Microchim Acta 155, 349–364 (2006)
DOI 10.1007/s00604-006-0511-3

Microchimica Acta
Printed in Austria

Review

Coupling Cloud Point Extraction to Instrumental Detection Systems for Metal Analysis

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Received October 22, 2005; accepted January 7, 2006; published online April 28, 2006

Abstract. The purpose of this article is to review and evaluate cloud point extraction of metals and its coupling to different contemporary instrumental methods of analysis. This review covers a selection of the literature published on this topic over the period mainly between 1997 and September 2005 (consisting of 50 publications). The current state of the art for CPE concerning metals and metal chelates is presented with special emphasis on the hyphenation of this interesting extraction/preconcentration approach mediated by surfactants to spectrophotometry, atomic spectroscopy and separation techniques. We present contemporary CPE developments concerning metal speciation and determination and their application to different environmental, clinical, geological and food samples. Strategies for method development as well as future perspectives are also covered.

Key words: Cloud point extraction; hyphenation; metal analysis; instrumental analysis.

Modern analytical chemistry is characterized by hyphenated analytical methods which involve a combination of a separation and/or sensitive detection technique with a preconcentration step. The latter is a consequence of the problems encountered in the chemical analysis of real analytical samples such as low analyte concentrations (in many cases incompatible with quantification limits of modern instrumental methods) and high concentrations of matrix interference species present in these complex samples, especially those of biological or environmental origin.

Consequently, there is an ongoing need for the development of alternative extraction/enrichment procedures which should be safe, rapid, convenient, and accurate. In the last two decades, there has been an increasing interest all over the world in developing surfactant-based methods in all fields of analytical chemistry.

During the past years, cloud point extraction (CPE) has become one of the most preferred preconcentration steps for enhancing the sensitivity in metal analysis thanks to the enormous potential, benefits and versatility offered by this particular technique. Since the first approaches of CPE presented by Watanabe and co-workers 28 years ago [1, 2], the number of publications appearing annually between 1990 and 2005 has been continually growing, as shown in Fig. 1, for the whole field of CPE and for CPE approaches concerning metals. The principles and relevant applications of this impressive separation methodology have been discussed in various interesting reviews [3–10].

CPE is based on surfactant aggregates which together with ionic liquids have been recognized as the solvents of a modern era. Undoubtedly, the most important property of micelles is their solubilization ability.

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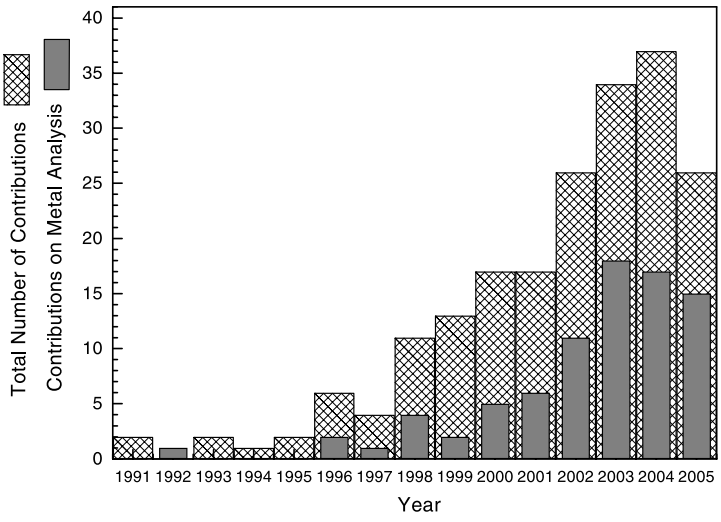

Fig. 1. Number of publications appearing annually in the field of CPE

Both bulk solvent-soluble and solvent-insoluble species can reversibly interact with and bind to the micellar assembly. Sparingly soluble or non-water-soluble materials can be solubilized in water due to their binding to the micelles in solution [3]. This explains the fact that many analytical and other applications of surfactant micellar media have been based upon their analyte solubilization proficiencies.

It has been demonstrated that non-ionic surfactant micelles provide the best general solubilization medium for the widest variety of solutes [3–5, 11]. In addition to the chemical nature of the solute, other factors that influence the extent of solubilization include type and structure of the surfactant, presence of electrolytes and other organic additives, and temperature.

The use of micellar systems as an alternative to other techniques of separation offers several advantages including low cost, safety and high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and very high concentration factors. From an analytical point of view, the surfactant-rich phase can be used to separate and/or preconcentrate different analytes before their injection into any hydrodynamic analytical system. Some additional advantages arising from sample/analyte storage (especially for speciation studies), further analyte detection enhancement provided by the micelle microenvironment, and favourable interactions with capillary walls will be discussed later.

Fundamentals of Cloud Point Extraction

Cloud point extraction (CPE) is an outstanding alternative to conventional solvent extraction because it produces high extraction efficiencies and preconcentration factors, and uses inexpensive, non-toxic reagents.

Aqueous solutions of some non-ionic and zwitterionic surfactants (at concentrations above the critical micelle concentration, cmc) or cyclodextrins [3] which behave like non-ionic surfactants are homogeneous and isotropic. Upon alteration of the conditions such as temperature, pressure, or additives, the solution becomes turbid due to the diminished solubility of the surfactant in water. Above the cloud point (temperature at which this phase separation behavior occurs), the single isotropic micellar phase separates into two isotropic phases: one, the aqueous phase that contains the surfactant at a concentration close to the cmc, and the other, the surfactant-rich phase, whose volume is very small. Any species initially present in the solution that interact with the micellar aggregates are thus extracted and may be preconcentrated in the small volume of the surfactant-rich phase.

Cloud-point extractions consist of three simple steps, illustrated in Fig. 2:

1. Solubilization of the analytes in the micellar aggregates (Fig. 2A and B);
2. Clouding (Fig. 2C);
3. Phase separation for analysis (Fig. 2D).

First, the surfactant is added to the aqueous solution containing the analyte(s) to be extracted and analyzed. The final surfactant concentration must be greater than its cmc. Surfactant aggregates are capable of selective interactions with different analytes and can strongly modify solubility, chemical equilibria, kinetics, and the spectroscopic properties [5]. Solubilization is a

