Universidad
de Córdoba

Departamento de
Química Analítica

EMPLEO DE NANOPARTÍCULAS HÍBRIDAS EN EL CONTEXTO DE LAS TÉCNICAS DE MICROEXTRACCIÓN

THE USE OF NANOCOMPOSITES IN THE MICROEXTRACTION CONTEXT

TESIS DOCTORAL

Emilia Mª Reyes Gallardo

Córdoba, 2017

TITULO: *EMPLEO DE NANOPARTÍCULAS HÍBRIDAS EN EL CONTEXTO DE LAS TÉCNICAS DE MICROEXTRACCIÓN*

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EMPLEO DE NANOPARTÍCULAS HÍBRIDAS EN EL CONTEXTO DE LAS TÉCNICAS DE MICROEXTRACCIÓN

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Trabajo presentado para aspirar al Grado de Doctor en Ciencias

LA DOCTORANDA,

Fdo. **Emilia Mª Reyes Gallardo**

Mª Soledad Cárdenas Aranzana, Catedrática del Departamento de Química Analítica de la Universidad de Córdoba; **Rafael Lucena Rodríguez**, Profesor Contratado Doctor del Departamento de Química Analítica de la Universidad de Córdoba, en calidad de directores de la Tesis Doctoral presentada por la Licenciada Emilia Mª Reyes Gallardo,

CERTIFICAN:

Que la Tesis Doctoral **"EMPLEO DE NANOPARTÍCULAS HÍBRIDAS EN EL CONTEXTO DE LAS TÉCNICAS DE MICROEXTRACCIÓN"** ha sido desarrollada en los laboratorios del Departamento de Química Analítica de la Universidad de Córdoba (España), y en el Departamento de Textiles de la Universidad de Gante (Bélgica), y que, a nuestro juicio, reúne todos los requisitos exigidos a este tipo de trabajo.

Que Emilia Mª Reyes Gallardo 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 los directores para evaluar y discutir los resultados obtenidos durante la Tesis Doctoral.

Y para que conste y surta los efectos pertinentes, expiden el presente certificado en la ciudad de Córdoba, a 10 de marzo de 2017.

Mª Soledad Cárdenas Aranzana **Rafael Lucena Rodríguez**

Mª Soledad Cárdenas Aranzana, Full Professor of Analytical Chemistry Department of the University of Córdoba; **Rafael Lucena Rodríguez**, Professor (Contratado Doctor) of Analytical Chemistry Department of the University of Córdoba, in quality of supervisors of the Doctoral Thesis of Emilia Mª Reyes Gallardo.

CERTIFIES THAT:

The Doctoral Thesis entitled **"THE USE OF NANOCOMPOSITES IN THE MICROEXTRACTION CONTEXT"** has been developed in the laboratories of the Department of Analytical Chemistry of the University of Córdoba (Spain) and in the Department of Textiles of the University of Ghent (Belgium), and, according to our judgement the Thesis fulfils all the requirements of this type of scientific work.

Emilia Mª Reyes Gallardo 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, 10th March 2017.

Mª Soledad Cárdenas Aranzana **Rafael Lucena Rodríguez**

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TÍTULO DE LA TESIS: Empleo de nanopartículas híbridas en el contexto de las técnicas de microextracción

DOCTORANDO/A: Emilia Mª Reyes Gallardo

INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

La licenciada Emilia Mª Reyes Gallardo cursó con distinción la Licenciatura de Ciencias Químicas y el máster en Química Fina Avanzada. En el año 2013 accedió a una beca de formación de profesorado universitario (FPU) para la realización de la Tesis Doctoral, cuya Memoria se presentará para su defensa bajo la modalidad de doctorado internacional y como compendio de publicaciones.

Su Tesis Doctoral evalúa el potencial de los nanocomposites poliméricos en el tratamiento analítico de la muestra. En ella, se diseñan métodos sencillos de síntesis de nanocomposites que después de ser caracterizados mediante diversas técnicas instrumentales se han aplicado para la extracción de analitos de diversa naturaleza en muestras ambientales, bioquímicas y de alimentos. El trabajo experimental se ha materializado en 7 artículos científicos, cinco de ellos experimentales. Además ha publicado dos artículos de divulgación científica. La doctoranda ha asistido a diez congresos nacionales e internacionales presentando un total de 11 comunicaciones en formato oral, flash o cartel. A lo largo de estos años, ha adquirido formación en técnicas de microextracción, tratamiento de muestras de agua, fluidos biológicos y alimentos para la determinación de distintas familias de compuestos. Entre las técnicas analíticas cabe destacar la cromatografía, tanto de líquidos como de gases, y la espectrometría de masas. La doctoranda ha desarrollado destrezas en la caracterización de materiales y se ha formado en el empleo de las técnicas microscópicas (microscopía electrónica de transmisión y de barrido) y espectroscópicas (infrarrojo).

Además de las competencias técnicas inherentes al trabajo en el laboratorio, ha demostrado iniciativa, capacidad para la resolución de problemas, planificación y dirección del trabajo a investigadores noveles así como para la innovación en la investigación. De hecho, ha llevado a cabo la supervisión de tres estudiantes, dos de ellos extranjeros, que se ha materializado en 4 artículos publicados o en vías de publicación.

La doctoranda ha colaborado en la impartición de clases prácticas en los Grados de Química y de Ciencia y Tecnología de los Alimentos durante los cursos académicos 2015/2016 y 2016/2017. Además, ha participado en la dirección de un trabajo fin de grado. De esta forma ha adquirido las competencias docentes asociadas a la beca de formación del profesorado universitario.

Además, ha participado en numerosas actividades de divulgación científica como "La noche europea de los investigadores", "Café con ciencia", "Paseo por la ciencia", "Jornadas de Introducción al laboratorio experimental de Química para alumnos de bachillerato" o "Espacios de ciencia".

Finalmente, y para poder optar a la mención de doctorado internacional, ha realizado una estancia de tres meses en Gante (Bélgica) bajo la supervisión de la profesora Karen de Clerck.

Por todo ello consideramos que la Tesis Doctoral cumple con los requisitos de calidad y novedad exigibles a este tipo de trabajo, por lo que se autoriza la presentación de la misma.

Córdoba, a 10 de marzo de 2017

Firma de los directores

Mª Soledad Cárdenas Aranzana

Rafael Lucena Rodríguez

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 Departamento de Textiles de la Universidad de Gante (Bélgica) gracias a la concesión de una ayuda para estancias en el extranjero de la Universidad de Córdoba.

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. Todas ellas se refieren a la categoría de Química Analítica (excepto para la revista de RSC Advances, que se encuentra en la categoría de Química Multidisciplinar).

Revista	Año	Factor de impacto	Cuartil
Journal of Chromatography A	2013	4,258	1
Journal of Chromatography A	2014	4,169	1
Bioanalysis	2015	2,813	2
Microchemical Journal	2016*	2,893	1
Trends in Analytical Chemistry	2016*	7,487	1
RSC Advances	2017*	3,289	2

** Para los datos de 2016 y 2017 se usaron los correspondientes a 2015.*

Otras publicaciones relacionadas con la Tesis Doctoral, en las que la doctoranda figura como segunda firmante.

Revista	Año	Factor de impacto	Cuartil
Journal of Chromatography A	2015	3,926	1
Bioanalysis	2016*	2,813	2

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

La única manera de descubrir los límites de lo posible es aventurarse un
poco más allá de ellos en lo imposible.

Arthur C. Clark

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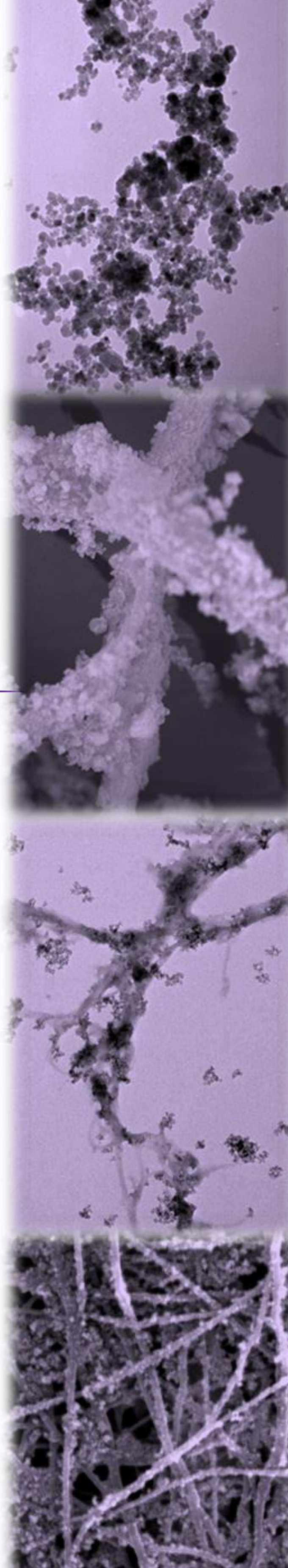
OBJETO

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. Dentro de las etapas del proceso de medida químico, cabe resaltar la de las operaciones previas, ya que debido a sus características intrínsecas así como a las dificultades que implica, la hacen una de las más importantes. Además, esta etapa se encuentra directamente relacionada con la calidad de los resultados generados.

Como consecuencia, uno de los retos que debe afrontar la Química Analítica en la actualidad está relacionado con las nuevas técnicas de tratamiento de muestra que permitan el desarrollo de metodologías analíticas más sencillas, automatizadas y miniaturizadas. Así, surgen las técnicas de microextracción que mejoran tanto la sensibilidad como la selectividad de las determinaciones gracias a la preconcentración de los analitos y a su aislamiento de la matriz de la muestra.

La investigación actual no sólo está centrada en el desarrollo de nuevas técnicas de microextracción, sino también en la síntesis de nuevos materiales sorbentes que posean unas altas capacidades extractivas. En este contexto, los materiales híbridos o composites, que son aquellos que combinan dos o más tipos de componentes, juegan un papel trascendental. Los objetivos de estos materiales pueden ser potenciar una determinada propiedad o combinar varias propiedades en un mismo material. Uno de los acoplamientos más versátiles en este área se produce al combinar polímeros con nanopartículas, ya que éstas pueden incrementar las capacidades extractivas de los polímeros (mediante el aumento de su superficie o incluyendo alguna nueva funcionalidad) o conferirles nuevas propiedades (por ejemplo, carácter magnético).

Teniendo en cuenta lo expuesto anteriormente, el objetivo principal de la Tesis Doctoral que se presenta en esta Memoria ha sido el desarrollo de nuevos materiales sorbentes híbridos que combinen polímeros y nanopartículas. Estos materiales se emplearán en nuevas técnicas de microextracción para la determinación de diferentes compuestos en muestras de distinta naturaleza. A raíz de este objetivo general, surgen diferentes objetivos más específicos, que se presentan a continuación:

- Desarrollo de nuevos procedimientos de síntesis de materiales híbridos que combinen polímeros con nanopartículas, y que se caractericen por su simplicidad, bajo precio y por ser respetuosos con el medio ambiente.

- Caracterización de los materiales sintetizados mediante diversas técnicas instrumentales.

- Evaluación del potencial sorbente de dichos materiales en técnicas de microextracción en fase sólida.

- Desarrollo de nuevas metodologías analíticas que combinen las técnicas de microextracción desarrolladas con técnicas cromatográficas.

- Aplicación de las metodologías desarrolladas para la determinación de compuestos de interés en muestras medioambientales, biológicas y agroalimentarias.

Todos estos objetivos se han abordado de manera transversal en cada uno de los capítulos que constituyen la presente Tesis Doctoral.

Analytical Chemistry is the science that develops, optimizes and applies measurement processes to obtain information about the composition and the (bio)chemical nature of matter. Preliminary operations are among the most important procedures in the analytical process due to their intrinsic characteristics and associated difficulties. Moreover, this stage is directly related to the quality of the final analytical results.

As a consequence, the development of new sample treatment techniques that allow the simplification, automation and miniaturization of analytical methods is one of the hot research topics in the Analytical Chemistry context. Microextraction techniques were developed under these three main trends to improve both the sensitivity and the selectivity of the determinations thanks to the preconcentration and isolation of the analytes from the sample matrix.

The current research is not only focused on the development of new microextraction techniques but also on the synthesis of new sorbent materials with high extraction capacities. In this context, hybrid materials or composites, which are those that combine two or more types of components, play a transcendental role. The aims of these materials can be to enhance a certain property or combine several properties in the same material. One of the most versatile unions in this area occurs when combining polymers with nanoparticles, since nanoparticles can increase the extraction capacities of the polymers (by increasing their surface area or including some new functionalities) or providing them with new properties (for example, magnetism).

Taking all of this into account, the main objective of this Doctoral Thesis was the development of new hybrid sorbent materials combining polymers and nanoparticles. These materials have been employed in new microextraction techniques for the determination of a variety of compounds in samples of different nature. From this general aim, several specific objectives can be established, as it is presented below:

- The development of easy, cheap and green procedures to synthesize hybrid materials combining polymers and nanoparticles.

- The characterization of the synthesized materials using different instrumental techniques.

- The evaluation of the sorbent capacities of these materials in solid phase microextraction techniques.

- The development of new analytical methodologies combining the developed microextraction techniques and chromatographic techniques.

- The application of these methodologies for the determination of compounds of interest in environmental, biological and agri-food samples.

All these objectives have been treated transversally in each of the chapters included in this Doctoral Thesis.

BLOQUE I

NANOCOMPOSITES EN LAS TÉCNICAS DE MICROEXTRACCIÓN

Nanocomposites en las técnicas de microextracción

1. Introducción

La Química Analítica puede definirse como la ciencia que desarrolla, optimiza y aplica procesos de medida para obtener información sobre la composición y la naturaleza (bio)química de la materia [1].

El proceso de medida químico (PMQ) es el conjunto de etapas que separan el sistema objeto de estudio de los resultados expresados según los requerimientos del cliente. Generalmente consta de cuatro etapas:

- Muestreo
- Operaciones previas
- Medida y transducción de la señal
- Adquisición y tratamiento de datos

La primera de ellas, el muestreo, tiene como objetivo la selección de una o varias porciones representativas del sistema objeto de estudio para someterlas posteriormente al resto de las etapas del PMQ. La representatividad permitirá asignarle al sistema objeto de estudio el resultado obtenido al analizar la muestra.

Las operaciones previas incluyen un conjunto de procedimientos que tienen como objetivo adaptar la muestra a la técnica instrumental. Las características intrínsecas de esta etapa, así como las dificultades que implica, hacen que sea una de las más importantes, pues de ella depende la calidad de los resultados generados. Además, las operaciones previas influyen directamente sobre propiedades analíticas supremas (exactitud), así como básicas (precisión, selectividad y sensibilidad) y complementarias (rapidez, bajo costo y factores personales).

Al contrario de lo que ocurre en las dos últimas etapas del PMQ, donde el grado de automatización y simplificación alcanzado es elevado, las operaciones previas del proceso de medida siguen siendo el cuello de botella de la mayoría de las metodologías analíticas. Las siguientes características generales de las operaciones previas dan constancia de ello:

- Variabilidad. Hay muchos factores que afectan al desarrollo de las operaciones previas, como por ejemplo, la muestra, el analito o la información requerida. Por tanto existe una amplia variedad de operaciones previas, lo que conlleva la necesidad de diseñar y optimizar cada uno de los procesos analíticos y la dificultad de usar soluciones generales.

- Complejidad. Estas operaciones son complejas y difíciles de automatizar debido a que suelen ser procesos multietapa y con una alta participación humana. Esto las convierte en una gran fuente de errores, tanto accidentales como sistemáticos, que influyen directamente en la calidad de los resultados finales.

- Lentitud. Suelen representar entre el 70% y el 90% del tiempo de un PMQ completo.

- Dificultad de ser controladas. Requieren de un contraste sistemático de todos los aparatos e instrumentos usados en esta etapa, además de asegurar su correcto empleo.

- Fuente de riesgos. Estas operaciones hacen uso de reactivos y disolventes que pueden ser peligrosos tanto para los operadores como para el medio ambiente.

Todas estas características hacen que las operaciones previas constituyan un aspecto crucial de los PMQ, ya que requieren de una gran atención por parte del personal del laboratorio así como de los sistemas de control y evaluación de la calidad. Por este motivo, en los últimos años, la investigación centrada en esta segunda etapa es cada vez más relevante.

Las técnicas analíticas de separación, que persiguen la separación física de una mezcla de sustancias, son de gran importancia en este contexto. Pueden clasificarse en técnicas cromatográficas y no cromatográficas. El primer tipo busca la separación de compuestos entre sí mientras que el segundo, tiene como objetivo aislar los compuestos de interés de la matriz de la muestra. La importancia de estas últimas radica en el hecho de que mejoran la sensibilidad a través de la preconcentración y la selectividad, gracias a la eliminación de interferencias (clean up).

Una técnica de separación no cromatográfica ideal debería ser simple, automática, fácilmente miniaturizable, rápida, barata y segura tanto para los operadores como para el medio ambiente [2]. En respuesta a estas necesidades, las cuales son díficiles de conseguir con las técnicas de extracción clásicas, surgieron las técnicas de microextracción, que se encuentran en continuo progreso. La investigación actual se centra principalmente en la propuesta de nuevas técnicas, en la mejora de las existentes así como en el desarrollo y la aplicación de nuevos materiales con capacidades extractivas superiores [3]. Además, la automatización, simplificación y miniaturización se consideran claves en la evolución de las mismas. La aplicabilidad de este tipo de técnicas se ha confirmado con el desarrollo de numerosos métodos de análisis en un amplio abanico de campos, como por ejemplo, el alimentario, forense, medioambiental o biomédico.

Las técnicas de microextracción pueden clasificarse en dos grandes grupos, microextracción en fase líquida (liquid phase microextraction, LPME) y microextracción en fase sólida (solid phase microextraction, SPME), según la naturaleza de la fase extractante. Cada modalidad, a su vez, engloba diferentes técnicas tal y como puede observarse en las Figuras 1 y 2 [4].

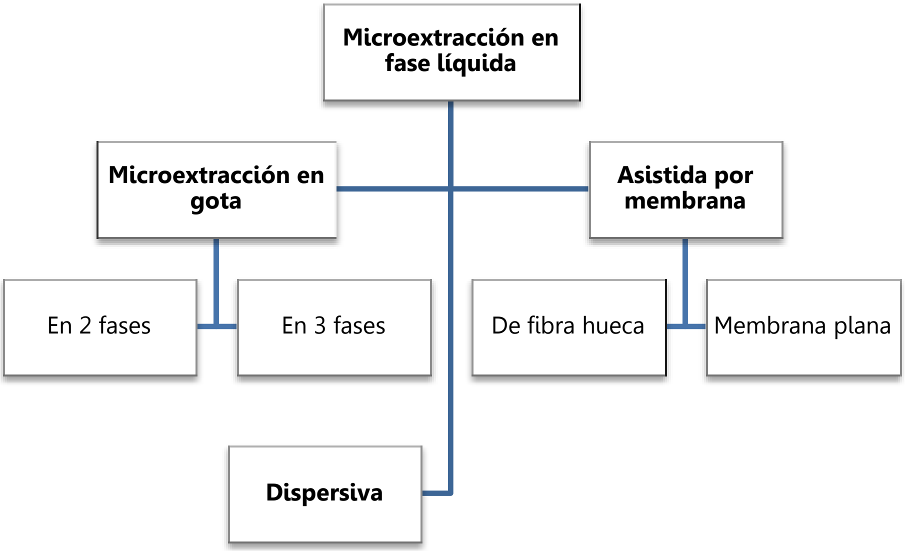


Figura 1. Clasificación de las técnicas de microextracción en fase líquida.

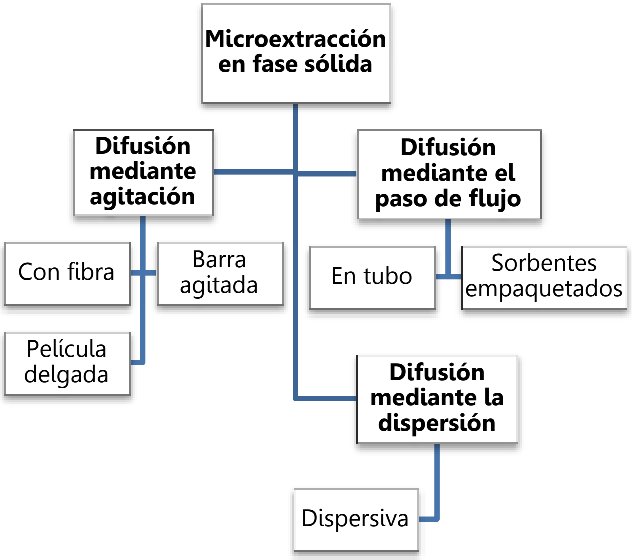


Figura 2. Clasificación de las técnicas de microextracción en fase sólida.

La microextracción en fase líquida surgió en 1995 cuando Liu y Dasgupta propusieron por primera vez el uso de pequeños volúmenes (una gota del orden de los µL) como extractantes en extracción líquido-líquido (ELL) [5]. Esta propuesta resolvía las principales limitaciones de la ELL convencional como el uso de grandes volúmenes de disolvente de alta pureza y de muestra [2]. Los tipos de LPME más destacados se describen a continuación:

- **Microextracción en gota (single-drop microextraction, SDME).** Esta técnica, descrita en 1997 por Jeannot y Cantwell, y He y Lee, se basa en la transferencia de los analitos desde la muestra hasta una pequeña gota (del orden de los micro o sub-microlitros) de fase aceptora situada en la punta de una microjeringa [6, 7]. Esta técnica es simple, barata y rápida. Además, permite el análisis de muestras de las que se dispone de poco volumen. Su principal inconveniente es la falta de precisión, debido a la inestabilidad de la gota. Así mismo, su compatibilidad con ciertas técnicas, como la cromatografía líquida, es limitada debido al pequeño volumen de fase extractante usada.

La microextracción en gota puede desarrollarse en dos o en tres fases. En el primer modo, se suele extraer una muestra acuosa con una gota de disolvente orgánico; mientras que en el segundo caso, el analito se transfiere de una muestra acuosa a una disolución aceptora de la misma naturaleza a través de una fase orgánica que las separa. Otra modalidad de microextracción en gota de tres fases sería la de espacio de cabeza (headspace SDME), donde el analito pasa de la muestra acuosa al espacio de cabeza, y tras esto al extractante [8].

- **Microextracción en fase líquida asistida por membrana (membrane-assisted LPME).** Esta técnica surgió para solventar los problemas de inestabilidad de la gota observados en SDME. Dentro de esta modalidad, pueden diferenciarse distintas configuraciones, de las cuales destacaremos las dos más importantes. La primera aproximación, propuesta por Pedersen-Bjergaard y Rasmussen en 1999 se denominó microextracción con fibra hueca (hollow-fiber LPME). Ésta hace uso de una fibra hueca para confinar la fase extractante, y cumple los requisitos de simplicidad, bajo coste y reproducibilidad [9]. En la modalidad de membrana plana, la membrana se impregna con un disolvente orgánico y se confina sobre dos piezas. La unidad de extracción se conecta a un sistema de flujo gracias al cual se bombea la disolución acuosa. Los compuestos pueden extraerse de forma selectiva de la disolución acuosa (fase donadora) al disolvente orgánico que impregna la membrana y posteriormente, a la segunda disolución acuosa (fase aceptora) [10].

- **Microextracción líquido-líquido dispersiva (dispersive liquid-liquid microextraction, D-LLME).** Rezaee y colaboradores propusieron por primera vez esta técnica basada en la dispersión en la muestra de un pequeño volumen de extractante asistida por un disolvente dispersante [11]. Tras la dispersión (extracción), el disolvente que contiene a los analitos preconcentrados se recupera mediante centrifugación. En esta alternativa, la superficie de contacto entre las fases es mucho mayor, lo que acelera el proceso de transferencia de materia permitiendo alcanzar el equilibrio más rápidamente.

La microextracción en fase sólida en fibra se propuso por primera vez en el 1990 por Pawliszyn, como alternativa a la extracción líquido-líquido [12]. Esta técnica se caracteriza por su simplicidad, rapidez, bajo coste y por requerir una cantidad casi nula de disolventes orgánicos. Aunque el acrónimo SPME se reserva para la extracción en fibra y técnicas derivadas, el término microextracción en fase sólida suele emplearse para definir aquellas técnicas caracterizadas por el uso de pequeñas cantidades de sorbente. Así, de forma general la microextracción en fase sólida se basa en la transferencia de los analitos desde la matriz de la muestra hasta una pequeña cantidad de sorbente, que puede encontrarse disperso en la muestra o inmovilizado en un soporte sólido. Para la posterior desorción de los analitos puede usarse un disolvente (desorción química) o bien un aumento de la temperatura (desorción térmica). Pueden diferenciarse varias modalidades dependiendo de cómo se produzca la difusión de los analitos hacia el sorbente:

(i) Difusión mediante agitación:

- **Microextracción en fase sólida con fibra (fiber solid-phase microextraction, fiber SPME).** En este caso, el sorbente se encuentra recubriendo un filamento, el cual está insertado en la aguja de una jeringa. La fibra puede sumergirse en la muestra o ponerse en contacto con su espacio de cabeza, tal como se indica en la Figura 3. Una de las ventajas de esta modalidad es su versatilidad, puesto que existen una gran variedad de fibras recubiertas por distintos polímeros (aunque la mayoría de ellas no comerciales), que pueden aplicarse para solucionar problemas analíticos de distinta naturaleza. Además, es simple de operar y fácilmente automatizable, lo que ha permitido su implantación en los laboratorios de rutina.

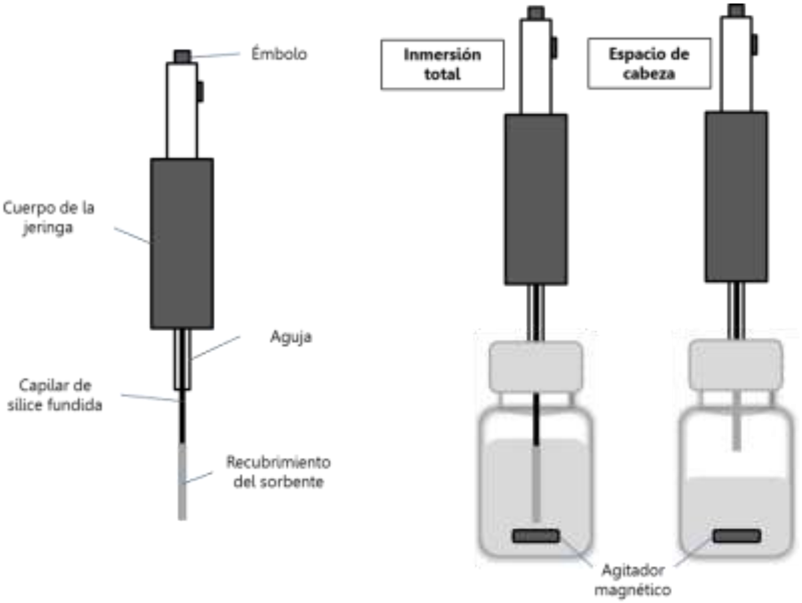


Figura 3. Dispositivo empleado para SPME en fibra y sus dos modalidades.

- **Microextracción en película delgada (thin-film microextraction, TFME).** Fue también propuesta por Pawliszyn como una alternativa a la SPME de fibra [13]. Esta técnica emplea una película de poli dimetilsiloxano (PDMS) como fase extractante y presenta mejores capacidades extractivas debido a su mayor área superficial en relación al volumen de muestra, permitiendo la extracción de una mayor cantidad de analitos en menos tiempo. Al igual que en la modalidad de SPME de fibra, la extracción de los analitos hacia la membrana puede realizarse introduciendo directamente la membrana en la muestra o en su espacio de cabeza. El uso de membranas como fase extractante ha abierto el campo de aplicabilidad de la técnica.

- **Extracción en barra agitada (stir bar sorptive extraction, SBSE).** Esta técnica se describió por primera vez por el grupo del profesor Sandra en 1999 [14]. En la SBSE, el sorbente se encuentra recubriendo una barra de agitación magnética, como puede observarse en la Figura 4. Esta barra se agita en el interior de la muestra, favoreciendo la transferencia de los analitos desde el seno de la muestra hacia la capa de sorbente (usualmente PDMS). Tras la extracción, la barra se recupera para llevar a cabo la desorción de los analitos, ya sea de forma térmica (para su posterior detección por cromatografía de gases) o mediante un disolvente (para cromatografía líquida). En comparación con la SPME, la técnica proporciona mayores rendimientos absolutos de extracción (ya que la cantidad de PDMS usada es mucho mayor) pero una cinética más lenta, debido a que el grosor del recubrimiento es mayor.

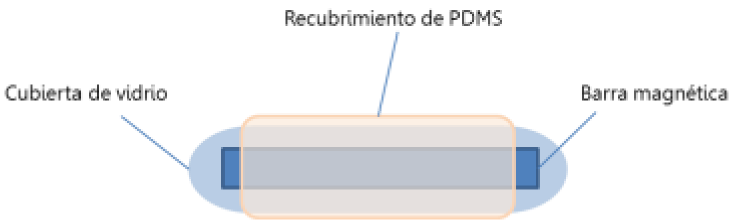


Figura 4. Esquema de una unidad de barra agitada.

(ii) Difusión mediante el paso de flujo:

- **Microextracción en fase sólida en tubo (in-tube solid phase microextración, in-tube SPME).** Esta técnica fue desarrollada por el grupo de Pawliszyn en el 1997 para facilitar el acoplamiento de SPME con cromatografía líquida [15]. La técnica original empleaba un segmento de una columna capilar de cromatografía de gases como sistema de extracción, por el que se hace pasar la muestra para que los analitos queden retenidos en el polímero sorbente. Tras la extracción, los analitos se desorben químicamente y el eluído se inyecta en un cromatógrafo de líquidos. Existen dos modalidades de trabajo, mediante la aspiración/expulsión de la muestra o con flujo continuo [16]. Una de las ventajas que posee esta técnica frente a la SPME es que presenta una mayor variedad de recubrimientos. Además, es posible elegir la longitud del capilar así como su espesor. La técnica sólo es aplicable a muestras libres de partículas, ya que de otra forma el capilar podría obturarse.

- **Microextracción con sorbentes empaquetados (microextraction by packed sorbent, MEPs).** Esta técnica se basa en el uso de una pequeña cantidad de material sorbente que se empaqueta en una jeringa o en una punta de pipeta. Al usar menores cantidades de sorbente, esta técnica permite trabajar con volúmenes de muestra y eluyentes menores. Además, puede conectarse en línea con cromatografía de líquidos o de gases sin introducir ninguna modificación [17]. Las etapas seguidas en este tipo de extracción son las mismas que en una SPE convencional, pero en este caso, la muestra y los disolventes se hacen pasar a través del sorbente mediante ciclos sucesivos de aspiración/expulsión.

(iii) Difusión mediante la dispersión:

- **Microextracción en fase sólida dispersiva (dispersive micro solid phase extraction, D-µSPE).** En 2003, Anastassiades y colaboradores propusieron esta técnica para mejorar la selectividad (mediante la retención de interferencias) de la determinación de pesticidas en muestras alimentarias [18]. Más tarde, Tsai y colaboradores adaptaron la técnica para la preconcentración de analitos [19]. Con este fin, el sorbente (en el orden de miligramos/microgramos) se añade directamente a la muestra sin procesar y se agita para favorecer su dispersión. Por último, la muestra se centrifuga para recuperar el sólido, y llevar a cabo la etapa de elución.

2. Uso de polímeros como sorbentes en las técnicas de extracción

Los polímeros, ya sean sintéticos o naturales, son sustancias cuya estructura molecular se construye mediante la combinación de varias unidades simples (monómeros) que se repiten de un modo concreto.

En medicina, los polímeros, y especialmente los biodegradables, han tenido un gran impacto, ya que pueden eliminarse una vez que hayan realizado su función clínica [20]. Además, constituyen la clase más versátil y abundante de materiales biocompatibles, ya que se pueden sintetizar fácilmente con una amplia variedad de estructuras y propiedades [21]. Por último, otras de sus ventajas serían su bajo coste y capacidad de reutilización. La mayoría de las aplicaciones están enfocadas a la ingeniería de tejidos y a la administración dirigida de fármacos, por lo que el estudio de nuevos materiales está en constante desarrollo [22, 23].

De igual forma, el empleo de estos polímeros en técnicas de extracción es un tema de creciente interés. La principal ventaja de los polímeros para su aplicación como sorbente radica en el hecho de que pueden sintetizarse ad-hoc, para favorecer un determinado tipo de interacción con los analitos. Además, su estabilidad química, mecánica y térmica, permite su uso en diversas técnicas de extracción, como: extracción en fase sólida (solid phase extraction, SPE), TFME o SPME.

Una clase de sorbentes para SPE de gran selectividad son los inmunosorbentes, que se basan en la interacción antígeno-anticuerpo. Sin embargo, estos sorbentes presentan una baja estabilidad, son difíciles de obtener y son caros. Por este motivo, surgieron los polímeros de impronta molecular (molecularly imprinted polymers, MIPs), que imitan dicho comportamiento [24]. Los MIPs son polímeros con sitios de unión específicos para un analito particular o un grupo de compuestos de estructura similar. Estos sitios se crean in-situ durante la copolimerización de los monómeros en presencia de la molécula modelo (template). Tras la polimerización, la molécula se retira, dejando una cavidad de reconocimiento que es complementaria a su tamaño, forma y funcionalidad. Los monómeros se eligen teniendo en cuenta la habilidad para interaccionar con los grupos funcionales de la molécula modelo. Los MIPs son estables, robustos y resistentes en un amplio intervalo de pH, disolventes y temperaturas. Su síntesis es barata y fácil, lo que los convierte en una clara alternativa al uso de biomoléculas de reconocimiento [25].

Los MIPs se basan en interacciones no covalentes, de manera que el complejo entre los monómeros y la molécula modelo se forma mediante puentes de hidrógeno, fuerzas electroestáticas, fuerzas de Van der Waals o interacciones hidrofóbicas [26]. Uno de los principales inconvenientes de los MIPs es la dificultad de eliminar por completo la molécula modelo tras la síntesis. En ocasiones, esta molécula puede contaminar los eluídos, dando lugar a errores por exceso, o bloquear los sitios de unión para los analitos. Para evitar esta contaminación, Andersson y colaboradores propusieron usar una molécula análoga (mimic) como template [27]. Estos polímeros también se han usado para el recubrimiento de fibras en SPME, para aumentar la selectividad de la técnica [28].

Las nanofibras, sintetizadas mediante la técnica de electrospinning [29], también se han usado como sorbentes, ya que la capacidad extractiva se ve mejorada gracias a su tamaño nanométrico. Los polímeros más usados son las poliamidas y el poliestireno, cuyos grupos funcionales les confieren una polaridad especial que puede ser explotada en este contexto. Gracias a esta técnica, pueden obtenerse membranas o recubrimientos de fibras de SPME.

Otro grupo de polímeros usados en extracción son los basados en poliestireno-divinilbenceno (PS-DVB), los cuales son eficientes, robustos y estables en un amplio intervalo de pH. A este tipo de polímeros, se les puede añadir diferentes grupos funcionales hidrofílicos, como los grupos acetil o hidroximetil, para hacer su superficie más hidrofílica. Se ha demostrado que estos polímeros son más eficientes al usarse como fase estacionaria en columnas de HPLC para la determinación de compuestos orgánicos polares, en comparación con las columnas sin modificar [30]. Estas modificaciones también se han empleado en la síntesis de membranas, que permiten tratar un mayor volumen de muestra. Las membranas de PS-DVB acetiladas proporcionan mejores resultados que las membranas sin modificar, al poseer una mayor polaridad, ya que mejoran el contacto entre el polímero y la muestra acuosa [31]. Igualmente ocurre cuando estas membranas se modifican con grupos sulfonados [32]. Del mismo modo, se han descrito monolitos de PS-DVB como fases estacionarias, pudiendo obtener materiales con distinta porosidad y área superficial dependiendo del disolvente usado durante la polimerización [33].

El Oasis HLB es un polímero comercializado por Waters ampliamente usado como sorbente. En éste, se combina un monómero hidrofóbico (divinilbenceno) con otro hidrofílico (n-vinilpirrolidona) y presenta hasta 5 veces más capacidad extractiva que los sorbentes de base silícea. Puede emplearse para la extracción de compuestos orgánicos tanto polares como apolares en muestras de agua. Además, presenta una amplia estabilidad frente al pH y los disolventes orgánicos, lo cual facilita la etapa de elución. Por otro lado, el Oasis MCX combina las propiedades del Oasis HLB con la capacidad de intercambio catiónico fuerte de los grupos sulfonados. Los grupos de ácido sulfónico que se añaden al polímero de PS-DVB, mejoran la selectividad y la extracción de analitos básicos [34]. De igual modo, Merck comercializa un sorbente polimérico de PS-DVB bajo el nombre de LiChrolut-EN. Éste posee una alta capacidad extractiva, debido a su alta superficie específica, y es especialmente útil para la extracción de compuestos orgánicos polares en muestras de diferente naturaleza [35, 36].

3. Las nanopartículas en el ámbito de la microextracción

Las nanopartículas (NPs) son grupos de átomos o moléculas (en la forma de óxidos metálicos) con una o más dimensiones en la escala nanométrica, tomando 100 nm como referencia arbitraria. Esta dimensión nanométrica dota al material con propiedades físico-químicas que no son observables a escala macroscópica [37]. En la Figura 5, se pueden observar las imágenes de microscopio de dos tipos de nanopartículas ampliamente usadas, los nanotubos de carbono y las NPs magnéticas.

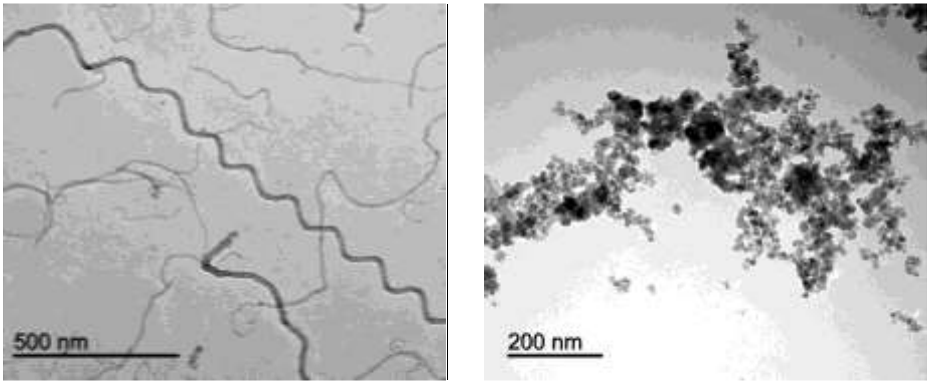


Figura 5. Imágenes de microscopía electrónica de transmisión de varios tipos de nanopartículas. A la izquierda, nanotubos de carbono; y a la derecha, nanopartículas magnéticas de Fe_3O_4.

Las NPs pueden clasificarse según:

- Su origen. Pueden ser naturales o generadas por la actividad humana, ya sea de manera voluntaria o involuntaria (como productos secundarios de una reacción).
- Su naturaleza química. Pueden ser inorgánicas o estar basadas en carbono.
- Su composición. Se dividen en puras o híbridas, según si están compuestas por uno o varios elementos diferentes.
- El número de dimensiones en la escala nanométrica (inferiores a 100 nm). En este caso, pueden ser de una, dos o tres dimensiones.
- Las propiedades más relevantes que se explotan de la nanomateria, ya sean químicas, eléctricas, ópticas, térmicas o magnéticas.

Las NPs se caracterizan por poseer una elevada área superficial, lo que les permite establecer interacciones múltiples con los analitos de interés. La naturaleza de estas interacciones varía según la composición química de las NPs empleadas. Éstas son fáciles de obtener comercialmente y muchas de ellas pueden sintetizarse en el laboratorio de forma simple. Todo esto hace de las NPs una poderosa herramienta en Química Analítica.

Las NPs pueden desarrollar diversas funciones dentro del proceso analítico, destacando su interés en la etapa del tratamiento de la muestra. Además, también se ha descrito su uso como fases estacionarias en cromatografía de gases y de líquidos y como fases (pseudo)estacionarias en electroforesis capilar [38].

Las NPs pueden jugar cuatro papeles fundamentales en la etapa de tratamiento de muestra [39]:

- Pueden actuar como sorbentes, interaccionando directamente con los analitos.

- Pueden emplearse como soportes inertes. En este caso, la interacción con los analitos se lleva a cabo a través de un grupo funcional o (bio)molécula que previamente se ha anclado a la superficie de la NP.

- Pueden aportar o mejorar ciertas propiedades complementarias, como el magnetismo o la porosidad.

- Pueden facilitar el acoplamiento de la técnica de tratamiento con la instrumental. Así, se ha descrito su uso como agentes ionizantes para el análisis directo de muestras mediante espectrometría de masas de ión secundario.

Las NPs más usadas en microextracción son las basadas en carbono (nanotubos, fullerenos y grafeno) y las inorgánicas (de oro, de plata o de óxidos metálicos).

Las formas alotrópicas del carbono más empleadas en el contexto de la microextracción son los nanotubos de carbono (CNTs), los fullerenos y el grafeno, cuyas estructuras se muestran en la Figura 6. Los CNTs fueron descubiertos por IIjima en el 1991 y consisten en una lámina de átomos de carbono dispuestos hexagonalmente que se enrolla para formar una estructura tubular con un diámetro exterior de entre 2.5 y 30 nm aproximadamente [40, 41]. Pueden estar formados por una única capa (nanotubos de carbono de pared simple, SWCNTs) o por un número de capas concéntricas (nanotubos de carbono de pared múltiple, MWCNTs), como se observa en la Figura 6 (a y b). Se caracterizan por tener una alta estabilidad térmica, por sus excelentes propiedades mecánicas y eléctricas, y por una alta fuerza de tensión. Además, su superficie hidrofóbica puede establecer interacciones no covalentes con compuestos orgánicos, mediante interacciones π-π o dipolo-dipolo, entre otras [42].

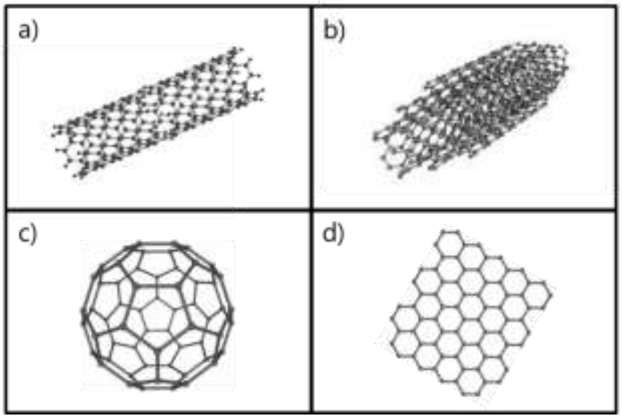


Figura 6. Modelos de las estructuras de los nanotubos de carbono de pared simple y de pared múltiple (a y b), fullereno (c) y grafeno (d).

Su potencial queda a veces limitado por su alta tendencia a la agregación que dificulta su dispersión eficiente en la muestra. Este aspecto impide explotar la totalidad de los sitios superficiales de interacción con los analitos. Para intentar solventar estos problemas, los CNTs pueden funcionalizarse covalentemente, inmovilizarse en soportes inertes o dispersarse mediante agentes anfifílicos [43].

Por otro lado, los fullerenos son estructuras de carbono compuestas por anillos pentagonales y hexagonales, que adoptan una forma cuasi-esférica. Fueron descubiertos por Kroto, Curl y Smalley en el 1985 [44], siendo el primer fullereno hallado el C_{60}, que consta de 12 pentágonos y 20 hexágonos. Si bien los CNTs han demostrado ser más eficientes para la extracción de compuestos orgánicos, los fullerenos son más adecuados para la determinación de metales [45].

Por último, el grafeno consiste en una estructura bidimensional que consta de una sola capa de átomos de carbono dispuestos en una red hexagonal. Fueron aislados por primera vez en 2004 por Novoselov y colaboradores [46]. El grafeno posee unas propiedades mecánicas, eléctricas, térmicas y ópticas excelentes, a parte de una gran área específica y fácil derivatización. Se obtiene principalmente de la oxidación de una lámina de grafito, de la cual se obtiene óxido de grafeno que tras su reducción con hidracina, se transforma en grafeno. Ambas estructuras, grafeno y óxido de grafeno, se han usado ampliamente en microextracción, aprovechando su carácter hidrofóbico e hidrofílico, respectivamente.

Las nanopartículas de oro (AuNPs) también se han empleado en el campo de la microextracción en numerosas ocasiones, gracias a su fácil síntesis, en la que puede controlarse tanto la forma como el tamaño de las NPs, y a su estabilidad frente al pH y a los disolventes [47]. Además, pueden funcionalizarse para poder interaccionar con analitos de diferente naturaleza [37]. Al igual que ocurre con los CNTs, las AuNPs tienden a formar agregados en agua, por lo que es necesario añadir un agente modificante para estabilizar su dispersión.

Las nanopartículas de plata (AgNPs) se han aplicado en diferentes campos, por ejemplo en catálisis, pero también han sido de gran interés en las técnicas de extracción, debido a su fácil síntesis y derivatización. Éstas pueden interaccionar con compuestos orgánicos no polares, además de mejorar la luminiscencia de ciertos analitos [48].

Los óxidos metálicos (TiO_2, Al_2O_3, SiO_2, ZrO_2, MnO, CeO_2, Fe_xO_y) presentan una elevada área superficial y una gran capacidad sorbente. Su reducida selectividad de interacción puede solucionarse mediante la modificación, ya sea física o química, de su superficie con moléculas orgánicas [49]. Estas NPs se han usado en numerosas ocasiones para la determinación de trazas metálicas en aguas residuales [50, 51].

El carácter magnético de ciertos óxidos metálicos, permite simplificar el proceso de extracción. Así, las nanopartículas magnéticas (MNPs), que se basan principalmente en la magnetita (Fe_3O_4), son fáciles de sintetizar, pudiendo modificar su forma, tamaño y composición eligiendo las condiciones de la síntesis. Las MNPs son superparamagnéticas sólo cuando poseen un tamaño de entre 30-40 nm, esto quiere decir que sólo presentan su carácter magnético bajo la influencia de un campo magnético externo, lo que es crucial para usarlas en microextracción. Aunque pueden usarse tal cual, normalmente, se recubren con una capa inorgánica para estabilizarlas en medio acuoso y servir de base para las posteriores funcionalizaciones [52].

4. Nanocomposites poliméricos

Los composites o materiales híbridos se definen como aquellos que combinan dos o más tipos de componentes con el objetivo de potenciar una determinada propiedad o de combinar varias propiedades en un mismo material. Cuando al menos uno de estos componentes se encuentra en la nanoescala, puede hablarse de nanocomposite o nanomaterial híbrido. Si se combinan dos materiales nanométricos, el resultado es una nanopartícula híbrida, cuyo tamaño también se encuentra en la nanoescala [53].

Pueden distinguirse dos tipos de materiales híbridos de acuerdo a la combinación de sus propiedades [39]:

(1) Las propiedades de los materiales por separado son diferentes pero complementarias. Éste es el caso de la combinación de NPs magnéticas con CNTs o chitosán para obtener un nuevo material que combine el comportamiento superparamagnético con una buena capacidad de extracción [54, 55].

(2) Las propiedades de los materiales que se combinan son de la misma naturaleza, pero al combinarlos se produce un importante efecto sinérgico, como ocurre con la combinación de los quantum dots [56].

Hoy en día, el gran potencial que presentan estos materiales en el campo de la microextracción es un tópico de creciente interés. La mayoría de las aplicaciones se centran en la combinación de materiales con propiedades de diferente naturaleza. Dentro de este tipo de composites, la combinación de polímeros con NPs es una de las más versátiles. La inclusión de NPs en la red polimérica puede mejorar la capacidad de extracción de esta (de forma general aumentando su superficie) o bien conferirle nuevas propiedades (como, por ejemplo, carácter magnético).

Los nanocomposites que combinan polímeros y NPs pueden ser de dos tipos atendiendo a su estructura (Figura 7). Por un lado, el polímero puede recubrir la superficie de las NPs, obteniendo un composite de tamaño nanométrico. Por otro lado, las NPs pueden encontrarse integradas en la red polimérica, dando lugar a composites micrométricos. En el primer caso, los polímeros pueden unirse a las NPs mediante interacciones covalentes (por ejemplo cuando se emplean MIPs o polipirrol) o electroestáticas (con biopolímeros como chitosán o alginato) [57]. En el segundo caso, la inmovilización de las NPs se lleva a cabo según los grupos funcionales disponibles en el polímero y pueden ser desde enlaces covalentes hasta fuerzas de Van der Waals, interacciones electroestáticas o puentes de hidrógeno.

