Double-Headed Amphiphile-Based Sponge Droplets: Synthesis, Characterization and Potential for the Extraction of Compounds over a Wide Polarity Range

S. González-Rubio[1], A. Ballesteros-Gómez [1*], D. García-Gómez[2], S. Rubio[1]

[1] Department of Analytical Chemistry. Institute of Fine Chemistry and Nanochemistry. Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie (anexo), E-14071 Córdoba, Spain.

[2] Department of Analytical Chemistry, Nutrition and Food Science, University of Salamanca, Spain.

[*]Corresponding author, email: ana.ballesteros@uco.es, Phone 34 957 218643

Abstract

Supramolecular solvents (SUPRASs) are gaining momentum in the multi-residue analysis of liquid samples thanks to the delimited hydrophilic and hydrophobic microenvironments in their nanostructures. In this work, SUPRASs with increased hydrophilicity were synthesized with the aim of enhancing the extractability of polar compounds. For this purpose, a double-headed amphiphile, 1,2-decanediol, was self-assembled in hydro-organic media in the presence and absence of sodium chloride. The SUPRASs formed, characterized by scanning electron microscopy, consisted of sponge droplets made up of a highly convoluted three-dimensional (3D) network of amphiphile. The network contained interconnected bilayers that were intersected by similarly interconnected aqueous channels with high and nearly constant water content (~30%, w/w). Both the inherently open structure of the sponge morphology and the increased hydrophilic-hydrophobic balance of the amphiphile, provided highly hydrophilic microenvironments into the aggregates that rendered in increased recovery factors for 15 perfluorinated compounds (PFCs, C_4-C_{18}, log P_{ow} values from 0.4 to 11.6) in natural waters. Extraction took 15 min without further clean-up or evaporation of extracts which were readily compatible with LC-MS/MS quantitation. Absolute recoveries for PFCs, at the level of a few ng L^{-1}, were in the range 70-120%, except for perfluoropentanoic acid (40%) and perfluorobutane sulfonic acid (51%). Detection limits for PFCs in water were in the range 0.01-0.02 ng L^{-1}, which allowed their determination in slightly polluted waters (0.07-2.33 ng L^{-1}). This work proves that hydrophilicity in SUPRASs can be tailored through the amphiphile and the morphology of their aggregates, and that this characteristic improves compound extractability in multi-residue analysis.

1. Introduction

The design and production of tailored solvents for analytical extractions has become a fruitful field in recent years because of the possibility they offer to improve yields, selectivity, sustainability and costs [1]. Innovative approaches based on tailored ionic liquids (ILs), deep eutectic solvents (DESs) or supramolecular solvents (SUPRASs) have enabled the development of unique sample treatment platforms for determination of organic compounds in a variety of food, environmental or biological matrices [2-4]. Thus, the development of new tailor-made solvents for their further application has experienced fast grown in the last few years [1].

Supramolecular solvents are nanostructured liquids made up of coacervate droplets that are produced from colloidal suspensions of amphiphiles by establishing environmental conditions that lead to their spontaneous self-organization into a new liquid phase (see **Figure S1** in Supplementary Information, SI) [5]. The ordered structures in SUPRASs can be tailored by proper design of the environment (e.g., selection of the driving force leading to coacervation) and/or the components (e.g., selection of the head group and hydrocarbon chain of the amphiphile) [6,7].

Considerable progress has been made in SUPRAS-based analytical extractions in the last two decades by just selecting the proper amphihile for each application [5,8]. Thus, the use of non-aromatic anionic amphiphiles overcame the main drawbacks of the traditional temperature-induced SUPRASs based on Triton X and PONPE surfactant series, such as high signal backgrounds in LC-UV/fluorescence detection, unacceptable

chromatographic peak widths and decomposition of thermally labile compounds [9]. Further advances in tailored SUPRASs have been related to the use of alkanols, which have allowed the production of densely packed water-induced SUPRASs that provides a huge number of binding sites for solute solubilization (up to 1 mg μL^{-1}) [10]. These SUPRASs also feature restricted access properties, thus allowing the integration of analyte extraction and interference removal in a single step, and have negligible signal in ESI-MS [11], which make them suitable for mass detection [12]. Likewise, some SUPRASs have been reported to provide novel solubilization mechanisms (e.g. halogen bonding [13], polar hydrophobicity [14]) that have been successfully exploited for developing innovative applications. Also, engineered SUPRASs have permitted to expand their scope of application to GC-MS [15,16], proteomic [17], wastewater treatment [18], biomass valorization [19], etc. However, despite all these advances, the production of SUPRASs with tailored properties is still in its infancy and there is a long way to go to exploit its full potential in analytical extractions.

In this work, progress in this area is intended by designing and producing engineered SUPRASs made up of double-headed amphiphile sponge droplets with the aim of rising the efficiency in the liquid-liquid extraction of compounds that feature a wide range of polarities, prior to their screening/quantification by LC-MS/MS. This is a demanding challenge in many fields where multi-component determination is routine (e.g., environmental, agri-food, anti-doping, etc.) and at which conventional solvents do not provide a suitable solution.

Sponge droplets (also called L3 phases) consist of a highly convoluted and interconnected three-dimensional network of amphiphile bilayers intersected by similarly interconnected nanometer-sized aqueous channels [20-22]. These bicontinuous phases offer enormous amphiphilic surface areas and highly hydrophilic, in addition to

hydrophobic, environments [22]. So they have the potential to solubilize compounds in a wide polarity range. The formation of sponge phases has been reported for a wide variety of amphiphilic systems but frequently they only exist within a narrow range of amphiphile concentration, pH, and temperature, and are highly sensitive to additives [20,23,24]. To the best of our knowledge, sponge phases have not been used for analytical purposes.

On the other hand, amphiphiles possessing more than one head group present unique aggregation behavior and important biological functions [25]. Thus, micellar sizes and aggregation numbers decrease as the number of head groups rises owing to the enhanced electrostatic repulsion and/or steric hindrance among the amphiphilic molecules. The hydrocarbon chains remain highly folded in the resulting micelles and this renders them wetter than their single-head group counterparts [26]. So, both the larger amphiphilic head group and the wetter micellar environment should result in more hydrophilic SUPRASs, thus facilitating the extraction of very polar compounds.

Here we investigate the production of water-induced SUPRASs with sponge morphology from the double-headed amphiphile 1,2-decanediol. Our working hypothesis was that combining both strategies (i.e., larger amphiphilic head groups and wetter SUPRAS nanostructures), the extractability of very polar compounds should increase while keeping good extraction rates for very non-polar compounds, thus facilitating multi-component extraction. These SUPRASs were characterized and their extraction potential for compounds covering a wide polarity range was tested by extracting perfluorinated compounds (PFCs) from environmental waters as model compounds prior to their determination by LC-MS/MS. The selected PFCs consisted in sulfate and carboxylate head groups and alkyl chain lengths in the range C4-C18. Results were compared to those provided by decanol, a single-head amphiphile SUPRAS consisting of

inverted hexagonal aggregates [10]. To the best of our knowledge, this is the first SUPRAS produced from the coacervation of alkanediols.

