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Vesicle-mediated high-performance liquid chromatography coupled to atomic detection for speciation of toxic elements

A. Sanz-Medel*, B. Aizpun, J.M. Marchante, E. Segovia, M.L. Fernandez, E. Blanco

Department of Physical and Analytical Chemistry, University of Oviedo, Julian Claveria 8, 33006 Oviedo, Spain

Abstract

High-performance liquid chromatographic (HPLC) separation followed by element-specific detection by plasma emission or atomic absorption is shown to be a synergic combination through the use of surfactant vesicles as mobile phases for metal speciation. Several species of As, Se and Hg of environmental and toxicological concern were separated by using vesicular mobile phases of the surfactant didodecyldimethylammonium bromide (DDAB) on a C_{18} reversed-phase column which was previously modified by DDAB molecules. A mobile phase of the surfactant dihexadecyl phosphate (DHP) containing methanol was used for the speciation of butyltin compounds. The usefulness of the proposed vesicle-mediated methods has been demonstrated for the separation and determination of As species in tap water and urine and Hg species in seawater. The fundamental basis of this emerging new strategy of vesicle-mediated coupling of HPLC separation to atomic detection is also discussed.

1. Introduction

The most reliable approaches today to tackle the problem of element speciation are hybrid analytical techniques resulting from the coupling of a powerful chromatographic separation technique with atomic (specific) spectroscopic detection. In particular, inductively coupled plasma atomic emission spectrometry (ICP-AES) has been frequently used as a "specific" detection method coupled to high-performance liquid chromatography (HPLC) for metal speciation [1–4]. Conventional mobile phases for HPLC utilize organic and hydro–organic solvents and unfortunately such solvents may be detrimental to the ICP analytical performance with conventional nebulization (e.g. higher plasma background, increased unstability and noise and even eventual extinction of the plasma) [5]. Therefore, the search for alternative mobile phases for HPLC is worthwhile and may result in improved analytical characteristics of new HPLC–ICP-AES hybrid methods.

Around ten years ago aqueous micelles have been introduced as a new effective mobile phase in HPLC giving rise to micellar liquid chromatography (MLC) [6,7]. Surfactant-based organized media, such as micelles, vesicles, bile salts, etc, are able to solubilize, concentrate and organize solutes at a molecular level [7–9] and these unique properties have been shown to be very useful for HPLC separations [7]. Although the use of organized media for separations has greatly increased in recent years only normal micelles mobile phases have been investigated extensively. The use of vesicles as mobile phases

* Corresponding author.

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in liquid chromatography seems to have been largely neglected so far.

Single typical surfactants are amphiphilic molecules (i.e. molecules in which a non-polar hydrophobic tail is attached to a polar or hydrophilic head group) that dynamically associate in aqueous solution upon reaching the so-called critical micellar concentration (CMC) to form large spherical aggregates of colloidal dimensions termed normal micelles [8,9]. The structure of micelles is such that the hydrophilic head groups are directed toward and in contact with the aqueous solution (thus forming a polar surface), while the hydrophobic tails are directed away from the water, forming a central non-polar core.

Other surfactants possessing of two or more hydrophobic tails per monomer form bilayer in water which under sonic dispersal by ultrasonic radiation leads to closed bilayer structures like spheric bags, referred to as vesicles (schematically depicted in Fig. 1) [7,10], such as didodecyldimethylammonium bromide (DDAB) and dihexadecyl phosphate (DHP).

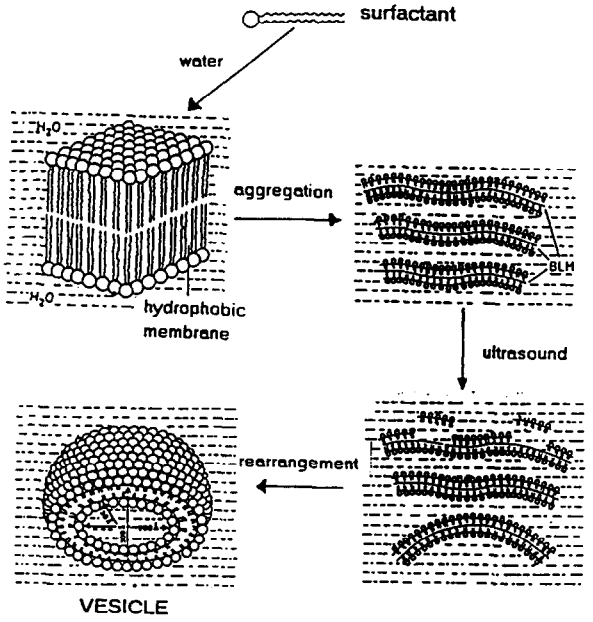

Fig. 1. Vesicle formation by ultrasonic treatment of bilayer membrane.

