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A review on electrochemical methods for trace metal speciation in environmental media

E. Companys^a, J. Galceran^{a,*}, J.P. Pinheiro^b, J. Puy^a and P. Salaün^c

^aDepartament de Química. Universitat de Lleida, and AGROTECNIO, Rovira Roure

191, 25198 Lleida, Catalonia, Spain;

^bUniversité de Lorraine et CNRS, LIEC, UMR7360, 15 Avenue du Charmois,

Vandœuvre-lès-Nancy F-54501, France;

^cSchool of Environmental Sciences, University of Liverpool, 4 Brownlow Street,

L693GP Liverpool, UK.

* corresponding author: galceran@quimica.udl.cat

Abstract

Trace metal speciation is key to understand/predict bioavailability and potential toxicity of metals to biota and will, undoubtedly, be incorporated in future regulations. Electroanalytical methods have a role to play in such development: they offer a wide range of advantages such as speed, portability, economy, solid interpretation backgrounds and low limits of quantification. This review focusses on three selected stripping techniques: Competitive-Ligand Exchange - Cathodic Stripping Voltammetry (CLE-CSV), Scanned Stripping Chrono-Potentiometry (SSCP) and AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) reporting their working principles, characteristics (strong and weak points) and recent applications to systems of environmental relevance (such as seawaters, freshwaters or soil extracts).

Keywords: speciation, availability, electroanalysis, electrochemistry, heavy metal, CLE-CSV, SSCP, AGNES

List of nomenclature and abbreviations:

AdCSV	Adsorptive Cathodic Stripping Voltammetry
AFS	Atomic Fluorescence Spectroscopy
AGNES	Absence of Gradients and Nernstian Equilibrium Stripping
APDC	Ammonium 1-pyrrolidinedithiocarbamate
ASV	Anodic Stripping Voltammetry
BiFE	Bismuth Film Electrode
BLM	Biotic Ligand Model
CLE	Competitive Ligand Exchange
CSV	Cathodic Stripping Voltammetry
DHN	2,3-dihydroxynaphthalene
DMEM	Dulbecco's modified Eagle's medium
DMG	Dimethylglyoxime
DMT	Donnan Membrane Technique
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DPP	Differential Pulse Polarogram
EDTA	Ethylenediaminetetraacetic acid
EPPS	4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid
EPS	Extracellular polymeric substances
EQS	Environmental Quality Standard
FIAM	Free Ion Activity Model
GSH	Glutathione
HEPES	N-2-Hydroxyethylpiperazine-N'-2'-ethanesulfonic acid
HMDE	Hanging Mercury Drop Electrode
HS	Humic Substances
<i>I</i>	Ionic strength
ISE	Ion Selective Electrode
LOD	Limit of Detection
MES	2-(N-morpholino)-ethanesulfonic acid
MOPS	3-(N-morpholino)-propanesulfonic acid
NICA	Non-Ideal Competitive Adsorption
NN	1-Nitroso-2-naphthol
NOM	Natural Organic Matter
NP	Nanoparticles
NTA	Nitrilotriacetic Acid
OECD	Organisation for Economic Cooperation and Development
PDCA	Pyridinedicarboxylic acid
PNIPAM	Poly(N-isopropylacrylamide)
POPSO	Piperazine-1,4-bis(2-hydroxypropanesulfonic acid)

QD	Quantum Dot
RT	Resin Titration technique
S	Salinity
SA	Salycilaloxime
SCP	Stripping ChronoPotentiometry
SIA	Sequential Injection Analysis
SPE	Screen Printed Electrode
SSCP	Scanned Stripping ChronoPotentiometry
SSV	Scanned Stripping Voltammetry
TAC	2-(2-Thiazolylazo)-p-cresol
TMFE	Thin Mercury Film Electrode
UV	Ultra Violet
VGME	Vibrating Gold Micro Electrode
VM	Visual Minteq

Introduction

Speciation (i.e. the distribution of an element among its different chemical forms[1]) has been recognised as a key factor for trace metal (bio)availability to biota, as postulated by hegemonic ecotoxicological paradigms such as the Free Ion Activity Model (FIAM) [2] or the Biotic Ligand Model (BLM)[3], both assuming that the free ion is the only bioavailable species. The limits of these models have recently been reviewed [4], highlighting numerous cases where specific fractions of metal complexes can be internalized, either directly or through the dissociation of labile complexes. Thus, there is a need to design, develop, apply and interpret analytical techniques able to provide reliable speciation information [5-7] that can be used by regulatory bodies.

Although there are numerous non-electrochemical techniques [5-13], those that are provide attractive possibilities such as speed of analysis, low-cost and miniaturization in view to on-site (and in-situ) measurements [14], and can also provide thermodynamic (i.e. equilibrium) information (such as the amount of the free metal, the amount of complexing sites, the stoichiometry of complexes and their stability constants [15]) as well as dynamic information (such as kinetic rate constants and diffusion coefficients [16]). However, the correct signal interpretation can be difficult in natural media [16;17]. Stripping techniques, due to their low detection limits, are well suited for environmental analysis. They are two-stage techniques, where the first stage is a deposition step that accumulates the analyte (at the electrode surface or in its volume), followed by a second stage that quantifies this analyte. Anodic Stripping Voltammetry (ASV), in its multiple variants, is the most popular and has the advantage to possibly be measuring a potentially bioavailable fraction of the metal, as shown again recently [18;19].

This critical evaluation focusses on three techniques that are known powerful tools for metal speciation: AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and SSCP (Scanned Stripping ChronoPotentiometry), that are relatively recent techniques, together with the more established CLE-CSV, Competitive Ligand Exchange (or Equilibration) - Cathodic Stripping Voltammetry. Principles, recent applications, weak and strong points are reviewed.

CLE-CSV (Competitive-Ligand Exchange - Cathodic Stripping Voltammetry)

CLE-CSV is an equilibrium-based method that determines complexation parameters, namely ligand concentrations and conditional stability constants, allowing indirect determination of the free metal ion concentration. The method consists in the addition of a ligand (L_{add}) that forms an electroactive complex ML_{add} with the metal M of interest. Once equilibrium between the natural ligands (L_x) and L_{add} is achieved (typically few hours), quantification of ML_{add} adsorbed on the sensor (Figure 1) by CSV in a minimum of 12 aliquots at increasing concentrations of M leads to a titration curve ($I_{\text{p}}(ML_{\text{add}})$ vs $c_{\text{T},M}$) that can be fitted with the presence of up to typically 2 classes of ligands of specific concentrations $[L_x]$ and conditional stability constant K_x' . Recent CLE-CSV studies published in the last 2 years were applied almost exclusively to marine systems, looking at the speciation of biologically important metals, mostly Cu[20-29] and Fe[30-36], but also Zn[37;38] or Co [39]. The analytical method is powerful and can give insights into potential biological limitation (e.g. free Cu^{2+} limitation for Cu-dependent ammonia oxidising archaea [40]), toxicity (e.g. high free Cu^{2+} in coastal waters[41]) or for studying the effect of ocean acidification on future Cu and Fe speciation [27]. It is also used to indirectly look at the biogeochemical cycling of the ligands, from their origins (e.g. sediments [22], peat [21], biological productivity in

water column [29], stormwater[42]), and identity (e.g. humics [24], polysaccharides[43]) to their seasonal [31] and spatial [35;36] variations, as well as stability [21].

Recent analytical developments to note include the catalytic detection of Fe by oxygen in presence of SA [44] and the use of reverse titrations [45;46], where the concentration of the ligand, not that of the metal, is varied. New methods for the determination of humic substances, HS (based on the adsorption and quantification of Cu-HS complex at the Hg drop electrode [28], similarly to the Fe-HS method[47]), have demonstrated that the same HS are involved in the complexation of both Cu and Fe in coastal waters of Liverpool Bay, accounting for 97 and 65% of the total ligands for Fe and Cu, respectively [28]. This competition for ligands implies that the complexation of Fe-HS might be dependent on Cu levels, or other metals, and vice versa. Various fitting methods can be used to determine speciation parameters from the titration curves (current vs. total metal concentrations). However, both the experimental [48] and fitting [49;50] procedures are far from trivial and latest recommendations [49] are, if time allows, to run multiple titrations at different detection windows (i.e. with different concentrations of the added ligand e.g. [51;52]) to decrease errors. Freely available software such as ProMCC [53] or others (see [49] and references therein) are available to help fitting the data correctly. However, no matter how well data are fitted, CLE-CSV does not give any indication related to potential kinetics and bioavailability of the various complexes. When internalization is fast enough to develop a free metal concentration profile close to the consuming interface, fast dissociating organic complexes (e.g. Cu-HS substances [54]) may also participate to the metal flux to organisms and methods based on anodic stripping voltammetry can estimate this bioavailable fraction [19;55].

CSV in presence of relatively high concentration of added ligand(s) is also widely used as a detection technique and is very helpful in gaining insights into the cycling of various metals, such as that of Zr, Mo, V and Ti at increasing salinities [56]. Although the Hg drop electrode is still used for developments of new CSV methods (e.g. for Ga [57], Te[58] or Fe [59]), a large amount of research is now devoted to greener and more user-friendly solid and/or film electrodes. Latest development for analysis of water samples include methods for Ni [60], Ag[61], Co [62], Mn[63], V[64;65], U[66], Ga[67] and Cr[68]. These methods are developed using common carbon paste electrodes [60;61] or screen-printed ones [69;70], but also use more recent substrates such as e.g. lithographically produced Bi microelectrode array [71], tin-bismuth alloy[62;66] or indium-oxide [63]. Although progress is coming fast, up to now, only the Hg drop electrode is used for CLE-CSV speciation studies (due to yet unrivalled stability over the time required to perform a titration).

SCP/SSCP (Scanned Stripping ChronoPotentiometry)

Stripping Chronopotentiometry (SCP) was developed in the early 2000's by Town and van Leeuwen [72] mainly to address the problems caused by electrodic adsorption of organic matter in trace metal analysis in environmental waters. The main difference in this technique is that, in the stripping step, a sufficiently small reoxidation current is used such that a small diffusion gradient inside the electrode is created. Therefore full depletion of the accumulated analyte is accomplished effectively, and the integrated recorded signal is essentially proportional to the accumulated charge, almost immune from electrodic adsorption of organic matter, distorted peaks [73] or heterogeneity of metal complexes [74]. SCP truly reaches its potential when a thin mercury film electrode is used [75] due to the low detection limit associated with these electrodes,

and ever since full depletion is always maintained, allowing the decrease of both the analysis time and the oxygen interference [76].

One of the limitations of SCP is the absence of an expression relating the peak potential and the metal complex speciation in the matrix solution. This led to the development in mid-2000's of an extension named Scanned Stripping Chronopotentiometry (SSCP), which is composed from a series of individual measurements performed at different deposition potentials (E_d), varying from values where no metal reduction occurs to the diffusion limited region where the free metal concentration at the electrode surface is nil [77].

In SSCP, the shift in half-wave deposition potential in the presence of complexing agents species can be interpreted straightforwardly, analogous to the DeFord-Hume expression for a voltammetric wave [78], while the decrease in limiting transition time (i.e. the stripping time when accumulation is under diffusion limited conditions) provides information on the dynamic nature of the metal complexes, namely their diffusion coefficients and association rate constants [79]. Additionally, the slope variation of the SSCP wave provides information on the degree of heterogeneity of the complexing ligands [80].

