UNC-WRRI-73-85

Determination of the Complexing Capacity of Natural Water

by

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The work upon which this publication is based was supported in part by funds provided by the Office of Water Resources Research, Department of the Interior, through the Water Resources Research Institute of the University of North Carolina as authorized under the Water Resources Research Act of 1964.

Project No. A-052-NC
Agreement No. 14-31-0001-3533

December 1973

ACKNOWLEDGMENTS

The Water Resources Research Institute of the University of North Carolina provided the bulk of the support for this work through a grant (Project A-052-NC) from its FY 1972 annual allotment fund. An extension of one year granted by the Institute allowed the project to develop to its present stage. During FY 1973 partial support was received from the research funds of the Chemistry Department of North Carolina State University.

Mr. James W. Dillard carried out the bulk of the experimental work associated with this project. He is seeking a Ph.D. in Analytical Chemistry; portions of the data in this report will also appear in Mr. Dillard's thesis. Mr. Dillard was supported for one year by WRRI Project A-052-NC. Sara Joyner Singer was employed as an undergraduate technician during the first year of the project.

The cooperation of David H. Howells, Director of the Water Resources Research Institute and the members of his staff particularly Mrs. Linda Kiger are gratefully acknowledged. Mrs. Sally Hobby, School of Physical and Mathematical Sciences, kept an accounting of the project finances. The staff of the Office of Water and Air Resources of the State of North Carolina was most cooperative and helpful in the process of selecting suitable sampling sites for this project.

Joyce Weatherspoon, Department of Chemistry, provided secretarial service for the project and typed the project report.

ABSTRACT

The role of natural complexing agents in the transport, toxicity and availability of trace metal ions has been the subject of considerable speculation in recent years. To more directly study these questions a reliable analytical procedure for measuring the complexing capacity of natural water is needed. The development and testing of such a procedure has been the major objective of this project.

Three methods were evaluated both theoretically and experimentally. Solutions of ethylenediaminetetraacetic acid, a synthetic complexing agent, were used to evaluate the sensitivity, accuracy and precision of the methods. Two methods were based on the concept of a micro scale complexometric titration using anodic stripping voltammmetry to locate the endpoint of the titration. Although very sensitive both methods were rejected in favor of a method involving the chemical conversion of the ligands in the sample to the corresponding cobalt(III) complex. Differential pulse polarography was used to monitor the decrease in cobalt(II) concentration after production of the cobalt(III) complexes and hence the complexing capacity of the sample.

The recommended procedure has a detection limit of 0.6×10^{-6} moles of ligand/liter. A relative error of 5% or less may be expected at the micromolar level. Ligands forming 1:1 complexes will be quantitatively determined if the formation constant of their Co(III) complex exceeds 10^{17}.

A sampling network was setup on Crabtree Creek, Walnut Creek and the Neuse River near Raleigh. The results indicate that complexing capacity is approximately 10^{-6} moles of ligand/liter. Several key trends were observed. Complexing capacity more than doubled in Walnut Creek after effluent from

Raleigh's sewage treatment plant was discharged into the creek. The complexing capacity of Crabtree Creek increases dramatically after entering the environs of Raleigh. Along the Neuse River, complexing capacity was observed to decrease during the 20 mile flow from the Falls of the Neuse to SR 1007. Whether this phenomenon is due to natural biodegradation/complexation of ligands or merely a dilution effect as the Neuse increases in volume could not be determined. Further work on the factors influencing complexing capacity is needed.

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SUMMARY and CONCLUSIONS

Three methods for the determination of the complexing capacity of natural water were examined. Tests using known samples of EDTA, a synthetic complexing agent, were used to determine the sensitivity, accuracy and precision of each method.

Method 1. A procedure in which the course of a micro-complexometric titration of natural water is followed with anodic stripping voltammetry was found, on theoretical grounds, to be quite satisfactory provided the metal ion titrant forms a complex with the natural ligands having a formation constant greater than 10^{10}. ASV cannot accurately distinguish between uncomplexed metal ion titrant and metal complex if the formation constant of the complex is less than 10^{10}. This method proved experimentally unsatisfactory because of systematically low results. The cause of this error was found to be complex dissociation during the time required to estimate the uncomplexed metal present using ASV.

Method 2. This method was also based on a complexometric titration but care was taken to minimize complex dissociation. A dual cell, temperature controlled apparatus was constructed in order to permit ASV determinations after a 50 second electrolysis at 0°C. The short electrolysis time and low temperature reduced the effect of complex dissociation significantly. In order for additional portions of metal ion titrant to react with the sample, it was necessary to warm the solution to 25°C. The cycling between 25°C for additions of titrant and 0°C for ASV runs increased the time of analysis beyond that felt to be practical.

Method 3. This method was not a titration but a chemical conversion of the ligands present in the sample to the corresponding cobalt(III) complex. The amount of cobalt(II) which remained unreacted was determined by differential pulse polarography. Subtraction of the amount of cobalt(II) left after the reaction from that initially added yielded the amount complexed by ligands in the sample. Since cobalt(III) complexes dissociate at a very slow rate, the kinetic problems of the type observed when using methods 1 and 2 were not observed with method 3.

Method 3 has been recommended as a satisfactory analytical method for measuring complexing capacity. The limit of detection was found to be 0.6 μmoles/liter. No serious interferences were found. A relative error of 5% or less may be expected at the micromolar level. Theoretical computations indicate that a ligand which forms a 1:1 complex with Co(III) will be quantitatively determined at the micromolar level if the formation constant of the corresponding cobalt(III) complex is 10^{17} or greater.

Natural Water Samples. Eight sampling stations in the vicinity of Raleigh were set up during the summer of 1973. Based on analysis of these samples, we have concluded that complexing capacity is a measurable parameter of water quality which increases dramatically in areas near discharge points from sewage treatment facilities. Complexing capacity also seems to be higher in regions of light industrial or commercial usage than in protected areas.

It was observed that the complexing capacity of the Neuse River decreased as the river flowed past Raleigh. Whether this phenomenon is caused by natural biodegradation/complexation or is merely the result of dilution of the

Neuse by relatively pure tributaries could not be determined. The results of our limited sampling program indicated a need for a year long program of sample collection and analysis to enable comparisons to be made between complexing capacity and other parameters of water quality such as TOC and trace metal content.

RECOMMENDATIONS

1. The procedure for estimating complexing capacity be made available to all scientists engaged in studies of trace metal transport, toxicity or availability. This should include those studying fresh water, sea water and the soil solution.

2. A sampling/analysis program of at least a years duration should be carried out to probe further the role of complexing capacity in determining water quality. Such a study should include a site near the discharge of secondary sewage effluent. The study should seek to establish a "material balance" of complexing capacity by monitoring the tributaries of the sampling area. Correlation of complexing capacity with trace metal content and TOC should be attempted.

3. Further refinement and improvement in the recommended analytical method should be encouraged with the eventual goal of a detection limit of 0.01 micromolar with the polarographic technique and/or an atomic absorption procedure applicable at the micromolar level. The interaction between the Co(II) reagent and ligands already bound to a metal ion should be explored both experimentally and theoretically.

INTRODUCTION

The role of complexing agents in natural water has recently become an important topic for discussion and research. Four symposiums dealing with organic matter in aquatic environments have been held in the past 5 years; all of these conferences included one or more papers on complexation by organic matter. Relatively little work has been done on the importance of complexation in determining the quality of fresh water; work in marine systems and in the soil solution, on the other hand, has progressed to the point of identifying the organic functional groups responsible for natural complexation and testing the use of synthetic complexing agents to improve the fertility of soil and sea water (Shapiro, 1964; Barber et al., 1971; Schnitzer, 1971; Siegel, 1971).

Organic ligands are known to form soluble complexes with a number of metals. They may in fact be an important mechanism for solubilizing potentially dangerous trace metals. It has been experimentally demonstrated that ppm quantities of NTA can leach significant amounts of lead from lake sediment (Gregor, 1972). Chau (1973) observed that complexing agents decreased the toxicity of copper and concluded that a knowledge of complexing capacity was essential in order to establish meaningful safe levels of trace metals such as copper, lead, cadmium and zinc in lake water.

Most of the information which is available on the levels of complexing agents present in natural water is of the one of a kind variety; a carefully controlled survey has not been performed. Studies seeking a correlation between complexing capacity and trace metal levels have not appeared; relatively little is known of the chemical forms of trace metals in natural water. Siegel (1971) observed that " . . . only rarely has a body of water

been examined with some hope of seeing the microstructure of the trace metals present; the chief reason for this state of affairs has been the lack of a simple, convenient and widely applicable tool which could distinguish between the various possible species of a metal ion."

One of the goals of this project was to develop a reliable analytical procedure capable of distinguishing between metal ions which are complexed and metal ions merely solvated by water molecules. We sought to apply this method to the determination of the complexing capacity of natural water and finally to determine whether the complexing capacity of North Carolina's natural water has been affected by man's use of water.

Analytical Methods for Determining Complexing Agents. The basic principle of most published procedures for determination of complexing agents is the measurement of the amount of metal complex which results when an excess of a metal is added to the sample. Several metals have been used to form the complex; the method employed to quantitate the amount of metal complex present and the chemical properties of the metal ion used are important keys to the applicability of existing procedures for the determination of complexing agents in natural water.

Thompson and Duthie (1968) proposed a spectrophotometric procedure based on measuring the absorbance of Zn zinconate. A variation of their technique has been adopted as a standard method for determining NTA in water (E.P.A., 1971). Several spectrophotometric procedures for the determination of EDTA at trace levels have been reported (Menis et al., 1956; Knight and Osteryoung, 1958; Roubalova and Dolezal, 1960; Kratochvil and White, 1965; Huber and Tallant, 1968); presumably these procedures could

be modified so as to determine any complexing agent. Interference from sample turbidity would seem to limit the usefulness of spectrophotometry as a tool in quantitating the concentration of metal complexes in natural water.

A number of electrochemical techniques have been used to determine trace levels of complexing agents. LeBlanc (1959) measured the concentration of $CdNTA^-$ and $CdEDTA^{2-}$ polarographically in order to determine NTA and EDTA at the millimolar level. Asplund and Wanninen (1971) also utilized cadmium complexes and DC polarography; their method was satisfactory for NTA at the 1-10 ppm level. Haberman (1971) used indium to form complexes and linear sweep voltammetry to quantitate the results; if preconcentrated by anion exchange, 0.02 ppm of NTA could be determined. Afghan and Goulden (1971) used dual cell cathode-ray polarography to determine NTA at the 10μgm/liter level; both lead and bismuth were investigated as metals for forming NTA complexes. An automated method employing a similar technique has been described (Afghan, Goulden and Ryan, 1972).

Matson and coworkers (Allen, 1970; Bender, 1970) were first to employ anodic stripping voltammetry to estimate complexing capacity. This technique is a very sensitive one and unlike previous methods measures the amount of uncomplexed metal present in the sample. Detection limits at the micromolar level were reported using Cu^{2+} to form the complexes. Chau (1973) employed a similar method but stressed the importance of allowing sufficient time (at least 2 hr.) for the complexation reactions to reach equilibrium before measuring the amount of free copper present. Ion selective electrodes have also been employed in determining complexing agents (Rechnitz and Kenny, 1970; Blay and Ryland, 1971; van't Riet and Wynn, 1969).

Several trace metals are known to catalyze the rate of chemical reactions; complexation of these metals reduces their catalytic effect. Several kinetic procedures sensitive in the sub-micromolar range which are based on the measurement of the decrease in reaction rate in the presence of complexing agents are available (Mottola, 1967; 1968 and 1970). The catalytic effect is inhibited by the presence of certain combinations of trace metals and enhanced by others. Since natural water contains a varied array of trace metals, serious matrix effects are likely if these procedures are applied to samples of natural water.

Kunkel and Manahan (1973) employed the enhanced solubility of copper hydroxide at pH 10 as a monitor of complexing capacity. Atomic absorption was used to monitor the level of soluble copper; the use of a pH 10 medium, however, disrupts the natural equilibria present in the sample.

Theory of Voltammetric Determination of Complexing Capacity. After reviewing available procedures, we elected to use a voltammetric method for measuring complexing capacity. Attempts have been made by other workers to apply voltammetric techniques to the determination of complexing agents in natural systems; the theoretical aspects of these methods have not been discussed nor have the expected accuracy and precision been reported. We felt it essential to fully understand what the method was measuring, what interferences might be expected and how reliable the numbers were before beginning an extensive sampling program. The theoretical basis of voltammetric methods for measuring complexing capacity will be briefly described here; the results of interference studies and accuracy measurements are found in the RESULTS section.

The ability of voltammetry to distinguish between complexed and uncomplexed metal ions lies in the fact that more energy must be supplied to reduce (or oxidize) a complexed metal ion than an uncomplexed one. Qualitatively the reduction of metal complex ML^{n+} can be viewed as shown below.

$$\text{step 1: } ML^{n+} \rightleftharpoons M^{n+} + L$$

$$\text{step 2: } M^{n+} + ne^- \rightleftharpoons M$$

Since ML^{n+} is a stable chemical species energy must be expended to free M^{n+} from the complex before reduction can take place. Uncomplexed metal, M^{n+}, is therefore reduced at a lower applied potential than ML^{n+}. This argument is somewhat oversimplified and applies only to methods in which the product of the electrode process contains fewer ligands than the initial material. Since most of the metal ions used by previous workers (and by us) are reduced to the zero valence state the simple model will apply.

The measurement base of all voltammetric methods is a current-voltage curve. This experimentally obtained curve is a plot of the current flowing in an electrochemical cell versus the voltage applied to the cell. Although each type of voltammetry obtains this curve using a slightly different experimental approach the results of all of the methods can be theoretically predicted from the same current-voltage relationship. The principles of the various voltammetric methods have been reviewed (Osteryoung and Osteryoung, 1972; Flato, 1972; Strobel, 1973, pp. 687-724).