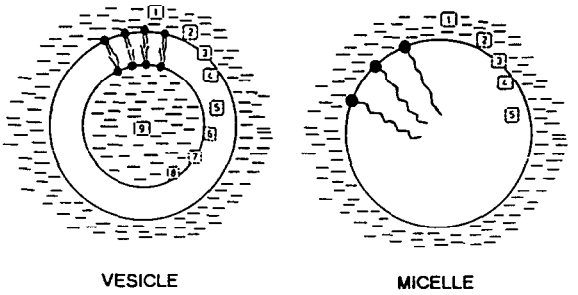

Fig. 2. Available sites for solute solubilization/interaction in vesicles and normal micelles.

It is important to recognise the chemical differences and potential between vesicles and micelles. As shown in Fig. 2 an aqueous vesicle displays nine different regions or compartments for solute localization [10]: the outer water volume (bulk volume), the hydration sphere, the outer head groups, the hydrophobic membrane close to the outer head groups and finally the similar four inner regions in the water pool direction. Micelles provide less available sites for interaction/solubilization of a given solute. In fact, localization of solute in normal micelles (Fig. 2) is restricted to the four first regions mentioned above and perhaps to a solubilization close to the hydrophobic core of the micelle [7].

Therefore, in principle, vesicles can provide a greater variety of interactions (hydrophobic, electrostatic, steric) with the solute present in the mobile phase, interactions which cannot be duplicated by any traditional pure or mixed solvent system. Moreover, the richness of possibilities in such interactions could be manipulated in order to achieve a desired separation. In fact, we have recently shown for the first time [11] that HPLC separation followed by hydride generation (HG)-ICP-AES detection can be a synergic combination for the speciation of toxic arsenic species through the use of vesicles as mobile phase.

Further work on the analytical potential and usefulness of this new strategy of vesicle-mediated HPLC separations coupled to atomic spectroscopic detection is discussed here in terms of mechanisms of separations and application to the speciation of several compounds of toxi-

cological and environmental concern derived from arsenic, mercury, tin and selenium.

2. Experimental

2.1. Instrumentation

The chromatographic system used for arsenic and mercury speciation consisted of a Knauer (Berlin, Germany) Model 6400 HPLC Pump with an attached sample injection valve equipped with a 100-μl loop.

Selenium and tin speciation studies were carried out by using an HPLC system constructed from LKB components (LKB, Bromma, Sweden), two Model 2150 HPLC pumps, a Model 2152 system controller and a Rheodyne sample injection valve (Berkeley, CA, USA) equipped with a 500-μl loop.

The analytical columns used (250 × 4.6 mm I.D.) were packed with 10-μm C_{18} bonded silica stationary phase (Spherisorb Phase Separations, Deeside, UK).

A four-channel peristaltic pump HP4 Minipuls 2 Gilson (Villiers-le-Bel, France) and a laboratory-made gas–liquid separator constituted the continuous hydride or cold vapor generator system [12].

An ultrasonic device from Sonics & Materials (CT, USA) Model VC (250 W) was used for the preparation of vesicles from surfactant solutions.

A UNICAM (Cambridge, UK) Model PU 9400X atomic absorption spectrometer, equipped with a T-shaped absorption quartz cell (12 cm × 8 mm I.D.) was used for absorption measurements of mercury vapour at 253.7 nm.

A Perkin-Elmer (CT, USA) ICP 5000 spectrometer interfaced with a microcomputer (Perkin-Elmer 3500) was used for ICP emission measurements of arsenic and tin hydrides. A computer program for transient ICP emission data acquisition and processing was written in BASIC.

A Perkin-Elmer atomic absorption spectrometer Model 3030 (EP-3030) equipped with HGA 500 furnace and AS-40 autosampler. Perkin-Elmer EDL lamp at a current of 6 mA and pyrocoated graphite tubes with a totally pyrolytic graphite L'Vov platform were used for selenium detection.

2.2. Reagents

Stock solutions (1000 mg/l of As) of arsenite was prepared by dissolving the appropriate amount of As_2O_3 (Merck, Darmstadt, Germany) in 25 ml of 0.5 *M* NaOH (Merck) solution and then diluting the solution to 1 l with 0.6 *M* HCl (Merck). Stock solutions (1000 mg/l of As) of monomethylarsonic (MMAs) and dimethylarsinic (DMAs) acids were prepared by dissolving the appropriate amount of MMAs (Carlo Erba, Milan, Italy) and DMAs (Sigma, St. Louis, MO, USA) directly in ultrapure Milli-Q water. An As(V) stock solution (1000 mg/l) was obtained from Merck.

