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Review

Analytical applications of organized assemblies for on-line spectrometric determinations: present and future

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Abstract

An amphiphile (surfactant) spread on water can lead to the formation of different aggregates: vesicles, miscelles, emulsions or microemulsions; depending on its concentration; its molecular structure and/or the experimental conditions. Such aggregates, (a) may concentrate products, reactants or analytes and so improve the analytical sensitivity and (b) may solubilize such substances and so favorably change the analytical selectivity. Bilayer membrane vesicles for instance, apart from their wide applications in cosmetic and pharmaceutical industries, have a great analytical potential due to their ability to (i) reversibly sequester metal ions avoiding matrix interference and (ii) improve cold vapor (Hg and Cd) and hydride (As, Se, Pb) chemical generation. Micellar solutions have also found wide applications in different areas of analytical chemistry, showing their capacity to concentrate and separate a significant variety of analytes. Among the numerous micelle-based separation techniques, cloud point extraction offers an excellent enrichment factor for metal ions, allowing their quantification at microgram/litre levels. Also agitating a mixture of water, oil and one or more surfactants under controlled experimental conditions, a cloudy mixture (emulsion) or a transparent solution (microemulsion) can be formed. Adequate formulation is necessary in order to obtain a stable organized media. To fulfill this requirement, a major effort is necessary in order to shorten the gap between the current knowledge on this topic and the promising field of applications that await development. Recent publications show that self-assembly structures from highly viscous samples can be accomplished on-line with the advantages of drastically reducing the time of analysis and assuring the absolute control over the stability of the aggregate. Flow systems allow effective mixing of samples with added surfactant and provide continuous pumping of the resulting mixture to sensitive detectors for the on-line determination of different analytes in complex samples.

Keywords: Organized media; Surfactants; Aggregates; Vesicles; Micelles; Emulsions; Microemulsions; Spectrometric determinations

1. Introduction

A surfactant (amphiphile) is a substance, which lowers the surface tension of the medium in which it is dissolved, and/or the interfacial tension with other phases, and accordingly, is positively adsorbed at the liquid/vapor and/or at the other interfaces [1]. This official definition identifies such a substance as a surface active agent, from which its name is derived. Surfactant molecules exhibit both a polar group, ionic or nonionic which is hydrophilic and an apolar group, which is often a long-chain hydrocarbon with at least 10 carbon atoms that is hydrophobic (Fig. 1a). The available surfactants could be classified in synthetic or naturally occurring and if their chemical structure is taken into account, in ionic (cationic or anionic), non-ionic and zwitterionic [2–9]. When an amphiphile is spread on water can lead, depending on the surfactant concentration, its molecular structure and/or the experimental conditions, to the formation of different aggregates governed by two fundamental phenomena: adsorption and self-assembly association of its monomers [2–7]. Adsorption phenomena give rise to modification of certain physical parameters, while self-assembly refers to structural agglomeration of surfactants monomers (Fig. 1b) in either hydrophobic or hydrophilic moieties. The simplest arrangements are the formation of mono- (Fig. 1c) or bilayers (Fig. 1d) at the water–air interface, which control the surface area and the surface pressure by the formation of a film or a membrane, respectively. These assemblies have been extensively used for investigating two-dimensional processes [5] and the transport mechanism across biological

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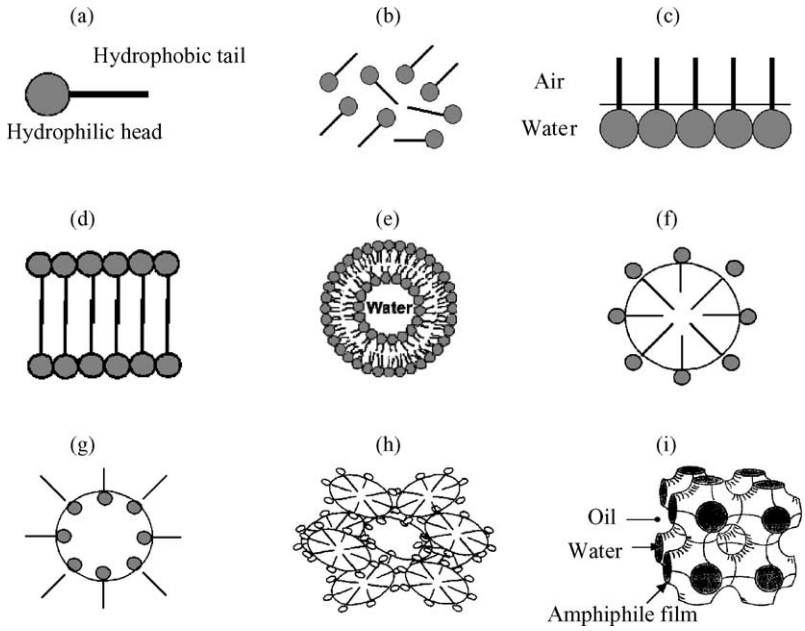

Fig. 1. Schematic representation of: (a) surfactant molecule; (b) monomers; (c) monolayer; (d) bilayer; (e) liposome or vesicle; (f) micelle; (g) reverse micelle; (h) microemulsion and (I) bicontinuous phase microemulsion.

membranes [9] using monolayers and bilayers, respectively. More complex self-assembly structures like liposome or vesicle (Fig. 1e), micelle Fig. 1f and g and microemulsion (Fig. 1h and i) have potential industrial applications in cosmetics, pharmaceuticals, food processing, oil-recovery, etc.

