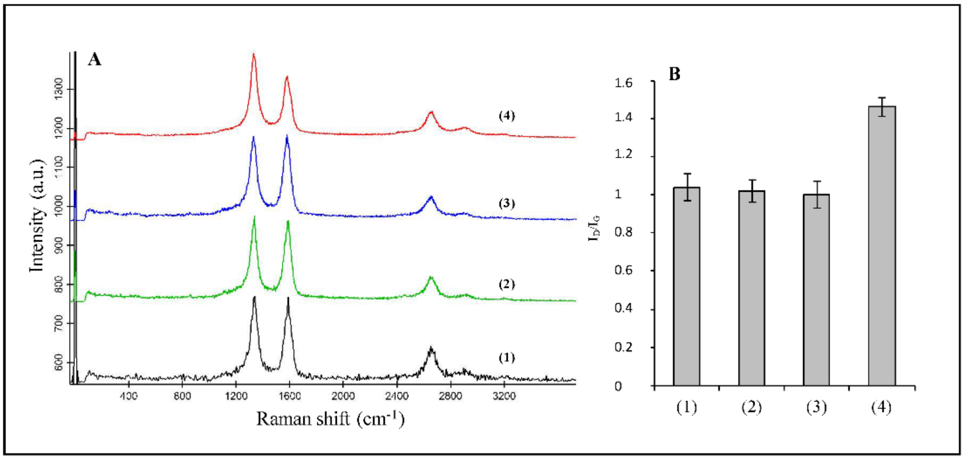


Figure 6. (A) Raman spectra of pristine SWNHs **(1)**, SWNHs dispersed in chloroform followed by a rapid evaporation of the solvent without sonication **(2)** and with a previous sonication step **(3)**, and the SWNH-monolith **(4)**. **(B)** Plot of the average value of the intensity ratio of the Raman D and G bands calculated from the Raman spectra measured of the different materials. Spectra at different positions within each sample were acquired and the average value calculated (n=5), with the error bars depicting the standard deviation within measurements.

A feature of SWNHs is that they are hardly dispersed into individual primary particles, even under sonication in surfactant solutions. This characteristic contrasts with that of other carbon nanostructures, such as single walled carbon nanotubes (SWCNTs), which can be easily dispersed into individual tubes, thus leading to the hypothesis that SWNH aggregation is owing not only to van der Waals interactions, but also chemical bonding, which may contribute to the stability of such aggregates [25]. As reported by Utsumi et al. [25], C–C single bonds between dahlia-aggregates contribute to the assembly structure, which is reflected by a strong D-band in the Raman spectrum. Thus, the high stability of the macroscopic monolithic solid may be the result of the formation of sp^3 single C–C bonds between aggregates, as reflected by an increase in the I_D/I_G ratio (Table 2), which is in agreement with the C=C and C–C bonds ratio determined by XPS (Figure 7). The relevance of the slow rate of the drying process at the polypropylene device should be pointed out, which enables the formation of bonds between aggregates. Interestingly, monolithic solids created from carbon nanotubes did not show the same stability towards water and solvents, the Raman spectra of the solid CNTs (Figure S6A), and the macroscopic tridimensional structure (Figure S6B), revealing a similar I_D/I_G ratio compared to the powder of CNTs, as shown in the Table 2.

Table 2. Ratio of the intensity of the D and G band from the Raman spectra of the pristine carbon nanomaterials, namely carbon nanotubes (CNTs) and single-walled carbon nanohorns (SWNHs) and their corresponding monoliths.

	Pristine CNTs	**CNT-monolith**	**Pristine SWNHs**	**SWNH-monolith**
I_D/I_G ratio	0.90 ± 0.09	0.90 ± 0.07	1.04 ± 0.07	1.46 ± 0.05

Figure 7 shows the C1s XPS spectra of pristine SWNHs and the SWNH-monolith. The C1s spectrum of pristine SWNHs shows a relatively broad peak with the maximum at about 285 eV, while the C1s spectrum of the SWNH-monolith presents a narrower band centered at about 285.2 eV. The deconvoluted C1s spectrum of pristine SWNHs (Figure 7A) shows two main contributions at about 284.5 eV and 285.2 eV. The peak at 284.5 eV is assigned to the double bonding carbons (C=C) corresponding to carbons with sp^2 hybridization typical of SWNHs [25]. On the other hand, the sub-peak at 285.2 eV can be assigned to single bonding carbons (C–C) of carbons displaying sp^3 hybridization [25]. Other sub-peaks are also observed at a higher binding energy, which can be ascribed to oxygen-containing functional groups, such as C–O at 286.1 eV and C=O at 287.0 eV.

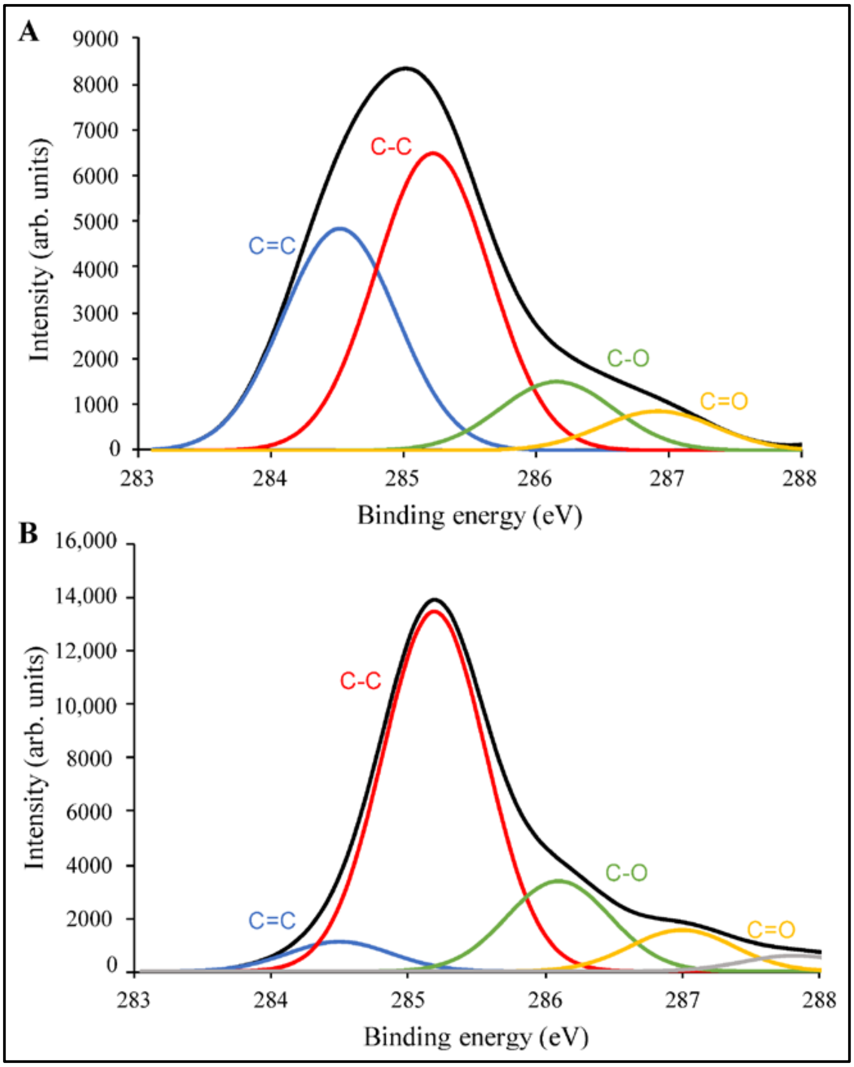


Figure 7. X-ray photoelectron spectroscopy C1s spectra and deconvolution results of **(A)** pristine SWNHs, and **(B)** SWNH-monolith.

Figure 7B depicts the deconvoluted C1s XPS spectrum of the SWNH-monolith. As can be seen, the narrower band centered at about 285.2 eV has a major contribution of the C–C bonding (sp^3 hybridization), while the contribution of the sub-peak at 284.5 eV corresponding to the sp^2 carbon has significantly decreased compared sp^3 carbon. This increase in the C–C bonds as regards C=C is in agreement with the increase in the D band value observed in the Raman spectrum of the SWNH-monolith. According to Kawai et al. [34], in junction structures within graphene sheets or tubes, the carbons may acquire sp^3 hybridization, which has been associated with the firm structure of SWNH aggregates.

3.4. Microextraction procedure

The microextraction procedure for the extraction of TEXS from water samples is described as follows. Aliquots of 50 mL of aqueous standards or water samples were placed in a 100 mL glass beaker and a magnetic stirrer was added. Next, the SWNHs monolith supported on a rubber platform fixed to a stainless-steel wire, was exposed to the sample headspace. Then, the standard/sample was magnetically stirred (15 min, 275 rpm) at room temperature. Next, analytes were eluted by direct immersion of the SWNH-monolith, enriched with the analytes, in a glass insert containing 200 μL of n-hexane (5 min, vortex agitation). Volumes of 2 μL of the final hexane phase with the extracted analytes were injected into the GC/MS for separation and detection. Between samples, the solid was washed with n-hexane and then dried in an oven at 100 °C.

The selection of the most favorable values for all the variables affecting the microextraction procedure was assessed by means of a univariate model. In this way, those variables having a major influence on the process could be clearly identified. For this aim, aqueous standards containing the six volatile analytes at a concentration of 10 $\mu g \cdot L^{-1}$ were prepared. The initial conditions of the extraction were: 25 mL of aqueous standard, and magnetic stirring during 20 min at 275 rpm at a temperature of 25 °C using 200 µL of n-hexane as the eluent.

Several parameters were optimized, namely: (a) sample volume; (b) stirring rate; (c) extraction time; and (d) extraction temperature, and the results are explained in detail in the *Electronic Supporting Information*. The optimum values were: (a) sample volume of 50 mL; (b) stirring rate of 275 rpm; (c) extraction time of 15 min; and (d) extraction temperature of 90 °C.

3.5. Analytical figures of merit and analysis of water samples

The headspace microextraction method, working under the selected experimental conditions, was characterized in terms of a linear interval, limits of detection and quantification, and precision. The values are summarized in Table 3. Calibration curves were calculated using nine working aqueous standards, with each one prepared in duplicate. The corresponding equations were obtained by plotting the peak areas of the characteristic m/z fragment ions versus the concentration for each target analyte and were linear from 0.1 to 10,000 $\mu g \cdot L^{-1}$. The limits of detection (LODs) and quantification (LOQs) were obtained using a signal-to-noise ratio (S/N) of 3 and 10, respectively. The calculated values were 0.01 $\mu g \cdot L^{-1}$ (LOD) and 0.1 $\mu g \cdot L^{-1}$ (LOQ) for all analytes. The precision of the method was studied under repeatability and reproducibility experimental conditions. Repeatability (intra-day conditions), expressed as relative standard deviation (RSD), was lower than 6.88% (n=5) for all the analytes. The reproducibility between days (inter-day conditions) ranged from 7.37% to 15.7% (n=5). The enrichment factors for all the analytes were calculated by a comparison of the slopes of the calibration graphs before and after the extraction process. They were in the range from 11.3 to 26.9.

Table 3. Analytical figures of merit of macroscopic SWNH-monoliths for the determination of the target TEXS.

Analyte	m/z	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)	Precision		EF
				Intra-day RSD (%, n=5)	Inter-days RSD (%, n=5)	
Toluene	91	0.01	0.1	3.3	10.8	15.0
Ethylbenzene	91	0.01	0.1	4.9	14.2	26.9
m- and p-Xylene	91	0.01	0.1	6.2	8.4	11.3
o-Xylene	91	0.01	0.1	6.5	15.7	22.6
Styrene	104	0.01	0.1	6.9	7.4	16.3

The proposed method was applied to the determination of TEXS in environmental waters (tap and river). The samples were analyzed under the optimized conditions. Since none of the analytes were detected, blank tap and river waters were enriched with the six target analytes at a concentration of 1 $\mu g \cdot L^{-1}$. The concentrations were calculated by interpolating the peak area obtained in the corresponding calibration graph. The recovery values (average of three replicates calculated dividing the concentration found by the concentration added, expressed in percentage) are shown in Table 4. They ranged from 85.9% to 116.4% and 81.5% to 89.8% for tap and river waters, respectively.

Table S2 compares the performance of the proposed microextraction method with that provided by recent approaches developed for the same analytical problem [35–39]. As it can be seen, the SWNH-monolith provides equal or better sensitivity (PDMS-grafted carbon nanospheres excepted) with comparable extraction recoveries. As an advantage, it allows processing large sample volumes in an intermediate time interval as regards fiber-based extraction. Regarding the method based on aerogels, the extraction capacity of the SWNH-monolith is higher, which results in better detection limits.

Table 4. Recovery study for the target TEXS spiked to water samples analyzed using the SWNHs monolith.

Analyte	Tap water (%, n=3)	River water (%, n=3)
Toluene	93.3 ± 6	89.7 ± 7
Ethylbenzene	86.8 ± 3	81.6 ± 16
m- and p- Xylene	116.4 ± 13	84.1 ± 14
o-Xylene	90.8 ± 5	81.5 ± 16
Styrene	85.9 ± 8	85.3 ± 15

4. Conclusions

A monolith based on carbon nanohorns has been synthesized for the first time. The procedure is quite simple as it only requires the preparation of a stable dispersion of pristine SWNHs in chloroform using ultrasound energy. The dispersion is placed in a polypropylene device with the desired geometry and left to stand for slow solvent evaporation for 48 h at 25 °C. The monolith thus obtained is hydrophobic in nature and stable in organic solvents. As an advantage over other synthetic procedures described in the literature for similar porous structures, no crosslinker or initiators (gelation promoters) are required. This results in a cleaner solid without any impurities from the synthesis. The solid was characterized by several techniques including SEM, confocal laser scanning microscopy, Raman spectroscopy, XPS, and nitrogen intrusion porosimetry, which proved the increase in sp^3 bonds within the monolithic material as regards pristine SWNHs, which accounts for the high stability of the porous macrostructure as regards other carbon nanomaterials with just van der Waals and π–π intermolecular interactions. Finally, the material has been evaluated in the microextraction context using toluene; m-, p-, and o-xylene; ethylbenzene; and styrene as model analytes. The excellent results obtained envisage an extended applicability of the carbon nanoparticles, and in particular of SWNHs, within the analytical field.

Supplementary materials

The following are available online at http://www.mdpi.com/2079-4991/8/6/370/s1, Table S1: Variables studied in the headspace microextraction indicating the initial value, the interval studied, and the selected value, Figure S1: Photographs of the solid monoliths after 2 h immersion in different solvents, namely water (a), methanol (b) and hexane (c), Figure S2: Effect of the sample volume on the analytical signal obtained after the microextraction procedure, Figure S3: Effect of the stirring rate on the analytical signal obtained after the microextraction procedure, Figure S4: Effect of the extraction time on the analytical signal obtained after the microextraction procedure, Figure S5: Effect of the extraction temperature on the analytical signal obtained after the microextraction procedure, Figure S6: Raman spectra of pristine CNTs (A), and CNT-monolith (B), Table S2: An overview on recently reported headspace methods for preconcentration and determination of BTEXs.

Author contributions

Investigation, B.F.-C., A.I.L.-L and S.C.; Data Curation, B.F.-C. and A.I.L.-L.; Writing-Original Draft Preparation, B.F.-C.; Writing-Review & Editing, A.I.L.-L and S.C.; Supervision, S.C.

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Conflicts of interest

The authors declare no conflict of interest.

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Electronic Supplementary Material

1. Stability of monoliths towards solvents

The stability of the SWNH-monoliths towards solvents was evaluated by their immersion in different solvents including water, methanol, and hexane for two hours. As shown in Figure S1, the monoliths remained stable when using different solvents.

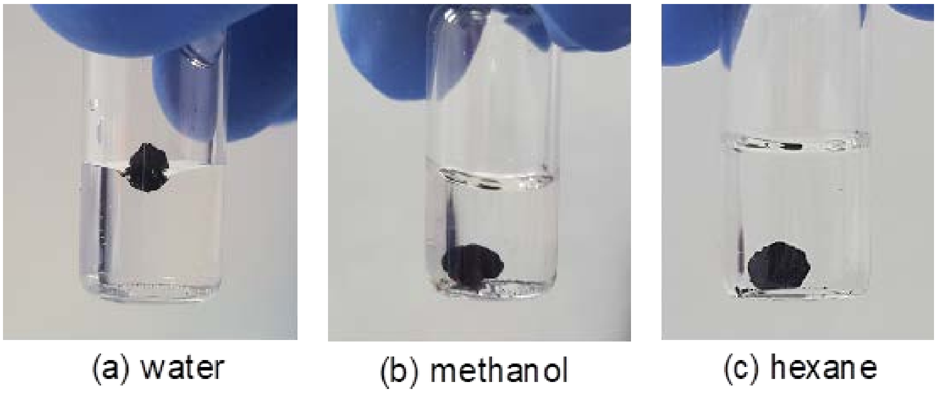
(a) water (b) methanol (c) hexane

Figure S1. Photographs of the solid monoliths after 2 h immersion in different solvents, namely water **(a)**, methanol **(b)**, and hexane **(c)**.

2. Variables affecting to the microextraction procedure

Different variables may affect the efficiency of the microextraction procedure and therefore their effect on the analytes extraction was considered in depth. Table S1 reflects their initial values, the interval studied and the optimum values for each variable. The optimization was performed under a univariate approach using an aqueous standard solution containing the six analytes at a concentration of 10 $\mu g \cdot L^{-1}$.

Table S1. Variables studied in the headspace microextraction indicating the initial value, the interval studied, and the selected value.

Variable	Initial value	Interval studied	Selected value
Sample volume (mL)	25	10-50	50
Stirring rate (rpm)	275	0-550	275
Extraction time (min)	20	10-30	15
Extraction temperature (°C)	25	25-90	90

Sample volume was studied in the interval 10–50 mL, keeping the analytes concentration constant. As it is shown in Figure S2, the analytical signal obtained for the target analytes increases up to 50 mL, and therefore it was found to be the most convenient value.

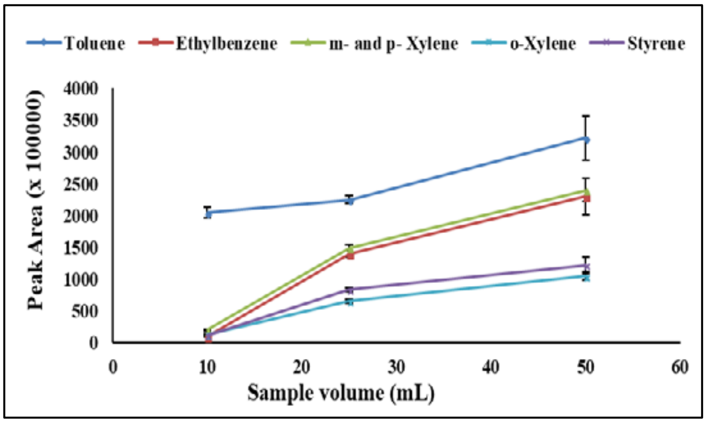

Figure S2. Effect of the sample volume on the analytical signal obtained after the microextraction procedure.

The next variable evaluated was the stirring rate of the sample within the interval 0–550 rpm. As it can be seen in Figure S3, the peak areas increased up to 225 rpm, reaching an almost steady state over this value.

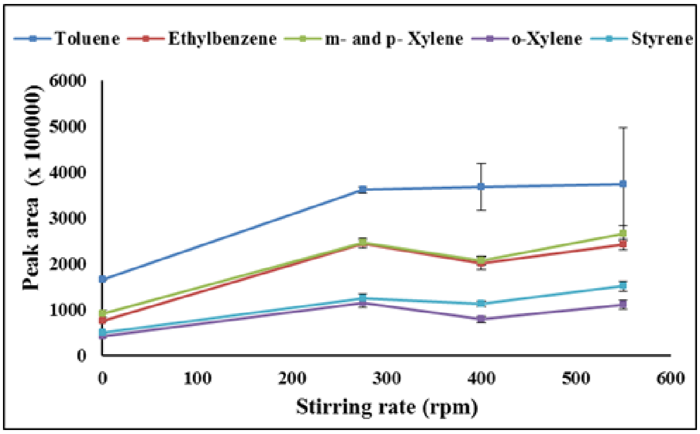

Figure S3. Effect of the stirring rate on the analytical signal obtained after the microextraction procedure.

Concerning the extraction time, this variable was evaluated between 10 and 30 min (Figure S4). The results pointed out that this variable positively affects the extraction up to 15 min, decreasing over this value.

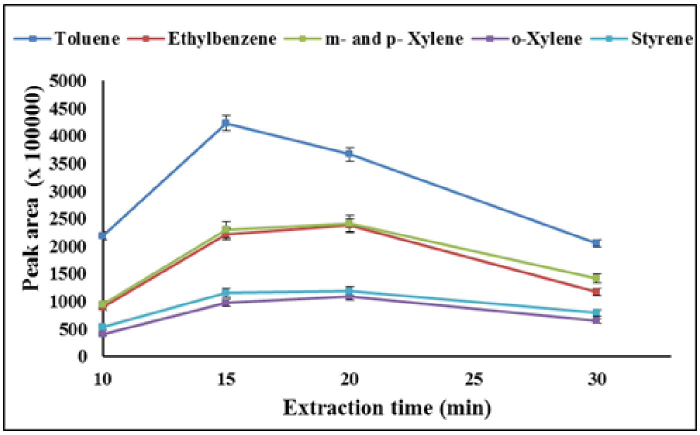

Figure S4. Effect of the extraction time on the analytical signal obtained after the microextraction procedure.

Finally, the influence of extraction temperature during the extraction was evaluated from 25 to 90 °C (Figure S5). An increase in the temperature facilitates the release of these analytes to the headspace of the vial and thus the adsorption at the surface of the SWNH-monolith. Temperatures higher than 90 °C were not evaluated considering the aqueous nature of the standard solutions and further application to water samples.

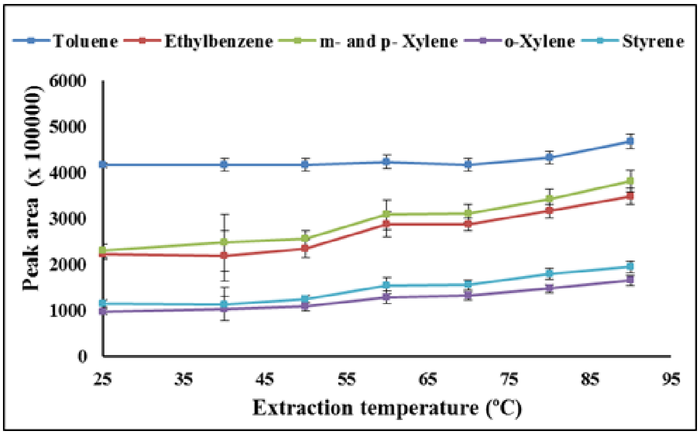

Figure S5. Effect of the extraction temperature on the analytical signal obtained after the microextraction procedure.

3. Raman spectroscopy characterization

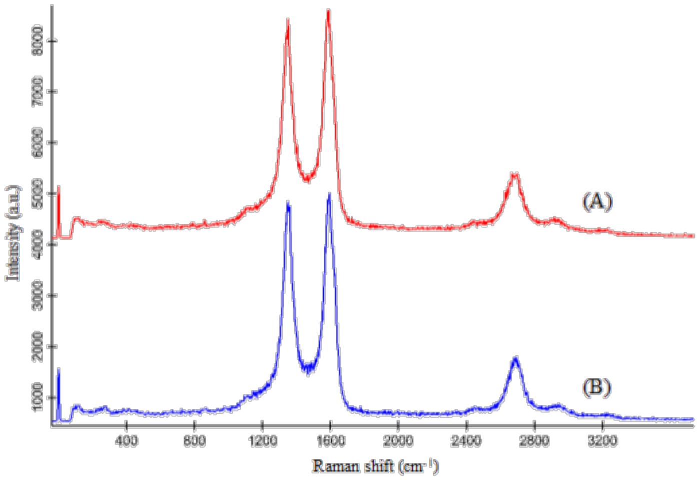

Figure S6. Raman spectra of pristine CNTs **(A)**, and CNT-monolith **(B)**.

Table S2. An overview on recently reported headspace methods for preconcentration and determination of BTEXs.

Material used	Sample	Sample volume (mL)	Extraction time (min)	Detection method	LODs ($\mu g \cdot L^{-1}$)	LOQs ($\mu g \cdot L^{-1}$)	R (%)	Ref
PDMS-grafted carbon nanospheres	Tap water, river water, well water and wastewater	15	25	GC-FID	0.001-0.01	0.003-50	92.5- 99.5	[28]
Polypyrrole-carbon nanotubes-titanium oxide (PPy-CNT-TiO_2)	Tap water, mineral water, river water, well water and wastewater	15	35	GC-FID	0.01-0.04	0.03-500	93.7-106.2	[29]
Poly(o-anisidine)/ graphene oxide nanosheets composite (PoA/GONSs)	River and agricultural well water	10	30	GC-MS	0.01-0.06	0.1-500	92.0-101.2	[30]
Metal-organic frameworks (MIL-101(Cr))	River water	10	3	GC-MS	0.32-1.7	10-20000	80.0-1130.0	[31]
Powdery polymer aerogel- carbon aerogel (PPA-PCA)	River, pond and tap water	-	7-9	GC-MS	0.2-0.9	5-5000	82.9-102.0	[32]
SWNHs monolith	Tap and river water	50	15	GC-MS	0.01	0.1-10000	81.5-116.4	This work

LOD limit of detection, LOQ limit of quantification, R recovery values, Ref references.

Capítulo 10

One-Pot Synthesis of Graphene Quantum Dot and Simultaneous Nanostructured Self-Assembly Via a Novel Microwave-Assisted Method: Impact on Triazines Removal and Efficiency Monitoring

En revisión en RSC Advances

One-pot synthesis of graphene quantum dot and simultaneous nanostructured self-assembly via a novel microwave-assisted method: Impact on triazines removal and efficiency monitoring

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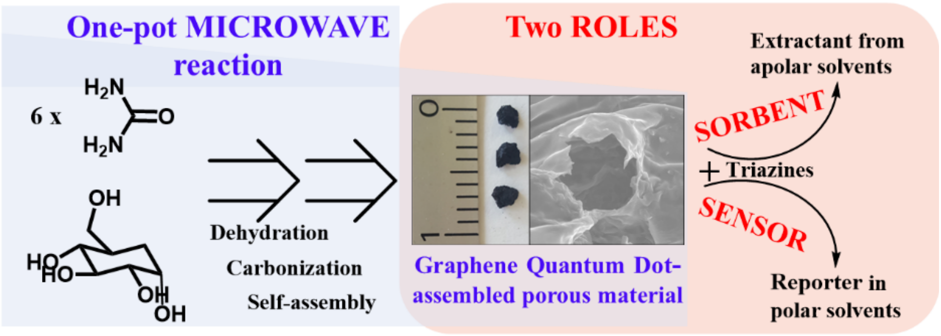

Graphical abstract

Preparation of porous continuous solid based on graphene quantum dots from nanoparticle precursors by a microwave-assisted organic solvent-free protocol.


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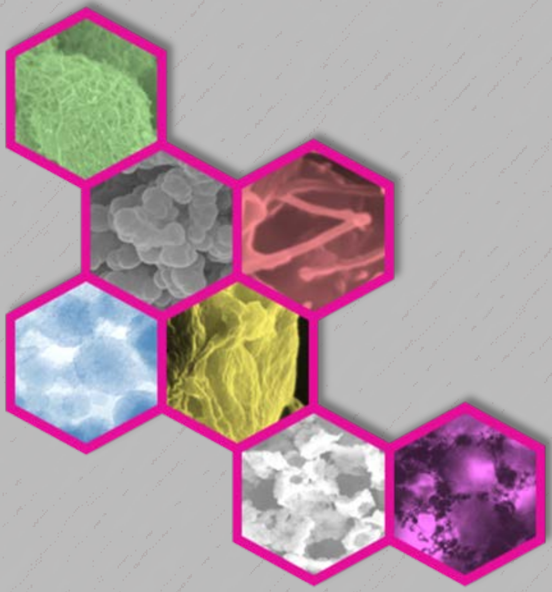
Conclusiones

Conclusions



La investigación recogida en esta Tesis Doctoral se centra en la etapa de tratamiento de la muestra. Concretamente, las aportaciones realizadas se han dirigido al diseño y empleo de nuevos sorbentes monolíticos en técnicas de separación. Siguiendo esta línea, la Memoria ha sido dividida en tres ***Bloques*** según el formato de microextracción usado y/o la utilización de las nanopartículas de carbono como componentes principales en la estructura monolítica.

A continuación, se describen las conclusiones principales de cada uno de los ***Bloques*** abordados en esta Tesis Doctoral.

1. Sólidos híbridos basados en la combinación de polímeros monolíticos y nanopartículas de carbono preparados en capilares

En este ***Bloque*** de la Tesis Doctoral se han sintetizado dos tipos de sólidos monolíticos; polímeros de naturaleza orgánica (generalmente metacrilatos) y de naturaleza inorgánica (sílice).

Para obtener columnas monolíticas basadas en metacrilato se ha utilizado la polimerización por radicales libres. En este caso, la mezcla de polimerización suele estar formada por monómeros, agente entrecruzante, disolventes porogénicos e iniciador. Así, el sólido se forma por la adición sucesiva de los monómeros y entrecruzador gracias a la elevada reactividad de los radicales libres, los cuales se generan a partir del iniciador por medio de diferentes mecanismos (térmico, químico o radiación UV). La modificación de las condiciones de la reacción de polimerización cambia, por tanto, el proceso de polimerización, y como consecuencia produce un efecto sobre las características morfológicas del monolito final.

Por este motivo, se ha evaluado la síntesis de capilares monolíticos mediante polimerización radicalaria térmica (***Capítulos 1 y 3***) y mediante radiación UV (***Capítulo 2***), obteniendo las siguientes conclusiones:

- La velocidad de polimerización, es decir, la completa formación de la estructura monolítica es más lenta cuando se usa energía térmica (~ 24 h) que al usar radiación UV (5–30 min) para iniciar la reacción. Esto puede afectar a la homogeneidad y morfología de los sólidos monolíticos híbridos ya que tiempos de polimerización largos pueden producir problemas de sedimentación de las nanopartículas en un lateral de la columna capilar. En cambio, polimerizaciones rápidas favorecen la estabilidad de la dispersión de las nanopartículas y evita la formación de agregados de mayor tamaño en las mezclas de reacción.

- Para llevar a cabo una iniciación mediada por radiación UV es necesario preparar los sólidos monolíticos en el interior de capilares de sílice fundida transparentes a la luz UV. Generalmente, los capilares que cumplen este requisito presentan diámetros internos inferiores, lo que puede derivar en una presión elevada durante el paso de los disolventes.

