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Technical Completion Report for Project W-958

Development of a Liquid Membrane Technique to Measure the Temporal Variation in "Bioavailable" Copper and Nickel in the South San Francisco Bay

Abstract

A supported liquid membrane (SLM) technique for the determination of free and labile copper in estuarine and coastal water was developed. The SLM consisted of 10 mM Lasalocid, a naturally occurring carboxylic polyether ionophore, dissolved in o-nitrophenyl octyl ether (NPOE), immobilized on a thin microporous solid membrane. The solid membrane was sandwiched between two aqueous phases: (1) a source or sample solution containing the analyte (in this case free/labile copper) to be extracted and (2) a strip or acceptor solution into which the analyte(s) is trapped. The membrane support was clamped between two circular Teflon blocks each with circular groves like an Archimedes spiral

The SLM was used to extract free and labile copper from water samples collected from two sampling stations, Dumbarton Bridge and San Bruno Shoals in South San Francisco Bay The copper concentration in the SLM extracts was determined off-line by GFAAS.

Between 90-97% of the total dissolved copper was bound to organic ligands, and therefore not "bioavailable" to phytoplankton. Thus only about 5-10% of the total dissolved copper existed as inorganic and/or labile organic copper species. The measurements were consistent with earlier copper speciation measurements that were made in South San Francisco Bay using electrochemical methods.

Introduction and Problem Statement

In response to concerns about the effects of pollutant metals on aquatic ecosystems, considerable resources are dedicated to the monitoring of point and non-point pollutants and the implementation of metal source control programs. Current environmental regulations are intended to maintain the total metal concentrations below levels considered by the EPA to be potentially toxic to sensitive aquatic organisms. This approach has been criticized because it fails to consider the decrease in toxicity that occurs when metals are adsorbed on to particles or are complexed by dissolved organic ligands. As the resources dedicated to controlling pollutant metals continue to increase, these concentration-based regulations are being re-evaluated. For example, there has been the emergence of Total Maximum Daily Load or TMDL as an important planning and regulatory decision-making tool in regional and local efforts to achieve continued improvement in water quality. TMDL establishes the allowable loadings of a pollutant that a water body can receive without violating applicable water quality standards or harming beneficial uses. In addition, monitoring agencies within the

San Francisco Bay area have used the Water-Effect Ratio (WER) calculation in an attempt to evaluate the extent to which the "binding capacity" of waters in the South Bay can decrease Cu and Ni toxicity. A WER value for Cu of 3 has been observed in the South San Francisco Bay and has been used as an argument to allow a higher TMDL.

Large spatial and temporal differences in Cu and Ni concentrations and chemical speciation can lead to wide variations in biological availability of these metals and their effects on phytoplankton. Copper is strongly chelated by organic ligands in estuarine and coastal seawaters and its biological availability is determined by the concentrations of the free metal ions or kinetically labile inorganic species (free ions plus inorganic complexes) rather than the total dissolved concentration. It is important to have methods that can determine the concentrations of the labile or bioavailable forms of Cu to complement the extensive monitoring of total metal concentrations.

South San Francisco bay has been designated as an impaired water body under the clean water act (San Francisco Estuary Institute, 1995) because the total concentration of dissolved Cu and Ni often exceeds the water quality objectives (Flegal et al., 1991). However, there is strong evidence to suggest that most of this Ni and possibly Cu is complexed to anionic hydrophilic ligands such as ethylenediamine tetraacetic acid (EDTA) that are not directly assimilated by microorganisms (Bedsworth and Sedlak, 1999; Phinney and Bruland, 1997). It is, therefore, important to measure the bioavailable fraction of the total dissolved concentration to provide better estimates of their TMDL.

Objectives

The aim of our study was:
1) to develop a liquid membrane-based, technique to measure the free/labile and hence bioavailable fraction of Cu and Ni in South San Francisco Bay

2) to compare the measurements made with the liquid membrane technique to those made using classical electrochemical techniques and hence asses the utility of liquid membrane for use in monitoring the bioavailable fraction of copper and other trace elements in estuarine waters.

Procedure

Supported liquid membrane (SLM) overview

The permeation of metal species through SLM involves a simultaneous

combination of an extraction and a stripping occurring in non-equilibrium conditions. The SLM system employs two aqueous phases: (1) a source (donor) solution containing the analyte(s) to be extracted and (2) a strip (acceptor) solution into which the analyte(s) is trapped. The two phases are separated by an immiscible organic solvent in the pores of a hydrophobic, supporting membrane. The organic liquid is held in the membrane pores by capillary forces. Because only hydrophobic species can diffuse through the membrane, metal ionic species must be neutralized. This is accomplished by incorporating an organic complexing agent or carrier C, in the membrane liquid. The carrier forms a hydrophobic complex MC with the metal ion species M (carrier or extractant mediated transport) (Ndung'u et al., 1999; Salaun and Buffle, 2004). Alternatively, the metal ion can form an uncharged complex with a complexing agent added to the sample, or already exist as such in the sample (passive diffusion, figure 1) (Papantoni et al., 1995; Parthasarathy et al., 2004).

In extraction mediated transport SLM extraction, the metal ions diffuse toward the membrane through the source diffusion layer and then through the hydrophobic membrane in the form of a complex MC formed with the organic carrier C present in the membrane. M dissociates at the interface and M is released in the acceptor and L is protonated to form HL. The flux of M through the SLM is induced by the pH difference between the strip and source solutions. Alternatively, M can also be trapped in the strip solution by forming a soluble charged complex with a complexing agent in the strip solution. The driving force then is the difference in electrochemical potential of M between the strip and source solutions (Figure 1).

