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Environment-Responsive Alkanol-based Supramolecular Solvents: Characterization and Potential as Restricted Access Property and Mixed-Mode Extractants

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ABSTRACT

Self-assembly, the process by which supramolecular solvents (SUPRASs) with an ordered structure are produced, provides unique opportunities to obtain tailored solvents with advanced functional features. In this work, environment-responsive (C_7–C_{14}) alkanol-based SUPRASs were synthesized and their potential for analytical extractions assessed. The global composition of the solvent, the size of the coacervate droplets that form it and the aqueous cavities of the inverted hexagonal arrangement of the alkanols can be tailored by controlling the environment (specifically, the THF:water ratio) for alkanol self-assembly. Interestingly, supramolecular solvents are highly adaptive and the previous features can all be reversed by modifying the environment. The spontaneous self-assembly of these solvents followed predictable routes and their composition and volume can be accurately predicted based on equations derived in this work. The solvents were structurally elucidated by light and cryo-scanning electron microscopy. Extractive applications exploiting the molecular size-based restricted access properties of SUPRASs were developed and their ability to engage in mixed-mode mechanisms for solute solubilization was established. Thus, solutes of increasing molecular weight were extracted from food and environmental samples with recoveries dependent on vacuole size in the SUPRASs, while macromolecules such as proteins, carbohydrates and humic acids were excluded. The ability of SUPRASs to establish hydrogen bonding and dispersion interactions was exploited to extract carcinogenic chlorophenols (CCPs) from environmental waters and a simple and fast method was developed with quantitation limits (e.g.0.21–0.23 μg L^{-1}) low enough to comply with legislation (e.g. maximum permitted levels for pentachlorophenol are in the range 0.4–1 μg L^{-1}).

INTRODUCTION

Tailored solvents represent a wholly new paradigm in manufacturing and processing.[1] Designing and producing solvents that meet programmed, specific requirements for a particular purpose greatly increases our ability to improve process economics, selectivity and yield. Revolutionary applications of these solvents have been developed in areas such as chemistry, chemical engineering, biotechnology, coating, energy, extraction[2] and various others in the last decade by using ionic liquids tailored by judiciously modifying the length and degree of branching of the alkyl chain and anionic precursor. Additional chemical tunability can be obtained with supercritical solvents (especially carbon dioxide containing appropriate additives)[3] and fluorous phases.[4]

Self-assembly, the process by which isolated components organize autonomously and spontaneously into ordered and/or functional structures, has become one of the most widespread and powerful strategies for the production of designed structures from components with sizes ranging from the molecular to the macro scale.[5] Self-assembly processes are giving access to advanced functional supramolecular materials (e.g. supramolecular polymers,[6] liquid crystals,[7] solid-state assemblies[8]) and providing an original approach to nanoscience and nanotechnology[9] (e.g. the bottom-up approach). However, such appealing power remains virtually unexplored for the production of tailored solvents although self-assembly has already proved an invaluable strategy for the energyless synthesis of ordered structure-based solvents from amphiphiles.

Ordered structure-based solvents have long been known in the fields of colloid and analytical chemistry in relation to the study and applications of the phenomenon observed in liquid–liquid phase separations in heated micellar solutions of nonionic surfactants (viz. the *cloud point*).[10–13] Also, surfactant-rich liquid phases have been successfully separated from micellar and vesicular solutions of zwitterionic[14] or ionic surfactants by effect of the addition of salts,[15,16] pH changes[17] or the presence of a non-solvent for the amphiphile[18] (viz. *coacervates*). Although coacervates are also referred to as L_3, *anomalous* and *sponge* phases, it is unclear whether these terms designate the

same systems.[19] Recently, the term *supramolecular solvent* (SUPRAS) was used to emphasize that amphiphiles form self-organized supramolecular structures in the solvent, this being the most distinctive feature compared to molecular solvents and ionic liquids.[20] One unique property of SUPRASs is the presence of different polarity regions in their constituents and hence their ability to dissolve, concentrate, compartmentalize, organize and localize solutes spanning wide polarity ranges, thus providing specific reaction environments and separation media. SUPRASs have so far been successfully used in analytical extractions of metals[21,22] and organic compounds[23] from environmental, biological and food samples.

In this work, the possibility of developing tailored supramolecular solvents by self-assembly with a view to developing new, outstanding analytical applications, was for the first time explored. The self-assembly process can be controlled via four key factors (viz. component structure, environment, and binding and driving forces)[24] and that results in multiple opportunities to get tailored SUPRASs. Amphiphiles are ubiquitous in both nature and synthetic chemistry, and contain a wide variety of alkyl chains and polar head groups; therefore, tailoring their structure can be expected to provide the most immediate means of obtaining tunable solvent properties. The solvent components must be embedded in a proper environment (e.g. a solution) for binding forces to act. By tailoring or dynamically altering the environment, aggregates can be transformed and made indistinguishable from the original to obtain highly adaptive materials. Thus, the potential exists to obtain solvents with properties that can be switched reversibly at will. For ordered, self-assembled structures to form, the binding forces involved (e.g. coulomb, van der Waals, π–π and π–cation interactions, hydrogen bonding, electromagnetic or capillary forces) must be reversible —and comparable to those tending to disrupt the aggregates. Lastly, the solvent components must be mobile for self-assembly to occur. In solution, thermal noise is the driving force for molecular self-assembly; in nano, mesoscopic and macroscopic self-assembly systems, Brownian motion rapidly becomes irrelevant, and gravity and friction prevail.

