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Flow injection flame atomic absorption determination of Cu, Mn and Zn partitioning in seawater by on-line room temperature sonolysis and minicolumn chelating resin methodology

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ABSTRACT

A flow-injection system is developed for Cu, Mn and Zn partitioning in seawater by flame atomic absorption spectrometry. The first approach is where the trace metal species are fractionated in situ, but analysis is performed by using a flow injection manifold in the laboratory. This operational mode is used for the determination of the dissolved labile metallic fraction and is based on the elution of this fraction from a minicolumn packed with a chelating resin containing iminodiacetic acid groups (Serdolit Chelite Che) loaded in situ with the sample. The second is used for the determination of total dissolved concentrations of trace metals. This last mode is based on the retention/preconcentration of total dissolved metals on the chelating resin after on-line sonolysis of seawater samples acidified with diluted nitric acid to breakdown the metal–organic matter complexes. The figures of merit for Cu, Mn and Zn determinations in both fractions are given and the obtained values are discussed. The fractionation scheme is applied to the analysis of coastal seawater samples collected in Galicia (Northwest, Spain). The results of fractionation showed that Mn and Zn are mainly in the labile fraction, while Cu was mainly present in the organic fraction.

1. Introduction

Metal ions can be present in aquatic systems in different physicochemical forms. Consequently, analytical information on the lability of metal species and their possible transformation into species of either higher or lower stability is of increasing relevance because the biological availability, and hence toxicity, of metals in aquatic systems is strongly dependent on the nature of the metal species present [1]. In addition, determining the chemical form of metals is essential, since the knowledge of distribution of metal species in natural waters helps understanding of geochemistry and distribution of metals in the aquatic environment [2]. Free ions, aquo-ions, inorganic complexes and weak metal–organic matter constitute the labile fraction, whereas metal bound to the strong binding sites forms strong humic-substance complexes, which are inert and not bio-available [3]. Humic substances (i.e., humic and fulvic acids) are high molecular weight compounds, their origin may be either terrestrial or marine and constitute from 10 to 30% of dissolved organic carbon in seawater [4,5].

One of the problems found in fractionation analysis is the development of analytical procedures that do not disturb the chemical equilibrium of the original forms existing in the matrix of the sample, for it is essential to avoid disturbing the system under study as much as possible. However, these problems cannot be solved simply by development of extremely sensitive techniques, but also require proper analytical methodologies and procedures that, for example, allow the sampling/preconcentration process in situ. Thus, in the last years, a variety of methods have been proposed for in situ metal fractionation to differentiate labile and inert metal species in aquatic systems, above all based on application of chelating resins in minicolumns [6–8], and on the technique of diffusive gradients in thin-films (DGT) [9–11]. In this way, accumulated metals are measured later in the laboratory mainly by stripping voltammetry or atomic absorption spectrometry. Nevertheless, the use of in situ sampling/preconcentration devices based on minicolumns is the simplest alternative for this purpose because of its relative easy to develop and exploit automated manifolds, which allow change in environmental conditions (such as water level or sample flow-rate), filter and preconcentrate the sample. As a result, this methodology preserves the sample for further laboratory pretreatment, and the analysis can be easily carried out by incorporating the sample loaded minicolumns into a flow injection

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manifold [12–14]. Iminodiacetate based chelating resins, such as Chelex-100 or resins possessing 1,4,7,10,13-pentaazatridecane, or the tetren group such as Chelamine, have been used to retain free metal ions and kinetically labile forms because they do not retain those metals when strongly complexed by organic ligands as humic substances [15–18]. Moreover, they have high selectivity for transition metal ions with minimal retention of alkali and alkaline earth metal ions.

