Figure 12. Comparison of effect of albumin on absorbance and derivation signals for bilirubin. Bilirubin concentration = 0.5 mg/L. a, Absorbance signal (456 nm). b, Derivative signal (483 nm). I, The ordinate is the ratio of the signal at a given albumin concentration to that extrapolated to zero albumin concentration

Bilirubin. Bilirubin is used as an example to illustrate potential advantages of the derivative method compared to more conventional absorbance measurements. Figures 10A and 10B include absorption and derivative spectra of bilirubin and albumin as well as similar spectra for a solution containing both species. It is observed that albumin contributes a significant absorbance at all wavelengths, but that the derivative spectra of the bilirubin and bilirubin-albumin mixtures (curves a and b in Figure 10B) are virtually superimposed on the long-wavelength side of the derivative spectrum. This suggests greater selectivity of the derivative signal for bilirubin over albumin than is possible with absorbance measurements. To evaluate this effect quantitatively, measurements were made on solutions containing the same bilirubin concentration and variable amounts of albumin. Results presented in Figure 12 indicate that the derivative signal is affected much less by the presence of albumin than is the absorbance signal.

Similar studies on the effects of hemoglobin and turbidity on bilirubin derivative spectra were carried out with the goal of determining bilirubin in biological fluids. Preliminary data for synthetic solutions were very promising. However, attempts to determine bilirubin in sera were unsuccessful because of significant variations in spectral properties of bilirubin in sera and in available standards. Because of the sensitivity of the derivative spectrometer to peak position, this instrument could be very useful in studies aimed at understanding these spectral changes.

Projections. Although a digital computer was used to compute absorbance derivatives from intensity and intensity derivative data, the operations in Equation 1 could be implemented with either analog or digital hardware to produce a "stand-alone" system. Also, derivative spectra can be computed from intensity data recorded in the conventional scanning mode (7). We are investigating these different approaches at this time and will report on the relative merits of each at a later date.

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Effect of Chloride Ion and Ionic Strength on the Response of a Copper(II) Ion-Selective Electrode

G. B. Oglesby, W. C. Duer,* and F. J. Millero

Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida 33149

The potentiometric response of the copper(II) ion-selective electrode (Ag₂S/CuS membrane) was investigated in aqueous solutions containing background electrolyte. For background electrolyte containing chloride, the electrode response, although linear with the logarithm of copper(II) activity, was not Nernstian. For background electrolyte which contained no chloride, the electrode response was Nernstian irrespective of background ionic strength.

The potentiometric response of the Ag$_2$S/CuS copper(II) ion-selective electrode has been the subject of some consideration in the literature (1–3). It has been shown that above

a certain copper(II) ion concentration, the electrode exhibits Nernstian response in binary aqueous solution (1, 3). It is also known that chloride ion in solution reduces the upper concentration limit of utility for the electrode because of a compositional change in the electrode's solid phase (1). Further, the electrode has been used to determine copper(II) in seawater although the response slope was larger than Nernstian (2). However, whether the increase in response slope is due solely to chloride ion or the accompanying increase in ionic strength has not been demonstrated.

It is the purpose of the present communication to document the response of the electrode in solutions which contain a single background electrolyte. To fulfill this purpose, the electrode responses were monitored as a function of ionic

strength for two background electrolytes: one containing chloride ion, NaCl; the other containing no chloride ion, KNO₃.

EXPERIMENTAL

Determination of the activity of the copper(II) ion in aqueous solutions were made with an Orion Model 94-29 Copper(II) Ion-Selective Electrode. An Orion Single Junction Ag/AgCl Electrode (Model 90-01) was used as the reference. The reference electrode was filled with Orion 90-00-01 filling solution (1 M KCl saturated with AgCl). An Orion Model 801 digital voltmeter was used to read potentials of 0.1 mV.

No special precautions were taken to minimize sorption of copper(II) on the reference electrode or contamination of the test solution with silver chloride. These phenomena did not manifest themselves in any obvious fashion.

Stock copper(II) solutions were prepared from the Baker Analyzed reagents: $CuCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Cu(NO_3) \cdot 3H_2O$. Electrogravimetric analyses (4) were performed on the stock copper sulfate and copper nitrate solutions, while gravimetric silver chloride analyses were performed on the stock copper chloride solution. The imprecision (one standard deviation) for all of these analyses was, at worst, 0.05%. Density measurements by employing a vibrating densimeter (5) were performed on each stock solution to enable the concentrations to be expressed in terms of molarity, M.

