Voltammetric methods for speciation analysis of trace metals in natural waters

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ABSTRACT

Trace metals play an important role in the regulation of primary productivity and phytoplankton community composition. Metal species directly affects the biogeochemical cycling processes, transport, fate, bioavailability and toxicity of trace metals. Therefore, developing powerful methods for metal speciation analysis is very useful for research in a range of fields, including chemical and environmental analysis. Voltammetric methods, such as anodic stripping voltammetry (ASV) and competing ligand exchange-adsorptive cathodic stripping voltammetry (CLE-AdCSV), have been widely adopted for speciation analysis of metals in different natural aquatic systems. This paper provides an overview of the theory of voltammetric methods and their application for metal speciation analysis in natural waters, with a particular focus on current voltammetric methods for the discrimination of labile/inert fractions, redox species and covalently bound species. Speciation analysis of typical trace metals in natural waters including Fe, Cu, Zn, Cd, and Pb are presented and discussed in detail, with future perspectives for metal speciation analysis using voltammetric methods also discussed. This review can elaborate the particular knowledge of theory, merits, application and future challenge of voltammetric methods for speciation analysis of trace metals in natural waters.

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1. Introduction

Trace metals, such as iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni) and molybdenum (Mo), are essential for various biological metabolic processes and biogeochemical cycling in natural waters such as marine, estuary, and freshwater environments [1,2]. Some trace metals, such as Fe, Cu, Zn, Mn, and Co are responsible for the functioning of metallo-protein enzymes and photosynthetic activity, serving as the limiting factors in primary production [2,3]. For example, in 30–40 % of the world oceans, Fe limits the growth of phytoplankton and regulates their community composition, especially in high nutrient-low chlorophyll (HNLC) regions [4–6]. It has also been reported that Cu, Zn, Mn, and Co have

essential roles in the regulation of phytoplankton community species composition [4,7–9]. However, some metals are toxic to organisms especially when their concentrations exceed a certain threshold [10,11].

Different species of metals have varying chemical and physical properties, affecting their mobility, bioavailability and toxicity [2,12]. According to International Union of Pure and Applied Chemistry (IUPAC), speciation is defined as the "distribution of an element amongst defined chemical species in a system", with species classified as a "specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure" [13]. The speciation of trace metals in natural waters is usually affected by the surrounding aquatic environment, such as ionic strength, pH, salinity and temperature [14]. Trace metals can be operationally classified as either particulate or dissolved forms (including both dissolved and colloidal species) using membrane filtration (0.45 or 0.2 μm) [15]. Furthermore, dissolved metals can be divided into free ions, inorganically complexed and organically complexed forms. Assessing the form of metal species is essential as this affects the degree of adsorption to suspended matter, the migration rate to sediments

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and overall transport within aquatic systems [16]. For example, researches have shown that compared with the total dissolved concentration, the free ion concentration of metals is more closely related to their bioavailability and toxicity to zooplankton and phytoplankton [17,18].

Dissolved metal species have historically been the main focus of research on trace metals in natural waters. However, the existing forms of dissolved metals are mainly controlled by organic ligands in aquatic systems. The conditional stability constant, K, can reflect the strength of metal complexes with organic ligands. According to the complexation strength, organic ligands are generally electrochemically divided into two categories: 1) ligands with stronger complexation ability (L_1), exhibiting $\log K$ values of 12–14; and 2) ligands with weaker complexation ability (L_2), exhibiting $\log K$ values of <10 [19,20]. These organic ligands combine with free metal ions, resulting in dissolved metals mainly existing in the form of organic complexes and therefore, reducing their bioavailability. For example, dissolved Cu is mostly complexed by organic ligands in natural waters, which reduces the fraction of Cu that is most bioavailable and toxic to organisms [11,16]. More than 99 % of Fe in surface seawater exists in an organically complexed form, resulting in the concentration of reactive Fe in ocean water often being far below the level required for the normal growth of algae [21,22]. This phenomenon of "iron limitation" is usually observed in HNLC regions. Studies assessing the effects of oceanic Fe addition have demonstrated that the addition of Fe can promote the growth of algae [5]. However, recent studies have shown that Fe complexed by some small molecule organic compounds, such as exopolymeric substances and siderophores, can also be absorbed and utilized directly by algae [23,24]. Therefore, in an attempt to simplify the classification of dissolved metals, they can be further divided into reactive (labile), inert, acid dissolved and total dissolved forms, according to the water sample pretreatment methods applied [25–27]. Trace metal chemistry regulates the primary productivity and species composition of phytoplankton, with phytoplankton having various feedback effects in response [4]. A diagram of the interactions between trace metals and phytoplankton is shown in Fig. S1. Therefore, speciation analysis of trace metals is essential to understand the biological and geochemical cycling in aquatic systems.

To date, speciation analysis of trace metals has been largely limited by the analytical and sampling methods applied, due to the low concentrations, extreme complexity and heterogeneity of natural water matrices [12,15]. Different strategies have been proposed for speciation analysis of metals to obtain thermodynamic (concentration of elemental forms) and dynamic (nonequilibrium) information [12,28]. Various techniques such as atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), inductively coupled plasma-mass spectroscopy (ICP-MS), and electrochemical methods have been developed for trace metal analysis [29,30]. Although spectrographic and mass spectrographic techniques exhibit advantages, such as high sensitivity and the potential to be applied to a large number of metals, they can only be used for the analysis of total metal concentrations [31]. To achieve detailed speciation analysis of trace metals, separation and extraction procedures must be coupled with these detection techniques. However, the chemical forms of trace metals are easily changed during separation and extraction steps, with the potential for sample contamination also significantly increased. In contrast, electrochemical techniques are highly suitable for direct metal speciation analysis with minimum sample preparation, reducing the risk of change in speciation or sample contamination, while also allowing *in situ* application [29,31]. Electrochemical methods are powerful enough to allow the determination of metal species, especially free metal ion concentrations, which is of significance for the assessment of environmental water quality. Based on the assumption that free ions are the only bioavailable species and that toxicity is mainly caused by free ion permeability through biological membranes, two toxicity models have been successfully established, the biotic ligand model (BLM) [32] and the free ion activity model (FIAM) [33]. However, as discussed previously, recent studies have shown that some organically complexed metals can also be absorbed and utilized either directly or through the dissociation of labile complexes [34,35]. Fortunately, kinetic studies of metal complex dissociation on the surface of electrodes can be supported well by the established theory, providing an effective model for the complexation of metals with different ligands and their bioavailability [16].