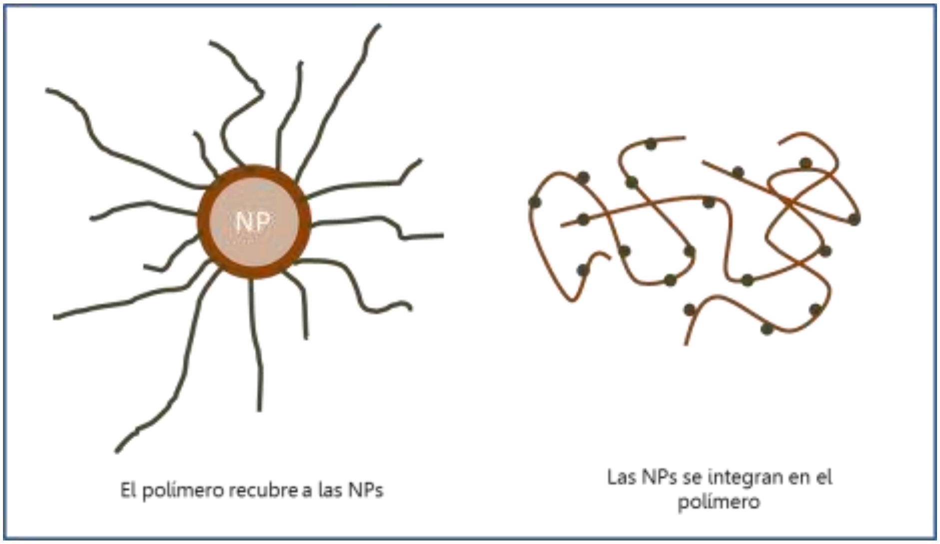


Figura 7. Esquema general de los tipos de nanocomposites de polímeros y NPs según su estructura.

En este sentido, los polímeros presentan una gran ventaja frente a otro tipo de materiales, ya que pueden ser diseñados a medida para favorecer la interacción con un determinado tipo de analitos. Con este fin, pueden introducirse en la red polimérica varios grupos funcionales con características químicas diferentes (desde grupos no polares hasta de intercambio iónico).

Las NPs más usadas para la síntesis de estos composites suelen ser las MNPs. Estas MNPs presentan una elevada relación superficie/volumen, gracias a su tamaño nanométrico, lo que es esencial para mejorar la cinética de extracción. Además, como ya se ha comentado anteriormente son superparamagnéticas, y sólo muestran sus propiedades magnéticas bajo la influencia de un campo magnético externo, lo que facilita tanto la dispersión como la recuperación de las mismas.

Este tipo de combinación de MNPs con polímeros se ha propuesto para la extracción de distintos compuestos en muestras de diferente naturaleza. Por ejemplo, se han usado MNPs combinadas con PS-DVB para la extracción en fase sólida de fenitrotión en orina y plasma [58] o combinadas con DVB y una molécula especial que permite aislar el radioisótopo ^{90}Sr en muestras de orina incluso en presencia de otros interferentes como el ^{90}Y [59].

Además de los polímeros convencionales, los polímeros conductores (aquellos que presentan un sistema π-conjugado en su estructura) también son de gran interés, gracias a su versatilidad y su facilidad de síntesis. De este modo, aquellos polímeros basados en el polipirrol o la polianilina se han usado para formar nanocomposites con distintas finalidades. Por ejemplo, se han empleado MNPs tratadas previamente con chitosán y recubiertas con polipirrol para extraer naproxeno en muestras de agua, plasma y orina [60]. También se ha usado este tipo de polímeros con NPs de plata para extraer furosemida en orina. En este composite, las NPs actúan de centros de nucleación que dotan al polímero de unas propiedades especiales.

Los MIPs, caracterizados por su alta selectividad, también se han combinado con distintos tipos de NPs para su uso en microextracción. Algunos ejemplos de ello pueden ser su combinación con MNPs, CNTs o sílice. Los MIPs magnéticos se han usado para la determinación de paracetamol y estrógenos en muestras de plasma, aprovechando la capacidad magnética del sorbente para ser recuperado tras la extracción [61]. Al usar CNTs como soporte, la eficiencia del sorbente se ve mejorada, ya que se facilita la accesibilidad de los analitos a los huecos selectivos del MIP. Con este objetivo, Zhan y colaboradores recubrieron la superficie de MWCNTs con una fina capa de un MIP para la determinación de un colorante (Sudán IV) en chile en polvo. Aquí, los MWCNTs sirven también como elementos de refuerzo para la síntesis del composite [62]. Por último, al sintetizar un composite de MIP-sílice, se obtiene un sorbente con mejores propiedades extractivas y más resistente, que se aplicó para la determinación de tetraciclinas en leche [63].

La combinación de nanofibras poliméricas obtenidas mediante electrospinning con NPs de diferente naturaleza es un tópico que aún no ha sido muy explotado en el campo de la microextracción. Estos composites se han usado principalmente en tres tipos de técnicas: SPME, TFME y SPE dispersiva [29]. Bagheri y Roostaie propusieron la combinación de NPs de sílice con poliamidas para recubrir una fibra de SPME y llevaron a cabo la determinación de clorobencenos en muestras de agua [64]. De igual modo, usaron diferentes óxidos metálicos en una disolución de tereftalato de polietileno (PET) para dotar al composite de una mayor porosidad y aumentar la extracción de BTEX en agua [65]. Estos composites también pueden sintetizarse en forma de membrana mediante electrospinning, lo que los hace ideales para su aplicación en el formato de película delgada (TFME). De hecho el profesor Huang y colaboradores han propuesto una membrana de poliestireno modificada con grafeno que mejora la capacidad extractiva del polímero [66]. En cuanto al modo de SPE dispersivo, se han descrito varias aplicaciones donde se aprovecha la mayor superficie de las nanofibras cuando se combinan con NPs. De esta forma, se llevó a cabo la determinación de algunos iones metálicos mediante una fibra de poliacrilonitrilo (PAN) combinada con NPs de sílice, que incrementan su superficie [67].

Los biopolímeros (aptámeros, péptidos y carbohidratos) también se han empleado para la síntesis de composites. Los aptámeros, que son moléculas sintéticas de ADN o ARN diseñadas específicamente para interaccionar con un determinado analito [68], se han combinado con MNPs para la extracción magnética de diferentes analitos [69, 70]. Su mecanismo de actuación es similar al de los anticuerpos, pero poseen una mayor estabilidad física y química. Por otro lado, los péptidos se han combinado con MNPs y NPs metálicas para distintos fines, pero su selectividad es limitada. Los composites de carbohidratos (chitosán o ciclodextrinas por ejemplo) y diferentes NPs se han usado en el contexto de la microextracción para aumentar la estabilidad de las NPs o incrementar la interacción con los analitos.

Los temas abordados en la introducción de esta Memoria de Tesis Doctoral ponen de manifiesto la relevancia de las técnicas de extracción en el diseño de nuevos procesos analíticos que presenten mejores propiedades analíticas supremas, básicas y complementarias. En este sentido, las propuestas que se han desarrollado en esta Tesis Doctoral, que se basan en la combinación de polímeros y nanopartículas para sintetizar nuevos materiales, han sido claves para esta mejora.

En los siguientes capítulos de la Memoria se van a describir las contribuciones desarrolladas en este ámbito, aplicadas a la resolución de problemas analíticos de carácter ambiental, agroalimentario y clínico. La investigación se divide en dos grandes bloques, según se emplee la microextracción en fase sólida dispersiva (Bloque III) o la microextracción en fase sólida con membrana (Bloque IV). Dentro del primero de ellos, se hará una introducción del papel que juegan los composites de polímeros y nanopartículas en el tratamiento de muestra y se describirán los diferentes trabajos en los que se proponen la síntesis de nuevos composites y su empleo en microextracción en fase sólida dispersiva (magnética y en jeringa). El Bloque IV, comenzará con una introducción sobre las membranas sintetizadas mediante electrospinning así como su empleo en microextracción, y finalmente se expondrá la síntesis de un composite mediante esta técnica y su posterior uso como sorbente en microextracción en fase sólida con membrana. Por último, en el Bloque V se resumirán las conclusiones más relevantes de la investigación desarrollada.

Referencias

[1] M. Valcárcel, Principios de Química Analítica, Springer-Verlag Ibérica, Barcelona, 1999.

[2] R. Lucena, M. Cruz-Vera, S. Cárdenas, M. Valcárcel, Liquid-phase microextraction in bioanalytical sample preparation, Bioanalysis 1 (2009) 135-149.

[3] M. Valcárcel, S. Cárdenas, R. Lucena, Microextraction techniques, Anal Bioanal Chem 406 (2014) 1999–2000.

[4] C. Silva, C. Cavaco, R. Perestrelo, J. Pereira, J. S. Câmara. Microextraction by Packed Sorbent (MEPS) and Solid-Phase Microextraction (SPME) as Sample Preparation Procedures for the Metabolomic Profiling of Urine, Metabolites 4 (2014) 71-97.

[5] S. Liu, P. K. Dasgupta, Liquid Droplet. A Renewable Gas Sampling Interface, Anal Chem 67 (1995) 2042-2049.

[6] M. A. Jeannot, F. F. Cantwell, Mass transfer characteristics of solvent extraction into a single drop at the tip of a syringe needle, Anal Chem 69 (1997) 235-239.

[7] Y. He, H. K. Lee, Liquid-phase microextraction in a single drop of organic solvent by using a conventional microsyringe, Anal Chem 69 (1997) 4634-40.

[8] K. Choi, J. Kim, D. S. Chung, Single-drop microextraction in bioanalysis, Bioanalysis 3 (2011) 799–815.

[9] S. Pedersen-Bjergaard, K. E. Rasmussen, Liquid-Liquid-Liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis, Anal Chem 71 (1999) 2650-2656.

[10] J. A. Jönsson, L. Mathiasson, Supported liquid membrane technique for sample preparation and enrichment in environmental and biological analysis, Trends Anal Chem 11 (1992) 106-114.

[11] M. Rezaee, Y. Assadi, M. R. Millani, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid-liquid microextraction, J Chromatogr A 1116 (2006) 1-9.

[12] C. L. Arthur, J. Pawliszyn, Solid phase microextraction with thermal desorption using fused silica optical fibers, Anal Chem 62 (1990) 2145–2148.

[13] I. Bruheim, X. Liu, J. Pawliszyn, Thin-film microextraction, Anal Chem 75 (2003) 1002-1010.

[14] E. Baltussen, P. Sandra, F. David, C. J. Cramers, A novel extraction technique for aqueous samples: theory and principles, J Microcol Sep 11 (1999) 737-747.

[15] R. Eisert, J. Pawliszyn, Automated in-tube solid-phase microextraction coupled to high-performance liquid chromatography, Anal Chem 69 (1997) 3140–3147.

[16] H. Kataoka, A. Ishizaki, Y. Nonaka, K. Saito, Developments and applications of capillary microextraction techniques: A review, Anal Chim Acta 655 (2009) 8-29.

[17] M. Abdel-Rehim, New trend in sample preparation: on-line microextraction in packed syringe for liquid and gas chromatography applications: I. Determination of local anaesthetics in human plasma samples using gas chromatography–mass spectrometry, J Chromatogr B, 801 (2004) 317–321.

[18] M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J AOAC Int 86 (2003) 412-431.

[19] W. H. Tsai, T. C. Huang, J. J. Huang, Y. H. Hsue, H. Y. Chuang, Dispersive solid-phase microextraction method for sample extraction in the analysis of four tetracyclines in water and milk samples by high-performance liquid chromatography with diode-array detection, J Chromatogr A 1216 (2009) 2263–2269.

[20] B. D. Ulery, L. S. Nair, C. T. Laurencin, Biomedical applications of biodegradable polymers, J Polym Sci B Polym Phys 49 (2011) 832–864.

[21] A. S. Kulshrestha, A. Mahapatro, Polymers for biomedical applications, ACS Symposium Series, American Chemical Society 977 (2008).

[22] X. Wen, P. A. Tresco, Fabrication and characterization of permeable degradable poly(DL-lactide-co-glycolide) (PLGA) hollow fiber phase inversion membranes for use as nerve tract guidance channels, Biomaterials 27 (2006) 3800–3809.

[23] A. S. Determan, B. G. Trewyn, V. S. Lin, M. Nilsen-Hamilton, B. Narasimhan, Encapsulation, stabilization, and release of BSA-FITC from polyanhydride microspheres, J Control Rel 100 (2004) 97–109.

[24] N. Masqué, R. M. Marcé, F. Borrull, Molecularly imprinted polymers: new tailor-made materials for selective solid-phase extraction, Trends Anal Chem 20 (2001) 477-486.

[25] F. G. Tamayo, E. Turiel, A. Martín-Esteban, Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: Recent developments and future trends, J Chromatogr A 1152 (2007) 32–40.

[26] C. He, Y. Long, J. Pan, K. Li, F. Liu, Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples, J Biochem Biophys Methods 70 (2007) 133–150.

[27] L. I. Andersson, A. Paprica, T. Arvidsson, A highly selective solid phase extraction sorbent for pre-concentration of sameridine made by molecular imprinting, Chromatographia 46 (1997) 57.

[28] E. H. M. Koster, C. Crescenzi, W. Den Hoedt, K. Ensing, G. J. de Jong, Fibers coated with molecularly imprinted polymers for solid-phase microextraction, Anal Chem 73 (2001) 3140-3145.

[29] X. Wang, B. S. Hsiao, Electrospun nanofiber membranes, Curr Opi Chem Eng 12 (2016) 20–28.

[30] J. J. Sun, J. S. Fritz, Chemically modified resins for solid-phase extraction, J Chromatogr 590 (1992) 197-202.

[31] J. S. Fritz, P. J. Dumont, L. W. Schmidt, Methods and materials for solid-phase extraction, J Chromatogr A 691 (1995) 133-140.

[32] P. J. Dumont, J. S. Fritz, D. F. Hagen, C. Markell, L. W. Schmidt, Recent developments in polymer-based sorbents for solid-phase extraction, US Pat Appl, (1994).

[33] Q. C. Wang, F. Svec, J. M. J. Fréchet, Macroporous polymeric stationary-phase rod as continuous separation medium for reversed-phase chromatography, Anal Chem 65 (1993) 2243-2248.

[34] C. W. Huck, G. K. Bonn, Recent developments in polymer-based sorbents for solid-phase extraction, J Chromatogr A 885 (2000) 51 –72.

[35] S. Castiglioni, R. Bagnati, D. Calamari, R. Fanelli, E. Zuccato. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters, J Chromatogr A 1092 (2005) 206–215.

[36] R. Lopez, M. Aznar, J. Cacho, V. Ferreira, Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection, J Chromatogr A 966 (2002) 167–177.

[37] G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel, Nanoparticle-based microextraction techniques in bioanalysis, Bioanalysis 3 (2011) 2533-2548.

[38] M. Valcárcel, S. Cárdenas, B. M. Simonet, Y. Moliner-Martínez, R. Lucena, Carbon nanostructures as sorbent materials in analytical processes, Trends Anal Chem 27 (2008) 34-43.

[39] R. Lucena, B. M. Simonet, S. Cárdenas, M. Valcárcel, Potential of nanoparticles in sample preparation, J Chromatogr A 1218 (2011) 620–637.

[40] J. M. Jimenez-Soto, R. Lucena, S. Cárdenas, M. Valcárcel, Solid phase (micro)extraction tools based on carbon nanotubes and related nanostructures, Carbon Nanotubes, Marulanda JR (Ed.), In-tech Capítulo 22 (2010) 409–428.

[41] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56-58.

[42] Y. Cai, G. Jiang, J. Liu, Q. Zhou, Multiwalled carbon nanotubes as a solid-phase extraction adsorbent for the determination of bisphenol A, 4-n-nonylphenol, and 4-tert-octylphenol, Anal Chem 75 (2003) 2517-2521.

[43] G. Z. Fan, J. X. He, S. Wang, Multiwalled carbon nanotubes as sorbent for on-line coupling of solid-phase extraction to high-performance liquid chromatography for simultaneous determination of 10 sulfonamides in eggs and pork, J Chromatogr A 1127 (2006) 12-17.

[44] H. W. Kroto, J. R. Heath, S. C. O'Brien, R. F. Curl, R. E. Smalley, C60: Buckminsterfullerene, Nature 318 (1985) 162-163.

[45] M. Gallego, Y. P. de Peña, M. Valcárcel, Fullerenes as sorbent materials for metal preconcentration, Anal Chem 66 (1994) 4074-4078.

[46] K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva, A. A. Firsov, Electric field effect in atomically thin carbon films, Science 306 (2004) 666-669.

[47] H. Wang, A. D. Campiglia, Determination of polycyclic aromatic hydrocarbons in drinking water samples by solid-phase nanoextraction and high-performance liquid chromatography, Anal Chem 80 (2008) 8202–8209.

[48] S. Y. Vasileva, A. Y. Olenin, G. I. Romanovskaya, Y. A. Krutyakov, V. I. Pogonin, A. S. Korotkov, B. K. Zuev, Adsorption preconcentration of pyrene by silver nanoparticles and its determination in aqueous solutions, J Anal Chem 64 (2009) 1214-1220.

[49] A. G. Howard, N. H. Khdary, Nanoscavenger based dispersion preconcentration; sub-micron particulate extractants for analyte collection and enrichment, Analyst 130 (2005) 1432-1438.

[50] J. Yin, Z. Jiang, G. Chang, B. Hu, Simultaneous on-line preconcentration and determination of trace metals in environmental samples by flow injection combined with inductively coupled plasma mass spectrometry using a nanometer-sized alumina packed micro-column, Anal Chim Acta 540 (2005) 333-339.

[51] Y. Hang, Y. Qin, J. Shen, Separation and microcolumn preconcentration of traces of rare earth elements on nanoscale TiO_2 and their determination in geological samples by ICP-AES, J Sep Sci 26 (2003) 957-960.

[52] P. Dou, L. Liang, J. He, Z. Liu, H. Y. Chen, Boronate functionalized magnetic nanoparticles and off-line hyphenation with capillary electrophoresis for specific extraction and analysis of biomolecules containing cis-diols, J Chromatogr A 1216 (2009) 7558–7563.

[53] A. I. López-Lorente, B. M. Simonet, M. Valcárcel, Analytical potential of hybrid nanoparticles, Anal Bioanal Chem 399 (2011) 43–54.

[54] G. Morales-Cid, A. Fekete, B. M. Simonet, R. Lehmann, S. Cárdenas, X. Zhang, M. Valcárcel, P.Schmitt-Kopplin, In situ synthesis of magnetic multiwalled carbon nanotube composites for the clean-up of (fluoro)quinolones from human plasma prior to ultrahigh pressure liquid chromatography analysis, Anal Chem 82 (2010) 2743-2752.

[55] M. Martínez-Cabanas, M. López-García, J. L. Barriada, R. Herrero, M. E. Sastre de Vicente, Green synthesis of iron oxide nanoparticles. Development of magnetic hybrid materials for efficient As (V) removal, Chem Eng J 301 (2016) 83–91.

[56] S. M. Sadeghi, The inhibition of optical excitations and enhancement of Rabi flopping in hybrid quantum dot–metallic nanoparticle systems, Nanotechnology 20 (2009) 225401-225406.

[57] L. Xie, R. Jiang, F. Zhu, H. Liu, G. Ouyang, Application of functionalized magnetic nanoparticles in sample preparation, Anal Bioanal Chem 406 (2014) 377-399.

[58] H. Eskandari, A. Naderi-Darehshori, Preparation of magnetite/poly(styrene-divinylbenzene) nanoparticles for selective enrichment-determination of fenitrothion in environmental and biological samples, Anal Chim Acta 743 (2012) 137-144.

[59] Z. Varve, E. P. C. Lai, C. Li, B. B. Sadi, Selective extraction of ^{90}Sr in urine using 4′4′′(5′′)di-tert-butyl dicyclohexano-18-crown-6 ether immobilized on polyacrylamide coated magnetic nanoparticles, J Radioanal Nucl Chem 303 (2015) 1053–1057.

[60] H. Bagheri, A. Roostaie, M. Y. Baktash, A chitosan-polypyrrole magnetic nanocomposite as µ-sorbent for isolation of naproxen, Anal chim acta 816 (2014) 1-7.

[61] S. Wang, R. Wang, X. Wu, Y. Wang, C. Xue, J. Wu, J. Hong, J. Liu, X. Zhou, Magnetic molecularly imprinted nanoparticles based on dendritic-grafting modification for determination of estrogens in plasma samples, J Chromatogr B 905 (2012) 105–112.

[62] Z. Zhang, H. Zhang, Y. Hu, S. Yao, Synthesis and application of multi-walled carbon nanotubes–molecularly imprinted sol–gel composite material for on-line solid-phase extraction and high-performance liquid chromatography determination of trace Sudan IV, Anal Chim Acta 661 (2010) 173–180.

[63] Y. K. Lv, L. M. Wang, L. Yang, C. X. Zhao, H. W. Sun, Synthesis and application of molecularly imprinted poly(methacrylic acid)–silica hybrid composite material for selective solid-phase extraction and high-performance liquid chromatography determination of oxytetracycline residues in milk, J Chromatogr A 1227 (2012) 48–53.

[64] H. Bagheri, A. Roostaie, Electrospun modified silica-polyamide nanocomposite as a novel fiber coating, J Chromatogr A 1324 (2014) 11–20.

[65] H. Bagheri, A. Roostaie, Roles of inorganic oxide nanoparticles on extraction efficiency of electrospun polyethylene terephthalate nanocomposite as an unbreakable fiber coating, J Chromatogr A 1375 (2015) 8–16.

[66] J. Huang, H. Deng, D. Song, H. Xu, Electrospun polystyrene/graphene nanofiber film as a novel adsorbent of thin film microextraction for extraction of aldehydes in human exhaled breath condensates, Anal Chim Acta 878 (2015) 102–108.

[67] A. Dastbaz, A. R. Keshtkar, Adsorption of Th^{4+}, U^{6+}, Cd^{2+} and Ni^{2+} from aqueous solution by a novel modified polyacrylonitrile composite nanofiber adsorbent prepared by electrospinning, Appl Surf Sci 293 (2014) 336–344.

[68] A. B. Iliuk, L. Hu, W. A. Tao, Aptamer in bioanalytical applications, Anal Chem 83 (2011) 4440-4452.

[69] S. P. Song, L. H. Wang, J. Li, J. L. Zhao, C. H. Fan, Aptamer-based biosensors, Trends Anal Chem 27 (2008) 108-117.

[70] X. Liu, L. Su, L. Zhu, X. Gao, Y. Wang, F. Bai, Y. Tang, J. Li, Hybrid material for enrofloxacin sensing based on aptamer-functionalized magnetic nanoparticle conjugated with upconversion nanoprobes, Sensor Actuat B Chem 233 (2016) 394–401.

BLOQUE II

HERRAMIENTAS ANALÍTICAS

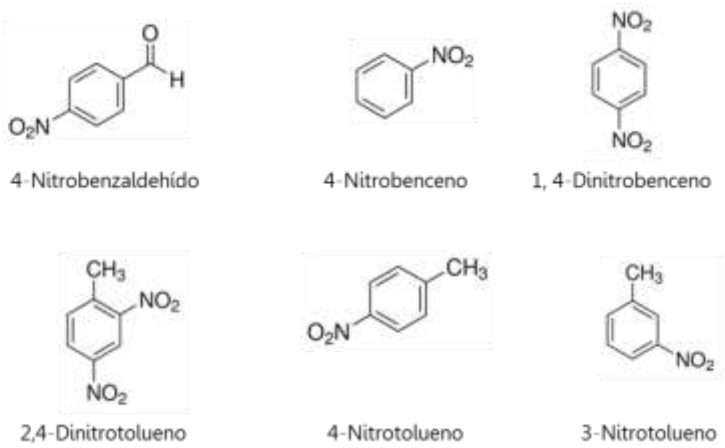
El desarrollo experimental de esta Tesis Doctoral ha requerido el empleo de diferentes herramientas analíticas, que se describen en este bloque de la Memoria. En éste, se enumeran dichas herramientas, señalando sus características más relevantes. Tal es el caso de analitos, reactivos, disolventes, materiales sorbentes, otros materiales y las muestras empleadas. Por último, se describen los aparatos y los instrumentos utilizados para el desarrollo de la Tesis Doctoral.

1. Analitos, reactivos, disolventes, materiales sorbentes y otros materiales

Todos los estándares, reactivos y disolventes empleados durante el desarrollo de la Tesis Doctoral fueron de pureza analítica o superior. A continuación se describen cada uno de ellos.

1. 1. Analitos. Los compuestos objeto de estudio se nombran agrupados por familias. Todos ellos fueron adquiridos en Sigma-Aldrich.

- **Hidrocarburos nitroaromáticos.** Estos compuestos se caracterizan por tener un anillo aromático y al menos un grupo funcional “nitro” en su estructura y se emplean en la industria de los explosivos. Son muy tóxicos incluso a bajas concentraciones, por lo que la Agencia de Protección Medioambiental de los EEUU (EPA) los considera como agentes contaminantes prioritarios. Se han utilizado 4-nitrobenzaldehído; nitrobenceno; 1,4-dinitrobenceno; 2,4-dinitrotolueno; 4-nitrotolueno y 3-nitrotolueno, cuyas estructuras se muestran en la Figura 1.

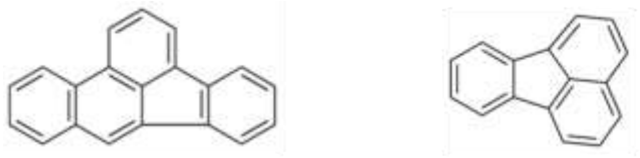


4-Nitrobenzaldehído 4-Nitrobenceno 1, 4-Dinitrobenceno

2,4-Dinitrotolueno 4-Nitrotolueno 3-Nitrotolueno

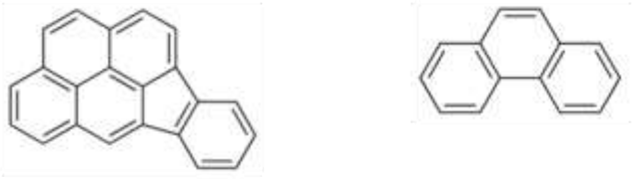
Figura 1. Estructura química de los hidrocarburos nitroaromáticos.

- **Hidrocarburos policíclicos aromáticos.** Son un grupo de compuestos orgánicos formados por anillos aromáticos condensados. Pueden ser generados de forma natural o por acción del hombre. En esta Tesis Doctoral se han usado cuatro hidrocarburos representativos como son: benzo[b]fluoranteno, fluoranteno, indeno[1,2,3-cd]pireno y fenantreno. Sus estructuras se muestran en la Figura 2.



Benzo[b]fluoranteno

Fluoranteno



Indeno[1,2,3-cd]pireno

Fenantreno

Figura 2. Estructura química de los hidrocarburos policíclicos aromáticos seleccionados en esta Memoria.

- **Bisfenol A.** Es un reactivo ampliamente usado en la industria de los plásticos. Es un disruptor endocrino por lo que su monitorización es importante en diferentes muestras como por ejemplo las agroalimentarias. En éstas puede encontrarse como consecuencia de su migración desde los plásticos empleados como envases. Su estructura se muestra en la Figura 3.

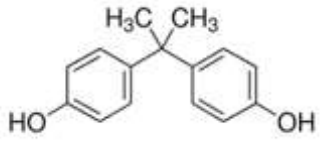
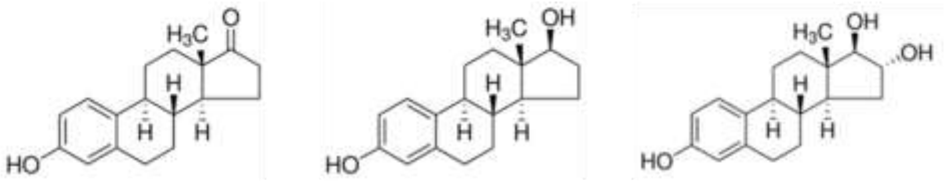


Figura 3. Estructura química del bisfenol A.

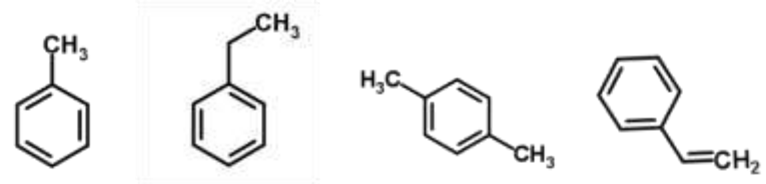
- **Estrógenos.** Son un tipo de hormonas sexuales esteroideas responsables del desarrollo de las características sexuales secundarias femeninas. Altos niveles de estos compuestos en mujeres postmenopáusicas se relacionan con el riesgo de sufrir cáncer de pecho. Se han utilizado: estrona (E1), 17β-estradiol (E2) y estriol (E3). En la Figura 4 aparecen sus estructuras.



Estrona (E1) 17β-Estradiol (E2) Estriol (E3)

Figura 4. Estructuras químicas de los estrógenos seleccionados en esta Memoria.

- ○ **Compuestos volátiles monoaromáticos.** Estos compuestos aparecen principalmente en el petróleo y sus derivados como la gasolina. Se encuentran como contaminantes en los distintos compartimentos ambientales, principalmente en aquellos lugares que están cercanos al almacenamiento de carburantes. Dentro de este grupo de analitos, se estudiaron el tolueno, el etilbenceno, el p-xileno, y el estireno, cuyas estructuras se muestran en la Figura 5.



Tolueno Etilbenceno p-Xileno Estireno

Figura 5. Estructuras químicas de los compuestos volátiles monoaromáticos empleados en esta Memoria.

- **Undecano.** Este compuesto se usó como un compuesto modelo de un hidrocarburo saturado de cadena lineal. Suele ser un contaminante en industrias de la construcción. Su estructura se muestra en la Figura 6.

Figura 6. Estructura química del undecano.

- **Halocarbonos o haloalcanos.** Este grupo de compuestos químicos está formado por alcanos con uno o más halógenos en su estructura. En la presente Tesis Doctoral se usaron diclorometano y cloroformo como compuestos modelo, cuyas estructuras se representan en la Figura 7. Dichos compuestos presentan efectos negativos en el medio ambiente.

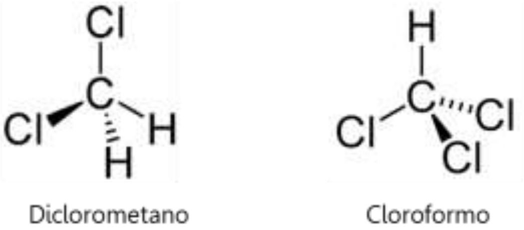


Figura 7. Estructuras químicas de los haloalcanos usados en esta Memoria.

1. 2. Reactivos. El trabajo experimental llevado a cabo ha requerido el empleo de reactivos de diferente naturaleza, todos ellos se adquirieron en Sigma-Aldrich o Panreac.

- **Ácidos:** ácidos clorhídrico, sulfúrico, nítrico, cítrico, etilendiaminotetraacético (EDTA) y fórmico.
- **Bases:** hidróxido sódico y amoniaco 25%.
- **Sales:** cloruro sódico y fosfato sódico dibásico dihidratado.
- **Nanopartículas de sílice:** con un tamaño de partícula de entre 10 y 20 nm.
- **Otros reactivos:** peróxido de hidrógeno 33% (w/v), nitrato de hierro (III) nonahidratado y nitrato de cobalto (II) hexahidratado, cloruro de hierro (III) hexahidratado, cloruro de hierro (II) tetrahidratado, tetraetilortosilicato y nylon 6.

1. 3. Disolventes. Los disolventes orgánicos que se han empleado a lo largo de la Tesis Doctoral son: Metanol (grado HPLC de J. T. Baker y grado LC-MS de Scharlab), acetonitrilo, etanol, (Panreac), agua Milli-Q ultrapura (tipo I) (Millipore).

Estos se han usado con cuatro finalidades: (a) para la preparación de las disoluciones estándar de los analitos, (b) para limpiar y acondicionar materiales sorbentes, (c) como eluyentes, y (d) como componentes de las fases móviles utilizadas en los sistemas cromatográficos.

1. 4. Materiales sorbentes. Se usaron diferentes sorbentes comerciales:

- LiChrolut-EN (40–120 µm, copolímero de estireno–divinilbenceno) de Merck.
- Oasis HLB (30 µm,copolímero de polivinilpirrolidona–divinilbenceno) de Waters.
- Oasis MCX (30 µm, copolímero de estireno–divinilbenceno sulfonado) de Waters.
- RP-C18 (20 µm) de Análisis Vínicos.
- Policloruro de vinilo de Fluka.

- Nanotubos de carbono multicapa (pureza >95%, diámetro entre 6–9 nm y longitud media de 5 μm) de Sigma–Aldrich.
- Nanocuernos de carbono de pared simple, de Carbonium srl (Padua, Italy). Los nanocuernos tienen un diámetro de 4-5 nm y una longitud de entre 40 y 50 nm, además forman agregados en forma de dalia con un diámetro medio de 60–80 nm.

1. 5. Otros Materiales

- Material de vidrio clase A. Empleado para la preparación de patrones, medida de volúmenes y desarrollo de algunas de las técnicas de microextracción presentadas en esta Memoria.
- Envases de politetrafluoroetileno (PTFE) y vidrio para el correcto almacenamiento de los estándares y las muestras.
- Filtros de nylon y de PTFE de 0.22 μm adquiridos en Análisis Vínicos.
- Imán de neodimio/hierro/boro con recubrimiento de níquel de dimensiones 60 x 30 x 15 mm, con una fuerza de sujeción de aproximadamente 550 N (Supermagnete, Alemania).
- Jeringas plástico de 1, 5 y 10 mL suministradas por Terumo.
- Micropipetas de diferentes volúmenes.
- Tubos de poliestireno para centrifugar (Deltalab).
- Contenedores de plástico de diferentes tamaños para la correcta gestión de residuos de laboratorio.

2. Muestras

Para evaluar la capacidad extractiva de los diferentes sorbentes sintetizados en esta Tesis Doctoral en el ámbito medioambiental, alimentario y clínico, se seleccionaron diferentes muestras de agua, leche, orina y saliva, respectivamente.

Las muestras de agua, una vez tomadas, se almacenaron en frascos de vidrio ámbar sin espacio de cabeza a 4 ºC hasta su análisis. Se utilizaron muestras de agua de diferente procedencia: de grifo, de río y de arroyo. Las muestras de agua de río pertenecen al río Guadalquivir a su paso por Córdoba (Andalucía, España). Asimismo, las muestras de agua de arroyo se recogieron de varios afluentes del mismo río. Por último, las muestras de agua de grifo se tomaron de la red municipal de aguas de Córdoba.

Las muestras de leche, con diferente contenido de grasa (entera, semi-desnatada y desnatada) se adquirieron en comercios locales. Se almacenaron en sus propios envases a 4 °C hasta su análisis. Previamente a su extracción, se sometieron a un proceso de precipitación de las proteínas con el uso de una disolución acetonitrilo/buffer EDTA-McIlvaine (80/20). Finalmente, se centrifugaron para obtener un suero libre de partículas.

Se usaron biofluidos de diferente naturaleza: orina y saliva. Ambas muestras se obtuvieron de voluntarios sanos. Las muestras de orina se congelaron a -18 °C en recipientes de PTFE y se diluyeron con una disolución acuosa de ácido fórmico 0,1% (1:1) para su posterior análisis. Por otro lado, las muestras de saliva, se recogieron mediante el uso de un dispositivo denominado Salivette® y se analizaron tras ser filtradas con algodón.

3. Aparatos

Durante el desarrollo del trabajo experimental realizado en esta Tesis Doctoral, se emplearon los siguientes aparatos:

- Equipo de electrospinning. Consta de una bomba de jeringa (Serie 100, KD Scientific) que impulsa la disolución polimérica contenida en una jeringa de plástico con una aguja metálica. Para obtener una alta diferencia de potencial, la aguja se conecta a una fuente de alto voltaje (modelo EH30, Glassman High Voltage) que permite alcanzar un intervalo de voltajes de 0 a 30 kV. Para recoger las nanofibras se usó papel de aluminio, el cual se colocó en una placa colectora o en un rotor giratorio, ambos conectados a tierra.

- Agitador magnético (Velp Scientifica).
- Agitador Vortex (modelo Reax top, Heidolph).
- Placa calefactora con agitación magnética Agimatic-N (J.P. Selecta).
- Cámara de secado (modelo ED 53, Binder).
- Baño de agua con control de la temperatura (Selecta).
- Equipo de agua Milli-Q (Millipore).
- Ultracentrífuga controlada por microprocesador (Centronic BL-II, J. P. Selecta).
- Evaporador centrífugo (modelo Concentrator plus, de Eppendorf).
- Baño de ultrasonidos (modelo 3510, Branson).

4. Instrumentos

En el desarrollo experimental de la Tesis Doctoral se ha hecho uso de diversos equipos instrumentales. Éstos pueden clasificarse en dos grandes grupos, según se hayan empleado para la identificación y cuantificación de los analitos o para la caracterización de los materiales sintetizados.

De este modo, con fines analíticos se usó:

- Cromatografía de líquidos de alta presión con detector UV-visible
- Cromatografía de líquidos de ultra-alta presión con detector UV-visible de diodos en fila
- Cromatografía de líquidos de alta presión acoplada a espectrometría de masas
- Cromatografía de gases acoplada a espectrometría de masas

Y con fines de caracterización se usó:

- Espectroscopía infrarroja
- Análisis elemental
- Microscopía electrónica de barrido (SEM)
- Microscopía electrónica de transmisión (TEM)
- Porosimetría
- Curvas de magnetización

A continuación se describe cada uno de estos equipos.

Cromatografía de líquidos de alta presión con detector UV-visible (HPLC-UV/Vis). El cromatógrafo de líquidos modelo HP1100 de Agilent está compuesto por una bomba binaria de alta presión (serie HP1200), un automuestreador, una columna LiChrosob® C18 (5 μm, 4.6 x 150 mm) (Análisis Vínicos) y un detector ultravioleta/visible (serie HP1100), para la detección de los analitos a una longitud de onda fija. El control del sistema, así como el análisis de datos se lleva a cabo a través del software HP ChemStation, de la misma casa comercial.

Cromatografía de líquidos de ultra-alta presión con detector UV-visible de diodos en fila (UPLC-DAD). El cromatógrafo de líquidos modelo Aquity de Waters, está compuesto por una bomba de ultra alta presión, un desgasificador, un automuestreador, un compartimento/horno para la columna y un detector ultravioleta-visible de diodos en fila (DAD) para la determinación de analitos a varias longitudes de onda. Se ha utilizado una columna Aquity UPLC® BEH C18 de 1.7 μm de tamaño de partícula, y de dimensiones 2.1 x 100 mm. El sistema se controla con el software Empore, de la misma casa comercial.

Cromatografía de líquidos de alta presión acoplada a espectrometría de masas (HPLC-MS/MS). El cromatógrafo de líquidos HP1260 de la casa comercial Agilent consta de una bomba binaria de alta presión, un desgasificador, un automuestreador y un compartimento para la columna. Se ha utilizado una columna Zorbax Eclipse XDB-C18 (5 μm, 4.6 x 150 mm) de Agilent. El sistema cromatográfico está acoplado a espectrometría de masas de triple cuadrupolo con una fuente de ionización por electrospray (ESI). Se usó el software Agilent MassHunter WorkStation para la toma y tratamiento de datos.

Cromatografía de gases acoplada a espectrometría de masas (GC/MS). Se usó el cromatógrafo de gases HP6890 acoplado a un espectrómetro de masas HP5973, ambos de Agilent. Este espectrómetro consta de una fuente de ionización de impacto electrónico, para fragmentar las moléculas (con una energía de ionización de 70 eV a una temperatura de 230 ºC) y un cuadrupolo para analizar las masas. Los cromatogramas se adquirieron en el modo SCAN, estableciéndose un intervalo de valores de m/z en los que se encontraban los analitos.

El control del sistema se lleva a cabo por el software HP1701CA MS ChemStation (Agilent Technologies). La separación cromatográfica se ha realizado en una columna capilar de sílice fundida HP-5ms (30 m x 0.25 mm d.i.) recubierta en su interior por una fase estacionaria de 0.25 μm de espesor de película y compuesta por un 5% de fenilpolisiloxano y un 95% de metilpolisiloxano (Agilent, Palo Alto, CA, USA). El inyector y el horno del cromatógrafo se programaron en términos de temperatura, presión y caudal, para realizar la separación bajo condiciones óptimas. Como gas portador, se seleccionó helio de pureza 6.0 (Air Liquide) a un caudal de 1 mL/min que se fijó mediante un regulador digital de presión y flujo.

La desorción térmica de los analitos se realizó con un automuestreador de espacio de cabeza MPS2 (Gerstel). Este dispositivo está compuesto por un módulo de inyección en el que se encuentra una jeringa de gases de 2.5 mL que se mantuvo a una temperatura de 150 ºC; dos bandejas donde se colocan los viales con las muestras a analizar; un brazo robotizado y un horno que permite agitar y calentar las muestras para la generación del espacio de cabeza.

Espectroscopía infrarroja (IR). Las medidas se realizaron en un espectrómetro FT-IR de Bruker, modelo Tensor 37. Éste está equipado con una unidad de reflectancia total atenuada (ATR) de diamante con una superficie circular de 3 mm de diámetro y tres reflexiones internas. Los espectros fueron recogidos entre 4000 y 600 cm^{-1} con resolución de 4 cm^{-1} y 64 scans. El detector empleado fue de sulfato de triglicina deuterada (DTGS). Los datos se recogieron y trataron con el software OPUS (Bruker).

Análisis elemental. Las medidas del contenido de carbono y nitrógeno de los materiales sintetizados se llevaron a cabo en un analizador elemental modelo EA3000 de EuroVector. Este equipo pertenece al "Servicio Central de Apoyo a la Investigación" (SCAI) de la Universidad de Córdoba (UCO).

Microscopía electrónica de barrido. Para caracterizar el material se usó el microscopio JEOL JSM 6300 (Isaza) con una capacidad de aumento de entre 10x y 300.000x y una resolución de 3~4 nm (a 30 Kv), perteneciente al SCAI de la UCO.

También se empleó el modelo JEOL Quanta 200 F FE-SEM perteneciente a la Universidad de Gante (Bélgica). Previamente, las muestras se recubrieron de oro usando un recubridor modelo SKD 030 (Balzers Union).

Microscopía electrónica de transmisión. Las imágenes se obtuvieron con dos microscopios distintos, PHILIPS CM-10, y JEOL JEM-1400. Ambos pertenecientes al SCAI de la UCO.

Porosimetría. El área superficial de los diferentes materiales sintetizados se midió mediante el uso de un analizador de área superficial y de tamaño de poros, Quantachrome® ASiQwinTM-Automated, el cual se basa en medidas de adsorción/desorción de un gas (nitrógeno) a -196 ºC. Los valores de áreas superficiales específicas se calcularon de acuerdo a la ecuación de Brunauer–Emmett–Teller (BET). El equipo pertenece al "Instituto Universitario de Investigación en Química Fina y Nanoquímica" (IUIQFN) de la UCO.

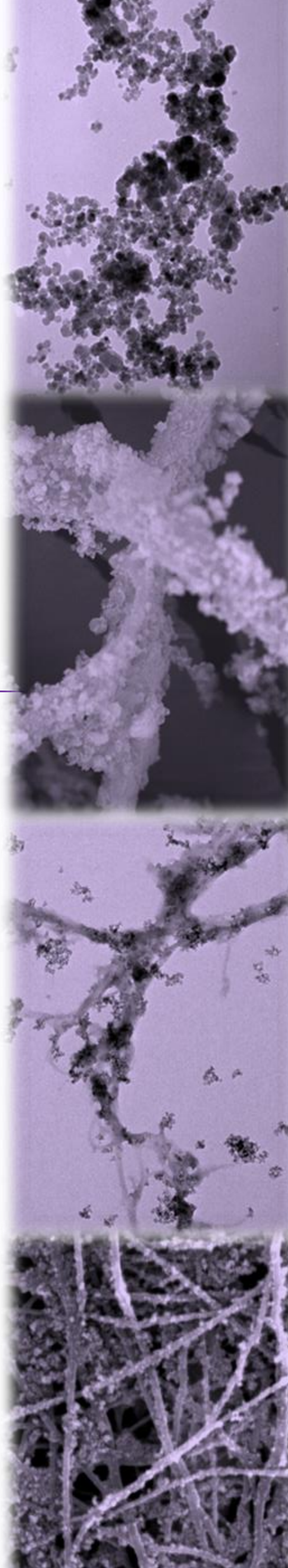
Curvas de magnetización. El comportamiento magnético se analizó mediante las curvas de histéresis, usando un dispositivo superconductor de interferencia cuántica (SQUID), modelo MPMS-XL-7T de Quantum Designs, a una temperatura constante de 27 °C. Las medidas se realizaron en el "Institut Catalá de Nanotecnología" (Barcelona, España).

Otros instrumentos. Se usaron un pH-metro (modelo Basic 20 pH, Crison) y una balanza analítica de precisión (modelo CPA225D de Sartorius).

Además, para medir la viscosidad y la conductividad de las disoluciones usadas en electrospinning, se usó un viscosímetro modelo LVDV-II (Brookfield) y un conductímetro modelo CDM210 (Radiometer Analytical).

BLOQUE III

MICROEXTRACCIÓN EN FASE SÓLIDA DISPERSIVA



Capítulo III. 1

Polymer-nanoparticles composites in bioanalytical sample preparation

Bioanalysis

7 (2015) 1723-1730

Polymer-nanoparticles composites in bioanalytical sample preparation

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The term composite refers to a class of synthetic materials made from different constituents which exhibit final properties which are different from those of the individual components. Composites have been extensively used in the sample treatment context as sorbents since the resulting solid present better extraction efficiency. In this realm, polymeric nanocomposites have raised as a powerful alternative. They can be tailored-synthesized for selectivity enhancement or include a magnetic core to simplify the extraction /elution process. This review article points out the relevance of such nanomaterials in bioanalysis. Several synergic combinations of nanoparticles (magnetic, carbon-based) as well as polymeric coatings (conventional, conductive or molecularly imprinted) are commented on. Finally, the potential of biopolymers in the microextraction field is briefly highlighted.

Keywords: *Polymer; nanoparticle; composite; sample treatment; molecularly imprinted polymer.*

Capítulo III. 2

Hybridization of commercial polymeric microparticles and magnetic nanoparticles for the dispersive micro-solid phase extraction of nitroaromatic hydrocarbons from water

Journal of Chromatography A

1271 (2013) 50-55

Hybridization of commercial polymeric microparticles and magnetic nanoparticles for the dispersive micro-solid phase extraction of nitroaromatic hydrocarbons from water

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In this article, the combination of commercial polymeric microparticles (OASIS MCX) and cobalt ferrite nanoparticles is evaluated in dispersive micro-solid phase extraction (D-μSPE) for the determination of six nitroaromatic hydrocarbons in water. The high affinity of the polymeric material toward the target analytes as well as the magnetic behavior of cobalt ferrite nanoparticles are combined in a synergic way to developed an efficient and simple D-μSPE approach. The sorptive performance of the hybrid material is compared with that most usual sorbents and the effect of its synthesis steps on the extraction capability is also evaluated in depth. After the optimization of selected variables, D-μSPE method was assessed in terms of linearity, sensitivity, precision and accuracy. The new extraction method allows the determination of the target compounds with limits of detection in the range from 0.12 to 1.26 μg/L and relative standard deviations lower than 9.6%. The recovery study was performed in two different water samples obtaining percentages from 71 to 103%, which demonstrated the applicability of the hybrid sorbent for the selected analytical problem.

Keywords: *Magnetic solid phase extraction; polymeric microparticles; superparamagnetic nanoparticles; nitroaromatic compounds; UPLC-UV.*

1. Introduction

In the last 15 years, extraction techniques have been intensely investigated in order to improve the isolation and preconcentration steps of the analytical procedures. The ideal extraction technique should be simple, automatic, suitable of miniaturization, rapid, inexpensive and safe to operators and environment. In this context, automation, simplification and miniaturization can be considered as key trends in this evolution [1]. The current research is mainly focused on the development of new techniques and the discovery/application of novel materials with enhanced extraction efficiencies.