Since the high concentration of alkali and alkaline earth metal ions in seawater makes direct analysis of seawater even by the most sensitive analytical techniques such as ICP-MS very difficult, a separation and preconcentration step to separate the trace metals of interest from these major ions is necessary. For this, to determine total dissolved trace metals, it is necessary to release the trace metals from the strong metal–organic complexes prior to analysis when a separation step is inserted in the analytical process [19]. Liquid–liquid extraction and solid-phase extraction with chelating resins have been two of the most popular separation techniques used for this purpose. However, chelating resins are preferred because organic solvents are avoided, providing simple, clean, green and environmentally friendly methodologies to trace metal separation [20]. As it is commented previously, chelating resins were used to extract only free metal ions and kinetically labile forms. A sample acidification could breakdown organic complexes, but as most experimental optimum conditions require that acidified water samples must have their pH adjusted up prior to the preconcentration step, some of metals can re-complex and become bound as kinetically inert complexes with organic ligands again. In consequence, unless the dissolved organic ligands are destroyed, they are not retained by the resin. The preferred approaches for the breakdown dissolved metal–organic complexes are wet digestion using chemical oxidants and treatment of the sample with ultraviolet (UV) radiation prior to trace metal determination. UV digestion is a trace metal clean sample pretreatment method, since it does not require the addition of large amounts of oxidants, and the procedure can be readily integrated in flow injection manifolds. In consequence, several procedures involving this methodology have been proposed [21–25]. Nevertheless, there is disagreement about experimental conditions applied for natural water digestion. Thus, water samples were acidified with hydrochloric acid or nitric (until pH about 1.7–2.2) with [22] and without [21,23] the presence of diluted hydrogen peroxide, and UV digestion time ranged between 0.5 and 8 h. On the other hand, flow injection procedures reduced this time considerably [25].

The emerging interest in better and fast environmental analytical methodologies forces to take into account rapid sample preparation procedures based on Green Chemistry (e.g., methodologies based on the application of ultrasonic energy) [26]. Accordingly, this energy was used by our investigation team in a continuous mode for acid or alkaline extraction of metals from several matrices [27–31]. As a result, application of ultrasonic energy can be an attractive alternative to seawater sample digestion for the breakdown dissolved metal–organic complexes because this technology may be used for water and wastewater off-line treatments as an advanced oxidation process in order to purify water by degradation or organic pollutants [32–35], for degradation of organomercurials to determine inorganic and total mercury [36] and in a continuous mode to digest urine samples [37,38].

In the present work, a rapid on-line approach for the determination of total dissolved and labile trace copper, manganese and zinc concentrations in seawater samples by flow injection (FI)-flame atomic absorption spectrometry (FAAS) is presented. The copper, manganese and zinc fractionation are based on chelating ion-exchange because the Serdolit Chelite Che chelating resin (containing iminodiacetic acid groups) is suitable to preconcentrate/separate only free and labile forms of trace metals. The decomposition of organic complexes is performed on-line by sonolysis for the determination of total dissolved concentrations of Cu, Mn and Zn in seawater samples.

2. Experimental

2.1. Apparatus

The experiments were performed with a Perkin Elmer Model 5000 (PerkinElmer Life and Analytical Sciences, USA) air–acetylene flame atomic absorption spectrometer attached to a Perkin Elmer Model 50 Servograph Recorder with a range of 5 mV. Hollow cathode lamps operated at recommended currents were utilized as radiation source. The on-line device used consisted of two Gilson Minipuls-3 peristaltic pumps (Gilson, France), five Rheodyne low-pressure injection valves (four of them adapted to work as selection valves) (Rheodyne, USA), an ultrasonic bath with an operating frequency of 40 kHz and a generator power 200 W (Selecta, Spain) and a digestion chamber (a glass minicolumn, 100 mm × 15 mm i.d., bed volume 12 mL, Omnifit, UK). PTFE tubing of 0.8 mm i.d. (Omnifit, UK) was used for connections. The laboratory-made minicolumn for the on-line preconcentration step was prepared filling Viton tubes (100 mm × 1.1 mm i.d.) with 50 mg of Serdolit Chelite Che chelating resin. All glasswares were decontaminated with 10% (v/v) nitric acid for at least 48 h and washed three times with Milli-Q water before use.