Methylmercury stock solution (100 mg/l of Hg) was obtained by dissolving the appropriate amount of methylmercury chloride salt (Merck) in 10 ml of acetone and the solution was made up to 100 ml with ultrapure Milli-Q water. This stock solution was stored in a glass bottle at 4°C. Inorganic mercury was obtained from Merck as a 1000 mg/l Hg solution.

Monobutyltin chloride, dibutyltin chloride and tributyltin chloride (Aldrich, Milwaukee, WI, USA) stock solutions (1000 mg/l as Sn) were prepared in HPLC-grade methanol and stored at 4°C.

Stock solutions of 10 mg/l of selenocystine, selenomethionine and selenoethionine all obtained from Sigma were prepared by dissolving the appropriate amount in water. Selenite stock solution (1000 mg/l) was prepared by dissolving Na_2SeO_3 (Merck) in water and Se(VI) stock solution (1000 mg/l) was obtained from Merck.

Working standards solutions of all the compounds investigated were freshly prepared daily by diluting the stock solutions with ultrapure Milli-Q water.

The DDAB vesicular solution (10^{-2} *M*) was prepared by dissolving 0.4626 g of DDAB (Fluka, Buchs, Switzerland) in 100 ml of Milli-Q water, and sonicating this solution during 10 min with a power output of 60 W [13,14].

The DHP vesicular solution (10^{-2} *M*) was prepared by dissolving 0.5469 g of DHP (Aldrich) in 100 ml of Milli-Q water and sonicating this solution (with a power output of 60 W) during 12 min at 80°C.

Sodium borohydride solution (1%, m/v) was prepared by dissolving 1 g of $NaBH_4$ (Probus, Barcelona, Spain) in 100 ml of 0.1% (m/v) NaOH (Merck) solution. Filtration of the solution through a Whatman grade 4 filter paper before use was carried out. This solution was stored at 4°C and prepared weekly.

The mercury-selective complexing agent, 2-mercaptoethanol was obtained form Merck and used as received without further purification.

HPLC-grade methanol and acetonitrile (Romile Chemicals, Loughborough, UK) were used.

All other chemicals were of analytical reagent grade and distilled and deionized (Milli-Q system; Millipore, MA, USA) water was used throughout all the work.

2.3. Procedures

The C_{18}-bonded silica reversed-phase columns were modified by passing a total of 500 ml of a 10^{-3} *M* surfactant aqueous solutions in 50% methanol at a flow-rate of 1 ml/min. Milli-Q water was then passed through the column during 30 min at the same flow-rate.

The optimal conditions found for the chromatographic separation of the different metal species investigated are summarized in Table 1 for each element investigated. Vesicular mobile phases were prepared by dissolving the appropriate amount of surfactant in water buffered at the desired pH and containing a small amount of organic modifier, as specified in Table 1 [e.g. for Hg species separation containing 0.005% (v/v) of 2-mercaptoethanol, 5% (v/v) acetonitrile and buffered with ammonia acetate (0.01 *M*) at pH 5]. These mobile phases were degassed by ultrasonicating during 30 min prior to use.

The effluent from the column was first mixed with a 1% (v/v) HCl solution through a mixing coil and then mixed with the sodium tetrahydroborate solution for As, Se or Sn hydrides, and for mercury cold vapor generation. A continuous stream of argon carries the generated volatile species directly into the ICP injector tube [11] or into the T quartz cell of the atomic absorption spectrometer (for mercury detection) [15]: when higher sensitivity is needed and hydride generation is not effective (e.g. some selenium species) off-line detection was carried out and fractions from the HPLC eluate were collected (250 μl) in the autosampler cups and immediately analysed for selenium by atomic absorption spectrometry with electrothermal atomisation (AAS-ETA). Fig. 3 shows schematically the on-line and off-line coupling used depending on the particular element (speciation problem).

Preconcentration of mercury species from water samples was carried out using Sep-Pak C_{18} [trichloro(octadecyl)silane, chemically bonded to Porasil A] cartridges, modified with a 2-mercaptoethanol solution [15].