Nanoparticles (NPs) are clusters of atoms or molecules (in the form of metal oxides) with one or more dimensions in the nanometric range considering 100 nm as an arbitrary reference. Furthermore, the nanometric dimension provides the material with new physicochemical properties that are not present in the macroscopic scale [2]. Nanoparticles are characterized by a high surface which may develop a wide variety of interactions, depending on the NP nature, with target analytes. Both aspects make NPs excellent extracting phases in solid phase extraction (SPE) and solid phase microextraction (SPME) approaches [3]. Apart from this active role, NPs can be also used as inert supports as well as coadjutants that simplify the extraction procedures.

When bare NPs are unable to interact with the target analytes, they can be coated with active extractive phases. The coating process employs covalent interactions (currently the most common approach) [4] and [5], electrostatic interactions [6] and [7], or simply physisorption [8].

Moreover, NPs can act as coadjutants simplifying the extraction procedure. In this case, magnetic NPs (MNPs) [9] are particularly helpful for processing large volume of samples since the recovery of the sorbent, usually performed by filtration or centrifugation, can be simply achieved using an external magnet.

Superparamagnetism, which involves that these NPs only present magnetic behavior under the influence of an external magnetic field, is crucial in reducing their aggregation due to magnetic attraction during the extraction [10]. Moreover, they offer high surface-to-volume ratio and easy surface modification, which convert them in a very versatile tool in this context. Extractions with these MNPs are generally based on electrostatic or hydrophobic interactions [11].

At present, the potential of hybrid materials [12] in the extraction field is a topic of growing interest. Hybrid NPs, obtained by the combination of two different materials in nature (organic–inorganic for example), can be classified in two types according to the properties of two materials merged [3]. The properties can be different but complementary as it is the case of the combination of carbon nanotubes (sorption capacity) and ferrite NPs (paramagnetic behavior) [13]. On the other hand, these properties can be of the same nature but their combination may produce an important synergistic effect as it is the case of the combination of quantum dots [14].

Hybrid NPs comprising polymers and magnetic NPs can be considered as powerful materials in the (micro)extraction context. In most of the cases, these hybrid NPs consist of a magnetic core (usually based on iron oxide) coated with a polymeric network which can be anchored covalently or by electrostatic interactions. Covalent bound are usually involved when molecularly imprinted polymers (MIPs) [15] and [16] or polypyrrole [17] are used as polymeric coating while electrostatic interactions take place when biopolymers, such as chitosan [18] or alginate [19], are employed. In the latter case, these biopolymers are usually employed to enhance the dispersibility of derivatized MNPs.

In this paper, the combination of commercial polymeric microparticles (OASIS MCX) and magnetic nanoparticles (cobalt ferrite) is presented. In this way, we combine the great capability of sorption of the polymeric material with the magnetism of the nanoparticles, which facilitates the sorbent isolation after the extraction. Moreover, the use of a commercial polymer clearly simplifies the synthesis process while ensures the reproducibility of the final hybrid material. As model analytical problem, six selected nitroaromatic hydrocarbons were determined in water samples.

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade or better. Nitroaromatic hydrocarbons (4-nitrobenzaldehyde; 1,4-dinitrobenzene; nitrobenzene; 2,4-dinitrotoluene; 4-nitrotoluene and 3-nitrotoluene) were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solutions were prepared in acetonitrile (Panreac, Barcelona, Spain) at 1000 mg/L and stored at 4 °C. Working solutions of the target analytes were prepared by the appropriate dilution of the stocks in Milli-Q water (Millipore Corp., Madrid, Spain) or methanol/water (1/1, v/v) as required.

For analyte extraction, different sorbents were evaluated, namely: LiChrolut-EN (40–120 μm, styrene–divinylbenzene copolymer) from Merck (Madrid, Spain); OASIS HLB (30 μm, polyvinylpyrrolidone–divinylbenzene copolymer) and OASIS MCX (30 μm, sulfonated styrene–divinylbenzene copolymer) both from Waters (Madrid, Spain), RP-C18 (20 μm) from Analisis Vínicos (Tomelloso, Spain), polyvinyl chloride from Fluka (Madrid, Spain) and multi-walled carbon nanotubes (purity >95%, diameter in the range of 6–9 nm and average length of 5 μm) from Sigma–Aldrich.

Sulfuric acid was used for LiChrolut-EN sulfonation. For the magnetization of sulfonated LiChrolut-EN and OASIS MCX, hydrogen peroxide 33% (w/v), sodium hydroxide, iron (III) nitrate-9-hydrate and cobalt (II) nitrate-6-hydrate were employed. All these reagents were purchased from Panreac.

Tap and creek water samples were collected in amber glass bottles without headspace and stored at 4 °C until analysis.

2.2. Apparatus

Chromatographic analyses of nitroaromatic hydrocarbons were carried out on a Waters Acquity™ Ultra Performance LC system (Waters Corp., Madrid, Spain) using an Acquity UPLC® BEH C18 column (1.7 µm, 2.1 mm × 100 mm) maintained at 54 °C. The separation was performed under a gradient elution program using acetonitrile (solvent A) and water (solvent B) as mobile phase components. The elution started at 10% of solvent A and it was linearly increased up to 55% in 5.0 min. The system was re-equilibrated for 1 min between analyses, and therefore the total run time was 6.5 min. During the separation, the flow rate was maintained at 0.5 mL/min and 1 µL was injected with partial loop mode. The separated analytes were measured at 260 nm using a PDA eλ Detector (Waters). System control was achieved with Empower software also from Waters.

Magnetic behavior was analyzed performing an hysteresis loop using a Superconducting Quantum Interference Device (SQUID) MPMS-XL-7T from Quantum Designs (San Diego, CA, USA) at constant temperature (27 °C). Measurements were performed at the "Institut Catalá de Nanotecnología" (Barcelona, Spain).

For the synthesis, a heating magnetic stirrer (Velp Scientifica, Milan, Italy) was required and a vortex (Reax top, Heidolph) was employed for sample agitation.

An external magnet (60 mm × 30 mm × 15 mm and 549.4 N of maximum magnetic force), purchased from Supermagnete (Gottmadingen, Germany), was employed for the sorbent recovery after D-µSPE.

2.3. Sorption curves

Single-component binding sorption curves for nitrobenzene on different sorbents were established by batch extraction experiments. 10 mg of the different sorbents were incubated for 24 h at 25 °C with 5 mL of methanol/water (1:1) solutions containing nitrobenzene. The initial concentration of nitrobenzene $[NB]_0$, was in the range from 1 to 50 mg/L. UPLC analysis of the supernatants of the equilibrated samples gave the concentrations of the free analyte $[NB]_{free}$. The amount of analyte bonded to the sorbent was calculated from the difference $[NB]_0 - [NB]_{free}$. The corresponding data were plotted in the form $[NB]_{bound}$ vs $[NB]_{free}$.

2.4. Synthesis of the hybrid particles

Hybrid particles consist of the combination of sulfonated copolymers and magnetic nanoparticles of cobalt ferrite ($CoFe_2O_4$) following a previously described procedure [20]. In brief, OASIS MCX and LiChrolut-EN, which were employed as polymeric precursors, have a different chemical composition since the OASIS MCX presents sulfonic groups in its structure while the LiChrolut-EN lacks of them. Therefore, LiChrolut-EN should be previously sulfonated. For this purpose, 1 g of the sorbent was introduced in 80 mL of concentrated sulfuric acid and stirred for 1 h at room temperature. Then, the particles were washed with 700 mL of cold milli-Q water in order to avoid overheating due to the mixture of sulfuric acid and water.

The incorporation of $CoFe_2O_4$ nanoparticles on the sulfonated polymeric network, which is hereinafter referred to as the magnetization process, was performed in different steps. First of all, sulfonated particles were immersed in 50 mL of an aqueous solution of Fe(III) and Co(II) nitrates (1 and 0.5 M, respectively) and stirred for 12 h. In this step, Fe(III) and Co(II) were retained by cation exchange interaction on the sulfonated polymer. Then, the solid was recovered and washed twice with 100 mL of milli-Q water to remove the excess of physisorbed ions. Then, the solid was dispersed in 250 mL of an aqueous solution of NaOH 1.2 M and stirred for 2 h at 80 °C, causing the precipitation of the corresponding hydroxides. Afterwards, 250 mL of an aqueous hydrogen peroxide solution (33%, v/v) was added dropwise over a period of 15 min and the reaction mixture was kept at 80 °C for 2 h with continuous stirring. In this step, the hydroxide was transformed into magnetic $CoFe_2O_4$. Finally, the solid was washed twice with 100 mL of Milli-Q water. The magnetization process was performed three times in order to obtain hybrid particles with an appropriate magnetic behavior.

The amount of cobalt ferrite introduced in the polymeric network was quantified by gravimetric analysis. For this purpose, the magnetized material was heated at 600 °C for 1 h in order to destroy the organic polymeric network. After the treatment, the solid was weighted.

2.5. Extraction procedure

The extraction procedure consists of several well-defined steps. First of all, 20 mg of magnetized OASIS MCX are added to 200 mL of sample. The addition of external ionic strength and pH adjustment is not necessary. The sorbent and the sample are stirred in a vortex for 2 min, favoring the extraction of the target analytes. Then, the sorbent is isolated from the sample by an external magnet without needing to centrifuge or filter. The isolated sorbent is subsequently washed twice with 10 mL of water and the analytes are finally eluted by means of 500 µL of acetonitrile, which are collected in a 2 mL glass vial for the further UPLC analysis.

3. Results and discussion

Magnetic nanoparticles tend to aggregate during their synthesis which makes difficult their size control. This shortcoming can be avoided using an inert matrix as silica [21] and [22], zeolites [23] and [24], or polymers [20] and [25] as templates. In this sense, the use of styrene–divinylbenzene has been reported to obtain MNPs with narrow size distribution.

This general strategy has been exploited in this paper to confer a commercial polymeric sorbent with magnetic properties. The influence of the different synthetic steps on the sorption capacity is evaluated in depth and finally the sorbent is applied to solve a model analytical problem.

3.1. Sorbents evaluation

The selection of the sorbent material for the extraction of nitroaromatic hydrocarbons is a crucial step in order to achieve their efficient isolation and preconcentration. This selection was performed by obtaining the sorption curves of different materials using nitrobenzene (NB) as model and representative analyte and methanol:water (1:1, v/v) as media. This medium was selected since very low sorption was observed in methanol while strong retention was obtained in water. Thus, in methanolic solution a negligible difference between $[NB]_{free}$ and $[NB]_0$ was observed, which involved a consequent error in the calculation of $[NB]_{bound}$. Nearly complete sorption of analytes from pure water decreases their concentrations under LOD of the method. As a mixed medium is employed, the term Langmuir isotherm has been avoided in the discussion although the behavior observed can be directly related.

The results obtained for unmodified sorbents (namely: LiChrolut-EN, OASIS HLB, carbon nanotubes, RP-C18 and PVC) are presented in Fig. 1. All the measurements were performed in duplicate and the overall standard deviation, following the guidelines for duplicate analyses [26], was better than 10%. The sorption curves show that polymeric materials provide better affinity and capacity values compared to the other sorbent assayed. In fact, LiChrolut-EN (ethylvinylbenzene–divinylbenzene polymer) and OASIS HLB (vinylpyrrolidone–divinylbenzene polymer) are demonstrated suitability for the extraction of moderate polar organic compounds as it is the case of nitroaromatic hydrocarbons and therefore they were employed for further analysis.

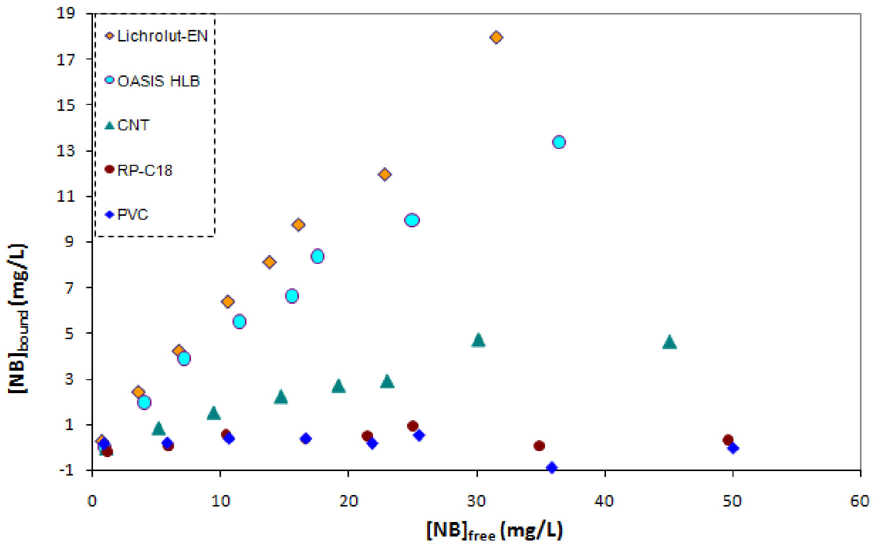

Figure 1. Sorption curves obtained for nitrobenzene in methanol/water (1:1) using different sorbents, namely: LiChrolut-EN, OASIS HLB, carbon nanotubes (CNT), octadecylsilica (RP-C18) and polyvinyl chloride (PVC).

3.2. Impact of sulfonation on sorption

The presence of sulfonic acid groups in the polymeric network is essential to the homogeneous distribution of magnetic nanoparticles. As LiChrolut-EN lacks of these functional groups, a previous sulfonation process with sulfuric acid is required. The sulfonation of LiChrolut-EN was evaluated at high (80 °C) and room temperature, and the effect of both conditions on the extraction of NB is presented in Fig. 2. The sorption curves of bare LiChrolut-EN, bare OASIS HLB and its commercial sulfonated version (OASIS MCX), are also presented for comparative purposes. As it can be inferred, when sulfonation is performed at high temperatures, the interaction with the analyte is markedly reduced. On the other hand, the sulfonation at lower temperatures modifies the sorption behavior of the polymeric material in the same way that occurs if the sorption curves of OASIS MCX and OASIS-HLB are compared.

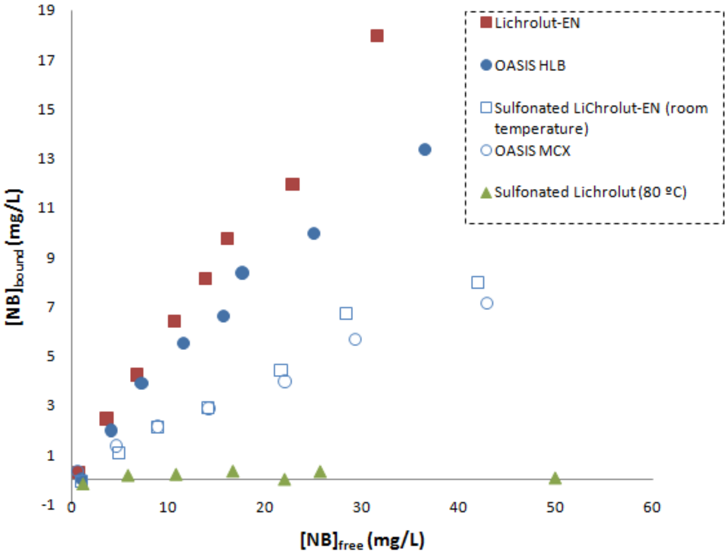

Figure 2. Effect of the sulfonation on the sorption curves for nitrobenzene in methanol/water (1:1) solutions. Different sorbents are presented, namely: bare LiChrolut-EN, sulfonated LiChrolut-EN in two different conditions, OASIS HLB and OASIS MCX.

3.3. Polymer magnetization

Once the polymer presents sulfonic acid groups in their structure, the introduction of MNPs of $CoFe_2O_4$ was performed following the procedure described in Section 2. The introduction of MNPs involves two different effects. On the one hand, the magnetic properties of the polymer are clearly enhanced when a large amount of NPs are introduced in the network. In fact, the recovery of the sorbent with the external magnet becomes easier in such conditions. On the other hand, as it is shown in Fig. 3, the introduction of the MNPs reduces the sorption capacity of the polymer. This aspect is due to the deposition of the MNPs on the polymer surface thus reducing its active extracting area. Therefore, although the magnetization cycle can be repeated several times in order to confer a more intense magnetic behavior to the polymer, the number of cycles also reduces the extraction capacity. Three magnetization cycles were selected as compromise between both aspects owing to the nature of the coating process. Despite the loss of extraction capabilities, the magnetized sorbents are still competitive sorbents compared for example with CNTs and RP-C18 and also present a magnetic behavior which is essential to simplify the overall D-μSPE procedure.

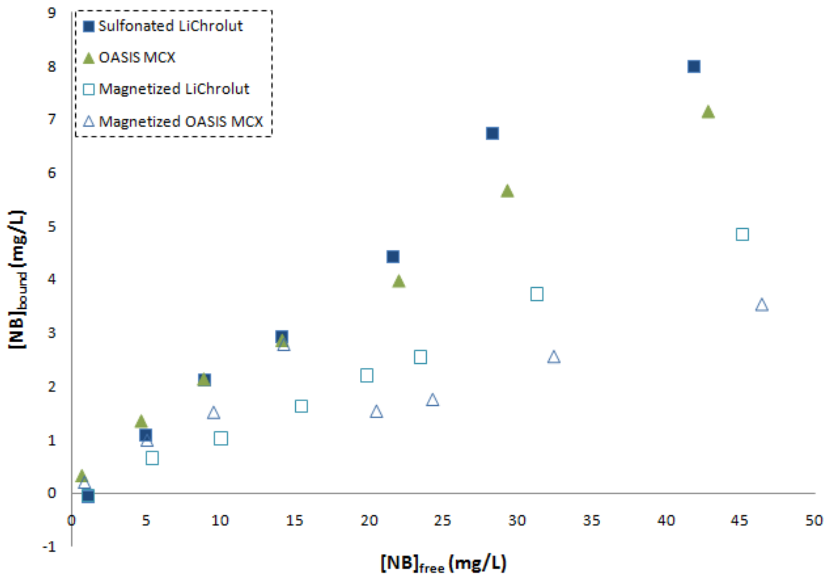

Figure 3. Effect of the magnetization on the sorption curves for nitrobenzene in methanol:water (1:1) solutions. Different sorbents are presented, namely: sulfonated LiChrolut-EN, OASIS MCX, magnetized LiChrolut-EN and magnetized OASIS MCX.

As it can be observed from Fig. 3, magnetized LiChrolut-EN and magnetized OASIS-MCX presents a similar sorption behavior, the latter presenting slightly lower extraction efficiency. OASIS MCX was selected for further studies since a previous sulfonation is not required thus reducing the number of steps of the synthetic procedure which is a greener and cheaper alternative.

The saturation magnetization of the hybrid material was investigated by magnetic hysteresis loop analysis (Fig. 4). The symmetrical shape of the curve suggests that there is no magnetic retentivity and therefore the hybrid material shows superparamagnetism, attaining a saturation magnetization value of 1 emu/g. This value is comparable to other hybrid polymeric materials [27]. Although it is not very high, the magnetic response allows the easy separation of the sorbent.

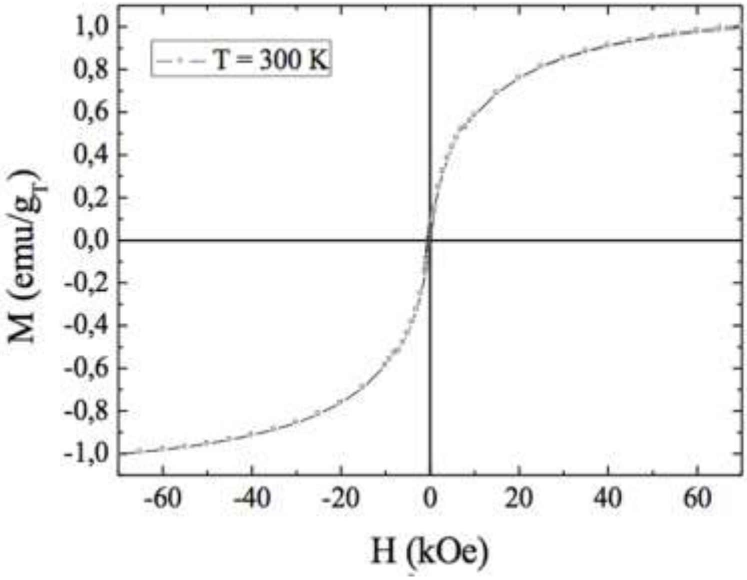

Figure 4. Magnetization curve of the magnetized OASIS MCX material (saturation magnetization is 1 emu/g).

The gravimetric analysis was finally carried out to quantify the amount of cobalt ferrite introduced in the polymeric network. After the treatment, a brown solid was obtained corresponding to ca. 9.8% of the initial weight. This solid presented paramagnetic behavior and it responds to the application of an external magnet.

3.4. Extraction optimization

After the selection of the optimum sorbent, the effect of several variables on the extraction of the target analytes were considered in depth, namely sample pH, ionic strength, stirring time, sorbent amount and sample volume. For this purpose, different extraction conditions were evaluated using aqueous solutions containing the six nitroaromatic hydrocarbons at a concentration of 100 µg/L. The initial experimental conditions were: 10 mL of sample, pH 7, 20 mg of sorbent, 2 min of vortex stirring and elution with 500 µL of acetonitrile. Sample pH, which was studied in the range from 2 to 10, presented a small influence on the analyte extraction. Although at acidic pH a slight improvement is observed for some analytes, for simplicity, environmental waters can be directly processed without a previous pH adjustment. Moreover, ionic strength (evaluated in the range from 0 to 200 g/L using sodium chloride as model electrolyte) and stirring time (evaluated from 1 to 5 min) present a negligible effect on the analytes extraction. The latter aspect is especially interesting since it indicates that the analytes transference is quick enough due to the efficient sorbent dispersion. So, no salt was added and a stirring time of 2 min was fixed for subsequent analyses.

Finally, the sorbent amount and the sample volume were evaluated as they have a critical effect on the preconcentration factors. The sorbent amount was evaluated at five different levels (5, 10, 20, 50 and 100 mg), the result being shown in Fig. 5. Taking into account that a similar behavior was observed for all the analytes, only four compounds are presented for simplicity. As it can be observed, the analytical signals increased up to 20 mg of sorbent, decreasing over this value. However, the analytical signals decrease for higher quantities probably due to a worse sorbent dispersion. Therefore, 20 mg was selected to evaluate the sample volume at seven different levels, from 1 to 200 mL, the results being presented in Fig. 6. The signals increased with the volume for all analytes, so that the 200 mL was fixed in order to improve the sensitivity of the method. Larger sample volumes were not investigated as the sorbent recovery becomes difficult and slow in such conditions.

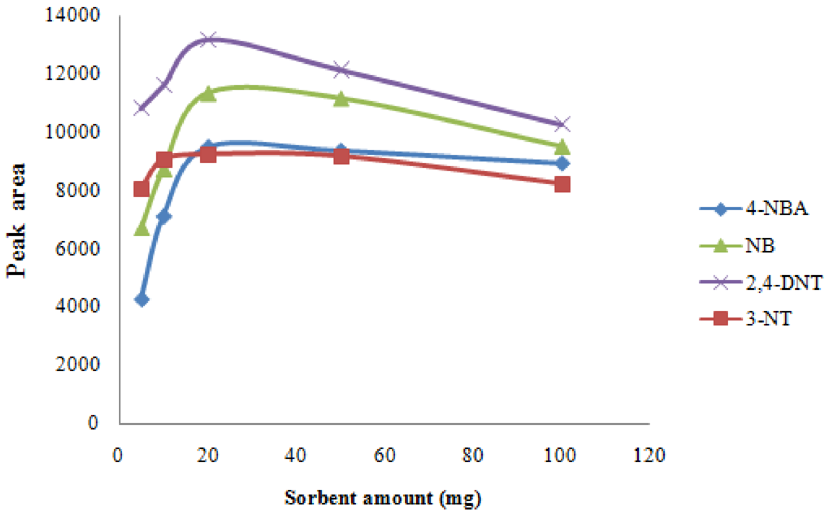

Figure 5. Effect of the hybrid particles amount on the extraction of target analytes. For simplicity, only 4-nitrobenzaldehyde (4-NBA), nitrobenzene (NB), 2,4-dinitrotoluene (2,4-DNT) and 3-nitrotoluene (3-NT) are shown.

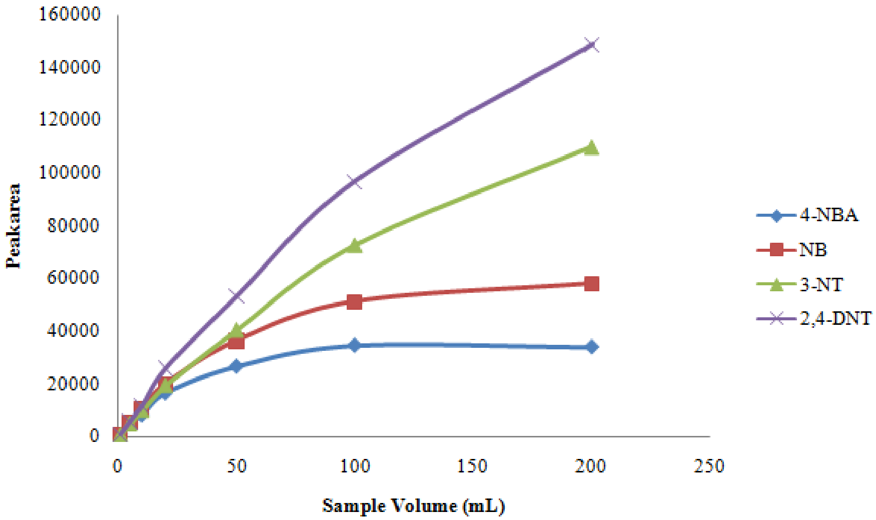

Figure 6. Effect of the sample volume on the extraction of target analytes. For simplicity, only 4-nitrobenzaldehyde (4-NBA), nitrobenzene (NB), 2,4-dinitrotoluene (2,4-DNT) and 3-nitrotoluene (3-NT) are shown.

3.5. Evaluation of extraction protocol

The optimized procedure was characterized in terms of linearity (linear ranges and correlation coefficients), precision (as repeatability), limits of detection and extraction efficiency (enrichment factors and absolute extraction recovery). The results are summarized in Table 1.

Table 1. Analytical figures of merit for the determination of nitroaromatic compounds in water samples.

Analyte	Linear range (µg/L)	R^{2a}	LOD[b] (µg/L)	RSD[c] (%)	EF[d]	ER[e]
4-Nitrobenzaldehyde	0.4-50	0.998	0.11	4.4	161	40.3
1,4-Dinitrobenzene	1.0-50	0.998	0.29	5.7	186	46.6
Nitrobenzene	1.5-50	0.999	0.46	9.5	184	46.0
2,4-Dinitrotoluene	1.7-50	0.999	0.53	4.6	292	73.1
4-Nitrotoluene	4.1-50	0.999	1.26	6.0	185	46.3
3-Nitrotoluene	2.7-50	0.999	0.81	6.4	197	49.2

[a] R^2, correlation coefficient.
[b] LOD, limit of detection.
[c] RSD, relative standard deviation.
[d] EF, enrichment factor.
[e] ER, absolute extraction recovery.

For each analyte, a calibration graph was constructed by extracting in triplicate six working aqueous standards with all the analytes at different concentrations in the range from 0.5 to 50 µg/L. For all the analytes, a good linearity ($R^2 > 0.998$) was observed. The limits of detection, which were calculated using a signal-to-noise ratio of 3, varied between 0.11 µg/L (4-nitrobenzaldehyde) and 1.26 µg/L (4-nitrotoluene). The repeatability of the method in terms of relative standard deviation (RSD, %) was evaluated at the limit of quantification in quintuplicate with results in the range from 4.4% (4-nitrobenzaldehyde) to 9.5% (nitrobenzene).

The enrichment factors for all the analytes, which were obtained by comparing the calibration graphs before and after the extraction process, were in the range from 161 (for 4-nitrobenzaldehyde) to 292 (for 2,4-dinitrotoluene). The absolute extraction recoveries, which refer to the percentage of total analyte that can be extracted efficiently by the sorbent and finally eluted with acetonitrile, were in the interval of 40.3–73.1%.

Since no positive samples were found, a recovery study was performed spiking water samples with the analytes at 10 μg/L. Table 2 shows that the recoveries were all in the range from 86 to 103 for tap and creek water. Only for 4-nitrobenzaldehyde, a lower recovery was obtained, although the value satisfies the 70–130% recovery criterion [28].

Table 2. Analyses of tap and creek water samples (n = 3) spiked with the target nitroaromatic compounds at a concentration of 10 μg/L.

Analyte	Tap (R±SD)[a]	Creek (R±SD)[a]
4-Nitrobenzaldehyde	89± 4	71 ± 3
1,4-Dinitrobenzene	92 ± 5	90± 5
Nitrobenzene	90 ± 8	93 ± 9
2,4-Dinitrotoluene	93 ± 4	96± 4
4-Nitrotoluene	86± 5	99 ± 6
3-Nitrotoluene	103± 6	97 ± 6

[a] R, relative extraction recovery expressed as percentage. SD, standard deviation.

4. Conclusions

In this article, the combination of commercial polymeric microparticles (OASIS MCX) and cobalt ferrite nanoparticles is evaluated in dispersive micro-solid phase extraction (D-μSPE) by the determination of six nitroaromatic hydrocarbons in water. The sorbent synergistically combines the extraction capabilities of the polymer and the magnetic nature of cobalt ferrite nanoparticles allowing the development of a simpler extraction procedure. Within the advantages of the presented extraction method, we can stand out the possibility of performing an analysis of large sample volumes with a small sorbent quantity, only 20 mg of sorbent for 200 mL of sample. Moreover, the relative short extraction time made the procedure simple and quick. In addition, the isolation of the sorbent is extremely easy since a simple magnet is required to separate it from the sample matrix. Finally, high enrichment factors, in the range from 161 to 292, can be obtained.

Being objective, the procedure also presents some shortcomings that should be overcome in future research. Limits of detection can be improved using lower elution volumes. Moreover, the use of MNPs with higher magnetic behavior than cobalt ferrite may enhance the sorption capacity of the hybrid material since the required magnetization cycles, and the inherent sorptive reduction, can be reduced.

Acknowledgments

Financial support from the Spanish Ministry of Science and Innovation (grant CTQ2011-23790) and P09-FQM-4801 from Junta de Andalucía are gratefully acknowledged. E.M. Reyes Gallardo would like to express her gratitude for the grant (“Iniciación a la investigación”) from the University of Cordoba. G. Lasarte-Aragonés would like to express his gratitude for the predoctoral grant (ref. AP2009-2850) from the Spanish Ministry of Education.

References

[1] R. Lucena, M. Cruz-Vera, S. Cárdenas, M. Valcárcel, Bioanalysis 1 (2009) 135.

[2] G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel, Bioanalysis 3 (2001) 2533.

[3] R. Lucena, B. M. Simonet, S. Cárdenas, M. Valcárcel, J. Chromatogr. A 1218 (2011) 620.

[4] Y. Sha, C. Deng, B. Liu J. Chromatogr. A 1198-1199 (2008) 27.

[5] P. Yu, Q. Wang, X. Zhang, X. Zhang, S. Shen, Y. Wang Anal. Chim. Acta 678 (2010) 50.

[6] L. Li, Y. Huang, Y. Wang, W. Wang, Anal. Chim. Acta 631 (2009) 182.

[7] H. Niu, Y. Cai, Y. Shi, F. Wei, S. Mou, G. Jiang, J. Chromatogr. A 1172 (2007) 113.

[8] A. Ballesteros-Gómez, S. Rubio, Anal. Chem. 81 (2009) 9012.

[9] A.-H. Lu, E. L. Salabas, F. Schüth, Angew. Chem. Int. Ed. 46 (2007) 1222.

[10] R.-H. Qin, W. Jiang, H.-Y. Liu, F.-S. Li, J. Funct. Mat. 38 (2007) 902.

[11] J.-H. Lin, Z.-H. Wu, W.-L. Tseng, Anal Methods 2 (2010) 1874.

[12] A. I. López-Lorente, B. M. Simonet, M. Valcárcel, Anal. Bioanal. Chem. 399 (2011) 43.

[13] G. Morales-Cid, A. Fekete, B. M. Simonet, R. Lehmann, S. Cárdenas, X. Zhang, M. Valcárcel, P. Schmitt-Kopplin, Anal. Chem. 82 (2010) 2743.

[14] S.M. Sadeghi, Nanotechnology 20 (2009) 225401.

[15] Z. Xu, L. Ding, Y. Long, L. Xu, L. Wang, C. Xu, Anal. Methods 3 (2011) 1737.

[16] Z. Lin, W. Cheng, Y. Li, Z. Liu, X. Chen, C. Huang, Anal. Chim. Acta 720 (2012) 71.

[17] J. Meng, J. Bu, C. Deng, X. Zhang, J. Chromatogr. A. 1218 (2011) 1585.

[18] X. Zhang, H. Niu, Y. Pan, Y. Shi, 357 Y. Cai, Anal. Chem. 82 (2010) 2363.

[19] S. Zhang, H. Niu, Y. Cai, Y. Shi, Anal. Chim. Acta 665 (2010) 167.

[20] L. A. García-Cerda, R. Chapa-Rodríguez, J. Bonilla-Ríos, Polym. Bull. 58 (2007) 989.

[21] V. Blanco-Gutiérrez, M. J. Torralvo-Fernández, R. Sáez-Puche, J. Phys. Chem. C 114 (2010) 1789.

[22] N. A. Fellenz, J. F. Bengoa, S. G. Marchetti, F. R. Sives, S. J. Stewart, R. C. Mercader, Hyperfine Interact. 170 (2006) 75.

[23] J.-S. Jung, J.-Y. Kim, W.-S. Chae, Y.-R. Kim, J.-H. Jun, L. Malkinski, W. Zhou, A. Kumbhar, M. L. Viciu, J. B. Wiley, C. J. O'Connor, Mater. Res. Soc. Symp. P. 676 (2001) Y3.45.1-Y3.45.5.

[24] N. A. Fellenz, S. G. Marchetti, J. F. Bengoa, R. C. Mercader, S. J. Stewart, J. Magn. Magn. Mater. 306 (2006) 30.

[25] S. M. Montemayor, L. A. García-Cerda, J. R. Torres-Lubián, Mater. Lett. 59 (2005) 1056.

[26] Z. Zhang, Y. Zhang, W. Tan, G. Li, Y. Hu, J. Chromatogr. A 1217 (2010) 6455.

Capítulo III. 3

Magnetic nanoparticles-nylon 6 composite for the dispersive micro solid phase extraction of selected polycyclic aromatic hydrocarbons from water samples

Journal of Chromatography A

1345 (2014) 43-49

Magnetic nanoparticles-nylon 6 composite for the dispersive micro solid phase extraction of selected polycyclic aromatic hydrocarbons from water samples

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In this article, the easy synthesis of magnetic nanoparticles-nylon 6 composite is presented, characterized and applied in the microextraction field. The one-step synthesis of the composite is performed by a solvent changeover playing with the different solubility of the polymeric network in formic acid and water. The new material has been characterized by different techniques including infrared spectroscopy, transmission and scanning microscopy. The extraction performance of the composite under a dispersive micro solid phase extraction format has been evaluated by determining four polycyclic aromatic hydrocarbons (benzo[b]fluoranthene, fluoranthene, indeno[1,2,3-cd]pyrene and phenanthrene) in water using ultra performance liquid chromatography (UPLC) combined with photo diode array detection. The developed methodology allows the determination of the analytes with limits of detection in the range from 0.05 μg/L (benzo[b]fluoranthene) to 0.58 μg/L (phenanthrene). The repeatability of the method was better than 6.9% at the limit of quantification level. The relative recoveries varied in the interval 80–111%.

Keywords: *Magnetic nanoparticles; nylon 6; composite; solid phase extraction; polycyclic aromatic hydrocarbons.*

1. Introduction

In the last years, the number of analytical techniques that involves the use of magnetic nanoparticles (MNPs) has exponentially increased. These nanoparticles (NPs) can be employed for three main tasks: extraction (isolation and preconcentration of target analytes); sensors and detection; and magnetic resonance imaging [1]. Due to their superparamagnetism, MNPs have a magnetic induced dipole only when a magnetic field is applied while they return to their native non-magnetic state when this field is removed [2]. This property is essential in magnetic solid phase extraction since it allows the easy dispersion of MNPs in aqueous or organic media and their simply recovery applying a magnetic field, usually in the form of an external magnet [3] and [4]. In addition, MNPs present a large surface-to-volume ratio thanks to their nanometric size which enhances the extraction kinetics.

The derivatization of the MNPs surface is a usual procedure to broad their application field since the inclusion of functional groups make the interaction with the analytes feasible and/or more selective. These coatings include non-magnetic, antiferromagnetic, or ferro/ferri-magnetic shells [5], the former being the most employed. Non-magnetic shells, like silica or alumina, are used to stabilize the magnetic properties of the MNPs providing also a surface than can be more easily functionalized. There are many examples of these types of NPs for dispersive micro solid phase extraction (d-µSPE) in environmental and bioanalytical applications [6], [7] and [8].

Hybrid MNPs comprising polymers are widely used in analytical chemistry considering that they are powerful materials in sample preparation. There are mainly two kinds of interaction whereby the polymer network is anchored to the MNP, by covalent bonding or by electrostatic interaction. The first one is normally concerned when polypyrrole or molecularly imprinted polymers (MIPs) are used as polymeric coatings, since biopolymers are immobilized by electrostatic interactions [3]. Magnetic molecularly imprinted polymers (MMIPs) have been recently used in this context since they also improve the selectivity of the extraction [9], [10] and [11]. These MMIPs have been used for the extraction of fluoroquinolones in milk, sterols in biological samples, β-agonists in pork and pig liver samples and auxins in plants, among others [12], [13], [14] and [15].

Hybrid NPs comprising polymers and MNPs can be classified in two types according to their structure. In most of the cases a polymeric layer is coated on the MNPs surface and the resultant hybrid maintains the nanometric size. On the other side, the MNPs are embedded on a polymer network giving rise to composites which combines nano and micrometric structures. In the last case, the immobilization of NPs depends on the functional groups of the polymer and can involve dispersive or Van der Waals, electrostatic, hydrogen or covalent bonds [16]. In a previous work, we anchored cobalt ferrite nanoparticles in a commercial polymeric network to synthesize a new sorbent with complementary properties for d-μSPE [17]. In addition, magnetic carbon nanotubes (mCNTs) and magnetic polypyrrole nanowires (mPPYs) have been also synthesized by a simple co-mixing method for their application as sorbents for estrogens in milk and pesticides in food and environmental samples respectively [18] and [19].

In this article, the synthesis, characterization and potential uses of magnetic nanoparticles-nylon 6 composites in sample preparation are proposed for the first time. The composite is easily synthesized by a solvent changeover playing with the different solubility of the polymeric network in formic acid and water. The determination of four polycyclic aromatic hydrocarbons (PAHs), as representatives of atmospheric pollutants, in aqueous samples has been used as model analytical problem.

2. Experimental

2.1. Reagents

All reagents were of analytical grade or better. The analytes: benzo[b]fluoranthene, fluoranthene, indeno[1,2,3-cd]pyrene and phenanthrene were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solution of each analyte was prepared at a concentration of 200 mg/L in methanol (Carlo Erba Reagents, Italy) and stored at 4 °C. A diluted solution containing all the analytes at a concentration of 20 mg/L was prepared in methanol and stored at 4 °C. Working solutions were prepared by the appropriate dilution of the stocks in Milli-Q water (Millipore Corp., Madrid, Spain).

Iron (III) chloride hexahydrate ($FeCl_3·6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2·4H_2O$) and ammonia were used for the synthesis of the magnetic nanoparticles (Fe_3O_4). Tetraethylorthosilicate (TEOS) and ethanol were employed for covering the NPs with a protective silica-based coating. Formic acid and nylon 6 were used for the preparation of the polymer network where the MNPs are embedded. All the reagents were purchased from Sigma–Aldrich.

2.2. Synthesis of the magnetic nanoparticles-nylon 6 composite

The first step was the synthesis of the Fe_3O_4 nanoparticles by a coprecipitation method. In this case, 24 g of $FeCl_3·6H_2O$ and 9.8 g of $FeCl_2·4H_2O$ were dissolved in 100 mL of Milli-Q water. The mixture was maintained at 80 °C for 30 min under nitrogen atmosphere. After that, 50 mL of ammonia (25 wt%) were added producing a black precipitate of iron oxide. The MNPs were separated by an external magnet, washed with water and methanol, and finally dried.

The second step was the coating with silica [20]. For this purpose, 1 g of MNPs was dispersed in 500 mL of ethanol and the dispersion was homogenized by sonication for 15 min. Then, 120 mL of Milli-Q water, 25 mL of TEOS and 15 mL of an aqueous solution of ammonia (pH 10) were added and stirred for 24 h under a nitrogen atmosphere. The obtained nanoparticles ($Fe_3O_4@SiO_2$) were separated with the help of an external magnet, washed with water (six times) and methanol, and finally dried. The silica coating is necessary to protect the magnetic core during the final step of the synthesis.

The third and last step was the synthesis of the nylon 6 polymeric network with embedded MNPs. For this purpose, 150 mg of nylon-6 were dissolved in 5 mL of formic acid and then 200 mg of $Fe_3O_4@SiO_2$ were dispersed into the solution by means of sonication for 5 min. Then, the dispersion was added with a syringe to a beaker containing 50 mL of water, to cause the gelation of the nylon-6 around the magnetite NPs. The gelation is produced by solvent changeover since nylon is soluble in formic acid but it is insoluble in water. The solid was washed with water and methanol (twice) using a magnet to separate the solid from the washing solution, and finally dried.

2.3. Characterization of the magnetic nanoparticles-nylon 6 composite

A Philips CM-10 transmission electron microscopy (TEM) was used to obtain the micrographs of the NPs which were used to characterize their particle size and morphology. A JEOL JSM 6300 scanning electron microscopy (SEM) was also used to obtain the micrographs of the synthesized composite. Micrographs were acquired in the Central Service for Research Support (SCAI) of the University of Córdoba.

Infrared measurements were performed in a Bruker Tensor 37 FT-IR spectrometer, equipped with a diamond ATR cell with a circular surface of 3 mm diameter and three internal reflections. A Deuterated Triglycine Sulfate (DTGS) detector was used for spectra acquisition. Spectra were collected between 4000 and 600 cm^{-1} at a 4 cm^{-1} resolution with 64 coadded scans each. Data collection was made using OPUS software (Bruker, Ettligen, Germany).

2.4. Sample extraction procedure

First of all, 40 mg of the composite are added to 25 mL of aqueous sample. The sorbent and the sample are stirred in a vortex for 1 min, sonicated for 30 min and finally stirred in a vortex (1 min) to favor the extraction of the target analytes. Then, the sorbent is isolated from the sample by an external magnet and is subsequently washed with 10 mL of water. Once the sorbent is totally dried, the analytes are finally eluted by means of 500 µL of acetone, which are collected in a 2 mL glass vial for the further UPLC analysis. A schematic diagram of the dispersive micro solid phase extraction procedure is shown in Fig. 1.

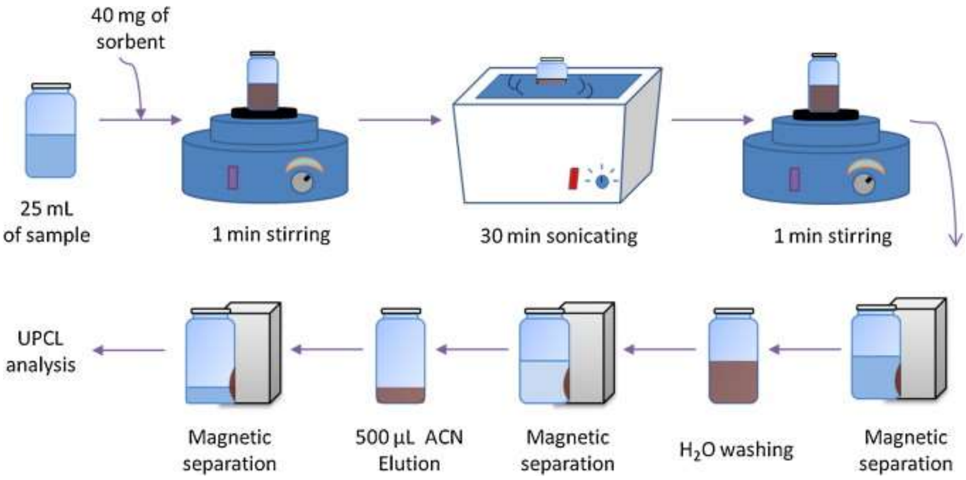

Figure 1. Schematic diagram of the dispersive micro solid phase extraction procedure.

2.5. Chromatographic analysis

Chromatographic analyses of PAHs were carried out on a Waters Acquity™ Ultra Performance LC system (Waters Corp., Madrid, Spain) using an Acquity UPLC® BEH C18 column (1.7 μm, 2.1 mm × 50 mm) maintained at 30 °C. The separation was performed under a gradient elution program using water (solvent A) and acetonitrile (solvent B) as mobile phase components. The elution started at 65% of solvent B and it was maintained for 1.6 min. After that, the percentage was linearly increased up to 75% in 2.4 min. The system was re-equilibrated for 0.5 min between analyses, and therefore the total run time was 4.5 min. During the separation, the flow rate was maintained at 0.5 mL/min and 2 μL was injected with partial loop mode. Detection was performed in the UV Maxplot mode (210–660 nm) using a PDA eλ Detector (Waters). Areas of the different analytes were obtained at their maximum absorption wavelength, 251 nm for phenanthrene, 236 nm for fluoranthene, 257 nm for benzo[b]fluoranthene and 249.7 nm for indeno[1,2,3-cd]pyrene. System control was performed with Empower software also from Waters.

3. Results and discussion

3.1. Characterization of the magnetic nanoparticles-nylon 6 composite

The composite was characterized by SEM, TEM and FT-IR spectroscopy. SEM images, which are shown in Fig. 2, were obtained in order to evaluate the composite structure. As it can be seen, after the synthesis, the surface of the polymer becomes rougher due to the formation of the composite with magnetic nanoparticles in the polymeric network.

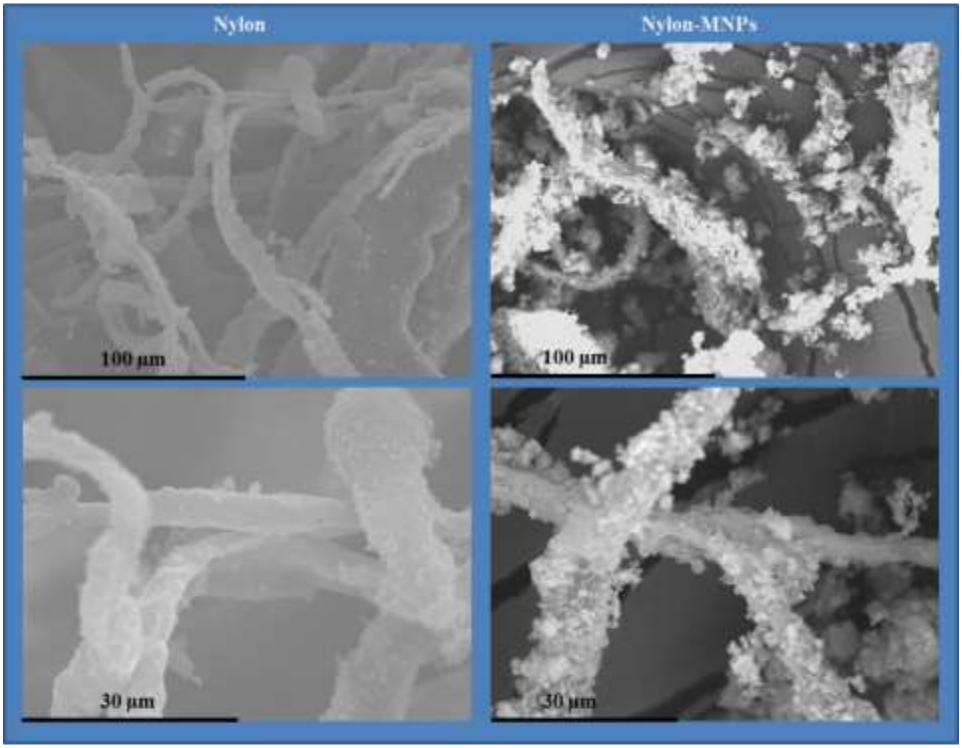

Figure 2. Scanning electron microscopy (SEM) images of nylon 6 (left) and magnetic nanoparticles-nylon 6 composites (right) at 500× and 1600×.