The use of voltammetry has developed rapidly for the determination and speciation analysis of trace elements in natural waters [12], with stripping methods such as anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) being sufficiently sensitive for the analysis of trace metals at environmental levels [28]. ASV can determine the total metal concentration and the reactive or labile (ASV-labile) fraction concentration, which is the fraction most relevant to the toxicity or bioavailability of trace metals [36]. CSV can be used for the speciation analysis of a wider range of elements, with the lability of metals defined by their competitive complexation with added and natural ligands. Generally, four fractions of metals can be distinguished using voltammetric methods: free ions, electroactive (reactive or labile), electroinactive (inert or immobile), and total (dissolved) metals [28]. Studies on voltammetric methods for speciation analysis were originally driven by marine research and were subsequently adapted for the analysis of freshwater environments. With continuous development, stripping voltammetry has become the most promising analytical method for speciation analysis of trace metals in natural waters.

Most of the reviews on electrochemical speciation analysis of metal elements are relatively old. The recent published review by Companys et al. in 2017 focuses on the working principles, strong and weak points and applications in environmental media of three selected stripping techniques [34]. This review paper focuses on the speciation analysis of trace metals in natural aquatic environments using voltammetric methods, with an overview of the existing voltammetric methods for speciation analysis of metals presented. The two most commonly used techniques, ASV and competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-AdCSV), are reviewed in particular for labile fraction determination and stability parameters. Subsequently, the speciation analysis of typical trace metals including Cu, Fe, Zn, Cd and Pb in natural waters (especially in seawater) using voltammetric methods is described. Future perspectives for voltammetric analysis of metal speciation in natural waters are also discussed.

2. Voltammetric methods

Stripping voltammetry includes two stages: (1) the deposition step, in which the electroactive analyte is accumulated from solution to the electrode surface; (2) the stripping step, in which the analyte is reduced or oxidized back into the solution. The signal to noise ratios (S/N) can be enhanced by up to four orders of magnitude through the addition of a deposition step prior to a potential scan. Different potential sweep techniques can be adopted for the voltammetric analysis of metals, such as linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). DPV and SWV have been used more frequently for metal analysis due to their higher S/N providing improved analytical sensitivity. Depending on the reduction or oxidation of analytes during a potential sweep,

stripping voltammetry can be classed as either ASV (oxidation) or CSV (reduction), with methods based on cathodic stripping (CLE-AdCSV and CSV) more commonly used for metal speciation analysis. Other voltammetric methods such as competitive ligand equilibration-anodic stripping voltammetry (CLE-ASV), reverse titration, absence of gradients and Nernstian equilibrium stripping (AGNES), and stripping chronopotentiometry (SCP) can also be used for metal speciation analysis. Fig. 1 shows the imposed/recorded functions and typical concentration ranges of commonly used voltammetric techniques for speciation analysis of trace metals [31]. Using different sample pretreatment processes, the total (after acidification or UV digestion) and labile metal concentrations can be established by stripping voltammetric methods [28]. Although the free metal ion concentration cannot

be directly determined using ASV or CSV, it can be obtained by determining the complexing capacity using the metal titration method.

2.1. Anodic stripping voltammetry

ASV is a stripping voltammetric technique that has been widely applied for the determination of different metal species such as Cu, Pb, Cd, and Zn in natural waters [10,25,30,37,38]. In ASV, a reductive deposition (pre-concentration) step is employed, in which the potential is kept sufficiently negative to accumulate and reduce metal ions onto the working electrode (such as a Hg electrode) surface. A significant advantage of ASV over other methods is that no pretreatment of water samples is required. The

Technique	Imposed function	Recorded function	Conc. range (mole L)
Linear sweep voltammetry (LSV) (cyclic voltammetry dotted line)			$10^{-2} - 10^{-6}$
Differential pulse voltammetry (DPV)			$10^{-4} - 10^{-7}$
Square wave voltammetry (SWV)			$10^{-4} - 10^{-8}$
Anodic Stripping Voltammetry (ASV) with linear scan (full line) or modulations (e.g. DP → DPASV or SW → SWASV; dotted line)			$10^{-6} - 10^{-11}$
Adsorptive stripping voltammetry (AdSV) (with or without modulation)			$10^{-6} - 10^{-12}$
Stripping Chronopotentiometry (SCP)			$10^{-5} - 10^{-9}$

Fig. 1. Commonly used voltammetric techniques for speciation analysis of trace metals. v, potential scan rate; i_p, peak current; t_p, pulse width; ΔE, pulse amplitude; f, frequency; I, deposition step; II, stripping step; t_d, accumulation time; τ, transition time.
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concept of 'ASV-labile' species was introduced at the end of 1970′s and has subsequently been widely applied for the speciation analysis of trace elements [29]. ASV-labile metals refers to the fraction that can be reduced and accumulated on the electrode (traditionally Hg electrode), which comprises free metal ions, as well as the inorganically bound and weakly organically bound metals [12]. Hg film electrodes are generally used for ASV determination of metal species in natural waters, especially seawater samples [39–42]. For example, Hg deposited Iridium (Ir) micro-electrode (array) with gel protective layer is exactly the main working electrode used in voltammetric *in situ* profiling (VIP) system for real-time monitoring of trace metals [41,42]. The labile fraction of the metal M is first reduced to M° in the form of a metal amalgam under a given deposition condition (potential and time), then oxidized from the Hg film into the solution with the oxidation current recorded. The standard addition method is used for the quantification of metals in ASV systems. Hu et al. (2018) investigated the species of Cu in seawater using ASV, establishing the concentrations of electroactive Cu (determined directly after filtration), acid-dissolved Cu (determined after filtration and acidification), inert Cu (the difference between acid-dissolved and electroactive Cu) and total dissolved Cu (determined after filtration, acidification and UV digestion) [25].

For speciation analysis, metal titration is usually adopted to obtain information about the complexation capacity of ligands L, with the complexation equilibrium obtained after the metal M is added to the sample using the equilibrium equation shown in Eq. (1):

$$M + L \rightleftarrows ML \tag{1}$$

The peak current (i_p) is considered relatively low when the concentration of M (c_M) is less than that of L (c_L), increasing proportionally with the increase of free metal ion concentration when $c_M > c_L$. Therefore, a curve can be obtained at $c_M \approx c_L$, allowing the complexation constant K_{ML}^{cond} to be obtained from the metal titration data. However, the current response obtained by ASV is contributed by the labile fraction which includes not only free metal ions, but also metal ions dissociated from complexes in the diffusion layer [29]. This makes it very difficult to unambiguously interpret titration curves. Although ASV has high sensitivity for determination of trace metals in natural waters, the adsorption of organics on the working electrode surface may cause interference to the i_p. Moreover, when metal titration is used, a long equilibration time of 20−40 min is needed to equilibrate the added metal ion with natural ligands.