Overview of SLM Cu speciation

The vast majority of copper (and other trace metals) speciation techniques aim at measuring the concentrations of a specific species, such as the free hydrated Cu^{2+} ion in equilibrium with all labile complexes. Ligand exchange techniques are among the most widely used for measuring the free hydrated Cu^{2+} ion concentration (and hence the overall degree of complexation) at the nano and pico molar concentration levels usually encountered in natural waters (Bruland et al., 2000; Moffett, 1995; Van den Berg, 1986). In such techniques, a known reference ligand, forming a detectable complex CuR with known stability constant, is added to the test sample, in substoichiometric concentration compared to total Cu concentration. The following reaction occurs:

$$\sum CuL_i + R \Leftrightarrow CuR + \sum L_i \qquad (1)$$

Where $\sum CuL_i$ represents only the copper complexes in the sample which can dissociate in response to the addition of R. CuR can then be measured by various techniques, either directly in solution, by adsorptive cathodic stripping voltammetry (Bruland et al., 2000; Twiss and Moffett, 2002)) or after separation by liquid-liquid extraction (Miller and Bruland, 1994). From the known equilibrium constant of CuR formation, the free Cu^{2+} concentration can then be computed.

In SLM extraction, an organic ligand R (in this case the extractant, lasalocid) is incorporated in the hydrophobic organic phase i.e. the membrane liquid. Lipophilic reference ligands are then used and the competition reaction occurs at the donor/membrane interface:

$$\sum CuL_{i(donor)} + R_{(membrane)} \Leftrightarrow CuR_{(membrane)} + \sum L_{i(donor)} \qquad (2)$$

The hydrophobic copper-lasalocid complex, $CuR_{(membrane)}$ diffuses through the organic membrane and at the membrane/acceptor interface the following reaction occurs:

$$CuR_{(membrane)} + H^+_{(acceptor)} \Leftrightarrow Cu^{2+}_{(acceptor)} + R_{(membrane)} \qquad (3)$$

Thus the flux through the SLM is also based on competition reaction (1) at the interface, but in contrast to stripping voltammetry or ion-selective electrode techniques, a flux is maintained through the membrane by means of a chemical gradient. This flux is speciation dependent and is thus the measured signal.

In this study, we developed a SLM technique to measure the dissolved copper speciation in water samples from San Francisco Bay, using a naturally occurring carboxylic polyether ionophore as the membrane extractant. The data was compared with copper speciation measurements using differential pulse anodic stripping voltammetry (DPASV) with a thin mercury film (TMF) rotating glassy carbon disk electrode (RGCDE).

Overview of differential pulse anodic stripping voltammetry (DPASV) copper speciation

DPASV copper speciation determinations involve titrating a sample (containing natural copper complexing organic ligands) with copper, and measuring the oxidation current of copper deposited in a TMF-RGDE as a function of added copper. During the DPASV deposition step, Cu^{2+} is reduced to elemental Cu^0 at the mercury electrode surface, where the Cu^0 will then amalgamate with the mercury electrode. After the deposition period, the potential of the mercury electrode is ramped positive, and the current resulting from the oxidation of the amalgamated copper is measured. These measurements of peak current are plotted against the total copper in the sample at the point in the titration at which the peak current was measured producing a titration curve.

The reaction for the formation of the complex can be represented it two ways:

$$Cu' + L' = CuL \qquad (4)$$

having the equilibrium expression

$$K'_{cond, Cu'} = [CuL]/ [Cu'][L'] \tag{5}$$

or

$$Cu^{2+} + L' = CuL \tag{6}$$

having the equilibrium expression

$$K'_{cond, Cu}{}^{2+} = [CuL]/ [Cu^{2+}][L'] \tag{7}$$

$[Cu']$ is the total concentration of dissolved inorganic Cu present (i.e. free hydrated Cu^{2+} plus inorganic species), $[L']$ is the concentration of organic ligands not already bound to Cu (but which have the potential to bind it), and $[CuL]$ is the concentration of Cu-organic complexes. $K'_{cond, Cu'}$ and $K'_{cond, Cu}{}^{2+}$ are the conditional stability constants with respect to inorganic copper (Cu') and with respect to free copper (Cu^{2+}) of the copper-organic ligand complex measured under specific conditions (ionic strength, pH, and major ion competition of seawater). $[Cu']$ is related to the concentration of the free, hydrated ion $[Cu^{2+}]$, by an inorganic side reaction coefficient, $\alpha_{Cu'}$, $[Cu'] = [Cu^{2+}].\alpha_{Cu'}$ (Ringbom, 1963; Turner et al., 1981). $[L']$ includes the concentrations of free dissolved ligand, $[L^n]$, protonated forms e.g. $[HL]$, and ligands which may be complexed by other cations e.g. $[CaL]$, but does not include L already bound to Copper (i.e. $[CuL]$). Thus the relevant mass balance equations are:

$$[Cu_T] =[Cu'] + [Cu^{2+}] \tag{8}$$

and

$$[L_T] =[L'] + [CuL] \tag{9}$$

$[Cu_T]$ and $[L_T]$ are the respective concentrations of total dissolved copper and Cu-complexing organic ligand(s).
From the above mass balance and conditional stability relationships, the titration data can be linearly transformed into

$$[Cu']/[CuL]= [Cu']/[L_T] + 1/ K'_{cond, Cu'} [L_T] \tag{10}$$

or

$$[CuL]/[Cu']= K'_{cond, Cu'} [CuL]/ [L_T] +K'_{cond, Cu'} [L_T] \tag{11}$$

Values for $[L_T]$ and $K'_{cond, Cu'}$ are obtained from a linear regression of a plot of $[CuL]/[Cu']$ as a function of $[CuL]$ (Langmuir transformation) or $[Cu']/[CuL]$ as a function of $[Cu']$ (Scatchard transformation). Then, using these values, the original concentrations of organically-complexed copper, inorganically-complexed copper, and free Cu^{2+} originally present in the sample can be

calculated. The conditional stability constants $K'_{cond, Cu'}$ were converted to stability constants expressed with respect to Cu^{2+} ($K'_{cond, Cu^{2+}}$) by multiplying $K'_{cond, Cu'}$ by the inorganic side reaction coefficient $\alpha_{Cu'}$, appropriate for the salinity and pH of the sample.