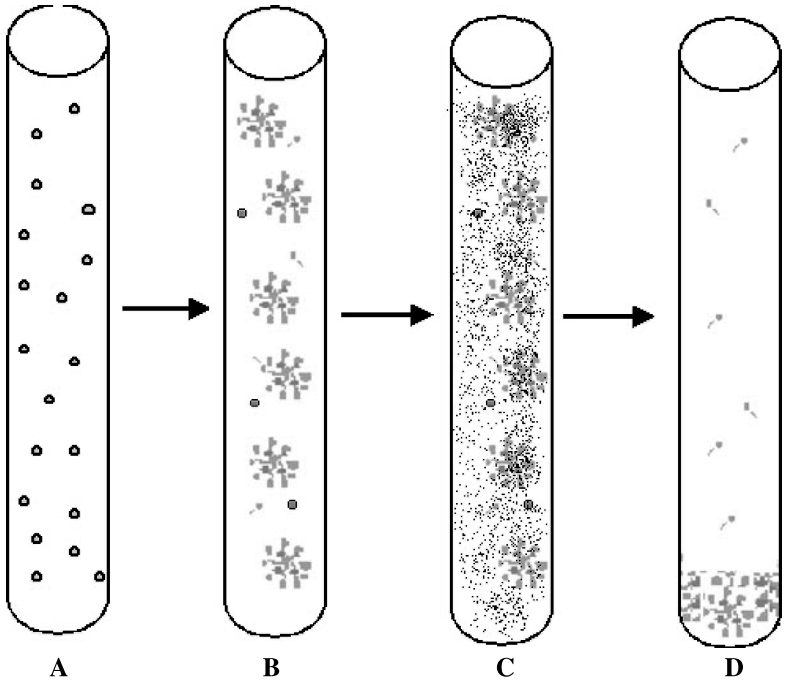

Fig. 2. Schematic representation of cloud point extractions. (A–B) Solubilization of the analytes in the micellar aggregates, (C) Clouding, (D) Phase Separation for analysis

dynamic process; analyte solubilization in micelles has traditionally been treated in terms of a two-phase process [12], the micellar phase (hydrophobic dissolved state in the micellar interior and/or a more polar adsorbed state at the micellar water interface) and the aqueous bulk state. Solutes that bind to micelles in solution are extracted to different extents, depending on the micelle-solute binding interactions [13]. The metallic ion species can bind electrostatically to the polar head of the surfactant, or the hydrophobic metallic chelates can remain referentially in the hydrophobic domain of the micelles in a surfactant-rich phase, thus being extracted and preconcentrated [14].

Next, the temperature is altered (raised to above the cloud point temperature of non-ionic surfactants or lowered to below the cloud point temperature in the case of zwitterionic surfactants) so that phase separation occurs. Several recent studies have been conducted in order to study the clouding phenomenon. According to Kjellander each surfactant molecule is surrounded by a water molecule lattice, through hydrogen bridges to the polar groups. When the temperature is raised, the lattice is destroyed by the entropy, the weak Van der Waals forces prevailing among the surfactant molecules, leading to the phase separation [15–17]. In conclusion, phase separation results from the competition between entropy, which favors miscibility of micelles in water, and enthalpy, which favors separation of micelles from water.

The mechanism by which this separation occurs is attributed to the rapid increase in the aggregation number of the surfactant's micelles, as a result of the increase of temperature, or to critical phenomena [18]. Ethylene oxide segments in the micelle repel each other at low temperatures, when they are hydrated, and attract each other as the temperature increases due to dehydration. This effect causes a decrease in the effective area occupied by the polar group on the micelle surface, increasing the size of the micelle that can be considered to become infinite at the cloud point, resulting in the phase separation [19, 20].

For non-ionic systems, the temperature-induced dehydration of the polyoxyethylene headgroups promotes micellar growth and demixing [3].

Besides, although regular solution theory predicts that partition constants of the metal chelates will be almost independent of the metal ion nature, they vary with the kind of extracted metal in the case of CPE with polyethyleneglycolmono-p-nonylphenylether (PONPE 7.5) with consequent potential increase of selectivity [12]. The mechanism in the variation of the partition constants could be explained in terms of the presence of microscopically ordered structures in the surfactant phase, such as those in liquid crystals, which can distinguish slight differences in molecular size, shape and structural factors [5].

The turbidity of the system stems from the presence of very large surfactant aggregates that scatter the

visible light passing through the solution. Phase separation typically occurs over a narrow temperature range.

The temperature at which phase separation occurs depends on the nature and concentration of surfactant and the presence and concentration of both organic and inorganic additives. For a homologous series of polyoxyethylated non-ionic surfactants, the cloud point increases with decreasing length of the hydrocarbon chain or increasing length of the oxyethylene moiety [3]. It should be noted that the presence of other surfactants, acids or bases, salts, and/or organic additives can dramatically alter the critical temperature of such aqueous surfactant solutions [5].

Although higher temperatures are commonly applied to attain the cloud point, other possibilities, such as pressure or the use of additives (including electrolytes), have been proposed.

Finally, the following step in the cloud-point process is the separation of the surfactant-rich phase from the aqueous bulk phase. The actual physical separation of the phases is facilitated by the difference in density between the two (dilute aqueous and surfactant-rich) phases but presents some practical problems. The phase separation process is reversible and, upon cooling the mixture to a temperature below the cloud point, the two phases again merge to form an isotropic, homogeneous solution.

Usually, quantitative extractions are obtained but, if necessary, the CPE procedure can be repeated by addition of more surfactant/additives to the aqueous phase, in order to achieve higher extraction efficiency.

Cloud point extraction has been used in the field of metal analysis to preconcentrate the analytes based on the formation of chelates in the surfactant aggregate. Nevertheless, it has been demonstrated that quantitative extraction of metal ions [21–23] is feasible even in the absence of chelating reagent. This behaviour could be explained in terms of the formation of a complex located in the micelle surface between the surfactant through its polyoxyethylene groups and the metal [24].

Luconi et al. [21] considered the following factors with the aim to establish the nature of extracting species and the location of the metal ion in the micelle: the nature of the amphiphilic media; metal distribution equilibria and the results obtained after the evaluation of the parameters affecting the process. PONPE 7.5 forms a cationic complex with $[Pb(OH)]^+$ through its polyoxyethylene groups. They concluded that a maximum amount of 60 metal ions can be associated/bound to each micellar entity.

Developing and Optimizing CPE Approaches in Batch and On-Line Modes

Over the past 30 years, trace metal ion determinations received a lot of attention because of their strong environmental impact of polluting or physical processes. Actually, the evaluation of the impact of human activity on the environment is one of the main goals of present-day analytical chemistry. Indeed, the most effective method for assessing chemical exposure is biomonitoring, or the analysis of biological fluids such as blood, urine, saliva or breath. Contemporary analytical scientists should develop, optimize and validate reliable analytical methods for routine environmental monitoring featuring high reproducibility, high recoveries and low interferences from the matrix constituents and the ability to determine total metal content and metal species even at $ng\,L^{-1}$ levels.

The relevance of trace metal determination in biological samples derives from their potentially toxic or positive effects on living beings. The analysis of trace elements in biological samples is particularly difficult because of the complex matrix and the usually low concentration of these elements in such samples. For this reason, metal ion preconcentration is an area of incessant research.

As has been stated, a scheme of preconcentration mediated by surfactants can be proposed. Among other micelle-based separation methods, cloud point extraction is an efficient extractive step for the enrichment of metal ions, allowing their quantification at trace and ultra-trace levels. The use of the CPE process for extraction of metals, metal chelates, biomolecules, many types of organic species and environmental clean-up procedures has been reported [25, 26]. Nevertheless, the coupling of CPE to instrumental methods is not an easy challenge. The effect of the experimental parameters on the extraction parameters and sensitivity has to be thoroughly evaluated and optimized. The optimal combination of experimental variables leads to higher extraction percentage, optimal stability, lower equilibration time and ease of phase separation.