SCP in environmental analysis was reviewed in 2007 [81], while SSCP applications in the development of chemodynamics theory and applications in seawater analysis were reviewed in 2012 [16]. We are focusing here on published works from 2012 which can be clustered in four groups: SCP/SSCP technical and methodological developments; trace metal interaction with humic matter; nanoparticle studies and, finally, trace metal bioavailability studies (see Table 1 for applications).

SSCP was first used with solid electrodes for the study of Pb(II) speciation using bismuth electrodes [82]. Pb(II) speciation in seawater required attention to the correct

interpretation of the calibration, due to the formation of PbCl_x complexes [83], while ligand size polydispersity was investigated using the binding of Pb(II) and Cd(II) to three different sizes of otherwise identical polymers [84]. The study of Cd(II) binding by clay minerals provided interesting dynamic information [85] and, finally, the effect of temperature in the lability of Cd(II) and Pb(II) complexes was investigated [86].

SCP methods were developed for the Hg(II) analysis using Sequential Injection Analysis (SIA) and screen printed gold electrodes [87]; for the metal detection in the acceptor solution of a DMT device [88]; for sulphide determination in hydrothermal seawater samples using a vibrating gold micro-wire [89]; and for the speciation of Cd(II) and Pb(II) in the acidified ultrafiltrated solution ($<1\text{kDa}$) [90].

SSCP was used in several works investigating the binding of trace metals with humic matter, namely the binding of Cu^{2+} and Ni^{2+} with aquatic fulvic and humic acids [91], the effect of Ca^{2+} on the chemodynamics of Pb(II) complexation by humic acid (HA)[92] or the effect of electric condensation on the HA binding of Cd^{2+} , Pb^{2+} , Cu^{2+} [93], among others.

There are several examples of SCP and SSCP studies to elucidate the speciation of metal ions in presence of nanoparticles and/or to study the dissolution of the nanoparticles. For instance, SSCP was used to investigate the structural effects on Cd(II) binding to multi-responsive carboxylated PNIPAM particles (temperature, pH, ionic strength) [94], the role of charged polymer coatings of nanoparticles on the trace metal speciation [95], the impact of electrostatics on the binding of Pb(II) and Cd(II) with highly charged nanoparticles [96] and the dissolution of Cd-based quantum dots in seawater [97].

There are several examples of SSCP utilization to determine trace metal speciation in exposure solutions of biouptake experiments, like the study of Cd(II) availability to

Saccharomyces cerevisiae in presence and absence of polyhydroxyfullerenes [98], while SCP was used, for example, to follow the bulk Cd(II) depletion due to biouptake by *Pseudomonas putida* [99]).

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping)

AGNES technique provides direct access to the free metal ion concentration in solution via a two-stage voltammetric procedure [100]. In the first stage, the metal ion M^{n+} in solution is reduced and amalgamated in the mercury electrode M^0 due to the application of a potential program [101] always ending with the deposition potential E_1 (for a sufficiently long time). By the end of the first stage, equilibrium has to be reached: i) absence of gradients in the concentration profiles of the reduced and oxidised species on both sides of the electrode interface and ii) metal concentrations at both sides of the electrode surface are related through Nernst relationship

$$Y = \frac{[M^0]}{[M^{n+}]} = \exp\left[-\frac{nF}{RT}(E_1 - E^0)\right] \quad (1)$$

where Y is the gain (or preconcentration factor), F the Faraday constant, R the gas constant, n the number of electrons, T the temperature and E^0 the standard formal potential of the redox couple. If at equilibrium, the amount of reduced species is directly proportional to the free metal ion concentration in the solution. The aim of the second stage is, thus, to quantify that amount of reduced M^0 accumulated. In the variant AGNES-I, M^0 is reoxidised under diffusion-limited conditions and the current (I) is measured. Other variants for the second stage quantify the stripped charge (Q) in the reoxidation step at a fixed potential (AGNES-Q) [102] or along a linear reoxidation potential scan (AGNES-LSV) [103]. A fourth variant (AGNES-SCP), especially useful when electroactive metals other than the analyte are present, computes the charge from the recorded potentials needed to sustain a fixed stripping current [104]. The state of

equilibrium at the end of the first stage can be checked by constant responses with longer deposition times at E_1 .

One of the most attractive characteristics of AGNES is its simplicity: there is a linear relationship between the measured signal and the free metal concentration. In AGNES-I

$$I = Y\eta[M^{n+}] \quad (2)$$

while in the other variants

$$Q = Y\eta_Q[M^{n+}] \quad (3)$$

where η and η_Q are proportionality factors found in calibrations (except in the case of indium [105]).

Due to the equilibrium situation, the free concentration determination is not affected by complexation, precipitation or induced electrodic adsorption [103]. A local free metal concentration in non-purged solutions can be found [102].

AGNES has been widely applied with the hanging mercury drop electrode (HMDE). Other mercury-based electrodes used are the Hg-Ir microelectrode [106], the rotating disk electrode [107] and the screen printed electrode, SPE [108]. The SPE is specially indicated for *in situ* applications [109]. Mercury-free options are the bismuth film deposited on the surface of a glassy carbon solid electrode [110] or the Vibrating Gold Microwire electrode (VGME) [111].

The capability of AGNES to determine free metal ion concentration has been validated with other techniques, such as ISE [103], DMT [8] [112], SSCP [113], CLE-CSV [38] as well as with speciation codes [101;114].

A list of applications is given in Table . Very low ionic strength systems, like river waters, can be dealt accurately with AGNES due to the low product of residual current times resistance when equilibrium is reached[90;102;115]. Higher ionic strength systems, such as estuarine [38] and seawater samples [83;116], have also been analysed

with AGNES. The extent of complexation of metals to Dissolved Organic Matter (DOM) [117-120], clays [85] or nanomaterials -from latex [96;113] to TiO₂ nanoparticles [121;122] - has been assessed. The solubility of different nanomaterials has been evaluated, i.e. CdTe/CdS quantum dots [123] and ZnO nanoparticles [124;125]. AGNES has also contributed to the elucidation of toxicity mechanisms, i.e. ZnO nanoparticles to *Daphnia magna* [126].

Conclusions and perspectives

The three selected techniques have been successfully applied to a large number of environmental systems. Table 1 presents an exhaustive list of AGNES applications with selected recent applications of SSCP and CLE-CSV. On the other hand, table 2 outlines *caveats* and drawbacks of each of those techniques, so that a comparison between them is possible. As general conclusions of such comparison, one can say: i) CLE-CSV is the most established technique, currently used mostly for Cu and Fe speciation in marine systems but has also been used for few other elements (e.g. Zn, Ni, Co); on the other hand, the adsorptive CSV method is applicable for the detection of numerous elements and CLE-CSV is, thus, also potentially applicable to those, assuming that the complexation between added ligand and metal of interest is well known. In CLE-CSV, sample throughput is limited by the often-long experimental time; the use of specifically developed fully automated system for the detection (and possibly the titration) would certainly help in this respect; ii) AGNES is powerful in measuring directly the free ion concentration; once limited to typical amalgamating elements (Cd, Pb, Zn), there are now promising results for less typical ones (Sn, In, Sb, Cu) as well as future candidates (e.g. Tl, Ga) and great potential development using solid electrodes (ex: Cu on gold and Pb on BiFE); iii) SSCP and CLE-CSV provide indirect access to the free ion concentration (as well as stability constant and ligand concentration), but the modelling

is based on several assumptions; iv) SSCP provides dynamic information on complexes. v) To avoid changes in the original speciation due to e.g. purging, mixtures of N₂ and CO₂ (or other strategies) might be used. vi) Miniaturization of equipment in the future should reduce the current minimum amount of needed sample (of the order of 10 mL). vii) The study of the impact of temperature effects on the application of the techniques have just started. viii) The improvement of mercury film electrodes (especially when combined with more effective and reproducible decreases of the diffusion layers due to some kind of stirring or moving) will lower their limit of detection. ix) 10% uncertainty in the retrieved concentration values is usual. x) Even though there is extensive published documentation, training with experienced practitioners is strongly recommended before using any of the 3 techniques.

These techniques have been extensively validated (independently and against each other), though further inter-comparison work in specific systems is timely. They can be complementary when applied to the same system (for instance coupling of SSCP and AGNES is important in environmental studies due to the presence of heterogeneous ligands [93;113;127]).

Electroanalytical techniques yield physicochemical information on the exposure medium, but this must be correlated with bioavailability and toxicity in interdisciplinary studies; these are needed for future implementation of adapted EQS at local level.

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Tables

Table 1. Compilation of AGNES and recent CLE-CSV and SSCP applications to selected systems, arranged in chronological order and by alphabetical order of the first author. Unless stated otherwise, $T=25^{\circ}\text{C}$

Analyte	System	Technique	Electrode Type	pH	$I / \text{mol L}^{-1}$ (electrolyte)	Range of concentrations	Comments	Reference
Cd	NTA	AGNES	HMDE	4.7	0.1 KNO_3	$[\text{Cd}^{2+}] = 0.14$ to $1.4 \mu\text{mol/L}$	Proposal of the basic principles of AGNES. Introduction of variant AGNES-I and 1 Pulse.	[100]
Zn	NTA, EDTA, Oxalate	AGNES	HMDE	4.0 to 6.2	0.1 KNO_3	$[\text{Zn}^{2+}] = 0.029$ to $23 \mu\text{mol/L}$	AGNES measurements are not hindered by some irreversibility or precipitation. AGNES 2P is introduced.	[101]
Pb	NTA PDCA	AGNES	HMDE	pH 3.0 to 6.0 pH 2.8 to 7.0	0.1 KNO_3	$[\text{Pb}^{2+}] = 0.010$ to $1.0 \mu\text{mol/L}$ $[\text{Pb}^{2+}] = 1.2$ to 88 nmol/L	Values of AGNES crossvalidated with RT	[114]
Cd Zn	Aldrich Humic Acid	AGNES	HMDE	4 to 7	0.1 KNO_3	$[\text{Cd}^{2+}] = 0.017$ to $96 \mu\text{mol/L}$ $[\text{Zn}^{2+}] = 78 \text{ nmol/L}$ to	NICA-Donnan fitting of AGNES results.	[117]