The current-voltage curves expected for a system containing 2×10^{-6} M M^{n+} and 10^{-6} M L are shown in figure 1. These curves were calculated for the voltammetric technique known as DC polarography; given sufficient computer time the curves appropriate to the other voltammetric methods could have

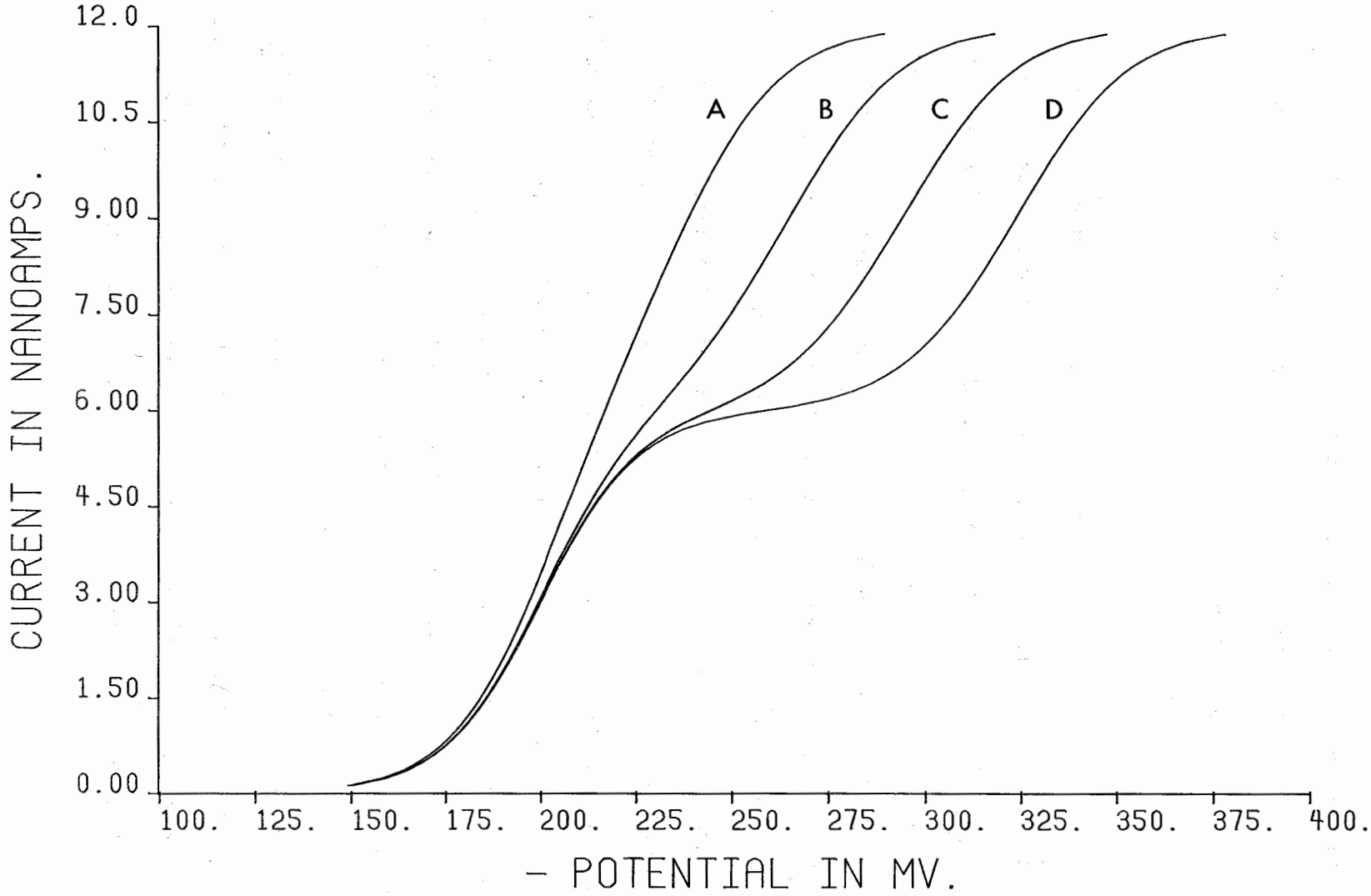

Figure 1. Effect of formation constant on theoretical current-voltage curve. Analytical concentration of M^{n+} = 2 x 10^{-6} M; analytical concentration of L = 10^{-6} M. Formation constant: A 10^{7}, B 10^{8}, C 10^{9}, and D 10^{10}.

been calculated but the polarographic case was chosen for its simplicity. The equations derived and discussed by Macovschi (1968) were used in calculating the curves shown in figures 1 and 2.

It is obvious from figure 1 that the presence of two species (i.e. M^{n+} and ML^{n+}) is not detected until the formation constant of the complex exceeds 10^8. To practically measure the current due to M^{n+} and that due to ML^{n+} the formation constant must be 10^{10} or greater.

Figure 2 illustrates how the current voltage curves change during the titration of 10^{-6} M L with M^{n+}. If the current indicative of M^{n+} is plotted versus the amount of M^{n+} added curves of the type shown in figure 3 are obtained. Experimentally the amount of complexing agent in a sample is obtained from the x-intercept of the titration curve (see figure 3). Clearly in order to determine the concentration of a complexing agent to within an error of 1-2% the formation constant of the metal complex used must exceed 10^{10}. Samples containing ligands capable of forming complexes weaker than 10^{10} will only be partially titrated (i.e. the x-intercept occurs before the theoretically predicted point). The fraction of weakly complexing materials which are determined will depend on the strength of the complex (figure 3); complexes weaker than 10^7 will not be measured at all. Table 1 compares the predicted accuracy with the formation constant of the complex.

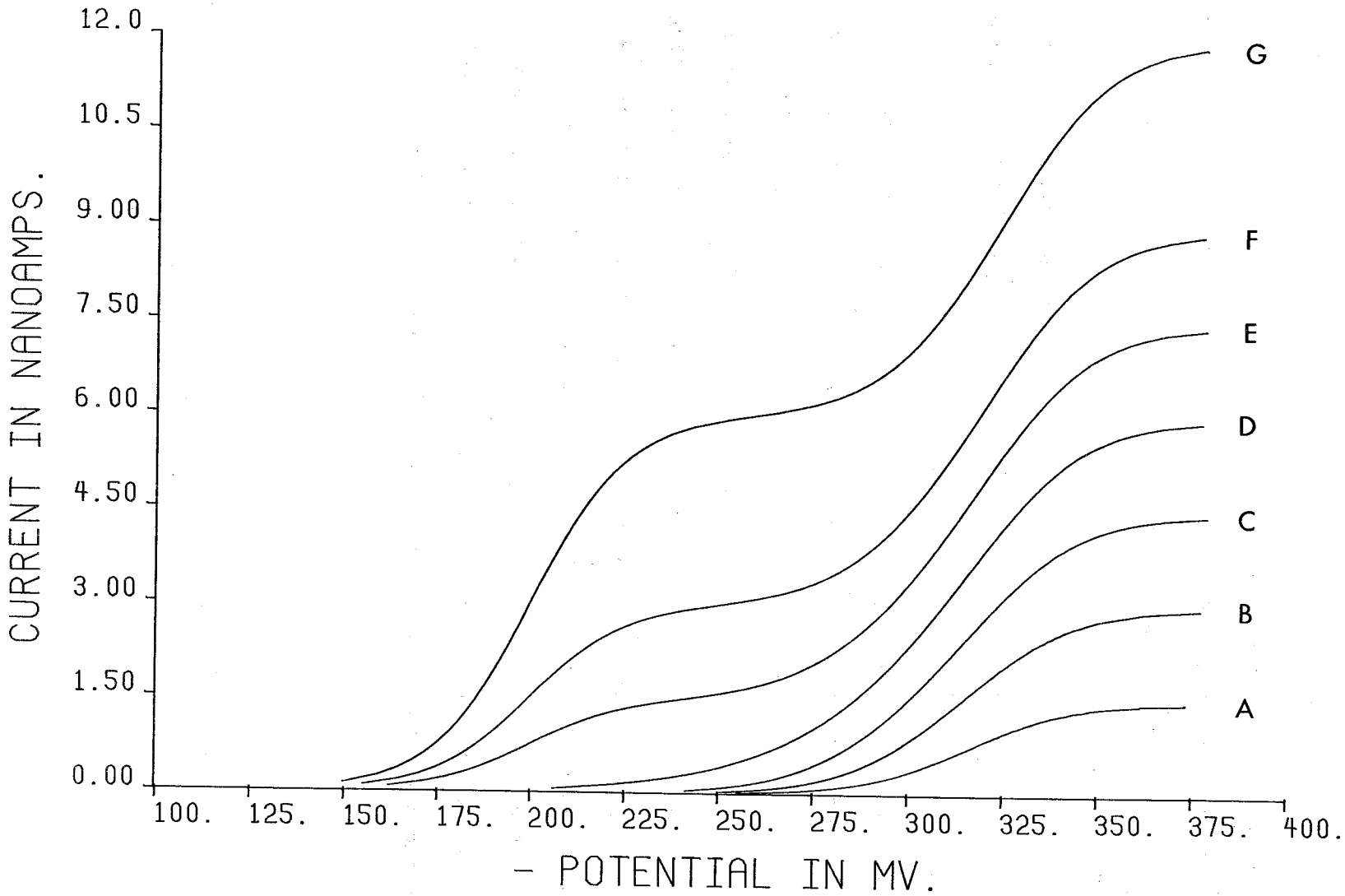

Figure 2. Theoretical current-voltage curves during titration of 10^{-6} M L with M^{+n}. Percent titrated: A 25%, B 50%, C 75%, D 100%, E 125%, F 150%, and G 200%.

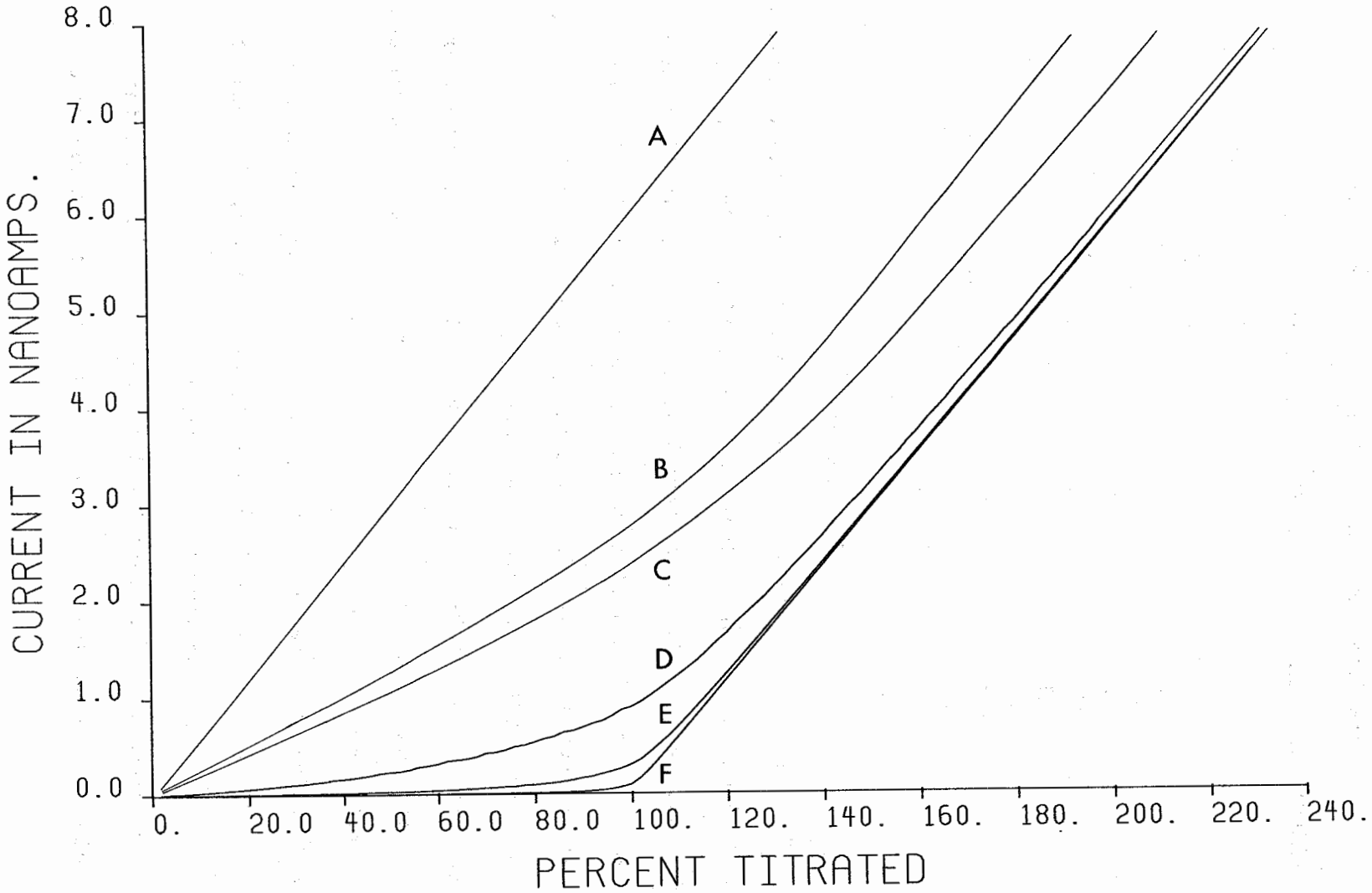

Figure 3. Theoretical titration curves for the titration of 10^{-6} M L with M^{n+}. Formation constant: A 10^7, B 7 x 10^7, C 10^8, D 10^9, E 10^{10} and F 10^{11}.

Table 1

Predicted Accuracy of Voltammetric Methods

Formation Constant of Complex	Percent Relative Error
10^7	no ligand detected
7×10^7	38.6
10^8	25.2
10^9	6.3
10^{10}	0.8
10^{11}	0.0

EXPERIMENTAL

Reagents. The water used in this work was deionized then passed through an activated charcoal column and deionized a second time. Finally the water was filtered through an 0.20 μm membrane filter. The specific resistance of the water was checked periodically and was consistently found to be greater than 17 megohm-cm. The deionization system was installed and maintained by Continental Water Conditioning Corporation.

Reagent grade EDTA (Fisher Scientific) was dried at 80°C for 1 hour before use. Eastman white label NTA was used without additional purification except drying for 1 hour at 80°C.

Stock solutions of metal ions were prepared by dissolving the appropriate amount of metal in nitric acid (ultrex, J. T. Baker); all reagent solutions were stored in polyethylene containers.

Potassium nitrate was recrystallized 5 times from water to eliminate a lead impurity. The phosphate and acetate salts used for preparation of

buffers were reagent grade (Fisher Scientific) and were not further purified. Acetate buffer was found to contain intolerable quantities of lead which was removed by controlled potential electrolysis; the technique and apparatus have been previously described (Barendrecht, 1967; Mitchell, 1973).

Solutions of catalase were prepared by dissolving 10 mg of the powder (Calbiochem, bovine liver 3100 units/mg) in 100 ml of previously autoclaved deionized water. One milliliter portions of this solution were pipetted into small glass bottles with screw caps which had been previously autoclaved. All catalase solutions were stored at 5°C; one small bottle was removed from the refrigerator just before use and returned immediately after use.

Stock solutions of ethylenediamine were prepared by diluting distilled anhydrous ethylenediamine (Fisher Scientific) 1:5 with water; stock solutions were stored at 5°C.

Hydrogen peroxide solutions were prepared by diluting 30% H_2O_2 (Fisher Scientific) with water. Stock solutions of H_2O_2 (typically 0.5 M) were stored at 5°C and periodically checked for H_2O_2 content by cerate titration.

Nitrogen gas (Air Products prepurified grade) was used to deaerate solutions and was further purified by passage through a column of Ridox (Fisher Scientific) to reduce its oxygen content.

Electrodes and cells. Corning saturated calomel reference electrodes (cat. no. 476002) were used. A dropping mercury electrode of conventional design was used; electrical contact was made using a commercially available mercury contact (Princeton Applied Research Corp. part no. 9308). Glassy carbon electrodes used in the anodic stripping studies were prepared by sealing a 2 cm length of GC-A glassy carbon (International Carbon Corp.) into 6 mm glass tubing with epoxy cement. Electrical contact was made through mercury

inside the glass tube. A 6 mm glass tube whose ends had been partially closed was inserted into the cell top; an appropriate length of 26 gauge Pt wire inserted through the tubing served as a counter electrode. Commercial gas dispersion cylinders were found to be bulky and inefficient; gas dispersion tubes prepared by sealing ca. 2 cm of a 10 mm medium porosity sealing tube to 10 cm of 7 mm glass tubing were used throughout this work.

