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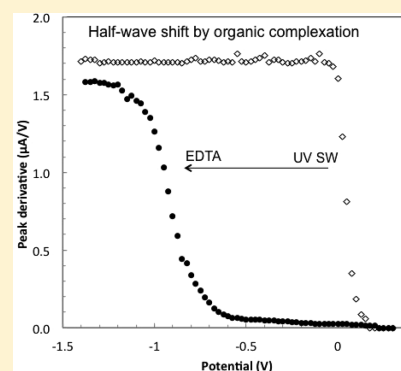
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Pseudopolarography of Copper Complexes in Seawater Using a Vibrating Gold Microwire Electrode

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ABSTRACT: Copper (Cu) in seawater can be determined by anodic stripping voltammetry using a vibrating gold microwire electrode (VGME) with a much lower limit of detection than using a mercury electrode, enabling detection of labile Cu at trace level. The possibility of pseudopolarography of Cu using the VGME is investigated here and is calibrated against known chelating agents. The sensitivity much (15-fold) improved by application of a desorption step to remove adsorbed organic substances and excess anions. The notorious tendency of solid electrodes to be affected by memory effects was overcome by a conditioning interval between measurements that stabilized the electrode response. Model ligands, including EDTA, humic substances (HS), and glutathione (examples of natural ligands) were analyzed to calibrate the half-wave shift to complex stability. The half-wave shift on the VGME is much greater ($\sim 2\times$) than that on the mercury drop electrode which is attributed to several parameters including a much (5-fold) thinner diffusion layer on the VGME. Experiments showed that the same procedure is suitable for pseudopolarography of zinc. Application of the new method to samples from the Irish Sea showed Cu occurring in several complexes, all strongly bound, and some occurring in the electrochemically reversible region of the pseudopolarogram. The humic substance complex of Cu was also found to occur in the reversible region of the pseudopolarogram. The pseudopolarograms of Cu in seawater were unaffected by sample filtration and did not require purging to remove dissolved oxygen, suggesting that this method can be readily used as part of an in situ measuring system.



INTRODUCTION

Biogenic trace metals copper (Cu) and zinc (Zn) are essential micronutrients, serving as cofactors in a number of enzymes essential to marine algae.¹ Cu and Zn are divalent metals; however, in seawater inorganic Cu is stabilized to some extent as Cu^I by complexation with chloride² and thiols.³ Complexation with organic matter amounts to 99% of all dissolved Cu,^{4,5} and similarly Zn for ~ 98 –99%.^{6,7} Knowing the distribution of a metal between its “free” inorganic fraction and its organic complexed fraction (the metal speciation) is important as bioavailability is generally increased when the metal occurs in the inorganic form,⁸ although this is a simplified view as complex lability plays a role in dynamic speciation and bioavailability.⁹

Complexing ligand concentrations in natural waters are determined by complexometric titration with detection of the metal by cathodic stripping voltammetry with ligand competition,¹⁰ or by anodic stripping voltammetry (ASV).^{11,12} Subsequently, the natural ligand concentration and its conditional stability constant are used to compute the speciation in the original sample. In reverse titrations the complexed metal is progressively drawn out of its complexes by titrating with a competing ligand.¹³ Pseudopolarography also probes the already complexed fraction, but uses increasingly negative electrode potentials until the metal, or the complex, is reduced. The process is similar to polarography using a dropping mercury electrode, but the response at each potential point is enhanced by a deposition step.^{14–16} Pseudopolarograms are

constructed using scanned-potential stripping techniques,¹⁷ primarily ASV and anodic stripping chronopotentiometry (ASC) in which the deposition potential of each individual scan is varied.

From the difference between the half-wave potentials of the inorganic metal and its electrochemically irreversible organic complexes, the *thermodynamic* stability constant of the organic species is calculated.¹⁸ This is different from complexing capacity titrations with detection by ASV or CSV from which *conditional* stability constants for chemically reversible complexes are obtained (the various competing reactions reach equilibrium within the time scale of the measurement). A species is electrochemically irreversible if its reduction potential is much more negative than its oxidation potential. Kinetic factors may make a species electrochemically irreversible (on a time scale of milliseconds) while it is reversible on the much longer chemical time scale of hours. Electrochemically reversible complexes cause a shift of the pseudopolarographic wave for the metal toward more negative potential,^{19,20} where the shift is related to the complex stability. The complex stability is related to the conditional stability constant (which takes side-reactions into account) and the ligand concentration.

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Reversible species are characterized by their dissociation in the diffusion layer followed by the reduction of the free metal ion, whereas irreversible species do not dissociate until the metal within the complex is reduced at a potential related to the thermodynamic stability constant. Half-wave potentials for reversible species are within at most a few 100 s of mV lower than that of the inorganic metal, whereas those of irreversible species tend to show a much greater shift: for this reason the two types of waves are easily separated.

Pseudopolarography has the potential to determine metal speciation in unmodified samples, without metal or ligand additions, if the limit of detection (LoD) is sufficiently low to detect a fraction of the metal concentration. Previous work on pseudopolarography has used mercury-based electrodes: a hanging mercury drop electrode (HMDE),^{21,22} a mercury thin film electrode,²³ and mercury hemisphere microelectrodes.^{24,25} Mercury electrodes have been used successfully for pseudopolarography of Zn in seawater,^{15,26} Pb in sulfidic and oxic seawater,²⁷ Cd in fresh water,²⁸ and Pb in porewaters.²⁵ Mercury electrodes do not have sufficient sensitivity for pseudopolarography of Cu in uncontaminated seawater as the peak for Cu is on the shoulder of that for Hg (LoD of 2.5 nM Cu²⁹), and pseudopolarography requires levels of 10× the LoD. The half-wave shift has previously been related to values for thermodynamic stability constants by calibration against a "chelate scale" of known complexing ligands.^{15,22}

We present here a method for pseudopolarography of Cu in uncontaminated seawater using a solid, vibrating gold microelectrode, which has a LoD <0.1 nM Cu^{30,31} which is 20× better than achievable by mercury electrodes. The method can also be used for Zn, and has possibility for in situ speciation of Cu as it is not affected by dissolved oxygen (DO). The method was tested in seawater samples from Liverpool Bay at trace levels of Cu and Zn.

■ EXPERIMENTAL SECTION

Reagents. Water used to prepare reagents and preliminary working solutions was deionized water (Millipore Elix 3 with Milli-Q gradient deionizer and UV) with a resistivity of 18 MΩ cm. Standard solutions of Cu^{II} and Zn^{II} were prepared by dilution of stock solutions (1000 mg·L⁻¹ in nitric acid, atomic absorption standard solutions, AnalaR BDH Chemicals Ltd., U.K.), acidified to pH 2 with HCl. Any unacidified stock solutions were prepared freshly for use. HCl was purified by sub-boiling distillation on a quartz condenser. 0.5 M H₂SO₄ used for electrode conditioning was from BDH (AnalaR grade). Humic acid and fulvic acid was Suwannee river fulvic acid and humic acid from the International Humic Substances Society. HEPES buffer (purity 99%, Acros organics, U.K.) contained 1 M (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Fisher scientific, U.K.) and 0.5 M NaOH, and had its pH adjusted to pH 7.85 using NaOH. POPSO buffer (purity 99%, Acros organics, U.K.) contained 1 M Piperazine-1,4-bis-(2-hydroxypropane-sulfonic acid) dihydrate and had its pH adjusted to pH 7.85 using NaOH. HEPPS buffer solution (purity 99%, Acros organics, U.K.) contained 1 M (3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid and had its pH adjusted to pH 8 using NaOH.

The model ligands nitrilotriacetic acid (NTA, 99%, Fluka, puriss p.a), 2-hydroxybenzaldehyde (Salicylaldoxime, Sigma, Germany), dodecane (99%, Fisher scientific, U.K.), Dopamine hydrochloride (sigma, Germany), 3, 4-dihydroxy-DL-phenyl-

alanine (DOPA, Sigma, Switzerland), *n*-nonane (>99%, Acros organics), ethylenediaminetetraacetic acid (EDTA, BDH, analaR, Poole, U.K.), dimethylglyoxime (DMG, Sigma), L-cysteine (>99%, Sigma), 1,2-cyclohexanedione dioxime (nioxime, Sigma), ethylenediamine (>99.5%, puriss p.a. absolute, Fluka), diethylenetriaminepentaacetic acid (DTPA, Acros organics), glutathione (>98%, BDH, analaR, Poole, U.K.), 1,2-diaminocyclohexanetetraacetic acid (CDTA, >98%, agross organics), and cyclen (97%, Fisher scientific) were added to UV digested seawater buffered with HEPES (pH 7.85).