Millipore (18 MΩ) water with a total copper concentration of less than 10^{-9} M (as determined by a Perkin-Elmer 403 atomic absorption unit using a graphite furnace) was used to prepare all solutions. Trials comparing results with those obtained from triply distilled water and Millipore water showed no observable differences in potentials. All subsequent solutions were prepared volumetrically from standarized stock solution. Oxford micropipets (10 μL, 0.5 mL, 1 mL) were used to transfer aliquots of stock for dilution to the working molarities. All labware was made of Teflon or polyethylene and was carefully rinsed with nitric acid (metal free) and dried before use. Working solutions were prepared fresh daily and used within 4 h of preparation.

Beakers containing the solutions to be measured were submerged in a large isothermal bath and maintained at 25.00 ± 0.01 °C. Measurement sequences were arranged so that potentials of increasingly concentrated solutions were being read during a series of runs. A reading of constant potential ± 0.2 mV was taken to mean that the electrode had come to equilibrium—the precise times required for equilibration are in basic agreement with those of other workers (2, 3); the rise to the equilibrium value was monitored on a Heath-Schlumberger (Model EU-205-11) strip chart recorder. Stirring was carried out at a constant speed (approximately 150 rpm) which was not varied throughout the series of experiments. The bath was shielded in such a way that ambient light remained constant over the entire series of experiments.

For the measurements made in NaCl and KNO₃ media, known volumes of the working stock of fixed Cu(II) concentration were prepared. Successive volumetric additions of either NaCl or KNO₃ concentrated solutions prepared from Baker Analyzed reagents, were then made to increase the ionic strength from 0.1 to 0.9 in increments of roughly 0.1 unit. The equilibrium response was measured after each incremental addition.

RESULTS AND DISCUSSION

The electrode response is to the activity of the copper(II) ion. It is therefore necessary to relate the formal molar concentration, c, to ionic activity, a, by means of a single ion activity coefficient, γ:

$$a = \gamma \cdot c \qquad (1)$$

One of the weaknesses of the present approach is that nonthermodynamic assumptions are necessary to obtain single ion activity coefficients from available data on mean ionic activity coefficients of electrolytes. In this paper, single ion activity coefficients for Cu(II) were estimated using the ionic strength principle (6) in combination with the MacInnes convention (7) and the mean ionic activity approximation. The underlying assumption involved is that the activity coefficients of individual ions, at a particular ionic strength,

Table I. Electrode Response in Pure Water

Salt	Intercept, mV	Slope, mV/decade	Std dev, mV
Cu(NO₃)₂	267.9	30.0	0.8[a]
	272.0	30.7	1.8[b]
CuSO₄	264.9	29.4	0.6[a]
	274.8	31.3	2.5[b]
CuCl₂	265.1	29.5	0.2[a]

[a] Concentration range (2×10^{-7}–9×10^{-4}), activity range (2×10^{-7}–7×10^{-4}). [b] Concentration range (2×10^{-7}–2×10^{-1}), activity range (2×10^{-7}–1×10^{-1}).

Table II. Electrode Response in Pure Water over an Extended Period of Time (for concn range 1.7×10^{-7}–8.7×10^{-4})

Salt	Intercept, mV	Slope, mV/decade	Std dev, mV
Cu(NO₃)₂	267.9	30.0	0.8
	274.3	30.3	0.2
CuSO₄	264.9	29.4	0.6
	271.4	29.3	0.8
CuCl₂	265.5	29.4	0.2
	273.6	28.5	0.9

do not depend on the chemical nature of the specific salts that contribute to the ionic strength of the solution. Thus, in $CuSO_4$ solutions,

$$\gamma_{Cu} = \gamma^2_{CuSO_4}(\gamma^2_{KCl}/\gamma^3_{K_2SO_4}) \qquad (2)$$

in $CuCl_2$ solutions,

$$\gamma_{Cu} = \gamma^3_{CuCl_2}(1/\gamma^2_{KCl}) \qquad (3)$$

and in $Cu(NO_3)_2$ solutions,

$$\gamma_{Cu} = \gamma^3_{Cu(NO_3)_2}(\gamma^2_{KCl}/\gamma^4_{KNO_3}) \qquad (4)$$

where the mean activity coefficients for the salts were obtained from least square fits of the data presented by Robinson and Stokes (8) in an extended Debye–Hückel equation (9). Minor changes due to conversion of activity coefficients to the molar scale were taken into account.

Table I summarizes the results obtained in pure water. The experimental data were fit by a least squares technique in the form

$$E(mV) = E°(mV) + K \log_{10} a \qquad (5)$$

(the theoretical Nernst slope at 25 °C is 29.58 for the divalent copper ion). The $CuCl_2$ concentration both in pure water and in the NaCl and KNO₃ solutions were kept below 10^{-3} M in order to avoid Cl⁻ interference with the electrode response (1, 10). Table II is provided to demonstrate long term effects (over a 1-month period) on the electrode.