The cells used were 100 ml tall form Pyrex beakers fitted into a commercially available polyethylene cell top (Leeds and Northrup cell cover no. 067513 and ring no. 127182).

Electronic apparatus. A Princeton Applied Research Model 174 Polarographic Analyzer was used to make most of the electrochemical measurements. In the early portions of the work a Heath EUW-401 Polarography System was utilized. A Hewlett-Packard Moseley 2001A X-Y recorder was used in conjunction with the P.A.R. 174. During the latter stages of the work the P.A.R. 174 was modified to allow semi-automatic differential pulse polarographic measurements. These modifications are shown in figure 4. A Heath EU-805 Universal Digital Instrument was used as a digital voltmeter to monitor the potential the P.A.R. 174 was applying to the cell.

The circuit of figure 4 was constructed using a Heath EU-801A Analog-Digital Designer as a source of power and as a support for printed circuit cards. The following Heath cards were used: EU-800-JC, EU-800-LA, EU-900-JA, EU-800-JD, EU-800-CD and (2) EU-800-DE. The FET switches on the EU-900-JA card were opened by a logic 0 and closed by a logic 1. The monostable output pulse was a logic 1 of 1 second duration. An SU-50-JA permanent patch accessory was used to hard wire the connections between cards. The principles of digital circuitry have been reviewed (Malmstadt and Enke, 1969).

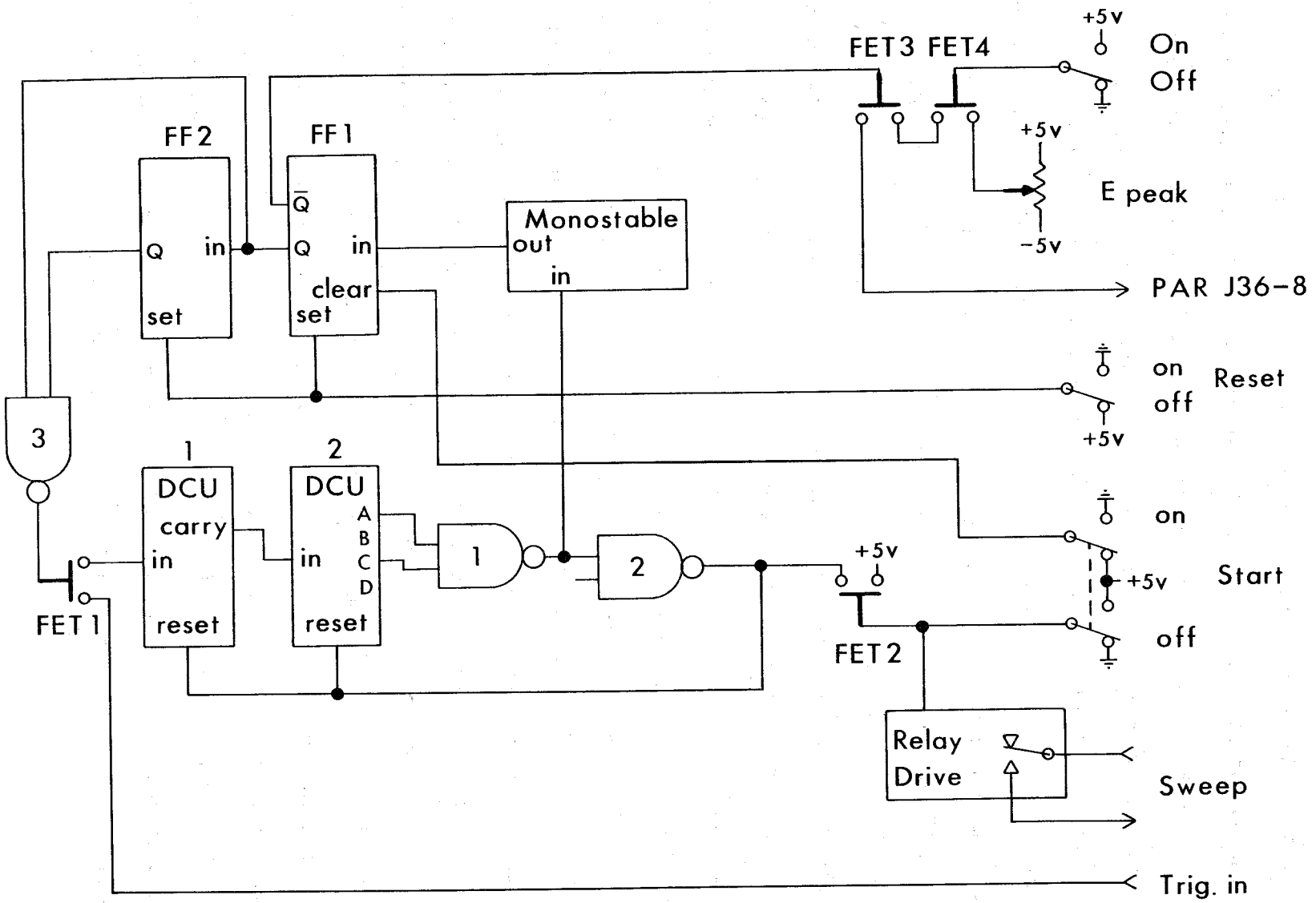

Figure 4. Schematic diagram of semi-automatic potential control accessory.

When making semi-automatic differential pulse measurements, an initial scan is made to determine the peak potential and the baseline potential. The circuit shown in figure 4 is activated by closing the ON/OFF switch. The baseline potential is adjusted to the desired value with the initial potential control of the P.A.R. 174; to prevent the modulation pulse from interfering with this measurement the drop time selector should be off. The peak potential is applied to the P.A.R. 174 by momentarily closing the start switch; the peak potential may be adjusted to the desired value with the E peak control. Measurements are begun by (1) setting the drop time selector to 2 seconds, (2) momentarily closing the reset switch, and (3) momentarily closing the start switch.

The P.A.R. 174 supplies a voltage spike approximately 75 milliseconds before each mercury drop is dislodged. Momentarily closing the reset switch sets FF1 and FF2. $\bar{Q}1$ is at logic 0 therefore FET 3 is open and the instrument applies the baseline potential to the cell. NAND gate 3 has a 0 output consequently FET 1 is open and timing spikes are not transmitted to the counters. When the start switch is momentarily closed, FET 2 closes thereby resetting the decade counting units and starting the recorder. FF1 is cleared, $Q1 = 0$ and $\bar{Q}1 = 1$ therefore FET 3 closes and the instrument applies the peak potential to the cell. FF2 also changes, $Q2 = 0$ forcing NAND 3 to a 1 output thereby turning FET 1 on and allowing timing pulses to be counted by DCU1 and DCU2. When 50 spikes have been counted (i.e. 50 drops dislodged) NAND 1's output changes from 1 to 0 triggering the monostable which in turn supplies a 1 pulse to FF1, Q1 is now 1 and $\bar{Q}1$ is 0 opening FET3 and the instrument applies the baseline potential to the cell. The $1 \rightarrow 0$ from NAND1 is inverted by NAND2, the resulting $0 \rightarrow 1$ resets the DCU's and the counting cycle repeats. After

an additional 50 drops the monostable is triggered again; Q1 = 0, $\bar{Q}1$ = 1, Q2 = 0, FET 3 is opened and the instrument applies the peak potential to the cell. After another 50 drops have been dislodged, the monostable triggers for the last time. Q1 = 1, $\bar{Q}1$ = 0, Q2 = 1, FET 3 is open and the instrument applies the baseline potential to the cell. NAND 3, however, has a 0 output which opens FET 1 preventing further cycling. To initiate a second sequence the start switch is depressed momentarily.

Procedures. The procedures employed to determine complexing capacity were based on measuring the amount of metal ion which remains uncomplexed when a known amount of the metal ion is added to the sample. Both anodic stripping voltammetry and differential pulse polarography were used to quantitate the uncomplexed fraction of metal ion titrant.

A rotated thin film Hg electrode (glassy carbon base) was used in the anodic stripping procedures; a Sargent synchronous rotator was used to rotate the electrode. The electrode was initially polished with 500A silicon carbide paper to remove the excess epoxy cement. Two grades of alumina (0.3 and 0.1 μm) were used for final polishing of the newly prepared electrode and for re-polishing previously plated electrodes. A 1 micron Hg film was electrodeposited on the electrode at a constant current of 1 mAmp in 0.05M mercurous nitrate in 0.1M $HClO_4$. The ASV electrolysis potential was selected by first obtaining a DC polarogram of the metal ion titrant or consulting the polarographic literature. A potential 120/n mV negative of the polarographic $E_{1/2}$ was used as an initial guess at a satisfactory electrolysis potential. Several ASV runs were made using solutions 10^{-5}M in metal ion titrant. The electrolysis potential was adjusted an additional 60/n mV in the negative direction and several ASV runs obtained. If the

average peak current of the two series differed by less than 10% the first potential was used as the ASV electrolysis potential; if agreement was not found the first set of runs was discarded and a third series run using an electrolysis potential 60/n mV negative of the potential used for the second series. The process was repeated until agreement was found. An electrolysis potential was obtained for each metal ion employed and for each buffer system. Details of operating an anodic stripping voltammeter are available (Barendrecht, 1967; Ellis, 1973).

The differential pulse polarographic procedure was developed to minimize some of the problems inherent in the ASV procedure. A detailed procedure suitable for use as a standard method is contained in appendix A. Briefly an aliquot of Co^{2+} was added to a measured portion of the sample which was buffered at pH 7. Hydrogen peroxide was added to oxidize the Co(II) complexes formed to Co(III) complexes. The excess peroxide was removed with the enzyme catalase. The uncomplexed Co^{2+} was measured in an ethylenediamine medium using differential pulse polarography. General operating instructions for the differential pulse polarograph are available (Princeton Applied Research, 1971 a).

RESULTS

Quantitation Using Anodic Stripping Voltammetry. The ionic content of natural water is too low to permit use of electroanalytical techniques. It was necessary to add an electrolyte to the sample which would not contribute to the complexing capacity of the sample nor disturb the natural equilibria present. The electrolyte should therefore change the pH of the sample as little as possible. Further the medium chosen must allow the ASV peak to be observed at a convenient

potential. Four electrolytes were evaluated: NaCl, KNO_3, acetate buffer (pH 5) and $Na_2C_4H_4O_6$ (sodium tartrate). The tartrate system proved unsatisfactory because of large levels of trace metal impurities which could be removed only by prolonged controlled potential electrolysis. The complexing power of the tartrate ion was found to be so high as to disturb natural equilibria. Sodium chloride was sufficiently pure but the ASV peak of Cu^{2+} (one of the metal ion titrants to be evaluated) was poorly resolved from the background current. Potassium nitrate was satisfactory electrochemically but did not permit rigorous pH control. Acetate buffer (pH 5) gave the best results and was adopted as the medium even though it disturbs natural equilibria somewhat because of the pH change and mild complexing power of the acetate ion. It was subsequently learned that another research group (Chau, 1973) had selected an acetate medium for its studies of metal complexation in natural water.

The metal ion titrant selected must form strong complexes with organic ligands and must be detectable by an electrochemical method. Four metal ion titrants were evaluated in pH 5 acetate buffer: Cu^{2+}, Pb^{2+}, Cd^{2+} and In^{3+}. The electrolysis potentials for each ion are shown in table 2.

Table 2

ASV Electrolysis Potentials

Ion	No. of Electrons	E^*_{peak}	$E^*_{electrolysis}$
Cu^{2+}	2	-0.075V	-0.250V
Cd^{2+}	2	-0.625V	-0.750V
Pb^{2+}	2	-0.430V	-0.600V
In^{3+}	3	-0.645V	-0.675V

*versus SCE

Indium was selected as the best metal ion titrant because of its strong complexing ability, low detection limit (3 electron process), low natural levels in water, and the ease with which it may be detected electrochemically.

The results summarized in table 3 are typical of the numerous synthetic samples of EDTA which were titrated with In^{3+}. The results were nearly always low suggesting the presence of a systematic error. The results of

Table 3

ASV Determination of EDTA

EDTA taken (μmolar)	EDTA found (μmolar)	Percent Relative Error
8.24	6.70	-18.7
2.19	2.17	-0.91
1.73	1.66	-4.05
1.59	1.52	-4.40
1.04	1.05	+0.96
0.82	0.64	-21.9
0.82	0.54	-34.1
0.21	0.18	-14.3
0.21	0.15	-28.6
0.16	0.13	-18.7

table 3 indicate the In^{3+} titration to be an unacceptable method for approximating the complexing capacity of a synthetic sample. Errors of 20% at the 10^{-7} molar level would be satisfactory if systematic errors were absent or at least controlled. The formation constant of the $InEDTA^{-}$ complex is approximately 10^{17} at pH 5, well in excess of the minimum value of 10^{10} needed for quantitative work.

The theory presented earlier (pg. 4) does not consider complex lability. The term lability refers to the rate at which a metal complex exchanges its ligands either with water, another ligand or a ligand identical to that in

the complex. Whether a metal complex is labile (i.e. exchanges ligands rapidly) or inert (i.e. exchanges ligands slowly) is predominately a function of the metal ion in the complex; the nature of the ligand exerts relatively little influence in determining complex lability. The electronic structure of all the metal ions tested using the ASV procedure is such that labile complexes will be formed with the natural ligands present in the sample.

A small fraction of metal ion is removed from solution while monitoring the fraction of metal ion titrant which is uncomplexed. Typically 2 minute electrolysis were used for the ASV measurements; the fraction of free metal ion plated into the electrode is approximately 1% under these conditions. The concentration of free metal ion at the electrode surface is very close to zero during the electrolysis. The loss of free metal ion from the bulk of solution and the concentration gradient near the electrode surface tend to drive the metal ion/metal complex equilibrium in the direction of the free metal ion. The result is that during the time the free metal ion concentration is being sampled the amount of free metal ion in the vicinity of the electrode surface increases because of the dissociation of the metal complex. Consequently the electrode "sees" more free metal ion than is actually there; the amount of complexing agent determined is therefore smaller than expected. If the rate of complex dissociation is rapid on the time scale of the measurement, the entire concentration of metal ion (i.e. both complexed and uncomplexed) will be sensed by ASV and no complexing capacity will be detected.

The effect of chemical reactions can be minimized by shortening the time of measurement and by lowering the temperature thus slowing the rate of

the reaction. It was not possible to obtain a useable current peak from ASV at the 10^{-6} M level unless the time of electrolysis exceeded a few minutes. To eliminate this problem a dual electrode ASV procedure was devised.

Two Hg plated electrodes were placed in the cell; one was rotated as before but the second electrode was stationary and was isolated from the bulk of the cell solution by a small tube capped with a glass frit. The two electrodes were interconnected using a P.A.R. model 174/51 Linear Sweep Module. Details on the operation of this device are available elsewhere (Princeton Applied Research, 1971b). By electronically subtracting the background current common to both electrodes the dual electrode procedure permitted ASV measurements after only 10 seconds of electrolysis.