Statistical analysis of the experimental designs was carried out by means of the Statgraphics Plus V.5.1 statistical package (Manugistic, Inc., USA).

2.2. Reagents and solutions

Metal stock solutions ($1000\,\mu\mathrm{g\,mL^{-1}}$) were purchased from Merck (Germany). The working solutions were prepared by series dilution of the stock solutions immediately prior to use. The nitric acid used for water acidification and the hydrochloric acid used to elute the metals from the chelating resin were from Merck (Germany). The chelating resin containing iminodiacetic acid groups used for trace metal preconcentration was from Serdolit Chelite Che (sodium form) (Serva Electrophoresis, Germany). Ammonia–ammonium chloride buffer solution (pH 8) was prepared by dissolution of 1.1 g of ammonium chloride (Merck, Germany) in 100 mL of ultrapure water and adjusting the pH to 8.0 by adding diluted ammonia ($3\,\mathrm{mol\,L^{-1}}$) (Merck, Germany). Aqueous solutions of humic acid (Sigma–Aldrich, Switzerland), ethylenediaminetetraacetic acid (EDTA), sodium chloride and sodium sulfate (Merck, Germany) were prepared as model natural ligands. Certified reference material SLEW-3 (estuarine water) from the National Research Council of Canada was used to verify the accuracy of the proposed methodology.

All chemicals used were of ultrapure grade. Solutions (reagents and standards) were prepared using ultrapure water of 18.2 MΩ cm resistivity, obtained from a Milli-Q water purification system (Millipore, USA).

2.3. Minicolumn preparation

The minicolumns were manufactured in the laboratory from Viton tubes (100 mm × 1.1 mm i.d.) and were packed with 50 mg (20 mesh) Serdolit Chelite Che chelating resin (a small amount of resin beads was injected into the minicolumn with a syringe). The ends of the tube were fitted with glass wool to keep the beads in the tube. The resin was equilibrated with $2\,\mathrm{mol\,L^{-1}}$ hydrochloric acid to convert it into the acid form, which was the one used to preconcentrate because it has the lowest blank values. The resin was washed with water until the pH was neutral.

2.4. Sampling and sample preparation

Superficial seawater samples used for the development of the analytical procedure were collected from the northwest Spanish coastal surface water (Galicia). For the determination of total dissolved trace metals, seawater samples were collected in pre-cleaned glass bottles, filtered through a 0.45 μm filter and immediately acidified with 10 mL of concentrated nitric acid (for a sample volume of 250 mL) to avoid metal adsorption on the container walls. The samples were stored in a refrigerator (4 °C) for no longer than two weeks. For the determination of the metal labile fraction, seawater samples (without addition of acid) were pumped at 4.0 mL min^{-1} (for 12.5 min) through the minicolumn containing the chelating resin (Serdolit Chelite Che) after on-line filtration with a 0.45 μm filter as described elsewhere [14]. As a result, the labile fraction of the trace metals was retained on the minicolumn, and the seawater matrix was sent to waste. After loading, the resin was washed with ultrapure water in order to remove the remaining sample from the minicolumn, and the residual internal fluid was drawn off passing an air stream (4 mL min^{-1}) through the minicolumn until it does not drip. The minicolumns were transported in a portable refrigerator, and returned to the laboratory where they were stored in a refrigerator until further analysis. Thus, the time of the whole sampling and washing cycle was ca. 15 min.

2.5. Determination of the metal labile fraction

With the purpose to determine the labile fraction of trace metals in seawater samples, the in situ loaded minicolumns were connected to the FI manifold. The minicolumn was located immediately before the detector. The analysis procedure consists of the injection of 110 μL of 3 mol L^{-1} hydrochloric acid into a carrier ultrapure water pumped at 5.0 mL min^{-1}. Thus, the labile fraction of trace metals is released directly into the nebulizer of the FAAS spectrometer.