2. Materials and methods

2.1. Chemicals

All solvents were LC-grade and were used as supplied. Tetrahydrofuran (THF) and methanol (MeOH) were purchased from VWR-Prolabo Chemicals (Bois, France) while isopropanol was obtained from Panreac (Barcelona, Spain). Ultra-high-quality water was generated from a Milli-Q water purification system (Millipore-Sigma, Madrid, Spain). 1-Decanol, 1,2-decanediol and sodium chloride were supplied by Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate was a Fluka reagent (Buchs, Stwitzerland). Certified native perfluorinated compounds (PFCs) and isotope-labeled internal standards (IS), all of them prepared in methanol at a concentration of 2 µg mL^{-1}, were purchased as custom solution mixtures from Wellington Laboratories (Guelph, ON, Canada). The target PFCs included three perfluoroalkane sulfonic acids (PFSAs) and twelve perfluoroalkyl carboxylic acids (PFCAs). **Table S1** in Supplementary Information shows the names and acronyms for the native PCFs and IS selected in this study. Two stock solutions of a mix of PFCs and a mix of ISs (both at a concentration of 0.2 µg mL^{-1} each), as well as intermediate and working solutions, were prepared in MeOH and stored at -20°C.

2.2. SUPRAS formation and characterization

The formation of water-induced 1,2-decanediol-based SUPRASs was investigated by constructing phase diagrams for the ternary mixtures THF:water:1,2-decanediol, THF: aqueous NaCl (1M):1,2-decanediol and isopropanol:water:1,2-decanediol. For this

purpose, the components of each ternary mixture were added at different percentages (w/w %) in centrifuge tubes, magnetically stirred (1000 rpm, 30 min) and centrifuged (3000 g, 5 min). Region boundaries in the phase diagrams for SUPRAS formation were assigned by visual inspection (i.e. existence of an isotropic solution, two liquid phases or solid-liquid phases).

The chemical composition of the SUPRASs was determined as follows. The water content was measured in a coloumetric Karl Fischer titrator from Metrohm (Herisaus, Switzerland). The percentage of 1,2-decanediol was calculated by weighting a SUPRAS aliquot of 200 µL before and after evaporation until obtaining a dry residue that corresponded to the amount of amphiphile. Finally, the THF or isopropanol content in the SUPRAS was calculated by difference.

The SUPRAS volumes obtained as a function of the composition of the ternary mixture of the bulk synthesis solution were measured in centrifuge tubes with narrow necks designed by authors. Pobel S.A. (Madrid, Spain, web page: www.pobel.com) constructed them from commercial heavy-duty glass cylindrical centrifuge tubes with round-bottom but reducing the diameter from a specified height. The measures were as follows: bottom: 34 mm of outside diameter and 57 mm height, neck: 8 mm of internal diameter and 35 mm height. Their capacity was between 41 and 42 mL. The volumes of SUPRASs were calculated by measuring their height in the cylindrical neck ($\pi \cdot r2 \cdot h$) of the centrifuge tubes with a digital caliper. Non-linear regression was used to fit a model for the prediction of the volume of SUPRAS within the SUPRAS region of analytical interest. The design of the model was carried out by using the statistical program Statgraphics Centurion XV.All experiments were performed in duplicate.

The presence of coacervate droplets in the SUPRAS was investigated with a light microscope (Leica model DME; Wetzlar, Germany) equipped with an automatic photocamera, using the bright field.

Investigation of the nanostructures in the SUPRAS was undertaken by scanning electron microscopy (SEM). For this purpose, the SUPRAS (~10 µL) was fixed with glutaraldehyde and then it was embedded with a 6% aqueous agarose solution. After that, the sample was washed three times with sodium cacodylate, and stained with OsO_4 (1%) for contrast enhancement. Samples were then dehydrated with a graded series of acetone (30, 50, 70, 80, 90, 100 %) and then they were dried using the critical point drying. Finally, samples were coated with gold and observed under SEM. The accelerating voltage was set at 10 kV.

2.3. Determination of PFCs in environmental waters

2.3.1. Samples

Water samples (reservoir, rivers and well) were collected in Córdoba province (South of Spain) in March 2021. The reservoir water was taken in La Breña, located in Almodóvar del Río village. River waters were collected in the Guadalquivir, flowing by Córdoba city, and Guadiato, located in a protected natural area close to Trassierra village (Baños de Popea), which has frequent hiking activity. The well water was also located in the latter area. All samples were collected in polypropylene bottles fitted with a polypropylene screw cap, and filtered through 0.45µm cellulose acetate filters (Millipore HNWP, Bedford, MA, USA), to remove suspended particles. Then, they were stored at 4°C until analysis.

2.3.2. SUPRAS-based microextraction of PFCs

Water aliquots (36 mL) containing 1 M NaCl were transferred to specially designed centrifugation tubes with narrow necks (i.d. 8 mm, 42 mL volume) which contained 175 mg of 1,2-decanediol dissolved in 4 mL of THF. After sealing the tubes with parafilm to avoid THF evaporation, the mixtures were magnetically stirred for 15 min at 1,000 rpm, and then they were centrifuged for 5 min at 3,000 g to speed up the separation of SUPRAS from the aqueous solution. The supramolecular extracts (about 230μL) were standing at the top of the solutions in the narrow necks of the tubes. An aliquot of 200 μL of each extract was withdrawn with a microsyringe, and it was transferred to a sealed glass vial with insert for subsequent LC-MS/MS analysis. **Figure S2** in SI shows a schematic of the SUPRAS-based extraction procedure. All experiments were done in triplicate.

2.3.3. Quantification of PFCs by LC(ESI-)MS/MS

All PFCs were separated and quantified using a liquid chromatograph (Waters, Acquity H-Class, Milford, MA, USA) coupled to a hybrid triple quadrupole/linear ion trap (Applied Biosystems MSD Sciex, 5500QTRAP, Four Valley, ON, Canada) equipped with a TurboIonSpray (TIS) interface. Separation was carried out on a phenyl-hexyl analytical column (100mm x 2mm, particle size: 3μm) from Phenomenex Luna (USA). An additional LC column (Agilent Eclipse Plus C8, 5 μm, 4.6 mm × 50 mm) was inserted between the pump and the injector in order to trap possible PFCs released from the instrument. All data were controlled and processed using the Analyst 1.6.2 Software. The mobile phase consisted of a mixture of (A) 70% water and 30% methanol containing 2 mM ammonium acetate and (B) 2 mM ammonium acetate in methanol. The flow rate was 250 μL min^{-1} and the injection volume was 5 μL. The column temperature was set at 30ºC. The elution program was as follows: isocratic conditions at 100% of A for the first

minute and linear gradient from 0% to 100% of B in 12 min. These isocratic conditions were maintained during 7 min. Finally, initial conditions were re-equilibrated for 10 min. Negative ionization mode was used for all PFCs. The most abundant fragment was used as quantifier ion while the second served as qualifier ion. Products ions and detection parameters of each MS/MS transition are shown in Table **S1**. Optimal source settings were the following: source gas temperature 400°C, capillary voltage -4500 V, nebulizer gas pressure 50 psi and curtain gas pressure 40 psi.