The vast approaches available in the literature in the field of CPE show that the optimization of the experimental conditions has to date been accomplished by the traditional method of one-at-a-time. No quimiometric methodology has been applied in this area of research.

The variables which have to be examined to make CPE successful are the chemical nature and concentration of the surfactant, the presence of additives

(inorganic, organic solvents, monomeric or polymers agents), the complexing agent concentration (if necessary), pH, buffer concentration, ionic strength, complex stability, equilibration temperature and time, centrifugation conditions, diluting agent of the surfactant rich-phase, effect of interferences, and specific variables affecting the resolution/sensitivity/robustness of the selected instrumental methodology.

Selection of the Surfactant

To date, non-ionic surfactants (mainly polyoxyethylenated alkylphenols, from the Triton and PONPE series) are those most widely employed for CPE metal analysis. They are all commercially available of high purity grade, stable, non-volatile, non-toxic and environmentally friendly [3, 5, 24]. Non-ionic surfactants do not contain a specific fixed number of ethylene oxide units, but instead consist of a statistical distribution of homologues (ethoxamer distribution) [5].

The extraction efficiency typically increases with a surfactant concentration up to a maximum value, with essentially quantitative recovery often being observed.

The surfactant concentration affects both the extraction and preconcentration factor. Thus, the minimum concentration that produces quantitative extraction should be chosen in order to obtain the best aqueous phase volume/surfactant-rich phase volume ratio.

Some authors have reported the experimental convenience of working with a surfactant mother solution containing ethanol [1, 21–23] which yielded better reproducibility.

Additives Prior to Phase Separation

In many cases, the addition of an organic solvent or a salt prior to the extraction step is necessary to reach efficient extraction. The presence of ethanol when working with surfactants showing low critical temperatures produces an adequate increase on the cloud point temperature of the system, higher preconcentration factors and a better kinetics of phase separation [12].

On the other hand, the presence of inorganic electrolytes decreases the cloud point temperature due to dehydration of the poly(oxyethylene) chains [27]. Additionally, inorganic salts enhance the hydrophobic interactions among the surfactant aggregates and the analytes, thus favouring their extraction from the aqueous to the micellar phase.

Effect of pH

The effect of pH on the sensitivity and extraction parameters has to be tested. In the case of metal chelates, the optimal pH range frequently matches the range of the most favorable complex formation.

The pH plays an important role in improving the extraction efficiency in CPE of metals without the addition of a chelating agent, since it affects the overall charges of the analyte, thus affecting the formation of the complex between the metal and the surfactant polyoxyethylene groups.

Equilibration Temperature and Time

The greatest analyte preconcentration factor is reached when the CPE process is conducted with equilibration temperatures well above the cloud point temperature of the system [4, 12]. It was observed that the volume of the surfactant-rich phase decreased by a factor of 5 when the temperature was increased from 25 to 90 °C working at a surfactant concentration of 1% (w:w) [21]. Nevertheless, for thermally labile metal chelates, the use of elevated temperatures could result in a decreased recovery due to decomposition. Equilibration times within the range of 4–15 min at 60–70 °C are often selected as optimal.

Physical Phase Separation

Several alternatives have been proposed in order to separate the surfactant-rich phase from the aqueous phase. After the centrifugation step, the system is cooled (frequently at −4 °C), and the surfactant-rich phase becomes a viscous gel, therefore facilitating physical phase separation which can simply be poured off by inverting the tubes [11]. Wuilloud et al. [28, 29] cooled the surfactant-rich phase in an ice-NaCl bath for 5 min. The removal of the aqueous phase was carried out by means of a peristaltic pump. Manzoori et al. [30] made use of an ice-acetone mixture, separating the supernatant aqueous phase with the help of a syringe. In another paper, the enriched phase was heated in a water bath at 100 °C to remove the remainder of water, and the enhancement factor increased 4-fold [31].

Paleologos et al. [7] reported that the residual water trapped in the condensed surfactant phase can be removed by oven drying at 100–120 °C.

Diluting Agent of the Surfactant Rich-Phase

In order to decrease the viscosity of the surfactant-rich phase and facilitate its handling and introduction into the instrument, it is necessary to search for the optimal diluting agent depending on the surfactant system employed, the instrumental detection system and the target analytes [32]. When working with AAS, organic solvents such as methanol or ethanol containing strong acids are frequently the diluting agent of choice, offering appropriate solution properties for aspiration and nebulization. In the case of ICP–OES, the enriched surfactant phase is diluted with concentrated acids. For absorptiometric measurements, the CPE-extracted phase can be measured without further treatment, while for flourometric determination with formic acid, 100% organic solvents, such as acetonitrile, are employed when injecting the phase into a CE instrument.

Effect of Interferences

It has to be pointed out that when this kind of extraction is implemented in conjunction with different analytical detection systems, the elimination of a great part of the saline content and several interfering species is possible due to the selectivity of the micelle towards the analyte and/or the limited tendency shown by other metals to form complexes with the chelating agent.

However, the presence of many metals in the final extract may be the reason for several spectral interferences, especially during AAS analysis, that require cautious treatment in order not to affect the accuracy of the measurements [32, 33].

Automated CPE Extractions

When preconcentration techniques are applied in a batch mode, the time of analysis increases and the operations are usually incompatible with the instrumental measurements. Furthermore, despite offering high sample throughput in routine analysis, these procedures depend on the analyst's efficiency, which may be a significant source of errors especially in the event of analysis at low $\mu g\,L^{-1}$ levels [33].

This situation has been significantly improved by utilizing flow injection (FI) associated with instrumental methods of analysis, such that the general drawbacks of batch preconcentration procedures have been largely eliminated and currently preconcentrations can be achieved almost as efficiently as with a simple instrumental determination. Reagent consumption is usually reduced to a small percentage of that in batch procedures. Sample contamination is also reduced, which becomes important when trace concentrations are determined. In fact, to date the most dramatic improvements achieved have been in the field of on-line preconcentration. The possibility of performing CPE