It should be noted that the labile fraction obtained by ASV generally corresponds well with the concentration of bioavailable metals [43–45]. The metals that can be determined by ASV must be capable of being reduced, accumulated and then reoxidized from the electrode surface under appropriate potentials. Therefore, ASV is not suitable for the determination of all trace metals, with some metal elements such as Fe, Co and Ni requiring determination using the CSV method [20,46–48].

2.2. Cathodic stripping voltammetry

In CSV systems, the metal ions or their complexes are first adsorbed onto the electrode surface, with the potential scan then performed in a negative direction to achieve the reduction of metal ions or complexes. The CSV process with adsorptive pre-concentration of metal complexes is referred to as adsorptive CSV (AdCSV). CSV has been widely used for the determination and speciation analysis of metals which cannot be reduced on electrode surface and determined by ASV. Han et al. (2021) determined the Fe concentration in coastal waters using CSV adopting a gold nanoclusters /poly(3,4-ethylenedioxythiophene)-poly(sodium 4-styrenesulfonate) modified micro-needle electrode [49]. Zhu et al. (2017) established an AdCSV method for Fe determination using 2-(5-bromo-2-pyridylazo)-5-diethylamino-phenol as the complexing ligand [50]. Subsequently, this method was used for the determination of different Fe species in coastal river water, with analysis of spatial and seasonal Fe species distribution [51].

Currently, CLE-AdCSV, is the most widely used voltammetric method for metal speciation analysis, especially in seawater matrices. The complexation capacity of water samples determined by CLE-AdCSV is a widely employed concept in speciation analysis. The principle of this method is the addition of a well-characterized artificial ligand (AL) with a known complexation constant to the sample, establishing a competitive balance between the AL and natural ligands (L). The electroactive complex between the metal M and AL is adsorbed on the electrode surface, then reduced and stripped through potential scan. The corresponding equilibration equation is shown in Eq. (2) as follows:

$$ML + AL \rightleftarrows MAL + L \tag{2}$$

The electroactive MAL complex can be determined by CSV, with the content of free metal ions [M] then calculated based on the MAL concentration. CLE-AdCSV has become a widely adopted voltammetric method for investigation of the complexation of different metals by natural ligands [52]. The competitive ligands widely used for metal speciation analysis are listed in Table 1.

Similar to ASV, the concentration of the metal-artificial ligand complex [MAL] can be experimentally determined using metal titration. Using this method, a certain volume of water sample (usually 100−150 mL) is mixed with a known concentration of AL

Table 1
Competitive ligands used for CLE-AdCSV speciation analysis of trace metals.

Competitive ligand	Metals	Reference
Tropolone	Cu	[53]
Benzoylacetone	Cu	[20,54]
Catechol	Cu	[55]
Salicylaldoxime (SA)	Cu, Fe	[56,57]
8-hydroxyquinoline (Oxine)	Cu, Pb, Cd	[58,59]
1-nitroso-2-napthol (NN)	Fe	[60]
2,3-dihydroxynaphthalene (DHN)	Fe	[61]
2-(2-thiazolylazo)-*p*-cresol (TAC)	Fe	[62]
1,2-cyclohexanedione dioxime	Co	[63]
Dimethylglyoxime (DMG)	Co	[47]
DMG	Ni	[48]
N-nitroso-N-phenylhydroxylamine (Cupferron)	Aluminium (Al)	[64]
Calcein-blue (CB)	Pb	[65]
Ammonium pyrrolidine dithiocarbamate (APDC)	Zn	[55,66]

under suitable pH conditions, then the solution is divided into 12–16 aliquots with increasing concentrations of metal ions added. The concentration of the [MAL] complex is then determined after a set equilibration period. Subsequently, the original ambient speciation of M in the water sample can be calculated according to the concentration of [AL] and [MAL], using the conditional stability constant K_{MAL}^{cond} [29]. The strength of the formed complex after the addition of AL can be defined based on its side reaction coefficient as shown in Eq. (3):

$$\alpha_{MAL} = \frac{[MAL]}{[M]} = \sum K_{MAL}^{cond}[AL] \qquad (3)$$

Using this method, the detection window depends on the characteristics and concentration of the added AL. To ensure that the α_{MAL} remains constant throughout the titration process, the AL should be supplied in excess, typically at least 10^3-fold greater than the M concentration in the water sample [29]. After the [MAL] is determined, the free metal ion concentration [M] in the presence of AL can be obtained if the α_{MAL} is known. Considering that only electroactive MAL can be measured by CSV, the side reaction coefficient of AL with M must be within a similar range to that of the natural complexes. Therefore, α_{MAL} defines the detection window of the method and natural ligands having α_{ML} values up to about 10-fold of α_{MAL} can be detected. If the side reaction coefficients of the complexes are much lower than the detection window, they will be completely decomposed by AL and the corresponding stability constants cannot be obtained, while the complexes with stronger side reaction coefficients cannot be detected unless the concentration of AL is increased or it is replaced by a different AL with higher α_{MAL}.

Linear regression and nonlinear regression methods can be used for the fitting of titration data to obtain the required information. Linear fitting methods mainly include the Langmuir/Ruzic/van den Berg method and the Scatchard method, while the Langmuir method is commonly used for nonlinear fitting [29]. Software can also be used for the processing of titration data, with ProMCC and KMS (KINETEQL Multiwindow Solver) recommended by the GEOTRACES Academic Committee [67,68].

The formation of mixed complexes due to the existence of a continuum of ligands could hinder the interpretation of CLE-AdCSV results. Additionally, CLE-AdCSV is an equilibrium technique which requires a sufficient duration (overnight is usually preferred) after addition of the metal or AL into the sample. Internal calibration is widely used for voltammetric determination and speciation analysis of metals, with the sensitivity determined by the upper portion of the titration curve, the slope of which is usually affected by the presence of weak ligands and surface-active material [29,69]. In addition, the *in situ* application of CLE-AdCSV is limited by the necessity for ligand addition, long equilibration times, and the low reliability of most electrodes [29].