TEM micrographs show, as it can be observed in Fig. 3A, that the nanoparticles are spherical and have an average diameter of 10 nm. In addition, as it is shown in Fig. 3B, the magnetic nanoparticles nylon 6 composite could be well dispersed in aqueous solution due to the amide polar groups of the outer nylon surface of the composite but it can be easily recover applying an external magnet in few minutes leaving a clear and transparent aqueous solution.

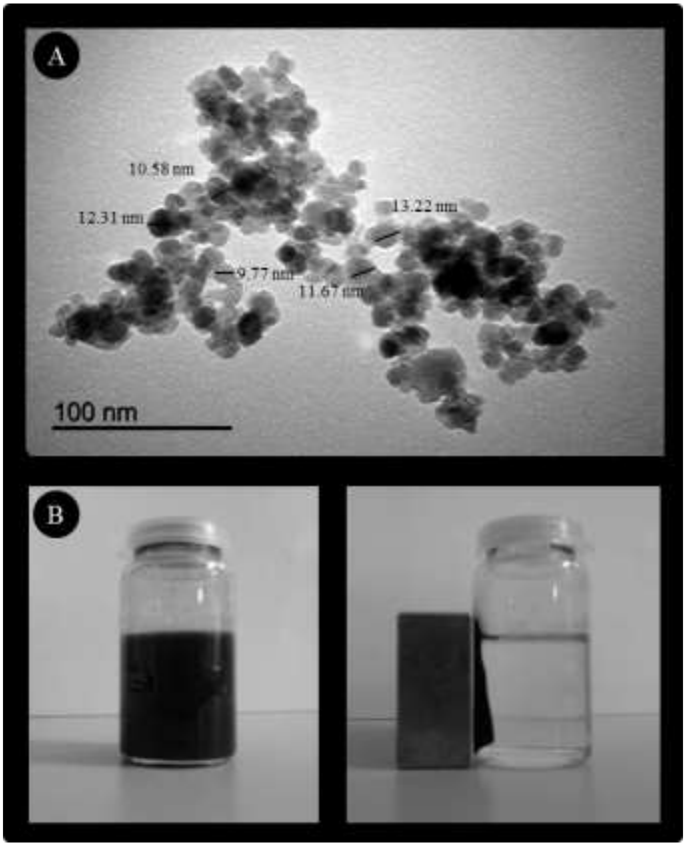

Figure 3. (A) Transmission electron microscopy (TEM) images of magnetic nanoparticles-nylon 6 composite (magnification 500k) and (B) solution of magnetic nanoparticles-nylon 6 composite before and after the magnetic separation.

FTIR was employed to confirm the chemical composition of the composite. An IR spectrum of each step of the synthesis was recorded. All the spectra show a characteristic band at 580 cm−1 which can be assigned to the Fe−O−Fe vibration. After the deposition of the silica, a strong absorbing region at 1200–1000 cm^{-1} appears, being characteristic of the Si−O−H and Si−O−Si bonds. The introduction of nylon in the structure involves the appearance of a band around 1642 cm^{-1} corresponding to the C=O stretching of the amide group. Moreover, another band around 1546 cm^{-1} is observed, which can be assigned to the N−H deformation band of the amide I. In Fig. 4, spectra of nylon 6, $Fe_3O_4@SiO_2$ and MNPs-nylon 6 composite are represented. As it can be observed, the MNPs-nylon 6 composite spectrum is the addition of nylon 6 and $Fe_3O_4@SiO_2$ spectra, and has the representative bands of both compounds.

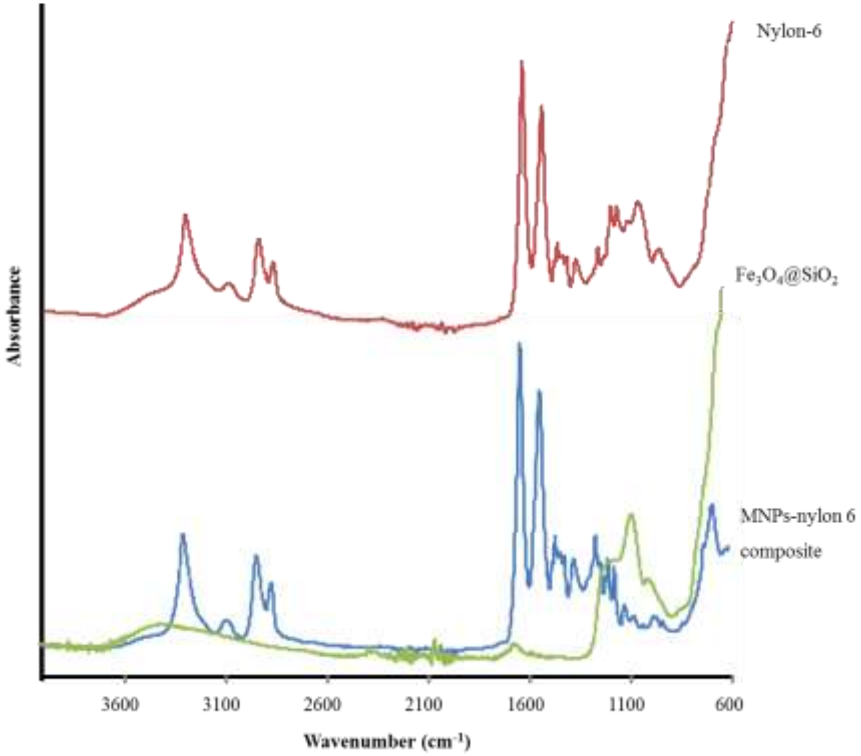

Figure 4. Infrared spectra of nylon 6, silica coated magnetic nanoparticles and magnetic nanoparticles-nylon 6 composite.

The magnetic behavior of the synthesized sorbent is key for the development of efficient procedures. The magnetic nature of the magnetic nanoparticles-nylon 6 composite in water and acetone is shown in Movie 1 (supplementary material). As it can be seen, the magnetic composite is easily recovered from both media although the recovery from water is a little bit slower due to the efficient dispersion of the sorbent in this solvent.

3.2. Optimization of the synthesis

The amount of nylon employed for the synthesis of the composite was evaluated in depth. For this purpose, three different composites were prepared using 200 mg of MNPs, 5 mL of formic acid and different amounts of nylon 6 (100, 150 and 200 mg). The procedure of the synthesis was carried out as it was previously explained (Section 2.2). For each composite, an IR spectrum was recorded where it was observed (data not shown) that the intensities ratio between nylon and SiO2 bands increases with the amount of nylon.

The extraction capability of each composite was also assessed. For this aim, 40 mg of composite was used to extract 25 mL of an aqueous standard solution containing the analytes at 20 µg/L. The mixture was stirred for 1 min in a vortex, sonicated for 30 min and stirred again for 1 min more. Then, the sorbent was isolated by a magnet and the sample was washed with 10 mL of water. Finally, the sorbent was dried and the analytes were desorbed with 500 µL of acetone and the eluent was analyzed in the chromatographic system. Attending to the results (Fig. 5), the composite containing 200 mg of nylon provided the best results in terms of extraction. Although 150 mg provides slightly lower signals than 200 mg, it results in a better precision and its separation from the aqueous phase is faster. Therefore, 150 mg was selected as optimum. From the results obtained, it can be concluded that there is a dual effect of the presence of nylon 6 in the composite network. On the one hand, it positively affects to the extraction capacity of the composite but higher amounts results in a reduction of the porosity. For that reason, the ER for the PAHs with higher molecular volume (indeno(1,2,3-cd)pyrene) decreased remained almost constant while the smallest PAHs are extracted more effectively with higher nylon amounts. 150 mg were selected as compromise between extraction capacity and porosity.

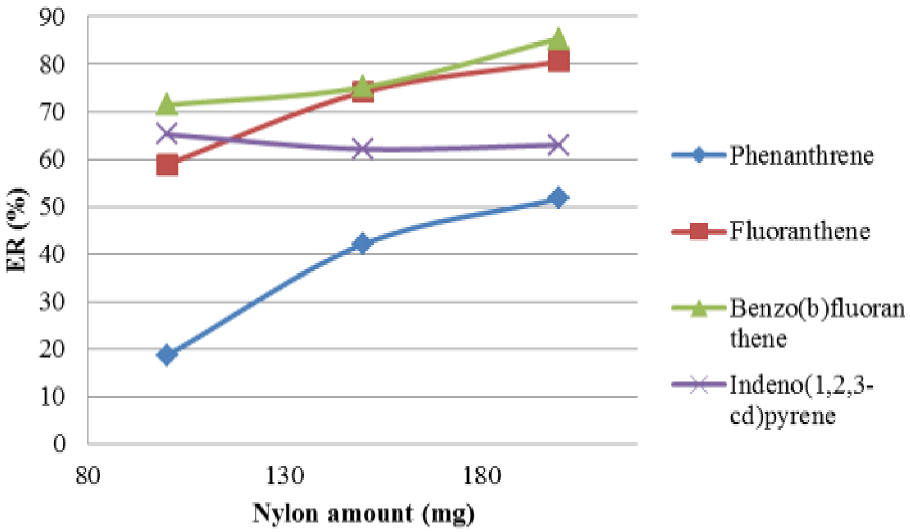

Figure 5. Effect of the nylon amount on the synthesis of the composite.

3.3. Optimization of the extraction process

Once the synthesis of the magnetic nanoparticles-nylon 6 composite was optimized, the effect of several variables on the extraction of the PAHs was considered. First, several solvents, namely: hexane, methanol, acetone, toluene, ethyl acetate, hexane/2-propanol and methanol/formic acid were evaluated as solvents. 500 μL of acetone provided the best elution in terms of precision and sensitivity.

The rest of the variables, sorbent amount and sample volume, were evaluated using aqueous solutions containing the analytes at a concentration of 20 μg/L. The initial experimental conditions were: 25 mL of sample, 20 mg of sorbent and elution with 500 μL of acetone. Sorbent amount was studied in the range from 10 to 40 mg, the result being shown in Fig. 6. As it can be observed, each analyte has a different tendency. The analytical signals of phenanthrene and fluoranthene increased with the sorbent amount while the signals for benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene have the opposite behavior. This unpredicted behavior, since an increase of the signal with the sorbent amount was expected, can be explained taking into account the analytes volume, their polarity and the polymeric network nature. At low sorbent amount, the composite is easily dispersed and the analytes are mainly extracted according to their polarity. Therefore, those analytes with a more non-polar nature (benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene) are extracted in a larger extent. When the sorbent amount is increased, the composite may present a higher aggregation tendency forming structures with a low porosity. In these circumstances, the analyte diffusivity plays an important role. Those analytes with a lower molecular volume (phenanthrene and fluoranthene) can easily diffuse to the core of the composite. Therefore 40 mg was selected as a compromise.

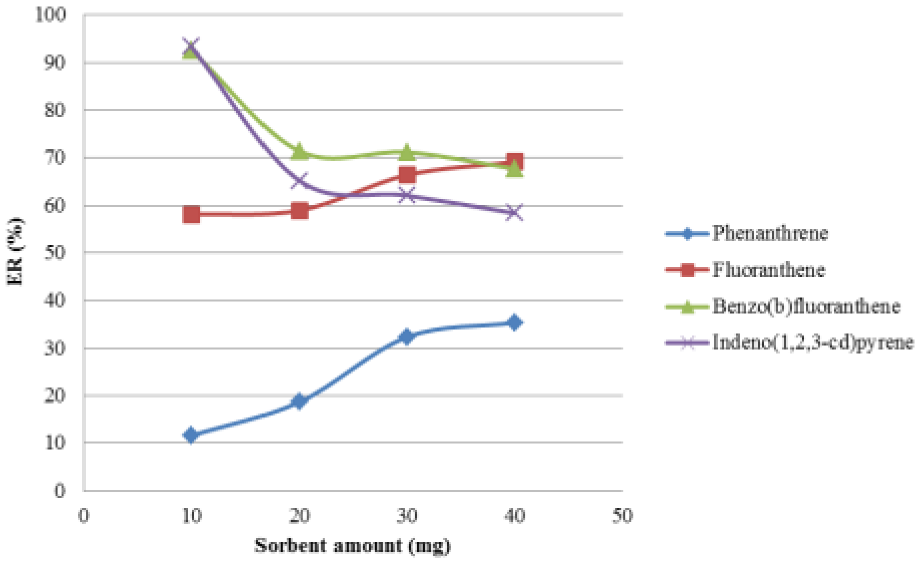

Figure 6. Effect of the sorbent amount on the extraction of target analytes.

The sample volume was evaluated maintaining the sorbent amount/sample volume ratio constant and equal to the previous optimized value (40 mg per 25 mL) in order to work in the best dispersibility conditions. The results are presented in Fig. 7. As it can be seen, the signals for the analytes increased up to 25 mL as it was expected. However, a decrease of the signal was observed at larger volumes due to the more difficult recovery of the sorbent after the extraction.

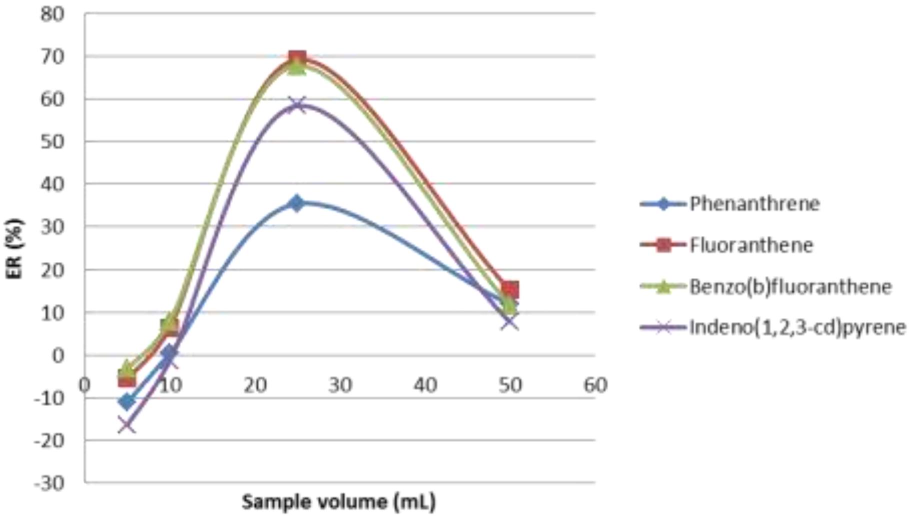

Figure 7. Effect of the sample volume on the extraction of target analytes.

Although the extraction was quick, 30 min was selected as extraction time to ensure the quantitative extraction of the analytes. This time has not a great influence on the overall procedure since several vials can be sonicated at the same time.

3.4. Analytical figures of merit

The optimized procedure was characterized in terms of linearity (linear ranges and correlation coefficients), precision (as repeatability), accuracy (relative extraction recoveries), sensitivity (limits of detection) and extraction efficiency (enrichment factors and absolute extraction recovery). The results are summarized in Table 1 and Table 2.

Table 1. Analytical figures of merit for the determination of the selected PAHs in water samples.

Analyte	Linear range (µg/L)	R^2 [a]	LOD [b] (µg/L)	MDL[c] (µg/L)	RSD[d]	EF[e]	ER[f]
Phenanthrene	0.6-50	0.996	0.58	0.19	3.8	18.1	36.2
Fluoranthene	0.4-50	0.998	0.39	0.12	3.4	36.5	73.0
Benzo(b)fluoranthene	0.5-50	0.998	0.05	0.16	6.8	43.5	87.0
Indeno(1,2,3-cd)pyrene	0.4-50	0.998	0.31	0.11	4.2	39.7	79.4

[a] R^2, correlation coefficient.
[b] LOD, limit of detection.
[c] MDL, method of detection limit.
[d] RSD, relative standard deviation.
[e] EF, enrichment factor.
[f] ER, absolute extraction recovery.

Table 2. Analysis of water samples spiked with the target analytes at a concentration of 5 µg/L.

Analyte	Creek 1 (R ± SD)[a]	Creek 2 (R ± SD)[a]	River (R ± SD)[a]
Phenanthrene	95 ± 3	105 ± 3	95,1 ± 0,4
Fluoranthene	89 ± 4	97 ± 3	85,5 ± 0,8
Benzo(b)fluoranthene	100 ± 2	111 ± 3	106 ± 5
Indeno(1,2,3-cd)pyrene	92 ± 2	90 ± 4	80 ± 25

[a] R, relative extraction recovery expressed as percentage. SD, standard deviation.

For each analyte, a calibration graph was built by extracting in duplicate five working aqueous standards with all the analytes at different concentrations in the range from 1 to 50 μg/L. For all the analytes, a good linearity (R2 > 0.996) was observed up to 50 μg/L. The limits of detection, which were calculated using a signal-to-noise ratio of 3, varied between 0.05 μg/L (benzo(b)fluoranthene) and 0.58 μg/L (phenanthrene). The method detection limit defined by the US EPA [21], was also used to estimate the minimum detectable concentration for PAHs, which varied from 0.11 μg/L (indeno(1,2,3-cd)pyrene) to 0.19 μg/L (phenanthrene). The repeatability of the method in terms of relative standard deviation (RSD, %) was evaluated at the limit of quantification level in septuplicate. The results were in the range from 3.4% (fluoranthene) to 6.8% (benzo(b)fluoranthene).

The enrichment factors for all the analytes were in the range from 18.1 (for phenanthrene) to 43.5 (for benzo(b)fluoranthene). The absolute extraction recoveries, which refer to the percentage of total analyte that can be extracted efficiently by the sorbent and finally eluted with acetone, were in the interval of 36.2–87.0%.

The performance of the method was evaluated through the analysis of different samples including river and creek waters. Since only two analytes were presented in two of the samples, a recovery study was performed spiking water samples with the analytes at 5 μg/L. Table 2 shows that the recoveries were all in the range of 80 to 111, therefore the 70–130% recovery criterion is satisfied in all the values.

4. Conclusions

In this article, a new sorbent based on magnetic nanoparticles-nylon 6 composite is synthesized. After its synthesis, the composite was characterized (SEM, TEM and FT-IR) and evaluated in dispersive micro solid phase extraction by the determination of four polycyclic aromatic hydrocarbons in water using ultra high performance liquid chromatography (UPLC) combined with an UV detector. The new material has the synergic combination between the extraction capabilities of nylon 6 and the magnetic nature of magnetite nanoparticles, allowing the development of a simpler extraction procedure. In comparison to other synthesis methods for composites, the proposed one-step method is extremely fast, simple and has a good reproducibility. The suggested μ-SPE procedure has the advantages of being sensitive and selective. In addition, a magnet is employed to isolate the sorbent from the sample which simplifies the procedure. The new extraction method allows the determination of the target compounds with limits of detection in the range from 0.05 to 0.58 μg/L and relative standard deviations lower than 6.9% at the limit of quantification level. The recovery study was performed in three different water samples obtaining percentages from 80 to 111%, which demonstrated the applicability of the hybrid sorbent for the selected analytical problem.

The proposed magnetic sorbent is compared with other counterparts [22], [23], [24] and [25] in Table 3. The comparison is performed on the basis of some operational (sample volume, sorbent amount and extraction time) and analytical (LOD, recovery and precision) properties. In summary, the magnetic nanoparticles-nylon 6 composite provides comparable recoveries than the other solids, requiring lower sample volumes and sorbent amounts. Although the new approach shows the second best precision values, its sensitivity is lower. This fact can be ascribed to the use of DAD as instrumental technique instead of MS or FD. In fact, Huang et al. have demonstrated that FD provides LODs 38-325 better than DAD for the determination of the PAHs evaluated in this work [26].

Table 3. Comparison of the proposed method with other magnetic extraction procedures previously reported for PAHs analysis.

Method	Sample volume (mL)	Sorbent amount (mg)	Extraction time (min)	LOD (ng/L)	Recovery (%)	RSD (%)	Reference
HPLC-FLD	350	200	15	0.1-0.25	88-93	3-6	[22]
GC-MS	10	50	1	14.1-70	53.5-119.1	3.8-12.1	[23]
HPLC-FLD	500	100	10	0.2-0.6	89-115	1-8	[24]
GC-MS	20	10	30	0.02-0.34	-	3.6-9.3	[25]
HPLC-DAD	25	40	32	50-580	80-111	3.4-6.8	Proposed method

Acknowledgments

Financial support from the Spanish Ministry of Science and Innovation (grant CTQ2011-23790) and Junta de Andalucía (grant P09-FQM-4801) are gratefully acknowledged. E. M. Reyes Gallardo expresses her gratitude for the predoctoral grant (refs FPU12/02670) from the Spanish Ministry of Education.

References

[1] J. S. Beveridge, J. R. Stephens, M. E. Williams, Annu. Rev. Anal. Chem. 4 (2011) 251.

[2] A.-H. Lu, E. L. Salabas, F. Schüth, Angew. Chem. Int. Ed. 46 (2007) 1222.

[3] L. Xie, R. Jiang, F. Zhu, H. Liu, G. Ouyang, Anal Bioanal Chem 406 (2014) 377.

[4] S. C. N. Tang, I. M. C. Lo, Water Res. 47 (2013) 2613.

[5] W. Liu, W. Zhong, Y. W. Du, J. Nanosci. Nanotechnol. 8 (2008) 2781.

[6] I. P. Román, A. Chisvert, A. Canals, J. Chromatogr. A 1218 (2011) 2467.

[7] P. Yu, Q. Wang, X. Zhang, X. Zhang S. Shen, Y. Wang, Anal. Chim. Acta 678 (2010) 50.

[8] L. Sun, C. Zhang, L. Chen, J. Liu, H. Jin, H. Xu, L. Ding, Anal. Chim. Acta 638 (2009) 162.

[9] L. Chen, B. Li, Anal. Methods 4 (2012) 2613.

[10] Y. Hu, J. Pan, K. Zhang, H. Lian, G. Li, TrAC 43 (2013) 37.

[11] A. Ríos, M. Zougagh, M. Bouriab, Anal. Methods 5 (2013) 4558.

[12] H.-B. Zheng, J.-Z. Mo, Y. Zhang, Q. Gao, J. Ding, Q.-W. Yu, Y.-Q. Feng, J. Chromatogr. A 1329 (2014) 17.

[13] Z. Zhang, W. Tan, Y. Hu, G. Li, J. Chromatogr. A 1218 (2011) 4275.

[14] Y. Hu, Y. Li, R. Liu, W. Tan, G. Li, Talanta 84 (2011) 462.

[15] Y. Zhang, Y. Li, Y. Hu, G. Li, Y. Chen, J. Chromatogr. A 1217 (2010) 7337.

[16] S. Mahouche-Chergui, M. Guerrouache, B. Carbonnier, M. M. Chehimi, Colloid Surface A 439 (2013) 43.

[17] E. M. Reyes-Gallardo, G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel, J. Chromatogr. A 1271 (2013) 50.

[18] J. Ding, Q. Gao, X.-S. Li, W. Huang, Z.-G. Shi, Y.-Q. Feng, J. Sep. Sci. 34 (2011) 2498.

[19] Q. Zhao, Q. Lu, Y.-Q. Feng, Anal. Bioanal. Chem. 405 (2013) 4765.

[20] A. L. Andrade, D. M. Souza, M. C. Pereira, J. D. Fabris, R. Z. Domingues, Cerâmica 55 (2009) 420.

[21] US Environmental Protection Agency (EPA), Guidance on Evaluation, Resolution and Documentation of Analytical Problems Associated with Compliance Monitoring, US Environmental Protection Agency (EPA), Washington, DC, 1993.

[22] A. Ballesteros-Gómez, S. Rubio, Anal. Chem. 81 (2009) 9012.

[23] J. Ding, Q. Gao, D. Luo, Z.-G. Shia, Y.-Q. Feng, J. Chromatogr. A 1217 (2010) 7351.

[24] S. Zhang, H. Niu, Y. Zhang, J. Liu, Y. Shi, X. Zhang, Y. Cai, J. Chromatogr. A 1238 (2012) 38.

[25] L. Bai, B. Mei, Q.-Z. Guo, Z.-G. Shi, Y.-Q. Feng, J. Chromatogr. A, 1217 (2010) 7331.

[26] Y. Huang, J. Wei, J. Song, M. Chen, Y. Luo, Chemosphere 92 (2013) 1010.

Capítulo III. 4

Dispersive micro-solid phase extraction of bisphenol A from milk using magnetic nylon 6 composite and its final determination by HPLC-UV

Microchemical Journal

124 (2016) 751-756

Dispersive micro-solid phase extraction of bisphenol A from milk using magnetic nylon 6 composite and its final determination by HPLC-UV

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Herein, a new method for the determination of bisphenol A (BPA) in milk samples is presented. This method comprises a precipitation process, bearing in mind that the analyte has a high tendency to interact with the fatty compounds of the milk, and a magnetic micro-solid phase extraction prior to the determination by high-performance liquid chromatography with UV detection. The presented precipitation process combines EDTA-McIlvaine buffer (the common precipitation agent) with acetonitrile to facilitate the transference of the analyte to the supernatant (only the 30% is transferred in the absence of acetonitrile). The employed sorbent, a magnetic nanoparticles-nylon 6 composite, was synthesized in a quick and one-step method, and was also characterized by several instrumental techniques (SEM, TEM, FT-IR, and SQUID). The developed methodology provides a limit of detection of 3.05 μg/L, which is in accordance with the specific migration limit (SML) established by the European Union. The repeatability of the method, in terms of relative standard deviation, was better than 9.1% (at 100 μg/L) and 16.8% (at 25 μg/L). The recovery study provided values in the range from 86 ± 7 to 99 ± 5%.

Keywords: *Bisphenol A; milk; dispersive solid phase extraction; magnetic composite.*

1. Introduction

Bisphenol A (BPA), 4,4'-(propane-2,2-diyl)diphenol, is an estrogenic endocrine-disrupting monomer which is extensively used in the plastic industry. BPA is mainly employed for two different purposes: a) as a monomer in the fabrication of polycarbonate plastic products and epoxy resins, and b) as an additive in polyvinyl chloride plastics and other products (for example, flame retardants) [1]. Although only a little percentage of these plastics is applied in the food and beverage containers industry, contaminated food intake is the major source (ca. 90%) of the overall BPA exposure [2]. The thermal treatment of these containers, that favors the leaching of BPA into food, is the reason behind that situation [3].

BPA has been determined in a variety of biological and food samples, including human colostrums [4], breast milk [5], water [6], soft drinks [7], and canned food [8], among others. Due to its potential risk, in 2002, the European Union established the specific migration level in food at 3 mg/kg, although this value was finally amended to a value of 0.6 mg/kg [9]. According to this legislation, the determination of BPA in cow milk, a usual commodity, is desirable. However, this determination is not simple due to the complexity of the sample. In fact, the fatty content of milk, which varies between 0.3 and 4% depending on the type (whole, defatted or skim), makes the BPA determination a real challenge since BPA tends to interact with the fat (due to its hydrophobic nature) making the sample matrix–analyte interaction more intense. This interaction still remains even during the precipitation process that is usually employed as milk pretreatment in analytical methodologies [10].

Several extraction techniques have been proposed for the isolation/preconcentration of BPA from milk sample including solid phase extraction (SPE) [11], solid-phase microextraction (SPME) [12] and [13], molecularly imprinted SPE [6], [14] and [15], or immunoaffinity chromatography [8].

The use of nanoparticles (NPs) in the microextraction context has improved the performance of the developed techniques. The larger superficial area of NPs (that enhances the extraction kinetic) and the variety of different interaction chemistries (that makes wider the applicability to different problems) can be considered among the main reasons. The combination of different types of NPs (hybrid NPs) as well as the combination of NPs with micrometric systems (composites) has increased the potential of this type of materials in analytical sciences. Within the composites, the combination of magnetic nanoparticles (MNPs) with micrometric polymers is especially interesting. In this sense, the composite presents high extraction capabilities due to the polymeric network while maintains a magnetic behavior that simplifies the overall extraction procedure [16].

Polyamide polymers, including nylon-6, are interesting sorbents that can be easily synthesized in the laboratory using common reagents. Polyamides present inter- and intra-molecular hydrogen bounds through their amide groups that allow the stacking of the linear polymer chains. These bounds may be developed with external carbonyl or amine groups permitting the interaction with target molecules [17]. In addition, non-polar interactions with the hydrocarbon polymer chain are also possible, increasing the versatility of the polymer. Taking this into account, polyamides have been previously reported for the extraction of BPA in water samples, using microfiltration membranes [18]. Here, the use of magnetic NPs-nylon 6 composite is proposed instead of membranes in order to obtain a better dispersion of the sorbent and to avoid the sealing of the membranes when using complex samples as milk. The synthesis of the composite is very simple, as it can be achieved by a simple solvent changeover [19].

In this article, a magnetic NPs-nylon 6 composite is employed for the dispersive micro-solid phase extraction (d-μSPE) of BPA from milk samples, the analyte being finally determined by HPLC-UV. The main aim is to provide a method, giving priority to simplicity over sensitivity, to fulfill the SML established by the EU legislation.

2. Experimental

2.1. Reagents

All reagents were of analytical grade or better. Bisphenol A was purchased from Sigma–Aldrich (Madrid, Spain). A stock standard solution of the analyte was prepared at a concentration of 400 mg/L in methanol (Carlo Erba Reagents, Italy) and stored at 4 °C. Working solutions were daily prepared by the appropriate dilution of the stocks in Milli-Q water (Millipore Corp., Madrid, Spain).

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), and ammonia were used for the synthesis of the magnetic nanoparticles (Fe_3O_4). Tetraethylorthosilicate (TEOS) and ethanol were employed for covering the NPs with a protective silica-based coating. Formic acid and nylon 6 were used for the preparation of the polymer network where the MNPs are embedded. Sodium phosphate dibasic dihydrate, citric acid monohydrate, and ethylenediaminetetraacetic acid (EDTA) were employed for the preparation of EDTA–McIlvaine buffer. All these reagents were purchased from Sigma–Aldrich.

EDTA–McIlvaine buffer solution (pH = 4) was prepared by dissolving 7.5 g of disodium hydrogen phosphate, 6.5 g of citric acid, and 1.86 g of EDTA in 500 mL of water.

Whole, defatted, and skim milk samples were purchased from local markets and were maintained in their own containers under normal storage conditions.

2.2. Synthesis of the magnetic nanoparticles-nylon 6 composite

The synthesis of the magnetic nylon 6 composite is described elsewhere [19], and it consists of different steps. In brief, Fe_3O_4 MNPs are synthesized by a coprecipitation method and coated with a silica shell ($Fe_3O_4@SiO_2$) [20] using TEOS as reagent. The magnetic nylon 6 composite was finally synthesized as follows: 150 mg of nylon-6 were dissolved in 5 mL of formic acid and then 200 mg of $Fe_3O_4@SiO_2$ were dispersed into the solution. After that, the dispersion was injected with a syringe into a beaker with water, to cause the gelation of the nylon-6 around the magnetic NPs. The gelation is produced by solvent changeover since nylon is soluble in formic acid but it is insoluble in water. The solid was washed, recovered using an external magnet, and finally dried.

2.3. Apparatus

Liquid chromatographic analyses of bisphenol A were performed using an Agilent 1100-system liquid chromatograph (Agilent®, Palo Alto, CA) equipped with a binary high-pressure pump 1200 series), a single wavelength photometer (1100 series), and an autosampler. Data analysis was carried out using HP ChemStation software. Chromatographic separations were developed on a LiChrosorb® C18 column (5 µm, 4.6 × 250 mm) (AnálisisVínicos, Tomelloso, Spain) under an isocratic program using 65% of acetonitrile (solvent B) and 35% of water (solvent A) as mobile phase components. The flow rate was set at 1 mL/min, the injection volume was 10 µL, and the analyte was monitored at 226 nm.

For the synthesis of the composite, a heating magnetic stirrer (VelpScientifica®,Milan, Italy) was used. A test tube shaker (ReaxtopTM Heidolph, Schwabach, Germany) and an ultrasonic bath (model 3510, Branson®, Danbury, CT, USA) were employed in the extraction procedure. A centrifuge (model Centronic BL-IITM, J.P. Selecta, Barcelona, Spain) was used during milk samples pretreatment and a vacuum concentrator (Eppendorf Concentrator plusTM, Eppendorf, Germany) was employed to evaporate the obtained extracts.

An external magnet (60 × 30 × 15 mm and 549.4 N of maximum magnetic force), purchased from Supermagnete (Gottmadingen, Germany), was employed for the sorbent recovery after D-µSPE.

2.4. Characterization of the magnetic nanoparticles-nylon 6 composite

Micrographs of the NPs were obtained in a Philips® CM-10 transmission electron microscopy (TEM) and a JEOL® JSM 6300 scanning electron microscopy (SEM).

Infrared measurements were performed in a Bruker® Tensor37 FT-IR spectrometer, equipped with a diamond ATR cell with a circular surface of 3 mm diameter and three internal reflections. A deuterated triglycine sulfate (DTGS) detector was used for spectra acquisition. Spectra were collected between 4000 and 600 cm^{-1} at a 4 cm^{-1} resolution with 64 coadded scans each. Data collection was made using OPUS software (version 4.2) (Bruker, Ettligen, Germany).

Magnetic behavior was analyzed performing a hysteresis loop using a Superconducting Quantum Interference Device (SQUID) MPMS-XL-7 T from Quantum Designs® (San Diego, CA, USA) at constant temperature (27 °C).

2.5. Sample extraction procedure

The extraction procedure of the milk samples involves several steps. First of all, milk samples are pretreated to induce protein precipitation. For this purpose, 20 mL of sample are mixed with the same volume of EDTA–McIlvaine buffer/acetonitrile solution (20/80, v/v) obtaining a final concentration of ACN of 40%. This medium is left to stand for 1 h and centrifuged (8000 rpm or 6439 g, 9 min). Then, 10 mL of the supernatant are four-fold diluted with McIlvaine buffer, and 80 mg of the composite are added subsequently to this medium. The sample is stirred in a test tube shaker for 1 min and sonicated for 30 min to favor the extraction of the analyte. After that, the sorbent is isolated from the sample by an external magnet and washed with 10 mL of milli-Q water. Once the sorbent is completely dried, the analyte is batch-mode eluted using 500 µL of methanol. After that, the liquid is recovered after the magnetic separation of the solid. To improve the sensitivity, the extract is evaporated to dryness in a vacuum concentrator and redissolved in 50 µL of methanol before the final HPLC analysis.

Due to its easy and cheap synthesis, a fresh amount of composite is employed for each extraction avoiding potential carry-over effects.

3. Results and discussion

3.1. Characterization of the magnetic nanoparticles-nylon 6 composite

The synthesized composite was characterized by SEM, TEM, FT-IR, and SQUID. SEM micrographs (Fig. 1A) show that the surface of the polymer becomes rougher after the synthesis. According to TEM images (Fig. 1B), the MNPs are spherical and have an average diameter of 10 nm.

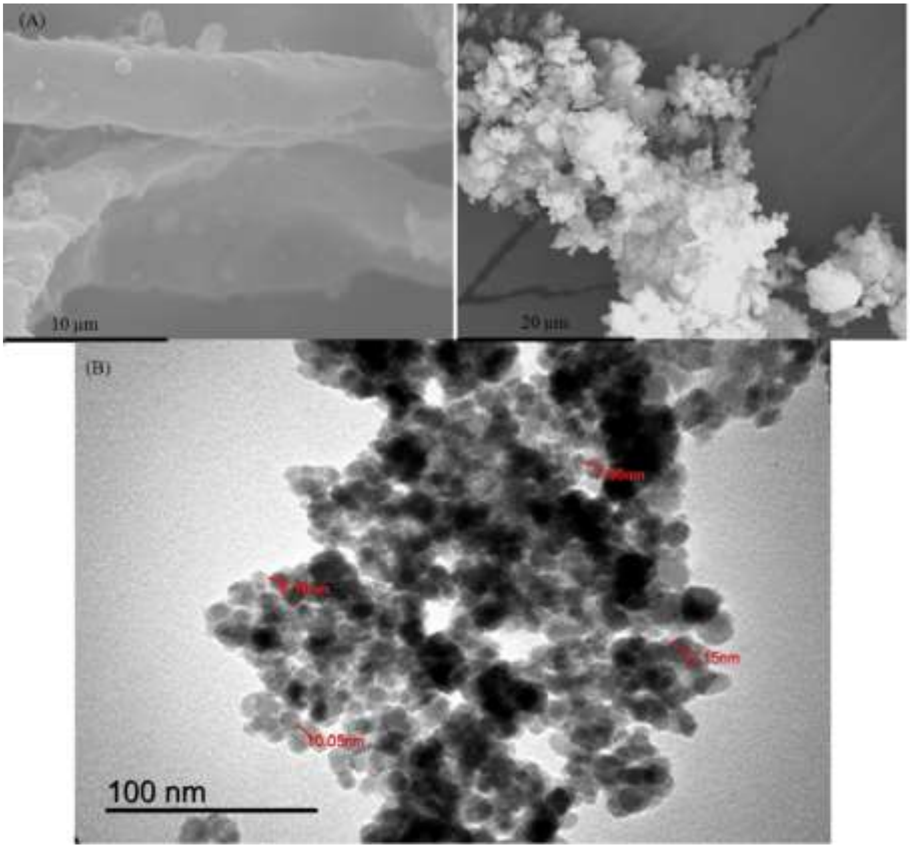

Figure 1. (A) Scanning electron microscopy (SEM) images of nylon 6 (left) and magnetic nanoparticles-nylon 6 composites (right). (B) Transmission electron microscopy (TEM) images of magnetic nanoparticles-nylon 6 composite.

FT-IR was used to confirm the chemical composition of the composite (Fig. 2). A characteristic band at 580 cm^{-1}, which can be assigned to the Fe—O—Fe vibration, can be observed. The Si—O—H and Si—O—Si bonds present a strong absorbing region at 1200–1000 cm^{-1}. The presence of nylon in the structure involves the appearance of a band around 1642 cm^{-1} (the C=O stretching of the amide group) and another band around 1546 cm^{-1} (the N—H deformation of the amide I).

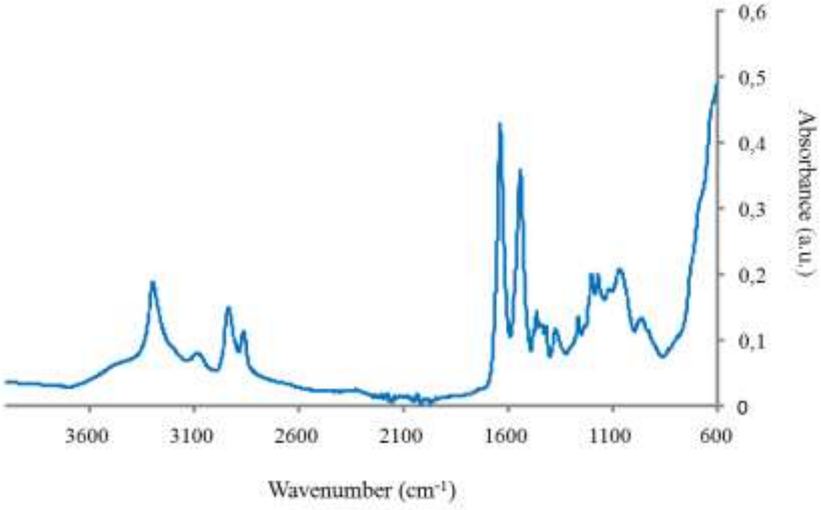

Figure 2. Infrared spectrum of magnetic nanoparticles-nylon 6 composite.

The magnetic behavior of the synthesized sorbent is key for the development of efficient and fast procedures. The saturation magnetization of the composite was investigated by magnetic hysteresis loop analysis (Fig. 3). The symmetrical shape of the curve suggests that there is no magnetic retentivity and therefore the composite shows superparamagnetism, obtaining a saturation magnetization value bigger than 40 emu/g. This value is much higher than other hybrid polymeric materials previously synthesized [21] and allows the easy and fast separation of the sorbent.

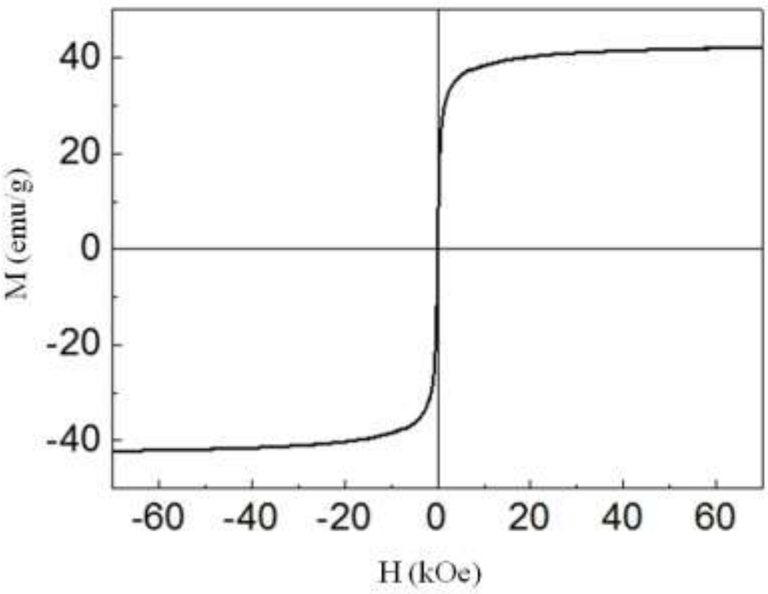

Figure 3. Magnetization curve of the MNPs-nylon 6 composite at 27 °C.

3.2. Sample pretreatment optimization

Protein precipitation is required to perform an efficient extraction protocol of milk samples. This precipitation, which can be performed using different alternatives (pH change, the addition of organic solvents, or the modification of the ionic strength), produces a protein clot where the fatty compounds of the milk are trapped [10]. Due to its hydrophobicity, bisphenol A presents a high tendency to interact with the fat and its transference to the supernatant might be compromised.

Taking this into account, the precipitation method should be optimized. Whole milk, due to its higher fat percentage, was employed as model sample. The use of EDTA–McIlvaine buffer, which is the common precipitation reagent, did not produce good results. Different buffer/sample ratios (Fig. 4A) were tested but only the 30% of the original BPA was transferred to the supernatant due to the strong retention of BPA in the protein/fat clot.

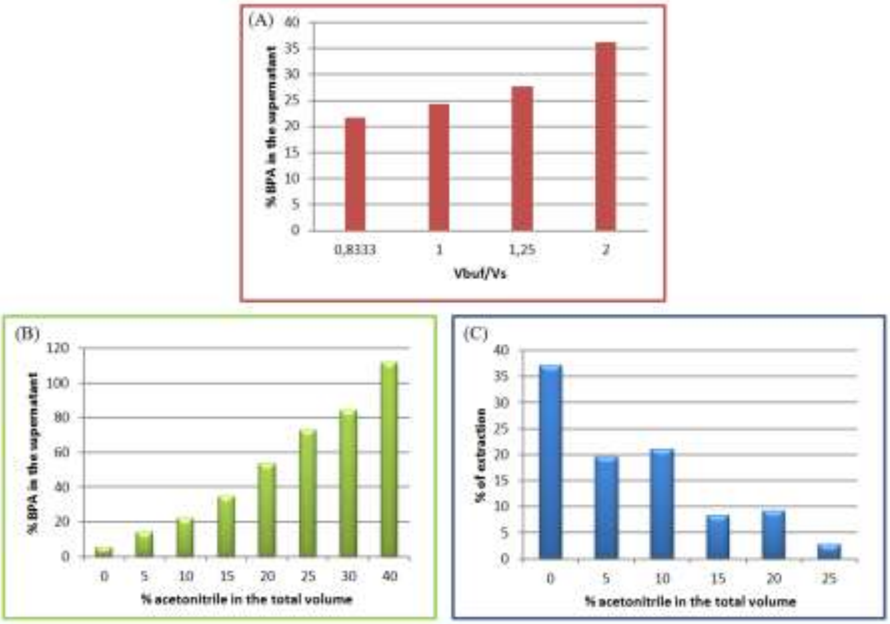

Figure 4. (A) Evaluation of the buffer/sample ratio (V_{buf}/V_s) for the transfer of the target analyte to the supernatant. (B) Evaluation of the percentage of acetonitrile in the transfer of the analyte. (C) Evaluation of the percentage of acetonitrile in the extraction of the analyte.

In order to increase this transference, acetonitrile was added to the buffer. The effect of the acetonitrile in the transference efficiency was evaluated in the following way. 5 mL of spiked whole milk (4 mg/L was used in order to measure the analyte without any preconcentration step) were deproteinized with 5 mL of a mixture of buffer and acetonitrile with different proportions and the supernatants were directly analyzed. According to the results, which are summarized in Fig. 4B, the transfer of the analyte increases with the acetonitrile percentage, the maximum value being obtained at 40% of the total volume. Under these conditions, the analyte is quantitatively transferred to the liquid phase. Higher acetonitrile concentrations were not evaluated since it negatively affects to the subsequent D-µSPE procedure increasing the analyte solubility in the liquid phase. To study this influence, several standards containing the analyte at the equivalent concentration (2 mg/L) were prepared in different McIlvaine buffer/acetonitrile proportions and extracted with 5 mg of the composite. All the standards were analyzed before and after the extraction process to calculate the analyte extraction recovery. The results, which are shown in Fig. 4C, corroborate the negative effect of acetonitrile in the transference of the analyte from the supernatant to the composite. To make compatible the milk extract with the subsequent D-µSPE, the precipitation extract was 4-fold diluted with buffer before the addition of the composite. In this way, the acetonitrile percentage is reduced from 40% (its concentration in the precipitation medium) to 10%, making possible the retention of the analyte on the composite.

3.3. D-µSPE optimization

The effect of several variables on the extraction of the BPA from milk was then considered in depth. All variables were evaluated using blank whole milk samples spiked with the analyte at a concentration of 500 µg/L. This concentration level was employed taking into account the legal SML and to avoid the saturation of the sorbent when real samples are processed. Before processing the spiked samples, they were pretreated following the optimum protein precipitation protocol previously described. To begin with the optimization, 30 min were fixed at the extraction time and the elution step was carried out with 500 µL of methanol, being the extract evaporated to dryness and the residue redissolved in 50 µL of methanol.

Sorbent amount and sample volume were studied jointly since they are directly related. Sample volume was optimized in the range from 1 to 10 mL, and sorbent amount between 20 and 80 mg, the result being shown in Fig. 5. The analyte signal increased with both the sorbent amount and the sample volume as it was expected. The maximum signal was obtained for 5 mL of sample (which corresponds to a final extract volume of 40 mL) and 80 mg of sorbent and therefore these conditions were selected for further analyses.

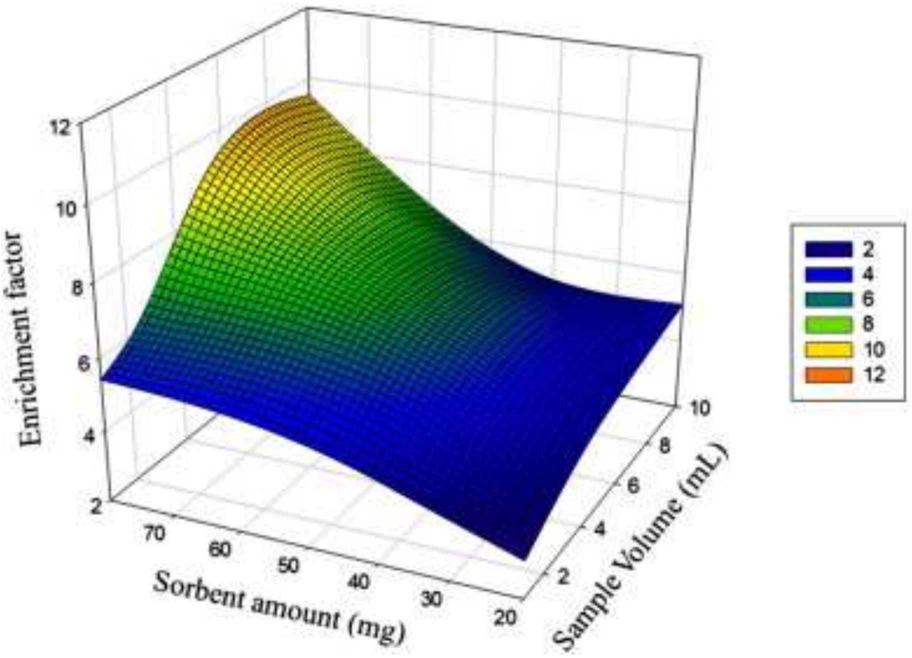

Figure 5. Effect of the sample volume and the sorbent amount on the extraction of the target analyte.