Table 4 summarizes the results obtained using dual electrode ASV to determine the complexing capacity of nine samples each containing 96.2 nmoles of EDTA.

Table 4

Effect of Electrolysis Time on ASV Results

	nmoles EDTA found				
Electrolysis Time (sec)	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
10	126.3	91.9	106.5	108.2	17.3
20	110.7	101.3	56.4	89.5	29.0
50	70.9	10.4	28.2	36.5	31.1

An analysis of variance of the data in table 4 at the 95% level indicated that electrolysis time significantly affected the nmoles of EDTA found.

Lowering the sample temperature to 0°C was found to be extremely effec-

tive in decreasing the lability of metal complexes. At 0°C the rate is so slow that the metal ion titrant does not complex the ligands in the sample at a reasonable rate; the lowered temperature slows both the rate of complex breakup and the rate of complex formation. It was necessary to add the metal titrant to a 25°C sample, allow the complexation reactions to reach equilbrium, and then cool the sample to 0°C before monitoring the free metal ion concentration. The sample must be warmed to 25° if another aliquot of titrant is added then cooled for the ASV measurement. Although this technique is quite time consuming reasonable results were obtained using it (table 5).

Table 5

Results of ASV Procedure with Temperature Variation

Sample No.	nmoles EDTA Found*
1	100.4
2	114.6
3	114.6
4	77.5
5	75.3
6	118.9
7	100.4
8	97.1
Mean	99.8 ± 13.8

*103.7 nmoles EDTA taken for each sample

At 0°C the height of an In^{3+} ASV peak is approximately one third that at 25°C. Lowering the temperature minimized the lability effects at the expense of sensitivity and length of analysis. Cycling the Hg film electrodes between 25° and 0° greatly reduced the lifetime of the electrodes; a

single plating at best lasted for only a single sample before the Hg film beaded up exposing unplated carbon. In spite of its simplicity and low cost we have concluded that ASV is not a satisfactory method for measuring the complexing capacity of natural water.

Quantitation Using Differential Pulse Polarography. Based on our experience with ASV we have concluded that the metal ion titrant approach is a good one provided the metal ion selected as titrant has certain properties. These are:

1. Reacts rapidly with ligands in the sample.
2. Forms stoichiometric complexes of high stability.
3. Forms kinetically inert complexes or can be converted to a kinetically inert form.
4. The concentration of free metal ion can be determined by DPP or ASV.

The Co^{2+} ion is the only readily available ion which satisfies each of the requirements. Although Co^{2+} can be determined using ASV (Hovsepian and Shain, 1966), the procedure requires considerable sample treatment; we have elected to quantitate Co^{2+} with DPP. Since a procedure for determining Co^{2+} with DPP has not been reported, a procedure was developed.

The reduction of Co^{2+} is usually not observable in aqueous electrolytes near pH 7; when the reduction can be observed it frequently overlaps with the reductions of Fe^{2+}, Zn^{2+} and Mn^{2+} (Meites, 1963). Cobalt(II) yields a well defined oxidation wave in solutions containing ethylenediamine. A typical differential pulse polarogram of Co^{2+} in 0.05M ethylenediamine is shown in figure 5. A calibration curve for the determination of Co^{2+} by DPP is shown in figure 6; four sets of data taken on different days were used to construct figure 6. Table 6 summarizes the least squares analysis

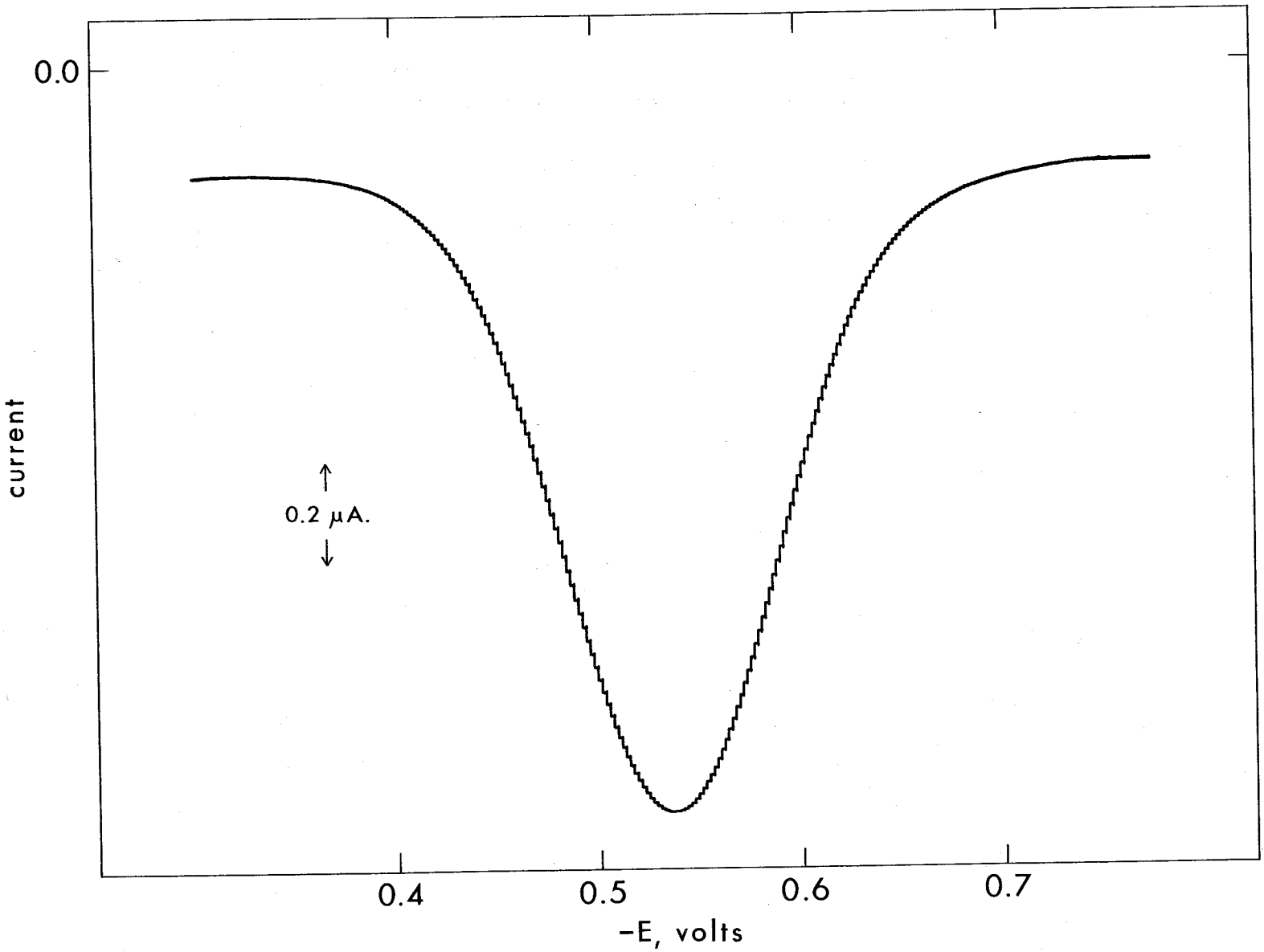

Figure 5. Differential pulse polarogram of 5.8×10^{-5} M Co(II) in 0.05 M ethylenediamine. Drop time = 2 seconds; modulation amplitude = 100 mV.

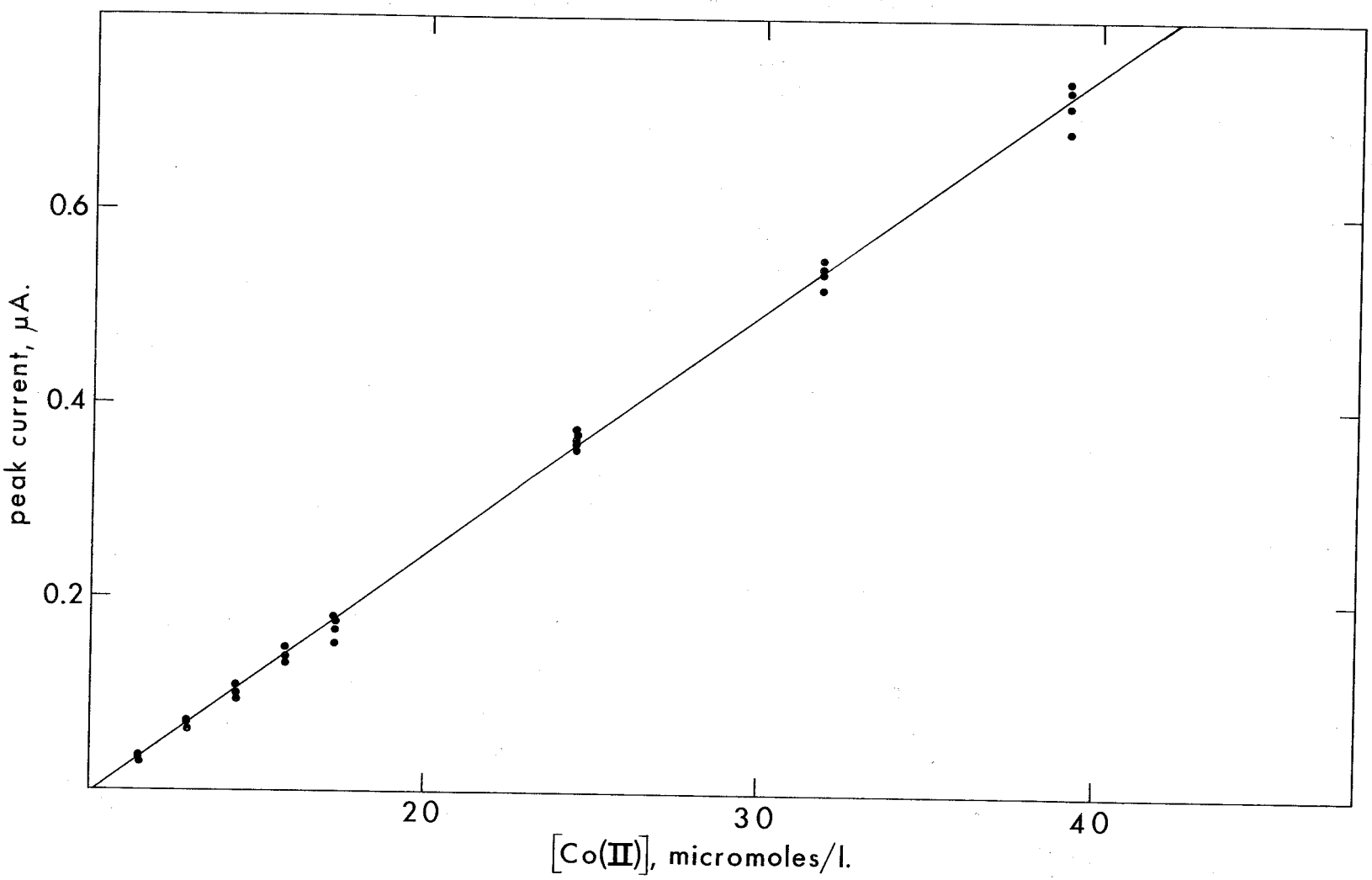

Figure 6. Calibration curve for the determination of Co(II).

of seven Co^{2+} calibration curves. In no case was a statistically significant x-intercept observed; the slopes of all seven runs are identical to within the uncertainity of measurement.

Table 6

Co^{2+} Calibration Data

Run	Slope	x-intercept(μmolar)
1	0.0033 ± 0.0004	-1.6 ± 7.1
2	0.0037 ± 0.0002	0.5 ± 2
3	0.0037 ± 0.0004	2.1 ± 5
4	0.0036 ± 0.0004	-0.2 ± 5
5	0.0038 ± 0.0001	+0.2 ± 0.6
6	0.0036 ± 0.0001	0.4 ± 0.7
7	0.0037 ± 0.0002	1.2 ± 1.4
Composite	0.0036 ± 0.0001	0.3 ± 1.9

Range of Co^{2+} concentration: 2.9 - 58 μmolar

Four 50 milliliter samples of a 5.14 μmolar solution of EDTA were determined with the DPP procedure; a composite plot of all four runs is shown in figure 7. Data from each run and for a second more dilute sample are given in table 7. The percent relative error of the mean for the samples are +0.19% and -3.8% respectively.

Analysis of Natural Water Samples. Eight sampling sites were selected after consultation with the N. C. Office of Air and Water Resources.

Table 7

Determination of Known EDTA Samples Using Co^{2+} and DPP

Sample 1: 5.140 μmoles EDTA per liter

Run	Slope	µmoles/ℓ found
1	0.0032 ± 0.0001	6.3 ± 1.9
2	0.0033 ± 0.0001	4.7 ± 2.2
3	0.0034 ± 0.0001	4.5 ± 1.2
4	0.0034 ± 0.0001	5.1 ± 2.1
Average		5.15 ± 1.28
Composite	0.0034 ± 0.0001	5.3 ± 2.8

Sample 2: 1.798 µmoles EDTA per liter

Run	Slope	µmoles/ℓ found
1	0.000482 ± 0.000007	1.4 ± 0.5
2	0.000486 ± 0.000006	1.4 ± 0.4
3	0.000434 ± 0.000013	2.8 ± 1.1
4	0.000440 ± 0.000013	1.3 ± 1.1
Average		1.73 ± 1.14
Composite	0.000461 ± 0.000018	1.7 ± 2.5

The sites were chosen to reflect industrial, commercial and municipal usage; a site in Umstead Park was chosen as a control.

Table 8

Sampling Sites

Site No.	Location	Comments	Classification*
1	Sycamore Creek at SR1647	Umstead Park	B
2	Crabtree Creek at SR 1669	Crabtree Valley Shopping Center	C
3	Crabtree Creek at SR 2030	Below all known industrial users	C
4	Neuse River at SR 2000	Above Burlington Industries	AII
5	Neuse River at SR2215	Below Burlington Industries	C
6	Neuse River at SR 1007	Above entrace of Walnut Creek	C

Site No.	Location	Comments	Classification*
7	Walnut Creek at SR 2551	Below Raleigh treatment plant	D
8	Walnut Creek at SR 2564	Above Raleigh treatment plant	D

*As determined by N. C. Bd. of Air and Water Resources (see appendix C for further details)

The sampling sites are mapped in figure 8.

Approximately 300 ml of water was collected at each site using a clean pyrex bottle with a polyethylene lined cap. No preservatives were used. All samples were stored at 5°C until analyzed. All eight samples from a single trip through the sites were analyzed within 48 hours of collection.

The results of analysis of 50.0 ml aliquots of each sample are shown in table 9.