Sample Collection and Pretreatment. Seawater used for optimization experiments was collected from the Irish Sea, filtered (0.2 μm filtration cartridge) and stored in an acid rinsed 50 L polyethylene container. Individual seawater samples for on-board and laboratory analysis were collected (7 m depth) during a survey of the Irish Sea with the RV Prince Madog (June 2010) using a rosette-mounted, Niskin bottle (General Oceanics, 5 L), modified to obtain samples uncontaminated with metals (Teflon coated spring on the outside, no rubber inside). Samples to be analyzed in the laboratory were filtered (0.45 + 0.2 μm Sartobran 600 filtration cartridge) and transferred to 500 mL acid washed Nalgene low-density polyethylene bottles stored frozen on board the ship. A second bottle of each sample was acidified to pH ~ 2 using HCl for later metal analysis. A composite sample was made up from water samples collected in Liverpool Bay (March 2010), filtered and kept in an acid cleaned 50 L polyethylene container. This water was used for method development. Natural organic matter in seawater was destroyed, when required, by UV irradiation of seawater samples (30 mL) using a 125 W UV lamp (home-built). The quartz sample tubes were conditioned using neutral pH seawater prior to use to minimize adsorption, and irradiation was for 45 min.

Instrumentation. Voltammetric instrumentation was a 696 VA electrode stand from Metrohm, Switzerland, connected via an interface (IME663, Autolab) to a μAutolab(III) (Metrohm, Switzerland) potentiostat. Data was processed with GPES 4.9 software. A 50 mL three-electrode cell (glass) was used containing the working electrode (WE) consisting of a gold microwire; a counter electrode (CE) consisting of a 200 μm iridium wire (from Goodfellow, U.K.); and a Ag/AgCl/KCl(3M)//KCl(3M) double junction reference electrode (RE). Both the WE and the CE were heat-sealed in a propylene pipet tip, with 3 mm protruding as adapted from before,^{32,33} where the fitting was adapted to facilitate tip replacement and vibration (patent applied for).³⁰ The cell was PTFE for analyses and a separate, glass, cell was used for electrode preparation in 0.5 M H₂SO₄. The gold microwire (99.99%, hard, Goodfellow) was fitted within a 100 μL polypropylene pipet tip (uncoloured). This tip was then fitted onto a 1 mL polypropylene pipet tip, which had a vibrator incorporated and a connecting electrical cable protruding ~1 cm at the bottom, which made contact with the WE. The vibrator was driven by a 1.5 V power supply (home-built converter of 5 to 1.5 V), which was interfaced to, and powered by, the IME (Autolab, Metrohm, Switzerland), and controlled by the stirrer on/off trigger in the software (GPES, Autolab). The diameter of the microwire electrode was 5 μm and the length approximately 0.5 mm, unless otherwise stated. The surface of the WE was cleaned electrochemically by hydrogen generation at -2 V for 60 s in 0.5 M H₂SO₄, followed by cyclic voltammetry (5 scans) between 0 and 1.5 V (100 mVs⁻¹) until

the response was stable,³¹ which was usually from the second scan.

The total surface area and the geometric area of the microwire electrode were determined as previously described.³¹ Briefly, an oxide monolayer was first formed at a positive potential in 0.5 M H₂SO₄ and its reduction charge used to calculate the total area, assuming a 450 $\mu\text{C cm}^{-2}$ charge density for an oxide monolayer. The length was calculated assuming a constant diameter of 5 μm (commercial specification and checked by scanning electron microscope) from the chronoamperometric current obtained in a solution of 10 mM ferricyanide (in 0.5 M KCl). The geometric area was calculated from the diffusion-limited current obtained under vibrated conditions.

For analysis conducted on board the Prince Madog (June 2010) the voltammetric cell was placed inside a Faraday cage (Windsor Scientific, U.K.) instead of the VA 696 stand.

Voltammetric Parameters Used for the Pseudopolarograms. Fifteen milliliters of seawater were transferred to the voltammetric cell, and the pH was buffered to 7.85 with 10 mM HEPES. The solution was purged with N₂ for 15 min, and a N₂ blanket maintained thereafter. The WE was conditioned prior to the start of the experiment using a sequence of 10 ASV scans, each preceded by a 30 s deposition time at -1.4 V and a 60 s cleaning step at $+0.5$ V, in the seawater that is to be used for the pseudopolarogram. The peak for Cu was measured, and it was checked that this peak height stabilized during the 10 scans.

Each pseudopolarogram was built up of a series of voltammetric or chronopotentiometric scans preceded by deposition at various potentials, the deposition potentials constituting the individual points in the pseudopolarogram. Each scan was preceded by a conditioning step to clean the electrode consisting of a conditioning potential at -1.4 V (30 s), followed by a cleaning step at $+0.5$ V (60 s). The deposition time was 60 s at each selected deposition potential (electrode vibration on) and was followed by a 1 s anion-desorption step at -1.2 V (vibration off) to remove adsorbed organics and anions.^{31,34} The -1.2 V anion-desorption step was unstirred and doubled as quiescence step. The voltammetric scan was initiated immediately thereafter and was always from -1 V to $+0.5$ V unless indicated differently. Electrode vibration was on during the initial N₂ purge (when used), the conditioning steps at -1.4 and $+0.55$ V, and the deposition step. A background scan was made after each analytical scan using the same parameters but with a deposition time of 1 s, and was subtracted from the analytical scan. Scan parameters were square-wave mode, step: 8 mV, amplitude: 50 mV, frequency: 50 Hz. The derivative of the background-corrected peak signal was used for quantification. The Zn peak was located at -0.55 V and the Cu peak at $+0.2$ V. The pseudopolarograms had a resolution of 25 mV between individual points. The individual points were obtained in a nonsystematic manner to avoid introducing systematic errors. The deposition potential steps of the pseudopolarograms followed the following sequence: -1.4 to 0.3 V in 100 mV steps, then 0.275 V to -1.325 V in 100 mV steps, then -1.35 to 0.25 V in 100 mV steps, and then 0.225 V to -1.375 V in 100 mV steps.

Pseudopolarograms of Model Ligands. Experiments with model ligands were carried out in filtered bulk seawater from Liverpool Bay collected in March 2010. The seawater was UV-digested (UV-SW) and 10 mM HEPES was added giving a final pH of 7.85. The Cu concentration was 17 nM, and model

complexing ligands were added in large excess of the Cu. The model ligands were selected to cover a broad range of functionality and range of complex stabilities, and most have been used previously in pseudopolarography of Cu at a mercury drop electrode.²² Thermodynamic stability constants for the complexes ($\log K^*$ values) ranged from 12.4 for CuNTA to 26.5 for Cu-cyclam.

THEORY

The theoretical background to pseudopolarography using mercury electrodes has been discussed in detail previously²² (and references therein). The same equations are used here to obtain half-wave potentials from pseudopolarograms, and to relate the shift in the half-wave potential to the value of the conditional stability constant for reversible complexes and to that of the thermodynamic stability constant for irreversible complexes.

Equation to Obtain Half-Wave Potentials from Pseudopolarograms. The modified Lingane equation^{22,35} was used to obtain values for half-wave potentials from pseudopolarograms:

$$\log[(i_{\max} - i)/i] = (E_d - E_{1/2})\alpha n/0.059 \quad (1)$$

where i_{\max} is the peak height of copper (which is a measure of the reduction current) obtained under diffusion-limited conditions at deposition potentials $E_{\text{dep}} < E_{1/2}$ of the relevant species, i is the peak height at each E_{dep} , α is the transfer coefficient, n is the number of exchanged electrons. By plotting $\log[(i_{\max} - i)/i]$ as a function of E_{dep} , $E_{1/2}$ is obtained at the potential where the log-term is zero ($i = i_{\max}/2$). The value for $E_{1/2}$ is independent of the transfer coefficient, α , which means that this procedure can be used to obtain $E_{1/2}$ values for reversible as well as irreversible systems. The slope equals the product of α with the number of electrons of the electrode reaction.

Equation to Calculate Conditional Stability Constants from the Shift in $E_{1/2}$ for Reversible Complexes.

$$E_{1/2,\text{complex}} = E_{1/2,\text{Cu}'} - (0.059/n)(\log K'_{\text{CuL}} - p \log[L'] + \log(i_1/i_{1,\text{complex}})) \quad (2)$$

where $E_{1/2,\text{Cu}'}$ = the half-wave potential for inorganic Cu in pH 8 seawater, $n = 2$ ($\text{Cu}^{\text{II}}/\text{Cu}^0$), and p is the number of ligands binding Cu^{2+} showing that the half-wave potential depends linearly on $\log[L']$ (simplified from ref 36, p 148)).