Extraction time was evaluated in the range from 5 to 60 min. The results showed that the signal of the analyte increased with the extraction time up to 30 min, after that, the signal remained constant.

3.4. Analytical figures of merit and sample analysis

The calibration graph for BPA was built by extracting several blank samples spiked with the analyte in the range from 10.2 μg/L to 4 mg/L, a good linearity (R > 0.996; $S_{y/x}$ = 5.6; MAD = 39) was observed. The sensitivity of the method was evaluated according to the limit of detection (LOD). The LOD, which was calculated by using a signal-to-noise ratio of 3, was 3.05 μg/L. The limit of quantification (LOQ), for a signal-to-noise ratio of 10, was 10.16 μg/L. The repeatability of the method, in terms of relative standard deviation (RSD, %), was evaluated in septuplicate at two different concentration levels: 100 μg/L and a concentration closer to the limit of quantification (25 μg/L). The results were 9.1% and 16.8%, respectively. Inter-day precision was also estimated at 100 μg/L over 3 days (n = 9), obtaining a RSD of 9.5%. These precision values involve also the synthesis reproducibility since 80 mg of fresh composite are used for each new extraction while the amount of composite synthesized per batch is ca. 300 mg.

The enrichment factor, which was obtained by comparing the calibration graphs before and after the extraction process, was 7.58 and the absolute extraction recovery, which refers to the percentage of total analyte that can be extracted efficiently by the sorbent and finally eluted with methanol in the fortified whole milk samples, was 7.58%. The EF is not too high due to the coexistence of two subsequent steps (precipitation and extraction) where the addition of acetonitrile plays an opposite effect.

The performance of the method was evaluated through the analysis of different kinds of milk samples including whole, defatted, and skim. Since no sample contained the analyte in a measurable concentration, a recovery study was performed spiking the samples with the analyte at 100 μg/L. The relative extraction recoveries (expressed as percentages with standard deviations) were 86 ± 7, 98 ± 4, and 99 ± 5% for whole, semi-skimmed, and skimmed milk, respectively, satisfying all of them the 70–130% recovery criterion. The increase in the percentage of extraction is directly related with the amount of fat that is contained in the sample since BPA is strongly retained in the protein/fat clot.

4. Conclusions

In this paper, an estrogenic endocrine-disrupting monomer, bisphenol A, is determined in different kind of milk samples (whole, defatted, and skim). The extraction procedure is carried out using a magnetic-nylon 6 composite in a dispersive micro-solid phase extraction format, the analyte being finally determined by HPLC-UV. Table 1 presents a comparison of the proposed method with other counterparts based in non-sophisticated instrumental techniques such as HPLC-UV or HPLC-FD [6], [12], [13], [14] and [15]. The proposal extraction method provides the higher LOD although it requires a lower sample amount. However, it is suitable to detect BPA below the SML established by the European Union (600 μg/kg) although BPA can be found in real samples at lower concentrations. The precision is in the same order of magnitude of several approaches [6], [14] and [15] although it is surpassed by the SPME approaches [12] and [13] due to the potential automation of the latter. Finally, it provides very good recoveries when real samples are processed.

Table 1. Comparison of the proposed method with other counterparts using non-sophisticated instrumental techniques.

Instrumental technique	Sample treatment	Sample amount	LOD (µg/L)	Precision (% RSD)	Relative recovery (%)	Reference
HPLC-UV	MIP-SPE	50 mL	0.16 µg/L	6.1-10.5	95-101	[6]
HPLC-DAD	SPME using polymeric ionic liquids	20 mL	0.09 µg/L	<4.69	74.8-107	[12]
HPLC-FD	SPME	10 mL	0.2 µg/L	6.6	93-102	[13]
HPLC-FD	Magnetic MIP-SPE	20 mL	0.3 µg/L	8.9-12.6	72-113	[14]
HPLC-FD	MIP-SPE	5 g	1.32 µg/kg	12-14	86-111	[15]
HPLC-UV	Magnetic SPE	5 mL	3.05 µg/L	9.1-16%	86-99	This work

HPLC, high performance liquid chromatography; UV, ultraviolet detection; DAD, diode array detection; FD, fluorescence detection; MIP, molecularly imprinted polymer; SPE, solid phase extraction; SPME, solid phase microextraction; LOD, limit of detection; RSD, relative standard deviation.

The main advantage of the technique is the simplicity of the sorbent obtaining over MIPs synthesis [6], [14] and [15]. The composite can be obtained in a simple, quick, and one-step method using commercially available polymers.

Acknowledgments

Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2014-52939R) is gratefully acknowledged. E. M. Reyes-Gallardo expresses her gratitude for the predoctoral grant (refs FPU12/02670) from the Spanish Ministry of Education.The authors would like to thank the Central Service for Research Support (SCAI) of the University of Córdoba and the "Institut Catalá de

Nanotecnología" (Barcelona, Spain) for the service provided to obtain the micrographs and magnetic measurements, respectively.

References

[1] F.S. VomSaal, W.V. Welshons, Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A, Environ. Res. 100 (2006) 50–76.

[2] T. Geens, D. Aerts, C. Berthot, J.P. Bourguignon, L. Goeyens, P. Lecomte, G. Maghuin-Rogister, A.M. Pironnet, L. Pussemier, M.L. Scippo, J. Van Loco, A. Covaci, A review of dietary and non-dietary exposure to bisphenol-A, Food Chem. Toxicol. 50 (2012) 3725–3740.

[3] K.V. Ragavan, N.K. Rastogi, M.S. Thakur, Sensors and biosensors for analysis of bisphenol-A, Trends Anal. Chem. 52 (2013) 248–260.

[4] R. Kuruto-Niwa, Y. Tateoka, Y. Usuki, R. Nozawa, Measurement of bisphenol A concentrations in human colostrum, Chemosphere 66 (2007) 1160–1164.

[5] S.M. Zimmers, E. Browne, P. O'Keefe, D. Anderton, L. Kramer, D. Reckhow, K. Arcaro, Determination of free Bisphenol A (BPA) concentrations in breast milk of U.S. women using a sensitive LC/MS/MS method, Chemosphere 104 (2014) 237–243.

[6] Y. Ji, J. Yin, Z. Xu, C. Zhao, H. Huang, H. Zhang, C. Wang, Preparation of magnetic molecularly imprinted polymer for rapid determination of bisphenol A in environmental water and milk samples, Anal. Bioanal. Chem. 395 (2009) 1125–1133.

[7] A. Khedr, Optimized extraction method for LC–MS determination of bisphenol A, melamine and di(2-ethylhexyl) phthalate in selected soft drinks, syringes, and milk powder, J. Chromatogr. B 930 (2013) 98–103.

[8] S. Sungur, M. Köroğlu, A. Özkan, Determination of bisphenol a migrating from canned food and beverages in markets, Food Chem. 142 (2014) 87–91.

[9] European Directive 2002/72/EC, Amendment 2004/19/EC, Relating to plastic materials and articles intended to come into contact with foodstuffs.

[10] R. Lucena, M. Gallego, S. Cárdenas, M. Valcárcel, Autoanalyzer for milk quality control based on the lactose, fat, and total protein contents, Anal. Chem. 75 (2003) 1425–1429.

[11] N.C. Maragou, E.N. Lampi, N.S. Thomaidis, M.A. Koupparis, Determination of bisphenol A in milk by solid phase extraction and liquid chromatography–mass spectrometry, J. Chromatogr. A 1129 (2006) 165–173.

[12] M. Mei, J. Yu, X. Huang, H. Li, L. Lin, D. Yuan, Monitoring of selected estrogen mimics in complicated samples using polymeric ionic liquid-based multiple monolithic fiber solid-phase microextraction combined with high-performance liquid chromatography, J. Chromatogr. A 1385 (2015) 12-19.

[13] X. Liu, Y. Ji, H. Zhang, M. Liu, Elimination of matrix effects in the determination of bisphenol A in milk by solid-phase microextraction–high-performance liquid chromatography, Food Addit. Contam. 25 (2008) 772–778.

[14] Z. Xu, L. Ding, Y. Long, L. Xu, L. Wang, C. Xu, Preparation and evaluation of superparamagnetic surface molecularly imprinted polymer nanoparticles for selective extraction of bisphenol A in packed food, Anal. Methods 3 (2012) 1737-1744.

[15] J. O'Mahony, M. Moloney, M. McCormack, I.A. Nicholls, B. Mizaikoff, M. Danaher, Design and implementation of an imprinted material for the extraction of the endocrine disruptor bisphenol A from milk, J. Chromatogr. B 931 (2013) 164-169.

[16] E.M. Reyes-Gallardo, G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel, Hybridization of commercial polymeric microparticles and magnetic nanoparticles for the dispersive micro-solid phase extraction of nitroaromatic hydrocarbons from water, J. Chromatogr. A 1271 (2013) 50–55.

[17] Q. Xu, X. Yin, S. Wu, M. Wang, Z. Wen, Z. Gu, Determination of phthalate esters in water samples using Nylon 6 nanofibers mat-based solid-phase extraction coupled to liquid chromatography, Microchim. Acta 168 (2010) 267-275.

[18] J. Han, S. Meng, Y. Dong, J. Hu, W. Gao, Capturing hormones and bisphenol A from water via sustained hydrogen bond driven sorption in polyamide microfiltration membranes, Water Res. 47 (2013) 197-208.

[19] E.M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel, Magnetic nanoparticles-nylon 6 composite for the dispersive micro solid phase extraction of selected polycyclic aromatic hydrocarbons from water samples, J. Chromatogr. A 1345 (2014) 43–49.

[20] A.L. Andrade, D.M. Souza, M.C. Pereira, J.D. Fabris, R.Z. Domingues, Synthesis and characterization of magnetic nanoparticles coated with silica through a sol-gel approach, Cerámica 55 (2009) 420-424.

[21] Z. Zhang, Y. Zhang, W. Tan, G. Li, Y. Hu, Preparation of styrene-co-4-vinylpyridine magnetic polymer beads by microwave irradiation for analysis of trace 24-epibrassinolide in plant samples using high performance liquid chromatography, J. Chromatogr. A 1217 (2010) 6455-6461.

Capítulo III. 5

Silica nanoparticles-nylon 6 composites: synthesis, characterization and potential use as sorbent

RSC Advances

7 (2017) 2308-2314

Silica nanoparticles-nylon 6 composites: synthesis, characterization and potential use as sorbent

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Composite materials based on the combination of nanoparticles (NPs) and polymers have attracted much attention in recent years thanks to their positive characteristics. In this article an easy, cheap and green procedure for the synthesis of silica nanoparticles–nylon 6 composites is proposed. Several materials comprising different NPs–polymer ratios have been synthesized and characterized. The evaluation of their extraction performances indicated that the inclusion of NPs into the polymeric network increases their superficial area, boosting their sorbent capacity, because the NPs modify the normal stacking of the polymeric chains. Among the tested composite materials, the best one has been applied for the dispersive micro-solid phase extraction of selected estrogens (estrone, β-estradiol and estriol) from aqueous samples as a model analytical problem. The approach allows the determination of the target compounds by LC-MS/MS at the low μg L^{-1} range with acceptable precision (better than 13.9%, expressed as relative standard deviation). Finally, the potential use of the composite for the isolation of the targets from more complex samples has been outlined.

1. Introduction

Synthetic polymers have been applied for decades in extraction techniques thanks to their advantageous properties [1]. Among these features, their chemical/mechanical stability, and their ability to interact with target analytes through different mechanisms can be highlighted. In addition, the physical/chemical architecture of synthetic polymers, in the so called molecularly imprinted polymers [2,3], can be adapted to the target analyte to achieve selective isolation from complex samples. The thermal stability of some polymers has allowed their use as extracting phase in solid phase microextraction (SPME), a research field where polymers have demonstrated prevalence over other materials [4–7].

In the same way, nanoparticles (NPs) have been extensively used in the microextraction context as supports or as extracting phases [8,9]. Their high surface to volume ratio, a direct consequence of the nanometric size, guarantees a high extraction rate if aggregation is not an issue [10]. Besides, the great variety of NPs, covering different interaction chemistries, and their potential functionalization increases even more the scope their applicability [11].

Although both synthetic polymers and NPs present a great applicability, their combination in a synergic way to create composite materials opens a door to interesting applications [12]. A deep revision of the scientific literature shows different alternative procedures for the synthesis of polymer nanocomposites.

The core shell approach consists of covering the nanoparticle surface with a polymeric layer in such a way that the resulting composite remains in the nanometric domain and it is characterized by a high superficial area. Magnetic NPs are used as the common support and the final magnetic nanocomposite is usually applied under the dispersive micro-solid phase extraction (dμSPE) format [13–16]. The opposite approach, the coverage of the bulk polymer surface with NPs, was evaluated by our research group [17]. The decoration of the polymer with magnetic NPs allowed the easy synthesis of a magnetic composite which resulted useful for the extraction of nitroaromatic compounds from water. The decoration of the surface resulted critical since an excessive coverage induced a reduction of the extraction surface.

Electrospun fibers containing NPs have attracted much attention in the last years due to the versatility of the resulting materials [18]. These composites can be obtained in two ways, namely: (a) electrospinning a solution containing the polymer and the NPs or (b) loading the electrospun fiber after their synthesis with the NPs. Electrospun nanocomposites can be obtained in the form of fibers or films that can be applied in SPME [19,20], thin film microextraction [21,22] and dμSPE [23].

The inclusion of NPs inside the polymeric network has been also proposed for extraction purposes. The NPs may play two different roles. On the one hand, they can act as modifiers modifying the normal stacking of the polymer and therefore increasing the porosity of the composite [20]. On the other hand, they can be the active extracting phase or they can complement the extraction capabilities of the polymer [24,25].

Despite the potential of the above mentioned synthetic procedures, some of them are multistep protocols that require resources (energy, reagents and solvents) or special manifolds. Recently, our group have proposed a quick, easy, cheap and green method to synthesize nanocomposite playing with the switchable solubility of polymers [26,27]. In this article, the procedure is extended to silica NPs and the role of these NPs in the extraction is evaluated using the determination of selected hormones as model analytical problem. In addition, the synthetic procedure has been modified to allow the synthesis of larger amount of material in a more reproducible way.

2. Experimental Section

2.1. Reagents

All reagents were of analytical grade or better. Estrone (E1), β-estradiol (E2) and estriol (E3) were purchased from Sigma-Aldrich (Madrid, Spain). Stock standard solutions of the analytes were prepared at a concentration of 1000 mg L^{-1} in methanol (Carlo Erba Reagents, Italy) and stored at 4 °C. Working solutions were daily prepared by the appropriate dilution of the stock in methanol or Milli-Q water (Millipore Corp., Madrid, Spain) as required.

Formic acid, nylon 6 (in the form of cubical pellets) and silica nanoparticles (10–20 nm particle size) were used for the synthesis of the nanocomposites. All these reagents were obtained from Sigma-Aldrich.

Saliva and urine samples were collected from volunteers who gave their informed consent prior to sampling.

2.2. Synthesis of the silica nanoparticles–nylon 6 composite

The composite is synthesized in an easy and rapid way. First of all, a defined amount of nylon 6 pellets (Table 1) is dissolved in 25 mL of formic acid under vigorous stirring in a magnetic stirrer (Velp Scientifica, Milan, Italy). In a second step, a weighted amount of silica nanoparticles (Table 1) are added to the solution. The resulted dispersion is stirred for 5 min to assess the homogeneous distribution of the nanoparticles. Finally, 25 mL of Milli-Q water are added dropwise into the solution inducing the gelation of the polymer around the nanoparticles.

The obtained material is recovered by filtration under vacuum using filter paper as it is capable, due to its larger pores, to separate the composite from those silica NPs which have not been successfully introduced in the polymeric network. The material is washed with acetone and methanol and dried in an oven at 80 °C. The synthesis is shown in movie 1 (Electronic supplementary information, ESI). Table 1 summarizes the different materials (A, B, C and D) that have been synthesized, based on different proportions of both components. Materials B, C and D are really composites (they combine silica NPs and nylon 6) while material A is just nylon subjected to the same synthetic procedure.

Table 1. Materials synthesized by the proposed method.

Material	% nylon 6	Amount of nylon 6 (g)	Amount of silica (g)	Carbon content[a] (%±SD)	Nitrogen content[a] (%±SD)
A	100	0.5	0	68.4±0.3	13.3±0.1
B	75	0.35	0.15	43.7±0.8	8.83±0.2
C	50	0.25	0.25	25±5	5±1
D	25	0.15	0.35	9±3	1.9±0.7

[a]Values obtained by elemental analysis. SD, standard deviation.

2.3. Characterization of the silica nanoparticles–nylon 6 composites

Micrographs of nylon 6, silica nanoparticles and their resulting composites were obtained by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) using a JEOL JSM 6300 and a Philips CM-10 microscopes, respectively. A EuroVector Elemental Analyser EA3000 (EuroVector SpA, Milan, Italy) was used for measuring the carbon and nitrogen content of the synthesized materials. Both the micrographs and elemental analysis were developed in the Central Service for Research Support (SCAI) of the University of Córdoba.

Infrared spectra were acquired in a Bruker Tensor 37 FT-IR spectrometer, equipped with a diamond ATR cell (circular surface of 3 mm diameter and three internal reflections) and a deuterated triglycine sulfate (DTGS) detector. Spectra were collected between 4000 and 600 cm^{-1} at a 4 cm^{-1} resolution with 64 coadded scans each. Data collection was done using OPUS software (Bruker, Ettligen, Germany).

The superficial area of the composites were measured using a Quantachrome® ASiQwin™-Automated Gas Sorption Data based on the nitrogen adsorption/desorption measures at 196 °C. The specific surface area values were calculated according to the BET (Brunauer–Emmett–Teller) equation.

2.4. Sample extraction procedure

The extraction unit, which is described elsewhere [26], consists of a 10 mL-syringe which is adapted to a pipette tip section where a cotton bead is located as frit. 40 mg of the composite is placed between the cotton bead and the syringe plunger (see Fig. 1).

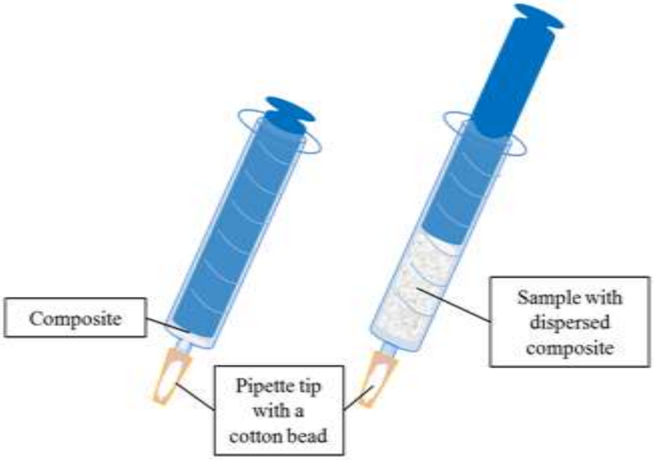

Figure 1. Schematic diagram of the extraction device used in this work.

The extraction procedure involves several steps. First of all, the sorbent is conditioned by means of 1 mL of methanol followed by 1 mL of milli-Q water. Secondly, 10 mL of the sample or standard (containing the analytes in the range from 0.5 to 200 µg L^{-1}) is drawn into the syringe inducing the composite dispersion. The sample is aspirated and ejected twice to increase the interaction between the analytes and the sorbent. Thirdly, a washing step is performed using 1 mL of milli-Q water.

Finally, the analytes are eluted with 500 mL of methanol. The eluate is evaporated to dryness in a vacuum concentrator (Eppendorf, Germany) and the final residue is redissolved in 50 mL of mobile phase before the final LC analysis (ESI).

3. Results and discussion

3.1. Characterization of the synthesized materials

Nylon 6 presents a chemical structure where two different polarity domains can be highlighted, namely: the polar amide group and the non-polar hydrocarbon backbone. Both domains may interact with target analytes favouring their extraction from the samples. Nylon 6 is usually marketed as pellets (or as granular solid) which presents a low surface to volume ratio due to its uniform surface as it can be observed in Fig. 2. This aspect hinders the extraction capacity of the polymer.

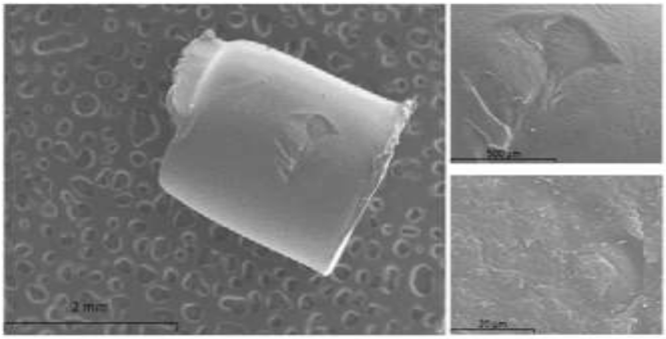

Figure 2. SEM micrographs of one nylon 6 pellet at different magnifications.

The synthetic procedure proposed in this article improves this aspect providing a rougher surface due to two different mechanisms: (a) the slow gelation of the nylon produced by the dropwise addition of water into the solution of nylon 6 in formic acid and (b) the introduction of silica NPs into the polymeric network. In this sense, the micrographs of a nylon pellet (Fig. 3A), material A (Fig. 3B) and material B (Fig. 3C) confirm this hypothesis. The superficial structures of the pellet and material A are dramatically different although both are just nylon. In addition, the inclusion of NPs in the polymeric network provides to the solid B with a spongier surface as it can be deduced from the superficial area values shown in the Fig. 3. The values for nylon 6 pellets were negligible (lower than the sensitivity of the technique).

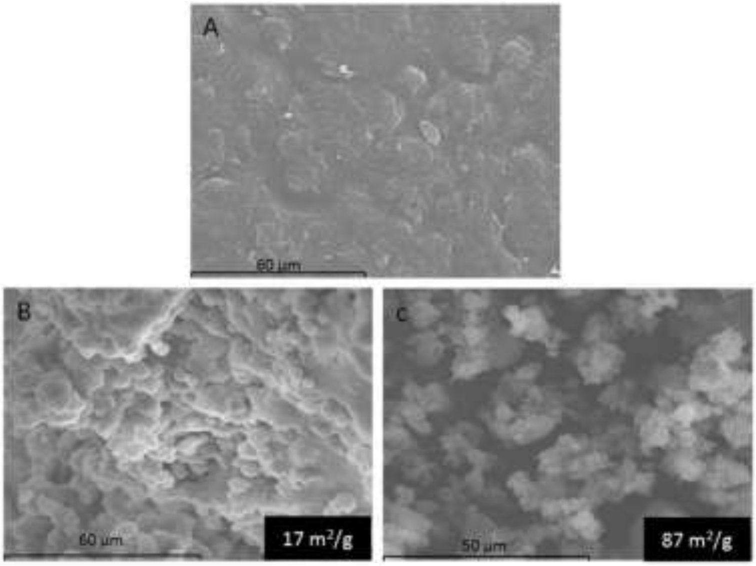

Figure 3. SEM micrographs of a nylon pellet at 800 magnifications (A), material A at 800 magnifications (B) and material B at 1000 magnifications (C). Surface area (m^2 g^{-1}) of B and C materials is also shown.

TEM micrographs of the different synthesized materials were obtained. For simplicity, Fig. 4 only presents the TEM images obtained for material A (Fig. 4A), material B (Fig. 4B) and silica NPs (Fig. 4C). Fig. 4A shows a dark spot that can be ascribed to the polymeric network of nylon 6. Besides, the presence of NPs into the polymeric network can be easily observed in Fig. 4B. Finally, Fig. 4C illustrates how the NPs are easily dispersed in the absence of the polymeric network which confirms, comparing with Fig. 4B, the efficient inclusion of the NPs in the polymer.

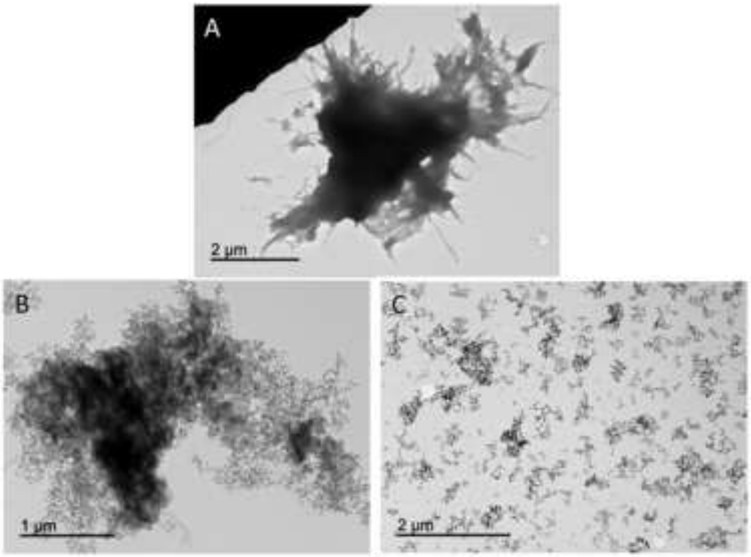

Figure 4. TEM micrographs of material A (A), material B (B) and silica NPs (C).

The elemental analysis of the synthesized materials, which is presented in Table 1, demonstrated, as it was expected, that the carbon and nitrogen content decreases when silica NPs are incorporated to form the composites. However, the precision of the measurements are lower for materials C and D. This lower precision, that we assume is not related to the elemental analysis itself, may indicate that when the amount of NPs is increased their inclusion in the polymeric network (due to the lower amount of nylon) is less reproducible.

ATR-FTIR spectra of the composites and the silica NPs were recorded and compared, as it can be seen in Fig. 5. In the silica NPs spectrum, a strong absorbing region at 1200–1000 cm^{-1} appears, being characteristic of the Si–O–H and Si–O–Si bonds. On the other hand, nylon 6 (material A) spectrum shows a characteristic band around 1642 cm^{-1} corresponding to the C=O stretching of the amide group. Furthermore, another band around 1546 cm^{-1} is observed, which can be assigned to the N–H deformation band of the amide. Finally, in the other composites spectra, the representative bands of both compounds can be noticed. It was also observed that the intensity of the characteristic bands of nylon 6 decreased from solid B to D as well as the characteristic band of silica NPs increased, what verify the composition of the different sorbents.

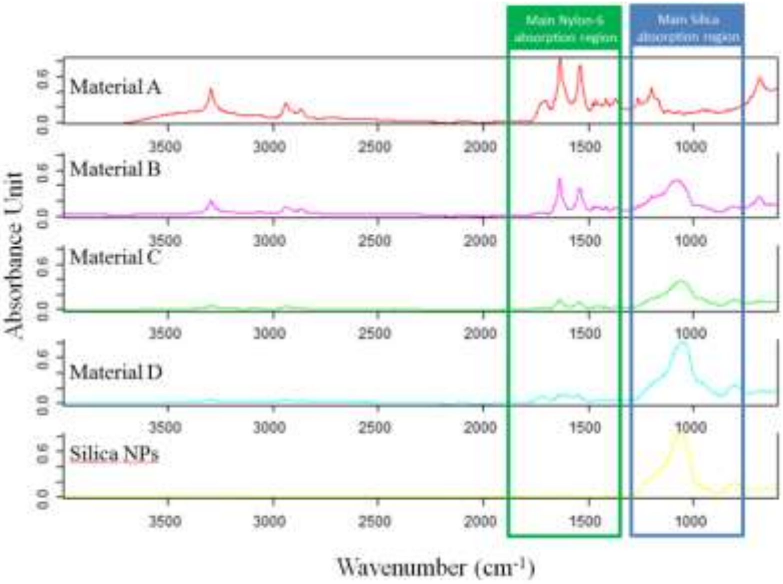

Figure 5. FT-IR spectra of the synthesized materials. The main absorption region of the components of the composites are highlighted in order to clearly observe how the ratio of each component varies.

3.2. Study of the extraction performance of the synthesized materials

As it has been explained, several materials comprising different proportions of nylon 6 and silica NPs (Table 1) were synthesized by the proposed method. All these materials were preliminary evaluated to know their sorption abilities. For this purpose, 1 mL of an aqueous standard solution containing the analytes at 1mg L^{-1} was extracted with 5 mg of each material. The retained analytes were eluted with 500 µL of methanol which was further analyzed. The chromatograms obtained for this test, which are shown in Fig. 6, lead to several conclusions.

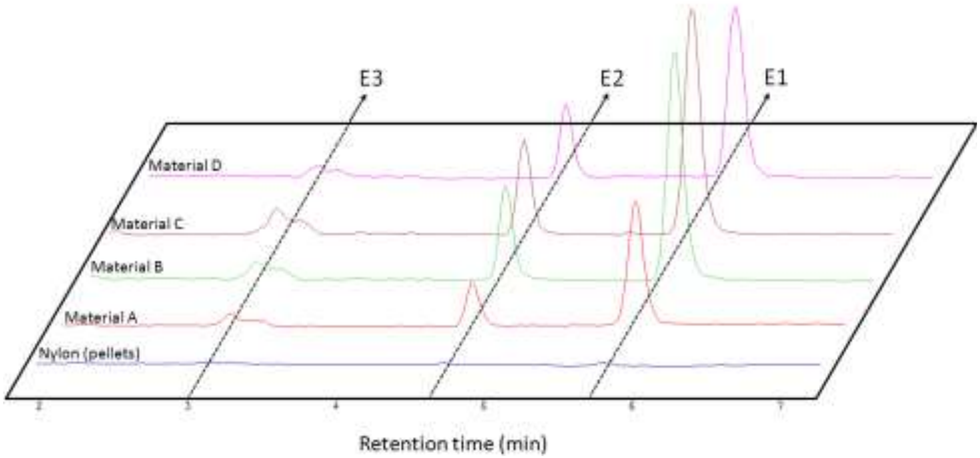

Figure 6. Obtained chromatograms for the extraction of 1 mL of aqueous standards containing the analytes [estrone (E1), β-estradiol (E2) and estriol (E3)] at the same concentration (1 mg L^{-1}) by different materials (see Table 1).

On the one hand, the synthetic procedure enhances the extraction performance as it can be concluded by comparing material A and nylon 6 pellets results and considering that both materials have the same composition. On the other hand, materials B and C provided the best results in terms of extraction as a consequence of the introduction of nanoparticles in the polymeric network. However, the results for material C present a lower precision probably due to the synthesis (see Table 1 where the precision obtained for several batches is lower for material C than for material B). Material D presents a slightly lower performance due to the lower content of nylon 6 in this composite. In the light of these results, material B was considered for further studies.

3.3. In syringe d-μSPE optimization

The extraction of the target analytes can be influenced by several factors such as the sample pH, the ionic strength, the amount of composite and the volume of sample. All these variables were studied in depth using aqueous standards spiked with the analytes at a concentration of 25 µg L^{-1}. The elution step was carried out using 500 µL of methanol.

First of all, pH was studied in the range from 3 to 10, showing a negligible effect on the extraction of the analytes. This behavior is in accordance with the octanol partition coefficient of these compounds which is almost constant in this range. Secondly, ionic strength was evaluated in the range from 0 to 300 g L^{-1} using sodium chloride as model electrolyte. The extraction recoveries slightly increased with the ionic strength up to 100 g L^{-1}, decreasing at higher concentrations due to an increase on the sample viscosity. Therefore, pH and ionic strength adjustment is not required for sample extraction which simplifies the overall procedure.

Sorbent amount, which defines the maximum sorption capacity, was evaluated at four different levels, namely: 5, 10, 20 and 50 mg using 5 mL of aqueous standard. The results show that the peak areas for all the analytes increased with sorbent amount until 20 mg, decreasing slightly for 50 mg. This final diminution of the signal can be assigned to the worse dispersion of the sorbent in such conditions. Finally, the sample volume was evaluated maintaining the sorbent amount/sample volume ratio constant and equal to the previous optimized value (20 mg per 5 mL) in order to work in the best dispersibility conditions. As it was expected (data not shown) the peak areas for all the analytes increased linearly with the sample volume. Therefore, 10 mL of standard was selected as optimum value.

In order to improve the enrichment of the analytes, an evaporation/redissolution of the eluates was implemented. The best values were obtained when the analytes were eluted from the composite with 500 µL of solvent, this solution being evaporated to dryness and redissolved in 50 µL of mobile phase. In such conditions, the enrichment factors resulted to be 80.2, 88.2 and 43.4 for E1, E2 and E3 respectively.

3.4. Analytical figures of merit and sample analysis

Once the procedure was optimized, it was characterized in terms of linearity, precision, sensitivity and extraction efficiency. For each analyte, a calibration graph was constructed by extracting in duplicate seven aqueous standards containing all the analytes at different concentrations. The calibration graphs were built by the representation of the two values obtained for each concentration level and were used for sample analysis. The analytical figures of merit, which are summarized in Table 2, show good linearity, limits of quantification in the low μg L^{-1} range and acceptable precision (lower than 13.9%, expressed as relative standard deviation, evaluated in septuplicate at 10 μg L^{-1}).

Table 2. Analytical figures of merit of the proposed method for the determination of estrogens in aqueous samples.

Analyte	EF[a]	MQL (μg L^{-1})	RSD (%)	Linear range (μg L^{-1})	R
E1	80.2	0.5	6.9	0.5-200	0.994
E2	88.2	1	2.8	1-200	0.995
E2	43.4	1	13.9	1-200	0.996

[a]EF, enrichment factor.

Finally, the selectivity of the composite for the extraction of the target compounds from more complex samples, like urine and saliva, was outlined. Direct infusion MS was selected for monitoring the selectivity, since it is more affected by the sample matrix. Urine and saliva samples were spiked with the analytes at 10 μg L^{-1} levels and were analyzed by direct infusion MS before and after the proposed sample treatment. The analysis of the raw samples provided no signal revealing a high ion suppression effect. However, after the sample treatment the characteristics transition of the analytes are again detected thanks to the clean-up ability of the extraction technique.

Although these results are promising about the potential of the composite in biofluids extraction, the quantitative evaluation of the results (data not shown) demonstrated that the intensities of the transitions are lower in biological samples than in aqueous standards even when the composite is used. This resulted especially marked for E2 and E3 extraction. This suggests that the extraction procedure should be further improved for bioanalytical applications.

The proposed method has been compared (see Table 3) with other counterparts published in the literature for the determination of the same compounds in several samples [28–34]. Our method provided similar [28,29] or even better [31] sensitivity than several approaches. However, these methods have been proposed for urine analysis which is not affordable by our method due to selectivity issues. In addition, it should be remarked that two LC-DAD methods provided similar LOQ than our proposal. This fact could be ascribed to a better extraction efficiency of the SBSE and cloud point extraction which are well developed techniques. In addition, those methods proposed for the analysis of environmental waters [32–34] are characterized by a better sensitivity due to the large volume of sample that is processed during the extraction technique.

Table 3. Comparison of the proposed extraction procedure with other published methods for the resolution of the same analytical problem.

Extraction	Instrument	Sensitivity[a]	RSD[b]	Ref.
Stir bar sorptive extraction	LC-DAD	LOQ (µg L^{-1}): 3.0 (E1), 3 (E2)	<7.6% (E1), <4.9% (E2)	28
Cloud point extraction	LC-DAD	LOD (µg L^{-1}): 0.2 (E1), 0.1 (E2)	<2.36 (E1), <3.86% (E2)	29
Magnetic solid phase extraction	LC-UV	LOQ (µg L^{-1}): 1.0 (E1), 0.8 (E2), 0.9 (E3)	<4.6% (E1), <4.0% (E2), <5.3% (E3)	30
Solid phase extraction	LC-FD	LOD (µg L^{-1}): 2.7 (E2), 8.3 (E3)	<3.3% (E2), <1.3% (E3)	31
Solid phase extraction	LC-MS/MS	LOD (ng L^{-1}): 15 (E1), 30 (E2), 30 (E3)	<6% (E1), <16.0% (E2), <15% (E3)	32
Immuno solid phase extraction	LC-MS	LOD (ng L^{-1}): 0.07 (E1), 0.18 (E2)	<5% (E1 and E2)	33
Stir bar sorptive extraction	LC-UV	LOD (µg L^{-1}): 0.29 (E1), 0.28 (E2)	<5.8% (E1), <4.5% (E2)	34
In-syringe dispersive SPE	LC-MS/MS	LOQ (mg L1): 0.5 (E1), 1.0 (E2), 1.0 (E3)	<6.9% (E1), <2.8% (E2), <13.9% (E3)	This work

[a]Sensitivity expressed as limit of detection (LOD) or limit of quantification (LOQ). [b]RSD, relative standard deviation.

4. Conclusions

In this article, different composites comprising nylon 6 and silica NPs have been synthesized, characterized and their extraction abilities towards selected hormones have been evaluated. Several advantages can be highlighted. First of all, the positive effect of the synthetic procedure on the extraction performance has been demonstrated. This effect relies on two facts, namely: (a) the solubilization/precipitation of nylon due to a solvent changeover infers a different superficial structure to the polymer and (b) the inclusion of silica NPs on the polymeric network enhances the extraction of the sorbent. In short, the composite presents better extraction performance than the raw polymer.

Second, the synthesis is easy and relatively cheap. The optimum composite has shown good extraction behaviour towards the target analytes in aqueous standards and their potential application to bioanalytical samples is promising according to the direct infusion profiles obtained for real samples. However, the quantitative results indicated a matrix effect even when the extraction procedure is applied. Therefore a further optimization should be done in order to develop an analytical method for the determination of these hormones in saliva or urine. Although the composite has demonstrated a great potential for the treatment of aqueous based samples, the chemical nature of nylon opens a door for processing non-aqueous ones. In fact, the amide group which does not prevail in a polar environment may be exploited in a hydrophobic one.

Acknowledgments

Financial support from the Spanish Ministry of Science and Innovation (grant CTQ2014-52939R) is gratefully acknowledged. E. M. Reyes Gallardo expresses her gratitude for the predoctoral grant (refs FPU12/02670) from the Spanish Ministry of Education. The authors would like to thank the Central Service for Research Support (SCAI) of the University of Córdoba for the service provided to obtain the micrographs and elemental analysis of the synthesized material.

Notes and references

[1] C. W. Huck and G. K. Bonn, J. Chromatogr. A, 2000, 885, 51–72.

[2] A. Martín-Esteban, Trends Anal. Chem., 2013, 45, 169–181.

[3] W. J. Cheong, S. H. Yang and F. Ali, J. Sep. Sci., 2013, 36, 609–628.

[4] A. Spietelun, M. Pilarczyk, A. Kloskowski and J. Namiesnik, Chem. Soc. Rev., 2010, 39, 4524–4537.

[5] L. Lin, H. Chen, H. Wei, F. Wang and J.-M. Lin, Analyst, 2011, 136, 4260–4267.

[6] F. Zhao, Y. Meng and J. L. Anderson, J. Chromatogr. A, 2008, 1208, 1–9.

[7] M. Zhang and J. Huang, RSC Adv., 2016, 6, 94098–94104.

[8] R. Lucena, B. M. Simonet, S. Cárdenas and M. Valcárcel, J. Chromatogr. A, 2011, 1218, 620–637.

[9] M. L. Castillo-García, M. P. Aguilar-Caballos and A. Gómez-Hens, Trends Anal. Chem., 2016, 82, 385–393.

[10] C. Carrillo-Carrión, R. Lucena, S. Cárdenas and M. Valcárcel, Analyst, 2007, 132, 551–559.

[11] A. H. Lu, E. L Salabas and F. Schüth, Angew. Chem., Int. Ed., 2007, 46, 1222–1244.

[12] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas and M. Valcárcel, Bioanalysis, 2015, 7, 1723–1730.

[13] Z. Huang and H. K. Lee, J. Chromatogr. A, 2015, 1414, 41–50.

[14] H. Bagheri, R. Daliri and A. Roostaie, Anal. Chim. Acta, 2013, 794, 38–46.

[15] A. Mehdinia, S. Dadkhah, T. B. Kayyal and A. Jabbari, J. Chromatogr. A, 2014, 1364, 12–19.

[16] M. C. Alcudia-León, R. Lucena, S. Cárdenas and M. Valcárcel, J. Chromatogr. A, 2016, 1455, 57–64.

[17] E. M. Reyes-Gallardo, G. Lasarte-Aragon´es, R. Lucena, S. Cárdenas and M. Valcárcel, J. Chromatogr. A, 2013, 1271, 50–55.

[18] E. M. Reyes-Gallardo, R. Lucena and S. Cárdenas, Trends Anal. Chem., 2016, 84, 3–11.

[19] H. Bagheri and A. Roostaie, J. Chromatogr. A, 2014, 1324, 11–20.

[20] H. Bagheri and A. Roostaie, J. Chromatogr. A, 2015, 1375, 8–16.

[21] K. Yoshimatsu, L. Ye, J. Lindberg and I. S. Chronakis, Biosens. Bioelectron., 2008, 23, 1208–1215.

[22] X. M. He, G. T. Zhu, J. Yin, Q. Zhao, B. F. Yuan and Y. Q. Feng, J. Chromatogr. A, 2014, 1351, 29–36.

[23] X. M. He, G. T. Zhu, H. B. Zheng, S. N. Xu, B. F. Yuan and Y. Q. Feng, Talanta, 2015, 140, 29–35.

[24] S. Tong, X. Zhou, C. Zhou, Y. Li, W. Li, W. Zhou and Q. Jia, Analyst, 2013, 138, 1549–1557.

[25] B. Fresco-Cala, S. Cárdenas and M. Valcárcel J. Chromatogr. A, 2016, 1468, 55–63.

[26] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas and M. Valcárcel, J. Chromatogr. A, 2014, 1345, 43–49.

[27] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas and M. Valcárcel, Microchem. J., 2016, 124, 751–756.

[28] C. Almeida and J. M. F. Nogueira, J. Pharm. Biomed. Anal., 2006, 41, 1303–1311.

[29] Y. Zou, Y. Li, H. Jin, H. Tang, D. Zou, M. Liu and Y. Yang, Anal. Biochem., 2012, 421, 378–384.

[30] S. Wang, R. Wang, X. Wu, Y. Wang, C. Xue, J. Wu, J. Hong, J. Liu and X. Zhou, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci., 2012, 905, 105–112.

[31] L. Mao, C. Sun, H. Zhang, Y. Li and D. Wu, Anal. Chim. Acta, 2004, 522, 241–246.

[32] M. Pedrouzo, F. Borrull, E. Pocurull and R. M. Marcé, Talanta, 2009, 78, 1327–1331.

[33] P. L. Ferguson, C. R. Iden, A. E. McElroy and B. J. Brownawell, Anal. Chem., 2001, 73, 3890–3895.

[34] C. Hu, M. He, B. Chen, C. Zhong and B. Hu, J. Chromatogr. A, 2013, 1310, 21–30.

ELECTRONIC SUPPLEMENTARY MATERIAL (ESI) FOR RSC ADVANCES

1. Chromatographic analyses

Two different systems were employed in this research. The optimization of the method was performed using a Waters AcquityTM Ultra Performance Liquid Chromatography system (Waters Corp., Madrid, Spain) using an Acquity UPLC®BEH C18 column (1.7 µm, 2.1 mm × 100 mm) maintained at 45 °C. The separation was performed under a gradient elution program using water (solvent A) and acetonitrile (solvent B) as mobile phase components. The elution started at 20% of solvent B and it was linearly increased up to 50% in 5 min. After that, the system was re-equilibrated for 2 min between analyses. During the separation, the flow rate was maintained at 0.4 mL/min and 5 µL was injected with partial loop mode. Detection was performed at 200 nm using a PDA eλ Detector (Waters). System control was performed with Empower software also from Waters. Since the extraction efficiency is calculated in relative terms (extraction recoveries and enrichment factors), the obtained values are independent of the employed instrument.

An Agilent 1260 Infinity HPLC system (Agilent, Palo Alto, CA, USA) coupled with mass spectrometric detector was employed for the validation of the method. It was equipped with a binary high-pressure pump for mobile phase delivery and an autosampler. Chromatographic separation was performed on a Zorbax Eclipse XDB-C18 (4.6 mm × 150 mm, 5 µm) column from Agilent, using LC-MS grade acetonitrile and water as mobile phase components in a 35/65 (v/v) ratio. A guard column (0.2 µm filter, 2.1 mm), also from Agilent, was used to preserve the integrity of the analytical column. The injection volume was 5 µL and the flow rate was maintained at 0.5 mL/min.

Quantification was performed on an Agilent 6420 Triple Quadrupole MS system equipped with an electrospray ionization source working in negative mode.

Data analyses were carried out using Agilent MassHunter Software (Version B.06.00). The mass spectrometer settings were fixed in order to improve the selected reaction monitoring signal. The flow and temperature of the drying gas (N_2) were 8 L/min and 350 °C, respectively. The nebulizer pressure was 20 psi, and the capillary voltage was 5500 V. The analytes were detected using the selected reaction monitoring transitions. Table S1 summarizes the optimized parameters for each analyte.

Table S1. Transitions and optimized potentials for LC–MS/MS analysis, including fragmentor, collision energy (CE) and cell accelerator voltage (CAV).

Analyte	Transition	Fragmentor (V)	CE (eV)	CAV (V)
Estrone	269.2 →145.2	133	37	7
β-Estradiol	271.3 → 145	130	40	7
	271.3 → 183.3	130	42	7
Estriol	287.3 → 145.1	137	47	7

BLOQUE IV

MICROEXTRACCIÓN EN FASE SÓLIDA CON MEMBRANA

Capítulo IV. 1

Electrospun nanofibers as sorptive phases in microextraction

Trends in Analytical Chemistry

84 (2016) 3-11

Electrospun nanofibers as sorptive phases in microextraction

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The development of new sorptive materials with improved properties over the existing ones is one of the main research fields in the microextraction context. Polymers, which have demonstrated a great potential for extraction purposes, are excellent candidates according to their versatility, stability and efficiency. The versatility of polymers can be seen from two different perspectives: (i) they may develop different interaction chemistries with targets even allowing the ad-hoc selection of the polymer and (ii) they can be obtained in different formats (like fibers, coatings, membranes or beads). Polymeric nanofibers, especially those obtained by electrospinning, are characterized by large surface to volume ratio which enhances the extraction kinetics and capacity. In this article, the potential of nanofibers as sorptive phase is presented and discussed with selected references. In addition, the impact of nanofibers composites (either formed by combination of different polymers or combining polymers and nanomaterials) in microextraction is therefore considered.

Keywords: *Nanofibers; electrospinning; microextraction; polymers; Nanomaterials.*

1. Introduction

Analytical Chemistry is in a constant evolution due to the continuous appearance of new challenges in different fields like environmental sciences, toxicology, agri-food…etc. These challenges are often related with analytical problems in which sensitivity and/or selectivity are issues of concern. The threshold concentrations in many of these scenarios are being decreased year by year and therefore, the existing analytical methodologies must be adapted or even substituted by more competitive counterparts. The combination of sample handling and sophisticated instrumental techniques (characterized by high sensitivity and selectivity levels) answers to these requirements.

Sample treatment has exponentially evolved in the last decades in accordance to three known trends, namely: automation, simplification and miniaturization [1]. The current research in this field is vast but it can be divided in well-defined lines. The theoretical development of the existing techniques, the design of new ones or the proposal of novel extracting phases can be considered among the most noteworthy ones. In the latter case, the use of nanoparticles, ionic liquids, monolithic materials or polymers can be highlighted.

Polymers have been extensively used in the microextraction context [2] as sorptive phases for several reasons. There is a wide variety of polymers covering different interaction chemistries that permits the selection of the polymer according to the target analytical problem intended to be solved. In addition, they usually present a better mechanical and chemical stability (specially related to the pH effect) and they may be produced in several formats (beads, membranes or fiber) allowing the development of different extraction procedures (conventional SPE, thin film microextraction or solid phase microextraction).

In the same way as nanoparticles, when a polymer fiber goes to the nanoscale, new characteristics appear, for instance, extremely large surface to volume ratio, improved mechanical properties and flexibility in surface functionalities [3]. In recent years, electrospinning has demonstrated to be a good technique to synthesize this kind of nanofibers. A typical electrospinning process consists of the application of a high voltage to a viscous polymeric solution to produce tunable diameter fibers from nano to micrometers [4]. This phenomenon is based on repulsive electrostatic forces since when a high voltage is applied between the solution and the collector, these forces overcome the surface tension and the polymeric jet is charged, producing a non-woven fibrous material on the surface of the conductive collector [5]. Although this is the typical procedure to fabricate electrospun nanofibers, other electrospinning methods such as magnetic field assisted electrospinning [6], melt electrospinning [7], core-sheath electrospinning [8], or bilayer electrospinnig [9] have been proposed for the synthesis of nanofibers.