Table 9

Complexing Capacity of Natural Water*

A. Sampling Network

Date collected	1	2	3	4	5	6	7	8
I (5/28/73)	1.2±0.7	2.1±0.5	1.5±0.7	2.1±0.5	2.4±0.6	1.7±0.7	2.0±0.2	1.5±0.3
II (6/4/73)	1.7±0.8	1.1±0.3	1.0±0.3	ND	ND	ND	1.4±0.3	0.6±0.5
III (6/11/73)	ND	1.6±0.8	1.8±0.4	1.9±0.2	ND	ND	0.5±0.4	0.7±0.4
IV (6/18/73)	ND	1.4±0.7	1.7±0.4	1.3±0.4	0.8±0.4	1.2±0.6	1.3±0.3	0.9±0.6
Averages	0.7	1.6	1.5	1.3	0.8	0.7	1.3	0.9

B. Miscellaneous Samples

Raleigh City tap water	N.D.	
Raleigh sewage effluent	4.1 ± 0.9	(5/11/73)
Raleigh sewage effluent	2.9 ± 1.2	(5/9/73)
Briery Swamp (Stokes, N.C.)	0.6 ± 0.1	
Tar River (Greenville,N.C.)	N.D.	
Aquarium water	7.5 ± 0.1	

*μmoles ligand/liter
ND = not detectable

The data in table 9 was obtained semi-automatically employing the circuit shown in figure 4. A conventional differential pulse polarogram of sample II-3 is shown in figure 9, the semi-automatic output of this sample is shown in figure 10. Clearly the peak current and baseline current are much easier to obtain from figure 10 than from figure 9.

Four known additions of Co^{2+} were used in obtaining each data point of table 9. Table 10 illustrates the effect of using only a single addition of Co^{2+} beyond the initial Co^{2+} spike.

Table 10

Effect of Using a Single Co^{2+} Addition

Date collected	1	2	3	4	5	6	7	8
I (5/28/73)	1.65	2.49	1.99	2.46	2.86	2.14	2.12	1.68
II (6/4/73)	2.13	1.29	1.01	1.78	2.05	0.06	1.57	0.78
III (6/11/73)	0.17	2.10	2.09	1.97	ND	0.34	0.67	0.63
IV (6/18/73)	0.61	1.67	1.74	0.98	0.84	1.62	1.31	1.38
Average	1.14	1.89	1.71	1.80	1.44	1.04	1.42	1.12

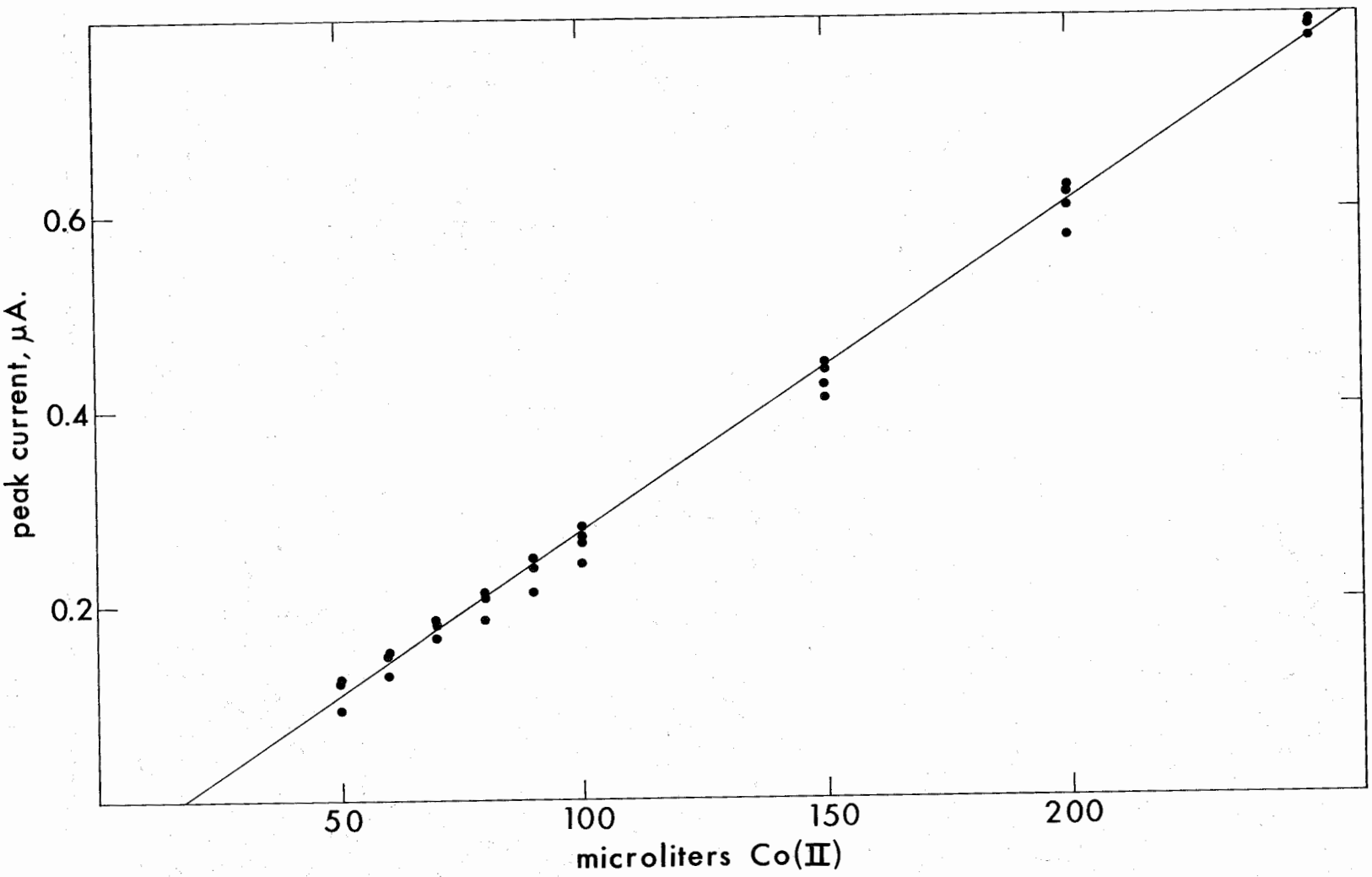

Figure 7. Standard addition curve for the determination of 5.14 μmolar EDTA. Sample volume 50.0 ml; concentration of Co^{2+} = 1.452×10^{-2}M.

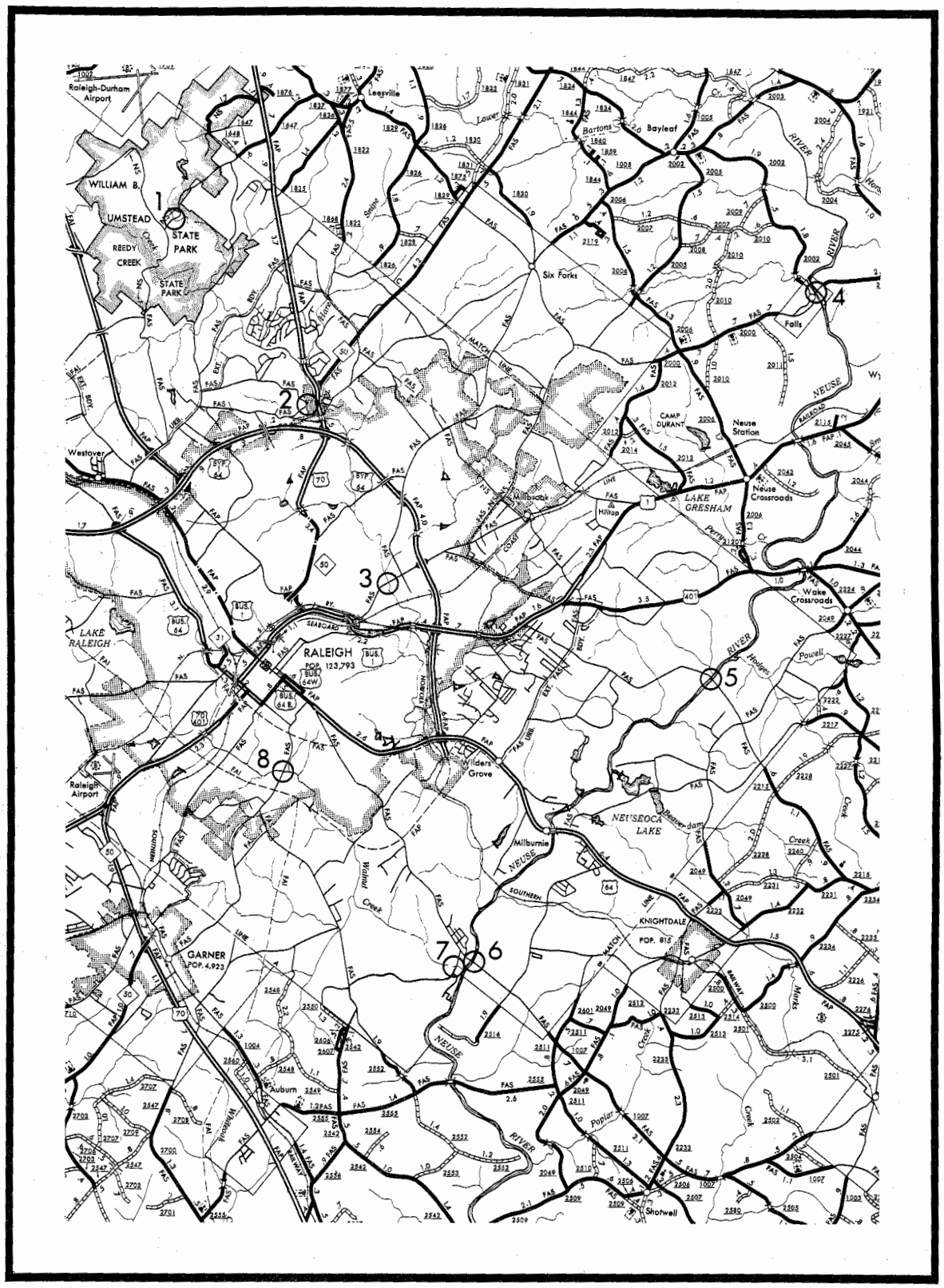

Figure 8. Location of sampling sites.

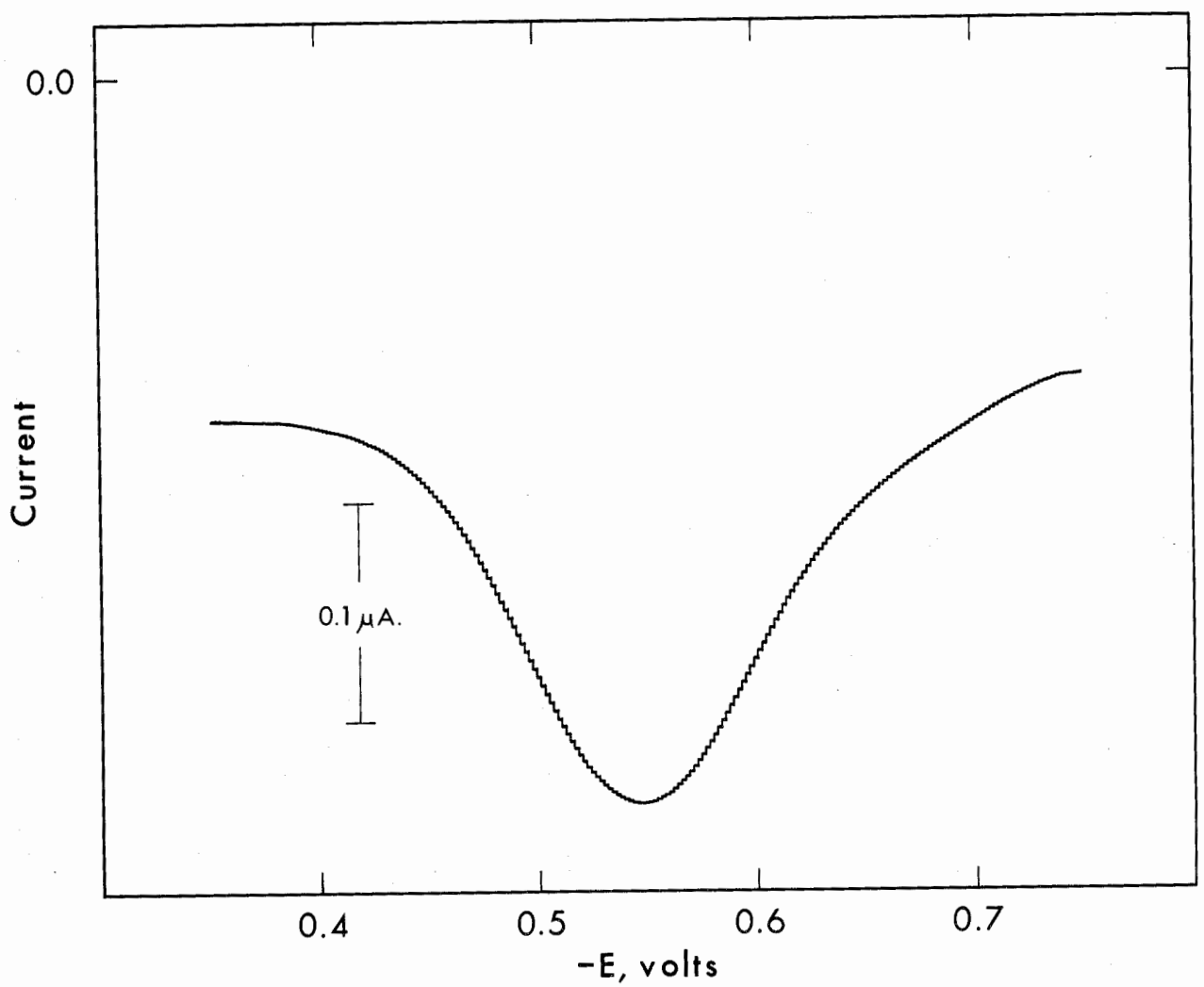

Figure 9. Differential pulse polarogram of sample II-3 after complexation by Co(III).