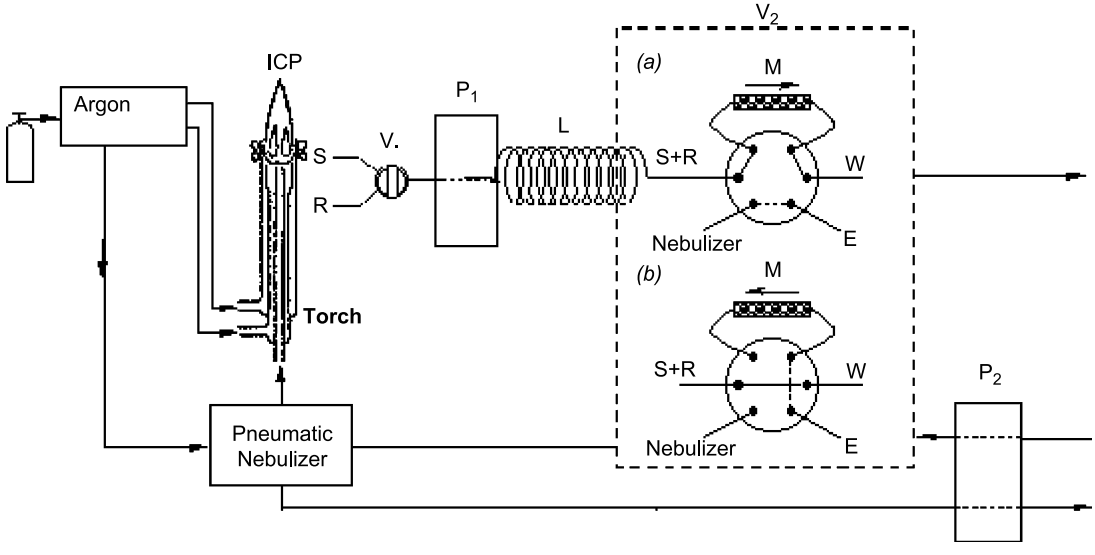

Fig. 3. Schematic diagram of the instrumental setup for on-line CPE preconcentration (reprinted with permission of Ref. [34]): *S* sample; *R* chelating reagent + extracting solution + buffer; *E* eluent; *W* waste; P_1 and P_2 peristaltic pump; *L* mixing coil, *M* microcolumn; V_1 two-way valve; V_2 load-injection valve ((*a*) *Load position*; (*b*) *Injection position*)

on-line opens up an attractive alternative in the field of automated separation methods, particularly in view of the excellent extraction efficiencies and preconcentration factors associated with the technique. Figure 3 shows a schematic diagram of the instrumental setup for an on-line CPE approach.

With regard to this kind of CPE system, further experimental parameters must be tested, especially those related to the collection column (such as chemical nature of the packing material, dimensions, loading and elution flow rates and architecture of the FI manifold).

To date, only a few automated CPE approaches have been reported. Ortega et al. [34] demonstrated the feasibility of the on-line CPE with FI–ICP–OES for the preconcentration-determination of total Gadolinium content in urine samples. The methodology is based on the complexation of Gd(III) with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol in the presence of non-ionic micelles of PONPE-7.5. The surfactant-rich phase was retained in a cotton-packed microcolumn and eluted with nitric acid directly in the nebulizer of the plasma. The detection limit value for the preconcentration of 10 mL of aqueous solution of Gd was $40\,ng\,L^{-1}$. Later, they presented a fully automated CPE approach for the determination of dysprosium in which the complex was formed on-line [35]. An enhancement factor of 50 was obtained, with a detection limit of $0.03\,\mu g\,L^{-1}$.

The on-line incorporation of CPE into flow injection analysis (FIA) associated with CE to simultaneously determine dysprosium and iron at ppb levels in urine was also presented [36]. Paleologos et al. described the on-line preconcentration of Cr-SDS-Triton X-114 (octylphenoxypolyethoxyethanol) system onto a cotton-packed reactor for the speciation of Cr [37]. Once eluted, the complex is mixed on-line with the lumiphore leading directly to the luminescence detector.

Hyphenation of CPE to Instrumental Methods of Analysis

Spectrophotometry: UV-Vis Spectrofluorimetry, Chemiluminescence

Table 1 summarizes some of the most recent literature in this area. Spectrophotometry continues to enjoy wide popularity. The common availability of the instrumentation and the simplicity of procedures, as well as speed, precision and accuracy of the technique still make photometric methods an attractive alternative. Most importantly, spectrophotometry has become the most widely used detection technique in flow-injection analysis.

Table 1. Coupling CPE to spectrophotometry

Metal ions	Matrix	Complex formation	Micellar system	Detection	LODs	P[a]	Comments	Ref.
Er(III)	synthetic superconducting materials and permanent magnets	3,5-diClDMPAP	PONPE 7.5	UV-Vis, 584 nm	$1.48\ 10^{-7}\,mol\,L^{-1}$	3.33	partition and dissociation constants calculation	[12]
Gd(III)	urine	3,5-diClDMPAP	PONPE 7.5	UV-Vis, 592 nm	$5.8\ 10^{-9}\,mol\,L^{-1}$	25	total and free Gd content determination	[38]
Al(III)	parenteral solutions	CAS-BDTAC	PONPE 7.5	UV-Vis, 554 nm	$1.12\ 10^{-7}\,mol\,L^{-1}$	50	FI system using an HPLC pump	[39]
V(IV) V (V)	tap water hair centrum tablets	8-quinolinol	TX-114	Fluo[b]	$0.0007\,\mu g\,L^{-1}$	50	FI-Fuo detection	[40]
Cr(III) Cr(VI)	centrum tablets, river, lake and sea water	8-HQ	TX-114	Fluo[b] 543 nm	$0.2\,\mu g\,L^{-1}$	75	FI-Fuo detection	[41]
Cr(III)	seawater, river water, wastewater certified material	luminol	TX-114-SDS clouding medium Na_2SO_4	Chemilu[c]	$0.5\,ng\,L^{-1}$		CPE on-line Sur. P. in a cotton-packed column	[37]

[a] Preconcentration factor = vol. surfactant-rich phase/vol. aqueous phase.
[b] Flourometric determination.
[c] Micellar chemiluminescence.

In 1997 an application of the hyphenation of CPE to UV-Vis absorptiometry was developed in our lab [12] for the determination of Er(III). We presented an exhaustive study of the variables affecting the extraction efficiency as well as the calculation of the partition and dissociation constants for the chelating agent 2-(3,5-dichloro-2-pyridylazo)-5-dimethylaminophenol (3,5-diClDMPAP) in the surfactant-rich phase of polyethyleneglycolmono-p-nonylphenylether (PONPE 7.5) by means of a successive approximation method using a least squares computer program. We also developed a methodology for the monitoring of total and free gadolinium contents in urine after the NMR imaging diagnostic examination with contrast agent [3]. Using the micro-scale procedure, a LOD for Gd(III) of $5.8 \cdot 10^{-9}\,\mathrm{mol\,L^{-1}}$ could be reached. Sombra et al. reported a combined cloud point preconcentration-flow injection analysis method for the determination of trace aluminum content in parenteral solutions [39]. The analyte complexed with Chrome Azurol S (CAS) in the presence of the cationic surfactant benzyldimethyltetradecylammonium chloride (BDTAC). The enriched analyte solution was injected into an FI system using an HPLC pump.