For analytical purposes, it is convenient to choose a surfactant with non-foaming properties, necessary to easily make up volume and to avoid the heterogeneous distribution of sample droplets in the bulk of the aggregate. Non-ionic emulsifiers satisfactorily join these requirements. Any hydrophobic species (organic compounds or metal ions after reaction with a suitable ligand) originally present in an aqueous solution can be entrapped into the hydrophobic core of an aggregate and get involved in preconcentration/separation processes before instrumental detection.

The principles and theoretical background as well as the structural and practical aspects regarding surfactants and their self-assemblies have been well documented in the specialized literature [2–10]. However, improving the analytical chemists knowledge on amphiphilic structures and their physico chemical properties, and understand the solute–surfactant interactions and their effects on chemical equilibrium and reactivity must be carefully considered [2]. This would avoid the implementation of empirical experiments regarding the correct formulation of self-assembly structures.

The purpose of this review is to offer an overview of the on-line coupling of organized media approaches with spectrometric detection techniques and to highlight their advantages and limitations.

2. Aggregates in analytical spectrometry

The use of organized media in analytical chemistry is limited by the stability of the particular aggregates, which should last during a time interval long enough to complete the determination procedure. The kinetic stability of the aggregates (formation and breakdown) varies from several weeks for vesicles to micro- or milliseconds for micelles or to, 10^{-4} to 10^{-6} s for a microemulsion. Addition of polar additives like aliphatic alcohols, called co-surfactants, can help to stabilize the aggregates [2–7]. The aggregates (a) may concentrate reactants at a molecular level and so conveniently may change thermodynamic and kinetic reaction constant and (b) may solubilize, in a selective manner, analytes and reactants, thus, the special microenvironment existing in these aggregates may change the reactions (for instance, interferences) observed in the bulk aqueous phase. These structures in general and micellar and microemulsion systems in particular are associated with exceptional changes in the physico chemical properties of complex samples, such as low interfacial tension, low viscosity and high solubilization. Such attributes, ease and shorten the otherwise tedious analytical process and are beneficial for sample introduction in most spectrometric detection systems. Surveys of the recent publications, discussed later in this review, show that such structures are increasingly used in analytical chemistry due to the fact that allow researchers to enhance the performance of certain analytical methods (selectivity and sometimes the sensitivity) and to develop a new generation of separation and/or preconcentration techniques which finds interesting applications in areas like biotechnology, environment remediation, oil recovery, etc.

Procedures involving the trapping of analytes inside liposomes or vesicles [11–15], extractions mediated by micelle [16,17,20–28] or emulsification of viscous samples [29–35] have many advantages over the classical separation and preconcentration techniques. This is extremely beneficial for most of the spectrophotometric, spectrofluorimetric or chemiluminescent detection techniques as well as for atomic absorption (atomic absorption spectrometry (AAS) with flame (FAAS) or electrothermal (ET AAS) atomization) or atomic emission (inductively coupled plasma optical emission spectrometry (ICP–OES) and ICP–mass spectrometry (ICP–MS)) techniques which require of aqueous standards for calibration and of water-like viscous samples for analysis. Flow systems combined with such sensitive detection systems allow effective mixing of samples with added surfactant and provide continuous pumping of the resulting aggregate mixture to the detector, with additional advantage of absolute control of the entire system, low cost and relative simplicity [27,28]. Real viscous samples of complex composition and low metallic content are usually submitted to tedious pretreatment procedures to destroy organic matrices. Simplifying this step of the analytical process was the dream of many experimentalists and apparently the adequate application of organized media could be the answer.

The current literature in this subject reveals that it is overwhelming the number of published papers related to "in-batch" applications of self-assembly structures for analytical purposes, but not so for on-line systems, although an increased interest is noticed in recent years [17,21,23–26,34,35].

2.1. Liposome and vesicle

The closed bilayer aggregates are called liposomes or vesicles (Fig. 1e) if derived from phospholipids or surfactants, respectively.

Depending on the processing conditions and the chemical composition, liposomes are formed with one or several concentric bilayers and are often distinguished according to their number of lamellae (uni- or multilamellar) and diameter (20–100 nm up to several microns). Given the small size and the ability to enclose certain substances in their inner core, liposomes are widely used in cosmetics as vehicles for controlled delivery of active ingredients through the skin [10].

On the other hand, surfactant bilayer membrane vesicles with sub-micron diameter are able to reversibly sequester metal ions. A lipophilic metal carrier through the vesicle wall facilitates their uptake, while a water soluble metal chelating agent dissolved in the aqueous vesicle interior provides the driving force for metal ion uptake. The carrier and chelator play important roles in selectivity; the vesicle will only take up ions for which the carrier have high affinity and to which the encapsulated chelator bind strongly. Vesicle encapsulating hydroquinones were used to selectively detect Cd^{+2} by following the change in the fluoresce intensity as the ions are transported cross the vesicle wall by ionomicyn, which does not fluoresce upon Cd binding [11]. Also using fura-2 as encapsulated chelator and Kryptofix 22DD (a microcycle) as ionophore, trace amounts of Pb^{+2} were determined, while Cd^{+2} shows no response in this system [12]. These publications show that such luminescence detection systems based on metal-sorbing vesicle technology are highly selective for the determination of toxic metal ions at trace levels and could be easily integrated for on-line process monitoring and also could conveniently be adapted for field measurements.

It has been experimentally demonstrated that vesicles have a great analytical potential to improve cold vapor (Hg and Cd) and hydride (As, Se, Pb) chemical generation [13–15]. Cadmium atoms are effectively formed in a continuously flowing system at room temperature by merging analyte with reductant ($NaBH_4$), both trapped inside didodecyldimethylammonium bromide (DDAB) vesicles, followed by cold vapor (CV)—AAS detection [13]. The authors claim that this vesicular volatile species generation facilitates Cd transport to the quartz cell, thereby improving by about 20 times the detectability of Cd.