3. Metal species detection using voltammetric methods

Voltammetric methods can be used to detect different kinds of species of trace metals such as labile/inert fractions, redox species and covalently bound species. The following section discusses the available voltammetric methods for each kind of metal species in detail, especially labile/inert fractions.

3.1. Labile/inert fractions

The labile (i.e. reactive or electroactive) metal content is usually expressed as a percentage of the total dissolved metal, with the difference between labile and total metal concentrations

attributed to the inert (unreactive, non-electroactive) metal content. An important issue for the determination of labile metals is how well this fraction is defined. In ASV (including ASV titrations), the determination of labile metals that can be reduced and accumulated on the electrode surface, comprises the free metal ions, inorganically bound and weakly organically bound metals. In CSV and CLE-AdCSV analysis, the labile fraction is clearly defined by thermodynamic constraints, referring to the fraction bound to the AL. Voltammetric methods including ASV, CLE-ASV, CSV, CLE-AdCSV, reverse titration, AGNES, and SCP can all be used for the determination of labile fractions.

3.1.1. Competitive ligand equilibration-adsorptive cathodic stripping voltammetry

In the CLE-AdCSV procedure, the MAL complex (i.e. the labile fraction) can be directly determined by CSV, resulting in this being the predominantly employed technique for speciation analysis of trace elements in natural waters. The CLE-AdCSV method for speciation analysis of metals is based on attaining an equilibrium between the metal, natural ligands and AL components. The usually employed overnight (14 h) equilibration time is the focus of some uncertainty. It has been concluded from coordination chemistry theoretical calculations, that long equilibration times are needed for Cu, Fe and Ni, whereas Pb, Cd and Zn are well within equilibrium conditions when "overnight" equilibration times are applied [12,52].

The development of suitable ALs is another issue that requires further research. It has been reported that CSV can be used for the determination of more than 30 elements, whereas CLE-AdCSV has been developed for the speciation analysis of only 8 elements (Table 1). Therefore, although CLE-AdCSV is widely used for the speciation analysis of trace metals, this method has been applied to a surprisingly limited number of elements. The requirements for long equilibration times, lower concentrations of added ligands and narrow pH range, might be the main difficulties in converting CSV methods to CLE-AdCSV procedures. A detailed discussion of this phenomenon is presented in a comprehensive review by Monticelli and Caprara [12].

3.1.2. Anodic stripping voltammetry

ASV-based metal speciation analysis methods were first introduced over 40 years ago [70]. This method involves a titration process, using increasing amounts of the target metal ion (as described above), with the complexation capacity and conditional stability constant then obtained from titration data. Traditionally, only metal elements that can be reduced on Hg or Hg film electrodes to form an amalgam, including Hg on a solid electrode, are suitable for ASV-based speciation analysis methods [71]. It is assumed that the complexes are inert and do not dissociate during the pre-concentration step [72]. Therefore, to obtain reliable results, a thorough evaluation of the kinetic features of complexes is needed. In addition, the sensitivity of ASV is relatively lower than that of CSV with adsorptive accumulation, which limits its widespread application for the speciation analysis of ultratrace metals. To obtain an operationally defined labile fraction, ASV is also employed without the titration process, establishing a labile fraction concentration under certain deposition conditions (potential and time). Mikkelsen et al. (2006) determined the labile Fe in estuarine and coastal waters by ASV using a silver alloy electrode (-1.5 V deposition potential and $300-900$ s deposition time) [73]. Furthermore, labile Cu concentrations in coastal seawater samples were previously determined by ASV using a functional micro-needle electrode with a deposition potential of -0.3 V and deposition time of 120 s [74].

3.1.3. Competitive ligand equilibration-anodic stripping voltammetry

The competitive ligand equilibration procedure can also be followed by ASV for the detection of labile metals. In this process, the selected AL is added to compete with natural ligands, with metal titration then performed for the detection of labile metal fractions. Through CLE-ASV, the complexation parameters, natural ligand concentration and conditional stability constant can all be obtained using thermodynamic calculations. When the AL is added, either labile or inert complexes may be formed. If an inert complex is formed, such as ethylenediaminetetraacetic acid (EDTA) for Zn speciation analysis, the labile fraction detected includes free ion and inorganic forms. In contrast, if a labile complex is formed, the detected labile fraction comprises free ions, inorganic forms and the MAL complex. When ethylenediamine and EDTA are used for Cu speciation analysis or ethylenediamine for Cd speciation analysis, labile complexes will be formed. Deposition potentials in excess of -1.0 V are usually used for the CLE-ASV speciation analysis of metals. Considering the lower sensitivity compared with CLE-AdCSV and the poorly defined detectable fraction, the CLE-ASV method has been rarely adopted for metal speciation analysis and has only been reported for the analysis of Cu, Cd and Zn speciation [12].

3.1.4. Reverse titration

Reverse titration was introduced by Nuester and van den Berg (2005) for the determination of metal speciation [75]. In this method, the natural ligand L is titrated by increasing doses of artificial ligand AL, with measurements performed after equilibrium conditions are obtained (20−30 min). The ligand concentration and conditional stability constant are calculated by linear or non-linear fitting of the titration data. A typical feature of the reverse titration method is that it can determine the strongest complexing ligands in natural waters, which presents a challenge for standard metal titration methods [75]. In CLE-AdCSV systems, the obtained results are unreliable when L is saturated by the metal being investigated, whereas the reverse titration method can be employed in this situation. To date, the reverse titration method has been successfully used for the speciation analysis of Cu and Fe [75–77].

3.1.5. Absence of gradients and Nernstian equilibrium stripping

The AGNES method was introduced in 2004 specifically for the determination of free metal ions [78]. AGNES was initially developed using a hanging mercury drop electrode (HMDE) and subsequently Hg coated Ir micro-electrodes, screen-printed electrodes and Hg film electrodes have also been utilized [79–81]. Similar to ASV, the AGNES process includes two stages, a deposition step and a stripping step. During the deposition step, a potential is applied for a sufficient duration to obtain the Nernstian equilibrium, at which point there is no concentration gradient between the amalgam and the solution. A pre-concentration factor Y in the amalgam concentration respective to the solution concentration is obtained, which obeys the Nernst law. Subsequently, the metal in the amalgam is stripped through the application of a constant potential and its concentration can be obtained based on the i vs. t function. The application of AGNES to natural waters is usually limited by its detection performance, especially for very low concentration metals. AGNES has been used for the determination of free Zn ion concentrations in coastal seawater and river water samples [82,83] and it has been shown that free metal ion concentrations obtained using AGNES are in good agreement with those obtained using other procedures [84].