3. Results and discussion

3.1.Synthesis and characterization of 1,2-decanediol-based SUPRASs

Among double headed alcohol-based amphiphiles, 1,2-decanediol was selected for SUPRAS formation because the length of its hydrocarbon chain is at an intermediate point in the range of the target PFCs (i.e. C_4-C_{18}). Colloidal suspensions of this amphiphile were obtained in both a protic (isopropanol) and an aprotic (THF) solvent and coacervation was investigated by the addition of water, a poor solvent for 1,2-decanediol.

3.1.1. *Phase diagrams*

Ternary phase diagrams for the mixtures 1,2-decanediol-THF-water and 1,2-decanediol-isopropanol-water are depicted in **Figures 1A and 1B**, respectively. Both ternary plots exhibited three different domains that were set by visual inspection: (*i*) the SUPRAS region, (*ii*) the isotropic solution domain at low and medium content of water, and (*iii*) the amphiphile precipitation region at low organic solvent content. SUPRASs in THF:water formed in a broader region than those prepared in isopropanol:water and, in

addition, the minimum percentage of THF required for coacervation (i.e. 8.5%, w/w) was lower than that of isopropanol (i.e. 10.1%, w/w).

This behavior was similar to that occurring in the coacervation of alkylcarboxylic acids in organic solvent:water media and it has been attributed to the different solvation ability of solvents for the amphiphile which can be guided by their Hildebrand solubility parameter (δ) [27]. Thus, solvents having lower δ value ($\delta_{THF} = 9.5$ cal$^{1/2}$cm$^{-3/2} < \delta_{Isopropanol} = 11.5cal^{1/2}cm^{-3/2}$) will have higher solvency for the amphiphile, and consequently, the coacervation will occur at less percentage of organic solvent. On the other hand, the minimum concentration of amphiphile required to observe coacervation was 0.25 % (w/w) in both THF:water and isopropanol:water media.

The influence of salts on the coacervation of 1,2-decanediol in THF:water was further investigated. Salts have long been known to influence the temperature-induced coacervation of non-ionic surfactants in water solutions, their effects being concentration-dependent [28]. Thus, salts destruct the hydration layer of amphiphile head groups at low concentrations (e.g. around 0.1-0.4 M), which causes decrease in the effective area per molecule at the interface and the corresponding increase in surfactant monomer packing and aggregate growth. At high salt concentration (e.g. 1-2 M), most of the water is captured at the ion hydration spheres and *salting out* occurs [29].
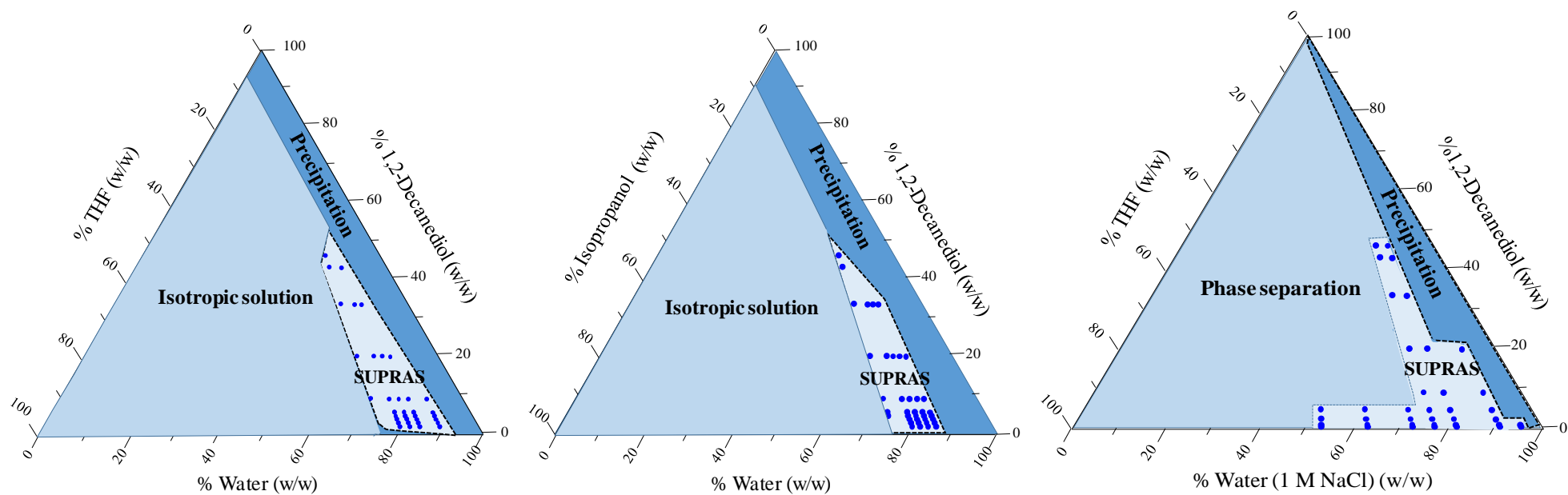
Figure 1. *Ternary phase diagrams for (A) 1,2-decanediol:water:THF, (B) 1,2-decanediol:water:isopropanol and (C) 1,2-decanediol:water (1M NaCl):THF mixtures. All ingredient concentrations are expressed as percentages (w/w).*

Figure 1C shows the phase diagram obtained for the ternary system 1,2-decanediol:THF: aqueous NaCl (1M). Addition of salt caused the broadening of the SUPRAS region and the solvent formed in a wider range of THF:water compositions, which suggests that salting out was an additional mechanism helping to 1,2-decanediol coacervation. The most valuable asset obtained by the addition of salt was the decrease in the minimum concentration required for coacervation of both amphiphile (0.125%, w/w) and THF (4.4%, w/w). This decrease is beneficial not only from an analytical point of view (i.e., higher analyte concentration factors can be achieved), but also with a view towards the environmental sustainability of the sample treatment process. It is worth mentioning that unlike the phase diagram for 1,2-decanediol in THF:water (Fig. 1A), there was not an isotropic region in the presence of salt, but a phase separation of THF owing to a salting-out effect, a phenomenon previously reported by our research group [30].

Given that SUPRAS formation in THF, both in the absence and presence of salt, was more favorable compared to isopropanol, we decided to further investigate the two first ones for determining their characteristics for PFC extraction.

3.1.2. SUPRAS volume

The volume of SUPRAS produced in the colloidal system was a function of both the concentration of amphiphile and the organic solvent. This volume increased linearly with the amount of 1,2-decanediol, independently of the concentration of THF, or the absence or presence of salt in the colloidal system (**Figure S3A and B, in SI**). On the other hand, the volume of SUPRAS varied linearly and exponentially with THF in the absence (**Figure S3C**) and presence (**Figure S3D**) of sodium chloride, respectively.