Vesicle mediated high performance liquid chromatography (HPLC) coupled on-line with microwave digestion (MW)–hydride generation (HG) systems, have been used for the organic and inorganic selenium speciation in human urine. Specific detection by off-line ET AAS [14] or on-line HG-AAS [14,15] and HG–ICP–MS [15] was performed. A vesicular solution of DDAB was prepared by sonicating the surfactant in water. While passing through the HPLC column filled with a conventional reversed-phase (C_{18}), the hydrophobic tails of DDAB are retained on the stationary phase surface by molecular interactions, so that the positively charged head of DDAB monomers remain on the surface of C_{18} material, directly exposed to the mobile phase flow. There are electrostatic repulsions and hydrophobic interactions, which allow anionic interchange with the selenium ions, depending on their polarity/hydrophobicity [14,15]. No significant degradation of the column package was observed after few months of daily usage if the mobile phase consisted on acetate buffer, methanol and DDAB vesicles at pH 5. Higher sensitivity was obtained for most of the selenocompounds assayed when compared to HPLC–ICP–MS with conventional nebulization without vesicle-mediated separation.

Some characteristics and figures of merit of such systems are given in Table 1.

2.2. Micelles

Another interesting process is the formation of micelles, which consist on the aggregation of a certain number of ionic amphiphiles monomers (40–200, called aggregation number, N_{AG}), although nonionic and zwitterionic surfactants are also prone to form micelles. The aggregation is done in such a way that the hydrophobic or the hydrophilic portions

Table 1
Recent on-line applications of organized media in spectrometric techniques

Sample	Analyte	Surfactant	Figures of merit	Procedure/method	Reference
Vesicles					
Aqueous standards	Cd	DDAB	DL: 0.08 $\mu g\,l^{-1}$	Off-line vesicle formation/FI–CV–AAS or ICP/OES	[13]
Urine (spiked samples)	Se species	DDAB	DL: 5 $\mu g\,l^{-1}$	Off-line vesicle formation; HPLC-off-line ET AAS;	[14]
			DL: 1 $\mu g\,l^{-1}$	Off-line vesicle formation; HPLC–MW–HG–AAS	
Urine	Se species	DDAB	DL: 1.0–5.3 $\mu g\,l^{-1}$; R.S.D.: 3.4–8.4%;	On-line vesicle formation; HPLC–MW–HG–ICP–MS	[15]
Micelles					
Selenium tablets, synthetic samples	Se (IV)	CPC	Calibration range: 5-1000 $\mu g\,l^{-1}$; DL: 1 $\mu g\,l^{-1}$; R.D.S. 0.76% for $n = 12$ at 100 $\mu g\,l^{-1}$ level);	Off-line micelle formation (Se + resorufin+S^{-2} + CPC) fed into FI/Spectrofluorimetry	[16]
Biological materials (CRM)	Pb	Triton X-114	DL: 44.6 $ng\,l^{-1}$; R.D.S.: 2.9% for $n = 11$ at 1 $\mu g\,l^{-1}$ level	On-line micelle formation (Pb + APDC + Triton X-114), trapped in silica gel, eluted with acetonitrile/off-line ET AAS	[17]
Parenteral solutions	Al	PONPE 7.5	Calibration range: 5.4–675 $\mu g\,l^{-1}$; DL: 3.0 $\mu g\,l^{-1}$;	Off-line-CPE of [Al(III)-CAS-BDTAC]/FI-Spectrophotometry	[20]
Tap water	Hg	PONPE 5	Calibration range: up to 50 $\mu g\,l^{-1}$; DL: 4 $ng\,l^{-1}$; R.D.S.: 3.4% for $n = 10$ at 0.5 $\mu g\,l^{-1}$;	Off-line CPE of [Hg(II)-(5-Br-PADAP)] complex/FI–CV–ICP/OES	[21]
Tap and river waters	U	Triton X-114	DL: 1.4 $\mu g\,l$ for V_i/V_f of 50; R.S.D.: 5.1% for $n = 8$ at 1.5×10^{-7} $mol\,l^{-1}$;	Off-line CPE of [U(VI)-PAN)] complex/FI spectrophotometry	[22]
Urine	Gd	PONPE 7.5	Calibration range: up to 50 $\mu g\,l^{-1}$; DL: 0.04 $\mu g\,l^{-1}$ for 10 ml; R.S.D.: 1.9% for $n = 10$ at 2.0 $\mu g\,l^{-1}$ level;	Off line CPE of [Gd(III)-5-Br-PADAP] complex, on-line retention on cotton, elution with HNO_3/FI–ICP/OES	[24]
Urine	Dy	PONPE 7.5	DL: 0.03 $\mu g\,l^{-1}$ for 10 ml; R.S.D.: 2.2% for $n = 10$ at 2.0 $\mu g\,l^{-1}$ level;	Off line CPE of [Dy(III)-5-Br-PADAP] complex, on-line retention on cotton, elution with HNO_3/FI–ICP/OES	[25]
Sea, river and waste waters	Cr	Triton X-114 and SDS	Calibration range: 2–200 $ng\,l^{-1}$; DL: 0.5 $ng\,l^{-1}$; R.S.D.: 0.9–1.6% for $n = 6$;	On-line mixed micelle formation/chemiluminescence	[26]
Microemulsions					
Commercial reagents and duralumin alloys	Mg, Zn	SPAN-80	Calibration range: Mg 20–200 and Zn 100–500 $\mu g\,l^{-1}$; DL: Mg 20 and Zn 100 $\mu g\,l^{-1}$ for 1 ml	On-line emulsification (W/O/W) surfactant + extractant + solvent + HCl + sample, demulsification/FI–FAAS	[32]
Fish–eggs oil	Hg species	Tween 20	Calibration range: 2–20 $\mu g\,l^{-1}$; DL: 0.11 $\mu g\,l^{-1}$; R.S.D.: 2.2 and 8.5 for $n = 5$ at 1.0 and 0.2 $\mu g\,l^{-1}$ level.	Off-line emulsification (O/W): sample + surfactant+water (mechanical stirring)/FI–CV–AAS	[33]