This paper reports the synthesis, characterization and use in analytical extractions of environment-responsive SUPRASs consisting of aggregates of C_7–C_{14} alkanols in THF:water mixtures. The composition and structure of the resulting SUPRASs was examined in the light of the environment for alkanol self-assembly. The knowledge thus gained was used to develop specific analytical applications involving the extraction of organic compounds based on the restricted access properties of these solvents and their ability to engage in mixed-mode mechanisms for interaction with solutes. Below are described and discussed the most salient results of this study.

EXPERIMENTAL SECTION

All the experimental details concerning the research here developed can be found in Supporting Information. The following topics are described there: chemicals, materials and instruments; methods followed to establish water:THF:alkanol ternary phase diagrams and to determine the composition of the SUPRASs synthesized and characterize their macro and microstructure. Mathematical and statistical procedures involved in the development of equations to predict SUPRAS composition and volume are also included. Likewise, the analytical procedures developed to prove the molecular size-based restricted access properties of SUPRASs (i.e. microextraction of Ochratoxin A from cereal baby food and dyes from sludge) and their ability to engage in mixed-mode mechanisms for solute solubilization (i.e. determination of carcinogenic chlorophenols in surface and ground water) are there described.

RESULTS AND DISCUSSION

Alkanol-based SUPRASs were synthesized by adding water to solutions of (C_7–C_{14})-alkyl alcohols in tetrahydrofuran. Water promoted self-assembly of the alkanols and caused the spontaneous formation of oily droplets (i.e. coacervate droplets) that flocculated through the formation of conglomerates of individual droplets. The overall density of such conglomerates was slightly lower than that of the solution in which they

formed, which facilitated creaming and phase separation (coacervate phase or SUPRAS) from the bulk solution (Fig. 1A). The process occurred throughout the aqueous pH range. The alkanol-rich SUPRAS was in equilibrium with the alkanol-poor bulk solution. Both the volume of SUPRAS produced and the size of the spherical droplets forming it as determined by optical microscopy decreased with increasing proportion of water in the bulk solution (Fig. 1B). This suggested that the SUPRAS composition and structure was environment-responsive. The phenomenon was reversible and both SUPRAS composition and aggregate size were successfully changed at will by adding water or THF to the equilibrium solution.

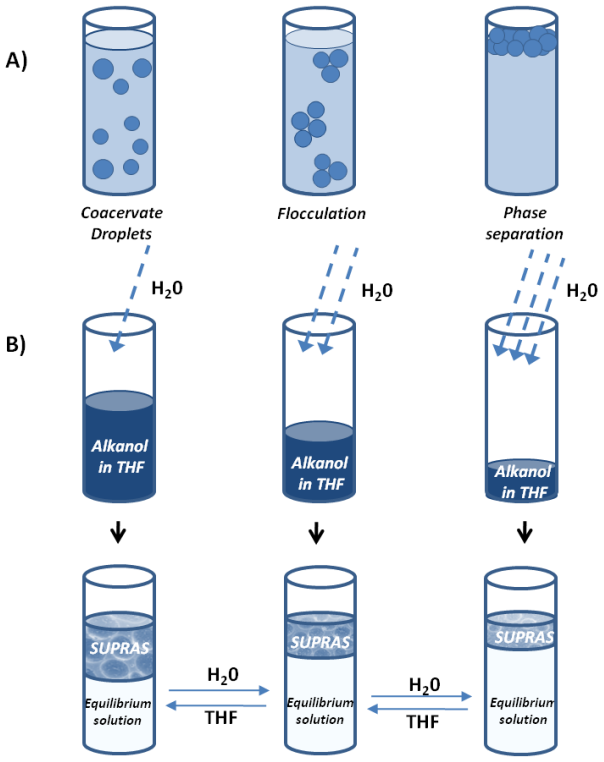

Figure 1. Schematics of (A) SUPRAS formation in the bulk solution and (B) the influence of the environment (water/THF ratio) on the size of coacervate droplets and volume of SUPRAS

Representative phase diagrams depicting the boundaries for the region where separation of two isotropic liquids from ternary mixtures of alkanol, THF and water

occurred can be found in Figure 1 of Supporting Information (S-1). Beyond these boundaries, alkanols became insoluble or gave a single isotropic solution —the latter by effect of the density of the SUPRAS and equilibrium solution approaching as the content of THF in the mixture was raised.

SUPRAS Composition Tuning

The composition of the solvents produced from various alkanols was investigated as a function of the initial composition of the self-assembling ternary mixture (alkanol, THF and water). SUPRASs consisting of surfactant, THF and water, at amphiphile concentrations between 10 and around 80% (w/w), and THF:water percent ratios (w/w) spanning a short range but dependent on the hydrocarbon chain length for each alkanol (e.g. 3.2–4.5, 3.3–6.9 and 4.8-9.2 for the C_7-, C_{10}- and C_{14}-alkyl alcohol, respectively) were obtained (see Figure S1, B as an example).

The solvent composition was not affected by the proportion of alkanol in the bulk solution up to ~5% w/w (the region of analytical interest), the volume of solvent produced being linearly dependent on it. However, altering the environment of the bulk solution led to the production of SUPRASs spanning a broad range of composition. Thus, increasing the THF:water percent (w/w) ratio from 0.05 to 1.3 caused the gradual incorporation of both THF and water into the SUPRAS, which thus became increasingly diluted with respect to the alkanol. The volume of solvent obtained increased exponentially with the content in THF of the bulk solution.

