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Effect of ethanol in the organic phase on liquid–liquid extraction in monosegmented flow analysis. Determination of zinc in drugs

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Abstract

In liquid–liquid extraction performed by monosegmented flow analysis (MSFA), the aqueous sample is introduced between two air bubbles and flows, under restricted dispersion, through a glass extraction tube where the analyte is retained, usually at pH higher than 8. The retained analyte is removed to a small volume of an organic phase containing a ligand which is introduced after the second air bubble. In this work, the effect of the organic phase composition on the extraction of Cu(II), Zn(II) and Cd(II) in MSFA systems was investigated by changing the ethanol content (0.1–4% v/v) in toluene, chloroform and carbon tetrachloride. The extracting efficiency of the organic phases containing ethanol was evaluated by using dithizone (DT), 1-2-pyridylazo-2 naphthol (PAN) and sodium diethyldithiocarbamate (DDTC) as ligands for the metals. The MSFA extraction system was improved by introducing a new syringe-based device for organic phase delivery. The presence of ethanol in the organic phase shows a remarkable (up to ten times) effect on the extraction efficiency of the flow system when DT is employed. Its presence is mandatory if DDTC is used, as it accounts for ligand solubility in the organic phase. The extraction efficiency also increases with the pH of the aqueous phase as a consequence of higher ionisation of the glass silanols, where the analytes are adsorbed before extraction. The system has been evaluated for determination of Zn(II) in drugs showing a mean R.S.D. of 2.2% and mean relative accuracy of 4.4%, when compared with atomic absorption spectrometry results. Typical sample frequency, sample and organic phase consumption are 30 samples per hour, 200 and 100 μl, respectively.

Keywords: Monosegmented flow analysis; Liquid–liquid extraction; Zinc determination; Effect of ethanol

1. Introduction

Liquid–liquid extraction is frequently employed in analytical methodologies to improve sensitivity, by pre-concentration and selectivity, by isolating

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the analyte from complex matrices. Usually, a suitable ligand is present in an organic phase to which the analyte is transferred from an aqueous sample. In conventional batch procedures, the effective contact between the two phases is achieved through vigorous shaking. Sometimes, the extracting agent is present in the aqueous phase and the compound, formed with the analyte, transferred to the organic phase.

In view of its broad utilisation, liquid–liquid extraction has been mechanised by using the flow analysis approach [1–4]. Mechanisation through flow analysis results in a higher sample throughput and, at the same time, in a reduction of the organic solvent volume necessary for the analysis. Effectively, the flow injection analysis concept entered into liquid–liquid extraction methodology with the pioneering works of Karlberg [1] and Bergamin et al. [2]. The subject has been extensively reviewed by Kuban [3].

Besides a higher sample throughput and solvent economy, liquid–liquid extraction made in flow systems requires a very well stabilised segmentation of the aqueous sample by the extracting organic phase, followed by some kind of phase separation, before detection. Although several devices and strategies have been described in the literature, reporting on the optimisation of such operations in flow systems [4–9], some works describe systems which perform flow extraction without phase separation [10,11] and also without phase segmentation [12–14].

Recently, the monosegmented flow approach (MSFA) [15] has been proposed to perform liquid–liquid extraction [16]. The monosegmented system can operate using either the single-phase [15] or the two-phase approach [17,18]. The resulting system does not require the use of either phase segmenters or separators. The monosegmented extraction system has been recently miniaturised to work with the typical apparatus of capillary electrophoresis, reducing even more the volume of organic phase necessary for the extraction [18].

This work was aimed at the investigation of the effect of the organic phase composition, altered by the addition of ethanol and of different ligands, on the efficiency of the MSFA-LLE system. A parallel objective was also to improve the knowledge on the extraction mechanism of those systems and to evaluate its utilisation in real samples. Within this last objective, the spectrophotometric determination of zinc in pharmaceuticals is proposed.

To improve the performance of the MSFA-LLE system, a syringe device was added to the previously described system in order to facilitate the use of organic solvents and to obtain better reproducibility for delivering the organic phase volume.

2. Experimental

2.1. Monosegmented liquid–liquid extraction system

Fig. 1 depicts the flow manifold employed in this work. Tygon® tubing and acrylic parts were employed as no organic solvent enters in contact with the pumping tubes or injection port device. The present MSFA-LLE system introduces a syringe-based (10.0 ml) delivery device driven by a stepper motor (RS 318-711) to improve the reproducibility of the volume of organic phase introduced and to avoid contact of the organic phase with non-resistant parts of the manifold. A volume in the range from 38 to 1350 μl can be added with an average S.D. of $\pm$ 1.0 μl ($n = 10$). The use of the syringe device improved the overall reproducibility of the extraction over the system previously described, which was based on time controlled delivery of the organic phase impelled by a peristaltic pump [17].