2.6. Determination of the total dissolved concentration of trace metals in seawater

In a first step is carried out the decomposition of metal organic complexes by sonolysis. Thus, 10 mL of seawater samples in 0.6 mol L^{-1} nitric acid medium are inserted into the flow system at 5.0 mL min^{-1} by means of a peristaltic pump (P2). Once all the sample volumes are located in the digestion cell (DC), which is immersed within the ultrasonic bath at room temperature, the pump controlling the sample stream (P2) was stopped and the sample stays under the action of ultrasound energy for a period of 120 s. After, the selecting valve (SV3) was switched to its other position to select the digestion cell downstream, and the pump P2 was again activated. In this way, the sonodigested seawater sample arrives at the part of the FI system where the preconcentration step takes place. For this, the sonodigested sample stream converged with the buffer stream (ammonia–ammonium chloride buffer solution, pH 8) in order to obtain the optimum pH value for metal retention in the chelating resin. Both streams are homogenized in the mixing coil, resulting a homogenized stream with a flow-rate of 4.0 mL min^{-1} and then, the resulting stream passed through the minicolumn containing the chelating resin (Serdolit Chelite Che). By means of SV2, the sample matrix is sent to waste, while ultrapure water flowing through the detector by the nebulizer suction. Finally, the retained metals were subsequently eluted by injection of 110 μL of 3 mol L^{-1} hydrochloric acid into a water carried stream, being continuously monitored by a FAAS instrument. In order to avoid carry-over, a washing step was included in the analysis cycle. Thus, between each sample analysis, the digestion cell was washed with ultrapure water during 30 s. For this, the selecting valve (SV4) was switched to select the ultrapure water stream channel, while the wash water was sent to waste through SV3. The time for the determination of total dissolved concentration of Cu, Mn and Zn was ca. 6.5 min.

3. Results and discussion

3.1. Effect of natural ligands

Competition for metals from ligands in solution will determine the distribution among labile and inert fractions. In this study were selected as representative ligands chloride, sulfate, EDTA and humic acid at a concentration of 19,000, 905, 30 and 30 mg L^{-1}, respectively. The inorganic ligands chosen are the major anionic components of seawater, and regarding the organic ligands, humic acids are the very important. EDTA is an organic ligand present in seawater, which is frequently used as organic ligand model because, has high affinity for metals, with which forms strong complexes [39]. The influence of these ligands on the complexation and retention of copper, manganese and zinc by the Serdolit Chelite Che resin was investigated at pH 8 by using solutions containing 1 μg L^{-1}, and a volume of 50 mL. The results of this study show that more than 95% Zn(II), Mn(II) and Cu(II) in chloride and sulfate solutions were retained by the resin. It is due to the formation of labile complexes with these anions. However, when the metallic solution contains humic acid or EDTA the recovery was reduced to 5.1% (with EDTA) and 3.9% (with humic acid) for Zn, to 5.1% (with EDTA) and 6.1% (with humic acid) for Mn and to 4.6% (with EDTA) and 3.4% (with humic acid) for Cu. This behavior can be explained by the formation of nonlabile complexes, which are not retained on the chelating resin. Thus, on the basis of these recovery values, the Serdolite Chelite Che resin can be used for separation of the free and the labile metallic fraction from inert complexes formed with organic ligands.