SLM-GFAAS

SLM-GFAAS also relies on the differences in the dissociation kinetics between inorganic metal complexes and strong organic metal complexes. The analytical time scale of the method is therefore dependent on the thickness of the donor diffusion layer. Thus the fraction of copper detected as labile by this method (hereafter called Cu_{slm}) will depend on the hydrodynamic conditions especially the sample flow-rate applied on the donor side that determine the thickness of the diffusion layer. The SLM-GFAAS titration data are linearized and interpreted in a manner analogous to that described above for the DPASV technique.

Experimental Details

Reagents

All solutions were prepared with de-ionized water (18 MΩcm^{-1}) from a Milli-Q$^{®}$ analytical reagent-grade water purification system (Millipore, Bedford, MA). Trace Metal grade nitric acid (Fisher Scientific, Pittsburgh, PA) was diluted with Milli-Q$^{®}$ to make up 10 mM HNO_3 acceptor eluent solution. The membrane liquid was prepared by dissolving six mg of Lasalocid sodium salt, in one ml of 2-nitrophenyl octyl ether (NPOE) (both from Sigma) in a 1.5 ml acid cleaned, capped Polypropylene vial to make ca. 10 mM Lasalocid solution in NPOE. It was shaken in a vortex shaker for 15 minutes until all the Lasalocid had dissolved. 0.25 ml of 3 M nitric acid was added and the mixture shaken for another five minutes to clean off any copper contamination. The mixture was then centrifuged for half an hour at 4000 rpm to separate the phases. The bottom aqueous phase was carefully pipetted out and discarded.Copper standard solutions were prepared by dilution of a 1000 μgl^{-1} commercial stock solution. Working standards solutions were made daily by spiking UV irradiated metal free seawater (vide infra) with known concentrations of copper.

UV irradiated metal free seawater (UV-SW)

The coastal surface seawater used in development of this technique was collected in an acid-cleaned polyethylene carboy from a rubber raft. In the laboratory, this

seawater was pumped at a flow rate of 2 ml/min through a continuous system consisting of a 0.4-μm polycarbonate filter (Nuclepore) to remove particulates, Duolite S-587 resin (Diamond Shamrock Corp.) to remove dissolved organics, Sumichelate Q-1OR resin (Sumitomo Chemicals) and Chelex-100 resin (Bio-Rad) to remove trace metal cations, an ultraviolet (UV) irradiation system (1.2-kW Hanovia Hg arc lamp, irradiation time 5 h) to destroy residual dissolved organics, two Chelex-100 resin columns in series to remove any dissolved trace metals previously bound to residual organics, and finally through a C-18 resin (Sep-PAK, Waters Associates) in an attempt to remove any remaining refractory dissolved organics. Seawater thus treated is hereafter termed "UV-SW" (Donat and Bruland, 1988).

Sample collection and handling

The equipment and procedure used to collect samples in the San Francisco Bay were described previously (Donat et al. 1994). The samples analyzed in this study were from two sampling events taken from the R/V David Johnston and collected on January 27-28 (San Bruno Shoals) and March 28 of 2003 (Dumbarton Bridge). All speciation samples were collected by filtering the sample through 0.45 μm pore size in-line cartridge filter (MSI) and stored in 2 or 1 liter acid-cleaned FEP Teflon bottles. The samples were stored in the dark on ice until they could be transferred to the refrigerator in the laboratory, where they were stored at 4°C until analysis. Filtered samples for nutrients and dissolved organic carbon (DOC) were collected and handled similarly to the speciation samples until they were placed in the freezer back in the laboratory. Subsequent electrochemical and SLM analyses of samples were performed at room temperature in the laboratory.

Supported Liquid Membrane

Membrane Device

The SLM device used (figure 2) was similar to the one described previously (Ndung'u et al., 1999). It consists of two circular Teflon blocks (with a diameter of 120 mm and a thickness of 30 mm). Each block has machined grooves like Archimedes spiral (depth 0.5 mm, width 1.5 mm and length 250 cm giving a total volume of ca 2 ml). The membrane used was a Celgard-2400 polypropylene hydrophobic membrane (porosity, 0.45; thickness 25 μm; pore diameter, 0.04 μm), a kind gift from Celgard (Celanese Plastic, Charlotte, NC). The micro porous membrane (cut into, 90 mm diameter, 254 cm^2), was placed in a Petri dish and impregnated with ca. 150 μL of 10 mM Lasalocid in NPOE solution. A well-soaked membrane is transparent (as opposed to translucent). The membrane was then rinsed three times on both sides with MQ water to remove excess organic

solvent. It was then placed between the two Teflon® blocks and clamped with six stainless screws fastened on two aluminum blocks. The stainless steel screws were covered with Teflon® tape to prevent any contact with the solution in the membrane channels (figure 2). The membrane device was then connected to the automated online SLM set-up (vide infra) and the donor and acceptor sides of the membrane were further flushed with ca 15-20 ml of 10 mM nitric acid on both the donor and acceptor sides. This was both to clean the system and flush out any remaining excess organic solvent.

SLM Flow system

The automated flow system used is similar to the one described previously (Djane et al., 1999). It consists of an eight channel peristaltic pump (Rainin Instruments, Oakland, CA) and three electrically actuated valves (two six port, two position valves, Valco Instruments, Houston, Texas). The third six-port multi-position valve served as a fraction collector where four ml of the enriched sample extract from the stagnant acceptor was collected. A simple computer program written in Pascal (Turbo Pascal v. 5.5 and 6.0, Borland, Scotts Valley, CA) controlled the pump and valve switching. The sample solution was pumped through the donor channels of the membrane device containing Lasalocid in NPOE, which extracts free/labile Cu species. After the extraction, the selectively enriched analyte solution in the stagnant acceptor channel was pumped into the fraction collector. Usually, four ml of solution was used to ensure a complete transfer of the enriched sample from the acceptor channel (volume ca 2 ml) to the vials. The donor and acceptor channels of the membrane were flushed with MQ water and 10 mM nitric acid respectively between samples to minimize memory effects. The tubing that eluted the enriched samples to the vials in the auto-sampler was also emptied before the next elution commenced. The flow system and SLM support unit are shown in (figure 2). Cu concentrations were then measured on a Perkin Elmer 5000 atomic absorption spectrophotometer equipped with a HGA 500 graphite furnace atomizer with a Zeeman background corrector and an AS-40 auto-sampler. Traversely heated pyrocoated graphite tubes with an integrated L`Vov platform were used with manufacturer recommended drying, ashing and atomization conditions.