The extracting reactor consists of a Pyrex® VBTR (boron-silicate termo-resistant) glass tube with a 3 mm i.d. and 60 cm in length. Connections between the various parts of the flow manifold were made with PTFE tubing of 0.8 mm i.d.

The in-line buffered aqueous sample is introduced as a monosegment between two air bubbles in the manifold by using an acrylic proportional sampling device [19] in a single operation, as previously described [17,19].

Spectrophotometric detection was made on-line in two ways. In the first, detection was perpendic-

ular to the glass tube by using an optical bundle to deliver the monochromatic light beam (obtained after an interference filter) or from a light emitting diode (LED) as the radiation source. The light transmitted through the tube was sensed by a photodiode (RS 308-067). The selected wavelengths for the different ligands studied were 530 nm (green LED, or interference filter) for DT and PAN complexes or 445 nm (interference filter) for DDTC complexes. Alternatively, for Cu(II) extraction using DDTC, a longer optical path was employed by moulding the glass tube at the end of the extracting reactor into a 'U' format. The estimated optical path was then 10 mm.

Four optical switches are present in the system allowing the computer to access the state of the injection port (sampling/introducing) and to locate the sample monosegment throughout the system [20].

2.2. System control and data acquisition

The system was controlled by an IBM compatible micro-computer (100 MHz, 16 Mbytes of RAM) running a controlling software written in Visual Basic 3.0. Spectrophotometric signals were obtained by digitising, with resolution of 12 bits, the analogue signal produced by the sensor. A PCL-711-S (Advantech, USA) interface was employed. This interface was also employed to access the logical state of the optical switches, to control the three-way solenoid valves and to drive the stepper motor of the syringe device.

2.3. System operation

The MSFA-LLE system described in Fig. 1 starts its operation by introducing a fixed volume of the aqueous sample (100–300 μl), defined by the loop size (LS) in between two air bubbles whose volumes are defined by La_1 and La_2 (typically 50 μl). The sample is buffered after a 1:1 confluence with an ammonia/ammonium chloride stream before entering the injection device. While the monosegment is inserted in a buffer carrier stream, the controlling micro-computer detects the sample introduction by sensing the change in the logical state of the optical switch O_1.

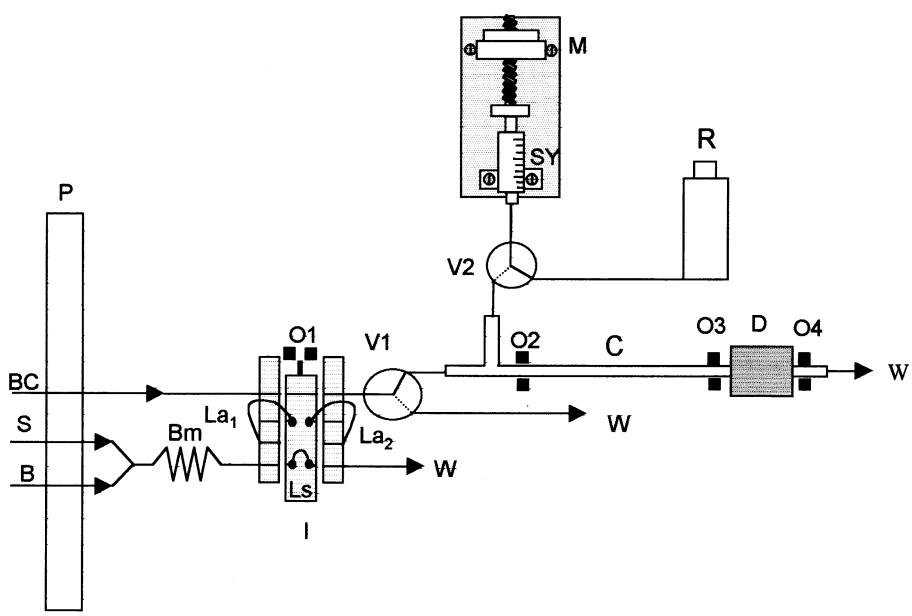

Fig. 1. Diagram showing the monosegmented flow analysis liquid–liquid extraction system. P, peristaltic pump; I, injection port; BC, buffer carrier stream; S, sample solution; B, conditioning buffer stream; Bm, homogenisation coil; Ls, sample loop; La_1 and La_2, air bubble loops; O_{1-4}, optical switches; V_{1-2}, three way solenoid valves; SY, 10 ml gas tight syringe; M, stepper motor; R, organic solution reservoir; C, extraction glass tube, D, spectrophotometric detector; and W, the discharged fluids reservoir.