Table 1
Chromatographic conditions

Column	C_{18}-bonded silica, 10 μm particle size, 250 × 4.6 mm I.D. (modified with 10^{-3} *M* DDAB or 10^{-3} *M* DHP)
Temperature	Room temperature
Sample volume	100 μl (or 500 μl for Se) for As and Hg
Mobile phase	
Arsenic	0.010 *M* Sodium phosphate buffer, pH 5.75 + 0.5% methanol + 10^{-5} *M* vesicles of DDAB
Selenium	(A) 0.01 *M* Ammonium acetate buffer, pH 5 + 0.5% methanol + 10^{-4} *M* vesicles of DDAB (B) 0.2 *M* Ammonium acetate buffer, pH 7.5 + 10^{-4} *M* vesicles of DDAB
Tin.	(A) 0.1 *M* Ammonium citrate + 5% acetic acid + 10^{-5} *M* vesicles of DHP, pH 4.5 (B) Methanol; gradient (B) 50–90%
Mercury	0.01 *M* Ammonium acetate buffer + 0.005% 2-mercaptoethanol + 2 · 10^{-4} *M* vesicles of DDAB + 5% acetonitrile
Flow-rate	1.0 ml/min (or 1.5 ml/min for Hg)

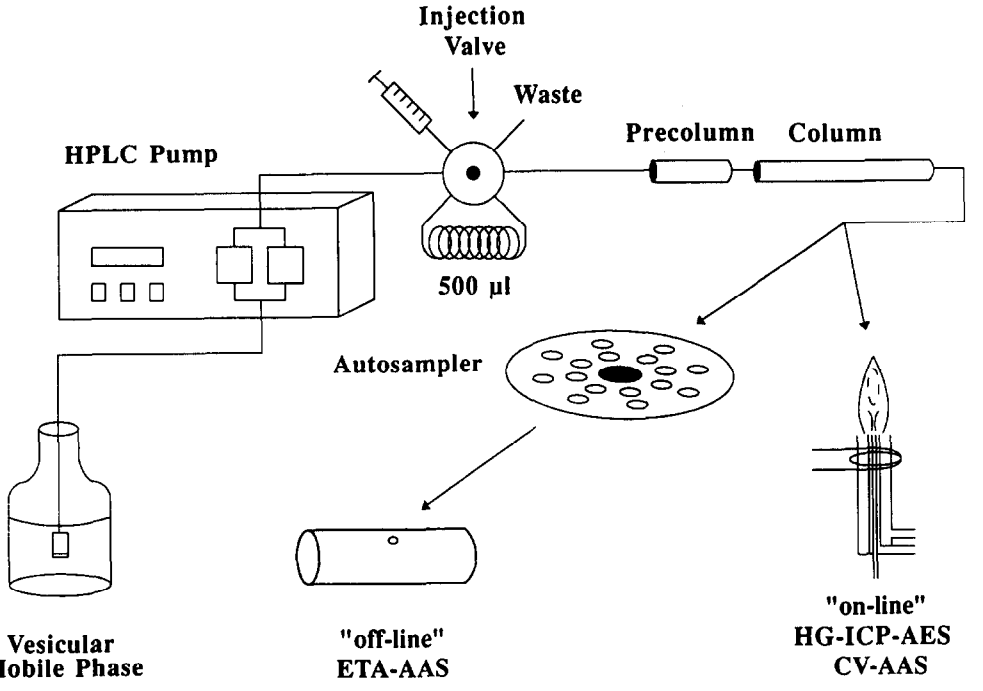

Fig. 3. Schematic diagram of the coupled HPLC–atomic detection systems for element speciation. CV = Cold vapour.

3. Results and discussion

As stated before, the obtained optimal conditions for separations of the different species have been summarized in Table 1. Optimal conditions for ICP-AES and ETA-AAS detection are given in Table 2.

3.1. Arsenic speciation

The feasibility of using vesicular mobile phases in HPLC coupled to ICP-AES was demonstrated for the first time by solving the problem of arsenic speciation [11]. As shown in Fig. 4 the more toxic arsenic species (including arseneous, arsinic, monomethylarsonic and dimethylarsinic acids), can be separated within 10 min by using a mobile phase of cationic vesicles of didodecyldimethylammonium bromide (BDDA) 10^{-5} *M* in phosphate buffer (pH 5.7) containing only 0.5% methanol. The column was a conventional C_{18}-bonded silica column previously modified by passing through the adequate surfactant solution (see *Procedures*). The detection was accomplished on-line by HG-ICP-AES.

The normalized detection limits observed for the different toxic species of As with this novel hybrid method were found to be below the ng level (0.5–1.2 ng of As).

The analytical potential of this vesicle-mediated hybrid technique was demonstrated by determination of toxic arsenic species in spiked samples of local tap water and human urine [11]. Satisfactory recoveries of the type illustrated in Table 3 (93–108%) were obtained for the four toxic arsenic species under investigation.