SLM System optimization Experiments

Experiments were carried out to investigate the effect of sample ionic strength, pH, and copper concentration on the extraction efficiency. Because the driving force for the extraction of Cu from the sample (source) solution into the strip is the $[H^+]$ gradient between the two phases, having a too low strip pH will change the sample pH and hence affect the Cu speciation. The concentration of HNO_3 in the strip solution needed to keep the donor pH as close as possible to the sample

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pH was also investigated In all the experiments a known volume (usually 30 ml) of UV-SW was pumped through the donor side of the SLM unit at a flow rate of 1 ml/min. Then 10 ml of MQ was pumped through the donor side to remove any salts after which the acceptor side was eluted with four ml of 10 mM HNO_3. Copper concentration in the eluates was determined by GFAAS.

In order to investigate the variation in SLM labile copper fraction, UV-SW spiked with 31 nM copper was titrated with EDTA, humic and fluvic acids.

Dissolved copper speciation experiments

Individual copper titrations were made by transferring an aliquot of the analyte to an acid cleaned 250 ml polypropylene sample bottle. This addition was allowed to equilibrate with the sample for about 15 minutes before SLM extraction as described above. Three replicate SLM extractions were performed for each copper titration. The copper in the eluates was determined by GFAAS and represents SLM labile copper in the samples. Blank runs (with UV-SW) and system calibration were performed periodically and after every membrane change. Copper concentration in the acceptor eluates was corrected for extraction efficiency and plotted against the total copper concentrations (sum of the concentrations of copper added + ambient copper) used in the titrations.

Nafion-coated thin mercury film electrode (NCTMFE)-DPASV

The details of the electrochemical system used to perform the NCTMFE-DPASV technique has been previously described (Bruland et al., 1985; Coale and Bruland, 1988). Preparation of the Nafion-coated mercury film electrode is described elsewhere (Hurst and Bruland, 2004). All measurements were made using a 6 mm diameter glassy carbon - rotating disk electrode (GC-RDE), a Ag/AgCl/sat'd KCl reference electrode, and a platinum wire counter electrode. The three-electrode system was connected to a Princeton Applied Research (PAR) 174A voltammetric analyzer and strip chart recorder. All laboratory work was performed in a Class-100 clean area.

Results

Choice of SLM extractant

One of the main advantages of SLM extraction is the ability to tune the extraction by the choice of the complexing agent (extractant) in the membrane. A good extractant should have a low water solubility, a high dielectric constant and the complex formed with metal ion should not have a too high K_d or high strength.

This is because too high a partition coefficient and too stable complex will make the back extraction from the organic membrane phase into the aqueous strip difficult. On the other hand, a too low Kd or unstable complex will be difficult to extract into the membrane. While the choice of possible extractants is wide for extraction of total dissolved copper, the choice is rather more restrictive for speciation studies. This is because of the added requirement of maintaining the sample pH. Most of the complexing agents suitable for use as SLM extrication are weak organic acids. This is because an exchangeable proton is necessary to provide the driving force for the metal extraction (see figure 1) into the strip phase, often against its concentration gradient. While this is not a problem for total dissolved metal extraction, a high proton gradient usually results in an appreciable pH drop of the sample, which would not be acceptable for speciation studies.

Diethylhexyl phosphoric acid (DEHPA) has been widely used for SLM trace metal extraction. In those extractions the strip solution is usually quite acidic (up to 1 M HNO3). This results in a lowering of sample pH by several pH units. The pH of most natural waters is between 6 and 8. An attempt to lower the strip nitric acid to pH 3 (1 mM HNO_3) while keeping the natural sample pH (7.5<pH<8) still resulted into a pH drop of more than three units. Maintaining a pH difference of two units (using a strip pH of about 5) still resulted into a unit sample pH drop and too low a flux.

Other studies have opted to use a different trapping mechanism i.e. having a neutral extractant in the membrane and a strong ionic complexant the strip to trap the metal ions. This is usually a carboxylic acid type e.g. EDTA. However, dilute HNO3 matrix is more compatible with final GFAAS determination.

Lasalocid, a naturally occurring carboxylic polyether ionophore has been used as a carrier in the SLM extraction of Cd, Pb and Zn (Canet and Seta, 2001) but not copper. The transport involves Lasalocid complexation with metal ions via loss of the ionaizable proton (figure1), driven by the proton gradient across the membrane. However, any other suitable cation gradient can be employed. Lindoy and Walker (Lindoy and Walker, 1990) used an NH_4^+ gradient to transport $Co(NH_3)_4^{3+}$ across a lasalocid liquid membrane. The ammonium ion was also tried in this study and found to provide slightly better flux than H^+. An ammonium ion concentration of 2 M at pH 9 in the strip was found to provide good copper flux across the SLM. However, H^+ was chosen because HNO3 provided a more compatible matrix for final GFAAS determination compared to NH_4^+

Optimization of SLM extraction parameters

In SLM extraction, it is important that the extraction efficiency i.e. fraction of analyte extracted into the strip solution for a chosen set of parameters (e.g. at constant flow rate, extractant concentration and support channel dimensions) be independent of sample properties such as initial copper concentration, sample pH

and salinity. This is especially important in estuaries where salinity gradients are common. We therefore investigated the effect of sample pH, Salinity and copper concentration on the extraction efficiency using spiked UV-SW and final determination by GFAAS. The extraction efficiency was found to be independent of sample pH, initial copper concentration and salinity in the ranges investigated. (Figure 3 A, B, C)

Extraction of SLM labile copper species

EDTA and Humic Acid Copper complexes

The ability of the lasalocid SLM to extract labile copper species was investigated by extracting copper from UV-SW spiked with varying concentrations of EDTA and humic acid. In these experiments, UV-SW containing 31 nM of copper was titrated with EDTA or humic acid and extracted by the SLM (Figure 4). The fraction of labile copper decreases with increasing EDTA and humic acid concentration as expected. The UV-SW used was free of organics and trace metal except the added copper but contained the alkaline earth ions of calcium and magnesium. The presence of calcium at seawater concentrations has been shown to significantly retard the reaction of copper with EDTA. This is due to competition between Copper and the alkaline earth ions and the slow direct attack of copper on EDTA complexed to alkaline-earth ions as compared with reactions with free or protonated EDTA species (Hering and Morel, 1988) This explains the shape of the EDTA titration curve which shows no decrease in SLM labile copper until after 50 nM EDTA had been titrated.