Equation That Relates the Shift in $E_{1/2}$ to the Value of the Thermodynamic Stability Constant of Irreversible Complexes. In case the complex does not dissociate in the diffusion layer, it is electrochemically irreversible (inert), and the complex is destroyed at a negative potential, releasing the metal to react with the electrode (forming an amalgam if the electrode is mercury²²). In this case the following equation is valid for the shift in the half-wave potential:¹⁸

$$E_{1/2} = E_0 - 0.059/n \log K^*_{\text{ML}} + (0.059/\alpha n) [\log(k_s) + \log(\delta/D)] \quad (3)$$

where K^*_{CuL} is the thermodynamic stability constant, δ is the diffusion layer thickness, D the diffusion coefficient, and k_s the rate constant. According to eq 3, the shift in the half-wave potential of inert complex species is independent of the ligand concentration. However, in addition to the thermodynamic

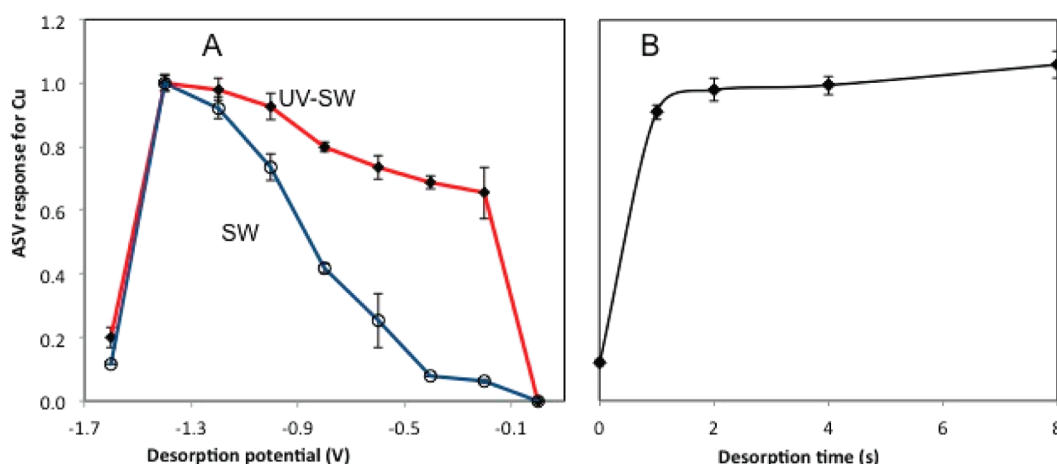


Figure 1. Optimization of the desorption potential that is applied after the deposition step immediately prior to the scan; $E_{\text{dep}} = -0.2$ V for 60 s. (A) Normalized-ASV response as a function of the desorption potential. The response was separately normalized to the maximum response at -1.4 V in each experiment. (B) Effect of varying the length of the -1.2 V desorption time prior to the scan.

stability constant this equation contains two parameters that affect the half-wave potential of the complexes: the diffusion layer thickness and the rate constant.

RESULTS

Preliminary experiments were carried out to optimize electrode and procedural sensitivity and stability at least over the duration of an experiment (several hours). Cu measurement does not require degassing, but solutions were nevertheless N_2 -purged to eliminate interference of DO with the peak for Zn. The purging also minimizes any changes in the pH at the electrode surface, which might affect the speciation, albeit only over a short time span of a few milliseconds when the copper species are in the diffusion layer during the deposition step. pH buffers were used to minimize this pH change although the carbonate in seawater already has a strong pH buffering effect. The shape and height of the copper peak were greatly improved by making use of a desorption potential to remove adsorbed organic matter and anions prior to the scan. Measurement stability was greatly improved by the use of a conditioning interval between measurements, which eliminated memory effects. This conditioning interval consisted in the imposition of a negative potential (-1.4 V) for 30 s, followed by a more positive, reoxidation, potential (60 s at 0.55 V) to reoxidize plated metals between measurements; the vibrator was on during those steps, and the timings are optimized for the deposition time used in this work (60 s).

Choice of Buffer. Buffer solutions were tested in UV digested seawater for interference with the voltammetric scans. POPSO (pH = 7.85) and HEPPS (pH = 8) added to 10 mM to UV digested seawater (UV-SW) were found to interfere by causing suppression of the pseudopolarogram in the shape of an elongated wave, indicating that an irreversible Cu complex was formed. No interference was caused by 10 mM HEPES (pH = 7.85), which was therefore chosen for optimization experiments. Pseudopolarograms of Cu in UV-SW with and without the HEPES buffer were the same suggesting that pseudopolarography of Cu can be carried out in seawater without the need for external buffering.

Desorption Potential. Adsorption of anions (chiefly bromide and chloride) on the surface of the gold electrode is known to adversely affect the ASV sensitivity for Cu in seawater. The sensitivity is increased and stabilized by

application of a desorption potential (thought to cause desorption of adsorbed anions) immediately prior to the scan.³¹ Voltammetric scans using the mercury drop electrode are similarly improved by a desorption step used to remove adsorbed surface-active substances.^{34,37} The peak for trace Cu using a relatively positive deposition potential is very small in the presence of organic complexation unless the deposition time is extended or an anion desorption step included. The value of the desorption potential (E_{des}) was varied in the range 0 to -1.6 V while measuring trace Cu in seawater, before and after UV-digestion to also test for effects of organic matter. The desorption time was 1 s (vibrator off) at each value of E_{des} and was preceded by 60 s deposition at -0.2 V (vibrator on), which is just negative of the reduction potential of $\text{Cu}^{\text{II}}/\text{Cu}^0$ in seawater at the mercury electrode (this would be independent of the underpotential adsorption effect on the gold electrode). The peak current was found to increase strongly with decreasing desorption potential, reaching a maximum at -1.4 V, followed by a strong decrease when $E_{\text{des}} \leq -1.6$ V possibly because of the reduction of sodium (Figure 1A). The increase in response was obtained in organic-free seawater (UV-digested), but the effect was much greater in the untreated seawater, indicating that the signal enhancement was mostly due to removal of adsorbed organic matter prior to the scan. The response in the untreated and organic-free seawater were both maximum at desorption potentials between -1.2 and -1.4 V (Figure 1A). The actual response was much greater in the untreated seawater due to organic complexation of Cu. The overall effect was very large in the untreated seawater, causing a 15-fold increase in peak intensity at E_{des} values between -1.2 and -1.4 V highlighting the importance of this desorption potential which much facilitated the pseudopolarography of natural copper species in seawater, and that should be considered for any ASV method using solid and mercury electrodes. A desorption potential of -1.2 V was adopted as the response was similar to that at -1.4 V and as it better avoids the steep decrease at potentials less than -1.4 V. Mercury drop electrodes also benefit from a desorption step³⁴ though the effect is less pronounced.

The length of the desorption step ($E_{\text{des}} = -1.2$ V) was varied from 0 to 8 s at a constant deposition time of 60 s in non-UV-digested seawater. The desorption effect on the peak height was found to be completed within 1 s after which no further

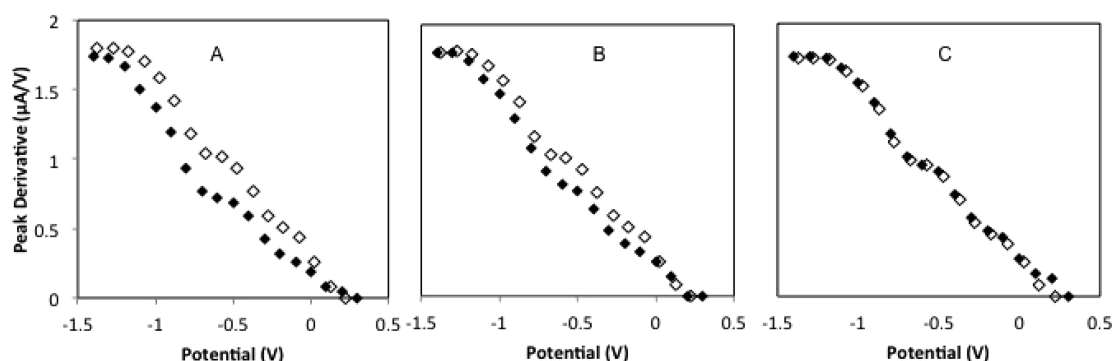


Figure 2. Effect of application of a conditioning step on the pseudopolarograms. Forward (negative going, filled symbols) and reverse (open symbols) pseudopolarogram are shown to highlight the effect. (A) Each scan preceded by a 60 s interval at 0.55 V to remove any copper plated in the previous scan; (B) 20 s at -1.4 V and 60 s at 0.55 V; (C) 30 s at -1.4 V and 60 s at 0.55 V. The selected optimized condition was $E_{\text{dep}} = -1.4$ V, $t_{\text{dep}} = 30$ s, $E_{\text{cond}} = 0.55$ V, $t_{\text{des}} = 60$ s; scan -1 to 5 V, $f = 50$ Hz, pulse height = 50 mV, step = 8 mV.

improvement was apparent. The 1 s desorption step at -1.2 V was employed from now on immediately prior to all voltammetric stripping scans. The duration of this step is much less than that of the deposition step of each point in the pseudopolarogram, and the vibration was switched off, so that it had a negligible effect on the amount of Cu that was being deposited. The short desorption time used here is possible because of the use of the vibrating electrode where hydrodynamic perturbations are localized within the close vicinity of the electrode. When the solution is stirred a typical period of 8–10 s is required to obtain a stripping scan free of hydrodynamic noise. In that case, Cu accumulation at the equilibration potential would be significant which would affect the outcome of the pseudopolarogram.