The optical switch O_2 is used to detect the moment when the second air bubble has passed the point of organic phase addition. The controlling software turns the three-way valve V_1 on. In consequence, the carrier flow is diverted and stops flowing through the extraction tube while the organic phase is delivered by the syringe device. The volume of organic phase added is defined by the number of steps sent to the syringe pump motor. Valve V_1 is then turned off and the monosegmented sample and the following organic phase are carried through the glass tube, towards the detection point. The optical switches O_3 and O_4 detect when the aqueous monosegment has passed the detector and the passage of the organic phase is monitored for a fixed pre-defined time interval. A washing cycle, made by injecting a blank, can be employed to reduce carry-over between successive samples.

2.4. Reagents and solutions

Stock solutions of the metal ions Cu(II), Cd(II) and Zn(II) were prepared to contain 100 μg ml^{-1} of the metal. The nitrate salts of the metal ions were employed. Organic extracting solutions of the ligands, sodium diethyldithiocarbamate (DDTC), 1-(2-pyridylazo)-2-naphthol (PAN) and dithizone (DT) were prepared in three solvents: toluene, chloroform (preserved with 20–50 mg l^{-1} of amylene) and carbon tetrachloride. The content of absolute (99.8% m/m) ethanol in the organic solutions was varied from 0.1 to 4% (v/v).

The carrier was a solution of 8.8×10^{-2} mol l^{-1} NH_3 and 2.0×10^{-2} mol l^{-1} of NH_4Cl (pH = 10.0). In order to investigate the effect of the pH in the extraction efficiency, this solution had its pH adjusted between 8 and 10.6 (using a pH meter) with the addition of HCl or NaOH solutions. Distilled-deionised water was used throughout.

The organic solutions containing DDTC and ethanol were prepared by taking 50 ml of the organic phase and shaking it vigorously in the presence of solid Na-DDTC. The saturated solution obtained was employed directly in the MSFA-LLE system.

The four commercial drugs (ZINCOPAN®, ZINCO FONTOVIT, LERIN®, BLUMEN®) were purchased from a local pharmacy.

2.5. Zinc determination by atomic absorption spectrometry

The instrumental conditions recommended by the manufacturers were employed for determination of zinc using a Perkin–Elmer AA 5400 atomic absorption spectrometer.

3. Results and discussion

When MSFA is employed for two-phase liquid–liquid extraction (MSFA-LLE) [17], the mechanism governing the analyte (metal ion) transfer to the organic phase includes its prior adsorption on the glass surface of the extraction tube. This adsorption is favoured by the alkaline buffers (pH > 8) in the aqueous sample and carrier solution. Fig. 2 shows the flow profile produced inside the glass extraction tube for a MSFA-LLE system and the suggested mechanism for analyte transference to the organic phase. The two main steps in analyte transference are its adsorption on the glass surface and its release from that surface after complexation by the ligand. The ligand, in its turn, is supplied by the organic phase and its concentration in the aqueous layer present on the hydrophilic surface of glass depends on the partition equilibrium between the organic and aqueous phase. Finally, the metal–ligand compound is transferred to the organic phase, due its higher solubility in that medium.

Considering the mechanism described above, it is predictable that the composition of the organic phase would affect the extraction efficiency of the MSFA-LLE system. The effect could be, in principle, related to the change in the partition coefficient of the ligand or to the change in the solubility of the ligand–metal complex, as observed for the conventional batch procedure. However, additional effects, such as an increase of the interaction of the organic/aqueous phases, due to the change in the polarity of the organic phase,