3.1.6. Stripping chronopotentiometry

Similar to ASV and AGNES, SCP is a two-step method that includes a deposition step and a stripping step. In the deposition step, metal ions are reduced at a constant potential, in an identical procedure to that of ASV. However, in SCP, a constant oxidizing current is applied to reoxidize the accumulated metals back into solution. SCP measures the change of potential as a function of time, with the main analytical parameter of each signal being the time required for reoxidation or the transition time (τ), corresponding to two consecutive waves or potential jumps [85]. The transition time can be obtained from integration of the inverse of the time derivative of the potential curve (dt/dE vs. E), which is proportional to the labile metal concentration [29]. SCP at scanned deposition potentials (SSCP) is the mainly adopted SCP method for metal speciation analysis. The SSCP method consists of SCP measurement of transition times at different deposition potentials. As with ASV, only metals that can be reduced on the electrode can be determined by SCP. Application of the SCP method for environmental analysis and SSCP for dynamic speciation analysis of nanoparticulate metal complexes have been comprehensively reviewed previously [85,86]. Table 2 shows the summary of available voltammetric methods for labile fractions determination of some typical trace metals.

3.2. Redox species

The toxicity, transfer and adsorption behavior of metal elements are drastically affected by redox state. For example, Cr (VI) exists in an anionic form (chromate) is highly toxic, while Cr (III) is non-toxic and exists as anionic or organic species. However, for some other elements such as As and Mn, lower valence states form more toxic species. Therefore, the determination of redox state is an important aspect of metal speciation analysis in natural waters. Voltammetric redox speciation analysis methods are based on the selective determination of a certain oxidation state of an element (which is electroactive). The electroactive labile species concentration is first determined, and then the total metal concentration can be established after converting the inert species into labile species by chemical treatment of the sample. Subsequently, the concentration of the inert redox state of the metal can be calculated based on the difference in measurements. The selection of pH conditions, addition of complexing agents, and pre-treatment of samples can all be used for the selective determination of specific labile oxidation states of metals. Therefore, voltammetric methods are perfectly suited for the determination of redox species of redox-sensitive metals. Table 3 summarizes the voltammetric methods and the ligands commonly added for redox speciation analysis of trace metals.

Table 2

Available voltammetric methods for the determination of labile fractions of some typical trace metals.

Voltammetric Methods	Trace Metals	Added ligands	Reference
CLE-AdCSV	Cu	Tropolone, Catechol	[53,55]
	Fe	NN, DHN, TAC	[60–62]
	Co	DMG	[47]
	Ni	DMG	[48]
ASV	Cu	–	[25,74]
	Pb	–	[87]
	Zn	–	[45]
CLE-ASV	Cu	EDTA	[88]
	Zn	EDTA	[89]
	Cd	ethylenediamine	[90]
Reverse titration	Cu	SA	[76]
	Fe	NN	[77]
AGNES	Zn, Cd, Pb	–	[80]
SCP	Cu	–	[91]
	Hg	–	[92]

Table 3
Voltammetric methods for redox speciation analysis of trace metals.

Metals	Redox state	Methods	Ligands	Reference
Antimony (Sb)	Sb(III)/Sb(V)	ASV	–	[93]
		AdCSV	Chloranilic acid	[94]
			Catechol	[95]
			Pyrogallol	[96]
Fe	Fe(II)/Fe(III)	AdCSV	NN	[97]
			2,2′-dipyridyl (Dp, masking agent)	
			Bromate (catalyst)	
Cr	Cr(III)/Cr(VI)	AdCSV	Diethylenetriaminepentaacetic acid (DTPA)	[98]
			NO_3^- (oxidizer)	
Thallium (Tl)	Tl(I)/Tl(III)	ASV	DTPA (masking agent)	[99]
Arsenic (As)	As(III)/As(V)	ASV	–	[100]
		CSV	–	[101]
			Copper	[102]
			Pyrrolidine dithiocarbamate	[103]

3.3. Covalently bound species

Covalent binding, such as methylation, has a significant effect on the toxicity of metal elements. To the best of our knowledge, only the direct determination of Cd and Hg has been reported for methylated species. By adjusting the pH of the supporting electrolyte to 8.0, methylcadmium (MeCd) and Cd^{2+} were determined simultaneously by DPASV using a Hg film electrode [104]. When a sufficiently long deposition time (30 min) was applied, a high sensitivity could be obtained which was suitable for the determination of MeCd in uncontaminated marine water. MeHg can be selectively determined by ASV using a nanostructured Au electrode after Hg^{2+} is masked by DTPA or reduced by stannous chloride [105,106]. The use of voltammetric methods for determination and speciation analysis of Hg in natural waters has been reviewed in detail previously [107,108]. However, since the introduction of gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS), these voltammetric methods have rarely been applied for the determination of covalently bound species, due to the long deposition time requirements [12].

4. Speciation analysis of typical trace metals in natural waters

4.1. Copper

In previous speciation analysis literature, Cu is the most widely studied trace metal in freshwater environments [12]. Cu is an essential element for the growth of phytoplankton, while it is toxic to algae and shellfish at free Cu^{2+} concentration ($[Cu^{2+}]$) greater than 0.01 nmol L^{-1}, thus inhibiting its growth [109,110]. As more than 99 % of Cu exists in the form of organic complexes, its bioavailability is significantly reduced. As a result, $[Cu^{2+}]$ are often considered to be low enough to pose no harm to the ecosystem [2,111]. However, recent studies have shown that organically complexed Cu can also be utilized by some phytoplankton, which indicates that the bioavailability and toxicity of Cu may be underestimated if only the $[Cu^{2+}]$ is considered [111,112]. The transformation of different Cu species and their uptake by plankton and filter feeders is presented in Fig. 2 [113].