Table 1 (*Continued*)

Sample	Analyte	Surfactant	Figures of merit	Procedure/method	Reference
Lubricating oils (new and used)	Cr	SDS	Calibration range: 7–50 $\mu g\,l^{-1}$; DL: 4.0 $\mu g\,l^{-1}$ (6.0 $ng\,g^{-1}$); R.S.D.: 0.5 and 0.8% for $n = 5$ at 30 and 10 $\mu g\,l^{-1}$ level.	On line emulsification: (sample + hexane) + SDS + sec-butanol/FI–ET AAS	[29]
Olive oils	Various e.g. Pb	Triton X-100	Calibration range: up to 60 $\mu g\,l^{-1}$; DL: 0.33 $\mu g\,kg^{-1}$; R.S.D: 2.37% for $n = 10$ at 10 $\mu g\,kg^{-1}$ level.	On-line emulsification: FI merging of sample + Triton X-100 (diluent) + Triton X-100 (emulsifier) + Rh solution (internal standard)/FI–ICP–MS.	[35]

DDAB: didodecyldimethylammonium bromide; CPC: cetylpyridinium chloride; BDTAC: benzyldimethyltetradecylammonium chloride; CAS: Chrom azurol S; PONPE 7.5: polyethylene glycol *p*-nonylphenylether; PONPE 5: polyethyleneglycolmono-*p*-nonylphenylether; PADAP: 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; SDS: sodium dodecyl sulfate.

of the surfactant molecule associate together to practically exclude the water (Fig. 1f) or the non-polar solvent (Fig. 1g), respectively. In other words, if the solvent is polar (aqueous phase), normal micelles are exhibited. Inverse or reverse micelle (have a polar core and the surfactant tails pointing outside) are formed in apolar media. When double tail surfactants are used, the inverse micell structure (Fig. 1g) is easier to accommodate and may be favored by steric hindrance effects [3,4]. The strong repulsions between the head groups, inherent in the aggregation of alike charges, is partially compensated by binding of counterions. The entrapment of metal ions in the micelle core is therefore governed by electrostatic interactions [2–4]. The behavior of zwitterionic micelles is different from that of common ionic aggregates; the micelle is equivalent to a double shell concentric spherical capacitor immersed in an electrolyte solution [2].

The structure of any type of micelle will depend upon the temperature and the surfactant concentration and molecular structure: size of head group, length and number of hydrocarbon chains, presence of branches, double bonds, aromatic rings, etc. The concentration at which micelles first form in water, called the critical micellization concentration (CMC), is sharply defined and its value can be determined by following the behavior of any physico chemical property of the solution like osmotic pressure, surface tension, turbidity, electrical conductance, spectral behavior, etc. with changing the surfactant concentration. These two parameters (N_{AG} and CMC) are intrinsic characteristics of each surfactant [8]. As the concentration of surfactant increases, spheroid micelles change to cylindrical and liquid crystalline structures by passing through hexagonal, spherical and lamellar phases. It was experimentally attained that when the CMC is reached, the solubility of certain substances in such preparations increases proportionally with the increase in the surfactant concentration. The term solubilization refers to the incorporation of the water soluble material(s) to the micellar system and also to the incorporation of the hydrocarbon insoluble material(s) to the reverse micelles. Solubilization of different substances in micellar systems can occur: (a) in the inner core of the micelle; (b) in the palisade layer between the hydrophilic head groups; (c) deeper in the palisade layer; or (d) adsorbed at the micellar surface. In the case of the non-ionic surfactants, substances are solubilized between the hydrophilic head groups [6,7].

Micellar solutions have found wide applications in different areas of analytical chemistry, showing several advantages over conventional separation techniques, like low cost, high concentration factors, capacity to concentrate a wide variety of analytes and applicability to samples of varying natures. Relevant figures of merit obtained for the determination of several metal ions in such systems are given in Table 1.

A simple FIA manifold combined with spectrofluorimetric detection was used to determine selenium (IV), based on the reduction of a buffered (pH 7) resorufin containing selenium solution by sulfide in a cationic micellar medium of cetylpyridinium chloride (CPC) [16]. Anionic sodium dodecylsulfate (SDS) and non-ionic (Triton X-100) surfactants have also been tested but only the presence of cationic micelle, prepared at CPC concentration above its CMC, enhanced the rate of the sulfide–resorufin–Se (IV) reaction. This finding confirmed the assumption that the catalytic effect arises essentially from electrostatic interactions between the negatively charged selenite and sulfide and the cationic micelle, since resorufin it is uncharged at the working pH. The selectivity was improved by passing the sample through a column filled with a cationic exchanger, allowing the application of the method to the determination of the analyte in selenium tablets.