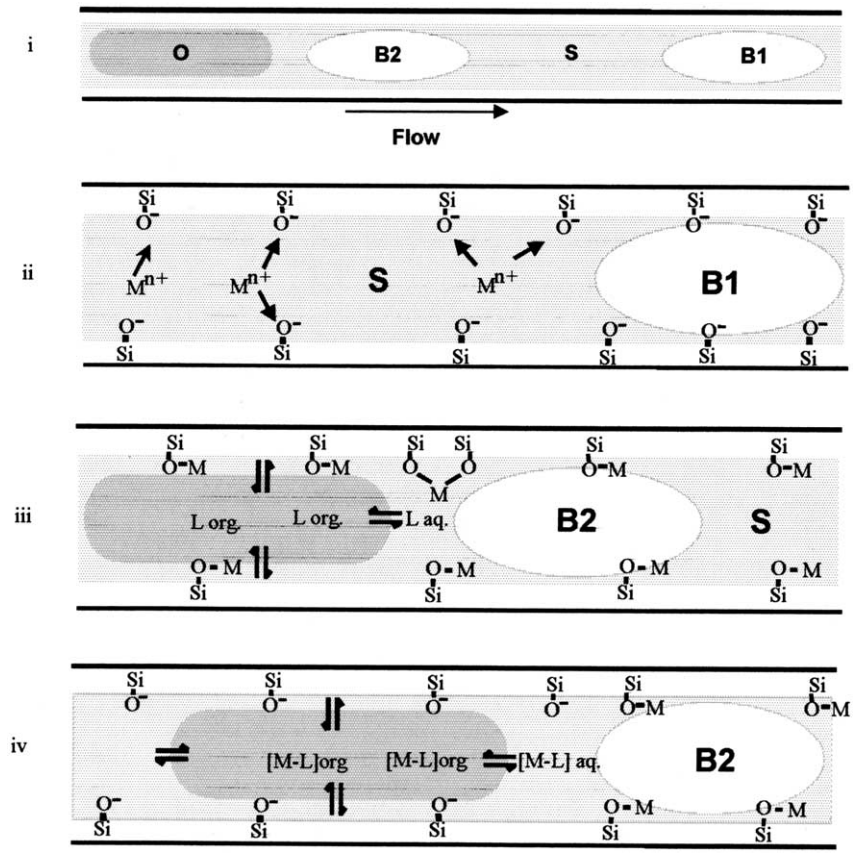

Fig. 2. Flow profile and steps of the extraction mechanism proposed for the monosegmented liquid–liquid extraction system. (i) Aqueous sample monosegment (S) in between two air bubbles (B1 and B2) and organic segment (O) inside the glass extraction tube; (ii) detail of analyte absorption by the active sites of the glass wall during the passage of the aqueous sample monosegment; M^{n+}, metal; (iii) detail showing the transport of ligand from the organic segment to the aqueous film; L_{aq} and L_{org}, ligand in the aqueous and organic phase, respectively; (iv) detail of analyte transport into the organic segment after complex formation and release from the glass surface; $[M–L]_{aq}$ and $[M–L]_{org}$, metal ligand complex in the aqueous and organic phase, respectively.

can be significant in the flow system. Furthermore, increasing the polarity of the organic phase can be used to increase the solubility of certain ligands, allowing extension of the use of the MSFA-LLE technique.

3.1. Effect of the ethanol content in the organic phase

Preliminary investigation showed that the maximum ethanol content that could be incorporated into any of the organic phases studied should not exceed 3% (v/v). Trials introducing more ethanol result in an irregular motion of the organic segment due strong to interactions with the glass tube. At higher flow rates, the segment can be disrupted. Therefore, the investigation of the effect of the ethanol content was made considering this limitation. Other flow parameters were initially fixed as described elsewhere [16]: carrier flow rate = 1.5 ml min^{-1}; pH = 10.0 (ammonium buffer). The ligand concentration was (1.0×10^{-1})% (w/v), (5.0×10^{-3})% (w/v) and saturated organic solution for PAN, DT and DDTC, respectively. The volume of sample and organic phase were fixed at 200 and 100 μl, respectively. The glass tube was 60 cm long and 3 mm i.d.

Fig. 3 shows how the efficiency of the extraction of Zn(II) is increased by adding small

amounts of ethanol to the organic solvent containing DT as extracting ligand. The same behavior was observed for the extraction of Cd(II) and Cu(II). For all solvents, the extraction practically does not occur in the absence of ethanol. The extraction efficiency rises significantly for toluene and carbon tetrachloride, reaching a maximum when the ethanol content is $>0.5\%$ (v/v). For chloroform, a minimum addition of 1.0% (v/v) of ethanol is necessary to achieve maximum efficiency.

By changing the ligand to PAN, the behavior of the extraction efficiency was altered. Fig. 4 shows that the efficiency increases only for toluene presenting a maximum at $\approx 0.25\%$ (v/v) of ethanol. In fact, the efficiency suffers a significant decrease when the ethanol content is $>0.4\%$ (v/v).

The behavior of the analytical signals, reflecting a homogeneity of the organic phase, is always better when ethanol is present in any of the phases. Fig. 5 shows the peak profile obtained for the organic phase in the absence (A) and the presence (B) of ethanol. Fig. 5(C) shows how the profiles of the organic phase are visually observed inside the glass tube in the absence and presence of ethanol. As can be observed, the interaction of the organic phase with the glass surface and the aqueous layer is improved in presence of ethanol. Due to the high solubility of ethanol in water it tends to be transported from the organic phase to the aqueous film present on the glass surface as the organic monosegment flows through the extraction tube. This fact promotes an interaction and a transition zone, between the frontier of the organic segment and the aqueous film retained on the glass wall, can be visually observed. The transition zone can improve the transport of both the ligand from and the complex to the organic phase, promoting a more effective contact between the ligand and the aqueous layer and the complex with the organic phase. The stronger interaction also improves the mass transport inside the single organic segment, due to convective intra-segment flow streams reinforced by that interaction. The final result is that the signals shown in Fig. 5(B) present a flat maximum along the organic segment while the signals produced in the absence of ethanol show a peak like profile (Fig. 5A).