Snow and rain sample analysis was conducted using the ASV method by Cheng et al. (1994), with results indicating that the proportion of ASV-labile species was 50 % and 64 % of the total Cu concentration in snow and rainwater samples, respectively [114]. Speciation analysis of Cu in a eutrophic lake (Lake Greifen, Switzerland) was performed by Xue et al. (1993) using CLE-AdCSV with catechol as the AL [115], resulting in two classes of ligand being identified, the stronger L_1 ($logK_{CuL}$ 13.9–14.9) and the weaker L_2 ($logK_{CuL}$ 11.8–12.9). The Cu^{2+} and total Cu concentrations were in
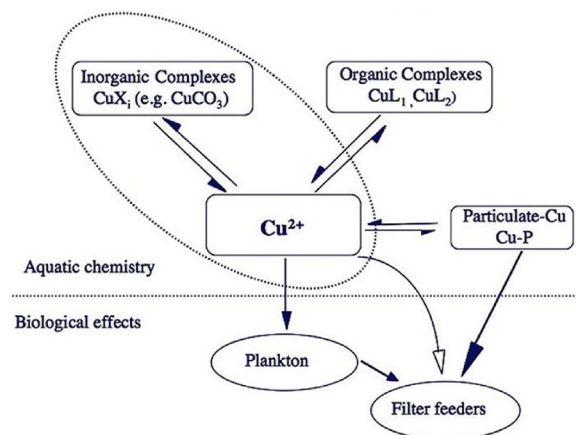

Fig. 2. The speciation, transformation and fate of Cu in natural waters. Reprinted with permission from Ref. [113] Copyright © 2012 American Chemical Society.

the range of 0.1–10 fmol L^{-1} and 5–28 nmol L^{-1}, respectively, indicating that the $[Cu^{2+}]$ was mainly controlled by natural ligands originating from organisms. The strong Cu ligands in glacial streams have also been determined by CLE-AdCSV using SA as the AL [116], showing that ligand concentrations and $logK_{CuL}$ were in the range of 0.2–8.9 nmol L^{-1} and 11.51–13.60, respectively.

L_1 and L_2 classes of ligands can also be detected in seawater samples [117]. L_1 usually exists in surface seawater samples with lower concentrations and $logK_{CuL}$ values of 11−14. Compared to L_1, the concentration of L_2 is generally relatively high with a uniform vertical distribution. One previous study failed to detect L_1 ligands in seawater samples from the Atlantic sector of the Southern Ocean, observing only L_2 ligands with a relatively uniform distribution [118]. The organic complexation of Cu was also investigated in surface seawater samples from the Eastern Mediterranean [119]. Evidence has been presented that Cu can be complexed with humic substances (HS), including humic acid (HA) and fulvic acid (FA) with $logK_{CuL}$ value of 12.08 [120,121]. Traditionally, it is widely accepted that L_1 ligands in seawater are produced by marine phytoplankton and bacteria [69,122]. However, terrigenous HS is another source of L_1 ligands in coastal seawater and studies have shown that river parameters have a significant influence on the speciation of Cu [123,124]. It is generally believed that the source of L_2 is synthetic ligands transported from freshwater environments to the sea, such as EDTA [125]. In recent years, the speciation of Cu in a submarine

hydrothermal environment has also been successfully investigated [126].

4.2. Iron

After discovery of the Fe limitation phenomenon, Fe speciation analysis has become a major issue in marine research due to its crucial role in marine biogeochemistry. As shown in Fig. 3, the vertical distribution of Fe in seawater exhibits a typical nutrient-like profile, with low surface concentration and high concentrations in lower regions, with Fe existing in two oxidation states (Fe(II) and Fe(III)) with different physico-chemical forms [127]. Correspondingly, Fe speciation is commonly investigated by voltammetric methods in terms of redox state and labile fraction concentration.

The oxidation state of Fe can be established using voltammetric methods with Hg electrodes or solid electrodes and masking reagents [46]. Gledhill and van den Berg (1995) adopted Dp as a masking agent for Fe(II) to determine Fe(III) selectively with NN as the complexing agent and bromate as the oxidizer [97]. Using this method, the concentration of Fe(II) can be calculated as the difference between the concentrations of Fe determined in the absence and presence of Dp. The redox speciation of Fe in the northern North Sea was investigated using this method, with Fe(II) present at concentrations up to 1.2 nmol L^{-1}. Aldrich et al. (2001) used 2,2′-bipyridyl (Bp) as a masking agent for the selective determination of Fe(III) [128]. Lin et al. (2016) adopted three-dimensional platinum nanoflowers/titanium carbide nanoparticles modified GCE with Bp as the complexing agent for selective determination of Fe(II) in coastal waters [129]. A Fe^{2+} selective sensor was also fabricated by modifying a carbon screen-printed electrode with polypyrrole and sodium dodecyl sulfate [130]. Labile fractions of Fe have been widely determined using CLE-AdCSV methods (see Table 2). Based on the commonly applied CLE-AdCSV methods, catalytic enhancement by oxygen was introduced in 2014, achieving a remarkable improvement in sensitivity [57].

The speciation of Fe has been investigated in different natural water samples. Nagai et al. (2008) extensively investigated the speciation of Fe in river water using the CLE-AdCSV method with NN as the AL [131]. Results showed that Fe concentration and speciation (organic Fe) were controlled by pH and algal growth, respectively, with the established quantitative model successfully used for the prediction of organically bound Fe concentration based on pH and algal cell density data. The same method was also used for the Fe speciation analysis in lakes [132]. The results of these studies suggested that Fe might be a limiting factor for phytoplankton communities. Compared with freshwater environments, Fe speciation in seawater has been studied more comprehensively. Abualhaija and van den Berg (2014) studied the chemical speciation of Fe and complex stability of natural ligands in Atlantic seawater samples by CLE-AdCSV with SA as the AL [57]. The concentrations of natural ligands and their conditional stability constants were established across the full water column depth profile of seawater samples collected from the North Atlantic (GEOTRACES GA03), using CLE-AdCSV with SA as the added competitive ligand [133]. Fe binding organic ligands and inorganic Fe concentrations in the West Atlantic Ocean were also investigated using CLE-AdCSV with TAC as a competitive ligand [134]. The speciation of Fe in samples from the Mersey River estuary and Liverpool Bay was studied using CLE-AdCSV in the presence of SA at multiple analytical windows [135]. Gledhill and Buck (2012) reviewed the organic complexation of Fe in marine environments in detail, using the CLE-AdCSV method [136].

