Towards field trace metal speciation using electroanalytical techniques and tangential ultrafiltration

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A B S T R A C T

In this work we propose a trace metal speciation methodology to determine the total, free and ultra-filtered (< 1 KDa) metal fractions using electrochemical methods (SCP and AGNES) and tangential ul-trafiltration (UF) experiments that can easily be carried out on-site. We tested our methodology spiking Cadmium ions into two natural waters samples from Itapanhau and Sorocabinha rivers in Sao Paulo State, Brazil. The limits of detection (LOD) was 1.6×10^{-9} M for the total Cd^{2+} determination performed by Stripping Chronopotentiometry (SCP) in the source and acidified ultrafiltered solution and 1.9×10^{-9} M for the free Cd^{2+} determination using Absence of gradients and Nernstian equilibrium stripping (AGNES), using a thin mercury film electrode. The total metal determination was performed by SCP in acidified samples and the results compared with graphite furnace atomic absorption spectroscopy (GF-AAS). The SCP results were adequate with a 96% of recovery from the known metal spike for the 12 samples tested. For the Itapanhau sample the free metal determined by AGNES and the ultrafiltered fraction are identical, while for the Sorocabinha the free metal in the source is significantly smaller than the ultrafiltered fraction, indicating that this sample must be rich in metal complexes with small in-organic ligands that are able to permeate the 1 kDa membrane. The proposed metal speciation metho-dology validated in the laboratory combining UF and SCP/AGNES is able to be used in on-site experiments providing valid information regarding the total and free metal concentrations and additionally some insight on the role of small inorganic ligands to the metal complexation.

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1. Introduction

The interest of studying trace metal ions in natural waters stems from their toxicity towards microorganisms and accumu-lation through the food chain. Toxic metallic elements, like Pb, Cd, Zn and Hg are found in trace amounts in environmental waters mostly as a result from anthropogenic activity. Their impact on the environment depends on their concentration and speciation since metals may be present as hydrated cations, dissolved molecular species or as parts of complex mineral particles. The dissolved fraction has a much higher bioavailability than the particulate one, thus a higher toxicity.

Currently the conventional notion in ecotoxicological studies is that the controlling species regarding trace metal bioavailability is the free hydrated cation (FIAM, BLM) [1]. Since the trace amounts of toxic metal present in natural water are difficult to measure; therefore the free cations are even more challenging, since their concentration often lies on the nanomolar range or below. Natural waters have a plethora of ligands able to bind the metal ions, like dissolved inorganic and organic ligands, colloids, suspended matter and the surfaces of organisms, hence only a small part of the total dissolved metal exists as free hydrated cations [2].

Environmental trace metal analysis is not a simple task due to the complexity of the environmental matrices. Additionally the collection and handling of natural waters samples can further in-duce changes in the distribution of species prior to laboratory measurements, thus on-site measurements are preferable since they avoid problems due to contamination during sampling and storage, metal adsorption to container walls and instability of metal species during transport and storage prior to measurements.

Over the years an extensive effort has been made to design chemical sensors for on-site measurements and there are several books on chemical sensors for aquatic measurements that describe

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various continuous flow analyzers as well as optical and electrochemical sensors [3]. Nevertheless these methods focus mostly on the determination of total metal; hence at this point there are very few affordable on-site systems for free trace metal quantification at the concentration levels present in natural waters.

In terms of portability for on-site trace metal detection system, electrochemical devices are the most advantageous due to their small size and low energy demand. Stripping electrochemical methods coupled with the correct electrode/flow systems present some of the lowest detection limits available for metal determinations, often as low as a few nmol L^{-1} [4].

Amongst the stripping electrochemical techniques one of the most suited for environmental analysis is Stripping Chronopotentiometry (SCP) [5] especially because the signal is not affected by adsorption of organic species at the surface of the electrode [6].

SCP is a two-step technique composed of a deposition step, where metal ions are reduced at a constant potential and a chosen fixed time, followed by a stripping step, where the amalgamated metal is reoxidized through the application of a constant oxidizing current. When a thin mercury film electrode is used in SCP, the rapid metal transport inside the thin film during stripping means that measurements are performed almost always under conditions of complete depletion which was explored by Parat et al. [7] to use much higher stripping currents and therefore remove the oxygen competition in this step.

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping technique) is an electrochemical technique that measures directly the free metal ion concentration [8]. In the deposition step the amalgamation of the metal in the mercury electrode is allowed to continue until the equilibrium value between oxidized and reduced forms in solution and in the mercury electrode is reached. This equilibrium is set by the deposition potential and follows Nernst equation. When this situation is achieved no concentration gradients will be present, either in solution or in the mercury amalgam and the amount of amalgamated metal will be proportional to the free metal ion concentration in the bulk solution. The main disadvantage of AGNES is the waiting time in the deposition step needed to attain equilibrium, however this might be mitigated by using a thin mercury film electrode to benefit from its larger area to volume ratio [9]. Worth mentioning is also the change in the stripping mode from a simple Cottrell decay to a stripping chronopotentiometry step [10].