Comparison between the efficiencies in the absence or presence of ethanol was made on the basis of the maximum of the observed signals. Therefore, the results obtained in the presence of

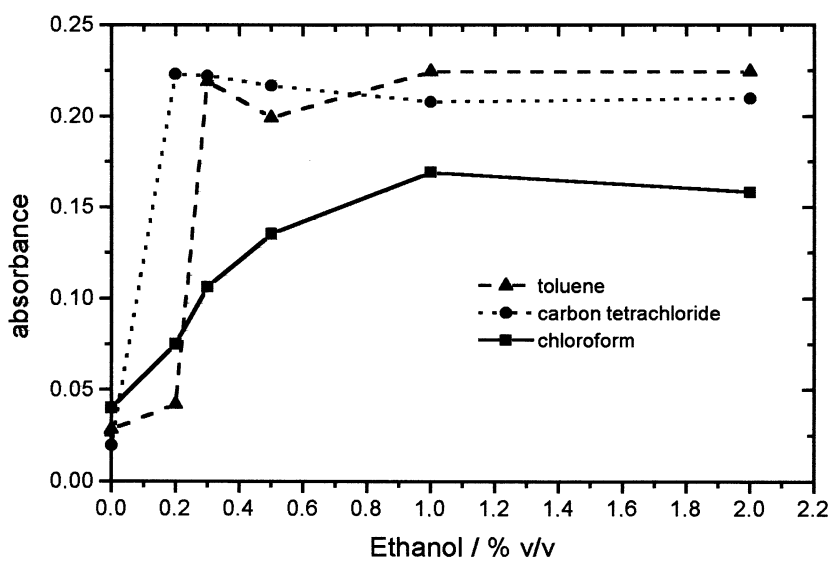

Fig. 3. Effect of the ethanol content in the organic phase on the extraction efficiency of Zn(II) with DT by the MSFA-LLE system. Zn(II) concentration = 1.0 μg l^{-1}, pH = 10.0, sample and organic phase volumes 200 and 100 μl, respectively.

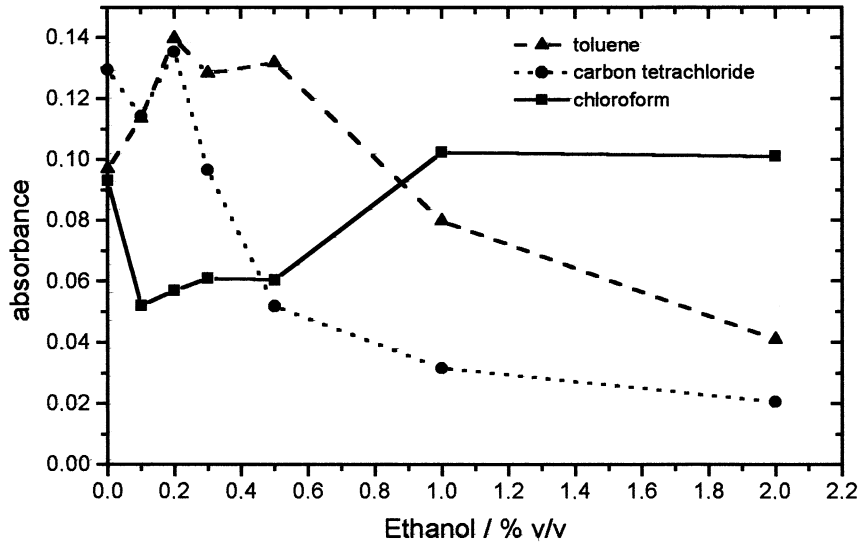

Fig. 4. Effect of the ethanol content on the extraction efficiency of Cd(II) with PAN. Cd(II) concentration = 1.0 μg ml^{-1}, pH = 10.0, sample and organic phase volumes 200 and 100 μl, respectively.

ethanol should reflect an even better efficiency because the ratio of the area of the analytical signals is greater than the peak ratio for both the presence and absence of ethanol. The repeatability of the signals was also improved in the presence of ethanol for all solvents studied, due to more homogeneous distribution of the extracted complex in the organic phase volume.