Paleologos et al. described a combined CPE-flourometric determination of vanadium based on the pyronine B-hydrogen peroxide reaction in a flow injection system [40]. The preconcentration step is performed by means of Triton X-114 micelles after complexation with 8-quinolinol in an acidic solution, and the surfactant-rich phase was diluted with formic acid before its injection into the FI manifold. Karayannis and co-workers performed a methodology for the determination of Cr species based on the complexation of Cr(III) with 8-hydroxyquinoline (8-HQ) in Triton X-114 micelles [41]. Cr(VI) was reduced with sulfite before its complexation, whereas the surfactant-rich phase was diluted with methanol before its introduction into the FI system.

As stated before, Paleologos at al. reported an automated CPE-chemiluminescence methodology for chromium at $\mathrm{ng\,L^{-1}}$ levels [37]. Both the carrier and the reagent stream contained EDTA masking the catalytic effect of interfering cations. This demonstrated that the emission is not only more intense in the presence of the micellar carrier but also retains its intensity for a longer period compared to that in the absence of the surfactant.

Atomic Spectroscopy: FAAS–ETAAS–ICP–OES–ICP–MS

The large majority of papers employing CPE for metal analysis have been devoted to its merging with atomic spectroscopy techniques. This is because ordered media inherently offer two important analytical advantages when combined with spectroscopic techniques: they change the physical properties (density, viscosity, surface tension) of the liquid sample solution to be fed into the atomizer and they alter the chemical properties of these solutions, customizing the sensitivity of the atomic detectors according to the requirements, and improve the nebulisation efficiency [14, 32].

Flame Atomic Absorption Spectrometry

Table 2 summarizes the available literature of applications of CPE to FAAS. The potential of hyphenating CPE to FAAS was demonstrated in 1998 by Curtius and co-workers in the enrichment and determination of Ag and Au in geological samples [42]. The analytes in the initial aqueous solution acidified with hydrochloric acid were complexed with ammonium *O*,*O*-diethyldithiophosphate (DDTP), and Triton X-114 was added as a surfactant. Giokas et al. described a combined CPE-FAAS procedure for the monitoring of six metal species as their pyrrolidinedithiocarbamate (APDC) complexes in natural waters [43]. The measured concentrations of dissolved metal species were deployed to estimate the pollution status of the ecosystems and reveal the mechanisms that contribute to the metal load. Coelho et al. reported the preconcentration of Cd after its complexation with DDTP in an acidic medium ($0.32\,\mathrm{mol\,L^{-1}}$ HCl) using Triton X-114 [44]. The cloud point is formed in the presence of 1% (w/v) NaCl as clouding agent. The method was applied to Cd determination in physiological solutions, mineral water, lake water and cigarette samples (tobacco). Manzoori et al. described cloud point extraction of silver as a diphenylthiocarbazone (dithizone) complex prior to flame atomic absorption spectrometric determination [45]. The proposed method was applied to the determination of silver in water samples. Chen and Teo developed a methodology for the determination of cadmium, copper, lead and zinc in water samples [46] after their complexation with 1-(2-thiazolylazo)-2-naphthol (TAN) in micelles of Triton X-114. The same authors presented the determination

Table 2. Coupling CPE to flame atomic absorption spectrometry (FAAS)

Metal ions	Matrix	Complex formation	Micellar system	SRP[a] diluting agent	LODs	EF[b]	Ref.
Ag Au	Reference geological materials	DDTP	TX-114	methanol	0.53 ng mL^{-1} Au 0.46 ng mL^{-1} Ag	130 Au 91 Ag	[42]
Multi-elemental	Natural waters	APDC	TX-114	methanolic solution 1 M HNO_3	NA[c]	NA	[43]
Cd	physiological solutions, mineral water, lake water cigarette samples	DDTP	TX-114	5% v/v ethanol 0.1 mol L^{-1} HNO_3	0.9 μL^{-1}	NA	[44]
Ag	water	dithizone	TX-114	tetrahydrofuran	0.56 ng mL^{-1}	43	[45]
Cd Cu Pb Zn	water reference materials	TAN	TX-114	methanol 0.1 mol L^{-1} HNO_3	0.099 ng mL^{-1} Cd 0.27 ng mL^{-1}Cu 1.1 ng mL^{-1} Pb 0.095 ng mL^{-1} Zn	57.7 Cd 64.3 Cu 55.6 Pb 63.7 Zn	[46]
Co Ni	water	TAN	TX-114	methanol 0.1 mol L^{-1} HNO_3	0.24 $\mu g\,L^{-1}$ Co 0.44 $\mu g\,L^{-1}$ Ni	57 Co 65 Ni	[47]
Cr(III) Cr(VI)	tap and river water	MBED	TX-114	methanol 0.1 mol L^{-1} HNO_3	0.17 $\mu g\,L^{-1}$	57	[48]
Fe(II) Fe(III)	water certified reference material	APDC	TX-114	NA	7 $\mu g\,L^{-1}$	NA	[49]
Ge	water	quercetin	TX-114	NA	0.59 $\mu g\,L^{-1}$	200	[50]
Pb Cd	human hair	DDTP	TX-114	methanol 0.1 mol L^{-1} HNO_3	2.86 $\mu g\,L^{-1}$ Pb 0.62 $\mu g\,L^{-1}$ Cd	43 Pb 22 Zn	[30]
Pb	human saliva	none	PONPE 7.5	Ethanol 0.1 mol $\cdot$ L^{-1} HNO_3	NA	10	[21]
Mn	tap water, river water, sea water	TAN	TX-114	methanol 0.1 mol L^{-1} HNO_3	0.28 $\mu.g\,L^{-1}$	57.6	[51]
Co	urine	PAN	TX-114	methanol	0.38 $\mu g\,L^{-1}$	115	[31]
Co	pharmaceutical samples (B12 vitamin)	PAN, PAR, 5-BrPADAP	TX-100-SDS	ethanol 0.1 mol $\cdot$ L^{-1} HCl	1.1 $\mu g\,L^{-1}$	27.5	[52]
Fe free bound	wine	APDC	NA	methanol HCl-KCl-8-HQ	0.02 mg L^{-1}	NA	[53]
Cu free bound	river water, raw and settled wastewater	APDC CTAB	TX-100 TX-45	methanol 0.1 mol L^{-1} HNO_3	0.9 $\mu g\,L^{-1}$	100	[54]
Cu	drinking and rainwater, serum and human hair	DDTP	TX-100	methanol 0.1 mol L^{-1} HNO_3	0.94 ng mL^{-1}	NA	[55]
Cu	tap water	capric acid octylamine	OP-10	water	0.01 $\mu g\,mL^{-1}$	NA	[56]
Mg	natural and mineral water	Trizma-Chl	TX-114	methanol 0.1 mol L^{-1} HNO_3	0.75 $\mu g\,L^{-1}$	50	[57]

[a] Surfactant-rich phase.
[b] Enhancement factor.
[c] Non-available data.

of cobalt and nickel in water samples also by means of TAN and TX-114 [47].