General equations were derived using a multifactorial non-linear regression model to predict the volume of SUPRAS produced as a function of composition of the colloidal system under conditions of analytical interest (i.e., low concentration of THF and amphiphile). The equations for the systems THF:water and THF:water:NaCl are displayed in Supporting Information.

3.1.3. SUPRAS chemical composition

The chemical composition of SUPRASs produced at increasing percentages of THF is shown in **Table 1**. In all cases, water contents were around 30% (w/w) and kept almost invariable while changing the THF/water ratio in the synthetic solution. This was a differential feature of decanediol-based SUPRASs compared to those based on 1-decanol, where the water content was lower and dependent on the THF:water ratio in the synthetic mixture (i.e., water content ~4-21 % w/w) [10]. This differential feature seems to be related to the higher hydration degree of the head of the surfactant as consequence of the presence of an extra –OH group. Furthermore, aggregates made up of double-headed surfactants are expected to be more open, as described in the Introduction, thus favoring the interactions with water molecules.

As can be seen in Table 1, the content of THF in SUPRAS increased (14-38% w/w) as the organic solvent increased in the synthetic solution. When NaCl was present, values for THF varied in a wider interval (6-49% w/w). The concentration of the amphiphile also changed accordingly. This means that SUPRAS composition was environment dependent and that it could be tuned for obtaining SUPRAS with different solubility properties to maximize recoveries for each analytical application.

Table 1. Chemical composition and percentage of incorporation of 1,2-decanediol into SUPRASs at representative experimental conditions

Synthetic mixture, (%, w/w)			[a]SUPRAS composition, (%, w/w)			% 1,2-decanediol in SUPRAS
1,2-decanediol	THF	Water	1,2-decanediol	THF	Water	
3.0	8.6	88.4	54.5±0.2	14±2	31±4	99.1±0.4
3.0	13.0	84.0	44.7±0.7	21±2	34±3	93.2±0.5
3.0	17.5	79.5	36.3±0.1	31±1	33±1	84.3±0.1
3.0	22.0	75.0	29.2±0.4	38±1	32.5±0.9	82±1
1,2-decanediol	THF	Water (NaCl 1M)	1,2-decanediol	THF	Water (NaCl 1M)	
4.9	8.6	86.5	59±1	6.0±0.5	35±2	99±2
4.9	17.3	77.8	38±1	32±1	30±1	96±3
4.9	21.8	73.3	30.6±0.7	39±4	30.1±0.2	100±4
4.9	26.3	68.8	23.4±0.3	49±2	27.61±0.01	99±5

[a]Mean values of three replicates ± standard deviation.

The amphiphile was almost fully incorporated into the SUPRAS phase in a wide range of compositions, being the incorporation more favourable in NaCl-containing aqueous solutions (Table 1). This behaviour is logical considering the salting out effect for the amphiphile and the consequent reduction in the *cac* value. So, under salty conditions, and at low THF/water ratios in the absence of salt, the synthesis occurred through an energy-saving process (spontaneous coacervation at room temperature) that had a high-atom economy (1,2-decanediol incorporated entirely into the SUPRAS).

3.1.4. SUPRAS structure

Figure **2A** shows a representative optical micrograph obtained from the synthesized SUPRASs. Optical microscopy studies clearly revealed that the liquid phases here reported were not continuous but made of coacervate droplets within the interval 4-16 µm (mean value 14 µm). So, as expected, these liquid phases were produced through self-assembly and coacervation.

The morphology of SUPRAS aggregates was investigated with SEM according to the procedure specified in section 2.2. **Figure 2B** shows a representative image for SUPRASs formed in THF-water-salt. Similar images were obtained for the SUPRASs in the absence of salt (data not shown). This figure clearly shows that the SUPRAS lacked any well-ordered internal structure and consisted in a random 3D amphiphile bilayer network separating water pores, which is consistent with the characteristics of a sponge phase [20-24]. An illustration of the porous and bilayer-rich nanostructure is given in Figure **2C**. Magnification of the SEM micrographs (Figure **2D**) showed typical features of the sponge morphology [31]; ellipsoidal structures nearly flat and surrounded by a network of curved areas with a smooth appearance. In different sections of this micrograph it was possible to follow the bilayer over large distances (several micrometers range), which is an indication that the bilayer was indeed continuous [31]. In concordance with the previously reported sponge coacervates, which were made up of ternary systems made up amphiphile-organic solvent-water (with and without salt) [20,31,32], it is probable that THF is incorporated at the SUPRAS hydrophobic region while water flows through the pores. This assumption is in good agreement with the nearly constant percentage of water into the SUPRASs (Table 1). The high water content, along with the double-headed groups of 1,2-decanediol provide a broad hydrophilic region for solubilization of polar compounds.
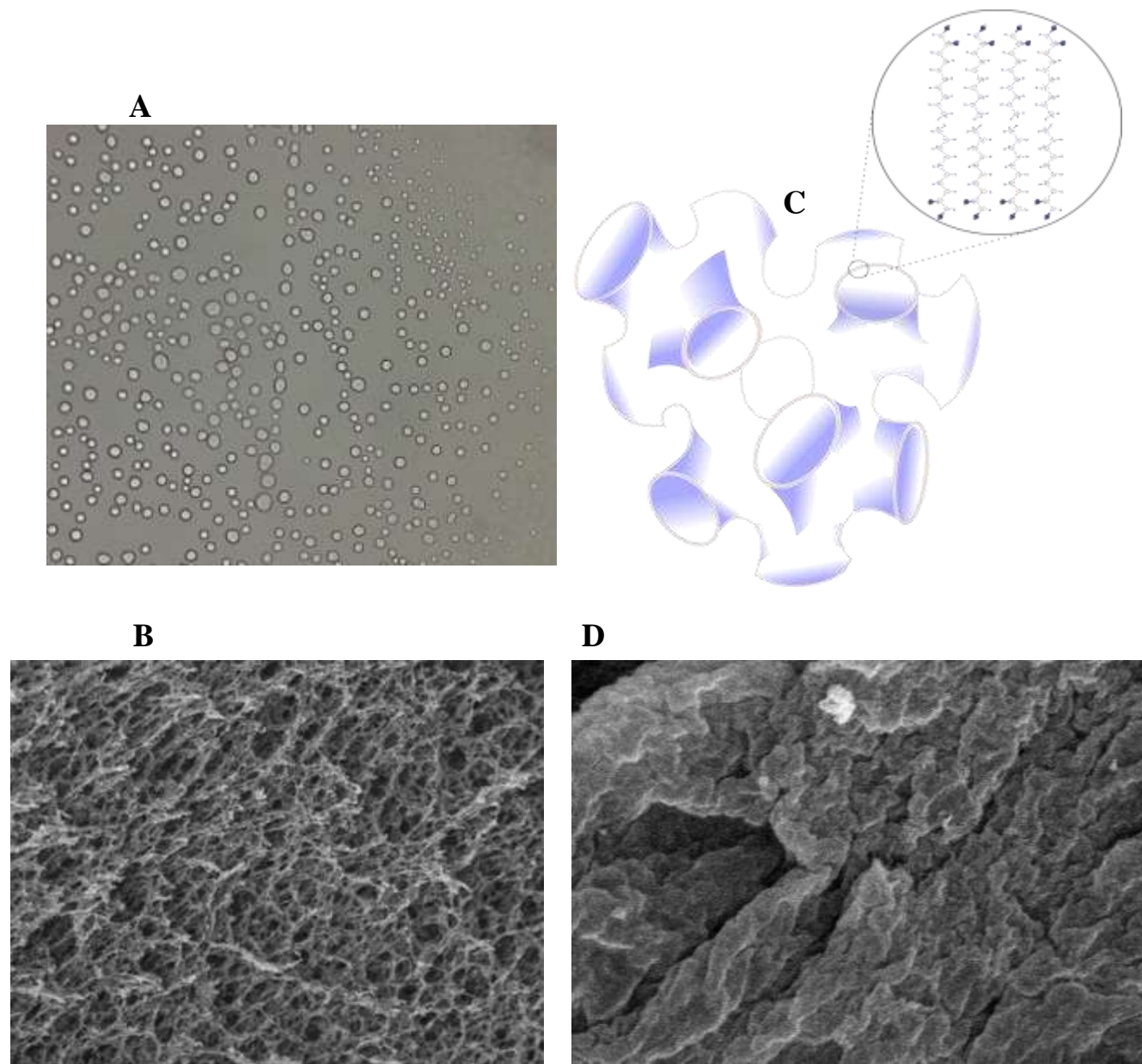
A