Por otro lado, el método de síntesis más usado para monolitos inorgánicos es el sol-gel, con el cual se crea una red continua por gelificación de una solución en el interior del capilar. Este proceso se basa en la hidrólisis y policondensación de los monómeros (alcoxisilanos) bajo unas condiciones controladas de temperatura y pH.

Las principales diferencias y peculiaridades observadas en este tipo de columnas monolíticas (***Capítulo 4***), respecto a las anteriores, son las siguientes:

- Este tipo de sólido monolítico presentó un elevado carácter polar e hidrofílico, el cual se modificó gracias a la inclusión de las nanopartículas de carbono en la matriz polimérica.

- A pesar de poseer una alta resistencia física y estabilidad química frente a diversos disolventes, a valores de pH superiores a 4 se producen interacciones secundarias entre los analitos y las cargas de los grupos silanoles presentes en la superficie del polímero. Esto repercute en una mayor irreproducibilidad entre extracciones.

- La incorporación de nanotubos de carbono (tanto de pared simple como múltiple) no produjo cambios morfológicos en la estructura polimérica. En cambio, en los monolitos con nanocuernos de carbono se observaron las estructuras esféricas tipo dalia características de este nanomaterial.

Por último, otro aspecto a destacar es la estrategia de inclusión de las nanopartículas de carbono en los polímeros monolíticos usada en cada uno de los artículos de este ***Bloque*** de la Memoria; anclaje químico (***Capítulo 1***) o fotoquímico (***Capítulo 2***) a la superficie del sólido y atrapamiento de las nanopartículas en el sólido (***Capítulos 3 y 4***). Las ventajas y desventajas de cada una de estas aproximaciones se comentan a continuación:

- La inmovilización de las nanopartículas de carbono en la superficie del sólido monolítico orgánico mediante reacciones químicas aumentó la superficie específica del material y por tanto se consiguió mejorar su capacidad sorbente (***Capítulo 1***). En este caso, todas las nanopartículas se encuentran disponibles para la interacción con los analitos. No obstante, el paso de dispersiones con concentraciones altas de nanopartículas durante la etapa de anclaje, condujeron al bloqueo y obstrucción de los poros del sólido como consecuencia de la reactividad y agregación entre las propias nanopartículas, especialmente al comienzo de la columna capilar.
- En el ***Capítulo 2*** se llevó a cabo una aproximación diferente y consistió en dos etapas; (1) activación de la superficie monolítica e (2) inmovilización de las nanoestructuras. Ambas se llevaron a cabo haciendo uso de energía procedente de una fuente UV. La principal diferencia con la inmovilización química es que una vez que se ha llenado el capilar monolítico con la dispersión de las nanopartículas se irradia con luz UV, lo que permite el anclaje de las nanopartículas a la superficie polimérica previamente activada.

Este procedimiento evita los problemas derivados de la formación de agregados de mayor tamaño en determinadas zonas del capilar.

- Como alternativa a la inmovilización o anclaje de las nanopartículas en la superficie monolítica, se procedió a la adición directa de éstas en la mezcla de polimerización. Esta estrategia se realizó tanto en polímeros de metacrilato (***Capítulo 3***) como de sílice (***Capítulo 4***), obteniendo materiales híbridos con propiedades sorbentes mejoradas en comparación con los monolitos preparados en las mismas condiciones, pero sin nanopartículas. El factor limitante en estos casos es, de nuevo, la cantidad de nanopartículas que se pueden adicionar sin interferir negativamente en la formación del monolito final. Sin embargo, se observó un comportamiento especial cuando se incorporaron nanocuernos de carbono a la mezcla de polimerización de sólidos de metacrilato. En este caso, la alta reactividad de este tipo de nanopartículas cónicas produjo un aumento de la velocidad de propagación de las cadenas poliméricas obteniendo glóbulos de menor tamaño y una mayor superficie específica total.

2. Sólidos híbridos basados en la combinación de polímeros monolíticos y nanopartículas de carbono preparados en otros formatos de microextracción

Cuando se preparan sólidos monolíticos modificados con nanopartículas en otros formatos de microextracción como *spin-columns* (***Capítulo 5***), *pipette-tips* (***Capítulo 6***), o *stir-bars* (***Capítulo 7***), hay que tener en cuenta que, a diferencia de los capilares monolíticos, se trata de polimerizaciones en masa (*bulk*) en moldes de mayor tamaño y diámetro interno. Generalmente, los sólidos monolíticos preparados en estas condiciones se ven más afectados por el efecto de contracción reduciendo su tamaño en un porcentaje mayor que los sintetizados en capilares. Aunque el anclaje químico del polímero a los sistemas de extracción reduce la contracción hacia el centro del sólido, al estar inmovilizado en la pared del soporte el sólido, tiende a quebrarse y romperse por la parte central. Por lo tanto, es importante llegar a una situación de compromiso entre la permeabilidad y la rigidez que presenta el material monolítico, para así obtener sólidos porosos que permitan el flujo de los disolventes sin producir una presión elevada pero suficientemente estables y robustos para poder ser reutilizados.

En este ***Bloque*** se ha descrito la síntesis de diferentes sólidos híbridos en este tipo de formatos de microextracción. También se ha evaluado la participación de las nanopartículas de carbono en la estructura y estabilidad del material final:

- Cuando se incorporan nanocuernos de carbono a polímeros de metacrilato se consigue un aumento de la velocidad de polimerización y se obtiene un sólido híbrido con mejores propiedades sorbentes y mecánicamente más estable que su homólogo sin nanopartículas (***Capítulos 5 y 7***). Además, el tamaño de los glóbulos del polímero final, es decir, su porosidad depende de la cantidad de nanocuernos de carbono presente en la polimerización. Gracias a las propiedades inherentes a este tipo de nanopartículas cónicas se puede controlar y modificar fácilmente la estructura y las características de las unidades de extracción.
- Otro tipo de combinación polímero orgánico/nanoestructuras de carbono evaluada han sido los monolitos de metacrilato con nanotubos de carbono de pared múltiple (***Capítulo 6***). En este caso, los nanotubos quedaron embebidos en la matriz polimérica siendo su superficie rodeada por láminas de polímero. A pesar de no estar completamente disponibles y libres en la superficie del monolito, la inclusión de nanotubos mejoró la eficiencia de extracción gracias a las interacciones π–π adicionales con los analitos.

Por último, cabe señalar que las unidades de microextracción monolíticas descritas en esta Memoria resultaron ser muy eficientes para la extracción de los analitos seleccionados, y mostraron una elevada estabilidad frente a una gran variedad de disolventes y pHs. Una ventaja derivada de la estabilidad mecánica de los sólidos híbridos desarrollados en este ***Bloque,*** es su exitoso anclaje a las paredes internas de los sistemas de extracción elegidos en los ***Capítulos 5 y 8***, o la integración del sistema de agitación en el propio sólido sin necesidad de etapas adicionales en la síntesis, como es el caso de la unidad descrita en el ***Capítulo 6***. Otra característica a destacar de las extracciones con *spin-columns* o *stir-bars* es la posibilidad de procesar varias muestras simultáneamente. Además, los procesos de síntesis desarrollados en esta Tesis Doctoral son sencillos y reproducibles. Todas estas razones facilitan su potencial desarrollo e implantación en laboratorios de rutina.

3. Sólidos monolíticos basados en el uso de nanopartículas de carbono como monómeros principales

El ***Bloque III*** se ha centrado en el desarrollo de sólidos monolíticos compuestos principalmente por nanopartículas. A diferencia de los sólidos sintetizados en los Bloques anteriores, en este caso la estructura tridimensional porosa la forman las propias nanopartículas de carbono, las cuales pueden estar entrecruzadas con un polímero (***Capítulo 8***) o directamente unidas entre sí (***Capítulos 9 y 10***). Este tipo de polímeros nanoestructurados presentan las propiedades especiales de la nanomateria, y son de gran interés en áreas como medicina, energía y medio ambiente. Sin embargo, a pesar de su gran potencial, hasta ahora han sido poco usados dentro del ámbito analítico.

Con el objetivo de ampliar el estudio y desarrollo de sólidos monolíticos basados en nanopartículas de carbono, en el ***Bloque III*** se describen y evalúan diferentes rutas de síntesis, así como tipos de nanopartículas. Asimismo, de los resultados obtenidos han derivado las siguientes conclusiones:

- Se ha diseñado una ruta de síntesis de monolitos de nanocuernos de carbono basada en la formación de una emulsión W/O y su posterior fotopolimerización en presencia de un iniciador radicalario (***Capítulo 8***). Gracias al carácter hidrofóbico de los nanocuernos, estos permanecen en la fase orgánica o exterior, mientras que el agua se introduce en las micelas formadas por el tensioactivo. Una vez formado el sólido, se invierten las micelas y se expulsa el agua de la estructura, generando las cavidades o poros. Dicho sólido monolítico se preparó en una punta de pipeta, y para evitar su desprendimiento durante el paso de los disolventes se inmovilizó a las paredes internas de la punta siguiendo la estrategia comentada en el ***Bloque II***. Aunque la rigidez y la porosidad del sólido puede optimizarse con la modificación de las cantidades de los diferentes componentes de la emulsión, para mejorar la robustez y estabilidad de la unidad de microextracción, se añadió también un entrecruzador (EDMA) a la fase orgánica.

- Se ha conseguido preparar sólidos monolíticos únicamente formados por nanopartículas de carbono, sin la presencia de iniciadores o entrecruzadores, mediante la preparación de dispersiones de dichas nanopartículas por encima de una concentración crítica (***Capítulo 9***). De esta forma, la distancia entre ellas es tan reducida que al eliminar el disolvente se mantienen unidas por interacciones π–π y de Van der Waals. Como peculiaridad, cabe resaltar la alta estabilidad y robustez de los monolitos basados en nanocuernos de carbono. Esto se consiguió gracias a la formación de enlaces C–C entre los anillos aromáticos de las dalias, que reforzaron la estructura monolítica.

- Por otro lado, en el ***Capítulo 10*** se preparó una estructura monolítica de *quantum-dots* de grafeno a partir de una mezcla de los precursores orgánicos de las nanopartículas. Mediante energía de microondas se generaron las nanopartículas y, debido a enlaces de hidrógeno y otras fuerzas no covalentes como interacciones de carácter π–π entre ellas, se formó un gel que al secarse en una estufa dio lugar a un sólido poroso nanoestructurado. De hecho, este monolito presenta las bandas de fluorescencia típicas de los *quantum dots* así como una marcada capacidad sorbente, incluso en medios no polares. La principal ventaja de esta aproximación es la posibilidad de obtener un sólido monolítico durante la propia síntesis de las nanopartículas, evitando etapas adicionales y garantizando la reproducibilidad entre sólidos.

La redacción de esta Memoria, junto con la experiencia adquirida durante el desarrollo de esta Tesis Doctoral, ha proporcionado una visión general y objetiva del trabajo presentado. De esta manera, a continuación, se presenta una autoevaluación de los resultados obtenidos con el fin de valorar, las principales aportaciones del trabajo a la comunidad científica, y por otro analizar las limitaciones con vistas a planificar futuras investigaciones.

Aunque se han cumplido todos los objetivos propuestos en esta Tesis Doctoral, sintetizando y caracterizando una amplia variedad de sólidos monolíticos con nanopartículas de carbono, así como desarrollando y aplicando las unidades de microextracción a muestras de carácter medioambiental, agroalientario y/o biológico, también pueden identificarse algunos aspectos a mejorar, como son:

- Los bajos factores de preconcentración obtenidos (~ 10–20) en la mayoría de las metodologías desarrolladas por la inviabilidad de usar mayores volúmenes de muestra. Esto no ha sido posible debido a los bajos caudales usados y, por consiguiente, a los largos tiempos de extracción requeridos.

- La falta de automatización de los procesos analíticos y su acoplamiento en línea con técnicas instrumentales para obtener metodologías altamente eficientes con un mínimo consumo de disolventes, reactivos, muestra, y tiempo, reduciendo así el coste de cada análisis.

- La poca o escasa selectividad de los monolitos desarrollados, ya que la retención de los analitos se basa principalmente en interacciones tipo π–π, hidrofóbicas, puentes de hidrógeno, y de Van der Waals. En este sentido, sería deseable la introducción de elementos de (bio)reconocimiento específico para aumentar la selectividad, tales como anticuerpos o aptámeros. No obstante, en todos los casos se hicieron estudios de recuperación en las matrices seleccionadas, encontrándose valores aceptables y, por tanto, demostrándose el gran poder de eliminación de interferencias que presentan los procedimientos analíticos miniaturizados propuestos.

- La limitada variedad de nanopartículas usadas a lo largo de la Tesis Doctoral. Sería positivo desarrollar una línea complementaria de trabajo basada en el uso de fases monolíticas modificadas con nanopartículas metálicas, o incluso extender los conocimientos y habilidades adquiridas a la preparación de estructuras monolíticas formadas por una mezcla de varios tipos de nanopartículas, consiguiendo así una sinergia de sus propiedades y características. Por ejemplo, así se podrían sintetizar monolitos cuya estructura porosa principal la formen nanocuernos de carbono que a su vez estén recubiertos con nanopartículas metálicas, dándole carácter magnético al sólido final.

The research performed in this Doctoral Thesis is centered on analytical sample preparation. In particular, these contributions are related with the design and use of new monolithic sorbents in separation techniques. In this context, this Report has been divided into three different ***Blocks*** according to the microextraction format and/or the use of carbon nanoparticles as main components in the monolithic structure.

Hereunder, the main conclusions of each of the ***Blocks*** addressed in this Doctoral Thesis are presented and described.

1. Hybrid solids based on the combination of monolithic polymers and carbon nanoparticles prepared in capillaries

Two types of monolithic solids have been synthesized in this ***Block*** of the Doctoral Thesis; organic polymers (usually methacrylates) and inorganic polymers (silica).

Free-radical polymerization has been used to obtain methacrylate monolithic columns. In this case, the polymerization mixture usually consists of monomers, crosslinker, porogenic solvents and initiator. Thus, the solid is formed by the successive addition of monomers and crosslinker thanks to the high reactivity of free radicals, which are generated from the initiator by means of different mechanisms (thermal, chemical or UV radiation). The modification of the conditions of the polymerization mixture lead to changes in the polymerization process, and as a result it produces an effect on the characteristics of the final monolith.

For this reason, the synthesis of monolithic capillaries, by thermal radical polymerization (***Chapters 1 and 3***) and under UV radiation (***Chapter 2***), has been evaluated obtaining the following conclusions:

- The polymerization rate, that is, the complete formation of the monolithic structure using thermal energy is slower (~ 24 h) than using UV energy (5–30 min). This fact can affect the homogeneity and morphology of the monolithic hybrid solids since long polymerization times can cause sedimentation problems of the nanoparticles on one side of the capillary column. In contrast, fast polymerizations favour the stability of the nanoparticle dispersions and avoid the formation of large aggregates in the reaction mixtures.

- In order to carry out a UV-mediated initiation, it is necessary to prepare the monolithic solids inside the fused silica capillaries which are transparent to UV light. Generally, this kind of capillaries has low internal diameters which can result in high pressure during the pass of solvents through them in the separation systems.

On the other hand, the most commonly used synthesis method for inorganic monoliths is the sol-gel, in which a continuous network inside the capillary is created by gelation of a solution. This process is based on the hydrolysis and polycondensation of the monomers (alkoxysilanes) under controlled conditions of temperature and pH.

The main differences and characteristics observed in this type of monolithic columns (***Chapter 4***), with respect to the previous ones, are the following:

- This kind of monolithic solid presented a high polar and hydrophilic character, which was modified thanks to the inclusion of carbon nanoparticles in the polymeric network.

- Despite having a high physical resistance and chemical stability against various solvents, secondary interactions with the analytes are produced at pH values above 4 because of the charges of the silanol groups present on the surface of the polymer. Therefore, it results in higher irreproducibility between the extractions.

- The incorporation of carbon nanotubes (both single- and multi-walled) did not produce morphological changes in the polymer structure. However, the unique spherical structures of the dahlia aggregates of carbon nanohorns were observed in the monoliths prepared with this nanomaterial.

Finally, another aspect which would be highlighted is the inclusion strategy of the carbon nanoparticles in the monolithic polymers used in the articles of this ***Block*** of the Doctoral Thesis; chemical (***Chapter 1***) or photochemical (***Chapter 2***) anchorage to the surface of the solid and entrapment of the nanoparticles in the solid (***Chapters 3 and 4***). Therefore, the advantages and disadvantages of each of these approaches are discussed below:

- The immobilization of the carbon nanoparticles on the surface of the organic monolithic solid by chemical reactions increased the specific surface area of the material and, therefore, its sorbent capacity was improved (***Chapter 1***). In this case, all of them were available to interact with the analytes. However, the pass of nanoparticle dispersions at high concentration in the anchoring stage, led to blockage and obstruction of the pores of the solid because of the reactivity and tendency to aggregation of the nanoparticles, which especially occurred at the beginning of the capillary column.
- A photografting approach, which consisted of two stages; (1) activation of the monolithic surface and (2) immobilization of the nanostructures, is described in ***Chapter 2.*** Both stages were carried out under UV radiation. The main difference with chemical immobilization is that once the monolithic capillary is filled with the dispersion of the nanoparticles, it is irradiated with UV light achieving the anchorage of the nanoparticles to the previously activated polymer surface. This approach helps to avoid problems of large aggregate formation in certain areas of the capillary.
- As an alternative to the immobilization or anchoring of the nanoparticles on the monolithic surface, they were directly added to the polymerization mixture. This strategy was carried out both in methacrylate (***Chapter 3***) and silica (***Chapter 4***) polymers, obtaining hybrid materials with enhanced sorbent

properties compared to monoliths prepared under the same conditions, but without nanoparticles. Here, the limiting factor is, again, the amount of nanoparticles that it is possible to add without negatively interfering in the formation of the final monolith. However, a particular behavior was observed when carbon nanohorns were incorporated into the polymerization mixture. In this regard, the high reactivity of these conical nanoparticles produced an increase in the propagation rate of the polymer chains, obtaining globules of smaller size and, therefore, a greater total specific surface area.

2. Hybrid solids based on the combination of monolithic polymers and carbon nanoparticles prepared in other microextraction formats

As regards the preparation of monolithic solids modified with nanoparticles in other microextraction formats such as spin-columns (***Chapter 5***), pipette-tips (***Chapter 6***), or stir-bars (***Chapter 7***), it is necessary to take into account that, in contrast to the monolithic capillaries, they are obtained by bulk polymerization in molds of larger sizes and internal diameters. Generally, the monolithic solids prepared in these conditions are more affected by the shrinkage effect reducing their size in a higher percentage than those synthesized in capillary columns. Although the chemical anchoring of the polymer to the extraction device prevents the shrinkage toward the centre of the solid, when the solids are immobilized to the inner wall of the device, they tend to break in the central part. Consequently, it is important to reach a compromise between the permeability and rigidity of the monolithic material in order to obtain porous solids that allow the pass of the solvents, without producing a high pressure, being sufficiently stable and robust to be reused as well.

This ***Block*** has described the synthesis of several hybrid solids in this kind of microextraction formats. The participation of carbon nanoparticles in the structure and stability of the final material has also been evaluated:

- When carbon nanohorns are incorporated into methacrylate polymers, an increase in the polymerization rate is achieved and, therefore, a more mechanically stable hybrid solid with enhanced sorbent capacity is obtained in comparison with its homologue without nanoparticles (***Chapters 5 and 7***). In addition, the size of the globules of the final polymer, i.e. its porosity, depends on the amount of carbon nanohorns in the polymerization mixture. Thus, the structure and characteristics of the extraction units can be easily controlled and modified thanks to the inherent properties of these conical nanoparticles.
- Another type of organic polymer/carbon nanostructure combination; a methacrylate monolith with multi-walled carbon nanotubes (***Chapter 6***), has also been evaluated. Here, the nanotubes were embedded in the polymer network being the nanoparticle surface practically surrounded by polymer sheets. Although they were not completely available on the surface of the monolith, the inclusion of nanotubes improved the extraction efficiency due to the additional π–π interactions with the analytes.

Finally, it would be noted that the monolithic microextraction units, which are described in this Doctoral Thesis, show high efficiencies for the extraction of the target analytes, as well as high stability in a great variety of solvents and pHs. Another feature to be highlighted of using these extraction formats is the possibility of processing several samples simultaneously. In addition, the proposed synthesis procedures are simple and reproducible. All these reasons facilitate its potential development and implementation in routine laboratories.

3. Monolithic solids based on the use of carbon nanoparticles as main monomers

Block III is focused on the development of monolithic solids formed mainly by carbon nanoparticles.

Contrary to the other solids synthesized in the previous ***Blocks***, in this case the porous three-dimensional structure is formed by the carbon nanoparticles themselves, which can be crosslinked with a polymer (***Chapter 8***) or directly self-assembled (***Chapters 9 and 10***). This type of nanostructured polymer has the unique properties of nanomaterials, and they have a great interest in areas such as medicine, energy and environment. However, despite their great potential, until now they have been less exploited in the Analytical field.

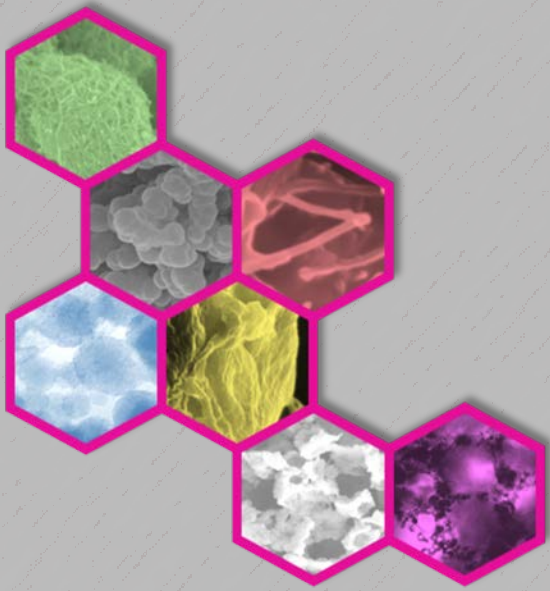
To expand the studies related to the development of monolithic solids based on carbon nanoparticles, various nanoparticles as well as different synthetic routes were described and evaluated in the ***Block III***. Likewise, the following conclusions have been derived from the results obtained:

- A synthetic route to obtain carbon nanohorn-monoliths based on the formation of a W/O emulsion and its subsequent photopolymerization in the presence of a radical initiator (***Chapter 8***) has been designed. Carbon nanohorns were trapped in the organic phase or external phase due to their hydrophobicity, while the water was contained in the droplets formed by the surfactant. Once the solid is formed, the surfactant and the aqueous phase were removed with methanol giving rise to the porosity of the resulting material. The monolithic solid was prepared in a pipette-tip and immobilized to the inner walls of the tip in order to avoid its detachment during the pass of the solvents through it. The strategy followed for this anchoring is discussed in the ***Block II***. Although the rigidity and porosity of the final solid can be optimized by the modification of the percentages of the emulsion compounds, a crosslinker (EDMA) was also added to the organic phase in order to improve the robustness and stability of the microextraction unit.
- Additionally, monolithic solids composed only by single walled carbon nanohorns, without the assistance of extra components such as crosslinkers or initiators, were obtained preparing dispersions of carbon nanohorns above a

- The low preconcentration factors obtained (~ 10-20) in most of the developed methodologies were the result of the low sample volumes used of using larger sample volumes. This was primarily due to the low flow rates used and, consequently, the long extraction times required.
- The lack of automatization of the analytical processes and their coupling in line with instrumental techniques to obtain highly efficient methodologies with a minimum consumption of solvents, reagents, sample and time, which would reduce the cost of each analysis.
- The low selectivity of the developed monoliths, since the retention of the analytes is based mainly on hydrogen bonds, hydrophobic, Van der Waals and π–π interactions. In this regard, the introduction of specific (bio)recognition elements to increase the selectivity, including antibodies and aptamers, would be desirable. Nevertheless, recovery studies in the selected matrices were performed yielding acceptable values in all cases and, therefore, demonstrating the efficiency of elimination of interferences of the proposed miniaturized analytical methods.
- The limited type of nanoparticles used throughout the Doctoral Thesis. It would be positive to develop a complementary research based on the use of monolithic phases modified with metallic nanoparticles, or even to extend the knowledge and skills acquired to the preparation of monolithic structures formed by a mixture of several types of nanoparticles, combining in a synergic way of their properties and characteristics. For example, synthesizing monoliths whose main porous structure is formed by carbon nanotubes which are functionalized with magnetic nanoparticles, giving magnetic properties to the final solid.

Anexos

Annexes



Anexo A

Artículos Científicos Derivados de la Tesis Doctoral

Scientific Articles Derived from the Doctoral Thesis

1. Improved microextraction of selected triazines using polymer monoliths modified with carboxylated multi-walled carbon nanotubes

Fresco-Cala, B.; Cárdenas, S.; Valcárcel, M.

Microchimica Acta, 183 (2016) 465-474

2. Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters

Fresco-Cala, B.; Cárdenas, S.; Valcárcel, M.

Journal of Chromatography A, 1468 (2016) 55-63

3. Preparation of porous methacrylate monoliths with oxidized single-walled carbon nanohorns for the extraction of nonsteroidal anti-inflammatory drugs from urine samples

Fresco-Cala, B.; Cárdenas, S.; Herrero-Martínez, J.M.

Microchimica Acta, 184 (2017) 1863-1871

4. Nanostructured hybrid monolith with integrated stirring for the extraction of UV-filters from water and urine samples

Fresco-Cala, B.; Cárdenas, S.

Talanta, 182 (2018) 391-395

5. Carbon nanotube-modified monolithic polymethacrylate pipette tips for (micro) solid-phase extraction of antidepressants from urine samples

Fresco-Cala, B.; Óscar Mompó-Roselló; Ernesto F. Simó-Alfonso; Cárdenas, S.; Herrero-Martínez, J.M.

Microchimica Acta, 185 (2018) 127

6. Carbon nanostructures incorporated on methacrylate monoliths for separation of small molecules by nano-liquid chromatography

Fresco-Cala, B.; Carrasco-Correa, EJ.; Cárdenas, S.; Herrero-Martínez, J.M.

Microchemical Journal, 139 (2018) 222-229

7. Monolithic solid based on single-walled carbon nanohorns: preparation, characterization and practical evaluation as sorbent

Fresco-Cala, B.; López-Lorente, A.I.; Cárdenas, S.

Nanomaterials, 8 (2018) 370

8. Potential of nanoparticle-hybrid monoliths as sorbents in microextraction techniques

Fresco-Cala, B.; Cárdenas, S.

Analytica Chimica Acta. DOI: 10.1016/j.aca.2018.05.069

9. Carbon nanohorns as porous monolithic extraction phases prepared in polypropylene tips by emulsion photopolymerization

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10. One-pot synthesis of graphene quantum dot and simultaneous nanostructured self-assembly via a novel microwave-assisted method: Impact on triazines removal and efficiency monitoring

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11. Effect of carbon nanohorns in the radical polymerization of methacrylate monolithic capillary columns and their application as extractant phases

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En revisión en Talanta

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ORIGINAL PAPER

Improved microextraction of selected triazines using polymer monoliths modified with carboxylated multi-walled carbon nanotubes

Beatriz Fresco-Cala [1] · **Soledad Cárdenas** [1] · **Miguel Valcárcel** [1]

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Abstract This article reports on the enhancement of the capacity of an acrylate-based monolithic solid sorbent by anchoring carboxylated multi-walled carbon nanotubes (c-MWCNTs) in its pores and on its surface. Monolithic poly(butyl acrylate-*co*-ethyleneglycol dimethacrylate) [poly(BA-*co*-EGDMA)] was synthetized inside a fused silica capillary via free-radical polymerization, and an ethanolic dispersion of c-MWCNTs was passed through the capillary. The resulting poly(BA-*co*-EGDMA-c-MWCNTs) monolith was characterized by scanning electron microscopy to confirm the presence of the c-MWCNTs. The effect of using three different kinds of carbon nanoparticles and the microextraction step were studied using triazine herbicides as model compunds. The use of c-MWCNTs resulted in best performance in terms of extraction enhancement (compared to carboxylated single-walled carbon nanotubes and oxidized single-walled carbon nanohorns). The use of these carbon nanoparticles improved the extraction of triazines in any case when compared to using a bare poly(BA-*co*-EGDMA) monolith. The triazines were then quantified by gas chromatography with mass spectrometric detection. Detection limits ranged from 0.03 to 0.1 $\mu g \cdot L^{-1}$ (except for simazine; 0.6 $\mu g \cdot L^{-1}$), and the precision (relative standard deviation) varied between 3.0 and 11.4 %. The reproducibility between units is <14.3 % (expressed as relative standard deviation) which demonstrates the robustness of the method. The method was applied to analyze an unknown sample of orange juice and gave a value of 0.18 $\mu g \cdot L^{-1}$ for prometryn. Finally, the analysis of spiked samples of water and orange juices yielded recoveries ranging from 81 to 113 % and 75 to 125 %, respectively.

Keywords Monolithic solid · Carboxylated multi-walled carbon nanotubes · (Micro)solid phase extraction · Herbicides · Gas chromatography · Triazines · Orange juice

Introduction

Sample preparation has been the focus of intense research in order to improve the isolation and preconcentration steps of the analytical procedures. Current trends in this context involve the simplification and miniaturization of separation techniques in both solid and liquid phase formats. The success of these tendencies depends on the efficiency of the extracting medium [1]. Nanostructured materials can be identified as a turning point on the development of new miniaturized approaches [2], as they are more efficient than silica-based or polymeric sorbents due to their high aspect ratio and chemical nature.

Monoliths are a continuous piece of a highly porous material, allowing solvents to flow through their large pores (>50 nm macropores, 2–50 nm mesopores). The monoliths can be classified in three types: polymer monoliths [3], silica-based monoliths [4] and organic-silica hybrid monoliths [5]. In the chromatographic and electrophoretic context, these materials have some advantages over particle packed columns such as: easy synthesis, mechanical stability and direct linkage of the solid with the inner walls of the support. Besides, they

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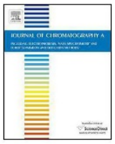

Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters

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ABSTRACT

A novel hybrid micro and meso porous silica monolith with embedded carbon nanoparticles (Si-CNPs monolith) was prepared inside a fused silica capillary (3 cm in length) and used as a sorbent for solid-phase microextraction. The hybrid monolithic capillary was synthetized by hydrolysis and polycondensation of a mixture of tetraethoxysilane (TEOS), ethanol, and three different carbon nanoparticles such as carboxylated single-walled carbon nanotubes (c-SWCNTs), carboxylated multi-walled carbon nanotubes (c-MWCNTs), and oxidized single-walled carbon nanohorns (o-SWNHs) via a two-step catalytic sol-gel process. Compared with silica monolith without carbon nanoparticles, the developed monolithic capillary column exhibited a higher extraction efficiency towards the analytes which can be ascribed to the presence of the carbon nanoparticles. In this regard, the best performance was achieved for silica monolith with embedded c-MWCNTs. The resulted monolithic capillaries were also characterized by scanning electron microscopy (SEM), elemental analysis and nitrogen intrusion porosimetry. Variables affecting to the preparation of the sorbent phase including three different carbon nanoparticles and extraction parameters were studied in depth using polycyclic aromatic hydrocarbons (PAHs) as target analytes. Gas chromatography-mass spectrometry was selected as instrumental technique. Detection limits range from 0.1 to 0.3 $\mu g\,L^{-1}$, and the inter-extraction units precision (expressed as relative standard deviation) is between 5.9 and 14.4%.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of non-polar organic compounds consisting of two or more benzene rings which are byproducts of petroleum processing or combustion of hydrocarbons. PAHs are of environmental concern due to their significant toxicity and potential carcinogenic properties at relatively low levels [1]. In this regard, the concentration of benzo[a]pyrene has been used as indicator of total contamination by PAHs being 0.2 $\mu g\,L^{-1}$ the maximum level established by Agency for toxic substances & Disease Registry (ATSDR) in waters [2].