Memory Effects. The optimized conditions were tested in pseudopolarograms of Cu in seawater. Pseudopolarograms carried out in forward and reverse directions (without using the conditioning procedure between scans) were found to show the expected polarographic wave. However, the forward and reverse pseudopolarograms were found to be displaced by up to 325 mV from each other, with a large, reproducible, hysteresis (Figure 2A), suggesting a persistent memory effect in the electrode response. The returning pseudopolarogram (in a positive potential direction) had the half-wave potential more positive, and the currents were greater than of the negative going pseudopolarogram. Repeated measurements after deposition at various potentials suggested that this hysteresis was caused by continued desorption of anions at the negative potentials, which was not resolved by the 1 s desorption step at -1.2 V. It is known that processes of adsorption/desorption on the gold polycrystalline electrode are slow to reach equilibrium, and their magnitude is highly dependent on the potential variations imposed at the electrode.³¹

The hysteresis was the cause of a major bias in the half-wave potentials determined from pseudopolarograms in opposite direction. Experiments were conducted to negate the memory effect by returning the electrode to a constant initial state with respect to anion and organics adsorption, and free of deposited metal prior to each subsequent voltammogram. After several experiments in which the electrode stability was determined by repeated ASV scans the following potential sequence was found to be optimal: an anion desorption potential of -1.4 V was applied for 30 s and this was followed by a cleaning step at $+0.55$ V for 60 s to remove any plated metals. This way at first anions were desorbed (and copper plated) for a significant time

at the most negative potential used in the pseudopolarograms and subsequently anions readsorbed at the most positive potential at which point the deposited Cu was reoxidized at the same time. This procedure was alternated with the scans used for the pseudopolarogram, which meant that the electrode was conditioned consistently for each subsequent measurement. Other ratios of the oxidizing and desorption potentials were tested, but did not have the same effect (Figure 2B and 2C). Thirty seconds at -1.4 V followed by 60 s at $+0.55$ V were found to be the minimum time necessary for the conditioning step to be fully effective. The memory effect of the scans was completely removed by this conditioning interval between each voltammetric scan. This interval was found to be suitable also when the deposition period was extended up to 300 s with no need for further optimization. The conditioning procedure was found to work equally well for gold microwires of diameters, 5, 10, and 25 μm ; however, a 100 μm wire was found to require longer periods of conditioning: -1.4 V, 90 s followed by 0.55 V for 180 s. The conditioning protocol of 10 voltammetric scans (plating at -1.4 V, 60 s, followed by a SWASV scan from -1 V to $+0.5$ V) was maintained between experiments and was carried out at the start of a new pseudopolarogram.

As a result of the stability of the electrode, pseudopolarograms could be constructed from voltammetric scans from variation in the deposition potential in either direction without causing significant change in the shape of the final pseudopolarogram, with a reproducible measurement of half-wave potentials of complex species. To prevent the possibility of experimental bias, pseudopolarograms were successively refined from 4 iterations, each with 100 mV difference between sequential scans, to build up a full pseudopolarogram of 25 mV resolution as described in the Experimental Section.

Effect of Oxygen. Pseudopolarograms were constructed in a seawater sample in the presence of DO and repeated after purging for 15 min with N_2 . The seawater was not UV-digested and organic matter was present that complexed the Cu. DO has no interference on the ASV peak of Cu because it is more positive ($+0.2$ V) than the peak of DO on the gold electrode (-0.2 V).³¹ Because of the reduction of DO during deposition at potentials less than -0.3 V, the pH at the surface of the electrode would tend to be raised in unbuffered solution though seawater has a natural pH buffering capacity of 2 mM HCO_3^- . A significant pH change could affect the half-wave potential of reversible species if the raise in pH were to significantly increase the complex stability. The half-wave

potential of irreversible species would not be affected in case of a pH change as it responds to the thermodynamic stability constant.

The ASV peak for Zn is affected by DO as it occurs under the oxygen wave, and pseudopolarograms for Zn therefore always required purging of the solution with N₂. Further experiments in the laboratory therefore always employed purging.

Stability of the Cu Signal. The ASV response for Cu was measured over 72 h, at hourly intervals in a 400 mL seawater sample containing 17 nM Cu in a 500 mL Nalgene bottle, using a deposition potential of -1 V, to evaluate the stability of response. The response was found to decrease 8% ($n = 72$) over this period. The original signal was recovered after cyclic conditioning in 0.5 M H₂SO₄. The result showed that the Cu signal is stable for 1 or 2 days, but that conditioning is required between measurements over longer periods. The effect of cleaning in H₂SO₄ suggests that the decrease was due to fouling of the electrode. The stability of response indicates that the gold wire electrode can be used for pseudopolarograms over extended periods of time. The lack of interference of DO means that pseudopolarograms of Cu can in principle be carried out in situ using an electrode submerged in the sea.

Pseudopolarography of Inorganic Cu in Seawater. The half-wave potential for inorganic copper in UV-SW on the gold microwire electrode ($E_{1/2} = 0.03 \pm 0.02$ V) (Figure 3) is

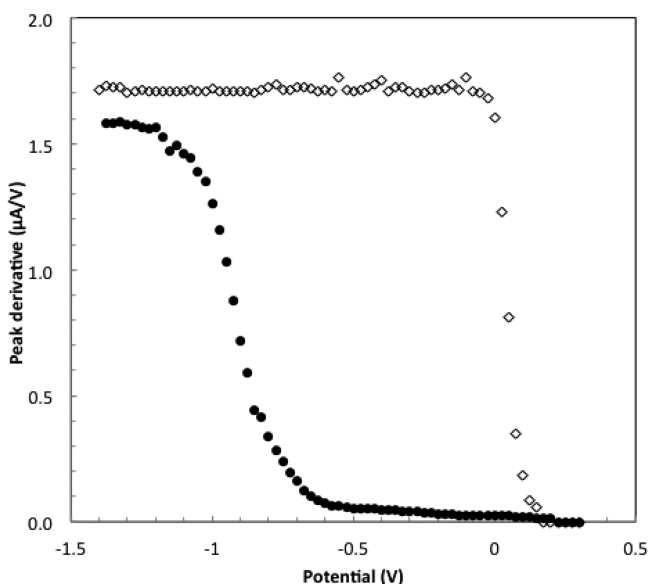


Figure 3. Pseudopolarograms of Cu (17 nM) in organic-free seawater (UV-digested) before and after addition of 200 μ M EDTA (solid symbols).

much more positive than that at the HMDE (-0.2 V).²² The oxidation of copper to Cu^{II} in seawater is a two-step process ($\text{Cu}^0 \rightarrow \text{Cu}^{\text{I}} \rightarrow \text{Cu}^{\text{II}}$) because of stabilization of Cu^I by complexation with chloride.³⁸ High stability of these chloride species causes a negative shift of the Cu⁰/Cu^I couple of about 0.25 V, causing a peak potential for Cu of about -0.2 V using the HMDE in seawater, equal to the $E_{1/2}$ found using the HMDE by pseudopolarography.²² The Cu^I/Cu^{II} peak cannot be seen using the HMDE as it is masked by the oxidation of Hg. Using the gold electrode also only one peak is apparent for Cu which has been shifted to $+0.2$ V from the expected -0.2 V because of adsorption stabilization on the gold, which is the

cause for the process of under-potential stabilization.³⁹ The half-wave potential of inorganic copper was 0.03 ± 0.02 V ($n = 4$), which is about 0.2 V more negative than its peak potential.

