

Analysis and toxicity of pCu_{free} in seawater

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In memory of my father Kjell Helge Eriksen (31.7.31 – 27.3.89)

&

In celebration of my sons Samuel and Lachlan.

*We have a mental habit which makes it much easier for us to explain the
miraculous in natural terms than to explain the natural in miraculous.*

T.S. Eliot

Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the Thesis. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text of the Thesis.

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Abstract

This thesis describes the development of an analytical technique to measure the free copper ion concentration (pCu_{free}) in seawater. Ion-Selective Electrode (ISE) potentiometry is the only analytical technique that can directly measure pCu_{free} without manipulating the sample matrix, or making assumptions about the thermodynamic behaviour of the metal species. Flow analysis techniques were investigated with the aim of overcoming problems inherent in batch analysis, including sluggish response at low analyte concentrations, cross contamination from one sample to another, contamination by membrane dissolution and/or silver chloride precipitation, and an offset in response due to adsorption of natural organic matter present in seawater.

Surface studies of a rotating disk ISE by Electrochemical Impedance Spectroscopy (EIS) confirmed that the effect of organic matter was small (~ 0.1 pCu units) and was unlikely to significantly effect the analysis of marine samples. Flow analysis studies showed that the chloride interference was less significant in a flow system than in batch mode, and that the chloride signal could be separated from the copper signal by kinetic discrimination.

On the basis of preliminary flow injection studies and the EIS results, Continuous Flow Analysis (CFA) was tested and a method for the routine analysis of open-ocean and contaminated samples was developed. CFA reduced the effect of membrane dissolution and silver chloride precipitation, and eliminated sample cross contamination, by virtue of the sample stream flowing past the electrode. Low sample throughput, a result of sluggish response at low concentrations of copper, was increased by using an extrapolation technique. This technique is based on the fitting of an empirical mathematical expression to the electrode time-response curve, enabling the prediction of the final steady-state potential. Predicted potentials fell within 0.5 mV of the true steady state potential, and sample analysis time was reduced by a factor of 3 – 6.

The CFA method was used to study the effect of copper on the unicellular benthic marine diatom *Nitzschia closterium* (Ehrenberg) W. Smith. *N. closterium* is extremely sensitive to low concentrations of copper, and a strong relationship between pCu_{free} and growth inhibition was established at two salinities. Growth inhibition bioassays of samples from a severely contaminated estuary showed no toxic effects, despite elevated levels of total and “labile” copper. Speciation measurements in salinity 20 waters showed that labile copper grossly overestimated the toxicity of the samples, while pCu_{free} measurements indicated that there was insufficient free copper to cause a toxic effect. Mild toxicity was observed in some samples at salinity 30, however this could not be explained by ISE measurements, as

pCu_{free} levels were below the toxic threshold. In contrast to earlier studies of the copper complexing capacity of the estuary, ISE results showed that more than 99.9% of the total copper was bound to ligands, probably originating from the surrounding button grass and hardwood forests in the catchment areas of the two major freshwater sources.

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If I have forgotten anyone, you have my permission to abuse me.

List of Publications

1. De Marco R., Eriksen R., & Zirino A. (1998) "An electrochemical impedance spectroscopy study of the response mechanism of the jalpaite copper(II) ion-selective electrode." *Analytical Chemistry* 70, 4683-4689.
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3. Eriksen R.S., Mackey D.J., Nowak B., & van Dam R. (submitted) "Copper speciation and toxicity in Macquarie Harbour, Tasmania." *Marine Chemistry*
4. Stauber J.L., Benning R.J., Hales L.T., Eriksen R.S., & Nowak B. (2000) "Copper bioavailability and amelioration of toxicity in Macquarie Harbour, Tasmania, Australia." *Marine and Freshwater Research* 51, 1-10.
5. Stauber, J.L., Ahsanullah, M., Nowak, B., Eriksen, R., & Florence, T.M. (1996) "*Toxicity Assessment of Waters From Macquarie Harbour, Western Tasmania, Using Algae, Invertebrates and Fish.*" Supervising Scientist , Report no.112, Barton, ACT.
6. Eriksen, R.S., Nowak, B., & Van Dam, R. (accepted) "*Copper Speciation and Toxicity in Estuarine Environments.*" Office of the Supervising Scientist Report Series.

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Chapter 1 General Introduction

BACKGROUND

Approaches to copper speciation in seawater

Copper is an essential trace element that is necessary for normal growth and metabolism in marine organisms. At concentrations above those required for normal physiological processes, copper is potentially toxic (Lewis & Cave 1982).

Knowledge of the chemical speciation that exists in seawater is fundamental to our ability to understand or predict copper toxicity in the marine environment. Chemical speciation governs the reactivity of copper, and its subsequent 'bioavailability' to marine organisms (Zorkin et al. 1986). For the purpose of this discussion, bioavailability is defined as that fraction of an element or group of elements that can be taken up by an organism, by active or passive mechanisms.

Different chemical forms arise from the equilibria between copper ions, and inorganic and organic ligands naturally present in seawater. The dominant chemical form will depend upon a complex combination of parameters, including pH, salinity, the type of ligands present, the affinity of the metal ion for the ligands and the extent of thermodynamic and kinetic control (Viarengo 1989; Mackey & Zirino 1994). Many different approaches have been used to gain a better understanding of the processes that dominate and control metal speciation, and the environmental significance of these processes. These include, but are not limited to, chemical speciation modelling, analytical speciation schemes, and bioassays for studying uptake and the effects of trace metals in aqueous media.

In general, speciation studies only consider the "dissolved" form of the metal, operationally defined as that fraction which passes through a 0.45 µm (membrane) filter, and the fraction remaining is defined as "particulate". Colloidally bound material may also pass through a 0.45 µm filter, incorrectly classifying colloidal matter with the dissolved fraction (Kramer 1986).

Chemical Speciation Modelling

Approximately 98-99% of copper in seawater is bound to naturally occurring organic material, and the remainder is accounted for by inorganic species. Less than 0.08 % may actually be present as the cupric ion, Cu^{2+} (Sunda & Hanson 1987). Given that typical oceanic values of total copper are around 1 – 3 nM (Mackey 1983), this means that the concentration of Cu^{2+} can be orders of magnitude lower. The exact composition of organic constituents in seawater is unknown, and the mathematical modelling of copper speciation in the presence of these compounds is difficult, although a few models consider metal-organic interactions (Kramer 1986;

Benedetti et al. 1996). In the absence of organic matter, the speciation of copper in seawater is markedly different. At pH 8.1, salinity 35 and a temperature of 25 °C, the inorganic speciation model of Millero and Sohn (1992) predicts over 88 % of copper is present as CuCO_3 , $\text{Cu}(\text{CO}_3)_2^{2-}$, and CuHCO_3^+ complexes, with less than 4 % present as the free copper ion Cu^{2+} . The remaining copper is distributed between hydroxide and sulphate species. Such inorganic speciation models are based on the well known major inorganic ions which can be assumed to dominate species distribution (Zirino & Yamamoto 1972; Dyrssen & Wedborg 1974). Even using this simplified model of copper speciation, it has been observed that the reliability of the stability constants and possible omission of potentially important equilibria leads to large discrepancies between investigators (Kramer 1986). Additionally, speciation models based on thermodynamic data do not take into account kinetic effects.

Speciation schemes

Speciation schemes based on analytical procedures can also be used to gain useful insight into the distribution of copper species in seawater. A typical speciation scheme may use filtration to separate particulate and dissolved forms, acidification and UV-oxidation to release bound metals, Anodic Stripping Voltammetry (ASV) at pH 4.7 to determine “labile” species, or competition with ion-exchange resins to determine strongly bound complexes (Florence 1989). A widely used technique is Cathodic Stripping Voltammetry (CSV) which utilises adsorptive deposition of metal complexes with specific chelating agents onto a hanging mercury drop to provide information on the copper complexing capacity of the water (Colombo and van den Berg, 1997). In all these instances, the fractions are operationally defined (e.g. filtration through 0.45 μm filters, decomposition by acidification to pH 2, deposition under defined conditions), and interpretation of results from different laboratories employing different speciation schemes and analytical methods can be difficult. Extrapolation of results to the natural environment should therefore be made cautiously, since empirical analytical fractions or separations will not necessarily correlate to biological processes or responses.

Bioassays

An approach used to investigate the *effects* of speciation, which can incorporate both mathematical speciation models and analytical schemes, is the use of bioassays. Bioassays study the effects of an element, at the cellular, organism or population level, and can be used to gain information about the uptake, nutrition or inhibitory effects of the toxicant (Walsh 1993). Typically, “simple” organisms are maintained in the laboratory in batch or flow-through cultures and exposed to various dilutions of the toxicant, in either real or artificial seawater. Algae are favoured because of their ease of culture, rapid growth rates and well defined inorganic media (Stauber

1995). Trace metal concentrations are typically controlled by the addition of chelation compounds, and cultures are maintained under conditions of constant pH, light, and temperature. *In situ* data on the effect of copper on marine organisms is scarce (Campbell 1995).

There is ample laboratory evidence that the total aqueous metal concentration is not a good indicator of the toxic effects of copper, and it is generally reported that the free cupric ion, Cu^{2+} is the most toxic or available form (Morel & Morel-Laurens 1983; Turner 1986). The Free Ion Activity Model (FIAM) was formulated to define the conditions under which changes in the activity of the free metal ion, or a particular metal complex could elicit a response in a biological system, be it perfused fish gills or a whole organism (Morel 1986). The FIAM model is based on the premise that for a system in chemical equilibrium, no single species in solution can be considered more or less 'available' than any other species (Campbell 1995). However, the free metal ion activity controls biological effects because it determines the reactivity of the metal in systems at equilibrium, and thus the extent of the metal reaction with a biological surface, and hence its bioavailability. For this reason, the metal ion activity has been termed a "master" variable for biological uptake (Zirino et al. 1998).

Fundamental research by Sunda and co-workers showed clearly that the activity of the free metal ion induced changes to physiological processes in marine bacteria (Sunda & Gillespie 1979) and algae (Sunda & Guillard 1976). Since those early studies, many researchers have gone on to demonstrate the same relationship for higher trophic levels and for many other species (Lewis & Cave 1982; Stearns & Sharp 1994; Campbell 1995; Sharp & Stearns 1997).

Significantly, there are also many documented exceptions to the FIAM, where lipid soluble metal species have caused sublethal or lethal responses in test organisms (Florence & Stauber 1986; Stauber & Florence 1987; Florence et al. 1992b), or where the presence of dissolved organic matter (commonly thought to ameliorate metal toxicity) have exacerbated physiological effects (Campbell 1995). Clearly our knowledge of trace metal speciation, bioavailability and toxicity is far from complete.

A lack of suitable analytical techniques for the direct determination of the free metal ion concentration in seawater has, in part, hindered the development of a better understanding of the toxicity of copper to marine organisms. As a result, many investigators are limited to measuring only the total metal concentration added in a series of dose-response type studies on marine organisms. To overcome the necessity to monitor variations in metal speciation, researchers have developed protocols for preparing seawater that contains only known inorganic and organic ligands, for which speciation models can be developed (Morel et al. 1979). Even so,

the accuracy of the speciation model is dependent on the quality of the thermodynamic constants used to calculate the metal speciation in solution. In addition, the observations made in such strictly defined media where the composition of a single element is varied cannot be extrapolated to real systems with a high degree of confidence (Kramer 1986).

Speciation measurements have been used in conjunction with bioassays to explore the role of speciation in metal toxicity and nutrition. ASV-labile copper is reported to show good correlation with toxicity in some instances (Florence 1989; Apte & Batley 1995). This technique assumes that only inorganic species are electrochemically labile. Recent toxicity tests with a suite of estuarine and marine organisms have shown that ASV-labile copper is a poor predictor of toxicity, and significantly overestimates the degree of bioavailable copper (Stauber et al. 1996; Stauber et al. 2000). The overestimation of toxicity is believed to be due to the reduction of strong organic complexes under the mildly reducing analysis conditions, and hence the larger fraction of “labile” copper measured (Croot et al. 1999).

Measurements of free copper in seawater

Many analytical methods have appeared in the literature for the estimation of the free cupric ion activity in seawater. Some have shown good correlation with growth inhibition or other sublethal physiological effects, but are difficult for other researchers to reproduce in their own laboratory. The ligand exchange technique and bacterial bioassay techniques of Sunda and co-workers (1979; 1987), whilst clearly supporting the FIAM, are not readily reproduced by researchers not set up to perform those specific analyses. Other techniques involve trace analysis protocols and the use of purposely built (i.e. clean room) facilities. There exists a real need for a simple, reliable analytical technique that responds directly to the free metal ion activity, which can be ideally used in the field, in long term monitoring, and toxicity studies.

Ion-Selective Electrodes (ISEs) have been recognised as an important tool for toxicology and environmental studies in freshwater, by virtue of their ability to measure free ion activities and complexation capacities (Lund 1986). Potentiometric measurements with ISEs can directly measure free copper, *in situ*, without influencing or perturbing the natural speciation of the sample (Belli & Zirino 1993). This is an important advantage over other electrochemical techniques, where the very act of measurement can cause changes in the sample composition (e.g. addition of buffers, degassing) or where the species measured are operationally defined (e.g. ASV-labile) and do not necessarily correlate with observed nutrition or toxicity effects. The ISE overcomes the major disadvantages of modelling and speciation schemes (viz. kinetic effects and arbitrarily defined fractions), as it can directly

measure the master variable, and it does not “see” the other forms present in solution. For marine studies, however, Cu(II)ISEs have not been used widely due to an abundance of literature reports claiming they are unsuitable for measurements in saline media. Many early mechanistic investigations of the Cu(II)ISE reported non-theoretical response, noisy and unstable potentials, extensive fouling of the sensor membrane by AgCl deposits and unfavourable limits of detection (Moody et al. 1979; Westall et al. 1979; Gulens 1987). These studies used chloride concentrations up to 3M, and Cu^{2+} concentrations as high as 10^{-2} M to demonstrate the “chloride effect”. Many more general papers appeared, often dismissing the use of the ISE for saline matrices in a single sentence. Thus the message delivered to the wider scientific community was the chloride interference was an insurmountable problem that did not warrant further attention.

In contrast, Jasinski et al. (1974) were able to show that the Cu(II)ISE does function normally in acidified seawater, and were able to measure total copper in the nanomolar range at pH 3. The non-theoretical (super-Nernstian) response typically observed in natural seawater at pH 8.2 was attributed to the presence of natural ligands which chelated Cu^{2+} added during standard addition analysis. Belli and Zirino (1993) showed that the assumption used in standard addition analyses that natural seawater binds the same constant fraction of added copper, independent of the copper concentration, is invalid (i.e. α does not remain constant). If the response is interpreted in terms of Cu^{2+} in solution (i.e. what the electrode senses), rather than the total copper added, the response is Nernstian.

Recent research by Belli and Zirino (1993), and De Marco and co-workers (1994; 1997) has demonstrated that under specific conditions of analysis, the copper ISE does function normally in saline media such as seawater. Earlier problems documented were found to be the result of

- 1) *misinterpreting the response of the ISE to total copper additions to seawater, which lead to the belief that the ISE was responding in a non-theoretical fashion i.e. super-Nernstian response.*

Jasinski et al. (1974), Belli and Zirino (1993), and De Marco et al. (1997) have shown unequivocally that this is not the case. When the electrode response is plotted as a function of the free copper added, normal Nernstian response is observed.

- 2) *using the ISE in samples where the chloride interference would be invoked (Gulens 1981; Gulens 1987; Catrall & De Marco 1992), which were not representative of seawater composition.*

It has since been demonstrated that contrary to common expectation, the “chloride effect” is less significant at very low concentrations of free copper than at the concentrations typically reported in the literature to demonstrate the interference

effect (De Marco 1994). This phenomenon is due to kinetic suppression of the interference at the chloride and copper concentrations found in seawater.

- 3) *failure to recognise that the ISE requires strict polishing, conditioning and calibrating protocols to function normally in seawater (De Marco 1994).*

Poor reproducibility as a result of electrode drift and sample contamination by membrane dissolution are significant problems encountered in ISE analyses. Both may be minimised by appropriate polishing and conditioning regimes. Electrode carryover and long-term electrode stability are also commonly cited problems, that may be overcome by employing a sacrificial buffer between measurements and using a stability criterion of 0.2 mV/min or better to record signals at the same juncture in the potential-time curve for all solutions (De Marco et al. 1997). Sensitivity may be improved by calibration with buffer systems, and the use of clean techniques.

- 4) *the fact that all commercially available ISEs are not identical, and some will be more suitable for seawater analysis than others (De Marco 1994; Mackey & De Marco 1997).*

Studies by Gulens (1981) and De Marco (1994) demonstrated that different membrane types respond differently to seawater and that not all are suitable for analysis of saline waters.

Since these initial reports appeared, it has been demonstrated that using the Orion 94-29 Cu(II)ISE, it is possible to measure free and total copper in a variety of environments, ranging from pristine open ocean to coastal and estuarine waters (Mackey & De Marco 1997; Zirino et al. 1998a; Zirino et al. 1998b; Eriksen et al. 1999; Eriksen et al. submitted). The potential for further development of the analytical technique as a tool for toxicology studies is enormous. No other analytical technique can measure the activity of the free metal ion in solution directly, without perturbing the natural speciation of the sample. A rapid method, ideally developed as a portable instrument for field measurements, based on the Cu(II)ISE would be an important advancement in the field of environmental chemistry and toxicology.

AIM

The main aim of this study was to develop instrumentation and suitable analytical protocols for the measurement of free copper in seawater, using the Orion Cu(II) 94-29 ion-selective electrode.

OVERVIEW OF THESIS

Although it has been clearly demonstrated (De Marco 1994) that the chloride interference is negligible at the very low concentrations of free copper found in the marine environment, two subsequent issues were highlighted as being worthy of further attention. These are

1. *dissolution or corrosion of the ISE membrane, and subsequent release of Cu^{2+} into the sample.*

Membrane corrosion may seriously contaminate seawater samples in which the natural range of copper concentrations is 1-3 nM (Mackey 1983), and hence needs to be reduced or eliminated to allow accurate analyses. De Marco (1994) demonstrated that this could be reduced by appropriate polishing and conditioning of the membrane, however it is desirable to totally eliminate the contribution of copper from the electrode.

2. *interactions between the ISE membrane and natural organic ligands present in seawater.*

It has been suggested that the presence of natural organic matter may cause an offset in electrode response, thereby bringing into question the concentration of free copper determined by ISE (Gulens 1981). The mechanism and extent of the interference must therefore be determined.

With these questions in mind, an analytical method for the determination of free copper in seawater was developed. Chapter 2 provides a brief discussion of ISE theory, focusing on the mechanism of the chloride ion interference, and other ions present in seawater which may influence the electrode response. Chapter 3 describes the practical aspects of potentiometric measurements, confirming the importance of polishing, conditioning and calibration protocols for making meaningful measurements. Electrochemical Impedance Spectroscopy was used to explore the mechanism and extent of interference by natural organic matter present in seawater, and using the technique of standard additions, it was demonstrated that the ISE has the required sensitivity to measure environmentally relevant concentrations of free and total copper. Flow analysis was identified as a way to overcome the problems inherent in batch analysis (such as electrode dissolution and cross-contamination), and Chapter 4 reports the results of preliminary investigations using Flow Injection

Analysis (FIA). Kinetic discrimination of the chloride signal from the copper signal was achieved, and a number of carrier solutions were tested with the aim of developing a method for free copper. It was ultimately found that the FIA approach used was not successful, however results strongly suggested that a Continuous Flow Analysis (CFA) system would be more applicable. The development and validation (for total copper) of the CFA method is described in Chapter 5.

The system developed was designed to be portable, enabling shipboard or field analyses to be undertaken. A major issue in the development of any analytical method is the need to validate against a widely accepted technique. In the absence of an independent technique to directly measure pCu_{free} in seawater (where $pCu = -\log Cu[(II)]$), it is not possible to validate the ISE technique in the traditional sense. It is possible however to reproduce complex analytical techniques such as the ligand exchange technique (Sunda & Hanson 1987) or to develop arbitrary speciation schemes, or to model seawater in an attempt to correlate the ISE results with other speciation methods. However, this approach is limited in value as all these methods are indirect, and each has particular disadvantages as discussed earlier.

An alternative approach is to look at the correlation between the direct chemical measurement of pCu_{free} and some biological effect i.e. combine speciation measurements by ISE with a bioassay. The benefits of this approach are twofold:

1. It demonstrates clearly that the biological response is correlated to the activity (or concentration) of the free metal ion in seawater, *by direct measurement*, and
2. It may ultimately allow the prediction of the potential toxicity or nutritive status of a water body.

Chapter 6 describes the application of the CFA method to a study of copper speciation and toxicity in Macquarie Harbour, a contaminated estuary in western Tasmania. Algal bioassays using the marine diatom *Nitzschia closterium* were conducted to examine the relationship between free copper and toxicity at two salinities. Measurements of free copper in Macquarie Harbour were made with the aim of predicting copper toxicity to the algae, and it was found that there was insufficient free copper to cause an effect in the test organisms. The ISE results gave useful information about the speciation and complexation of copper in the harbour. Chapter 7 contains the summary of the research conducted, and reviews the results obtained in the context of where major improvements could be made

Chapter 2 The Use of the Cu(II)ISE for Seawater Analyses

INTRODUCTION

Until recently, doubt still existed as to the suitability of the copper(II)ISE for measurements of free copper in saline matrices such as seawater, due to interference by the chloride ion, and a reported lack of sensitivity. Early work by Jasinski et al. (1974) however, suggested that it may be possible to measure copper in seawater with the Cu(II)ISE, and that the electrode was capable of detecting very small additions of Cu^{2+} . Other researchers reported that ISE measurements indicated higher levels of free copper in polluted waters than in pristine sites, and found variations in electrode response consistent with oceanographic models of copper distribution in open ocean seawater profiles (Zirino & Seligman 1981). More recently Belli and Zirino (1993) demonstrated good agreement between theoretical values of free copper in synthetic seawater and results obtained by ISE.

The purpose of this chapter is to demonstrate that most of the reports of chloride interference are inapplicable to the analysis of seawater, by virtue of the type of sensor used or the conditions under which experiments were performed. While the chloride interference undoubtedly occurs, the observations do not apply to the critical range of chloride and Cu^{2+} concentrations found in seawater, under specific conditions of analysis. Sensitivity can be dramatically improved by calibration of the electrode with suitable metal-ion buffers, again under specific conditions of analysis. These conditions are discussed further in Chapter 3. A basic description of ISE theory is given here, along with a discussion of the mechanism of electrode response in the presence of chloride and other interferences. Non-theoretical behaviour observed in seawater is explained in terms of the electrode response, and copper speciation in seawater.

Ion-Selective Electrode theory

Ion-selective electrodes are electrochemical sensors that respond directly to changes in the activity of certain ions, usually in aqueous solution. In pure solutions, an ISE ideally responds to the species to be determined according to the Nernst equation. For analyte X, the Nernst equation may be written as follows

$$E = E^0 + \frac{RT}{zF} \ln a_x \quad (1)$$

where E = potential measured in solution (mV)

E^0 = standard potential of the cell (mV)

z = charge on x

a_x = activity of ion x

R = gas constant

T = temperature (K)

F = Faraday constant

Thus the electrode response E is proportional to the logarithm of the activity of ion X. For a monovalent ion, the theoretical Nernst response at 25 °C is 59.2 mV change in potential per 10-fold change in activity (decade), while for a divalent ion, the “Nernstian” response is 29.6 mV/decade (Figure 2.1). The sign in the equation will be positive for cations and negative for anions. The term “sub-Nernstian” is used to describe electrode response where the proportionality constant is less than 29.6 mV/decade for a divalent ion. “Super-Nernstian” response describes the situation where the proportionality constant is greater than the theoretical value of 29.6 mV/decade (Bailey 1976).

The activity a_x of an ion X in solution is related to its concentration, m_x by the equation

$$a_x = \gamma_x \cdot m_x \quad (2)$$

where γ_x = activity coefficient

m_x = molality (moles X / kg of solvent)

The activity coefficient is dependent upon the ionic strength, I, of the test solution. The ionic strength can be calculated by the following relationship

$$I = \frac{1}{2} \sum m_x \cdot z_x^2 \quad (3)$$

where the summation is taken over all the ions in the solution, and z = the charge on each ion. Concentrations are more commonly reported in the molarity scale than the molality scale. In aqueous solutions, except for very strong concentrations of X, m_x may be approximated by [X] so that Equation 2 becomes

$$a_x = \gamma_x \cdot [X] \quad (4)$$

without significant error for most analytical work. The activity coefficient cannot be measured for individual ions, so the mean activity coefficient is determined for X and a counter ion Y in solution, viz.

$$\bar{\gamma}_{xy} = \sqrt{\gamma_{x^+} \cdot \gamma_{y^-}} \quad (5)$$

The Debye-Hückel theory describes the dependence of $\bar{\gamma}_{xy}$ on the ionic strength,

$$\log \bar{\gamma}_{xy} = \frac{-A |z_x z_y| \sqrt{I}}{1 + Bd \sqrt{I}} \quad (6)$$

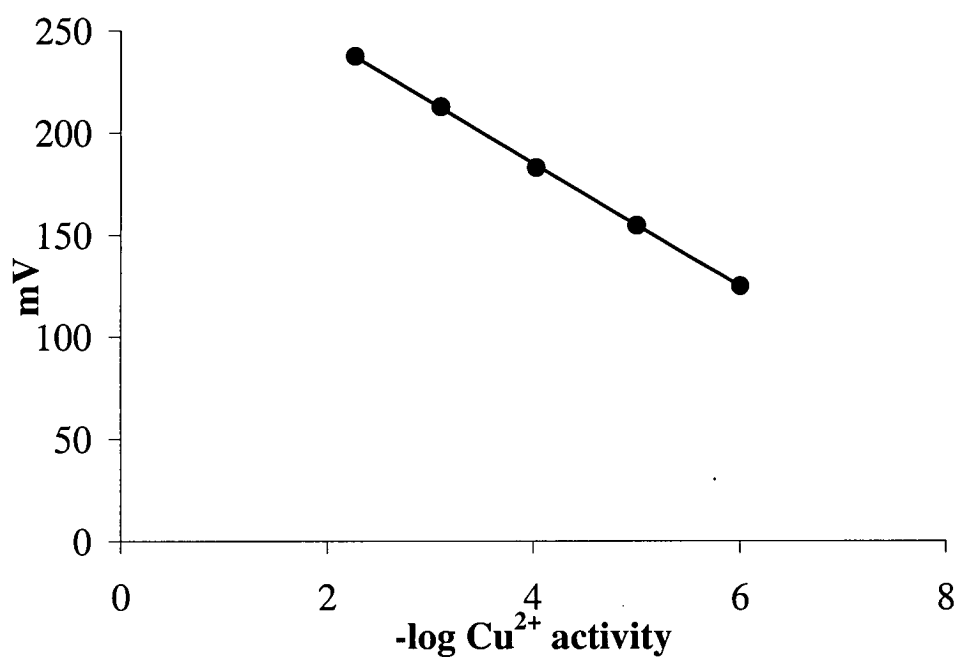


Figure 2.1 Electrode response to Cu^{2+} standards prepared by serial dilution in deionised water, showing a near-Nernstian slope of 30.18 mV/decade and E° of 305.79 mV.

where A and B are conditional constants, dependent upon the temperature, density and dielectric constant of the solvent

z_X and z_Y are charges on ions X and Y

d = average effective diameter of the ions

I = ionic strength as defined in (3)

and may be calculated from equation 6, which is a practical approximation for the mean ion activity coefficient, since single ion activity coefficients can not be measured

For pure solutions of XY , the activity coefficient is close to unity, so for solutions less than 10^{-4} M, activity \cong concentration (Bailey 1976). At concentrations above 10^{-4} M, the activity coefficient decreases significantly causing a calibration graph prepared by serial dilution of a concentration standard to curve at high concentrations (Camman 1977) as illustrated in Figure 2.2.

Equation 1 holds for solutions in which there are no interfering ions, that is, it describes the ideal response of the electrode to species X . Ideal selectivity is rarely achieved with real electrodes and, in practice, the activity range over which an electrode will give Nernstian response is also limited. If an interfering ion is present, the electrode will respond to both the primary and the interfering ions. The degree of selectivity exhibited by the electrode for the primary ion A with respect to the interfering ion B is expressed by the potentiometric selectivity coefficient $k_{A,B}^{pot}$

$$E = E^o \pm \frac{2.303RT}{z_A F} \log_{10}(a_A + \sum_B k_{A,B}^{pot} a_B^{z_A/z_B}) \quad (7)$$

where z_A and z_B are the charges on ions A and B .

The sign is determined by the sign on the primary ion. For an interferent ion B , the magnitude of the interference effect will be described by the magnitude of $k_{A,B}^{pot}$. If $k_{A,B}^{pot}$ is $\ll 1$, the electrode is much more selective for species A . If $k_{A,B}^{pot} > 1$, then the electrode will respond preferentially to species B (Bailey 1976).

Copper(II) ISEs

A variety of copper(II) ISEs are available commercially, or can be produced relatively easily in the laboratory. Broadly speaking, there are two main types of membranes: these are solid state comprising glass or a single crystal of pressed powder, and polymer membranes (Cattrall & De Marco 1992). This discussion will only focus on solid state electrodes.

Many of the copper ISEs in use have been discussed in extensive reviews by Gulens (1981; 1987). Membranes may be based on copper(I) sulfide (e.g. Cu_2S , chalcocite),

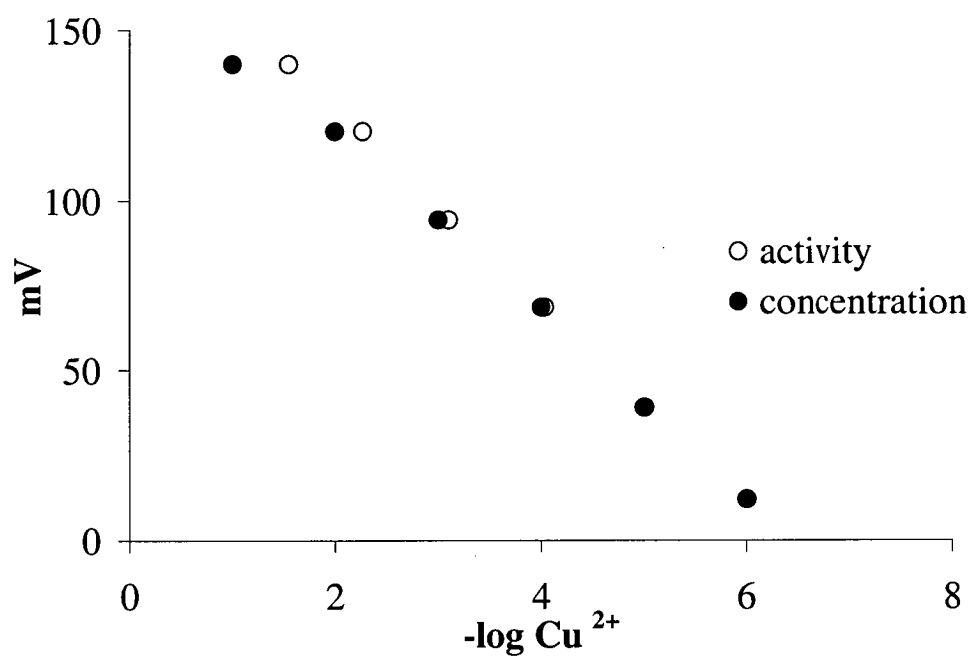


Figure 2.2 Calibration plot showing ISE response vs. activity and concentration standards in deionised water, over the range 10^{-1} M to 10^{-6} M .

copper(II) sulfide (e.g. CuS, Cu_{1.92}S, Cu_{1.77}S, Cu_{1.60}S, Cu_{1.31}S), a mixture of CuS and Ag₂S, ternary compounds of Cu, S, and Ag (e.g. Cu_{0.45}Ag_{1.55}S, jalpaite; Cu_{0.8}Ag_{1.20}S, macinstyrite; Cu_{1.07}Ag_{0.93}S, stromeyerite) or copper selenide (e.g. CuSe, Cu_{1.8}Se, Cu₂Se, Cu₃Se₂) and others. Each of these electrode types will have different performance characteristics depending upon membrane type, electrode construction, method of preparation, presence of impurities, sample matrix, and analysis history. Significantly, the susceptibility to interferences will also depend upon these factors, so sweeping statements about the inability of the Cu(II)ISE to measure environmentally relevant concentrations in seawater is an oversimplification of the situation (Mackey & De Marco 1997).

Interferences

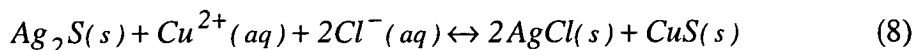
For the Cu(II)ISE, the most significant interferences are the chloride ion, other divalent metal cations, strong oxidants and the cuprous ion.

Chloride ion

The observation of apparent non-theoretical behaviour of copper(II) ISEs in the presence of chloride has led to the declaration that the electrode is unsuitable for the analysis of seawater (Midgley 1976; Gulens 1981; Bond et al. 1982; Lund 1986; Gulens 1987; Batley 1989; Xue & Sunda 1997). The study of surface effects has been fundamental in gaining an understanding of the electrode response in the presence of interfering ions such as chloride, and the use of surface analysis techniques such as Auger spectroscopy (Moody et al. 1979), X-ray diffractometry (Heijne & van der Linden 1977; Siemroth & Hennig 1981), and X-ray photoelectron spectroscopy (Ebel 1981; Catrall & De Marco 1992; De Marco et al. 1992) has greatly advanced our understanding of the response mechanism of the chloride interference. These mechanistic studies also identify under what conditions the interference effects can be minimized or overcome.

Early studies investigating the chloride interference reported the formation of a silver chloride layer on the electrode surface, when the CuS/Ag₂S electrode was used in a solution containing sufficient concentrations of both Cu²⁺ and chloride ions (Moody et al. 1979). The observed effects of this poisoning was to give the electrode a dull appearance, and to change the response of the electrode to the concentration of chloride ions in solution rather than the cupric ion, and to alter the standard potential of the electrode. An increase in the response time of the electrode was also noted, accompanied by a significant reduction in sensitivity (Hoyer & Loftager 1988)

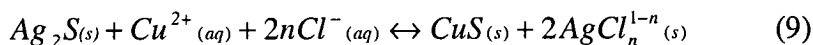
The AgCl poisoning is the result of a very slow reaction at the electrode surface, which occurs when the membrane is exposed to chloride for long periods of time (> 7 days) and/or high concentrations of Cu^{2+} i.e. $> 10^{-3}$ M (De Marco 1996)



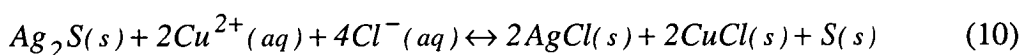
The limit of Nernstian response and the extent of chemical interferences are not always consistent with theoretical predictions (Gulens 1981). The onset of AgCl formation is predicted to occur at $\sim 10^{-11}$ M Cu^{2+} , based on solubility product calculations for silver sulfide, silver chloride, and copper(II) sulfide. Despite this thermodynamic prediction, the fouling process only occurs at copper concentrations $> 10^{-3}$ M, or over long periods of time.