Another commonly used approach to study the speciation of metals in natural waters is the fractionation of the sample based on tangential ultrafiltration. The procedure is generally carried out with a low pore size membrane (e.g. 1 kDa) to distinguish the free species from the colloidal ones. The permeate is generally defined as the free fraction (FF) although the metal cations here may also be complexed with small organic or inorganic ligands that are small enough to pass the membrane [11,12].

It is interesting to compare the free metal ions and the small species able to permeate through the 1 kDa membrane which effectively constitute the most mobile metal fraction in natural waters, thus in this work we propose a methodology to determine the total, free and ultrafiltered (< 1 kDa) metal fractions using electrochemical methods and tangential ultrafiltration experiments that could easily be carried out on-site. In this work, this methodology was tested in laboratory by spiking cadmium ions into two natural waters samples from Itapanhau and Sorocabinha rivers in Sao Paulo State, Brazil and the results obtained by the electrochemical techniques were compared with GF-AAS in the source waters and ultrafiltered samples for validation of the methodology.

2. Materials and methods

2.1. Reagents

Chemicals used in this work were of analytical reagent grade and used as received, unless stated otherwise. Ultrapure milli-Q water (resistivity 18.2 MΩ cm) was employed in all experiments. Nitrogen (> 99.99% pure) for the electrochemical experiments and argon (> 99.99% pure) for the atomic absorption experiments were provided by White Martins. Standard pH 4.00 ± 0.02 and pH 7.0 ± 0.2 solutions were obtained from MS Tecnopon Instrumentação, Brasil. Sterile 0.45 μm and 47 mm diameter acetate of cellulose membranes were used to filtrate the samples. KH_2PO_4, Na_2CO_3 and $NaHCO_3$ all 1000 mg L^{-1} were obtained from Merck. Nitric acid 65% (p.a) and phosphoric acid 85% (p.a) were purchased from Merck. The standard stock solutions of mercury nitrate (1005 ± 4 mg L^{-1}), cadmium nitrate (1000 ± 4 mg L^{-1}), and multi element standard solution G16V ICP (100 mg L^{-1}, NIST-USA rastreable) were purchased from Specsol. Cd(II) solution was prepared from dilution of the certified standard using milli-Q water. Palladium and Magnesium matrix modifiers (10.0 ± 0.2 g L^{-1}) used in GFAAS measurements were purchased from Merck. Sodium nitrate electrolyte solutions (0.1 M) and MES (2-(N-morpholino)ethanesulfonic acid) buffer (0.2 mol L^{-1}) were prepared from the solids (Merck). Solutions prepared from nitric acid and sodium hydroxides (0.1 M standard, Merck) were used to adjust the pH when necessary. Ammonium acetate, hydrochloric acid, potassium thiocyanide, potassium chloride (all p.a. from Merck) was used to prepare the solution for the cleaning of the working electrode and re-dissolution of the mercury film. The working electrode was screen-printed using a carbon commercial ink Electrodag® PF-407A. Tangential ultrafiltration was performed using a regenerated cellulose acetate membrane of 1 kDa and 76 mm diameter (Millipore, Billerica, U.S.A).

2.2. Equipment

An Ecochemie Autolab PGSTAT10 potentiostat (controlled by GPES 4.9 software) was used in conjunction with a Metrohm 663VA stand (Metrohm, Switzerland). A three electrode configuration was used comprising a Hg thin film plated onto a home-made screen-printed electrode as the working electrode, a glassy carbon rod counter electrode (6.1247.000), and an Ag/AgCl reference electrode (6.0728.020) with a salt bridge (6.1245.010) all from Metrohm.

A Marconi Instrument (model MA-522) and an Analyzer (model 2A14) combination pH electrode, calibrated with MS tecnopon buffers (Merck) were used to measure pH in the laboratory.

A Teflon home-made tangential ultrafiltration device was built according to Burba et al. [12] and used in conjunction with a peristaltic pump, Gilson Minipuls 3 with Agilent tygon tubing.

The atomic absorption experiments were carried out in a Varian Zeeman Atomic Absorption Spectrometer model AA240z with a PSD 120 auto sampler, Zeeman correction and graphite furnace with integrated L'vov platform. The hollow cathode lamp was obtained from Agilent (λ=228,8 nm (Cd) e i=10 mA), and the argon flow used was 300 mL min^{-1}. The signal was measured in peak integration mode.