This article tries to give a general overview of the potential of these nanofibers as sorptive phase in microextraction. The applications presented are divided in two main groups depending on the nature of the electrospun material. In this sense, those application based on just one polymer are described and commented at the beginning while those applications based on composite material are finally presented.

2. Electrospun nanofibers as sorbents in microextraction techniques

The main application of electrospun nanofibers made of a single polymer as sorbent are summarized in Table 1. These contributions are divided in three main sections as follow.

Table 1. Applications of electrospun nanofibers in the (micro)extraction context.

Nanofiber composition[a]	Extraction technique[b]	Instrumental technique[c]	Analytes	Samples	Reference
N6	SPE	LC-UV	Dimethyl phthalate, diethl phthalate, di-n-butyl phthalate, di-(2-ethylhexyl) phthalate and dioctyl phthalate	Environmental water	[11]
N6	SPE	LC-UV	Estradiol, ethinylestradiol and estrone	Environmental water	[12]
N6	SPE	LC-UV	Docetaxel	Rabbit plasma	[13]
N6	On-line µSPE	LC-UV	Clodinafop, Propargyl	Water, Soil and Wheat	[14]
N6	SPME	GC-MS	Phenol and chlorophenols	Environmental water	[15]
PS	PFSPE	LC-FD	Cortisol	Saliva	[16]
PS	PFSPE	LC-MS/MS	Diethylstilbestrol, hexestrol, and dienestrol	Milk products	[17]
PS	PFSPE	LC-UV	Twenty different drugs	Plasma samples	[18]
poly(styrene-co-methacrylic acid), poly(styrene-co-p-styrene sulfonate), PS	SPE	LC-UV	Nitrobenzene, 2-naphthol, benzene, n-butyl p-hydroxybenzoate, naphthalene, p-dichlorobenzene	Environmental water	[19]
poly(styrene-co-methacrylic acid)	PFSPE	LC-FD	Retinol and α-tocopherol	Plasma	[20]
styrene-co-p-styrene sodium sulfonate	PFSPE	LC-UV	Hydrocortisone, cortisone acetate, ethinylestradiol and estradiol	Hair samples	[21]

Nanofiber composition[a]	Extraction technique[b]	Instrumental technique[c]	Analytes	Samples	Reference
styrene-co-p-styrene sodium sulfonate	PFSPE	LC-UV	Hydrocortisone, cortisone acetate, ethinylestradiol and estradiol	Hair samples	[21]
PS and dithizone	PFSPE	LC-UV	Lead	Water plasma	[22]
PS	PFSPE	LC-UV	Trazodone	Plasma samples	[24]
PS	SPE	UHPLC-MS	Sulfonamide residues	Environmental water	[25]
PS	SPME	GC-MS	Pesticides	Honey	[26]
Cellulose-g-oxolane-2,5-dione	SPE	ICP-OES	Cd, Cu, Fe, Pb and Zn	Gasoline	[27]
PET	SPME	UV	Chromium(VI)	Environmental water	[28]
PI	TFME	GC-MS	Phenols	Environmental water	[29]
PEI	SPME	GC-MS	Selected PAHS	Water	[30]

[a] Nanofibers composition acronyms: N6, nylon-6; PS, polystyrene; PET, polyethylene terephthalate; PI, polyimide; PEI, polyetherimide.

[b] Extraction techniques acronyms: SPE, solid phase extraction; μSPE, micro-solid phase extraction, SPME, solid phase microextraction, PFSPE, packed-fiber solid-phase extraction; TFME, thin film microextraction.

[c] Separation and instrumental techniques acronyms: LC, liquid chromatography; GC, gas chromatography; UHPLC, ultrahigh pressure liquid chromatography; UV, photometric detection; MS, mass spectrometry; FD, fluorescence detector; ICP-OES, inductively coupled plasma- optical emission spectrometry.

2.1. Polyamides

Polyamides are polymers obtained by the controlled reaction of a dicarboxilic acid and a diamine compound giving rise to a hydrocarbon chain with amide groups regularly distributed through the structure. Although some polyamides are commercially available, their synthesis, which is simple enough to be developed with conventional glassware, opens the door to the inclusion of special functionalities in the polymer that may assist the extraction of a given compound or structure-related substances [10]. The in-lab synthesis of polyamides consists of a biphasic system where the dicarboxilic acid (in the form of acylchloride to favor the reaction) is dissolved in an organic solvent while the diamine compound is dissolved in an alkaline aqueous solution. In the interface of both solutions, the reaction takes places and the final polymer can be easily collected with a wire.

The amide groups are the responsible for the stacking of the polymers chains by hydrogen bonding, providing strength to the resulting fibers [11]. The combination of hydrocarbon chain and amide groups confers polyamides with special polarity balance that can be exploited for extraction purposes. The hydrocarbon chain allows the interaction with the compounds by different chemistries depending on the polyamide monomers while the amide groups allow the wetting of the fiber by water which is critical to enhance the transference of the target compounds from the bulk sample to the fibers.

Electrospun polyamides can be obtained in the form of membranes consisting of a mat of nanofibers. These membranes, thanks to their high cross-sectional area, can process larger volumes of sample since the backpressure provided by the membrane is low enough. The use of membranes as extracting phase can be developed using a similar device to that depicted in Fig. 1. The membrane is located in a special holder where it is tightly sealed to assess the sample passing through the mat. The procedure follows the conventional solid phase extraction (SPE) workflow and the final eluate can be analyzed by different instrumental techniques. This approach was firstly proposed for the extraction of three estrogens from environmental waters [12]. Besides the interesting application, several statements were proved. On the one hand, electrospun nanofibers presented better extraction performances than commercial polyamides filters (based on microfibers) which turns electrospinning as a powerful strategy to boost the extraction potential of these polymers. On the other side, the authors demonstrated that this type of polymers is not stable at acidic pH as it induces the protonation of the nitrogen in the polymeric chain modifying the polymer structure. The versatility of this membrane based devices has been demonstrated by its application for the analysis of large and low-volume samples. In the first case, phthalates has been successfully extracted from waters [11] using nanofibers (with diameters in the range from 100 nm–300 nm), as those presented in Fig. 2, to process up to 50 mL of sample at high flow rates (up to 4 mL /min). In fact, this material showed better performance than conventional C18 sorbent. In the second case, the extraction device has been adapted to extract docetaxel from 500 µL of rabbit plasma [13].

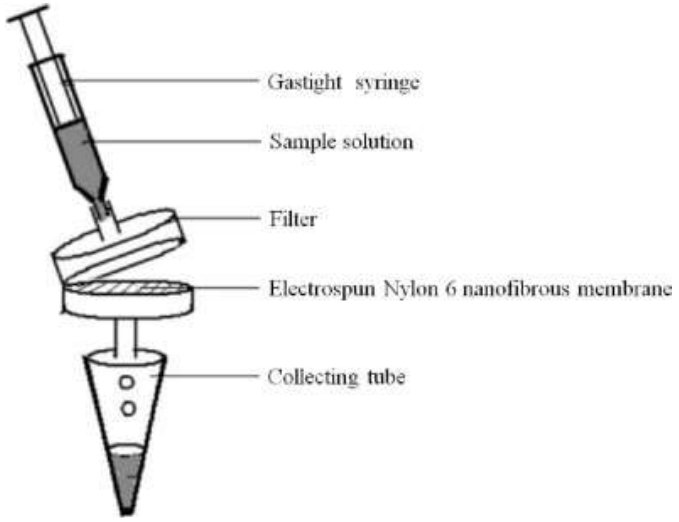

Figure 1. Extraction device for the use of electrospun nanofibers membrane. Reproduced with permission of Springer from reference [12].

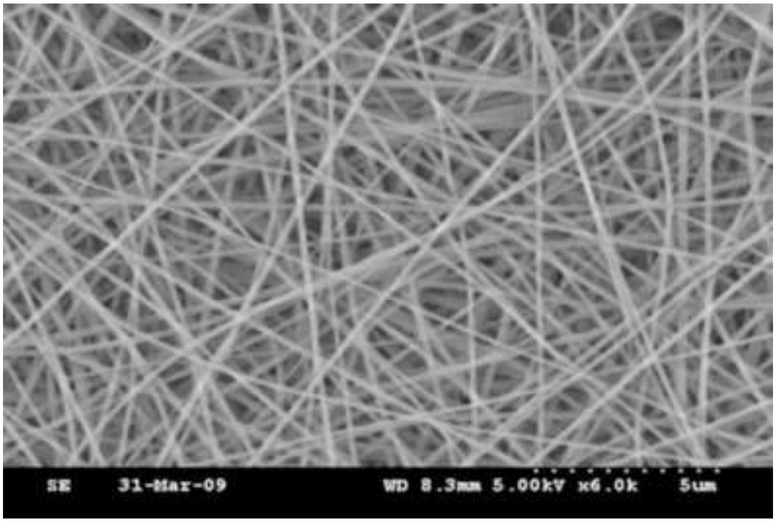

Figure 2. SEM images from a polyamide nanofibers mat. Reproduced with permission of Springer from reference [11].

The off-line coupling of the extraction and analysis stages hinders the preconcentration factors achieved since a larger volume of eluent is required. In addition, only a fraction of the final eluate is effectively introduced in the instrument. This shortcoming can be overcome if the nanofibers sheet is located in the loop of the chromatographic injection valve [14] using a special pressure-resistant holder. In this case, the sample is passed through the nanofibers for analyte enrichment and after a defined time the valve is switched to the injection position in order to inject (the own mobile phase elute the analytes) the target into the column.

Just a slight modification of the electrospinning equipment is enough to change the sorbent format. In this sense, if a continuously spun wire is used as collector during electrospinning a solid phase microextraction (SPME) fiber can be obtained as can be seen in Fig. 3. Nylon-6 has been used to design this type of fiber for the headspace (HS)-SPME of phenol and several chlorophenols from water [15]. The fabricated fibers are thermally stable, up to 250°C although a slight color change of the fiber is observed at high temperatures. This is a critical aspect when they are used in combination with gas chromatography (GC). During the synthesis, the concentration of polymer in the original electrospinning solution seems to play a crucial role. In fact, this concentration should be as high as possible to increase the sorbent capacity, although an excessive amount decreases the extraction kinetics due to the formation of thicker coatings. The synthesized fibers were effective enough, providing relative recoveries in the range from 72 to 98%, fulfilling the EPA criterion.

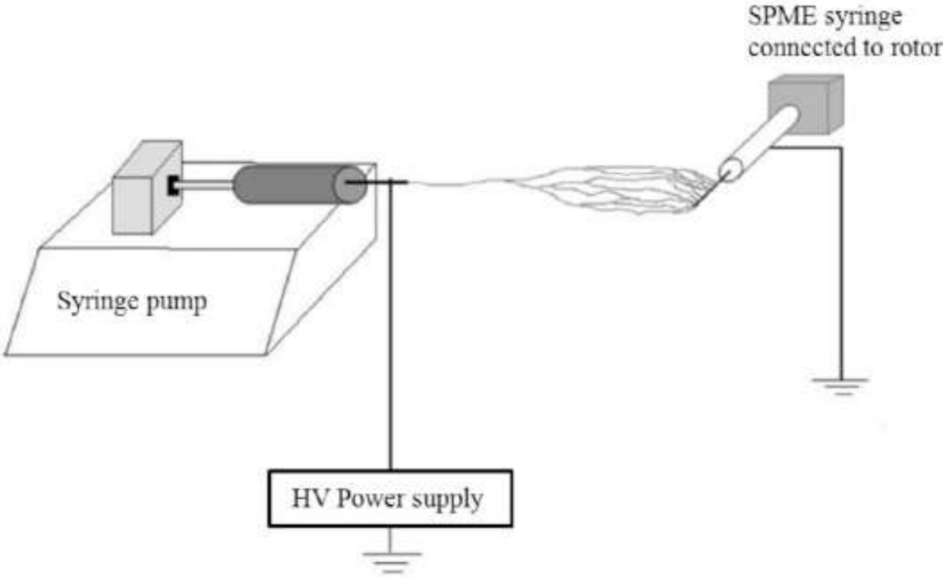

Figure 3. Electrospinning manifold for the synthesis of SPME fibers. Reproduced with permission of Elsevier from reference [15].

2.2. Polystyrene

Polystyrene (PS), which has been successfully employed as sorbent in conventional solid phase extraction, can be also used to prepare electrospun fibers. In most of the PS nanofibers applications, the material is packed in the low part of a pipette tip which acts as extraction device. Pipette tip extraction is a miniaturized SPE mode especially adapted for processing low sample volumes which is a normal requirement in bioanalysis due to the sample availability. This device also requires a low eluent volume (usually in the medium microliter range) which has a clear positive effect on the preconcentration factors. Fig. 4a shows the extraction device designed for the determination of cortisol in saliva [16]. This determination is a challenge due to its low concentration and it requires a very sensitive instrumental technique (mainly mass spectrometry, MS). However, the reaction of cortisol with sulfuric acid produces a compound which emits an intense fluorescence that can be advantaged to avoid the use of MS. The compound is not stable enough in such acid conditions and this media is not compatible with HPLC, a previous extraction being required to overcome both limitations. In that scenario, PS pipette tips have been compared with conventional SPE and LLE methodologies providing by far the best ER.

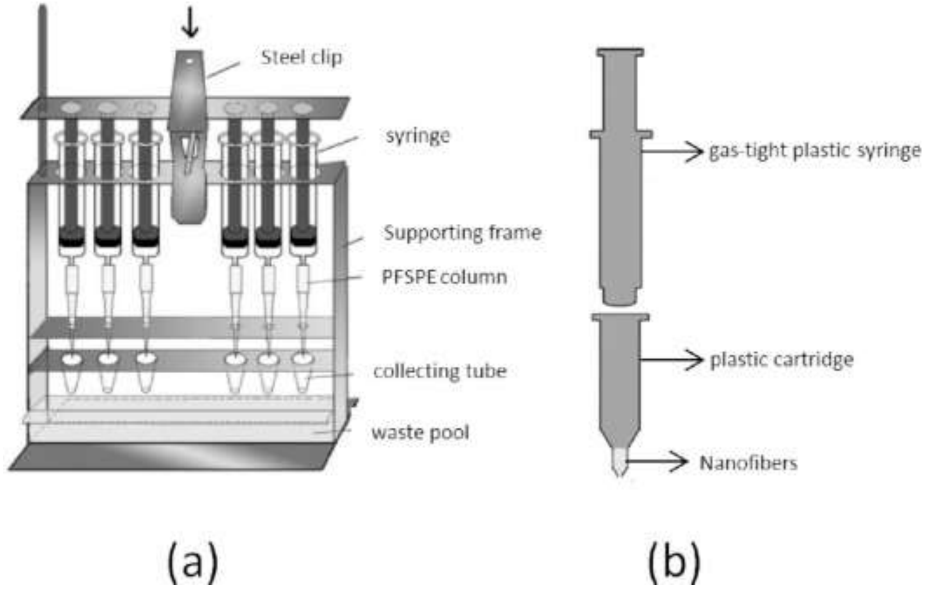

Figure 4. Devices for packed fiber solid phase extraction using (a) pipette tips (Reproduced with permission of Wiley from reference [16]) and (b) special cartridges (Reproduced with permission of Elsevier from reference [17]).

Although pipette tip extraction is the preferred mode for PS nanofibers, other alternatives, like packed fiber SPE, has been also proposed [17]. In this case, as it is indicated in Fig. 4b, the nanofibers are located in the lower part of a special cartridge.

The chemical structure of PS makes it especially useful for the extraction of moderate polar or non-polar compounds. The extraction recoveries obtained with this material are competitive for those substances presenting a logarithm of the octanol/water coefficient 1.5 or higher [18]. The hydrophobic nature may hinder slightly its performance for aqueous samples as it involves a poor wettability of the nanofibers. However, the introduction of styrene copolymers with a marked polarity has been used to overcome this initial limitation. The inclusion of methacrylic acid (MAA) as copolymer has been proposed to enhance the extraction of some aromatic hydrocarbons from water samples [19] and vitamins A and E from plasma samples [20]. The introduction of such polar groups may induce conformational and structural changes in the PS fibers as well as they increase the wettability of the fibers. A similar approach, synthesizing in this case a styrene-co-p-styrene sodium sulfonate polymer, has demonstrated superior performance over PS for the extraction of several hormones from waters [21].

The non-polar nature of the PS backbone may limit the versatility of these nanofibers. The addition of some modifiers to the electrospinning solution has been proposed to make the application of PS nanofibers wider. After electrospinning, the modifiers are incorporated to the nanofibers being responsible for the extraction of the target compounds. The addition of dithizone, a classic metal ligand, to PS has allowed the extraction of lead from aqueous samples [22] while the addition of a potassium salt of imidazole-1-carbodithioate has been used for the extraction of a organochlorine pesticide [23].

Extraction techniques are surface-dependent processes as the kinetics of the analyte transference between phases is directly dependent on the superficial area of the extracting phase. This fact is also applicable to nanofibers where a high superficial area per amount of material is highly desirable. The diameter of the nanofibers and their porosity are critical factors in this context. In this sense, as it was demonstrated by Kang et al., the diameter of the nanofibers is inversely related to their extraction capabilities [24]. This diameter can be controlled, among other factors, by the concentration of the PS during electrospinning. In addition, the porosity of the fibers has a beneficial effect on their extraction potential [25]. This porosity can be tuned controlling the humidity or playing with the solvents (especially with their vapor pressures) used in electrospinning. For example, just changing the solvent from pure THF to a mixture THF/DMF 1:3 v/v the specific surface can be increased from 3.75 m2/g to 70.59 m2/g with the inherent enhancement of the extraction recoveries.

As it was described for polyamides, electrospinning can be used for the fabrication of SPME fibers. In this sense, Zali et al. have recently proposed the use of PS nanofibers as alternative coating in HS-SPME for the extraction of multiple pesticides from honey samples [26]. The fibers presented a high stability allowing their continuous use (more than 100 extractions per fiber) which reduced the costs associated to the analysis.

2.3. Other polymers

Although polyamides and polystyrene have found a clear applicability as precursors for electrospun nanofibers in extraction techniques, other polymers have been also proposed in this context.

Cellulose, which has a great potential for the extraction of metals by a cation-exchange mechanism, has been proposed as precursor for electrospinning [27].

However, the low solubility of raw cellulose in conventional solvents makes this application challenging, making necessary a slight modification of the synthetic procedure. In this sense, cellulose should be transformed into cellulose acetate, which presents an enhanced solubility, prior to be electrospun. Once the nanofibers are obtained, they can be reverted to the raw cellulose state using a strong alkaline reaction and finally derivatized to include special extraction groups on the surface (oxalone-2,5-dione). The resulting material, that presents a specific area around 14.31 m^2/g, provided exceptional recovery values for 5 cations from gasoline. Polyethylene terephtalate (PET) nanofibers have also been proposed for the extraction of cations although the hydrophobic nature of the polymer required a previous complexation of the metal [28].

Polyimide mats have been used as sorptive phase in thin film microextraction, an evolution of SPME based on the use of membranes instead of fibers [29]. The planar shape increases the contact area with the samples making easier the extraction of the target compounds. Polyimide presents a balance polarity structure with aromatic groups, carbonyl groups and nitrogen atoms that can be exploited as well for extraction purposes. The wettability of the membrane seemed to depend critically on the solvent used in the activation. This membrane has been used for the extraction of some phenols from water and their subsequent determination by thermal desorption in combination with gas chromatography.

Thermal and mechanical stability are essential when a polymer is intended to be used in SPME. In this case, especially when SPME is combined with GC (which is for sure its more powerful combination), thermal stability is critical to assess the reusing of the fiber or to avoid the appearance of ghost peaks on the chromatogram since the injection port of the GC is usually fixed at temperatures higher than 200 °C. On the other hand, the mechanical stability avoids the breakage of the fiber which is pretty common when other phases are employed. The use of polyetherimide (PEI) which has a great thermal (it melts in the range from 340 to 427 °C, and decomposes at higher temperatures than 527 °C) and chemical stability is advantageous in this context. Electrospun PEI fibers have been proposed for the extraction of some aromatic hydrocarbons from water by HS-SPME [30]. Although PEI presents several functional groups that may develop different interaction chemistries, π-π interactions seem to play a key role in the extraction.

In addition, polyurethane (PU) fibers have been also proposed [31] to build SPME fibers. This coating resulted to present a high superficial area and mechanical stability and was used for the extraction of chlorobenzenes from environmental samples where π-π interactions seemed to play a key role.

3. Composites

3.1. Combination of electrospun nanofibers of different nature

Electrospinning has also been used to prepare nanofibers combining different polymers with the aim of increasing the versatility of the final structure while controlling the diameter, polarity and porosity of the nanofibers. When a mixture of polymers are used, the structural and mechanical properties of the nanofibers are different from those obtained using a single polymer. In these polymer blends, the components interact by secondary forces (e.g. hydrogen bonding or dipole-dipole interaction) [32]. In comparison with electrospun nanofibers obtained by copolymerization, this alterative is more simple (as regards the synthetic pathway) and economical, with minimal reagents waste. Moreover, they can be prepared in different formats. The main recent contributions will be briefly described in this section and are summarized in Table 2.

Table 2. Applications of electrospun nanofibers containing polymers of different nature in the (micro)extraction context.

Composite elements[a]	Extraction technique[b]	Instrumental technique[c]	Analytes	Samples	Reference
PPy/PA	μSPE	GC-MS	Malathion	Aqueous samples	[33]
PPy/PA	MEPS	GC-MS	Organophosphorous pesticides	Aquatic samples	[34]
PPy/PA	Disks SPE	LC-DAD	Acid yellow 9 (CAS: 74543-21-8), acid orange 7 (CAS: 633-96-5) and metanil yellow	Environmental water	[35]
PANI/N6	HS adsorptive ME	GC–MS	Chlorobenzenes	Water samples	[36]
PMMA/PS	SPE	CD-IMS	Tramadol	Urine and plasma	[37]
PS/PAn	Pipetter tip extraction	LC–MS/MS	Gibberellin A3, gibberellin A7, jasmonic acid, abscisic acid, p-hydroxycinnamic acid	Tissues	[38]

[a] Composite elements acronyms: PPy, polypyrrole; PA, polyamide; PANI, polyaniline; N6, nylon-6; PMMA, Poly(methylmethacrylate), PS, polystyrene; PAn, polyaniline; PEG, polyethylene glycol; PBT, polybutylene terephthalate.

[b] Extraction techniques acronyms: μSPE, micro-solid phase extraction; MEPS, microextraction in packed syringe; SPE, solid phase extraction; HS, headspace; ME, microextraction; SPME, solid phase microextraction.

[c] Separation and instrumental techniques acronyms: GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; DAD, diode array detector; CD-IMS, corona discharge ion mobility spectrometry.

Nylon-6 has been selected by far as one of the components of the polymeric mixture, thanks to its well-known extraction capabilities. Bagheri et al. have evaluated the combination polypyrrole/Nylon-6 (PPy-N6) in both nanofiber sheet [33] and MEPS [34]. As it is stated by the authors, the polymeric blend exhibited the high surface area and π functional groups of the PPy as well as the polar functional groups of the nylon-6. The fibers are highly porous, which results in an increased surface area with smooth morphology. Another valuable property of this polymeric mixture is its stability in organic solvents commonly used as eluent in solid phase extraction. Also, its extracting capacity remains almost unaltered after 200 uses. The ratio PPy-N6 is important as it has been demonstrated that both polymers play a synergic role in the extraction of the analytes. The isolation of malathion from waters using a nanofiber sheet requires the presence of a 30% (w/v) of N6 and 0.75 g of PPy. However, for the extraction of organophosphorous pesticides from the same matrix, the MEPS of PPy-Nylon-6 were composed of 25% (w/v) nylon-6 and 0,5 g PPy.

For both applications, the authors optimized the preparation of the nanofibers, including the electrospinning voltage and the collecting time. If low voltages are applied, few fibers are obtained which results in lower extraction capacity. However, higher voltages transform the fibers in beads, which exhibit lower surface area. The collecting time affects to the final thickness of the fiber sheet which directly defines its mechanical properties. Longer times (4h) produces fibers of ca. 100 µm in thickness. The final nanofiber can be immersed in the solution [33] or transferred to a microsyringe (three layers) where they laid flat in the syringe barrel using two frits [34].

Qui et al. have used the same polymeric blend in a disk format to extract sulfonated azo dyes from environmental waters [35]. Taking into account the polar nature of the analytes, the PPy allows, in addition to the previously mentioned π-π interactions, base-acid, ion exchange and hydrogen bonding properties. In this case, the Nylon-6 nanofibers provide the final sorbent with excellent mechanical and chemical stability. In this case, the mixture was prepared by in-situ chemical oxidative polymerization to obtain a N6-PPy core-shell nanofibers. The authors compare their behavior with that obtained with Nylon-6 nanofibers and conclude that, although negligible differences were obtained for less polar compounds, the high polar analytes were better extracted (ca. 4 times) in the presence of PPy. Again, the mechanical stability of the nanofibers was a critical issue. In this case, a nanofiber amount higher than 2 mg is needed to allow the processing of large sample volumes (up to 50 mL) and therefore, 2.5 mg were fixed as optimal. After an exhaustive optimization of the extraction procedure, a recovery study was carried out to demonstrate the accuracy of the method. The analytes were spiked to lake water samples at the low microgram per liter level and the recoveries were between 87.6 and 112.3%, which demonstrates the very little interference of the sample matrix in the determination of the sulfonated azo dyes.

Nylon-6 has also been combined with polyaniline (PANI) and the resulting nanofiber sheet was evaluated for the extraction of chlorobenzenes from waters [36]. The conjugated structure of the PANI would favour the interaction of these analytes via π-π and hydrophobic interactions. In this case, PANI was synthesized inside the Nylon-6 solution and the mixture was then electrospun into an aluminum foil which acts as collector electrode. 16 kV and 12 h were needed to obtain the desired thickness and sheets of 1 cm × 1 cm were cut from the central part and used for extraction. It contained ca. 2 mg of nanofibers. Taking into account the volatility of the analytes, the PANI-N6 nanofiber sheet was used in the headspace mode. The extraction was carried out from 10 mL of water at 30 °C during 30 min and the chlorobenzenes were eluted with chloroform (30 min). The presence of NaCl (30% w/v) led to a higher sorbent/sample distribution of the analytes, which resulted in an increased sensitivity. The favorable analytical features achieved (limits of detection between 19–33 ng/L and precision in the range 5–14%) derive from the fibrous and web structure of the PANI-N6 nanofiber, which results in a higher specific surface area, loading capacity, mechanical stability and sheet reusability (up to 100 times).

Another hydrophilic/hydrophobic polymeric mixture based on poly(methylmethacrilate) and polystyrene (PMMA/PS) has been proposed for the extraction of tramadol from urine and plasma samples [37]. The nanofiber was synthesized using a PMMA/PS 25/75 wt% dissolved in dimethylformamide and tetrahydrofurane (4:6, v/v). For this application, the nanofibers were packed in a pipette tip and confined by means of two frits. Although PS has been extensively used as sorbent phase, its hydrophobic nature makes it incompatible with aqueous samples, such as biological ones. In this context, the polar groups of PMMA facilitate the interaction of the analytes with the sorbent, thus increasing the extraction capacity. As it has been previously mentioned for other polymeric mixtures, the resulting nanofiber is highly porous, which indicates a high surface to volume ratio. This fact increases the number of active sites and ensures a higher mass transfer during extraction. The PMMA-PS nanofiber was stable in alkaline medium and therefore, the extraction was carried out at a pH of 9.41. The limits of detection were 1.6 and 9.4 ng/mL for urine and plasma, respectively using 2 mL of sample and 200 µL of methanol as eluent. Detection of the analytes was carried out by ion mobility spectrometry.

Polystyrene has also been used in combination with another polar polymer such as polyaniline (PA) for in vitro extraction of acidic phytohormone from plant tissue [38]. The presence of the polar groups of PA in combination with the sorbent capacity of PE is crucial to achieve the highest extraction of the analytes. The matrix selected, plant tissue, adds an additional difficulty in the extraction process as it has higher viscosity and mass transfer resistance in comparison with other biological materials. In this specific application, core-sheath electrospun nanofibers were prepared using a PS core and a collagen PA sheath. The collagen was further removed to leave a pure PA shell. The obtained nanofibers were compressed into an integrated monolithic tiny bar (1 mm diameter, 2 mm length). The bar was directly introduced into the living aloe leave through a hole (1 mm diameter, 3 mm depth) were the analytes extraction took place for 20 min. Then, the tiny bar was withdrawn by means of a pointed tips tweezers. It is worth noting the resistance of the compressed nanofibers to the aloe leave matrix (e.g. pH and matrix component, salt concentration, among others). The selectivity of the extraction towards weak organic acids was attributed by the authors to specific weak exchange groups and various hydrophobic groups of PA. In addition to the higher selectivity, the biocompatibility is a remarkable property of the extraction method.

Ultrafine nanofibers involve the production of a core-shell nanofiber using a mixture of polymers (two or more) with the further removal of either the core or the shell. However, the procedure is complex to implement. An elegant alternative to obtain such nanofibers has been proposed by Bagheri et al. [39]. The authors formed PA-PEG nanofibers directly on a rotating statinless steel SPME needle. The direct electrospinning of PA is difficult and this could be related to its low molecular weight and low solubility in volatile solvents. In this regard, the presence of PEG facilitates the electrospinning owing to an enhancement of the electrical conductivity of the polymeric solution. Longer, narrower and higher aspect-ratio nanofibers are obtained as result. PEG is further removed by washing the system with double distilled water and the modified PA SPME nanofiber was conditioned at 200 °C in an inert atmosphere for 2 h. Headspace SPME mode was selected as the optimal approach to extract chlorophenols from waters. The interaction between PA and the analytes is associated to the NH and $C=O$ functional groups of the polymeric network. The comparison of the performance of PEG-PA, PA and modified PA nanofibers showed the higher extraction capabilities of the latter, thanks to its more condensed phase which facilitates the mass transfer process during extraction/desorption. The analytical features obtained lead to the conclusion that the modified PA SPME fiber is a suitable candidate for extracting polar organic compounds.

In addition to those examples previously commented on, some other combinations of polymers have been proposed to synthesize nanofibers for micro solid phase extraction.

Core-shell polyvinylpyrrolidone-polybutylene terephthalate/polypyrrole (PVP-PBT/PPy) nanofibers have been synthesized and the PVP core was further removed by immersing the fibers in double distilled water [40]. PBT/PPy hollow nanofibers were obtained as a result. The electrospinning method required the separate preparation of the core (PVP) and shell material (PTB/PPy) solutions which were simultaneously ejected to the aluminum collector by means of a single syringe pump. TFA was selected as solvent on account of its capability to dissolve the polymers and adequate volatility which permits an easy evaporation during the elongation of the fibers. The extraction performance of the hollow nanofibers was evaluated using triazines as model analytes under direct immersion microextraction format. The method requires only 8 mg of nanosorbent to reach limits of detection as low as 50 ng L^{-1} with a precision, expressed as relative standard deviation, better than 8%. The authors demonstrated that the better performance of their material as compared to conventionally synthesized PBT/PPy one is due to its hollow structure, which provides the nanofibers with a higher number of adsorptive sites and porosity.

Inorganic species can be removed from waters using a nanofiber membrane composed of polyvinyl alcohol and natural gum karaya (PVA/GK) [41]. The hydrophobicity of the membrane was achieved by a methane plasma treatment. Different PVA/GK ratios were evaluated in order to select the best conditions to obtain a good spinability with a uniform nanofiber size distribution. 80:20 or 90:10 ratios were considered as optimum. The nanofiber membrane was characterized by different instrumental techniques, including microscopy and infrared spectroscopy. Surface area and wettability were determined before and after the plasma thermal treatment and the authors concluded that the treated membrane exhibits better adsorption capacity due to its increased specific surface area.

3.2. Combination of electrospun nanofibers with nanomaterials

The combination of nanofibers and nanomaterials to form hybrid composites has been extensively used in several scientific fields like the fabrication of lithium ion batteries. For instance, Z. L. Xu et al. [42] developed a Si/Ni/CNF composite with ultrafine Ni particles surrounded by graphene layers what acted as catalyst for the graphitization of the polymer (polyvynil alcohol, PVA). The encapsulated Si nanoparticles served as the conducting medium as well as buffer to remove the stress and prevent the re-aggregation. Eventually, graphene layers provided better electrical conductivity and increased the Li-ion storage sites with the production of micropores.

Despite the potential of these composites in other fields, their use as sorptive phase in microextraction procedures has been scarcely proposed. These applications, which are summarized in Table 3, cover three main microextraction techniques, namely: SPME, TFME and dispersive solid phase extraction (d-SPE).

Table 3. Applications of electrospun nanofibers-nanomaterials composites in the (micro)extraction context.

Composite elements[a]	Extraction technique[b]	Instrumental technique[c]	Analytes	Samples	Reference
PET/SiO_2 NPs	SPME	GC-MS	Benzene, toluene, ethylbenzene and xylene	Water	[44]
PVA/ CNTs@SiO_2	SPME	GC–CD-IMS	Diazinon, fenthion, parathion, chlorpyrifos	River water and agricultural wastewater	[45]
PET/nanoMIP	SPE	LC-MS/MS	Atenolol, metopronolol, timolol, pindololand acebutolol	Tap water	[46]
PS/o-CNTs	TFME	MALDI-ToF-MS	Benzo[a] pyrene and 1-hydroxypyrene	Urine	[47]
PS/graphene	TFME	LC-UV	Aldehydes	Exhaled breath condensates	[48]
PANI/ SiO_2 nanofibers	d-SPE	LC-FD	Fluoroquinolones	honey samples	[50]
PAN/ CH_3MOF5	SPE	LC-UV	Levonorgestrel and megestrol acetate	Urine	[53]

[a] Composite elements acronyms: PET, polyethylene terephthalate; NPs, nanoparticles; PBT, polybutylene terephthalate; PVA, polyvinyl alcohol; CNTs, carbon nanotubes; MIP, molecularly imprinted polymer; PS, polystyrene; o-CNTs, oxidized carbon nanotubes; PANI, polyaniline; PAN, polyacrylonitrile; CH_3MOF5, metal–organic framework-5.
[b] Extraction techniques acronyms: SPME, solid phase microextraction, SPE, solid phase extraction; TFME, thin film microextraction; d-SPE, dispersive solid phase extraction.
[c] Separation and instrumental techniques acronyms: GC, gas chromatography; LC, liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; CD-IMS, corona discharge ion mobility spectrometry; ToF, time of flight; UV, photometric detection; FD, fluorescence detector.

As it has been previously described, electrospinning can be used for the fabrication of SPME fibers using a metallic wire, with an enhanced mechanical stability compared to silica fibers, as support. If nanomaterials are dispersed in the precursor solution of the polymer, they can be easily incorporated to the final fibers enhancing the extracting performance of the material. In this sense, Bagheri and Roostaie proposed the combination of silica NPs of different nature with polyamides as sorptive composite for the extraction of chlorobenzenes from aqueous samples [43]. The composite containing silica nanoparticles modified by cis-9-octadecenoic acid (OA) provided higher extraction recoveries than those obtained with pristine silica, methyl-silica or Si-silica. This can be ascribed to the better dispersion of OA-silica nanoparticles in the polymer or to its chemical structure that enhances the hydrophobic and π-π interactions with the analytes. Later on, the same group evaluated the role of some inorganic oxide NPs (Fe_3O_2, SiO_2, CoO and NiO) on the extraction efficiency of polyethylene terephthalate (PET)-based nanocomposites for the SPME of some volatile organic compounds (BTEX) from water [44]. The NPs induced an increasing in the superficial area and porosity of the composites, which resulted to have a positive effect on the extraction efficiency of the resulting composites. SiO_2 showed the best results because of its better dispersion, giving rise a porous and homogeneous coating film around the polymer network. The extraction capabilities of the composite were improved with the amount of silica NPs, although the electrospinning process became more difficult when high amount of NPs were employed. In addition, the combination of nickel oxide and polybutylene terephthalate (PBT) solution has been used for the extraction of several volatile organic compounds (VOCs) from aqueous samples [5]. The nanocomposite presented higher surface area and reinforcement properties in comparison with raw PBT coating, since NPs generate pores in the structure. Finally, Saraji and co-workers developed a new SPME coating based on a porous carbon nanotubes-silicon dioxide (CNTs-SiO_2) nanohybrid for the determination of some pesticides in vegetables, fruits and water samples [45]. To immobilize the nanohybrid to the stainless-steel wire, electrospinning of PVA was carried out, and glucose were employed to connect CNTs and SiO_2. The new composite presented better extraction efficiency than the commercial SPME fibers.

These nanocomposites can be also electrospun in the form of membranes which are especially interesting for TFME. In 2007, Yoshimatsu et al. encapsulated molecularly imprinted NPs into a PET polymer by electrospinning for the determination of drug residues in water [46]. After encapsulation, the binding sites in molecularly imprinted NPs remain easily accessible and provide good extraction recoveries for the selected analytes. Molecularly imprinted microspheres were also tested, but due to their bigger size, they form aggregates and produce larger beads in the fiber. Later, polystyrene/oxidized carbon nanotubes (PS/oCNTs) film was also synthesized by electrospinning [47]. In this case, the obtained film was additionally used as matrix for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for the determination of benzo[a]pyrene (BaP) and 1-hydroxypyrene (1-OHP) from water and urine samples. With the traditional organic matrices of MALDI, the analysis of low-molecular-weight compounds is limited since they produce high background interference in this region. Previously in this group, oCNTs was used as sorbent and matrix for the determination of BaP, taking advantages of their high surface area, the effective desorption/ionization ability and their hydrophobicity. Nevertheless, the obtained matrix was heterogeneous because oCNTs tends to form aggregates. To avoid this, oCNTs were incorporated to the electrospun nanofibers to produce stable PS/oCNTs film where oCNTs were attached firmly and homogeneously on the MALDI plate. The results show that oCNTs and PS/oCNTs films have similar desorption/ionization capabilities towards BaP but the second one is more stable and homogeneous. Another SPME application was developed by Huang et al. [48] in which six aldehydes in human breath condensates were determined with a polystyrene/graphene (PS/G) composite nanofiber film. Three different graphene concentrations were tested and compared with the PS nanofiber. The results showed that the extraction efficiencies were higher when graphene was in the structure. With the increase of graphene amount, the efficiency was higher; however, when the concentration is too high the stacking of the nanofibers was produced and the surface area as well as the extraction efficiency was reduced.

Finally, some authors have described several applications of NPs-polymer composites by electrospinning for dispersive solid phase extraction (d-SPE). As mentioned before, the high surface area of the electrospun nanofibers is increased when NPs are homogeneously dispersed in its surface, for this reason, this kind of composite is quite interesting in microextraction. For instance, for the adsorption of some heavy metal ions from aqueous solutions, an electrospun nanofiber was made combining modified SiO_2 NPs with polyacrylonitrile (PAN) [49]. Once the amount of silica of the composite was optimized, amine groups were introduced into the silica NPs by a treatment with 3-aminopropyltriethoxysilane (APTES), enhancing the interaction with the analytes. Surface area of the composite was higher with the amount of silica NPs up to 20%, after that, the extraction efficiency became worse because of the agglomeration of the NPs that decrease the surface area. He et al. used silica nanofibers as template for the polymerization of aniline to obtain PANI/SiO_2 nanofibers avoiding aggregation of PANI nanoparticles [50]. The sorbent was employed in an in-syringe d-SPE for the determination of fluoroquinolones (FQs) from honey samples. When PANI was synthesized without silica nanofibers, they form aggregates and the extraction of FQs was much worse, probably due to the ineffective extraction sites.

Metal organic frameworks (MOFs), defined by the IUPAC as coordination polymers with an open framework containing potential voids, present a high porosity which confers them with outstanding superficial areas [51]. The joint electrospinning of MOFs with conventional polymers provides composites with enhanced properties. In this case, MOFs can provide the composite with a higher porosity and therefore a larger superficial area while MOFs can act as well as sorptive material enhancing the extraction capacity of the composite. Osterman et al. have evaluated this possibility using zeolitic imidazolate framework (ZIF) and polyvinylpyrrolidone as MOF and polymer, respectively [52]. The dual electrospinning resulted to boost the superficial area of the polymer from 10 m^2/g to 180–530 m^2/g depending on the ZIF concentration. Undoubtedly, this aspect has a clear impact on microextraction techniques where the extraction kinetics and capacity dramatically depends on the superficial area of the sorbent. In fact, methyl derived MOF-5 (a porous carbon) has been already proposed in combination with PAN as sorbent for the SPE of two estrogenic drugs from urine samples [53]. The introduction of MOF-5 in the electrospun fiber increased 8–10 times the extraction recovery of the target compounds probably by a double mechanism: the increasing of the superficial area and the interaction of MOF-5 with the target analytes.

Although the improvement of the fiber porosity by nanomaterials has been the common strategy, other dopants can also be used. As a way of example, Bagheri et al. demonstrated the positive effect of magnetic ionic liquids during the electrospinning of polyamides on the extraction efficiency of the nanofibers [54].

4. Concluding remarks

Electrospun nanofibers have demonstrated a great potential as sorptive phases in different microextraction techniques including solid phase extraction, solid phase microextraction and thin film microextraction. This potential is based on several and well defined facts:

i. They can be synthesized using relatively cheap and simple manifolds.

ii. They can be obtained from different polymers covering a wide variety of functionalities and therefore they can be adapted to a defined analytical problem.

iii. Electrsopun nanofibers can be obtained in different formats including membranes or coatings.

The versatility of these nanofibers has been even increased in the last years with the development of composites. These composites can be obtained by the combination of two different polymers or combining polymers with nanoparticles. The latter composite will be the focus of an extensive research in the next years.

Acknowledgments

Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2014-52939R) is gratefully acknowledged. E. M. Reyes Gallardo expresses her gratitude for the predoctoral grant (refs FPU12/02670) from the Spanish Ministry of Education.

References

[1] R. Lucena, Making biosamples compatible with instrumental analysis, J. Appl. Bioanal. 1 (2015) 72–75.

[2] C.W. Huck, G.K. Bonn, Recent developments in polymer-based sorbents for solid-phase extraction, J. Chromatogr. A 885 (2000) 51–72.

[3] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Compos. Sci. Technol. 63 (2003) 2223–2253.

[4] X. Wang, B.S. Hsiao, Electrospun nanofiber membranes, Curr. Opin. Chem. Eng. 12 (2016) 20–28.

[5] H. Bagheri, A. Roostaie, Polybutylene terephthalate-nickel oxide nanocomposite as a fiber coating, Anal. Chim. Acta 863 (2015) 20–28.

[6] Y. Liu, X. Zhang, Y. Xia, H. Yang, Magnetic field-assisted electrospinning of aligned straight and wavy polymeric nanofibers, Adv. Mater. 22 (2010) 2454–2457.

[7] J. Lyons, C. Li, F. Ko, Melt-electrospinning part I: processing parameters and geometric properties, Polymer (Guildf.) 45 (2004) 7597–7603.

[8] L. Ji, Z. Lin, Y. Li, S. Li, Y. Liang, O. Toprakci, et al., Formation and characterization of core-sheath nanofibers through electrospinning and surface-initiated polymerization, Polymer (Guildf.) 51 (2010) 4368–4374.

[9] J. Pu, F. Yuan, S. Li, K. Komvopoulos, Electrospun bilayer fibrous scaffolds for enhanced cell infiltration and vascularization in vivo, Acta Biomater. 13 (2015) 131–141.

[10] T. Ballesteros-Esteban, E.M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel, Determination of carvedilol and propranolol in urine samples using a magnetic polyamide composite and LC-MS/MS, Bioanalysis (2016) (submitted).

[11] Q. Xu, X. Yin, S. Wu, M. Wang, Z. Wen, Z. Gu, Determination of phthalate esters in water samples using nylon6 nanofibers mat-based solid-phase extraction coupled to liquid chromatography, Microchim. Acta 168 (2010) 267–275.

[12] Q. Xu, S.-Y. Wu, M. Wang, X.-Y. Yin, Z.-Y. Wen, W.-N. Ge, et al., Electrospun nylon6 nanofibrous membrane as SPE adsorbent for the enrichment and determination of three estrogens in environmental water samples, Chromatographia 71 (2010) 487–492.

[13] Q. Xu, N. Zhang, X. Yin, M. Wang, Y. Shen, S. Xu, et al., Development and validation of a nylon6 nanofibers mat-based SPE coupled with HPLC method for the determination of docetaxel in rabbit plasma and its application to the relative bioavailability study, J. Chromatogr. B 878 (2010) 2403–2408.

[14] H. Bagheri, S. Asgari, H. Piri-Moghadam, On-line micro solid-phase extraction of clodinafop propargyl from water, soil and wheat samples using electrospun polyamide nanofibers, Chromatographia 77 (2014) 723–728.

[15] H. Bagheri, A. Aghakhani, M. Baghernejad, A. Akbarinejad, Novel polyamidebased nanofibers prepared by electrospinning technique for headspace solid-phase microextraction of phenol and chlorophenols from environmental samples, Anal. Chim. Acta 716 (2012) 34–39.

[16] L.-Q. Chen, X.-J. Kang, J. Sun, J.-J. Deng, Z.-Z. Gu, Z.-H. Lu, Application of nanofiber-packed SPE for determination of salivary-free cortisol using fluorescence precolumn derivatization and HPLC detection, J. Sep. Sci. 33 (2010) 2369–2375.

[17] W.Y. Hu, X.J. Kang, C. Zhang, J. Yang, R. Ling, E.H. Liu, et al., Packed-fiber solid-phase extraction coupled with high performance liquid chromatography tandem mass spectrometry for determination of diethylstilbestrol, hexestrol, and dienestrol residues in milk products, J. Chromatogr. B 957 (2014) 7–13.

[18] X.-J. Kang, L.-Q. Chen, Y.-Y. Zhang, Y.-W. Liu, Z.-Z. Gu, Performance of electrospun nanofibers for SPE of drugs from aqueous solutions, J. Sep. Sci. 31 (2008) 3272–3278.

[19] D. Qi, X. Kang, L. Chen, Y. Zhang, H. Wei, Z. Gu, Electrospun polymer nanofibers as a solid-phase extraction sorbent for the determination of trace pollutants in environmental water, Anal. Bioanal. Chem. 390 (2008) 929–938.

[20] Z. Liu, X. Kang, F. Fang, Solid phase extraction with electrospun nanofibers for determination of retinol and α-tocopherol in plasma, Microchim. Acta 168 (2010) 59–64.

[21] Y. Zhang, X. Kang, L. Chen, C. Pan, Y. Yao, Z.Z. Gu, Fiber-packed SPE tips based on electrospun fibers, Anal. Bioanal. Chem. 391 (2008) 2189–2197.

[22] J. Deng, X. Kang, L. Chen, Y. Wang, Z. Gu, Z. Lu, A nanofiber functionalized with dithizone by co-electrospinning for lead (II) adsorption from aqueous media, J. Hazard. Mater. 196 (2011) 187–193.

[23] D. Adeyemi, J. Mokgadi, J. Darkwa, C. Anyakora, G. Ukpo, C. Turner, et al., Electrospun nanofibers sorbents for pre-concentration of 1,1-dichloro-2,2 bis-(4-chlorophenyl)ethylene with subsequent desorption by pressurized hot water extraction, Chromatographia 73 (2011) 1015–1020.

[24] X. Kang, C. Pan, Q. Xu, Y. Yao, Y. Wang, D. Qi, et al., The investigation of electrospun polymer nanofibers as a solid-phase extraction sorbent for the determination of trazodone in human plasma, Anal. Chim. Acta 587 (2007) 75–81.

[25] R. Chen, Y. Yang, N. Wang, L. Hao, L. Li, X. Guo, et al., Application of packed porous nanofibers-solid-phase extraction for the detection of sulphonamide

residues from environmental water samples by ultra high performance liquid chromatography with mass spectrometry, J. Sep. Sci. 38 (2015) 749–756.

[26] S. Zali, F. Jalali, A. Es-Haghi, M. Shamsipur, Electrospun nanostructured polystyrene as a new coating material for solid-phase microextraction: application to separation of multipesticides from honey samples, J. Chromatogr. B 1002 (2015) 387–393.