4.3. Zinc

The concentration of dissolved Zn in open ocean water is generally in the range of $0.05-10$ nmol L^{-1} with vertical distribution patterns exhibiting nutrient-like profiles [137]. Zn is an essential metal element in metabolic enzymes of phytoplankton and the limitation of reactive Zn may lead to changes in the phytoplankton community [17,138]. Similar to Cu, the speciation of
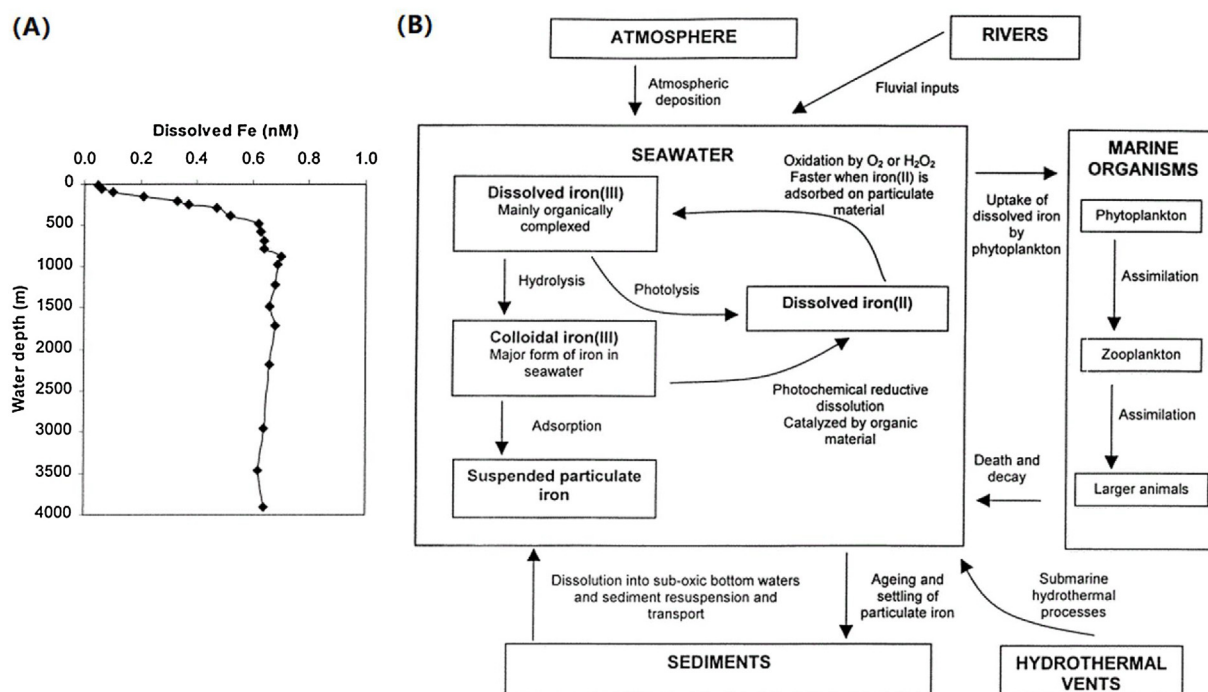

Fig. 3. The typical vertical distribution (A) and biogeochemical cycle process (B) of Fe in seawater.
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Zn can also be determined by ASV and CLE-AdCSV. Considering the low solubility of Zn in Hg, Hg film electrodes are generally adopted when the ASV method is used for the direct determination of reactive Zn. The use of Hg film electrodes can also reduce the reduction of Zn organic complexes on the electrode surface, increasing the accuracy of determination. CLE-AdCSV has been increasingly used with APDC as the added competitive ligand to improve the detection sensitivity and reproducibility for Zn speciation analysis [137]. CLE-AdCSV has become the most commonly applied method for Zn speciation analysis in seawater.

High ASV-labile percentages in the range of 76 %–93 % were found for rain and snow samples [114]. The CLE-AdCSV method was also used for Zn speciation analysis in lakes, showing that the ligand concentration exceeded total Zn with $\log K_{ZnL}$ values in the range of 10.0–11.1 [139]. As a result, the free Zn^{2+} concentration ($[Zn^{2+}]$) was very low, in the range of $5-48\,\mathrm{pmol\,L^{-1}}$, which might be a limiting factor for algal growth. Speciation analysis of Zn in rivers showed that the ASV-labile Zn percentage is very variable [140]. ASV-labile Zn could also be detected *in situ* using an automated system, generating similar results to diffusive gradients in thin films (DGT) in freshwater [141,142]. The speciation of Zn has also been widely studied in seawater, which has been shown to vary greatly with latitude. In high latitude environments such as the Southern Ocean and western North Pacific, the content of Zn complex ligand is low, with more dissolved Zn existing in an inorganic form [137,143]. However, about 98 % of dissolved Zn in surface seawater at low latitudes is organically complexed, with the concentrations of total dissolved Zn and ligands all being lower than those in high latitude seawater environments [17,144]. The organic ligands complexed with Zn in seawater may originate from HS, bacterial secretions, or phytoplankton, which have no relationship with colloidal organic matter [137,145]. The complexes of Zn with organic ligands in the ocean is strong with $\log K_{ZnL}$ values in the range of 10–11, while the concentration of Zn ligands in the marginal sea is higher with relatively lower $\log K_{ZnL}$ values of about 9.5 [137,144,145].

4.4. Cadmium

Cd is an essential element for the growth of phytoplankton which can replace Zn in biochemical reactions under Zn limited conditions. However, Cd is toxic to phytoplankton at high concentrations [146]. The speciation analysis of Cd in water samples can only be determined using the ASV method with similar parameters to Zn [145,147]. Hg film electrodes are usually suggested as the working electrode for Cd speciation analysis. The ligand competitive equilibrium method can be combined with ASV to improve the sensitivity of metal speciation analysis when their concentrations are very low. In CLE-ASV, ethylenediamine is widely adopted as the competitive ligand to increase the concentration of reactive Cd complexes [12].

The speciation of Cd has been studied in fresh and saline waters. In freshwater environments, rivers and lakes have been more comprehensively investigated, with results showing that Cd speciation is highly regulated by the presence of strong natural organic ligands [148]. The vertical distribution of Cd in oceans exhibits a nutrient-like profile, which suggests that it can be taken up by phytoplankton at the surface and remineralized at lower depths [146]. As a result, the concentration of dissolved Cd is generally low (pmol L^{-1} levels) in surface seawater environments. The vertical variation in reactive Cd concentrations is also significant [149]. It has been reported that about 70 %–99 % of the dissolved Cd in open ocean surface waters exists in an organically complexed form, while the complexation degree in estuarine and coastal waters ranges from 20 %–80 % [145,147,150]. Interestingly, almost all of the dissolved Cd exists in reactive form

in the South China Sea and northern coastal waters of France [151,152]. The organic ligands of Cd in seawater are usually classified as only one group which can form strong Cd complexes with $\log K_{CdL}$ values of 9–12 [147]. Humus from marine phytoplankton may be the only source of weak organic ligands for Cd [145,147].