Analytical methods including gas chromatography (GC) [3], gas chromatography coupled with mass spectrometry (GC–MS) [4], high-performance liquid chromatography with fluorescence detection (HPLC-FLD) [5], thin-layer chromatography (TLC) [6], and spectrofluorimetry [7] have traditionally been used to determine PAHs. However, preconcentration and clean-up steps are needed to achieve the required sensitivity and selectivity. In this context, new microextraction techniques have been recently developed based on the simplification and the miniaturization of classical separation techniques in both solid and liquid phase formats. The incorporation of nanostructured materials as well as hybrid sorbents is behind the consolidation of these new approaches particularly due to their high superficial area and variety of interactions [8].

Monolithic solids have gained prominence as new separation material because of their unique properties including fast dynamic transport, simplicity of their preparation, frit-free construction, good loading capacity and low backpressure. To date, both organic polymer and silica-based monoliths have been used in several formats in the extraction context such as micropipette-tips, spin columns, microfluidics chips, and capillary columns [9,10]. Carbon nanoparticles, especially carbon nanotubes, have received special research attention since their discovery thanks to their unique and outstanding properties. Their excellent properties in terms

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native fluorescence of PAHs. Nevertheless, it should be highlighted that present hybrid sorbent has been optimized for achieve the concentration level established by ATSDR, and therefore, a better sensitivity is not required in water samples.

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ORIGINAL PAPER

Preparation of porous methacrylate monoliths with oxidized single-walled carbon nanohorns for the extraction of nonsteroidal anti-inflammatory drugs from urine samples

Beatriz Fresco-Cala[1] · **Soledad Cárdenas**[1] · **José Manuel Herrero-Martínez**[2]

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Abstract A copolymer was prepared from glycidyl methacrylate, ethylene glycol dimethacrylate and oxidized single-walled carbon nanohorns via photo-polymerization and used in spin columns for the extraction of nonsteroidal anti-inflammatory drugs (NSAIDs) from human urine samples. All microextraction procedures (loading, washing and elution) can be performed by centrifugation. The hybrid monolithic polymers were characterized by scanning electron microscopy and nitrogen intrusion porosimetry. Following elution with methanol, the NSAIDs (naproxen, fenbufen, flurbiprofen, and ibuprofen) were quantified by reversed-phase HPLC with UV detection. The detection limits varied between 0.1 and 10 $\mu g \cdot L^{-1}$, and the precision (relative standard deviation) ranged from 3.5 to 11.8%. Relative recoveries between 81 and 106% were found when analyzing spiked urine samples.

Keywords Centrifugal spin columns · Photo-polymerization · (micro)solid-phase extraction · High-performance liquid chromatography · Biological samples

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Introduction

Since the first synthesis of monolithic polymers at the end of the twentieth century [1] organic monoliths derived from methacrylates, acrylamides, and styrenes have been successfully applied as stationary phases in chromatography [2], and as sorbents in sample treatment techniques [3] but to a lesser extent. Among the microextraction formats where monolithic sorbents have been used, spins columns can be cited as one of the most advantageous. Thus, the spin-column format offers a simple operation procedure, allows a high-throughput sample, requires a low eluate volume and does not involve solvent evaporation, all features in rough agreement with the principles of green analytical chemistry. Monolithic silica spin column was first introduced by Namera and Saito in 2008 [4, 5]. The monolithic solid is packed in the bottom of the column unit without using frits, and then solvents are passed through the sorbent phase by centrifugation. This approach has been used to extract target compounds from biological matrices [6, 7]. While there are already several monolithic silica spin columns used to isolate and preconcentrate target analytes, a potential growth area of interest may be the development of polymer-based monoliths. In this context, Güzel et al. have developed an erbium phosphate doped poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate (poly(GMA-*co*-EDMA)) spin column for selective enrichment of phosphopeptides [8].

To achieve more specific interaction of the analytes with the polymeric networks, nanomaterials including graphene [9], carbon nanotubes [10, 11], and carbon nanohorns [12], have been combined with monolithic material to prepare novel stationary phases or sorbents with enhanced performances.

Single-walled carbon nanohorns (SWNHs) were firstly discovered by Iijima et al. in 1999 [13]. Concretely, SWNHs are cone-shaped carbon structures with a single wall, which have natural tendency to form dahlia-shaped aggregates [14]. Their

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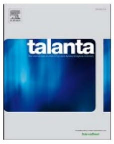

Nanostructured hybrid monolith with integrated stirring for the extraction of UV-filters from water and urine samples

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Microextraction

ABSTRACT

This article presents a monolithic extraction unit with integrated stirring using carbon nanohorns and methacrylate-based compounds as monomers. The hybrid monolithic material was prepared by thermal polymerization at 70 °C for 24 h, and was applied for the extraction of UV-filters from waters and human urine samples. To achieve the integrated stirring unit, the monolith was grown over an ironware. Variables dealing with the polymerization mixture composition and the microextraction procedure were studied in depth. The resulting hybrid monolithic polymer was also characterized by scanning electron microscopy (SEM) and nitrogen intrusion porosimetry. The target analytes were quantified by UPLC-DAD, and the limits of detection were between 1 and 10 µg/L. The precision of the method (inter extraction units) expressed as relative standard deviation ranged from 5.4% and 7.9%. Also, relative recoveries values of the analyte spiked to swimming pool water and urine samples varied in the interval 72–124 and 71–114%, respectively.

1. Introduction

Benzophenones (BPs) are a family of compounds which have the ability to absorb and dissipate ultraviolet light, and therefore, are used in the cosmetic industry as UV-filters to protect the skin against solar UV radiation [1]. Thus, these compounds may be absorbed through the skin, accumulate in the body and/or excreted in the urine [2]. In addition, studies have shown that UV-filters may be significantly accumulated in swimming pools through direct contact [3]. However, a maximum concentration has been established for each country legislation due to their dermatological implications [4]. Moreover, they have been also linked to the development of endocrine and reproductive problems. For all these reasons, it is important to develop new methods for the determination of UV-filters both in biological samples and in waters.

Microextraction techniques are processes which depend on thermodynamic and kinetics factors. Therefore, it is crucial to enhance the mass transference during the extraction in order to improve the extraction yield [5]. In such conditions, the developed new extraction modes with integrated stirring in the same device such as stir bar [6], stir cake [7], or stir membrane [8] are good choices as they simplify the extraction to a large extent. Apart from the commercial ones, in most of these cases, the preparation of the microextraction unit involves an additional step for the attachment of the material to the surface of the selected device. As a main drawback, detachment of part of the material due to the friction can occur during the stirring. This is especially relevant when a thin film of the extraction medium is covered the support. However, this problem can be solved using stir cake sorptive extraction modality which possesses an excellent longevity because the sorbent does not come in contact with the vessel wall during stirring [9].

Monolithic materials, which consist of a rigid macroporous structure, are very attractive in this context [7,10,11]. They can be easily prepared and their interconnecting pores allow the solvents flow through them, resulting in a good permeability [12]. In addition, to improve the sorbent capacity and the specific surface area of the material as well, nanomaterials such as carbon nanotubes [13,14], or carbon nanohorns [15,16], have been incorporate into the polymer networks. In a previous work, our group demonstrated the participation of oxidized single-walled carbon nanohorns (o-SWNHs) as monomer in polymerization reactions when lauroyl peroxide (LPO) is used as free-radical initiator [15]. Thus, a uniform, rigid and robust hybrid monolithic solid is obtained by the copolymerization of organic monomers and carbon nanohorns. The principal difference between carbon nanoparticles entrapped into the monolithic network and copolymerized is that in this case, the most of the nanoparticles are accessible to interact with the analytes.

In this work, we describe the synthesis of a hybrid solid with

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ORIGINAL PAPER

Carbon nanotube-modified monolithic polymethacrylate pipette tips for (micro)solid-phase extraction of antidepressants from urine samples

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Abstract

This work evaluates the potential of methacrylate monoliths with multi-walled carbon nanotubes incorporated into the polymeric network for the extraction of antidepressants from human urine. The method is based on a micropipette solid-phase extraction tip containing a hybrid monolithic material covalently attached to the polypropylene housing. A polymer layer made from poly(ethylene dimethacrylate) was bound to the inner surface of a polypropylene tip via UV grafting. The preparation of the monolith and the microextraction steps were optimized in terms of adsorption capacity. Limits of detection ranged from 9 to 15 $\mu g \cdot L^{-1}$. The average precision of the method varied between 3 and 5% (intra-tips), and from 4 to 14% (inter-tips). The accuracy of the method was evaluated through a recovery study by using spiked samples.

Keywords Carbon nanoparticles · Hybrid monolith · Microextraction · Porous polymer · Photografting process · UV attachment · Drugs · Liquid chromatography · Biological samples

Introduction

Tetracyclic antidepressants (TCAs) are commonly used for treatment of psychiatric disorders such as depression and anxiety [1–3]. To minimize the risk of overdose and adverse effects of these drugs, their measurement in biological fluids is mandatory [4]. Several methods have been reported for the extraction of TCAs from human body fluids using conventional sample pretreatment techniques including liquid-liquid extraction [5], and solid-phase extraction (SPE) [6]. However, these approaches present main shortcomings such as large volumes of solvents as well as tedious manual operating procedures. In the last years, new microextraction techniques based on the simplification and the miniaturization of classical separation techniques have been developed [7–9].

Nanostructured materials can be identified as a turning point on the development of these new techniques particularly due to their high superficial area and variety of interactions [10, 11]. In this context, carbon nanotubes have been used as SPE sorbents for the analytical extraction of drugs, pesticides or natural compounds in different media such as biological fluids, drug preparations, environment, plants or animal organs [12, 13]. From an analytical point of view, carbon nanotubes are especially suitable for analytical applications derived from their nanometric size [14]. First, they exhibit interesting chemical properties and a high specific surface when used as a sorbent. In addition, they open up new approaches, providing exceptional possibilities for further miniaturization. Therefore, in order to benefit from their sorbent capacity, carbon nanotubes have to be efficiency embedded into a material or immobilized on a surface/support, such as disk [15], porous-hollow fiber [16], and polymer monoliths [17]. Among these alternatives, the combination of monolithic solids and the specific features of carbon nanotubes is an

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Carbon nanostructures incorporated on methacrylate monoliths for separation of small molecules by nano-liquid chromatography

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ABSTRACT

Carbon nanostructures have been incorporated to polymethacrylate monoliths to develop novel stationary phases with enhanced separation performance in nano-liquid chromatography (nano-LC). Two incorporation approaches of these nanomaterials in monoliths were investigated. Single-walled carbon nanohorns (SWNHs) were first incorporated to the polymerization mixture, followed by UV initiation. The resulting stationary phases with embedded SWNHs led to an increase in the separation performance of analytes compared to the parent monolith. Alternatively, a two-step photo-grafting approach was also adopted to attach the carbon nanoparticles onto the polymer monolith surface. The effect of the amount of SWNHs and irradiation time on the performance of the resulting composites was investigated. Moreover, these novel stationary phases were characterized by scanning electron microscopy. The photografted modified with SWNHs led to an enhanced separation performance (up to 6000 plates m^{-1}) for test analytes. Besides, stationary phases containing oxidized SWNHs and multi-walled carbon nanotubes (MWCNTs) grafted on polymeric monoliths were prepared and a comparison in terms of separation performance was accomplished. The photografted columns with SWNHs also showed satisfactory reproducibility in terms of retention time and efficiency (RSD values below 8.6 and 1.4%, respectively).

1. Introduction

Polymethacrylate-based monolithic columns developed by Svec et al. [1–3] have several good features as easily adjustable polarity, fine control for pore characteristics and high stability under extreme pH conditions (pH 2–12). These stationary phases have proven ideal media for the efficient separation of biopolymers (e.g. proteins) using gradient elution in high-performance liquid chromatography (HPLC) [4,5] or simply isocratic elution in capillary electrochromatography (CEC) [6–8]. However, these macroporous polymeric monoliths suffer from low surface areas due to the absence of a mesoporous structure and in turn limited retention toward small solutes in HPLC.

In any case, the ideal flow features of these polymer-based monoliths such as their high permeability and through-pores make them suitable supports for the incorporation of nanoparticles, which can be exploited to develop stationary phases that can improve the low surface area (by increasing the surface-to-volume ratio) as well as to afford distinct selectivity toward a wide range of solutes.

In this context, the preparation of polymer-based monolithic columns modified with carbon nanostructures has gained relevance in the field of chromatographic separations. This fact is due to unique properties of carbon nanoparticles, such as their large specific surface and hydrophobic nature, which improves in both selectivity and retention of small molecules. Along this topic, Li et al. [9] have studied the incorporation of pretreated single-walled carbon nanotubes (SWCNTs) into a vinylbenzyl chloride-based monolithic column for micro-LC and CEC. The presence of these nanostructures in the polymeric matrix improved the efficiency and enhanced retention of capillary/nano-LC separations. Also, multi-walled carbon nanotubes (MWCNTs) have been embedded into polymeric matrices containing glycidyl methacrylate (GMA) [10,11] and benzyl methacrylate [12] as bulk monomers. Other types of carbon nanoparticles less common studied such as single-walled carbon nanohorns (SWNHs) have been recently described. Thus, Zhao et al. [13] have entrapped single-walled nanohorns (SWNHs) into a poly(styrene-*co*-divinylbenzene) monoliths using thermally initiated polymerization to afford capillary columns for CEC.

In all these works, the composites have been usually prepared by thermal polymerization, which involves rather long reaction times (20–24 h). This undoubtedly favours the aggregation and sedimentation phenomena of these carbon nanomaterials in the polymerization mixture, being a drawback for fully displaying their

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Monolithic Solid Based on Single-Walled Carbon Nanohorns: Preparation, Characterization, and Practical Evaluation as a Sorbent

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Abstract: A monolithic solid based solely on single walled carbon nanohorns (SWNHs) was prepared without the need of radical initiators or gelators. The procedure involves the preparation of a wet jelly-like system of pristine SWNHs followed by slow drying (48 h) at 25 °C. As a result, a robust and stable porous network was formed due to the interaction between SWNHs not only via π-π and van der Waals interactions, but also via the formation of carbon bonds similar to those observed within dahlia aggregates. Pristine SWNHs and the SWNH monolith were characterized by several techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal laser scanning microscopy, Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and nitrogen intrusion porosimetry. Taking into account the efficiency of carbon nanoparticles in sorption processes, the potential applicability of the SWNH-monolith in this research field was explored using toluene; m-, p-, and o-xylene; ethylbenzene; and styrene, as target analytes. Detection limits were 0.01 $\mu g \cdot L^{-1}$ in all cases and the inter-day precision was in the interval 7.4–15.7%. The sorbent performance of the nanostructured monolithic solid was evaluated by extracting the selected compounds from different water samples with recovery values between 81.5% and 116.4%.

Keywords: carbon monolith; carbon nanoparticles; volatile organic compounds; headspace microextraction; single-walled carbon nanohorns; gas chromatography

1. Introduction

The number of publications in the field of nanoscience and nanotechnology has greatly increased in the last years due to the exceptional physical and chemical properties of the nanomatter [1–4]. Within the sample preparation context, nanoparticles have been extensively used to improve the performance of extractant phases [5–7]. In particular, carbon nanostructures have demonstrated a unique sorbent capacity due to their extraordinary surface-volume ratio and the possibility to interact with organic molecules via non-covalent forces. However, most of them are non-porous in nature and possess a high tendency to aggregate when packed as a powder, which requires immobilization on the surface of particles or polymers which are then packed in conventional separation units [8]. These stationary phases present the main problems associated with this type of packing, including the need for frits, bubble formation, and poor mass transfer.

Monolithic solids are porous polymeric networks capable of providing fast mass transport and a high permeability [9]. Many efforts have been devoted to the development of carbon nanoparticle-modified monolithic polymers, which combine the unique and inherent advantages of both materials [10–13]. Moreover, interconnected porous structures formed in their majority or

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Review

Potential of nanoparticle-based hybrid monoliths as sorbents in microextraction techniques

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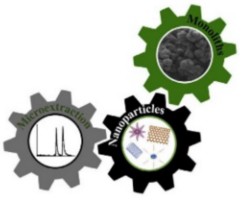
HIGHLIGHTS

- Synergic combination of monoliths and nanoparticles.
- Enhanced sorption capacity of the hydrid material.
- Preparation in different micro-extraction formats.
- Wider applicability than separate sorbent phases.

GRAPHICAL ABSTRACT



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Carbon nanoparticles
Micro-solid phase extraction

ABSTRACT

This article reviews the synthesis and uses of nanoparticle-based hybrid monoliths in microextraction techniques. Synthesizing monolithic solids containing nanoparticles allows the advantages of the two sorbent phases to be synergistically combined. The resulting hybrid material exhibits outstanding capabilities for isolating the target compounds from the samples. In addition to conventional in-cartridge solid-phase extraction, the monoliths can be easily adapted for use in most of the usual formats of micro-solid phase extraction including capillaries, pipette tips, spin columns, microfluidic chips and stirred units. The most salient uses devised so far are described and critically discussed in this article, which deals with both silica and organic monolithic solids modified with nanostructured materials including carbonaceous and metal or metal oxide nanoparticles.

Contents

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Beatriz Fresco Cala is a doctoral researcher at the Department of Analytical Chemistry, University of Córdoba (UCO, Spain) working with an official research grant (FPU). She finished her Bachelor's degree in Chemistry with the Extraordinary End-of-Studies Award in 2012. She obtained a research grant during her Master in Fine Advanced Chemistry (2013). Her research is focused in the assessment of new microextraction units based on monolithic solids modified with different carbon nanostructures and their application to different problems. She participates in the training of research beginners. She is part of the Editorial board of "Advances in Food Processing and Technology" journal.

Soledad Cárdenas Aranzana is Full Professor of Analytical Chemistry and head of the research group FQM-215 from the Department of Analytical Chemistry, University of Córdoba. Her research interests comprise microextraction techniques, including the developing of new approaches, synthesis of novel nanomaterials based on carbon, metallic nanoparticles and polymers. She has coauthored 190 scientific articles (h-index = 39) and co-supervised 16 Doctoral Thesis, 12 with european/international mention and 4 with extraordinary doctorate award. She has participated in several national and international evaluation programs. Currently, she is president of the A3 Committee (Chemistry) of the Spanish National Agency for Quality Assessment and Accreditation (ANECA).

Please cite this article in press as: B. Fresco-Cala, S. Cárdenas, Potential of nanoparticle-based hybrid monoliths as sorbents in microextraction techniques, Analytica Chimica Acta (2018), https://doi.org/10.1016/j.aca.2018.05.069

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Anexo B

Publicaciones Científicas Relacionadas con la Tesis Doctoral

Scientific Articles Related to the Doctoral Thesis

Artículos científicos:

1. Single-walled carbon nanohorns immobilized on a microporous hollow polypropylene fiber as a sorbent for the extraction of volatile organic compounds from water samples

Fresco-Cala, B.; Jiménez-Soto, J.M.; Cárdenas, S.; Valcárcel, M.

Microchimica Acta, 181 (2014) 1117-1124.

2. Carbon nanohorns suprastructures coated over paper, a new and simple sorptive phase

Ríos-Gómez, J.; Fresco-Cala, B.; García-Valverde, M.T.; Lucena, R.; Cárdenas, S.

Molecules (Special Issue Solid Phase Extraction: State of the Art and Future Perspectives), 23 (2018) 1252.

Capítulos de libro:

1. Recent nanomaterials-based separation processes

Fresco-Cala, B.; López-Lorente, A.I.; Soriano, M.L.; Lucena, R.; Cárdenas, S.

Chapter 9 in the Handbook of smart materials in Analytical Chemistry, Volume I; new materials for sample preparation and analysis that will be published by Willey in 2018.

Microchim Acta (2014) 181:1117–1124
DOI 10.1007/s00604-014-1211-z

ORIGINAL PAPER

Single-walled carbon nanohorns immobilized on a microporous hollow polypropylene fiber as a sorbent for the extraction of volatile organic compounds from water samples

Beatriz Fresco-Cala · Juan Manuel Jimenez-Soto ·
Soledad Cardenas · Miguel Valcarcel

Received: 30 October 2013 / Accepted: 14 February 2014 / Published online: 12 March 2014

Abstract We have evaluated the behavior of single-walled carbon nanohorns as a sorbent for headspace and direct immersion (micro)solid phase extraction using volatile organic compounds (VOCs) as model analytes. The conical carbon nanohorns were first oxidized in order to increase their solubility in water and organic solvents. A microporous hollow polypropylene fiber served as a mechanical support that provides a high surface area for nanoparticle retention. The extraction unit was directly placed in the liquid sample or the headspace of an aqueous standard or a water sample to extract and preconcentrate the VOCs. The variables affecting extraction have been optimized. The VOCs were then identified and quantified by GC/MS. We conclude that direct immersion of the fiber is the most adequate method for the extraction of VOCs from both liquid samples and headspace. Detection limits range from 3.5 to 4.3 ng L^{-1} (excepted for toluene with 25 ng L^{-1}), and the precision (expressed as relative standard deviation) is between 3.9 and 9.6 %. The method was applied to the determination of toluene, ethylbenzene, various xylene isomers and styrene in bottled, river and tap waters, and the respective average recoveries of spiked samples are 95.6, 98.2 and 86.0 %.

Electronic supplementary material The online version of this article (doi:10.1007/s00604-014-1211-z) contains supplementary material, which is available to authorized users.

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Keywords Oxidized single-walled carbon nanohorns · (Micro)solid phase extraction · Waters · Volatiles compounds · Nanotechnology

Introduction

Volatile pollutants are an environmental problem because they are widely distributed on account of their easy transport through the media. The origin of these compounds can be either natural or anthropogenic. Contamination of surface and ground waters with volatile aromatic compounds is one of the main environmental problems today. Benzene, toluene, ethylbenzene, xylene isomers and styrene (BTEX–S) constitute the most representative family of the volatile aromatic compounds. They are routinely used in the industry as raw materials and as solvents and they can be present in many oil derivatives. These types of aromatic compounds are widespread pollutants and they are ubiquitous in the different environmental compartments. BTEX–S are highly toxic and they have low biodegradability. Indeed, they can easily penetrate in humans through ingestion, inhalation or absorption, producing different effects for health and comprising a high toxicity [1]. The effects negatives depend on the nature of each compound and the degree and period of exposure.

The analytical methods designed for the analysis of environmental samples for the determination of BTEX–S must reach the legal limits fixed for these compounds. Taking into account the low values established, the direct analysis of the samples is unfeasible, being necessary the inclusion of a preconcentration step [2, 3].

Solid phase microextraction (SPME) was first proposed in 1990 by Arthur and Pawliszyn [4]. It can be considered as a miniaturized sample pretreatment technique sensitive than the chemical elution as no analyte dilution occurs after preconcentration on the solid support.

Acknowledgements Financial support from the Spanish DGICyT (Grant CTQ2011-23790) is acknowledged.

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Article

Carbon Nanohorn Suprastructures on a Paper Support as a Sorptive Phase

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Abstract: This article describes a method for the modification of paper with single-wall carbon nanohorns (SWCNHs) to form stable suprastructures. The SWCNHs form stable dahlia-like aggregates in solution that are then self-assembled into superior structures if the solvent is evaporated. Dipping paper sections into a dispersion of SWCNHs leads to the formation of a thin film that can be used for microextraction purposes. The coated paper can be easily handled with a simple pipette tip, paving the way for disposable extraction units. As a proof of concept, the extraction of antidepressants from urine and their determination by direct infusion mass spectrometry is studied. Limits of detection (LODs) were 10 ng/L for desipramine, amitriptyline, and mianserin, while the precision, expressed as a relative standard deviation, was 7.2%, 7.3%, and 9.8%, respectively.

Keywords: carbon nanohorns; sorptive phase; paper; microextraction; antidepressants

1. Introduction

Solid-phase microextraction (SPME) is a consolidated sample treatment technique that combines isolation, preconcentration, and sample introduction into one step [1]. This miniaturized technique, which can easily be automated, is based on the distribution of the analytes between the sample and the fiber coating. In this context, the reversible chemical interactions between the analyte and the sorptive phase are of paramount importance to define the efficiency and selectivity of the microextraction. SPME is in a continuous development following several evident tendencies like the development of new coatings [2,3] or the direct coupling with instrumental techniques like mass-spectrometry (MS) [4,5]. All these trends make SPME the predominant technology for microextraction.

The adaptation of the SPME principles to a specific field such as environmental analysis drove the development of thin film microextraction (TFME) [6]. Although both techniques share the same foundations, they differ in their application. TFME uses a thin sheet of a polymeric phase as a sorptive phase that may adopt several shapes [7] like a flat membrane [8] or a coated blade [9]. These formats present a higher extraction capacity compared to traditional fibers due to an increased surface to volume ratio, which has positive connotations for the thermodynamic and kinetics. Also, TFME can be automated to allow the simultaneous extraction of several samples, thus increasing the sample throughput [10]. In the typical procedure, the thin film is immersed in the sample, which is stirred to favor the analyte transference to the sorptive phase [11]. However, the thin film can also be stirred into the sample. The use of planar sorptive phases integrated into stirring units has allowed for

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Sample Availability: Samples of the membranes are available from the authors.

Recent nanomaterials-based separation processes

Beatriz Fresco-Cala, Ángela I. López-Lorente, M. Laura Soriano, Rafael Lucena, Soledad Cárdenas.

Departamento de Química Analítica, Instituto Universitario de Investigación en Química Fina y Nanoquímica IUIQFN, Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie Anexo, E-14071 Córdoba, España.

Abstract

Analytical separation processes aim to isolate/separate the target analytes from a complex environment. Sample preparation is focused on the selective isolation of the targets from the sample while chromatography/electrophoresis seeks the individual separation of the compounds from a mixture for their subsequent instrumental determination. Nanomaterials show a great potential in this field thanks to their outstanding properties. Their high surface to volume ratio makes them exceptional sorption materials and the wide range of available nanomaterials provides the analyst with a set of interaction chemistries that can be selected to solve a given analytical problem. This versatility can be even improved by the chemical modification of the nanomaterials surfaces with special functional groups or by the synthesis of hybrid materials and composites. The most salient contributions in the microextraction and chromatographic-electrophoretic realms will be discussed in this chapter. Taking into account the large number of references on this topic, it has been mainly limited to the last five years.

University of Valencia - Department of Analytical Chemistry

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Prof. Cárdenas has been invited to participate in the writing of a chapter included in the Handbook of smart materials in Analytical Chemistry, Volume 1: New materials for sample preparation and analysis that will be published by Willey in 2018.

1 am pleased to inform you about the acceptance of the book chapter entitled: **"Chapter 9. Recent nanomaterials-based separation processes"** by Beatriz Fresco-Cala, Ángela l. López-Lorente, M. Laura Soriano, Rafael Lucena, and Soledad Cárdenas.

Yours sincerely,

Burjassot, March 12th, 2018

Francesc A. Esteve-Turrillas, Ph.D.

Handbook co-editor

Anexo C

Presentación de Comunicaciones a Congresos

Communications to Conferences

Comunicaciones a congresos internacionales:

1. Oral

Macroscopic three dimensional carbon nanohorns monolith for (micro)solid-phase extraction of volatile organic compounds.

Fresco-Cala, B.; Cárdenas, S.

45th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2017). Praga, República Checa. Junio-2017.

2. Oral

Potential of the synergic combination of polymeric phases and nanoparticles in the microextraction context.

Fresco-Cala, B.; Reyes-Gallardo, E.; Cárdenas, S.

19th International Symposium on Advances in Extraction Technologies (Extech). Santiago de Compostela, España. Junio-2017.

3. Oral

Paper-based sorptive phases.

Fresco-Cala, B.; Ríos-Gómez, J.; Lucena, R.

19th International Symposium on Advances in Extraction Technologies (Extech). Santiago de Compostela, España. Junio-2017.

4. Póster

Improved microextraction of selected triazines from orange juices using polymer monoliths modified with carboxylated multi-walled carbon nanotubes.

Fresco-Cala, B.; Cárdenas, S.; Valcárcel, M.

XVI Latin-American Congress on Chromatography and 9th National Meeting on Chromatography. Lisboa, Portugal. Enero-2016.

5. Póster

Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters.

Fresco-Cala, B.; Cárdenas, S.