On the contrary, there is no similar lag in the humic acid titration curve where the SLM labile fraction decreases smoothly with increasing humic acid concentration. This is because the rate of copper complexation with humic acid is not retarded by the presence of seawater calcium probably because copper and calcium do not compete for the same binding sites in the humic molecules(Hering and Morel, 1988). The different nature of EDTA and humic ligands is also reflected in the nature of the humic titration curve where the kinetics of Cu-HA dissociation are slower in comparison to Cu-EDTA (Figure 4)

Dissolved copper speciation in San Francisco Estuary samples

Table 1 summarizes the nutrient and other ancillary measurements data for the two samples used in this study. Both samples were collected from two sites in South San Francisco Bay. The high pH value in the Dumbarton Bridge sample is an indication of the productivity occurring in the San Francisco Bay waters and was most pronounced during nutrient drawdown during a phytoplankton bloom in

the South Francisco Bay in March 2003. The inorganic side reaction conditional stability constant, log α_{Cu}, which is a measure of the competition strength of inorganic species, is strongly influenced by pH. The larger conditional stability constant reported in the Dumbarton Bridge site, which was sampled in March, can be contributed to an increase in pH. That increased pH was due to a drawdown of CO_2 created by the phytoplankton bloom, which causes an equilibrium shift to more $[CO_3^{2-}]$ and less $[HCO_3^-]$. The net result is that the pH rise from January to March is due to the phytoplankton bloom and net photosynthesis. Consequently, the large log α_{Cu} results in a decrease in the calculated $[Cu^{2+}]$.

The samples were titrated with Cu and the labile fraction extracted by the lasalocid SLM plotted against the total copper. The total copper in the original sample was determined after UV irradiation of the samples. Representative titrations of San Bruno Shoals and Dumbarton Bridge are presented in figure 5 and 6 Together with their Langmuir linearization linearization. The SLM labile fraction $[Cu_{slm}]$, determined on zero-addition aliquots was 0.9 nM and 2.5 nM for the Dumbarton Bridge and San Bruno shoals samples respectively. Thus the SLM measured 3% and 11% of the total copper as labile in the Dumbarton Bridge and San Bruno shoals sites respectively. The corresponding DPASV labile copper fractions were < 0.4% and < 0.9% for Dumbarton Bridge and San Bruno shoals respectively (Figure 5 and 6).

Donat et al. (1994) used four different analytical approaches to measure the dissolved copper speciation in South San Francisco Bay: (i) competitive ligand equilibration-cathodic stripping voltammetry [CLE-CSV], (ii) differential pulse anodic stripping voltammetry using a thin mercury film rotating glassy carbon disk electrode [DPASV(TMF-RGCDE)], (iii) DPASV using a hanging mercury drop electrode [DPASV(HMDE)], and (iv) chelating resin column partitioning-graphite furnace atomic absorption spectrometry [CRCP-GFAAS].
CLE-CSV technique, involves establishing a competitive equilibrium for the test metal ion between the natural organic metal-complexing ligands and a well-characterized reference ligand added to the sample in a set concentration analogous to the extractant in SLM. The amount of metal complexed by the added competing ligand is measured by CSV. That amount is related to both the concentrations and strengths of the added reference ligand and also the natural metal-complexing ligands originally in the sample
DPASV techniques and the CRCP-GFAAS method measure the equilibrium concentrations of free metal ions and labile metal complexes that dissociate to free metal ions during the analytical time scales of these methods. The metal fractions detected by DPASV techniques and CRCP-GFAAS includes the inorganic metal fraction (i.e. free hydrated ions and inorganic metal species e.g. $CuCO_3^0$) and, depending on the analytical time scale of the particular technique, may include a fraction of the relatively labile organic complexes as well.

The labile fraction in SLM is determined by the diffusion layer thickness, which

in turn is set by the operating flow rate. Thus lability in SLM can be tuned by changing the flow-rate. Tomaszewski et al. (2003) reported a diffusion layer thickness in the order of about 100 μm at a flow-rate of about 1ml/min using an SLM flow system similar to the one we used. This value is a bout 20 times that of a TMF-RGCDE) at a rotation speed of 5000-6000 RPM and ca. five times that of a hanging mercury drop electrode(Donat et al., 1994). The conditional stability constants and natural copper binding ligand concentrations calculated from Langmuir and Scatchard linearization of the titration data using SLM and DPASV-NCTMFE techniques are summarized in table 2.

The concentrations of the strong copper binding ligand L_1 for both samples were similar to those obtained by DPASV-NCTMFE. The better agreement in L1 values is expected since this class of ligands binds copper more strongly than L2 and therefore not likely to dissociate appreciably in the diffusion layer.
The corresponding conditional stability constants $K_1'_{cond, Cu^{2+}}$ (converted to $K_1'_{cond, Cu^{2+}}$ or $K_2'_{cond, Cu^{2+}}$ by multiplying $K_1'_{cond, Cuslm}$ or $K_2'_{cond, Cuslm}$ with $\alpha_{Cu'}$) were also lower than those determined by DPASV-NCTMFE. The SLM technique thus underestimated the strengths of both ligand classes compared to DPASV-NCTMFE.