Nan et al. [17] determined lead in several certified reference materials of biological nature in a FI micelle-mediated preconcentration/separation system followed by ET AAS detection. For the on-line coupling of the preconcentration/separation system to ET AAS, previously developed air-segmented and air-transported operational sequences were adopted [18,19]. The analyte-entrapped surfactant micelles were obtained by on-line merging of the analyte

solution with an ammonium pyrrolidine dithiocarbamate (APDC) solution, which meets downstream with the surfactant (Triton X-114) solution. The resulting mixture is directed to a microcolumn packed with silica gel to retain the micelles, while airflow is used to remove any residual solution in the column. The micro column packed with the polar sorbent (silica gel) acted well for the adsorption of the analyte-containing micelles because of the hydrophilic and polar nature of the outer sphere of the micelle. At the same time, a 50 μl loop is filled with acetonitrile while the delivery tip of the ET AAS autosampler was moved into the dosing hole of the graphite tube. At this stage, airflow is introduced into the system in order to drive the acetonitrile into the minicolumn for eluting the analyte and deliver it to the graphite furnace. The capillary tip moved out of the furnace just before the temperature program started for ET AAS determination. A concentration factor of 22.5 were obtained at a sample throughput of 30 samples h^{-1}.

Among the numerous micelle-based separation techniques, cloud point extraction (CPE) offers an excellent enrichment factor for metal ions, allowing their quantification at microgram per litre ($\mu g\,l^{-1}$) levels. Metal ions aqueous solutions mixed with non-ionic surfactants become turbid just above the CMC (as a result of a decrease in the solubility of the surfactant in water), and over a very narrow temperature range called cloud point temperature (CPT). Above the CPT the solution separates into two phases: one rich in surfactant into which the ions are concentrated and the bulk aqueous solution which mainly contains surfactant monomers, providing an attractive alternative to the use of relatively large volumes of toxic and expensive solvents normally used in liquid–liquid extraction [20–22].

Off-line CPE approaches coupled to FI systems have been successfully used to determine several metal ions in different types of samples. For instance, aluminum was determined in parenteral solutions without previous treatment by complexing the analyte with Chrome azurol S (CAS) in the presence of the cationic surfactant benzyldimethyltetradecylammonium chloride (BDTAC). The ternary complex [Al(III)–CAS–BDTAC] formed was extracted in a non-ionic surfactant (PONPE 7.2) and the surfactant-rich phase was injected in an optimized FI–Spectrophotometric system [20]. A 99.9% extraction for a preconcentration factor of 50 was achieved.

Similar methodologies were developed for trace levels determination of mercury in tap water [21] and uranium in tap and river waters [22]. The mercury was extracted as mercury-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complex [Hg(II)-(5-Br-PADAP)], at pH 9.2 mediated by micelles of the non-ionic surfactant polyethyleneglycolmono-*p*-nonylphenylether (PONPE 5) prior to the determination by ICP–OES coupled to a FI–CV generation using $SnCl_2$ solution as reductant. The pre-concentrated sample solution ended-up with an enrichment factor of 200-fold.

Instead, uranium (VI) was complexed with 1-(2-pyridylazo)-2-naphtol (PAN) to form the hydrophobic species which were readily concentrated in the surfactant –rich phase of a non-ionic surfactant (Triton X-114). In all the experiments the solutions were buffered at pH 9.2 and the analytical signal was a function of the initial volume (V_i) of preconcentrated sample and the final volume (V_f) chosen for the measurement. For a V_i/V_f ratio of 50, the limit of detection was of 1.4 $\mu g\,l^{-1}$. The samples analyzed were uranium free and the recovery test was carried out only to show the reliability of the procedure developed.

CPE incorporated into a FI system has been used for the first time by Fang et al. [23] for the analysis of coproporphyrine after inducing phase separation with ammonium sulfate and filtration through a cotton-packed column. This otherwise original approach cannot be applied to metals preconcentration, as a complexation step is required in order to form the hydrophobic chelate between the metal and a suitable ligand. Latter, Ortega et al. [24] determined gadolinium (Gd) in urine using a sequential ICP spectrometer. The preconcentration step, mediated by micelles of the non-ionic surfactant poly (ethylene glycol) mono-*p*-nonylphenyl ether (PONPE 7.5) was performed by means of the formation of Gd(III)-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol, [Gd(III)-5-Br-PADAP] complexes. The micellar system was thermostated at 25 °C and the surfactant rich-phase retained in a microcolumn packed with cotton at pH 9.2, followed by elution with diluted nitric acid directly into the plasma nebulizer. The overall procedure lasts less than 3 min, allowing a throughput of approximately 20 samples h^{-1}. An enhancement factor of 20 was obtained for the preconcentration of 10 ml of sample solution.

Based on the hyphenation of CPE–FIA associated with ICP–OES, the same group of researchers applied the same system to the determination of dysprosium (Dy) in urine [25]. They promoted the formation of a micellar system containing the complex [Dy(III)-5-Br-PADAP], and claim an enrichment factor of 50 for the preconcentration of 50 ml of sample solution.

In a recent publication, Paleólogos et al. [26] used a mixed micellar medium to extract and preconcentrate chromium, previous to its chemiluminescent detection based on the catalytic activity of the metal on the luminol-hydrogen peroxide reaction. A mixture of two surfactants, one anionic (SDS) and another non-ionic (Triton X-114) was clouded by the addition of sodium sulfate and also filtered through a cotton-packed minicolumn which retain the micelle phase. Another nonionic surfactant (Triton X-100) dissolved the metal-containing micellar phase and carry it to the detector. Just in front of the cell, this stream merged with the chemiluminescence-forming reagent. Both streams contained EDTA to mask the catalytic effect of foreign ions and the procedure was applied to the determination of Cr in seawater and wastewater. This method offers a mean time of analysis of ca. 5 min, for a sample throughput of 12 h^{-1}.