On the other hand, the nature of the ligand can impart distinct results of the extraction efficiency in function of the presence of ethanol, as observed comparing DT and PAN. It is very difficult to fully explain the different behavior of DT and PAN. It is probable that the total effect observed results from more than one factor affecting the transport of the ligand and the partition coefficient of the ligand and its complex between the organic and aqueous phase. Anyway, it is important to say that the monosegmented system is unique in its mechanism of extraction (see Fig. 2) as the only segment employed must supply the ligand to the aqueous phase and, at the same time, collect the formed complex from that phase to the organic phase. The more intense interaction between phases, promoted by the presence of ethanol, can improve the transportation. On the other hand, a change in the kinetics of the complex formation could be assumed to explain why DT suffer a positive effect in the presence of ethanol, while PAN has shown a less impressive gain in the extraction efficiency. Obviously, the information obtained so far is insufficient to support a full explanation of the factors affecting the monosegmented extraction system in the presence of ethanol and additional investigation is certainly necessary.

Another important effect of the presence of ethanol in the organic phase was observed when DDTC is employed as ligand. In this case, the ligand is not soluble in any of the pure organic phases. This fact forbids the use of DDTC in the monosegmented system because, in this approach, the ligand needs to be present in the organic phase, according to the mechanism shown in Fig. 2. However, the addition of small amounts of ethanol to the organic phase allows a small but sufficient increase of the solubility of DDTC, in order to employ this ligand in an MSFA-LLE extraction system. The advantage in this case is that the DDTC is highly selective for Cu(II) ions because the complexes formed with Cd(II) and Zn(II), for instance, do not absorb in the visible spectral range.

A remarkable effect of the presence of ethanol in all three organic solvents for DDTC extraction was observed. In the absence of ethanol, no signal is obtained using carbon tetrachloride, chloroform or toluene, because the ligand cannot be dissolved in the organic phase. The signal intensity rises until the ethanol content is $\approx 1.0\%$ (v/v) in the organic phase. Due the low molar absorptivity of the complex formed between Cu(II) and DDTC, a 1 cm optical path flow cell was employed for these experiments. Ethanol content $> 4.0\%$, although it can still increase the solubility of DDTC, produces a turbid emulsion in the organic phase and precludes signal acquisition, although the extraction is performed, as can be observed with the naked eye.

3.2. Effect of the pH

As previously observed [17] and based on the mechanism of the extraction in the MSFA system, the pH affects all the common parameters of the batch extraction procedure (mainly the partition equilibrium of ligand and its complex) plus another which is specific for the MSFA-LLE system. This is related to the release of protons from the silanol (Si–OH) groups present on the glass surface, providing the sites for adsorption of the metal ions. In this way, it is not surprising that the extraction occurs with low efficiency below pH = 8, even if the batch procedure for a given ligand (such as PAN) indicates that the efficiency is still good at pH values below 7.0 [21].

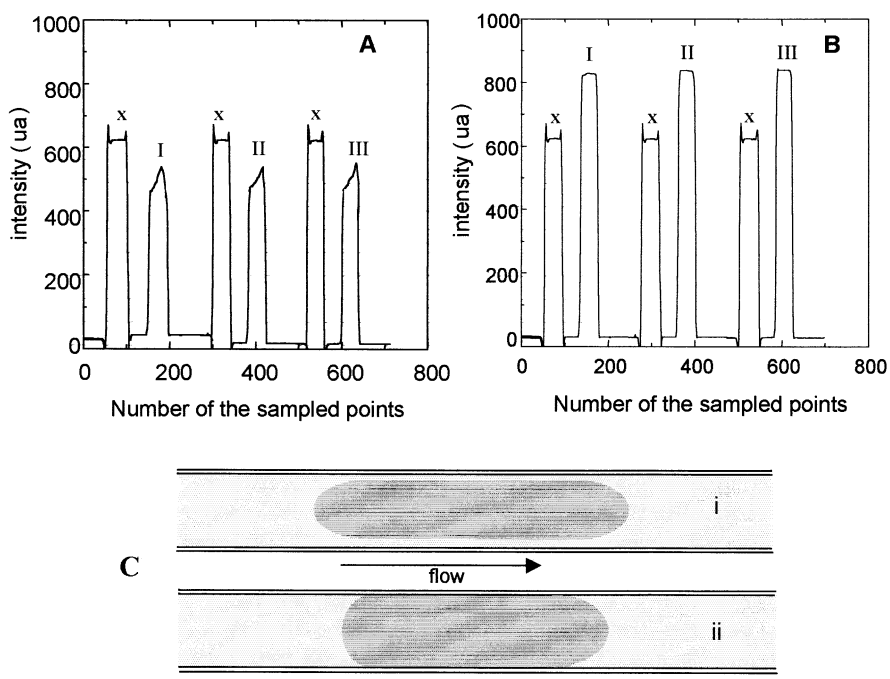

Fig. 5. Signals profiles for Zn(II) extraction using chloroform with DT as ligand. (A) Organic phase without ethanol; (B) with 1.0% (v/v) ethanol present in the organic phase. X, signal produced by the passage of the second air bubble of the sample monosegment; I, signal registered for the organic phase containing the extracted complex. Zn(II) concentration = 1.0 μg ml^{-1}, pH = 10.0, sample and organic phase volumes 200 and 100 μl, respectively. (C) Appearance of the organic phase inside the glass extracting tube; (i) without ethanol; (ii) with added ethanol.