Pseudopolarography of CuEDTA. EDTA was used as a model ligand to evaluate whether the half-wave potential of its Cu complex can be determined reproducibly and its shift can be modeled using the theory. The $E_{1/2}$ for CuEDTA was determined 5 \times in UV-SW containing 0.01 M HEPES buffer (pH 7.85) and 1 μ M EDTA (Figure 3). The electrode was conditioned in H₂SO₄ between pseudopolarograms.

The addition of EDTA caused the pseudopolarographic wave to shift toward a more negative half-wave potential of $E_{1/2} = -970 \pm 15$ mV. This shift is greater than expected as the expected shift for $\log K^*_{\text{CuEDTA}} = 18.7$ ⁴⁰ is 18.7×30 mV = 560 mV, instead of the actual shift of 770 mV relative to the reduction potential for Cu in seawater (-0.2 V), and is much greater than found previously using a HMDE ($E_{1/2} = -0.46$ V, a negative shift of 0.21 V)²² where the shift was less than expected. The difference with the peak potential of Cu is even greater as this is shifted positive on gold because of underpotential deposition.³⁹ The halfwave potential of pseudopolarograms on micromercury electrodes with a very small volume has been predicted to shift in a negative direction.⁴¹ By analogy this could contribute to the large negative shift seen in the halfwave potentials found here on the gold microwire electrodes.

In diffusion limited conditions ($E_{\text{dep}} = -1.4$ V), the current for Cu with EDTA in seawater was 10% lower than without EDTA (Figure 2), in general agreement with a lower diffusion coefficient for CuEDTA than for inorganic Cu.⁴²

The peak potential for Cu in the SWASV scans was not affected by the presence of EDTA: the peak potential might have been expected to shift in an analogue manner to the half-wave potential, but the copper concentration in the diffusion layer is much greater than in the bulk solution, and possibly greater than that of EDTA, because of the electrochemical preconcentration step prior to the scan. This means that the inorganic copper may well be in excess of the EDTA. In addition, the rate of complex formation of metals with EDTA in seawater is slow because of major ion complexation of any free EDTA,⁴³ much slower than the rate of Cu oxidation during the square-wave modulation of the scan, which takes a 0.5 s in our experimental condition, while for instance the complexation of lead by EDTA in seawater has a half-life of hours.⁴³

Scanning Mode: SWASV versus Anodic Stripping Chronoamperometry (ASC). The scan rate of ASC is reduced when the potential scan reaches the oxidation potential of the metal, which is thought to minimize ligand saturation in the diffusion layer and potentially enable the possible use of the peak potential for the purpose of speciation.⁴⁴ In seawater it is not possible to use the peak potential of the scan to assess the complex stability because of low ligand concentrations and slow reaction kinetics due to major ion competition.⁴³ Nevertheless, SWASV was compared to anodic stripping chronoamperometry (ASC) as scanning method in pseudopolarography of Cu in seawater with and without EDTA. The potentials used for deposition, desorption, and conditioning were the same so that only the stripping methods differed. The peak area was used for quantification in ASC, while the peak derivative was used for quantitation in the SWASV method. In the UV-digested seawater the $E_{1/2}$ values obtained from ASV and ASC were 0.03 and 0.04 V, within 10 mV of each other, whereas in the presence of EDTA the $E_{1/2}$ was 60 mV more negative using

Table 1. Experimentally Derived Half-Wave Potentials ($E'_{1/2}$), Tafel Plot Slopes, and Thermodynamic Stability Constants for Copper-Ligand Complexes with Selected “Model” Ligands in Seawater at pH 7.85^a

organic ligand	[L] (μ M)	functional group	$\log [(i_{\max} - i)/i]$ vs E (V)	$E'_{1/2}$	literature $\log K^*$
UV seawater		Cl ₂	0.07	0.03	
NTA(1:1)	5	COOH-NH ₂	0.07	0.01	12.37
salicylaldoxime	50	CN(OH)	0.4	-0.30	12.64
dodecane	100	-NH	0.43	-0.36	13.2
dopamine	100	-C(OH)-NH ₂	0.06	-0.48	14.04
DOPA	100	-C(OH)-COOH-NH ₂	0.14	-0.52	14.4
<i>n</i> -nonane	10	-NH	0.16	-0.71	15.84
NTA(1:2)	2000	COOH-NH ₂	0.31	-0.70	17.13
EDTA	200	COOH-NH ₂	0.46	-0.90	17.94
DMG	500	CN(OH)	0.44	-0.91	18.05
cysteine	100	-SH	0.27	-0.79	18.5
nioxime(1:2)	200	CN(OH)	0.09	-0.65	19.11
ethylenediamine(1:2)	25	NH ₂	0.37	-1.04	19.6
DTPA	200	COOH-N	0.15	-0.90	20.43
glutathione	50	-SH	0.24	-0.97	21
CDTA	100	COOH-N	0.21	-1.05	21.24
cyclam			not detected		

^a10 mM HEPES buffer). $\alpha n = 0.059 \log [(i_{\max} - i)/i]$.

ASV. A negative shift relative to ASC was expected because of the 50 mV modulation of the SWASV method. The difference in the half-wave potentials using the two procedures was relatively small. Therefore the voltammetric stripping method was chosen for this work as it was facilitated by automation of the peak measurement.

Effect of Varying Microwire Length and Diameter. The experimental value of $E_{1/2}$ for quasi-reversible reactions obtained from pseudopolarograms is affected by the size of the electrode and the deposition time,⁴⁵ while it is independent of these parameters in the case of irreversible complex formation. Experiments were conducted to assess the effect of microwire size (length and diameter) on the $E_{1/2}$ found using ASV for the CuEDTA wave in UV-SW containing 200 μ M EDTA and 10 mM HEPES buffer (pH 7.85). Fourteen gold electrode tips were prepared, 3 of 5 μ m, 4 of 10 μ m, 4 of 25 μ m, and 3 of 100 μ m diameter. The diffusion layer thickness of these electrodes was respectively 1.2, 2.2, 3, and 4.9 μ m during the deposition step. The $E_{1/2}$ of CuEDTA was found to average -0.91 ± 0.01 V ($n = 11$) for electrode diameters of 5–25 μ m, showing that electrode diameter did not affect the experimental $E_{1/2}$ in this range. Electrode length was also not found to have an effect. The 100 μ m diameter electrodes were found to give a $E_{1/2}$ of -0.83 ± 0.01 V ($n = 3$), indicating a systematic offset of 80 mV possibly in connection with a thicker diffusion layer. Ten micrometer electrodes were used for the remainder of this work to maintain a constant and minimized diffusion layer thickness, and as they are more robust than the 5 μ m variety.

The experiments in this work made use of low metal concentrations; the natural ligand concentrations were low in the natural water samples but comparatively high for the added ligands including of EDTA. The lack of an electrode size effect on $E_{1/2}$ is in-line with expectation as these species are irreversible in view of the large shift in $E_{1/2}$.

Effect of Varying the Ligand Concentration on the Half-Wave Potential of Reversible and Inert Species. The half-wave potential of reversible, labile, complex species is directly dependent on the ligand concentration.^{19,36} On the other hand, when an inert complex is directly reduced at the electrode surface without prior dissociation of the complex, the

half wave potential is only dependent on the thermodynamic stability constant of ML and not on the ligand concentration.¹⁸ We tested this theory by measuring the effect of the ligand concentration on $E_{1/2}$ of CuEDTA in UV-SW containing 17 nM Cu, 10 mM HEPES buffer and EDTA at concentrations of 1, 10, 100, and 1000 μ M. In agreement with the theory for irreversible systems, the $E_{1/2}$ was found to be -0.92 ± 0.01 V, independent of the concentration of EDTA.

Effect of Variation of the Metal Concentration. The effect of variation in the Cu concentration on $E_{1/2}$ was tested for CuEDTA in UV-SW containing 200 μ M EDTA, 10 mM HEPES buffer (pH 7.85) and at 17, 37, and 77 nM Cu. The increase in Cu concentration gave no shift in the $E_{1/2}$ for CuEDTA, in agreement with previous work using an HMDE.⁴⁶

Effect of Varying the Deposition Time. The deposition time was varied to evaluate whether this affects the $E_{1/2}$ for the Cu wave in UV digested seawater containing 17 nM Cu and 10 mM HEPES buffer (pH 7.85). After normalization of the pseudopolarograms by plotting the ratio of the peak current over the maximum current as a function of the deposition time a negative shift in $E_{1/2}$ was apparent with increasing deposition time. This is in agreement with the theory which predicts $E_{1/2}$ for a reversible electrode reaction (and the reduction of inorganic Cu is reversible) to shift negative with increasing deposition time.⁴⁶

$$E_{1/2,2} - E_{1/2,1} = 0.059 \times \log(t_{\text{acc},1}/t_{\text{acc},2})/n$$

The half wave potential of Cu was found to shift linearly with the logarithm of the deposition times with a slope of 57 mV close to that expected for 59/n mV, if n , the number of electrons transferred, is 1, in accordance with reduction of Cu to Cu^I: this makes sense as the Cu^I state is stabilized by chloride complexation, causing the Cu to be oxidized to Cu^{II} in two separated steps,³⁸ each via a one-electron charge transfer.