3.2. Mercury and tin speciation

This new strategy of vesicle-mediated HPLC separation coupled to on-line atomic spectroscopy detection was extended to the speciation of other elements which, as As, are able to form volatile species, e.g. mercury and tin. Experiments showed that a mobile phase consisting of cationic vesicles of DDAB ($2 \cdot 10^{-4}$ *M*), 0.005% 2-mercaptoethanol and 5% of acetonitrile, buf-

Table 2
Instrumental conditions

HG	
HCl^+	10% (m/v)
KI^+	0.1% (m/v)
Vesicle	$1 \cdot 10^{-3}$ *M* DDAB
$NaBH_4$	2% (m/v) (stabilized by 0.1%, m/v, NaOH)
Flow-rate	1 ml/min
Ar carrier	70 ml/min
ICP-AES	
Analytical line	193.69 nm
Radio frequency forward power	1 kW
Reflected power	<5 W
Viewing height	15 mm
CV	
$NaBH_4$	1% m/v (stabilized by 0.1% NaOH)
HCl	1% (v/v)
Flow-rate	1 ml/min
Ar carrier	Flow-rate 250 ml/min
AAS	
Wavelength	253.7 nm
Lamp current	6 mA
Slit	0.5 nm
AAS-ETA	
Wavelength	196.0 nm
Slit	0.7 nm
Intensity	6 mA
Integration time	4 s
Background	On. Deuterium
Signal processing	Integrated absorbance
Type tube	Pyrolitic graphite coated graphite with L'vov platform

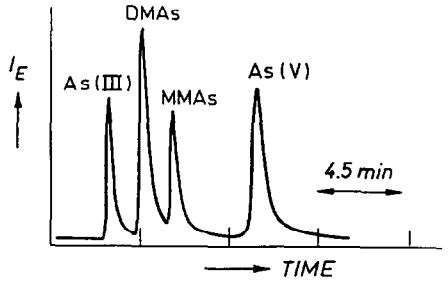

Fig. 4. Arsenic speciation by vesicle-mediated HPLC coupled to HG-ICP-AES. Experimental conditions as in Tables 1 and 2. I_E = Emission intensity (arbitrary units).

Table 3
Recoveries of arsenic species added into the tap water and human urine samples

Samples	Arsenic	Recovery (%)[a]	
		Spiked 150–250 ng/ml	Spiked 50–100 ng/ml
Tap water	As(III)	95	97
	DMAs	98	102
	MMAs	102	100
	As(V)	103	104
Human urine	As(III)	96	101
	DMAs	105	108
	MMAs	102	99
	As(V)	100	93

[a] Mean of two analyses.

fered at pH 5 with acetate, allowed the isocratic separation of Hg^{2+} and methylmercury on a C_{18} column previously modified with DDAB molecules in a similar way to that described previously. The detection limits obtained by on-line cold vapour AAS detection were 10–16 μg/l [15]. These results can be substantially improved after preconcentration of the aqueous sample in C_{18} cartridges impregnated with 2-mercaptoethanol solutions [15]. Preconcentration factors of 100 were easily achieved and with that pretreatment detection limits of 0.1–0.2 μg/l of mercury were easily achieved. The whole approach was applied to the determination of inorganic and methylmercury in spiked seawater and recoveries obtained ranged between 89 and 92% using extremely low concentrations in the toxic species (down to 5 μg/l).

Conversely, attempts to use only anionic vesicles of DHP as mobile phases for the HPLC separation of organometallic tin compounds (monobutyl-, dibutyl- and tributyltin) have been unsuccessful so far owing to the high hydrophobicity of these solutes. The separation was attempted at room temperature on a C_{18} column modified with DHP molecules under several different combinations of organic modifier (10–75% methanol), DHP ($0–6 \cdot 10^{-5}$ *M*), ammonium citrate (0.05–0.2 *M*) and acetic acid (0–

5%). The best chromatographic resolution of the various set of conditions tested was obtained using gradient elution and the separation conditions are summarized in Table 1. It is clear that the solvent strength of aqueous vesicular solutions seems to be lower than that of classical hydroorganic mobile phases [7].

In order to obtain a reasonable retention time for tributyltin on the C_{18} column modified with the surfactant, it was necessary the presence of at least 60% methanol in the mobile phase, which will probably destroy the vesicular aggregates. In fact, the retention times of butyltin species increased with surfactant concentrations in the hydro–organic mobile phase and this behavior is typical of ion-pair surfactant chromatography where the surfactant concentrations and/or conditions in the mobile phase are such that no micellar aggregates form [7]. A typical chromatogram obtained using a mobile phase containing 0.1 *M* ammonium citrate, 5% acetic acid and 10^{-5} *M* DHP and a methanol gradient 50–90% in 10 min is shown in Fig. 5.