						95 μM		
Zn	Coastal Mediterranean seawater	AGNES	HMDE	8.2	natural ionic strength	$[\text{Zn}^{2+}] = 1$ to 3 nmol/L	Introduction of the shifted blank for AGNES. First application of AGNES to seawater. Use of 2P variant.	[116]
Pb	PDCA	AGNES	mercury-coated iridium microelectrode	6.1	0.1 KNO_3	$[\text{Pb}^{2+}] = 0.0040$ to $1.2 \text{ } \mu\text{mol/L}$	Access to lower free ion concentrations with smaller electrodes.	[106]
Zn	Wine	AGNES	HMDE	3.6	natural ionic strength	$[\text{Zn}^{2+}] = 0.45$ to $0.72 \text{ } \mu\text{mol/L}$	Introduction of the EDTA blank. Methodology for hydroalcoholic systems.	[128]
Pb	Latex nanoparticles	AGNES and SSCP	HMDE	5.1 to 7.1	0.01 NaNO_3	$[\text{Pb}^{2+}] = 0.25$ to $1.0 \text{ } \mu\text{mol/L}$	Values of AGNES crossvalidated with SSCP and ISE.	[113]
Pb	Purified Aldrich Humic Acid	AGNES	HMDE	4 to 9	0.1 KNO_3	$[\text{Pb}^{2+}] = 13 \text{ pmol/L}$ to 0.11 mmol/L	Interpretation of Pb binding through the Conditional Affinity Spectrum distributions.	[118]
Zn	Cu and Oxalate	AGNES	HMDE	6	0.05 KNO_3	$[\text{Zn}^{2+}] = 0.6$ to 2.3 mmol/L	$[\text{Zn}^{2+}]$ measured in presence of Cu. The impact of intermetallic compounds was evaluated.	[129]
Zn Cd	analyte and background electrolyte	AGNES	HMDE	3 to 5	0.05 to $1.0 \text{ } \text{KNO}_3$	$[\text{Zn}^{2+}] = 9.4 \text{ } \mu\text{mol/L}$ to 70 mmol/L	Effects of high metal ion concentrations. Response function charge might be more accurate than current (AGNES-I). Introduction of AGNES-Q.	[130]
Cd	NTA/glycine/MOPS NTA/citric	AGNES and SSCP	HMDE	8.00	0.1 NaNO_3	$[\text{Cd}^{2+}] = 2.7$ to 2.9 nmol/L $[\text{Cd}^{2+}] = 2.0$	By unambiguously providing the free metal ion concentration, AGNES contributed to the understanding of the interplay of labilities.	[131]

	acid/MOPS					to 2.9 nmol/L		
Pb	Latex nanospheres IDA	AGNES	TMFE	5.50 to 6.12 5.5	0.01 NaNO ₃	[Pb ²⁺]= 0.087 to 0.28 μmol/L [Pb ²⁺]= 0.029 to 0.18 μmol/L	Use of the Rotating Disc Electrode to reduce the deposition times.	[107]
Zn	Besòs river water	AGNES	HMDE	7.7	natural ionic strength	[Zn ²⁺]= 12.8 nmol/L	First implementation of AGNES to a natural freshwater.	[132]
Cd, Zn, Pb	Oxalate	AGNES	HMDE, SPE	2 to 7	0.1 KNO ₃	[Cd ²⁺]= 0.062 to 1.1 μmol/L [Zn ²⁺]= 6.8 nmol/L to 0.57 μmol/L	Suitability of AGNES-Q for SPE.	[108]
Cd, Zn, Pb	Oxalate NTA	AGNES	HMDE, SPE	4.8 and 6	0.1 KNO ₃	[Cd ²⁺]= 53 to 162 nmol/L	Introduction of the variant AGNES-SCP.	[104]
Pb	Hydrophobic, transphilic and hydrophilic DOM	AGNES	HMDE	4 to 9	0.1 KNO ₃	[Pb ²⁺]= 0.11 nmol/L to 10 μmol/L	Evaluation of the binding properties of each DOM fraction (extracted from effluent of a wastewater treatment plant and from the river Seine) to Pb. General flow diagram to optimise gains and deposition times.	[119]

Zn	Rhine water	AGNES	HMDE	7.9	natural ionic strength	[Zn ²⁺]= 84 nmol/L	Values of AGNES crossvalidated with DMT.	[8]
Zn	Dutch soil extracts			4.9 to 6.8	10 mM CaCl ₂	[Zn ²⁺]= 0.8 to 2.4 μmol/L		
Zn	ZnO nanoparticles	AGNES	HMDE	7.5 to 8.8	0.1 KNO ₃ or KCl	[Zn ²⁺]= 0.83 μmol/L to 0.64 mmol/L	The kinetics of dissolution of the nanoparticles could be followed in few-minutes intervals.	[124]
Zn Cd	MOPS, Glycine MES, NTA	AGNES	SPE	7.6 5–6.7	0.01 KNO ₃ and MES	[Zn ²⁺]= 0.112 to 0.759 μmol/L [Cd ²⁺]= 0.45 to 1.31 μmol/L	Model for the increase of local pH in the vicinity of the electrode when the solution is not purged. Free local metal concentration can be estimated from AGNES measurements.	[115]
Zn	Glycine	AGNES	HMDE	4–7.5	0.001 to 0.1 KNO ₃	[Zn ²⁺]= 0.113 to 3.07 μmol/L	Low salinity systems can be accurately tackled using DPP peak potentials at higher ionic strengths to compute AGNES gains.	[102]
Zn	Wine	AGNES	HMDE	3.4	0.0560 KNO ₃	[Zn ²⁺]= 1.49 μmol/L to 0.0182 mol/L	Determination of the complexation capacity of wine. Measurements and calibrations at different ionic strengths.	[133]
Cd	Polyacrylate-stabilized CdTe/	AGNES and SSCP	HMDE	4.5 to 8.5	0.01 NaNO ₃	[Cd ²⁺]= 0.01 to 1.0 μmol/L	Evaluation of Cd dissolution from the QD between pH 4.5 to 8.5 and exposed to different concentrations of citric acid, glycine and	[123]

	CdS quantum dots (QD). Effect of citric acid, glycine and histidine.						histidine.	
Cd and Pb	highly charged carboxylated latex nanoparticles	AGNES and SSCP	TMFE	6 to 7 for Cd 5 to 6 for Pb	NaNO ₃ from 6 to 100 mM	[Cd ²⁺]= 1.0 μmol/L [Pb ²⁺]= 1.0 μmol/L	Study of the electrostatic effects on the dynamic behaviour of Pb and Cd binding to highly charged nanoparticles.	[96]
Fe	Celtic Sea for method development	CLE-CSV	HMDE	8.0	S = 35	c _{T,Fe} = 3 nmol/L	5 μmol/L SA + Borate buffer. Catalytically enhanced Fe peak through the use of dissolved oxygen.	[44]
Zn	<i>Daphnia magna</i> in OECD medium	AGNES	HMDE	7.8–8.2	ionic strength of the OECD medium	[Zn ²⁺]= 0.0046 to 2.4 μmol/L	Role of released free Zn concentration in the toxicity of ZnO NP to <i>Daphnia magna</i>	[126]
Pb	Seawater NOM 2 mg/L	SSCP and AGNES	HMDE	7.0 and 8.6	seawater	[Pb ²⁺]= 0.6 μmol/L	Combined study of Pb in seawater by SSCP and AGNES. Influence of lead chloride in the SSCP signal interpretation.	[83]
Cd	Iodide	AGNES	HMDE	6	0.1 KNO ₃	[Cd ²⁺]= 0.82 to 7.8 μmol/L	Free metal concentration correctly determined in presence of electrodic adsorption. General classification of variants.	[103]
Cd	polyacrylic acid			4 to 7	0.1 KNO ₃	[Cd ²⁺]= 0.79 nmol/L		
Pb	xyleneol			6.1	0.1	[Pb ²⁺]= 0.46		

Zn	orange ZnO nanoparticles			8.3	KNO ₃ 0.1 KCl	to 2.6 μmol/L [Zn ²⁺]= 10 μmol/L		
Cu	Puget Sound, Washington	CLE- CSV	HMDE	Natural pH (from 7.3 to 8.4)	ca. 22 < S < 30	c _{T, Cu} = 5.5 nmol/L to 12.5 nmol/L	5 μmol/L SA with MOPS, HEPES or EPPS. Show that Cu limitation may be the reason for slow nitrification rates because of limiting Cu- dependent ammonium oxidising archaea.	[40]
Zn	ZnO nanoparticles	AGNES	HMDE	7.7	ionic strength of the DMEM medium	[Zn ²⁺]= 0.053 to 2.5 μmol/L	ZnO NP solubility in the dispersion media, DMEM, under cell culture conditions (37 °C, 5% CO ₂).	[125]
Pb Cd	Clay (Wyoming Montmorillo nite SWy-2)	AGNES and SSCP	TMFE	5.5 to 7.1 3.2 to 7.2	0.005 NaNO ₃	[Pb ²⁺]= 0.1 to 1.0 μmol/L [Cd ²⁺]= 0.1 to 1.0 μmol/L	Determination of diffusion coefficient of clay nanoparticles. Determination of binding parameters of Cd and Pb. Assessment of lability and heterogeneity.	[85]
Cu	Seawater Tasman sea	CLE- CSV	HMDE	8.1	34.4 < S < 35.6	c _{T, Cu} = ca. 0.3 to 3.3 nmol/L	0.5 μmol/L SA + 1.9 mmol/L EPPS. Production of ligands attributed to cyanobacteria <i>Synechococcus spp</i> and coccolithophores.	[29]
Cd	TiO ₂ nanoparticles	AGNES and SSCP	TMFE	7.0 and 7.5	0.01 NaNO ₃	[Cd ²⁺]= 0.37 to 0.71 μmol/L	Assessment with AGNES of the impact of TiO ₂ NP addition on [Cd ²⁺] and its toxicity for <i>Corbicula fluminea</i> .	[121]
Cu	Estuarine and Coastal	CLE- CSV	HMDE	8.2	18.8 < S < 32.2	c _{T, Cu} = Cu from 11.6	5 μmol/L SA (Fe)/ 1, 2 or 10 μmol/L SA (Cu) + 10 mmol/L borate buffer.	[28]

Fe					$4.9 < S < 18.8$	to 54.5 nmol/L $c_{T,Fe}$ from 4.9 to 86 nmol/L	HS responsible for almost 100% of Fe complexation, and 70% for Cu. Same HS ligands are involved in the complexation of Cu and Fe.	
Co	Eastern Tropical North Atlantic	CLE-CSV	HMDE	9.1	$34.5 < S < 36.5$	$c_{T,Co}$ up to 80 pmol/L	0.2 or 2 $\mu\text{mol/L}$ Nioxime (or DMG) + 32 $\mu\text{mol/L}$ bromate + NH_3 buffer. Co(II) complexed with weak organic ligands, while Co(III) is complexed to strong inert ligand.	[39]
Fe	North Atlantic Geotraces cruise	CLE-CSV	HMDE	8.2	$34.5 < S < 38$	$c_{T,Fe}$ from 0.3 nmol/L up to typically 1.5 nmol/L	25 $\mu\text{mol/L}$ SA + Ammonium-borate buffer.	[35]
Fe	Estuarine and nearby coastal waters	CLE-CSV	HMDE	8.2	$0 < S < 26$	$c_{T,Fe}$ between 7 and 131.5 nmol/L	SA (9 to 33 $\mu\text{mol/L}$) + borate buffer. Multiple analytical windows. Flocculation of weak ligands at $S < 7$ while strong ligand present at all salinities. [Fe] and [L] tightly coupled along the whole salinity gradient.	[34]
Fe	West Atlantic (North & South) Geotraces cruise	CLE-CSV	HMDE	8.05	$34 < S < 37.4$	$c_{T,Fe}$ from 0.3 nmol/L up to typically 1.8 nmol/L	10 $\mu\text{mol/L}$ TAC + NH_3 /borate buffer. Study shows that Fe ligands have a surprisingly constant $\log K'$ of 22.3 throughout most of the West Atlantic.	[36]
Cu	Seawater collected across the South Atlantic/Drak	CLE-CSV	HMDE	Natural pH	$34.1 < S < 34.8$	$c_{T,Cu} = 1.9$ to 2.6 nmol/L	1-2 $\mu\text{mol/L}$ SA + 10 mmol/L EPPS. Uniform (25-50 nmol/L) concentration of 1 relatively weak ligand.	[26]