C

B

D

Figure 2. (A) Micrograph of a SUPRAS using light microscopy in the bright field (magnification: 20X). (B) SEM micrograph of a SUPRAS at the magnification 400X. (C) Illustration of the sponge morphology and magnification of the 1,2-decanediol bilayer. (D) SEM micrograph of a SUPRAS at the magnification 4.97 KX. SUPRAS were obtained from 1,2-decanediol in 10% THF (v/v) and 1M NaCl.

3.2. Extraction of PFCs from water by sponge droplets of 1,2-decanediol

In order to check our working hypothesis (*the recoveries of compounds in a wide polarity range should improve with the use of SUPRASs featuring increased hydrophilicity*), we compared the results obtained for the extraction of perfluoroalkyl sulfonates (C_4-C_{10}) and perfluoroalkylcarboxylates (C_5-C_{18}), with log P_{ow} values from 0.4 to 11.6, with SUPRASs made up of 1,2-decanediol and 1-decanol. As described in

previous sections, the first one consists of a double-headed amphiphile arranged in sponge droplets, while the second one is made up of a single-head amphiphile arranged in inverse hexagonal aggregates [10]. Both SUPRASs were synthesized in THF-water media under the same experimental conditions (150 mg of amphiphile, 4 mL of THF and 36 mL of Milli-Q water spiked with 9 ng L^{-1} of each PFC). **Figure 3A** shows that the recoveries for PFCs greatly improved for 1,2-decanediol compared to 1-decanol. Thus, they were above 60% for PFSAs and PFCAs in the range C_8-C_{10} and C_6-C_{18}, respectively. Under the same conditions, the applicability of 1-decanol-based SUPRAS was much more limited for both most polar and most apolar PFCs, i.e., recoveries above 60% were found only for PFCAs with carbon atoms in the range 12-16 and were always more than 10% lower than those obtained for 1,2-decanediol-based SUPRAS.

The extractability of PFCs with the use of sponge droplets synthesized from 1,2-decanediol-THF-water and 1,2-decanediol-THF-water (1M NaCl) was also investigated. SUPRASs were synthesized by adding 36 mL of Milli-Q water spiked with 9 ng L^{-1} of each PFC, both in the absence and presence of NaCl to 4 mL of THF containing 250 mg of 1,2-decanediol. The results are shown in Figure **3B**. Recoveries for PFCs improved in around 10-20% for SUPRASs that were synthesized in the presence of NaCl due to the salting-out effect. Under these conditions, recoveries were above 70% for all the compounds except for the most polar PFPeA (39±1%) and PFBS (50±5%).
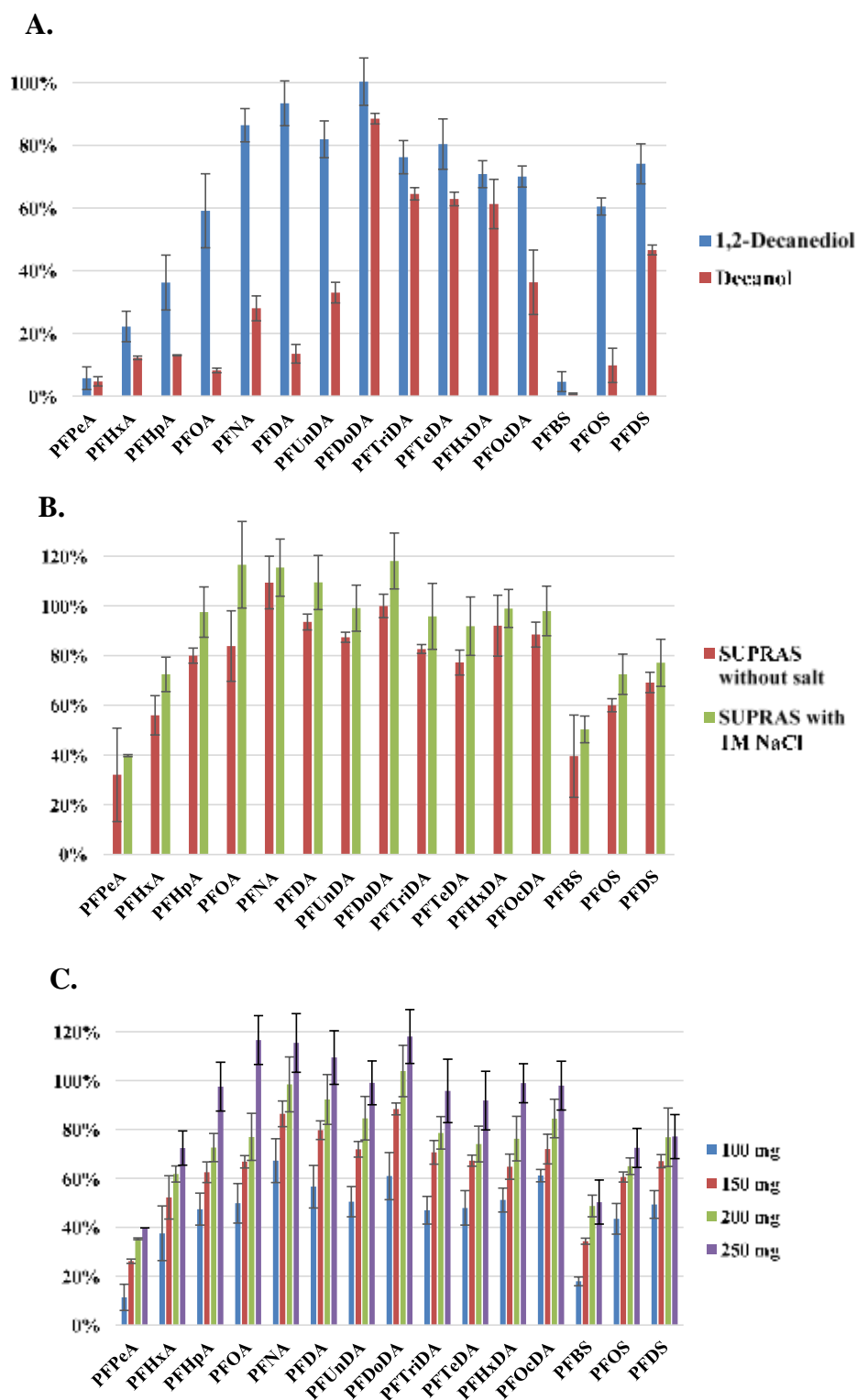
Figure 3. *Recoveries ± SD (in %) of PFCs extracted with SUPRAS formed from (A)1-decanol and 1,2-decanediol in THF-water; (B) 1,2-decanediol in THF-water and THF-aqueous NaCl (1M); and (C) 1,2-decanediol in THF-aqueous NaCl. Water sample: 36 mL spiked with 9 ng L^{-1} of each PFC. Amount of amphiphile: (A) 150 mg; (B) 250 mg. THF: 10%.*