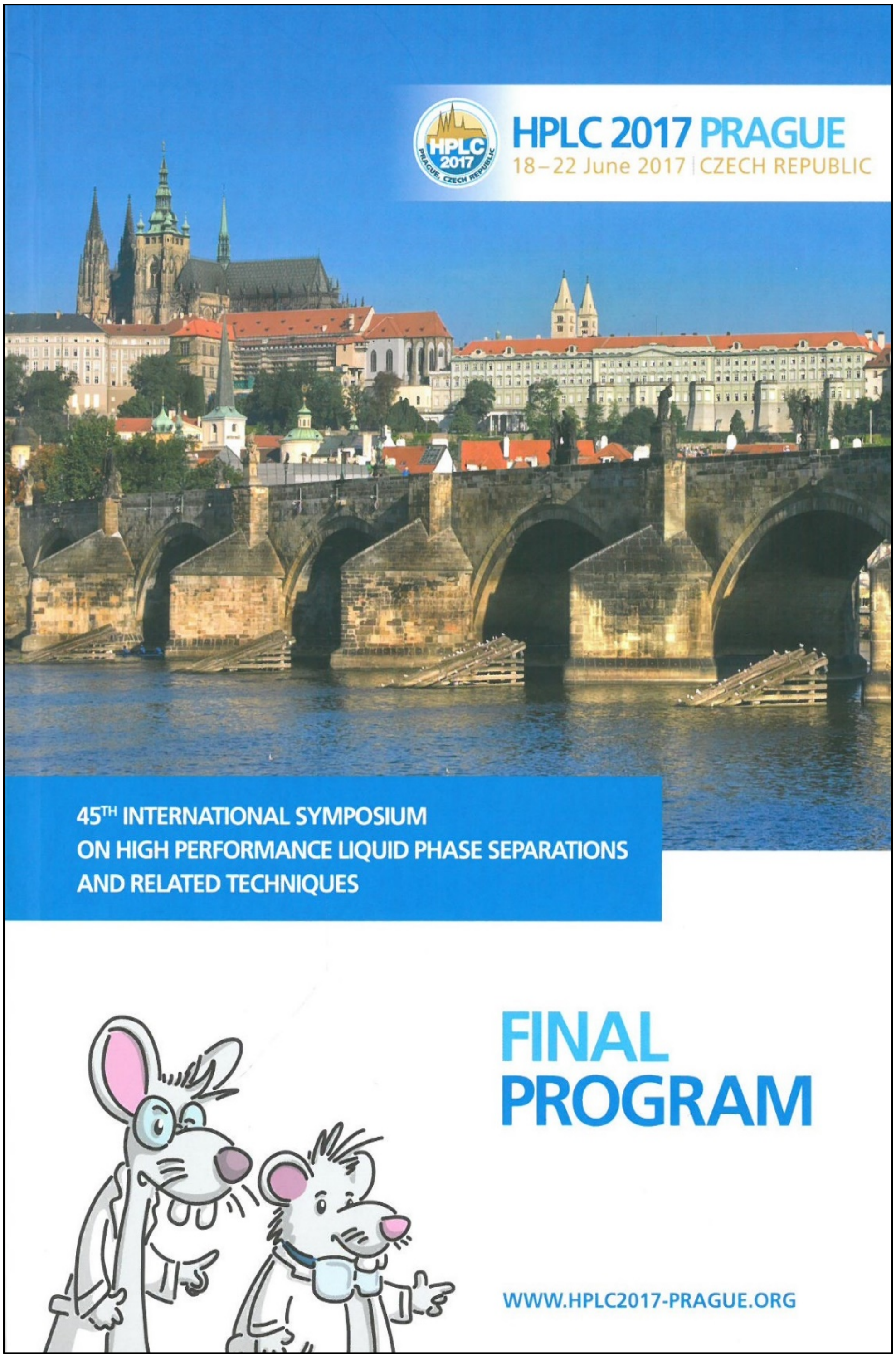
2nd Caparica Christmas Conference on Sample Treatment. Caparica, Portugal. Diciembre-2016.

6. Póster

Incorporation of single-walled carbon nanohorns in methacrylate monoliths for nano-liquid chromatography.

Fresco-Cala, B.; Carrasco-Correa, E.J; Cárdenas, S.; Herrero-Martínez, J.M.

45th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2017). Praga, República Checa. Junio-2017.



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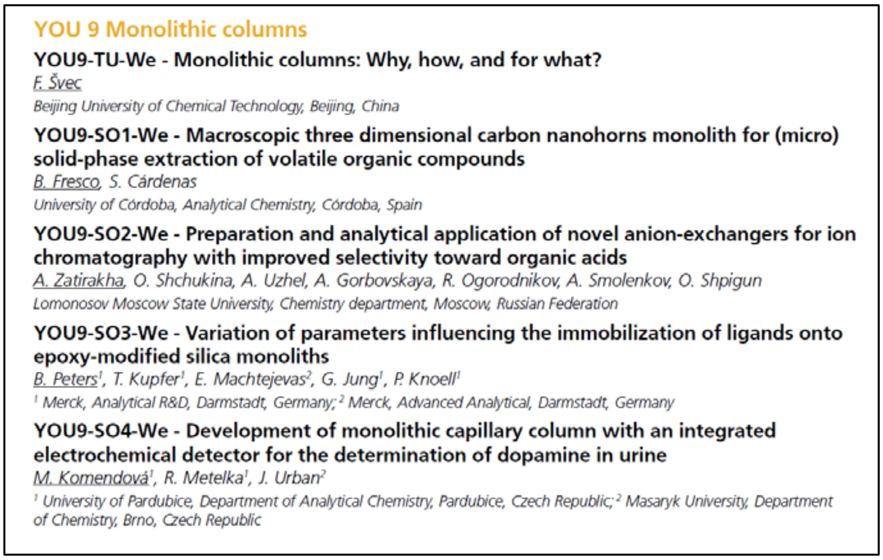
Macroscopic three dimensional carbon nanohorns monolith for (micro)solid-phase extraction of volatile organic compounds

Beatriz Fresco Cala [a], *Soledad Cárdenas* [a].

[a]*Department of Analytical Chemistry, Institute of Fine Chemistry and Nanochemistry, Marie Curie Building, Campus of Rabanales, University of Córdoba, 14071, Córdoba, Spain.*

A macroscopic three dimensional single-walled carbon nanohorns (SWNHs) monolith was synthesized without the need for radical initiators or gelators. The characteristic of these nanostructures is that they naturally form stable aggregates and we have exploited this property to prepare the first macroscopic pristine SWNHs monolith. The preparation involves the formation of a wet gel of pristine SWNHs followed by slow drying (48 h) at 25 ºC. The gelation is driven by physical interactions including van der Waals forces and π-π stacking forming a robust 3D network. The 3D monolith was characterized by scanning electron microscopy (SEM), confocal laser scanning microscopy, Raman spectroscopy, and nitrogen intrusion porosimetry. As a proof of concept, its potential as sorbent phase has been evaluated using toluene as model compound. The excellent results obtained allow us to encourage a great applicability in this specific field.



YOU 9 Monolithic columns

YOU9-TU-We - Monolithic columns: Why, how, and for what?
F. Švec
Beijing University of Chemical Technology, Beijing, China

YOU9-SO1-We - Macroscopic three dimensional carbon nanohorns monolith for (micro) solid-phase extraction of volatile organic compounds
B. Fresco, S. Cárdenas
University of Córdoba, Analytical Chemistry, Córdoba, Spain

YOU9-SO2-We - Preparation and analytical application of novel anion-exchangers for ion chromatography with improved selectivity toward organic acids
A. Zatirakha, O. Shchukina, A. Uzhel, A. Gorbovskaya, R. Ogorodnikov, A. Smolenkov, O. Shpigun
Lomonosov Moscow State University, Chemistry department, Moscow, Russian Federation

YOU9-SO3-We - Variation of parameters influencing the immobilization of ligands onto epoxy-modified silica monoliths
B. Peters[1], T. Kupfer[1], E. Machtejevas[2], G. Jung[1], P. Knoell[1]
[1] Merck, Analytical R&D, Darmstadt, Germany; [2] Merck, Advanced Analytical, Darmstadt, Germany

YOU9-SO4-We - Development of monolithic capillary column with an integrated electrochemical detector for the determination of dopamine in urine
M. Komendová[1], R. Metelka[1], J. Urban[2]
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ExTech 2017

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BOOK OF ABSTRACTS

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MABEGONDO

Deputación
DA CORUÑA

O_06: PAPER BASED SORPTIVE PHASES

Julia Ríos-Gómez [1], Beatriz Fresco-Cala [1], Rafael Lucena [1*], Soledad Cárdenas [1]

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Paper based analytical devices have attracted much attention in the last decade since their first proposal in 2007 by Whitesides et al [1]. Paper, as material, presents several advantages (e.g low price, high porosity, variable thickness) that make it attractive in the microextraction context. In addition, it can be easily modified in order to introduce special moieties to enhance the interaction with target analytes. In this communication, our recent studies on the topic are described and discussed while the main research lines for the near future are outlined.

Although the modification of paper or cellulose fibres in order to obtain sorptive phases for microextraction purposes have been already proposed [2,3], the procedure described in this communication does not require any complex synthetic workflow. It uses paper as simple support of the sorptive phase which is coated by simple dipping. In the first approach, polymeric phases were evaluated as coatings [4]. The procedure requires a solution of the polymer in an appropriate solvent where a segment of paper is introduced. The solution wets the paper and the evaporation of the solvent leaves a polymer coating over the support. The coating thickness may be selected controlling the number of dips and/or the concentration of the polymer in the original solution. In addition, the resulting material can be easily extended to a pipette tip which is used as extraction device. This general procedure can be adapted to any polymer soluble enough in a volatile solvent. Moreover, the potential of this workflow in order to immobilize nanomaterials have been also studied demonstrating a high versatility. In this case, carbon nanohorns have been coated on a paper support in a stable way and they have been used as sorptive phases in bioanalysis.

Acknowledgement Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2014-52939R) is gratefully acknowledged.

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O_08: POTENTIAL OF THE SYNERGIC COMBINATION OF POLYMERIC PHASES AND NANOPARTICLES IN THE MICROEXTRACTION CONTEXT

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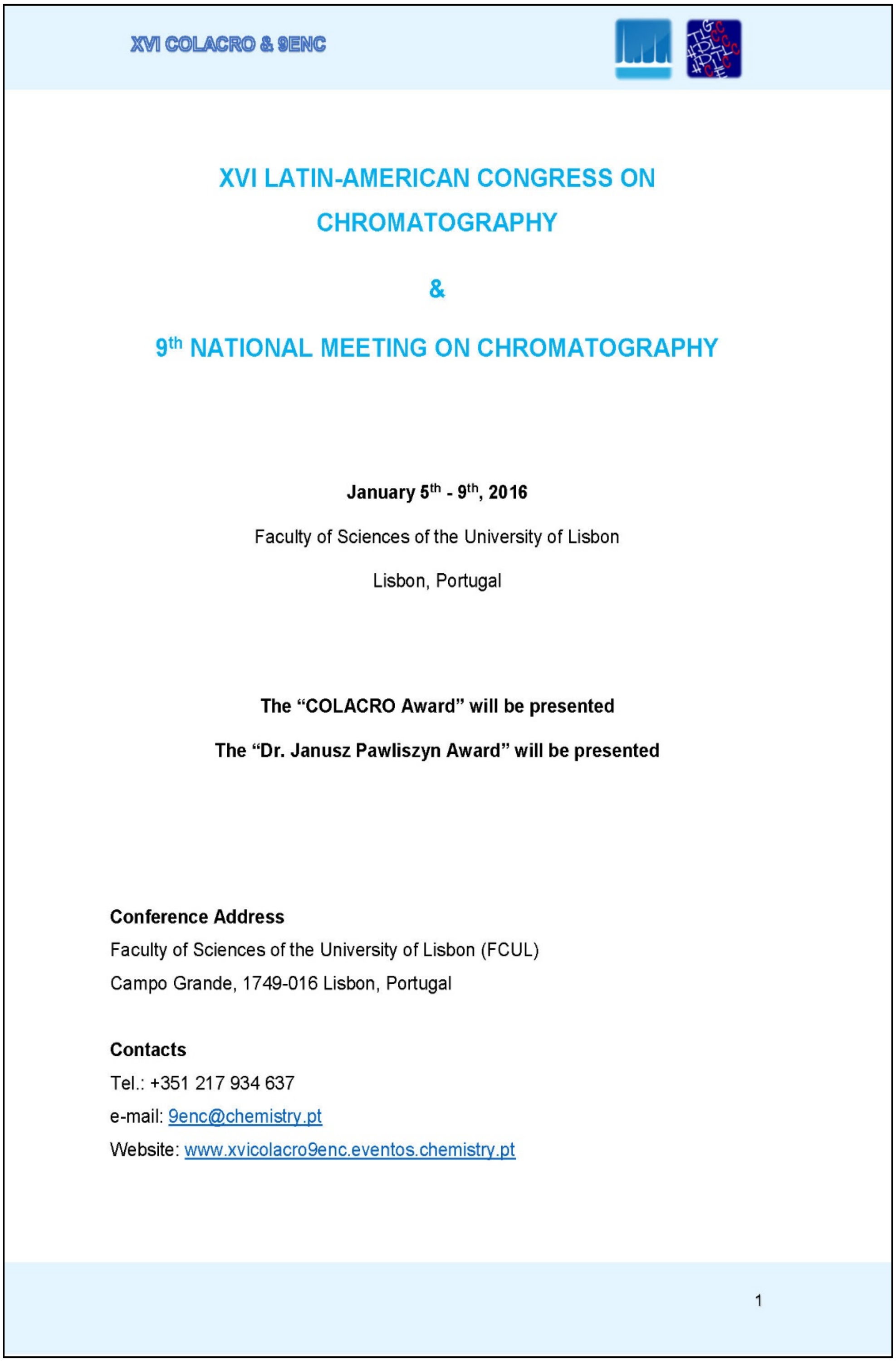
The development of highly efficient sorbent phases is a main research topic in Analytical Chemistry, and specifically in the microextraction context. The advantage of these novel extractants over conventional sorbents should comprise an enhanced sorption capacity, taking into account the reduced amount usually employed in micro solid-phase extraction techniques (µ-SPE). Among the existing alternatives, the joint use of polymers and nanoparticles has been identified as a powerful alternative. The selectivity of the hybrid solid material can be tailored by the proper selection of the monomers used to synthesize the polymeric network while the presence of nanoparticles can provide the resulting composite with other complementary properties such as superparamagnetic character or higher porous surface. This communication presents our experience in this context.

First, the synthesis of composite materials using polyamide-based polymers and silica or magnetic nanoparticles will be discussed. The introduction of nanoparticles in the polymeric network has demonstrated to be an excellent alternative to improve the extraction capacity of the composites as well as their mechanical properties. Nanoparticles disturb the normal stacking of the polymer increasing their superficial area which results critical to improve both thermodynamics and kinetics aspects of the extraction. In addition, the use of magnetic nanoparticles adds singular properties to the resulting composites. Next, the advantages of including carbon nanoparticles on monolithic phases will also be outlined. Monoliths are unique and continuous structures which contain large pores, whose dimensions can be controlled by selecting the most convenient experimental conditions. In addition, their chemistry can be easily tailored considering the nature of the monomers and cross-linker used. As a drawback, their specific surface area tends to be lower than that of other sorbents employed in microextraction techniques. The incorporation of nanoparticles during or after the synthesis of the monolithic sorbent may circumvent this limitation expanding their applicability in the microextraction context.

Acknowledgement Financial support from the Spanish Ministry of Science and Innovation (CTQ2014-52939-R) is gratefully acknowledged



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Improved microextraction of selected triazines from orange juices using polymer monoliths modified with carboxylated multi-walled carbon nanotubes

XVI COLACRO

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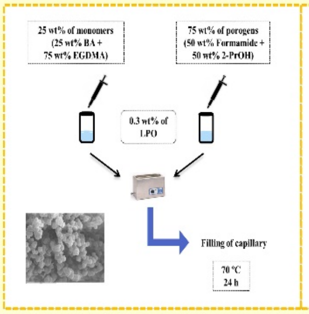
ABSTRACT

This communication reports on the enhancement of the capacity of an acrylate-based monolithic solid sorbent by anchoring carboxylated multi-walled carbon nanotubes (c-MWCNTs) in its pores and on its surface. Monolithic poly(butyl acrylate-*co*-ethyleneglycol dimethacrylate) [poly(BA-*co*-EGDMA)] was synthetized inside a fused silica capillary via free-radical polymerization, and an ethanolic dispersion of c-MWCNTs was passed through the capillary. The resulting poly(BA-*co*-EGDMA-c-MWCNTs) monolith was characterized by scanning electron microscopy to confirm the presence of the c-MWCNTs. The effect of using three different carbon nanoparticles and the microextraction step were studied using triazine herbicides as model compounds. The use of c-MWCNTs resulted in best performance in terms of extraction enhancement [compared to carboxylated single-walled carbon nanotubes (c-SWCNTs) and oxidized single-walled carbon nanohorn (o-SWNHs)]. The use of these carbon nanoparticles improved the extraction of triazines in any case when compared to using a bare poly(BA-*co*-EGDMA) monolith. The triazines were then quantified by gas chromatography with mass spectrometric detection. Detection limits ranged from 0.03 to 0.1 μg·L^{-1} (except for simazine; 0.6 μg·L^{-1}), and the precision (relative standard deviation) varied between 3.0 and 11.4%. The reproducibility between units is <14.3% (expressed as relative standard deviation) which demonstrates the robustness of the method. The method was applied to analyze an unknown sample of orange juice and providing a value of 0.18 μg·L^{-1} for prometryn. Finally, the analysis of spiked samples of orange juices yielded recoveries ranging from 81 to 113% .

EXPERIMENTAL SECTION

1. Preparation of monolithic solid



25 wt% of monomers (25 wt% BA + 75 wt% EGDMA)

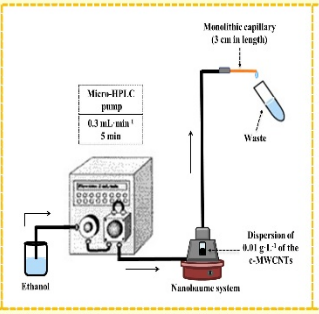
75 wt% of porogens (50 wt% Formamide + 50 wt% 2-PrOH)

0.3 wt% of LPO

Filling of capillary

70 °C
24 h

2. Immobilization of c-MWCNTs



Micro-HPLC pump
0.3 mL·min^{-1}
5 min

Monolithic capillary (3 cm in length)

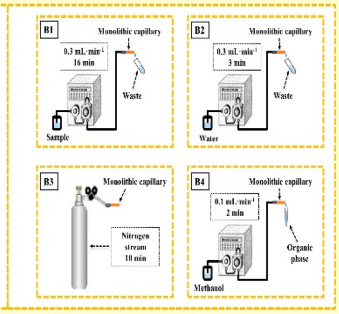
Waste

Ethanol

Nanobaume system

Dispersion of 0.01 g·L^{-1} of the c-MWCNTs

3. Microextraction procedure



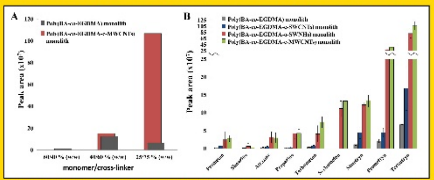
B1: Monolithic capillary; 0.3 mL·min^{-1}, 16 min; Waste; Sample

B2: Monolithic capillary; 0.3 mL·min^{-1}, 3 min; Waste; Water

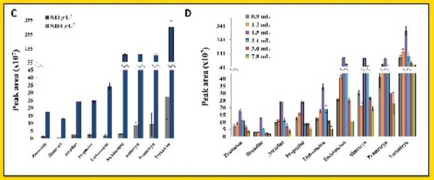
B3: Monolithic capillary; Nitrogen stream 10 min

B4: Monolithic capillary; 0.1 mL·min^{-1}, 2 min; Organic phase; Methanol

Variables affecting to the preparation of the poly(BA-co-EGDMA-c-MWCNTs) monolith



(A) The relative extraction efficiency for terbutryn as model compound, using the bare poly(BA-co-EGDMA) monolith and the poly(BA-co-EGDMA-c-MWCNTs) monolith as microextraction unit. (B) Comparison of the analytical performance of poly(BA-co-EGDMA) monolith without nanoparticles and the monolith with c-MWCNTs, o-SWNHs and c-MWCNTs immobilized on its pores for 20/80 % (w/w) and 25/75% (w/w) proportions of monomers/porogens and monomers/cross-linker ratios, respectively



(C) Influence of the concentration of the c-MWCNTs dispersion on the triazines retention. (D) Effect of the volume of the 0.01 g·L^{-1} c-MWCNTs dispersion passed through the poly(BA-co-EGDMA) monolith at a flow rate of 0.3 mL·min^{-1}.

ACKNOWLEDGMENTS

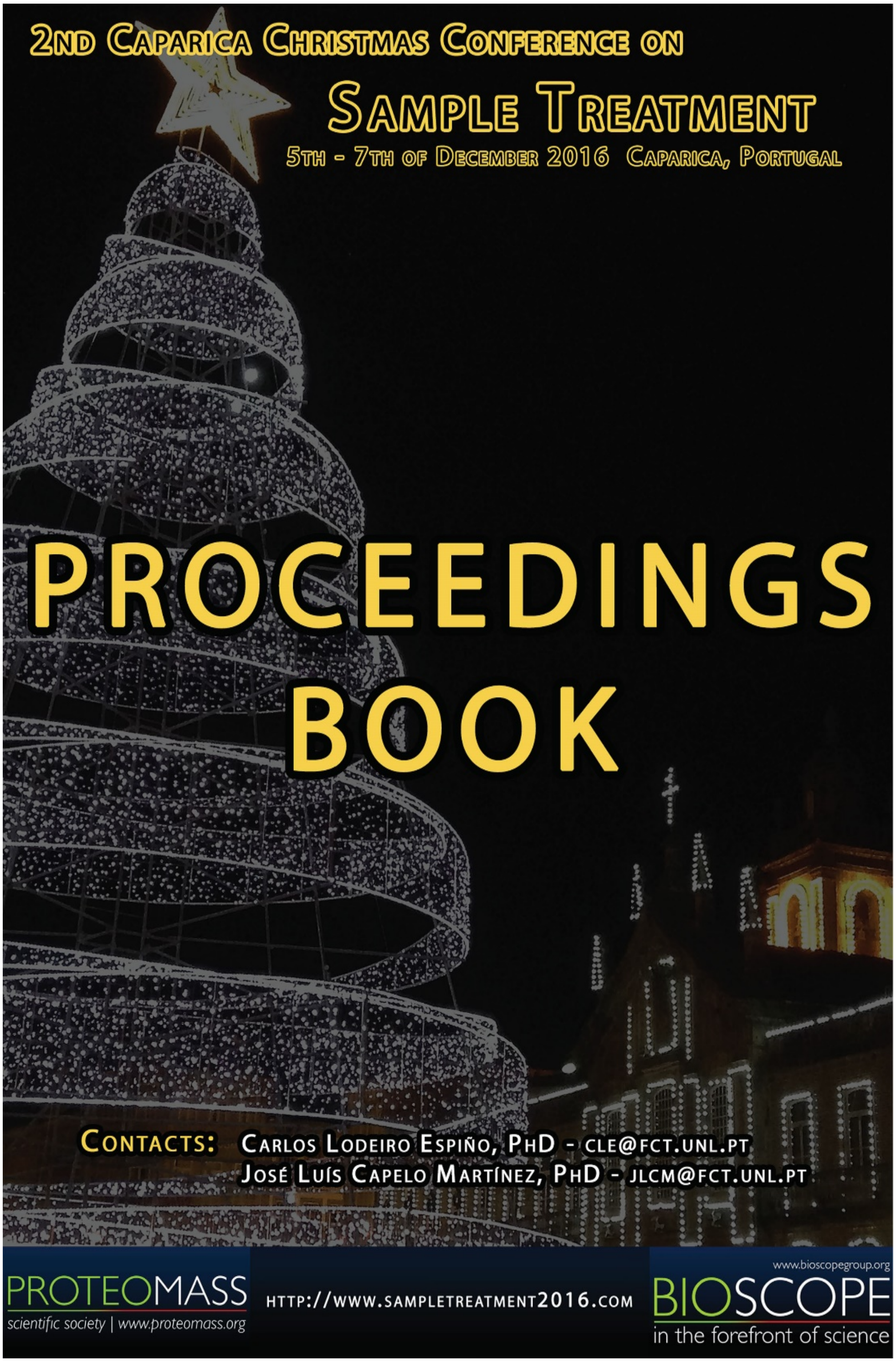
Financial support from the Spanish Ministry of Science and Innovation (CTQ2014-52939-R) is gratefully acknowledged. B. Fresco-Cala expresses her gratitude for the predoctoral grant (ref FPU13/03896) from the Spanish Ministry of Education.

ANALYTICAL FIGURES OF MERIT

Analyte	m/z	LOD (μg/L)	LOQ (μg/L)	Precision		
				Intra-day RSD (%, n=5)	Inter-day RSD (%, n=5)	Inter- units RSD (%, n=5)
Prometon	210	0.10	0.4	9.4	11.4	6.8
Simazine	201	0.60	1.0	4.2	7.1	7.9
Atrazine	200	0.10	0.4	11.4	9.9	3.9
Propazine	214	0.10	0.4	6.0	5.3	8.2
Terbumeton	210	0.10	0.4	7.2	10.3	8.7
Secbumeton	196	0.03	0.1	3.0	7.4	10.0
Simetryn	213	0.03	0.1	4.1	8.6	9.0
Prometryn	241	0.03	0.1	5.9	8.1	13.8
Terbutryn	226	0.03	0.1	10.5	11.3	14.3

ANALYSIS OF ORANGE JUICE SAMPLES

Analytes	Spiked (μg·L^{-1})	Commercial orange juice		Squeezed orange juice	
		Detected (μg·L^{-1})	Recoveries (%, n=3)	Detected (μg·L^{-1})	Recoveries (%, n=3)
Prometon	0	ND		ND	
	1	1.06	106 ± 10	0.84	84 ± 11
Simazine	0	ND		ND	
	1	0.75	75 ± 9	1.25	125 ± 10
Atrazine	0	ND		ND	
	1	1.03	103 ± 10	1.25	125 ± 11
Propazine	0	ND		ND	
	1	0.75	75 ± 10	0.94	94 ± 11
Terbumeton	0	ND		ND	
	1	0.91	91 ± 10	0.76	76 ± 10
Secbumeton	0	ND		ND	
	1	1.49	121 ± 8	0.76	76 ± 8
Simetryn	0	ND		ND	
	1	0.98	98 ± 6	1.21	121 ± 8
Prometryn	0	0.18		ND	
	1	1.18	100 ± 13	0.98	98 ± 12
Terbutryn	0	ND		ND	
	1	0.99	99 ± 13	1.15	115 ± 14



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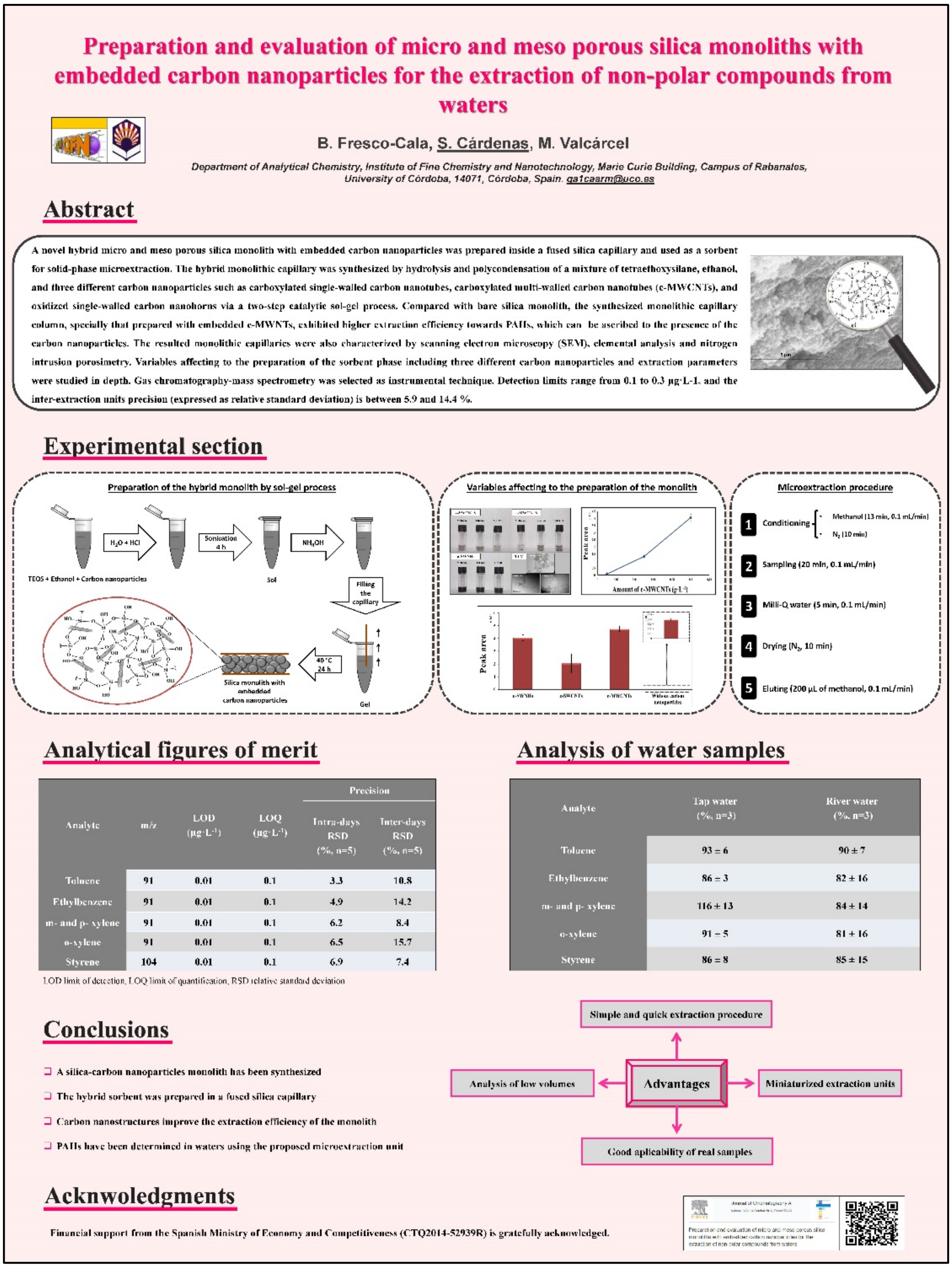

Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters

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Abstract

A novel hybrid micro and meso porous silica monolith with embedded carbon nanoparticles was prepared inside a fused silica capillary and used as a sorbent for solid-phase microextraction. The hybrid monolithic capillary was synthesized by hydrolysis and polycondensation of a mixture of tetraethoxysilane, ethanol, and three different carbon nanoparticles such as carboxylated single-walled carbon nanotubes, carboxylated multi-walled carbon nanotubes (c-MWCNTs), and oxidized single-walled carbon nanohorns via a two-step catalytic sol-gel process. Compared with bare silica monolith, the synthesized monolithic capillary column, specially that prepared with embedded c-MWNTs, exhibited higher extraction efficiency towards PAHs, which can be ascribed to the presence of the carbon nanoparticles. The resulted monolithic capillaries were also characterized by scanning electron microscopy (SEM), elemental analysis and nitrogen intrusion porosimetry. Variables affecting to the preparation of the sorbent phase including three different carbon nanoparticles and extraction parameters were studied in depth. Gas chromatography-mass spectrometry was selected as instrumental technique. Detection limits range from 0.1 to 0.3 µg·L-1. and the inter-extraction units precision (expressed as relative standard deviation) is between 5.9 and 14.4 %.