Equations were derived to calculate the proportion by weight of water and THF in the SUPRAS obtained from a specific alkanol as well as the resulting solvent volume produced (see Supporting information).These equations allow one to estimate the maximum concentration factors that can be achieved in extractions with alkanol-based SUPRASs, thus facilitating the choice of the most suitable preparation method and its optimization. Interestingly, SUPRASs of specific composition (Fig. S-1, B) can be prepared by direct mixing of their components. This can save reactants (e.g. THF) in

applications not requiring equilibration of the SUPRAS with the aqueous solution (e.g. reaction media, extractants for solid matrices).

Coacervate Droplet Size Tuning and Microstructure

Careful analysis of the alkanol-based SUPRASs by light and cryo-scanning electron microscopy (cryo-SEM) revealed that they consisted of a cluster of hexagonal droplets (Fig. 2, panels A–C), the images closely resembling those for inverted hexagonal phases in liquid crystals[25,26] (i.e. hexosomes or H_2 phases). Droplet size was found to depend on the environment of self-assembly and liquid phase separation of the alkanols. By way of example, Fig. 2 (panels D–F) shows typical light micrographs for SUPRASs consisting of decanol synthesized in environments containing variable amounts of THF. To the best of our knowledge, no SUPRASs with coacervate droplets exhibiting hexagonal structures had previously been observed.

The microscopic analysis exposed two clear facts, namely: (*a*) that the size of the coacervate droplets increased gradually with increasing proportion of THF in the bulk solution; and (*b*) that the droplets spanned very narrow size ranges (e.g. ~ 1–5, ~5–20, ~20–40, ~40–60, ~60–100 and ~100–200 μm for decanol self-assembled in environments containing 10%, 20%, 30%, 40%, 50% and 60% THF, respectively). Using dynamic light scattering (DLS) to establish the coacervate droplet size distribution failed to improve the accuracy since the concentration of alkanols in the SUPRAS ranged from 0.07 to 0.68 mg μL^{-1} and this technique requires diluted solutions to make interparticle interactions negligible and avoid multiple scattering phenomena as a result. Also, diluting the SUPRASs to lower alkanol concentrations was completely unacceptable because their constituent aggregates were environment-sensitive.

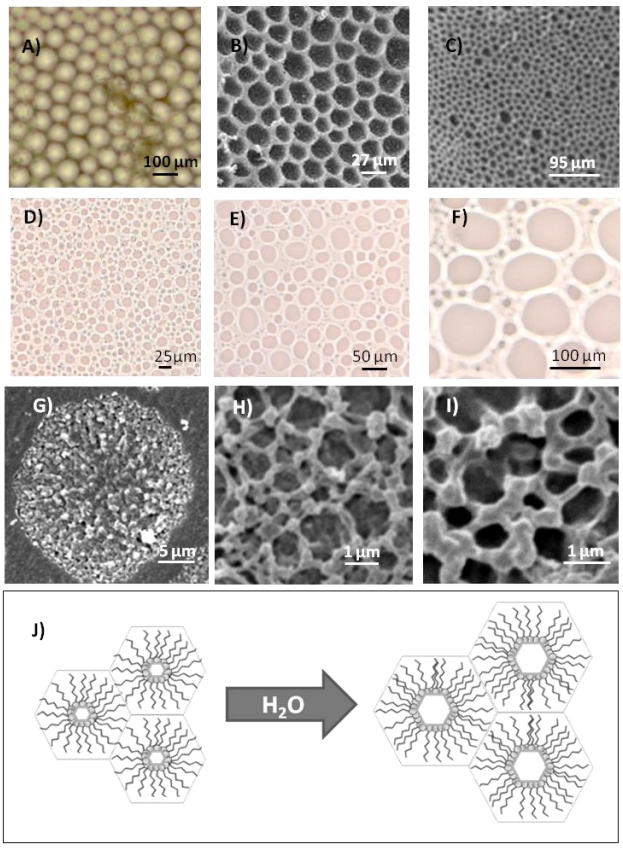

Figure 2. Micrographs of coacervate droplets showing (A–C) the hexagonal arrangement of alkanols and (D–F) the coacervate droplet size dependence on the self-assembly environment. Micrographs showing (G) the porous microstructure of the coacervate droplets and (H, I) the hexagonal pore structure. (J) Effect of water addition on the size of the polar core of the inverted hexagonal structures. Micrographs obtained by (A, D–F) light microscopy in the bright field and (B, C, G–I) cryo-SEM. Experimental conditions: Decanol-based SUPRAS produced in environments containing THF in the following proportions by volume: (A) 50%, (B, C, E) 30%, (D) 20%, (F) 60%; SUPRAS additionally containing Rhodamine B at 10 µM (D, E, F). (G–I) Tetradecanol-based SUPRAS prepared from 30% v/v THF in bulk solution

Cryo-SEM micrographs obtained at higher magnifications exposed the porous morphology of the droplet surface (Fig. 2, panel G), its structure clearly showing a hexagonal arrangement in the alkanols (Fig. 2, panels H and I). The hexagonal holes, with apparent diameters ranging from approximately a few tenths of a micron to about 0.5 µm, were assumed to be openings of the water channels from the inside of the matrix, and THF to be the continuous phase for dispersion of the coacervate droplets

forming the SUPRAS. Vacuoles with apparent diameters ranging from 0.2 to 3 μm have been reported for gum Arabic–chitosan coacervates with a sponge-like microstructure.[27]