Conclusion

We developed a supported liquid membrane (SLM) technique to determine free and labile copper in estuarine and coastal waters. This, rather than the total dissolved copper concentration, is the bioavailable fraction to phytoplankton. We found that between 90-97% of the total dissolved copper from two sampling stations located in South San Francisco Bay was bound to organic ligands, and therefore not "bioavailable" to phytoplankton. Thus only about 5-10% of the total dissolved copper existed as inorganic and/or labile organic copper species. The measurements were consistent with earlier copper speciation measurements that were made in South San Francisco Bay using electrochemical methods (Donat et al 1994).

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Tables

Sample site	Sampling Date	Salinity	pH	NO_3^-	HPO_4^{2-}	H_2SiO_3	$\log \alpha_{Cu}$
Dumbarton Bridge	March 2003	22.8	8.5	0	2.1	4.1	1.61
San Bruno Shoals	January 2003	22.9	7.7	35.4	4.9	96.1	0.94

Table 1: Salinity (0/00), pH and the corresponding $\log \alpha_{Cu}$, and nutrient data (μM) for January and March 2003 samplings in San Francisco Bay.

Site	Technique	Scratchad					Langmuir		[Cu] nM	
		$[L_T]$	$[L_1]$	$[L_2]$	$\log K_1$	$\log K_2$	$[L_T]$	$\log K_2$	$[Cu_T]$	labile Cu
Dumbarton Bridge	SLM	471	78	393	10.4	8.8	476	8.8	27	0.9
	DPASV	354	60	295	11.4	8.9	360	8.9	27	< 0.1
San Bruno Shoals	SLM	76	38	38	9.5	8.7	85	8.4	23	2.5
	DPASV	220	37	183	10.9	8.2	213	8.3	23	< 0.2

Table 2: Ligand concentrations, conditional stability constants and labile copper concentration calculated from Langmuir and Scratchard linearizations of the SLM and NFCTMF-DPASV copper titration data. The conditional stability constants $K'_{cond, Cu'}$ were converted to stability constants expressed with respect to Cu^{2+} ($K'_{cond, Cu^{2+}}$) by multiplying $K'_{cond, Cu'}$ by the inorganic side reaction coefficient α_{Cu}

Figure legends

Figure 1
Transport of copper across supported liquid membrane (SLM). (A) extractant mediated transport with lasalocid, dissolved in o-nitrophenyl octyl ether (NPOE) as the extractant ligand (b) lasalocid molecule. The hydrophobic copper-lasalocid complex formed at the interface diffuses through the organic membrane to the aqueous acceptor (pH =2) where the lasalocid molecule complexes with a proton and diffuses back through the membrane to the aqueous donor. There is a net transport of copper from the donor or sample side to the acceptor and reciprocal transport of protons on the other direction. The driving force is the pH gradient across the membrane. (c) passive diffusion transport where copper forms a neutral complex in the aqueous donor side with oxine that diffuses though the membrane. Copper is trapped on the acceptor side by forming a charged complex with EDTA

Figure 2
Automated set-up used for SLM copper extraction and membrane support unit. Final copper analysis of the SLM extract done by GFAAS.

Figure 3
SLM optimization experiments to investigate the effect of; (a) ionic strength (with dilution of UV-irradiated sea water), (b) initial sample copper concentration and (c) sample pH on copper recovery. Copper concentration in the ionic strength and sample pH experiments was 31 nM with a 30 min SLM extraction time. The SLM in all cases was 10 mM lasalocid in NPOE.

Figure 4
SLM extraction of 31 nM copper in UVSW spiked with increasing concentrations of EDTA (a) and humic acid (b). SLM conditions as in figure 3.

Figure 5
Copper titration of San Francisco Bay water samples. Titration of UVSW and a sample from Dumbarton Bridge station with a Langmuir transformation of the titration data.

Figure 6
Titration of San Bruno Shoals sample and Langmuir transform of the titration data.

Figures

A

B

Passive Diffusion

C

Figure 1

Recycled back
to sample bottle

recycle

waste

Membrane

GFAAS Analysis

Fraction
collector

acceptor
recycle

waste

Acceptor solution
(10 mM HNO$_3$)