Beside these performances, such procedures are often arduous and time consuming; probably this is the reason why the number of publications describing on line approaches is

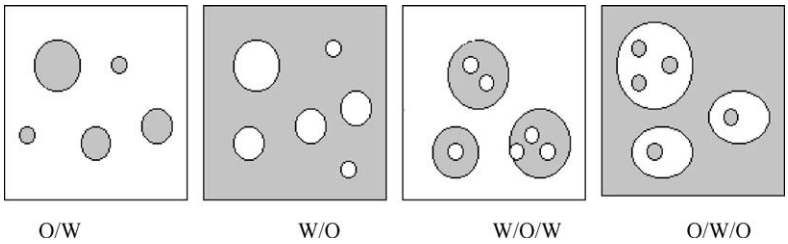

Fig. 2. Different types of emulsions. White and gray areas represent water and oil, respectively.

limited. Table 1 summarizes the most relevant features of the systems described so far.

2.3. Emulsions and microemulsions

Whenever two immiscible liquids are stirred, a macroemulsion is obtained, either O/W (droplets of oil in water) or W/O (droplets of water in oil) depending on the dispersed phase, situation related mainly to the formulation and to a lesser degree to the W/O ratio. Its droplets are very fine (10 nm) and are usually named miniemulsions. However, agitating a mixture of water, oil and one or more surfactants under controlled experimental conditions, a cloudy mixture (emulsion) or a transparent solution (microemulsion) can be formed [2–6]. The emulsions contain relatively large particles, which scatter light causing the cloudiness; they are either dispersion of oil droplets in water or of water droplets in oil, in which the internal phase is oil and water, respectively (Fig. 2). The conventional procedure for the preparation of these aggregates consists of stirring the components at a controlled temperature. Once formed, the emulsion's droplet structure remains fairly constant but its size increases with time due to droplet fusion; the emulsion eventually separates into its basic components: oil and water. Choosing the right surfactant and the appropriate experimental conditions can radically decrease the rate of fusion, although the instability of emulsions can, sometimes be advantageous and are commonly deliberately formulated with poor stability for specific industrial uses.

The microemulsion is a kind of emulsion in which the droplets are much smaller thus, the light passes through without much scattering. However, they are dynamic systems that form, disintegrate and reform in fractions of milliseconds. Microemulsions are thermodynamically stable solutions, which means that they form spontaneously when the components are brought together and stay stable as long as the ingredients are intact. Their water microdomain have characteristic structural dimensions between 5 and 100 nm. Aggregates of this size are poor scatters of visible light and hence these solutions are optically clear and suitable for analytical uses. As a rule, a hydrophilic surfactant is needed to produce an O/W emulsion; a hydrophobic surfactant to make a W/O emulsion and a surfactant of intermediate hydrophilicity gives a microemulsion. The main advantage of microemulsions is their long term stability although they require a high amount of surfactant (five times the one needed to create an emulsion) and are very sensitive to temperature changes. The best experimental way to obtain a microemulsion is to (i) increase the temperature in order to increase the molecular motion and debilitate the directional interactions and (ii) add an alcohol because its molecules get inserted in between surfactant molecules and push them apart, with a corresponding reduction in polar interactions and rigidity [3,4].

When the system obtained by mixing the three components (oil, water, surfactant) reaches equilibrium, two or more phases can be formed, which can conveniently be represented in a so called Winsor diagram which indicates the lipophilic and the hydrophilic interactions at O/W interphase [6]. Winsor introduced the ratio R, which is a measure of the affinity of surfactant for oil or for water. The diagram presents a single-phase region close to the S (surfactant) vertex, which contain liquid crystal lamellae, and near the OW side, two biphasic regions are located on both sides of the diagram and one triphasic region is located in its center (Fig. 3). If $R < 1$, the surfactant has more affinity for water, the hydrophilic interactions will be stronger and a micellar solution or a type S_1 emulsion will be obtained. If $R > 1$, the surfactant has more affinity for oil, the lipophilic interactions are stronger and an inverse micellar solution or a type S_2 microemulsion is obtained. They are also represented as Winsor Type I and Type II systems or are indicated with the mnemonic symbols $\underline{2}$ or $\bar{2}$, respectively. When these interactions are in equilibrium, $R = 1$ and it is assumed that the optimum formulation has been reached or a Winsor Type

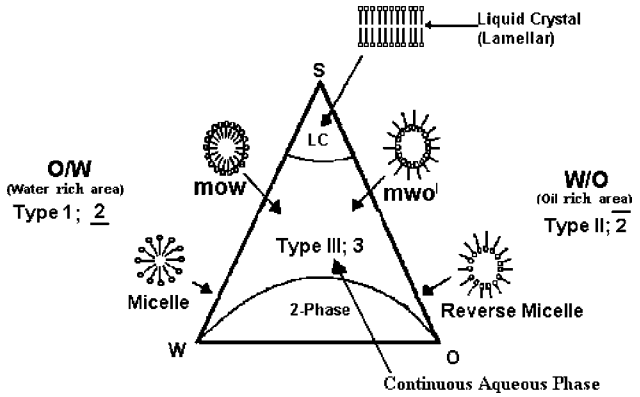

Fig. 3. Winsor diagram for surfactant (S)/water (W)/oil (O) mixture. For other abbreviations see text.