An inductively coupled plasma atomic emission spectrometer (ICP-OES) model 700 Agilent Technologies was used to quantify the following elements: Al, Co, Cr, Cu, Fe, Mn, Ni, and Zn. Dissolved organic carbon was determined using an Analytik Jena Carbon Analyzer (model N/C 3100). The field measurements of pH, conductivity and turbidity were performed using a Hanna multi-parameter probe model HI9829. UV/vis spectroscopic measures were performed in a spectrophotometer Varian model Cary 50.
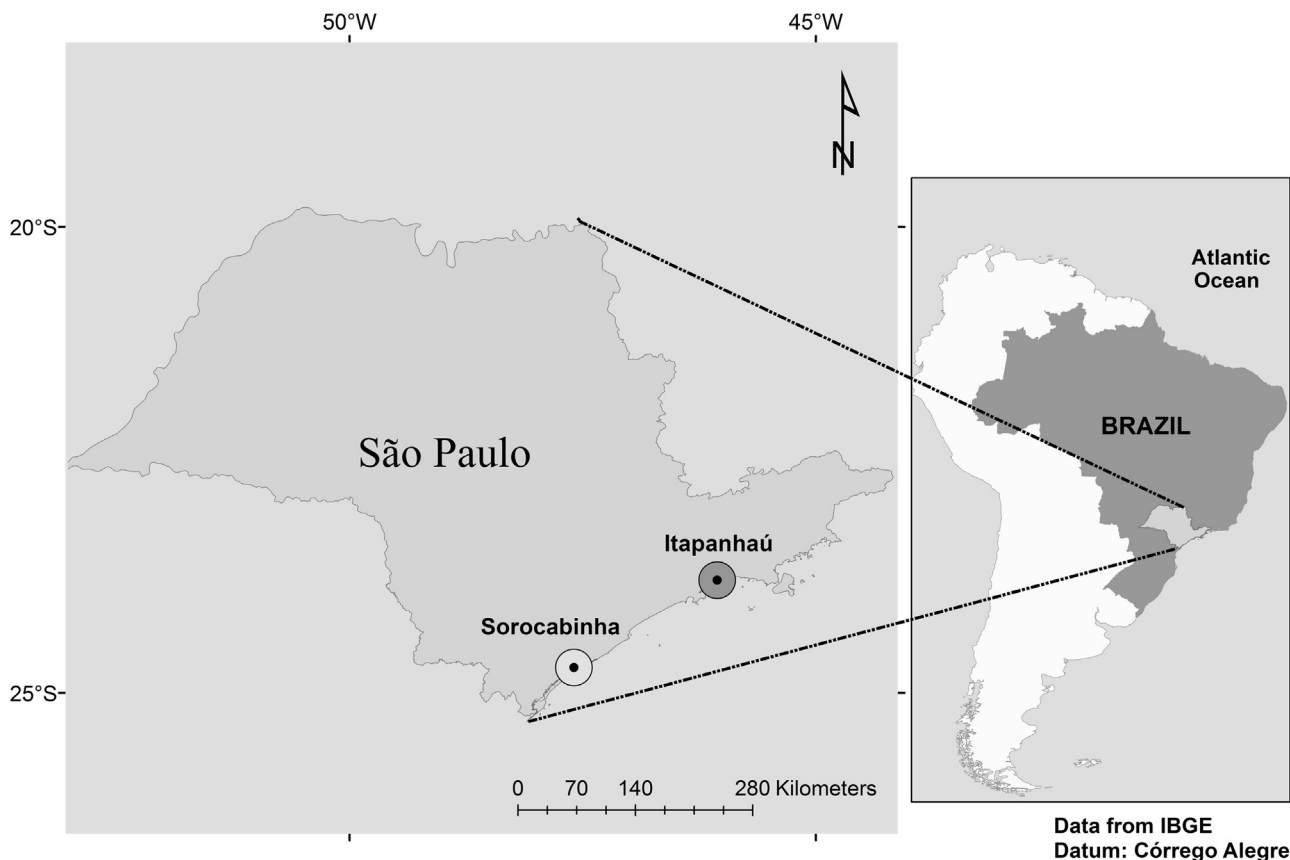
Fig. 1. Map of the state of São Paulo indicating the collection points in the Sorocabinha and Itapanhau rivers.

2.3. Natural water collection and characterization experiments

The natural water samples were collected in the Itapanhaú and Sorocabinha Rivers located in São Paulo state in Brazil (Fig. 1) in November 2014. The geographical coordinates of the sampling points are: latitude 24 °41′59″ and longitude 47°35′43.68″ for the Itapanhaú river and latitude 23°50′23″ and longitude 46 °08′21″ for the Sorocabinha river [16].

The samples were collected from the surface layer using polyethylene flasks which were rinsed thoroughly with the water sample before sampling. The samples were stored at 4 °C until the analysis. The pH, the conductivity and the dissolved oxygen were measured *in situ*. Roughly 10 L of water sample were taken to the laboratory where DOC, sulfate, chloride and dissolved metal were analyzed within 48 h.

Details of ICP-OES analysis, Dissolved organic carbon (DOC) and UV/vis Spectroscopy experiments are given in the electronic support information.

2.4. Graphite furnace atomic spectroscopy (GF-AAS) experiments

The pyrolysis temperature and atomization temperature for the Cd measurements in the current samples were 700 and 1600 °C. These values resulted from an optimization procedure using 10 μg Cd^{2+} L^{-1} (prepared from dilution of the standard solution 1000 mg L^{-1}) in HNO_3 2% (v/v) in presence of the chemical modifiers 5 μL $Pd(NO_3)_2 + 3$ μL $Mg(NO_3)_2$. The calibration plot was carried out for Cd^{2+} concentrations of 0.89; 1.78; 4.45; 7.12 and 8.90×10^{-8} mol L^{-1}. All measurements were performed in triplicate and when necessary the samples were diluted using milli-Q water. The obtained LOD and LOQ were respectively 0.6 μg L^{-1} (0.5×10^{-8} mol L^{-1}) and 1.9 μg L^{-1} (1.7×10^{-8} mol L^{-1}).