Shemirani et al. reported a methodology for the speciation of chromium in tap water samples [48]; Cr is preconcentrated as (bis(2-methoxybenzaldehyde) ethylene diimine (MBED) complex; Cr(VI) determination is based on its reduction to Cr(III) by the addition of concentrated H_2SO_4 and ethanol. In 2002, Giokas et al. presented an interesting approach to the speciation of Fe(II) and Fe(III) by the modified ferrozine method [49]. The method involves cloud-point extraction (CPE) of both species with ammonium pyrrolidinecarbodithioate (APDC) under standard conditions, which facilitates the in-situ complexation and extraction of both species. Differentiation of the oxidation states of iron is achieved by using mathematical equations to overcome the interference of Fe(III) in the FI-spectrophotometric determination of Fe(II) when they are both present in the same solution. Böyukbayram reported a CPE-HGAAS method for the preconcentration of germanium based on its complexation with quercetin [50]. Manzoori et al. reported a method for the determination of Cd an Pb in human hair [30] using *O*,*O*-diethyldithiophosphate (DDTP) and Triton X-114 as hydrophobic ligand and non-ionic surfactant, respectively.

Our research group described a methodology for the determination of lead in human saliva by using non-ionic PONPE 7.5 without added chelating agents [21]. We studied the nature of the extracting species as well as the location of lead in the micelle. Teo et al. reported a CPE-FAAS methodology for the determination of manganese in water samples after the formation of a complex with 1-(2-thiazolylazo)-2-naphthol (TAN) using octylphenoxypolyethoxyethanol (Triton X-114) as surfactant [51]. In 2003 Manzoori et al. described a method for the determination of cobalt in urine samples [31] based on the formation of 1-(2-pyridilazo)-2-naphtol (PAN) complexes in micelles of Triton X-114. Nascentes et al. presented a cloud point formation based on mixed micelles (Triton X-100 and SDS) in the presence of electrolytes for cobalt extraction and determination in pharmaceutical samples containing B12 vitamin [52]. Phase separation was induced by HCl or NaCl addition. A micelle-mediated methodology for the determination of free and tannin-bound iron in wines was demonstrated by Paleologos et al. [53] in 2002. The method employs precipitation of the tannins and other phenolic and insoluble compounds in the micelles of a non-ionic surfactant mixture (TX-100 and TX-45), and they are subsequently separated from the initial solution by centrifugation. The supernatant is submitted to the same cloud point extraction procedure for the determination of free iron species in the presence of a chelating agent, ammonium pyrrolidine dithiocarbamate (APDC), in order to form water-insoluble complexes with free iron. Giokas et al. reported a methodology for the speciation analysis of free and organically complexed metal species in natural waters (river water, raw and settled wastewater) [54] based on the neutralization of the electrostatic charge of the humate-metal complexes with a positively charged surfactant (cetylatrimethylammonium bromide (CTAB)) in a high ionic strength solution environment. Labile metal ions are then extracted by the same preconcentration technique after complexation with an excess of ammonium pyrrolidinecarbodithioate (APDC).

Manzoori et al. developed a combined methodology for the determination of Cu as *O*,*O*-diethyldithiophosphate (DDTP) complex in drinking and rainwater, serum and human hair samples [55]. In 2003 Kulichenko et al. described a method for Cu quantification in water samples [56]. Cu was complexed with monocarboxylic acids. Also, the extraction degree and the distribution coefficient of copper in the water-surfactant-rich phase-phase system were calculated. Giokas et al. presented a CPE-FAAS methodology for the determination of Mg in natural and mineral water after the formation of Mg-trizma-chloranilate chelate (Trizma-Chl) [57].

Electrothermal Atomic Absorption Spectrometry (ETAAS)

Table 3 summarizes the available literature on applications of CPE to ETAAS. One of the first applications was the on-line preconcentration and determination of lead in certified biological reference materials by Yan and co-workers [58] in 2003. The procedure involved the formation of the analyte-entrapped surfactant micelles by online merging of the analyte solution with an ammonium pyrrolidine dithiocarbamate (APDC) solution and a Triton X-114 solution sequentially, the adsorption of the resultant analyte-entrapped surfactant micelles onto a microcolumn packed with silica gel, and online elution of the adsorbed analyte acetonitrile for ETAAS detection. To facilitate the on-line coupling of the CPE system to ETAAS, an ‘‘air-segmented and air-transported

Table 3. Coupling CPE to electrothermal atomic absorption spectrometry (ETAAS)

Metal ions	Matrix	Complex formation	Micellar system	SRP[a] diluting agent	LODs	EF[b]	Ref.
Pb	certified biological reference materials	APDC	TX-114	acetonitrile	44.6 ng L^{-1}	22.5	[55]
As(III) As(V)	tap water hair and nail	molybdate sulfuric acid	TX-114	methanol	0.01 ng mL^{-1}	52.5	[56]
Bi	tap water urine and hair	dithizone	TX-114	THF	0.02 ng mL^{-1}	196	[57]
Cd Pb	biological reference materials	DDTP	TX-114	methanol 0.1 mol L^{-1} HNO_3	6 ng g^{-1} Cd 40 ng g^{-1} Pb	129 Cd 18 Pb	[58]
Cr(VI) Cr(II)	water	Br-PF	TX-100	methanol 0.1 mol L^{-1} HNO_3	0.01 μg L^{-1}	NA	[59]
Cd, Pb Pd	certified blood reference samples	DDTP	TX-114	methanol 0.1 mol L^{-1} HNO_3	0.02 μg L^{-1} Cd 0.08 μg L^{-1} Pb 0.014 μg L^{-1} Pd	71 Cd 34 Pb 34 Pd	[60]
Cd	sea water	DDTC	TX-114	NA	2.0 ng L^{-1}	51.6	[61]
Sn	water	8-HQ	TX-114	NA	0.012 ng mL^{-1}	96.2	[62]

[a] Surfactant-rich phase.
[b] Enhancement factor.
[c] No-available data.

operational sequence'' was adapted to the FI manifold design.

Shemirani et al. reported a CPE–ETAAS method for the determination of arsenic(III) and arsenic(V) in tap water and total arsenic in biological samples [59]. The method is based on the Triton X-114 mediated phase separation after reaction of As(V) with molybdate towards a yellow heteropoly acid complex in sulfuric acid. Total inorganic arsenic (III, V) was extracted similarly after oxidation of As(III) to As(V) with $KMnO_4$. Shemirani et al. described a methodology for the quantification of bismuth in tap water and biological samples (urine and hair) [60]. Dithizone-Bi complex was preconcentrated in Triton X-114 micelles, and the surfactant-rich phase was diluted using tetrahydrofuran (THF). Recently, Curtius and co-workers developed a method for the quantification of cadmium and lead in certified biological reference materials [61]. The analytes were complexed with *O*,*O*-diethyldithiophosphate (DDTP) in hydrochloric acid medium and extracted by means of Triton X-114 micelles.