It was established by Oglesby et al. (1977) that these effects were attributable to the presence of the chloride ion, and were not simply an artifact of variations in the ionic strength of the solutions. The effects were reversible, and normal sensitivity could be restored by polishing the electrode, washing the electrode with dilute sulphuric acid (Blaedell & Dinwiddie 1974) or cleaning the surface with ammonia (Lanza 1979).

Lanza (1979) proposed the formation of soluble silver chloride complexes, AgCl_n^{1-n} , as a mechanism for chloride ion interference

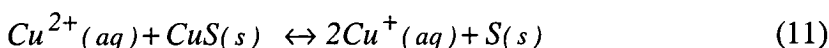


where n is the average coordination number at the particular chloride concentration, but his theory is only applicable to CuISEs containing Ag_2S (Gulens 1981). Super-Nernstian response to Cu^{2+} for an electrode exposed to high concentrations of Cl^- was explained by the following reaction

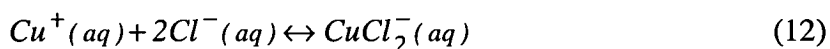


At the same time, Westall et al. (1979) presented a theory to explain the chloride interference for copper electrodes, and significantly this theory could be extrapolated to describe the response of all $\text{CuS}/\text{Ag}_2\text{S}$, Cu(I)S and Cu(II)S electrodes. It was also applicable to the observations of Jasinski et al. (1974) and Oglesby et al. (1988).

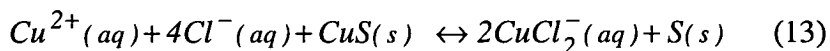
The Westall-Morel-Hume theory proposed that for a solution containing both Cu^{2+} and chloride ions, a redox reaction occurred at the solution-membrane interface, producing Cu^+ and elemental sulphur:



The driving force for this mechanism is the stabilisation of cuprous ions by complexation with chloride ions



Which can be summarised in the overall equation



The net effect of this mechanism is that in the presence of chloride, complexation of Cu(I) released into the electrode diffusion layer causes a negative shift in potential, and super-Nernstian response (De Marco 1996). Gulens (1981) extrapolated this theory to describe the response of CuISEs to other ligands capable of complexing cuprous and cupric ions. Lewenstam et al. (1990) confirmed the mechanism of Equation 13 by identifying elemental sulphur on the surface of a CuS electrode which had been exposed to solutions containing chloride and Cu^{2+} .

The mechanisms proposed by Lanza (1979) and by Westall et al. (1979) predict essentially the same effects, which depend on the absolute and relative concentrations of Cu^{2+} and chloride (Mackey & De Marco 1997). These mechanisms have been used to explain the phenomenon observed in chloride media (up to 3 M) using Cu^{2+} concentrations above 10^{-5} M.

The significant factor in these mechanistic studies is that the solutions studied bear no resemblance to the composition of seawater, that is moderate chloride ion concentration (0.6 M) and very low copper concentrations, typically $1\text{--}3 \times 10^{-9}$ M (Mackey 1983). Additionally, the experiments of Westall et al. (1979) were conducted in unstirred, oxygen free solutions, with a Radiometer electrode of unknown composition.

Hoyer and Loftager (1988) showed that the application of the cation-exchange resin Nafion onto the surface of a $\text{Cu}_{1.8}\text{Se}$ electrode was effective in preventing the chloride interference in solutions containing 0.5 M NaCl and 10^{-6} M Cu^{2+} . De Marco (1994) showed that this approach was also effective for the jalpaite ISE over the range 10^{-8} to 10^{-16} M using metal ion buffers, and also demonstrated that the ligand used to control free copper (ethylenediamine) had no effect on the electrode response. However, equilibration times were increased considerably, and the application of the coating was difficult to do in a reproducible manner.

Other interferences

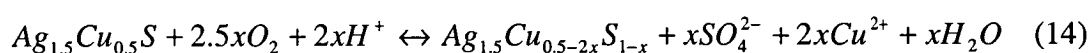
Some known selectivity coefficients for the Cu(II)ISE are shown in Table 2.1. Jasinski et al. (1974) investigated the potential interference of metal cations which form sulfides more insoluble than CuS e.g. Fe(III) and Ag(I) on the CuS/Ag₂S ISE. In contrast to data from Cammann (1977), they determined that due to the low soluble ferric and silver ion content of seawater, their presence presented no practical problem. Mercury concentrations should also be too low to be of

Table 2.1 *Selectivity data for Cu(II) selective electrodes at 25 °C.*

Interferent	Selectivity co-efficient	Reference
Pb ²⁺	2×10^{-3}	Bailey (1976)
Cd ²⁺	3×10^{-4}	“ “
Zn ²⁺	$2-5 \times 10^{-4}$	“ “
Ni ²⁺	2×10^{-4}	“ “
Mn ²⁺	1.6×10^{-4}	“ “
Fe ³⁺	10	Cammann (1979)
Cu ⁺	1	“ “
Hg ²⁺	Must be absent	“ “
Ag ⁺	Must be absent	“ “

consequence for seawater analysis (Zirino & Seligman 1981). Strong oxidants such as cerium(IV) and permanganate have been observed to cause a shift in the standard potential and a decrease in slope (Gulens 1981) while weaker oxidising agents (e.g. dissolved oxygen) may have an affect at low copper concentrations.

Gulens (1981) and Hoyer and Loftager (1988) specified that dissolved oxygen must be removed from solution for low level copper measurements, in order to minimize mixed potentials contributing to the electrode response. This approach is questionable for environmental analysis, however, as it does not allow for *in situ* measurements, it complicates field analysis, and any procedure involving manipulation of the sample may significantly increase the chances of sample contamination. The mechanism of membrane photooxidation for the Orion CuS/Ag₂S electrode as reported by De Marco (1994)



results in the release of Cu²⁺ into solution. This further reacts with AgCl via Equation 10 to produce AgCl on the membrane.

More recently, Tani et al. (1999) reported that significant electrode dissolution in the presence of dissolved oxygen made it mandatory for solutions to be deaerated before a meaningful sample measurement could be made. It is important to note here that the CuS/Ag₂S membrane they used is not jalpaite (i.e. Ag_{1.5}Cu_{0.5}S), so the results are not applicable to the analysis of seawater with the Orion 94-29 Cu(II)ISE. Additionally, mixtures of CuS/Ag₂S used by Tani et al. (1999) have been shown by Heijne and van der Linden (1977) to be inferior to jalpaite for construction of Cu(II)ISEs. Long-term exposure to oxygen (hours) does contribute to electrode dissolution of the Orion 94-29 ISE. However Zirino et al. (1998a) showed that the presence of oxygen itself in a seawater sample did not cause a significant error in measurement.

The presence of Cu(I) in seawater has been proposed as a potential interferent in seawater analyses. However, Cu(I) is photochemically produced in the euphotic zone, and rapidly complexed by chloride. Whilst this *may* present a problem for *in situ* analyses of surface waters, for measurements in the laboratory under artificial lighting, the production of Cu(I) will not be a problem.

Calibration of the ISE with metal-ion buffers

Copper ion buffers can be prepared using a ligand to control the activity of the free metal ion, in a similar manner to the regulation of hydrogen ion activity by pH buffers (Perrin & Dempsey 1974). The purpose of the buffer is to extend the linear range of response below that which can be achieved by serial dilution. This is essential for the analysis of seawater in which total copper concentrations are typically in the nano-molar region (Mackey 1983), while the free copper ion

concentration may be many orders of magnitude lower (Sunda & Hanson 1987). The ligand chosen must fulfill two criteria; firstly the stability constant should be accurately known, and secondly the ligand itself should not affect the electrode response (Svehla 1975). Castro Peñuelas (1996) has shown that five ligands commonly used as metal-ion buffers (CDTA, NTA, ethylenediamine, glycine, and EDTA) have no adverse affect on the response of the ISE. This can be confirmed by the fact that a calibration curve constructed from metal-ion buffers is co-linear with a calibration curve prepared from deionised water standards (see Chapter 3). Avdeef et al. (1983) reported the detection limit of a Beckman CuS/Ag₂S ISE was extended significantly to 10⁻¹⁹ M using metal ion buffers, with rapid response and excellent linearity (99.2 % of theoretical Nernstian slope). It should be noted that linear response in the range 10⁻¹⁵ to 10⁻⁸ M reported by De Marco (1994) for the Orion Cu(II)ISE in saline copper(II) ethylenediamine buffers indicates that the chloride interference reactions is kinetically limited under these conditions.

Analytical measurements using this calibration system are limited by the accuracy with which the assigned buffer value of free copper is known. Uncertainty in the temperature and ionic strength dependence of the stability constants means that the ISE values are, at best, within 0.2 pCu units of the true value.

Analysis of seawater

The suitability of a number of types of copper(II) ISEs for the analysis of seawater has recently been investigated by De Marco (1994). He found that the Orion Model 94-29 electrode, consisting of a pressed disk of the polycrystalline powders CuS and Ag₂S, was the most suitable. X-ray powder diffraction (XRD) analysis of the Orion membrane by Siemroth and Hennig (1981) showed that it mainly consists of the ternary compound Ag_{1.5}Cu_{0.5}S, known as jalpaite. De Marco also showed that a homemade copper sulfide (CuS) electrode was too insensitive to copper to be suitable for the analysis of free copper in seawater, with a detection limit of 10⁻⁴ M. The Radiometer F112 comprising a single crystal of copper selenide (Cu_{1.8}Se) was also unsuitable due to an offset in the standard potential of the electrode when used in the presence of metal ion buffers, and it released an unacceptably high amount of copper into solution by membrane dissolution. Studies by De Marco and co-workers (De Marco 1996; De Marco et al. 1997; Mackey & De Marco 1997; Zirino et al. 1998; De Marco et al. 1998; Eriksen et al. 1999) have shown that many of the concerns traditionally associated with the use of the CuISE in seawater are invalid, or can be overcome sufficiently so that routine analysis of marine waters for free copper is possible. These factors are addressed in Chapters 3, 4 and 5 as part of the development of an automated method for the determination of free copper in seawater.

In a wider context, Cu(II)ISEs are also being used successfully for the analysis of soil contamination and potential toxicity (Dumestre et al. 1999), to study the role of natural organic matter and metal oxides in copper speciation (McBride & Martinez 2000), and to study the kinetics of copper speciation, complexation and toxicity to invertebrates in natural waters (Ma et al. 1999). Other application in natural waters include the investigation of copper complexation characteristics by Rozan and Benoit (1999) and a study of copper interactions with fulvic acid by Wolf et al. (1999).

In seawater at natural pH, additions of copper result in super-Nernstian response (Jasinski et al. 1974) when the response is calculated in terms of the added total copper. However, Belli and Zirino (1993) demonstrated that additions of copper to artificial seawater was Nernstian when the response was expressed as a function of the calculated equilibrium levels of free copper. Thus the observation of non-theoretical response was an artifact of attributing the electrode response to a parameter it could not detect i.e. total copper (De Marco 1996). Belli and Zirino (1993) also showed that acidification of seawater to pH 2 resulted in Nernstian response to the total copper added. The difference in response was attributed to the complexation of copper by natural ligands at pH 8, which are released upon acidification. This phenomenon was observed even when the sample was acidified in the middle of a titration, summarised by Zirino et al. (1998a) as “further weakening the argument for super-Nernstian response in seawater”.

De Marco (1994) observed encouraging agreement ($\pm 0.2 - 0.3$ pCu units) between experimental and theoretical values of free copper determined by ISE in artificial seawater samples with similar complexing capacity to seawater. These results implied that the ISE could be used to detect environmentally relevant free Cu^{2+} concentrations in a defined matrix. Close agreement between total copper determined by ISE in UV-oxidised acidified, seawater and GFAAS confirmed that this was so. The analysis of seawater with a Nafion-coated electrode yielded similar results to a bare electrode, confirming that the jalpaite ISE is unaffected by the presence of chloride ions at low free copper concentrations.

As discussed earlier, dissolved oxygen has been flagged as an interferent for low level copper measurements by ISE. De-oxygenation of samples does not seem to be as important for the jalpaite ISE as it has been shown that oxidative dissolution of the Orion 94-29 can be reduced by conditioning the electrode before immersion in a sample, and the rate of dissolution is less significant than for other electrodes (De Marco 1994). Gulens (1981) also noted that the $\text{CuS-Ag}_2\text{S}$ electrode is less susceptible to direct redox interference than Cu(I)S electrodes.

Detection limits and accuracy of ISE measurements

The IUPAC definition of detection limit is the concentration of analyte A at which the extrapolated linear portion of the calibration graph at extreme dilution of the analyte intersects the extrapolated Nernstian part of the graph (Bailey 1976). Reported detection limits for copper ISEs in unbuffered systems is usually between 10^{-4} M and 10^{-8} M (Pungor et al. 1979), though it has long been recognised that rigorous analytical techniques are required to obtain response at the lower concentrations. The solubility products of copper sulfide ($10^{-35.2}$) and silver sulfide (10^{-49}) suggest that the detection limit for the CuS/Ag₂S membrane should be significantly lower than is observed. Variations from theoretical detection limits in unbuffered systems can be attributed to impurities in the membrane itself (Gulens 1987), contamination of the solutions, and oxidation of the copper sulfide causing dissolution of the membrane (Cattrall & De Marco 1992). These limitations can be overcome by the correct choice of electrode, the use of clean laboratory facilities, and fastidious attention to the storage, preparation, calibration and use of the copper(II) ISE (see Chapter 3). De Marco et al. (1997) have shown that it is possible to obtain Nernstian behaviour in deionised water down to 10^{-9} M total copper, using the Orion 94-29 Cu(II)ISE. Apparent limits of detection can be extended many orders of magnitude by the use of cation or anion buffers. In both cases (buffered and unbuffered) the limit of detection will depend upon the solution matrix, the presence of interfering ions, the analysis conditions and the membrane condition. Avdeef et al. (1983), Belli and Zirino (1993) and De Marco (1994) have demonstrated that it is possible to measure as low as 10^{-19} M Cu²⁺ by using metal ion buffers, however no detection limits were reported.

De Marco et al. (1997) cited the accuracy of the ISE measurements to be 0.1 - 0.3 pCu units and this is mainly due to uncertainties in the thermodynamic data used to calculate equilibrium speciation in the metal-ion buffers. In the absence of an independent, validated technique for the determination of free Cu²⁺ in seawater, it is not possible to assign a definitive pCu value or assess the accuracy of free Cu²⁺ measurements by ISE (Belli & Zirino 1993).

CONCLUSIONS

Whilst there is a significant body of research on the fabrication and response mechanisms of ISEs, there are fewer papers on actual applications. Fewer still focus on ISEs for environmental monitoring, which is a reflection of the difficulty in using ISEs in a reliable and dependable fashion. ISEs are complex instruments, and given the wide variety of Cu(II)ISEs available, generalisations about their suitability for particular analyses can not be made.

This chapter demonstrates that the two major problems cited in the analysis of saline matrices, i.e. the chloride interference and lack of sensitivity, are not applicable to the jalpaite ISE. Suitable buffer systems have been developed for the calibration of the ISE over the range of environmental values of interest (i.e. 10^{-15} – 10^{-8} M) and the ligands used do not interfere with the electrode response. Kinetic limitation of the chloride interference over this range means that the ISE is capable of measuring free copper in seawater. Good agreement with theoretical or known copper concentrations can be achieved using the jalpaite ISE. The observation of super-Nernstian response in seawater can be explained by complexation of added copper, and when the electrode response is interpreted in terms of the free copper in solution, Nernstian response is achieved. This is confirmed by acidification of seawater to pH 2 (conditions under which copper is released from complexation by natural ligands) where normal Nernstian response to copper additions is observed. The jalpaite ISE has been identified as the most suitable electrode for seawater analysis, and it has been demonstrated that ISE results are consistent with data generated by other techniques (i.e. GFAAS). These findings form the basis of using the Orion 94-29 Cu(II)ISE for method development in these studies, and further details on analytical protocols required for measurements in natural waters are presented in Chapter 3.

Chapter 3 Potentiometric Measurements with the Cu(II)ISE

EIS results from this chapter are published in “Electrochemical Impedance Spectroscopy Study of the Response Mechanism of the Japaite Copper^{II} Ion-Selective Electrode in Seawater.” Roland De Marco, Ruth Eriksen, and Alberto Zirino, Analytical Chemistry, (1997) 70, 4683–4689.

INTRODUCTION

The major advantage of the Cu(II)ISE for environmental analyses is that the electrode measures the free ion activity, a physiologically important parameter in nutritional and toxicological studies (Bailey 1976). Additionally, the measurement is non-destructive, and the method makes no assumptions about the degree of control thermodynamic or kinetic factors exert on the metal speciation. However, the technique has several inherent practical disadvantages, including the fact that ions other than Cu^{2+} can influence the potential of the electrochemical cell (Warner 1975). As discussed in Chapter 2, the metals that exert an interference effect on the ISE response e.g. Hg^{2+} , Fe^{3+} and Ag^{+} are present in seawater at concentrations orders of magnitude lower than Cu^{2+} , and if they were causing a significant problem, good agreement between ISE analyses and GFAAS (for total copper) would not have been achieved. Chloride interference can be ignored using the Orion 94-29 Cu(II)ISE, under well defined conditions of analysis (Belli & Zirino 1993; De Marco 1994).

The accuracy and precision of ISE analyses is poorer than some other techniques (e.g. GFAAS, ASV) but they remain unaltered over several decades of activity and it has been proposed that in general, ISEs are more suited to the relatively imprecise analysis of samples containing a wide range of Cu^{2+} activities, rather than the precise analysis of samples containing the analyte in a narrow range (Bailey 1976). However, Zirino et al. (1998a) recently described an analytical technique whereby the japaite Cu(II)ISE could be used to discriminate small time-dependent changes in pCu in a contaminated estuary, with a precision of 0.06 pCu units. Slow response, limited sensitivity and inaccuracy are also frequent complaints of ISEs, however they all can be improved by control over the experimental conditions. Recent work on the japaite Cu(II)ISE has demonstrated that adhering to rigid protocols for the preparation, calibration and use of the ISE can result in superior performance to that often cited in the literature (De Marco 1994; De Marco et al. 1997; Mackey & De Marco 1997; Zirino et al. 1998a).

The field of potentiometry has expanded rapidly in the last 20 years, with significant advancements in the number and type of ISE's available, and the use of new analytical techniques to gain a better understanding of response and interference mechanisms. The purpose of this chapter is to establish the conditions necessary for making accurate and meaningful potentiometric measurements and to demonstrate the effect of a number of parameters on the electrode response. Electrochemical Impedance Spectroscopy (EIS) was used to explore the mechanisms and effect of natural organic matter on the ISE. This is possible as EIS measures the rate of the charge-transfer occurring at the membrane solution-interface, which is widely accepted as the process controlling the potential of the Cu(II)ISE (De Marco et al. 1998). EIS has not previously been used to study the response of the jalpaite Cu(II)ISE in seawater.

Confirmation that normal function of the ISE can be extended into the nM range in unbuffered solutions and archived seawater samples demonstrates that the ISE is suitable for environmental studies of speciation. The analysis of total copper is principally dealt with here, since this is a parameter that can easily be validated by other techniques. Those parameters affecting ISE response are addressed in terms of the ultimate goal of developing an automated method for speciation analyses with the ISE.

EXPERIMENTAL

Potentiometry

Two Cu(II)ISE's (Orion Model 94-29) were used during the course of this study. Electrode 1 was approximately 2 years old at the commencement of the project (March 1995) and was used for all initial experiments. Electrode 2 was purchased new in August 1995. An Orion double junction sleeve-type reference electrode (Model 900200) was used for static measurements. The inner (saturated KCl) and outer (10 % KNO₃) filling solutions of the reference electrode were replaced before each set of experiments. Potentials of the electrochemical cell were recorded using an Orion Expandable Ion Analyser (Model EA940). A stability criterion of 0.2 mV/min was employed for all studies, unless otherwise specified. Output was recorded on a YEW Type 3056 chart recorder.

Standard solutions

Potentiometric response curves were measured in pure salt solutions, using analytical grade reagents. Deionised water ("Milli-Q") from a Millipore 4 cartridge system with a "Milli-Ro 4" pretreatment system was used to prepare all solutions. All glassware and plasticware (low density polyethylene, LDPE) involved in the preparation and analysis of standards and samples was acid washed in a 10% HCl bath for 1 week, and rinsed extensively with deionised water. Glassware and plastic ware was air-dried and stored sealed until use.

The ion scale pCu, where $pCu = -\log[Cu^{2+}]$ is employed for this study, and $pCu = pCu_{free}$ unless otherwise stated. pCu_a refers to the $-\log Cu^{2+}$ activity. The logarithmic notation can disguise small changes in concentration, so where appropriate molar units have also been used. Where the ionic strength of the standards is fixed and known, the ISE can be calibrated in terms of activity or concentration. The same applies for samples, assuming the ionic strength is the same as the calibrating standards. In this work, buffers were made up to have approximately the same ionic strength as seawater (0.6M NaCl).

Standard solutions were prepared by serial dilution of a stock 10^{-1} M Cu(NO₃)₂ solution. Serial dilution standards covered the range $2 < pCu < 7$. Standards above pCu 3 were prepared daily to reduce errors incurred by adsorptive losses of copper to the glass, and because they are more likely to be contaminated. Calibrated A-grade volumetric ware was used for all dilutions, and a calibrated Eppendorf Multipipette was used for dispensing standards.

pCu buffers

Saline copper ethylenediamine (Cu(II)-en) buffers were prepared in accordance with Avdeef et al. (1983) and comprised 0.6 M NaCl, 10^{-3} M Cu(NO₃)₂, and 1.5×10^{-2} M ethylenediamine (en). pH adjustment was made with analytical grade NaOH (50 % w/v solution) and H₂SO₄ (50 % v/v solution).

Calculations of pCu were made using the stability constants reported in De Marco (1994), and by modelling the solutions in MINTEQA2 (Allison et al. 1996) or MINEQL (Westall et al. 1986). Details of thermodynamic databases for the speciation software are described in Appendix 1.

pCu was also calculated from an empirical relationship reported in Eriksen et al. (1999)

$$\text{pCu} = 125.39 - 86.958 \times \text{pH} + 22.788 \times \text{pH}^2 - 2.7572 \times \text{pH}^3 + 0.16079 \times \text{pH}^4 - 3.6683 \times 10^{-3} \times \text{pH}^5$$

The relationship between pH and pCu fits the MINEQL data to within 0.02 pCu units for $6.5 < \text{pCu} < 17$.

pH

pH was measured using a Radiometer Precision pH meter, Orion 8103 combination pH electrode, and a Radiometer T801 temperature sensor. pH 7 buffers were prepared using disodium hydrogen phosphate/ potassium dihydrogen phosphate, and pH 4 buffers were prepared using potassium hydrogen phthalate. Buffers were stored in acid washed LDPE bottles, and renewed frequently to prevent biological growth.

Electrode pretreatment and storage

Polishing

Polishing was done using small felt pads (Buehler) and two grades of alumina powder (BAS Inc.). The pads were scrubbed clean with a toothbrush before use to remove particulate matter, including traces of jalpaite which can scratch the surface of the electrode. Pads were then rinsed clean with deionised water. The ISE first received a coarse polish with 0.3 μm alumina, by moving the electrode in a figure-of-eight pattern. After copious rinsing with deionised water to remove jalpaite particles, the procedure was repeated on a separate moistened felt pad with 0.05 μm alumina. The ISE was then rinsed extensively with deionised water and blotted dry with a lint free tissue. The surface was inspected for pits and scratches before use, as the surface should be as smooth as possible to reduce problems with carryover and

memory effects. The whole polishing procedure was repeated if the surface of the electrode was deemed unsatisfactory.

For EIS studies, the electrodes were polished using a Struers Dap-V polishing wheel with 2 grades of diamond polishing spray. The wheel was fitted with a Struers DP Nap cloth, which was scrubbed clean with a toothbrush prior to use. The pad was sprayed with 3 μm Struers DP-Spray and Struers DP-Lubricant Red spray, and the electrode polished for several minutes at high speed. The ISE was rinsed copiously with deionised water, and the procedure was repeated on a separate Nap cloth with Struers 1 μm diamond spray and lubricant. Finally the electrode was gently blotted dry after rinsing, with a lint free cloth. The surface of the electrode was then inspected for pits and scratches before use.

Severe fouling of the electrode by AgCl deposits required polishing with SiC paper, but this was done sparingly as the jalpaite membrane is soft enough to be damaged by extended polishing on such an aggressive surface. The electrode was then sonicated for 10 minutes to remove any traces of SiC. Polishing was then completed as described above using either the polishing wheel or hand polishing on felt pads.

Conditioning

After polishing, the ISE was conditioned by immersion in about 250 mL of the medium to be analysed (generally 0.45 μm filtered seawater) for 24 hours, as per De Marco et al. (1997). This process stabilises the electroactive species ($\text{Ag}_{1.5}\text{Cu}_{0.5-2x}\text{S}_{1-x}$) on the outermost surface layer (De Marco et al. 1992), improves electrode stability, and reduces Cu^{2+} contamination resulting from membrane corrosion by decreasing the response time of the electrode. In seawater the membrane will consist of the copper deficient sulfide with traces of AgCl and AgI, and it is important to note that the E^0 of this surface is peculiar to jalpaite alone. Prior to calibration and immediately before sample analysis, the ISE was rinsed with filtered seawater and dipped in a sacrificial buffer (pCu 14 - 15) for 15 minutes, as this has been shown to minimise carryover of adsorbed Cu^{2+} (De Marco 1994).

Electrode storage

The protocol for electrode storage was as follows:

- Short term (1 hour- 1 day) clean sample or pCu 14 buffer
- Medium term (1 day -1 week) pCu 14 buffer
- Long term (1 week - ?) store dry with cap on

Electrode calibration

A series of 4 - 5 pCu buffers ($2 < \text{pCu} < 6$, or $9 < \text{pCu} < 14$) was used for each calibration, beginning and ending with a sacrificial buffer (pCu 14 - 15). Calibration parameters (slope, E^0 , R^2) were calculated in Microsoft Excel. Frequent standardisation is necessary for a divalent ion electrode, as an error of only 0.2mV will produce a relative concentration error of 1.6% (Smith & Manahan 1973).

Standard addition potentiometry

Standard addition analyses were carried out in accordance with the method of Smith and Manahan (1973). In this method, the original concentration of Cu^{2+} in solution is calculated from the potential shift caused by adding a known small volume of copper to a relatively large, accurately measured volume of sample. The method assumes that the activity coefficient of the ion does not change and that the liquid junction potential remains constant. Significantly, the method also assumes that the fraction of copper that is complexed (if any) does not change with the addition of standard. Details of the calculations are described in Appendix 2.

For measurements of deionised water and seawater, the complexing antioxidant buffer (CAB) described by Smith and Manahan (1973) was not used. Samples were stirred using a magnetic flea and a “Magnetir” stirrer on medium speed (Lab-Line Instruments). A thick piece of insulating foam was inserted between the beaker and the stirrer to prevent the solution heating up. The electrode was conditioned for 24 hours in the same matrix as the sample (i.e. Milli-Q or seawater) and then exposed to sacrificial buffer (pCu 15) for 15 - 30 minutes to cleanse the membrane of adsorbed Cu^{2+} salts, resulting in minimal electrode carryover of Cu^{2+} . All standard addition analyses were performed in the CSIRO Class-100 clean room.

Sample history and pretreatment

Seawater for these studies was collected by the author from the Southern Ocean, onboard Aurora Australis during voyage A9407, along WOCE transect SR3. Water was collected in acid washed LDPE carboys from 10L Niskin bottles deployed on a CTD. Water was filtered through 0.45 μm HA Millipore filters using acid washed Millipore filtration apparatus, on return to Hobart. Water was subsequently stored at 4 °C until required.

Archived seawater samples which had previously been analysed by GFAAS for total copper, total dissolved copper, and/or copper complexing capacity were also analysed for total copper by ISE. These samples were collected, analysed and stored by CSIRO Marine Research (Hobart) using trace metal techniques, and therefore represent an extremely useful resource for validating the response of the ISE to total copper. Archived samples were acidified to pH 2 with 1 mL/L of high purity HCl immediately after collection (Mackey 1983). Sub-samples of the archived water

were decanted and analysed in the clean room. This environment is crucial to the analysis of copper in open ocean samples, and prevents the cross-contamination of samples and solutions. The clean room has the additional advantage of providing an environment of constant temperature and light regime, both important factors in potentiometric analysis.

UV-photo oxidation

Selected samples were UV-oxidised to dissociate non-labile Cu-organic species still present after acidification (Armstrong et al. 1966). Batch photo-oxidations were performed in the CSIRO clean room. Individual samples were UV-oxidised in 60 mL aliquots by 5 hours exposure to a 1200 W mercury arc lamp. Each sample was spiked with 80 μ L of H₂O₂ prior to UV-oxidation. Samples were allowed to cool before being removed from the unit, and were stored in acid washed 60 mL or 125 mL LDPE bottles until analysis. Samples were previously acidified to pH 2 with 1 mL/L of high purity HCl, unless otherwise stated.

Larger volumes of seawater were UV-oxidised as required using a flow through system. A 2 m quartz coil (3 mm I.D.) wrapped around a 1200 W mercury arc lamp was connected to a peristaltic pump to enable continuous production of UV-treated seawater. Residence time in the coil was calculated to be 25 minutes. The system was flushed with seawater for 40 minutes before collection began. The seawater stream was segmented with oxygen from a nearby cylinder to facilitate the oxidation reaction. In between runs, the quartz tubing was stored full of seawater. The feed water was stored in an acid washed 25 L LDPE carboy at room temperature. When the feedwater supply was exhausted, the system was cleaned with 10 %HCl, followed by Milli-Q, and the carboy refilled with seawater. UV-treated water was collected and stored in 1 L acid washed LDPE bottles until required.

Graphite Furnace Atomic Absorption Spectroscopy

Acidified seawater samples were analysed for total and/or total dissolved copper by GFAAS in the Class-100 clean room, using the methods described in Mackey (1983) and O'Sullivan et al. (1994). This method uses a solvent extraction procedure to pre-concentrate the metals from 50 mL of sample buffered to pH 4. The extraction is followed by back-extraction into 1 mL of Milli-Q resulting in a 50-fold concentration factor. The extraction agent is ammonium pyrrolidenedithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDDC) with metals extracted into double-distilled 1,1,2-trichloro-1,2,2-trifluoroethane (Freon TF). Standards were carried through the same extraction procedure and extraction efficiency calculated by comparing extracted to non-extracted standards. Samples and standards were analysed at 324.8 nm on a Perkin Elmer 5000 GFAAS with Zeeman background correction, using an injection volume of 20 μ L. Certified NASS

–3 open ocean seawater reference or SLEW-1 estuarine water reference was run with each batch of samples to check the efficiency and accuracy of the extraction and analysis process.

Preparation and characterisation of jalpaite powder

Jalpaite powder was prepared according to the method of Heijne and van der Linden (1977). The method involves simultaneous precipitation of copper(II) sulfide and silver(I) sulfide with an excess of sodium sulfide as the precipitating agent. All reagents used were AR grade, and all glassware and plasticware (LDPE) involved were acid washed in a 10 % HCl bath. The method gives a mole ratio of 67:33 for Ag:Cu, and a 50:50 ratio of $\text{Ag}_2\text{S}:\text{CuS}$ formed. Using an 80 % excess of Na_2S to co-precipitate copper and silver sulfide produces a precipitate consisting predominantly of jalpaite ($\text{Ag}_{1.5}\text{Cu}_{0.5}\text{S}$), with traces of covellite (CuS). Using a 20 % excess of Na_2S also produces jalpaite with a greater yield of covellite.

80 % excess of Na_2S

0.02 mole of copper nitrate and 0.04 mole of silver nitrate were dissolved in 50 mL of deionised water. This solution was added dropwise, with vigorous stirring, to 0.072 mole of sodium sulphide (1 M solution). Both solutions were cooled to about 2 °C before mixing. The addition was completed in about 3 - 4 minutes, and during this time the temperature rose to about 15 °C. The solution was then heated to about 70-75 °C over a 40 minute period, stirring vigorously all the time. The solution was held at this temperature for 30 minutes. After settling and decantation of the supernatant liquid (or centrifugation at moderate speed) the precipitate was washed thoroughly: 4 times with 200 mL of deionised water at 70 °C, once with 100 mL of 0.1 M HNO_3 , and twice with 200 mL of redistilled water.

Each time the precipitate was stirred for 5 minutes and supernatant liquid removed after centrifugation. Finally the precipitate was washed with acetone and dried overnight at 80 °C in air. The yield was about 5 g of powder. The dry precipitate was then stored over silica gel in a light proof container until required.

20 % excess of Na_2S

The method is as above except that the mixed silver/copper solution was added to 0.048 mole of sodium sulfide. The yield was about 6 g of powder.

X-Ray Diffractometry

The precipitate prepared by the method of Heijne and van der Linden (1977) was characterised using X-ray diffraction (XRD) analysis at the Department of Chemistry, University of Tasmania. XRD data was acquired on a Philips PW

1010/25 X-ray generator using a Cu X-ray tube and Cu K(alpha) radiation. Cu K(beta) was filtered out with a Nickel filter. A Philips PW 1050/25 goniometer and Philips PW 1965/40 proportional detector probe were employed. Voltage and current settings were 40 kV and 20 mA respectively. Data collection was via in-house software written by staff at the University of Tasmania. X-ray powder diffraction data for binary and ternary sulfides of copper and silver were obtained from the JCPDS data files (1972) and Heijne and van der Linden (1977).

Preparation of rotating ISE

Rotating Cu(II) electrodes were made using jalpaite powder prepared as described above. Approximately 1 gram of jalpaite powder was pressed under vacuum in a SPECAC punch and stainless steel 13 mm die at 10 tons to give a disk ~ 3 mm thick. The powder was held at 5 ton for 2 minutes before ramping up to full pressure (10 ton) for 5 minutes.