Experimental section

Preparation of the hybrid monolith by sol-gel process

Variables affecting to the preparation of the monolith

Microextraction procedure

1. Conditioning
   - Methanol (13 min, 0.1 mL/min)
   - N_2 (10 min)
2. Sampling (20 min, 0.1 mL/min)
3. Milli-Q water (5 min, 0.1 mL/min)
4. Drying (N_2, 10 min)
5. Eluting (200 µL of methanol, 0.1 mL/min)

Analytical figures of merit

Analyte	m/z	LOD (µg·L⁻¹)	LOQ (µg·L⁻¹)	Precision	
				Intra-days RSD (%, n=5)	Inter-days RSD (%, n=5)
Toluene	91	0.01	0.1	3.3	10.8
Ethylbenzene	91	0.01	0.1	4.9	14.2
m- and p- xylene	91	0.01	0.1	6.2	8.4
o-xylene	91	0.01	0.1	6.5	15.7
Styrene	104	0.01	0.1	6.9	7.4

LOD limit of detection, LOQ limit of quantification, RSD relative standard deviation

Analysis of water samples

Analyte	Tap water (%, n=3)	River water (%, n=3)
Toluene	93 ± 6	90 ± 7
Ethylbenzene	86 ± 3	82 ± 16
m- and p- xylene	116 ± 13	84 ± 14
o-xylene	91 ± 5	81 ± 16
Styrene	86 ± 8	85 ± 15

Conclusions

- ❑ **A silica-carbon nanoparticles monolith has been synthesized**
- ❑ **The hybrid sorbent was prepared in a fused silica capillary**
- ❑ **Carbon nanostructures improve the extraction efficiency of the monolith**
- ❑ **PAHs have been determined in waters using the proposed microextraction unit**

Advantages

Simple and quick extraction procedure

Analysis of low volumes

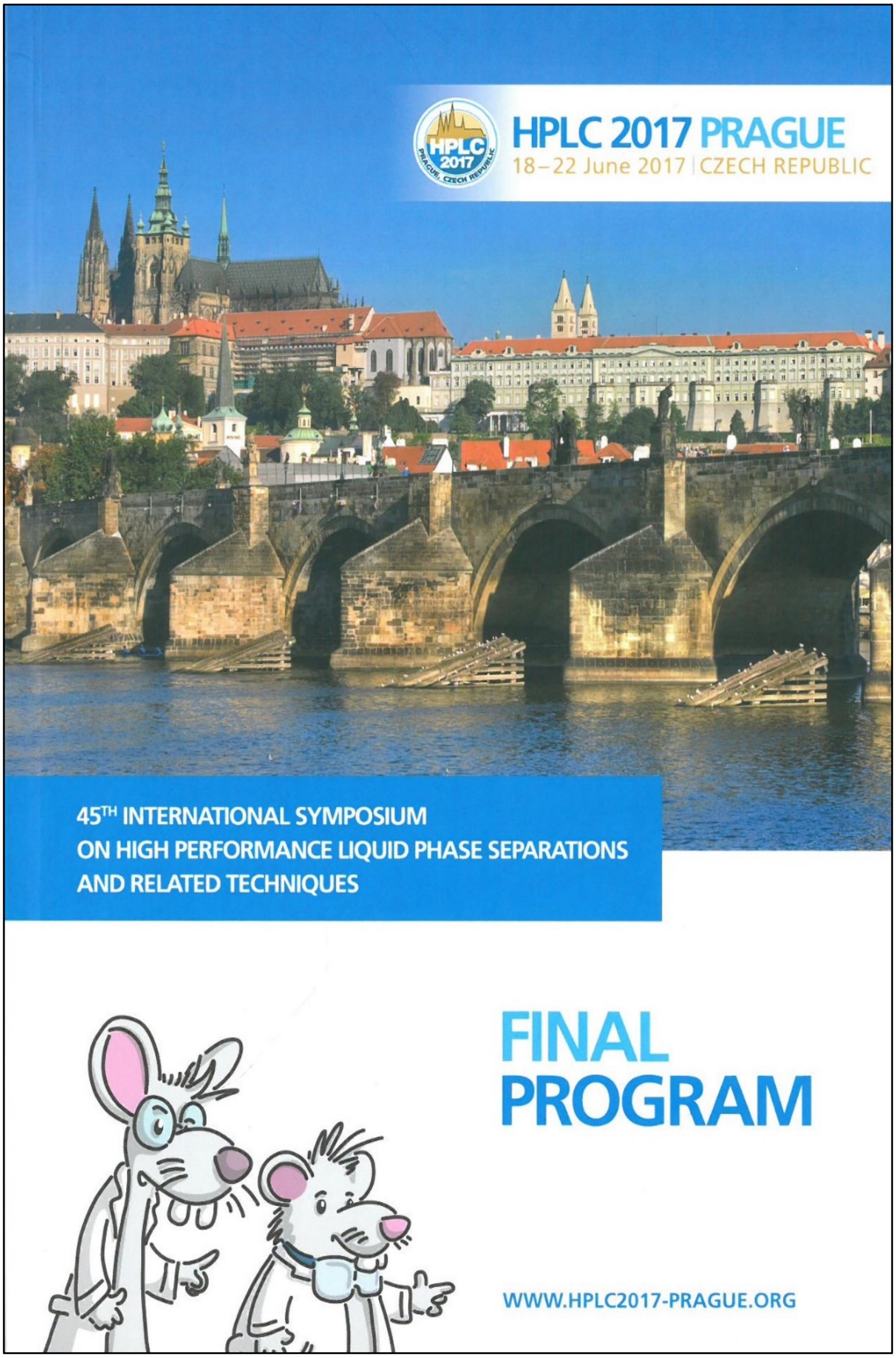
Miniaturized extraction units

Good aplicability of real samples

Acknowledgments

Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2014-52939R) is gratefully acknowledged.

Journal of Chromatography A
Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters



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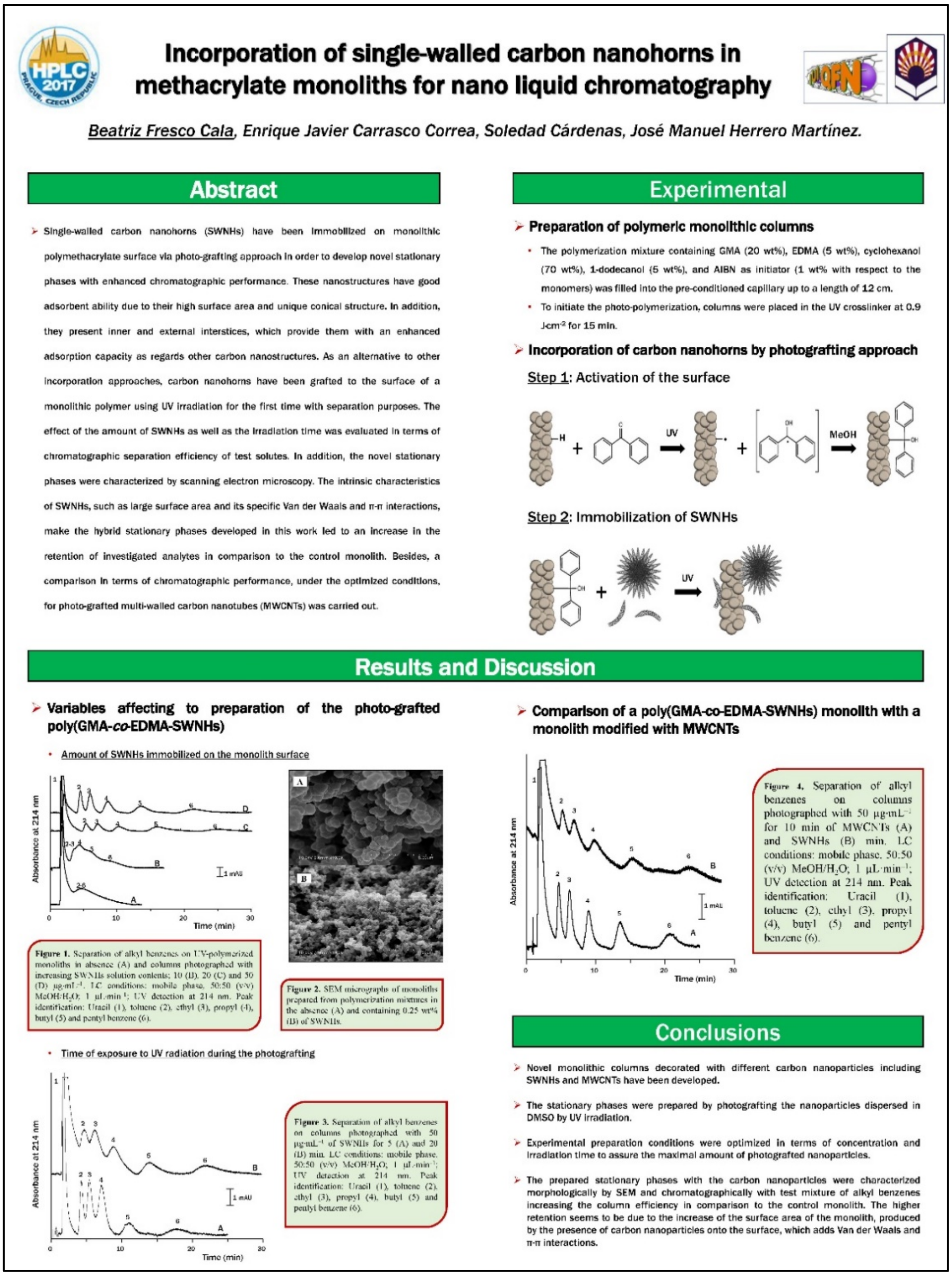


Incorporation of single-walled carbon nanohorns in methacrylate monoliths for nano liquid chromatography

Beatriz Fresco Cala, Enrique Javier Carrasco Correa, Soledad Cárdenas, José Manuel Herrero Martínez.

Abstract

- Single-walled carbon nanohorns (SWNHs) have been immobilized on monolithic polymethacrylate surface via photo-grafting approach in order to develop novel stationary phases with enhanced chromatographic performance. These nanostructures have good adsorbent ability due to their high surface area and unique conical structure. In addition, they present inner and external interstices, which provide them with an enhanced adsorption capacity as regards other carbon nanostructures. As an alternative to other incorporation approaches, carbon nanohorns have been grafted to the surface of a monolithic polymer using UV irradiation for the first time with separation purposes. The effect of the amount of SWNHs as well as the irradiation time was evaluated in terms of chromatographic separation efficiency of test solutes. In addition, the novel stationary phases were characterized by scanning electron microscopy. The intrinsic characteristics of SWNHs, such as large surface area and its specific Van der Waals and π-π interactions, make the hybrid stationary phases developed in this work led to an increase in the retention of investigated analytes in comparison to the control monolith. Besides, a comparison in terms of chromatographic performance, under the optimized conditions, for photo-grafted multi-walled carbon nanotubes (MWCNTs) was carried out.

Experimental

Preparation of polymeric monolithic columns

- The polymerization mixture containing GMA (20 wt%), EDMA (5 wt%), cyclohexanol (70 wt%), 1-dodecanol (5 wt%), and AIBN as initiator (1 wt% with respect to the monomers) was filled into the pre-conditioned capillary up to a length of 12 cm.
- To initiate the photo-polymerization, columns were placed in the UV crosslinker at 0.9 J·cm^{-2} for 15 min.

Incorporation of carbon nanohorns by photografting approach

Step 1: Activation of the surface

Step 2: Immobilization of SWNHs

Results and Discussion

Variables affecting to preparation of the photo-grafted poly(GMA-*co*-EDMA-SWNHs)

- Amount of SWNHs immobilized on the monolith surface

Figure 1. Separation of alkyl benzenes on UV-polymerized monoliths in absence (A) and columns photographed with increasing SWNHs solution contents: 10 (B), 20 (C) and 50 (D) μg·mL^{-1}. LC conditions: mobile phase, 50:50 (v/v) MeOH/H$_2$O; 1 μL·min^{-1}; UV detection at 214 nm. Peak identification: Uracil (1), toluene (2), ethyl (3), propyl (4), butyl (5) and pentyl benzene (6).

Figure 2. SEM micrographs of monoliths prepared from polymerization mixtures in the absence (A) and containing 0.25 wt% (B) of SWNHs.

- Time of exposure to UV radiation during the photografting

Figure 3. Separation of alkyl benzenes on columns photographed with 50 μg·mL^{-1} of SWNHs for 5 (A) and 20 (B) min. LC conditions: mobile phase, 50:50 (v/v) MeOH/H$_2$O; 1 μL·min^{-1}; UV detection at 214 nm. Peak identification: Uracil (1), toluene (2), ethyl (3), propyl (4), butyl (5) and pentyl benzene (6).

Comparison of a poly(GMA-co-EDMA-SWNHs) monolith with a monolith modified with MWCNTs

Figure 4. Separation of alkyl benzenes on columns photographed with 50 μg·mL^{-1} for 10 min of MWCNTs (A) and SWNHs (B) min. LC conditions: mobile phase, 50:50 (v/v) MeOH/H$_2$O; 1 μL·min^{-1}; UV detection at 214 nm. Peak identification: Uracil (1), toluene (2), ethyl (3), propyl (4), butyl (5) and pentyl benzene (6).

Conclusions

- Novel monolithic columns decorated with different carbon nanoparticles including SWNHs and MWCNTs have been developed.
- The stationary phases were prepared by photografting the nanoparticles dispersed in DMSO by UV irradiation.
- Experimental preparation conditions were optimized in terms of concentration and irradiation time to assure the maximal amount of photografted nanoparticles.
- The prepared stationary phases with the carbon nanoparticles were characterized morphologically by SEM and chromatographically with test mixture of alkyl benzenes increasing the column efficiency in comparison to the control monolith. The higher retention seems to be due to the increase of the surface area of the monolith, produced by the presence of carbon nanoparticles onto the surface, which adds Van der Waals and π-π interactions.

Comunicaciones a congresos nacionales:

1. Oral

Evaluación de la capacidad sorbente de los nanocuernos de carbono de pared simple (SWNHs) inmovilizados sobre una fibra hueca en microextracción en fase sólida.

Fresco-Cala, B.; Jiménez-Soto J.M.; Cárdenas, S.; Valcárcel, M.

IV Encuentro sobre Nanociencia y Nanotecnología de Investigadores Andaluces (IV NANOUCO). Córdoba, España. Febrero-2013.

2. Flash/Oral

Evaluation of a carboxylated multi-walled carbon nanotube monolithic microextraction unit for the determination of selected triazines from waters.

Fresco-Cala, B.; Cárdenas, S.; Valcárcel, M.

VII Workshop on Analytical Nanoscience and Nanotechnology (VII NYNA). Salamanca, España. Julio-2015.

3. Oral

Preparation of methacrylate porous monoliths with oxidized single-walled carbon nanohorns into spin columns for the extraction of nonsteroidal anti-inflammatory drugs from urine samples.

Fresco-Cala, B.; Cárdenas, S.; Herrero-Martínez, J.M.

VI Encuentro sobre Nanociencia y Nanotecnología de Investigadores Andaluces (VI NANOUCO). Córdoba, España. Enero-2017.

4. **Oral invitada**

Effect of carbon nanohorns in the polymerization of methacrylate monolithic capillaries and their application as extractant phases.

Fresco-Cala, B.

XXVII Reunión Bienal de Química Orgánica. Premio Lilly de Investigación 2018. Santiago de Compostela, España. Junio-2018.

5. **Póster**

Nanocuernos de carbono de pared simple inmovilizados en una fibra hueca microporosa de polipropileno como sorbentes para la extracción de compuestos orgánicos volátiles.

Fresco-Cala, B.; Jiménez-Soto, J.M.; Cárdenas, S.; Valcárcel, M.

XIV Reunión del Grupo Regional Andaluz de la Sociedad Española de Química Analítica (XIV GRASEQA). Baeza, España. Junio-2014.

6. **Póster**

Evaluación del potencial de sólidos monolíticos modificados con nanopartículas en técnicas de microextracción

Fresco-Cala, B.

III Congreso Científico de Investigadores en Formación en Agroalimentación ceiaA3. Córdoba, España. Noviembre-2014.

7. **Póster**

Evaluación de una unidad de microextracción basada en un sólido monolítico modificado con nanotubos de carbono multicapa carboxilados.

Fresco-Cala, B.; Cárdenas, S.; Valcárcel, M.

V Encuentro sobre Nanociencia y Nanotecnología de Investigadores Andaluces (V NANOUCO). Córdoba, España. Febrero-2015.

8. Póster

Evaluación del potencial de sólidos monolíticos modificados con nanopartículas en técnicas de microextracción.

Fresco-Cala, B.

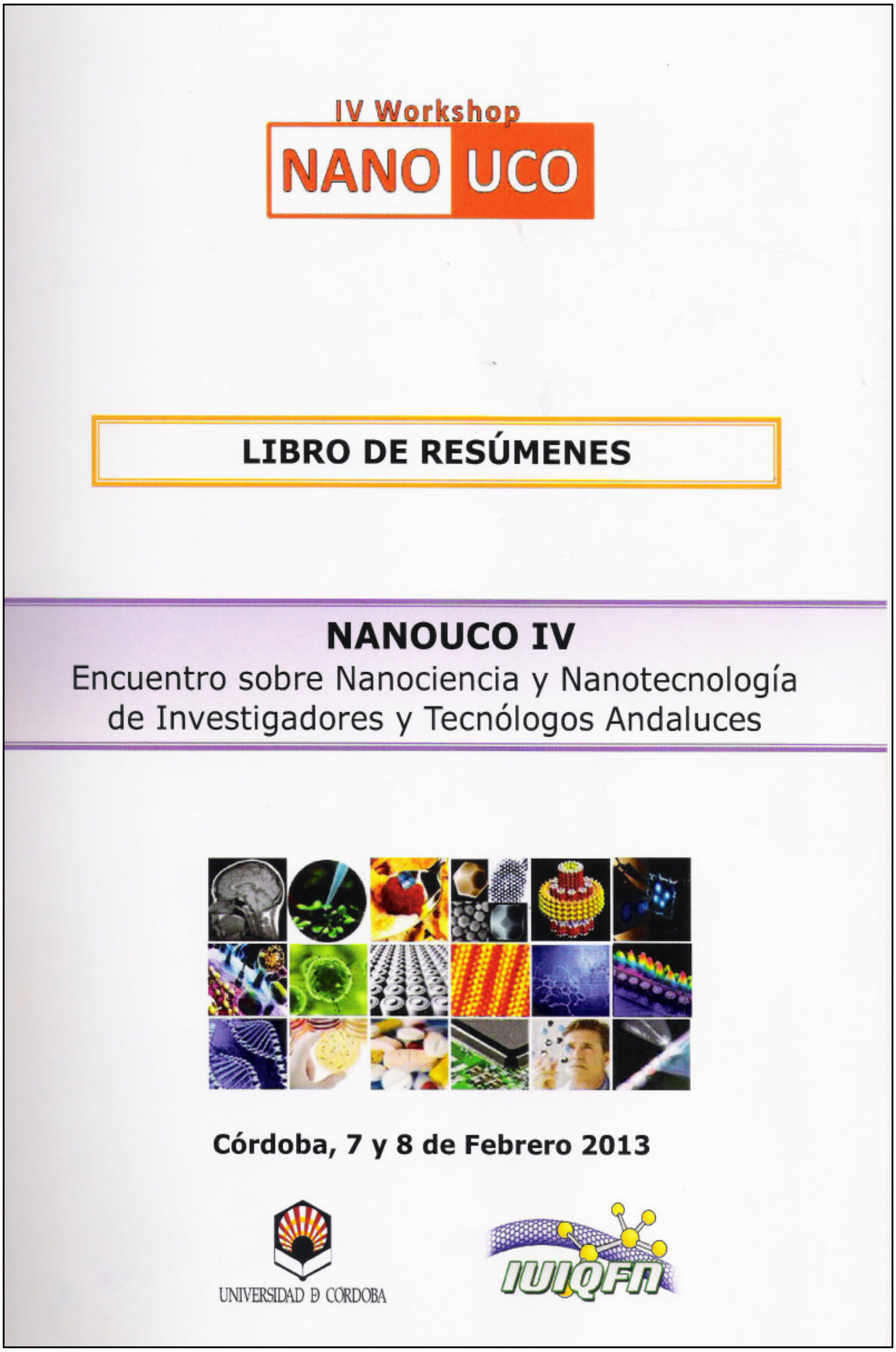
V Congreso Científico de Investigadores en Formación de la Universidad de Córdoba. Córdoba, España. Noviembre-2016.

9. Póster

Monolithic solids based on carbon nanoparticles as solid extraction phases.

Fresco-Cala, B.

VI Congreso Científico de Investigadores en Formación de la Universidad de Córdoba. Córdoba, España. Enero-2018.



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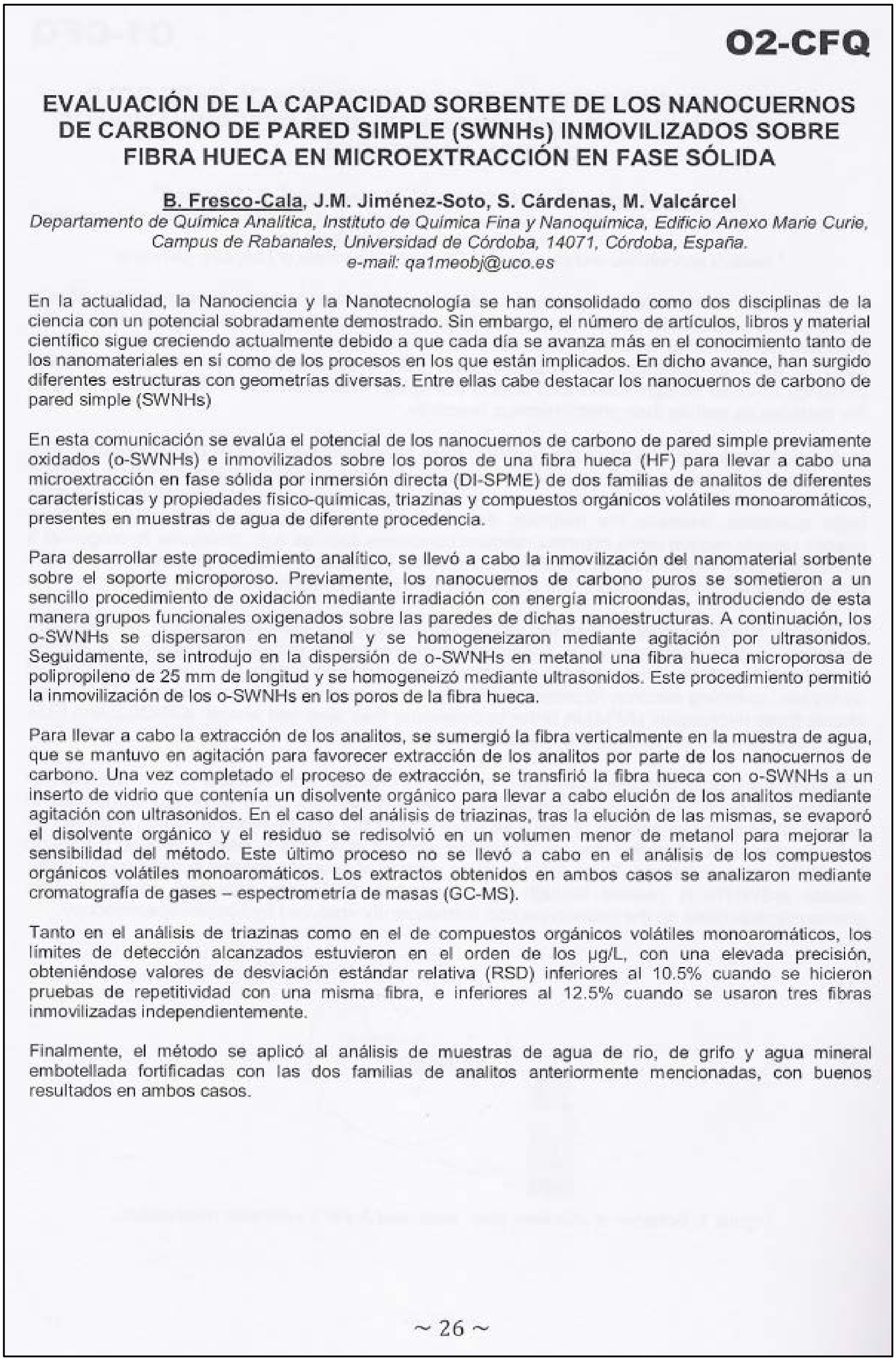

O2-CFQ

EVALUACIÓN DE LA CAPACIDAD SORBENTE DE LOS NANOCUERNOS DE CARBONO DE PARED SIMPLE (SWNHs) INMOVILIZADOS SOBRE FIBRA HUECA EN MICROEXTRACCIÓN EN FASE SÓLIDA

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En la actualidad, la Nanociencia y la Nanotecnología se han consolidado como dos disciplinas de la ciencia con un potencial sobradamente demostrado. Sin embargo, el número de artículos, libros y material científico sigue creciendo actualmente debido a que cada día se avanza más en el conocimiento tanto de los nanomateriales en sí como de los procesos en los que están implicados. En dicho avance, han surgido diferentes estructuras con geometrías diversas. Entre ellas cabe destacar los nanocuernos de carbono de pared simple (SWNHs)

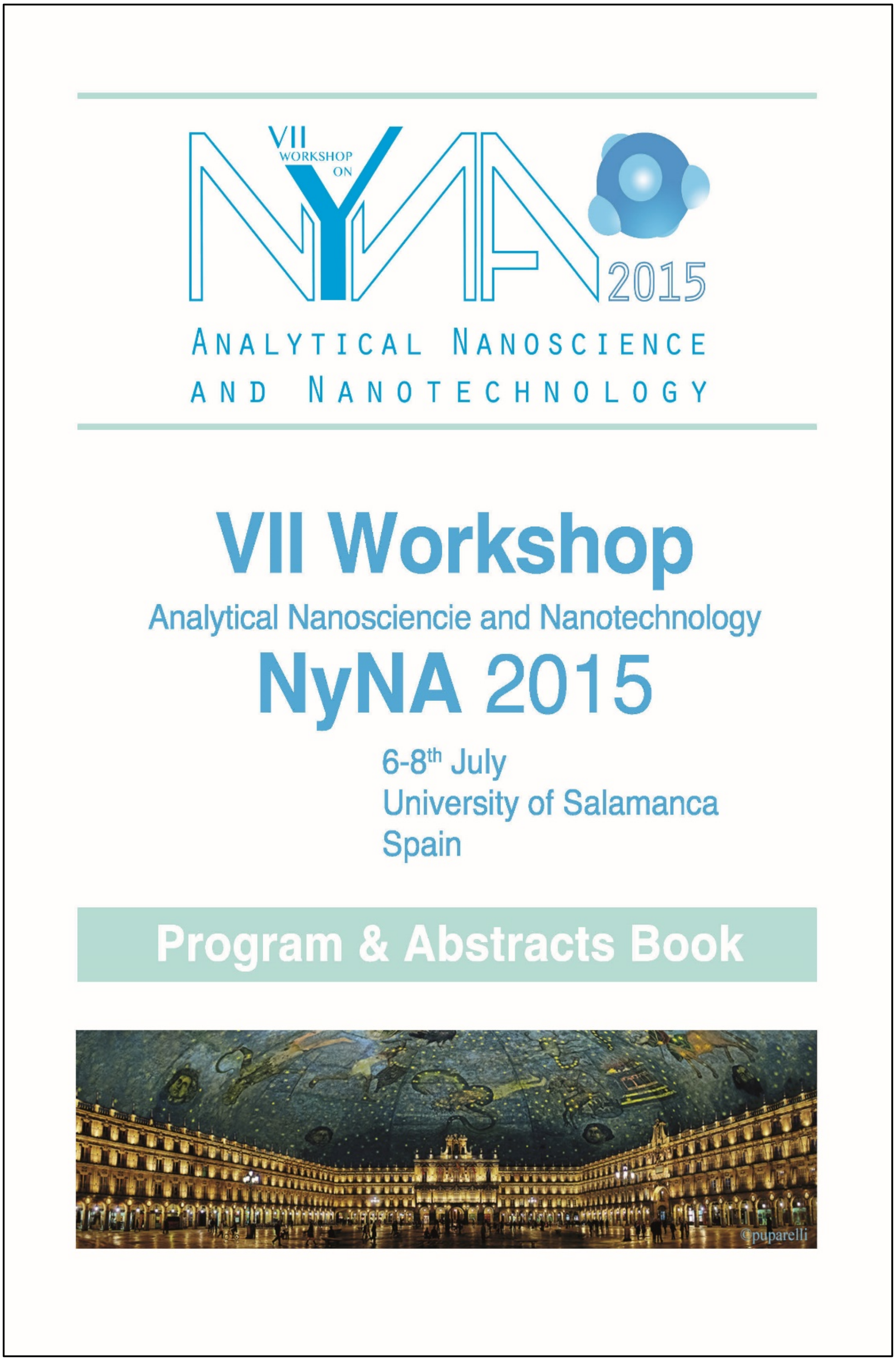
En esta comunicación se evalúa el potencial de los nanocuernos de carbono de pared simple previamente oxidados (o-SWNHs) e inmovilizados sobre los poros de una fibra hueca (HF) para llevar a cabo una microextracción en fase sólida por inmersión directa (DI-SPME) de dos familias de analitos de diferentes características y propiedades físico-químicas, triazinas y compuestos orgánicos volátiles monoaromáticos, presentes en muestras de agua de diferente procedencia.

Para desarrollar este procedimiento analítico, se llevó a cabo la inmovilización del nanomaterial sorbente sobre el soporte microporoso. Previamente, los nanocuernos de carbono puros se sometieron a un sencillo procedimiento de oxidación mediante irradiación con energía microondas, introduciendo de esta manera grupos funcionales oxigenados sobre las paredes de dichas nanoestructuras. A continuación, los o-SWNHs se dispersaron en metanol y se homogeneizaron mediante agitación por ultrasonidos. Seguidamente, se introdujo en la dispersión de o-SWNHs en metanol una fibra hueca microporosa de polipropileno de 25 mm de longitud y se homogeneizó mediante ultrasonidos. Este procedimiento permitió la inmovilización de los o-SWNHs en los poros de la fibra hueca.

Para llevar a cabo la extracción de los analitos, se sumergió la fibra verticalmente en la muestra de agua, que se mantuvo en agitación para favorecer extracción de los analitos por parte de los nanocuernos de carbono. Una vez completado el proceso de extracción, se transfirió la fibra hueca con o-SWNHs a un inserto de vidrio que contenía un disolvente orgánico para llevar a cabo elución de los analitos mediante agitación con ultrasonidos. En el caso del análisis de triazinas, tras la elución de las mismas, se evaporó el disolvente orgánico y el residuo se redisolvió en un volumen menor de metanol para mejorar la sensibilidad del método. Este último proceso no se llevó a cabo en el análisis de los compuestos orgánicos volátiles monoaromáticos. Los extractos obtenidos en ambos casos se analizaron mediante cromatografía de gases – espectrometría de masas (GC-MS).

Tanto en el análisis de triazinas como en el de compuestos orgánicos volátiles monoaromáticos, los límites de detección alcanzados estuvieron en el orden de los µg/L, con una elevada precisión, obteniéndose valores de desviación estándar relativa (RSD) inferiores al 10.5% cuando se hicieron pruebas de repetitividad con una misma fibra, e inferiores al 12.5% cuando se usaron tres fibras inmovilizadas independientemente.