Table 2
Effect of aqueous phase salinity on phase behavior (lubricating oil emulsification)

Salinity (NaCl% w/v)	Phase		
	New oil	Used oil	Hexane
3.4	2	2	2
3.5	2	2	3
3.6	2	3	3
3.7	3	3	3
3.8	3	3	3
3.9	3	$\bar{2}$	3
4.0	$\bar{2}$	$\bar{2}$	3
4.1	$\bar{2}$	$\bar{2}$	$\bar{2}$
4.2	$\bar{2}$	$\bar{2}$	$\bar{2}$
4.3	$\bar{2}$	$\bar{2}$	$\bar{2}$

The mnemonic symbols 2 and $\bar{2}$ correspond to the O/W and W/O emulsion types, whereas, 3 corresponds to the microemulsion (mow) [29].

III (3) system has been obtained. This situation corresponds to a bicontinuous phase associated with the lowest possible interfacial tension and viscosity values. For a better understanding, the sponge-like model can explain the bicontinuous structure; if a sponge is filled with a liquid, resembles a bicontinuous structure since the liquid forms a continuous phase and the sponge material another continuous phase although they appear to be one [2–6].

The parameters involved in an optimum formulation of a microemulsion have been extensively studied and are correlated in the formulae [3]:

$$\ln S - \mathrm{K} \times \mathrm{ACN} - f(A) + \sigma - a_\gamma \times \Delta T = 0$$

(for ionic surfactants)

$$\alpha - \mathrm{EON} + bS - \mathrm{K} \times \mathrm{ACN} - f(A) + c \times \Delta T = 0$$

(for nonionic surfactants)

where: ln S is the natural logarithm (ln) of the average salinity (S) expressed as the weight percentage of NaCl in the aqueous phase or the equivalent salinity of another electrolyte; ACN is the number of carbon atoms of alkane oil or its equivalent if a different type of oil is used; the function $f(A)$ accounts for the alcohol effect; σ and α accounts for surfactant hydrophilicity, ΔT is the temperature variation with respect to 25 °C while K, a_γ, b and c are empirical constants which depend on the type of system and EON is the average number of ethylene oxide groups/molecule of a non-ionic surfactant.

Any of these parameters can be modified with consequent change in R value and each can be used as a guide to the optimum formulation, which corresponds to the three-phase system, identified by Type III or 3 in Fig. 3. Typical variations of phase behavior with the salinity of the aqueous phase [29] and with the EON [30] are given in Table 2 and Fig. 4, respectively. The rows indicated in bold in Table 2 and arrow in Fig. 4 refer to optimum formulation that assures long term stable systems.

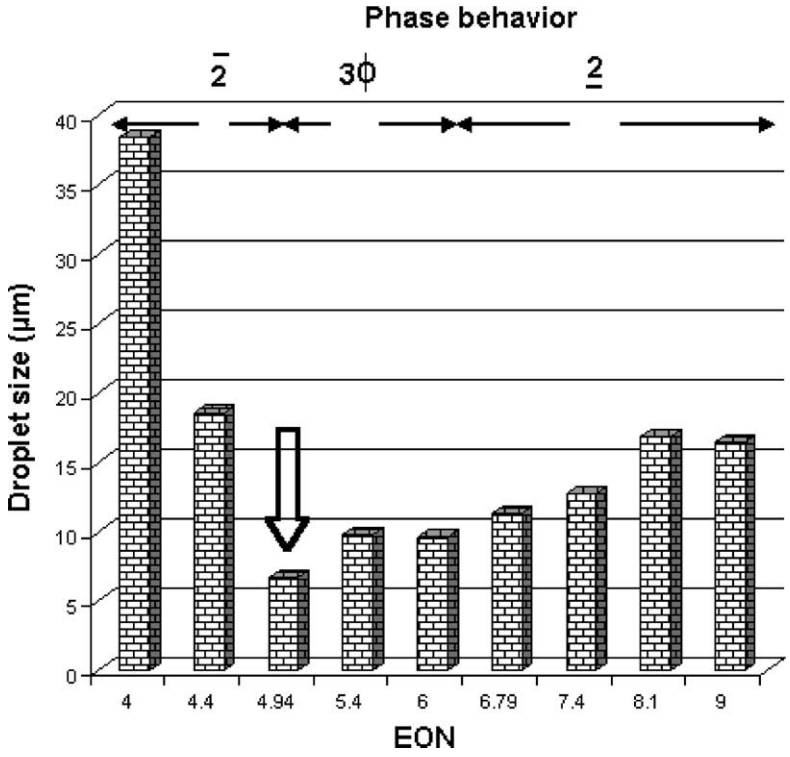

Fig. 4. Scanning for microemulsion formulation: EON vs. droplets size. W/O ratio of 70/30 at pH 10; 1% lignin and crude oil:kerosene of 10:20; bentonite 0.25 g/tubing [30].

One of the most important parameters to be considered on the selection of the adequate surfactant is the hydrophilic–lipophilic balance (HLB). Its value goes arbitrarily from 0 to 20 and depends on the sample nature. If HLB < 7 the surfactant is lipophilic and is likely to produce systems related to an $R > 1$ situation (see later), particularly W/O emulsions, and conversely if HLB > 13. For example, an HLB in the range 8–18 is recommended in order to obtain stable O/W emulsions from petroleum derivatives like lubricating oils [29]. This range is quite wide and very often, surfactants with the same HLB number exhibit different behavior. It is obvious that other formulation variables should be considered, such as surfactant affinity difference (SAD), which includes the ethylene oxide number (EON), parameter strictly related to HLB according to the formulae [3]:

$$\text{HLB} = \frac{100}{5} \times \frac{\text{weight of polyethylene oxide chain}}{\text{total molecular weight}}$$

Scanning EON with respect to the emulsion particle size (Fig. 4) obtained from a Venezuelan crude oil, the smallest particle size corresponds to an EON for the optimal formulation of the triphasic system.