2.5. Tangential ultrafiltration experiments

Tangential ultrafiltration experiments were carried out using the collected samples from the rivers Itapanhau and Sorocabinha at the natural pH and ionic strength under constant stirring and pH monitoring. All measurements were carried out at room temperature (23–26 °C).

Each experiment started with 1 L of sample water (source) and the system was equilibrated for 15 min for membrane conditioning using a speed of 6.0 rpm. The valve controlling the return flow is then set at 1 mL min^{-1} while the exit valve of the ultrafiltration device was set at a flow of approximately 0.3 mL min^{-1}. At this point a 25 mL aliquot was collected from the source and the collection of first ultrafiltered aliquot (also 25 mL) was initiated.

After the first permeate is collected, four additions of standard solution of $Cd(NO_3)_2$ 8.9×10^{-4} mol L^{-1} were carried out in order to obtain a total concentration of Cd(II) of 0.7, 1.5, 2.4 and 3.2×10^{-7} mol L^{-1} in the source. Following each addition the source pH is adjusted back to the original value using NaOH 1.0 mol L^{-1} due to the small variation induced by the acid present in the standard solution of Cd(II).

The tests performed on the system showed that a waiting period of 20 min is necessary to achieve equilibrium prior to the collection of the 25 mL of sample. At this point the collection of ultrafiltered aliquots is initiated with 5 mL destined for GF-AAS measurements and 20 mL for electroanalytical quantification. All metal additions were computed taking into account the volume variation of the source solution.

For the total metal determination using SCP in both, the source and ultrafiltered aliquots, the 20 mL of solution were acidified with 0.2 mL de HNO_3 1.0 mol L^{-1} and subsequently named Source acidified and UF acidified.

2.6. Working electrode preparation

The working electrodes were screen-printed on 1 mm-thick polystyrene support for serigraphy (Sericol) using inks purchased from Acheson Colloids. The electrode was screen-printed and allowed to dry (1 h at room temperature) and cure (1 h in the oven at 60 °C). After completion of the screen-printing process, an insulating layer was spread manually over the conductive tracks leaving a working disk area of 9.6 mm^2 [10].

Prior to deposition of the Hg film the screen-printed carbon electrode was conditioned using an electrochemical pre-treatment consisting on fifty successive cyclic voltammetry scans between -0.8 and $+0.8$ V at 0.1 V s^{-1} in NH$_4$CH$_3$COO (1 mol L^{-1})/HCl (0.5 mol L^{-1}) solution [13]. The thin Hg film was prepared ex-situ in 0.24 mmol L^{-1} Hg(II) nitrate in nitric acid 0.73 mmol L^{-1} (pH 1.9) by electrodeposition at -1.3 V for 700 s using a rotation rate of the stirrer of 1000 rpm. After each working day the charge associated with the deposited Hg (Q_{Hg}) was determined to assess the state of the mercury film. This was carried out by electronic integration of the linear sweep stripping peak of Hg with a scan rate $v = 0.005$ V s^{-1} in 5 mmol L^{-1} of ammonium thiocyanide (pH 3.4) using a stripping step from -0.15 V to $+0.4$ V [9].

2.7. Electrochemical experiments

The measurements were performed in triplicate in the laboratory. The solution was purged for 15 min at the beginning of every experiment and the electrochemical cell was continuously purged over the solution with nitrogen gas during the measurement. All measurements were carried out at room temperature (23–26 °C). The experiments were carried out in 40 mL disposable polystyrene (PS) beakers.

A three or four point calibration is daily performed prior to the sample measurements using known metal concentrations in the interval between 3×10^{-9} and 3×10^{-7} mol L^{-1}. The limits of detection (LOD) and quantification (LOQ) were estimated from the calibrations curve using the following equation:

$$\text{LOX} = kS_y/m \tag{1}$$

where $k = 3$ for the LOD and 10 for the LOQ, b is the intercept of the regression line, S_y is the standard deviation of the residuals and m is the slope of the calibration graph.

2.8. Stripping chronopotentiometry experiments in acid media (total metal in the source acidified and UF acidified)

Stripping chronopotentiometry measurements were carried out in 20 mL acidified source solutions of Cd(II). The experimental conditions used were: deposition potential -0.78 V (vs Ag/AgCl), deposition time (t_d) 45 s, stripping current (I_s) 2.5×10^{-6} A applied until the potential reached -0.30 V, rotation speed 1000 rpm (only during the deposition step).

The SCP signal is the characteristic transition time τ^* which is given by [5]:

$$\tau^* = \frac{I_d^* t_d}{I_s} = \frac{\alpha c_{M,T}^* t_d}{I_s} \tag{2}$$

where I_d^*, the limiting diffusion current, is directly proportional to the metal concentration in the bulk $c_{M,T}^*$.