Each pressed disk was attached to a Scanning Electron Microscopy (SEM) stub with threaded arm using a silver loaded epoxy adhesive (RS Components) as drawn in Figure 3.1. The stub and disk were then mounted in a perspex mould, and set in resin (Araldite) and left to cure for 48 hours. The mould was then removed and the resin ground back to the width of the SEM stub, and to within about 0.1 mm of the jalpaite surface. The jalpaite was then exposed by sanding on wet emery paper (1200 grit), and polished to a smooth surface using the Struers polishing wheel and diamond spray.

The electrode disk was attached to a stainless steel, perspex covered rod, which could then be screwed onto a rotator arm of a Pine Instrument Co Analytical Rotator (Model AFCPRB) for EIS studies.

Electrochemical Impedance Spectroscopy

EIS studies on polished Cu(II)ISEs were undertaken using an EG&G Princeton Applied Research Model 5120 potentiostat equipped with a lock-in amplifier at the Corrosion Testing Facility at Curtin University, WA. The EIS spectra were recorded using an AC voltage amplitude of ± 10 mV, and a frequency range of 100 kHz to 1 mHz. A conventional three electrode cell was used in the EIS studies with the Cu(II)ISE as the working electrode, a platinum wire as the counter electrode, and a saturated calomel electrode (SCE) as the reference. The glass cell was acid washed overnight in 10 % HCl, rinsed with deionised water, followed by a rinse with sample

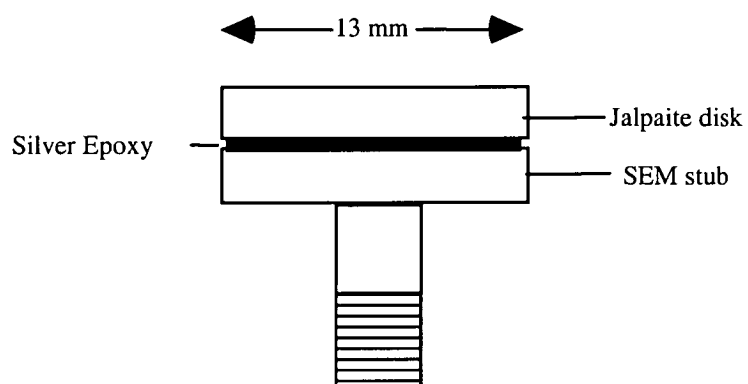


Figure 3.1 Schematic diagram of the jalpaite rotating disk electrode (RDE) used for EIS studies.

prior to filling. The electrode was rinsed with isopropylalcohol and ethanol so that no film was present when the ISE was immersed in the sample. The potential was allowed to stabilise (criterion 0.5 mV/ min) before the EIS profile was recorded.

EIS measurements are made from high frequency (10^5 Hz) to low frequency (10^{-2} Hz). High frequency data represents the film formed on the electrode surface, mid frequency data represents the corrosion or electrochemical process and low frequency data represents the diffusion process (EG & G Princeton Applied Research 1985). A “Warburg” interference is the element of the equivalent circuit that represents diffusional processes, and may be so large as to obscure the data at higher frequencies. Data is presented as complex-plane impedance plots, where the imaginary component of impedance (Z'') is plotted versus the real component of the impedance (Z') for each excitation frequency. The ohmic impedance under the experimental conditions is determined by fitting a semi-circle to the data and measuring the diameter. The charge transfer impedance R_t is inversely proportional to the rate of the charge transfer process.

The rotating electrodes described above were conditioned in the sample matrix for 24 hours before use in EIS studies. Technical grade sodium salt of humic acid (Aldrich Chemical Company) was used for EIS studies, as a readily available analogue of natural organic matter in seawater, since 50% or more of organics in seawater are classified as humic substances (Piotrowicz et al. 1983).

RESULTS AND DISCUSSION

Electrode performance in unbuffered Cu²⁺ solutions

Comparison of electrodes 1 and 2

Typical calibration curves for electrodes 1 and 2 are shown in Figure 3.2, and their respective calibration parameters are shown in Table 3.1. Electrode 1 was “inherited” for this study, and was approximately 2 years old at the time this data was collected. Figure 3.2 shows the first calibration performed with the new electrode after polishing in the prescribed manner. It is obvious that there was a large offset in the E^0 of the two electrodes (120.48 mV). The outer housing of electrode 1 was cracked, the response time of the electrode was much slower than electrode 2, and there was much more noise associated with measurements made using the older electrode. There may have been some internal damage to electrode 1 as a result of the cracked housing, however, Jasinski et al. (1974) also noted a difference of 60 mV in the E^0 of otherwise identical electrodes.

Variation in calibrations with time

Data from repeat calibrations over a 2 month period using electrode 1 are shown in Figure 3.3. It can be seen that the electrode response and sensitivity changes considerably with time, and this is dependent upon the history of use, the type of sample analysed and the condition of the electrode surface. As a result, strict protocols for polishing, conditioning and calibrating are necessary to make accurate and meaningful measurements of free copper.

Effect of polishing

The effect of polishing an aged electrode surface can be seen in Figure 3.4. The linear range of response is increased by 2 pCu units as a result of polishing which removes the build-up of deposits on the surface of the electrode. Electrode response was much faster for the polished electrode, and the signal was much more stable. Polishing of the electrode to achieve a mirror finish means a low electrode surface area and lower corrosion rates than a pitted surface.

Effect of chloride ion on ISE response

The effect of ionic strength and chloride ion concentration on the ISE is shown in Figure 3.5. There was no significant difference in the calibration parameters of the electrode calibrated in pCu_a standards prepared in Milli-Q, and 1 M KNO₃. If the standards are expressed as concentrations, however, the calibration curves will

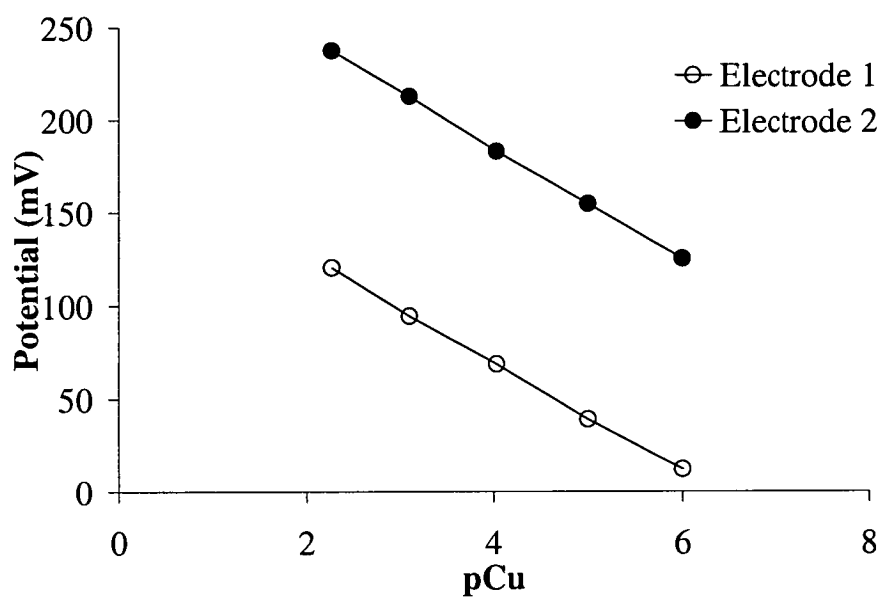


Figure 3.2 Comparison of calibrations using old (electrode 1) and new (electrode 2) Orion 94-29 Cu(II)ISEs.

Table 3.1 *Calibration parameters for Orion 94-29 Cu(II)ISEs in deionised water standards.*

Electrode	Slope (mV/decade)	E^0 (mV)	Linear range	R^2
1	28.99	185.31	$2 < \text{pCu} < 6$	0.9995
2	30.18	305.79	$2 < \text{pCu} < 6$	0.9997

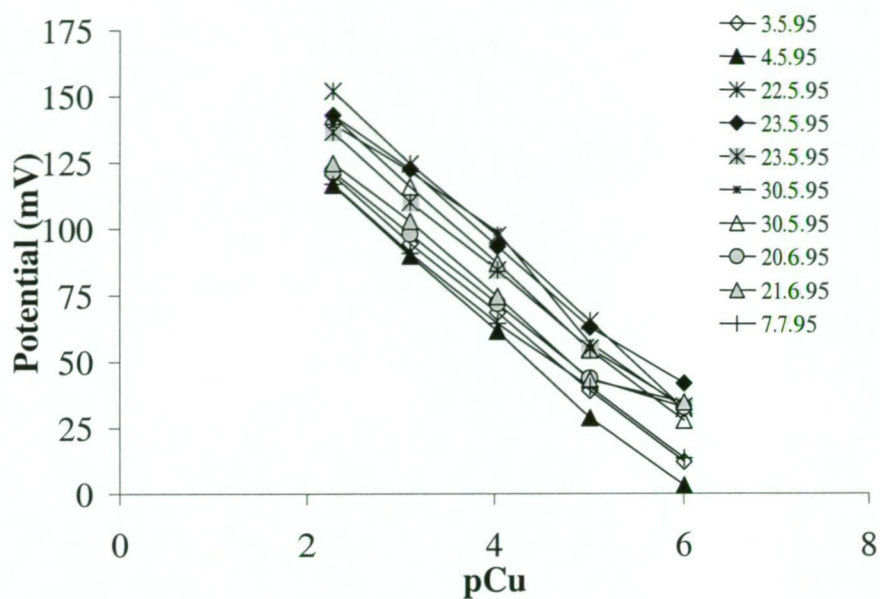


Figure 3.3 Variation in calibration curves for deionised water standards over a 2 month period using ISE#1. Mean slope and E^0 were 28.61 mV/decade and 195.62 mV respectively. Standard error for mean and slope were 0.75 and 5.54 mV respectively ($n=10$).

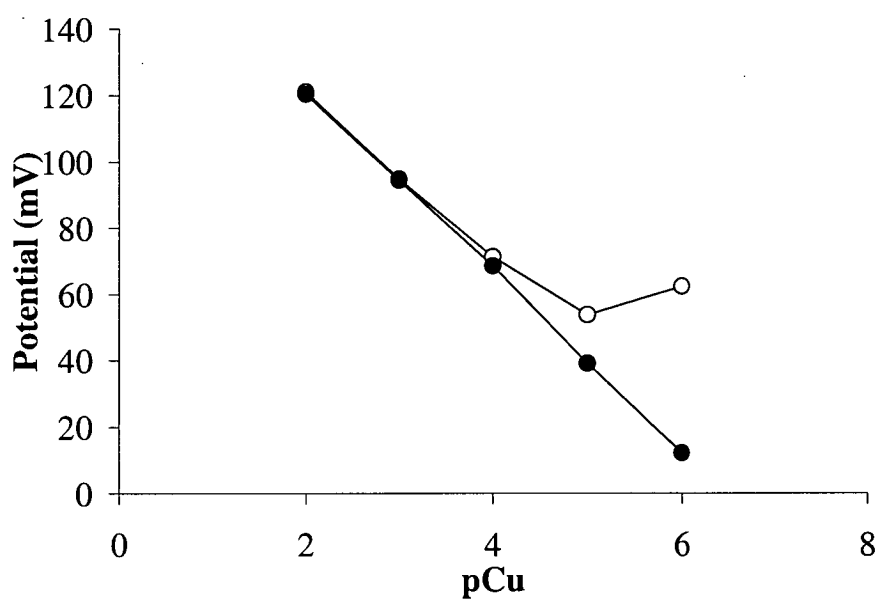


Figure 3.4 Effect of polishing on electrode response to Cu^{2+} in deionised water standards for an aged electrode before (○) and after (●) polishing with alumina.

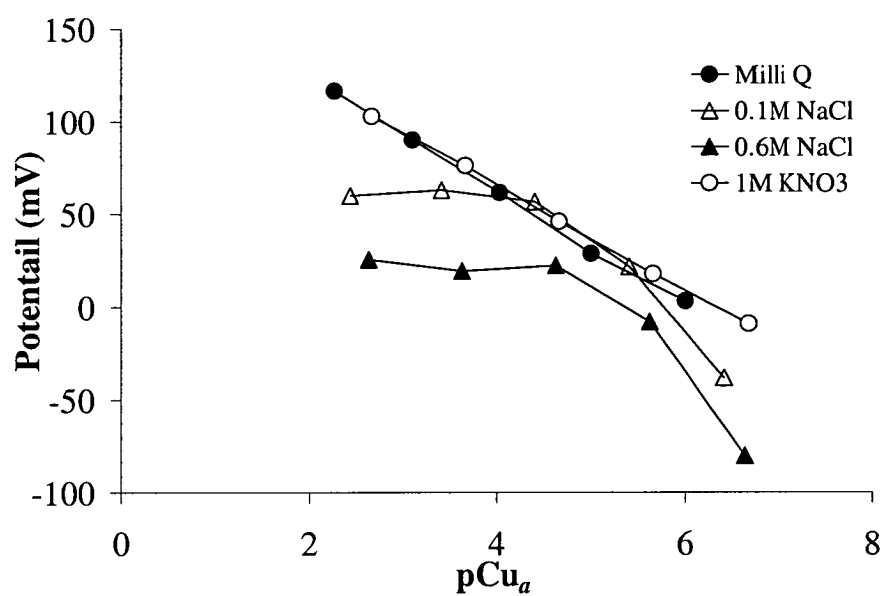


Figure 3.5 Effect of ionic composition and ionic strength on Cu(II)ISE response in standards prepared by serial dilution.

appear to be offset, with the higher ionic strength standards giving a more negative response. The electrode response to standards prepared in 0.1 M NaCl and 0.6 M NaCl demonstrate that the chloride interference is not simply due to ionic strength effects. There is an obvious lack of sensitivity above pCu_a 4.5, and evidence of super-Nernstian response between pCu_a 6.7 and pCu_a 5 (slope ~ 52 mV/decade). The lack of sensitivity is due to the formation of AgCl on the surface of the electrode, which then senses a constant concentration of chloride, rather than the change in free copper activity, in line with the observations of Moody et al. (1979). The negative offset in the electrode response to 0.6 M NaCl standards is due to greater complexation of Cu^+ in the electrode diffusion layer by chloride, and this is consistent with observations by De Marco et al. (1997) that the magnitude of the chloride interference increases with salinity.

Calibration with Cu(II)-en buffers

The linear calibration range can be extended significantly by the use of Cu(II)-en buffers (Figure 3.6). The electrode can be calibrated over the range $2 < pCu < 14$ by combining standards prepared in Milli-Q by serial dilution and Cu(II)-en buffers in a single calibration. The single most important observation on this data is that the electrode is unaffected by the presence of the chloride ion, demonstrated by the fact that the calibrations in the presence and absence of chloride are co-linear (Belli and Zirino, 1993; De Marco 1994) (Table 3.2). Also significant is the fact that the electrode E^0 is not affected by the presence of the ligand. The electrode response obeys theoretical Nernstian behaviour as the chloride interference is kinetically limited in the range $9 < pCu < 14$ (De Marco 1994).

The copper ion buffers have a number of advantages for the analysis of environmental samples. The reported linear range of the ISE with Cu(II)-en buffers is extended significantly to pCu 19 (Avdeef et al. 1983), with rapid stabilisation and excellent linearity (99.2% of theoretical Nernstian slope). De Marco (1996) has shown that the use of en as a calibrating ligand cleanses the membrane surface of adsorbed copper. Additionally, the pCu buffers are highly stable, and the pCu can be regulated simply by adjusting the pH of the solution. The Cu(II)-en system is well characterised and the relevant stability constants are known. Other buffer systems that have been used to extend the calibration range are ammonia, EDTA, NTA, trien (Avdeef et al. (1983) and references therein), CDTA and glycine (Castro Peñuelas 1996).

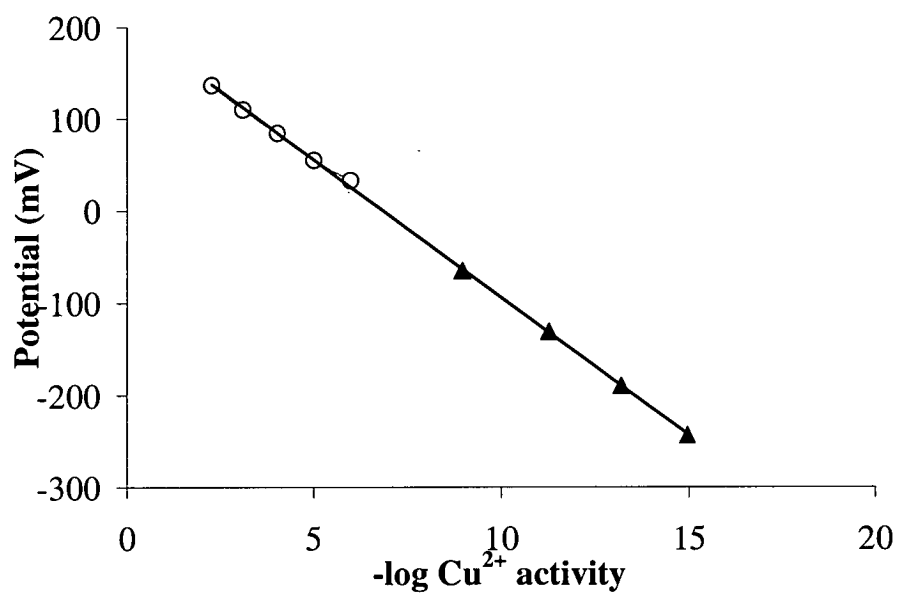


Figure 3.6 Electrode response to Cu^{2+} standards prepared in deionised water (o) over the range $2 < p\text{Cu} < 6$, and saline ethylenediamine buffers (▲) over the range $9 < p\text{Cu} < 15$.

Table 3.2 *Comparison of calibration parameters for standards prepared in deionised water and ethylenediamine buffers in 0.6 M NaCl.*

Standard series	Slope	E ⁰	Regression coefficient
Milli-Q water	29.45	202.74	0.9993
Cu(II)-en buffers	29.87	204.05	0.9997
Combined data	29.87	204.11	0.9999

Analysis of total copper

Unbuffered media

The level of sensitivity and linear response in unbuffered media can be extended quite significantly by applying the technique of standard additions. Using the method of Smith and Manahan (1973) it is possible to obtain Nernstian response (28.9 mV/ decade) in deionised water to a level of 0.38 nM (Figure 3.7). The criteria for no change in the fraction of copper complexed by the sample is met, with $\alpha = 0$. These results are in agreement with Mackey and De Marco (1997), who reported 1 – 3 nM for the same analyses. It is imperative to follow the protocol of polishing, conditioning, and exposure of the electrode to sacrificial buffer if representative measurements in the nM range on unbuffered samples are to be made.

Seawater analyses

Normally, total metal by ISE is determined by direct analysis of acidified samples. However De Marco et al. (1997) reported that the magnitude of an organic interference at pH 2 was so great as to cause a significant error using this approach (typically 1 pCu unit). The interference is due to the adsorption of protonated carboxylic acids organics and phenol functional groups at low pH, and can be removed by photo-oxidising the samples and destroying the organic material. Resultant analyses on acidified, UV-oxidised samples were within 0.1 – 0.3 pCu units of the GFAAS value. An alternative method for the determination of total copper by ISE is standard addition. When seawater is acidified to pH 2, the inorganic ligands are present in large excess and equilibrate rapidly with added Cu^{2+} , while the formation of organic complexes does not occur (α is constant).

A small number of archived samples were sub-sampled for analysis by standard addition potentiometry. The electrode was polished and conditioned in seawater prior to analysis, as described earlier. Results for these analyses are shown in Table 3.3. The total copper determined by Cu(II)ISE falls within 0.04-0.21 pCu units of the value determined by GFAAS, and this in agreement with data published by De Marco et al. (1997). Nernstian response was observed, despite the fact that organics are adsorbed to the electrode at pH 2, as this affects E^0 , not the slope.

De Marco et al. (1997) estimated the dissolution rate of a polished and conditioned electrode exposed to seawater after cleansing in a high pCu buffer (~ 15) to be

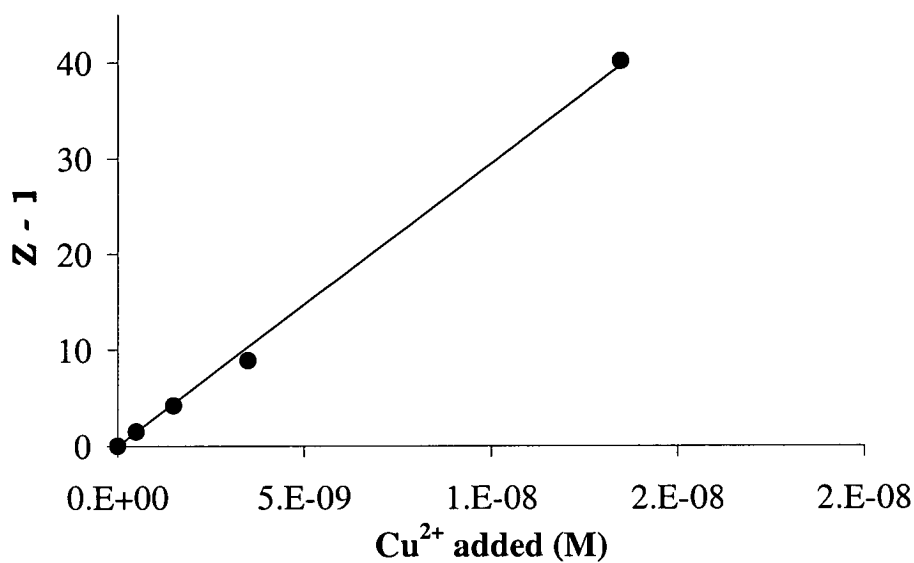


Figure 3.7 Standard addition analysis of deionised water by Cu(II)ISE. Using the method of Smith and Manahan (1973), the original concentration of copper present in the sample (C_o) is calculated from the inverse of the slope of the regression through all data points, and the experimentally determined slope (28.9 mV/decade). $C_o = 0.38$ nM, correlation coefficient = 0.9967.

Table 3.3 *Comparison of total copper in seawater by GFAAS and Cu(II)ISE. Samples were collected on R.V Franklin FR 07/89, and were unfiltered prior to acidification to pH2. GFAAS analysis was performed by dithiocarbamate extraction at pH 4.6.*

Sample I.D	pCu _{GFAAS}	pCu _{ISE}	Difference
T46	8.42	8.22	+0.20
T51	8.95	8.91	-0.04
T59	8.73	8.59	-0.14
T61	8.60	8.81	+0.21

0.072-0.18 nM/min (7.2 – 18 pmol/min). Zirino et al. (1998a) similarly measured the dissolution rate of the jalpaite Cu(II)ISE to be 0.017 nM/min (4.3 pmol/min) over a period of 1000 minutes. After this point, the level of copper contamination by electrode dissolution increased significantly.

The dissolution rate of a jalpaite ISE that has not been polished or conditioned, or exposed to the “sacrificial” buffer prior to analysis of seawater would be expected to be significantly higher than the dissolution rates reported above. Under these conditions, a dissolution rate of 0.37 nM/min (37 pmol/min) was calculated over a half hour period, and this represents significant contamination of the sample in a short period of time. This is 2 – 5 times higher than the dissolution rate reported by De Marco et al. (1997) of 0.072- 0.18 nM min, and 20 times greater than the rate reported by Zirino et al. (1998a) for conditioned electrodes exposed to seawater after cleansing in the sacrificial buffer.

The significance of this is twofold. Firstly, treatment of the electrodes in the appropriate manner will significantly reduce the contamination of the sample by electrode dissolution. Secondly, using the data from Zirino et al. (1998a), it can be seen that the dissolution rate is negligible over the time-frame of an analysis i.e. 0.017 nM/min up to 1000 minutes.

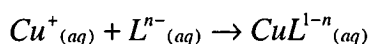
Electrochemical Impedance Spectroscopy studies of a ISE response to organic matter

Hulanicki et al. (1984), Hoyer (1991) and Gulens (1981) reported that the presence of organic ligands in solution has a deleterious effect on the response of the electrode. It is possible therefore that natural organic matter present in seawater would also exert an interference effect, and preclude the use of the Cu(II)ISE for analysis of marine samples. In contrast to Gulens observations, Castro Peñuelas (1996) showed that a number of organic ligands (CDTA, NTA, glycine, en, and EDTA) had no effect on the response of the Orion 94-29 Cu(II)ISE. The presence of natural organic matter in seawater may actually have a beneficial effect on the electrode performance, by reducing membrane dissolution or reducing the poisoning of the membrane by AgCl precipitation on the membrane surface (De Marco 1996). XPS data has shown that negligible AgCl is formed in natural seawater where organic ligands assist with AgCl removal. A possible mechanism for this could be by blocking those sites at which the poisoning reaction occurs, similar to the beneficial role of en which promotes the nucleation and peptization of AgCl (De Marco 1996). Peptization refers to the process of dispersion of the AgCl to form a stable colloidal solution.

Additions of humic acid were made to organic free (UV-oxidised) seawater, using concentrations similar to those found in the marine environment (1 mg/L) and

greater. De Marco et al. (1998) demonstrated that the addition of humic acid to organic-free seawater resulted in a -3 mV (or 0.1 pCu unit) change in potential of the Cu(II)ISE, indicative of a change occurring at the solution-membrane interface. Significantly, the magnitude of the organic interference at the typical level of humic acid in seawater is weak. Preliminary EIS experiments conducted on a earlier occasion showed a potential drop of 2.5 mV after the addition of 1 mg/L humic acid, equivalent to 0.08 pCu units. It was not possible to determine the charge transfer impedance (R_t) for these preliminary results, due to the presence of a large Warburg impedance at low frequency, which obscured the EIS response at higher frequencies. Thus it was not possible to fit a semi-circle to the data (see Experimental). Subsequent experiments used a refined measurement protocol, which was essentially dependent upon obtaining as stable and reproducible a membrane surface as possible i.e. fastidious attention to polishing and conditioning, and equilibrating the electrode in the sample to be measured, before acquiring EIS spectra. The frequency range over which data was collected was also optimised to allow semi-circle fitting.

In subsequent experiments, De Marco et al. (1998) found that the charge transfer impedance R_t was reduced with increasingly higher concentrations of humic acid (up to 100 mg/L), while the rate of charge transfer was increased (Figure 3.8). This correlation shows that the rate of the charge transfer reaction is the potential determining process for the organic ligand interference effect. They proposed the following reaction to explain the interference by organic ligands



where Cu(I) is formed via reductive ion exchange, in accordance with the accepted theory for the response of the Cu(II)ISE (Gulens 1981; Gulens 1987).

Although the potential shift observed in both the above cases was negative, complexation of Cu^{2+} by humic acid is considered unlikely in the time frame of the measurements, since the chelation of copper by organic material is under kinetic control (Mackey & Zirino 1994). If complexation were significant, the results would have shown a decrease in the rate of the charge transfer, while an increase was observed (De Marco et al. 1998).

The implication of these measurements is that the presence of natural organic matter in seawater, at natural pH, will have a negligible effect on the electrode response. The magnitude of the organic ligand interference is weak, (evidenced by a small change in potential) with 1 mg/L inducing an error of approximately 0.01 pCu units. This is supported by FIA studies (see Chapter 4) which showed no change in slope or E^0 in the presence and absence of similar quantities of humic acid.

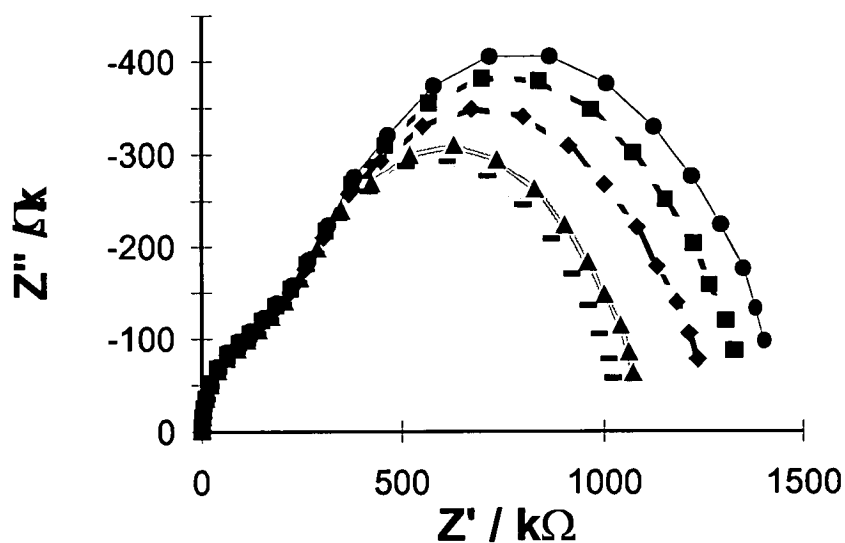


Figure 3.8 EIS spectra (complex plane impedance plots) for a static Cu(II)ISE in organic free (UV-oxidised) seawater spiked with different levels of commercial humic acid. Humic acid additions are 0 mg/L (●), 1 mg/L (■), 10 mg/L (◆), 50 mg/L (▲), and 100 mg/L (-). Data collected by R. De Marco and published in De Marco et al. (1998).

Rotating disk electrode EIS studies of jalpaite in seawater

X-Ray Diffraction Analysis of Jalpaite

The composition of the jalpaite prepared by the method of Heijne and van der Linden (1977) using an 80 % excess of Na_2S was determined using XRD. The composition was found to be jalpaite, with traces of covellite present. The powder precipitated with 20 % sodium sulfide was mostly jalpaite, with quantities of covellite and acanthite (Ag_2S). Powder prepared with an 80 % excess of Na_2S was subsequently incorporated into an RDE for further studies.

Effect of rotation speed

The effect of rotation speed on the jalpaite Rotating Disk Electrode (RDE) in unfiltered seawater (i.e. natural ligands present) is shown in Figure 3.9. As the rotation speed increased, the charge transfer resistance decreased, which is what we would expect for a diffusion controlled process, and De Marco et al. (1998) concluded that the organic ligands present in unfiltered seawater at pH 8 were strongly adsorbed (via chemisorption) to the electrode surface. Desorption of the ligands at high rotation speed suggests that the small offset in response noted for a static electrode (~ 0.1 pCu in the presence of humic acid), can be ameliorated by incorporating the electrode into a flow system with high flow rates, or by using an RDE for routine analysis. Indeed, Zirino et al. (1998a) have applied an RDE technique to the analysis of San Diego Bay waters at natural pH, and found that it was possible to follow small time-dependent changes in seawater pCu. Incidentally, the R_t value at high rotation speed (6000 rpm) in natural seawater is very similar to the R_t recorded for a static Orion electrode in organic free seawater.

It is interesting to note that decreasing the rotation speed to 1000 rpm after conducting the experiment at 4000 rpm resulted in a hysteresis effect, that is the R_t measured at 1000 rpm before and after a higher rotation speed was not the same (Table 3.4). This is due to the ablation of adsorbed organics at high speed, while the re-establishment of adsorbed organics at lower rotation speeds is not as effective as in static solutions. Zirino et al. (1998a) also observed a hysteresis effect with the jalpaite ISE (Orion 94-29) at high rotation speeds, and noted that the same effect did not appear in acidified seawater, presumably because the protonation of natural organic matter results in physisorption rather than chemisorption.

The Levich equation describes the effect of several variables, including rotation rate on the response of an RDE. De Marco et al. (1998) noted that the data acquired in “raw” seawater (Figure 3.9, slope 1.1) did not agree with that predicted by the Levich equation (slope 0.5) for thinning of the diffusion layer at elevated rotation

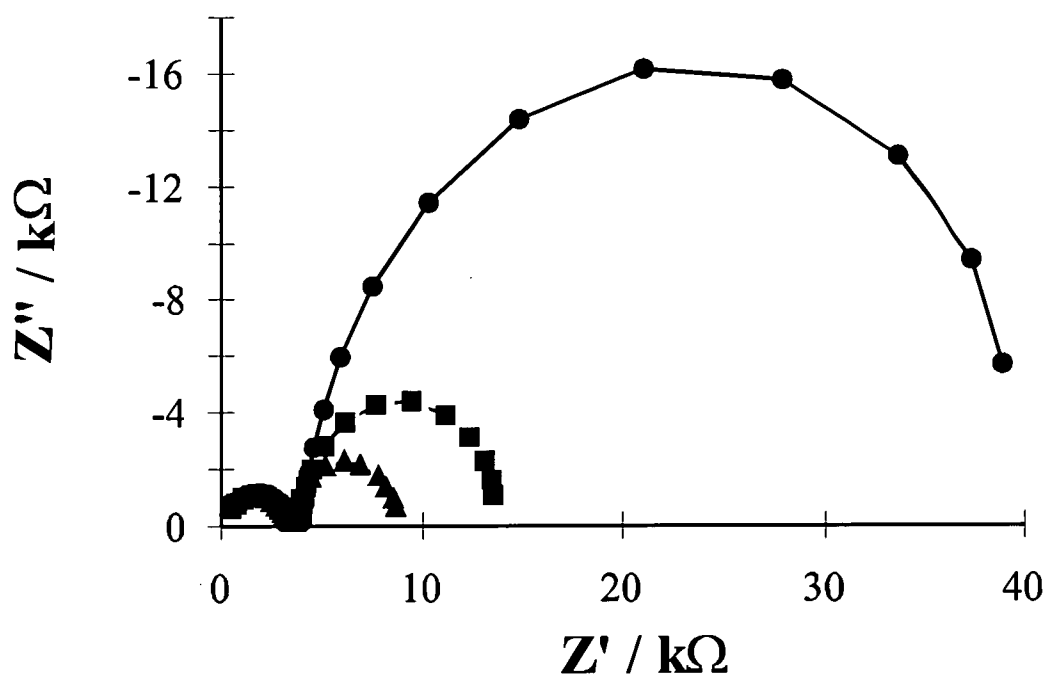


Figure 3.9 EIS spectra (complex-plane impedance plots) for the jalpaite RDE in natural seawater at various rotation speeds. Rotation speeds are 1000 rpm (●), 4000 rpm (■) and 6000 rpm (▼) (from De Marco et.al. 1998)

Table 3.4 *Effect of rotation speed on charge transfer impedance R_t of the jalpaite RDE in unfiltered seawater, showing the hysteresis effect.*

Rotation speed (rpm)	R_t (k Ω)
1000	38.46
4000	10.43
6000	5.37
1000	15.32

speed i.e. when $\ln(1/R_t)$ is plotted as a function of \ln (rotation speed).