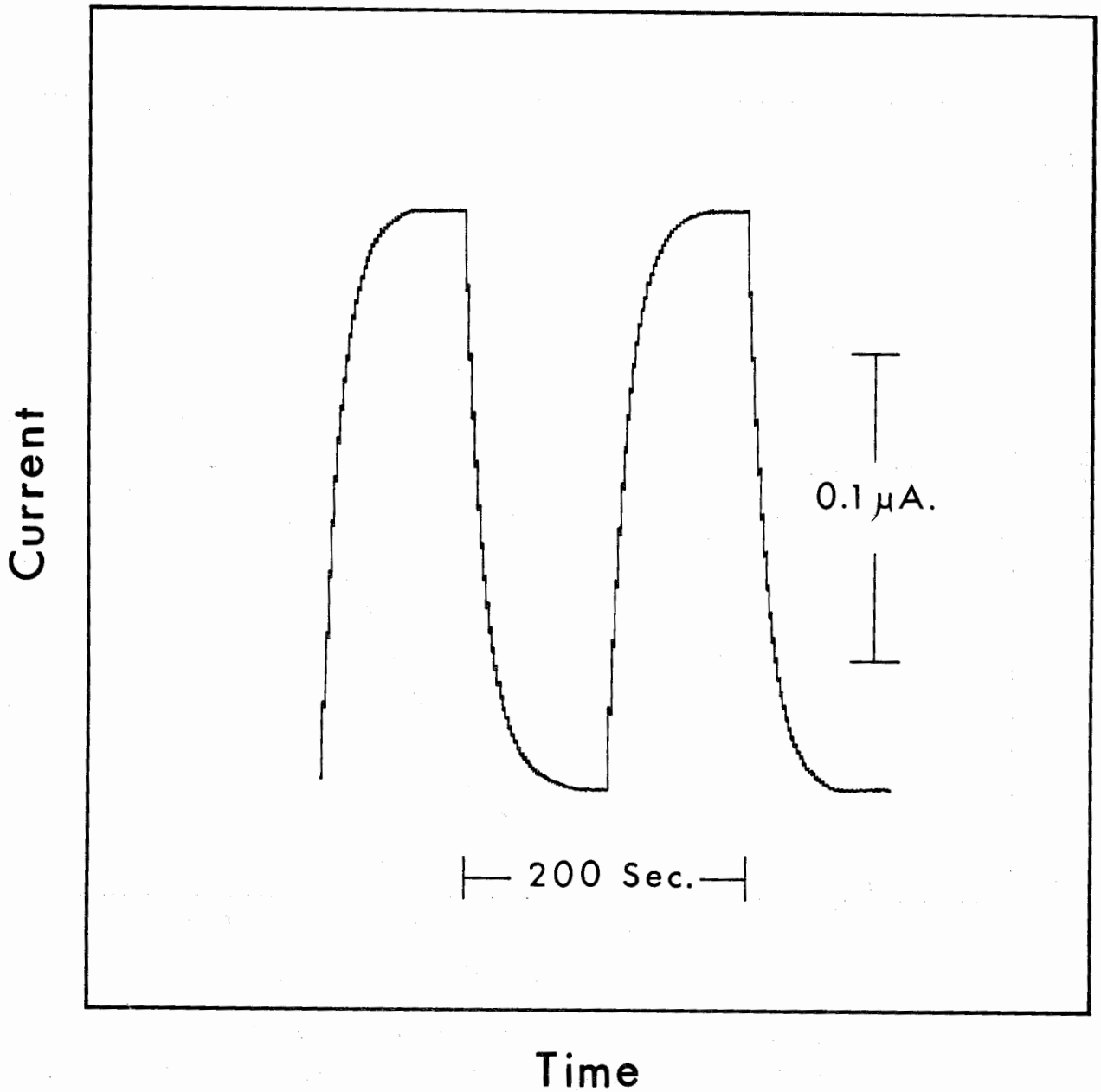

Figure 10. Semi-automatic readout of differential pulse polarogram of sample II-3.

Using only one calibration point instead of four results in a saving of time at the expense of accuracy; nearly every analysis reported in table 10 is higher than its counterpart in table 9.

Each sample collected from the sampling network on 6/4/73 was split; one fraction was sent to the N. C. State University Department of Biological and Agricultural Engineering for carbon analysis; the complexing capacity of the remaining fraction was determined in our laboratory. The results are compared in table 11.

Table 11

Correlation of Complexing Capacity with Carbon Content

Sample	TOC	TC	Complexing Capacity (μmolar)
II.1	13.5	16.5	1.7
II.2	12.0	16.0	1.1
II.3	11.5	16.5	1.0
II.4	11.0	14.5	ND
II.5	11.5	15.5	ND
II.6	18.0	22.0	ND
II.7	25.0	47.0	1.4
II.8	14.0	22.0	0.6

Complexing capacity does vary with TOC but the correlation is not strong enough to permit an estimate of the fraction of TOC which is capable of binding metals. The instrumentation used for the TOC and TC measurements was not designed for samples below 15 mgC/liter; the variations observed may reflect the uncertainity in the carbon measurements more than the variations of TOC or TC between samples.

DISCUSSION

Method Development. Three distinct procedures for determining complexing capacity have been evaluated. The general characteristics of each are summarized in table 12.

Table 12

Evaluation of Methods for Determining Complexing Capacity

Quantitation Method	Electrode	Titrant	Accuracy at the 1μmolar level	Man-hours per sample	Competence Required
ASV	Hg thin film on glassy carbon	In^{3+}	±5%	1.0	Expert
ASV with temp. variation and dual cell	Hg thin film on glassy carbon	In^{3+}	±5%	2.0	Expert
DPP semi-automatic mode	DME	Co^{2+}	±5%	1.4	Technican

Both of the procedures employing ASV possess idiosyncrasies which limit their use to expert electroanalytical chemists. The Hg thin film electrodes are needed for sufficient sensitivity, but they are relatively difficult to prepare reproducibly. The dual cell/temperature variation technique is particularly touchy as the two electrodes must be closely matched in order for the dual cell apparatus to function well. Location of a satisfactory baseline for an anodic stripping voltammogram is somewhat arbitrary, consequently ASV measurements are difficult to automate. Peak currents frequently increase

between one run and the next; reproducibility can be obtained but only by careful concern for details such as time of electrolysis, uniform stirring during electrolysis, potential of the electrode between runs and quality of the thin Hg film.

Some of the problems observed with the ASV techniques could be solved by using hanging mercury drop electrodes instead of thin mercury film electrodes. The peak currents obtained with these electrodes are much smaller than those obtained with thin film electrodes. This is due mainly to the low area to volume ratio of a sphere of Hg compared to the area to volume ratio of a planar film of Hg a few micrometers thick. Initial experiments with the HMDE indicated insufficient sensitivity for titrations at the micromolar level particularly if electrolysis time was held below 1 minute. The stripping peaks obtained with an HMDE are quite broad and tail off slowly on the anodic side of the peak potential, complicating measurement of the baseline current.

Recently Schuman and Woodward (1973) titrated EDTA with Cd^{2+} using ASV with an HMDE to locate the equivalence point of the titration. The lowest concentration studied was 4.45×10^{-5} M which is more than ten times higher than the highest chelate levels which we found in natural water. In spite of the relatively high levels, several anomalous effects were observed including significant dissociation of the metal complex during the deposition step. Our data using ASV also indicate the presence of complex dissociation during deposition. Schuman and Woodward offer a correction procedure which if properly applied allows estimation of the formation constant of a metal complex from ASV data taken during a complexometric titration.

A comparison of the expected and observed volume of Cd^{2+} consumed by samples of EDTA were not reported.

The differential pulse method eliminates the problem of complex lability by using a labile metal ion (Co^{2+}) to complex the ligands in the sample then chemically producing a non-labile metal complex by oxidizing the Co(II) complexes to Co(III) complexes which are non-labile. Although sample preparation time is increased, the reliability of the procedure is greatly improved and the need to mathematically correct the data for dissociation is eliminated.

The differential pulse instrument is simple enough to be used by a technician. The DME is the most reliable voltammetric electrode; it is not subject to the problems of the thin Hg film electrodes. Measurement of peak current can be as automatic as one wishes to make it. Our instrument displays the peak and baseline current on a recorder; it would not be difficult to incorporate additional digital circuitry so that the peak and baseline currents are subtracted and printed on paper tape or sent directly to a computer for analysis.

Our initial calculations indicated that voltammetric techniques are capable of accurately determining the complexing capacity of ligands which form complexes with the metal ion titrant having formation constants greater than 10^{10}. In systems having formation constants below 10^{10}, voltammetry cannot accurately distinguish between free metal and complexed metal. The figure of 10^{10}, however, cannot be applied to the method we have adopted. Our calculations assumed that the electrode process used to quantitate the amount of free metal present was the reduction (or oxidation) of M^{n+}; the species M^{n+} was assumed to be in equilbrium with the metal complex ML^{n+}. In

the DPP method the electrode process used for quantitation is the oxidation of tris-ethylenediamine cobalt(II) to tris-ethylenediamine cobalt(III); the complicating factors introduced by adding ethylenediamine and by chemically oxidizing CoL^{2+} to CoL^{3+} were not considered earlier and will be discussed here.

The reaction sequence in the DPP method is believed to involve two steps:

$$Co^{2+} + mL \rightleftharpoons CoL_m^{2+}$$

$$2CoL_m^{2+} + H_2O_2 + 2H^+ \rightleftharpoons 2CoL_m^{3+} + 2H_2O$$

The net reaction is the sum of the two steps:

$$2Co^{2+} + 2mL + H_2O_2 + 2H^+ \rightleftharpoons 2CoL_m^{3+} + 2H_2O$$

The overall equilbrium constant for this reaction is:

$$(K_{eq})_{o.a.} = \frac{[CoL_m^{3+}]^2}{[Co^{2+}]^2[L]^{2m}[H_2O_2][H^+]^2}$$

In order to gain an insight into the magnitude of the formation constants required for a quantitative determination, $(K_{eq})_{o.a.}$ must be estimated. Three principles of thermodynamics will be utilized in developing an estimate for $(K_{eq})_{o.a.}$. First the standard free energy of reaction $\Delta G°$, is related to the equilbrium constant of the reaction by:

$$\Delta G° = -RT\ln K$$

Second, for an oxidation/reduction reaction $\Delta G°$ is related to the standard potentials of the cell involved:

$$\Delta G^\circ = -nFE^\circ$$

Finally, ΔG° of a reaction does not depend on the path taken to achieve a chemical change; hence ΔG°'s for intermediate chemical reactions may be summed to yield ΔG° for the net reaction:

$$Co^{2+} + mL \rightleftharpoons CoL_m^{2+}; \qquad \Delta G^\circ_1$$

$$Co^{2+} + mL \rightleftharpoons CoL_m^{2+}; \qquad \Delta G^\circ_1$$

$$2CoL_m^{2+} + H_2O_2 + 2H^+ \rightleftharpoons 2CoL_m^{3+} + 2H_2O; \qquad \Delta G^\circ_2$$

Net: $2Co^{2+} + 2mL + H_2O_2 + 2H^+ \rightleftharpoons 2CoL_m^{3+} + 2H_2O; \qquad (\Delta G^\circ)_{o.a.}$

ΔG°_1 may be estimated from the formation constant of CoL_m^{2+}; this formation constant will be given the symbol K_{f2} (the subscript 2 indicates that the complex involves Co(II)):

$$\Delta G^\circ_1 = -RT\ln K_{f2}$$

ΔG°_2 may be estimated from the standard potential of the H_2O_2/CoL_m^{2+} cell:

$$\Delta G^\circ_2 = -2F(E^\circ_{H_2O_2} - E^\circ_{CoL_m^{2+}})$$

$$E^\circ_{H_2O_2} = 1.77 \text{ volts}; \quad E^\circ_{CoL_m^{2+}} = 1.84 + \frac{RT}{F}\ln\left(\frac{K_{f2}}{K_{f3}}\right)$$

Thus $$\Delta G^\circ_2 = -2F\left[1.77 - 1.84 - \frac{RT}{F}\ln\left(\frac{K_{f2}}{K_{f3}}\right)\right]$$

Finally: $$(\Delta G^\circ)_{o.a.} = -2RT\ln(K_{f2}) + 2F\left[0.07 + \frac{RT}{F}\ln\left(\frac{K_{f2}}{K_{f3}}\right)\right]$$

$$-RT\ln(K_{eq})_{o.a.} = -2RT\ln(K_{f2}) + 2F\left[0.07 + \frac{RT}{F}\ln\left(\frac{K_{f2}}{K_{f3}}\right)\right]$$

$$\log(K_{eq})_{o.a.} = 2\log(K_{f2}) - \frac{(2)(F)}{(2.303RT)}\left[0.07 + \frac{2.303RT}{F}\log\left(\frac{K_{f2}}{K_{f3}}\right)\right]$$

$$\log(K_{eq})_{o.a.} = \frac{-2(F)(0.07)}{2.303\ RT} + 2\log(K_{f3})$$

$$\log(K_{eq})_{o.a.} = -2.37 + 2\log(K_{f3}) \qquad \text{(at 25°C)}$$

The magnitude of the overall equilbrium constant and hence the quantitativeness of the determination depends on K_{f3}, the formation constant of CoL_n^{3+}.

If the H_2O_2 reaction scheme converts 95% of the ligand in the sample to CoL_n^{3+}, a 5% error will occur. Such an error does not seem unreasonable at the 10^{-6} molar level. One may compute $(K_{eq})_{o.a.}$ by evaluating all of the concentration terms. Assuming a 95% conversion of ligand to CoL_n^{3+}, an initial ligand concentration of $10^{-6}\underline{M}$, an initial Co^{2+} concentration of $10^{-5}\underline{M}$ and a buffer of pH 7 minimum $(K_{eq})_{o.a.}$ values for n = 1, 2, 3 and 6 were evaluated (Table 13). The minimum values of K_{f3} needed to achieve $(K_{eq})_{o.a.}$ and hence a minimum of 5% error are also tabulated.

Table 13

Variation of Equilbrium Constant with m

m	$(K_{eq})_{o.a.}$ (5% error)	Minimum Values of K_{f3}	
		5% error	95% error
1	4.6×10^{31}	1.0×10^{17}	2.6×10^{14}
2	4.1×10^{45}	1.0×10^{24}	1.4×10^{20}
3	6.2×10^{60}	3.8×10^{31}	9.5×10^{25}
6	1.0×10^{103}	4.7×10^{52}	5.5×10^{43}

Ligands forming complexes with Co(III) weaker than indicated in table 13 will be only partially determined. The weaker the ligand the smaller its percent

conversion to CoL_m^{3+} and the larger the error in the analysis. The minimum value of K_{f3} that a ligand must have to be detected may be estimated by computing a $(K_{eq})_{o.a.}$ based on a minimum conversion of L to CoL_n^{3+}; a value of 5% conversion was used to compute the data in table 13. Thus a 1:1 complex will be detected at the 10^{-6} M level if $K_{f3} \geq 2.6 \times 10^{14}$ and satisfactorily determined if $K_{f3} \geq 1.0 \times 10^{17}$.

Unfortunately relatively few Co(III) formation constants have been tabulated (Sillen and Martell, 1964; Yatsimirskii and Vasil'ev, 1960) making it difficult to predict which ligands could be determined. K_{f3} for EDTA (m = 1) is 4.8×10^{32} at pH 7; K_{f3} for NTA (m = 2) is unknown. Both EDTA and NTA have been titrated successfully. Cyanide should be determinable as a 6:1 complex since $K_{f3} = 10^{64}$; ammonia, however, forms too weak a complex to determine accurately.

The value of K_{f3} needed for determination may be decreased by increasing the initial concentration of Co^{2+} or H_2O_2. The sensitivity of the method depends on observing a loss in the amount of Co(II) because of the formation of CoL_m^{3+}. If the initial Co^{2+} level is high very little decrease will be observed. If the decrease is of the same order of magnitude as the uncertainity in measuring the Co^{2+} concentration, no loss will be detected and hence no complexing capacity found. Our experience has been that a 10 fold excess of Co^{2+} over ligand is as high as is feasible. If the initial H_2O_2 level is increased, complicating side reactions involving oxidizable organic matter may appear. As the H_2O_2 level is increased the amount of time required for its catalatic destruction increases proportionally. An addition of 60 units of catalase was found to satisfactorily decompose 50 ml of 10^{-5} M H_2O_2 in 10 minutes at 37°C. Increasing the amount of H_2O_2 used by a factor

of 10 will cause $(K_{eq})_{o.a.}$ to decrease by about a factor of 10 but the time needed to decompose the excess H_2O_2 will increase proportionally. The decomposition time could be reduced by adding more units of catalase but at the risk of introducing errors because of Co(II) binding to the protein structure of catalase; 60 units of catalase represent approximately 10^{-10} moles of protein (Samejima, Kamata and Shibata, 1962).