Effect of pH on Pseudopolarography of CuEDTA in Seawater. As the half-wave potential is related to the thermodynamic stability constant, the experimental conditions with respect to solution composition should have no significant effect, as long as the metal remains largely complexed. Here we test this part of the theory by variation of the pH during

pseudopolarography of UV-SW containing Cu complexed by EDTA. The half-wave potential found for CuEDTA in UV-SW (17 nM Cu, 200 μ M EDTA) was -0.91 V at pH 7.85 (10 mM HEPES buffer) and at pH 5.8 (10 mM acetate buffer): the half-wave potential was therefore not affected by the pH in line with expectation.

Pseudopolarograms of Model Ligands to Obtain a Practical Chelate Scale. The relationship between the half-wave potential and the thermodynamic complex stability is given by eq 3. Because of uncertainties in the diffusion coefficient and the rate constant it is not possible to use a measurement of the half-wave potential to obtain a value for the thermodynamic stability constant of unknown organic species in natural waters. However, an operational relationship (chelate scale) has been obtained in constant experimental conditions using the hanging mercury drop electrode.²² Here we determined half-wave potentials of Cu complexes with model ligands to calibrate the shift in the half-wave potential as a function of the thermodynamic stability constant using the VGME, with the aim of estimating the thermodynamic complex stability of unknown organic metal species in seawater. Compounds used for this experiment (Table 1) are the same as used previously using the HMDE,²² thus enabling intercomparison. The half-wave potentials were found to range from 0.01 V for CuNTA to -1.05 V for CuCDTA. No wave was obtained for Cu-cyclam which had the greatest complex stability with a $\log K'_{\text{Cu-Cyclam}}$ of 26.5,²² as the complex was not reduced at potentials greater than -1.4 V.

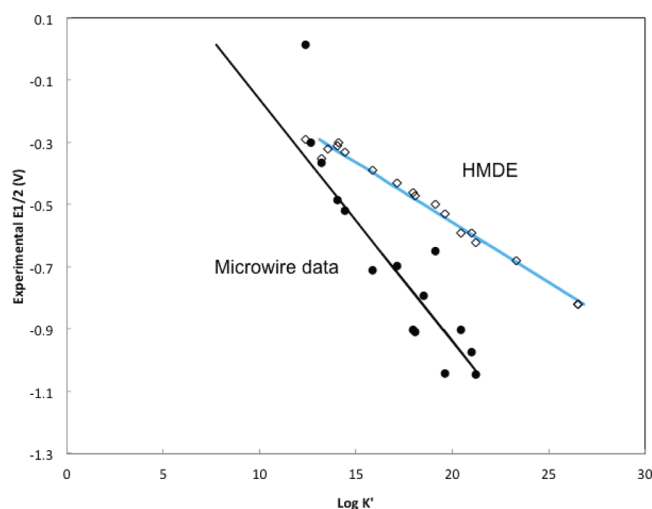


Figure 4. Calibration of the shift in the halfwave potential of pseudopolarograms using the VGME (closed symbols) of Cu (17 nM) irreversibly complexed with ligands added to seawater. Halfwave potentials are shown as a function of the thermodynamic stability constant ($\log K'$ values). Halfwave potentials obtained using a HMDE²² are shown for comparison (open symbols).

The half-wave potentials are shown as a function of literature values for $\log K'_{\text{CuL}}$ in Figure 4. A fit of the data to eq 3 gave the following result:

$$E_{1/2} = E_0 + 0.059(an)^{-1} \log(k_s \delta D^{-1}) - 0.090(\pm 0.011) \log K'_{\text{CuL}}$$

where $(E_0 + 0.059 (an)^{-1} \log(k_s \delta/D)) = 0.85 (\pm 0.2)$ V.

This means that $E_{1/2}$ varies with a slope of 90 mV/ $\log K'_{\text{CuL}}$ which is much greater than the slope of 30 mV expected for a 2-electron reaction in eq 3. Similar measurements using the HMDE had a slope of 39 mV²² and are also shown in Figure 4.

The larger shift in $E_{1/2}$ using the gold microwire is surprising. However, there are fundamental differences between gold and mercury electrodes that might give ground for this. Diffusion plays no role in the oxidation of Cu plated on the gold wire, while it does play a role on the mercury electrode. Second, the Cu is stabilized on the surface of the gold by means of adsorption processes with involvement of the major anions. This effect is absent on mercury. Third, the diffusion layer thickness on the microwire electrode ($\sim 1 \mu\text{m}$) is much less than on the mercury drop electrode (~ 8 to $9 \mu\text{m}$).⁴⁶ Previous work using a rotating disk electrode (RDE) has shown that an increase in the rotation rate (decrease in diffusion layer thickness) causes the half-wave potentials to shift negative faster than predicted by the theory.⁴⁷ The diffusion layer thickness of the vibrating electrode is less than that of the RDE, which suggests that this effect would be greater. Finally the process of electron transfer from the Cu complex to the electrode might differ on the gold and mercury electrodes. A combination of these effects could be an explanation for the greater than expected shift in the half-wave potentials seen here.

The data using the VGME shows more scatter than that obtained using the HMDE (Figure 4) suggesting greater variability of either the transfer rate constant, the transfer coefficient, or the diffusion layer thickness than at the HMDE.

Pseudopolarography of Humic Substances. Humic substances (HS) are known complexing ligands for Cu and other metals in seawater.^{48,49} Because they are also thought to occur in seawater,⁵⁰ we tested their effect on the pseudopolarography of Cu in seawater. Suwannee River humic acid (SRHA) and fulvic acid (SRFA) were added to UV digested seawater containing 12 nM Cu and 10 mM HEPES buffer (pH 7.85). Pseudopolarograms for this seawater with additions of between 0 and 4 mg L⁻¹ SRHA, and 0 and 8 mg L⁻¹ SRFA, are shown in Figure 5. The wave for the Cu-HS species was found to be located in the labile potential region (within 0.2 V of inorganic Cu), in spite of the known high stability of the Cu complexes with these HS in seawater ($\log K'_{\text{CuHA}} \sim 12$),⁴⁹ which is greater than that for EDTA ($\log K'_{\text{CuEDTA}} = 10.3$) which has a large shift in $E_{1/2}$ because of a high value of the thermodynamic constant. A likely explanation is that the HA and FA are much less strongly complexed with the major cations (Ca^{2+} and Mg^{2+}) which would mean that the thermodynamic constant for these Cu-HS species is much smaller than that for CuEDTA ($\log K'_{\text{CuEDTA}} = 18.7$).

In line with expectation for a reversible species, the $E_{1/2}$ was found to shift in a negative direction with increasing concentrations of HS (Figure 5). The diffusion limited current in the presence of the SRHA and SRFA was smaller than that of free Cu as a result of the slower diffusion of the Cu-HS species.

Conditional stability constants calculated from the half-wave shift were in the range of $\log K'_{\text{CuL}} \sim 9$ (Cu-SRFA) and ~ 10 (Cu-SRHA), valid for pH 7.9 seawater (Table 2). These values are much greater than those found by pseudopolarography of CuSRFA in freshwater ($\log K'_{\text{CuL}}$ around 7, at pH 5, 10^{-7} M Cu) which could be caused by saturation of strong complexing sites at the high concentration of Cu used in that work.⁵¹ The value found by us for CuSRHA in seawater is less than that ($\log K'_{\text{Cu-SRHA}} = 12$) found using CLE/CSV in seawater,⁴⁹ and is slightly larger than that ($\log K'_{\text{Cu-RSHA}} = 9.3$) found by CSV and