3.3. Selected MSFA-LLE conditions for Cu(II) and Zn(II) extraction

The best conditions for extraction and determination were established for Cu(II) and Zn(II), in view of the good results obtained for selectivity and sensitivity in preliminary studies. The length and diameter of the extraction glass tube was kept constant at 60 cm and 3 mm, respectively, since previous investigations suggest that these conditions are the most suitable for MSFA-LLE, as a compromise between extraction efficiency and sample throughput [17].

The adopted conditions for Cu(II) determination using DDTC as extracting ligand were: carbon tetrachloride containing 3.0% (v/v) of ethanol and saturated with DDTC; carrier flow rate equal to 1.0 ml min^{-1}; sample and organic phase volume equal to 300 and 100 μl, respectively; and pH = 10 (NH_3/NH_4Cl buffer). Under these conditions, a linear analytical curve was obtained for the copper ion concentration [Cu(II)] (mg l^{-1}) as a function of the absorbance of the extracted complex (A): $[Cu(II)] = 0.0033(\pm 0.034) + 0.1495(\pm 0.0039)A$, with a correlation coefficient equal to 0.9994. The detection limit (three times the signal-to-noise ratio) was estimated as 0.13 μg ml^{-1} of Cu(II) in the aqueous solution. Although this detection limit is not as low as that reported previously using PAN as complexing agent (0.060 μg ml^{-1}) [17], the selectivity of the DDTC is an advantage to be considered.

The best performance for zinc extraction and determination was obtained by using DT ligand in carbon tetrachloride containing 1.0% (v/v) of ethanol. The selected flow rate was 1.5 ml min^{-1}, considering the best compromise between the extraction efficiency and the analytical sample throughput. At this flow rate, the efficiency is $\approx 50\%$ lower than that obtained for the batch procedure.

Other conditions for Zn(II) extraction in the MSFA-LLE system are: DT concentration in the organic phase = $(5.0 \times 10^{-3})\%$ (w/v); sample and organic phase volume equal to 200 and 100 μl, respectively. The sample and carrier solutions were buffered (NH_3/NH_4Cl) at pH = 10.0. The analytical curve is linear from 0.3 to 2.5 μg ml^{-1} of Zn(II) and the equation relating the Zn(II) concentration [Zn(II)] with the absorbance of the organic phase (A) is: $[Zn(II)] = -0.0074(\pm 0.054) + 0.2197(\pm 0.0044)A$, with a correlation coefficient equal to 0.9994. The detection limit (three times the signal-to-noise ratio) was estimated as 0.20 μg ml^{-1}. The performance of DT as extracting agent for Zn(II) determination in MSFA-LLE is many times superior to the use of PAN [17] and the behavior of the analytical curve shows a better performance for low Zn(II) concentrations.

3.4. Effect of concomitants on zinc determination

Employing the selected conditions described for Zn(II) determination, the effect of some common metal ions was investigated. The results are summarised in Table 1. The main interfering species are Cu(II), Cd(II), Hg(II) and Ni(II). These ions, however, are not present in the drug samples analysed by the MSFA-LLE system and should not present a drawback to its use for this kind of determination.

Distinct interferences effects are observed in function of the concomitant added to the Zn(II) solution. In general, the effects can be explained considering the adsorption/desorption mechanism of the monosegmented flow and the saturation of the active sites achieved at higher metal concentration [17]. A given concomitant may produce a negative interference when the desorption mechanism governs the kinetic of complex formation and its transference to the organic phase. The competition for the ligand present in the aqueous phase and a slower kinetic for the desorption process can result in a lower income of the analyte and concomitant complexes into the organic phase. However, for higher concentrations of metal, this competition is not significant because the fraction of metal, present only in the aqueous layer and not adsorbed [17], becomes more important and the interference assumes a positive tendency. In addition to these probable interfering effects, the instability of certain complex, as that formed between DT and Fe(II), can account for the observed behavior of this concomitant.