In order to be employed in atomic spectrometry, the emulsions must meet certain requirements: (1) to be stable during the time of analysis; (2) be physically suitable for introduction into the atomizer; (3) provide identical results as standards of identical analyte concentration. Therefore, it is necessary to adequately select the emulsifying agent (the structure of its hydrophilic group which will interact with the analyte and the structure of its non-polar part which must be similar to the solvents to be emulsified) and to take into account several factors like the nature of the sample to be analyzed; the type of emulsion to be prepared apart from its stability and homogeneity, which are in turn related to the size of the dispersed droplets. The control of such parameters can improve the reproducibility of the analytical signals.

3. On-line emulsification

Emulsification of highly viscous or complex matrix samples can be accomplished on-line with the advantages of drastically reducing the time of analysis and assuring the absolute control over the stability of the emulsion. Flow systems allow effective mixing of samples with added surfactant and continuous pumping of the resulting emulsion to the detector [31]. The papers revealing research in this area are scarce, probably because of the difficulty of incorporating the emulsification system on-line with the spectroscopic detectors.

One of the first publications reporting an on-line preconcentration method based on W/O/W emulsions has been developed for the determination of Mn and Zn in commercial reagents and in duralumin alloys by FAAS [32]. The manifold consisted of three peristaltic pumps to propel the reagents solutions, three coils: one used to mix an oil phase which consisted on a mixture of surfactant (Span 80), extractant (palmitic acid) and solvent (kerosene) with HCl in the inner aqueous phase to form W/O emulsion; a second one in which the sample previously added as an outer aqueous phase will form the W/O/W emulsion and where the extraction take place and a last one located in a dry bath, used to demulsify or clarify the final solution. The system also included two gas phase separators: one to waste the outer aqueous phase after the formation of the W/O/W emulsion and the other one to waste the oil phase after demulsification, an injection valve to introduce the sample and an air pump to deliver the concentrated sample solution to the detector. Once all the experimental parameters (type of surfactant, concentration and flow rates of the reagents, dimensions of coils, demulsification temperature, etc.) have been optimized, the method proved to provide reproducible results in shorter time and less sample consumption than in batch systems. Enrichment factors of 2.4 and 1.8 were obtained for Mg and Zn, respectively, and the system allowed sample solutions to be continuously injected at 4 min intervals.

When dealing with more complex and viscous samples, like fish–eggs oil samples [33], lubricating [29], crude [34] or edible oils [35], there are experimental difficulties in obtaining uniform flow pattern of sample solutions in FI systems without back flush due to high built-up pressure within the conduits. Emulsification proved extremely advantageous for samples processing without the need of organic matter destruction.

In a first attempt to improve the sample pretreatment step of an analytical process, Burguera et al. prepared an O/W emulsion off-line by mechanically mixing the sample (fish–eggs oil) with Tween 20 and water. The resulting emulsion was subsequently introduced in a FI system to determine inorganic and total mercury by CV–AAS [33]. Such procedure drastically reduced the time for sample pretreatment and allowed the introduction of highly viscous samples into closed systems, preventing the decomposition of the organic mercury species.

Later, the same group succeeded in designing a completely on-line emulsification system in a computer controlled FI manifold to determine Cr in new and used lubricating oil samples by ET AAS [29]. For the first time, with solid knowledge about the parameters involved in the emulsification process, extensive studies have been carried out in order to elucidate the optimum formulation of emulsions for analytical purposes. The experimental assembly consisted on a peristaltic pump, a home-made time-based solenoid injector which regulates the introduction of reagents in the system (hexane as sample diluent and as carrier, surfactant (SDS), co-surfactant (sec-butanol) and NaCl to adjust the salinity of the aqueous phase), two injection valves, one to introduce the sample in the hexane stream and the other to waste the carrier when necessary, an ultrasonic bath which aided the emulsification, a home-made sampling arm assembly which

permitted the selection of a sub-sample from the emulsion and delivery of the adequate microvolume into the atomizer platform and the detector (ET AAS with Zeeman-effect background corrector).

Recently, a Spanish group of researchers used an automated procedure for the emulsification of olive oil samples and multielement (Al, Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn and V) determination by ICP–MS. The olive oil sample injected in a Triton X-100 carrier merges with a stream of emulsifier (also Triton X-100) containing a rhodium aqueous solution as internal standard [35]. A coil located in an ultrasonic bath serves as emulsification reactor and the emulsion formed is introduced into the ICP–MS instrument. The optimized method was applied to determine the metal content of different Spanish virgin olive oil samples.

Table 1 gives some details on the analytical performances of these systems.

4. Conclusions

The self-assembly structures mentioned in this review are fascinating topics that offer new possibilities as far as the analytical applications are concerned. The up-to-date literature survey show that much progress has been made in recent years to develop hybrid methods for trace element determination in samples with high organic content. The on-line systems described in this review: (a) involve automation of the sample preparation process which is considerably less time consuming than conventional approaches; (b) allow the use of aqueous standards for the determination of metallic and non-metallic species in complex samples; (c) prevent contamination of samples or losses of analytes by performing all the analytical operations in totally closed systems.

Organized assemblies like vesicles and micelles protect the analyte from foreign ions and organic matrix interference by readily trapping it in a controlled environment. Also, the correct formulation of microemulsions for analytical purposes might open new research horizons with unlimited applications.

A major effort from researchers working in this field is needed in order to shorten the gap between the current knowledge and the applicability of organized media to real samples.

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