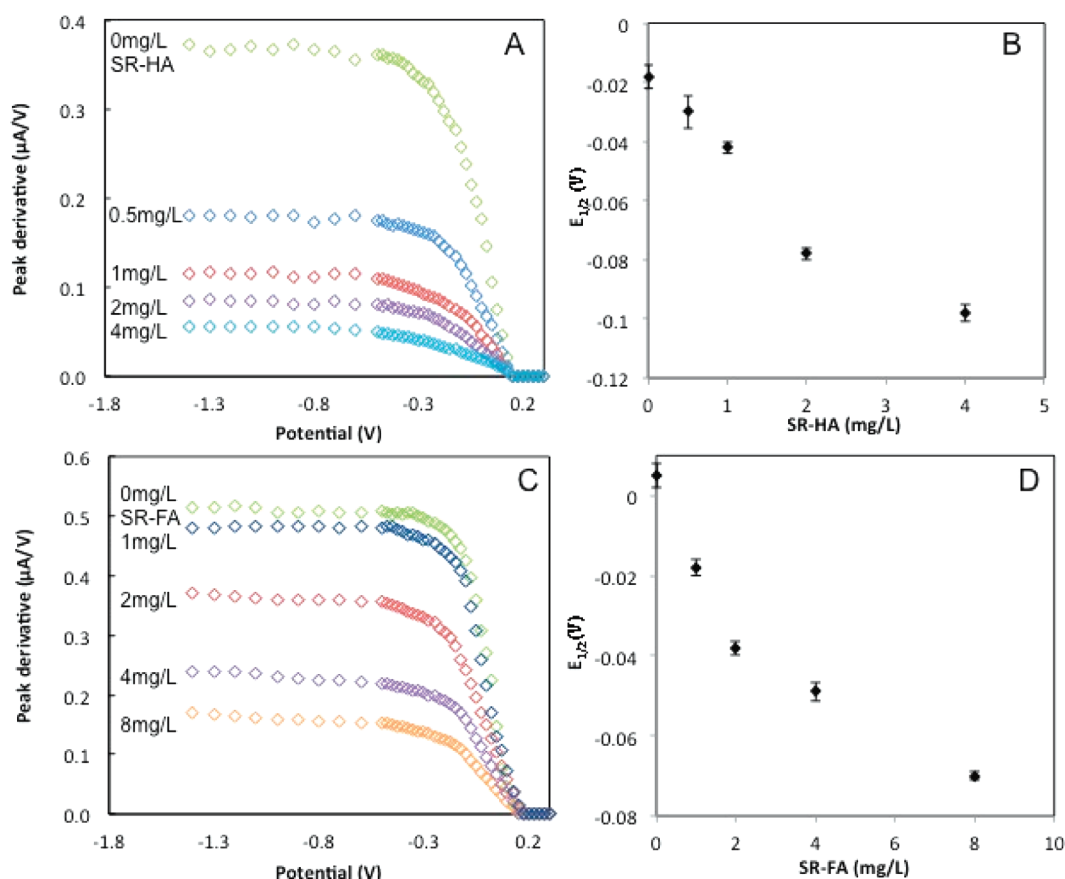


Figure 5. Pseudopolarograms for SRFA and SRHA added to organic-free seawater containing 12 nM Cu. (A) Pseudopolarograms at increasing concentrations of SRHA; (B) The shift in the half wave potential for increasing concentrations of SRHA; (C) Pseudopolarograms of increasing concentrations of SRFA; (D) The shift in the half wave potential for increasing concentrations of SRFA.

Table 2. Pseudopolarography of Humic Substance Complexes with Cu^a

mg L ⁻¹	C _L (nM)	half-wave potential (V)	log K' _{CuL}	log B' _{CuL2}
Fulvic Acid				
0	0	0.005		
1	16.7	−0.018	8.6	16.4
2	33.4	−0.038	9.1	16.6
4	50.1	−0.049	9.4	16.6
8	66.8	−0.07	9.9	16.8
Humic Acid				
0	0	−0.018		
0.5	32	−0.03	8.5	16.3
1	64	−0.042	8.8	16.3
2	96	−0.078	9.9	17.1
4	128	−0.098	10.5	17.4

^aThe equivalent ligand concentration (molar scale) associated with the concentrations of FA and HA was calculated from respectively 16.7 and 31.9 nmol mg^{−1} binding sites HS.⁵⁰ Conditional stability constants for FA and HA with Cu were calculated from the shift in the $E_{1/2}$.

iron competition.⁴⁸ The pseudopolarography shows that Cu is complexed by SRFA and SRHA added to seawater, and that the species is electrochemically reversible.

Pseudopolarograms of Natural Complexes of Cu and Zn in Seawater Samples. Seawater from the Irish Sea (Liverpool Bay, salinity 32) was used to test the new pseudopolarographic method. The first sample (Station 1,

March 2010) had been filtered and stored frozen until analysis. The concentration of dissolved Cu was 17 nM and zinc 13 nM.

A voltammetric scan for Cu and Zn is shown in Figure 6A and voltammograms for Cu and Zn in the seawater, with and without UV-digestion, in Figure 6B. Waves were apparent for 3 species of Cu, with half-wave potentials at 0.07, −0.35, and −0.80 V (Table 3). The first wave corresponds to a reversible wave for copper, occurring in an electrochemically reversible complex. Assuming similar diffusion coefficients for all species, this reversible species amounted to about 30% of all Cu.

The two irreversible species amounted to 30 and 40% respectively, and had half-wave potentials well in the irreversibility region. The slope of these species is flatter than that for labile copper, and it is possible that the third, most stable, species consists of more than one complexing ligand.

Thermodynamic stability constants were estimated for these species making use of the calibration shown in Figure 4, and are summarized in Table 3.

After UV-digestion of the sample all Cu occurred in the inorganic form, and the pseudopolarogram (Figure 5B) returned a half-wave potential of 0.07 V. The UV digestion of the sample leads to the conversion of the organic Cu to inorganic Cu. The Cu signal after UV was 12% greater than that in the untreated seawater, which was caused by a lower diffusion rate of the organic Cu species, or because of incomplete reduction of all organic Cu at −1.4 V.

Figure 6A shows the peak potentials of Cu at +0.2 V and Zn at −0.5 V in the same seawater sample after being accumulated for 60 s at −1.4 V. The peak for Cu is much narrower than that

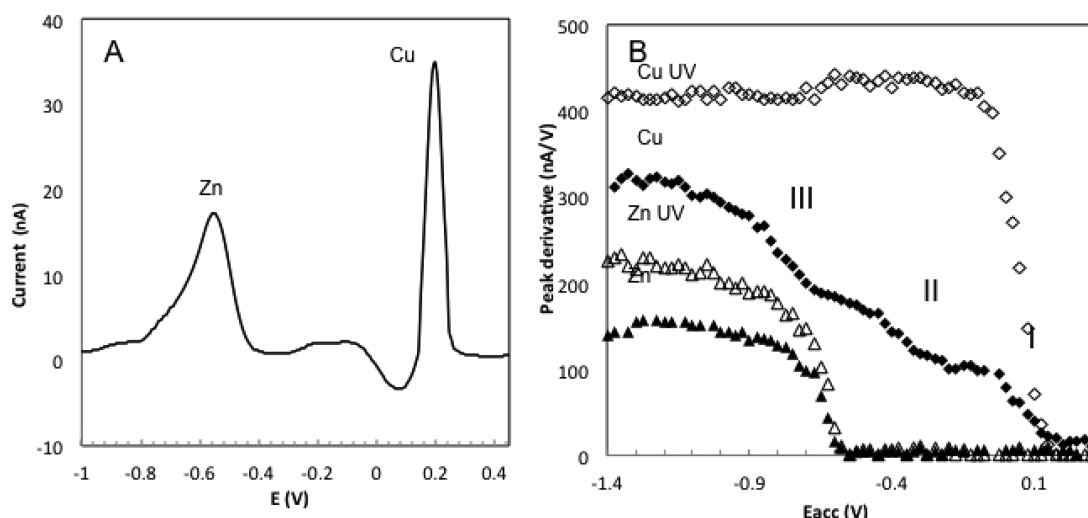


Figure 6. Application to seawater samples from the Irish Sea. (A) Square wave ASV scan for Zn (20 nM) and Cu (17 nM) in seawater (station 1) with 10 mM HEPES, pH 7.85, $E_{\text{dep}} = -1.4$ V, $t_{\text{dep}} = 60$ s, $E_{\text{des}} = -1.2$ V, $t_{\text{des}} = 1$ s, $t_{\text{eq}} = 2$ s. (B) Pseudopolarograms for Cu and Zn in seawater (station 1) with 10 mM HEPES buffer, pH 7.85, before and after UV digestion. Numerals relate to waves for irreversible organic Cu species in the seawater; the half wave potentials are given in Table 2.