The water/alkanol mole ratios in the global composition of the SUPRASs ranged approximately from 0.5 to 15, which is typical of reverse micelles.[28,29] Mole ratios were found to be environment-dependent; thus, they increased as the water content in the bulk solution was lowered (see equation 1) and larger aqueous cores formed as a result. Figure 2 (panel J) illustrates the effect of adding water to the hexagonal phase lattice: the addition inevitably caused the interfacial area per alkanol to increase.[30] The lability of the aggregates and their consequently difficult management in cryo-SEM precluded the accurate determination of the sizes of the holes in the SUPRASs under the different environmental conditions used. The water/alkanol mole ratio was also alkanol-dependent: it decreased with increasing chain length (see equation 1 in supporting information) and hence with decreasing size of the aqueous holes. This was the likely result of the electron-releasing inductive effect of the alkyl groups diminishing the proton-donor ability of the higher alkanols, thereby reducing molecular interactions in number and strength. The THF/alkanol mole ratio in the SUPRAS increased with increasing size of the coacervate droplets and length of the hydrocarbon chain, consistent with the hypothesis that THF provides a continuous medium for hexagonal aggregates to disperse.

Water was the directing agent for the hexagonal arrangement in the alkanols. In fact, refractive index[31] and density and ultrasonic speed measurements[32] have revealed that these surfactants do not adopt ordered structures in THF. The same conclusions were drawn from our experiments with variable concentrations of alkanol (0-80 mM) in THF in the presence of the fluorescent probes rhodamine B, pyrene, and 1-pyrene butyric acid.

Capabilities and Mechanisms of Alkanol SUPRAS-based Extractions

SUPRASs consisting of alkanols possess unique structure-derived properties that provide excellent opportunities to develop outstanding applications in analytical

processes. Thus, water and THF, two miscible solvents, do not mix in SUPRAS; rather, they establish distinct, highly polar and nonpolar microenvironments that endow them with a high flexibility as reaction and separation media. Also, the fact that the sizes of the aqueous cavities in alkanol-based SUPRASs are environment-dependent can facilitate their use as materials with restricted access properties which can be adjusted depending on the molecular size of the solutes. In fact, the potential exists for directly using these SUPRASs to enrich low-molecular mass polar and ionic compounds while excluding high-molecular mass components in solid matrices. In addition, alkanol-based SUPRASs provide a mixed-mode mechanism for dissolving medium polar and nonpolar solutes (viz. hydrogen bonding in the surfactant polar group and hydration water, and dispersion forces in the surfactant hydrocarbon chain), thus providing an effective means for isolating and concentrating such solutes from solid and liquid matrices. One useful asset of these solvents is their ability to extract solutes throughout the pH range by virtue of *n*-alkanols not dissociating in water (pK_a ~ 15) and retaining their ordered structure as a result. In this work, we assessed the flexibility of alkanol-based SUPRASs as extractants.

Molecular Size-based Restricted Access Properties of SUPRASs: Extraction and Clean-up of Polar/Ionic Solutes in Solid Matrices

Restricted access matrix sorbents (so-called "restricted access materials", RAMs) have been used in the past two decades for the enrichment of drugs, endogenous substances and xenobiotics in biological fluids without interference from proteins and other matrix components that are excluded by physical, chemical, or physico–chemical means.[33] To the best of our knowledge, no solvents with restricted access properties have to date been reported.

We assessed the ability of alkanol-based SUPRASs to behave as restricted access property solvents by extracting polar/ionic analytes with variable molecular weights from food (e.g. ochratoxin A, OTA, in cereal baby food, MW = 403.8) and environmental solid samples (e.g. Acid Red 97, MW = 698.6, and Brilliant Blue G,

MW = 825.9, in sludge), using decanol-based SUPRASs as models. Both OTA[34] and dyes[35] are deemed contaminants of concern in the selected matrices. SUPRASs produced in water/THF environments containing increasing proportions of THF by volume, and hence vacuoles of increasing size, were studied. For comparison, two solvents consisting of decanol and THF at amphiphile concentrations similar to those in the SUPRASs were also assessed as extractants.

Table 1. Recovery and standard deviations for the extraction of ochratoxin A from cereal-based baby food and dyes from sludge with SUPRASs obtained in different THF:water environments and decanol in THF

SUPRAS Formation (THF, % v/v)	**SUPRAS Composition**		**Recovery ± [a]Standard Deviation (%)**		
	Decanol (mg μL^{-1})	*H_2O/ Decanol (M/M)*	*OTA*	*AR 97*	*BBG*
[b]10	0.57	0.5	23 ± 5	−	−
[b]20	0.41	1.2	45 ± 9	18 ± 3	−
[c]30	0.28	2.6	80 ± 1	36 ± 1	5±3
[c]50	0.16	7.7	109 ± 4	100 ± 1	54±1
Decanol in THF (mg μL^{-1})					
0.28			11 ± 5	−	−
0.16			8 ± 4	−	−

[a]*n* = 3; SUPRASs made with [b]300 or [c]200 mg of decanol and 10 mL of THF:water solution; sample: 400 mg of baby-food or 300 mg of sludge; fortification level: 10 μg kg^{-1} for OTA and 1.7 mg kg^{-1} for the dyes

The recoveries for the target analytes were strongly dependent on the water/decanol mole ratios in the SUPRAS (Table 1), and hence on the size of their aqueous cavities. Quantitative recoveries for OTA and Acid Red 97 were only obtained with SUPRASs containing vacuoles with water/decanol mole ratios of 7.7 (Table 1), which were large enough to allow these analytes to efficiently diffuse through them. The size of the vacuoles did not afford complete recovery of Brilliant Blue G, the bulkiest analyte. The restricted access properties of alkanol-based SUPRASs also reflected in the results obtained by enriching a specific solvent (e.g. that formed in an

environment containing 30% THF) with the target compounds: recoveries decreased linearly with increasing molecular weight of the analytes. Decanol in pure THF, a disordered-structure solvent, was unable to extract the target compounds from the selected matrices (see Table 1), which confirms that the presence of vacuoles in the SUPRAS is essential to solubilize the analytes.