The influence of the amount of amphiphile employed for the synthesis of the sponge droplets on the extractability of PFCs was investigated by synthesizing SUPRAS from 100, 150, 200 and 250 mg of 1,2-decanediol. For this purpose, the amphiphile was dissolved in 4 mL of THF and it was added to spiked Milli-Q water samples (36 mL, 9 ng L^{-1}) containing 1 M NaCl. Given the low concentration of PFCs in most of natural waters (at the low ng L^{-1} level), selection of the optimal conditions was guided by concentration factors for PFCs, in addition to recoveries. **Figure 3C** shows the results. The SUPRAS volumes generated for the tested amounts of amphiphile were 106±3, 190±4, 275±4 and 360±7 µL for 100, 150, 200 and 250 mg of 1,2-decanediol, respectively, which gives theoretical concentration factors of 340, 189, 131 and 100, respectively. As expected, absolute recoveries of PFCs progressively increased as the amount of 1,2-decanediol did. Thus, only 2 out of 15 PFCs were out the range of what are generally considered acceptable mean recoveries (70-120%) with 250 mg of 1,2-decanediol. Finally, we proposed the use of 175 mg of 1,2-decanediol for the extraction of PFCs, as a compromise between absolute recoveries, which were below 60% only for three of them (i.e. PFPeA 31.1±0.9, PFHxA 57±1 and PFBS 41±1), and concentration factors, which were between 54 and 174 without the need for evaporation steps.

3.3. Analytical performance

Calibration curves were prepared by extracting 36 mL of Milli-Q water, fortified with PFCs in the initial range 0.05-10 ng L^{-1}, according to the procedure specified in the section materials and methods. Deuterated internal standards (10 ng L^{-1}, Table S1) were added to the SUPRAS extracts before measurement. Calibrations were adjusted to linear functions ($y_i = b_1 x_i + b_0$) being b_1 and b_0 the estimates of the slope and the intercept, respectively. Study of the linearity in the calibration range was performed through different statistical

graphical approaches (i.e. visual inspection of the calibration plot, the residuals plot, the response factors plot and the representation of percent relative errors of back-calculated concentrations against the concentration of calibration standards) [33]. The analysis of these plots showed that the predicted concentrations of perfluorinated compounds at 0.05 and 0.075 ng mL^{-1} had associated errors above 20%. So, these points were removed and new calibration curves were constructed for the interval 0.1-10 ng mL^{-1}. **Table 2** shows the values obtained for the slopes and intercepts, along with the respective standard deviations, for each of the perfluorinated compounds investigated. Correlation coefficients were in the interval 0.988-0.99. The different statistical graphical approaches used for the analysis of linearity of these calibration curves are shown in **Figure S4.**

Studentized residuals mostly appeared normally distributed and centered around zero and they were between around ±2 limits over the whole calibration range, thus indicating that the selecting functions fitted well the data. The response factors showed that sensitivity at the lowest concentrations was usually different compared to the highest ones, which occurs in a number of real situations [33]. However, the relative errors of the back-calculated concentrations were distributed within the calculated acceptance limits (± 15%), except for some concentrations, for which the error did not exceed ± 20%. Taking into account these results, linear response can be assumed within the calibration range proposed (0.1-10 ng mL^{-1}) according to the fit for its purpose.

Method detection (MDL) and quantification (MQL) limits were calculated considering a signal to noise ratio of 3 and 10 and were in the range 0.01-0.02 and 0.04-0.06 ng L^{-1}, respectively.

Table 2. Analytical performance of the proposed method for the determination of PFCs in natural waters

Calibration parameters	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFTeDA	PFHxDA	PFOcDA	PFBS	PFOS	PFDS
Intercept ± SD	0.02 ±0.03	0.9 ±0.3	0.9 ±0.6	0.4 ±0.3	-1.5 ±0.7	-0.07 ±0.13	-0.6 ±0.3	-0.006 ±0.005	0.2 ±0.2	0.08 ±0.05	-0.03 ±0.02	0.013 ±0.006	0.02 ±0.02	-0.06 ±0.02	-0.012
Slope ± SD $(ng^{-1}L)$	1.026 ±0.006	4.39 ±0.06	17.1 ±0.1	4.97 ±0.07	7.6 ±0.2	2.77 ±0.03	8.19 ±0.07	0.072 ±0.001	3.45 ±0.04	0.68 ±0.01	0.475 ±0.004	0.067 ±0.001	0.149 ±0.005	0.243 ±0.005	0.024 ±0.001
Correlation coefficient	0.999	0.997	0.999	0.998	0.995	0.998	0.999	0.998	0.998	0.998	0.999	0.995	0.988	0.995	0.989
Intra-day precision (RSD %)															
Guadalquivir river	5	5	13	13	8	6	17	3	3	3	3	3	8	5	5
La Breña reservoir	2	2	10	10	5	4	7	5	5	5	5	5	5	8	8
Guadiato river	10	10	15	17	5	7	16	7	7	7	7	7	1	8	8
Well	10	10	17	19	9	5	8	4	4	4	4	4	5	6	6

The selectivity for the quantification of PFCs was roughly assessed by comparing the recoveries of the corresponding standards extracted from Milli-Q water and four natural water samples spiked at a concentration of 5 ng L^{-1}. The results obtained are shown in **Figure S5**. Recoveries for all PFCs were quantitative (in the range 90-118%; median 100%; mean 100.9%) thus indicating the absence of matrix effects.