Finalmente, el método se aplicó al análisis de muestras de agua de rio, de grifo y agua mineral embotellada fortificadas con las dos familias de analitos anteriormente mencionadas, con buenos resultados en ambos casos.



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Evaluation of a carboxylated multi-walled carbon nanotubes monolithic microextraction unit for the determination of selected triazines from waters

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Carboxylated multi-walled carbon nanotubes (c-MWCNTs) have been immobilized on a poly(butyl acrylate-co-ethylene glycol dimethacrylate) monolith for the extraction of triazines from water samples. The hybrid monolithic phase was packed inside a fused silica capillary (3 cm in length, 320 µm i.d.) via one-step initiated free-radical polymerization, followed by the anchorage of the c-MWCNTs on its pores and surface. The hybrid c-MWCNTs monolithic solid was characterized by scanning electron microscopy (SEM) to confirm the presence of the c-MWCNTs through it. Variables affecting to the preparation of the sorbent phase, including three different carbon nanoparticles, and extraction parameters were studied in depth. Gas chromatography-mass spectrometry was selected as instrumental technique. Compared with poly(butyl acrylate-co-ethylene glycol dimethacrylate) without carbon nanoparticles, the developed monolithic capillary columns exhibited a higher extraction efficiency towards the analytes which can be ascribed to the presence of the c-MWCNTs. Detection limits range from 0.03 to 0.6 µg/L, and the precision (expressed as relative standard deviation) is between 3.0 and 11.4 %. The proposed method was applied to the determination of prometon, simazine, atrazine, propazine, terbumeton, secbumeton, simetryn, prometryn and terbutryn in tap and river waters, and the recoveries ranged from 80 to 113 %.

1. Pretreatment of the fused silica capillary

Pretreatment to favor the covalent binding of the monolithic phase to the capillary inner wall

2. Preparation of monolithic solid

20 wt% of monomers (25 wt% BA – 75 wt% EGDMA)

80 wt% of porogens (50 wt% Formamide + 50 wt% 2-PrOH)

0.5 wt% of LPO

Nitrogen stream 10 min

Sonication 20 min

Filling of capillary

To seal with a septum at both ends

70 °C 24 h

Variables studied	Interval	Value selected
Monomers/porogens ratio	20/80 - 80/20 % w/w	20/80 % w/w
BA/EDMA ratio	75/25 – 25/75 % w/w	25/75 % w/w

3. Immobilization of carboxylated multi-walled carbon nanotubes on the monolith

Variables studied	Interval	Value selected
Amount of the c-MWCNTs	0.001 – 0.05 g/L	0.01 g/L
Volume of dispersion of the c-MWCNTs	1 – 7.5 mL	1.5 mL

i. Methanol (10 min 0.2 mL/min)
ii. N$_2$ (10 min)
iii. Ethanolic solution of ethylenediamine 7% (v/v) (60 min, 50 µL/min)
iv. N$_2$ (10 min)

0.5 mL/min 5 min

Dispersion of 0.01 g/L of the c-MWCNTs

4. Microextraction procedure

1 Monolithic solid was conditioned with
- Methanol (10 min, 0.1 mL/min)
- N$_2$ (10 min)
- Milli-Q water (5 min, 0.3 mL/min)

2 Water sample (10 min, 0.2 mL/min)

3 Milli-Q water (2 min, 0.2 mL/min)

4 N$_2$ stream (10 min)

5 Elution with 200 µL of methanol (0.1 mL/min). Evaporation–reconstitution step (20 µL of methanol)

Variables studied	Interval	Value selected
Sample flow rate	0.3 – 0.5 mL/min	0.2 mL/min
Sample volume	1.5 – 5 ml	3 ml
Elution volume	100 – 500 µl	200 µl

Analytical features of merit

Analyte	m/z	LOD (µg/L)	LOQ (µg/L)	Precision		
				Intra-day RSD (%, n=3)	Inter-day RSD (%, n=5)	Inter- units RSD (%, n=5)
Prometon	210	0.30	1.0	9.4	11.4	6.8
Simazine	201	0.40	1.0	4.2	7.1	7.9
Atrazine	200	0.60	1.0	11.4	9.9	3.9
Propazine	214	0.60	1.0	6.0	5.3	8.2
Terbumeton	210	0.40	1.0	7.2	10.3	8.7
Secbumeton	196	0.03	0.1	3.0	7.4	10.0
Simetryn	213	0.03	0.1	4.1	8.6	9.0
Prometryn	241	0.03	0.1	5.9	8.1	13.8
Terbutryn	226	0.03	0.1	10.5	11.3	14.3

Analysis of water samples

Analyte	Tap water (%, n=3)	River water (%, n=3)
Prometon	107 ± 11	85 ± 8
Simazine	111 ± 9	95 ± 7
Atrazine	80 ± 7	91 ± 11
Propazine	97 ± 8	96 ± 3
Terbumeton	113 ± 15	85 ± 9
Secbumeton	89 ± 10	87 ± 16
Simetryn	99 ± 5	98 ± 8
Prometryn	81 ± 14	98 ± 14
Terbutryn	93 ± 5	101 ± 8

Conclusions

- A monolithic poly(BA-*co*-EGDMA-c-MWCNTs) solid was evaluated as sorbent for the preconcentration of nine target triazines from water samples.
- The monolithic solid was prepared in a fused silica capillary by in situ polymerization, and then carbon nanostructures were immobilized on its pores.
- Different types of carbon nanoparticles (o-SWNHs, c-SWCNTs and c-MWCNTs) were evaluated as sorbents, obtaining the best results for the poly(BA-*co*-EGDMA-c-MWCNTs) monolithic unit.
- This study demonstrates that the immobilization of c-MWCNTs onto the pore surface of poly(BA-*co*-EGDMA) monoliths, significantly increases the extraction efficiency for the target triazines.
- The proposed microextraction method shows favorable analytical features for the analytical problem selected.

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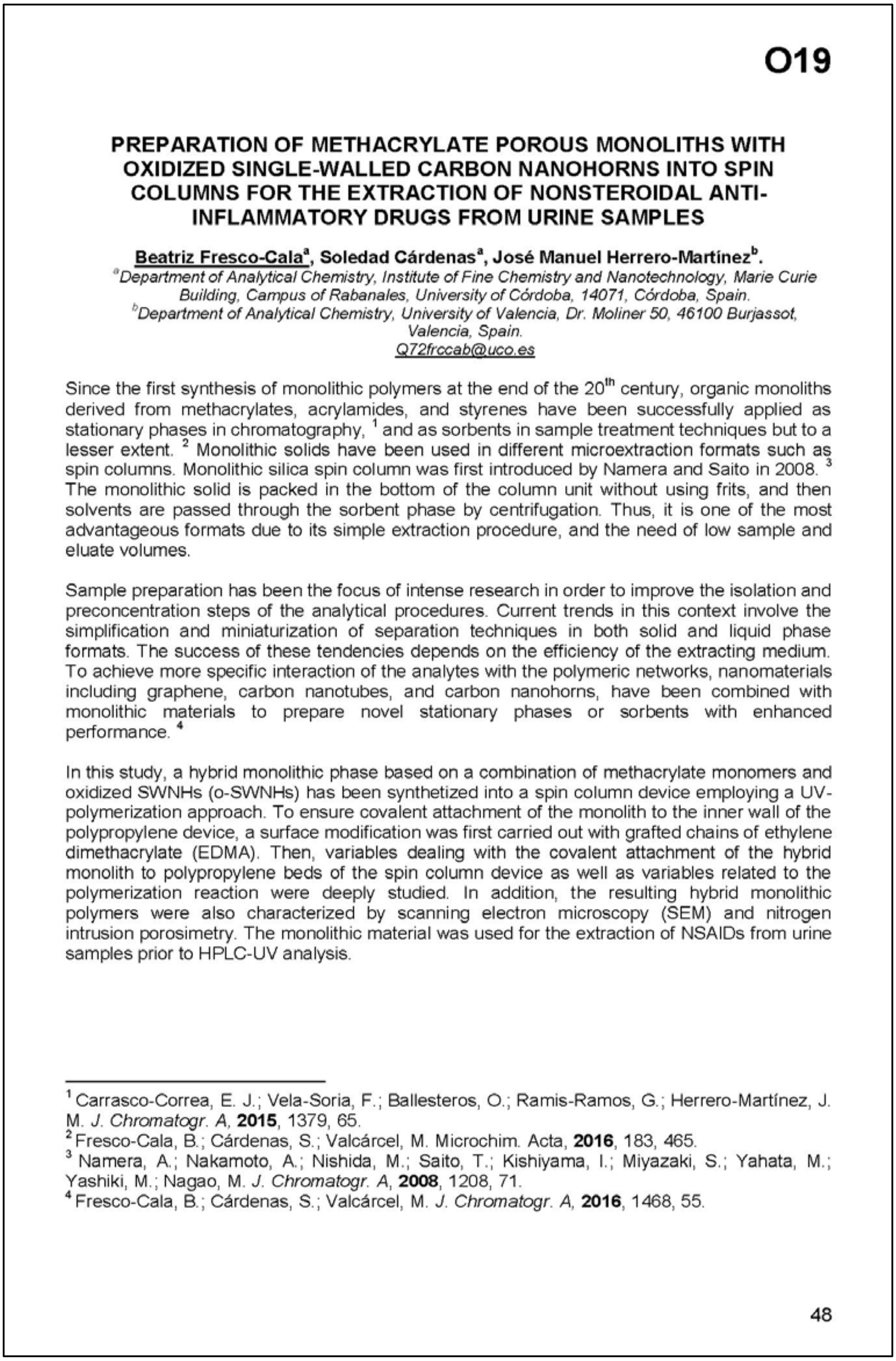

O19

PREPARATION OF METHACRYLATE POROUS MONOLITHS WITH OXIDIZED SINGLE-WALLED CARBON NANOHORNS INTO SPIN COLUMNS FOR THE EXTRACTION OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS FROM URINE SAMPLES

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Since the first synthesis of monolithic polymers at the end of the 20th century, organic monoliths derived from methacrylates, acrylamides, and styrenes have been successfully applied as stationary phases in chromatography, [1] and as sorbents in sample treatment techniques but to a lesser extent. [2] Monolithic solids have been used in different microextraction formats such as spin columns. Monolithic silica spin column was first introduced by Namera and Saito in 2008. [3] The monolithic solid is packed in the bottom of the column unit without using frits, and then solvents are passed through the sorbent phase by centrifugation. Thus, it is one of the most advantageous formats due to its simple extraction procedure, and the need of low sample and eluate volumes.

Sample preparation has been the focus of intense research in order to improve the isolation and preconcentration steps of the analytical procedures. Current trends in this context involve the simplification and miniaturization of separation techniques in both solid and liquid phase formats. The success of these tendencies depends on the efficiency of the extracting medium. To achieve more specific interaction of the analytes with the polymeric networks, nanomaterials including graphene, carbon nanotubes, and carbon nanohorns, have been combined with monolithic materials to prepare novel stationary phases or sorbents with enhanced performance. [4]

In this study, a hybrid monolithic phase based on a combination of methacrylate monomers and oxidized SWNHs (o-SWNHs) has been synthetized into a spin column device employing a UV-polymerization approach. To ensure covalent attachment of the monolith to the inner wall of the polypropylene device, a surface modification was first carried out with grafted chains of ethylene dimethacrylate (EDMA). Then, variables dealing with the covalent attachment of the hybrid monolith to polypropylene beds of the spin column device as well as variables related to the polymerization reaction were deeply studied. In addition, the resulting hybrid monolithic polymers were also characterized by scanning electron microscopy (SEM) and nitrogen intrusion porosimetry. The monolithic material was used for the extraction of NSAIDs from urine samples prior to HPLC-UV analysis.

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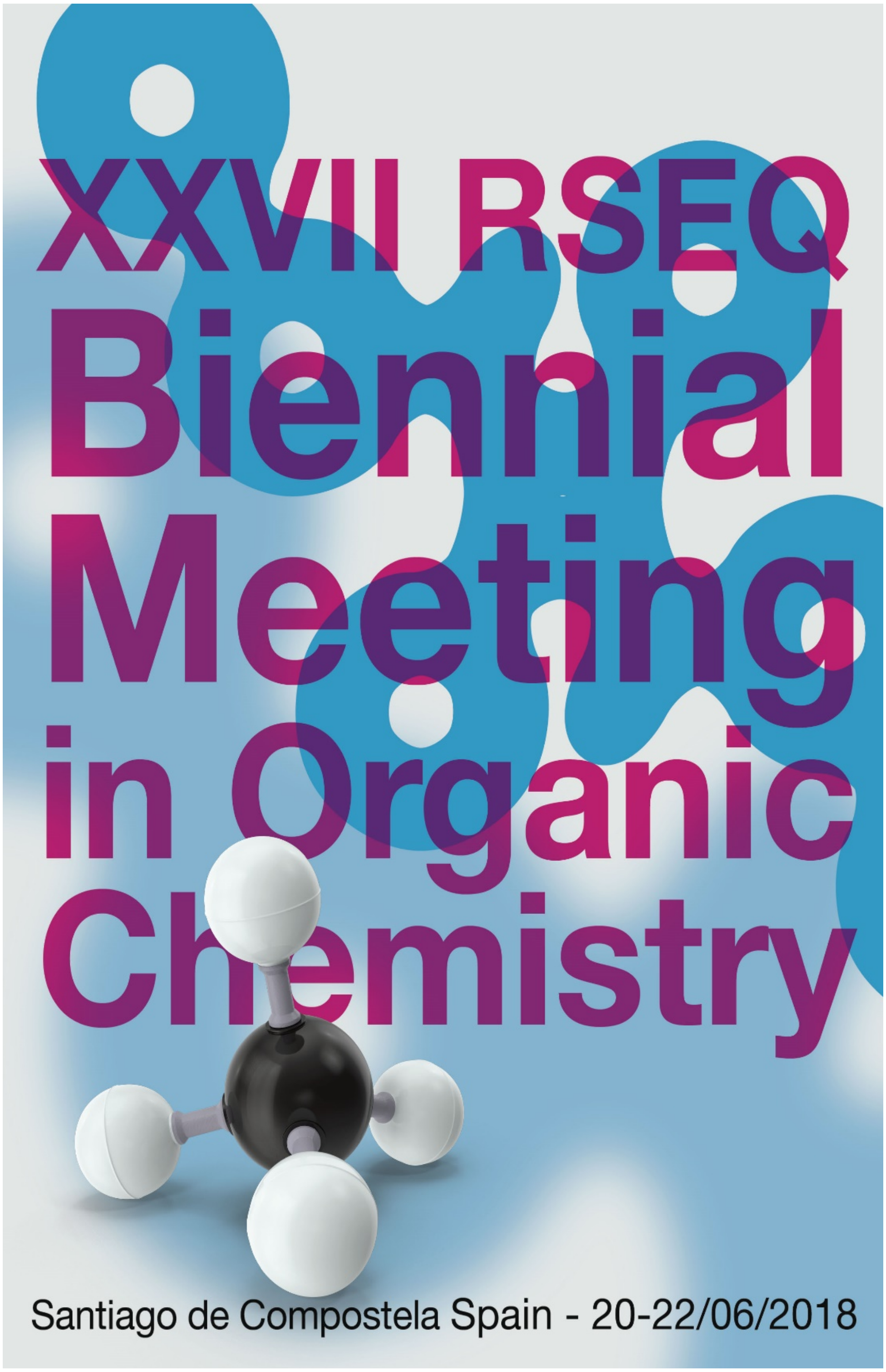
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2018 Lilly Thesis Award

LL1 • Effect of Carbon Nanohorns in the Polymerization of Methacrylate Monolithic Capillaries and their Application as Extractant Phases

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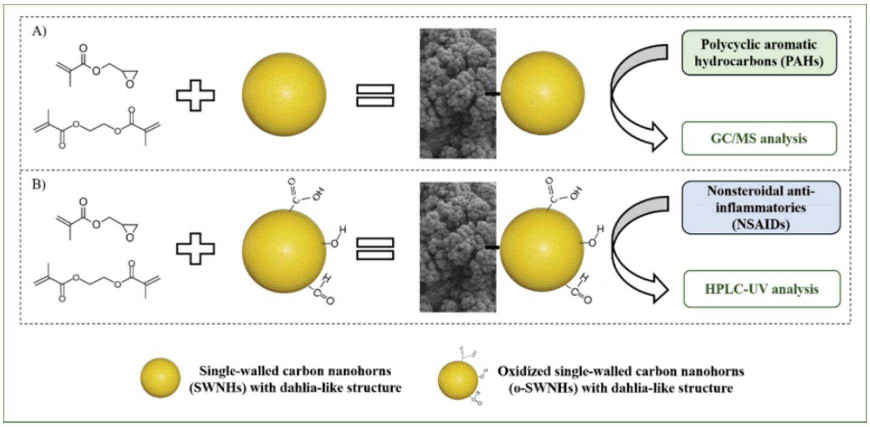
Keywords Carbon nanoparticles • conical nanostructures • hybrid monolith • microextraction • polycyclic aromatic hydrocarbons • nonsteroidal anti-inflammatory drugs

Monolithic solids are materials, which have a continuous and a highly porous structure, with excellent properties to be used them as stationary phases both in chromatographic and non-chromatographic techniques. In addition, it is worth noting that its easy preparation as well as the possibility of controlling the porosity of the final solid. However, their main disadvantage in comparison with other traditional sorbents is their low specific surface area. In this way, a new hybrid material based on the combination of a monolithic polymer and carbon nanostructures has been developed in order to solvent this limitation. In this context, a successful copolymerization of the single-walled carbon nanohorns (SWNHs) with methacrylate monomers was achieved via thermal initiated free-radical polymerization because of the high reactivity of the SWNHs over other carbon nanostructures. The effect of the incorporation of the bare or oxidized (o-SWNHs) carbon nanohorns at different percentages (0-0.5 wt%) in polymerization mixtures to obtain hybrid monolithic capillaries has been evaluated. In addition, their impact both in the polymerization step and in the extraction capacity was deeply studied. The hybrid solids were deeply characterized in terms of morphology, by scanning electron microscopy (SEM). Final hybrid monoliths were applied for the extraction of polycyclic aromatic hydrocarbons and nonsteroidal anti-inflammatory drugs in water and biological samples, respectively.



XIV REUNIÓN DEL GRUPO REGIONAL ANDALUZ DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA

BAEZA, 26 y 27 junio 2014

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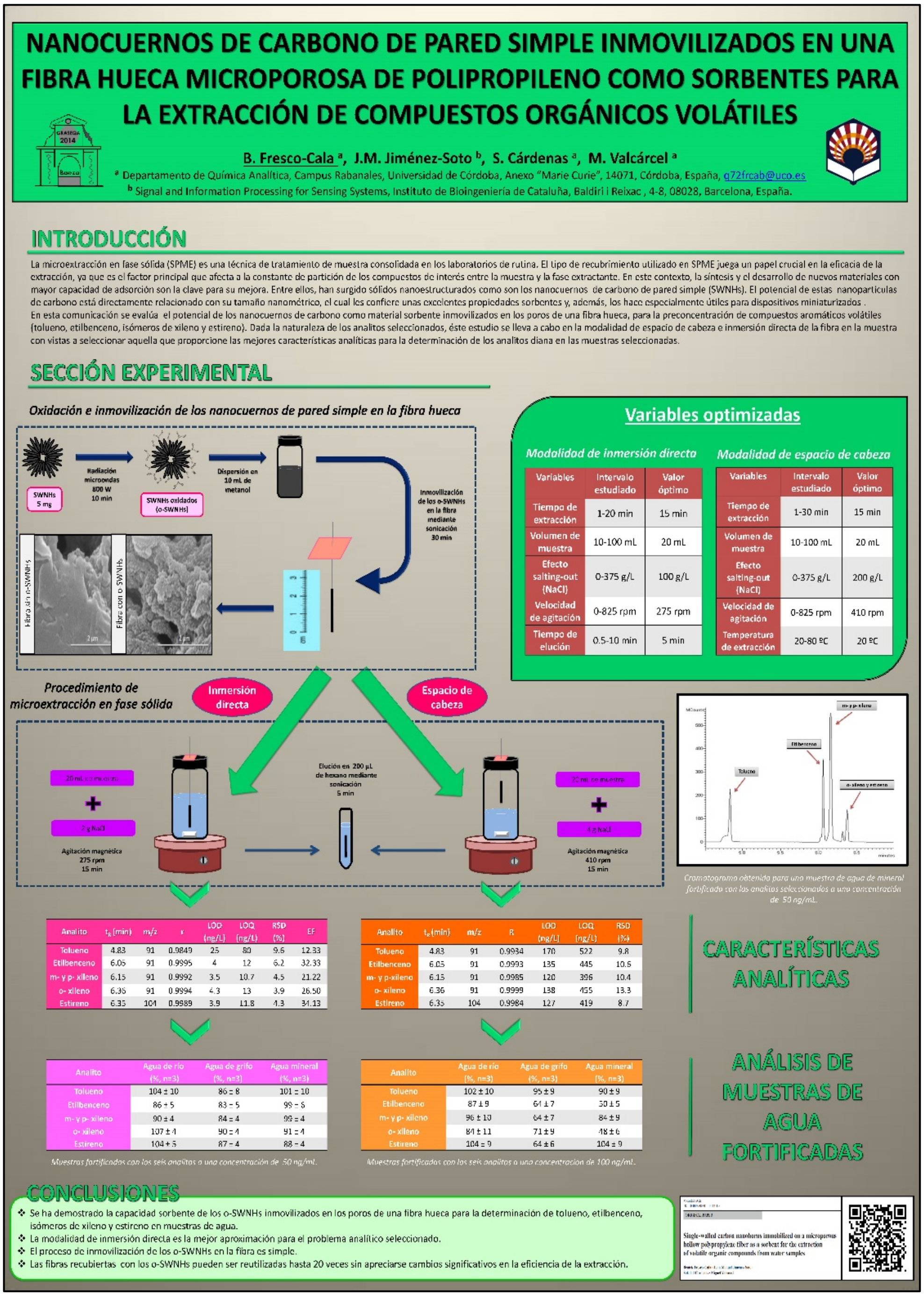

NANOCUERNOS DE CARBONO DE PARED SIMPLE INMOVILIZADOS EN UNA FIBRA HUECA MICROPOROSA DE POLIPROPILENO COMO SORBENTES PARA LA EXTRACCIÓN DE COMPUESTOS ORGÁNICOS VOLÁTILES

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INTRODUCCIÓN

La microextracción en fase sólida (SPME) es una técnica de tratamiento de muestra consolidada en los laboratorios de rutina. El tipo de recubrimiento utilizado en SPME juega un papel crucial en la eficacia de la extracción, ya que es el factor principal que afecta a la constante de partición de los compuestos de interés entre la muestra y la fase extractante. En este contexto, la síntesis y el desarrollo de nuevos materiales con mayor capacidad de adsorción son la clave para su mejora. Entre ellos, han surgido sólidos nanoestructurados como son los nanocuernos de carbono de pared simple (SWNHs). El potencial de estas nanopartículas de carbono está directamente relacionado con su tamaño nanométrico, el cual les confiere unas excelentes propiedades sorbentes y, además, los hace especialmente útiles para dispositivos miniaturizados .

En esta comunicación se evalúa el potencial de los nanocuernos de carbono como material sorbente inmovilizados en los poros de una fibra hueca, para la preconcentración de compuestos aromáticos volátiles (tolueno, etilbenceno, isómeros de xileno y estireno). Dada la naturaleza de los analitos seleccionados, éste estudio se lleva a cabo en la modalidad de espacio de cabeza e inmersión directa de la fibra en la muestra con vistas a seleccionar aquella que proporcione las mejores características analíticas para la determinación de los analitos diana en las muestras seleccionadas.

SECCIÓN EXPERIMENTAL

Oxidación e inmovilización de los nanocuernos de pared simple en la fibra hueca

Variables optimizadas

Modalidad de inmersión directa

Variables	Intervalo estudiado	Valor óptimo
Tiempo de extracción	1-20 min	15 min
Volumen de muestra	10-100 mL	20 mL
Efecto salting-out (NaCl)	0-375 g/L	100 g/L
Velocidad de agitación	0-825 rpm	275 rpm
Tiempo de elución	0.5-10 min	5 min

Modalidad de espacio de cabeza

Variables	Intervalo estudiado	Valor óptimo
Tiempo de extracción	1-30 min	15 min
Volumen de muestra	10-100 mL	20 mL
Efecto salting-out (NaCl)	0-375 g/L	200 g/L
Velocidad de agitación	0-825 rpm	410 rpm
Temperatura de extracción	20-80 ºC	20 ºC

Procedimiento de microextracción en fase sólida

Cromatograma obtenido para una muestra de agua de mineral fortificada con los analitos seleccionados a una concentración de 50 ng/mL.

CARACTERÍSTICAS ANALÍTICAS

Analito	t_R (min)	m/z	r	LOD (ng/L)	LOQ (ng/L)	RSD (%)	EF
Tolueno	4.83	91	0.9849	25	80	9.6	12.33
Etilbenceno	6.05	91	0.9995	4	12	6.2	32.33
m- y p- xileno	6.15	91	0.9992	3.5	10.7	4.5	21.22
o- xileno	6.36	91	0.9994	4.3	13	3.9	26.50
Estireno	6.35	104	0.9989	3.9	11.8	4.3	34.13

Analito	t_R (min)	m/z	R	LOD (ng/L)	LOQ (ng/L)	RSD (%)
Tolueno	4.83	91	0.9934	170	522	9.8
Etilbenceno	6.05	91	0.9993	135	445	10.5
m- y p-xileno	6.15	91	0.9985	120	396	10.4
o- xileno	6.36	91	0.9999	138	455	13.3
Estireno	6.35	104	0.9984	127	419	8.7

ANÁLISIS DE MUESTRAS DE AGUA FORTIFICADAS

Analito	Agua de río (%, n=3)	Agua de grifo (%, n=3)	Agua mineral (%, n=3)
Tolueno	104 ± 10	86 ± 8	101 ± 10
Etilbenceno	86 ± 5	83 ± 5	99 ± 6
m- y p- xileno	90 ± 4	84 ± 4	99 ± 4
o- xileno	107 ± 4	90 ± 4	91 ± 4
Estireno	104 ± 5	87 ± 4	88 ± 4

Muestras fortificadas con los seis analitos a una concentración de 50 ng/mL.

Analito	Agua de río (%, n=3)	Agua de grifo (%, n=3)	Agua mineral (%, n=3)
Tolueno	102 ± 10	95 ± 9	90 ± 9
Etilbenceno	87 ± 9	64 ± 7	30 ± 5
m- y p- xileno	96 ± 10	64 ± 7	84 ± 9
o- xileno	84 ± 11	71 ± 9	48 ± 6
Estireno	104 ± 9	64 ± 6	104 ± 9

Muestras fortificadas con los seis analitos a una concentración de 100 ng/mL.

CONCLUSIONES

- Se ha demostrado la capacidad sorbente de los o-SWNHs inmovilizados en los poros de una fibra hueca para la determinación de tolueno, etilbenceno, isómeros de xileno y estireno en muestras de agua.
- La modalidad de inmersión directa es la mejor aproximación para el problema analítico seleccionado.
- El proceso de inmovilización de los o-SWNHs en la fibra es simple.
- Las fibras recubiertas con los o-SWNHs pueden ser reutilizadas hasta 20 veces sin apreciarse cambios significativos en la eficiencia de la extracción.

Single-walled carbon nanohorns immobilized on a microporous hollow polypropylene fiber as a sorbent for the extraction of volatile organic compounds from water samples

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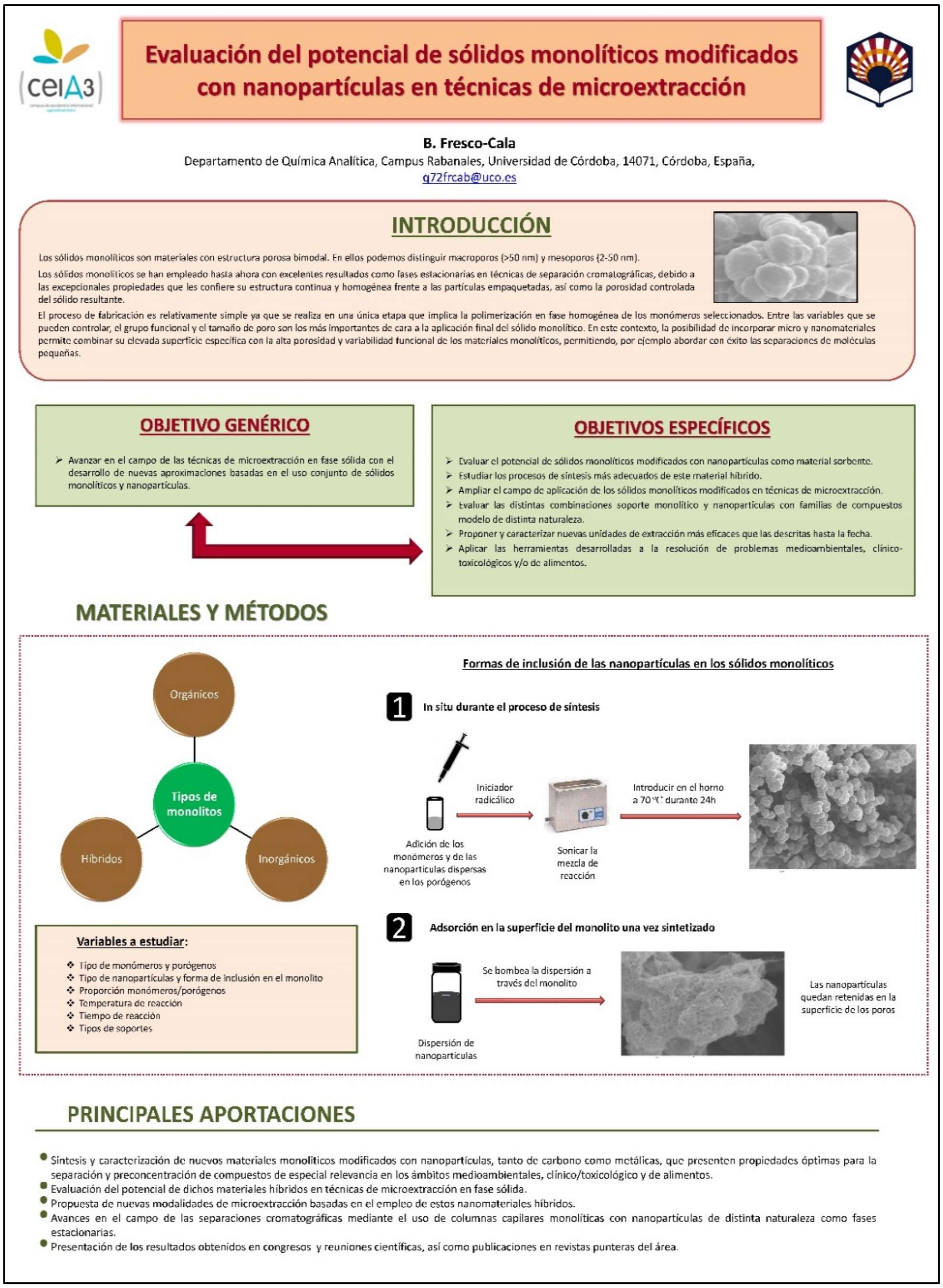

Evaluación del potencial de sólidos monolíticos modificados con nanopartículas en técnicas de microextracción

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INTRODUCCIÓN

Los sólidos monolíticos son materiales con estructura porosa bimodal. En ellos podemos distinguir macroporos (>50 nm) y mesoporos (2-50 nm).