The ability of alkanol-based SUPRASs to exclude high-molecular mass matrix components typically found in food, environmental and biological samples (e.g. proteins, polysaccharides, humic substances) was examined with the aim of assessing the potential of these solvents for combined analyte enrichment and sample cleanup. Similarly to RAM sorbents, both chemical and physical means to exclude the extraction of macromolecules were considered. Experiments were conducted by extracting 20 to 100 mg amounts of pure standards of the target molecules in SUPRASs produced from 200 mg of decanol in aqueous environments containing 10 and 50% THF (which gave SUPRAS volumes of 330 and 1437 μL, respectively).

The behavior of proteins in the extraction process was investigated by extracting aqueous solutions containing albumin from bovine serum, albumin and lysozyme from chicken egg white and gluten from wheat. THF in the solvent caused protein denaturation and the alkanol protein flocculation. As a result, proteins formed a white precipitate that separated from the SUPRAS as a thin layer in the bottom upon centrifugation. An identical white layer was clearly observed between the insoluble matrix components and the SUPRAS after extraction and centrifugation of solid food samples such as wheat. The amount of precipitate formed increased with increasing protein content of the sample and volume of THF in the SUPRAS. These results suggest that proteins do not incorporate into the SUPRAS, and also that THF and the alkanols are the solvent components causing their exclusion from the extracts.

The behavior of polysaccharides in the extraction process was studied by using two polymers with a different function in living organisms, namely: chitosan (MW = 50 000–190 000), which plays a structure-related role, and starch (MW not stated by the supplier), which plays a storage-related role. Neither chitosan nor starch was dissolved

by the studied SUPRASs. Extractions were also carried out under experimental conditions known to enhance the solubility of chitosan (1% acetic acid) and starch (40 ºC), but no extraction in the SUPRASs was observed. Therefore, the SUPRASs also acted as RAMs for polysaccharides.

The extraction of humic acids (MW = 2000–500 000), which are a major component of environmental samples such as soils and sediments, was SUPRAS composition-dependent. Thus, humic acids were only partially extracted in SUPRASs containing a high proportion of THF (e.g. 50%). This allows the self-assembly environment to be exploited for enhanced selectivity against these macromolecules.

In summary, alkanol-based SUPRASs are good extractants for low-molecular weight ionic and nonpolar contaminants in solid food, environmental and biological samples, where macromolecular matrix components are not expected to interfere. This allows simultaneous enrichment of analytes and cleanup to be conveniently accomplished in a single step.

Mixed-mode Mechanisms for Solute Solubilization in SUPRASs: Extraction of Medium Polar/NonPolar Solutes from Liquid Matrices

Medium polar and nonpolar analytes can be dissolved in alkanol-based SUPRAS by effect of hydrogen bonding and dispersion forces. The effects of the variables influencing the extraction efficiency of these solutes by SUPRASs were examined with the aim of developing predictable extraction schemes avoiding the very time-consuming optimization involved in trial-and-error testing. Carcinogenic chlorophenols and aqueous matrixes were used as models for this purpose. The choice of chlorophenols (Table 1 of Supporting Information, S-1) was based on their ability to establish hydrogen bonding and hydrophobic interactions, wide polarity range, acid–base properties, environmental significance and the need for developing simple and rapid concentration schemes affording their quantitation below the ultra-trace levels set up by the European Union as water quality criteria for surface waters (e.g. 0.4–1 μg L^{-1} for

pentacholorophenol).[36] Liquid matrices were used to determine the actual concentration factors achieved and the distribution of analytes between the SUPRAS and sample. All SUPRASs studied were formed *in situ* (i.e. by adding the alkanol and THF to the water sample).

Table 2. Mean recoveries and standard deviations (R ± SD, %)[a] obtained in the extraction of carcinogenic chlorophenols with SUPRASs consisting of various alkyl alcohols

	Aliphatic alcohol				
	[b]C7	**[b]C8**	**[b]C10**	**[c]C12**	**[d]C14**
Carcinogenic chlorophenol	R±[a]SD	R±[a]SD	R±[a]SD	R±[a]SD	R±[a]SD
2,4 DCP	51 ± 2	63 ± 3	88 ± 5	89 ± 1	69 ± 1
2,4,6 TCP	58 ± 2	80 ± 3	92 ± 3	89 ± 2	74 ± 1
2,4,5 TCP	59 ± 5	83 ± 4	93 ± 1	91 ± 1	71 ± 2
2,3,4,6 TTCP	63 ± 1	84 ± 5	95 ± 2	98 ± 1	79 ± 3
PCP	60 ± 1	85 ± 5	97 ± 2	96 ± 1	81 ± 1

Spiking level: 10 μg L^{-1}; [a]n = 3; SUPRAS formation: 100 mg of alkyl alcohol and 40 mL of a THF:water mixture containing a percentage by volume of THF of [b]7.5, [c]10, [d]12.5