[27] P.N. Nomngongo, J.C. Ngila, S.M. Musyoka, T.A.M. Msagati, B. Moodley, A solid phase extraction procedure based on electrospun cellulose-g-oxolane-2,5-dione nanofibers for trace determination of Cd, Cu, Fe, Pb and Zn in gasoline samples by ICP-OES, Anal. Methods 5 (2013) 3000–3008.

[28] H. Sereshti, F. Amini, H. Najarzadekan, Electrospun polyethylene terephthalate (PET) nanofibers as a new adsorbent for micro-solid phase extraction of chromium(VI) in environmental water samples, RSC Adv. 5 (2015) 89195–89203.

[29] S. Li, D. Wu, X. Yan, Y. Guan, Acetone-activated polyimide electrospun nanofiber membrane for thin-film microextraction and thermal desorption-gas chromatography-mass spectrometric analysis of phenols in environmental water, J. Chromatogr. A 1411 (2015) 1–8.

[30] H. Bagheri, A. Akbarinejad, A. Aghakhani, A highly thermal-resistant electrospun-based polyetherimide nanofibers coating for solid-phase microextraction, Anal. Bioanal. Chem. 406 (2014) 2141–2149.

[31] H. Bagheri, A. Aghakhani, Novel nanofiber coatings prepared by electrospinning technique for headspace solid-phase microextraction of chlorobenzenes from environmental samples, Anal. Methods 3 (2011) 1284–1289.

[32] S. Islam, J.H. Yeum, A.K. Das, Effect of pullulan/poly(vinyl alcohol) blend system on the montmorillonite structure with property characterization of electrospun pullulan/poly(vinyl alcohol)/montmorillonite nanofibers, J. Colloid Interface Sci. 368 (2012) 273–281.

[33] H. Bagheri, A. Aghakhani, M. Akbari, Z. Ayazi, Electrospun composite of polypyrrole-polyamide as a micro-solid phase extraction sorbent, Anal. Bioanal. Chem. 400 (2011) 3607–3613.

[34] H. Bagheri, Z. Ayazi, A. Aghakhani, N. Alipour, Polypyrrole/polyamide electrospun-based sorbent for microextraction in packed syringe of organophosphorous pesticides from aquatic samples, J. Sep. Sci. 35 (2012) 114–120.

[35] F. Qi, X. Li, B. Yang, F. Rong, Q. Xu, Disks solid phase extraction based polypyrrole functionalized core–shell nanofibers mat, Talanta 144 (2015) 129–135.

[36] H. Bagheri, A. Aghakhani, Polyaniline-nylon-6 electrospun nanofibers for headspace adsorptive microextraction, Anal. Chim. Acta 713 (2012) 63–69.

[37] S. Mirmahdieh, T. Khayamian, Electrospun nanofibers of poly(methylmethacrylate)/polystyrene blend as a microcolumn extraction sorbent followed by corona discharge ion mobility spectrometry for analysis of tramadol in biological fluids, Chromatographia 76 (2013) 541–548.

[38] Q. Wu, D. Wu, Y. Guan, Polyaniline sheathed electrospun nanofiber bar for in vivo extraction of trace acidic phytohormones in plant tissue, J. Chromatogr. A 1342 (2014) 16–23.

[39] H. Bagheri, H. Najarzadekan, A. Roostaie, Electrospun polyamide-polyethylene glycol nanofibers for headspace solid-phase microextration, J. Sep. Sci. 37 (2014) 1880–1886.

[40] H. Bahgeri, O. Rezvani, S. Banihashemi, Core-shell electrospun polybutylene terephthalate/polypyrrole hollow nanofibers for micro-solid phase extraction, J. Chromatogr. A 1434 (2016) 19–28.

[41] V. Vellora, T. Padil, M. Cernik, Poly (vinyl alcohol)/ gum karaya electrospun plasma treated membrane for the removal of nanoparticles (Au, Ag, Pt, CuO and Fe_3O_4) from aqueous solution, J. Hazard. Mater. 287 (2015) 102–110.

[42] Z.L. Xu, B. Zhang, Z.Q. Zhou, S. Abouali, M.A. Garakani, J. Huang, et al., Carbon nanofibers containing Si nanoparticles and graphene-covered Ni for high performance anodes in Li ion batteries, RSC Adv. 4 (2014) 22359–22366.

[43] H. Bagheri, A. Roostaie, Electrospun modified silica-polyamide nanocomposite as a novel fiber coating, J. Chromatogr. A 1324 (2014) 11–20.

[44] H. Bagheri, A. Roostaie, Roles of inorganic oxide nanoparticles on extraction efficiency of electrospun polyethylene terephthalate nanocomposite as an unbreakable fiber coating, J. Chromatogr. A 1375 (2015) 8–16.

[45] M. Saraji, M.T. Jafari, M. Mossaddegh, Carbon nanotubes@silicon dioxide nanohybrids coating for solid-phase microextraction of organophosphorus pesticides followed by gas chromatography-corona discharge ion mobility spectrometric detection, J. Chromatogr. A 1429 (2016) 30–39.

[46] K. Yoshimatsu, L. Ye, J. Lindberg, I.S. Chronakis, Selective molecular adsorption using electrospun nanofiber affinity membranes, Biosens. Bioelectron. 23 (2008) 1208–1215.

[47] X.M. He, G.T. Zhu, J. Yin, Q. Zhao, B.F. Yuan, Y.Q. Feng, Electrospun polystyrene/oxidized carbon nanotubes film as both sorbent for thin film microextraction and matrix for matrix assisted laser desorption/ionization timeof-flight mass spectrometry, J. Chromatogr. A 1351 (2014) 29–36.

[48] J. Huang, H. Deng, D. Song, H. Xu, Electrospun polystyrene/graphene nanofiber film as a novel adsorbent of thin film microextraction for extraction of aldehydes in human exhaled breath condensates, Anal. Chim. Acta 878 (2015) 102–108.

[49] A. Dastbaz, A.R. Keshtkar, Adsorption of Th^{4+}, U^{6+}, Cd^{2+} and Ni^{2+} from aqueous solution by a novel modified polyacrylonitrile composite nanofiber adsorbent prepared by electrospinning, Appl. Surf. Sci. 293 (2014) 336–344.

[50] X.M. He, G.T. Zhu, H.B. Zheng, S.N. Xu, B.F. Yuan, Y.Q. Feng, Facile synthesis of polyaniline-coated SiO2 nanofiber and its application in enrichment of fluoroquinolones from honey samples, Talanta 140 (2015) 29–35.

[51] J.L.C. Rowsell, O.M. Yaghi, Metal–organic frameworks: a new class of porous materials, Micropor. Mesopor. Mater. 73 (2004) 3–14.

[52] R. Ostermann, J. Cravillon, C. Weidmann, M. Wiebcke, B.M. Smarsly, Metalorganic framework nanofibers via electrospinning, Chem. Commun. 47 (2011) 442–444.

[53] M. Asiabi, A. Mehdinia, A. Jabbari, Preparation of water stable methyl-modified metal-organic framework-5/polyacrylonitrile composite nanofibers via electrospinning and their application for solid-phase extraction of two estrogenic drugs in urine samples, J. Chromatogr. A 1426 (2015) 24–32.

[54] H. Bagheri, H. Piri-Moghadam, S. Rastegar, Magnetic and electric field assisted electrospun polyamide nanofibers for on-line µ-solid phase extraction and HPLC, RSC Adv. 4 (2014) 52590–52597.

Capítulo IV. 2

Polyamide-carbon nanohorns electrospun composite membrane: synthesis and application for solid phase extraction

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In progress

Polyamide-carbon nanohorns electrospun composite membrane: synthesis and application for solid phase extraction

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In this article, the synthesis of a new electrospun nanocomposite membrane, comprising polyamide 6 and carbon nanohorns, is presented. A dedicated lab-made device has been designed to achieve the confinement of the membrane during the extraction and analysis steps. For extraction, the sample flows through the membrane enhancing the interaction of the analytes with the sorptive phase. After that, the device is closed and directly analyzed by headspace-gas chromatography-mass spectrometry. The performance of the membrane is evaluated using several contaminants in water (toluene, ethylbenzene, p-xylene, styrene, chloroform, dichloromethane and undecane) as model analytical problem. The effect of the nanohorns concentration on the extraction performance has been also evaluated showing an increase on the sorption capacity with the nanohorns concentration. This is probably due to the synergic effect of a higher membrane porosity and the sorption properties of these nanostructures.

1. Introduction

Nanocomposites are blended materials that combine the properties of their constituents, being at least one of them in the nanometric range. Hybrid nanoparticles, resulting from the combination of nanomaterials of different chemical nature can be highlighted as nanocomposites with a high analytical potential [1]. In addition, the combination of micrometric systems and nanoparticles (NPs) is gaining importance not only in Analytical Chemistry but also in material sciences. Polymer-NPs composites have been practically used in sample preparation [2, 3] and also in instrumental analysis [4, 5]. In the sample preparation context, these composites are especially interesting and they combine the sorption capacity of polymers with the advantages of NPs. These NPs may play different roles. On the one hand, they can provide the composite with special properties (e.g. magnetic behavior) [6, 7]. On the other hand, they can modify the normal stacking of the polymeric chains generating imperfections on the packing which results in a porosity and surface enhancement [8, 9]. The same occurs if the NPs surface is used as support for polymer growing [10, 11]. Finally, the NPs can also introduce a primary or secondary (if the polymer is active in the analyte sorption) interaction chemistry towards the target analytes [12].

Polymer nanocomposites can be synthesized by several methods that can be summarized in two main workflows. They can be obtained by chemical anchoring of the polymer over the NP surface [13]. This approach takes advantage of the high superficial area of the NP although it usually needs a complex synthetic pathway. In addition, they can be produced embedding or incorporating the NPs into the polymeric network [14, 15]. The resulting material may present different looks, powder or film, depending on the procedure.

Polymeric films (or membranes) containing NPs are especially attractive in sample extraction as they allow the easy and rapid processing of large sample volume maintaining a high sorption capacity. Depending on the case, the polymer, the NPs or both of them are the responsible for the analyte extraction. Electrospinning is the main technique used for the preparation of these membranes [16]. The inclusion of the NPs can be done loading the nanomaterial over the electrospun fibers or adding the NPs to the electrospinning solution.

In this article, a new electrospun nanocomposite containing polyamide 6 and carbon nanohorns as constituents is synthesized and practically evaluated. The performance of the membrane is tested using several contaminants in water as model analytical problem. In order to process the sample, a dedicated device that allows the confinement of the membrane has been designed. After the sample extraction, the device can be directly transformed for its headspace-gas chromatographic analysis. This design allows to handle the unit as a conventional headspace glass vial.

2. Experimental

2.1. Reagents

All reagents were of analytical grade or better. Toluene, ethylbenzene, p-xylene, styrene, chloroform, dichloromethane and undecane were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solution of each analyte was prepared at a concentration of 1 g L^{-1} in methanol (Carlo Erba Reagents, Italy) and stored at 4 °C. A diluted standard solution containing all the analytes at a concentration of 100 mg L^{-1} was prepared in methanol and also stored at 4 °C. Working solutions were prepared by the appropriate dilution of the stocks in Milli-Q water (Millipore Corp., Madrid, Spain).

Polyamide 6 (PA-6, MW 10,000 g/mol), formic acid and acetic acid were obtained from Sigma–Aldrich. Single-walled carbon nanohorns (SWNHs) were purchased from Carbonium srl (Padua, Italy). SWNHs presented lengths in the range from 40 to 50 nm and diameters between 4 and 5 nm. SWNHS purity was better than 90%.

2.2. Synthesis of the electrospun doped nanofibers

The electrospinning setup consists of a 10 mL syringe (15.9 mm internal diameter, Norm-jet of Henke StassWolf) equipped with a 15.24 cm long needle (1.024 mm internal diameter) that moves along the axis of a rotating drum collector (fixed at 21 cm), while electrospinning sideways. The speed of the drum (RPM) was set at 3% to allow the production of thick nonwoven samples. All the experiments were performed during 2 hours and with a flow rate of 1 mL/h, regulated with an infusion pump (KD Scientific Syringe Pump Series 100). The tip to collector distance (TCD) was fixed at 6 cm. In order to obtain a high potential difference, the needle is connected with a high voltage source (Glassman High Voltage Series EH30), which can deliver an output voltage over the range from 0 to 30 kV. The nanofibrous are collected on aluminium foil, which was placed on the grounded rotating drum collector. All electrospinning experiments were performed at room temperature and humidity, and the voltage was adjusted in order to obtain steady state conditions. A diagram of the setup is shown in Figure 1.

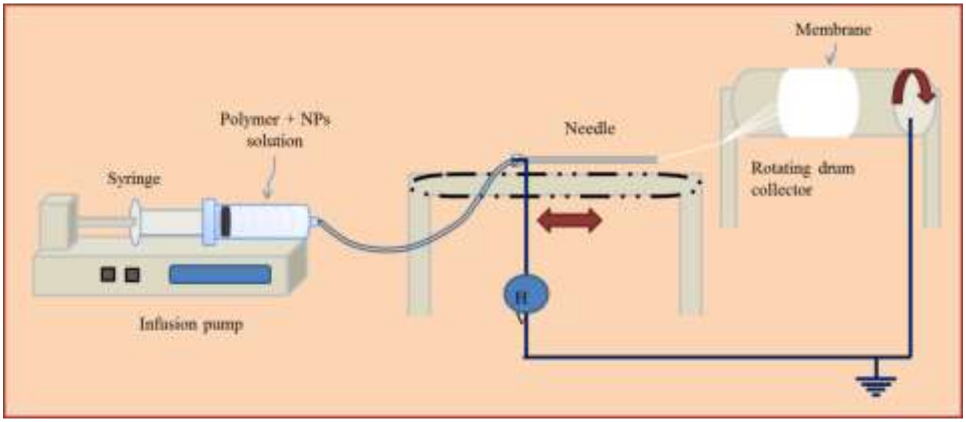

Figure 1. Diagram of the electrospinning setup.

For the electrospinning of naked fibers, a PA-6 solution (16%wt) was prepared in a formic acid/acetic acid mixture (50/50, v/v). This solution was loaded in the plastic syringe and connected to the electrospinning setup. The stability of the electrospinning process was controlled by visually evaluating the Taylor cone and by verifying the absence of droplets and beads on the SEM images. All the reported experiments showed steady state for the full duration of the 2 hours of the process, except for the first start-up minute. The doped nanofibers were obtained by adding a defined amount of nanohorns (0.2, 0.5 or 1 g L^{-1}) to the PA-6 solution prior to the electrospinning process. The homogeneous dispersion of the nanoparticles was obtained by sonicating the solution for one hour.

2.3. Characterization of the fibers

Prior to electrospinning, the viscosity and conductivity of the polymer solutions (with and without nanoparticles) were examined using a Brookfield viscometer LVDV-II and a CDM210 conductivity meter of Radiometer Analytical, respectively. Moreover, room temperature and humidity were checked to avoid marked changes during the electrospinning processes.

A Jeol Quanta 200 F FE scanning electron microscope was used to examine the fiber morphology and diameters at an accelerating voltage of 20 kV. Before this analysis, samples were coated with gold using a Balzers Union SKD 030 sputter coater. The average diameters of the nanofibers were calculated using ImageJ software by taking 50 measurements for one set of parameters.

2.4. Apparatus

Headspace analyses were performed on a MPS2 32-space headspace autosampler (Gerstel, Mülhein and der Ruhr, Germany) which includes a robotic arm and an oven. An automated injector fitted with a gastight HS-syringe (maintained at 150 °C) was used for the introduction of 0.5 mL of the headspace from the vial into the HP6890 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with an HP5973 mass spectrometric detector based on a quadrupole analyzer and an electronmultiplier detector. System control was achieved with an HP1701CA MS ChemStation (Agilent Technologies).

The analytes were separated using a HP5MS fused silica capillary column (30 m x 0.25 mm i.d.) coated with 5% phenylmethyl-polysiloxane (film thickness 0.25 µm) (Agilent, Palo Alto, CA, USA). The chromatographic temperature program began at 40 ºC for 3 min, was raised up to 70 ºC at 5 ºC/min, ramped at 40 ºC/min up to 280 ºC and kept finally at 280 ºC for 2 min.. A column split ratio 1:10 was selected for injection. Helium (6.0 grade purity, Air Liquide, Seville, Spain), at a flow rate of 1 mL/min, regulated by a digital pressured and flow controller, was used as carried gas. Electron impact ionization (70 eV) was used for analyte fragmentation. The MS source and quadrupole temperatures were maintained at 230 and 150 ºC, respectively. The mass spectrometer detector operated in Scan mode, recording the m/z fragment-ions from 50 to 106. The chromatograms were acquired and processed by using G1701BA Standalone Data Analysis Software (Agilent Technologies) on a Pentium 4 computer, which also controlled the whole system. The analytes were identified according to the retention time and the qualifying ions, while the peak area was used for quantification.

2.5. Description of the extraction unit

The extraction procedure is carried out using a lab-made unit which can be directly connected to the headspace autosampler of the gas chromatograph. The unit is built in commercial brass pipe elements (Inyectometal S.A.U., Spain). Brass is selected as material due to its lightness, which makes easier the unit handling, especially by the headspace autosampler arm. The unit operates in two different modes, namely: the extraction and analysis modes.

2.5.1. Unit under the extraction mode

In this mode, the membrane is hold between (i) a male-male fitting and (ii) a female-male fitting, to create (iii) the membrane holder (Figure 2.A). The membrane is orthogonally oriented to the unit entrance allowing the sample to pass through the membrane. In order to aspirate the sample, the holder is connected to a vacuum system and to a glass tube by means of a female-female fitting, as it is shown in Figure 2.B.

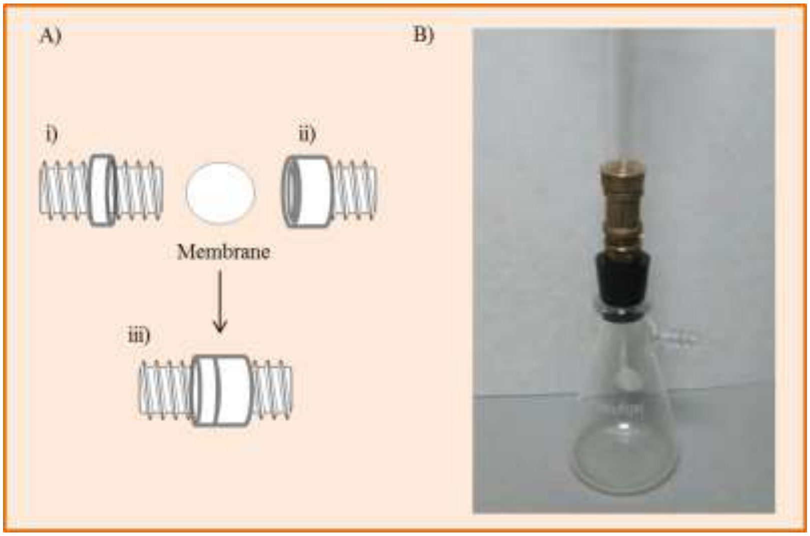

Figure 2. Description of the unit (sampling mode). Diagram of the elements (A) and how they are arranged to create the final sampling unit (B) are shown.

2.5.2. Unit under the analysis mode

In this mode, the unit acts like a conventional headspace vial. It allows the headspace analysis of the membrane as well as its storage between sampling and analysis, avoiding the evaporation of the analytes as well as the contamination of the membrane. This unit (Figure 3.A) requires two additional elements, namely: (i) a pierced female cap with an iron washer and a PTFE septum and (ii) a female cap. The iron washer in necessary for the autosampler to move the vial by the magnetic arm while the septum maintains the unit sealed until the sample injection in the chromatograph. After screwing all the elements, the resulting unit can be placed in the headspace autosampler, as it is shown in Figure 3.B.

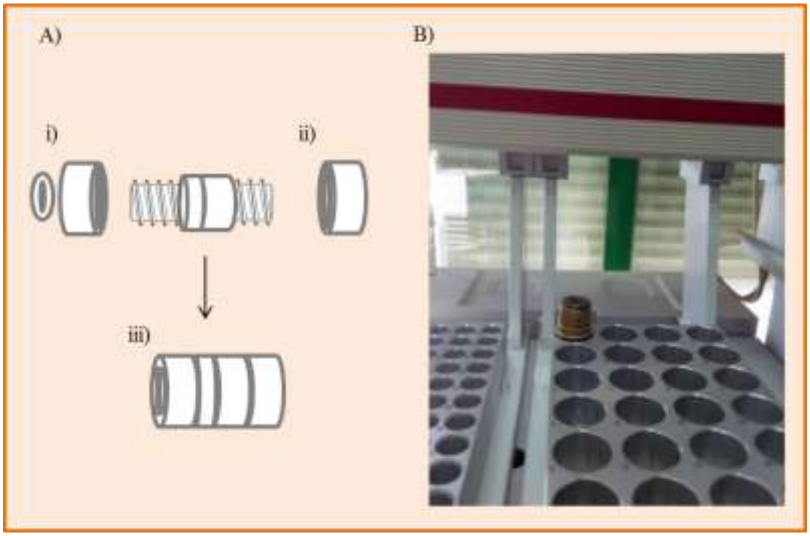

Figure 3. Description of the unit (analysis mode). Diagram of the main elements (A) and how the unit is located into the autosampler (B) are shown

2.6. Analytical procedure

Water samples or standards are processed in the unit under the extraction mode. In these conditions, a defined volume of sample is passed through the unit, filtered through the membrane where the analytes are trapped. After the extraction, the unit is transformed into the analysis mode and finally placed into the headspace autosampler tray of the gas chromatograph. The robotic arm automatically takes each unit from the tray and transferred it into the oven. After being pressurized with He, the unit is heated at 50 °C for 45 min under mechanical stirring at 750 rpm to ensure the release of the analytes to the headspace. Then, 0.5 mL of the headspace is introduced by means of a syringe into the GC–MS, for separation and identification/quantification.

3. Results and discussion

3.1. Characterization of the PA 6-nanohorns membrane

The synthesized membranes were characterized by scanning electron microscopy (SEM). This technique allows to verify the absence of beads or drops in the structure thus validating the steady state during the electrospinning process, as it can be seen in Figure 4. In addition, the average diameters of the nanofibers (calculated using ImageJ software by taking 50 measurements for one set of parameters) were obtained. Although there is a slightly difference between both diameters, 152 ± 35 nm for PA-6 membrane and 132 ± 30 nm for the one with nanohorns, this variance is not stadistically relevant.

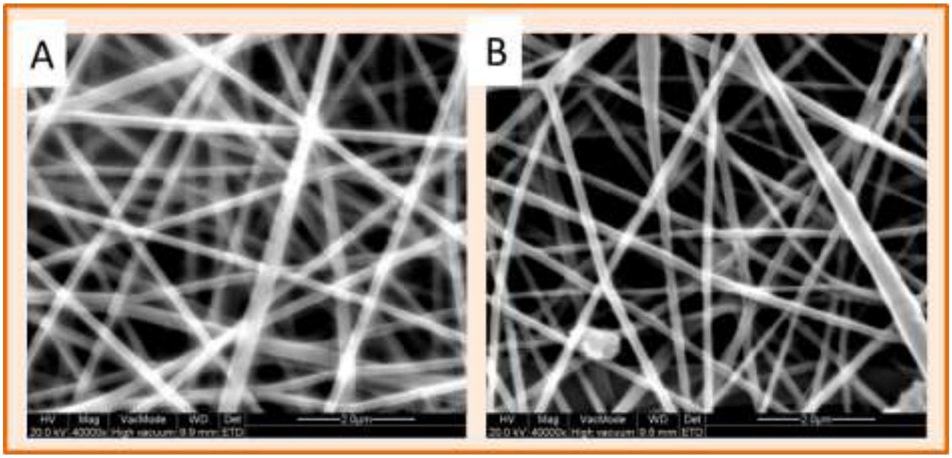

Figure 4. SEM images for (A) PA-6 membrane and (B) PA 6- nanohorns (1 g L^{-1}) membrane at 40000 magnifications.

3.2. Effect of the carbon nanohorns concentration on the sorptive performance of the membrane

The influence of the different SWNHs concentration (0.2, 0.5 and 1 g L^{-1}) was evaluated by submerging a piece of each membrane (1 x 1 cm) into a 5 mL aqueous solution containing the analytes at a defined concentration (from 0.25 to 5 mg L^{-1}). After 45 min of incubation (agitated at 750 rpm at 50 °C), 0.5 mL of the headspace of the vial was injected into the GC-MS. For comparative purposes, the signals obtained in the absence of membrane at each concentration were also evaluated. The results obtained for all the membranes are shown in Figure 5. For simplification, a factor F is defined as:

$$F = \frac{slope\ of\ the\ calibration\ graph\ in\ the\ presence\ of\ each\ membrane}{slope\ of\ the\ calibration\ graph\ in\ the\ absence\ of\ membrane} x100$$

This factor provides information about the analyte-membrane interaction. Some aspects should be considered to get proper conclusions. The sorption temperature (50 °C) is selected taking into consideration the type of analysis (HS). As sorption is an exothermic process, better results would be expected at room temperatures. The direct analysis of the vials require the use of concentrations in the mg L^{-1} range to leave analyte in the HS for further analysis. In this respect, higher sorption percentages would be expected at lower analytes concentrations. Moreover, this experiment is done in different hydrodynamic conditions than those anticipated for the final extraction. In fact, the extraction device is designed to force the flow through of the sample.

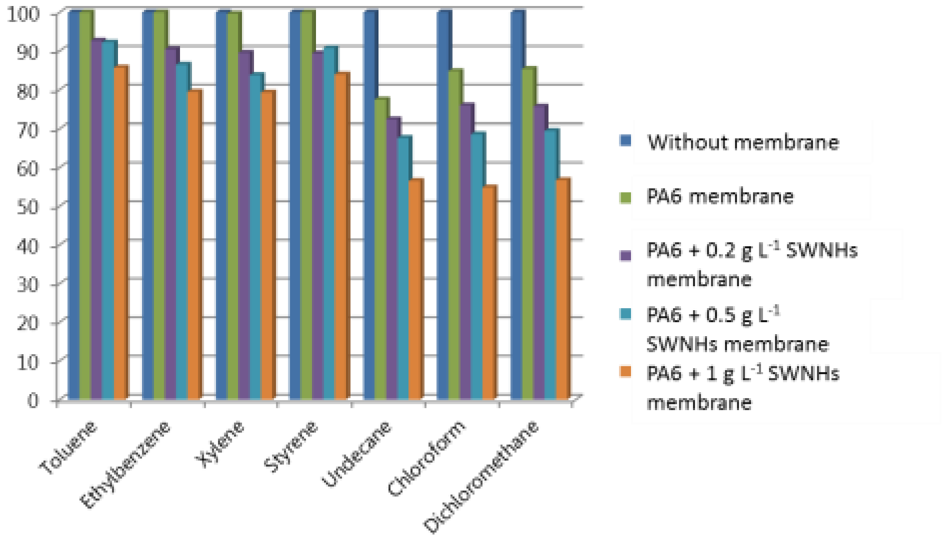

Figure 5. Effect of carbon nanohorns amount in the synthesis procedure.

Keeping in mind all these considerations, F factor informs us about the relative membrane-analyte interaction as a consequence of the inclusion of carbon nanohorns. However, F factor cannot be used to compare the sorption among analytes. This is a consequence of the different vapour pressure of the analytes. It would be expected (and further confirmed) that those analytes prefer the HS to the membrane.

In conclusion, the results show that the inclusion of carbon nanohorns in the polymeric network provides better capacity values compared to the membrane without nanoparticles. With the increase of the nanohorns amount, the extraction capabilities are higher.

3.3. Thermal desorption parameters

The effect of several instrumental variables that affect the thermal desorption of the analytes have been deeply studied. For this purpose, the unit in the analysis mode was employed, using the PA-6 membrane with 1 g L^{-1} of carbon nanohorns. In this way, 25 µL of a standard solution containing the analytes at 100 mg L^{-1} were added directly to the membrane, allowing the close interaction between the analytes and the sorptive media. After that, the unit is closed and transferred to the HS-autosampler for the analysis. Firstly, desorption temperature was studied in the range from 50 to 120 °C, using a desorption time of 45 min. The best results were obtained for 50 °C, since the analytes have a very high volatility. The second variable was desorption time, studied between 5 and 60 min. In this case, the analytical signal increased up to 45 min, decreasing over this value. Therefore, 45 min was selected for further analysis.

4. Conclusions

In this article, different electrospun nanocomposites membranes comprising polyamide 6 and carbon nanohorns have been synthesized, and their extraction capabilities towards selected model analytes have been evaluated. The positive effect of the inclusion of nanoparticles into the structure is demonstrated. Moreover, this new membrane is easily synthesized, since nanoparticles are dispersed into the PA 6 solution before electrospinning.

Although quantitative results with real samples are not yet obtained, the suggested methodology is simple and allows the direct connection with the HS-GC autosampler. The lab-made device proposed to confine the membrane is easily converted into a compatible headspace glass vial to its HS-GC-MS analysis, which simplifies the overall procedure. By way of example, Figure 6 shows the chromatogram obtained from a real sample spiked with the analytes at 250 µg L^{-1} after the treatment with the proposed membrane.

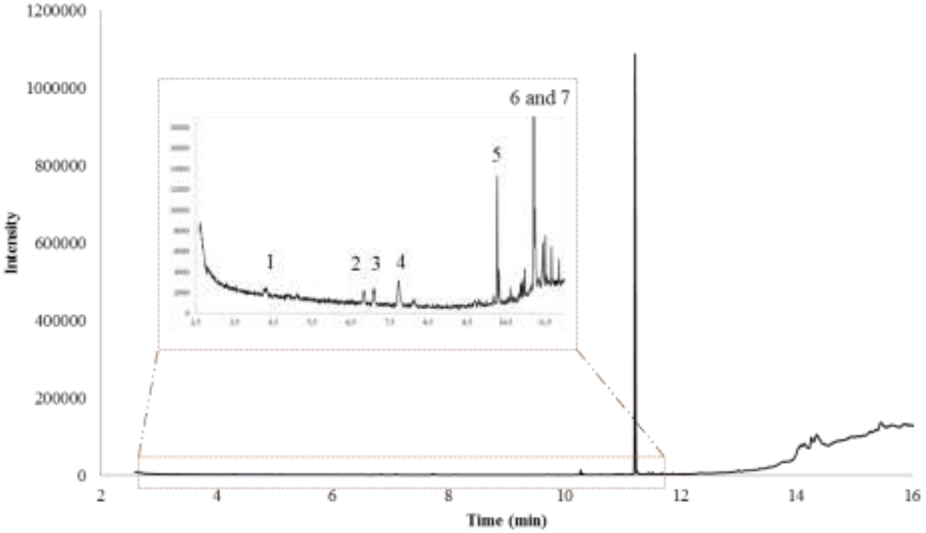

Figure 6. Chromatogram of a real sample spiked with the analytes at 250 µg L^{-1} after the treatment with the membrane. Peaks from 1 to 7 are toluene, ethylbenzene, p-xylene, styrene, undecane, chloroform and dichloromethane, respectively.

Acknowledgments

Financial support from the Spanish Ministry of Economy, Industry and Competitiveness (grant CTQ2014-52939R) is gratefully acknowledged. E. M. Reyes Gallardo expresses her gratitude for the predoctoral grant (refs FPU12/02670) from the Spanish Ministry of Education and the funding of her stay in Ghent (Belgium) from the University of Córdoba to conduct the research reported in this paper.

References

[1] A. I. López-Lorente, B. M. Simonet, M. Valcárcel, Analytical potential of hybrid nanoparticles, Anal Bioanal Chem 399 (2011) 43–54.

[2] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel, Polymer–nanoparticles composites in bioanalytical sample preparation, Bioanalysis 7 (2015) 1723–1730.

[3] Z. Ayazi, Application of nanocomposite based sorbents in microextraction techniques: A review, Analyst (2017) Accepted Manuscript.

[4] Z. Liu, Z. Yan, L. Jia, P. Song, L. Mei, L. Bai, Y. Liu, Gold nanoparticle decorated electrospun nanofibers: A 3D reproducible and sensitive SERS substrate, Appl Surf Sci 403 (2017) 29–34.

[5] M. Balamurugan, J. Yang, Three-dimensional surface-enhanced raman scattering substrate fabricated by chemical decoration of silver nanoparticles on electrospun polycarbonate nanofibers, Appl Spectrosc 0 (2016) 1-9.

[6] H. Bagheri, R. Daliri, A. Roostaie, A novel magnetic poly(aniline-naphthylamine)-based nanocomposite for micro solid phase extraction of rhodamine B, Anal Chim Acta 794 (2013) 38-46.

[7] M. Khan, E. Yilmaz, B. Sevinc, E. Sahmetlioglu, J. Shah, M. R. Jan, M. Soylak, Preparation and characterization of magnetic allylamine modified graphene oxide-poly(vinyl acetate-co-divinylbenzene) nanocomposite for vortex assisted magnetic solid phase extraction of some metal ions, Talanta 146 (2016) 130-137.

[8] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, Silica nanoparticles–nylon 6 composites: synthesis, characterization and potential use as sorbent, RSC Adv 7 (2017) 2308-2314.

[9] H. Bagheri, A. Roostaie, Roles of inorganic oxide nanoparticles on extraction efficiency of electrospun polyethylene terephthalate nanocomposite as an unbreakable fiber coating, J Chromatogr A 1375 (2015) 8–16.

[10] Q. Wen, Y. Wang, K. Xu, N. Li, H. Zhang, Q. Yang, A novel polymeric ionic liquid-coated magnetic multiwalled carbon nanotubes for the solid-phase extraction of Cu, Zn-superoxide dismutase, Anal Chim Acta 939 (2016) 54-63.

[11] S. Carrasco, E. Benito-Peña, F. Navarro-Villoslada, J. Langer, M. N. Sanz-Ortiz, J. Reguera, L. M. Liz-Marzán, M. C. Moreno-Bondi, Multibranched gold–mesoporous silica nanoparticles coated with a molecularly imprinted polymer for label-free antibiotic surface-enhanced raman scattering analysis, Chem Mater 28 (2016) 7947–7954.

[12] K. T. Peter, J. D. Vargo, T. P. Rupasinghe, A. De Jesus, A. V. Tivanski, E. A. Sander, N. V. Myung, D. M. Cwiertny, Synthesis, optimization, and performance demonstration of electrospun carbon nanofiber–carbon nanotube composite sorbents for point-of-use water treatment, ACS Appl Mater Interfaces 8 (2016) 11431–11440.

[13] M. C. Alcudia-León, R. Lucena, S. Cárdenas, M. Valcárcel, Selective extraction of Bactrocera oleae sexual pheromone from olive oil by dispersive magnetic microsolid phase extraction using a molecularly imprinted nanocomposite, J Chromatogr A 1455 (2016) 57-64.

[14] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel, Magnetic nanoparticles-nylon 6 composite for the dispersive microsolid phase extraction of selected polycyclic aromatic hydrocarbons from water samples, J Chromatogr A, 1345 (2014) 43–49.

[15] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel, Dispersive micro-solid phase extraction of bisphenol A from milk using magnetic nylon 6 composite and its final determination by HPLC-UV, Microchem J 124 (2016) 751–756.

[16] E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, Electrospun nanofibers as sorptive phases in microextraction, Trends Anal Chem 84 (2016) 3-11.

BLOQUE V

CONCLUSIONES

CONCLUSIONS

En este último apartado se resumen los principales resultados obtenidos, haciendo una justificación de la evolución seguida en el desarrollo experimental de la Tesis Doctoral.

La investigación recogida en esta Tesis Doctoral ha estado centrada en la etapa de tratamiento de la muestra. Concretamente, las aportaciones realizadas se han dirigido al diseño y empleo de nuevos materiales sorbentes en microextracción en fase sólida.

Todos los materiales sintetizados pueden considerarse híbridos o composites, ya que se obtienen por combinación de dos o más tipos de componentes. En concreto se han combinado polímeros (Oasis MCX y poliamidas) con nanopartículas de diferente naturaleza (nanopartículas magnéticas, nanopartículas de sílice y nanocuernos de carbono).

A continuación, se describen cronológicamente cada una de las aportaciones realizadas. Estos trabajos pueden clasificarse en dos grandes grupos según empleen microextracción en fase sólida dispersiva o microextracción en fase sólida con membrana.

1. Microextracción en fase sólida dispersiva

La microextracción en fase sólida dispersiva se basa en la dispersión eficiente del material sorbente en el seno de la muestra. Este proceso permite aprovechar al máximo la superficie extractante del sorbente, mejorando los rendimientos y las cinéticas de extracción.

Aunque las técnicas dispersivas se asemejan en las etapas iniciales, difieren en la etapa de recuperación del sorbente tras la extracción. Si el sorbente tiene propiedades magnéticas, el esquema general de extracción puede ser como el que se indica en la Figura 1. En primer lugar, el sorbente se añade a la muestra y se dispersa usando algún tipo de agitación como vórtex o ultrasonidos para incrementar el contacto entre ambos. Tras la extracción, el sólido se recupera con la ayuda de un imán, y la matriz de la muestra se desecha. Antes de la elución final con un disolvente adecuado, el sorbente se lava para eliminar interferentes co-extraídos.

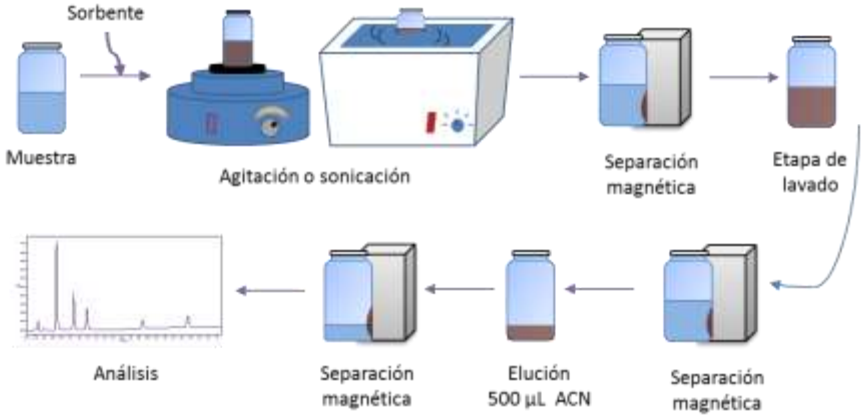

Figura 1. Esquema general del procedimiento de microextracción en fase sólida dispersiva usando sólidos magnéticos.

En esta Tesis Doctoral se han sintetizado dos tipos de sorbentes magnéticos. En el **Capítulo III.2** de la Memoria se describe la síntesis de un composite formado por un polímero sulfonado comercial (Oasis MCX) modificado con nanopartículas de ferrita de cobalto. Como ya se comentó en el Bloque I, esta combinación genera un sólido con una elevada capacidad extractiva debida al polímero y con características magnéticas gracias a las nanopartículas. De este modo, el sorbente puede recuperarse fácilmente, sin necesidad de centrifugar o filtrar.

Para seleccionar el mejor polímero, se realizaron curvas de sorción con diferentes materiales sin modificar: Lichrolut-EN, Oasis HLB, nanotubos de carbono, RP-C18 y PVC. Los mejores resultados se obtuvieron para el Lichrolut-

EN y Oasis HLB, ya que proporcionaban el mejor equilibrio de polaridad para la extracción de los hidrocarburos nitroaromáticos. Una vez seleccionados, estos materiales se sulfonaron para garantizar una distribución homogénea de las nanopartículas en la superficie. El Lichrolut-EN se sulfonó en el laboratorio, empleando dos temperaturas (temperatura ambiente y 80 °C), y para el Oasis HLB, se usó su versión sulfonada comercial, el Oasis MCX. Una vez sulfonados, ambos materiales se sometieron al proceso final de magnetización, mostrando resultados similares en las curvas de sorción. El material seleccionado fue el Oasis MCX, ya que al poder obtenerse de forma comercial el proceso de síntesis se simplifica enormemente.

La síntesis de este composite magnético se llevó a cabo en tres ciclos consecutivos de magnetización. Se comprobó que en cada ciclo el carácter magnético del material aumentaba pero la capacidad extractiva disminuía, ya que las NPs cubrían superficialmente el polímero. Por tanto, se seleccionó un número de ciclos como compromiso con ambos aspectos.

Una de las ventajas que presenta esta metodología es la posibilidad de llevar a cabo el análisis de un gran volumen de muestra usando una pequeña cantidad de sorbente. Además, el tiempo de extracción, de tan sólo dos minutos, permite tratar un número elevado de muestras a la hora. Por último, los factores de preconcentración que se consiguen son elevados.

Por otro lado, también pueden identificarse algunos aspectos a mejorar, como (i) el empleo de menores volúmenes de eluyente para alcanzar unos límites de detección aún más bajos; o (ii) el uso de otras nanopartículas con un mayor carácter magnético, ya que la síntesis de este composite requiere de varios ciclos de magnetización que hacen el proceso general complejo.

Así, en los **Capítulos III.3 y III.4**, se estudia la simplificación del proceso de síntesis para obtener composites magnéticos. Esta síntesis consta de un solo paso y emplea nylon 6 y magnetita como elementos. La síntesis aprovecha la diferente solubilidad del nylon 6 en ácido fórmico (soluble) y agua (insoluble). Además, al emplear magnetita en lugar de ferrita de cobalto, el poder magnético es mayor. Este sorbente se empleó para la determinación de hidrocarburos policíclicos aromáticos en muestras de agua y de bisfenol A en muestras de leche (Capítulo III.3 y III.4, respectivamente).

Si bien en el composite con Oasis MCX las nanopartículas magnéticas se sintetizaban sobre la red polimérica, en el caso del composite de nylon se sintetizan previamente. Por tanto, el primer paso de la síntesis consiste en la preparación de la magnetita, mediante un proceso de co-precipitación a partir de sus sales de Fe^{2+} y Fe^{3+}. Tras esto, se recubren de sílice para evitar su degradación cuando se dispersen en ácido fórmico, ya que de otro modo no serían estables en el medio ácido.

Para obtener el composite, sólo hay que disolver una cierta cantidad de nylon 6 en ácido fórmico y dispersar en esa disolución las nanoparticulas de magnetita recubiertas. Al añadir esta mezcla en agua, con la ayuda de una jeringa, se produce la precipitación del nylon (debido al cambio de disolvente), quedando las nanopartículas atrapadas en la red polimérica. En la Figura 2, se muestra un esquema de la síntesis del material.

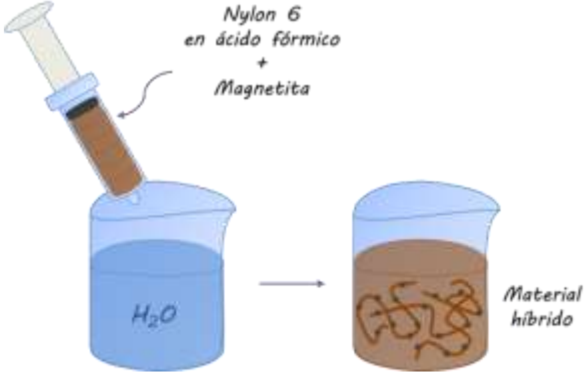

Figura 2. Síntesis del composite de nylon 6 y nanopartículas magnéticas.

La síntesis es barata y simple, ya que usa un polímero disponible comercialmente.

Para la determinación de hidrocarburos policíclicos aromáticos en muestras de agua (Capítulo III.3), se obtuvo una buena sensibilidad y selectividad, demostrándose la aplicabilidad del sorbente para ese problema analítico.

En el caso de la determinación de bisfenol A en muestras de leche (Capítulo III.4), el método proporciona unos límites de detección más elevados. A pesar de ello, es válido para la detección del analito por debajo del límite establecido por la Unión Europea. Por último, los resultados obtenidos al llevar a cabo el análisis de muestras de leche comerciales son buenos.

En el **Capítulo III.5** se presenta otra nueva síntesis en la que se combina nylon 6 con nanopartículas de sílice, que hacen que la superficie específica del polímero aumente, y como consecuencia, sus capacidades extractivas mejoren. Al introducir las nanopartículas en la red polimérica, el empaquetamiento normal de las cadenas de nylon 6 se ve distorsionado lo que incrementa la porosidad de la red polimérica. Este nuevo sorbente se usó para la extracción de estrógenos en muestras de agua, orina y saliva.

A diferencia de los trabajos anteriores, este material no presentaba carácter magnético, y por tanto, el método de extracción es diferente. En este caso, el sorbente se sitúa en una jeringa que contiene algodón en la punta para evitar la pérdida del material. El procedimiento de extracción conlleva una etapa de acondicionamiento con metanol y agua; la aspiración de la muestra, que produce la dispersión del sorbente; una etapa de lavado y por último, otra de elución.

La síntesis difiere de la descrita para el composite magnético. En primer lugar se prepara una disolución de polímero en ácido fórmico en la que se dispersan las nanopartículas de sílice. Tras esto, y con una bureta, se añade agua gota a gota para producir la precipitación controlada del polímero alrededor de las nanopartículas, tal y como se muestra en la Figura 3. Por último, el sólido se recoge mediante filtración a vacío con papel de filtro, lo que permite separar el polímero de las nanopartículas gracias a su gran tamaño de poro, y se lava con agua y metanol.

Figura 3. Síntesis del composite de nylon 6 y nanopartículas de sílice.

La síntesis del composite se llevó a cabo usando diferentes proporciones de nylon 6 y nanopartículas de sílice, para ver cuál presentaba mayor capacidad extractiva. Al comparar la superficie específica de las síntesis realizadas con y sin nanopartículas, se observa un incremento de 17 a 87 m^2/g, lo cual demuestra el importante papel que juegan las nanopartículas. La proporción de nylon 6/nanopartículas finalmente seleccionada fue de 75/25, ya que proporcionaba la mejor combinación entre capacidad y reproducibilidad de la síntesis.

La metodología propuesta se aplicó en muestras más complejas, como orina y saliva. Para comprobar la selectividad, se analizaron por infusión directa las muestras reales con y sin tratamiento, demostrándose el gran poder de eliminación de interferencias que presenta el sorbente propuesto. A pesar de ello, haría falta una mejora en el procedimiento de extracción para poder aplicarlo cuantitativamente en bioanálisis.

Los aspectos positivos del sólido sintetizado son la mejor capacidad de extracción respecto al material sin tratar, y la simplicidad y bajo coste de su síntesis.

Otro tipo de sorbente usado en microextracción en fase sólida dispersiva es el desarrollado en el **Anexo B.2**, donde se combinan las nanopartículas magnéticas con una poliamida sintetizada en el laboratorio. La ventaja que presenta esta síntesis es que los monómeros pueden seleccionarse con los grupos funcionales que se requieran, para favorecer un determinado tipo de interacción con los analitos. En este caso, se sintetizaron tres poliamidas usando hexametilendiamina y tres diácidos diferentes (isoftaloil, tereftaloil y oxalil). El sorbente que mejores resultados proporcionaba para la extracción de β-bloqueantes en muestra de orina fue el que contenía tereftaloil, ya que los anillos aromáticos de su cadena favorecían las interacciones π-π con los analitos.

Para concluir este apartado, se presenta una tabla resumen (Tabla 1) con las características principales de los cuatro trabajos discutidos en el mismo.

Tabla 1. Comparación de las metodologías propuestas en los Capítulos III.2, III.3, III.4 y III.5.

Capítulo	**III.2**	**III.3**	**III.4**	**III.5**
Muestra	Aguas		Leche	Agua, orina y saliva
Analitos	Hidrocarburos nitroaromáticos	Hidrocarburos policíclicos aromáticos	Bisfenol A	Estrógenos
Modalidad	Dispersiva magnética			Dispersiva en jeringa
Polímero	Oasis MCX	Nylon 6		
NPs	Ferrita de cobalto	Magnetita		Sílice
Caracterización	Curvas de magnetización, análisis gravimétrico	IR, TEM y SEM	IR, TEM, SEM y curvas de magnetización	IR, TEM, SEM, porosimetría y análisis elemental
Volumen de muestra (mL)	200	25	20	10
Cantidad de sorbente (mg)	20	40	80	40
Tiempo de extracción (min)	2 (vórtex)	2 (vórtex) + 30 (ultrasonidos)	1 (vórtex) + 30 (ultrasonidos)	-
Sistema detección	UPLC-DAD		HPLC-UV	HPLC-MS/MS
Límite de detección (µg/L)	0.12-1.26	0.05-0.58	3.05	0.15-0.3

Desviación estándar relativa (%)	4.4-9.5	3.4-6.8	9.1	2.8-13.9
Factor de preconcetración	161-292	18.1-43.5	7.58	43.4-88.2
Recuperación absoluta (%)	40.3-73.1	36.2-87.0	7.58	43.4-88.2
Recuperación relativa (%)	71-103	80-111	79-104	-

2. Microextracción en fase sólida con membrana

En este apartado, se comenta un nuevo tipo de sorbente que se emplea en microextracción en fase sólida con membrana. Esta modalidad de microextracción en fase sólida se considera un tipo de microextracción con película delgada (thin-film microextraction). De este modo, en el **Capítulo IV.2**, se describe la síntesis de una membrana polimérica de nylon 6 que contiene nanocuernos de carbono. La membrana sintetizada se empleó para la determinación de compuestos de diferente naturaleza (incluyendo compuestos volátiles monoaromáticos, un hidrocarburo saturado de cadena lineal y dos compuestos de la familia de los haloalcanos) en muestras de agua.