4.5. Lead

Pb is a highly toxic metal element, which is strongly affected by human activities and is a hazard to human health due to the risk of bioaccumulation [30]. ASV is the main voltammetric method for speciation analysis of Pb with high sensitivity and good reproducibility. In fresh waters, CB can be used as the competitive ligand to achieve Pb speciation analysis by CLE-AdCSV method [65]. In seawater, only the ASV method can be adopted for the determination of Pb species. Hg electrodes are commonly utilized for ASV determination of Pb. Considering the toxicity of Hg and therefore, the difficulty in handling processes, a series of environmentally friendly electrodes such as micro-electrodes have been developed for Pb speciation analysis [30,153].

A large number of studies on Pb speciation analysis have been performed on lake or river environments with different degrees of Pb contamination [65,140,142,154]. ASV-labile fractions were found to mostly range from 5 %–15 % of the total Pb content, with only a few studies detecting larger labile fraction contributions. The concentration of dissolved Pb in seawater is usually in the range of $10-80\,\mathrm{pmol\,L^{-1}}$ with the vertical distribution exhibiting a scavenging-type profile, decreasing with depth [155]. It has been reported that about 55 %–95 % of Pb in seawater is organically complexed, which significantly reduces the toxicity of Pb to marine organisms [150,156]. In contrast to Cu, unstable Pb organic complexes are usually formed, which are readily available for biological uptake and geochemical scavenging [153]. Although two kinds of Pb ligands have been reported, the organic ligands of Pb in seawater are generally classified as a single group with $\log K_{PbL}$ values ranging from 8.5–14 [65,117,150,157].

4.6. Other metals

The speciation analysis of other metals such as Co, Ni, Sb, Al, Cr, Tl and As, can also be accomplished by voltammetric methods. The determination and speciation analysis of Co and Ni are commonly conducted by AdCSV with DMG as the competitive ligand [47,48]. Saito and Moffett (2001) investigated the complexation of Co by natural ligands in the North Atlantic with $\log K_{MAL}^{cond} = 16.3 \pm 0.9$ obtained [47]. The similar CLE-AdCSV was also adopted for the kinetic studies of Ni speciation in aqueous environmental samples [48]. 1,2-cyclohexanedione dioxime was also reported as competitive ligand for speciation analysis of Co [63]. Different complexing reagents, such as chloranilic acid, catechol and pyrogallol, were adopted for the redox speciation of Sb using AdCSV [94–96]. Quentel and Filella (2002) also determined the inorganic Sb species, Sb(III) and Sb(V), in seawater rapidly with ASV [93]. Wang et al. (2001) determined five species (labile monomeric, total monomeric, acid reactive, non-labile monomeric and acid soluble) of Al in natural waters by AdCSV with solochrome violet RS as complexing reagent [158]. CLE-AdCSV was also used for speciation analysis of Al to obtain the electroactive fraction and complexing parameters with cupferron as competitive ligand [64]. Bobrowski et al. (2009) reviewed the voltammetric methods for Cr determination and speciation analysis, focusing on the catalytic AdCSV in the presence of DTPA and nitrate [98]. The redox speciation analysis of Tl, Tl(I) and Tl(III), can be achieved by indirect ASV in the presence of DTPA (masking agent) and acetic buffer [99]. Inorganic As in the environment is of considerable because of its

toxicity and frequently higher concentration level than the maximum allowable value. ASV and CSV are the two main techniques for the redox speciation analysis of As in natural waters [100,101]. Mays and Hussam (2009) reviewed the voltammetric determination and speciation analysis of inorganic As in the environment in detail [159].

5. Conclusions and future perspectives

Trace metals are essential for biological metabolic processes and biogeochemical cycling in natural waters. The toxicity and bioavailability of metals have a closely relationship with their species. The chemical forms of dissolved metals are mainly controlled by organic ligands in natural waters, significantly reducing their bioavailability. Speciation analysis seems to be an important route to study feedback interactions between metals and phytoplankton in natural waters. At present, voltammetric techniques are the most suitable and widely adopted methods for metal speciation analysis with high sensitivity and well-established reaction mechanisms.

This review summarizes the theory and application of voltammetric methods for speciation analysis of trace metals in natural waters. The voltammetric methods including ASV and CLE-AdCSV for speciation analysis were discussed in detail. The application of voltammetric methods in discriminating between labile/inert fractions, redox species and covalently bound species was also presented. Determination of labile/inert fractions is one of the most important aspects of metal speciation analysis in natural waters. Different methods such as ASV (CLE-ASV), CSV (CLE-AdCSV), reverse titration, AGNES, and SCP can be used for this purpose. Finally, the speciation analysis of typical trace metals including Fe, Cu, Zn, Cd and Pb in natural waters (especially seawater) using voltammetric methods was described.

Although voltammetric methods exhibit excellent performance for speciation analysis of a large number of metal ions, there remain some issues and limitations that need to be resolved. The relationship between bioavailability and speciation of metals remains unclear and needs to be further studied. Research in voltammetric speciation analysis of metals is mainly driven by oceanographic scientific studies, and then subsequently adapted to freshwater environments. Compared with seawater, less is known on speciation of trace metals in freshwaters. Furthermore, most speciation analysis of metals is conducted in laboratory environments and there is an urgent demand for new sensing methods and devices that allow *in situ* and real-time speciation analysis in natural waters.

The development of voltammetric analysis methods and novel working electrodes is also urgently needed. Hg electrodes such as HMDE and Hg film electrodes are commonly adopted for determination of different metal species due to their high sensitivity and stability. Considering the toxicity of Hg, it remains necessary to develop novel environmentally friendly electrodes as a substitute for Hg electrodes. Moreover, strategies to reduce the purging time, complexation time, deposition time and operator time in voltammetric speciation analysis of metals are also required. Finally, to achieve a more detailed and comprehensive description of the speciation of trace metals in natural waters, different complementary analytical methods with specific advantages should be combined effectively.

In summary, voltammetric methods have the potential to be powerful analytical techniques for metal speciation analysis and are likely be developed rapidly in the future. Combining all the improvements discussed in this study, the physicochemical characteristics of trace metals in natural waters established using voltammetric methods can be correlated with bioavailability in interdisciplinary studies. This will result in a more detailed and comprehensive understanding of the relationship between bioavailability and speciation of trace metals in natural water environments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.teac.2021.e00119.

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