This indicates organic ligands are tenaciously adsorbed to the electrode surface in natural seawater. Better agreement with the Levich equation was obtained for UV-oxidised water (slope = 0.3), confirming ligand adsorption does have an effect (albeit small) in seawater containing natural ligands.

Effect of Humic Acid and pH

It has already been established in static EIS measurements that the addition of humic material had a small effect on the analysis of copper in seawater (equivalent to an error of ~ 0.1 pCu units). Flow conditions may also ameliorate the effect of organic ligands in solution on the electrode response. The effect of humic acid additions using the jalpaite RDE was therefore investigated at various rotation speeds. At 1000 rpm, R_t in organic-free water (UV-oxidised control) was about 3 times greater than for the same experiment using a static Orion electrode. Additions of 1 mg/L and 10 mg/L humic acid reduced the charge transfer resistance by a factor of 1.7 and 2 respectively (Table 3.5). At 4000 rpm, a similar trend was evident, that is addition of 1 mg/L and 10 mg/L humic acid reduced the charge transfer resistance by a factor of about 1.6 and 1.9 respectively, however the magnitude of the resistance was decreased as rotation speed increased. This is consistent with the thinning of the diffusion layer at high rotation speeds, resulting in a lower concentration of humic acid in the immediate vicinity of the electrode-solution interface. Hence the magnitude of the interference is reduced.

The EIS profile of UV-oxidised seawater at natural pH was measured at 1000 rpm. The sample was then acidified to pH 2, and the EIS spectra recorded. Finally, the acidified sample was spiked with 1 mg/L of humic acid. This sequence was repeated for a UV-oxidised sample at 4000 rpm (Table 3.6). At both rotation speeds, the acidification process resulted in a halving of the charge transfer resistance, consistent with an increase in the level of free Cu^{2+} as protonation of seawater ligands dissociates Cu^{2+} complexes. Addition of humic acid to the acidified solution only slightly decreased the charge transfer resistance at both rotation speeds, and the interference phenomenon is believed to be the same process which causes an error in the direct analysis of pCu in acidified seawater samples i.e. suppression of the cathodic reaction by adsorption of natural organic matter at the cathodic sites on jalpaite. The electrode potential did not change on addition of humic acid, however the potential drifted + 7 mV over the course of the experiment (1 hour).

Table 3.5 *Rt (kΩ) as a function of rotation speed and concentration of humic material added to UV-oxidised seawater.*

<i>Rotation speed</i>	No humic acid (kΩ)	1 mg/L HA (kΩ)	10 mg/L HA (kΩ)
1000 rpm	30	18.1	15
4000 rpm	22	13.5	11.5

Table 3.6 *Effect of pH and presence of organic matter on R_i ($k\Omega$) for jalpaita RDE using UV-oxidised seawater.*

Rotation speed (rpm)	pH	Humic acid (mg/L)	$k\Omega$
1000	8	0	27.32
1000	2	0	12.83
1000	2	1	10.06
4000	8	0	29.89
4000	2	0	14.28
4000	2	1	12.27

The purpose of the EIS studies was to establish the magnitude of the organic interference effect and provide mechanistic information on the process occurring at the membrane surface. Studies with the RDE (here, Zirino et al. 1998a) indicate that the problem will be less severe in a flow analysis system. This is assuming that the flow of solution past the electrode will reduce the extent of interferences that occur at the solution-membrane interface in much the same way as the electrode rotating in a “static” solution. It has been established by Zirino (unpublished results) that a badly fouled electrode can be restored to normal operation by exposing the membrane to flowing seawater. Gradual ablation of surface products indicates that the use of flow analysis would overcome the problem of electrode fouling in static solutions. Additionally the problem of electrode dissolution would be minimised by insertion in a flow cell, as the time frame of an analytical measurement may be in the realm of 5 seconds to 1 minute, as opposed to 10 – 60 minutes by batch analysis. Using the corrosion rate calculated by De Marco (1997) of 0.072 - 0.18 nM/min, this could mean as little as 0.006 nM total copper affecting the sample analysis.

As discussed earlier, many of the perceived problems with ISE analyses (poor precision, electrode drift, slow response etc) may be improved by judicious control of the experimental conditions, and an improved knowledge of the extent and mechanism of interferences. This situation may be improved further by incorporating the ISE into an automated flow system as it has been demonstrated that flow conditions are beneficial to the analysis of Cu^{2+} by ISE, as the magnitude of the organic interference is reduced. Other inherent properties of flow analysis such as precise timing, reproducible conditions and fewer manual operations will also be advantageous.

CONCLUSIONS

This chapter described experiments which showed the effect of various analytical parameters on ISE performance, including electrode age, polishing, calibration history, membrane dissolution, and chloride interference. The linear range of response of the Cu(II)ISE was extended significantly in unbuffered solutions by adhering to strict protocols for the polishing, conditioning, and calibration of the electrode, and by using standard addition analysis. Potential drift may be the result of copper contamination or the membrane aging with the formation of products such as AgCl. Thus it is necessary to condition the electrode in the matrix of interest to establish a stable surface, and to calibrate the electrode frequently enough to detect changes in the electrode response.

Use of metal-ion buffers to calibrate the electrode demonstrated that the electrode has the required sensitivity for environmental measurements, and that the presence of en had no effect on the electrode E^0 . Kinetic suppression of the chloride interference means that the Cu(II)ISE will function normally in the presence of chloride ions in the range $9 < pCu < 15$.

This chapter described the first mechanistic studies using EIS to investigate the potential interference by dissolved organic matter on the Cu(II)ISE. Significantly, the magnitude of the interference is limited at natural pH, and the EIS studies also showed that solution flow past the membrane is a crucial factor in reducing the effect of organic matter on ISE response. A natural progression in method development then is to combine the Cu(II)ISE with a flow analysis system. It is also proposed that the limitations of batch analyses (namely sluggish response at low activities, cross contamination from one sample to another, contamination by membrane dissolution and/or silver chloride precipitation) may be overcome by the incorporation of the ISE into a flow analysis system. Flow injection analysis (FIA) has the potential to provide a method for the rapid measurement of free and total copper in seawater, as long as the fundamental elements of electrode pretreatment and calibration are incorporated.

Addendum

RDE-EIS results presented here were collected by the author while visiting Roland De Marco (at Curtin University), who was my PhD supervisor and designed the investigation. Al Zirino contributed data, and co-wrote the manuscript.

Chapter 4 Preliminary Investigations of the Copper(II)ISE Response in Flow Injection Analysis Mode

INTRODUCTION

It was suggested in the previous chapter that a rapid technique for copper analysis by ISE may be achieved by incorporating the electrode into a Flow Injection Analysis (FIA) system. Even without the potential to overcome the problems specified in Chapter 2, the advantages of FI techniques over batch analyses are quite considerable. Analysis speed, solution containment, automated standardisation, sample dilution, matrix removal, analyte pre-concentration and the ability to control the time/concentration domain of any solution chemistry are well recognised and exploited features (Ruzicka 1992). Significantly, flow systems allow most sensors to be used in a more reproducible manner than when used in batch mode, since contact time with the sensing surface can be precisely controlled. Risk of contamination is reduced when the number of manipulations involved in the analysis is reduced, and the precision and accuracy of the analyses are usually improved (Midgley and Torrance 1991). Potentiometric flow methods can enhance sensitivity significantly compared to classical measurements in a static solution (Pungor et al. 1979). However, serious deviations from Nernstian behaviour (narrower linearity range and higher detection limits) have also been observed (Neshkova 1993). When the analyte is near the electrode's limit of detection, flow systems have the advantage of being able to produce quasi-equilibrium conditions in a much shorter time frame. Flow systems can also be miniaturised by small-bore tubing, and therefore are more economical with reagents and samples than batch measurements.

ISEs are robust, readily available and relatively inexpensive, and the development of portable FIA equipment and flowcells is so far advanced that any commercially available ISE may be tested for FIA applications without the need for expensive and time consuming equipment development (Dimitrakopoulos 1994). Just as with static measurements, the chemical and phase composition of the membrane is crucial to determining the performance characteristics of the ISE, and jalpaite has been recognised as having produced better FIA results than other solid-state membranes (Neshkova 1993).

The advantages of FIA for the analysis of free copper by Cu(II)ISE are that simple systems can be developed as portable analysers, allowing real time measurements to be made on-site. The potential here is to overcome significant problems which may be encountered when storing trace metal samples for speciation analysis (Batley 1989). Another significant advantage is the reduction of contamination from the sensing membrane itself, as sample exposure to the membrane is reduced by virtue of the flowing solution, so no build-up of corrosion products is possible (Hoyer

1992). This can be further enhanced by utilising flowcells with a very small dead volume. Problems with liquid junction potentials of reference electrodes can be overcome by placing the reference downstream of the indicator electrode, or in its own reference stream (Blaedell & Dinwiddie 1974; Dimitrakopoulos 1994).

Another feature of FIA is the ability to resolve kinetic processes which occur at the electrode surface. This can be used to advantage to separate the effect of an interfering ion from the ISE response to the desired analyte (Gordon et al. 1989). Neshkova (1993; 1998) used electrochemically prepared chalcogenide membranes and kinetic discrimination to study the effect of chloride interference on the copper response. She found that under flow-injection conditions, a linear copper calibration curve with Nernstian response was obtained in the presence of 0.5 M KCl, although signal height was depressed. Similar kinetic studies have not been performed using the Orion 94-29 Cu(II)ISE.

Cu(II)ISEs have been evaluated in FIA type systems in numerous publications. Van der Linden and Oostervink (1978) prepared a flowthrough type electrode to monitor copper concentrations in tapwater, down to about 1 μ M. Van Staden and Wagener (1987) described a coated tubular solid-state Cu(II)ISE which had a linear response of over 5 orders of magnitude. The method was developed for the analysis of effluent and tap water samples, and gave excellent reproducibility and accuracy. High sample throughput (80 samples/hr) was a feature of the method, although the electrode was not suitable for the analysis of samples containing chloride, due to the failure of the membrane to discriminate against chloride under FIA conditions. Slanina (1980) and Pungor et al. (1979) described extremely simple FIA systems with potentiometric detection which significantly improved the sample throughput, and they discussed many of the criterion necessary for developing an automated flow system. Few, if any reports of FIA-ISE methods for the analysis of seawater, have appeared in the literature.

The aim of this work was to establish the suitability of FIA for the measurement of free and total copper in seawater. Initial studies were conducted with unbuffered standards in the range $2 < \text{pCu} < 6$, to determine the ISE performance in the absence of chloride, and in the realm of fast electrode response and linearity. Kinetic discrimination was investigated, and the effect of organic matter was also briefly studied. The use of the Cu(II)ISE for seawater analysis under FIA conditions is complicated by the interaction between the carrier and the injected samples or standards, and these observations are discussed in the context of developing a flow method for the analysis of free and total copper in seawater.

EXPERIMENTAL

Reagents

Analytical grade reagents were used as received from the suppliers (BDH AnalaR). Deionised water ("Milli-Q") from a Millipore 4 cartridge system, with a Milli-Ro 4 pretreatment system was used to prepare all solutions. Standards were prepared daily by serial dilution of a stock 10^{-1} M copper nitrate solution. Serial dilution of standards covered the range pCu 2 to pCu 7. Standards containing less than 10^{-3} M Cu^{2+} were prepared daily to reduce errors incurred by adsorptive losses of copper to the glass, and because they are more likely to be contaminated. All plastic and glassware used in analyses was acid washed in 10% HCl and rinse with Milli-Q prior to use.

Saline copper-ethylenediamine (Cu(II)-en) buffers were prepared according to the method described in Chapter 3. Calculation of pCu_{free} was as previously described.

Artificial seawater samples were prepared according to De Marco (1994). Solutions containing 0.6 M NaCl, 2×10^{-4} M $\text{Cu}(\text{NO}_3)_2$, and 10^{-3} M glycine were adjusted to pH 9.87 to give a pCu_{free} value of 11.93, which is comparable to values reported in open ocean samples. Filtered seawater for method development was collected by deploying acid washed Niskin bottles on a CTD Rosette. Seawater was collected in acid washed 1 L LDPE bottles. Archived samples from CSIRO were collected and preserved using trace metal techniques, as described in Chapter 3.

Electrode preparation and conditioning

The Orion Cu(II)ISE (Model 94-29) was polished on felt pads as described in Chapter 3. For seawater analyses, the electrode conditioning process was modified to reduce the time required (i.e. 24 hours for static exposure) to develop a stable membrane surface. The conditioning step was incorporated into the daily start-up procedure inserting the electrode in the flowcell and exposing the polished electrode to flowing seawater for two to three hours, until a stable potential was recorded (stability criterion 0.1 mV/ minute).

Portable flow injection system

The FIA studies described herein are based on a portable battery powered system as shown in Figure 4.1 (Alexander et al. 1996). Carrier and reference stream solutions were pumped through the system using a 2-channel peristaltic pump (A.I. Scientific Pty Ltd, Model OEM-S2). Flow rates between 1 and 5 mL/min were used, and all tubing (other than peristaltic pump tubing) was 0.5 mm I.D. Teflon. Samples and standards were injected using a Hamilton 2-way injector valve (Model HV 86728) or a Rheodyne six-way Type 50 Rotary valve (Figure 4.2) with various size loops

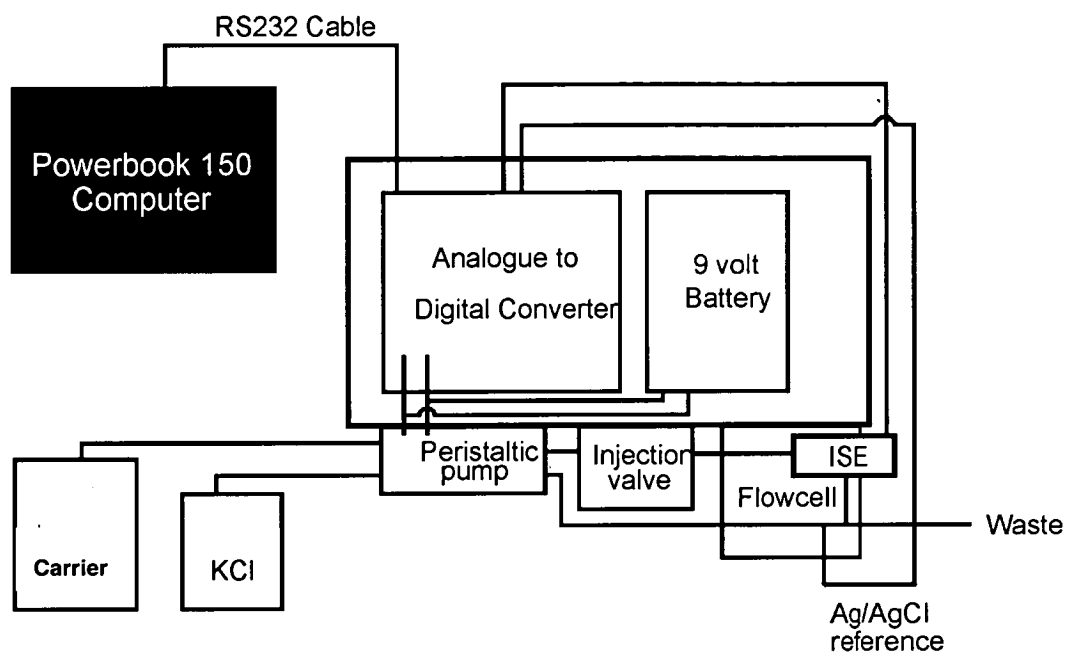


Figure 4.1 Schematic diagram of portable flow injection analysis system used in this study (not to scale).

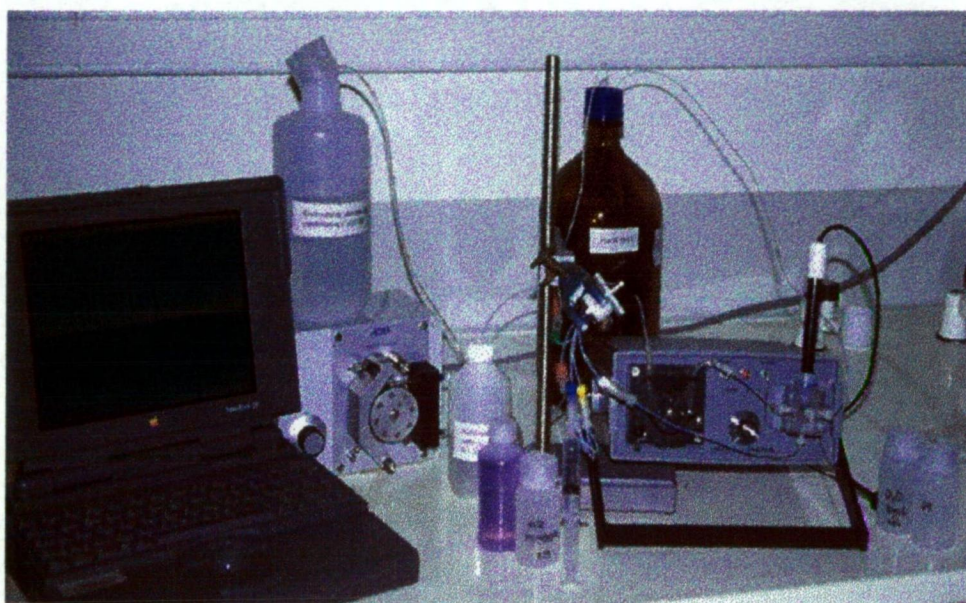


Figure 4.2 FIA/Cu(II)ISE system used for preliminary flow analysis studies. The peristaltic pump and FIP-3 flowcell are mounted on the portable battery-powered datalogger. Data storage and processing was performed using a Macintosh PowerBook laptop computer.

(100 – 500 μL). The sample was presented to the electrode in a wall-jet configuration flowcell (Figure 4.3), which has a separate stream for the Ag/AgCl reference electrode and a very small dead volume (18 μL).

The Ag/AgCl reference electrode was prepared by immersing a freshly polished silver wire in a solution of 1 M HCl. The silver wire was attached to the positive terminal of a 1.5 V battery, and a platinum wire electrode was attached to the negative terminal. After 15 minutes, the AgCl coated silver wire was rinsed with Milli-Q and soaked in 0.1 M KCl until required. Comparison of the anodised electrode with a standard double-junction reference electrode (in static measurements) did not give significantly different results with respect to slope and E° .

Data acquisition was undertaken using an A/D card with 12-bit resolution, in conjunction with a serial output (9600 baud rate) connected to a Macintosh 145B PowerBook computer, via an RS-232 cable. "Satod" (UNSW) software was used to record potentiometric data. The analogue signal from the system was also connected to a Radiometer PHM85 precision pH meter, and the output recorded on a YEW Type 3056 Pen Recorder.

Several carrier solutions were investigated; for initial studies 0.05 M KNO_3 spiked with a background concentration of 5×10^{-7} M Cu^{2+} to enhance the rate of return to baseline was used; later a saline Cu(II)-en buffer solution adjusted to pH 8 ($\text{pCu}_{\text{free}} \sim 15$) was used. "Reverse" FIA was also investigated for the measurement of free and total copper where pCu buffers were injected into a carrier of the sample.

Calibration procedure

The ISE was calibrated by injecting a series of pCu buffers ($9 < \text{pCu} < 15$), or a series of unbuffered copper standards that had been prepared by serial dilution ($2 < \text{pCu} < 7$). Replicate injections were made for all calibrations and analyses.

Kinetic discrimination studies

A study of kinetic discrimination of the chloride ion and cupric ion signals was performed under conditions similar to those described in Neshkova (1993). A carrier of 0.05 M KNO_3 and 5×10^{-7} M Cu^{2+} was employed at a flow rate of 4.2 mL/minute. A LDC Cheminert Metering Pump replaced the peristaltic pump, and an injection loop of 100 μL was used.

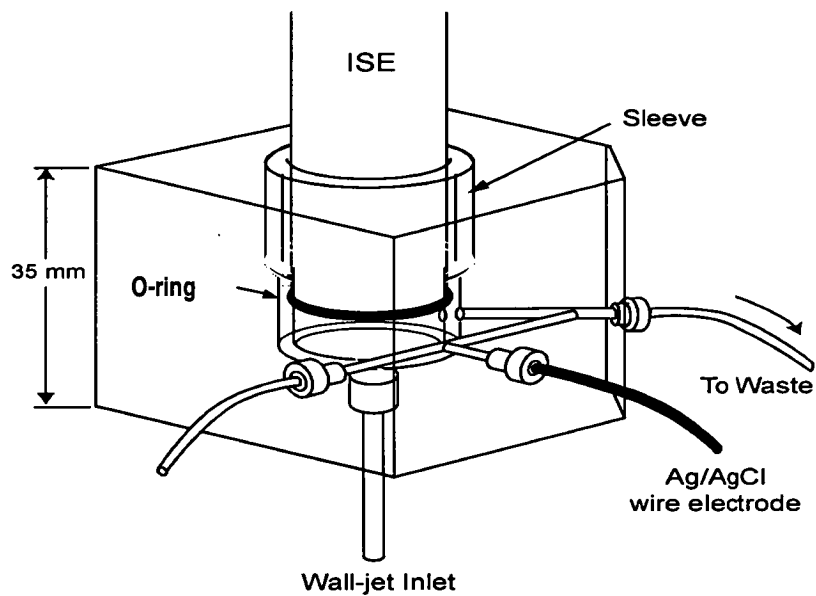


Figure 4.3 Perspex wall-jet flowcell used for FIA studies. For clarity, the electrode illustrated is not seated fully into the sleeve of the flow cell. (Picture by Michael Bessell).

RESULTS AND DISCUSSION

Conditioning trials

For seawater analysis, conditioning is crucial as it reduces the rate of corrosion by forming a stable electrode film. Gulens (1981) observed that sensitivity of ISEs could be improved by utilising an appropriate conditioning regime, and response times may also be improved (De Marco 1994). In order to reduce the time required for ISE analyses, the recommended electrode conditioning time (24 hours) was addressed. The conditioning step was incorporated into the flow procedure, by inserting the electrode into the flowcell immediately after routine polishing. This reduced the conditioning time from 24 to 3 hours. The advantages of shortening the conditioning time is that it is possible to polish the electrode daily if necessary, and since the step is automated, it can be much more easily incorporated into a portable analysis system. Additionally, the flowing solution will facilitate the desorption and diffusion of membrane surface products away from the diffusion layer.

ISE/FIA response to unbuffered standards

The response of the Cu(II)ISE in FIA mode using a non-complexing carrier of 0.05 M KNO₃ and 5×10^{-7} M Cu²⁺ was Nernstian in the range $2 < \text{pCu} < 6$ (Figure 4.4). The presence of the copper spike in the carrier increased the rate of return to baseline, but also limited the linear range.

Various flow rates were investigated in order to optimise the sensitivity of the system. For low ionic strength carriers (≤ 0.05 M), varying the flow rate caused the baseline to drop approximately 3 mV per 1mL/min increase in flow rate, a commonly observed phenomenon due to the thinning of the diffusion layer at the electrode surface. At higher ionic strengths (≥ 0.05 M), increasing flow rate had little effect on baseline stability (Figure 4.5). Streaming potentials are more significant in low ionic strength solutions, thus variations in flow rate will have a greater effect.

The optimal flow rate was found to be 2.5 mL/min, which gave good sensitivity, low RSD for replicate injections (0.33%), linearity over a wide dynamic range ($2 < \text{pCu} < 6$), and high sample throughput (40 samples/hr). The flow rate was found to have some effect on the electrode slope above 2 mL/min, with values decreasing at elevated flow rates (Table 4.1). The electrode E^0 was also affected by variations in flow rate. The linear range of the calibration was not affected by flow rates above 1mL/min.

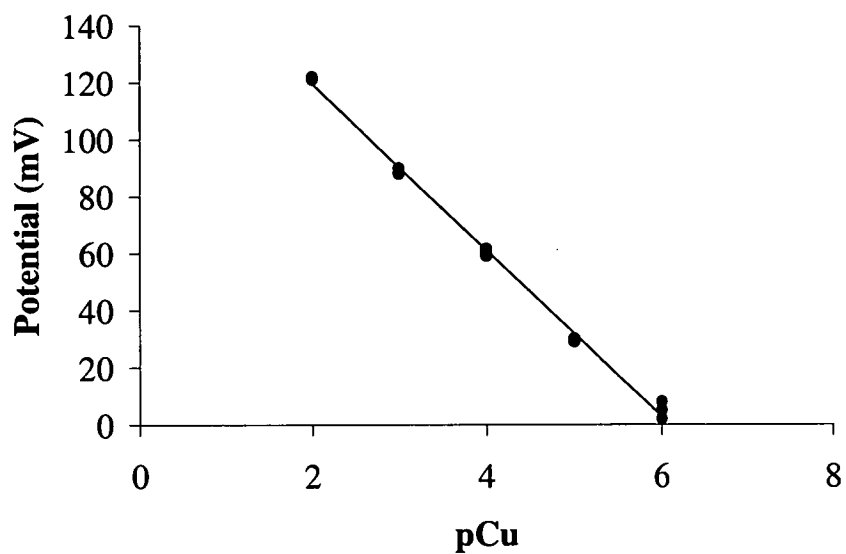


Figure 4.4 Response of the Cu(II)ISE #1 in FIA mode to triplicate injections (100 μ L) of standards prepared in Milli-Q. The carrier was 0.05M KNO₃ with a background spike of 5×10^{-7} M Cu(NO₃)₂. The response curve yielded a slope of 29.23 mV/decade and E^0 of 177.93 mV ($R^2 = 0.9970$).

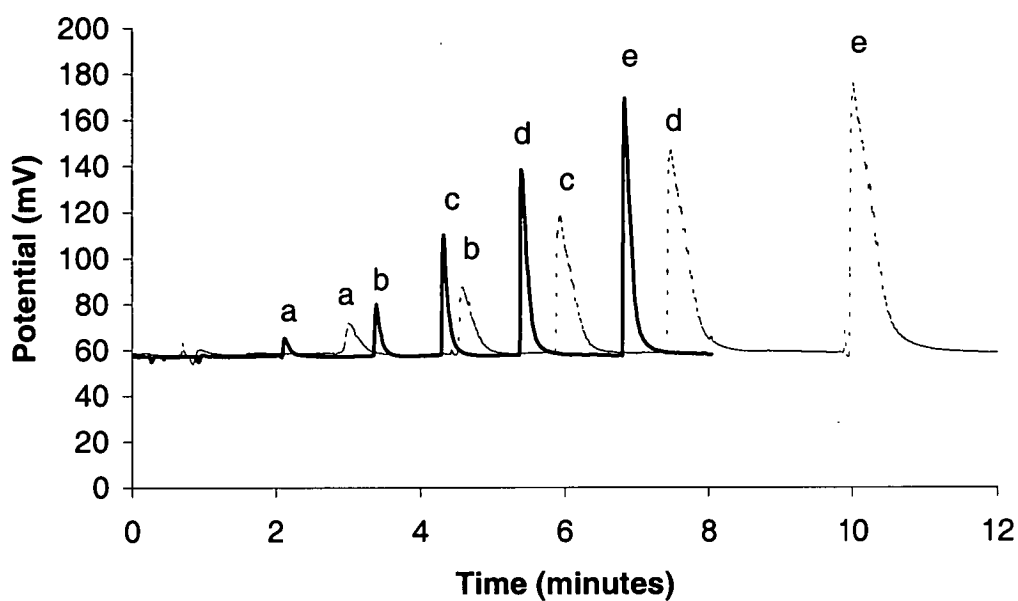


Figure 4.5 Effect of flow rate on baseline stability, peak height and shape of a) pCu 6 b) pCu 5 c) pCu 4 d) pCu 3 e) pCu 2 standards in deionised water. Carrier was 0.05 M KNO_3 and 5×10^{-7} M Cu^{2+} . The dashed line represents 1 mL/min, while the solid line is 4 mL/min. Single injections are shown for clarity.

Table 4.1 *Effect of flow rate on Cu(II)ISE calibration parameters.*
(Carrier 0.05 M KNO₃ and 5 x 10⁻⁷ M Cu(NO₃)₂ , ISE #2 and injection volume 100 μL).

Flow rate (mL/min)	Slope (mV/decade)	E ⁰ (mV)	Linear range	R ²
1.0	29.34	234.64	pCu 2-5	0.9996
2.0	30.45	239.41	pCu 2-6	0.9993
3.0	30.02	232.43	pCu 2-6	0.9999
4.0	29.49	227.41	pCu 2-6	0.9997

Effect of humic acid on FIA response

EIS studies of static and rotating potentiometric measurements revealed that the presence of humic material did not have a significant impact on the ISE response at pH 8, and that flow analysis may be beneficial. Consequently, the effects of humic material in FIA mode was investigated. The addition of 1 mg/L of humic acid to the carrier solution comprising 0.05 M KNO₃ and 5×10^{-7} M Cu(NO₃)₂ caused the baseline to drop by 13 mV in 30 minutes. This is a larger offset than was observed for the same humic acid addition for static EIS studies (2.5 – 3.0 mV). The negative offset may have been due to humic acid complexation of the copper spike in the carrier and copper present due to contamination of the KNO₃, but this is unlikely as the rate of complexation by humic acid is very slow (Mackey & Zirino 1994). The offset may also be due to some interaction between the membrane and the added organic matter, causing the electrode E° and slope to change. However, the slope and E° of the electrode remained essentially unaltered (Table 4.2), in fact the slope was closer to the theoretical value in the presence of humic acid. It is interesting to note that the lower limit of linear response for the electrode was extended from pCu 5 to pCu 6 when humic material was added to the carrier. The improvement in linearity may be due to the humic material reducing the corrosion of the jalpaite membrane. However, we expect that corrosion will already be minimal by virtue of the constant removal of dissolved copper as the carrier flows past the electrode. The resumption of a humic-acid free carrier resulted in the baseline potential stabilising to within 2 mV of its original value. For the analysis conditions used in this study, the presence of humic material did not have a deleterious effect on the response of the Cu(II)ISE.

Effect of chloride ion on ISE/FIA response

The presence of 0.1 M chloride in the standards reduced the slope of the electrode, causing a significant decrease in the E° (Figure 4.6). The slope was reduced to 25.05 mV/decade with an E° of 169.70 mV. Increasing the chloride ion concentration of the standards to 0.5 M reduced the slope of the electrode further over the range pCu 6 - 3, whilst pCu values ≤ 3 invoked super-Nernstian response, with a slope of 61.33 mV/decade. The E° offset between electrode 1 and 2 observed in static measurements was less significant in the FIA mode (Table 4.3), and the slope of electrode 1 was closer to the expected Nernstian response than electrode 2.

These results are markedly different to those obtained in static solutions by batch analysis. For static measurements, the presence of 0.1 M KCl in the standards resulted in a complete loss of sensitivity below pCu 4.5. Tarnishing of the membrane, which was very evident for static measurements, was negligible in the FIA study, indicating that the flow of solution past the membrane was having a beneficial effect by cleansing the electrode. At the higher chloride ion concentration,

Table 4.2 *Effect of humic acid on the Cu(II)ISE calibration parameters. (Flow rate 2.5 mL/min, ISE # 2, injection volume 100 µL.)*

Carrier composition	Slope (mV/decade)	E° (mV)	Linear range	R ²
0.05 M KNO ₃ + 5 x 10 ⁻⁷ M Cu ²⁺	28.83	231.61	pCu 2 - 5	0.9995
0.05 M KNO ₃ + 5 x 10 ⁻⁷ M Cu ²⁺ , 1 mg/L humic acid	29.61	234.61	pCu 2 - 6	0.9995

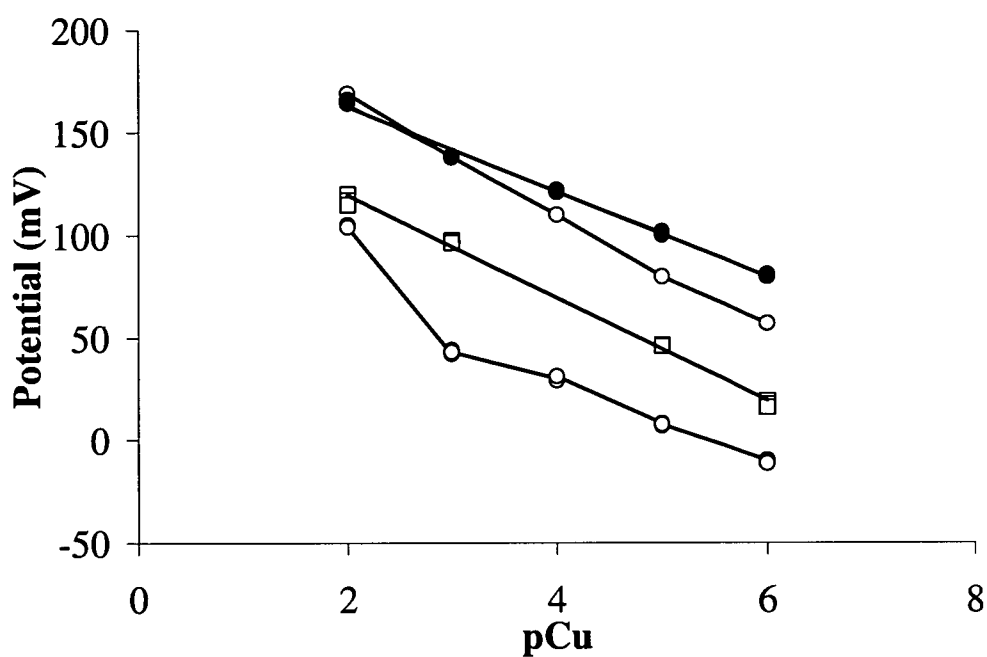


Figure 4.6 Effect of chloride on the Nernstian response of the Cu(II)ISE under FIA conditions. Data shown are ISE#2, chloride free standards (●); ISE#2, standards in 0.1 M KCl (●); ISE#2, standards in 0.5 M KCl (○); and ISE#1, standards in 0.1M KCl (□). (Carrier 0.05 M KNO₃ and 5 x 10⁻⁷ M Cu²⁺, flow rate 4.2 mL/min, and injection volume 100 μL.)