	e Passage							
Cu	Seawater collected across the North Atlantic	CLE-CSV	HMDE	Natural pH	$34.5 < S < 38$	$c_{T,Cu} = 0.4$ to 3.1 nmol/L	2 or 2.5 $\mu\text{mol/L}$ SA + HEPES/EPPS. Cu^{2+} found to range from 1.54 fmol/L to 1.07 pmol/L showing high complexation throughout the water column in the Atlantic. On-board analysis.	[25]
Zn	Western North Pacific	CLE-CSV	HMDE	8.2	$32.4 < S < 35.3$	$c_{T,Zn}$ from 0.03 to 2.11 nmol/L	25 $\mu\text{mol/L}$ APDC + Borate buffer. Some of the ligands attributed to bacteria and phytoplankton.	[37]
Fe	Eastern Tropical South Pacific oxygen minimum zone	CLE-CSV	HMDE	8.1		$c_{T,Fe}$ up to 1.5 nmol/L	7.3 $\mu\text{mol/L}$ TAC + 5 mmol/L EPPS. No significant difference of log K between anoxic and oxic waters	[33]
Fe	Antarctic, Sea ice, brine, snow and seawater	CLE-CSV	HMDE	8.3		$c_{T,Fe} = 0.3$ (brine) to 81.0 nmol/L (sea ice)	5 $\mu\text{mol/L}$ NN + borate buffer. <99% Fe complexed, possibly by polysaccharides.	[32]
Zn	GSH and root extracts of <i>Hordeum vulgare</i>	AGNES	HMDE	3.5 to 8	0.1 KNO_3	$[\text{Zn}^{2+}] = 20$ nmol/L to 1.5 mmol/L	Device using N_2/CO_2 saturated with water to control the evaporation and to fix the pH.	[134]
Fe	Mersey estuary + Liverpool Bay (UK)	CLE-CSV	HMDE	8.15	$18.8 < S < 30.9$	$c_{T,Fe} = 4.80$ to 84 nmol/L	5, 10, 15, 25 and 50 $\mu\text{mol/L}$ SA + Borate buffer. Agreement between multiple and single analytical windows suggests the presence of only 1 strong class of ligand.	[51]
Fe	Seawater enriched with	CLE-CSV	HMDE	8.1	seawater	Varying	10 $\mu\text{mol/L}$ TAC + EPPS buffer. Look at the potential role of EPS in	[135]

	EPS						contributing as a major Fe ligand in marine waters.	
Zn	Waters from rivers Garonne, Gave de Cauterets and Gave de Pau in the Pyrenees	AGNES	SPE	7.25 to 8.16	natural ionic strength with an addition of 0.01 M KNO ₃	[Zn ²⁺]= 2.0 to 451 µg/L	Methodology for on-site measurement in freshwaters. Crossvalidation of on-site and laboratory results.	[109]
Pb	Polystyrene sulfonate and Iminodiacetate	AGNES	Bi film electrode (BiFE)	4 6	0.01 NaNO ₃	[Pb ²⁺]= 20.4 to 63.6 nmol/L [Pb ²⁺]= 0.084 to 0.188 µmol/L	Application of AGNES with mercury-free electrodes. Theoretical framework to apply AGNES when the activity is related to a surface concentration (instead of a volumic one).	[110]
Fe	Estuary, In-shore and off-shore stations in the China Sea	CLE-CSV	HMDE	8.1	31.7 < S < 34.1	c _{T,Fe} from 3 to 126 nmol/L	100 µmol/L DHN + POPSO and bromate. 11 < Log K' _{FeL} < 14 Higher surface concentrations of the ligands observed in the spring.	[30]
Cd	TiO ₂ nanoparticles	AGNES and SSCP	TMFE	6 to 8.5	0.01 NaNO ₃	[Cd ²⁺]= 0.01 to 1.0 µmol/L	AGNES and SSCP determined a homogeneous and labile adsorption complex of Cd ²⁺ to TiO ₂ nanoparticles.	[122]
Cu	Seawater	CSV	HMDE	8.2	S = 35	Saturation of HS with addition of Cu	No added ligand. New method for the determination of Cu-HS substances based on their specific adsorption on the Hg electrode.	[24]

Cu	Aqueous extract of <i>Eichhornia crassipes</i>	CLE-CSV	HMDE	8	Not given	$c_{T, Cu} = 144-480$ nmol/L	SA ligand + borate buffer. Comparison of forward and reverse titrations.	[23]
Cu	UV seawater equilibrated with sediments	CLE-CSV	HMDE	8.1	Natural salinity. Coastal seawater	$c_{T, Cu}$ ca. 2 nmol/L	5 μ mol/L SA + 10 mmol/L Borate buffer. Cu complexing ligand released from sediments may account for 13% of the total ligand budget in coastal waters of the Tasman Sea.	[22]
Cd and Pb	Natural organic matter from two rivers in Brasil	AGNES and SSCP	TMFE on screen printed electrode	pH below 3 for total and 6.2 and 6.7 for the two rivers.	0.01 NaNO ₃	[Cd ²⁺]= 3 to 300 nmol/L	Development of a speciation methodology for total metal determination by SCP (acidification), free metal by AGNES and small complexes in the ultrafiltered solution (<1 kDa).	[90]
Cu	Malonic Iminodiacetic acids	AGNES	VGME	4 to 6	0.01 NaNO ₃	[Cu ²⁺]= 15 to 90 nmol/L [Cu ²⁺]= 26 to 92.7 nmol/L	First use of Au-electrode in AGNES. Crossvalidation of AGNES with SSV.	[111]
Zn	Tamar river estuary	AGNES and CSV	HMDE	7.2 to 8.6	natural ionic strength	[Zn ²⁺]= 4 to 26 nmol/L	Values of AGNES crossvalidated with CLE-AdCSV. Application of AGNES with various salinities.	[38]
Cd Zn	Laurentian Fulvic Acid	AGNES	TMFE	6 to 8	NaNO ₃ 10 mM	[Cd ²⁺]= 0.005 and 5 μ mol/L [Zn ²⁺]= 0.005 and 5 μ mol/L	AGNES was used to measure the free metal and obtain the NICA-Donnan parameters for Cd and Zn for Laurentian Fulvic Acid.	[120]

Cu	Estuarine waters	CLE-CSV	HMDE	8.2	Varying from freshwater to seawater	$c_{T, Cu} = 2.2$ nmol/L ($S = 34.5$) to 27.5 nmol/L ($S = 4.8$)	2.5 μ mol/L SA + Borate-ammonium buffer. Use of reverse titration. Look at the size-fractionated alteration of Cu complexing capacity of peat-land humics with residence time and along S gradient	[21]
Cu	Estuarine waters	CLE-CSV	HMDE	7.8	Varying from freshwater to seawater	$c_{T, Cu} = 8$ to 189 nmol/L	SA ligand (2 and 10 μ mol/L) + HEPES buffer. Shows that CLE-CSV results match VM prediction within 1 order of magnitude.	[20]
Cd Cu	Aldrich Humic Acid and MES	AGNES and SSCP	HMDE	6	10 mM or 100 mM KNO_3 or $Ca(NO_3)_2$	Not specified in the article	SSCP and AGNES used to determine the concentration of free metal ion in the bulk.	[127]
Cd Pb Cu	Aldrich Humic Acid and MES	AGNES and SSCP	HMDE	6	10 mM or 100 mM KNO_3 or $Ca(NO_3)_2$	$[Cd^{2+}] = 3.2$ to 0.3 μ mol/L $[Pb^{2+}] = 0.77$ μ M to 65 pmol/L $[Cu^{2+}] = 1.2$ μ mol/L to 0.4 nmol/L	SSCP and AGNES used to determine the concentration of free metal ion in the bulk.	[93]
Zn	Wine and synthetic solutions	AGNES	HMDE	3.4	natural ionic strength	$[Zn^{2+}] = 1.7$ μ mol/L and $[Zn^{2+}] = 6.5$ μ mol/L	Values of AGNES crossvalidated with DMT.	[112]
In	NTA	AGNES	HMDE	3.0	0.1	$[In^{3+}] = 9.84$	First application of AGNES with a trivalent	[105]

	oxalate			3.0 to 3.1	KNO ₃ 0.1 KNO ₃	μmol/L to 158 pmol/L [In ³⁺]= 87.9 μmol/L to 217 pmol/L	cation.	
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Table 2. Comparison of strong and weak characteristics of the selected electroanalytical techniques.

Issue	CLE-CSV	SSCP	AGNES
Pre-treatment	Buffer addition + Added ligand + possibly purging	Purging is necessary to avoid local pH increase.	Purging is necessary to avoid local pH increase [112;115;132]
Time of experimental determination	Each titration requires a minimum of 12 samples that must be prepared, equilibrated and analysed. Depending on those, experimental time can be significant. Recent recommendation includes the use of multiple analytical windows (i.e. multiple titrations) which will further this analysis time [49]. The use of fully automated apparatus would help increasing sample throughput.	A well-defined full SSCP wave needs roughly 15 individual SCP determinations. Due to the nature of the reoxidation step, the more adequate electrodes are thin-film electrodes and for those the reoxidation time is negligible when compared with the deposition time. Typical deposition times are of the order of 45-180s, so that an SSCP curve requires between 20 min and 1h. [75]	Depends on free concentration, on lability of complexes and on the size of the electrode. In the worst scenario, when complexes do not contribute at all, with HMDE, a rough rule is a deposition time (in seconds) 7 times the gain (Y) [101]. Zn from dissolving ZnO nanoparticles (in excess) at pH 7 could be measured every 5 minutes [124].
Interpretation of signal	Various methods (linear, non-linear, direct) for data fitting exist and it can be difficult to know which one to use. A recent inter-comparison of these methods has identified best procedures [49] and some software tools are recommended (e.g. [53] here). Strong experience is needed.	The potential shift in SCP follows a DeFord-Hume type equation providing the stability constants. The reoxidation time depends on kinetic and mass transport in solution. Comparison between the two signals provides experimental information on the lability of the complexes[79].	Very easy. The free concentration is just proportional to the analytical signal (e.g. current or charge) with a proportionality factor from a calibration
Limit of Detection	Low, due to accumulation at the surface of the sensor (no diffusion within the sensor during stripping).	Depends on the electrode geometry and hydrodynamic nature of the electrochemical cell. Best lower detection limit reported for	Depends on same characteristics as "Time of experimental determination" and on the time one can endure. pM range was achieved with Humic Acids

		SSCP is on the low nM level with 90s deposition time using a TMFE. For SCP the detection limit can reach pM levels[90].	[117].
On-field deployment	No. This is a laboratory technique that uses Hg drop electrode	Possible as long as the three electrode system uses a true reference electrode	Possible with SPE. [109]
Interferences from other metals or organic matter	DOC might interfere, possibly affecting the sensitivity at ligand saturation and, thus, affecting final results.	Possible signal superposition between Cd, Pb and In in natural media especially in presence of NOM.	Non-existing or avoidable with AGNES-SCP (except for In when Pb is at a similar level or very high interferent/analyte ratio) [104].
Electrode adsorption interference	High DOC might interfere with the sensitivity, thus affecting results.	Negligible effect whenever the charge for a full coverage is negligible with respect to the total deposited charge[73].	Non-existing, except if there is total blockage of the electrode surface. [103].
Impact of ionic strength	Must be kept constant. Measurements provide speciation information at specific ionic strength and pH.	Needs a calibration performed at the exact ionic strength of the determination.	Can be predicted, so there is portability of calibration across ionic strengths[102]
Elements to which can be applied	Current elements are Fe, Cu, Zn, Co, Ni, Pb but potentially applicable to many others.	Zn, Pb, Cd, Hg, Cu, Sn, In, Tl.	Existing results for: Zn, Pb, Cd, Cu, Sn, In[105], Sb. Future candidates: Tl, Ga.