3.3. Selenium speciation

As said before, if the attainable sensitivity of on-line conventional nebulization ICP-AES detection is not enough for speciation studies and hydride generation cannot be employed to increase this sensitivity, off-line ETA-AAS detection of the emerging fractions from the HPLC column can be called for. In fact, the utility of this atomic detector has been advantageously demonstrated for speciation of several seleno compounds which were separated by HPLC using vesicular mobile phases similar to those used for As [16].

The separation of inorganic selenium (selenite and selenate) and different selenoaminoacids (selenocistine, selenoethionine, selenomethionine) was achieved on a C_{18} column modified with molecules of the cationic surfactant DDAB with a sodium acetate gradient (0.005 to 0.2 *M*) and pH gradient (5 to 7.5) in the presence of DDAB vesicles (10^{-4} *M*) and 0.5% methanol. Fig. 6 shows the type of separation. Detection limits for these compounds were 5 μg/ml of Se.

3.4. Separation mechanisms

Once the analytical potential of vesicular-mediated HPLC separations in connection with atomic detection was demonstrated, particularly in connection with plasma detectors, we investigated the possible separation mechanisms operating in vesicular liquid chromatography. To

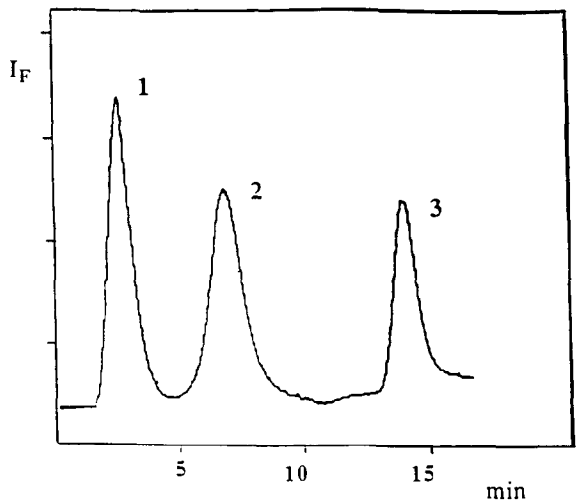

Fig. 5. Typical chromatogram showing tin speciation in water. Experimental conditions are given in Table 1. Peaks: 1 = monobutyltin; 2 = dibutyltin; 3 = tributyltin.

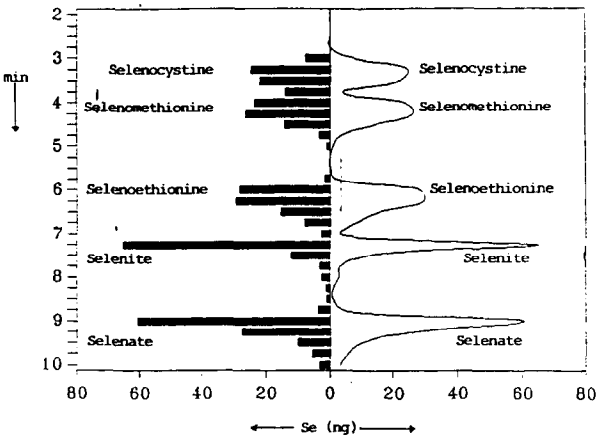

Fig. 6. Separation of inorganic selenium and different selenoaminoacids by vesicle-mediated HPLC with "off-line" ETA-AAS detection. Experimental conditions as in Tables 1 and 2.

do so the dependence of the solute retention time on the concentration of DDAB surfactant vesicles in the mobile phase was investigated for each element species. The results observed in these studies are summarised in Table 4–6 for arsenic, mercury and selenium species. The detailed observation of these results demonstrates that the effect of vesicle concentration depends on the nature of the solutes to be chromatographed. For instance, none of the retention times of any ionizable arsenic species did change at all with increasing vesicle concentration in the mobile phase (Table 4). Conversely, the retention times of hydrophobic mercury-mercaptoethanol complexes decreased with DDAB vesicles presence in the mobile phase (Table 5). However, as can be seen in Fig. 7 the retention time decrease observed with surfactant addition only takes place if monodispersed DDAB vesicles are present in the mobile phase (i.e. with previous sonicating of the surfactant solutions).

A similar effect of DDAB presence was found for the most hydrophobic selenium compounds (selenomethionine and selenoethionine) as the observed retention times decreased with DDAB presence. However, no retention time changes were observed for the ionic selenite and selenate species (Table 6).