Los sólidos monolíticos se han empleado hasta ahora con excelentes resultados como fases estacionarias en técnicas de separación cromatográficas, debido a las excepcionales propiedades que les confiere su estructura continua y homogénea frente a las partículas empaquetadas, así como la porosidad controlada del sólido resultante.

El proceso de fabricación es relativamente simple ya que se realiza en una única etapa que implica la polimerización en fase homogénea de los monómeros seleccionados. Entre las variables que se pueden controlar, el grupo funcional y el tamaño de poro son los más importantes de cara a la aplicación final del sólido monolítico. En este contexto, la posibilidad de incorporar micro y nanomateriales permite combinar su elevada superficie específica con la alta porosidad y variabilidad funcional de los materiales monolíticos, permitiendo, por ejemplo abordar con éxito las separaciones de moléculas pequeñas.

OBJETIVO GENÉRICO

- Avanzar en el campo de las técnicas de microextracción en fase sólida con el desarrollo de nuevas aproximaciones basadas en el uso conjunto de sólidos monolíticos y nanopartículas.

OBJETIVOS ESPECÍFICOS

- Evaluar el potencial de sólidos monolíticos modificados con nanopartículas como material sorbente.
- Estudiar los procesos de síntesis más adecuados de este material híbrido.
- Ampliar el campo de aplicación de los sólidos monolíticos modificados en técnicas de microextracción.
- Evaluar las distintas combinaciones soporte monolítico y nanopartículas con familias de compuestos modelo de distinta naturaleza.
- Proponer y caracterizar nuevas unidades de extracción más eficaces que las descritas hasta la fecha.
- Aplicar las herramientas desarrolladas a la resolución de problemas medioambientales, clínico-toxicológicos y/o de alimentos.

MATERIALES Y MÉTODOS

Variables a estudiar:

- Tipo de monómeros y porógenos
- Tipo de nanopartículas y forma de inclusión en el monolito
- Proporción monómeros/porógenos
- Temperatura de reacción
- Tiempo de reacción
- Tipos de soportes

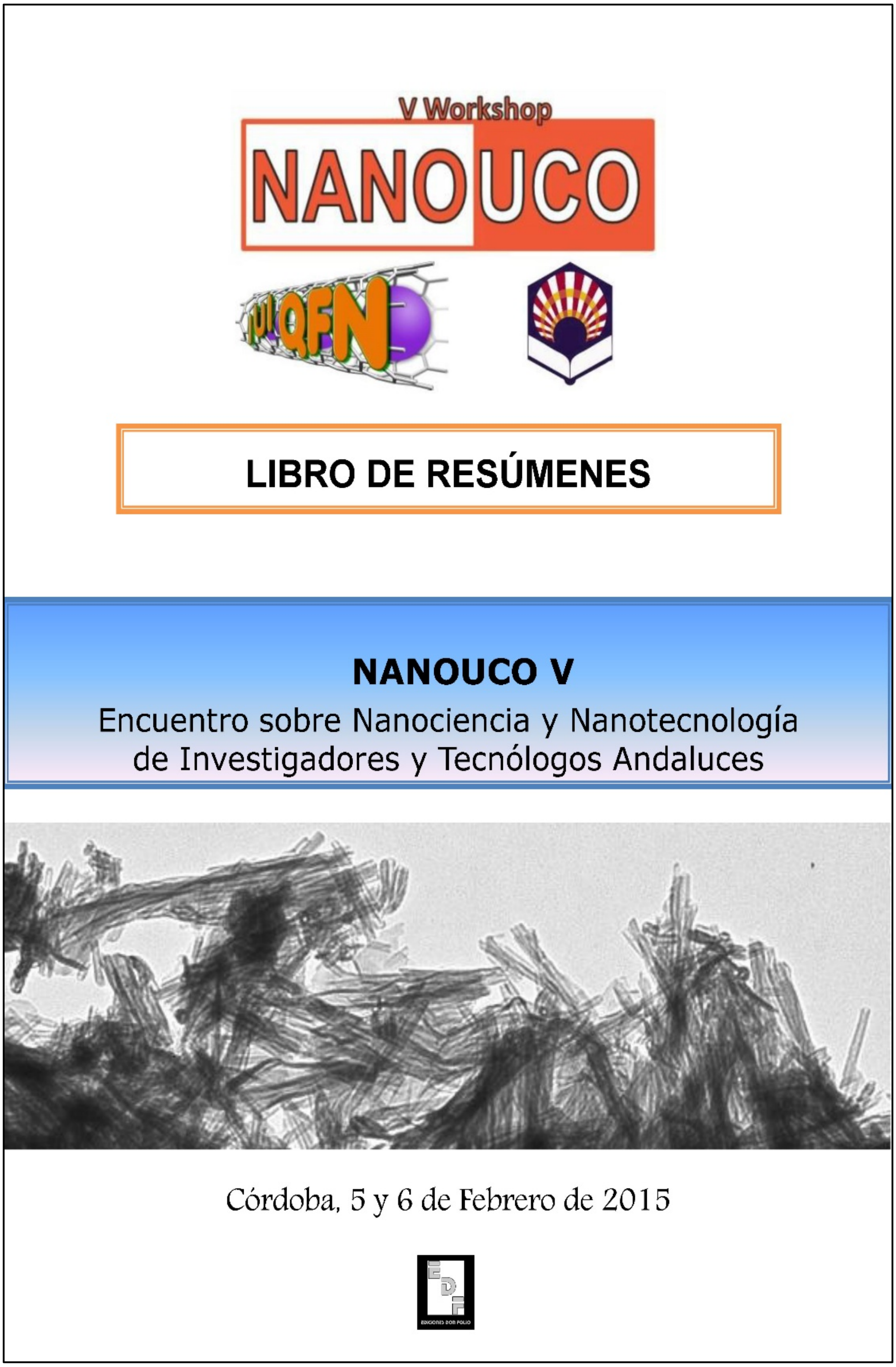
Formas de inclusión de las nanopartículas en los sólidos monolíticos

1 In situ durante el proceso de síntesis

2 Adsorción en la superficie del monolito una vez sintetizado

PRINCIPALES APORTACIONES

- Síntesis y caracterización de nuevos materiales monolíticos modificados con nanopartículas, tanto de carbono como metálicas, que presenten propiedades óptimas para la separación y preconcentración de compuestos de especial relevancia en los ámbitos medioambientales, clínico/toxicológico y de alimentos.
- Evaluación del potencial de dichos materiales híbridos en técnicas de microextracción en fase sólida.
- Propuesta de nuevas modalidades de microextracción basadas en el empleo de estos nanomateriales híbridos.
- Avances en el campo de las separaciones cromatográficas mediante el uso de columnas capilares monolíticas con nanopartículas de distinta naturaleza como fases estacionarias.
- Presentación de los resultados obtenidos en congresos y reuniones científicas, así como publicaciones en revistas punteras del área.



V Workshop

NANOUCO

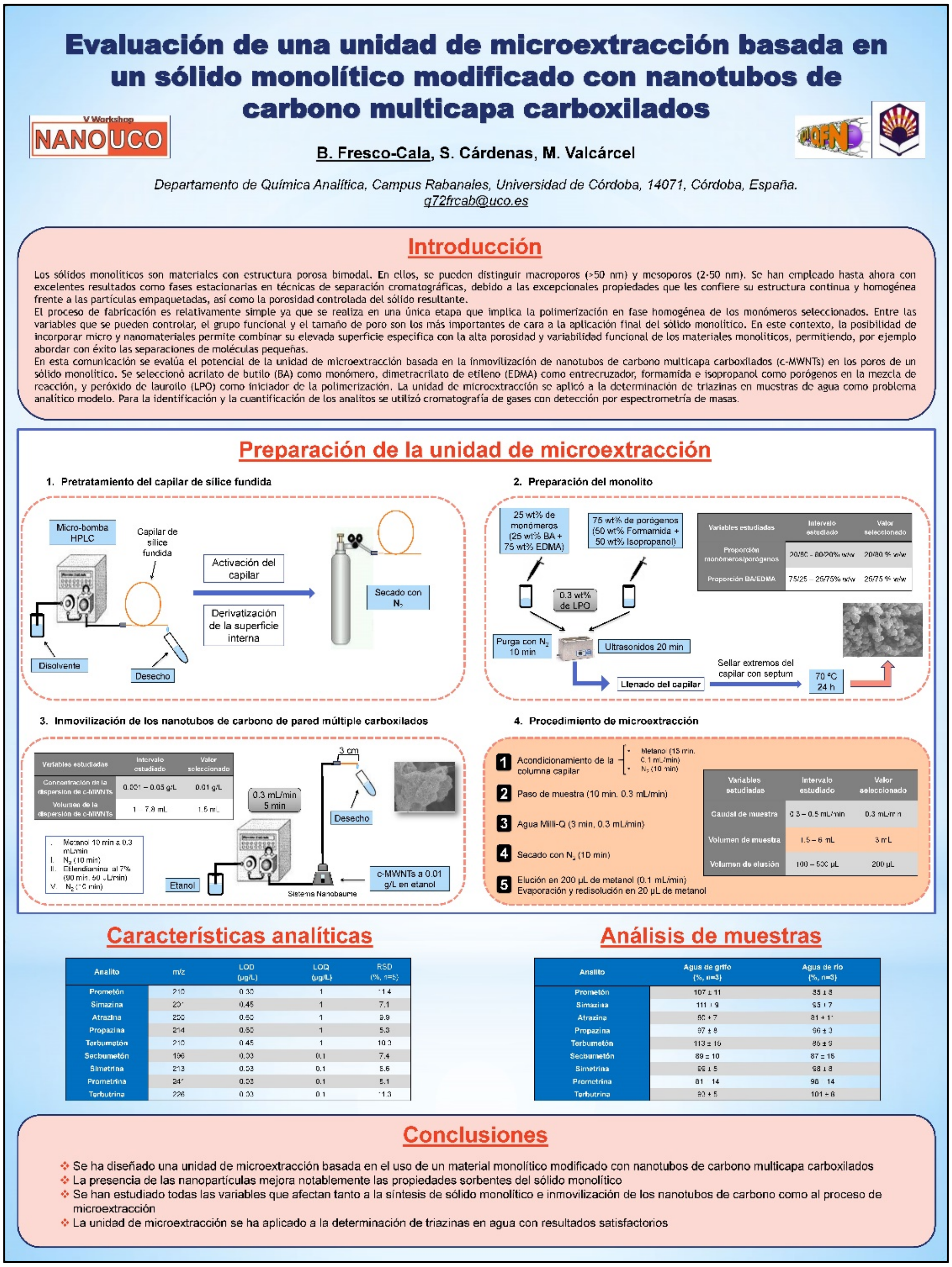
LIBRO DE RESÚMENES

NANOUCO V

Encuentro sobre Nanociencia y Nanotecnología
de Investigadores y Tecnólogos Andaluces

Córdoba, 5 y 6 de Febrero de 2015

EDICIONES DON FOLIO



Evaluación de una unidad de microextracción basada en un sólido monolítico modificado con nanotubos de carbono multicapa carboxilados

V Workshop NANOUCO

B. Fresco-Cala, S. Cárdenas, M. Valcárcel

Departamento de Química Analítica, Campus Rabanales, Universidad de Córdoba, 14071, Córdoba, España.
q72frcab@uco.es

Introducción

Los sólidos monolíticos son materiales con estructura porosa bimodal. En ellos, se pueden distinguir macroporos (>50 nm) y mesoporos (2-50 nm). Se han empleado hasta ahora con excelentes resultados como fases estacionarias en técnicas de separación cromatográficas, debido a las excepcionales propiedades que les confiere su estructura continua y homogénea frente a las partículas empaquetadas, así como la porosidad controlada del sólido resultante.

El proceso de fabricación es relativamente simple ya que se realiza en una única etapa que implica la polimerización en fase homogénea de los monómeros seleccionados. Entre las variables que se pueden controlar, el grupo funcional y el tamaño de poro son los más importantes de cara a la aplicación final del sólido monolítico. En este contexto, la posibilidad de incorporar micro y nanomateriales permite combinar su elevada superficie especifica con la alta porosidad y variabilidad funcional de los materiales monolíticos, permitiendo, por ejemplo abordar con éxito las separaciones de moléculas pequeñas.

En esta comunicación se evalúa el potencial de la unidad de microextracción basada en la inmovilización de nanotubos de carbono multicapa carboxilados (c-MWNTs) en los poros de un sólido monolítico. Se seleccionó acrilato de butilo (BA) como monómero, dimetracrilato de etileno (EDMA) como entrecruzador, formamida e isopropanol como porógenos en la mezcla de reacción, y peróxido de lauroilo (LPO) como iniciador de la polimerización. La unidad de microextracción se aplicó a la determinación de triazinas en muestras de agua como problema analítico modelo. Para la identificación y la cuantificación de los analitos se utilizó cromatografía de gases con detección por espectrometría de masas.

Preparación de la unidad de microextracción

1. Pretratamiento del capilar de sílice fundida

Micro-bomba HPLC
Capilar de sílice fundida
Activación del capilar
Derivatización de la superficie interna
Secado con N_2
Disolvente
Desecho

2. Preparación del monolito

25 wt% de monómeros (25 wt% BA + 75 wt% EDMA)
75 wt% de porógenos (50 wt% Formamida + 50 wt% Isopropanol)
0.3 wt% de LPO
Purga con N_2 10 min
Ultrasonidos 20 min
Llenado del capilar
Sellar extremos del capilar con septum
70 ºC 24 h

Variables estudiadas	Intervalo estudiado	Valor seleccionado
Proporción monómeros/porógenos	20/80 - 80/20% w/w	20/80 % w/w
Proporción BA/EDMA	75/25 – 25/75% w/w	25/75 % w/w

3. Inmovilización de los nanotubos de carbono de pared múltiple carboxilados

Variables estudiadas	Intervalo estudiado	Valor seleccionado
Concentración de la dispersión de c-MWNTs	0.001 – 0.05 g/L	0.01 g/L
Volumen de la dispersión de c-MWNTs	1 – 7.8 mL	1.5 mL

I. Metanol 10 min a 0.3 mL/min
II. N_2 (10 min)
III. Etilendiamina al 7% (90 min, 50 µL/min)
IV. N_2 (10 min)

3 cm
0.3 mL/min 5 min
Desecho
Etanol
Sistema Nanobaume
c-MWNTs a 0.01 g/L en etanol

4. Procedimiento de microextracción

1. Acondicionamiento de la columna capilar
   - Metanol (15 min, 0.1 mL/min)
   - N_2 (10 min)
2. Paso de muestra (10 min, 0.3 mL/min)
3. Agua Milli-Q (3 min, 0.3 mL/min)
4. Secado con N_2 (10 min)
5. Elución en 200 µL de metanol (0.1 mL/min)
   Evaporación y redisolución en 20 µL de metanol

Variables estudiadas	Intervalo estudiado	Valor seleccionado
Caudal de muestra	0.3 – 0.5 mL/min	0.3 mL/min
Volumen de muestra	1.5 – 6 mL	3 mL
Volumen de elución	100 – 500 µL	200 µL

Características analíticas

Analito	m/z	LOD (µg/L)	LOQ (µg/L)	RSD (%, n=5)
Prometón	210	0.30	1	11.4
Simazina	201	0.45	1	7.1
Atrazina	200	0.60	1	9.9
Propazina	214	0.60	1	5.3
Terbumetón	210	0.45	1	10.0
Secbumetón	196	0.03	0.1	7.4
Simetrina	213	0.03	0.1	8.6
Prometrina	241	0.03	0.1	5.1
Terbutrina	226	0.03	0.1	11.3

Análisis de muestras

Analito	Agua de grifo (%, n=3)	Agua de río (%, n=3)
Prometón	107 ± 11	85 ± 8
Simazina	111 ± 9	93 ± 7
Atrazina	90 ± 7	81 ± 11
Propazina	97 ± 8	96 ± 3
Terbumetón	113 ± 16	85 ± 9
Secbumetón	89 ± 10	87 ± 16
Simetrina	99 ± 5	98 ± 8
Prometrina	81 ± 14	96 ± 14
Terbutrina	90 ± 5	101 ± 8

Conclusiones

- Se ha diseñado una unidad de microextracción basada en el uso de un material monolítico modificado con nanotubos de carbono multicapa carboxilados
- La presencia de las nanopartículas mejora notablemente las propiedades sorbentes del sólido monolítico
- Se han estudiado todas las variables que afectan tanto a la síntesis de sólido monolítico e inmovilización de los nanotubos de carbono como al proceso de microextracción
- La unidad de microextracción se ha aplicado a la determinación de triazinas en agua con resultados satisfactorios

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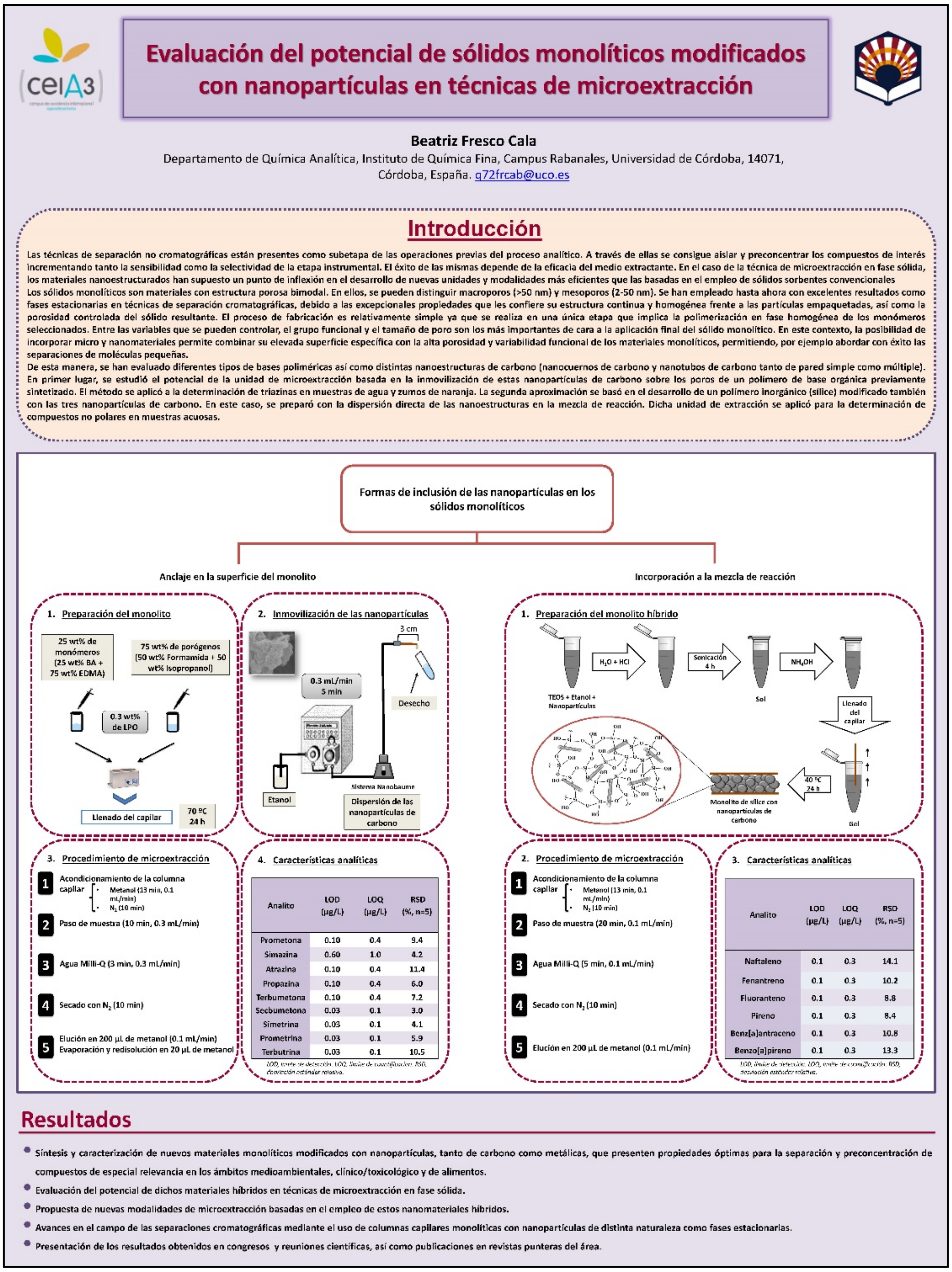
V Congreso Científico de Investigadores en Formación de la Universidad de Córdoba

CREANDO REDES – La generación del conocimiento

Córdoba, 30 de noviembre y 1 de diciembre de 2016



Evaluación del potencial de sólidos monolíticos modificados con nanopartículas en técnicas de microextracción

Beatriz Fresco Cala
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Córdoba, España. q72frcab@uco.es

Introducción

Las técnicas de separación no cromatográficas están presentes como subetapa de las operaciones previas del proceso analítico. A través de ellas se consigue aislar y preconcentrar los compuestos de interés incrementando tanto la sensibilidad como la selectividad de la etapa instrumental. El éxito de las mismas depende de la eficacia del medio extractante. En el caso de la técnica de microextracción en fase sólida, los materiales nanoestructurados han supuesto un punto de inflexión en el desarrollo de nuevas unidades y modalidades más eficientes que las basadas en el empleo de sólidos sorbentes convencionales

Los sólidos monolíticos son materiales con estructura porosa bimodal. En ellos, se pueden distinguir macroporos (>50 nm) y mesoporos (2-50 nm). Se han empleado hasta ahora con excelentes resultados como fases estacionarias en técnicas de separación cromatográficas, debido a las excepcionales propiedades que les confiere su estructura continua y homogénea frente a las partículas empaquetadas, así como la porosidad controlada del sólido resultante. El proceso de fabricación es relativamente simple ya que se realiza en una única etapa que implica la polimerización en fase homogénea de los monómeros seleccionados. Entre las variables que se pueden controlar, el grupo funcional y el tamaño de poro son los más importantes de cara a la aplicación final del sólido monolítico. En este contexto, la posibilidad de incorporar micro y nanomateriales permite combinar su elevada superficie específica con la alta porosidad y variabilidad funcional de los materiales monolíticos, permitiendo, por ejemplo abordar con éxito las separaciones de moléculas pequeñas.

De esta manera, se han evaluado diferentes tipos de bases poliméricas así como distintas nanoestructuras de carbono (nanocuernos de carbono y nanotubos de carbono tanto de pared simple como múltiple). En primer lugar, se estudió el potencial de la unidad de microextracción basada en la inmovilización de estas nanopartículas de carbono sobre los poros de un polímero de base orgánica previamente sintetizado. El método se aplicó a la determinación de triazinas en muestras de agua y zumos de naranja. La segunda aproximación se basó en el desarrollo de un polímero inorgánico (sílice) modificado también con las tres nanopartículas de carbono. En este caso, se preparó con la dispersión directa de las nanoestructuras en la mezcla de reacción. Dicha unidad de extracción se aplicó para la determinación de compuestos no polares en muestras acuosas.

Formas de inclusión de las nanopartículas en los sólidos monolíticos

Anclaje en la superficie del monolito

1. Preparación del monolito

25 wt% de monómeros (25 wt% BA + 75 wt% EDMA)

75 wt% de porógenos (50 wt% Formamida + 50 wt% Isopropanol)

0.3 wt% de LPO

Llenado del capilar

70 ºC
24 h

2. Inmovilización de las nanopartículas

3 cm

0.3 mL/min
5 min

Desecho

Etanol

Sistema Nanobaume

Dispersión de las nanopartículas de carbono

3. Procedimiento de microextracción

1. Acondicionamiento de la columna capilar
    - Metanol (13 min, 0.1 mL/min)
    - N_2 (10 min)
2. Paso de muestra (10 min, 0.3 mL/min)
3. Agua Milli-Q (3 min, 0.3 mL/min)
4. Secado con N_2 (10 min)
5. Elución en 200 µL de metanol (0.1 mL/min)
Evaporación y redisolución en 20 µL de metanol

4. Características analíticas

Analito	LOD (µg/L)	LOQ (µg/L)	RSD (%, n=5)
Prometona	0.10	0.4	9.4
Simazina	0.60	1.0	4.2
Atrazina	0.10	0.4	11.4
Propazina	0.10	0.4	6.0
Terbumetona	0.10	0.4	7.2
Secbumetona	0.03	0.1	3.0
Simetrina	0.03	0.1	4.1
Prometrina	0.03	0.1	5.9
Terbutrina	0.03	0.1	10.5

LOD, límite de detección; LOQ, límite de cuantificación; RSD, desviación estándar relativa.

Incorporación a la mezcla de reacción

1. Preparación del monolito híbrido

TEOS + Etanol + Nanopartículas

H_2O + HCl

Sonicación
4 h

Sol

NH_4OH

Llenado del capilar

Gel

40 ºC
24 h

Monolito de sílice con nanopartículas de carbono

2. Procedimiento de microextracción

1. Acondicionamiento de la columna capilar
    - Metanol (13 min, 0.1 mL/min)
    - N_2 (10 min)
2. Paso de muestra (20 min, 0.1 mL/min)
3. Agua Milli-Q (5 min, 0.1 mL/min)
4. Secado con N_2 (10 min)
5. Elución en 200 µL de metanol (0.1 mL/min)

3. Características analíticas

Analito	LOD (µg/L)	LOQ (µg/L)	RSD (%, n=5)
Naftaleno	0.1	0.3	14.1
Fenantreno	0.1	0.3	10.2
Fluoranteno	0.1	0.3	8.8
Pireno	0.1	0.3	8.4
Benz[a]antraceno	0.1	0.3	10.8
Benzo[a]pireno	0.1	0.3	13.3

LOD, límite de detección; LOQ, límite de cuantificación; RSD, desviación estándar relativa.

Resultados

- Síntesis y caracterización de nuevos materiales monolíticos modificados con nanopartículas, tanto de carbono como metálicas, que presenten propiedades óptimas para la separación y preconcentración de compuestos de especial relevancia en los ámbitos medioambientales, clínico/toxicológico y de alimentos.
- Evaluación del potencial de dichos materiales híbridos en técnicas de microextracción en fase sólida.
- Propuesta de nuevas modalidades de microextracción basadas en el empleo de estos nanomateriales híbridos.
- Avances en el campo de las separaciones cromatográficas mediante el uso de columnas capilares monolíticas con nanopartículas de distinta naturaleza como fases estacionarias.
- Presentación de los resultados obtenidos en congresos y reuniones científicas, así como publicaciones en revistas punteras del área.

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VI Congreso Científico de Investigadores en Formación de la Universidad de Córdoba

CREANDO REDES – La generación del conocimiento

Córdoba, 18 y 19 de enero de 2018

Monolithic solids based on carbon nanoparticles as solid extraction phases

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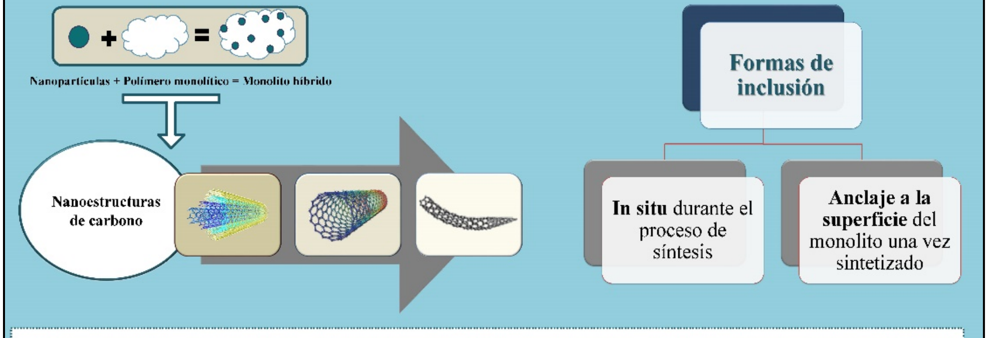
Introducción

Las técnicas de separación no cromatográficas están presentes como subetapa de las operaciones previas del proceso analítico. A través de ellas se consigue aislar y preconcentrar los compuestos de interés incrementando tanto la sensibilidad como la selectividad de la etapa instrumental. El éxito de las mismas depende de la eficacia del medio extractante. En el caso de la técnica de microextracción en fase sólida, los materiales nanoestructurados han supuesto un punto de inflexión en el desarrollo de nuevas unidades y modalidades más eficientes que las basadas en el empleo de sólidos sorbentes convencionales.

Los sólidos monolíticos son materiales con estructura porosa bimodal. En ellos, se pueden distinguir macroporos (>50 nm) y mesoporos (2-50 nm). Se han empleado hasta ahora con excelentes resultados como fases estacionarias en técnicas de separación cromatográficas, debido a las excepcionales propiedades que les confiere su estructura continua y homogénea frente a las partículas empaquetadas, así como la porosidad controlada del sólido resultante. El proceso de fabricación es relativamente simple ya que se realiza en una única etapa que implica la polimerización en fase homogénea de los monómeros seleccionados. Entre las variables que se pueden controlar, el grupo funcional y el tamaño de poro son los más importantes de cara a la aplicación final del sólido monolítico. En este contexto, la posibilidad de incorporar micro y nanomateriales permite combinar su elevada superficie específica con la alta porosidad y variabilidad funcional de los materiales monolíticos, permitiendo, por ejemplo abordar con éxito las separaciones de moléculas pequeñas.