Table 4.3 *Effect of chloride ion concentration on calibration parameters of the Cu(II)ISE under FIA conditions. (Carrier 0.05 M KNO₃ and 5 x 10⁻⁷ M Cu²⁺, flow rate 4.2 mL/min, and injection volume 100 µL.)*

Electrode	Standard composition	Slope (mV/decade)	E° (mV)	R ²
1	0.1M Cl ⁻	25.05 (pCu 2 - 6)	169.70	0.9962
2	0.1M Cl ⁻	20.64 (pCu 2 - 6)	203.74	0.9947
2	0.5M Cl ⁻	18.40 (pCu 3 - 6)	100.22	0.9893
2	0.5M Cl ⁻	61.33 (pCu 2 - 3)	227.40	0.9995

the electrode gave linear but sub-Nernstian response from pCu 6 to 3, significantly improved performance over static analyses. Super-Nernstian response was not invoked until $\text{pCu} \geq 3$, in contrast to Neshkova's work (1987). She reported an absence of interference with 0.5 M KCl, over the same concentration range, using an electrochemically prepared Cu_{2-x}Se electrode. Only above 0.5 M KCl, did Neshkova (1998) observe sub-Nernstian response and reduced linear range.

Kinetic discrimination

The phenomenon of kinetic discrimination is based on the different response times of the membrane to the interfering ion and the primary ion (Neshkova 1993). A typical FIA response of the Cu(II)ISE to solutions containing a fixed chloride ion concentration with increasing copper concentration are presented in Figure 4.7.

As noted by Neshkova (1993), in solutions containing both chloride and cupric ion, the FIA signal is indicated by a negative overshoot for chloride, followed by a positive Cu^{2+} signal. The magnitude of the chloride signal is dependent on the ratio of cupric ion to chloride ion, and at 0.1 M chloride, the chloride response is negligible above pCu 3. Baseline stability was good, and Neshkova et al. (1998) proposed that this is indicative of rapid membrane response to the transient chloride signal, and that the overall time constant of the ISE must therefore be smaller than the time scale of the processes occurring at the membrane surface while it is exposed to the sample.

In the absence of (added) copper, the magnitude of the negative peak varies with the chloride ion concentration, and a sub-Nernstian response towards chloride was observed (Figure 4.8). A small positive peak of unknown origin appeared after each negative peak, and the size of this peak did not vary with changing chloride ion concentration, flow rate or injection volume. Injections of raw seawater into the KNO_3 carrier produced a very large negative peak, similar in magnitude to 0.6 M NaCl, which was followed by the small positive peak. The magnitude of the positive peak did not correspond to the concentration of free copper in the solution, as this would have meant significant contamination of the seawater (signal equivalent to pCu 6).

The analysis of seawater requires that the electrode be calibrated over a much higher range of pCu values, typically pCu 9 to 14. In order to extend the linear range of the calibration, saline Cu(II)-en buffers adjusted to various pCu values were injected into the carrier of 0.05 M KNO_3 and 5×10^{-7} M Cu^{2+} . This data, in addition to chloride free standards in the range $2 < \text{pCu} < 6$ is shown in Figure 4.9. For the chloride free standards, a linear calibration curve was obtained, with Nernstian response (Table 4.4). Sub-Nernstian response was obtained for the saline buffers. Since the baseline response was to 5×10^{-7} M Cu^{2+} , both the chloride signal and

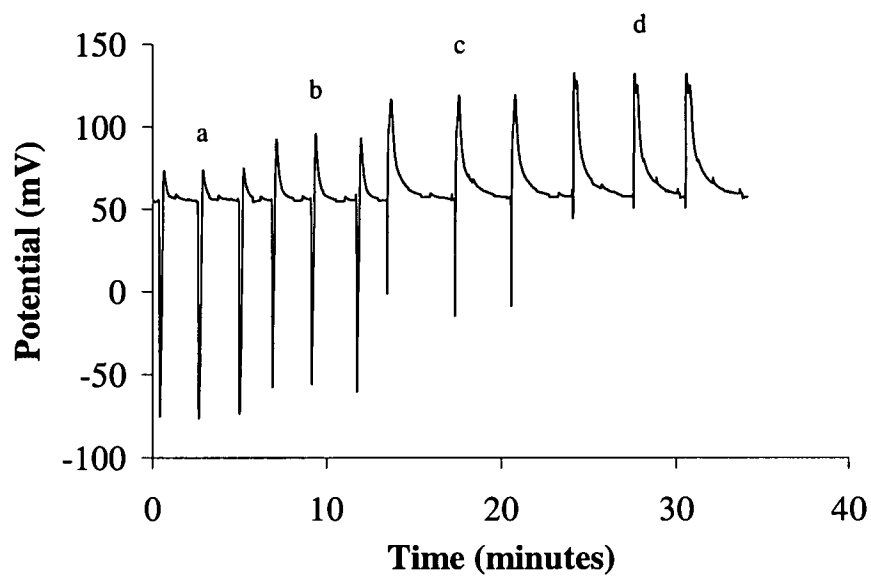


Figure 4.7 Response of jalpaite Cu(II)ISE to triplicate injections of a) pCu 6 b) pCu 5 c) pCu 4 d) pCu 3 standards in the presence of 0.1 M KCl. (Carrier 0.05 M KNO₃ and 5×10^{-7} M Cu²⁺, flow rate 4.2 mL/min, and injection volume 100 μ L.)

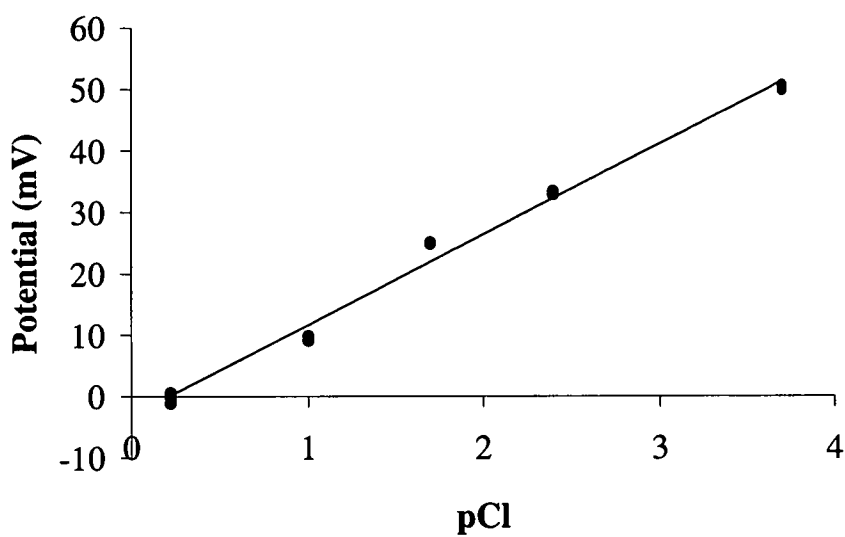


Figure 4.8 Effect of chloride ion concentration on Cu(II)ISE response in the absence of added copper. (Carrier 0.05 M KNO₃ and 5 x 10⁻⁷ M Cu²⁺, flow rate 4.2 mL/min, injection volume 100 µL. Regression equation $y = 14.74x + 3.12$, $R^2 = 0.9893$.)

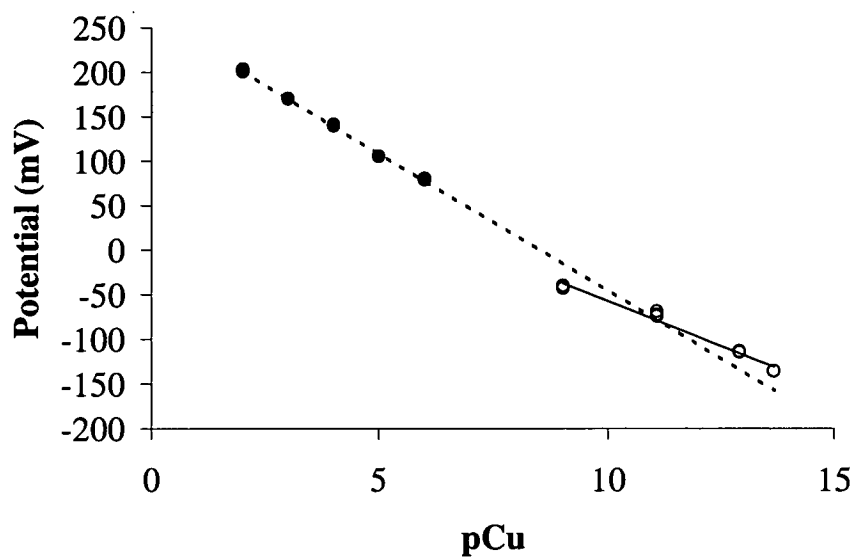


Figure 4.9 Calibration of Cu(II)ISE #2 with chloride free standards ($2 < pCu < 6$) and saline Cu(II)-en buffers ($9 < pCu < 13.6$). (Carrier 0.05 M KNO_3 and $5 \times 10^{-7}\text{ M Cu}^{2+}$, flow rate 4.2 mL/min , and injection volume $100\text{ }\mu\text{L}$.)

Table 4.4 *Calibration parameters for Cu(II)ISE #2 using chloride free and saline pCu buffers injected into a carrier of 0.05 M KNO₃ and 5 x 10⁻⁷ M Cu²⁺, at a flow rate of 4.2 mL/min, and injection volume 100 µl.*

Standard composition	Slope (mV/decade)	E° (mV)	R ²
Chloride free, 2 < pCu < 6	30.93	263.15	0.9980
Saline Cu(II)-en, 9.02 < pCu < 13.61	20.34	146.13	0.9814

the copper signal of the injected Cu(II)-en standards will appear below the baseline. The resulting negative peak was attributed to the electrode measuring the combined potentials in the time frame of the injection. This is plausible since the slope was not co-linear with the chloride free standards, and we know that the chloride interference is kinetically limited in the range $9 < \text{pCu} < 14$ (De Marco 1994) i.e. chloride interference is not responsible for the non-Nernstian response. It is likely therefore that the negative peak observed for seawater injected into a KNO_3 carrier is also a result of combined signals for both Cl^- and Cu^{2+} .

Since the electrode could not differentiate between the interferent and the analyte when saline pCu buffers or seawater were injected into KNO_3 use of kinetic discrimination is not warranted, under the conditions investigated here. It was therefore proposed to investigate alternative carriers for calibration with saline pCu buffers.

pCu buffer as carrier

The ideal carrier solution should have the following characteristics. It is easy to obtain or prepare; it has a high pCu so that injections of real samples give a positive peak (since electrode response is faster for positive change in potential); and it should maintain a stable membrane surface so that potentially interfering species or compounds present in the sample will not shift the E° . Additionally, the carrier should be compatible with the use of the pCu buffers for calibrating the electrode. The Cu(II)-en metal ion buffer meets all these requirements. The advantages of using a saline pCu buffer as carrier are a) the electrode is always exposed to free copper concentrations similar to those expected in open ocean samples, resulting in negligible carryover of adsorbed copper; b) the presence of en in the carrier will have a beneficial effect on the electrode response, as it has been shown that AgCl formation is reduced in the presence of en and c) the need for investigating optimal conditions for kinetic discrimination is overcome. A typical calibration run using the Cu(II)-en buffer as the carrier is shown in Figure 4.10. Sample throughput using the analysis conditions shown was 50 samples/hr. Larger injection volumes were used, with 500 μL determined as the optimal loop size. The electrode response was linear over the range $10.2 < \text{pCu} < 14$, with a slope of 28.38 mV/decade (theoretical response 28.9 mV/decade @19 °C), and E° of 232.04 mV ($R^2 = 0.9998$). The response to replicate injections was highly reproducible (RSD 0.11%). Due to the rapid response of the electrode to pCu buffers, a slightly lower flow rate of 1.7 mL/min was employed.

The FIA method was tested by injecting a sample of artificial seawater prepared as per De Marco (1994). Injections of 500 μL samples resulted in an assigned pCu value of 13.16 (± 0.03). Larger injection volumes were tested, and a 1200 μL loop

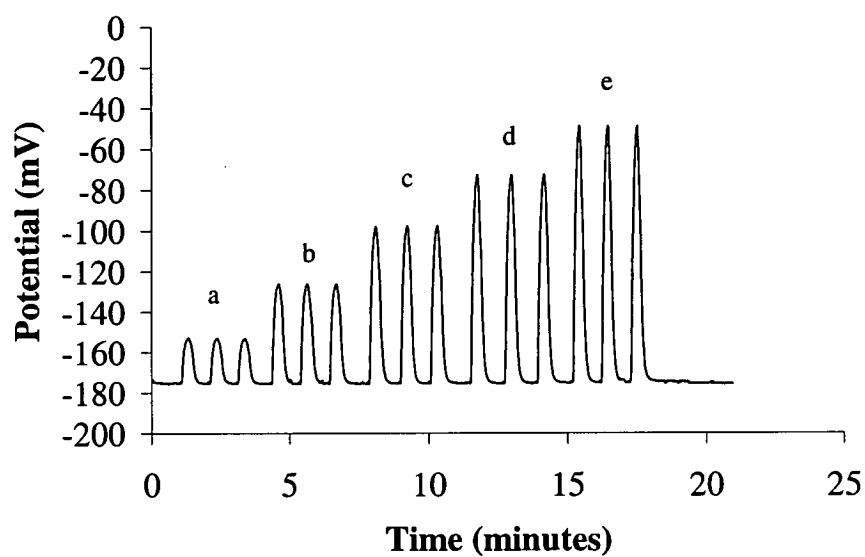


Figure 4.10 FIA trace showing calibration of Cu(II)ISE with pCu buffers. (Carrier pCu 14.89, standards a) pCu 14.01; b) pCu 13.01; c) pCu 12.01; d) pCu 11.11; e) pCu 10.22. Injection volume was 500 μ L.)

was found to give maximum signal size. The calculated pCu of the sample was 11.93, and was measured as 12.08 (± 0.01) after recalibration of the system with 1200 μL injections of Cu(II)-en buffers. It was proposed that the en buffer is “carried over” when the artificial seawater sample was injected, and the stronger complexing capacity of the en buffer was affecting the composition of the glycine “slug”. By injecting larger volumes, this effect was overcome. There may also be some contribution of en adsorbed onto the surface of the membrane, as Zirino et al. (1998a) found evidence of the ligand being tenaciously adsorbed onto the electrode surface. Following good agreement with theoretical values for the artificial seawater, natural seawater was injected into the Cu(II)-en carrier. This resulted in a small positive peak, equivalent to pCu 14, however, the size of the peak did not vary when the same seawater was UV-oxidised, or when injection volume was varied. The ISE response was therefore not representative of the free copper in solution, since we would expect that a UV-oxidised sample would contain more free copper than an untreated sample. Likewise, a larger injection volume should produce a larger signal. A series of seawater samples spiked with up to 1000 nM total copper were prepared to investigate the lack of response further. The samples were equilibrated for at least 24 hours before analysis. The electrode was calibrated in the usual way, by injections of pCu buffers into the Cu(II)-en carrier. Significantly, the electrode response to each sample was identical, that is the positive peak recorded at each injection was independent of the concentration of free or total copper present in the sample (Figure 4.11). Increasing injection size did not alter the situation.

It appears that in the time frame of an FIA measurement, and in the presence of the Cu(II)-en buffer as carrier, the electrode cannot respond rapidly enough to the free copper in seawater (at pH 8) for a representative measurement to be made. This is in contrast to the kinetic discrimination studies where high concentrations of copper and chloride injected into a non-complexing carrier could be resolved as two separate signals. To investigate if the effect was pH dependent, acidified samples (pH 2) from the CSIRO archive were sub-sampled. $\text{pCu}_{\text{total}}$ for these samples varied from 8.38 to 9.11. Injections of the acidified samples into the Cu(II)-en carrier resulted in an enormous positive peak, roughly equivalent to pCu 3. Excessive tailing followed, and it took several hours for the baseline to return to its original value. This is attributed to interaction between the carrier and the sample slug. The pH of the sample would be sufficient to protonate all the en, hence all the copper in the carrier/sample interface would be present as free copper i.e. pCu 3. There may also have been some contribution of copper from membrane dissolution at low pH,

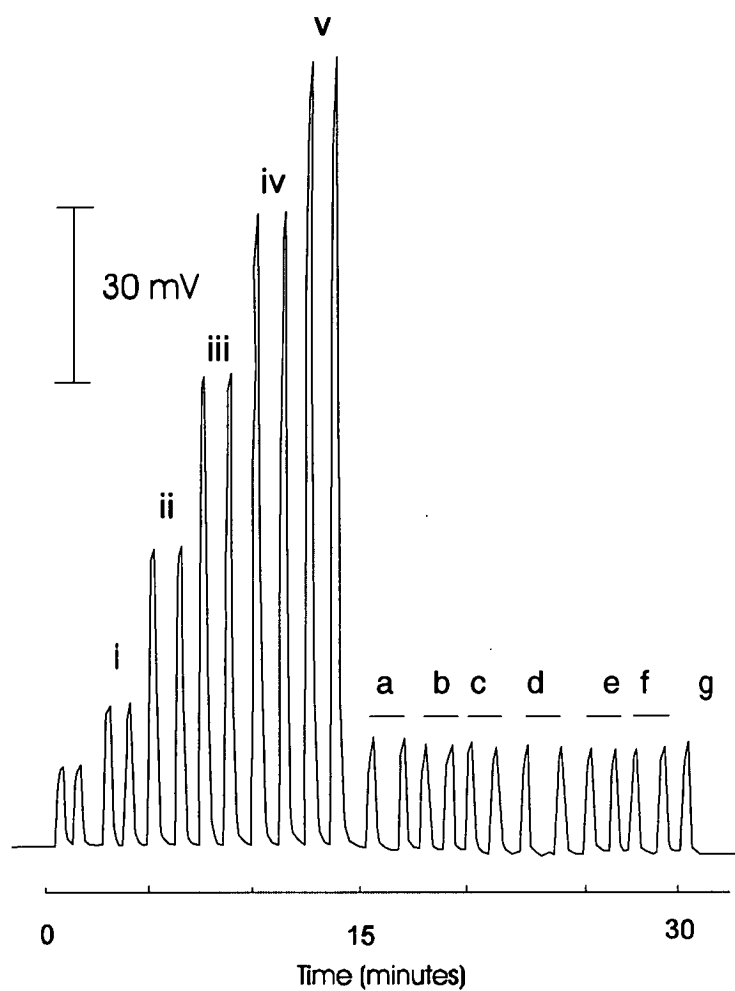


Figure 4.11 ISE calibration with pCu buffers and subsequent injection of copper spiked samples. Standards i) pCu 14.01 ii) pCu 13.01 iii) pCu 12.01 iv) pCu 11.11 v) pCu 10.21. Samples a) raw seawater; b) seawater + 50 nM total copper c) + 100 nM d) + 200 nM; e) + 300 nM; f) + 500 nM; g) + 1000 nM. (Carrier pCu 15 buffer, flow rate 1.7 mL/min, and injection volume 500 μ L)

However this will have been completely masked by the pH effect on the buffer composition. The tailing is most likely to be due to formation of AgCl, in the presence of high concentrations of free copper and 0.6 M NaCl. The flow of solution past the electrode gradually removed the AgCl, and the electrode response returned to its previous value as a clean membrane surface was restored.

From these results, it is obvious that the ISE response is affected by interaction between the carrier and the injected samples or standards. In the case of glycine, the complexing capacity of the ligand is high enough that simply increasing the sample volume will overcome the competition for copper by en. This is not effective for seawater samples at natural pH where the buffering capacity is many orders of magnitude lower than the carrier. The interaction between samples and carrier is most clearly demonstrated by injections of acidified samples into the Cu(II)-en carrier.

It is significant to note that the full chloride interference could be invoked by injecting chloride-free copper standards into a saline pCu 11 carrier. The only way AgCl could be formed is by carryover of chloride into the sample slug, i.e. the carrier is causing the interference reaction to affect the determination of pCu in the sample. As a result, a negative offset in the sample signal was observed, followed by excessive tailing as AgCl formed and was slowly removed by ablation from the flowing system. Visual inspection of the ISE during a tailing peak confirmed the formation of AgCl as a dull grey coating on the membrane surface. These results are additional evidence that components of the carrier are being tenaciously bound to the membrane in the diffusion layer and are reacting with the Cu^{2+} in the sample slug. The result is unwanted side reactions e.g. formation of AgCl, and protonation of en.

Sample as carrier

An alternative method of injecting pCu buffers into the sample (i.e. “reverse FIA”) was tested. The rationale for this method was that since the electrode responds rapidly to the buffers, the calibration procedure could be incorporated into the process of waiting for a steady state response to the seawater sample. The suppression of the copper signal by Cu(II)-en when seawater is injected may also be overcome by reducing the electrode exposure to the buffers.

A sample from a contaminated estuary (unfiltered, S=20) was run through the FIA system until a stable baseline was acquired. A series of pCu buffers ($10.21 < \text{pCu} < 14.01$) were injected, and the sample baseline allowed to re-establish before changing to the next sample (Figure 4.12). Using this method the sample baseline corresponded to a value of $\text{pCu } 10.53 \pm 0.28$. The result is feasible as it is higher than data reported for free copper in estuarine and coastal marine waters. Sunda and

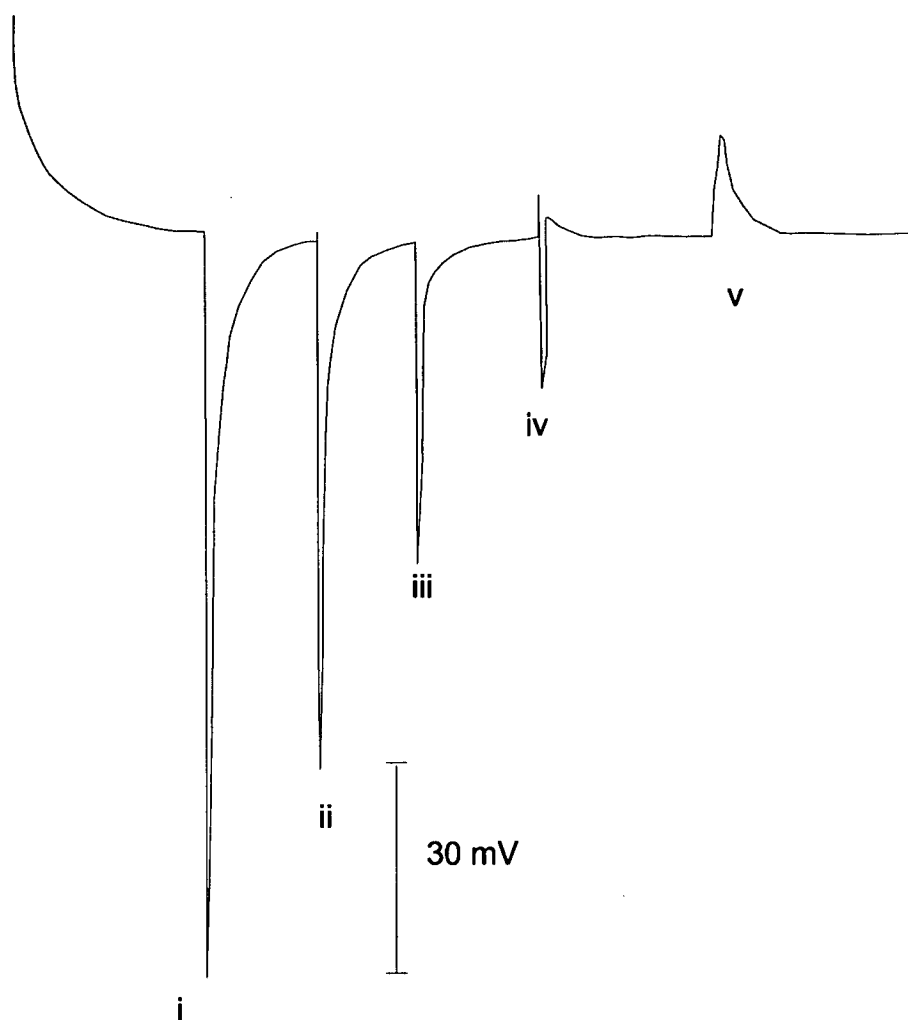


Figure 4.12 Calibration of Cu(II)ISE by injection of i) pCu 14.01; ii) pCu 13.01; iii) pCu 12.01; iv) pCu 11.11; v) pCu 10.21 buffers into a carrier of seawater collected from a contaminated estuary. Note that the slope was 28.42 mV/decade, and E° 246.75 mV, with $R^2 = 0.9956$. Total analysis time was 40 minutes. (Flow rate was 1.7 mL/min, and injection volume was 500 μ L.).

Ferguson (1983) estimated ambient pCu to be 11.5 in Peruvian coastal waters, and 11.3 to 11.5 for samples from the Northern Gulf of Mexico. Water collected from the seawater intake of an aquaculture facility was also measured by this method, and pCu determined to be 10.72 ± 0.19 . Repeat analyses with UV-oxidised seawater as carrier gave pCu values of 11.07 ± 0.33 , and after correction for inorganic complexation at pH 8 ($\alpha = 1.9$), (De Marco et al. 1997) gave a pCu_{total} value of 9.17. This corresponded well to the value of 9.02 determined by GFAAS, however the method proved to be time consuming, required a large volume of sample, and sample throughput was reduced to 1 to 2/hr. The tailing observed was indicative of a sluggish process occurring at the membrane after each injection, indicating that interaction between the carrier and the injected standard had not been eliminated.

It is evident from these studies that two primary factors are affecting the ISE response under FIA conditions

- 1) the response time of the electrode is vastly different for pCu buffers and natural samples (at natural pH and UV-oxidised)
- 2) Interaction between the sample slug and the carrier solution causes problems that are not encountered in batch type analyses.

Cross contamination from the carrier to the sample slug means that the content of the carrier and the standards should be identical with respect to salinity, pH and buffering capacity. It is also recommended that samples should be injected into an alternative carrier of seawater i.e. like with like. This should be combined with the successful approach of injecting the Cu(II)-en buffers into a Cu(II)-en carrier to calibrate the electrode. In this way, the rapid response to pCu buffers for calibration purposes is optimised, without compromising the sample analysis by unwanted side reactions.

CONCLUSIONS

In this chapter, the FIA experimental work in copper standards in the range $2 < \text{pCu} < 6$ was undertaken to establish the ISE response under various flow regimes, and to optimise the system for further studies. The presence of organic matter had no negative effect on the calibration parameters of the electrode, and may indeed be beneficial as the linear range was extended in the presence of humic acid. It was established that the chloride interference is ameliorated under flow conditions, with loss of sensitivity occurring at lower pCu values than observed in static experiments. A wider calibration range was obtained in the presence of chloride using FIA, although the response was sub-Nernstian in most cases. It was also established that kinetic discrimination can resolve the chloride ion interference from the copper signal using the jalpaite ISE.

For the analysis of seawater however, it is necessary to calibrate the electrode over the range pCu 9 -14, and calibration with saline Cu(II)-en buffers is required. A method was developed using a Cu(II)-en buffer (pCu 15) as carrier, with a range of pCu buffers injected into the carrier. Nernstian response was obtained, and the calibration procedure was rapid and extremely reproducible. The system was validated using artificial seawater prepared in glycine. Injections of copper spiked seawater samples revealed that the response time of the electrode to raw seawater was too slow for analysis by FIA. Using saline Cu(II)-en solutions as the carrier prevented the analysis of total copper in acidified samples, as the acidic slug interacted with the pCu buffer, causing a large positive signal that obscured the sample signal. It was possible to analyse total copper in UV-oxidised seawater by using the sample as carrier, and injecting pCu buffers into the sample. An alternative method for the determination of total copper would be the use of standard additions to an acidified carrier, however this is unlikely to offer any advantages with respect to the time taken for analysis.

Other examples of sample-carrier interaction (e.g. competition for free copper between en and glycine; formation of AgCl by injection of chloride-free copper standards into a saline carrier) confirm that carryover of carrier solution species is a significant limitation for the further development of a FIA method.

The slow response of the electrode to low concentrations of copper in seawater at natural pH suggests that continuous flow techniques would be more applicable, as the technique can be optimised to allow electrode contact with the sample for a much longer period of time. It was therefore decided to develop a continuous flow system that incorporated the rapid calibration with pCu buffers demonstrated in FIA, but using a seawater carrier to overcome the other problems described here.

Chapter 5 Continuous Flow Methods for Evaluating the Response of a Copper Ion Selective Electrode to Total and Free Copper in Seawater

This chapter is a modified version of 'Continuous Flow Methods for Evaluating the Response of a Copper Ion Selective Electrode to Total and Free Copper in Seawater.' Ruth Eriksen, Denis Mackey, Peter Alexander, Roland De Marco, and Xue Dong Wang, published in the Journal of Environmental Monitoring (1999) 1, 483-487.

ABSTRACT

This chapter describes the development of an instrument for measuring free and total copper in seawater by continuous flow analysis (CFA) with an Orion copper (II) ion selective electrode. Sample analysis times are reduced considerably by using an extrapolation technique based on the fitting of an empirical mathematical expression to the electrode time-response curve, enabling a prediction of the final equilibrium potential. CuISE measurements in seawater samples containing nanomolar levels of total copper can be very time consuming, and this predictive approach significantly reduces sample analysis time, and improves sample throughput. The time taken to measure pCu in seawater to a precision of ± 0.1 pCu, using conventional potentiometry, varies considerably depending on the condition of the electrode membrane but can be reduced by a factor of 3 to 6 (typically from 60 minutes to 10 minutes) by using the extrapolation technique in conjunction with CFA. Details of the protocols used for preconditioning the CuISE are given. The system can be used as a portable instrument for field measurements or for shipboard measurements of free copper in seawater. Extrapolated equilibrium potentials are within ± 0.5 mV of true steady state values.

INTRODUCTION

Considerable work has been done on incorporating ISEs into portable field monitoring devices (Di Benedetto et al. 1996; Dimitrakopoulos et al. 1995; Alexander et al. 1996) using techniques such as flow injection analysis (FIA) and continuous flow analysis (CFA). It has already been established in Chapter 4 that flow methods offer particular advantages as they overcome most of the problems inherent in batch ISE measurements. For the Cu(II)ISE, these include sluggish response at low activities, cross contamination from one sample to another, contamination by membrane dissolution and/or silver chloride precipitation, and an observed alteration in response due to adsorption of natural organic matter present in seawater (De Marco 1996; Mackey & De Marco 1997; De Marco et al. 1998).

Preliminary FIA studies revealed that slow response of the Cu(II)ISE at the very low Cu^{2+} activities found in seawater, and interaction between the carrier solution and injected sample, were significant factors that limited the usefulness of this approach. CFA flow methods are inherently slower than FIA techniques, but still offer distinct advantages over 'dip' type measurements. Reported improvements in reproducibility, precision and accuracy in FIA studies should be equally applicable to CFA methods. As in the case of FIA, electrode dissolution should be significantly reduced in CFA, and the risk of sample contamination is further reduced by the absence of sample transfers or exposure to the ambient laboratory environment.

This study was designed to determine if the sample throughput in CFA could be improved by using an extrapolation method. By acquiring the initial portion of the time-response curve in a CFA system, the final equilibrium potential may be accurately predicted without waiting for the establishment of a steady state signal. In this way, a sample measurement that may take an hour or more to reach a steady state signal under flow conditions, may be made within a matter of minutes. This approach has recently been used successfully with the fluoride (Wang et al. 1995), cadmium, lead, iodide, chloride and nitrate ISEs (Watkins 1997) and the results suggest that the technique should be equally applicable to Cu(II)ISE measurements at low free copper concentrations in natural waters. However, studies of other ISEs have usually been limited to concentrations between 10^{-1} M and 10^{-5} M, the operating range over which of many commercial ISEs give linear and relatively rapid response. For the technique to be applicable to marine and estuarine studies of copper speciation, the extrapolation technique must be robust in the nanomolar and sub-nanomolar range.

A number of semi-theoretical relationships describing potential-time response transients of ISEs have been published elsewhere in the literature. In this chapter, the extrapolation techniques described by Buffle and Parthasarathy (1977) and

Müller (1969) are investigated, and an experimental protocol for the rapid measurement of free and/or total copper in seawater using a CuISE in a CFA system is described.

EXPERIMENTAL

Chemicals and instrumentation

Analytical grade reagents (Ajax) were used to prepare all solutions, and high purity water was obtained from a Millipore “Milli-Q” water purification system. The CFA system employed in this study is based on the portable FIA field analyser described in Chapter 4, except that the flowcell, injection valve and peristaltic pump were mounted on a perspex stand and baseboard. Where possible, metal-free components were used in the construction of the system to reduce the potential for contamination. Initially the CFA work was carried out using a Hamilton 2-way valve (Model HV 86728) to switch between sample and standard solutions. Later, a Rheodyne 6-way Type 50 Teflon rotary valve was used to switch between pCu buffers, samples and pre-conditioning solutions (Figure 5.1).

All studies were conducted using the Orion Cu(II)ISE (Model 94-29). The perspex Chemflow Devices FIP-3 wall-jet flowcell described in Chapter 4 was used for all CFA measurements. A Ag/AgCl electrode was located in a separate carrier stream of 0.1 M KCl. This flowcell design minimizes streaming potentials and liquid junction potentials (Dimitrakopoulos 1994). Flow rates for both streams were optimized, with KCl and the carrier/sample stream pumped at flow-rates of 0.5 mL/min, and 2 mL/min respectively, using an Alitea peristaltic pump (Model C-XV). Data was recorded via a laptop PC using a Pico Technology “Picolog” datalogger and software. For the portable system, potentials were recorded on a battery powered A/D system connected to a Macintosh laptop computer. For laboratory measurements, the analogue signal from the system was also connected to Radiometer PHM85 Precision pH meter and the output measured on a YEW Type 3056 Pen Recorder. Processing of data for extrapolation studies was undertaken off-line using Microsoft Excel and software written in MS-VisualBASIC for Windows. Data were acquired at 100 ms intervals, although a data acquisition rate of 1 hz is adequate for testing of the extrapolation procedure.

Total copper was measured on archived samples which had been stored at pH 2 and previously analysed by conventional dithiocarbamate extraction and GFAAS (Mackey 1983). Before ISE analysis, the archived samples were UV-photooxidized to destroy organic matter which may interfere with the electrode response at pH 2 (De Marco et al. 1997). Metal ion buffers containing ethylenediamine were prepared in accordance with the method described in Chapter 3.