3.2. Optimization of the experimental conditions for the FI preconcentration of trace metals

Preconcentration step determines the efficiency of the final method and then, experimental work was initially focused on the optimization of the variables implied on the utilization of the chelating resin Serdolite Chelite Che to retain and elute the target trace metals. This optimization was made using an experimental design approach. Six factors were selected and studied at two levels: sample pH, sample flow-rate, eluent concentration (hydrochloric acid), elution flow-rate, eluent volume and minicolumn diameter. The factors selected and their levels are presented in Table 1. The factors and their levels were selected according to available data and experience gathered in previous screening experiments. A factorial screening Plackett–Burman $2^6 \times 3/16$ experimental design with one centerpoint, which studied the effects of the six factors in 13 experiments was run. Six degrees of freedom allowed estimating the experimental error [40]. All experiments were performed using a sample volume of 20 mL and

Table 1
Factors and levels considered in the factorial screening Plackett–Burman $2^6 \times 3/16$ experimental design applied for optimization of the preconcentration step.

Factor	Code	Low level	High level	Continuous
Sample pH	A	1	8	Yes
Sample flow-rate (mL min^{-1})	B	1.0	5.0	Yes
Eluent concentration (HCl) (mol L^{-1})	C	0.1	3.0	Yes
Elution flow-rate (mL min^{-1})	D	3.0	5.0	Yes
Eluent volume (μL)	E	70	110	Yes
Minicolumn diameter (mm)	F	1.1	2.3	Yes

Table 2
Analysis of variance (ANOVA) showing the significance of main effects.

Factors	A		B		C		D		E		F	
	F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Cu	13.71	0.0107	2.73	0.1496	11.97	0.0135	0.09	0.7724	10.54	0.0175	0.02	0.8940
Mn	2.23	0.1863	2.80	0.1455	6.53	0.0432	0.04	0.8428	6.31	0.0457	0.01	0.9426
Zn	12.73	0.0118	2.32	0.1788	10.22	0.0187	0.02	0.8877	5.25	0.0418	0.26	0.6300

introducing standard solutions of 1 μg L^{-1} for Cu(II) and Mn(II) and 0.5 μg L^{-1} for Zn(II) determination into the FI manifold. The factorial design was evaluated using the recovery as analytical response. Table 2 summarizes the analysis of variance for the six factors studied. A main factor or a factor interaction is significant when its *P*-value is lower than 0.05 at a 95% confidence level. As can be seen in this table, both factors concentration and volume of eluent were significant for the recovery of target trace metals. The sample pH was significant for copper and zinc, while the other variables were not significant factors. The main effects for the target trace metals explain that both elution flow-rate and the minicolumn diameter do not affect the recovery because their influence is much lower than that of other factors. In summary, the experimental conditions that could be selected after the study of the factorial design results involved the use of a sample pH of 8.0 (within the normal pH range of seawater, 8.0–8.3, which implies that the pH does not have to be modified for the determination of the metal labile fraction), 3 mol L^{-1} and 110 μL for the concentration and volume, respectively of the eluent solution, 5.0 mL min^{-1} for eluent flow-rate and 1.1 mm for minicolumn diameter. These two last were chosen at indicated values because a minicolumn diameter of 1.1 mm facilitates the FI connections and an eluent flow-rate of 5.0 mL min^{-1} increases sample throughput and analytical sensitivity. Under the selected experimental conditions, recovery was calculated for each metal modifying the sample flow-rate (between 1.0 and 4.0 mL min^{-1}). As can be seen in Table 3, average recoveries for three replicates ranged from 95.3 to 100.2%. Therefore, with a sample flow-rate of 4.0 mL min^{-1} can be obtained a recovery considered as quantitative, what is positive because increases extremely sampling frequency, decreasing sampling time.