Figure

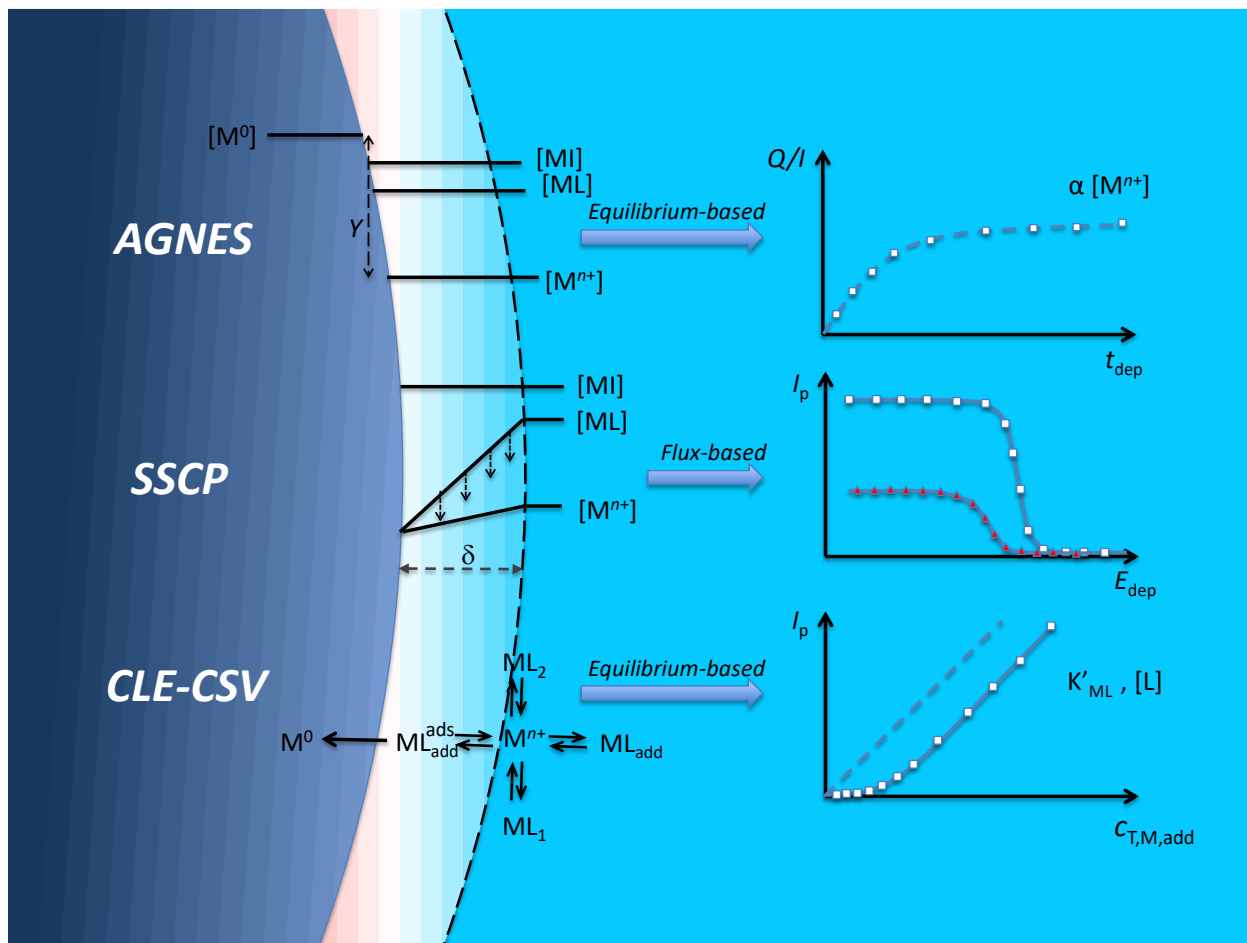


Fig 1 : Schematic outline of the selected electrochemical techniques of this review. A mercury electrode is represented on the left. Some relevant concentration profiles are depicted at the interphase with the solution, while typical outputs can be seen on the right.

COMMENTED REFERENCES IN ALPHABETICAL ORDER

* of special interest

** of outstanding interest

Abualhaija MM, Whitby H, van den Berg CMG: **Competition between copper and iron for humic ligands in estuarine waters.** 2015, **172**:46-56.

** Demonstrates that the same humic substances are involved in the complexation of both Fe and Cu, highlighting competition effects and how the presence of one metal can affect the bioavailability of another one.

Aguilar D, Parat C, Galceran J, Companys E, Puy J, Authier L, Potin-Gautier M: **Determination of free metal ion concentrations with AGNES in low ionic strength media.** *J.Electroanal.Chem.* 2013, **689**:276-283.

* Development of the methodology to apply AGNES in low salinity systems. Expressions are provided to compute the gain across media with different ionic strengths.

Buck KN, Sohst B, Sedwick PN: **The organic complexation of dissolved iron along the US GEOTRACES (GA03) North Atlantic Section.** *Deep-Sea Research Part Ii-Topical Studies in Oceanography* 2015, **116**:152-165

* Fe speciation is determined across the North Atlantic and shows the presence of at least 3 classes of ligands, the dissolved Fe mostly matching the concentration of the strongest L1 ligand throughout the water column.

Costa Monteiro AS, Parat C, Rosa AH, Pinheiro JP: **Towards field trace metal speciation using electroanalytical techniques and tangential ultrafiltration.** *Talanta* 2016, **152**:112-118.

* Development of field methodologies combining tangential ultrafiltration, SCP and AGNES to perform field speciation.

David C, Galceran J, Rey-Castro C, Puy J, Companys E, Salvador J, Monné J, Wallace R, Vakourov A: **Dissolution kinetics and solubility of ZnO nanoparticles followed by AGNES.** *J.Phys.Chem.C* 2012, **116**:11758-11767.

** Very relevant results for the field of nanoparticles, specially their ecotoxicology. AGNES could distinguish the free Zn concentration in equilibrium with nanoparticles of sufficiently different primary sizes and also follow a relatively fast kinetics of dissolution.

Duval JFL, Farinha JPS, Pinheiro JP: **Impact of Electrostatics on the Chemodynamics of Highly Charged Metal-Polymer Nanoparticle Complexes.** *Langmuir* 2013, **29**:13821-13835.

* A deep insight into the complexation of Pb^{2+} with latex nanoparticles followed with the combined application of AGNES and SSCP

Galceran J, Huidobro C, Companys E, Alberti G: **AGNES: a technique for determining the concentration of free metal ions. The case of Zn(II) in coastal Mediterranean seawater.** *Talanta* 2007, **71**:1795-1803.

* Application of AGNES to seawater samples. Development of the "shifted blank", useful for very complex matrices.

Galceran J, Lao M, David C, Companys E, Rey-Castro C, Salvador J, Puy J: **The impact of electrodic adsorption on Zn, Cd or Pb speciation measurements with AGNES.** *J.Electroanal.Chem.* 2014, **722-723**:110-118.

** This article contains a recent review of all the variants of AGNES. Adsorption impact is minimum in AGNES. A strategy is developed to measure solubility of ZnO nanoparticles containing a dispersant.

Kim JM, Baars O, Morel FMM: **Bioavailability and Electroreactivity of Zinc Complexed to Strong and Weak Organic Ligands.** *Environmental Science & Technology* 2015, **49**:10894-10902.

* The ASV measured Zn at $E_{\text{dep}} = -1.2$ V is shown to correlate with biouptake fluxes at a model diatom, built up from inorganic zinc and weak organic complexes.

Omanovic D, Gamier C, Pizeta I: **ProMCC: An all-in-one tool for trace metal complexation studies.** *Mar.Chem.* 2015, **173**:25-39

* A user-friendly freely available software for reliable quantification of titration data with possibility to incorporate multiple detection windows.

Parat C, Authier L, Aguilar D, Companys E, Puy J, Galceran J, Potin-Gautier M: **Direct determination of free metal concentration by implementing stripping chronopotentiometry as second stage of AGNES.** *Analyst.* 2011, **136**:4337-4343.

* Development of the variant AGNES-SCP specially useful to avoid interferences from other analytes and to spare blank measurements.

Parat C, Authier L, Castetbon A, Aguilar D, Companys E, Puy J, Galceran J, Potin-Gautier M: **Free Zn²⁺ determination in natural freshwaters of the Pyrenees: towards on-site measurements with AGNES.** *Environ.Chem.* 2015, **12**:329-337.

* The use of Screen Printed Electrodes allows the performance of AGNES on the field.

Pearson HBC, Comber SDW, Braungardt C, Worsfold PJ: **Predicting Copper Speciation in Estuarine Waters-Is Dissolved Organic Carbon a Good Proxy for the Presence of Organic Ligands?** *Environmental Science & Technology* 2017, **51**:2206-2216.

* Comparison of free ion concentration obtained by CLE-CSV and the speciation code Visual Minteq for estuarine samples obtained along the full salinity gradient were found to agree within 1 order of magnitude.

Pinheiro JP, Rocha LS, Goveia D, Town RM: **Scanned stripping chronopotentiometry at bismuth film rotating disc electrodes: a method for quantitative dynamic metal speciation.** *Environ.Chem.* 2014, **11**:150-157.

** First SSCP analysis using a mercury-free solid electrode, opening the perspective of speciation analysis for metal ions that do not form amalgam with mercury.

Pizeta I, Sander SG, Hudson RJM, Omanovic D, Baars O, Barbeau KA, Buck KN, Bundy RM, Carrasco G, Croot PL, Garnier C, Gerringa LJA, Gledhill M, Hirose K, Kondo Y, Laglera LM, Nuester J, Rijkenberg MJA, Takeda S, Twining BS, Wells M: **Interpretation of complexometric titration data: An intercomparison of methods for estimating models of trace metal complexation by natural organic ligands.** *Mar.Chem.* 2015, **173**:3-24.

** There are various methods that are commonly used for quantification of titration data. This in-depth inter-comparison provides recommendations of how best performing and analysing titration curves.

Rocha LS, Companys E, Galceran J, Carapuca HM, Pinheiro JP: **Evaluation of thin mercury film rotating disc electrode to perform Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) measurements.** *Talanta* 2010, **80**:1881-1887.

* The use of a very stable Rotating Disk Electrode can reduce the deposition times in AGNES.