2.9. AGNES experiments (free metal in the source)

Previous to the measurements, the solutions were set at the desired pH using MES (2-N-morpholino-ethanesulfonic acid) buffer. According to Soares et al. [14] MES does not bind Cd(II). In the case of the Sorocabinha we used the natural ionic strength but for the Itapanhau water sample, the ionic strength was set at 0.010 mol L^{-1} adding NaNO$_3$ and following the conductivity of the solution. This is necessary since a sufficiently high ionic strength (≥ 10 mmol L^{-1}) and a well buffered system are required to obtain accurate results in AGNES [15].

The free metal ion concentration was determined by the AGNES method according to the procedure developed by Parat et al. [10] where the quantification of the metal amalgamated in the equilibrium step is quantified using a chronopotentiometric measurement. The measurements were performed applying a deposition potential E_1 of -0.72 V for a period of time t_1 of 240 s. The stripping step was performed by SCP using an oxidizing current I_s of 2.5×10^{-6} A applied until the potential reached -0.30 V. The rotation speed used was 1000 rpm.

In this implementation the AGNES signal is the charge corresponding to the amalgamated metal in the mercury electrode Q which is given by:

$$Q = \tau^* I_s = h c_M^* \tag{3}$$

where τ^* is the reoxidation time, I_s is the reoxidation current applied in the stripping step, h is a constant and c_M^* is the free metal concentration in the bulk.

3. Results and discussion

The proposed methodology for on-site analysis was tested in the laboratory by spiking Cd(II) ions into two natural waters samples from Itapanhau and Sorocabinha rivers in Sao Paulo State-Brazil. These rivers were chosen due to their different characteristics especially regarding conductivity, since the Sorocabinha sampling is relatively close to the sea..

3.1. Natural river waters characterization

Table 1 shows the major parameters that allow us to characterize both rivers. Their pH and DOC are quite close, while the conductivity of the Sorocabinha river is much higher than the Itapanhau one due to its proximity to the ocean. This is also reflected in the higher contents of major cations like K$^+$, Mg^{2+} and Ca^{2+} and anions like Cl$^-$. Cadmium, Pb, Co and Ni are below the detection limit in both rivers, a small amount of both Cu and Cr

Table 1
Sorocabinha and Itapanhau rivers characterization.

		Itapanhau	Sorocabinha
pH		6.2	6.7
Conductivity (μS cm^{-1})		189	1043
DOC (mg L^{-1})		14.0	16.8
Turbidity (NTU)		3.7	6.1
Dissolved oxygen (mg O L^{-1})		1.8	1.4
Cl$^-$ (mol L^{-1})	3.6×10^{-4}		8.5×10^{-4}
SO$_4^{2-}$ (mol L^{-1})	5.0×10^{-6}		2.2×10^{-6}
Al (mol L^{-1})	4.9×10^{-6}		1.5×10^{-6}
Ca (mol L^{-1})		4.2×10^{-5}	3.0×10^{-4}
Cd (mol L^{-1})	< LOD		< LOD
Co (mol L^{-1})	< LOD		< LOD
Cr (mol L^{-1})	< LOD		6.5×10^{-9}
Cu (mol L^{-1})	< LOD		3.7×10^{-8}
Fe (mol L^{-1})	13.0×10^{-6}		6.1×10^{-6}
K (mol L^{-1})	5.5×10^{-5}		2.1×10^{-4}
Mg (mol L^{-1})	6.7×10^{-5}		8.4×10^{-4}
Mn (mol L^{-1})	1.3×10^{-6}		1.9×10^{-6}
Ni (mol L^{-1})	< LOD		< LOD
Pb (mol L^{-1})	< LOD		< LOD
Zn (mol L^{-1})	2.3×10^{-8}		2.0×10^{-7}

Table 2
Ratio A_{254}/A_{436} for the Itapanhaú and Sorocabinha water samples.

		Sorocabinha	Itapanhaú
Absorbance	254 nm	0.81	0.79
	436 nm	0.07	0.07
Ratio	A_{254}/A_{436}		11.3
		11.9	

appears in the Sorocabinha, while Zn appears in both.

Table 2 reports the UV/vis spectroscopic ratio (A_{254}/A_{436}), i.e. the absorption values taken at 254 and 436 nm in the UV/vis spectra, which is a semi-quantitative method to characterize humic substances in natural waters. This ratio is used as an indicator of the origin and the modifications of natural organic matter in aqueous systems. For instance, absorbance at 254 nm is typical for aromatic groups. Due to the absorption of visible light by humic acids, this ratio rises with increasing contents of humic acids in solution. These two samples showed very similar values, which means that the origin of their dissolved organic matter is likely similar.