Table 3. $E_{1/2}$ and $\log K^*$ Values from Pseudopolarograms for Organic Cu Species in Irish Sea Water^a

	wave	Tafel slope	$E_{1/2}$ (V)	error on $E_{1/2}$	estimated $\log K^*$
station 1 (March 2010)	I	0.15	0.067	0.003	reversible wave
	II	0.12	-0.349	0.004	12.9
	III	0.1	-0.797	0.005	17.9
station 27 (August 2010)	I	0.17	0.005	0.002	reversible wave
	II	0.12	-0.441	0.01	13.9
	III	0.23	-0.992	0.02	20.1
composite SW (June 2010) pH 7.85	I	0.13	0.09	0.1	reversible wave
	II	0.24	-0.7	0.1	15
	III	0.24	-1.2	0.1	22
pH 5.8	I	0.16	0.08	0.1	reversible wave
	II	0.25	-0.6	0.1	16
	III	0.12	-1.0	0.1	20
pH 3	I	0.15	0.11	0.1	reversible wave
	II	0.19	-0.5	0.1	12
	III	0.25	-1.0	0.1	20

^aThe slopes of the Tafel plots were calculated from $\log [(i_{\text{max}} - i)/i]$ vs E_D (V).

for Zn, which is surprising if the Cu peak corresponds to a 1-electron reaction and the Zn peak to a 2-electron reaction. The wider peak for Zn may be due to kinetic effects as electron transfer is relatively slow for Zn in solutions of high ionic strength.⁵²

Pseudopolarograms of Zn in the same seawater showed only one wave, corresponding to either free, or reversibly complexed, Zn at -0.5 V, which was steep and leveled off quickly. The peak height for Zn increased when the water was UV-digested, suggesting that the Zn was organically complexed. However, the same half-wave potential was obtained suggesting that the Zn complex was weak.

On-Site Analysis of Coastal Waters Liverpool Bay (Irish Sea). Pseudopolarography was used to determine the copper speciation in freshly collected seawater, on-board ship, without adding a pH buffer, before and after filtration ($0.2 \mu\text{m}$), and with and without purging to remove DO. Differences between the pseudopolarograms were insignificant indicating that neither filtration nor purging is required for pseudopolarography of Cu in seawater. This means that this method can be used without sample treatment, in principle by means of electrode insertion in situ in seawater.

As for the first, stored frozen, sample, the pseudopolarograms showed three waves for Cu, with half-wave potentials of 0.0, -0.44 , and -0.99 V (Table 2). Again a significant proportion of the Cu (between 20 and 30% on basis of the uncalibrated diffusion current) was found to occur in the reversible fraction with a half-wave potential of 0.0 V. The irreversible organic Cu species amounted to 30 and 40% each based on the magnitude of the diffusion current.

Using the calibration scale (Figure 4) the $\log K^*$ values of the organic Cu species were 12.9 and 13.9 for the type II species, and 18 and 20 for the type III species.

Effect of Variation of the pH on Pseudopolarography of Natural Organic Cu Complexes. Pseudopolarograms were made of seawater from the Irish Sea at 3 pH values: pH 7.85, pH 5.8, and pH 3, equilibrated for 24 h (Figure 7). There was no difference in the half-wave potentials of the pseudopolarograms at pH 7.85 or 5.8, but the limiting current at pH 5.8 had increased by 20% at a deposition potential of -1.4 V. The half-wave potential of the main Cu-organic species (III Figure 6S) was -0.8 V at both pH values. A second, more shallow and poorly resolved, wave had a half-wave potential at -0.3 V. The shift to pH 3 caused major changes in the speciation, the labile fraction (potential range 0 to -0.1 V) approximately doubling and its half-wave potential shifting from 0.09 to 0.1 V, the same as that for free (inorganic) Cu. The increase of the labile, organic, fraction of Cu is as expected from proton competition. The remaining shallow wave at -0.3 V was much increased, now representing about 40% of total Cu, whereas the wave at -0.8 V became much smaller to about 20% of total Cu. These shifts suggest that the Cu speciation at pH 3

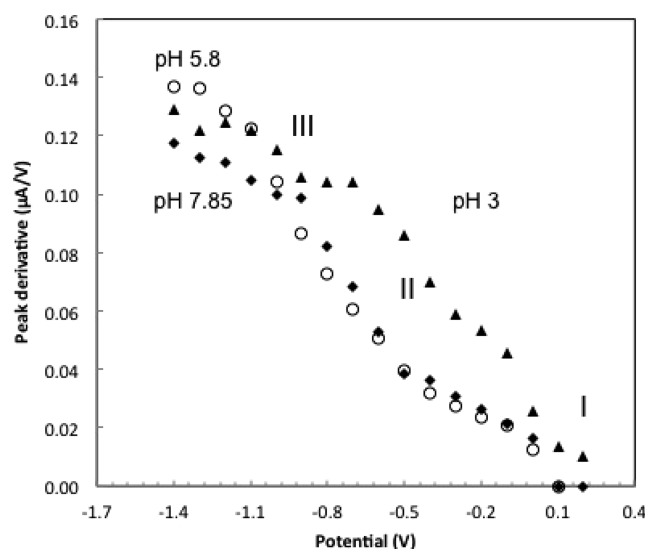


Figure 7. Effect of varying the pH on pseudopolarography of Cu in Irish Sea water. pH 3 using 3 mM HCl, pH 5.8 using 0.01 M NaAc buffer (open circles); pH 7.85 using 0.01 M HEPES buffer (closed diamonds). The location of halfwave potentials (listed in Table 3) is indicated by Roman numerals.

caused approximately 40% to become free, while the stronger bound Cu shifted in part to a weaker species or to a weaker complexing site on the same organic matter that became available because of the release of some of the Cu by the pH drop. The lack of changes in the pseudopolarogram between pH 7.9 and 5.8 indicates that the Cu speciation is not significantly affected by pH changes within this range, and similarly, that pseudopolarography is little affected over this pH range.

DISCUSSION

Chelate calibration scales were previously established for mercury based electrodes for iron (Fe) (Lewis et al., 1995a; Taylor et al., 1994), Cu (Croft et al., 1999), cadmium (Cd) (Tsang et al., 2006), and Zn (Lewis et al., 1995b; Vega et al., 1995). Our work extends the technique of pseudopolarography for the first time to speciation of Cu at trace level in seawater by using the VGME. The much thinner diffusion layer thickness of the VGME than that of the HMDE, and the different type of electrode (solid, gold) much lower the LoD for Cu, but also affect the half-wave potentials that are detected. Typically the half-wave potentials found using the VGME are considerably more negative than those found using the HMDE, and second, the slope of the plot is steeper. The presence of δ in eq 3 explains why the half-wave potentials (Figure 4) on the microwire electrode ($\delta = \sim 10^{-6}$ m)³¹ are more negative than on the mercury drop electrode ($\delta = \sim 10^{-5}$ m);⁴⁶ however, this effect should amount to no more than ~ 30 mV (for a 2-electron reaction and $\alpha = 1$). The presence of the rate constant in eq 3 means that the half-wave potential is affected by the rate of complex reduction which in its turn is affected by complex dissociation and electron exchange across the electrode surface. The diffusion coefficient has variability between species but probably not more than by a factor of 2, which would translate to a variability of about 10 mV for a 2-electron reaction between species. On the other hand the rate constant varies over orders of magnitude between species and could therefore cause shifts of 100 s of mV between species. This latter effect is

probably the reason for the variability of the data points obtained by the microwire electrode along the calibration line (Figure 4).

It remains uncertain why the variability in the data points is greater on the microwire electrode than on the mercury drop electrode. The overall effect of using the microwire electrode is that the half-wave potentials are shifted generally to more negative potentials on the microwire electrode and the slope of the shift is about double that of the HMDE: this means that a greater shift is obtained for all species and that the resolution for weaker species is greater than on the HMDE. On the other hand the very strongest species tested (Cu-cyclam) was not reduced (it was inert) within the accessible potential range and was off-scale for the microwire.

CONCLUSIONS

Experiments using model ligands, including EDTA, humic acids, and glutathione showed that pseudopolarography using a solid, gold, microwire electrode can be used to determine the chemical speciation of copper in seawater. The good sensitivity of the VGME, with a LoD of 0.1 nM inorganic Cu, means that the pseudopolarography can be carried out at trace level of copper in uncontaminated seawater. Preliminary measurements of samples from the Irish Sea (Figure 7) indicate that the copper occurs in at least 3 different complex species. One of these is an electrochemically reversible complex and the other two are electrochemically irreversible species. Separate experiments using humic substances showed that these formed electrochemically reversible species, consistent with the presence of humic substance-related complexing ligands in these waters.⁵⁰ As the Cu reduction wave on the VGME is at more positive potential than that for DO, the pseudopolarography could be carried out in freshly collected, unaltered seawater from Liverpool Bay, thus facilitating the possibility of in situ copper speciation in future work.

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