Zhu et al. described a method based on CPE separation, and ETAAS detection was proposed for the determination of chromium species [62]. Cr(VI) was preconcentrated in micelles of TX-100 as a dibromophenyluorone (Br-PF) complex, while Cr(II) remained in the aqueous phase. Curtius and co-workers reported an interesting approach to the determination of Cd, Pb and Pd from certified blood reference samples (lyophilized bovine blood and reconstituted human blood) after microwave-assisted digestion [63]. The analytes were preconcentrated in micelles of TX-114 after complexation with *O*,*O*-diethyldithiophosphate (DDTP) in hydrochloric acid medium.

In 2004, Yuan et al. developed a methodology for the quantification of cadmium at the nanogram per liter level in seawater [64]. Diethyldithiocarbamate (DDTC) was used as the chelating reagent to form Cd-DDTC complex in micelles of TX-114. A method for the determination of Sn in water samples after the formation of Sn-8-hydroxyquinoline (8-HQ) complex was presented by the same group [65].

Inductively Coupled Plasma Optical Emission Spectrometry (ICP–OES)

Table 4 summarizes the most recent literature in the area of hyphenation of CPE to ICP–OES. In 2002 our research group reported a combined methodology for mercury at trace levels in tap water samples [28]. CPE was employed for the preconcentration of mercury prior to inductively coupled plasma optical emission spectrometry coupled to a flow injection with a cold vapor generation system. The analyte was extracted as

Table 4. Coupling CPE to inductively coupled plasma optical emission spectrometry (ICP–OES)

Metal ions	Matrix	Complex formation	Micellar system	SRP[a] diluting agent	LODs	EF[b]	Ref.
Hg	tap water	5-Br-PADAP	PONPE 5	ethanol HNO_3 (conc.)	4 ng L^{-1}	200	[28]
Cr Cu	water	8-HQ	TX-100	NA[c]	1.29 ng mL^{-1} Cr 1.31 ng mL^{-1} Cd	NA[c]	[66]
Al	parenteral solutions	–	PONPE 7.5	HCl (conc.)	0.25 μg L^{-1}	200	[22]
Pb	tap water certified reference material	–	PONPE 7.5	HCl (conc.)	0.077 μg L^{-1}	>300	[23]
Cr(II) CrVI)	natural water samples	PMBP	TX-100	NA	0.81 μg L^{-1}	20	[67]
V	parenteral solutions	5-Br-PADAP	PONPE 5	ethanol	16 ng L^{-1}	250	[29]
Gd	urine	5-Br-PADAP	PONPE 7.5	nitric acid	40 ng L^{-1}	20	[34]
Dy	urine	5-Br-PADAP	PONPE 7.5	nitric acid 4 mol L^{-1}	0.03 μg L^{-1}	50	[35]
Clay minerals	clay	–	Pluronic L-61	–	NA[c]	NA	[68]
La Gd	–	HQ K100	TX-100	nitric acid 0.01 mol L^{-1}	NA	37.5	[69]

[a] Surfactant-rich phase.
[b] Enhancement factor.
[c] Non-available data.

mercury-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol Hg(II)-(5-Br-PADAP) complex, at pH 9.2 mediated by micelles of the non-ionic surfactant polyethyleneglycolmono-p-nonylphenylether (PONPE 5). Li et al. were able to determine chromium and copper in water samples by inductively coupled plasma optical emission spectrometry after CPE [66]. The method is based on the complexation of metal ions with 8-hydroxyquinoline (8HQ) in the presence of non-ionic micelles of Triton X-100.

In our group, we developed surfactant-mediated extraction methodologies coupled to flow injection inductively coupled plasma optical emission spectrometry in the absence of chelating agents. The latter is possible due to the fact that some metal ions under optimal experimental conditions form a complex with PONPE 7.5 through their polyoxyethylene groups which is consequently located in the micelle surface. Aluminum was determined in parenteral solutions [22] without any previous treatment. Aluminum contamination may be a potential hazard to patients with prolonged parenteral nutrition; patients may inadvertently receive significant amounts of aluminum present as contaminant introduced during the manufacture of the pharmaceuticals. The monitoring of lead in tap water was also possible [23].

The speciation of chromium in natural water, based on cloud point extraction and inductively coupled plasma optical emission spectrometry was presented by Liang and Li [67]. Cr(III) reacts with 1-phenyl-3-methyl-4-benzoylpyrazol-5-one (PMBP), yielding a hydrophobic complex which is entrapped in the surfactant-rich phase, whereas Cr(VI) remains in the aqueous phase. Total chromium was determined after reduction of Cr(VI) to Cr(III) with ascorbic acid, the reducing reagent.

A hyphenated methodology CPE–FI–ICP–OES for vanadium determination at trace levels in parenteral solutions was developed in our lab [29]. The vanadium was extracted as vanadium-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol [V-(5-Br-PADAP)] complex, at pH 3.7 mediated by micelles of the non-ionic surfactant polyoxyethylene (5.0) nonylphenol (PONPE 5.0).

Takahashi and co-workers presented a CPE–ICP–OES methodology for the separation of clay minerals [68]. They used an industrial non-ionic triblock copolymer surfactant, Pluronic L-61, consisting of polyethylene oxide and polypropylene oxide parts to recover artificial clay of saponite from its aqueous

solutions. They demonstrated that a cooling treatment of the surfactant-rich solution enhances the affinity of clay for surfactants.

In 2004 Foos and co-workers presented cloud point preconcentration mediated by micelles of TX-100 coupled to ICP–OES for the separation of Lanthanum and gadolinium [69]. The methodology used is based on the formation of lanthanide(III) organic complexes that are soluble in a micellar phase of a non-ionic surfactant, Triton X-114. Gadolinium complexes are extracted by means of Kelex 100 (K100). The selectivity of the extraction could be explained by the differences between the stability constants of the lanthanides(III) with 8-hydroxyquinoline.

As stated previously, our research group was able to develop automated methodologies involving the coupling of on-line cloud point preconcentration using a micro-column packed with cotton to flow injection inductively coupled plasma optical emission spectrometry [34, 35].

Inductively Coupled Plasma Mass Spectrometry (ICP–MS)

The use of Cloud Point Extraction in combination with Inductively Coupled Plasma Mass Spectrometry has appeared in few publications (Table 5).

The first combined CPE–ICP–MS methodology was presented by Curtius and co-workers in 2000. They proposed CPE-ultrasonic nebulization inductively coupled plasma mass spectrometry for the determination of Ag, As, Au, Cd, Cu, Pb and Se in water [70]. The analytes in the initial aqueous solution, acidified with hydrochloric acid, are complexed with ammonium *O*,*O*-diethyl-dithiophosphate, and 0.05% m/v Triton X-114 is added as surfactant. The same authors proposed a method for noble metal quantification in biological samples consisting of a preconcentration step mediated by micelles of TX-114 after complexation with *O*,*O*-diethyldithiophosphate (DDTP) and determination by electrothermal vaporization inductively coupled plasma mass spectrometry [71].

Giné and co-workers reported CPE-isotope dilution inductively coupled plasma mass spectrometry for the quantification of molybdenum in plants [72]. Mo was preconcentrated as 8-hydroxyquinoline (HQ) complex in micelles of TX-100.