The pCu was calculated using the empirical equation reported in Chapter 3. The pCu results are consistent with equilibrium speciation calculations performed using

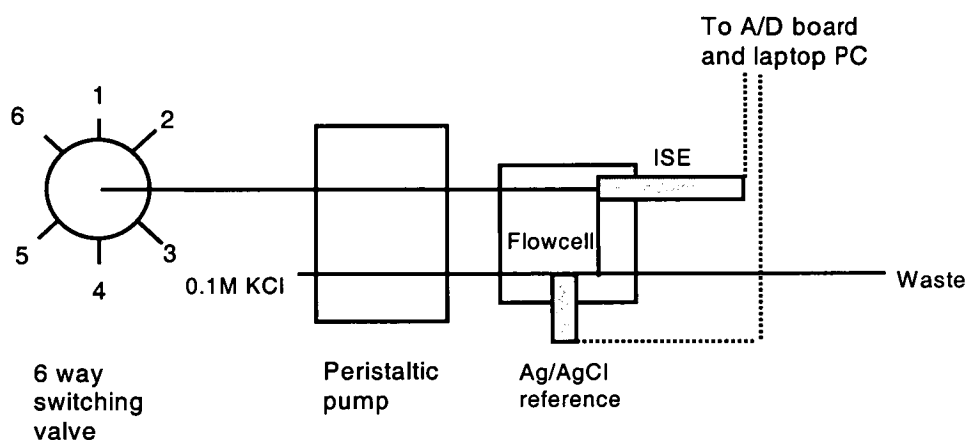


Figure 5.1 Schematic diagram of the CFA/Cu(II)ISE system. Switching valve positions were as follows: positions 1 - 4, calibrating buffers; position 5 filtered seawater or sample, and ; position 6, sacrificial buffer (pCu 14 - 15). A portable field kit was assembled by employing a simple injection system, a battery operated A/D converter, a peristaltic pump, and a Macintosh laptop computer.

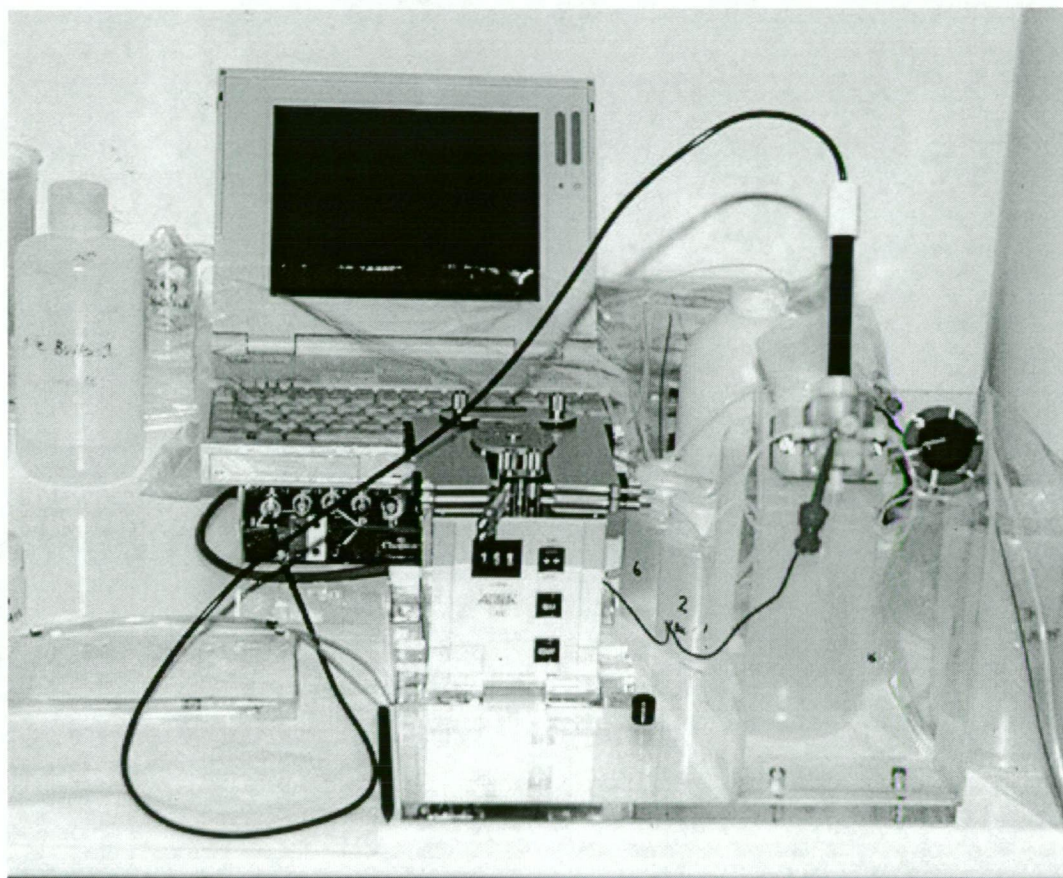


Figure 5.2 CFA/Cu(II)ISE system developed for ship board use, showing perspex baseboard and stand for clean room analyses. The ISE is positioned in the FIP-3 flowcell. The 6-way injection valve is mounted to the right of the flowcell, and calibrating buffers and filtered seawater are stored behind. Laptop is mounted on top of the datalogger.

MINTEQA2 (Allison et al. 1996) and the stability constants reported in De Marco (1994) and Appendix 1.

For method development, seawater samples were collected without clean protocols and stored in polyethylene containers that had been acid-washed. Basic precautions were taken to prevent significant contamination, however, the pCu values for these samples were not indicative of the true values in the original water masses. For samples that had been collected under clean conditions (Mackey 1983), all laboratory analyses were carried out in a Class-100 clean room to prevent sample contamination.

All glass and plasticware involved was acid washed in 10% HCl for 7 days, before copious rinsing with Milli-Q.

Analysis procedure

The Cu(II)ISE was polished before each series of experiments using alumina powder (0.3 and 0.05 μm) as described in Chapter 3. The polished ISE was then conditioned in the CFA system with filtered, open-ocean seawater until a steady state signal was observed, usually within 3 hours. A stability criterion of 0.1 mV/min was used to define the steady state. The system was calibrated by switching between a series of pCu buffers (typically $9 < \text{pCu} < 15$). The ISE response was linear over 6 orders of magnitude and agreed with the predicted Nernstian response for a divalent ion of ~ 30 mV per decade change in Cu^{2+} . After calibration of the CFA system, the electrode was exposed to filtered seawater again, until a steady state potential had been achieved. At this point the system is ready for sample analysis.

For overnight storage, the ISE was left in the flowcell in a high pCu buffer (12 - 14) with the pumps turned off. Between analysis runs the ISE was left in the flowcell with filtered seawater running between 1 and 2 mL/min, or in standby mode with a high pCu buffer on minimal flow. Storage protocols were designed to prevent fouling of the membrane that occurs when the ISE is left for long contact periods in static seawater. Seawater filtered through 0.45 μm filters (Millipore HA) was used for all conditioning procedures, and by preparing a large batch of seawater it could be used as a daily check on the response of the ISE. For a polished ISE, a seawater sample typically produced the same potential to within ± 2 mV from day to day. Deviations in ISE response of larger than a few mV indicated that the condition of the electrode had deteriorated and repolishing was required.

Equilibrium potential extrapolation

Buffle and Parthasarathy (1977) proposed that the electrode potential E at time t is related to the final equilibrium potential E_{eq} by the expression:

$$E = E_{eq} - 1/(At + B) \quad (1)$$

where A and B are empirical constants. Equation 1 can be expanded to a linear expression:

$$(Et - E_s t_s)/\pm(E - E_s) = E_{eq} (t - t_s)/\pm(E - E_s) - B/A \quad (2)$$

where E_s is the electrode potential taken at an arbitrarily chosen starting time t_s , generally chosen as close to zero (t_0) as possible. By evaluating and plotting $(Et - E_s t_s)/\pm(E - E_s)$ against $(t - t_s)/\pm(E - E_s)$, the slope of the line will provide the value of E_{eq} . The sign of the slope will be positive for an increase in potential (i.e. increase in activity at the electrode surface), and negative for a decrease in potential.

Müller (1969) proposed that the potential-time transient generated by the Orion Ag₂S electrode could be described by the empirical hyperbolic relationship:

$$E = t/(A + Bt) \quad (3)$$

where E is the increase in potential caused by a change in the primary ion concentration detected at the electrode membrane at time t , and A and B are constants. The linearized form of this equation is:

$$t/E = A + Bt. \quad (4)$$

By evaluating and plotting t/E vs. t , a straight line with slope B and intercept A is obtained. The predicted equilibrium value of E , E_{eq} , is obtained from the reciprocal of the slope, $1/B$. In both instances, the predictive power of the equation can be assessed by the degree of linearity of Equation 2 or 4, and by investigating the effect of small changes in t on the calculated value of E_{eq} .

Wang et al. (1995) addressed the problem of selecting t_0 in continuous flow systems where there is a gradual concentration change at the electrode-solution interface, rather than the sudden change in immersion-type measurements. There is a lag between switching the valve to a new solution, and that solution reaching the electrode membrane. This lag is dependent on the manifold setup and the dead volume of the flow cell, and therefore t_0 is defined as the time at which the electrode potential starts to change. Wang et al. (1995) recommended that t_0 be determined as the second t_i value for which $|E_{i+1} - E_i| > 0.5$ mV and that t_s be as close to t_0 as possible. These criteria were used for the application of both equations to the test data sets. In some cases, obvious noise spikes were filtered out of the data set prior to curve fitting. Wang et al. (1995) automated the data acquisition so that evaluation of E_{eq} was continuously calculated as data was acquired by PC. In this study, evaluations of Equations 2 and 4 were performed post data acquisition.

RESULTS AND DISCUSSION

Advantages of continuous flow analysis

It is widely reported that pre-treatment history and conditioning can drastically affect the response time and linearity of many ISEs (Mertens et al. 1976; van Oort & van Eerd 1983; Gulens 1987). Regular polishing and daily conditioning of the jalpaite membrane are crucial to producing a theoretical Nernstian response, reducing electrode response times, and establishing a stable reproducible surface for sample analysis (De Marco 1994). Incorporation of the ISE into a flow system significantly reduced the time required for a stable surface to form on the jalpaite membrane. The ISE was ready to use within 2 to 3 hours, as opposed to a 24 hour conditioning period when making static measurements. Toth et al. (1986) noted that when an ISE was incorporated into a flow system, electrode response was faster and detection limits and measurement precision were often improved. These observations stem from the fact that the thickness of the diffusion layer at the electrode-solution interface is significantly reduced in a flow system, and delivery of the analyte to the ISE surface is the rate determining step in the electrode response. Selectivity may also be enhanced by incorporating ISEs into flow systems since there may be a separation of the analyte ion signal from that of other interfering ions (Pacey et al. 1986). A separation of the chloride signal from the copper response has been noted when using the jalpaite ISE under FIA conditions (Chapter 4) and for chalcogenide electrodes (Neshkova et al. 1998).

Another advantage of including the Cu(II)ISE in a flow system is that the effect of membrane dissolution, which can significantly contaminate a sample in the static measurement mode, is negligible or non-existent. The solution at the electrode surface is constantly being replenished, preventing any build up of copper released from the ISE itself. The flowing solution also prevents the build-up of a silver chloride precipitate which can otherwise foul a jalpaite ISE if it is left standing in seawater. The influence of adsorbed organic ligands on the ISE response can be alleviated by either the use of a rotating ISE, or by using high carrier flow rates in a CFA system (De Marco et al. 1998). Whilst an organic interference effect does exist, it is relatively weak and does not pose a serious problem at the flow rates used in this study (De Marco et al. 1998).

Zirino et al. (1998a) demonstrated that en is retained on the surface of the electrode when it is removed from a calibrating buffer solution and placed in a seawater sample, and that repeated rinsing or sample exchange is required to completely remove all of the adsorbed ligand. They recommended analysis conditions under which all of the adsorbed ligand was removed (by rotating the electrode at high speed) so that a true equilibrium pCu of the seawater sample could be measured.

The method reported here similarly removes all of the adsorbed calibrating ligand, by changing the carrier solution to seawater once the calibration has been completed.

The instrument shown in Figures 5.1 and 5.2 was designed to be used in remote or field locations, and is suitable for use on research vessels. The system was designed to be operated in a small laminar flow hood onboard the CSIRO Research Vessel *Franklin*, and in the mobile clean-room (converted shipping container) onboard the Antarctic Research Vessel *Aurora Australis*. The system has been used by other researchers for studying real time copper speciation in the equatorial Pacific (D. Mackey, cruise FR0897,) and to calibrate the response of CTD mounted Cu(II)ISEs in Antarctic waters i.e. *in situ* analyses (D. Mackey and R. Edwards, voyage A9706) however those results are not discussed here.

Response to pCu buffers

The Orion ISE displays theoretical response in pCu buffers containing 0.6 M NaCl, over a wide range of free copper concentrations. The ISE response in ethylenediamine buffers is co-linear with standards prepared in deionised water over the range $1 < \text{pCu} < 6$, indicating that the chloride present in the buffers is not causing an interference reaction over the range $9 < \text{pCu} < 15$ (De Marco 1994). The lack of interference from chloride is due to the fact that below 10^{-8} M free copper, the kinetics of the interference reaction are so slow that it does not occur over the time of an analytical measurement. The range of pCu values that we expect to encounter in marine samples is in the region where the chloride interference is negligible ($9 < \text{pCu} < 14$).

Table 5.1 compares measurements on ethylenediamine buffers using steady state data (E_{eq}), collected after an equilibration time of 5 - 8 minutes (stability criterion 0.1 mV/min), and the values of E_{eq} predicted by both models using only the first 2 minutes of data. For Equation 2, the predicted E_{eq} values are within ± 0.4 mV of the true equilibrium potential, and the correlation coefficients for the linearized data are better than 0.9994 in all but one case. For Equation 4, the predicted E_{eq} values are within ± 1 mV of the true equilibrium potential, slightly poorer performance than using Equation 2. The R^2 values for Equation 4 are slightly lower than for Equation 2 which is largely due to small but progressive deviations from linearity for the lower values of t in which the range of experimental conditions are ill-defined (Müller 1969; Mertens et al. 1976). However, the overall effect on the calibration parameters is negligible with both equations giving slopes within 0.05 mV/decade and intercepts within 0.6 mV of those obtained using the equilibrium values (Table 5.2), 30.7 mV/decade and 272.7 mV respectively. These differences have a

Table 5.1 *Steady state equilibrium potentials, time taken to reach equilibrium and predicted E_{eq} values for pCu buffers, obtained using equation 2 (Buffle and Parthasarathy, 1977) and equation 4 (Müller, 1969). The predicted values were calculated from an extrapolation of the first 2 minutes of data.*

Buffer pCu	Steady state (mV)	Time (min)	Equation 2	Equation 4
12.94	-125.8	7.93	-125.8	-125.3
11.97	-92.5	8.00	-92.6	-91.7
11.03	-67.0	7.84	-66.6	-66.2
10.06	-38.8	5.80	-38.7	-37.3
9.0	-2.6	5.14	-2.2	-2.3

Table 5.2 *Comparison of slope and E^0 values using calibration curves constructed from steady state equilibrium potentials and predicted equilibrium potentials for Equation 2 (Buffle and Parthasarathy, 1977) and Equation 4 (Müller, 1969).*

Test	Slope (mV)	E^0 (mV)	R^2
Steady state values	30.72	254.27	0.9985
Equation 2	30.77	254.87	0.9983
Equation 4	30.70	254.74	0.9987

negligible impact on the calculated pCu. The accuracy could be improved by choosing a value for $t > 2$ min, but this would be at the cost of sample throughput.

Figure 5.3 shows a typical calibration run using pCu buffers ranging from 15 to 9.5. The total time to calibrate the electrode was approximately 12 minutes.

Response to seawater samples

In unbuffered solutions, it has frequently been observed that the time take to reach equilibrium increases as the concentration of the analyte ion decreases (Lindner et al. 1981). In addition, the response time of the Cu(II)ISE is always faster when the concentration step is positive, as is typical for many ISEs (Mertens et al. 1976). The response time of the Cu(II)ISE increase from minutes to hours when changing from the analysis of saline ethylenediamine buffers to samples of natural seawater and this variation in response rates was also observed by Zirino et al. (1998a).

Consequently, data must be acquired for longer periods to obtain accurate values of equilibrium potentials. In order to test the response of the CFA/Cu(II)ISE system at a low concentrations of copper, a sample of seawater was run through the CFA system until the steady state criterion of 0.1 mV/min drift was satisfied. The system had previously been equilibrated with a high pCu buffer (14) following calibration in the usual way. Several values of t were used to compare the predicted equilibrium potential with the true steady state value (Table 5.3). The raw data and the data fitted to Equation 2 (for $t = 8$ min) are shown in Figure 5.4. The accuracy of the predicted equilibrium potential improves as the value of t increases and, after acquiring data for 10 minutes, the predicted E_{eq} falls within 0.2 mV of the true value. This would, however, significantly reduce the sample throughput. The accuracy required is thus a compromise between the acceptable error and the speed of sample analysis. An error of 1.2 mV, obtained after 8 minutes, will be reflected as a 0.04 error in calculated pCu, which is acceptable in terms of the overall uncertainty of the analytical method. The pCu_{free} of the seawater sample was calculated to be 12.04.

In Table 5.4, we have shown a comparison between GFAAS and CFA/Cu(II)ISE analyses of total copper for open ocean seawater samples collected from the Tasman Sea on cruise FR07/89 of *R V Franklin*. After correcting for chloride and sulfate complexation of copper in the acidified seawater ($\log \alpha = 0.8$), the extrapolated values are within 0.2 pCu units of the GFAAS data. The response of the CFA/Cu(II)ISE system was similar to that found for natural seawater samples (no acidification, no UV-photooxidation) at pCu_{free} values in the range 11 to 14. Acquisition of steady state data in this range could typically take up to an hour. These samples could be reproducibly analyzed in 10-15 minutes using the experimental protocol described above.

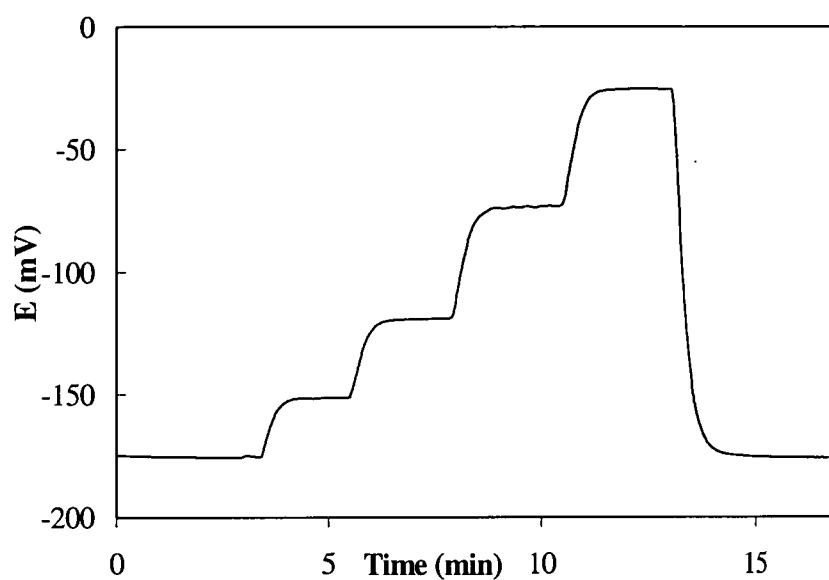


Figure 5.3 Typical calibration run for a conditioned CuISE using the CFA system. The pCu values of the buffer solutions were 14.99, 14.17, 12.99, 11.46, 9.59, and 14.99 respectively. Data was acquired for 2 minutes for each of the 4 calibrating buffers.

Table 5.3 *Potential of the Cu(II)ISE detector (E) as a function of time (t) after the sample was changed from pCu 14 buffer to a seawater sample at natural pH. Also shown are the potentials (E_{eq}) predicted at time t using equation 2, the errors between the predicted E_{eq} and the steady state value (-115.5 mV), which was obtained after 27 minutes and the corresponding errors in pCu. The pCu_{free} of the seawater sample was calculated to be 12.04.*

t (min)	E (mV)	Predicted E_{eq} (mV)	R^2	Error (mV)	Error in pCu
2	-143.8	-120.9	0.9873	-5.4	0.18
5	-128.6	-120.3	0.9973	-4.8	0.16
6	-126.8	-122.3	0.9982	-6.8	0.23
7	-125.0	-117.5	0.9981	-2.0	0.07
8	-123.9	-116.7	0.9990	-1.2	0.04
10	-121.8	-115.7	0.9990	-0.2	0.01
15	-118.6	-116.0	0.9994	-0.5	0.02
20	-116.8	-115.1	0.9995	0.4	-0.01

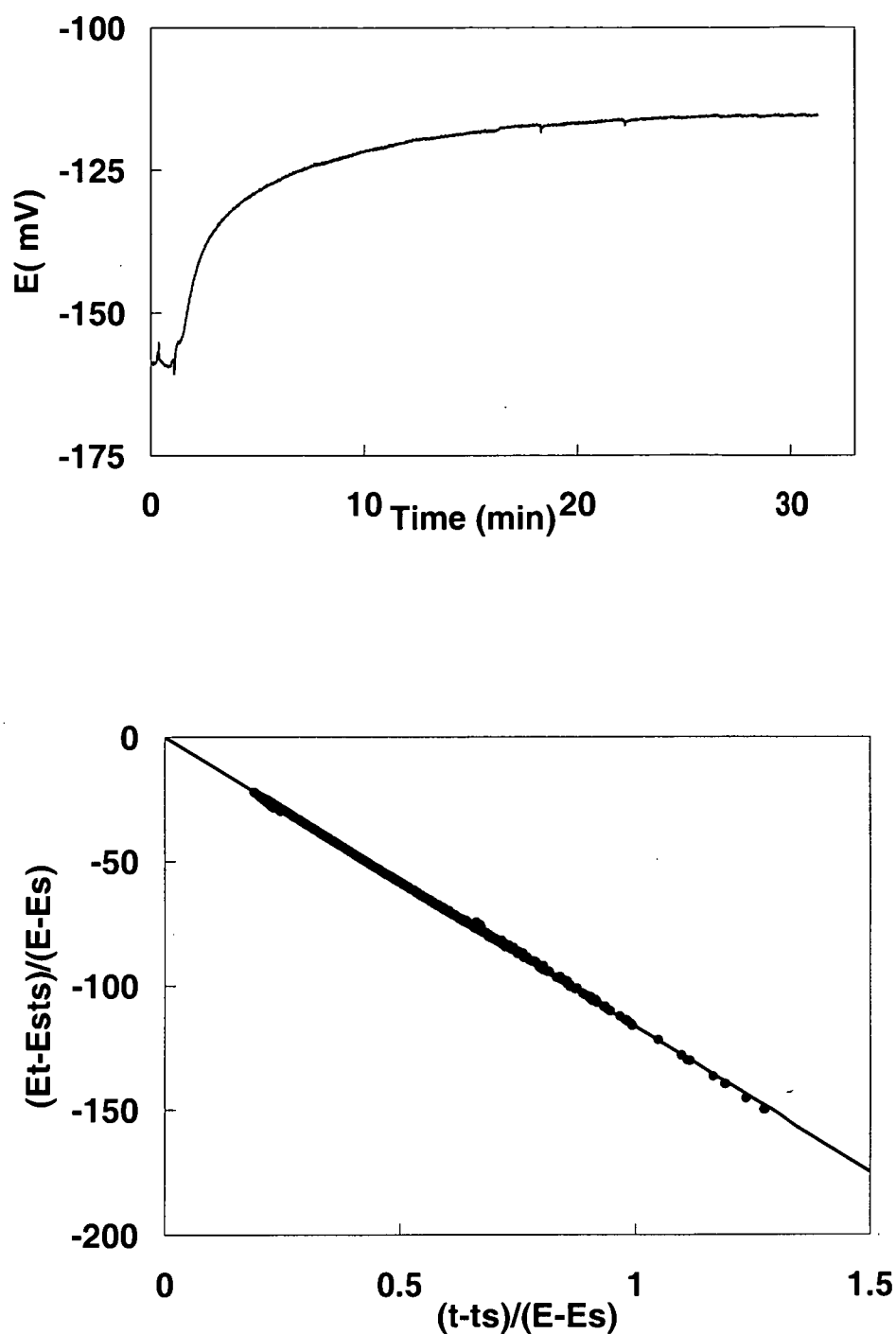


Figure 5.4 Response time transient for the CFA/Cu(II)ISE system for a stepwise change from a high pCu buffer to a seawater sample at natural pH. The linearized data were used for the determination of E_{eq} using equation 2, and an analysis time of $t = 8$ minutes. The pCu_{free} of the seawater sample was calculated to be 12.04.

Table 5.4 *Comparison of pCu_{free} measured by GFAAS and CFA/Cu(II)ISE measurements on acidified UV-oxidised seawater collected from 46°S, 167°E using clean protocols on cruise FR07/89 of R.V. Franklin. The CFA/Cu(II)ISE results have been corrected for complexation by chloride and sulfate using a side reaction coefficient of $\log \alpha = 0.8$.*

Sample	Depth (m)	pCu (CFA/Cu(II)ISE	pCu(GFAAS)
T85	1600	8.67	8.48
T88	1590	8.74	8.88
T99	300	8.55	8.74
T135	50	8.63	8.84

Table 5.5 *Comparison of predicted E_{eq} and the steady state value using Equation 2 (Buffle & Parthasarathy 1977) and Equation 4 (Müller 1969) expressed as ΔE , with examples of a) a small change in E (< 5 mV) and a large change in E (> 100 mV).*

Steady State (mV)	Equation 2 E_{eq} (mV)	ΔE (mV)	Equation 4 E_{eq} (mV)	ΔE (mV)
a) -19.6	-19.9	- 0.3	- 17.9	+ 1.7
b) -43.2	-43.3	- 0.1	-43.7	- 0.5

The extrapolation technique works equally well for samples where a small change in potential (less than 5 mV) or a large change between subsequent samples is observed (Table 5.5). The equation reported by Buffle and Parthasarathy (1977) gave superior results in terms of the difference between the predicted potential and the steady state value for real seawater samples. Replicate analyses of the same sample (Southern Ocean seawater collected under clean conditions) yielded a mean pCu_{free} of 12.22, with a standard deviation of 0.08 pCu units ($n = 12$). Thus a method for the rapid analysis of pCu is possible using the Cu(II)ISE, even at the low concentrations of copper found in marine waters.

CONCLUSIONS

The CFA instrument described here was designed to be suitable for field and shipboard analyses i.e. battery powered, simple design and lightweight construction, automated data capture and built from materials that will minimise the potential for sample contamination. The low reagent consumption, simple and reliable reference electrode and simple analysis procedures are also advantageous for operation in remote environments, as well as in the laboratory. Incorporation of the ISE into a CFA system has distinct advantages, overcoming several of the concerns encountered when using the CuISE in static measurements. Electrode dissolution is reduced in the flow system, sluggish response is addressed, cross contamination is eliminated, and the organic interference is reduced under flow conditions.

The CFA method has also addressed the problems encountered in the FIA studies, notably interaction between species in the carrier and sample slug, and the slow response rate at very low concentrations of Cu^{2+} .

Conditioning of the jalpaite membrane is essential for obtaining reproducible results and reducing the response time of the Cu(II)ISE by providing a stable membrane surface. Conditioning times are reduced significantly in CFA, from 24 hours to 3 hours, making routine analysis far more practicable. Analysis time can be reduced further by the application of an extrapolation technique for predicting the final equilibrium potential. The equation proposed by Buffle and Parthasarathy (1977) has been shown to be applicable to the Cu(II)ISE, and can accurately predict equilibrium potentials in copper ion buffers, in acidified UV-photooxidized seawater and in natural seawater samples. The approach used by Wang et al. (1995) for applying the extrapolation technique to CFA has also been successful. Increasing the flow rate in the CFA system will improve the response time of the Cu(II)ISE and further reduce any fouling of the membrane surface.

As for many analytical techniques, there is a trade-off between analysis time, consumption of reagents, sample size and accuracy of the measured value of pCu. Importantly, the CFA method allows for the analysis of copper in the nanomolar range, and is the first demonstration of the applicability of the extrapolation technique to these concentrations. The method is suitable for environmental monitoring, speciation studies and toxicity studies. Measurements may be made either in real-time in the field, or in the laboratory.

Addendum

The research in this paper was carried out by myself. Co-authors Denis Mackey, Roland De Marco and Peter Alexander played a supervisory role. These co-authors were my PhD research supervisors. Xue Dong Wang wrote the software for the post-

acquisition data analysis, and reviewed the manuscript. The staff of the CSIRO Marine Research workshop are thanked for building the perspex baseboard and stand for clean room analyses. The Australian Academy of Science provided a travel scholarship to enable me to conduct part of this research at Curtin University, WA.

Chapter 6 A case study of copper speciation and toxicity in Macquarie Harbour, Tasmania.

This chapter is a modified version of "Copper speciation and toxicity in Macquarie Harbour, Tasmania." Ruth S. Eriksen, Denis J. Mackey, Barbara Nowak and Rick van Dam, submitted to Marine Chemistry, May 2000.

ABSTRACT

Growth inhibition bioassays on the marine diatom *Nitzschia closterium* were used to determine the relationship between the reduction in growth rate and the concentration of free copper ion (pCu_{free}), as measured with a copper Ion Selective Electrode (ISE). At a salinity of 20, no toxicity was found for $pCu_{free} = 10^{-11.79}$ M while, for $pCu_{free} = 10^{-8.20}$ M, the growth rate was reduced to < 8 % of the control. Copper was found to be less toxic at $S = 30$ and the growth rate was reduced to 40 % of the control at $pCu_{free} = 10^{-8.50}$ M. These results are comparable to other estimates of the toxicity of copper to marine organisms. Measurements of pCu_{free} in Macquarie Harbour, a large semi-enclosed bay that is heavily polluted with copper, showed that pCu_{free} ranged from $10^{-11.37}$ M to $10^{-12.04}$ M even though total copper concentrations were as high as 25 $\mu\text{g/L}$. In line with these low concentrations of pCu_{free} , Macquarie Harbour waters ($S = 20$) were not found to be toxic to *Nitzschia closterium*. A reduction in growth rate was observed in some Macquarie Harbour waters at $S = 30$ but this was attributed to some factor other than copper toxicity. These results are in direct contrast with ASV measurements which predicted high toxicity for all water samples. More than 99.9% of the total copper was bound to ligands that were probably based on the high concentrations of humics entering the harbour and we propose that at least some of the copper was associated with colloids containing humic matter and iron and manganese (hydr)oxides. The CFA method developed for the analysis of free copper is assessed for environmental monitoring and toxicity studies.

INTRODUCTION:

It is widely accepted that the bioavailability or potential toxicity of copper is directly related to its physico-chemical speciation (Campbell 1995). The term bioavailability, as used here, refers to that fraction of the copper that is directly available to an organism and has been shown (Sunda and Guillard 1976) to be dependent on the concentration of the free copper ion (pCu_{free}). Florence and Stauber (1986) demonstrated that a number of lipophilic copper-organo complexes were more toxic to the marine diatom *Nitzschia closterium* than free copper alone. However, it is not clear that such complexes are significant in natural waters since, if they do occur, they are likely to be adsorbed on to particles and therefore not directly available to phytoplankton and there is no unambiguous evidence for the uptake of copper-organic complexes from natural waters or of copper-complexes used in laboratory culture experiments (Campbell 1995). In contrast, there is strong evidence that pCu_{free} determines copper toxicity and a relationship between pCu_{free} and growth inhibition in nearly 40 clones of marine phytoplankton has been established (Brand et al. 1986). If natural copper-organic complexes are not taken up by phytoplankton, then the biologically available fraction of copper is equivalent to either pCu_{free} or the total concentration of inorganic copper complexes, or any one of the inorganic copper complexes, since we cannot distinguish between these possibilities. In seawater at pH 8.1, the concentration of inorganic copper is equal to $10^{1.8} \times pCu_{free}$ regardless of the total concentration of copper (Sunda and Gillespie, 1979).

Analytical techniques for measuring or estimating pCu_{free} can be operationally defined e.g. ASV-labile copper, (Florence, 1989), complex and time consuming e.g. ligand competition, (Sunda and Hanson, 1987), difficult to extrapolate to the field e.g. computer modelling of synthetic solutions, (Turner and Whitfield, 1987), or some combination e.g. suite of speciation measurements plus modelling (Xue and Sunda, 1997). Direct measurement of pCu_{free} would facilitate the study of toxicity mechanisms and lead to a greater understanding of processes controlling speciation in natural waters

The Cu(II)ISE has been shown to respond to pCu_{free} in seawater (Belli & Zirino 1993; De Marco 1994; De Marco et al. 1997) and has been used to investigate copper speciation in a number of marine and estuarine locations (Zirino et al. 1998a; Zirino et al. 1998b). It has been shown that kinetic suppression of the chloride interference below 10^{-9} M copper enables the jalpaite ISE to be used to measure pCu_{free} in marine and estuarine samples (De Marco 1994). Good correlation between total copper determined by GFAAS and ISE proves that the ISE is sensitive enough

to measure copper in saline samples at environmentally relevant concentrations (De Marco et al. 1997; Eriksen et al. 1999). As for potentiometric measurements of pH, the ISE should be calibrated using external buffers. A method for the routine analysis of environmental samples has recently been developed (Eriksen et al. 1999) but the technique has not yet been used to determine copper toxicity in algal cultures, nor to relate laboratory studies to field measurements of toxicity.

In this study, toxicity tests were conducted with the benthic diatom *Nitzschia closterium* and the naked dinoflagellate *Dunaliella tertiolecta* using a 72-hour static exposure protocol. A combination of sterile and trace metal techniques were used to perform growth bioassays using surface ocean water as the growth medium. pCu_{free} in the culture solutions was measured with a copper ISE and we demonstrate that these measurements do indeed provide a measure of toxicity and that the results are in agreement with published data on the toxicity of copper to marine phytoplankton.

The ISE was used to measure pCu_{free} in samples collected from Macquarie Harbour which is a copper-contaminated estuary adjacent to a World Heritage Area on the west coast of Tasmania. Copper concentrations up to 500 $\mu\text{g/L}$ have been recorded entering the Harbour as a result of acid mine drainage, mill tailings, and smelter slag from the nearby mine (Koehnken, 1997). Our ISE results are compared with measurements of ASV-labile copper which had previously shown potentially toxic levels of copper, in contrast to bioassays on a range of marine species which indicated little or no toxicity (Stauber et al. 1996; Stauber et al. 2000).