Intra-day precision, expressed as relative standard deviation (RSD), was calculated from the analysis in triplicate of four natural water samples fortified with 5 ng L^{-1} of PFCs, by following the procedure described in the section materials and methods. Results are also shown in Table 2. In all cases, RSD values were between 1 and 19% (median 6%, mean 7%). Inter-day precision was evaluated from the analysis of a river water sample, fortified with 5 ng L^{-1} of PFCs and analyzed during three days, each by duplicate. RSD values for inter-day precision were in the range 4-16%.

Table 3 compares different extraction and quantification features of representative LC-MS/MS methods reported for PFCs with those obtained for the method based on sponge droplets [34-39]**.** Criteria for the selection of these methods were their applicability to both carboxylate and sulfate PFCs in natural waters, the use of LC-MS/MS for PFC determination, and their publication date (i.e. only those published within the last three years were considered). Additionally, the only previously reported method based on SUPRAS was included [39]

According to the data shown in Table 3, the extraction of PFCs from waters is mainly done with SPE owing to the inefficiency of water-immiscible organic solvents to carry out liquid-liquid extractions. This inefficiency arises from the broad polarity range and amphiphilic character of these compounds. Because of the low concentration at which PFCs are present in natural waters [40-42], the study of their occurrence in these matrices requires very sensitive methods, which demands for high concentration factors. Thus,

treatment of 1 L of sample is common [34-36], which takes several hours (5.6 to 8.5 h) excluding the evaporation steps. Although the use of lower water volumes with SPE have been also proposed [37,38], these methods are not sensitive enough for application to low-contaminated environmental waters. Recoveries for PFC methods based on SPE are usually within the acceptable range (70-120%), however the use of high water volumes in SPE may cause the loss of the strongly retained PFCs which results in decreased recoveries (e.g. [35,36]). Extraction with SUPRAS is simpler, faster and cheaper, as previously proved for SUPRASs based on dodecanol [39], however the high detection limits reported (10-80 ng L^{-1}) makes it non applicable to studies in natural waters. The method here reported is highly sensitive (LODs in the range 0.01-0.02 ng L^{-1}) while keeping the excellent operational features of SUPRAS for application in the determination of the distribution of PFCs in environmental waters.

Regarding greenness and sustainability of PFC methods, we have not data about how green and sustainable is the process of sorbent production, so we only may compare the organic solvent consumed during sample treatment. Thus, except for μ-SPE [38], between 2- and 13-fold more organic solvent was consumed per sample treated compared to SUPRAS. With regard to cost, conventional LLE is usually much cheaper than SPE and this also applies to SUPRAS.

Table 3. Comparison of the method developed in this work with LC/MS-based methods reported in the literature in the last three years for the determination of PFCs in natural waters

Compounds	Sample type and Volume	Sample Treatment	Organic Solvents and Solutions Involved in Sample Treatment	Separation, Detection, Calibration	[a]Extraction time per sample	Linearity ng L^{-1}	LOD ng L^{-1}	Sample to Solvent Extract Volume Ratio	Relative Recovery (%)	Ref.
7 PFCAs (C$_4$-C$_{10}$) and 3 PFSAs (C$_4$, C$_6$, C$_8$)	River water (1000 mL)	• SPE (Oasis HLB) • Evaporation Reconstitution	• Methanol (15 mL)	UPLC-MS/MS (Isotopically labelled IS)	5.6 h	0.025-2	0.01-0.14	500	87-102	(34)
11 PFCAs (C$_4$-C$_{14}$) and 5 PFSAs (C$_4$, C$_6$,C$_7$,C$_8$,C$_{10}$)	Seawater (1000 mL)	• SPE (Oasis HLB) • Evaporation • Reconstitution	• Methanol (27 mL)	LC-MS/MS (Isotopically labelled IS)	8.5 h	0-25000	0.0046-0.16	1000	54-117	(35)
8 PFCAs (C$_4$-C$_6$, C$_8$, C$_{13}$) and 3 PFSAs (C$_4$, C$_6$, C$_8$)	River water (1000 mL)	• SPE (Oasis WAX) • Evaporation • Reconstitution	• A solution of NH$_4$OH/CH$_3$OH (0.5%, v/v) (4 mL) • Methanol (4 mL)	LC-MS/MS (Isotopically labelled IS)	5.6 h	200-50000	0.03-0.2	1000	61-118	(36)
10 PFCAs (C$_4$-C$_{13}$) and 4 PFSAs (C$_4$,C$_6$, C$_8$, C$_{10}$)	Superficial/ underground water (5 mL)	• Online SPE (Oasis WAX) • Evaporation • Reconstitution	• Water with NH$_4$OH (0.05%, v/v) (24 mL) • Methanol with NH$_4$OH (0.05%, v/v) (52.8 mL)	LC-MS/MS (Isotopically labelled IS)	12 min	0.2-250	0.2-5	1	80-120	(37)
10 PFCAs (C$_4$-C$_{12}$, C$_{14}$) and 3 PFSAs (C$_4$, C$_6$, C$_8$)	Surface water (2 mL)	• μ-SPE • Evaporation • Reconstitution	• 10 mM NaOH in methanol (250 μL) • Methanol (250 μL) • 1% Acetic acid (250 μL) • 10 mM NaOH in methanol (100 μL)	LC-MS/MS (Isotopically labelled IS)	5 min	10-9000	0.3-6.6	1	86-111	(38)
12 PFCAs (C$_4$-C$_{14}$) and	Drinking, tap and river	• Dodecanol-based SUPRAS microextraction	• Tetrahydrofuran (1 mL) • Dodecanol (250 μL)	LC-Orbitrap (Matrix-matched calibration)	1 min	500-500.000	10-80	20	73-118	(39)

6 PFSAs (C$_4$ - C$_{10}$)	water (20 mL)		• NaCl (4 g)							
12 PFCAs (C$_5$-C$_{18}$) and 3 PFSAs (C$_4$, C$_8$, C$_{10}$)	River, reservoir and well water (36 mL)	• Double Head Amphiphile-Based Sponge Droplet microextraction	• Tetrahydrofuran 4 mL	LC-MS/MS (Isotopically labelled IS)	15 min	0.1-10	0.01-0.02	174	90-118	This work

[a]Only the time required for SPE or LLE is considered (evaporation, centrifugation, etc. is not included)

3.4.Determination of PFCs in natural waters

Four natural water samples collected in the province of Córdoba, South of Spain (i.e., La Breña reservoir, a well located in Trassierra and Guadalquivir and Guadiato rivers) were analyzed in triplicate in order to validate the applicability of the proposed method. **Table 4** shows the results obtained and **Figure 4** shows the extracted ion chromatograms of the PFCs found in the Guadalquivir river sample.