Rocha LS, Galceran J, Puy J, Pinheiro JP: **Determination of the Free Metal Ion Concentration Using AGNES Implemented with Environmentally Friendly Bismuth Film Electrodes.** *Anal.Chem.* 2015, **87**:6071-6078.

** First application of AGNES with mercury-free electrodes to determine Pb, based on the activity of the adsorbed analyte.

Rotureau E, Waldvogel Y, Present RM, Pinheiro JP: **Addressing temperature effects on metal chemodynamics studies using stripping electroanalytical techniques. Part 1: Lability of small complexes.** *J.Electroanal.Chem.* 2015, **752**:68-74.

* Effect of temperature on lability investigated by SSCP

References

1. Templeton DM, Ariese F, Cornelis R, Danielsson LG, Muntau H, van Leeuwen HP, Lobinski R: **Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000)**. *Pure Appl.Chem.* 2000, **72**:1453-1470.
2. Anderson MA, Morel FMM, Guillard RRL: **Growth limitation of a coastal diatom by low zinc ion activity**. *Nature* 1978, **276**:70-71.
3. Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F, Gensemer RW, Goss GG, Hogstrand C, Janssen CR, McGeer JC, Naddy RB, Playle RC, Santore RC, Schneider U, Stubblefield WA, Wood CM, Wu KB: **The biotic ligand model: a historical overview**. *Comp.Biochem.Physiol.C* 2002, **133**:3-35.
4. Zhao CM, Campbell PGC, Wilkinson KJ: **When are metal complexes bioavailable?** *Environ.Chem.* 2016, **13**:425-433.
5. Pesavento M, Alberti G, Biesuz R: **Analytical methods for determination of free metal ion concentration, labile species fraction and metal complexation capacity of environmental waters: A review**. *Anal.Chim.Acta* 2009, **631**:129-141.
6. Feldmann J, Salaun P, Lombi E: **Critical review perspective: elemental speciation analysis methods in environmental chemistry - moving towards methodological integration**. *Environ.Chem.* 2009, **6**:275-289.
7. Filella M: **Food for Thought: A Critical Overview of Current Practical and Conceptual Challenges in Trace Element Analysis in Natural Waters**. *Water* 2013, **5**:1152-1171.
8. Chito D, Weng L, Galceran J, Companys E, Puy J, van Riemsdijk WH, van Leeuwen HP: **Determination of free Zn²⁺ concentration in synthetic and natural samples with AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique)**. *Sci.Total Envir.* 2012, **421-422**:238-244.

9. Fortin C, Campbell PGC: **An ion-exchange technique for free-metal ion measurements (Cd²⁺, Zn²⁺): Applications to complex aqueous media.** *Int.J.Environ.Anal.Chem* 1998, **72**:173-194.
10. Cremazy A, Leclair S, Mueller K, Vigneault B, Campbell P, Fortin C: **Development of an In Situ Ion-Exchange Technique for the Determination of Free Cd, Co, Ni, and Zn Concentrations in Freshwaters.** *Aquat.Geochem.* 2015, **21**:259-279.
11. Alberti G, Biesuz R, Pesavento M: **Determination of the Total Concentration and Speciation of Metal Ions in River, Estuarine and Seawater Samples.** *Anal.Sci.* 2008, **24**:1605-1611.
12. *Diffusive Gradients in Thin-Films for Environmental Measurements.* Edited by Davison W. Cambridge (UK): Cambridge University Press; 2016.
13. Gonzalez A, Armenta S, Cervera ML, de la Guardia M: **Non-chromatographic speciation.** *Trac-Trend Anal Chem* 2010, **29**:260-268.
14. Bansod B, Kumar T, Thakur R, Rana S, Singh I: **A review on various electrochemical techniques for heavy metal ions detection with different sensing platforms.** *Biosens.Bioelectron.* 2017, **94**:443-455.
15. Dytrtova JJ, Jakl M, Schroder D, Navratil T: **Electrochemical and spectrometric detection of low-molecular weight organic acids and their complexes with metals.** *Curr.Org.Chem.* 2011, **15**:2970-2982.
16. Mota AM, Pinheiro JP, Goncalves MLS: **Electrochemical Methods for Speciation of Trace Elements in Marine Waters. Dynamic Aspects.** *J.Phys.Chem.A* 2012, **116**:6433-6442.
17. Hunter K, Frew R, Sander SG: **Sampling and measurements of trace metals in seawater.** In *Practical Guidelines for the Analysis of Seawater.* Edited by Wurl O. Boca Raton (FL,USA): CRC Press; 2009:305-328.
18. Sanchez-Marin P, Slaveykova VI, Beiras R: **Cu and Pb accumulation by the marine diatom *Thalassiosira weissflogii* in the presence of humic acids.** *Environ.Chem.* 2010, **7**:309-317.
19. Kim JM, Baars O, Morel FMM: **The effect of acidification on the bioavailability and electrochemical lability of zinc in seawater.** *Philosophical Transactions of the Royal Society A-Mathematical Physical and Engineering Sciences* 2016, **374**.

20. Pearson HBC, Comber SDW, Braungardt C, Worsfold PJ: **Predicting Copper Speciation in Estuarine Waters-Is Dissolved Organic Carbon a Good Proxy for the Presence of Organic Ligands?** *Environmental Science & Technology* 2017, **51**:2206-2216.
21. Muller FLL, Cuscov M: **Alteration of the Copper-Binding Capacity of Iron-Rich Humic Colloids during Transport from Peatland to Marine Waters.** 2017, **51**:3214-3222.
22. Al-Farawati R, El Sayed MA, Shaban YA, Turki AJ: **Occurrence of copper-complexing ligands in the coastal sediments of eastern Red Sea.** *Arabian Journal of Geosciences* 2016, **9**.
23. Abdelraheem WHM, Rabia MKM, Ismail NM: **Evaluation of copper speciation in the extract of Eichhornia crassipes using reverse and forward/CLE voltammetric titrations.** 2016, **9**:S1670-S1678.
24. Whitby H, van den Berg CMG: **Evidence for copper-binding humic substances in seawater.** *Mar.Chem.* 2015, **173**:282-290.
25. Jacquot JE, Moffett JW: **Copper distribution and speciation across the International GEOTRACES Section GA03.** *Deep-Sea Research Part II-Topical Studies in Oceanography* 2015, **116**:187-207.
26. Heller MI, Croot PL: **Copper speciation and distribution in the Atlantic sector of the Southern Ocean.** *Mar.Chem.* 2015, **173**:253-268.
27. Gledhill M, Achterberg EP, Li KQ, Mohamed KN, Rijkenberg MJA: **Influence of ocean acidification on the complexation of iron and copper by organic ligands in estuarine waters.** *Mar.Chem.* 2015, **177**:421-433.
28. Abualhaija MM, Whitby H, van den Berg CMG: **Competition between copper and iron for humic ligands in estuarine waters.** 2015, **172**:46-56.
29. Thompson CM, Ellwood MJ, Sander SG: **Dissolved copper speciation in the Tasman Sea, SW Pacific Ocean.** *Mar.Chem.* 2014, **164**:84-94.
30. Su H, Yang RJ, Zhang AB, Li Y: **Dissolved iron distribution and organic complexation in the coastal waters of the East China Sea.** *Mar.Chem.* 2015, **173**:208-221.

31. Sander SG, Tian F, Ibisani EB, Currie KI, Hunter KA, Frew RD: **Spatial and seasonal variations of iron speciation in surface waters of the Subantarctic front and the Otago Continental Shelf**. 2015, **173**:114-124.
32. Lannuzel D, Grotti M, Abelmoschi ML, van der Merwe P: **Organic ligands control the concentrations of dissolved iron in Antarctic sea ice**. 2015, **174**:120-130.
33. Kondo Y, Moffett JW: **Iron redox cycling and subsurface offshore transport in the eastern tropical South Pacific oxygen minimum zone**. *Mar.Chem.* 2015, **168**:95-103.
34. Bundy RM, Abdulla HAN, Hatcher PG, Biller DV, Buck KN, Barbeau KA: **Iron-binding ligands and humic substances in the San Francisco Bay estuary and estuarine-influenced shelf regions of coastal California**. *Mar.Chem.* 2015, **173**:183-194.
35. Buck KN, Sohst B, Sedwick PN: **The organic complexation of dissolved iron along the US GEOTRACES (GA03) North Atlantic Section**. *Deep-Sea Research Part II-Topical Studies in Oceanography* 2015, **116**:152-165.
36. Gerringa LJA, Rijkenberg MJA, Schoemann V, Laan P, de Baar HJW: **Organic complexation of iron in the West Atlantic Ocean**. 2015, **177**:434-446.
37. Kim T, Obata H, Kondo Y, Ogawa H, Gamo T: **Distribution and speciation of dissolved zinc in the western North Pacific and its adjacent seas**. 2015, **173**:330-341.
38. Pearson HBC, Galceran J, Companys E, Braungardt C, Worsfold P, Puy J, Comber S: **Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) for the determination of [Zn²⁺] in estuarine waters**. *Anal.Chim.Acta* 2016, **912**:32-40.
39. Baars O, Croot PL: **Dissolved cobalt speciation and reactivity in the eastern tropical North Atlantic**. *Mar.Chem.* 2015, **173**:310-319.
40. Jacquot JE, Horak REA, Amin SA, Devol AH, Ingalls AE, Armbrust EV, Stahl DA, Moffett JW: **Assessment of the potential for copper limitation of ammonia oxidation by Archaea in a dynamic estuary**. *Mar.Chem.* 2014, **162**:37-49.
41. Oldham VE, Swenson MM, Buck KN: **Spatial variability of total dissolved copper and copper speciation in the inshore waters of Bermuda**. *Marine Pollution Bulletin* 2014, **79**:314-320.

42. Nason JA, Sprick MS, Bloomquist DJ: **Determination of copper speciation in highway stormwater runoff using competitive ligand exchange - Adsorptive cathodic stripping voltammetry.** *Water.Res.* 2012, **46**:5788-5798.
43. Hassler CS, Norman L, Nichols CAM, Clementson LA, Robinson C, Schoemann V, Watson RJ, Doblin MA: **Iron associated with exopolymeric substances is highly bioavailable to oceanic phytoplankton.** 2015, **173**:136-147.
44. Abualhaija MM, van den Berg CMG: **Chemical speciation of iron in seawater using catalytic cathodic stripping voltammetry with ligand competition against salicylaldoxime.** 2014, **164**:60-74.
45. Hawkes JA, Gledhill M, Connelly DP, Achterberg EP: **Characterisation of iron binding ligands in seawater by reverse titration.** *Anal.Chim.Acta* 2013, **766**:53-60.
46. Hawkes JA, Connelly DP, Gledhill M, Achterberg EP: **The stabilisation and transportation of dissolved iron from high temperature hydrothermal vent systems.** 2013, **375**:280-290.
47. Laglera LM, Battaglia G, van den Berg CMG: **Determination of humic substances in natural waters by cathodic stripping voltammetry of their complexes with iron.** *Anal.Chim.Acta* 2007, **599**:58-66.
48. Laglera LM, Filella M: **The relevance of ligand exchange kinetics in the measurement of iron speciation by CLE-AdCSV in seawater.** *Mar.Chem.* 2015, **173**:100-113.
49. Pizeta I, Sander SG, Hudson RJM, Omanovic D, Baars O, Barbeau KA, Buck KN, Bundy RM, Carrasco G, Croot PL, Garnier C, Gerringa LJA, Gledhill M, Hirose K, Kondo Y, Laglera LM, Nuester J, Rijkenberg MJA, Takeda S, Twining BS, Wells M: **Interpretation of complexometric titration data: An intercomparison of methods for estimating models of trace metal complexation by natural organic ligands.** *Mar.Chem.* 2015, **173**:3-24.
50. Gerringa LJA, Rijkenberg MJA, Thuroczy CE, Maas LRM: **A critical look at the calculation of the binding characteristics and concentration of iron complexing ligands in seawater with suggested improvements.** 2014, **11**:114-136.
51. Mahmood A, Abualhaija MM, van den Berg CMG, Sander SG: **Organic speciation of dissolved iron in estuarine and coastal waters at multiple analytical windows.** *Mar.Chem.* 2015, **177**:706-719.