3.2. Proposed analytical methodology for on-site analysis

The methodology proposed is depicted in Fig. 2. A sample is collected and filtered through a 0.45 μm filter to separate the particulate and dissolved fractions. Three different treatments are applied on the filtered fraction: (a) the filtered fraction is acidified with HNO$_3$ before being analyzed by SCP to get the total dissolved metal concentration, (b) the filtered fraction is directly analyzed by using the AGNES method to get the free metal concentration and (c) the filtered fraction is ultrafiltered at 1 kDa then acidified with HNO$_3$ before being analyzed by SCP to get the metal concentration including both, the free metal ion and the metal complexed to small ligands. For each fraction, the pH, the conductivity and the temperature are measured and aliquots are then taken for different analyses.

Total metal determination: First the electroanalytical system is calibrated to obtain the value of the proportionality constant; α (Eq. (2)) using three or four metal additions measured in triplicate. The calibration solution is prepared using 20 mL NaNO$_3$ at the same conductivity as the sample concentrations, which is then acidified by addition of 0.1 mL of HNO$_3$ 1 mol L^{-1}.

Following the calibration procedure the electrodes are thoroughly cleaned using milli-Q water and the polystyrene beaker is changed. One aliquot of 20 mL of sample is placed directly in the PS beaker and acidified with 0.1 mL of HNO$_3$ 1 mol L^{-1} after which the total metal is measured in triplicate.

Ten calibration curves were measured in the range 3×10^{-9}–1×10^{-7} mol L^{-1} for which the LOD obtained was in the interval 0.8 to 2.4×10^{-9} and seven calibration curves were taken in the range 3×10^{-9} to 1×10^{-7} mol L^{-1} to 3×10^{-7} mol L^{-1} yielding LOD in the interval 3.6–9.8×10^{-9} which is of the same order of magnitude than the LOD obtained for GF-AAS (5.0×10^{-9} mol L^{-1}). Note that these values were obtained for a deposition time of 45 s, hence they can still be improved by a significant margin since traditionally deposition times used in anodic stripping voltammetry range between 60 and 600 s, as long as the blank solution has no metal impurities.

Free metal determination: the first step is the verification of the equilibrium condition for AGNES measurements for the chosen deposition potential. This should be done regularly due to possible variations in the potential of the reference electrode. A serie of measurements is done using different deposition times, usually from 30 s to 360 s and the equilibrium conditions are reached when the reoxidation time becomes the same for increasing deposition time [8]. After this step a calibration is performed to obtain the value of the proportionality constant; h (Eq. (3)) using three or four metal additions measured in triplicate. The calibration solution is prepared using 20 mL NaNO$_3$ at the same ionic strength as the sample concentrations (being the minimum value used of 10 mmol L^{-1}) and adding 1 mmol L^{-1} of a suitable buffer.

Following the calibration procedure, the electrodes are thoroughly cleaned using milli-Q water and the PS beaker is changed. One aliquot of 20 mL of sample is placed directly in the PS beaker, then the buffer is added (set to a final concentration of 1 mmol L^{-1}) and if necessary the ionic strength is set to 10 mmol L^{-1} by addition of NaNO$_3$ 1 mol L^{-1} and then the free metal is measured in triplicate.

Nine calibration curves were measured in the range 0.65×10^{-9}–1×10^{-7} mol L^{-1} for which the LOD obtained was in the interval 0.7–3.6×10^{-9} mol L^{-1}, which is a noteworthy result since this is the detection limit for the free metal and should be compared with the values obtained for ion selective electrodes (ISE) which are usually two to three orders of magnitude higher. In contrast this value will not be easier to improve ever since we are already operating at a deposition potential close to the limiting current.
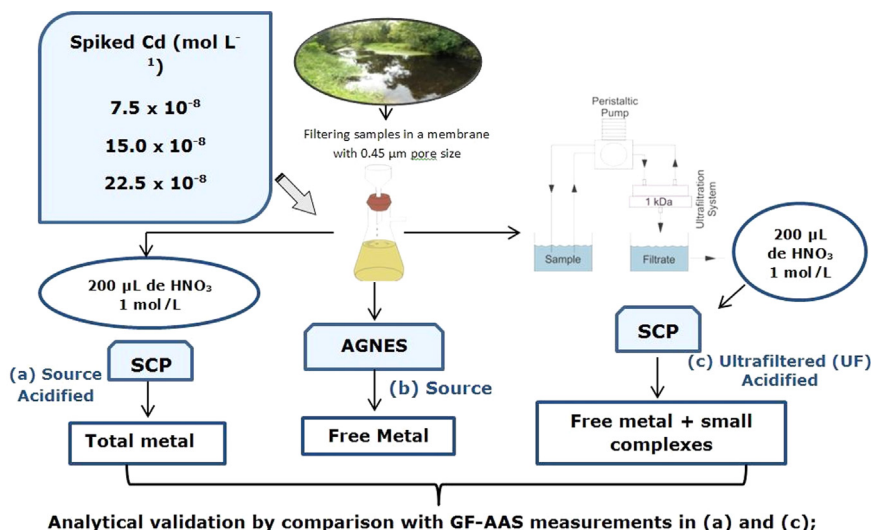