3.3. Optimization of the experimental conditions for the on-line sonolysis procedure

Once optimized the preconcentration process and confirmed that the target trace metals can be quantitatively on-line retained/eluted by the chelating resin Serdolit Chelite Che, the sonolysis step was studied. For this, a central composite design (2^3 +star with 11 error degree of freedom, 16 randomized experiments and three replicates) was used to optimize the variables affecting the on-line sonolysis procedure: concentration of nitric acid (acid sample medium), sonication time and sonolysis temperature. The tested values for each variable are shown in Table 4. The response variable was the recovery calculated according to the following equation: $\%R = (M1/M2) \times 100$, where $M1$ is the concentration of the target analytes obtained by the proposed procedure and $M2$ is the concentration obtained for the same sample, but performing the sample digestion procedure by traditional way using ammonium peroxydisulfate at elevated temperature [41]. After performing the experiments, it is proven that sonolysis temperature is not a significant variable, resulting in low estimated effects for the three analytes. In fact, some experiments result in quantitative recoveries when the sonolysis temperature is 20 °C. Thus, analyzing the design only for the sonication time and for the acid sample medium variables, which were the factors that showed a maximum of the response surface was possible to choose a concentration of nitric acid of 0.6 mol L^{-1} and a sonication time of 120 s as optimum conditions.

Table 3
Recovery of Cu, Mn and Zn at three sample flow-rates.

Sample flow-rate (mL min^{-1})	%Recovery		
	1.0	3.0	4.0
Cu	99.2	96.2	96.2
Mn	100.2	97.8	95.3
Zn	99.7	98.3	96.8

3.4. Performance of the method

Using the described optimized system, calibration graphs were run for standard solutions of the analytes. The sample volume used for the determination of the total dissolved concentrations of trace metals was 10 mL, and the sample volume used for the determination of the dissolved labile metallic fraction was 50 mL. The results are shown in Table 5. Detection limits, calculated as the concentration leading to a signal corresponding to three times the standard deviation of the noise, are suitable for the usual concentrations found in seawater samples. The precision of the method, expressed as relative standard deviation, was evaluated by eleven independent measurements of 50 mL of standard solutions containing 1 and 4 μg L^{-1} of Cu and Mn and 0.5 and 2 μg L^{-1} of Zn, and 10 mL of standard solutions containing 1 and 5 μg L^{-1} of Cu and Mn and 1 and 2 μg L^{-1} of Zn. The preconcentration factors calculated as the ratio of the slope of the calibration graph with and without preconcentration and sample throughputs were also shown in Table 5. Analytical recovery was assessed after spiking with metal aliquots of a seawater sample (for the determination of total dissolved concentration) and an artificial seawater without organic matter [42] (for the determination of the metal labile fraction) (Table 5). It can be concluded that a complete analytical recovery (within the 95.3–101.5% range) was reached for the target trace metals at all concentration levels studied. A certified reference material (SLEW-3) with certificate values of 1.55 ± 0.12, 1.61 ± 0.22 and 0.201 ± 0.07 μg L^{-1} for Cu, Mn and Zn, respectively was used to verify the accuracy of the proposed methodology on

Table 4
Experimental field definition for the central composite design used to optimize the on-line sonolysis procedure.

2^3 + star central composite design							
Variable	Code	Low level	High level	Continuous	Optimum		
					Cu	Mn	Zn
Acid sample medium (HNO_3) (mol L^{-1})	A	0.2	1.0	Yes	0.6	0.6	0.6
Sonication time (min)	B	30	180	Yes	120	120	120
Sonolysis temperature (°C)	C	20	50	Yes	20	20	20

Table 5
Analytical figures of merit of the determination of total and labile dissolved copper, manganese and zinc in coastal seawater samples.

	Copper		Manganese		Zinc	
	Labile	Total	Labile	Total	Labile	Total
Linear range ($\mu g\,L^{-1}$)	0.15–11.28	0.77–56.60	0.12–4.54	0.61–22.60	0.041–2.29	0.19–11.40
Correlation coefficient (r^2)	0.9998	0.9986	0.9999	0.9995	0.9999	0.9998
Preconcentration factor	446.1	88.9	440.8	88.7	440.5	88.3
Sample throughput (samples h^{-1})	4	8	4	8	4	8
Detection limit ($\mu g\,L^{-1}$)	0.049	0.25	0.034	0.18	0.013	0.055
Precision (RSD, %)	1.6–5.2	2.6–5.2	1.1–2.3	1.8–4.4	1.1–2.4	1.2–2.7
Recovery (%)[a]	99.2	97.2	97.8	95.3	99.1	97.8
Recovery (%)[b]	98.6	98.8	99.3	101.5	97.9	100.3