Separation Techniques: Capillary Electrophoresis (CE)

Over the past two decades, CE and related techniques have rapidly developed into a powerful analytical technique for the separation of a wide range of analytes, ranging from large protein molecules to small inorganic ions. Metal analysis by CE has the advantages of robustness and ruggedness, low-cost, rapidness and versatility. However, CE suffers from poor concentration sensitivity when using UV detection because of the small injection volumes (typically $<1\%$ capillary length) and narrow optical pathlength. This presents a significant obstacle for routine analyses of metal ions at ppb levels in real samples. The need for enhanced sensitivity has induced the development of highly sensitive detection and enrichment methods. Approaches to improve detection sensitivity include bubble and z-shaped cells, and other detectors such as laser-induced fluorescence, amperometry,

Table 5. Coupling CPE to inductively coupled plasma mass spectrometry (ICP–MS)

Metal ions	Matrix	Complex formation	Micellar system	SRP[a] diluting agent	LODs	EF[b]	Ref.
Ag, As Au, Cd Cu, Pb Se	riverine and sea water enriched water reference materials	DDTP	TX-114	Methanol 60% 1% HNO_3	NA[c]	40	[70]
Pt Rh Ru Au Pd	urine hair	DDTP	TX-114	methanol 60% 0.125 mol L^{-1} HCl	Ru 0.7 ng L^{-1} Rh 3.0 ng L^{-1} Pd 6 ng L^{-1} Pt 0.6 ng L^{-1} Au 0.8 ng L^{-1}	Ru 44 Rh 7 Pd 40 Pt 60 Au 35	[71]
Mo	plant certified materials	HQ	TX-100	water	0.8 ng g^{-1}	NA[c]	[72]

[a] Surfactant-rich phase.
[b] Enhancement factor.
[c] Non-available data.

conductometry and mass spectrometry. However, in most cases these approaches are inadequate for metal analysis, or the low concentration levels especially in environmental or clinical samples are not compatible with the detection limits of these techniques. Consequently, preconcentration techniques in conjunction with CE represent a promising tool especially in the area of simultaneous determination of metals at sub-trace levels. Considering the numerous advantages of micelle-mediated extractions, one logical approach to increase sensitivity in CE is its hyphenation to cloud point extraction. Moreover, CPE uses surfactants that inhibit the absorption of nonpolar analytes to glass surfaces.

Table 6 summarizes some of the most recent applications of CPE of metallic ions coupled to CE. We recently demonstrated the feasibility of hyphenating CPE to CE for the first time, for instance. The on-line incorporation of cloud point extraction (CPE) into flow injection analysis (FIA) associated with Capillary Zone Electrophoresis for the simultaneous determination of dysprosium and iron at ppb levels in urine was presented and evaluated [36]. The preconcentration step was mediated by micelles of the non-ionic surfactant PONPE 7.5 with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP). The micellar system containing the complex was loaded into the FIA manifold, and the surfactant rich-phase was retained in a microcolumn packed with cotton. The surfactant-rich phase was eluted with 50 µL acetonitrile directly into the CE sample vial.

Tang et al. presented a CPE–CE methodology for the determination of Cu(II) and Co(II) [73]. The preconcentration of Cu(II) and Co(II) in aqueous solution was achieved by CPE with 1-(2-pyridylazo)-2-naphthol (PAN) as the chelating agent and Triton X-114 as the extractant surfactant.

Two other CPE–CE methodologies were developed in our lab for the simultaneous determination of Pt and Pa [74] and Pb in human saliva [75]. We demonstrated the feasibility of the incorporation of a cloud point extraction step prior to CE for the simultaneous determination of platinum and palladium at sub-µg L^{-1} levels in water [74]. The analytes were extracted as 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) complexes, at pH 2.0, mediated by micelles of the non-ionic surfactant polyethyleneglycolmono-p-nonylphenylether (PONPE 7.5). Detection was performed at 576 nm.

A micelle-mediated phase separation without added chelating agents to preconcentrate trace levels of lead in human saliva as a prior step to its determination by capillary electrophoresis with indirect detection was developed [75]. The enrichment step is based on cloud point extraction of lead with the non-ionic surfactant PONPE 7.5 in the absence of chelating agent. Indirect detection was performed at 205 nm.

Table 6. Coupling CPE to capillary electrophoresis

Metal ions	Matrix	Complex formation	Micellar system	SRP[a] diluting agent	BGE[b]	LODs	PF[c]	Ref.
Gd Fe	human urine	5-Br-PADAP	PONPE 7.5	acetonitrile	20 mM sodium tetraborate 13% acetonitrile, pH 9.00	0.20 µg L^{-1} Dy 0.48 µg L^{-1}Fe	200	[36]
Cu Co	tap and snow water wines	PAN	TX-114	methanol	50 mM NH_4Ac 0.2 mM of PAN 80% acetonitrile, pH 8.0	0.12 µg L^{-1}Co 0.26 µg L^{-1}Cu	15.9 Co(II) 16.3 Cu(II)	[73]
Pt Pd	water	5-Br-PADAP	PONPE 7.5	acetonitrile	20 mM monobasic sodium phosphate 30% acetonitrile, pH 4.53	0.04 µg L^{-1} Pt 0.08 µg L^{-1} Pd	250	[74]
Pb	human saliva	–	PONPE 7.5	acetonitrile	20 mM imidazole 30% acetonitrile, pH = 6.20	11.4 µg L^{-1}	NA[d]	[75]

[a] Surfactant-rich phase.
[b] Background electrolyte.
[c] Preconcentration factor.
[d] Non-available data.

Future Perspectives

Undoubtedly, method optimization is crucial considering the numerous experimental factors to be tested and their significant effects on extraction efficiency. Chemometric methodologies would clearly support to this highly important point.

Efforts should be made to develop new approaches in the field of capillary electrophoresis. The analytical potential of CPE–CE lies in the possibility of simultaneous screening for a number of metal ions in complex matrices.

New developments can be expected regarding automatization. For instance, the use of new column packing materials (knotted reactors, chromatographic stationary phases, etc.) or monolithic columns could increase enhancement factors, selectivity and sample throughput.

The inclusion of CPE in advanced analytical chemistry courses [76] could be a potential future trend owing to the various aspects that may be discussed when a CPE experiment is demonstrated (green chemistry principles, effect of organic solvents and surfactants in spectroscopic analysis, background signal correction, trace analysis, the physico-chemical aspects related to phase separation, etc).

Concluding Remarks

Some general conclusions can be made regarding the hyphenation of cloud point extraction to different instrumental methods of analysis. The results shown in this study demonstrate the potential of this impressive micellar phase separation technique.

Considering the high preconcentration efficiency, versatility and low cost encountered in CPE, it is expected that analytical scientists will keep on doing research in this interesting area, not only in the field of analytical applications but also with respect ot basic studies in order to reach complete elucidation for phase separation behavior.

Acknowledgements. This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID) and Universidad Nacional de San Luis (Argentina).

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