Except for the sample collected in the well, all the target PFCs were found in the samples at the low ng L^{-1} level. In all cases, excepting for la Breña reservoir, concentrations for PFCAs were lower than those for PFSAs. Values for PFCs were lower in Guadiato river (natural park location) and especially in the well water. On the contrary, higher concentrations of PFCs were found in water collected from Guadalquivir river (0.17-2.33 ng L^{-1}), which flows by Córdoba city and it is more exposed to industrial contamination and in La Breña reservoir (0.11-0.74 ng L^{-1}), in which recreational activities such as fishing and boating are common. Levels were consistent with the values generally found in the literature (0.01-5 ng L^{-1}) [40-42].

Table 4. Concentrations ± SD (*n*=3) of PFCs in natural waters (ng L^{-1})

| Sample	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFTeDA	PFHxDA	PFOcDA	PFBS	PFOS	PFDS
Guadalquivir river	0.30±0.03	0.53±0.03	0.17±0.02	0.44±0.04	0.38±0.02	0.34±0.03	0.39±0.01	0.18±0.02	0.45±0.05	0.48±0.02	0.44±0.04	0.15±0.01	2.33±0.02	0.97±0.10	0.41±0.06
La Breña reservoir	0.32±0.05	0.73±0.10	0.11±0.01	0.4±0.04	0.61±0.02	0.60±0.03	0.67±0.07	0.13±0.02	0.74±0.08	0.65±0.07	0.20±0.03	0.19±0.02	0.24±0.03	0.22±0.03	0.38±0.01
Guadiato river	0.07±0.01	0.18±0.02	0.09±0.01	0.16±0.01	0.18±0.03	0.15±0.01	0.17±0.02	0.13±0.02	0.14±0.01	0.15±0.02	0.17±0.02	<LOD	0.48±0.05	0.41±0.04	0.59±0.09
Well	<LOD	<LOD	0.15±0.02	<LOD	<LOD	<LOD	<LOD	0.08±0.01	<LOD	<LOD	<LOD	0.15±0.02	<LOD	0.22±0.06	0.40±0.01
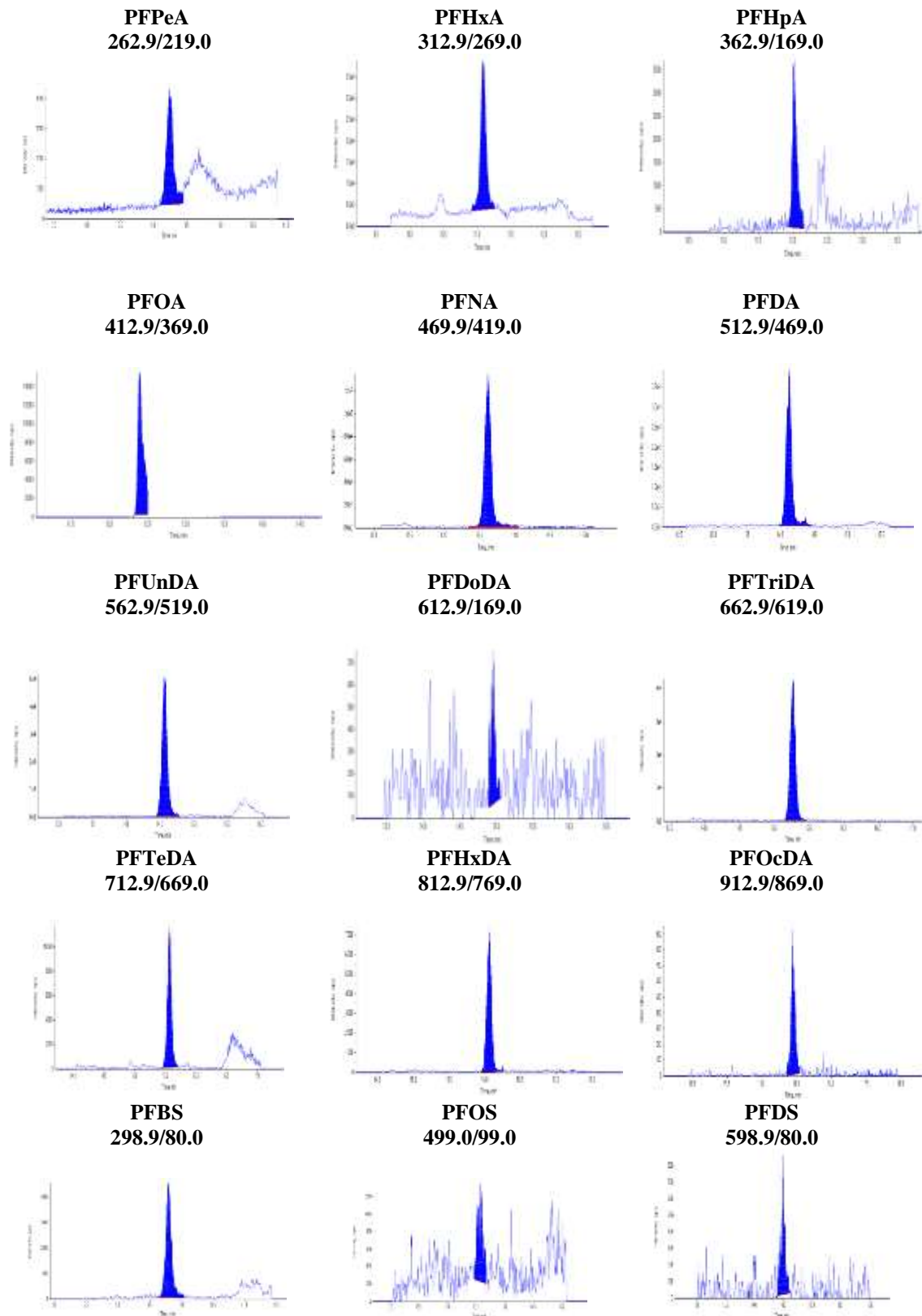
Figure 4. *Extracted ion chromatograms of detected PFCs (quantifiers) in the sample for the river Guadalquivir.*

Conclusions

By the selection of double-headed amphiphiles and water as coacervation-inducing agent, SUPRASs with extended hydrophilic regions in their nanostructures have been tailored. Combination of both strategies has resulted in high water content sponge droplets that feature increased recoveries for compounds over a wide polarity range, such as PFCs from waters, here selected as analytes for a proof of concept study. Sponge droplets are synthesized through spontaneous and energy-saving self-assembly processes that have high atom economy (practically all the amphiphile is incorporated into the SUPRAS), thus fitting the green chemistry synthetic principles. Regarding the application developed for PFCs, some valuable analytical and operational characteristics have been obtained compared to previously reported methods. Thus, PFCs are quantified at similar sensitivity that those methods requiring the treatment of high water volumes (e.g. 1 L) but taking far less time (e.g. 15 min instead of 5.6-8.5 h) and avoiding the evaporation step. This will all result in an increased sample throughput.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Credit author statement

Soledad González-Rubio: Investigation, Writing – original draft, Writing – review & editing; Ana Ballesteros-Gómez: Supervision, Writing-Review & Editing, Conceptualization; Diego García-Gómez: Supervision, Writing-Review & Editing, Conceptualization; Soledad Rubio: Conceptualization, Project administration, Funding acquisition.

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