

COPPER COMPLEXING CAPACITY OF SEAWATER: A CRITICAL APPRAISAL OF THE DIRECT ASV METHOD

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ABSTRACT

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The break-point in the Cu(II) titration curve obtained by anodic stripping voltammetry (ASV) is usually attributed to the formation of strong organo-copper complexes. This interpretation is the basis for the direct determination by ASV of the copper complexing capacity of natural waters. The present study suggests, however, that the break in the Cu(II) titration curve in seawater, as determined by ASV, could be due to the formation of a Zn-Cu intermetallic compound (IC) formed in the mercury during the deposition stage of the ASV procedure.

INTRODUCTION

It is generally accepted that copper is toxic to a diverse range of biological systems, even in minute quantities. Steeman Nielsen and Wium-Anderson (1970) have shown, for example, that even copper ion concentrations in the range common for natural waters can be toxic to phytoplankton. It has further been shown by Davey et al. (1973) that copper toxicity is apparently related to the concentration of free copper ion, as chelating agents detoxify copper-spiked seawater. This conclusion was subsequently confirmed by Jackson and Morgan (1978) who used a chemical speciation model to estimate the concentration of free copper ions in the experiments of Davey et al. (1973).

If toxicity is indeed related to the concentration of uncomplexed copper ions, natural chelating and adsorbing agents can play an important role in detoxifying the waters even if the total (free and complexed) concentration of the copper ion is relatively high. Thus, many investigators apply the parameter: Apparent Complexing Capacity (ACC) to quantify the total amount of copper (or other heavy metal ions) that will be complexed, and hence rendered non-toxic, by the chelating agents present in the water. It should be emphasized, though, that the ACC parameter is an operational parameter and as such is incapable of characterizing the forms in which copper (or any other metal) is present in the waters (Florence and Batley, 1977).

Virtually all methods for estimating the ACC are based on procedures in which the chelating agents are saturated with the heavy metal ions by spiking the sample with the metal. The ACC is then estimated either by separating the organo-metal complexes or by estimating the concentration of excess free-metal ion. Ultrafiltration (Smith, 1976) and gel filtration (Sugai and Healy, 1978) were used to estimate the ACC of various molecular weight fractions of dissolved organic matter in estuarine and interstitial water of marine sediments. Chelex resin was used by Stolzberg and Rosin (1977). A similar method was used by Figura and McDuffie (1979) to determine the amounts of Zn, Cd, Pb and Cu that were retained by various organic chelates in 0.02 M Tris pH 7.8 buffer. Hanck and Dillard (1977), applied the fact that Co(III) forms a stable organo-metal complex to estimate the Co(II) ACC. This was accomplished by oxidizing the Co(II) complexes formed by spiking the sample with Co(II). Davey et al. (1973) estimated copper ACC in artificial seawater by measuring the effect of copper addition on the growth rate of the diatom *Thalassiosira pseudonana* and assuming that the growth rate is inversely proportional to the concentration of uncomplexed copper ions.

Anodic stripping voltammetry (ASV) permits direct determination of the ACC as opposed to the indirect methods mentioned above. The method relies on the assumption, which is supported by numerous studies (i.e. Chan and Lum-Shue-Chan, 1974, and many others as reviewed by Whitfield, 1975) that the ASV response is related only to labile ionic species. The ACC can thus be determined by plotting the ASV peak current (I_p) versus the concentration of the added metal (Fig. 1). The sharp break-point of this curve is interpreted as the titration endpoint of the strong ligands. Hence, the added-metal concentration necessary to reach this break-point is, by definition, the ACC. This method has been used to determine copper ACC in synthetic solutions (O'Shea and Mancy, 1976; Figura and McDuffie, 1979), natural freshwater (Chau et al., 1974) and seawater (Whitfield, 1975; Batley and Florence, 1976; Duinker and Kramer, 1977; Florence and Batley, 1977;

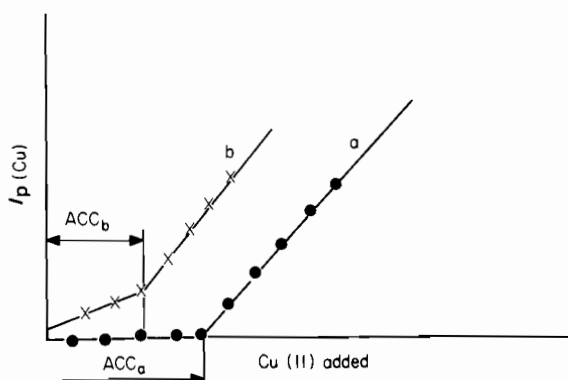


Fig. 1. Schematic representation of two types of ASV copper titration curves: type (a) curve was reported by O'Shea and Mancy (1976) and Figura and McDuffie (1979); type (b) curve was reported by Chau et al. (1974) and Duinker and Kramer (1977).