The sensitivity of the method toward ligands forming weak complexes with Co(III) may be improved by changing the pH. A decrease in pH will cause a decrease in the $(K_{eq})_{o.a.}$ needed for quantitative conversion of L to CoL_n^{3+} and a corresponding decrease in the minimum value of K_{f3} a ligand must have to be determined. The effective formation constant for ligands containing carboxyl or other acid groups is also a function of pH; as pH is decreased the effective formation constant decreases. Thus the effect of decreasing the pH can not be readily predicted unless the identity of the sample ligands is known. An increase in pH will have the opposite effect. Although a higher $(K_{eq})_{o.a.}$ is required an increase in pH will also increase the K_{f3} of ligands containing acidic functional groups. The effect of pH on the determination of EDTA are shown in table 14.

Table 14

Effect of pH on Determination of EDTA

pH	$(K_{eq})_{o.a.}$ *	K_{f3} *	K_{f3} $CoEDTA^-$
6	4.6×10^{29}	1.0×10^{16}	2.2×10^{31}
7	4.6×10^{31}	1.0×10^{17}	4.8×10^{32}
8	4.6×10^{33}	1.0×10^{18}	5.4×10^{33}

* 95% conversion of 10^{-6} M L to CoL_3^{3+}

The practical pH range is 5 to 10 because the efficiency of catalase in decomposing H_2O_2 decreases rapidly below pH 5; the catalase molecule decomposes above pH 10.

The effect of ligand concentration on the feasibility of determination is similar to that of increasing the initial concentration of Co^{2+} or H_2O_2. The product of $(K_{eq})_{o.a.}$ and C_L^{2m+1} will be constant provided the initial concentration of Co^{2+} and H_2O_2 are made equal to $10C_L$ (see table 15).

Table 15

Effect of Ligand Concentration on Minimum K_{f3}

m	$(K_{eq})_{o.a.}(C_L)^{2m+1}$	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
1	4.6×10^{13}	3.3×10^{15}	1.0×10^{17}	1.0×10^{18}	1.0×10^{20}
2	4.1×10^{15}	3.1×10^{21}	1.0×10^{24}	3.1×10^{26}	9.8×10^{28}
3	6.2×10^{18}	1.2×10^{28}	3.8×10^{31}	3.8×10^{37}	3.8×10^{38}
6	1.0×10^{25}	1.5×10^{46}	4.7×10^{52}	1.5×10^{59}	4.8×10^{65}

The results of table 15 imply that ligands having a high K_{f3} can be determined at lower concentrations than ligands of low K_{f3}. At pH 7 K_{f3} for EDTA is 4.8×10^{32}, consequently a 3.6×10^{-17} M solution should be determinable! In order to perform such an analysis an analytical method sensitive at the 10^{-16} molar level is required; since DPP is not reliable at concentrations below 10^{-7} M, determination of ligands at concentrations below 10^{-7} moles/liter is impossible regardless of pH or initial concentration of Co^{2+} and H_2O_2.

The detection limit of the DPP method under the conditions we employed may be estimated from the variance of the x-intercept of the "standard addition" plot by application of the t test. If four additions are made the

null hypothesis that the x-intercept equals zero (i.e. no ligands detected) may be rejected in favor of the alternate hypothesis that the x-intercept is greater than zero (i.e. ligands detected) if the computed statistic t exceeds 1.947 (95% confidence level). The variance of the x-intercept is about 0.10 for solutions of EDTA below 2 μmolar. The minimum concentration of ligands which can be detected with 95% confidence is therefore 0.6×10^{-6} moles/liter.

The variance of the x-intercept of the "standard addition" plot may be reduced by making more than four additions or by increasing the number of replicate measurements after each addition (Laisen, Hartmann and Wagner, 1973). Either step will increase the length of time required for an analysis; the recommended procedure represents a reasonable sacrifice of precision for shortened analysis time in view of the many analyses that are needed before conclusions may be drawn.

The DPP technique is not affected by the presence of chloride, carbonate, bicarbonate, Mg^{2+} or Ca^{2+} at levels normally found in groundwater. Two ions which can cause an interference are Cu^{2+} and Fe^{3+} (or Fe^{2+}). These substances exhibit electrochemical behavior in 0.05M ethylenediamine at nearly the same potential as that of Co^{2+}. It was for this reason that a blank was run for each natural water sample; the blank consisted of 50 ml of natural water sample plus ethylenediamine. If a peak appears the peak current is subtracted from each of the peaks obtained when the sample plus Co^{2+} and H_2O_2 is run. No blank peaks from Cu or Fe were observed in actual natural water samples.

Substances which inhibit the action of catalase could, if present, cause serious errors. If the excess H_2O_2 is not properly and completely decomposed by catalase, high results will be obtained because H_2O_2 will oxidize $Co(en)_3^{2+}$

to $Co(en)_3^{3+}$ thus decreasing the concentration of $Co(en)_3^{2+}$ seen by DPP; this decrease in Co(II) level cannot be distinguished from that caused by complexation with sample ligands. Chloroform, carbon monoxide, hydrogen cyanide, hydrogen sulfide, hydroxylamine and ascorbate are known to inhibit catalase (Bergmeyer 1963). These substances are not normally present in natural water in sufficient concentration to cause erroneous results.

Effect of Complexing Capacity on Water Quality. Table 9 summarizes our data; appendix B contains the raw data from which table 9 was constructed. In computing the concentration of ligands in a sample in which the chemical identity of the ligands is unknown a value of m must be assumed. We have assumed that m = 1 (i.e. one Co^{2+} binds one ligand molecule). Most of the ligands for which m would be greater than 1 form Co(III) complexes which are too weak to be detected. Since complexing capacity is equal to the product of m and the x-intercept of the standard addition plot, assumption of any value of m greater than 1 would increase the observed complexing capacities. Our primary purpose was to compare complexing capacities the value of m assumed is therefore of minor importance.

The data from our sampling network falls into two groups: sites 1, 5, 6, 8 and sites 2, 3, 4, 7. The average complexing capacity of each site in the first group was about 0.8 µmoles/l while that of the second group was about 1.4 µmoles/l.

Sites 1, 5, 6 and 8 are located on streams which receive relatively little industrial or muncipal use. Sampling station 1, for example, is located on a tributary of Crabtree Creek in Umstead State Park. Sites 2, 3, 4 and 7 are located on streams which receive moderate industrial and municipal

use. Station 7 is located on Walnut Creek just below the Raleigh sewage treatment plant. Based on the limited number of samples examined, complexing capacity is higher in streams which receive waste effluents than in streams free of such effluent.

A number of interesting trends were observed. The complexing capacity of Walnut Creek is increased by the effluent from the Raleigh treatment plant; on only one occasion (6/11/73) was the capacity below the plant smaller than that above the plant. This is not unexpected since the capacity of pure effluent from the Raleigh treatment plant was more than double that of the highest natural water sample.

Station 4, located above Burlington Industries on the Neuse River, was consistently higher than station 5, located below Burlington Industries; the average capacity of station 6, located about 7.5 miles downstream from station 5, was lower than that of station 5. The decrease in complexing capacity as the Neuse flows from site 4 to 6 (ca. 19.5 miles) is curious. Natural biodegradation or complexation of trace metals from sediments is a possible rationale for the decrease. A dilution effect as the volume of the Neuse is swelled by numerous tributaries including Crabtree Creek cannot be ignored. Clearly further work in this area is needed to determine whether the decrease is real and if so what causes it.

Certain samples in table 9 were designated N.D. This designation was used whenever the uncertainity in the x-intercept was greater than the value of the intercept. It is most unlikely that the complexing capacity of these samples is zero. The general trends are changed very little if the N.D. designation is replaced by a capacity of 0.6 μmolar (the detection limit of

our method); the average capacities of the stations become 1.0, 1.6, 1.5, 1.5, 1.1, 1.0, 1.3 and 0.9 respectively.

The results of our study are similar in magnitude to those reported by other workers. Chau (1973) reported values ranging from 0.7 to 1.0 in water from Hamilton Harbor, 1.8 to 2.5 in sewage effluent and 0.6 in water from the Niagara River near Niagara Falls. Bender, et al. (1970) reported values ranging from 1.0 to 8.0 in sewage effluent. Kunkel and Manahan (1973), using an atomic absorption technique, found capacities ranging from 15.4 to 23.3 in natural water and from 14.2 to 53.4 in sewage effluent. The high capacities found by Kunkel and Manahan may be due to the pH 10 medium employed; weak ligands which would not be determined by our method at pH 7 may form strong enough complexes to be determined at pH 10.

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GLOSSARY

A. Terms and Abbreviations

ASV: anodic stripping voltammetry. An electroanalytical technique in which a trace metal is concentrated by electrolytic reduction into an amalgam electrode then reoxidized by a linear potential scan. The maximum current resulting from the reoxidation is directly proportional to the trace metal concentration.

Complexing Capacity: The ability of a sample of water to complex metal ions; measured by forming complexes with Co^{3+}. Usually expressed as μmoles of Co^{3+} complexed per liter of sample.

DCU: decade counting unit. An electronic circuit capable of counting in base 10 arithmetic. Each unit contains several binary counters appropriately connected so that after every tenth event the counter is reset to zero.

DME: dropping mercury electrode. An apparatus consisting of a 40-50 centimeter length of 7 mm glass tubing connected to a 5-10 centimeter length of capillary tubing (0.06-0.08 mm ID, 6-7 mm OD). When the tubing is filled with mercury, drops of mercury fall from the end of the capillary once every 4-6 seconds depending on the height of the mercury head. Electrical contact is usually made through a Pt wire sealed in the wall of the glass tubing.

DPP: differential pulse polarography. An electroanalytical technique in which a slowly changing potential is applied between a DME and an appropriate reference electrode. The current flowing is mea-

sured just before and approximately 40 milliseconds after a 50 millisecond square wave pulse (5-200 millivolts in height) is applied on top of the slowly changing potential. The difference between the two currents is measured and displayed as a function of the potential of the DME at the time the voltage pulse was applied. The displayed current is directly proportional to concentration.

EDTA: ethylenediaminetetraacetic acid. A synthetic complexing agent which forms strong 1:1 complexes with most alkaline earth and transition metal ions.

Equilibrium constant: A mathematical expression relating the equilibrium concentrations of the reactants and products of a chemical reaction. For the reaction - $aA + bB \rightleftharpoons cC + dD$ the equilibrium constant is given by K

$$K = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$ where [ ] symbolizes an equilibrium concentration.

FET: field effect transistor. A solid state electronic device which functions with only one type of current carrier, either holes or electrons, but not both. An FET may function as an electrical switch by application of the appropriate voltage to the gate.

Free energy: Usually taken to mean Gibbs free energy which is the maximum amount of useful work that can be accomplished by a chemical reaction at constant temperature and pressure.

HMDE: hanging mercury drop electrode. An electrode fashioned by suspending a drop of mercury onto the end of a Pt wire which has been sealed into glass and ground flush with the glass. Commercial electrodes are available which function by squeezing a drop from the end of capillary tubing by forcing a plunger into a mercury reservoir.

Inorganic carbon: The materials in water which upon being raised to 300°C or treated with hydrochloric acid give off carbon dioxide.

Labile: A term referring to the rate at which a metal complex exchanges its ligands with other ligands. If the rate is rapid, the complex is said to be labile.

Ligand: A substance capable of forming a metal complex with a metal ion.

Logic level: The means by which voltage magnitudes are categorized in digital electronics.

Logic level 0: Any voltage smaller than 0.2V; frequently abbreviated logic 0.

Logic level 1: Any voltage greater than 3.3V; frequently abbreviated logic 1.

NAND gate: An electronic circuit capable of performing the Boolean logic operation NAND; a logic 0 output will be obtained when all logic input signals are logic 1.

NTA: Nitrilotriaacetic acid.

Q: Symbol frequently used in digital electronics for the output of a flip-flop.

TC: Total carbon. The total quantity of carbonaceous materials in water as determined by combustion to CO_2 at 900°C. Total carbon includes both organic and inorganic carbon materials.

TOC: Total organic carbon. The difference between TC and inorganic carbon.

B. Mathematical symbols

F = Faradays constant; 96,487 coulombs/equivalent or 22,061 calories/volt-equivalent.

ΔG° = the standard Gibbs free energy change for a chemical reaction; the change in free energy accompanying the conversion of reactants in their standard states to products in their standard states.

$(K_{eq})_{o.a.}$ = the equilbrium constant for the overall reaction between Co(II), ligand and H_2O_2 yielding the Co(III) complex of the ligand.

K_{f2} = the equilbrium constant for the formation of a complex between a ligand and Co(II).

K_{f3} = the equilbrium constant for the formation of a complex between a ligand and Co(III).

m = the number of ligand molecules which interact with one metal ion to form a metal complex.

n = the number of electrons transferred per molecule of reactant in an electro-reduction or electro-oxidation.

R = gas constant; 1.9872 calories/degree-mole or 8.3143 joules/degree-mole.

T = absolute temperature.

APPENDIX A

Determination of Complexing Capacity Using Differential Pulse Polarography

Basic Principles

Complexing agents are converted to the corresponding Co(III) complex by the reaction:

$$2Co^{2+} + 2mL + H_2O_2 + 2H^+ \rightleftharpoons 2CoL_m^{3+} + 2H_2O$$

A known amount of Co^{2+} is added; the concentration of Co^{2+} remaining after the reaction has reached equilibrium is determined with differential pulse polarography in an ethylenediamine medium. The amount of cobalt consumed by ligands in the sample is best determined by the standard addition method but may also be determined by subtracting the micromoles of Co left after the reaction from the micromoles of Co added initially.

Reagents

a. Phosphate Buffer, 0.1M; Dissolve 8.801g Na_2HPO_4 and 13.799g $NaH_2PO_4 \cdot H_2O$ in deionized water and dilute to one liter. Adjust to pH 7 with strong acid (12M HCl) or strong base (NaOH, 50%). Store at 5°C in polyethylene container.

b. Cobalt solution, 0.01M; Dissolve 0.238g $CoCl_2$ in deionized water and dilute to 100 ml. Store in polyethylene container and standardize before use.

c. Hydrogen Peroxide solution, 0.5M; Pipet 10 ml 30% H_2O_2 and dilute to 250 ml with deionized water. Store at 5°C in polyethylene container and check strength periodically.

d. Catalase solution, 3100 units/mg; Dissolve 10 mg Catalase in autoclaved deionized water and dilute to 100 ml. Store at 5°C in 1 ml portions. Autoclave all glassware used and check Catalase activity periodically by monitoring H_2O_2 disappearance as a function of time.

e. Ethylenediamine, 3.38M; Distill as 70% azeotrope and dilute 5ml of distillate to 25 ml with deionized water. Store in polyethylene container at 5°C.