It may be seen that the response for all of the salts is Nernstian (i.e., in agreement with the Nernst law form) within experimental error. The poorer fit (as reflected in the increased standard deviation) over the wide concentration range is most likely due to the assumption made in calculating single ion activity coefficients at the higher ionic strength. Although there is a drift in the intercept (E°) with usage, the response remains Nernstian and, within experimental error, shows the Nernst slope to be constant. This long term drift in E° is in general agreement with the observation of Jasinski, Trachtenberg, and Andrychuk (2). Clearly, the electrode must be calibrated prior to each use if results obtained over extended periods of time are to be correlated.

The response of the electrode was studied next in NaCl solutions. In order to treat the experimental data in a consistent fashion at total ionic strengths, I, it was necessary

Table III. Electrode Response in Varying NaCl Background

I, ional	Response slope, mV/decade	
	CuSO$_4$	CuCl$_2$
0.1	33.5	27.6
0.15	35.9	31.4
0.2	37.6	34.9
0.4	45.1	. . .
0.6	46.2	42.6
0.7	46.6	44.4
0.8	47.1	46.0
0.9	47.6	. . .
1.0	47.7	49.2

to prepare least squares fits of log a vs. $I^{1/2}$ and E(measured) vs. $I^{1/2}$. Here I is taken as $^1/_2 \sum_i Z_i^2 c_i$ (where Z_i is the charge of the ith species), the sum being over both background electrolyte and the copper salt species. Both dependent variables, log a and E, yielded linear relationships with $I^{1/2}$ and were fit by first degree polynomials to better than 0.1% (standard deviation). This technique of generating a smooth set of E vs. log a at rounded ionic strength accounts for the varying Cu(II) concentration on dilution by the added increment of NaCl solution in addition to the increased ionic strength.

Table III presents the response slope determined at rounded ionic strengths from least squares fits of E vs. log a data sets. The slopes at I = 0.7 (46.6 for CuSO$_4$ and 44.3 for CuCl$_2$) agree quite well with the reported "Nernstian slopes" (2) in seawater solutions of approximately the same chloride molarity, which in the present case is essentially the same as I.

The question arises as to the origin of this variation of response slope with background electrolyte concentration. The approach to the problem taken in the present paper was to test the electrode in a background electrolyte to which the electrode material is insensitive; in this case, the electrolyte was KNO$_3$. The approach both experimentally and in data treatment was analogous to that in the NaCl medium. The electrode response could be examined over the full concentration range (of the pure water experiments) for Cu(NO$_3$)$_2$ and CuSO$_4$ since the Cl$^-$ interference was absent. There was a lower limit of activity at each ionic strength at which the electrode responses became erratic. This "cutoff" value is, in general, above the cutoff in pure water (pCu = 6.7) and possibly results from salting-in or an increased solubility of the electrode material.

Again, solutions of the three copper(II) salts, Cu(NO$_3$)$_2$, CuSO$_4$, and CuCl$_2$, were examined individually with KNO$_3$ as the background electrolyte. The responses for each copper(II) salt were obtained at seven concentrations of KNO$_3$ (I = 0.1 to 1.0).

Several interesting observations resulted from examination of the data. A plot of E vs. log a at fixed ionic strength yielded slopes between 28.6 and 31.2 for all three salts. There were no systematic changes in response slope with increasing I, in contrast to NaCl as the background electrolyte. Further, all results for one particular copper(II) salt could be combined without affecting regression error. Combining all of the data for a given salt in varying KNO$_3$ backgrounds yielded the following least square results:

Cu(NO$_3$)$_2$ $\quad E = 268.7 + 30.6 \log a$

$\qquad$ (std dev = 2.8) $\hfill$ (6)

CuSO$_4$ $\quad E = 279.4 + 29.2 \log a$

$\qquad$ (std dev = 1.3) $\hfill$ (7)

CuCl$_2$ $\quad E = 276.3 + 28.8 \log a$

$\qquad$ (std dev = 0.7) $\hfill$ (8)

The uncertainties in the fits represented by Equations 6, 7, and 8 are virtually indistinguishable from the results with no background electrolyte (Table I). Thus, at each ionic strength within the range 0.1 to 1.0, the measured results are in agreement with the theoretical Nernst equation inasmuch as they exhibit slopes near 29.6.

In conclusion, the variation of the response slope with background electrolyte observed in NaCl solutions, is seen to result from the presence of chloride ion rather than to the ionic strength variations. The increase in the observed slope with increasing chloride concentration may be partially rationalized by considering the complexation of copper(II) ion by chloride ion. However, the range of estimated complexation constants (11) prohibits quantization of this effect.

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