52. Bundy RM, Biller DV, Buck KN, Bruland KW, Barbeau KA: **Distinct pools of dissolved iron-binding ligands in the surface and benthic boundary layer of the California Current.** *Limnol.Oceanogr.* 2014, **59**:769-787.
53. Omanovic D, Gamier C, Pizeta I: **ProMCC: An all-in-one tool for trace metal complexation studies.** *Mar.Chem.* 2015, **173**:25-39.
54. Gibbon-Walsh K, Salaun P, van den Berg CMG: **Pseudopolarography of Copper Complexes in Seawater Using a Vibrating Gold Microwire Electrode.** *J.Phys.Chem.A* 2012, **116**:6609-6620.
55. Kim JM, Baars O, Morel FMM: **Bioavailability and Electoreactivity of Zinc Complexed to Strong and Weak Organic Ligands.** *Environmental Science & Technology* 2015, **49**:10894-10902.
56. Schneider AB, Koschinsky A, Kiprotich J, Poehle S, do Nascimento PC: **An experimental study on the mixing behavior of Ti, Zr, V and Mo in the Elbe, Rhine and Weser estuaries.** *Estuarine Coastal and Shelf Science* 2016, **170**:34-44.
57. Grabarczyk M, Wardak C: **A new voltammetric strategy for sensitive and selective determination of gallium using cupferron as a complexing agent.** *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 2014, **49**:1142-1148.
58. Biver M, Quentel F, Filella M: **Direct determination of tellurium and its redox speciation at the low nanogram level in natural waters by catalytic cathodic stripping voltammetry.** *Talanta* 2015, **144**:1007-1013.
59. Monticelli D, Laglera LM, Caprara S: **Miniaturization in voltammetry: Ultratrace element analysis and speciation with twenty-fold sample size reduction.** 2014, **128**:273-277.
60. Saidin MI, Isa IM, Ahmad M, Hashim N, Ab Ghani S: **Analysis of trace nickel by square wave stripping voltammetry using chloropalladium(II) complex-modified MWCNTs paste electrode.** *Sensors And Actuators B-Chemical* 2017, **240**:848-856.
61. Zhiani R, Ghanei-Motlag M, Razavipanah I: **Selective voltammetric sensor for nanomolar detection of silver ions using carbon paste electrode modified with novel nanosized Ag(I)-imprinted polymer.** *Journal of Molecular Liquids* 2016, **219**:554-560.
62. Zhang L, Pan DW, Liu YS: **Rapid and sensitive determination of cobalt by adsorptive cathodic stripping voltammetry using tin-bismuth alloy electrode.** *Ionics* 2016, **22**:721-729.

63. Rusinek CA, Bange A, Warren M, Kang WJ, Nahan K, Papautsky I, Heineman WR: **Bare and Polymer-Coated Indium Tin Oxide as Working Electrodes for Manganese Cathodic Stripping Voltammetry.** *Anal.Chem.* 2016, **88**:4221-4228.
64. Rojas-Romo C, Arancibia V, Moreno-da Costa D, Tapia RA: **Highly sensitive determination of vanadium (V) by catalytic adsorptive stripping voltammetry. Substituent effect on sensitivity III.** *Sensors And Actuators B-Chemical* 2016, **224**:772-779.
65. Schneider AB, Nascimento PC, Bohrer D, de Carvalho LM, Guarda A, Krause C, Wiethan BA, Koschinsky A: **Determination of Zirconium and Vanadium in Natural Waters by Adsorptive Stripping Voltammetry in the Presence of Cupferron, Oxalic Acid and 1,3-Diphenylguanidine.** *Electroanal.* 2015, **27**:1864-1870.
66. Zhang L, Wang CZ, Tang HB, Wang L, Liu YS, Zhao YL, Chai ZF, Shi WQ: **Rapid Determination of Uranium in Water Samples by Adsorptive Cathodic Stripping Voltammetry Using a Tin-Bismuth Alloy Electrode.** *Electrochim.Acta.* 2015, **174**:925-932.
67. Grabarczyk M, Wasag J: **Adsorptive Cathodic Stripping Voltammetric Method for Determination of Gallium Using an In Situ Plated Lead Film Electrode.** *Electroanal.* 2015, **27**:2596-2600.
68. Ouyang RZ, Zhang WY, Zhou SL, Xue ZL, Xu LN, Gu YY, Miao YQ: **Improved Bi film wrapped single walled carbon nanotubes for ultrasensitive electrochemical detection of trace Cr(VI).** *Electrochim.Acta.* 2013, **113**:686-693.
69. Dal Borgo S, Sopha H, Smarzewska S, Hocevar SB, Svancara I, Metelka R: **Macroporous Bismuth Film Screen-Printed Carbon Electrode for Simultaneous Determination of Ni(II) and Co(II).** *Electroanal.* 2015, **27**:209-216.
70. Bobrowski A, Krolicka A, Maczuga M, Zarebski J: **A novel screen-printed electrode modified with lead film for adsorptive stripping voltammetric determination of cobalt and nickel.** *Sensors And Actuators B-Chemical* 2014, **191**:291-297.
71. Kokkinos C, Economou A: **Microfabricated chip integrating a bismuth microelectrode array for the determination of trace cobalt(II) by adsorptive cathodic stripping voltammetry.** *Sensors And Actuators B-Chemical* 2016, **229**:362-369.
72. Town RM, van Leeuwen HP: **Fundamental features of metal ion determination by stripping chronopotentiometry.** *J.Electroanal.Chem.* 2001, **509**:58-65.

73. Town RM, van Leeuwen HP: **Effects of adsorption in stripping chronopotentiometric metal speciation analysis.** *J.Electroanal.Chem.* 2002, **523**:1-15.
74. Town RM, van Leeuwen HP: **Significance of wave form parameters in stripping chronopotentiometric metal speciation analysis.** *J.Electroanal.Chem.* 2002, **535**:11-25.
75. Rocha LS, Pinheiro JP, Carapuca HM: **Evaluation of nanometer thick mercury film electrodes for stripping chronopotentiometry.** *J.Electroanal.Chem.* 2007, **610**:37-45.
76. Parat C, Schneider A, Castetbon A, Potin-Gautier M: **Determination of trace metal speciation parameters by using screen-printed electrodes in stripping chronopotentiometry without deaerating.** *Anal.Chim.Acta* 2011, **688**:156-162.
77. Town RM, van Leeuwen HP: **Depletive stripping chronopotentiometry: A major step forward in electrochemical stripping techniques for metal ion speciation analysis.** *Electroanal.* 2004, **16**:458-471.
78. Town RM, van Leeuwen HP: **Stripping chronopotentiometry at scanned deposition potential (SSCP) - Part 2. Determination of metal ion speciation parameters.** *J.Electroanal.Chem.* 2003, **541**:51-65.
79. Pinheiro JP, van Leeuwen HP: **Scanned stripping chronopotentiometry of metal complexes: lability diagnosis and stability computation.** *J.Electroanal.Chem.* 2004, **570**:69-75.
80. Serrano N, Díaz-Cruz JM, Ariño C, Esteban M, Puy J, Companys E, Galceran J, Cecilia J: **Full-wave analysis of stripping chronopotentiograms at scanned deposition potential (SSCP) as a tool for heavy metal speciation: Theoretical development and application to Cd(II)-phthalate and Cd(II)-iodide systems.** *J.Electroanal.Chem.* 2007, **600**:275-284.
81. Serrano N, Diaz-Cruz JM, Arino C, Esteban M: **Stripping chronopotentiometry in environmental analysis.** *Electroanal.* 2007, **19**:2039-2049.
82. Pinheiro JP, Rocha LS, Goveia D, Town RM: **Scanned stripping chronopotentiometry at bismuth film rotating disc electrodes: a method for quantitative dynamic metal speciation.** *Environ.Chem.* 2014, **11**:150-157.

83. Diaz-de-Alba M, Galindo-Riano MD, Pinheiro JP: **Lead electrochemical speciation analysis in seawater media by using AGNES and SSCP techniques.** *Environ.Chem.* 2014, **11**:137-149.
84. Rocha LS, Botero WG, Alves NG, Moreira JA, da Costa AMR, Pinheiro JP: **Ligand Size Polydispersity Effect on SCCP Signal Interpretation.** *Electrochim.Acta.* 2015, **166**:395-402.
85. Rotureau E: **Analysis of metal speciation dynamics in clay minerals dispersion by stripping chronopotentiometry techniques.** *Colloids Surf.A* 2014, **441**:291-297.
86. Rotureau E, Waldvogel Y, Present RM, Pinheiro JP: **Addressing temperature effects on metal chemodynamics studies using stripping electroanalytical techniques. Part 1: Lability of small complexes.** *J.Electroanal.Chem.* 2015, **752**:68-74.
87. do Nascimento FH, Masini JC: **Sequential Injection Assisted Stripping Chronopotentiometry at Screen Printed Gold Electrodes for Determination of Hg(II) in Adsorption Studies.** *Anal.Lett.* 2016, **49**:699-710.
88. Parat C, Pinheiro JP: **ISIDORE, a probe for in situ trace metal speciation based on Donnan membrane technique with related electrochemical detection part 1: Equilibrium measurements.** *Anal.Chim.Acta* 2015, **896**:1-10.
89. Aumond V, Waeles M, Salaun P, Gibbon-Walsh K, van den Berg CMG, Sarradin PM, Riso RD: **Sulfide determination in hydrothermal seawater samples using a vibrating gold micro-wire electrode in conjunction with stripping chronopotentiometry.** *Anal.Chim.Acta* 2012, **753**:42-47.
90. Costa Monteiro AS, Parat C, Rosa AH, Pinheiro JP: **Towards field trace metal speciation using electroanalytical techniques and tangential ultrafiltration.** *Talanta* 2016, **152**:112-118.
91. Town RM, van Leeuwen HP, Buffle J: **Chemodynamics of Soft Nanoparticulate Complexes: Cu(II) and Ni(II) Complexes with Fulvic Acids and Aquatic Humic Acids.** *Environ.Sci.Technol.* 2012, **46**:10487-10498.
92. Town RM: **Metal ion complexation by soft nanoparticles: the effect of Ca²⁺ on electrostatic and chemical contributions to the Eigen-type reaction rate.** *Environ.Chem.* 2015, **12**:130-137.