Table 1
Effect of concomitants on zinc determination by MSFA-LLE using dithizone in carbon tetrachloride containing 1.0% (v/v) of ethanol

	Interference factor							
	Concomitant concentration (μg ml^{-1})							
Concomitant	–	0.1	0.2	0.4	1.0	2.0	6.0	10.0
Cu(II)	1.0	1.1	1.3	1.5	1.9	2.2	2.7	3.3
Cd(II)	1.0	1.0	1.1	1.2	1.4	1.7	2.5	3.2
Fe(II)	1.0	0.8	0.7	1.0	1.2	0.8	0.8	0.7
Ni(II)	1.0	0.8	0.8	0.9	1.0	1.8	1.7	1.7
Hg(II)	1.0	1.0	1.0	1.4	1.3	1.6	1.2	1.6
Pb(II)	1.0	0.9	0.9	0.8	0.8	0.8	1.1	1.6

Interference factor = S_a/S_p, where S_a is the absorbance signal in the absence of the concomitant and S_p is the signal in its presence, at a given concentration. Zinc concentration = 1.0 μg ml^{-1}.

3.5. Determination of Zn(II) in drugs

Four commercial drugs containing zinc as part of their active substance were analysed by the proposed MSFA-LLE methodology. The samples were prepared through acid digestion (HCl) and the solution introduced, after pH adjustment, into the flow system. Table 2 shows comparative results obtained by the proposed methodology and by atomic absorption determination under factory recommended instrumental conditions. There is no significant difference between the results obtained by these techniques at the 95% confidence level.

4. Conclusion

The effect of the organic phase composition, altered in its polarity by addition of ethanol, on the efficiency of the MSFA-LLE methodology has been demonstrated. The effect can be ascribed to the better interaction between the extracting organic phase and the aqueous layer containing the analyte adsorbed on the glass tube wall. The effect depends on the ligand employed. A low ethanol content (0.25% (v/v)) can impart up to ten times improvement in the extraction efficiency, as seen in the case where DT is employed with carbon tetrachloride and toluene.

When the effect is not so intense, as in the case when PAN is used as extracting ligand, the spectrophotometric signals obtained present a better repeatability due to the better homogenisation achieved by the organic segment containing ethanol.

Another important effect of the use of ethanol in the organic phase is observed when the ligand is not soluble in the pure organic solvent, as in the case of sodium DDTC. In this case the addition of a polar solvent, such as ethanol, allows the ligand to dissolve in the organic phase in an amount sufficient to be used in the MSFA-LLE system. An additional gain, considering MSFA-LLE, is in the fact that the selectivity in these systems cannot be fully achieved by pH control, as the absorption of the analyte on the glass surface can only be produced at pH >8. Therefore, selectivity needs to be obtained by choosing the most appropriate ligand, as is the case for Cu(II) determination using DDTC.

The MSFA-LLE system has a good cost-to-benefit ratio and can operate with very low consumption of sample and reagent. Samples can be automatically processed at a rate of 30 per hour. The overall repeatability, expressed as the mean R.S.D. observed for all signals in the whole concentration range investigated during this work is $\approx 1.5\%$. The system does not employ phase segmentation or a phase separator and, therefore, is simpler than other flow approaches, previously described. Under optimised conditions, the MSFA-LLE system consumes ≈ 100 μl of organic phase and 200 μl of sample, as well as 20 μmol of

Table 2
Comparative results for zinc determination in drugs by the MSFA-LLE system and by atomic absorption spectrometry (AAS)

Sample	Concentration AAS	Concentration MSFA-LLE	Relative difference (%)
ZINCOPAN®	$(31.9 \pm 0.38)^a$	$(33.1 \pm 0.77)^a$	3.5
ZINCO FONTOVIT	$(56.9 \pm 0.44)^a$	$(55.8 \pm 0.60)^a$	−2.0
LERIN®	$(0.0267 \pm 0.0003)^b$	$(0.0281 \pm 0.0005)^b$	5.0
BLUMEN®	$(0.0530 \pm 0.0004)^b$	$(0.0570 \pm 0.0011)^b$	7.0

[a] Concentration of zinc in mg g^{-1}.
[b] Concentration of zinc in mg ml^{-1}.
The values are presented with their estimated S.D. for four replicates.

DT, 30 μmol of PAN or 10 μmol of DDTC, per determination. This low consumption of chemicals is compliant with the requirement for waste production reduction in analytical laboratories.

The use of the MSFA-LLE system for determination of Zn(II) in drugs has demonstrated its potential for use in routine analysis.

Finally, this work shows the importance of organic phase composition on the efficiency of the liquid–liquid extraction performed under flowing conditions. The dynamic conditions found in these systems, require investigation of the means to produce a more effective interaction between the organic and aqueous phase. In the present case, this interaction was improved by simply adding a polar solvent to the organic extracting solution. Although only the MSFA-LLE system has been investigated, it is probable that this effect may be significant for other flow approaches as well.

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