[a] Total dissolved metal: sample spiked with 2 $\mu g\,L^{-1}$. Labile dissolved metal: artificial seawater sample spiked with 2 $\mu g\,L^{-1}$ (Cu and Mn) and 0.5 $\mu g\,L^{-1}$ (Zn) ($n = 3$).
[b] Total dissolved metal: sample spiked with 3 $\mu g\,L^{-1}$. Labile dissolved metal: artificial seawater sample spiked with 4 $\mu g\,L^{-1}$ (Cu and Mn) and 2 $\mu g\,L^{-1}$ (Zn) ($n = 3$).

Table 6
Determination of total and labile dissolved Cu, Mn and Zn in superficial coastal seawater samples (SWS) from Galicia (north-western Spain).

Sample location	Copper ($\mu g\,L^{-1}$)					Manganese ($\mu g\,L^{-1}$)				Zinc ($\mu g\,L^{-1}$)			
	Salinity (‰)	Labile	Total	%LF[a]	$\log K_d$ [b]	Labile	Total	%LF[a]	$\log K_d$ [b]	Labile	Total	%LF[a]	$\log K_d$ [b]
Baiona (AO)[c]	35.2	n.d.[f]	0.97 ± 0.11	–	4.7	2.79 ± 0.05	4.53 ± 0.09	61.6	4.1	1.27 ± 0.01	2.12 ± 0.03	59.9	5.0
Foz (CS)[d]	35.7	0.17 ± 0.02	1.83 ± 0.18	9.3	4.6	1.29 ± 0.06	1.97 ± 0.09	65.5	4.2	2.17 ± 0.01	3.62 ± 0.05	59.9	4.8
Cariño(AO)[c]	34.6	0.24 ± 0.01	2.63 ± 0.11	9.1	4.3	2.04 ± 0.05	3.28 ± 0.15	62.2	4.1	1.86 ± 0.01	3.03 ± 0.05	61.4	4.9
Malpica (AO)[c]	35.4	0.17 ± 0.01	1.40 ± 0.11	12.4	4.6	1.56 ± 0.03	2.42 ± 0.09	64.5	3.8	1.54 ± 0.03	2.64 ± 0.03	58.3	4.8
Fisterra(AO)[c]	35.6	n.d.[f]	0.84 ± 0.11	–	4.8	1.02 ± 0.03	1.57 ± 0.09	65.0	4.2	0.98 ± 0.01	1.64 ± 0.05	59.8	4.8
Ribeira (AO)[c]	35.2	0.21 ± 0.02	2.07 ± 0.11	10.1	4.6	3.90 ± 0.08	5.68 ± 0.15	68.7	4.1	2.15 ± 0.02	3.52 ± 0.07	61.1	4.8
Noia (MNE)[e]	34.2	0.39 ± 0.02	4.04 ± 0.18	9.7	4.2	2.86 ± 0.03	4.48 ± 0.15	63.8	4.1	2.22 ± 0.03	3.66 ± 0.03	60.7	4.8
Porto do Son (MNE)[e]	34.5	0.45 ± 0.02	4.78 ± 0.18	9.4	4.2	3.20 ± 0.03	5.03 ± 0.09	63.6	3.9	2.01 ± 0.03	3.44 ± 0.05	58.4	4.8
Muros (MNE)[e]	34.3	0.43 ± 0.02	4.22 ± 0.18	10.2	4.2	2.46 ± 0.05	3.83 ± 0.09	64.2	3.9	2.00 ± 0.02	3.27 ± 0.07	61.2	4.8
Burela (CS)[d]	35.1	0.50 ± 0.02	5.02 ± 0.11	10.0	4.3	4.15 ± 0.05	6.79 ± 0.09	61.1	4.1	2.25 ± 0.01	3.78 ± 0.01	59.5	4.8

n.d.: not detected.
[a] LF: percentage of labile fraction.
[b] $\log K_d$: log(partitioning coefficient).
[c] AO: Atlantic ocean.
[d] CS: Cantabrian sea.
[e] MNE: Muros-Noia estuary.