Table 2 shows the recoveries of the target chlorophenols as a function of the length of the alkanol hydrocarbon chain over the range C_7–C_{14}. The maximum extraction efficiency was obtained with hydrocarbon chains of medium length (viz. decanol and dodecanol), which can be qualitatively interpreted as follows: the two binding forces driving the extraction process (viz. hydrogen bonding, δ_h, and dispersion, δ_d) decrease and increase, respectively, with increasing length of the hydrocarbon chain[37] (Fig. S-2, A, although no reported δ_h and δ_d values for C_{12}–C_{14} alkyl alcohols could be found, they can be expected to continue to decrease and increase, respectively, with increasing chain length.). Short-chain alcohols are better proton donors than are longer alcohols and accordingly, the results of Table 2 represent a compromise between

the strength of the two extraction forces. It seems reasonable to assume that other analytes in the polarity range considered (e.g. log K_{ow} = 2.9–4.8, Table 2) will behave similarly. Also, polar analytes falling outside this range but having hydrogen bonding capabilities will be more efficiently extracted by SUPRASs consisting of short-chain alcohols. This assumption was confirmed by extracting phenol (log K_{ow} = 1.5); thus, recoveries in a heptanol-based SUPRAS (70 ± 4%) were higher than those in solvents containing decanol (35 ± 2%) or tetradecanol (22 ± 3%) under the same conditions (SUPRASs prepared from a bulk solution containing 200 mg of alkyl alcohol in 40mL of THF:water 12.5:87.5)

The influence of the solvent composition on the extraction efficiency of chlorophenols was evaluated by using decanol-based SUPRASs obtained from aqueous solutions containing variable proportions of THF (2.5–30%, v/v). Because the volume of SUPRAS is exponentially dependent on the proportion of THF (see eq. 3 in supporting information), not only recoveries, but also concentration factors, should be considered in examining this variable. As can be seen from Table S-2 of Supporting Information, recoveries were maximal at THF proportions over the range 7.5–15%; on the other hand, the actual concentration factors (i.e. ACF = Recovery × V_{sample}/V_{SUPRAS}) decreased gradually above a THF proportion of 7.5%. Therefore, a solvent obtained in an environment containing 7.5% THF was deemed optimal for extraction.

In order to gain some insight into the influence of the solvent composition on extraction recoveries, the Hildebrand solubility parameter (δ_t) for the two sites expected to compete for solubilizing chlorophenols (viz. decanol–water in the SUPRAS, and THF–water in the equilibrium solution) was plotted as a function of the proportion of THF in the SUPRAS (Fig. S-2, B).[37] δ_t is a measure of the relative solvency of a solvent and of the combined van der Waals forces resulting from the additive effect of dispersion, polar and hydrogen bonding forces. As can be seen in the figure, δ_t increased in the SUPRAS and decreased in the equilibrium solution as the proportion of THF was raised. The solvency behaviour of the two solubilization sites was similar with THF proportions in the region of 30% (Fig. S-2, B); this caused the chlorophenols to

distribute between them and their recoveries to significantly decrease as a result (Table S-2). In the optimum THF content ranges for extraction of chlorophenols (7.5–15%), dispersion forces (δ_d) were higher in the SUPRAS than in the equilibrium solution (16.95–16.7 $MPa^{1/2}$ vs 15.7–15.8 $MPa^{1/2}$); on the other hand, hydrogen bonding forces (δ_h) were higher in the equilibrium solution (39.7–37.1 $MPa^{1/}2$ vs 19.7–22.9 $MPa^{1/2}$). We can therefore reasonably conclude that, although hydrogen bonding contributed to extraction, dispersion was the main driving force for the process. The pH dependence of the chlorophenol extraction efficiency further confirmed this conclusion; thus, recoveries were significantly lower at pH 9 (see pK_a values in Table S-1) as a result of the increased solubilization of the ionic form in the equilibrium solution (e.g. 47 ± 1, 8.51 ± 0.01, 41.9 ± 0.4, 2.7 ± 0.2 and 14.3 ± 0.3 % for 2,4-DCP, 2,4,6-TCP, 2,4,5-TCP, 2,3,4,6-TCP and PCP, respectively). One can therefore expect SUPRASs obtained at low concentrations of THF to be especially suitable for extracting medium polar and nonpolar analytes owing to the different solvency of SUPRAS and the equilibrium solution (Fig. S-2, B). Also, aqueous back-extraction of the ionic forms of solutes from SUPRAS should be feasible and facilitate the use of SUPRAS-based treatments prior to, for example, capillary electrophoresis. One useful feature of SUPRASs obtained with low proportions of THF is that, based on equation 3 included in supporting information, they can be expected to provide the highest concentration factors.

The effect of the volume of SUPRAS obtained with a 7.5% proportion of THF on the extraction efficiency of chlorophenols was also assessed. For this purpose, SUPRAS volumes of 61–1209 µL were *in situ* prepared from amounts of decanol ranging from 40 to 800 mg. Table S-3 shows the results. As usual, recoveries increased gradually and then leveled off, and the actual concentration factors exhibited a steady decrease, as the solvent volume used for extraction increased. A solvent volume of 151 µL prepared from 100 mg of decanol was selected as optimal.

The equilibrium conditions for extraction were rapidly attained (3 min) and variables such as the ionic strength (NaCl concentration up to 1 M) and temperature (up to 50 ºC) had no effect on the analyte extraction efficiency.