M-Q H$_2$O

Sample

Computer control of
valve switching and pump

Peristaltic
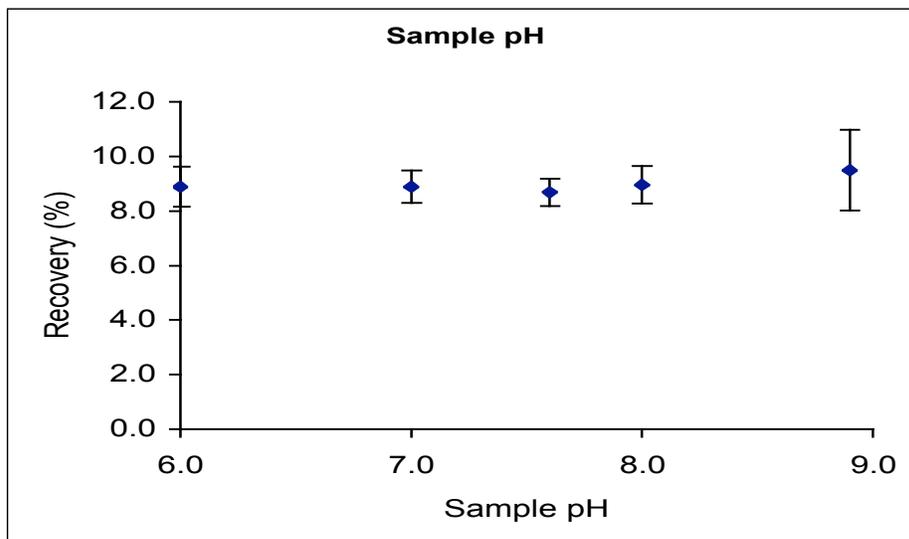pump
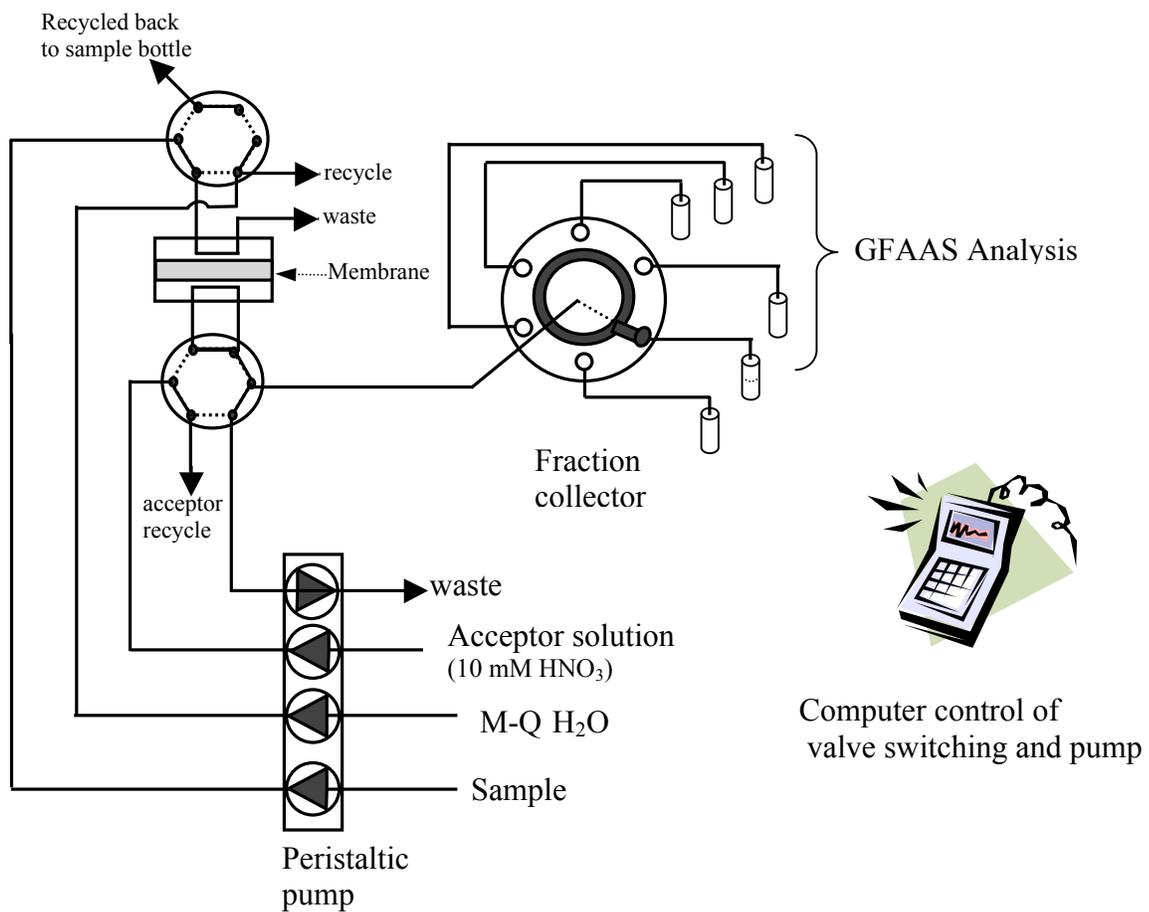
A: aluminium blocks

B: channelled PTFE
   blocks

C: PTFE membrane

A

B

C
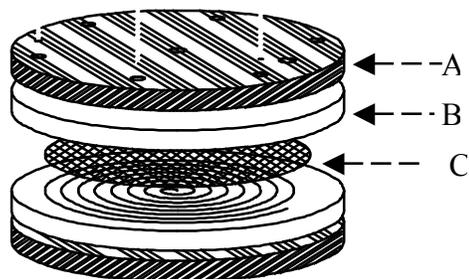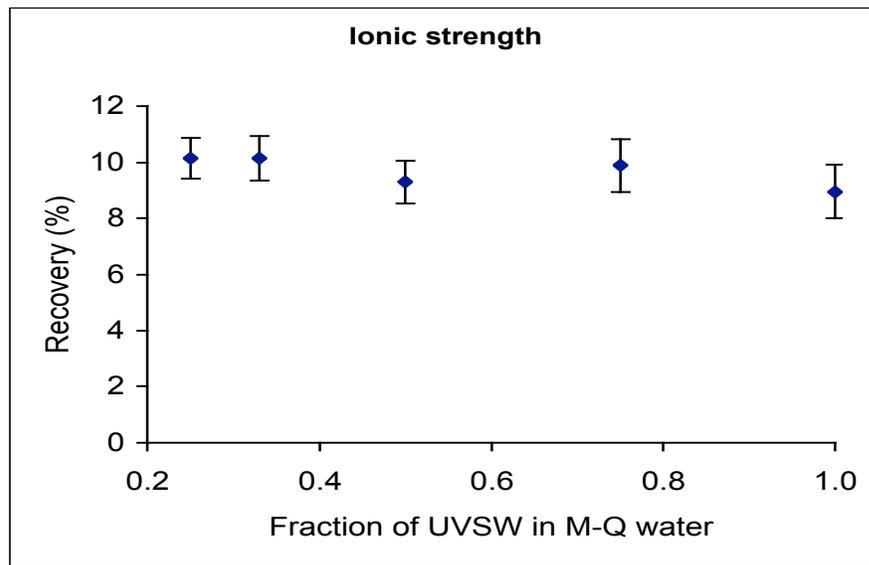
Figure 2