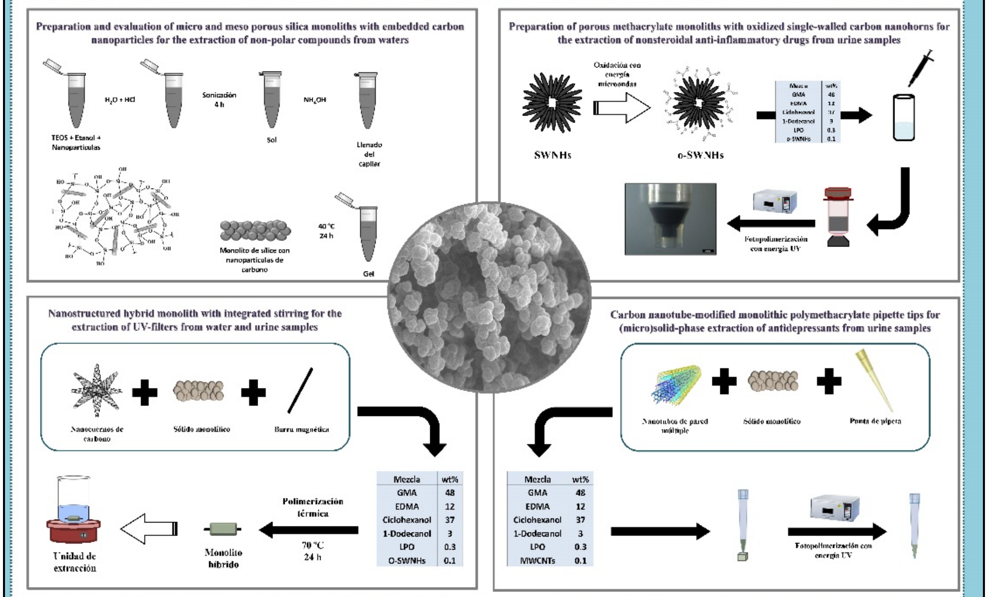
Nanopartículas + Polímero monolítico = Monolito híbrido

Nanoestructuras de carbono

Formas de inclusión

In situ durante el proceso de síntesis

Anclaje a la superficie del monolito una vez sintetizado



Preparation and evaluation of micro and meso porous silica monoliths with embedded carbon nanoparticles for the extraction of non-polar compounds from waters

Preparation of porous methacrylate monoliths with oxidized single-walled carbon nanohorns for the extraction of nonsteroidal anti-inflammatory drugs from urine samples

Mezcla	wt%
GMA	48
EDMA	12
Ciclohexanol	37
1-Dodecanol	3
LPO	0.3
o-SWNHs	0.1

Nanostructured hybrid monolith with integrated stirring for the extraction of UV-filters from water and urine samples

Mezcla	wt%
GMA	48
EDMA	12
Ciclohexanol	37
1-Dodecanol	3
LPO	0.3
O-SWNHs	0.1

Carbon nanotube-modified monolithic polymethacrylate pipette tips for (micro)solid-phase extraction of antidepressants from urine samples

Mezcla	wt%
GMA	48
EDMA	12
Ciclohexanol	37
1-Dodecanol	3
LPO	0.3
MWCNTs	0.1

Anexo D

Actividades de Divulgación Científica

Scientific Divulgation

1. Participación en la Actividad “Paseo por la Ciencia”.

Universidad de Córdoba. 2013, 2015 y 2017.

2. Ponente en las Jornadas de Café con Ciencia.

Universidad de Córdoba. 2014, 2015, 2016 y 2017.

3. Participación en el Comité Organizador del IV Congreso Científico de Investigadores en Formación de la Universidad de Córdoba y III Congreso Científico de Investigadores en Formación en Agroalimentación ceiA3.

Universidad de Córdoba. Noviembre-2014.

4. Asistencia a las III Jornadas de Divulgación Científica “#divulgA3. Más allá de los papers”.

Universidad de Córdoba. Marzo-2015 y 2016.

5. Asistencia al XVII Seminario Internacional de Ciencia, Periodismo y Medioambiente.

Enresa. Octubre-2015.

6. Participación en las Novenas Jornadas de Introducción al Laboratorio Experimental de Química para Alumnos de Bachillerato.

Departamento de Química Analítica, Universidad de Córdoba. Enero-2016.

7. Participación en las Actividades de Difusión de las Líneas de Investigación e Infraestructuras del Departamento de Química Analítica.

Plan Integral de Difusión de la Facultad de Ciencias y Plan Anual de Captación de Estudiantes (PACE) de la Universidad de Córdoba. Curso 2015-2016.

8. Participación en las Actividades de Difusión de las Líneas de Investigación e Infraestructuras del Departamento de Química Analítica.

Programa ORIENTAUCO. Curso 2015-2016.

9. Participación en las Jornadas de Introducción al Laboratorio Experimental de la Facultad de Ciencias (X Jornadas de Química).

III Plan Anual de Captación de Estudiantes (PACE). Curso 2016-2017.

10. Ponente en las Jornadas de la Noche Europea de los Investigadores.

Universidad de Córdoba. Septiembre-2016 y 2017.

11. Ponente en la Actividad “Espacios de Ciencia”.

Programa “Semana de la Ciencia 2016” dentro del III Plan Anual de Divulgación Científica. Unidad de Cultura Científica de la Universidad de Córdoba (UCC). Curso 2016-2017.

12. Ponente en la Actividad “Ingenios en ruta”.

IV y V Plan Anual de Divulgación Científica. Unidad de Cultura Científica de la Universidad de Córdoba (UCC). Curso 2016-2017 y 2017-2018.

Anexo E

Actividades de Innovación Docente

Teaching Innovation

1. **Participación a las II Jornadas sobre Estrategias para la Innovación de la Actividad Docente en Química Analítica: Contenidos y Herramientas.**

Alcalá de Henares, España. Julio-2016.

2. **Participación en Proyecto de Innovación Docente en la Modalidad 2: Proyectos de Innovación para Formación en Innovación Docente.**

Implementación de *Personal Response Systems* en asignaturas de los grados de Química y Bioquímica. Curso académico 2016-2017.

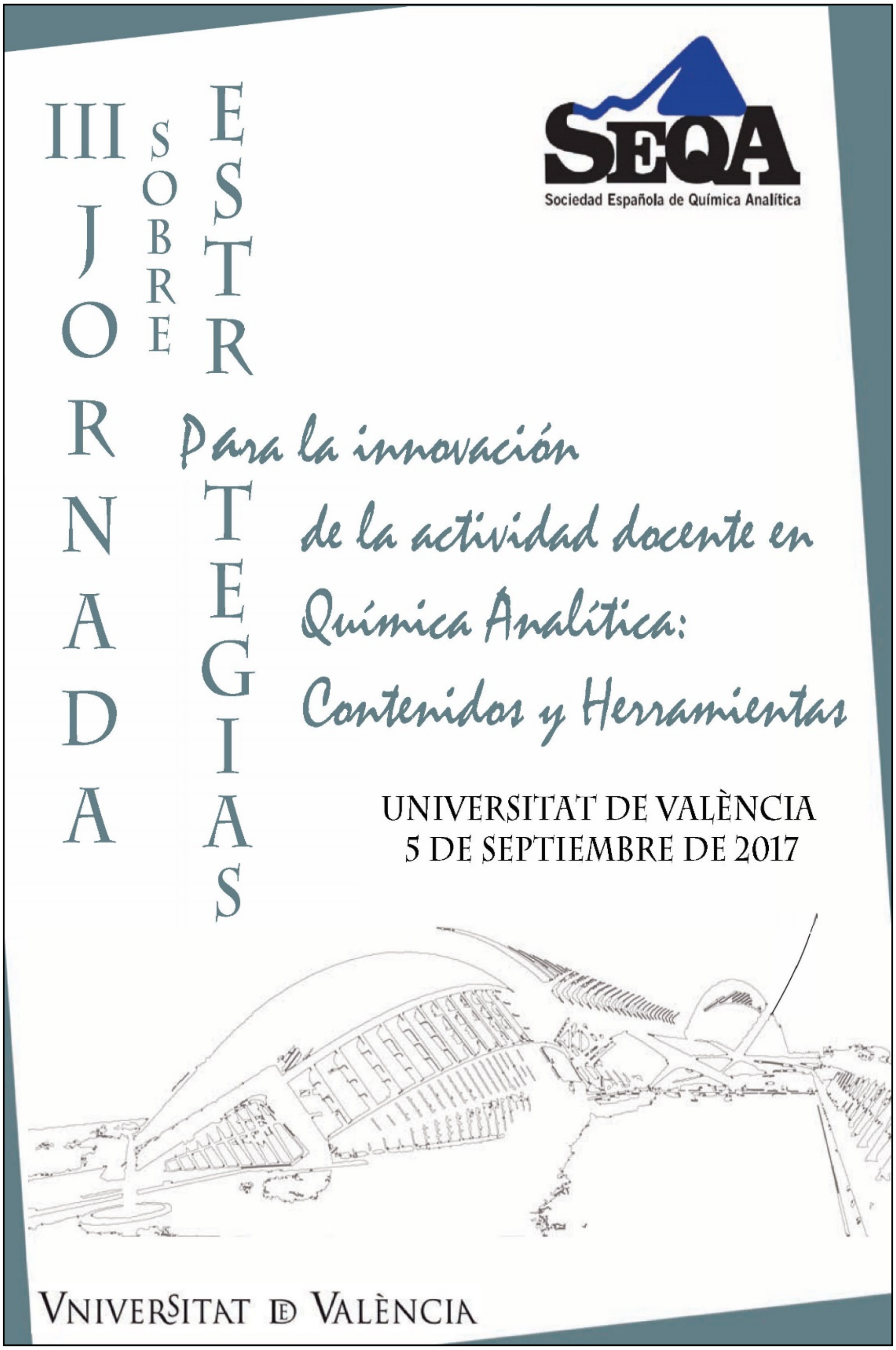
3. **Comunicación Póster y Oral "*Implementación de Personal Response Systems en asignaturas de los grados de Química y Bioquímica*" en las III Jornadas sobre Estrategias para la Innovación de la Actividad Docente en Química Analítica: Contenidos y Herramientas.** Valencia, España. Septiembre-2017.

Salatti-Dorado, J.A.; Fresco-Cala B.; López, M.I.; Ríos-Gómez, J.; Cárdenas, S.; Fernández-Romero J.M.; Romero, F.J.

4. **Publicación en el Boletín de la Sociedad de Química Analítica (SEQA).** Número 59. Septiembre-2017.

Implementación de Personal Response Systems en asignaturas de los grados de Química y Bioquímica.

Salatti-Dorado, J.A.; Fresco-Cala B.; López, M.I.; Ríos-Gómez, J.; Cárdenas, S.; Fernández-Romero J.M.; Romero, F.J.



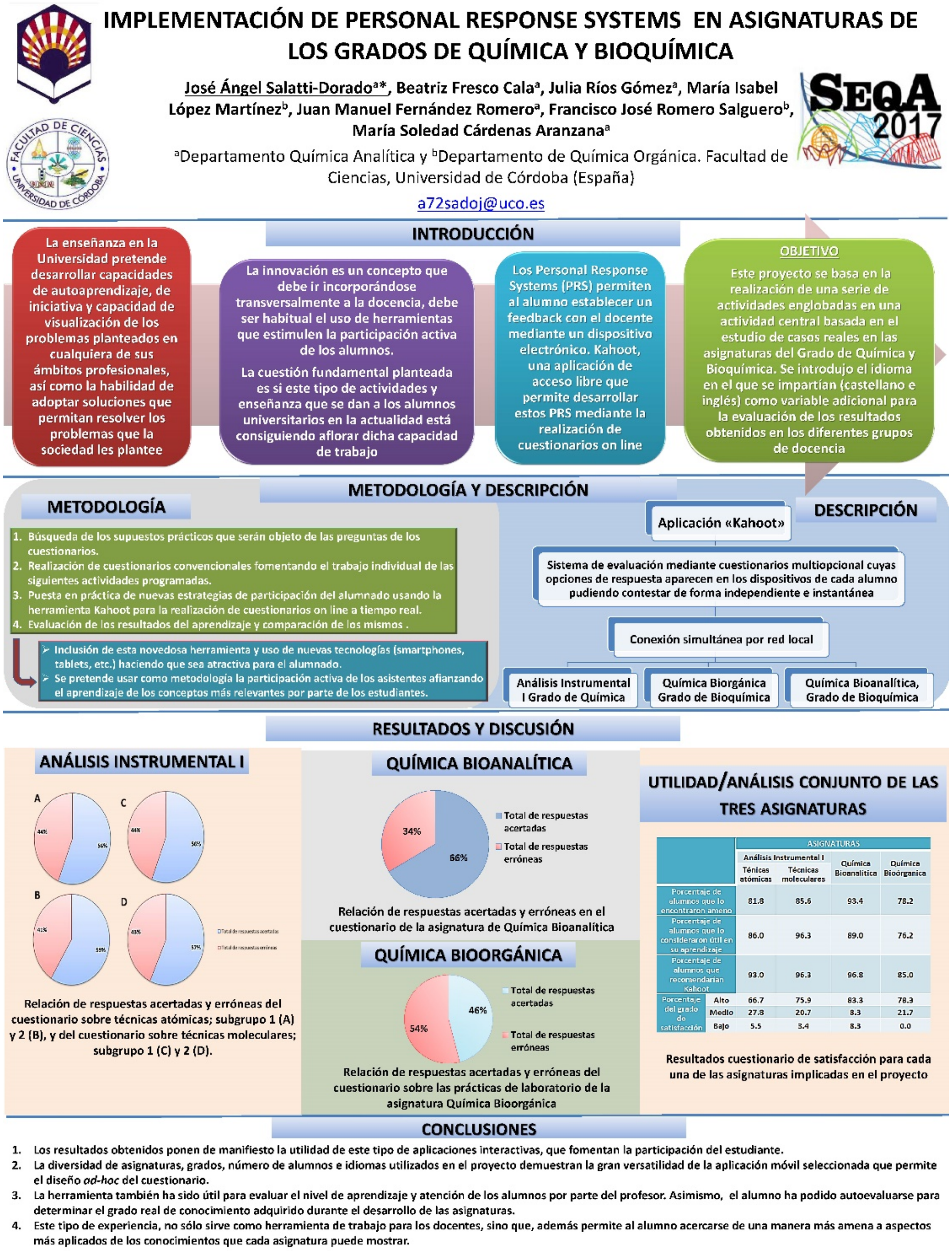
Sociedad Española de Química Analítica

III JORNADA SOBRE ESTRATEGIAS

para la innovación de la actividad docente en Química Analítica: Contenidos y Herramientas

UNIVERSITAT DE VALÈNCIA
5 DE SEPTIEMBRE DE 2017

VNIVERSITAT DE VALÈNCIA



IMPLEMENTACIÓN DE PERSONAL RESPONSE SYSTEMS EN ASIGNATURAS DE LOS GRADOS DE QUÍMICA Y BIOQUÍMICA

José Ángel Salatti-Dorado[a]*, Beatriz Fresco Cala[a], Julia Ríos Gómez[a], María Isabel López Martínez[b], Juan Manuel Fernández Romero[a], Francisco José Romero Salguero[b], María Soledad Cárdenas Aranzana[a]

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INTRODUCCIÓN

La enseñanza en la Universidad pretende desarrollar capacidades de autoaprendizaje, de iniciativa y capacidad de visualización de los problemas planteados en cualquiera de sus ámbitos profesionales, así como la habilidad de adoptar soluciones que permitan resolver los problemas que la sociedad les plantee

La innovación es un concepto que debe ir incorporándose transversalmente a la docencia, debe ser habitual el uso de herramientas que estimulen la participación activa de los alumnos.

La cuestión fundamental planteada es si este tipo de actividades y enseñanza que se dan a los alumnos universitarios en la actualidad está consiguiendo aflorar dicha capacidad de trabajo

Los Personal Response Systems (PRS) permiten al alumno establecer un feedback con el docente mediante un dispositivo electrónico. Kahoot, una aplicación de acceso libre que permite desarrollar estos PRS mediante la realización de cuestionarios on line

OBJETIVO

Este proyecto se basa en la realización de una serie de actividades englobadas en una actividad central basada en el estudio de casos reales en las asignaturas del Grado de Química y Bioquímica. Se introdujo el idioma en el que se impartían (castellano e inglés) como variable adicional para la evaluación de los resultados obtenidos en los diferentes grupos de docencia

METODOLOGÍA Y DESCRIPCIÓN

METODOLOGÍA

1. Búsqueda de los supuestos prácticos que serán objeto de las preguntas de los cuestionarios.
2. Realización de cuestionarios convencionales fomentando el trabajo individual de las siguientes actividades programadas.
3. Puesta en práctica de nuevas estrategias de participación del alumnado usando la herramienta Kahoot para la realización de cuestionarios on line a tiempo real.
4. Evaluación de los resultados del aprendizaje y comparación de los mismos .

- Inclusión de esta novedosa herramienta y uso de nuevas tecnologías (smartphones, tablets, etc.) haciendo que sea atractiva para el alumnado.
- Se pretende usar como metodología la participación activa de los asistentes afianzando el aprendizaje de los conceptos más relevantes por parte de los estudiantes.

DESCRIPCIÓN

Aplicación «Kahoot»

Sistema de evaluación mediante cuestionarios multiopcional cuyas opciones de respuesta aparecen en los dispositivos de cada alumno pudiendo contestar de forma independiente e instantánea

Conexión simultánea por red local

Análisis Instrumental I Grado de Química

Química Biorgánica Grado de Bioquímica

Química Bioanalítica, Grado de Bioquímica

RESULTADOS Y DISCUSIÓN

ANÁLISIS INSTRUMENTAL I

Relación de respuestas acertadas y erróneas del cuestionario sobre técnicas atómicas; subgrupo 1 (A) y 2 (B), y del cuestionario sobre técnicas moleculares; subgrupo 1 (C) y 2 (D).

QUÍMICA BIOANALÍTICA

Relación de respuestas acertadas y erróneas en el cuestionario de la asignatura de Química Bioanalítica

QUÍMICA BIOORGÁNICA

Relación de respuestas acertadas y erróneas del cuestionario sobre las prácticas de laboratorio de la asignatura Química Bioorgánica

UTILIDAD/ANÁLISIS CONJUNTO DE LAS TRES ASIGNATURAS

		ASIGNATURAS			
		Análisis Instrumental I		Química Bioanalítica	Química Bioórganica
		Ténicas atómicas	Técnicas moleculares		
Porcentaje de alumnos que lo encontraron ameno		81.8	85.6	93.4	78.2
Porcentaje de alumnos que lo consideraron útil en su aprendizaje		86.0	96.3	89.0	76.2
Porcentaje de alumnos que recomendarían Kahoot		93.0	96.3	96.8	85.0
Porcentaje del grado de satisfacción	Alto	66.7	75.9	83.3	78.3
	Medio	27.8	20.7	8.3	21.7
	Bajo	5.5	3.4	8.3	0.0

Resultados cuestionario de satisfacción para cada una de las asignaturas implicadas en el proyecto

CONCLUSIONES

1. Los resultados obtenidos ponen de manifiesto la utilidad de este tipo de aplicaciones interactivas, que fomentan la participación del estudiante.
2. La diversidad de asignaturas, grados, número de alumnos e idiomas utilizados en el proyecto demuestran la gran versatilidad de la aplicación móvil seleccionada que permite el diseño *ad-hoc* del cuestionario.
3. La herramienta también ha sido útil para evaluar el nivel de aprendizaje y atención de los alumnos por parte del profesor. Asimismo, el alumno ha podido autoevaluarse para determinar el grado real de conocimiento adquirido durante el desarrollo de las asignaturas.
4. Este tipo de experiencia, no sólo sirve como herramienta de trabajo para los docentes, sino que, además permite al alumno acercarse de una manera más amena a aspectos más aplicados de los conocimientos que cada asignatura puede mostrar.

P-1

Implementación de Personal Response Systems en asignaturas de los Grados de Química y Bioquímica

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[a]Departamento Química Analítica y [b]Departamento de Química Orgánica. Facultad de Ciencias, Universidad de Córdoba, Campus de Rabanales, 14071, Córdoba (España), a72sadoj@uco.es

La imagen de la docencia universitaria tradicional se ha visto modificada por los principios que definen el Espacio Europeo de Educación Superior. La enseñanza en la Universidad pretende desarrollar capacidades de autoaprendizaje, de iniciativa y capacidad de visualización de los problemas planteados en cualquiera de sus ámbitos profesionales, así como la habilidad de adoptar soluciones que permitan resolver los problemas que la sociedad les plantee. En este sentido, se requiere que el alumno adquiera las competencias para aplicar los conocimientos adquiridos en las clases magistrales a supuestos prácticos. Por parte del profesor, esto implica una adaptación de su metodología de manera que se incremente el trabajo más personalizado con los alumnos en los grupos de docencia reducidos. Por otra parte, la innovación es un concepto que debe ir incorporándose transversalmente a la docencia, es por ello que cada vez más habitualmente se están usando herramientas que estimulan la participación activa de los alumnos. La cuestión fundamental planteada es si realmente con este tipo de actividades y enseñanza que se dan a los alumnos universitarios en la actualidad se está consiguiendo aflorar dicha capacidad de trabajo autónomo y autocrítica.

Los Personal Response Systems (PRS) permiten al alumno establecer un feedback con el docente mediante un dispositivo electrónico donde se realiza una serie de preguntas y los resultados se obtienen e interpretan en tiempo real. Los primeros PRS fueron los denominados clickers, el problema que presenta este tipo de dispositivos es la necesidad de un software específico, conocimiento previo por parte del profesor y la necesidad de llevar al aula un material concreto. En 2013 aparece Kahoot, una página web de acceso libre que permite desarrollar la actividad descrita anteriormente, pero con algunas ventajas añadidas: la realización de cuestionarios on line siendo solo necesario dispositivos electrónicos con acceso a internet que hoy en día todo el alumnado posee.

Se pretende con este proyecto de innovación docente disponer de una herramienta que permita conocer en tiempo real y de una forma simple si el alumno es capaz de responder a cuestiones sencillas, con un marcado carácter aplicado para evaluar el grado de comprensión de la materia. En este sentido, el uso de nuevas tecnologías, en concreto las aplicaciones de móvil parecen un entorno amigable para los alumnos que les predispondrán favorablemente al desarrollo de esta actividad. Este proyecto se basa en la realización de una serie de actividades englobadas en una actividad central basada en el estudio de casos reales relacionados con diferentes temáticas las cuales pertenecen al área de estudio en el que se basa dicha asignatura. Con dicha actividad los alumnos podrán de una manera muy positiva incrementar tanto su capacidad de síntesis así como su “madurez laboral” en aquellos ámbitos en los cuales se basa en enfrentamiento de un problema en un ambiente laboral real. El cuestionario se aplicó en asignaturas del Grado de Química y Bioquímica en las preguntas se realizaron sobre aspecto teóricos y prácticos de las mismas así como sobre

ACTUALIDAD ANALÍTICA

SEQA
Sociedad Española de Química Analítica

BOLETÍN
de la
Sociedad Española de Química Analítica

SEQA 2017
XXI Reunión de la Sociedad Española de Química Analítica
Valencia, 5-7 Septiembre 2017

III Jornada sobre estrategias para la innovación de la actividad docente en Química Analítica: Contenidos y Herramientas
UNIVERSITAT DE VALÈNCIA
5 DE SEPTIEMBRE DE 2017

Grupo de Especiación Analítica
VNIVERSITAT DE VALÈNCIA
Pre-symposium on speciation and regulations in food

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IMPLEMENTACIÓN DE *PERSONAL RESPONSE SYSTEMS* EN ASIGNATURAS DE LOS GRADOS DE QUÍMICA Y BIOQUÍMICA

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La enseñanza en la Universidad pretende, entre otros objetivos, desarrollar capacidades de autoaprendizaje, iniciativa, y capacidad de visualización de los problemas planteados en cualquiera de sus ámbitos profesionales, así como la capacidad de adoptar soluciones que permitan resolver los problemas que la sociedad plantea. La imagen de la docencia universitaria tradicional se ha visto modificada por los principios que definen el Espacio Europeo de Educación Superior. La enseñanza en la Universidad pretende desarrollar capacidades de autoaprendizaje, de iniciativa y capacidad de visualización de los problemas planteados en cualquiera de sus ámbitos profesionales, así como la habilidad de adoptar soluciones que permitan resolver los problemas que la sociedad les plantee. En este sentido, se requiere que el alumno adquiera las competencias para aplicar los conocimientos adquiridos en las clases magistrales a supuestos prácticos. Por parte del profesor, esto implica una adaptación de su metodología de manera que se incremente el trabajo más personalizado con los alumnos en los grupos de docencia reducidos. Por otra parte, la innovación es un concepto que debe ir incorporándose transversalmente a la docencia, es por ello que cada vez más habitualmente se están usando herramientas que estimulan la participación activa de los alumnos. La cuestión fundamental planteada es si realmente con este tipo de actividades y enseñanza que se dan a los alumnos universitarios en la actualidad se está consiguiendo aflorar dicha capacidad de trabajo autónomo y autocrítica.

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A lo largo del curso académico 2016-2017 se ha usado dicha herramienta para el seguimiento de los conocimientos adquiridos por diferentes grupos de alumnos pertenecientes a distintos niveles y asignaturas. Este sistema ha permitido la comparación de los resultados obtenidos tanto dentro de una misma clase como entre diferentes tipos de docencia presencial (prácticas de laboratorio y/o seminarios teóricos y prácticos), en grupos de docencia en castellano e inglés. Se pretende disponer de una herramienta que permita conocer en tiempo real y de una forma simple si el alumno es capaz de responder a cuestiones sencillas, con un marcado carácter aplicado para evaluar el grado de comprensión de la materia. En este sentido, el uso de nuevas tecnologías, en concreto las aplicaciones de móvil parecen un entorno amigable para los alumnos que les predispondrán favorablemente al desarrollo de esta actividad. Se basa en la realización de una serie de actividades englobadas en una actividad central basada en el estudio de casos reales relacionados con diferentes temáticas que pertenecen al área de estudio de dicha asignatura. Con esta actividad los alumnos podrán de una manera muy positiva incrementar tanto su capacidad de síntesis como su “madurez laboral” en aquellas cuestiones basadas en la resolución de un problema en un ambiente laboral real. El cuestionario se aplicó en asignaturas del Grado de Química y Bioquímica y las preguntas se realizaron sobre aspectos teóricos y prácticos de las mismas así como sobre exposiciones preparadas por los alumnos. También se introdujo el idioma en el que se impartían (castellano e inglés) como variable adicional para la evaluación de los resultados obtenidos en los diferentes grupos de docencia. El plan de trabajo a seguir establece una serie de pautas, que se van uniendo y autoalimentándose a lo largo de período de duración de cada asignatura. El plan de trabajo se concreta en los siguientes aspectos:

- Búsqueda de los supuestos prácticos que serán objeto de las preguntas de los cuestionarios.
- Realización de una serie de cuestionarios convencionales que se llevarán a cabo en diferentes sesiones conforme se vaya impartiendo la materia. Dichas sesiones fomentarán el trabajo individual, siendo de esta manera preparativas de las siguientes actividades programadas.
- Puesta en práctica de nuevas estrategias de participación del alumnado. Consistirá en una sesión final donde se usará la herramienta Kahoot para la realización de un cuestionario *online* a tiempo real donde todos los alumnos participarán individual y activamente. Las preguntas de dicho cuestionario serán extraídas en un porcentaje significativo de los cuestionarios realizados durante la primera etapa.
- Evaluación de los resultados del aprendizaje y comparación de estos con los del nivel alcanzado por cada estudiante para detectar mejoras en aquellos aspectos que se hayan identificado como deficientes en las PRS. Por tanto, la inclusión de esta herramienta y la

compatibilidad con las nuevas tecnologías (smartphones, tablets, etc.) hacen que esta actividad sea atractiva para el alumnado. Con este nuevo formato de docencia se pretende usar como metodología la participación activa de los asistentes, que hará que el aprendizaje de los conceptos más relevantes de la materia sean más fácilmente asimilables por parte de los estudiantes.

Los resultados obtenidos en cada asignatura impartida para la realización de este proyecto se muestran a continuación en las diferentes figuras realizadas.

ANÁLISIS INSTRUMENTAL I

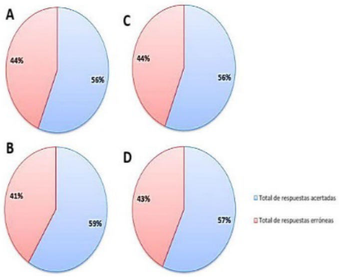

Figura 1. Relación de respuestas acertadas y erróneas del cuestionario sobre técnicas atómicas; subgrupo 1 (A) y 2 (B), y del cuestionario sobre técnicas moleculares; subgrupo 1 (C) y 2 (D).

QUÍMICA BIOANALÍTICA

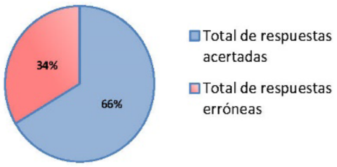

Figura 2. Relación de respuestas acertadas y erróneas en elcuestionario de la asignatura de Química Bioanalítica.

QUÍMICA BIOORGÁNICA

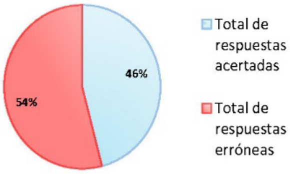

Figura 3. Relación de respuestas acertadas y erróneas del cuestionario sobre las prácticas de laboratorio de la asignatura Química Bioorgánica.

El menor porcentaje de aciertos en la asignatura de Química Bioorgánica puede deberse a la dificultad añadida de la realización del cuestionario en inglés..

La utilidad de este proyecto se ha determinado mediante el cuestionario de satisfacción de la actividad, cuyos resultados pueden verse en la Tabla 1.

		ASIGNATURAS			
		Análisis Instrumental I		Química Bioanalítica	Química Bioórganica
		Ténicas atómicas	Técnicas moleculares		
Porcentaje de alumnos que lo encontraron ameno		81.8	85.6	93.4	78.2
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	Medio	27.8	20.7	8.3	21.7
	Bajo	5.5	3.4	8.3	0.0

Tabla 1. Resultados cuestionario de satisfacción para cada una de las asignaturas implicadas en el proyecto.

A modo de conclusión se puede decir que el análisis de los resultados obtenidos permite poner de manifiesto la utilidad de este tipo de aplicaciones interactivas, que fomentan la participación del estudiante. El modelo se ha aplicado a diferentes asignaturas de características educativas muy variadas tanto en el tipo de alumnos (estudiantes de los grados de Química y Bioquímica de diferentes cursos), la lengua utilizada para la impartición de la misma (inglés y español) y número de alumnos. Esto ha sido posible dada la versatilidad de la aplicación móvil que permite el diseño *ad-hoc* del cuestionario. La herramienta también ha sido útil para evaluar el nivel de aprendizaje y atención de los alumnos por parte del profesor. Asimismo, el alumno ha podido autoevaluarse para determinar el grado real de conocimiento adquirido durante el desarrollo de las asignaturas. En nuestra opinión, este tipo de experiencia, no sólo sirve como herramienta de trabajo para los docentes, sino que, además permite al alumno acercarse de una manera más amena a aspectos más aplicados de los conocimientos que cada asignatura puede mostrar.

Agradecimientos: Los autores agradecen el apoyo de la Universidad de Córdoba (UCO) por la concesión del proyecto 2016-2-2005, Modalidad 2, en el marco del Programa de Formación para la Innovación Docente (Curso 2016/2017).