The simplicity and expeditiousness of the microextraction of chlorophenols with alkanol-based SUPRAS led us to consider the possibility of combining this sample treatment with LC-UV(DAD) in order to develop a sensitive, selective method for quantifying carcinogenic chlorophenols in compliance with the EU quality criteria for surface waters. Calibration curves for the target chlorophenols were run from standard solutions prepared in methanol since no differences in peak area or retention time were observed when the analytes were dissolved in an organic solvent or SUPRAS for injection. Responses were linear over the concentration range 50–2000 μg L^{-1}, and correlation coefficients ranged from 0.9992 to 0.9998. Instrumental detection (LOD) and quantitation (LOQ) limits were calculated from blank determinations, using a signal-to-noise ratio of 3 and 10, respectively, and were similar for all chlorophenols (LOD = 25 μg L^{-1}, LOQ = 50 μg L^{-1}). Method quantitation limits were estimated from the instrumental limits and actual concentration factors, and found to be 0.21–0.23 μg L^{-1}. Therefore, the proposed approach affords the quantitation of chlorophenols below the ultra-trace levels in surface water set as water quality criteria by the European Union (0.4–1 μg L^{-1}). Recoveries for CCPs varied from 88% to 97% throughout the studied concentration range (~0.5–50 μg L^{-1}), and standard deviations from 1 to 6%.

The potential interference of matrix components eluting with the chlorophenols was assessed by comparing the slopes of the calibration curves (n = 7) obtained from standards in distilled water with those for seawater and river water spiked with known amounts of CCPs and subjected to the whole procedure. The slopes of the calibration curves in distilled water were 12 874 ± 146, 12 846 ± 96, 13 060 ± 128, 10 324 ± 104 and 9683 ± 269 mV L μg^{-1} for 2,4-DCP, 2,4,6-TCP, 2,4,5-TCP, 2,3,4,6-TTCP and PCP, respectively. The difference between these slopes and those obtained from environmental samples were not statistically significant as per Student's t-test[38]. Thus, t_{calc} values ranged from 0.4–2.1 and fell below t_{crit} (3.17) at p = 0.01. Therefore, matrix components are expected not to interfere with the determination of the target compounds.

The precision of the method was evaluated by extracting 11 independent samples of seawater (n = 2) and river water (n = 2) spiked with CCPs at a 2 μg L^{-1} concentration each. The relative standard deviations (RSD) thus obtained for the five CCPs ranged from 2% to 8%.

The proposed method was used to determine CCPs in samples collected from two rivers, a reservoir and three coastal locations. None was contaminated with CCPs. As can be seen in Table 3, the recoveries obtained from samples spiked with CCPs at concentrations of 0.5–5 μg L^{-1} ranged from 84 to 99%, and their relative standard deviations from 1 to 7%. Figure S-3 (Supporting Information) shows the chromatograms at two different wavelengths for a standard solution and a spiked seawater sample. No interference from the matrix components was detected in any sample.

Table 3. Recoveries (%) ± standard deviations (n = 3) obtained from natural water samples spiked with carcinogenic clorophenols at variable levels (no target analytes were detected in any sample)

Sampling Location	**2,4-DCP**	**2,4,6-TCP**	**2,4,5-TCP**	**2,3,4,6-TTCP**	**PCP**
River Guadalquivir, (site 1)[a]	84±1	93±1	89±1	90±1	89±1
River Guadalquivir, (site 2)[b]	96±2	98±2	90±6	92±5	91±4
River Guadajoz[c]	91±4	96±6	85±5	84±1	92±1
Navallana reservoir[a]	92±5	96±1	97±2	99±1	93±1
Los Álamos beach[b]	91±1	94±3	96±1	95±1	92±1
La Malagueta beach[c]	93±4	87±5	88±4	80±1	85±1
Puerto de Santa María beach[d]	88±1	96±1	98±1	95±1	95±1

Spiking levels: [a] 2 μg L^{-1}, [b] 5 μg L^{-1}, [c] 1 μg L^{-1}, [d] 0.5 μg L^{-1}

One valuable asset of alkanol-based SUPRASs is the negligible absorbance and fluorescence of their amphiphiles, which facilitate chromatographic determinations. By way of example, Fig. S-4 compares the chromatograms obtained by injecting methanol, and SUPRASs consisting of decanol, decanoic acid and triton X-100. Whereas the chromatograms for decanol and methanol were similar, those for the other SUPRASs exhibited chromatographic peaks that precluded the determination of a number of coeluted solutes.

Conclusions

Although the phenomenon of coacervation has been extensively studied over the last 50 years, the nature of coacervates remains poorly understood owing to the lack of systematic studies with modern techniques. The delicate nature of the network structure of coacervates is the source of many artifacts which have so far prevented the obtainment of accurate electron micrographs for their structural elucidation. A comprehensive understanding of these fluids is no doubt essential with a view to their rational exploitation in a number of fields.

To the best of our knowledge, this is the first time environment-responsive supramolecular solvents consisting of coacervate droplets with an inverted hexagonal structure have been identified. The information gained from fundamental studies has allowed us to use these SUPRASs as solvents with restricted access properties —a feature previously observed in solid materials only. These properties allow SUPRASs to be used as RAM extractants for the direct isolation of low-molecular analytes from solid matrices (e.g. food, sludge) with no interference from high-molecular mass matrix components.

One essential feature of SUPRASs is that they remain in thermodynamic equilibrium with the environment where the amphiphiles self-assemble and from which they separate. Therefore, developing comprehensive, efficient extraction schemes based on these solvents requires a sound knowledge of the types of interactions the SUPRAS and equilibrium solution can establish with solutes. In this work, we considered

dispersion forces, hydrogen bonding and Hildebrand solubility to explain the distribution of carcinogenic chlorophenols between SUPRAS and their equilibrium solutions, and also to gain essential insight with a view to predicting the extraction efficiency for other solutes in liquid matrices.

The applications developed here testify to the suitability of alkanol-based SUPRAS for analytical extractions involving real liquid (sea, river and reservoir water) and solid matrices (food, sludge) containing solutes spanning a wide polarity range (ionic, polar, medium polar and nonpolar).