Fig. 2. Schema of the proposed analytical methodology for on-site analyses.
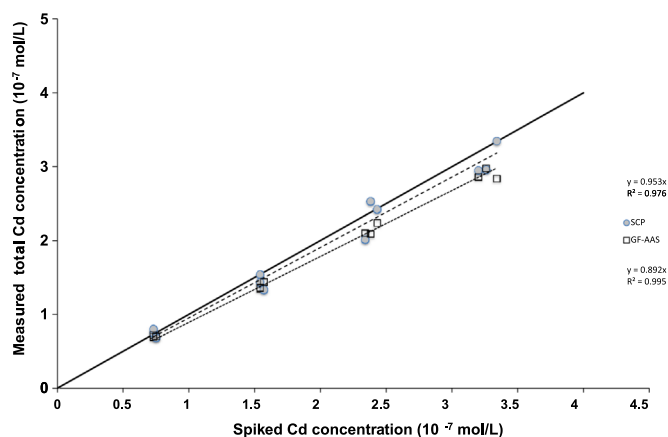
Fig. 3. Comparison of the SCP and GFAAS total measured metal with the real spiked concentration.

Table 3
Tangential UF free Cd and small complexes metal measured by SCP and GFAAS in acidified solutions.

Spiked total metal source (10^{-7} M)	UF SCP (M) (10^{-7} M)	UF AAS (M) (10^{-7} M)	Total metal Source (10^{-7} M)	UF SCP (M) (10^{-7} M)	UF AAS (M) (10^{-7} M)
Itapanhau 1			Itapanhau 2		
0.7	0.3	0.2	0.7	0.3	0.2
1.5	0.5	0.5	1.5	0.4	0.5
2.4	1.0	0.9	2.4	0.9	0.9
3.2	1.3	0.9[a]	3.2	1.3	1.0[a]
Sorocabinha 1			Sorocabinha 2		
0.7	0.4	0.5	0.7	0.5	0.5
1.5	0.9	0.9	1.5	0.8	1.1[a]
2.4	1.4	1.4	2.4	1.3	1.5
3.2	1.8	1.9	3.2	1.8	2.0

[a] Points in disagreement.

Tangential ultrafiltration (1 KDa): free metal and small complexes: The membrane is conditioned for 15 min with the source solution and the return and ultrafiltrated flow valves are set to 1 and 0.3 mL min^{-1} respectively. 25 mL of ultrafiltered solution are collected and 5 mL are taken for the GF-AAS measurements. The remaining 20 mL are acidified with 0.1 mL of HNO$_3$ 1 mol L^{-1} after which their metal concentrations are measured in triplicate by SCP.

The determination of total metal and ultrafiltered metal performed by SCP is validated by comparison with the samples measured by GF-AAS.

3.3. Total metal determination in the source solution

Due to the absence of Cd in the natural water samples, four spikes of Cd of approximately 0.7, 1.5, 2.4 and 3.2 × 10^{-7} mol L^{-1}, were carried out in each experiment. Fig. 3 shows the concentrations, percentage of recovery and differences obtained by SCP and GFAAS in two spiked natural water solutions from Sorocabinha and one from Itapanhau rivers.

The % of recovery obtained for SCP is quite good averaging 96% in a range varying from 85% to 109% while for GF-AAS are slightly worse with an average of 91% in an interval from 85% to 97%. These percentages of recovery are quite good since these environmental matrices are often difficult to measure reproducibly (Table B in Electronic Support information).

The slope values in Fig. 3 show that SCP (0.953) is slightly more accurate than GFAAS (0.892) while the correlation coefficient values show that SCP is less precise than GFAAS (0.976 *vs* 0.995). These results confirm the SCP ability to accurately determine the total metal in these spiked natural samples.

In the electrochemical measurements, no destruction of the organic matter was necessary for the total metal determination which is probably due to the weak binding of Cd(II) to the organic matter, which are mostly from the fulvic acid fractions. For other metals like Cu(II) or Pb(II) this simplification of the methodology assumed here must be validated.

3.4. Ultrafiltered (< 1 kDa) metal determination

Table 3 shows the metal concentrations measured by SCP and GFAAS in the filtered solution for four experiments carried out in the Sorocabinha and Itapanhau rivers. First the results obtained for the two replicas are in good agreement. A second observation is that the results obtained by SCP and GFAAS are mostly concordant, with the exception of the three GFAAS points marked with asterisks. Again the results obtained by SCP in an acid media are in

good agreement with the GFAAS confirming the good performance of this technique.

An interesting result is that although the DOC contents and pH are quite similar for both samples, the metal measured in the ultrafiltrated samples is significantly higher for the Sorocabinha than for the Itapanhau samples.