La membrana se sintetiza por electrospinning, una técnica ampliamente usada para la obtención de fibras poliméricas de tamaño nanométrico. La membrana sintetizada combina fibras de nylon 6 con nanocuernos de carbono, gracias a los cuales se mejora la capacidad extractiva del polímero.

Para estudiar el efecto que tiene la introducción de los nanocuernos en la red polimérica, se ensayaron diferentes proporciones de éstos (0.2, 0,5 y 1 g/L). Al llevar a cabo la extracción de los analitos con cada una de las membranas obtenidas, se comprobó que la capacidad extractiva aumentaba con la cantidad de nanocuernos de carbono introducidos. Esto es debido probablemente a la mayor porosidad de la membrana cuando los nanocuernos de carbono se encuentran en su interior, lo que favorece la interacción entre los analitos y la red polimérica.

Esta metodología se aplicó al análisis de muestras reales mediante el uso de un dispositivo que permite la conexión directa con el cromatógrafo de gases para la desorción térmica de los analitos de la membrana y su posterior análisis por espectrometría de masas.

En este caso, la síntesis propuesta es simple y rápida, aunque requiere de instrumentación sofisticada, al contrario de lo que ocurría en las anteriores síntesis. Otra de las ventajas que presenta es su facilidad para sintetizar nuevos composites, ya que sólo habría que añadir las nanopartículas a la disolución polimérica y someterla al proceso de electrospinning.

This last section of the doctoral Thesis tries to summarize the obtained results and also describes the evolution of the scientific developments.

The research that has been played out in this doctoral Thesis was focused on analytical sample preparation. In particular, these contributions are related with the design and use of new sorbent materials in solid phase microextraction.

All the synthesized materials can be considered as composites or hybrid materials, since they have been obtained by the combination of two or more types of components. Specifically, two types of polymers (Oasis MCX and polyamide) have been combined with nanoparticles of different nature, including magnetic nanoparticles, silica nanoparticles and carbon nanohorns.

Hereunder, each of the research work developed in this doctoral Thesis is described in chronological order. These contributions can be classified into two main groups depending on the extraction format: dispersive micro solid phase extraction or membrane solid phase microextraction.

1. Dispersive micro solid phase extraction

The dispersive micro solid phase extraction is based on the efficient dispersion of the sorbent material into the sample matrix. The extracting surface of the sorbent is totally exploited, improving the extraction efficiencies and kinetics.

Although the initial steps of dispersive techniques are quite similar, they differ in the way that the sorbent is recovered after the extraction. If the sorbent has magnetic properties, the general extraction procedure is similar to that shown in Figure 1. Firstly, the sorbent is added to the sample and it is dispersed with the help of an auxiliary stirring such as vortex or ultrasound, to increase the contact with the analyte. After the extraction, the sorbent is recovered with an external magnet, the sample matrix being discarded. Prior to the final elution with a suitable solvent, the sorbent is washed to remove the co-extracted substances.

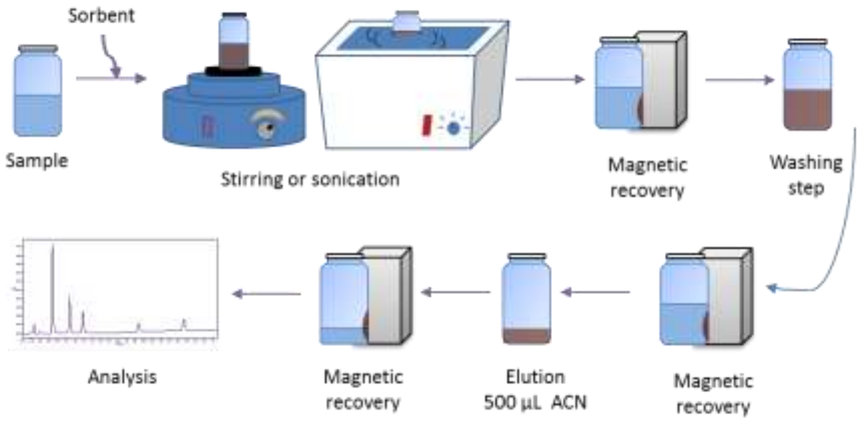

Figure 1. Schematic diagram of the dispersive micro solid phase extraction procedure when magnetic sorbents are employed.

In this doctoral Thesis, two different types of magnetic sorbents have been synthesized. In **Chapter III.2** of the Thesis, the synthesis of a composite made of a commercial sulfonated polymer (Oasis MCX) and cobalt ferrite nanoparticles is described. As it was already mentioned in Section I, this combination produces a solid with high extraction capabilities due to the polymer and with magnetic characteristics ascribed to the presence of the nanoparticles. In this way, the sorbent can be easily recovered from the sample, without needing a centrifuge or filter.

To select the optimum polymer, sorption curves of different materials (namely: Lichrolut-EN, Oasis HLB, carbon nanotubes, RP-C18 and PVC) were performed. The best results were obtained for Lichrolut-EN and Oasis HLB, since they provided the best polarity balance for the extraction of nitroaromatic hydrocarbons. Once the sorbents were selected, these materials were sulfonated to ensure the homogeneous distribution of the nanoparticles on the polymer surface. Lichrolut-EN was sulfonated in the laboratory using two different temperatures (room temperature and 80 °C), and for the Oasis HLB, its commercial sulfonated version, Oasis MCX, was employed. Once sulfonated, both materials were submitted to the final magnetization process, showing similar results in the sorption curves. Finally, Oasis MCX was selected as the optimum material since the previous sulfonation is not required. This fact simplifies the synthesis procedure.

The synthesis of this magnetic composite was carried out in three consecutive cycles of magnetization. Although each cycle increased the magnetic behaviour of the material, the extraction capacity was decreased since the NPs covered the surface of the polymer. Therefore, the number of cycles were selected as a compromise with both aspects.

The possibility of processing large sample volumes with a small sorbent quantity is one of the advantages of this methodology. In addition, a large number of samples per hour can be processed due to the short extraction time (ca. 2 min). Moreover, high enrichment factors were obtained.

On the other hand, the procedure can be also improved in several ways. (i) The use of lower elution volumes would allow reaching even lower limits of detection. (ii) The use of other nanoparticles with a higher magnetic behaviour would also improve the extraction capacity since a lower amount of NPs would be required to infer the magnetic behaviour on the composite.

Chapters III.3 and **III.4** face the simplification of the synthesis procedure to obtain magnetic composites. The proposed synthesis is single-step and makes use of nylon 6 and magnetite as elements. The procedure takes advantage of the different solubility of nylon 6 in formic acid (soluble) and water (insoluble). In addition, the magnetic power of the composite is higher due to the use of magnetite instead of cobalt ferrite. This sorbent was employed for the determination of polycyclic aromatic hydrocarbons in water and bisphenol A in milk samples (Chapter III.3 and III.4, respectively).

In this procedure the MNPs are embedded into the polymeric network, in contrast to the previous composite where the magnetic nanoparticles were synthesized over the polymer. The first step of the synthesis consists of the preparation of the Fe_3O_4 MNPs by the co-precipitation of Fe^{2+} and Fe^{3+}. After that, these nanoparticles are coated with silica to protect them during the final step of the synthesis.

To obtain the composite, a defined amount of nylon 6 is dissolved in formic acid and the coated MNPs are dispersed in this solution. By adding this mixture in water, with the help of a syringe, nylon precipitation occurs (due to solvent changeover), leaving the nanoparticles trapped into the polymeric network. Figure 2 shows a schematic diagram of the synthesis.

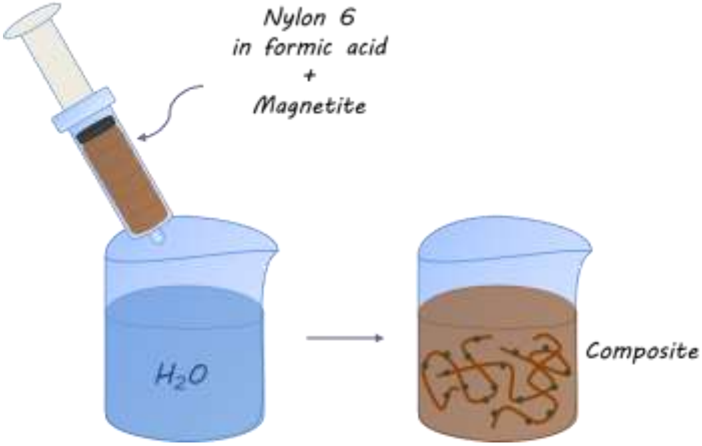

Figure 2. Synthesis of the composite of nylon 6 and magnetic nanoparticles.

The synthesis is cheap and simple, since it uses a commercially available polymer.

Good sensitivity and selectivity were obtained for the determination of polycyclic aromatic hydrocarbons in water samples (Chapter III.3), demonstrating the applicability of the sorbent.

In the case of the determination of bisphenol A in milk samples (Chapter III.4), the method provides higher limits of detection. However, it is acceptable for the detection of the analyte below the legal limit established by the European Union. Finally, it provides good recoveries when commercial milk samples are processed.

A new synthesis, where nylon 6 and silica nanoparticles are combined, is presented in **Chapter III.5.** These nanoparticles increase the specific surface area of the polymer, and as a consequence, they improve the extraction capabilities. The presence of the nanoparticles modify the normal stacking of the nylon 6 chains, creating imperfections in the network which provides the composite with a higher porosity. This new sorbent was employed for the extraction of estrogens in water, urine and saliva samples.

This material does not present magnetic behaviour, and therefore, the extraction procedure is quite different. In this case, the extraction unit consists of a plastic syringe which is adapted to a pipette tip section where a cotton bead is located to prevent the loss of the material. The composite is placed between the cotton bead and the syringe plunger. The extraction process involves a conditioning step with methanol and water; the aspiration of the sample, which produces the dispersion of the sorbent; a washing step and a final elution.

In addition, the synthesis is slightly different from the magnetic composite one although it is based in the same principle. Firstly, a solution of the polymer in formic acid is prepared, in which the silica nanoparticles are dispersed. After this, water is added dropwise with the help of a burette to produce the controlled precipitation of the polymer around the nanoparticles, as it is shown in Figure 3. Then, the solid is collected by vacuum filtration with filter paper, which allows the separation of the polymer from the not embedded nanoparticles thanks to its large pore size. Finally, the composite is washed with water and methanol, before being used.

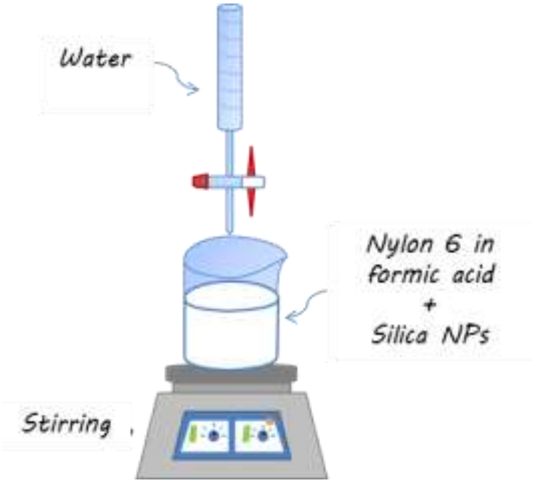

Figure 3. Diagram of the synthesis of the nylon 6-silica nanoparticles composite.

The synthesis of the composite was carried out using different proportions of nylon 6 and silica nanoparticles, to check which one provided a greater extraction capability. If the specific surface area of the solids are compared, an increase from 17 to 87 m^2/g is observed as a result of including NPs in the network. A nylon 6/nanoparticles proportion of 75/25 was finally selected as it provided the best combination of extraction capability and reproducibility of the synthesis.

The proposed methodology was applied in complex samples, such as urine and saliva. Direct infusion MS was selected for monitoring the selectivity. The analysis of the raw samples provided no signal but after the sample treatment the characteristics transition of the analytes were again detected thanks to the clean-up ability of the extraction technique. In spite of this, the extraction procedure should be further improved for bioanalytical applications.

The positive aspects of the synthesized solid are the better extraction capacity compared to the untreated material, and the simplicity and low cost of its synthesis.

Another type of composite is described in **Annex B.2** comprising the combination of magnetic nanoparticles and in-lab synthesized polyamides. The main advantage of this synthesis is that monomers can be selected with a certain functional group to favour different types of interactions with the analytes. In this case, three polyamides were synthesized using hexamethylenediamine and three different diacids (isophthaloyl, terephthaloyl and oxalyl). The composite containing terephthaloyl provided the best results for the extraction of β-blockers in urine sample, since its aromatics rings favor the π-π interactions with the analytes

To conclude this section, a summary table (Table 1) is presented with the main characteristics of the four research works presented.

Table 1. Comparison of the methodologies proposed in Chapters III.2, III.3, III.4 y III.5.

Chapter	**III.2**	**III.3**	**III.4**	**III.5**
Sample	Water		Milk	Water, urine and saliva
Analytes	Nitroaromatic hydrocarbons	Polycyclic aromatic hydrocarbons	Bisphenol A	Estrogens
Microextraction type	Magnetic (dispersive)			In-syringe (dispersive)
Polymer	Oasis MCX	Nylon 6		
NPs	Cobalt ferrite	Magnetite		Silica
Characterization	Magnetization curves, gravimetric analysis	IR, TEM, SEM	IR, TEM, SEM, magnetization curves	IR, TEM, SEM, porosimetry, elemental analysis
Sample volume (mL)	200	25	20	10
Sorbent amount (mg)	20	40	80	40
Extraction time (min)	2 (stir)	2 (stir) + 30 (sonicate)	1 (stir) + 30 (sonicate)	-
Instrumental technique	UPLC-DAD		HPLC-UV	HPLC-MS/MS
Limit of detection (µg/L)	0.12-1.26	0.05-0.58	3.05	0.15-0.3
Relative standard deviation (%)	4.4-9.5	3.4-6.8	9.1	2.8-13.9

Enrichment factor	161-292	18.1-43.5	7.58	43.4-88.2
Absolute extraction recovery (%)	40.3-73.1	36.2-87.0	7.58	43.4-88.2
Relative extraction recovery (%)	71-103	80-111	79-104	-

2. Membrane solid phase microextraction

In this section, a new type of sorbent that was employed in membrane solid phase microextraction is described. This mode of solid phase microextraction is considered a type of thin-film microextraction. Thus, in **Chapter IV.2**, the synthesis of a polymeric membrane containing carbon nanohorns is presented. The synthesized membrane was employed for the determination of some compounds of different nature (including monoaromatic volatile compounds, undecane and two compounds of the haloalkanes family) in water samples.

The membrane is synthesized by electrospinning, a widely used technique to obtain polymeric fibers of nanometric size. The synthesized membrane combines nylon 6 fibers with carbon nanohorns, which improve the extraction capability of the polymer.

In order to study the effect of the nanohorns in the polymeric network, different amounts of them were tested (0.2, 0.5 and 1 g/L). When these membranes were employed for the extraction of the analytes, it was confirmed that carbon nanohorns improves the extraction capability of the composites. This is probably due to the higher porosity of the membrane when carbon nanohorns are in its structure, which favors the interaction between the analytes and the polymeric network.

This methodology was applied to the analysis of real samples by the use of a new device which allows the extraction of samples and favours the direct analysis of the membrane by headspace-gas chromatography-mass spectrometry.

In this case, the proposed synthesis is simple and fast, nevertheless it requires sophisticated instrumentation, in contrast to the previous syntheses. Another advantage is the ease of synthesize new composites, since different kind of nanoparticles can be added to the polymer solution prior to the electrospinning process.

ANEXOS

PRODUCCIÓN CIENTÍFICA

Anexo A

Publicaciones científicas derivadas de la Tesis Doctoral

1. Hybridization of commercial polymeric microparticles and magnetic nanoparticles for the dispersive micro-solid phase extraction of nitroaromatic hydrocarbons from water

E. M. Reyes-Gallardo, G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel

Journal of Chromatography A, 1271 (2013) 50– 55

2. Magnetic nanoparticles-nylon 6 composite for the dispersive microsolid phase extraction of selected polycyclic aromatic hydrocarbons from water samples

E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

Journal of Chromatography A, 1345 (2014) 43-49

3. Polymer–nanoparticles composites in bioanalytical sample preparation

E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

Bioanalysis (2015) 7(14), 1723–1730

4. Dispersive solid phase extraction of bisphenol A from milk using magnetic nylon 6 composite and its final determination by HPLC-UV

E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

Microchemical Journal 124 (2016) 751-756

5. Electrospun nanofibers as sorptive phases in microextraction

E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas

TrAC Trends in Analytical Chemistry 84 (2016) 3-11

6. Silica nanoparticles-nylon 6 composites: synthesis, characterization and potential use as sorbent

E.M. Reyes-Gallardo, R. Lucena, S. Cárdenas

RSC Advances, 2017

7. Polyamide-carbon nanohorns electrospun composite membrane: synthesis and application for solid phase extraction

E.M. Reyes-Gallardo, M. C. Alcudia-León, R. Lucena, K. de Clerck, S. Cárdenas

In Progress

Anexo B

Publicaciones científicas relacionadas con la Tesis Doctoral

1. Octadecyl functionalized core–shell magnetic silica nanoparticle as a powerful nanocomposite sorbent to extract urinary volatile organic metabolites

Z. Qiao, R. Perestrelo, E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, J. Rodrigues, J. S. Câmara

Journal of Chromatography A, 1393 (2015) 18–25

2. Determination of propranolol and carvedilol in urine samples using a magnetic polyamide composite and LC–MS/MS

T. Ballesteros-Esteban, E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

Bioanalysis 8 (2016) 2115-2123

3. Recycling polymer residues to synthesize magnetic nanocomposites for dispersive micro-solid phase extraction

H. Ghambari, E. M. Reyes-Gallardo, R. Lucena, M. Saraji, S. Cárdenas

Submitted to Talanta

Journal of Chromatography A

1393 (2015) 18-25

Octadecyl functionalized core–shell magnetic silica nanoparticle as a powerful nanocomposite sorbent to extract urinary volatile organic metabolites

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In this present study, magnetic Fe_3O_4@SiO_2 nanoparticles (MNPs) functionalized with octadecyl groups (Fe_3O_4@SiO_2-C18NPs) were synthesized, characterized and employed, for the first time, as power fulnanosorbent to extract endogenous volatile organic metabolites (EVOMs) namely, hexanal, heptanal, decanal, benzaldehyde, 4-heptanone, 5-methyl-2-furfural and phenol, described as potential biomarkers of cancer, from human urine. By using co-precipitation, surface modification methods, the carbon-ferromagnetic nanocomposite was synthesized and characterized by infrared spectrum (IR) and transmission electron microscopy (TEM). By coupling with gas chromatography–mass spectrometry (GC–qMS), a reliable, sensitive and cost-effective method was validated. To test the extraction efficiency of the carbon-ferromagnetic nanocomposite toward urinary EVOMs experimental variables affecting the extraction performance, including nanosorbent amount, adsorption time, elution time, and nature of elution solvent, were investigated in detail. The extraction process was performed by dispersingFe_3O_4@SiO_2-C18 NPs into working solution containing targeted VOMs, and into urine samples, and then eluted with an adequate organic solvent. The eluate was collected, concentrated and analyzed by GC–qMS. Under the optimized conditions, the LODs and LOQs achieved were in the range of 9.7–57.3 and 32.4–190.9 ng/mL, respectively. Calibration curves were linear ($r^2 \geq$ 0. 988) over the concentration ranges from 0.25 to 250 ng/mL. In addition, a satisfying reproducibility was achieved by evaluating the intra- and inter-day precisions with relative standard deviations (RSDs) less than 3 and 11%, respectively. The method also afforded satisfactory results in terms of the matrix effect (72.8–96.1%) and recoveries (accuracy) higher than 75.1% for most of the studied EVOMs. The $Fe_3O_4@SiO_2$-C18NPs-based sorbent extraction combined with GC–qMS revealed that the new nanosorbent had a strong ability to retain the target metabolites providing a new, reliable and high throughput strategy for isolation of targeted EVOMsin human urine, suggesting their potential to be applied in other EVOMs.

Keywords: *Endogenous volatile organic metabolites (EVOMs); magnetic nanoparticles; magnetic solid phase extraction; urine samples; cancer biomarkers.*

Bioanalysis

8 (2016) 2115-2123

Determination of propranolol and carvedilol in urine samples using a magnetic polyamide composite and LC–MS/MS

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Aim: β-blockers are compounds that bind with adrenoreceptors hindering their interaction with adrenalin and noradrenalin. They are clinically relevant and they are also used in some sport as doping agents. **Results:** A new method based on the combination of dispersive micro-solid phase extraction and LC–MS/MS has been developed to determine propranolol and carvedilol in urine samples. For this purpose a magnetic-polyamide composite is synthesized and used as sorbent. Working under the optimum conditions, the method provides limits of detection and quantification in the range of 0.1–0.15 μg/l and 0.3–0.5 μg/l, for carvedilol and propranolol, respectively. The precision, expressed as RSD, was better than 9.6% and the relative recoveries varied between 73.7 and 81.3%. **Conclusion:** The methodology is appropriate for the determination of β-blockers in urine samples at the low microgram per liter range for therapeutic purposes.

Keywords: *β -blockers; composite; dispersive micro-solid phase extraction.*

Talanta

Submitted

Recycling polymer residues to synthesize magnetic nanocomposites for dispersive micro-solid phase extraction

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The ubiquitous presence of plastics, an obvious consequence of their usefulness and low price, has turned them into a problem of environmental and safety concern. The new plastic economy, an initiative recently launched by the World Economic Forum, promotes a change in the use of plastic worldwide around three main pillars: redesign, reusing and recycling. Recycled plastics, with the aim of extending their life spam, can be used to synthesize materials for analytical purposes. In this article polystyrene (PS) trays, previously used for food packaging, are proposed as polymeric source for the synthesis of magnetic nanocomposites. The synthesis plays with the solubility of PS in different solvents in such a way that PS is gelated in the presence of cobalt ferrite nanoparticles which are finally embedded in the polymeric network. The extraction capability of the magnetic PS nanocomposite was evaluated using the determination of four parabens (methylparaben, ethylparaben, propylparaben and butylparaben) in water using liquid chromatography-tandem mass spectrometry as model analytical problem. Under the optimum conditions, limits of detection and quantification were in the range of 0.05-0.15 and 0.15-0.5 ng/mL, respectively. The precisions, expressed as relative standard deviation

(RSD), varied between 4.4 and 8.5% and the relative recoveries for analysis of the water samples were in the interval 81.2-104.5%.

Keywords: *Dispersive micro-solid phase extraction, magnetic polystyrene nanocomposite, polymer recycling, sustainable chemistry, parabens, LC–MS/MS.*

Anexo C

Actividades de divulgación científica

1. Evaluación de un nuevo composite de nanopartículas magnéticas y nylon-6 como sorbente en técnicas de microextracción en fase sólida dispersiva

E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

Boletín del Grupo Regional Andaluz de Química Analítica (GRASEQA) nº 11-Septiembre de 2015

2. MNPs-Nylon 6 composite as a new sorbent for microextraction

E. M. Reyes-Gallardo

Blog de divulgación científica de Química Analítica "Microextraction Tech" http://microextraction.blogspot.com/ (9 de noviembre de 2016)

3. Participación en "Paseo por la Ciencia", ediciones de 2010, 2011, 2012 y 2015

4. Participación en "La noche europea de los investigadores", ediciones de 2014 y 2016

5. Ponente en "Café con Ciencia", ediciones 2014 y 2016

6. Participación en "III Jornadas de divulgación científica #divulgA3. Más allá de los papers" 2015

7. Participación en "Actividades de difusión de las líneas de investigación e infraestructuras (laboratorios e instrumentación científica) del Departamento de Química Analítica, dirigidas a estudiantes de centros de enseñanza secundaria". Del Programa "ORIENTAUCO" y del "Plan Integral de Difusión de la Facultad de Ciencias y el programa PACE de la Universidad de Córdoba", durante el curso 2015/2016

9. Participación en "Espacios de Ciencia" actividad organizada por la Unidad de Cultura Científica de la Universidad de Córdoba dentro del programa de la Semana de la Ciencia 2016

10. Coordinadora en las "IX Jornadas de Introducción al laboratorio experimental de Química para alumnos de bachillerato". Ediciones de 2016 y 2017

Boletín GRASEQA nº 11 – Septiembre de 2015

Investigación GRASEQA: Contribuciones XX SEQA

Depósito Legal: J 1559-2011
ISSN: 2254-12

Evaluación de un nuevo composite de nanopartículas magnéticas y Nylon-6 como sorbente en técnicas de microextracción en fase sólida dispersiva

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1. Introducción

Las técnicas de microextracción han evolucionado en los últimos años siguiendo dos tendencias bien definidas: por un lado, el desarrollo de nuevas modalidades o la mejora de las ya existentes y por otro, el diseño/empleo de nuevos materiales que permitan mejorar la eficiencia de estos procesos [1]. El potencial de los materiales nanoestructurados en este segundo contexto está, a día de hoy, fuera de toda duda. Además, la gran variedad existente de nanopartículas (NPs), que proporcionan diferentes químicas de interacción, permite aplicarlas a un gran número de problemas analíticos de diferente naturaleza [2].

En los últimos años se ha incrementado el uso de nanopartículas magnéticas (magnetic nanoparticles, MNPs) en las técnicas de microextracción. Este hecho se fundamenta en tres de sus características más relevantes. En primer lugar, su elevada área superficial (derivada de su tamaño nanométrico) permite mejorar la cinética del proceso al maximizarse el contacto extractante/muestra. En segundo lugar, este tipo de NPs son de fácil síntesis y pueden derivatizarse en superficie para mejorar su interacción con los analitos diana, pudiendo diseñarse ad hoc para un determinado analito o familia de analitos. Por último, por debajo de un cierto tamaño, que depende del tipo de MNPs que se considere, estas partículas son superparamagnéticas. Este superparamagnetismo, que hace que dichas nanopartículas sean magnéticas sólo bajo la influencia de un campo magnético externo, es esencial para entender su relevancia en técnicas de microextracción dispersiva ya que permite que estas MNPs puedan mantenerse en íntimo contacto con la muestra durante la etapa de interacción con los analitos y separarlas fácilmente a continuación mediante el uso de un imán externo. Esta característica simplifica sobremanera los procesos de extracción al soslayar etapas tediosas como la centrifugación o filtración.

La funcionalización de las MNPs permite incrementar la eficacia y la selectividad del proceso de extracción. Normalmente, esta etapa incluye la formación de un recubrimiento de sílice del core magnético para protegerlo y generar una superficie más fácilmente funcionalizable. Los recubrimientos de estas MNPs son diversos y engloban tanto las funcionalidades comunes en extracción en fase sólida como otras más novedosas.

Los recubrimientos poliméricos presentan una gran versatilidad debido a que existe una gran variedad de polímeros disponibles que pueden contener grupos funcionales muy diversos. Además, muchos de ellos presentan características mecánicas y químicas (como la estabilidad en pH extremos) que no se observan con los recubrimientos convencionales. Los nanomateriales composite que se obtienen tras la combinación de MNPs y polímeros pueden presentar dos morfologías diferentes. Por un lado, se pueden recubrir las MNPs con una capa del polímero seleccionado de manera que se mantienen sus dimensiones nanométricas. Por otro lado, se pueden incluir las MNPs dentro de la red polimérica de manera que el material resultante combina dimensiones nano y micrométricas. En ambos casos, se pretende combinar las propiedades favorables de ambos componentes del composite para mejorar el proceso de extracción [3].

2. Composites magnéticos de poliamidas

Las poliamidas son polímeros obtenidos por la síntesis controlada de un ácido dicarboxílico y una diamina. La reacción de ambos monómeros genera una cadena polimérica en la que se encuentran regularmente enlaces amidas (consecuencia directa de la reacción) que le dan nombre a estos polímeros. Estos enlaces amida juegan un papel fundamental en la estabilidad del polímero ya que permite el apilamiento de las cadenas poliméricas mediante enlaces por puentes de hidrógeno. Las poliamidas pueden comprarse ya sintetizadas pero también es posible sintetizarlas a partir de ciertos monómeros. Esta segunda opción es especialmente interesante si se quiere introducir funcionalidades especiales.

La obtención de un composite magnético de poliamida se realiza en dos etapas bien diferenciadas. La primera etapa es la síntesis de las nanopartículas y su recubrimiento, si procede, con una capa protectora de sílice. Se pueden emplear para esta etapa diferentes métodos descritos en la bibliografía, si bien, nosostros hemos optado por un proceso de coprecipitación [4]. Para ello, se disuelven 24 g de $FeCl_3{\cdot}6H_2O$ y 9,8 g de $FeCl_2{\cdot}4H_2O$ en 100 mL de agua destilada, manteniendo la disolución a 80 °C durante 30 min en atmósfera de nitrógeno. A continuación, se añaden 50 mL de hidróxido amónico (25% v/v) gota a gota generando las MNPs de Fe_3O_4. Las MNPs se separan mediante un imán de neodimio externo y se lavan secuencialmente con agua y metanol, y finalmente se secan. El recubrimiento de las MNPs con una capa de sílice para proteger el core magnético se realiza dispersando 1 g de MNPs en 20 mL de etanol. A continuación, se añaden 120 mL de agua destilada, 25 mL de trietoxisilicato (TEOS) y 15 mL de una disolución amoniacal de pH 10. La mezcla se agita durante 24 h en atmósfera de nitrógeno. Las nanopartículas ($Fe_3O_4@SiO_2$) se separan con la ayuda del imán, se lavan y se secan.

La segunda etapa es la más novedosa y explota la solubilidad diferencial de las poliamidas en ácido fórmico y agua. En esta etapa, se disuelven 150 mg de poliamida (Nylon-6 en nuestro caso) en 5 mL de ácido fórmico y se dispersan 200 mg de $Fe_3O_4@SiO_2$ en esta disolución con la ayuda de ultrasonidos. Esta dispersión se inyecta mediante una jeringa en un vaso de precipitados que contiene 50 mL de agua destilada. El cambio de disolvente (de un medio 100 % de ácido fórmico a otro acuoso en su mayoría) produce la gelificación del polímero alrededor de las MNPs. El sólido resultante se recoge, se lava secuencialmente con agua destilada y metanol y se separa del líquido de lavado con la ayuda de un imán. Finalmente, se seca quedando listo para su uso. Una vez sintetizado, el composite se caracterizó mediante distintas técnicas. La estructura del material se evaluó mediante microscopía de barrido (scanning electron microscopy, SEM). Como se puede ver en la Figura 1, tras la síntesis, la superficie se vuelve más rugosa como consecuencia de la formación de la red polimérica con las MNPs incluidas en la estructura.

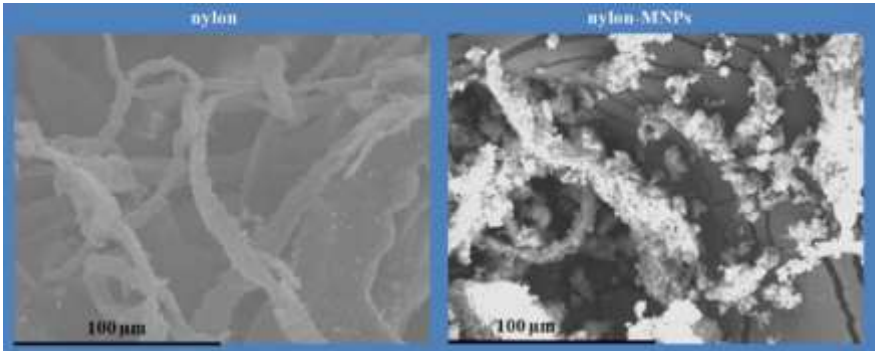

Figura 1. Imágenes de microscopía de barrido (SEM) del Nylon 6 (izquierda) y el composite Nylon-6-MNPs (derecha) a 500× y 1600×. Reproducida de la referencia 4 con permiso.

La composición química del composite se corroboró mediante espectroscopía de infrarrojo, registrando el espectro de infrarrojo del material obtenido en cada una de las etapas de la síntesis. Como puede verse en la Figura 2, el composite resultante combina las bandas de absorción correspondientes a cada uno de los componentes del composite.

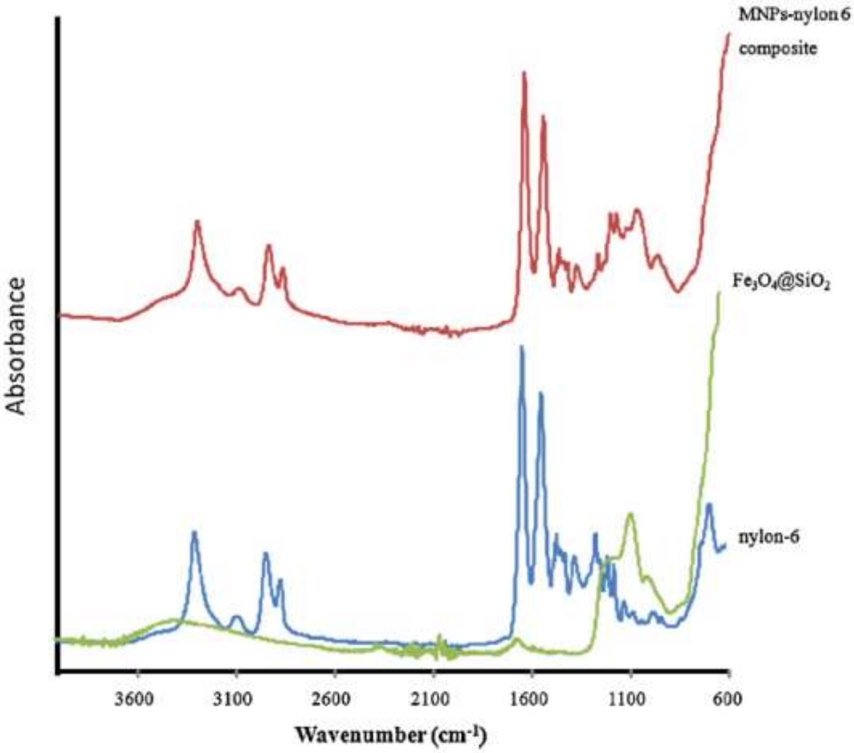

Figura 2. Espectros de infrarrojo del Nylon 6, MNPs recubiertas de sílice y del composite Nylon-6-MNPs. Reproducida de la referencia 4 con permiso.

3. Extracción de hidrocarburos policíclicos aromáticos (PAHs) de muestras de agua

Los PAHs constituyen un grupo de contaminantes prioritarios, dado su efecto prejudicial sobre la salud de los seres vivos y el medio ambiente. La determinación en compartimentos ambientales como suelo y agua es fundamental para garantizar que no se exceden los niveles máximos establecidos. Por ello, se seleccionó este problema analítico para evaluar la capacidad extractiva del sólido composite sintetizado [4]. El procedimiento de microextracción en fase sólida dispersiva optimizado es el siguiente. Se añaden 40 mg del composite Nylon-6-MNPs a 25 mL de estándar acuoso o muestra de agua. La mezcla se agita secuencialmente en un vortex, un baño de ultrasonidos y finalmente en un vortex (1/30/1 min) para favorecer la interacción entre los analitos y el sólido. El sorbente, enriquecido con los analitos, se separa de la fase líquida con la ayuda de un imán externo y se lava. Los PAHs se eluyen con 500 μL de acetona que se transfieren a un vial de 2 mL para su análisis por cromatografía de líquidos de ultra-alta resolución y detección UV-visible.

En estas condiciones se establecieron las características analíticas del método propuesto para los PAHs seleccionados. Los límites de detección se calcularon siguiendo dos aproximaciones. En primer lugar se consideró la relación señal/ruido de 3, oscilando los valores entre 0,05 μg/L (benzo(b)fluoranteno) y 0,58 μg/L (fenantreno). Se consideró también el límite de detección del método (MDL, method detection limit), calculado según la definición de la EPA, para establecer la mínima concentración detectable. En este caso, los valores estuvieron comprendidos en un intervalo más estrecho, 0,11μg/L (indeno(1,2,3cd)pireno) y 0,19 μg/L (fenantreno). La repetibilidad del método, expresada como desviación estándar relativa y calculada a un nivel de concentración igual al límite de cuantificación para cada analito, estuvo comprendida entre 3,4% (fluoranteno) y 6,8% (benzo(b)fluoranteno). Los factores de enriquecimiento (18,1-43,5) así como las recuperaciones absolutas (32,6-87,0%) fueron aceptables en todos los casos. Finalmente, se llevó a cabo un estudio de recuperación de los analitos en diferentes muestras de agua fortificadas a una concentración de 5 μg/L. Todos los valores de recuperación estuvieron comprendidos en el intervalo 80-111%, cumpliendo el requisito del 70-130% para todos los analitos y muestras.

4. Extracción de bisfenol A (BPA) de muestras de leche

El bisfenol A es un disruptor del sistema endocrino que se utiliza habitualmente en la industria de fabricación de plásticos. De hecho, está presente en muchos de los envases destinados a contener alimentos, por lo que existe un riesgo elevado de que éste contamine el alimento mediante un proceso de migración durante el almacenamiento.

Se seleccionó la determinación de bisfenol A en muestras de leche para evaluar la versatilidad del composite sintetizado. El principal problema que presenta esta aplicación es que el marcado carácter hidrofóbico del analito favorece su interacción con la fracción grasa de la leche, lo que reduce notablemente el rendimiento del proceso de extracción. Usando el buffer McIlvaine para precipitar la fracción proteica de la muestra, que es una etapa inevitable en la mayoría de los procedimientos que se encuentran en la bibliografía, no se consiguió extraer más del 40% del BPA añadido a una muestra de leche. Este hecho se debe a que parte del analito se queda interaccionando con la grasa de la leche, que como se ha comprobado queda ocluida en el precipitado proteico. Se optó entonces por añadir acetonitrilo al medio de precipitación, pero aunque la presencia de disolvente orgánico reducía la interacción BPA-grasa, disminuía a la vez su interacción con el composite. Como solución de compromiso se optó por realizar un proceso de dos etapas. En la primera etapa se precipitan las proteínas en un buffer que además contiene acetonitrilo. Una vez separada la fracción proteica y la grasa ocluida, se diluye el medio con agua (reduciendo así el porcentaje de acetonitrilo) ante de la extracción final con el composite.

El procedimiento de microextracción optimizado permitió la detección de BPA en leche a una concentración de 3.05 µg/L, inferior al SML establecido por la Unión Europea. La repetibilidad del método fue del 9.1%, a una concentración de 100 µg/L. Finalmente, se analizaron distintas muestras de leche con distinto contenido graso (entera, semidesnatada y desnatada) en las que no se detectó la presencia de BPA. Para evaluar la exactitud del método, se llevó a cabo un estudio de recuperación añadiendo el BPA a una concentración de 100 µg/L a muestras de leche con distinto contenido graso, encontrándose todos los valores entre 86-99 %, lo que demuestra la aplicabilidad del método para el problema analítico seleccionado.

5. Conclusión

En este artículo, se ha presentado la síntesis de un nuevo material composite Nylon-6-MNPs que combina las excelentes propiedades sorbentes del polímero con la simplificación del proceso de extracción aportado por las MNPs. El sólido se caracterizó mediante SEM, TEM, curva de magnetización y FT-IR y se evaluó en la modalidad de microextracción en fase sólida dispersiva determinando PAHs y BPA en muestras de agua y leche, respectivamente.

Las poliamidas, en su combinación con MNPs, generan composites con dos propiedades fundamentales: (a) carácter superparamagnetico que permite simplificar los procesos de extracción y (b) selectividad modulable mediante la selección de la poliamida adecuada para la resolución del problema analito abordado.

6. Agradecimientos

La investigación que se ha presentado en este artículo ha sido financiada por el proyecto CTQ 2014- 52939-R.

Referencias

[1] Lucena, R., Cárdenas S., Valcárcel, M. Analytical Microextraction Techniques. Special issue. Analytical and Bioanalytical Chemistry, 6 (8) (2014).

[2] Lucena, R., Simonet, B.M., Cárdenas S., Valcárcel, M. Potential of nanoparticles in sample preparation. Journal of Chromatography A 1218 (2011) 620-633.

[3] Mahouche-Chergui, S., Guerrouache, M., Carbonnier, B., Chehimi, M.M. Polymerimmobilized nanoparticles. Colloids and Surfaces. A 439 (2013) 43-68.

[4] Reyes-Gallardo, E., Lucena, R., Cárdenas S., Valcárcel, M. Magnetic nanoparticles-nylon 6 composite for the dispersive microsolid phase extraction of selected polycyclic aromatic hydrocarbons from water samples. Journal of Chromatography A 1345 (2014) 43-49.

Blog de divulgación científica de Química Analítica
"Microextraction Tech"

http://microextraction.blogspot.com/
9 de noviembre de 2016

MNPs-Nylon 6 composite as a new sorbent for microextraction

Emilia M. Reyes-Gallardo

In 2014 a new method for the synthesis of magnetic nanoparticles (MNPs)-nylon 6 composite was proposed. This method is simple and quick since only one step is needed. It is based on a solvent changeover, taking advantage of the different solubility of nylon 6 in formic acid and water. In Figure 1, it can be observed how this synthesis is carried out. The solution of MNPs and nylon-6 in formic acid is added to a beaker with water using a plastic syringe, inducing the precipitation of the polymer around the MNPs. To collect the composite after the synthesis, a magnet is used.

Figure 1. Scheme of the synthesis.

When MNPs are combined with a polymer, the resulting composite has the properties of both materials, combining the high extraction capabilities of the polymer and the magnetic behaviour due to the nanoparticles. Moreover, another advantage of using polymers is their wide versatility since they can be tailored-synthesized and different functional groups can be added, to react with different kind of analytes.

The synthesized composite was characterized in terms of infrared spectroscopy, electron microscopy, porosimetry, magnetization curves and elemental analysis. Scanning electron micrographs are shown in Figure 2. It is easy to observe how the MNPs are homogeneously distributed around the polymeric network, giving rise to a rougher surface.

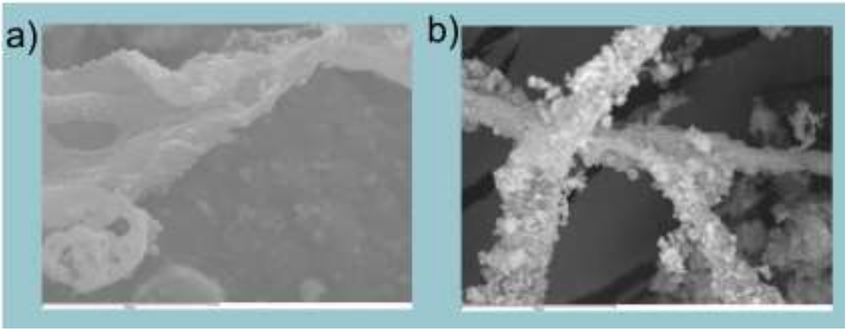

Figure 2. SEM pictures of nylon-6 (a) and MNPs-nylon-6 composite (b).

This new sorbent was employed for the resolution of two different analytical problems. The first one was the determination of polycyclic aromatic hydrocarbons (PAHs) in water samples (1), obtaining a limit of detection (LOD) between 0.05 µg/L for benzo(b)fluoranthene and 0.58 µg/L for phenanthrene. The relative standard deviations (RSD) were in the range from 3.4% (fluoranthene) to 6.8% (benzo(b)fluoranthene). The enrichment factors were in the range from 18.1 (phenanthrene) to 43.5 (benzo(b)fluoranthene), and the absolute extraction recoveries between 36.2 to 87.0%. The recovery study was performed spiking water samples with the analytes (5 μg/L), obtaining extraction recoveries from 80 to 111.

After that, bisphenol A (BPA) was determined in milk samples (2), obtaining a LOD of 3 μg/L and a RSD of 9%. The analysis of different kinds of milk samples verified the applicability of the method, with extraction recoveries of 86, 98 and 99 for whole, semi-skimmed and skimmed milk, respectively.

Referencias

(1) E.M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel. Magnetic nanoparticles-nylon 6 composite for the dispersive micro solid phase extraction of selected polycyclic aromatic hydrocarbons from water samples. Journal of Chromatography A, 1345 (2014) 43-49.

(2) E.M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel. Dispersive micro-solid phase extraction of bisphenol A from milk using magnetic nylon 6 composite and its final determination by HPLC-UV. Microchemical Journal, 124 (2016) 751-756.

Anexo D

Presentación de comunicaciones a congresos

1. IV Encuentro sobre Nanociencia y Nanotecnología de Investigadores Andaluces (NANOUCO) (Córdoba, febrero de 2013)

Comunicación oral titulada "Magnetización de un polímero comercial para la extracción en fase sólida dispersiva (D-µSPE) de compuestos nitroaromáticos en agua", E. M. Reyes-Gallardo

2. III Congreso Científico de Investigadores en Formación y II Congreso Científico de Investigadores en Formación en Agroalimentación (Córdoba, abril de 2013)

Comunicación oral titulada "Empleo de nanopartículas híbridas en el contexto de las técnicas de (micro)extracción", E. M. Reyes-Gallardo

3. XVII Euroanalysis. European Conference on Analytical Chemistry (Varsovia, Polonia, agosto de 2013)

Póster titulado "Hybridization of commercial polymeric microparticles and magnetic nanoparticles for the dispersive micro-solid phase extraction of nitroaromatic hydrocarbons from water", E. M. Reyes-Gallardo, G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel

4. XIV Reunión del Grupo Regional Andaluz de la Sociedad Española de Química Analítica (GRASEQA) (Baeza, Jaén, junio 2014)

Póster titulado "Síntesis de un material híbrido de nanopartículas magnéticas y nylon 6 para la extracción de hidrocarburos policíclicos aromáticos en muestras de agua", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

5. III Congreso Científico de Investigadores en Formación y II Congreso Científico de Investigadores en Formación en Agroalimentación (Córdoba, noviembre 2014)

Póster titulado "Síntesis de un material híbrido de nanopartículas magnéticas y nylon 6 para la extracción de hidrocarburos policíclicos aromáticos en agua", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

6. V Encuentro sobre Nanociencia y Nanotecnología de Investigadores y Tecnólogos Andaluces (NANOUCO V) (Córdoba, febrero de 2015)

Póster titulado "Extracción de Bisfenol A en muestras de leche mediante el uso de un material híbrido de nanopartículas magnéticas y nylon 6", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

7. XX Reunión de la Sociedad Española de Química Analítica (SEQA) (Santiago de Compostela, junio de 2015)

Comunicación oral titulada "Magnetic nanoparticles-nylon 6 composite as a novel sorbent for dispersive micro solid phase extraction", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

8. VII Workshop en Nanociencia y Nanotecnología Analíticas (Salamanca, julio de 2015)

Comunicación flash y póster titulados "Determination of Bisphenol A in milk using a magnetic nanoparticles-nylon 6 composite", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

9. VI EuCheMS Chemistry Congress (Sevilla, septiembre de 2016)

Comunicación oral titulada "Silica nanoparticles-nylon 6 composite for the in-syringe d-μSPE of estrogens from urine samples by HPLC-MS/MS", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Valcárcel

10. VI Encuentro sobre Nanociencia y Nanotecnología de Investigadores y Tecnólogos Andaluces (NANOUCO V) (Córdoba, enero de 2017)

Póster titulado "Síntesis de membranas de poliamida 6 y nanocuernos de carbono mediante electrospinning para su posterior uso en la extracción de compuestos de distitna naturaleza", E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas

Póster titulado "Simple synthesis of magnetic polystyrene composite for the dispersive micro-solid phase extraction of parabens from water samples followed by LC-MS/MS determination", H. Ghambari, E. M. Reyes-Gallardo, R. Lucena, S. Cárdenas, M. Saraji