Gurtisen et al., 1977). The direct ASV method, however, is apparently subject to a number of errors. Brezonic et al. (1976) pointed out, for example, that a major error source of this method is an interference by surface-active compounds sorbed to the electrode. Since these organic compounds also affect the ACC of the sample, the results could be erroneous.

Copper is preferred as a spiking agent in most ACC studies for several reasons:

- (1) copper ACC is an important parameter in many biological systems;
- (2) a submicromolar concentration of copper forms strong organic complexes even in the presence of competing major cations (Reuter and Perdue, 1977; Jackson and Morgan, 1978);
- (3) heavy metals which form stronger complexes than copper will hydrolyze at the pH of seawater and subsequently form strong chloride complexes (Stumm and Morgan, 1970);
- (4) copper is easily determined by various analytical methods.

The underlying assumption of the direct ASV method for the determination of copper ACC is that the break in the copper titration curve (Fig. 1) marks the ligands' titration endpoint. Indeed, a number of studies demonstrated that a characteristic break-point is obtained when the solutions are prepared with organic compounds which are known to complex heavy metals (O'Shea and Mancy, 1976). This, however, does not exclude the possibility that such a break-point in the titration curve (Fig. 1) is caused by yet another process not related to the metal complexing capacity of the waters. Should this be the case, it might lead to an erroneous result when determining copper ACC by the ASV method.

In this study, the break-point of the copper titration curve in seawater is explained by the formation of Zn-Cu intermetallic compounds (IC). These compounds are known to form in the mercury during the deposition stage of the ASV procedure (Kemula et al., 1958) and may give heavy interference. For example, application of the standard addition method for calibrating the Zn(II) response of ASV analysis could be erroneous if Cu(II) is also present in solution (Copeland et al., 1974). Furthermore, it will be shown that Zn-Cu IC formation will cause a break point in the copper titration curve in seawater, even when deposition potentials are strongly anodic with respect to Zn(II). This being the case, extreme caution must be exercised when attempting to estimate copper ACC of seawater by the direct ASV method.

EXPERIMENTAL

Instrumentation

Measurements were made with a Ben Gurion University (BGU) Model EI224 Polarographic Analyzer developed and built at the Department of Electrical Engineering of BGU. The analytical mode used in this study was differential pulse anodic stripping voltammetry (DPASV). In this mode the

polarographic analyzer uses a staircase type waveform on which a pulse is superimposed (Lazar and Ben-Yaakov, 1980). A Packard Auto Gamma Scintillation Spectrometer (Model 578) was used to count ^{65}Zn -spiked samples.

Electrodes and Cell

Measurements were carried out in a 100 ml Teflon beaker fitted with a Teflon cover through which the electrodes and the tube carrying the purging gas (CO_2) were inserted. The solution was stirred with a Teflon-coated stirring bar. A Radiometer type K-401 saturated calomel electrode equipped with a Coleman fiber-liquid junction (Perkin Elmer C003-0702) filled with KCl (saturated) served as a reference electrode and a coiled platinum wire was used as an auxiliary electrode. The working electrode was a thin mercury-film electrode (TMFE) deposited on glassy carbon (GC) 3 mm in diameter (Rignsdorf Werke S-10). A detailed description of the working electrode is presented elsewhere (Lazar and Ben-Yaakov, 1980). The ASV analysis of ^{65}Zn -spiked samples were done in the scintillation vials.

Reagents and Solutions

Gulf of Elat (Red Sea) seawater was sampled and stored in 15 l containers which had been cleaned with 1N HNO_3 and rinsed several times with the sampled water. Copper, zinc and cadmium spiking solutions were prepared from certified atomic absorption standard reference solutions (Fisher Scientific Company, Fair Lawn, NJ, U.S.A.). The mercury plating solution was approximately $2 \times 10^{-5}\text{ M}$ mercuric nitrate prepared by dissolving the salt (Baker Analyzed Reagent) in seawater. Commercial CO_2 gas, treated with vanadous chloride solution, was used to purge the sample during the entire analysis cycle. The ^{65}Zn solution had an activity of $0.1\ \mu\text{Ci}$, and was prepared from 3 mCi stock solution (The Radiochemical Centre Ltd., Amersham, Gt. Britain).