By analogy with MLC [7,17] a vesicular mobile phase can be considered as composed of both the surfactant vesicular aggregate (pseudophase) and the aqueous bulk solvent (Fig. 8). A dissolved solute may thus distribute (a) between the bulk solvent and the (surfactant-modified) stationary phase (distribution coefficient, P_{SS}); (b) between the bulk solvent and the pseudophase (or vesicular aggregates, with distribution coefficient P_{SV}) as shown schematically in Fig. 8. Consequently, there are now two partition coefficients (P_{SS} and P_{SV}) which can influence the separation. The two defined partition coefficients have opposing effects on solute retention: as P_{SS} increases retention time increases; conversely, a high value of P_{SV} favours a decrease in retention due to increased partition into the vesicle moving with the mobile phase. The relative magnitude of both factors will depend upon the nature of solutes for a given vesicular solution and stationary phase. For instance, when charged ions of ionizable solutes such as the arsenic species (Table 4) and some selenium species (Table 6) investigated here are chromatographed with an oppositely charged vesicle-forming surfactant (DDAB) it seems that electrostatic attractions between charged solutes and the surfactant clearly predominate. To understand such interaction

Table 5
Effect of DDAB vesicle concentration on retention time

Concentration of DDAB vesicle (M)	Retention time (min)	
	Inorganic mercury	Methylmercury
0	11.33 ± 0.14	7.75 ± 0.11
10^{-5}	9.93 ± 0.13	7.38 ± 0.11
10^{-4}	9.98 ± 0.13	7.55 ± 0.11
$5 \cdot 10^{-4}$	9.92 ± 0.13	7.47 ± 0.11

Table 4
Effect of DDAB vesicle concentration on the retention times

Concentration of DDAB vesicle (M)	Retention time (min)			
	As(III)	DMAs	MMAs	As(V)
0[a]	2.82	4.55	5.96	9.80
10^{-6}	2.83	4.54	5.98	9.82
$5 \cdot 10^{-6}$	2.83	4.55	5.96	9.82
10^{-5}	2.83	4.55	5.94	9.82
10^{-4}	2.83	4.56	5.95	9.81

[a] The column behaviour was not reproducible due to the continuous DDAB desorption and release from the stationary phase in the column.

Table 6
Effect of BDDA vesicle concentration on retention times

Concentration of DDAB vesicle (M)	Retention (min)			
	Se cystine	Se methionine	Se ethionine	Selenite
0	3.2	4.1	6.1	6.5
10^{-6}	3.2	4.0	5.9	6.5
10^{-5}	2.9	3.7	5.5	6.5
10^{-4}	3.0	3.8	5.6	6.6

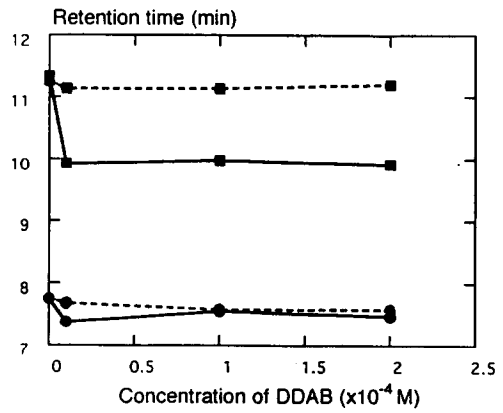

Fig. 7. Effect of DDAB concentration in the mobile phase on retention times: DDAB vesicles (solid lines), DDAB without sonicating (broken lines); ■ = inorganic mercury; ● = methylmercury.

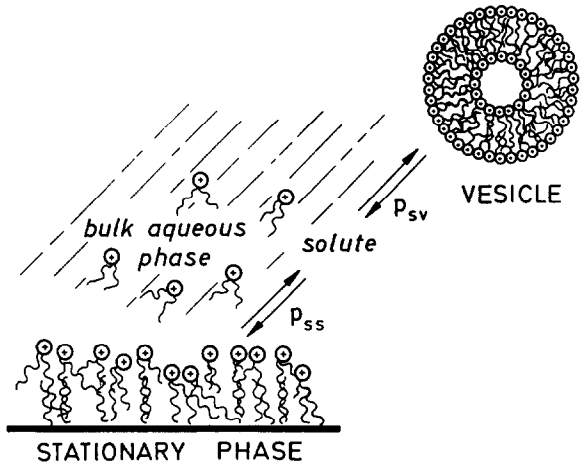

Fig. 8. Schematic representation of interactions of the solute in the aqueous bulk solvent with the (surfactant-modified) stationary phase and the (pseudophase) vesicular aggregates.