93. Town RM, van Leeuwen HP: **Intraparticulate speciation analysis of soft nanoparticulate metal complexes. The impact of electric condensation on the binding of Cd²⁺/Pb²⁺/Cu²⁺ by humic acids.** *Phys.Chem.Chem.Phys.* 2016, **18**:10049-10058.
94. Rotureau E, Waldvogel Y, Pinheiro JP, Farinha JP, Bihannic I, Present RM, Duval JFL: **Structural effects of soft nanoparticulate ligands on trace metal complexation thermodynamics.** *Phys.Chem.Chem.Phys.* 2016, **18**:31711-31724.
95. Domingos RF, Franco C, Pinheiro JP: **The role of charged polymer coatings of nanoparticles on the speciation and fate of metal ions in the environment.** *Environ.Sci.Pollut.Res.* 2015, **22**:2900-2906.
96. Duval JFL, Farinha JPS, Pinheiro JP: **Impact of Electrostatics on the Chemodynamics of Highly Charged Metal-Polymer Nanoparticle Complexes.** *Langmuir* 2013, **29**:13821-13835.
97. Rocha TL, Gomes T, Pinheiro JP, Sousa VS, Nunes LM, Teixeira MR, Bebianno MJ: **Toxicokinetics and tissue distribution of cadmium-based Quantum Dots in the marine mussel *Mytilus galloprovincialis*.** *Environ.Pollut* 2015, **204**:207-214.
98. Pradhan A, Pinheiro JP, Seena S, Pascoal C, Cassio F: **Polyhydroxyfullerene Binds Cadmium Ions and Alleviates Metal-Induced Oxidative Stress in *Saccharomyces cerevisiae*.** *Appl.Environ.Microbiol.* 2014, **80**:5874-5881.
99. Rotureau E, Billard P, Duval JFL: **Evaluation of Metal Biouptake from the Analysis of Bulk Metal Depletion Kinetics at Various Cell Concentrations: Theory and Application.** *Environ.Sci.Technol.* 2015, **49**:990-998.
100. Galceran J, Companys E, Puy J, Cecília J, Garcés JL: **AGNES: a new electroanalytical technique for measuring free metal ion concentration.** *J.Electroanal.Chem.* 2004, **566**:95-109.
101. Companys E, Cecília J, Codina G, Puy J, Galceran J: **Determination of the concentration of free Zn²⁺ with AGNES using different strategies to reduce the deposition time.** *J.Electroanal.Chem.* 2005, **576**:21-32.
102. Aguilar D, Parat C, Galceran J, Companys E, Puy J, Authier L, Potin-Gautier M: **Determination of free metal ion concentrations with AGNES in low ionic strength media.** *J.Electroanal.Chem.* 2013, **689**:276-283.
103. Galceran J, Lao M, David C, Companys E, Rey-Castro C, Salvador J, Puy J: **The impact of electrodic adsorption on Zn, Cd or Pb speciation measurements with AGNES.** *J.Electroanal.Chem.* 2014, **722-723**:110-118.

104. Parat C, Authier L, Aguilar D, Companys E, Puy J, Galceran J, Potin-Gautier M: **Direct determination of free metal concentration by implementing stripping chronopotentiometry as second stage of AGNES.** *Analyst.* 2011, **136**:4337-4343.
105. Tehrani MH, Companys E, Dago A, Puy J, Galceran J: **Free Indium concentration determined with AGNES.** *Sci. Total Environ.* 2017. In press.
106. Huidobro C, Companys E, Puy J, Galceran J, Pinheiro JP: **The use of microelectrodes with AGNES.** *J.Electroanal.Chem.* 2007, **606**:134-140.
107. Rocha LS, Companys E, Galceran J, Carapuca HM, Pinheiro JP: **Evaluation of thin mercury film rotating disc electrode to perform Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) measurements.** *Talanta* 2010, **80**:1881-1887.
108. Parat C, Aguilar D, Authier L, Potin-Gautier M, Companys E, Puy J, Galceran J: **Determination of Free Metal Ion Concentrations Using Screen-Printed Electrodes and AGNES with the Charge as Response Function.** *Electroanal.* 2011, **23**:619-627.
109. Parat C, Authier L, Castetbon A, Aguilar D, Companys E, Puy J, Galceran J, Potin-Gautier M: **Free Zn²⁺ determination in natural freshwaters of the Pyrenees: towards on-site measurements with AGNES.** *Environ.Chem.* 2015, **12**:329-337.
110. Rocha LS, Galceran J, Puy J, Pinheiro JP: **Determination of the Free Metal Ion Concentration Using AGNES Implemented with Environmentally Friendly Bismuth Film Electrodes.** *Anal.Chem.* 2015, **87**:6071-6078.
111. Domingos RF, Carreira S, Galceran J, Salaün P, Pinheiro JP: **AGNES at vibrated gold microwire electrode for the direct quantification of free copper concentrations.** *Anal.Chim.Acta* 2016, **920**:29-36.
112. Lao M, Companys E, Weng L, Puy J, Galceran J: **Speciation of Zn, Fe, Ca and Mg in wine with the Donnan Membrane Technique.** *Food Chem.* 2018, **239**:1143-1150.
113. Domingos RF, Huidobro C, Companys E, Galceran J, Puy J, Pinheiro JP: **Comparison of AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and SSCP (Scanned Stripping Chronopotentiometry) for Trace Metal Speciation Analysis.** *J.Electroanal.Chem.* 2008, **617**:141-148.

114. Alberti G, Biesuz R, Huidobro C, Companys E, Puy J, Galceran J: **A comparison between the determination of free Pb(II) by two techniques: Absence of Gradients and Nernstian Equilibrium Stripping and Resin Titration.** *Anal.Chim.Acta* 2007, **599**:41-50.
115. Aguilar D, Galceran J, Companys E, Puy J, Parat C, Authier L, Potin-Gautier M: **Non-purged voltammetry explored with AGNES.** *Phys.Chem.Chem.Phys.* 2013, **15**:17510-17521.
116. Galceran J, Huidobro C, Companys E, Alberti G: **AGNES: a technique for determining the concentration of free metal ions. The case of Zn(II) in coastal Mediterranean seawater.** *Talanta* 2007, **71**:1795-1803.
117. Companys E, Puy J, Galceran J: **Humic acid complexation to Zn and Cd determined with the new electroanalytical technique AGNES.** *Environ.Chem.* 2007, **4**:347-354.
118. Puy J, Galceran J, Huidobro C, Companys E, Samper N, Garcés JL, Mas F: **Conditional Affinity Spectra of Pb²⁺-Humic Acid Complexation from Data Obtained with AGNES.** *Environ.Sci.Technol.* 2008, **42**:9289-9295.
119. Pernet-Coudrier B, Companys E, Galceran J, Morey M, Mouchel JM, Puy J, Ruiz N, Varrault G: **Pb-binding to various dissolved organic matter in urban aquatic systems: Key role of the most hydrophilic fraction.** *Geochim.Cosmochim.Ac.* 2011, **75**:4005-4019.
120. Janot N, Pinheiro JP, Botero WG, Meeussen JC, Groenenberg JE: **PEST-ORCHESTRA, a tool for optimising advanced ion-binding model parameters: derivation of NICA-Donnan model parameters for humic substances reactivity.** *Environ.Chem.* 2017, **14**:31-38.
121. Vale G, Franco C, Diniz MS, dos Santos MMC, Domingos RF: **Bioavailability of cadmium and biochemical responses on the freshwater bivalve *Corbicula fluminea* - the role of TiO₂ nanoparticles.** *Ecotox.Environ.Safe.* 2014, **109**:161-168.
122. Vale G, Franco C, Brunnert AM, dos Santos MMC: **Adsorption of Cadmium on Titanium Dioxide Nanoparticles in Freshwater Conditions - A Chemodynamic Study.** *Electroanal.* 2015, **27**:2439-2447.
123. Domingos RF, Franco C, Pinheiro JP: **Stability of core/shell quantum dots-role of pH and small organic ligands.** *Environ.Sci.Pollut.R.* 2013, **20**:4872-4880.
124. David C, Galceran J, Rey-Castro C, Puy J, Companys E, Salvador J, Monné J, Wallace R, Vakourov A: **Dissolution kinetics and solubility of ZnO nanoparticles followed by AGNES.** *J.Phys.Chem.C* 2012, **116**:11758-11767.

125. Mu QS, David CA, Galceran J, Rey-Castro C, Krzeminski L, Wallace R, Bamiduro F, Milne SJ, Hondow NS, Brydson R, Vizcay-Barrena G, Routledge MN, Jeuken LJC, Brown AP: **Systematic Investigation of the Physicochemical Factors That Contribute to the Toxicity of ZnO Nanoparticles.** *Chem.Res.Toxicol.* 2014, **27**:558-567.
126. Adam N, Schmitt C, Galceran J, Companys E, Vakourov A, Wallace R, Knapen D, Blust R: **The chronic toxicity of ZnO nanoparticles and ZnCl₂ to *Daphnia magna* and the use of different methods to assess nanoparticle aggregation and dissolution.** *Nanotoxicology* 2014, **8**:709-717.
127. Town RM, van Leeuwen HP: **Intraparticulate Metal Speciation Analysis of Soft Complexing Nanoparticles. The Intrinsic Chemical Heterogeneity of Metal-Humic Acid Complexes.** *J.Phys.Chem.A* 2016, **120**:8637-8644.
128. Companys E, Naval-Sanchez M, Martinez-Micaelo N, Puy J, Galceran J: **Measurement of free zinc concentration in wine with AGNES.** *J.Agric.Food Chem.* 2008, **56**:8296-8302.
129. Chito D, Galceran J, Companys E: **The Impact of Intermetallic Compounds Cu_xZn in the Determination of Free Zn²⁺ Concentration with AGNES.** *Electroanal.* 2010, **22**:2024-2033.
130. Galceran J, Chito D, Martinez-Micaelo N, Companys E, David C, Puy J: **The impact of high Zn⁰ concentrations on the application of AGNES to determine free Zn(II) concentration.** *J.Electroanal.Chem.* 2010, **638**:131-142.
131. Pinheiro JP, Salvador J, Companys E, Galceran J, Puy J: **Experimental verification of the metal flux enhancement in a mixture of two metal complexes: the Cd/NTA/glycine and Cd/NTA/citric acid systems.** *Phys.Chem.Chem.Phys.* 2010, **12**:1131-1138.
132. Zavarise F, Companys E, Galceran J, Alberti G, Profumo A: **Application of the new electroanalytical technique AGNES for the determination of free Zn concentration in river water.** *Anal.Bioanal.Chem.* 2010, **397**:389-394.
133. Chito D, Galceran J, Companys E, Puy J: **Determination of the Complexing Capacity of Wine for Zn Using the Absence of Gradients and Nernstian Equilibrium Stripping Technique.** *J.Agric.Food Chem.* 2013, **61**:1051-1059.
134. Lao M, Dago A, Serrano N, Companys E, Puy J, Galceran J: **Free Zn²⁺ determination in systems with Zn-Glutathione.** *J.Electroanal.Chem.* 2015, **756**:207-211.

135. Norman L, Worms IAM, Angles E, Bowie AR, Nichols CM, Pham AN, Slaveykova VI, Townsend AT, Waite TD, Hassler CS: **The role of bacterial and algal exopolymeric substances in iron chemistry.** *Mar.Chem.* 2015, **173**:148-161.