Procedures

Metal Titration Procedures

The teflon cover carrying the electrodes was transferred to a 100 ml teflon beaker containing the Hg plating solution and a -1400 mV potential was applied to the working electrode (W.E.) for 20 min. Following the plating time, the potential was switched to -100 mV for another 3 min to strip any trace metal deposited during plating. The electrodes were then rinsed with double-distilled water and then placed in the tested solution (stored seawater) which was spiked with 12.5 ppb Cd(II) for a reproducibility check. The solution was then deaerated for 7 min after which the ASV cycle was begun.

The analysis consisted of 5 steps: (1) application of deposition potential (-1400 mV or -800 mV) to the W.E. in a stirred solution for 90 s; (2) rest

period of 30 s in unstirred solution at the deposition potential; (3) scanning the deposition potential to -100 mV (E pulse = 80 mV, E step = 10 mV, $T = 640$ ms) and recording the current; (4) addition of a known amount of Cu(II) spike by a micropipette; (5) stripping the W.E. for 30 s at -100 mV in a stirred solution. Cu was added in each experiment until the Cu/Zn concentration ratio in the solution was greater than 2.

⁶⁵Zn deposition experiments

A 5 min deposition step at -700 mV was carried out in a 100 ml beaker filled with seawater which had been spiked with $0.1 \mu\text{Ci } ^{65}\text{Zn}$. The three-electrode assembly, especially designed to fit a scintillation vial, and the stripping gas tube were then transferred to a scintillation vial filled with 15 ml of $1 M \text{KNO}_3$. The stripping stage was carried out for 2 min at a potential of -100 mV while the CO_2 bubbling served as the stirring agent. Blank estimation was obtained by a similar procedure except that the W.E. was disconnected from the potentiostat during the deposition stage.

RESULTS AND DISCUSSION

Experiments carried out with ⁶⁵Zn (Table I) reveal that Zn(II) will deposit at -700 mV and that the efficiency of this undervoltage deposition is about 30% with respect to deposition at -1400 mV. It is thus evident that Zn—Cu IC compounds form even if the deposition potential is anodic with respect to Zn(II), as proposed earlier for Zn(II) (see Haenny and Mivelaz, 1948; Haisinsky and Coch, 1949).

Copper titration curves for experiments which were run at -1400 mV deposition potential, are composed of two linear segments with a break-point at a given Cu(II) addition (Fig. 2). The position of the break point is a

TABLE I

Summary of ⁶⁵Zn ASV experiments

Experimental conditions	Counts/5 min	Repetitions
$0.1 \mu\text{Ci } ^{65}\text{Zn}$	$38423 \pm 155^*$	8
Vial with 15 ml $1 M \text{KNO}_3$	1416 ± 9	5
5-min immersion of disconnected W.E. in $0.1 \mu\text{Ci } ^{65}\text{Zn}$ followed by stripping in $1 M \text{KNO}_3$	3086 ± 73	5
Deposition at -1400 mV in $0.1 \mu\text{Ci } ^{65}\text{Zn}$ followed by stripping in $1 M \text{KNO}_3$	6782 ± 685	10
Deposition at -700 mV in $0.1 \mu\text{Ci } ^{65}\text{Zn}$ followed by stripping in $1 M \text{KNO}_3$	4080 ± 286	9

* Counted for 30 s only.

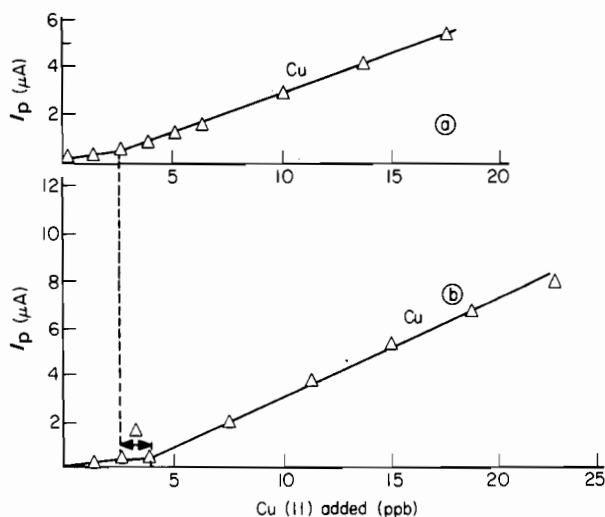


Fig. 2. Cu(II) titration experiments on stored seawater with a deposition potential of -1400 mV: (a) titration of original seawater; and (b) after addition of 6.25 ppb Zn(II). The break-point in the titration curve is shifted to the right (Δ) as Zn(II) concentration is increased.

function of the initial concentration of Zn(II) in the seawater (Fig. 2a, b). A similar phenomenon is observed with a deposition potential which is positive with respect to Zn(II) (Fig. 3). Regression analysis on the ASV data obtained with a deposition potential of -800 mV (Table II) seem to rule out the possibility that the breaks only represent data scatter. A summary of the analysis is given in Table III.