3.5. Free metal determination in the source solution

Fig. 4a and b shows the comparison of the total (spiked) metal with both the metal determined in the ultrafiltered samples and
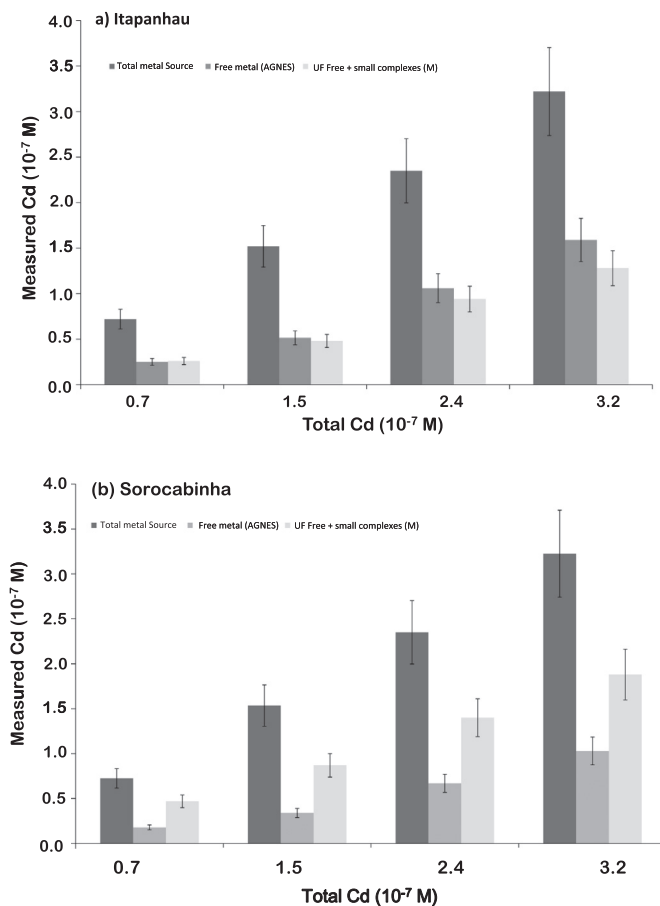

Fig. 4. Comparison of the total (spiked) metal with both the metal determined in the ultrafiltered samples and the free metal determined directly in the Itapanhau (a) and Sorocabinha (b) rivers samples.

the free metal determined directly in the Itapanhau (a) and Sorocabinha (b) river samples. The free metal is determined directly in the river solution by AGNES and upon comparison with the free plus small metal complexes measured in the ultrafiltered samples. Three interesting results are evident: i) for the Itapanhau the free metal fraction determined by AGNES and the ultrafiltered fraction are quite similar; ii) for the Sorocabinha the free metal is significantly smaller than the metal measured in the ultrafiltered fraction; iii) the metal concentration in the ultrafiltered fraction of the Sorocabinha is roughly twice that of the Itapanhau while its free metal in the source is actually smaller.

Starting from the first observation the agreement between the free and ultrafiltered fraction for the Itapanhau River is quite remarkable indicating that apart from the natural organic matter, represented by the DOC there are few small inorganic ligands in solution that are able to permeate the 1 kDa membrane. Regarding the methodology, this is an interesting observation since the agreement between ultrafiltered and free metal determinations implies a negligible contribution from small inorganic ligands for the metal binding.

The second observation means that the Sorocabinha River must be more concentrated in small inorganic ligands that are able to permeate the 1 kDa membrane, so that the metal content measured in the ultrafiltered fraction is higher than the free metal content measured in the source. This is supported by the much higher conductivity of the Sorocabinha ($1043\ \mu S\ cm^{-1}$) as compared with the Itapanhau ($189\ \mu S\ cm^{-1}$).

The third observation suggests that the Cd speciation is different in both river samples being controlled mostly by the organic matter in the Itapanhau River while in the Sorocabinha river the small inorganic ligands and the competition from other cations, namely a roughly $10\times$ higher concentration of calcium and magnesium ions in solution must be taken into account. Note that for Cd binding with DOC is mostly electrostatic so the competition of Ca and Mg is very effective. Regarding the DOC even if the concentration is similar in both rivers the nature of the organic matter present might be different, namely in the relative amounts of fulvic and humic fractions present. The higher ionic strength and the cations present in the Sorocabinha mean that the humic fraction of the DOC will be less stable or already coagulated where in the Itapanhau this humic fraction can still be significant. In future works we plan to investigated this issue in detail.

To confirm these observations we carried out an AGNES measurement in the non-acidified ultrafiltered fraction of two Sorocabinha river samples (spiked at 2.4 and 3.2×10^{-7} mol L^{-1}) to determine the free Cd proportion in the ultrafiltered solution. The results showed that only 37–45% of the total Cd of this fraction is free, thus confirming the metal is indeed complexed in the non-acidified ultrafiltered sample, most likely by small inorganic ligands.

We validated both the SCP total Cd^{2+} determination in the source and acidified ultrafiltered solution with a LOD of 1.6×10^{-9} M and the AGNES free Cd^{2+} determination with a LOD of 1.9×10^{-9} M. We demonstrated that the proposed metal speciation methodology is able to provide valid information regarding the total and free metal while being able to additionally afford some insight on the contribution of small inorganic ligands to the metal complexation in the source.

We plan to follow this proof-of-concept with the development of the same methodology for other metals like Pb and Zn and their mixtures, to further investigate the nature of the DOC in both rivers and finally to apply this methodology on-site and obtain experimental data to model the binding of metal ions in these rivers.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.01.053.

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