A
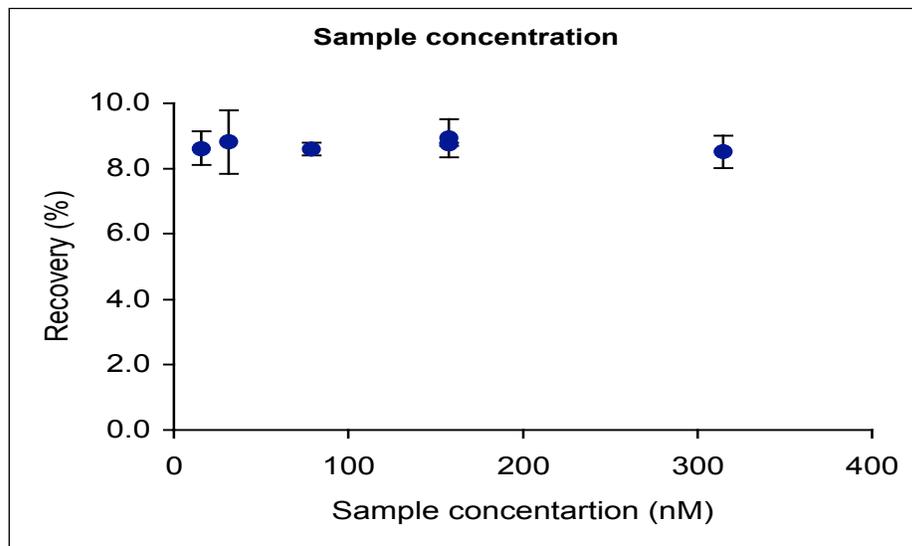
B

C

Figure 3
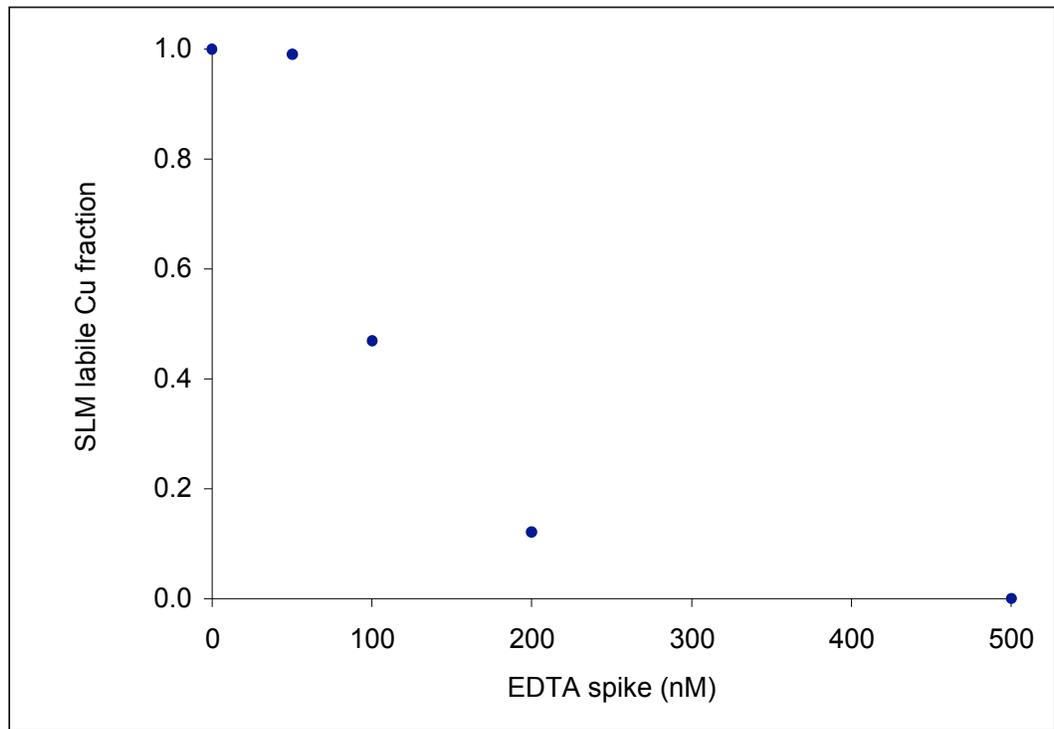
A
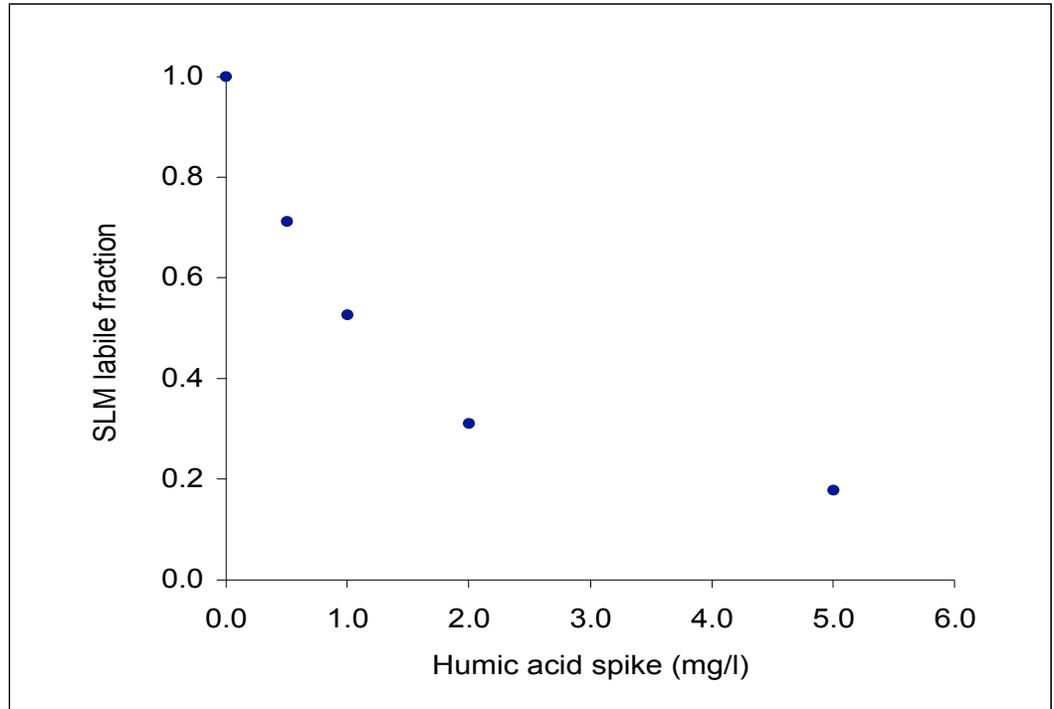
B
Figure 4

Figure 5

Figure 6