Standardization

Cobalt solution - Titrate with standard EDTA solution (10^{-2}M) at pH 5-6 (hexamethylenetetramine buffer) and 80°C using xylenol orange as indicator.

Hydrogen Peroxide - Titrate with standard cerium(IV) solution using ferroin as indicator.

EDTA solution - Titrate with standard copper(II) prepared by dissolving copper wire in nitric acid and diluting with deionized water. Use hexamethylinetetramine buffer at pH 5-6 and xylenol orange as indicator.

NTA solution - Titrate with standard cobalt solution at pH 9.2 using murexide as indicator.

Procedure

Samples should be stored at 5°C and allowed to stand until sediments have settled. Pipet a 50 ml aliquot into a Berzelius beaker (cell) and adjust the pH by adding 4 ml of 0.1M Na_2HPO_4 - NaH_2PO_4 buffer (pH 7). Then add 50 µl of 0.01M stock cobalt solution and 50 µl of 0.5M H_2O_2. Stir and allow 15 minutes for completion of reaction. Add 200 µl of Catalase solution, followed by incubation at 36°C in a water bath for at least 15 minutes. Longer incubation may be necessary depending on the Catalase activity. Stir

or agitate the sample cell.

Support sample cell on the dropping mercury electrode stand and purge the solution of dissolved oxygen by bubbling purified nitrogen through a medium porous diffusion tube for 15 minutes. Purge the standard cobalt solution and stock ethylenediamine solution in the same manner. Add 0.5g of KNO_3 and 1 ml of 3.38M ethylenediamine to the sample solution. Place electrodes in the cell and position the gas diffusion tube just above liquid surface. Using the PAR Polarographic Analyzer in the differential pulse mode, scan positive from -0.75 volts with 100 mv. modulation amplitude and 2 second drop time. From the recorded current-voltage curve, select a base line and peak potential to set the potential step control accessory as described in the experimental section. After a set of current cycles has been recorded, add 50 µl of deaerated 0.01M cobalt solution, stir for a minute and repeat measurements. At least three standard additions are recommended.

Blank

A blank of each sample is determined by pipeting a 50 ml aliquot into a Berzelius beaker (cell) and adjusting the pH by adding 4 ml of 0.1M Na_2HPO_4 - NaH_2PO_4 buffer (pH 7). Support the sample cell on the dropping mercury electrode stand and purge the solution of dissolved oxygen by bubbling purified nitrogen through a medium porous diffusion tube for 15 minutes. Add 0.5 g of KNO_3 and 1 ml of 3.38M ethylenediamine to the sample solution. Place electrodes in the cell and position the gas diffusion tube just above liquid surface. Using the PAR Polarographic Analyzer in the differential pulse mode, scan positive from -0.75 volts with 100 mv. modula-

tion amplitude and 2 second drop time. Record the current-voltage curve and determine the peak current of any inpurities which are oxidized or reduced in the potential region corresponding to the cobalt oxidation. The determined blank peak current should be subtracted from the observed cobalt oxidation currents.

Calculation of Results

A plot of the recorded currents versus the microliters of cobalt solution added will be linear with an intercept at the origin if no complexing agents are present. The shift of the intercept along the abscissa is a measure of the quantity of ligands in the sample. The concentration of complexing agents in the sample is given by:

$$\mu\text{moles ligand per liter} = \frac{-20 \times c \times C}{m}$$

where c = the y-intercept, m = the slope, and C = the concentration of Co(II) solution (moles/liter). From a linear regression fit of the data, the uncertainity of the complexing capacity may be computed in the form of a confidence interval (Larsen, Hartmann and Wagner, 1973):

$$\pm \frac{20C}{m} t_{(\alpha,n-2)} \cdot s_{y/x} \cdot \sqrt{\frac{n+1}{n} + \frac{[(-c/m + \bar{x})]^2}{(n-1)s_x^2}}$$

where $t_{(\alpha,n-2)}$ = the value of the statistic t at confidence level $(1-\alpha/2)\cdot 100\%$ and n-2 degrees of freedom, n = the number of current values plotted, $s_{y/x}$ = the standard error of estimate, s_x = the standard deviation of the volumes of cobalt solution added and $\bar{x}$ = the mean of the volumes of cobalt solution added. If the confidence interval is greater than the complexing capacity, the result is considered below the detection limit of the method; otherwise the confidence interval is a measure of the uncertainity of the determination.

APPENDIX B

Raw Data from Complexing Capacity Measurements

Sampling Site 1

µl Co^{2+}	Current (µAmp)			
	I	II	III	IV
50	0.195	0.189	0.236	0.215
	0.195	0.188	0.233	0.216
100	0.430	0.430	0.475	0.445
	0.427	0.426	0.471	0.445
150	0.649	0.665	0.674	0.669
	0.645	0.660	0.673	0.668
200	0.876	0.886	0.880	0.884
	0.866	0.880	0.880	0.884

Sampling Site 2

50	0.182	0.201	0.192	0.202
	0.179	0.197	0.192	0.203
100	0.422	0.429	0.438	0.445
	0.420	0.426	0.432	0.446
150	0.652	0.649	0.669	0.687
	0.650	0.649	0.665	0.685
200	0.884	0.876	0.894	0.920
	0.878	0.874	0.890	0.910

Sampling Site 3

50	0.193	0.197	0.189	0.194
	0.190	0.196	0.189	0.195
100	0.431	0.415	0.429	0.430
	0.430	0.415	0.427	0.430
150	0.665	0.638	0.655	0.669
	0.662	0.635	0.653	0.670
200	0.886	0.850	0.890	0.896
	0.884	0.850	0.890	0.900

Sampling Site 4

µl Co^{2+}	Current (µAmp)			
	I	II	III	IV
50	0.180	0.208	0.192	0.201
	0.179	0.207	0.192	0.202
100	0.419	0.460	0.432	0.425
	0.416	0.460	0.430	0.425
150	0.642	0.680	0.671	0.657
	0.645	0.675	0.668	0.655
200	0.876	0.900	0.906	0.890
	0.872	0.898	0.904	0.890

Sampling Site 5

50	0.177	0.193	0.245	0.208
	0.177	0.191	0.247	0.207
100	0.426	0.435	0.475	0.436
	0.424	0.432	0.474	0.432
150	0.657	0.665	0.703	0.664
	0.653	0.662	0.700	0.663
200	0.896	0.870	0.920	0.890
	0.890	0.876	0.920	0.884

Sampling Site 6

50	0.191	0.242	0.214	0.196
	0.190	0.242	0.212	0.196
100	0.435	0.487	0.435	0.430
	0.431	0.484	0.432	0.430
150	0.668	0.728	0.652	0.655
	0.665	0.724	0.650	0.658
200	0.894	0.960	0.860	0.876
	0.892	0.946	0.860	0.878

Sampling Site 7

µl Co^{2+}	Current (µAmp)			
	I	II	III	IV
50	0.186	0.191	0.215	0.192
	0.185	0.191	0.216	0.192
100	0.422	0.415	0.450	0.414
	0.420	0.420	0.443	0.412
150	0.654	0.640	0.673	0.638
	0.652	0.642	0.670	0.638
200	0.886	0.864	0.900	0.856
	0.884	0.864	0.900	0.854

Sampling Site 8

50	0.200	0.205	0.210	0.201
	0.196	0.210	0.207	0.205
100	0.437	0.430	0.432	0.438
	0.435	0.435	0.430	0.439
150	0.670	0.650	0.660	0.665
	0.670	0.654	0.660	0.665
200	0.904	0.876	0.878	0.886
	0.900	0.870	0.878	0.886

Concentration of Co^{2+} solution was exactly 0.0100 M.

APPENDIX C

North Carolina Fresh Surface Water Classifications

Class A-I. Source of water for drinking, culinary, or food processing purposes or any other usage requiring water of lower quality. This class is intended primarily for waters having watersheds which are uninhabited and which require only approved disinfection in order to meet the "Public Health Service Drinking Water Standards".

Quality Standards Applicable to Class A-I Waters

Items	Specifications
a. Floating solids; settleable solids; sludge deposits; taste or odor-producing substances.	None attributable to sewage, industrial wastes or other wastes.
b. Sewage, industrial wastes, or other wastes.	None.
c. Toxic wastes; oils; deleterious substances; colored or other wastes.	None.
d. Organisms of coliform group.	Not to exceed 50/100 ml (either MPN or MF count) as a monthly average value.
e. Radioactive Substances.	Gross beta activity (in the known absence of Strontium-90 and alpha emitters) not to exceed 1,000 picocuries per liter at any time.

Class A-II. Source of water supply for drinking, culinary or food processing purposes or any other usage requiring water of lower quality. The waters, if subjected to approved treatment equal to coagulation, sedimentation, filtration and disinfection, will meet the "Public Health Service Drinking Water Standards."

Quality Standard Applicable to Class A-II Waters

Items	Specifications
a. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, make the waters unsafe or unsuitable as a source of water supply for drinking, culinary, or food-processing purposes, or injurious to fish and wildlife.
b. Sewage, industrial wastes, or other wastes.	None which are not effectively treated to the satisfaction of the Board of Water and Air Resources and in accordance with the requirements of the State Board of Health.

c. Odor-producing substances contained in sewage, industrial wastes, or other wastes.	Only such amounts, whether along or in combination with other substances or wastes, as will not, after reasonable opportunity for dilution and mixture of same with receiving waters, cause taste and odor difficulties in water supplies which cannot be corrected by treatment as specified above, or impair the palatability of fish.
d. Phenolic compounds.	Not greater than 0.001 mg/l (phenols).
e. pH	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5 except that swamp waters may have a low of 4.3.
f. Total hardness.	Not greater than 100 parts per million as $CaCO_3$.
g. Dissolved oxygen.	Not less than 6.0 mg/l for natural trout waters; 5.0 mg/l for put-and-take trout waters; not less than a daily average of 5.0 mg/l with a minimum of not less than 4.0 mg/l for non-trout waters, except that swamp waters may have lower values if caused by natural conditions.

h.	Toxic wastes; oils; deleterious substances; colored or other wastes.	Only such amounts, whether alone or in combination with other substances or wastes as will not render the waters unsafe or unsuitable as a source of water supply for drinking, culinary, or food-processing purposes, injurious to fish and wildlife or adversely affect the palatability of same.
i.	Organisms of coliform group.	Not to exceed 5,000/100 ml as a monthly average value (either MPN or MF count); nor exceed this number in more than 20% of the samples examined during any one month; nor exceed 20,000/100 ml in more than 5% of such samples, Fecal Coliforms (MPN and MF count) not to exceed a log mean of 1,000/100 ml based on at least five consecutive samples examined during any 30-day period; nor exceed 2,000/100 ml in more than 20% of the samples examined during such period. (not applicable during or immediately following periods of rainfall.)

j. Temperature.	Not to exceed 5°F above the natural water temperature, and in no case to exceed 84°F for mountain and upper piedmont waters and 90°F for lower piedmont and coastal plain waters. The temperature of natural trout waters shall not be significantly increased due to the discharge of heated liquids and shall not exceed 68°F; however, the temperature of put-and-take trout waters may be increased by as much as 3°F but the maximum may not exceed 70°F.
k. Radioactive substances.	Same as class A-I.

Class B. Best usage of waters is bathing or any other purpose except as source of water supply for drinking, culinary or food processing purposes. The waters, under proper sanitary supervision by controlling health authorities, will meet accepted standards of water quality for outdoor bathing places.

Quality Standards Applicable to Class B Waters

Items	Specifications
a. Floating solids; settleable solids, sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving

		waters, make the waters unsafe or unsuitable for bathing, or injurious to fish and wildlife.
b.	Sewage, industrial wastes, or other wastes.	None which are not effectively treated to the satisfaction of the Board of Water and Air Resources in determining the degree of treatment required for such waste when discharged into waters to be used for bathing, the Board will take into consideration the quantity and quality of the sewage and wastes involved and the proximity of such discharges to the waters in this class.
c.	Phenolic compounds.	Same as class A-II.
d.	pH.	Same as class A-II.
e.	Dissolved oxygen	Same as class A-II.
f.	Toxic wastes; oils; deleterious substances; colored or other wastes.	Only such amounts, whether alone or in combination with other substances or wastes as will not render the waters unsafe or unsuitable for bathing, injurious to fish and wildlife or adversely affect the palatability of same.

g.	Organisms of coliform group. (Applicable only during the months of May through September. During other months the coliform organism standard for Class "C" Waters shall apply.)	Fecal coliforms not to exceed a log mean of 200/100 ml (either MPN or MF count) based on at least five consecutive samples examined during any 30-day period and not to exceed 400/100 ml in more than 20% of the samples examined during such period. (Not applicable during or immediately following periods of rainfall).
h.	Temperature.	Same as class A-II.

Class C. Best used for fishing, boating, wading and any other purpose except for bathing or as a source of water supply for drinking, culinary or food processing purposes. The waters will be suitable for fish and wildlife propogation.

Quality Standards Applicable to Class C Waters

	Items	Specifications
a.	Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters or make the waters unsafe or unsuitable for fish and wildlife.
b.	pH.	Same as class A-II.
c.	Dissolved oxygen	Same as class A-II.

d.	Toxic wastes; oils; deleterious substances; colored or other wastes.	Only such amounts, whether alone or in combination with other substances or wastes as will not render the waters injurious to fish and wildlife or adversely affect the palatability of same.
e.	Organisms of coliform group.	Fecal coliforms not to exceed a log mean of 1,000/100 ml (MPN or MF count) based upon at least five consecutive samples examined during any 30-day period; nor exceed 2,000/100 ml in more than 20% of the samples examined during such period. (Not applicable during or immediately following periods of rainfall).
f.	Temperature.	Same as class A-II.

Class D. Best used for agriculture, industrial cooling and process water supply, navigation and any other usage, except fishing, bathing, or as a source of water supply for drinking, culinary or food processing purposes. The waters without treatment will be suitable for agricultural uses and will permit fish survival.

Quality Standards Applicable to Class D Waters

	Items	Specifications
a.	Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or

		other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, render the waters unsuitable for agriculture, industrial cooling purposes and fish survival, or cause an offensive condition.
b.	pH.	Same as class A-II.
c.	Dissolved oxygen.	Not less than 3.0 mg/l.
d.	Toxic wastes; oils; deleterious substances; colored or other wastes.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not render the waters unsuitable for agriculture, industrial cooling purposes, navigation, fish survival, or cause offensive conditions.
e.	Organisms of coliform group. (applicable only to waters designated by the Board for irrigation of fruits and vegetables).	Fecal coliforms not to exceed a log mean of 1,000/100 ml (MPN or MF count) based upon at least five consecutive samples examined during any 30-day period; nor exceed 2,000/100 ml in more than 20% of the samples examined during such period. (Not applicable during or immediately following periods of rainfall).
f.	Temperature.	Not to exceed 5°F above the natural water temperature and in no case to

exceed 84°F for mountain and upper piedmont waters and 90°F for lower piedmont and coastal plain waters.

Taken from "Rules, Regulations, Classifications and Water Quality Standards Applicable to the Surface Waters of North Carolina," adopted by Board of Water and Air Resources, Office of Water and Air Resources, Department of Natural and Economic Resources, 1972.