EXPERIMENTAL

Study site

Macquarie Harbour is located on the west coast of Tasmania (Figure 6.1), an area noted for its combination of rich metal ore bodies and mining activities, and World Heritage Area classification. The Harbour has two major freshwater inputs, the King River at the northern end and the Gordon River at the southern end. The lower King River has carried waste from the Mt Lyell copper mine from 1893 to 1994 when the mine ceased operation. Between 1994 and 1995 the mine was closed with the result that there was a reduction in particulate load, at the expense of a dramatic increase in acidity and dissolved heavy metals in the King River (Koehnken 1997). The mine recommenced operations in 1995, with modified operating and environmental management procedures (Koehnken 1997). Macquarie Harbour has received more than 100 million tonnes of mine tailings and smelter slag, along with unknown quantities of acid mine drainage via the King and Queen Rivers (Featherstone & O'Grady 1997). Flow in the King River system is regulated by a hydroelectric power station approximately half a kilometre above the confluence of the King and Queen Rivers. The Gordon River drains a near-pristine catchment and is typical of many Tasmanian rivers with high concentrations of humic substances, low pH and low ionic loads (Carpenter et al. 1991). The environmental impact of the mine operations is clearly shown in Figures 6.2 to 6.5.

Tidal flushing is restricted by the narrow, shallow entrance at Macquarie Heads and the Harbour has a well-defined salinity gradient, with the $S = 20$ isohaline typically occurring at a depth of 5 - 7 metres (Koehnken 1996). Mid-depth waters ($S = 20$) were chosen for chemical and toxicity testing as they lie between the overlying freshwater layer and the more marine layer, and this salinity has previously been used for toxicity tests on flounder, algae and invertebrates (Stauber et al. 1996). Bottom waters ($S = 30$) have not previously been used for toxicity testing, typically contain less copper and would therefore be expected to be less toxic. At depths below the $S = 30$ isohaline, the water mass is fairly uniform with respect to salinity (Koehnken 1996).

Collection of water samples

Sampling was conducted over two days in July 1997 and two days in December 1997. Samples for toxicity testing and chemical analysis were collected in 1 L or 5 L LDPE bottles. New bottles were pre-soaked with a 0.1% Triton-X 100 (Sigma Chemical Co.) solution for one week, rinsed copiously with de-ionised water to remove any traces of surfactant, and then placed in a 10% v/v HCl bath for a further

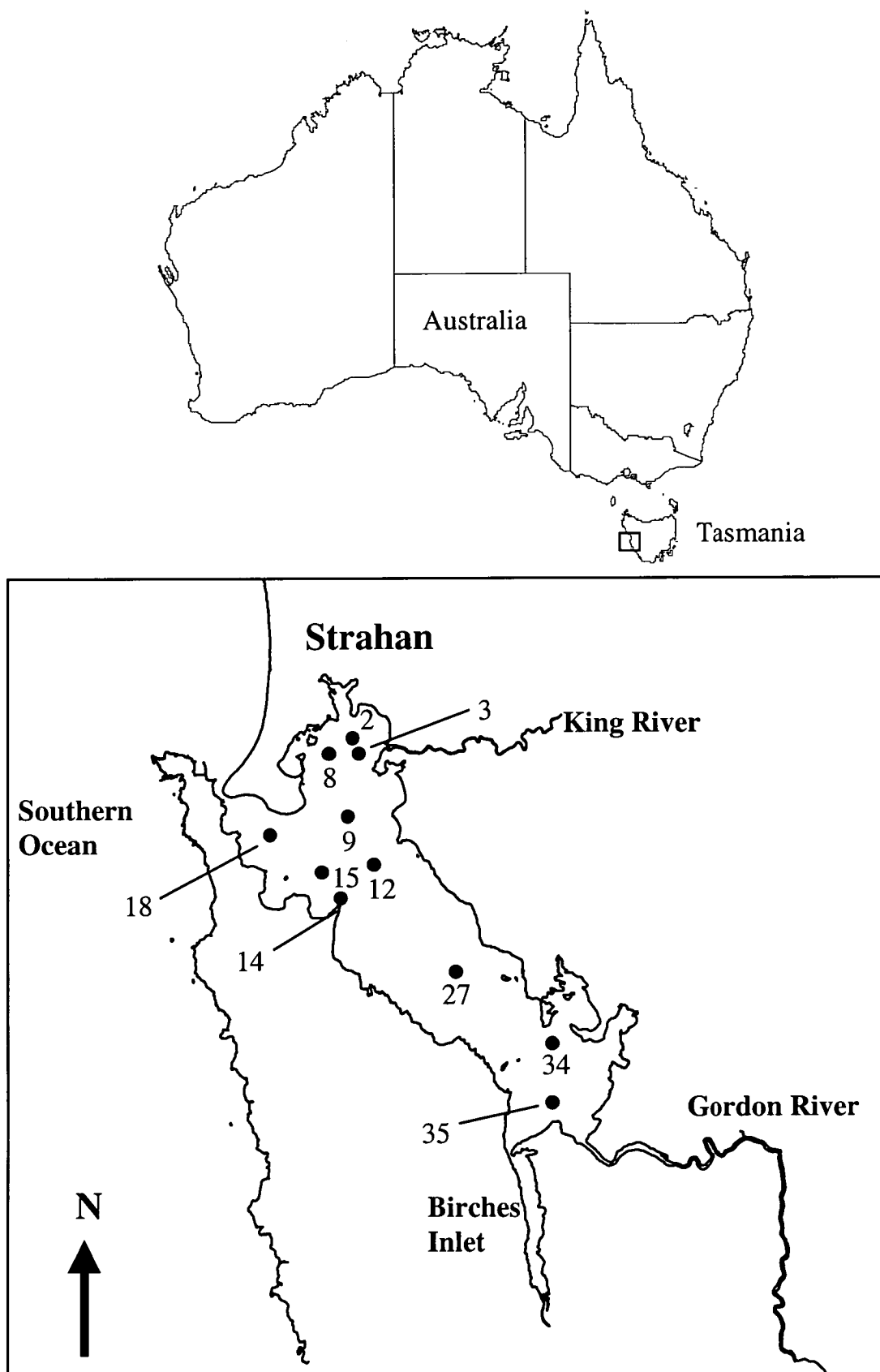


Figure 6.1 Map of Australia showing location of the study site. Inset, Macquarie Harbour sampling sites for chemical and toxicological tests.



Figure 6.2 *Confluence of the King and Queen Rivers, western Tasmania. Contaminated water from the Queen River is shown mixing with the clean, tannin-rich waters of the King River. Macquarie Harbour is approximately 12 kms downstream from this site. Photo courtesy Lois Koehnken.*



Figure 6.3 Lower King River carrying waste from the Queen River, during high flow conditions as a result of the operation of the hydroelectric power station. Photo courtesy Lois Koehnken.



Figure 6.4 Tailings banks along the lower King River, with forest die-back evident in the foreground. Healthy rain forest is visible in the background. Photo courtesy Lois Koehnken.



Figure 6.5 Aerial photo of the tailings delta at Macquarie Harbour, western Tasmania. A plume of contaminated water can be seen beyond the edge of the delta. The narrow entrance to the harbour, Hells Gates, can be seen in the upper right hand corner. Photo courtesy Lois Koehnken.

week. Sample bottles were then rinsed five times with deionised water, left to dry, capped tightly and bagged until required. All cleaning procedures were performed in a Class-100 clean room.

Salinity, temperature and pH profiles of the water column at each site were collected by deploying a Hydrolab Data Sonde 3 multiprobe from the boat and recording the data on a Hydrolab Surveyor 3 datalogger. This allowed us to determine sampling depths for subsequent hydrocasts using a polypropylene rope and a 5 L Niskin bottle. Niskin bottles were acid soaked with 5% HCl for one week, rinsed with deionised water, wrapped in plastic and kept bagged until the sample sites were reached. Samples were collected (inset Figure 6.1) from the King River which carries the mine waste (tailings, smelter slag, and acid mine drainage) and topsoil into the Harbour (Stations 2, 3, 8, 9, 12); along a transect towards the Gordon River at the southern end of the Harbour (Stations 27, 34, 35); near the fish farms (salmonid netpens) on the western shore (Stations 14, 15) and; near the entrance to the Harbour (Station 18), where the influence of clean coastal water is greatest. Sampling sites follow the Department of Primary Industry Water and Environment's (DPIWE) long term monitoring stations (Koehnken, 1996).

Total copper and ASV-labile copper

Samples were filtered (0.45 µm, Millipore HA), acidified to a pH of about 2 using high purity hydrochloric acid (1 mL/L) and left for at least a month before total dissolved copper (pCu_D) was measured by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), in accordance with the method described by Mackey (1983). Unfiltered samples were analysed for total copper by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) at the DPIWE laboratory. Some filtered samples were analysed by both GFAAS and ICP-MS and there was good agreement between the two techniques, despite different protocols for sample collection, preservation, filtration, storage time, analysis and reporting limits (Regression $y = 0.9579x - 0.702$, $R^2 = 0.858$). A limited number of ASV-labile copper measurements were made in accordance with the method described by Stauber (1996) using a Metrohm 646VA Processor with hanging mercury drop. Samples were purged with nitrogen for 300 seconds, then a new mercury drop was formed and deposition carried out for 300 seconds at -0.6 V (vs. Ag/AgCl). After 20 seconds without stirring, a potential scan was initiated (scan rate 3.3 mV/sec, pulse height 50 mV, pulse step 2 mV) and the copper oxidation wave recorded between -0.2 and 0.2 V. All samples analyses and manipulations were conducted in a Class-100 clean room to prevent contamination. These results represent free copper, plus any labile or colloidal complexes that dissociate or are reduced at the electrode.

Free copper

ISE measurements were made on filtered samples (0.45 μm , Millipore HA) at natural pH in accordance with the protocols described in Eriksen et al. (1999) and Chapter 5. An Orion 94-29 Cu (II)ISE was incorporated into a continuous flow analyser (CFA), in order to eliminate the contamination of samples by the electrode. The electrode was calibrated using copper/ethylenediamine buffers as described in Chapter 5. Calculation of pCu_{free} for each buffer was also as previously described. The buffers were made up to have approximately the same ionic strength as seawater (0.6 M NaCl) and so the ISE could be calibrated in terms of concentration. An extrapolation technique was used to process the CFA data, so as to reduce the time required for sample analysis (Eriksen et al. 1999). Samples were stored at 4 °C in the dark and analysed as soon as practicable after collection to minimise the effect of speciation changes. Since the response of the ISE is logarithmic, the results are expressed in pCu_{free} units and it should be noted that this can disguise significant changes in pCu_{free} .

The electrodes were regularly polished with 0.3 and 0.05 μm Al_2O_3 to ensure a reproducible membrane surface that had a rapid response. The electrode was conditioned with clean filtered seawater until a stable potential was recorded (stability criterion 0.2 mV/min) and the system was calibrated with a series of pCu buffers ($9 < \text{pCu}_{\text{free}} < 14$) before each set of samples was analysed. All calibration slopes were in the range 27.7 - 29.4 mV/decade. Two ISEs were used for analyses during this study and there were no significant differences found in either the slopes or measured values of pCu_{free} . The pCu_{free} values in this paper are reported to 2 decimal places since we can readily detect changes of less than 0.1 units even though the absolute values of pCu_{free} are probably accurate to no better than 0.2 units.

Nutrients, pH, salinity and temperature

Nitrate + nitrite and orthophosphate were measured using an Alpkem AutoAnalyser, using standard colourimetric methodologies (Eriksen 1997). Filtered samples were frozen until analysis. Laboratory measurements of pH were made using an Orion precision pH meter and Orion/Ross pH electrode. Salinity and temperature were measured in the laboratory using a WTW Microprocessor Conductivity Meter, Model LF196.

Algal culture and bioassay protocols

The unicellular marine diatom *Nitzschia closterium* (Ehrenberg) W. Smith (Strain CS 5) and the marine dinoflagellate *Dunaliella tertiolecta* (Strain CS-175) were

obtained from the CSIRO Marine Research microalgal culture collection. The cultures were maintained axenically using a modified f-media at 20 °C, on a 12-h light : dark cycle using fluorescent daylight bulbs (Stauber & Florence 1989). Cultures were transferred weekly so that the cells in the exponential growth phase were continually available.

Five day old *Nitzschia closterium* cells were inoculated axenically into flasks containing 100 mL of filtered (0.22 µm, Millipore Millipak 40) seawater collected from Maria Island, Tasmania. Dilution waters at S = 20 and 30 were prepared by diluting seawater with Milli-Q water. The flasks were coated with Coatasil (Ajax) to minimise adsorption of added copper, acid washed, and rinsed copiously with Milli-Q water prior to use in bioassays. The algal cells were washed to remove all traces of the culture medium, as this has been shown to affect the degree of copper toxicity (Stauber & Florence 1989). Flasks were spiked with 1.0 mL of 1.3 mM NaH₂PO₄ and 1.0 mL of 26 mM NaNO₃, to give final nutrient concentrations of 130 µM phosphate and 255 µM nitrate. They were then spiked with 0, 2.5, 5.0, 10.0, 20.0, 40.0, or 80.0 µg/L of copper, with two or three replicates at each test concentration. The total copper in the flasks was corrected for the initial concentration of copper in the Maria Island seawater. Initial concentrations ranged from 0.092 µg/L to 0.109 µg/L. Flasks were mixed thoroughly and placed in culture cabinets at an initial cell density of 2 - 5 x 10⁵ cells/mL.

Samples for bioassay studies were collected in July 1997 from the S = 20 isohaline at Stations 3, 8, 9, 12, 14, 15, 27, 34 and 35. Nutrients were determined in the samples, and an adjustment made to the amount of nutrient stock added so that the final nutrient concentrations were the same as in the control (J. Stauber, pers. comm.). Culture conditions were identical to those described for the ionic copper addition experiments. Temperature, pH and light irradiation were monitored throughout the tests as these parameters may have a significant impact on toxicity (Mayer et al. 1998). Salinity was checked for each sample in the lab, and the small variations (Table 6.1; 19.7 < S < 21.1) were due to difficulties in sampling the very sharp salinity gradients often found in the Harbour. In order not to change the chemistry of the samples, salinity was not adjusted to bring the samples to exactly 20, since Stauber et al.(1996) found no effect of salinity on the toxicity of ionic copper to *Nitzschia* for 15 < S < 20. Bioassays were also conducted on S = 30 samples from Stations 3, 9, 12, 15, 27 and 34. The concentration of pCu_{free} in each of the copper spiked flasks and the Macquarie Harbour samples was measured according to the method described in Eriksen et al.(1999). In both bioassays, the pCu_{free} values tested ranged over about 3.5 orders of magnitude (11.89 - 8.50 for S = 30 and 11.79 - 8.20 for S = 20).

Since different algal species can vary greatly in their sensitivity to toxicants, a growth inhibition bioassay was performed comparing growth of *Nitzschia closterium* and the dinoflagellate *Dunaliella tertiolecta*. Additional water samples ($S = 30$) were collected from Station 3 (close to the King River) and Station 34 (distant from the King River) in December 1997. In the July tests, Station 3 waters ($S = 30$) resulted in significant growth inhibition in *Nitzschia*, while Station 34 caused no toxicity (Dunnett's test, $\alpha = 0.05$). The protocol for tests using *Dunaliella tertiolecta* was identical to that described for *Nitzschia*. Both species are shown in Figure 6.6

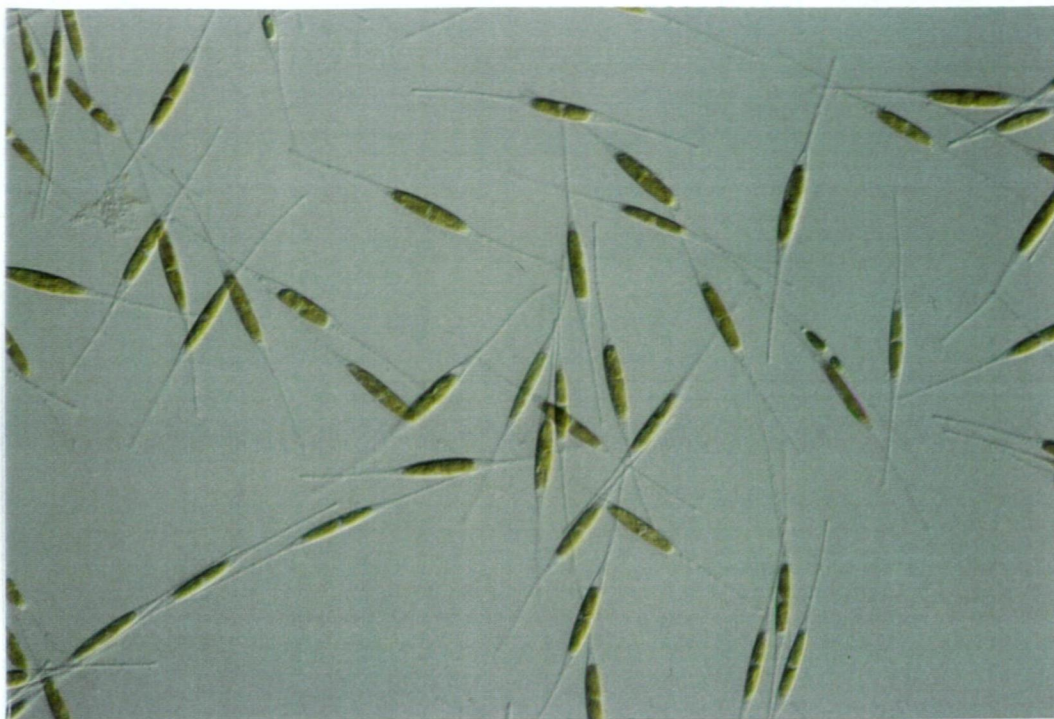
Cell counts

Cells were counted on an Improved Neubauer Hemacytometer, with a Leitz Wetzlar compound microscope, at $100\times$ magnification. One mL of sample was removed axenically from each flask on day 1, 2 and 3 of the test. Cell densities on day 0 were assumed to be identical in all flasks. Flasks treated with Coatasil were found to be coated with *Nitzschia* cells, which were difficult to remove from the bottom of the silanised flasks. Gentle stirring with a Teflon coated magnetic stirrer lifted the algae from the bottom of the flask and stirred the contents of the flask at the same time. Replicate counts (4 to 6) were made on all flasks. At the conclusion of the test, growth rates in all flasks were calculated by plotting cell density against time (hours), and determining the slope of the line for each flask by regression analysis. The slope of the line is equivalent to the growth rate per hour (μ) for each treatment. Daily doubling times are then calculated from $24 \times \mu \times 3.32$. Acceptable control growth rates vary with salinity (Eriksen et al. submitted). Population growth in each treatment was then expressed as a percentage of the controls to determine the relationship between speciation and toxicity.

Statistical analyses

Data was tested for homogeneity of variance using Cochran's test. Dunnett's test was used to determine which treatments were significantly different ($\alpha = 0.05$) from the controls (Snedecor & Cochran 1967).

a)



b)

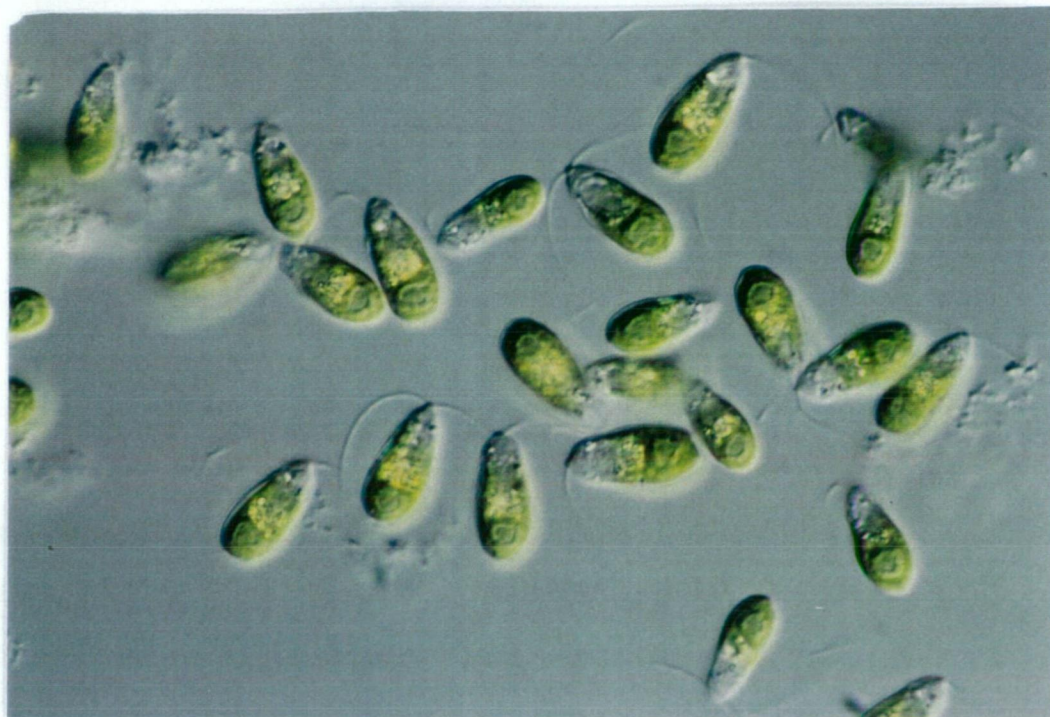


Figure 6.6 Algal species used for copper bioassays in Macquarie Harbour; a) *Nitzschia closterium* clone CS-5, x 40 magnification and b) *Dunaliella tertiolecta* clone CS-175, x 100 magnification. Photos courtesy Tony Rees.

RESULTS

Growth inhibition bioassays with ionic copper

As expected, the pCu_{free} was highest (i.e. lowest free copper) in the controls ($pCu_{free} = 11.79$), and decreased as the concentration of total copper increased in the $S = 20$ *Nitzschia* cultures. There was a strong correlation ($R^2 = 0.943$, $p < 0.01$) between the decrease in pCu_{free} (i.e. increase in pCu_{free}) and the decrease in the growth rate of *Nitzschia*, under the test conditions (Figure 6.7). Growth rates decreased from 100 % (controls, 0.1 $\mu\text{g/L}$ copper) to less than 8 % in the highest copper treatment (80.1 $\mu\text{g/L}$ copper). The ISE tracked the increase in pCu_{free} in solution and its effect on the algae over almost 4 orders of magnitude. Copper reacts at the cell membrane, affecting cell permeability and division (Stauber & Florence 1987), and a high incidence of swollen cells or incomplete division was observed in the higher copper concentrations from day 1.

The growth inhibition bioassay was repeated at $S = 30$, and a similar trend was observed to that found at salinity 20 (Figure 6.8). As the concentration of pCu_{free} , measured by ISE, increased there was a strong correlation with the decrease in the growth rate of the algae ($R^2 = 0.9073$, $p < 0.01$). The toxicity of copper to *Nitzschia* was less at the higher salinity with 80.1 $\mu\text{g/L}$ copper reducing growth rates of *Nitzschia* to 40 % of the controls, compared with less than 8 % in the $S = 20$ experiment.

No published data could be found for growth rates of *Nitzschia closterium* at $S = 30$, under the test conditions used here. However, data for growth rates at several other salinities (ranging between 15 and 35) was available (Stauber et al. 1994; Stauber et al. 1996) and growth at $S = 30$ was predicted from a regression of doublings/day against salinity (regression $y = 0.0253x + 0.506$, $R^2 = 0.9017$, $p < 0.02$) shown in Figure 6.9. The predicted rate of 1.27 doublings/day was in excellent agreement with our observed rate of 1.29 doublings/day confirming that there were no toxic effects in the control samples. Uncertainty for daily doubling rates for *Nitzschia closterium* is in the order of ± 0.3 doublings/day (Stauber, pers. comm.).

Growth inhibition bioassays on Macquarie Harbour samples

The bioassays revealed no significant difference between the growth of *Nitzschia closterium* in (salinity adjusted) Maria Island controls and Macquarie Harbour samples at $S = 20$. Growth rates of *Nitzschia* varied between 87 % and 107 % of the controls (Table 6.1), though neither the growth enhancement nor inhibition were significant using Dunnett's test ($\alpha = 0.05$). Within experimental accuracy, Macquarie Harbour waters at $S = 20$ were not toxic to *Nitzschia*.

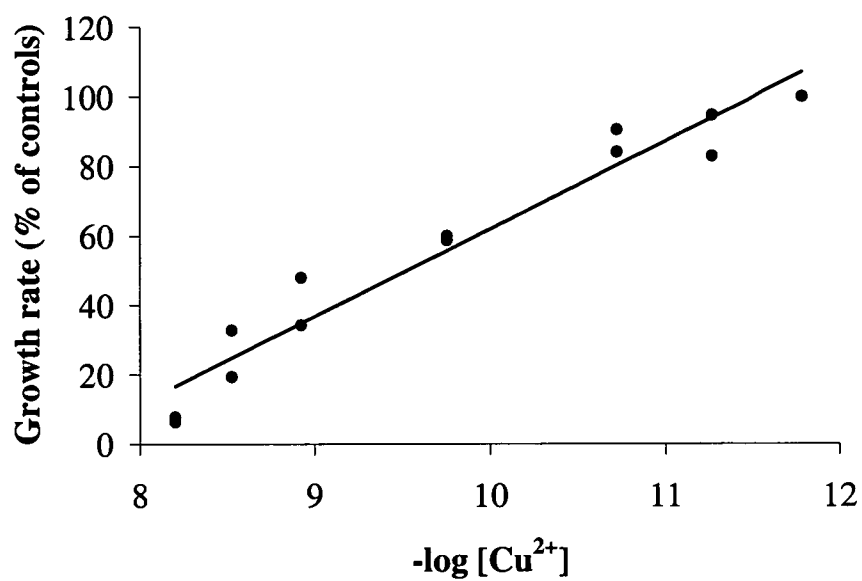


Figure 6.7 Effect of free copper, measured by ISE, on growth of *Nitzschia closterium* in spiked seawater at salinity 20. Regression $y = 25.22x - 190.25$, $R^2 = 0.943$, $p < 0.01$.

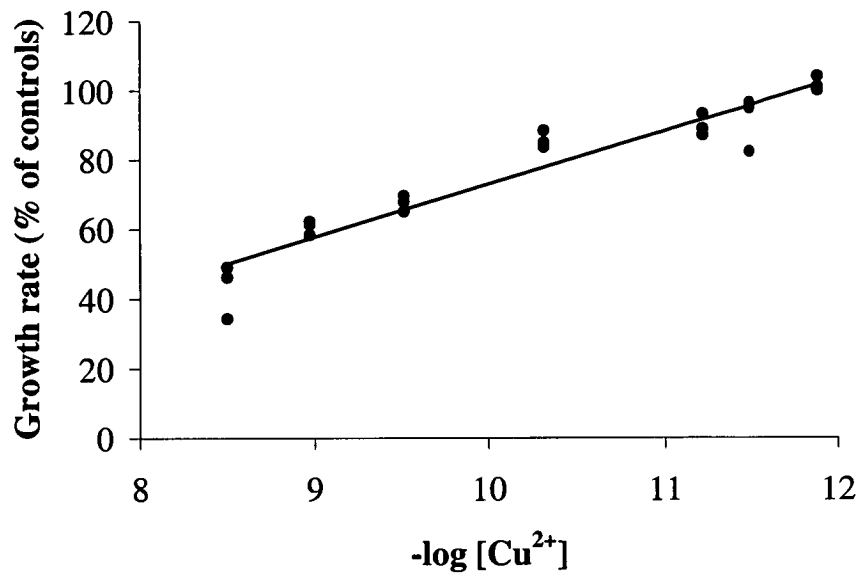


Figure 6.8 Effect of free copper, measured by ISE, on growth of *Nitzschia closterium* in spiked seawater at salinity 30. Regression $y = 15.23x - 79.34$, $R^2 = 0.9073$, $p, 0.01$.

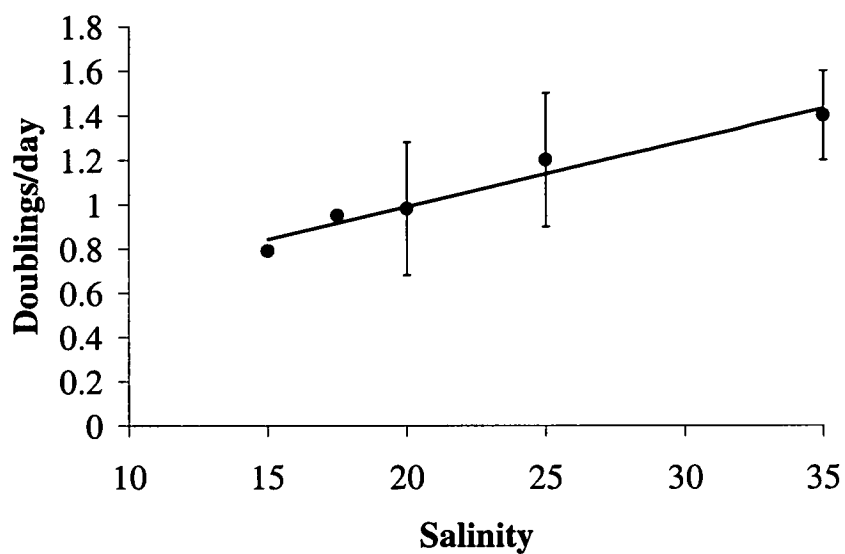


Figure 6.9 Variation in doubling rate per day of *Nitzschia closterium* with salinity, after Stauber (1994; 1996). Error bars included where data provided.

Table 6.1 *Physical and chemical data from Macquarie Harbour on July 10-11, 1997. Total filtered copper was determined by GFAAS, total unfiltered copper was determined by ICP-AES, and pCu_{free} was measured using an Orion Ion Selective Electrode. Toxicological results refer to the Nitzschia closterium algal bioassay, B denotes growth significantly different from controls (Dunnett's test α , = 0.05). No salinity 30 isohaline was present at Station 14, 18 and 35)*

Station	Depth (m)	Salinity ^A	Temp (°C)	pH	Total Cu unfiltered (µg/L)	Total Cu filtered (µg/L)	ASV Cu (µg/L)	pCu _{free}	% growth inhibition
3	5	20.2	10.7	7.4	19	17.1	11.5	11.37	87
	16	30.2	13.4	7.5	13	6.5		11.72	80 ^B
8	4	20.2	11.4	7.5		13.4		11.58	95
	15	31.4	13.3	7.6		4.8		11.90	
9	5	20.8	10.8	7.4	23	14.2	10.7	11.52	97
	32	30.9	13.6	7.6	6	4.2		11.91	80 ^B
12	5	21.1	10.5	7.3	16	13.5	14.9	11.70	101
	35	30.9	13.7	7.6	7	3.9		12.02	82 ^B
14	5	20.6	11	7.5	19	14.9	10.8	11.37	104
	10	27	12.7	7.5	13	7.7		11.65	
15	5	21.1	11.0	7.3	19	13.5	12.5	11.57	97
	32	30.2	13.7	7.6	7	4.1		11.94	81 ^B
18	4	20.9	10.8	7.5	19	12.2		11.75	107
27	6	19.7	10.5	7.4	23	14.6		11.41	93
	34	30.8	14.0	7.4	11	3.3		12.04	88
34	5	20.5	10.5	7.2	17	13.4	11.3	11.67	98
	21	30.8	14.0	7.3	8			11.81	86
35	6	20.9	11.5	7.2	7	10.6		11.97	97

Table 6.2 *Physical and chemical data from Macquarie Harbour on December 10-11, 1997. All analyses of total unfiltered copper and total filtered copper were determined by ICP-AES, and pCu_{free} was measured using an Orion Ion Selective Electrode. Toxicological results refer to the Nitzschia closterium algal bioassay.*

Station	Depth (m)	Salinity	Temp (°C)	pH	Total Cu unfiltered (µg/L)	Total Cu filtered (µg/L)	ASV Cu (µg/L)	pCu _{free}	% growth inhibition
2	6	20.6	14.3	7.1	25	17		11.29	
	20.2	30.4	13.2	7.4	10	5			
3	6	16.6	14.5	6.8	25	19		11.27	
	16	29.4	13.2	7.4	13	9		11.67	103
14	6	21.7	14.4	7.0	22	15		11.58	
	8.1	27.5	13.2	7.3	15	12		11.49	
18	2	18.9	16.5	7.1	19	13			
	5	33.8	13.6	7.7	4	3			
34	7.8	20.2	13.9	7.2	18	15		11.50	
	20	30.4	13.2	7.1	7	5		11.80	91

In contrast to the $S = 20$ samples, the bioassays for the six $S = 30$ samples revealed a significant difference between the growth rate of *Nitzschia* in the controls and the growth in four of the samples (Dunnett's test, $\alpha = 0.05$). A very reproducible reduction in growth rate (80 % to 82 % of controls; CV 4.9 %) was found for samples from stations 3, 9, 12 and 15 (Table 6.1). No significant toxicity was found in $S = 30$ water from stations 27 or 34, in the southern end of the harbour. The bioassays were repeated on $S = 30$ samples collected in December 1997 from Stations 3 (toxic response in July) and 34 (no toxic response in July), this time using both *Nitzschia* and *Dunaliella*. In contrast to the bioassays from the same locations in July, where reduced growth rate and normal growth rate were found respectively, no inhibition of growth was found for either *Nitzschia* or *Dunaliella* (Table 6.3).

Free and total copper in Macquarie Harbour

As expected, the highest concentrations of total copper (25 $\mu\text{g/L}$), dissolved copper (19 $\mu\text{g/L}$) and free copper ($\text{pCu}_{\text{free}} = 11.27$) in Macquarie Harbour were all found at the mouth of the King River (Table 6.1), while the lowest concentrations of dissolved copper (3.3 $\mu\text{g/L}$) and free copper ($\text{pCu}_{\text{free}} = 12.04$) were found in the marine dominated, high salinity waters in the middle of the Harbour (Table 6.2). The lowest concentration of total metal (4 $\mu\text{g/L}$) was found in high salinity waters near the mouth of the Harbour in the (austral) summer. Dissolved copper and free copper both ranged over a factor of almost 6 ($19/3.3$ and $10^{12.04 - 11.27}$ respectively). In general, the concentrations of all three forms of copper, at a given location, decreased with increasing salinity. The only exception was for pCu_{free} at Station 14 in December. pCu_{free} was also unusually high ($\text{pCu}_{\text{free}} = 11.37$) for Station 14 ($S = 20$) waters in July and it is interesting to note that the fish farms are located at this site, and that the salmon are fed a copper enriched pelletised feed (Koehnken, pers. comm.). The overall concentrations of all species did not differ greatly between the July and December samplings (Table 6.1 and 6.2). It should also be noted that the correspondence between pCu_{free} and ASV-labile copper is weak ($R^2 = 0.3875$). Excellent agreement between ISE and ASV has previously been reported for open ocean samples (Zirino & Seligman 1981) however conversion from potentials to pCu_{free} was not made.