the modification of the C_{18} stationary phase by the monomers of DDAB has to be considered: as shown by Fig. 9 it is clear that during conditioning of the C_{18} column the stationary phase is modified by adsorbed surfactant molecules, preferently via its hydrophobic tails, leaving its charged groups in contact with the bulk mobile phase. In this way we may have some hydrophobic tails but also ion-exchange interactions where the solutes in the mobile phase would be attracted by the oppositely charged heads of the surfactant adsorbed to the phase. At the low vesicle concentrations used (10^{-5}–10^{-4} M) these electrostatic attractions of the stationary phase for charged solutes predominate. Thus, the mechanism of separation resembles a typical ion-exchange mechanism. Therefore the retention times observed for solutes are not related to the presence of vesicles in the mobile phase (see Table 4 for As and Table 6 for selenium).

In the case of more hydrophobic solutes, such as the neutral mercury–dimercaptoethanol complex, only hydrophobic interactions would be operative for solutes (insoluble in the aqueous phase) partitioning between the modified stationary phase (hydrophobic tails) and the vesicles. Here the presence of vesicles in the mobile phase reduced the retention times of these solutes probably because they increase the P_{SV} partition coefficient. In brief, both the stationary phase and the mobile phase characteristics can be changed by resorting to vesicles and in this way taylored separations of charged and uncharged compounds can be achieved. However, the vesicular effect on retention times is more modest than that reported in MLC [7,17] probably because the concentration of surfactant in the

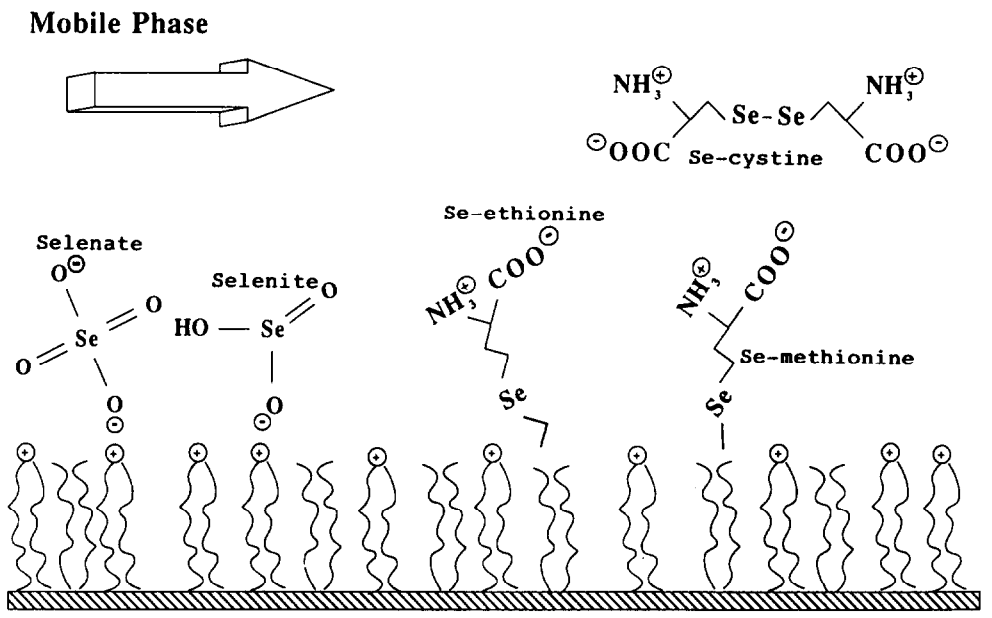

Fig. 9. Schematic representation of interactions of the selenium compounds in the aqueous bulk solvent with the (surfactant-modified) stationary phase.

mobile phase (10^{-5}–10^{-4} M) are lower than those commonly employed using micelles. In any case, no problems of viscosity were observed in all HPLC experiments with such concentrations in DDAB.

4. Conclusions

It has been demonstrated that aqueous vesicular media can be advantageously employed as mobile phases in HPLC. Although their solvent strength is lower than that of hydro–organic solvents, the vesicle-mediated HPLC procedures developed so far in our laboratory have proved to be fairly robust, chromatographic behaviour was durable and reliable and no degradation behaviour was noticed after months of daily use.

In brief, the results obtained so far indicate that vesicle-mediated HPLC separation coupled to atomic spectroscopy detection provides a competitive, low-cost, efficient and robust separation which at the same time can substantially enhance the performance of atomic spectroscopic detectors, specially plasma detectors [18]. This vesicle-enhanced hybrid techniques constitute a most useful analytical strategy to tackle the modern problem of metal speciation.

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