The breaks in the titration curves, for both -1400 mV and -800 mV deposition potentials, are clearly related to the initial concentration of Zn(II) in solution. An increase in the initial concentration of Zn(II) results in a shift in the break point toward a higher concentration of Cu(II) addition. The possibility that the shift in the break point was caused by contamination with chelating agents when Zn(II) was added or by electrode sorbing of surface acting agents (Brezonic et al., 1976) should be ruled out considering the good reproducibility of the I_p (Cd) in the range of $\pm 5\%$ throughout the experiments. Organo-metal complexation and surface sorbing should also affect Cd(II) (Brezonic et al., 1976). Hence, the stability of I_p (Cd) supports the conjecture that the shift in the copper titration curve is not related to a change in the concentration of chelating agents but to Zn-Cu IC formation in the amalgam which is an artifact of the ASV procedure.

The fact that an increase in Zn(II) concentration in seawater causes a shift to the right in the break point of the copper titration curves (Figs. 2 and 3) also seems to be in conflict with the assumption that the break point is related to copper ACC. The present experimental results seem to suggest that an increase in Zn(II) concentration increases the copper ACC. This, however, is

TABLE II

Experimental data of ASV on original and modified Mediterranean Sea waters (deposition potential: -800 mV)

Cu(II) added	ASV Copper peak current, I_p (Cu) (μ A)									
	Original seawater					Seawater spiked with 30 ppb Zn(II)				
	Average					Average				
0	0.198	0.201	0.201	0.200	(± 0.002)	0.151	0.137	0.160	0.149	(± 0.015)
1	0.324	0.322	0.314	0.320	(± 0.007)	0.260	0.280	0.248	0.263	(± 0.016)
2	0.505	0.535	0.520	0.520	(± 0.020)	0.335	0.372	0.355	0.375	(± 0.020)
3	0.790	0.810		0.800	(± 0.015)	0.508	0.538	0.493	0.513	(± 0.025)
4	1.070	1.040	1.040	1.050	(± 0.016)	0.818	0.775	0.756	0.783	(± 0.032)
5	1.245	1.235	1.268	1.249	(± 0.017)	0.957	0.876	0.868	0.900	(± 0.051)
6	1.525	1.486	1.490	1.500	(± 0.021)	1.388	1.328		1.358	(± 0.042)
7	1.720	1.765	1.765	1.750	(± 0.022)	1.662	1.608		1.635	(± 0.037)
8						1.878	1.845	1.837	1.853	(± 0.026)
9	2.255	2.230	2.650	2.250	(± 0.025)	2.248	2.208	2.220	2.225	(± 0.022)

unreasonable since any additional Zn(II) should neutralize some of the chelating agents and hence decrease the copper ACC.

The data presented here suggest that Zn—Cu IC which apparently forms in the amalgam during the deposition stage of ASV may cause a break in the copper titration curves. The break is interpreted as a transition between the region in which the amalgam is rich in the IC compound and a region in which the amalgam mainly consists of copper and relatively little IC. The stripping current peak I_p (Cu) is evidently related to both the oxidation of copper and the IC off the amalgam. The current contribution due to the IC oxidation is apparently lower than the current contribution of pure copper in the amalgam. Consequently, when Cu(II) concentration is relatively low with respect to Zn(II) concentration the amalgam is rich in IC and the titration slope is low. A steeper slope is obtained when Cu(II) concentration is higher than Zn(II) concentration, i.e. when most of the copper in the amalgam is in the free form. This model was investigated recently by computer simulation of the ASV (Lazar, Nishri and Ben-Yaakov, in preparation) process. The good agreement between the experimental and simulated titration curves, which were obtained in that study, seem to support the conjecture that the break-points in copper titration curves are due to Zn—Cu IC formation.