In Figure 6.10, we have plotted pCu_{free} against pCu_D ($-\log_{10} [\text{Cu}]$ dissolved) and it is clear that there is no seasonal change in the relationship between the two species of copper. However, there is a marked difference in the relationships at $S = 20$ and $S = 30$ and the slopes for the corresponding regression lines are 2.71 ($R^2 = 0.8606$) and 0.963 ($R^2 = 0.9581$) respectively.

Table 6.3 *Comparison of toxicity of selected Macquarie harbour samples to Nitzschia closterium (July, December data) and Dunaliella tertiolecta (December data only) at Stations 3 and 34. A - Growth was not significantly different from the controls (Dunnett's test, $\alpha = 0.05$). B – Growth was significantly different from the controls (Dunnett's test, $\alpha = 0.05$)*

Station	Total Cu unfiltered ($\mu\text{g/L}$)	pCu _{free}	% growth inhibition <i>N. closterium</i>	% growth inhibition <i>D. tertiolecta</i>
<i>July</i>				
3	13	11.72	80 ^B	-
34	8	11.81	86 ^A	-
<i>December</i>				
3	13	11.67	103 ^A	96 ^A
34	7	11.80	91 ^A	108 ^A

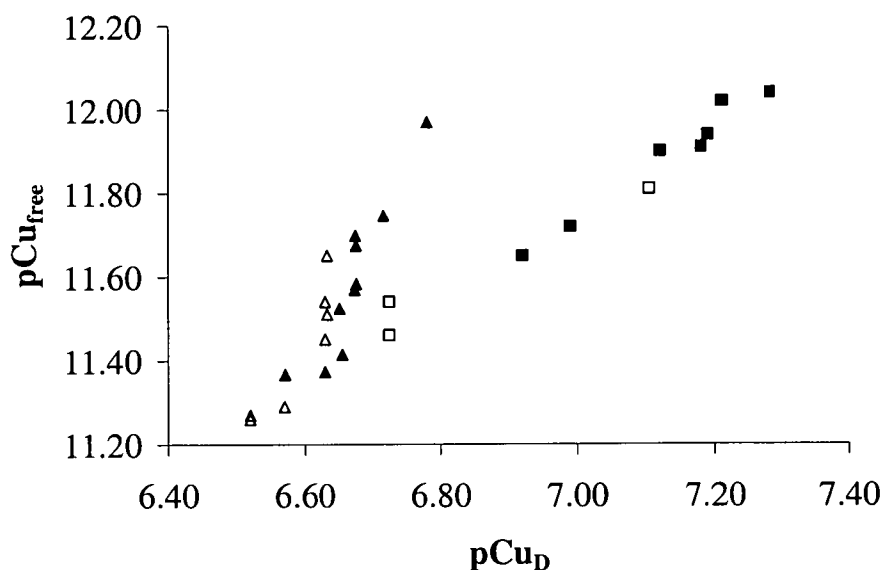


Figure 6.10 Relationship between free copper (pCu_{free}) measured by Cu(II)ISE and total dissolved copper (pCu_D) measured by GFAAS in Macquarie Harbour for salinity 20 and 30 waters. Triangles salinity 20 data, squares salinity 30 data. Open symbols December, closed symbols July. Regression for all salinity 20 data $y = 2.713x - 6.649$, $R^2 = 0.8606$, $p < 0.01$. Regression for all salinity 30 data $y = 0.963x - 5.016$, $R^2 = 0.9581$, $p < 0.01$.

DISCUSSION

Growth inhibition bioassays and ISE measurements

A number of assumptions are made when conducting laboratory toxicity tests and then attempting to extrapolate the results to the field. The copper addition experiments assume: that the ionic metal spikes equilibrate rapidly with the natural pool of metal species (Florence et al, 1992a); that the toxicity of the samples is due to copper alone and; that there are no synergistic or antagonistic effects contributed by other heavy metals or potential toxicants. In Macquarie Harbour, other heavy metals, apart from copper, are present in concentrations far in excess of natural background levels (Carpenter et al.1991, Stauber et al.1996) and could equally well be a contributing factor in determining toxicity. While algal growth has been shown to be a sensitive indicator of toxicity, conditions in the laboratory are not a perfect replication of environmental conditions since cell density is much higher than in naturally occurring populations, and this has been shown to affect inhibition of algal growth rates (Florence & Stauber 1986). In addition, the test uses a single species, whereas multiple species occur in the wild (Stauber 1995). Nevertheless, the bioassay provides a very useful and fundamental tool to investigate the relationship between speciation and toxicity in environmental samples. Algae are primary producers, and as such form the basis of many food chains. Any adverse impact on algae may therefore directly or indirectly affect organisms from higher trophic levels (Stauber 1995). Certain species have been shown to be very sensitive to copper and standardised bioassays have been developed for these species.

ISEs have tended to be overlooked for metal speciation studies, as they have been reported to be too insensitive to measure concentrations present in marine waters or have been subject to severe interference effects which rendered the electrode unusable (Morel & Morel-Laurens 1983; Florence et al. 1992a; Mackey & De Marco 1997). However, an Orion Cu(II)ISE has been used to measure the reduction in fluorescence intensity in a culture of *Dunaliella* sp. as pCu_{free} was decreased from 11.5 to 9.3 (Castro Peñuelas 1996). Most speciation studies use ASV, ion-exchange, solvent extraction, dialysis, ultrafiltration, gel permeation chromatography, computer modelling, or a combination of these techniques to determine the toxic fraction of metal. Each method has its limitations. ASV, probably the most widely used of these techniques, measures the labile fraction of the total dissolved metal that can be deposited at the electrode under carefully defined conditions (Florence et al. 1992a). The underlying assumption is that the toxic fraction of the dissolved metal is similar to, or equal to, the labile metal fraction. In reality, ASV cannot distinguish between free metal ions and labile complexes or colloidal particles that dissociate or are

directly reduced at the electrode surface (Sunda & Hanson 1987; Florence 1989). Reduction of copper-complexes can be substantial, particularly if large overpotentials are used in the deposition step (Mackey & Zirino 1994; Croot et al. 1999). ISE potentiometry is the only analytical technique that can directly measure pCu_{free} without manipulating the sample matrix or making assumptions about the thermodynamic behaviour of the metal species. We do not necessarily know all the relevant thermodynamic stability constants or how a given species will react under the experimental conditions, nor can we always be confident that the system is in thermodynamic equilibrium.

In this study, the concentration of pCu_{free} measured in clean seawater controls ($pCu_{free} = 11.79 - 11.89$) is comparable to other studies of pCu_{free} in estuarine and coastal marine waters. Using bioassays, Sunda and Ferguson (1983) estimated ambient pCu_{free} to be 11.5 in water from the coast of Peru, and 11.3 and 11.5 for samples from the Northern Gulf of Mexico. Zirino et al. (1998a), using an Orion 94-29 Cu(II)ISE, reported pCu_{free} values of 11.6 for waters outside the mouth of San Diego Bay.

Using a NTA-cupric ion buffer system, Brand et al. (1986) found reduced rates of reproduction in seven species of marine diatoms at $pCu_{free} = 10.5 - 11.0$, with an EC_{50} of $pCu_{free} = 10$. They also found reduced rates of reproduction in two species of dinoflagellates (*Prorocentrum* sp. and *Peridinium* sp.) at $pCu_{free} = 12$. Anderson and Morel (1978) also used artificial organic ligands to control the free ion activity in solution and found that for $pCu_{free} < 12$ there was a significant effect on the motility of *Gonyaulax tamarensis*. Cultures of *Thalassiosira pseudonana* were inhibited at $pCu_{free} < 10.5$ when Tris was used to buffer both pH and pCu_{free} (Sunda & Guillard 1976) and this was corroborated by Castro-Peñuelas (1996). Gavis et al. (1981) showed that there was a wide variability in the response of phytoplankters to pCu_{free} , both among different species and among clones within a single species.

Our results demonstrate a very strong correlation between the concentration of pCu_{free} measured by ISE and the reduction of algal growth at both salinities tested (Figures 6.7 and 6.8). On the basis of the work reported above, we find no copper toxicity at $pCu_{free} = 12$ and substantial toxicity at $pCu_{free} = 8$. We are therefore confident that the Cu(II)ISE is indeed measuring pCu_{free} in the cultures and providing a direct indication of copper toxicity. Our results also indicate that *Nitzschia* is less stressed by ionic copper additions at $S = 30$ than at $S = 20$ in agreement with Florence (1989) but in contrast to Stauber et al. (1994) who reported that *Nitzschia* is more sensitive to copper at $S = 35$ than at $S = 20$. However, as discussed later, other factors may also be important.

The dose-response model used here is a simple linear regression of growth rate as a function of the measured concentration of free copper by ISE. It is common for dose-response data to be plotted showing a characteristic sigmoidal shape, usually showing a region of marginal (or unnoticeable) inhibition followed by a clearly linear region (Peleg et al., 1997). If the range of concentrations tested is sufficiently wide, a region of zero growth will also be noted at very high concentrations. The test concentrations used here appear to just bracket the range in which a linear response occurs, hence the fitting of a straight line to the data to describe the change in growth rate with cupric ion concentration.

Growth inhibition bioassays on Macquarie Harbour samples

The growth inhibition bioassays conducted on water collected from the S = 20 isohaline revealed no toxicity to the algae with growth rates varying from 87 % to 107% of the controls. Although there was a good correlation between inhibition of growth and our ASV-labile copper measurements ($R^2 = 0.8248$, $p < 0.01$) the ASV measurements predicted growth inhibition of between 42 and 48% of salinity matched controls (S = 20). Our measurements of ASV-labile copper significantly overestimated the toxicity of the samples. This is in agreement with the earlier study by Stauber et al. (1996), where none of the sites sampled at S = 18 proved to be toxic and growth rates varied from 88 % to 110 % of the controls although the waters contained 14 - 24 $\mu\text{g/L}$ ASV-labile copper (26 - 42 $\mu\text{g/L}$ dissolved copper). This was despite evidence that in clean copper-spiked seawater, significant growth inhibition in *Nitzschia* occurred at total copper concentrations as low as 5 $\mu\text{g/L}$. Our ASV measurements on Macquarie Harbour samples also showed no correspondence between reduction in growth rate of *Nitzschia* and ASV-labile copper even though this would be expected at concentrations of 10.7 to 14.9 $\mu\text{g/L}$ copper (Table 6.1). It is clear that both our ASV measurements and those of Stauber et al. (1996) overestimate the availability of copper, presumably by determining species such as weakly bound organic complexes that can reduce the free copper concentration but cannot be directly taken up by phytoplankton. Recently Croot et al. (1999) showed that even moderately strong copper complexes are electroactive at the reducing potential commonly used in speciation studies (-0.4 – 0.75 V vs. Ag/AgCl), causing significant overestimation of bioavailable species.

Using the relationship between pCu_{free} and growth of *Nitzschia* established in the bioassays (Figures 6.7 and 6.8), the predicted inhibition of algal growth was calculated using the free copper concentration at each site for which toxicity tests were conducted. For S = 20, the range of predicted growth rates in Macquarie Harbour samples was very narrow (96 – 111 %), with no statistically significant difference between the predicted growth rates and the observed growth rates.

Significantly, the ISE results showed that pCu_{free} concentrations in Macquarie Harbour were not high enough to pose a threat to the algae.

For $S = 30$, the predicted values varied from 99 % to 104 % in contrast to the observed results where *Nitzschia* growth was reduced to 80 - 82% of the controls at four sites (Stations 3, 9, 12 and 15) and was 86 % and 88 % at the other two sites. However, no reduction in growth rate was observed when the bioassay was repeated for a sample collected from Station 3 in December 1997 even though the concentrations of total copper, dissolved copper and, most significantly, pCu_{free} , were essentially the same as those found in July 1997 (Table 6.3). Since the measurements of pCu_{free} are completely independent of the nature of any other species of copper present in the sample (organic, inorganic, colloidal, particulate, labile or inert), the most obvious explanation for the result is that there is some antagonistic or synergistic interaction between copper and some other chemical element or compound present in the water or that the toxicity is due to some other chemical element or compound.

Copper speciation in Macquarie Harbour

The pCu_{free} values in Macquarie Harbour are higher than the pCu_D values by about 4.7 - 5.2 units (Figure 6.10). If the copper were complexed only by inorganic ligands, this difference would be 1.8 since the ratio of inorganically complexed copper (largely carbonate and hydroxide species) to pCu_{free} is $10^{1.8}$ for seawater at pH 8.1 (Zirino & Yamamoto 1972; Sunda & Gillespie 1979). The free copper concentrations in Macquarie Harbour are lower than predicted by inorganic complexation by a factor of more than 10^3 and therefore > 99.9% of the copper must be complexed by other ligands in line with the high concentrations of humic matter entering the Harbour from the King and Gordon Rivers. This is true regardless of the total concentration of copper, confirming that the ligands are present in excess of the amounts required to bind copper. We believe that some of the complexed copper may exist as colloidal matter (see later) but, for the purpose of this discussion we will not distinguish between colloidal matter and dissolved, organically complexed copper and we will just use the term 'ligands'.

If the ligands (L) are present in large excess and, if there is no change in the effective stability constant (K_{eff}) for the formation of copper complexes, then the ratio of pCu_D to pCu_{free} should be equal to the product of the effective stability constant and the excess ligand concentration ($pCu_D/pCu_{free} = K_{eff}[L] \gg 1$) and a plot of pCu_{free} against pCu_D should give a straight line with unit slope while the intercept gives the value of $\log_{10} K_{eff}[L]$. For the data at $S = 30$ (Figure 6.10), the slope is close to unity (0.963) confirming that there is a large excess of copper-complexing ligands in

Macquarie Harbour despite the high ambient concentrations of copper while the intercept (5.02) implies that the ligands bind strongly to copper.

Zirino et al.(1998a) measured a range of copper species by ASV and GFAAS in San Diego Bay as well as pCu_{free} using an Orion ISE. When their values of pCu_{free} are plotted against the negative logarithm of their total exchangeable copper, a straight line is also obtained with a slope of 1.05 although there is more scatter in the data since they only report pCu_{free} to one decimal place and their samples were not filtered. Their results are therefore very similar to our data at $S = 30$ confirming that the copper ISE is capable of providing meaningful data on copper in marine and estuarine waters.

For the $S = 20$ waters from Macquarie Harbour, the slope is much greater than unity (2.71) and the results are therefore not consistent with an excess of ligand having a constant stability constant. If the copper were effectively 'titrating' a range of ligands, then as progressively weaker ligands were complexed by copper, pCu_{free} would increase more rapidly than the total copper, that is pCu_{free} would decrease more rapidly as pCu_D decreased. This would give a curve, approximated by a straight line with a slope of greater than unity when pCu_{free} was plotted against pCu_D as was observed. Since the copper in Macquarie Harbour is also more strongly complexed at $S = 20$ than at $S = 30$ (pCu_{free} is higher at $S = 20$ for the same value of pCu_D , Figure 6.10), our results seem to suggest that copper in the intermediate waters ($S = 20$) is complexed by a low concentration of strong ligands while the copper at depth ($S = 30$) is complexed by a high concentration of weaker ligands. Note that the strong ligands must be derived from the freshwater source so that the situation is quite different from other reports of strong and weak ligands existing in seawater (Moffett et al. 1997).

Koehnken's (1996) generalised circulation model of the Harbour shows the $S = 20$ water mass is largely created by mixing of polluted water from the King River and salt water which enters the Harbour through the heads, with some contribution of freshwater from the Gordon River. $S = 30$ water is created by mixing of the deep saltwater layer with Gordon water, with some contribution from the overlying polluted brackish layer. One possible explanation for the two types of ligand is that the waters of the Gordon River may contain weaker ligands than the King River but this is considered unlikely given the similarity in the drainage basins of the two rivers.

The $S = 20$ waters are formed by mixing high ionic strength and high pH seawater with low ionic strength, low pH water containing high concentrations of humics, manganese and iron. These conditions would favour the formation of colloids

containing organic ligands as well as iron and manganese (hydr)oxides. These colloids could form as successive layers which could readily trap heavy metal ions and essentially isolate them from the bulk seawater (Mackey & Zirino 1994). The net result would be that much of the copper would be removed from true solution (although it would still contribute to pCu_D) by kinetic processes and so lead to the high values of pCu_{free} observed at pCu_D values between 6.5 and 6.8. On the other hand, if the formation of colloids were limited in some way then, as the concentration of pCu_D increased, less copper would be incorporated into colloids and the pCu_{free} vs. pCu_D relationship would approach that at $S = 30$ in line with observations (Figure 6.10).

The copper complexing capacity of a water is the ability of the natural complexing agents in the water to bind copper and thus ameliorate or prevent toxicity. Using a technique based on competition with Chelex-100, Carpenter et al. (1991) found that the strong complexing capacity of Macquarie Harbour waters decreased with increasing salinity and, although it was high compared to coastal and open ocean values, it was always less than the concentration of dissolved copper. This was in contrast to the situation found in the pristine environment of Bathurst Harbour / Port Davey, approximately 100 km to the south of Macquarie Harbour (Mackey et al. 1996), which had similar values of complexing capacity but much lower concentrations of copper. Since the Chelex-100 technique only measures strongly bound copper, the ISE results imply that there are also high concentrations of ligands present which can form copper complexes which are dissociated by Chelex-100. Earlier studies of chemical complexing capacity using ASV found that the waters of Macquarie Harbour had no excess complexing capacity even though copper additions as high as 40 $\mu\text{g/L}$ still did not cause significant toxicity to *Nitzschia* (Stauber et al. 1996). It appears that the ASV technique, like the Chelex-100 technique, also dissociates weakly bound copper complexes so that it overestimates the concentration of free or inorganic copper and underestimates the ability of ligands (including colloids) to complex the high concentrations of copper that occur throughout Macquarie Harbour.

Whilst this study and the work by Stauber et al. (1996; 2000) has shown limited or no toxicity in the harbour waters to selected species of algae, invertebrates and fish, several issues remain unanswered. One of these is the issue of whether filter-feeders or benthic organisms may be affected by high concentrations of copper in the sediments or the particulate copper in the water column and it is clear that more work needs to be done to establish the potential toxicity status of these fractions. The cause of the toxicity observed in some samples is also unknown, and it is important

to identify them, in the light of pending remediation works and subsequent altered physico-chemical parameters in the harbour.

CONCLUSIONS

The CFA method described in this thesis was successfully used to conduct a study of speciation and toxicity on Macquarie Harbour. We have shown that the CFA/Cu(II)ISE technique can be used to measure pCu_{free} in seawater and that it provides a reproducible measure of the toxicity of copper in cultures of marine phytoplankton. The measured values of pCu_{free} in field samples and in algal cultures, and the observed reductions in growth in the algal bioassays, are in agreement with values expected on the basis of literature reports. More than 99.9% of the dissolved copper in Macquarie Harbour is complexed by strong ligands. These ligands are almost certainly based on humic compounds and our results are consistent with the presence of colloidal species (particularly at $S = 20$) which could be partly inorganic due to the high concentrations of iron and manganese present in the Harbour and originating in the King and Gordon Rivers (Carpenter et al. 1991; Stauber et al. 1996; Stauber et al. 2000).

In contrast to predictions of toxicity based on ASV measurements (this study; (Stauber et al. 2000) and possible toxicity based on ligand exchange techniques (Carpenter et al. 1991), our copper ISE measurements clearly demonstrate that the concentration of pCu_{free} in Macquarie Harbour is reduced to levels that should not be toxic to most marine organisms. This is in agreement with bioassays on the marine phytoplankton *Nitzschia* and *Dunaliella* (this study); and earlier bioassays using flounder and amphipods (Stauber et al. 1996; Stauber et al. 2000).

Addendum

The research in this paper was conducted by myself. Co-authors D. Mackey and B. Nowak were my PhD advisors and played a supervisory role. This work was made possible in part by a grant from the Environmental Research Institute of the Supervising Scientist, administered by R. van Dam. Jenny Stauber and Jeanie-Marie Leroi are thanked for expert advice on algal bioassays. Jeff Ekert, Daniel Ray and Lois Koehnken from DPIWE Tasmania are thanked for assistance with sampling and data from Macquarie Harbour. John Sherwood and Al Zirino kindly reviewed the manuscript.

Chapter 7 Summary and conclusions

This study consisted of a series of investigations on the Orion 94-29 Cu(II)ISE, in order to develop a suitable analytical technique for the analysis of free copper in seawater. A flow analysis method was developed, based on the results of static ISE and preliminary flow injection analysis experiments. A primary advantage of flow analysis is the reduction or elimination of sample contamination from electrode dissolution. Two factors contribute to a reduction in the buildup of corrosion products from electrode dissolution. Firstly, the cleansing action of the continuous flow of solution past the membrane which also reduces the formation of AgCl and secondly, employing a flow cell with low dead-volume, resulting in only a minimal buildup of products in the immediate vicinity of the electrode. Flow analysis also offers more reproducible analytical conditions, and reduces the time taken for analysis by incorporating the conditioning process into the instrument start-up time. Cross contamination between samples is eliminated in flow analysis. Thus, the limitations of batch analyses are greatly reduced.

The effect of organic matter on the electrode response was investigated by EIS. Importantly, the magnitude of the interference is weak and unlikely to significantly affect the analysis of marine samples at natural pH. On the basis of the rotating ISE studies, analysis under flow conditions should serve to reduce the effect further. Indeed flow studies showed an improved limit of detection in the presence of humic acid. We know from XPS studies that the presence of organic matter reduces the formation of AgCl, thus the presence of organic matter does not preclude the analysis of marine samples.

FIA studies revealed the complex nature of the ISE response. Electrode response to chloride can be used to advantage by kinetic discrimination of the copper signal from the interferent. This was possible over a concentration range where the chloride effect is a problem in static analysis. Slow electrode response to samples with nM - μ M total copper limited the usefulness of FIA using the Cu(II)-en buffer as carrier, and the interaction between the carrier and the sample slug strongly suggested that a CFA method would be more applicable. CFA has all the advantages of FIA but relies on the development of a potential-time curve. This is a time consuming approach (comparable to static analyses) but the use of an empirical equation to predict the equilibrium potential increases the throughput of the samples by a factor of 3-6. Rapid sample throughput is important for field studies where the rate and extent of speciation changes in a sample is largely unknown. Thus the method can be applied to routine environmental monitoring, toxicology studies and other applications where knowledge of copper speciation is required.

Direct measurement of the free copper ion in seawater is a significant development in speciation studies. The role of pCu_{free} in toxicity was investigated in Macquarie Harbour as previous chemical and biological studies of this estuary showed an apparent anomaly between levels of bioavailable copper and toxicity in a suite of marine bioassays. Laboratory cultures of *Nitzschia closterium* exposed to clean, copper spiked seawater showed a strong correlation between growth inhibition and pCu_{free} measured by ISE. This relationship was demonstrated for two salinities, in a study of the intermediate harbour waters ($S = 20$) and the deeper more saline waters ($S = 30$). Toxicity was greater at $S = 20$, although both salinities revealed a linear relationship between growth and free copper. The range of pCu_{free} concentrations measured in the bioassays was consistent with literature reports of toxicity in other marine phytoplankton, and this confirms the usefulness of the ISE for speciation measurements in toxicity studies.

Copper measurements from Macquarie Harbour mid-depth waters revealed that despite elevated levels of total copper, free copper was extremely low and no toxicity was observed in the 72 hour algal bioassay. On the basis of the pCu_{free} measurements no toxicity was predicted in the intermediate waters, in line with our observation of no toxicity. However, the bioassays in more saline waters revealed mild toxicity that could not be explained on the basis of pCu_{free} measurements alone. This predictive approach assumes that the toxicity of the samples is due to copper alone, and that there are no synergistic or antagonistic effects contributed by other heavy metals or potential toxicants. We can speculate that other chemicals and compounds must be involved since repeat bioassays revealed no toxicity, despite essentially the same concentrations of total copper, dissolved copper and most significantly, pCu_{free} . Phylogenetic differences in copper response were explored using *Dunaliella tertiolecta*, however no toxicity was observed in this species either.

It is possible that the toxicity observed in the harbour was due to copper and that the ISE did not reflect this because of some offset in response or some interferent. This is unlikely as it has been demonstrated that the presence of well known interferents chloride, silver, mercury, iron and organic matter have negligible effect if the analysis protocols described here are employed. It is also possible that the results of the interference studies conducted at high rotation speed cannot be extrapolated to analyses conducted under conditions of continuous flow (i.e. lower effective flow of solution over the electrode surface). However this is unlikely as FIA studies showed that even under conditions of low flow, the ablation of surface products was possible, albeit at a slower rate. Additionally, both static, FIA and RDE experiments showed that the presence of humic material had little or no impact on the electrode response.

Examination of the relationship between pCu_{free} and total dissolved copper revealed that the waters of Macquarie Harbour have significant copper complexing capacity. Additionally, the ratio of $pCu_{free} : pCu_D$ in the two water masses studies implies the nature of the complexing ligands at each salinity is quite different. This is in contrast to estimates of copper complexing capacity determined by ASV and by competition with Chelex-100, which revealed the complexing capacity of the samples was exceeded, and it is assumed that these techniques dissociate weakly bound copper complexes, and thus overestimate the concentration of free or inorganic copper, and underestimate the ability of the ligands to complex high concentrations of copper entering the harbour from the King River. The role of colloidal material is likely to be critically important in the ability of the harbour waters to “buffer” the metal load and this is again is a parameter not taken into account in more conventional means of measuring complexing capacity. Significantly, the ISE measurements are unaffected by the presence of colloidal, or indeed any other complexes, a significant advantage over other analytical techniques. This speciation study clearly demonstrates that direct measurement of pCu_{free} by ISE gives important information that could not be determined by analytical speciation schemes.

In the absence of a benchmark technique for the direct analysis of pCu_{free} , it is not possible to determine the absolute accuracy of the ISE measurements. However, we can examine the following evidence for the use of the ISE to determine pCu_{free} in seawater: There is a good correlation between GFAAS and ISE results for total copper, confirming that the ISE has the required sensitivity, and that other ions present in seawater are not interfering. Additionally, the pCu_{free} concentrations reported here are consistent with literature values determined by indirect methods. The correlation between free copper and the toxic response of a marine diatom is also consistent with studies on other species, employing a variety of analytical approaches to determining free copper. The speciation trends reported here (i.e. relationship between copper complexation, free copper and total copper) have been reported elsewhere by investigators also using the Orion Cu(II)ISE.

In conclusion, the investigation was successful in developing an analytical method that addressed the two issues raised in Chapter 1 i.e. electrode dissolution and the unknown effect of organic matter. The CFA method can provide useful information on speciation for environmental and toxicology studies by calibration of the electrode with Cu(II)-en buffers. It has been demonstrated that this approach is valid under laboratory conditions. However, the accuracy of the ISE measurements is dependent on the quality of the thermodynamic data used to calculate pCu in the buffers. For greater accuracy, especially for real time measurements in the field we need a detailed knowledge of the stability constants for all species in the calibration buffers as a function of temperature and ionic strength (salinity). Better models for

determining speciation in complex media such as seawater would also increase our confidence in calculations of pCu_{free} .

Further developments of the method may include the use of higher flow rates, since work on rotating electrodes has shown effects of organics can be completely overcome at very high rotation speeds. Alternatively, modifications to the flow cell design, or even the electrode itself may improve performance under flow analysis conditions. The CFA method could be incorporated into long-term studies of the toxicity of Macquarie Harbour waters to marine and estuarine organisms, by incorporating it into turbidostat or 'cage-culture' experiments. This may overcome some of the problems of short-term bioassays, and give a truer picture of the health of aquatic organisms in the estuary. Such an approach would also be useful for investigations on the effects of the various remediation options proposed for the mine effluent. Finally, more *in situ* and field speciation data would allow a better understanding of the processes governing speciation. On the basis of the analytical protocols and toxicity results described here, this is now achievable.

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Appendix 1 Equilibrium speciation modelling

The purpose of this appendix is to describe the programs and databases used in the calculation of copper speciation in seawater and in the Cu(II)-en buffer system. These buffers are described in Chapters 2 and 3.

The task of calculating chemical equilibrium concentrations of trace metal species in seawater is made considerable easier by the use of computer models such as MINEQL (Westall et al. 1986) and MINTEQA2 (Allison et al. 1996)

MINTEQA2 is a geochemical assessment model, distributed by the USEPA. This equilibrium speciation model can be used for modelling equilibria among dissolved adsorbed, solid or gas phases of environmental systems. The thermodynamic database is taken from the US Geological Survey's WATEQ3 model. Version 3 was used throughout this study. MINEQL allows modelling of trace metal speciation using specified inorganic and organic ligands, and recent versions use the same database as MINTEQA2.

An excellent article by Nordstrom et al. (1979) compared the consistency of 13 different computerised models (including MINEQL) which were all used to perform two test case calculations on a typical seawater sample, and a diluted river water sample. The primary limitation of the models was inconsistencies in the thermodynamic databases, and the equations and correction used for such factors as pressure, temperature, and activity coefficients. More recently, an article by Serkiz et al. (1996) appeared documenting errors in the MINTEQA2 thermodynamic database file for ligands including EDTA, ethylenediamine, and glycine.

For this study, the model databases were carefully evaluated, firstly to ensure the most applicable value was used, and secondly to ensure consistency with calculations performed by other members of the research team. Table A1.1 summarises the chosen values for frequently performed calculations such as buffer values for pCu_{free} . The sources of the thermodynamic data for each case are noted. Data from Smith and Martell (1975) are also noted for reference. Table A1.2 summarises the inorganic model for seawater composition used for predicting speciation in UV-oxidised seawater. A simplified model was often used for "quick and dirty" calculations.

Table A1.1 Comparison of overall stability constants used in this study with the thermodynamic database of MINTEQA2 (Version 1.0) and MINEQL (Version 3.0). Data from Smith and Martell (1975) is included for reference. Values for this study taken from De Marco (1994). Buffer compositions were a) 0.6M NaCl, 15 mM ethylenediamine (en), 1 mM Cu(NO₃)₂ or b) 0.6M NaCl, 1 mM glycine (gly) , 2 x 10⁻⁴ M Cu(NO₃)₂. All data reported is for ionic strength = 0, and at 25°C.

	Species	MINTEQA2	MINEQL	Smith & Martell	This study
a)	Cuen	10.49	10.50	10.36	10.48
	Cu(en) ₂	19.62	19.50	19.32	19.55
	enH	9.96	9.70	9.93	9.92
	en(H ₂)	16.85	16.50	16.85	16.76
b)	Cugly	8.62	8.70	8.53	8.56
	Cu(gly) ₂	15.64	16.00	15.60	15.64
	Hgly	9.78	9.90	9.55	9.78
	H ₂ gly	12.12	12.20	Nc	12.13

Nc-not considered.

Table A1.2 Seawater composition used for modelling chemical speciation in MINTEQA2 and MINEQL from Dyrssen and Wedborg (1974). Salinity = 35, $Cl = 19.734$ and $\sigma = 1.02336 \text{ g/cm}^3$ and 25°C . pH chosen was 8.1, and copper concentration was $5 \times 10^{-9} \text{ M}$.

Component	Mol/kg (Mw)	Mol/L (M)	Mg/kg	Used for simple model?
Na^+	0.468(38)	0.479(32)	107(68)	✓
K^+	0.01021	0.01045	399.1	✓
Mg^{2+}	0.05315	0.05439	1291.8	✓
Ca^{2+}	0.01029	0.01053	412.3	✓
Sr^{2+}	0.000093	0.000095	8.14	
Cl^-	0.54587	0.55862	19353	✓
Br^-	0.00084	0.00086	67.3	
F^-	0.0000734	0.0000751	1.39	✓
SO_4^{2-}	0.02823	0.02889	2712	✓
Alkalinity	0.00238	0.00244		
Total carbonate	0.00230	0.00235	27.6 (as C)	
HCO_3^-	0.00188	0.00192		
CO_3^{2-}	0.00023	0.00024		✓
Total boron	0.000412	0.000421	4.45 (as B)	
B(OH)_4^-	0.000081	0.000083		✓
Formal ionic strength	0.69765	0.71395		

Appendix 2 Standard addition analysis of total copper in seawater.

This appendix contains the full calculations for the determination of total copper by standard addition analysis, using the method of Smith M.J. & Manahan S.E. (1973) "Copper determination in water by standard addition potentiometry." *Analytical Chemistry* 45, 836-839.

This method was used to determine the total copper concentration in archived seawater samples, as described in Chapter 3. A 100 mL sample was used, and the Cu(II)ISE was polished and conditioned in the manner described in the Experimental section of Chapter 3. All analyses were conducted in the CSIRO Class-100 clean room. A series of small copper additions were made initially to determine C_o , and a series of larger additions were made to the same sample to determine the experimental slope for equation 1.

To calculate the concentration of copper in the sample, the following equation is used

$$E_1 = E'_a + S \log C_o \quad (1)$$

where C_o is the concentration of copper in the sample

S is the theoretical Nernst factor $2.303 \frac{RT}{2F}$

The term E'_a contains a correction for the activity coefficient, and in some cases the fraction of cupric ion complexed.

If a known volume V_s of a standard Cu(II) solution of concentration C_s is added to a sample of initial volume V_o , the new potential E_2 is given by

$$E_2 = E'_a + S \log \left[\frac{C_o V_o}{V_o + V_s} + \frac{C_s V_s}{V_o + V_s} \right] \quad (2)$$

if V_s is very much smaller than V_o , equation 2 can be simplified

$$E_2 = E'_a + S \log \left[C_o + \frac{C_s V_s}{V_o} \right] \quad (3)$$

The shift in potential $E_2 - E_1$ is related to the original sample concentration by the following equation

$$E_2 - E_1 = S \log \left[1 + \frac{C_s V_s}{C_o V_o} \right] \quad (4)$$

If the term Z is defined as follows

$$Z = \text{antilog} \left[\frac{E_2 - E_1}{S} \right] \quad (5)$$

and equation 5 is substituted into equation 4, we have

$$\left[Z - 1 = \frac{C_s}{C_o V_o} \times V_s \right] \quad (6)$$

Equation 6 is in the form $y = mx + c$, therefore a plot of $Z-1$ vs. V_s will allow the calculation of C_o from the slope of the line, where a series of additions have been made.