total concentration determination. The concentrations found were 1.58 ± 0.11, 1.62 ± 0.12 and 0.201 ± 0.037 $\mu g\,L^{-1}$, for Cu, Mn and Zn, respectively. As can be seen, they are in concordance with the certified values.

3.5. Minicolumn stability

With the aim to establish the time that, once in situ loaded with seawater samples, the minicolumns could be stored before Cu, Mn and Zn determination, stability studies at optimum conditions were made. For this, Cu, Mn and Zn retained on minicolumns were determined at the same day of the sampling, and after 10 and 15 days stored at 4 °C (refrigerator). The obtained results indicated that minicolumns were stable for at least 15 days and could be stored during this time without any significant losses.

3.6. Determination of total and labile dissolved copper, manganese and zinc in coastal seawater samples

The proposed methodology has been applied to coastal seawater samples from Galicia (north-western Spain) and the results obtained are summarized in Table 6 (mean ± standard deviation, $n = 3$). The highest concentrations for Cu, Mn and Zn were located at the port of Burela, while the lowest were found at Fisterra. The percentages for the labile fraction of each metal are also presented in Table 6. Thus, it has demonstrated that in the analyzed seawater samples, copper is mainly present forming organic metal complexes (the labile fraction found was 10.0 ± 1.0%). On the other hand, manganese and zinc are found mainly in the labile dissolved fraction (64.0 ± 2.2 and 60.0 ± 1.1%, for Mn and Zn, respectively). These results agree well with published results for seawater samples. Thus, it is generally accepted that copper is predominantly organically complexed in seawater [43,44], while Mn and Zn are principally present in ionic forms [16,25,44].

The behavior of metals in natural water systems depends significantly of their distribution in the particulate and dissolved phases. The partitioning coefficient (K_d) is the most commonly employed means of describing the solid–solution partitioning in aquatic systems, and it is defined as the ratio of particulate concentration (w/w) to dissolved concentration (w/v) [45]. So, the relative affinity of Cu, Mn and Zn for the particulate and dissolved phases can be evaluated using K_d. To calculate K_d, the filter-retained particulate matter was analyzed as described elsewhere [46]. As can be seen in Table 6, the $\log K_d$ values obtained varied from 4.2 to 4.8 for Cu, from 3.8 to 4.2 for Mn and from 4.8 to 5.0 for Zn. Although the samples have similar salinity (34.2–35.7‰), the partitioning coefficient for each seawater sample was related to its salinity. Thus, the results obtained reflect that K_d increases slightly with salinity, indicating affinity of metal to be associated and transported with the solid phase. These results are in agreement with those reported by other authors for estuarine and seawaters [45,47–51].

4. Conclusions

A simple, automatic FI system for the fractionation of copper, manganese and zinc in seawater samples by chelating ion-exchange preconcentration/separation-FAAS has been developed. Sonolysis has been used for the decomposition of organic complexes of metals from seawater. This sample pretreatment allowed the determination of total dissolved concentrations of the target trace metals in seawater samples accelerating and simplifying the analysis time. The reliability of the proposed methodology has been validated by performing a recovery study on real samples and furthermore by the analysis of a certified reference material. Furthermore, the proposed FI manifold meets the best analytical requirements: simple design and instrumentation with an easy functioning and a low cost of acquisition and maintenance as FAAS. However, it should take into account that the procedure could be easily applied with other techniques such as inductively coupled plasma mass spectrometry or inductively coupled plasma atomic emission spectrometry.

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