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References

[1] Torimoto, T.; Tsuda, TT.; Okazaki, K-I. *Adv. Mater.* **2010**, *22*, 1196.

[2] Poole, C.F.; Poole, S.K. *J. Chromatogr. A* **2010**, *16*, 2268.

[3] Eckert, C.A.; Liotta, C.L.; Bush, D.; Brown, J.S.; Hallett, J.P. *J. Phys. Chem*. **2004**, *108*, 18108.

[4] Yu, M.S.; Curran, D.P.; Nagashima, T. *Org. Lett*. **2005**, *7*, 3677.

[5] Ariga, K.; Kunitake, T. *Supramolecular Chemistry, Fundamentals and Applications;* Springer: Berlin, 2006.

[6] Armstrong, G.; Buggy, M. *J. Mater. Sci*. **2005**, *40*, 547.

[7] Kato, T.; Mizoshita, N.; Kishimoto, K. *Angew. Chem. Int. Ed*. **2006**, *45*, 38.

[8] Brammer, L. *Chem. Soc. Rev.* **2004**, *33*, 476.

[9] Steed, J.W.; Turner, D.R.; Wallace, K.J. *Core Concepts in Supramolecular Chemistry and Nanochemistry;* John Wiley & Sons: Chichester, 2007.

[10] Hinze, W.L.; Pramauro, E. *Crit. Rev. Anal. Chem*. **1993**, *24*,133.

[11] Carabias-Martínez, R.; Rodríguez-Gonzalo, E.; Moreno-Cordero, B.; Pérez-Pavon, J.L.; García-Pinto, C.; Fernández- Laespada, E.; *J. Chromatogr. A* **2000**, *902*, 251.

[12] Rubio, S. ; Pérez-Bendito, D. *Trends Anal. Chem*. **2003**, *22*, 470.

[13] Yazdi, A.S. *Trends Anal. Chem*. **2011**, *30*, 918.

[14] Saitoh, T.; Hinze, W.L. *Anal. Chem*. **1991**, *63*, 2520.

[15] Jin, X.; Zhu, M.; Conte, E.D. *Anal. Chem.* **1999**,*71*, 514.

[16] Ruiz, F.; Rubio, S.; Pérez-Bendito, D. *Anal. Chem*. **2006**, *78*,7229.

[17] Casero, I.; Sicilia, D.; Rubio, S.; Pérez-Bendito, D. *Anal. Chem*. **1999**, *71*, 4519.

[18] Ruiz, F.J.; Rubio, S.; Pérez-Bendito, D. *Anal. Chem*. **2007**, *79*,7473.

[19] Menger, F.M.; Sykes, B.M. *Langmuir*, **1998**, *14*, 4131.

[20] Ballesteros-Gómez, A.; Rubio, S.; Pérez-Bendito, D. *J. Chromatography A* **2009**, *1216*, 530.

[21] Bosch-Ojeda, C.; Sánchez-Rojas, F. *Anal. Bioanal. Chem.* 2009, 394, 759.

[22] Paleólogos, E.K.; Giokas, D.L.; Karayannis, M.I. *Trends Anal. Chem.* 2005, 24, 426.

[23] Ballesteros-Gómez, A.; Sicilia, M.D.; Rubio, S.; D. *Anal. Chim. Acta* **2010**, *677*, *108.*

[24] Pelesko, J.A. *Self-Assembly: The Science of Things that Put Themselves Together;* Chapman and Hall/CRC: London, 2007, Chapter 4.

[25] Boyd, B.J.; Rizwan, S.B.; Dong, Y-D.; Hook, S.; Rades, T. *Langmuir* **2007**, *23*, 12461.

[26] Yaghmur, A.; Glatter, O. *Adv. Colloid Interf. Sci*. **2009**, *333*, 147.

[27] Espinosa-Andrews H.; Sandoval-Castilla, O.; Vázquez-Torres, H.; Vernon-Carter, E.J.; Lobato-Calleros, C. *Carbohyd. Polym*. **2010**, *79*, 541.

[28] Fendler, J.H.; *Acc. Chem. Res.* **1976,** *9*, 153.

[29] Wong, M.; Thomas, J.K.; Grätzel, M. *J. Am. Chem. Soc.* **1976,** *98*, 2391.

[30] Seddon, J.M. *Biochim Biophys. Acta* **1990**, *1031*, 1.

[31] Piñeiro, A.; Brocos, P.; Amigo, A.; Pintos, M.; Bravo, R. *J. Solution Chem*. **2002**, *31*, 369.

[32] Ali, A.; Nain, A.K.; Sharma, V.K.; Ahmad, S. *Indian J. Pure Appl. Physics*, **2004**, *42*, 666.

[33] Sadílek, P.; Satínský, D.; Solich, P. *Trends Anal. Chem*. **2007**, *26*, 375.

[34] Duarte, S.C.; Pena, A.; Lino, C.M. *Food Microbiol*. **2010** , *27*, 187.

[35] Song, W.X.; Qing, L.H. *Desalin. Water Treat.* **2011**, *29*, 10.

[36] Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy; *Official J. Eur. Union*, **2008**, *348*, 84.

[37] Burke, J. Solubility Parameters: Theory and Application. In *The Book and Paper Group Annual;* The American Institute for Conservation, 1984, Vol. 3. Available on http://cool.conservation-us.org/coolaic/sg/bpg/annual/v03/index.html

[38] Cuadros-Rodríguez, L.; García-Campaña, A. M.; Alés-Barrero, F.; Jiménez-Linares, C.; Román-Ceba, M. *J. AOAC Int.* **1995**, *78*, 471.