The magnitude of the ratio of the two slopes in each copper titration experiment can perhaps be used as an indicator for distinguishing between breaks which are caused by ACC and those which may be due to IC formation. The ratios in the present seawater experiments for deposition potentials of -1400 mV and -800 mV are 4.5 and 2.2, respectively. On the other hand, the data of Chau et al. (1974) reveal a ratio of 11 for lake water when using a deposition potential anodic to Zn(II). The high ratio of the slope results in

TABLE III

Summary of regression analysis on ASV data given in Table II and Fig. 3

Data points used	Regression line ^a	Correlation coefficient (r^2)	χ^2 test and corresponding confidence limits	Calculated original Cu(II) concentration (ppb)
Original Mediterranean seawater				
All	$I_p(\text{Cu}) = 0.136 + 0.0229 \times C_{\text{Cu}}$	0.99562	47.74 (<0.5%)	(0.59) ^c
Last seven	$I_p(\text{Cu}) = 0.054 + 0.243 \times C_{\text{Cu}}$	0.99917	2.13 ^b (>97.5%)	(0.22) ^c
First two	$I_p(\text{Cu}) = 0.200 + 0.120 \times C_{\text{Cu}}$	0.99714		1.19
Mediterranean seawater spiked with 30 ppb Zn(II)				
All	$I_p(\text{Cu}) = 0.014 + 0.229 \times C_{\text{Cu}}$	0.97639	1408.74 ($\leq 0.5\%$)	$(6.11 \times 10^{-5})^c$
Last five	$I_p(\text{Cu}) = -0.344 + 0.282 \times C_{\text{Cu}}$	0.99616	2.86 ^b (>90%)	$(-1.22)^c$
First four	$I_p(\text{Cu}) = 0.146 + 0.120 \times C_{\text{Cu}}$	0.99797		1.22

^a Regression lines calculated from average values of Table II.^b Calculated for the data points of both lines.^c Incorrect values due to the use of incorrect extrapolation lines.

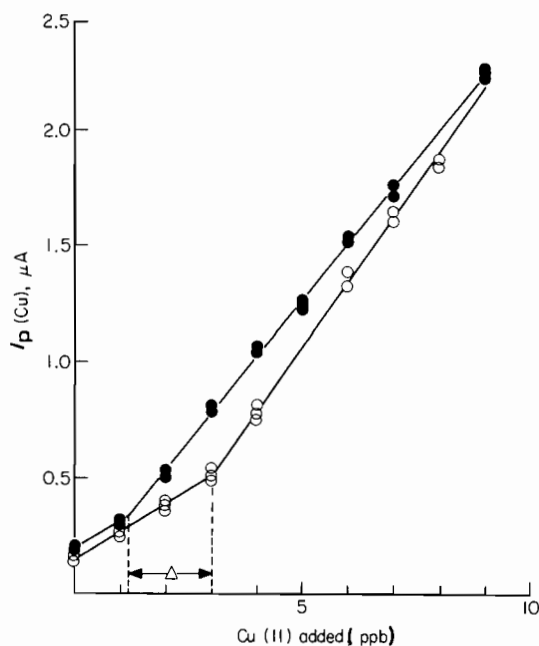


Fig. 3. Cu(II) titration experiments on stored seawater with a deposition potential -800 mV (data from Table II): (a) titration of original seawater; and (b) after addition of 30 ppb Zn(II). Note the shift in break-point (Δ) as Zn(II) concentration is increased.

a titration curve which is similar in shape to Fig. 2a whereas the lower ratio corresponds to a shape similar to Fig. 2b. The very large difference between the ratio obtained here (2.2) and the one derived from Chau et al. (1975) data (11) seems to suggest that, unlike in the present case, the data of Chau et al. apparently reflect copper ACC. However, since the slope ratio derived from the data of Duinker and Kramer (1977) for seawater is only ~ 2 , the break in their copper titration curve may not reflect copper ACC as interpreted by them.

CONCLUSIONS

The characteristic break-point in the $I_p(\text{Cu})$ curve vs. Cu(II) addition could be caused by Zn-Cu IC formation in the amalgam during the deposition stage of the ASV analysis. Since Zn(II) is plated out, even at deposition potentials which are anodic with respect to Zn(II), Zn-Cu IC could form at these deposition potentials and may cause a break in the copper titration curve.

The ratio between the two slopes of the titration curve may serve as an indicator for the cause of the break, low ratios suggesting an IC origin, whereas high ratios are probably due to ACC.

Direct ASV analysis can probably be used to determine copper ACC in lake water, but the method may not be valid for normal seawater.

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