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Trace metal ion activities from liquid-liquid partitioning measurements

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TRACE METAL ION ACTIVITIES FROM LIQUID-LIQUID PARTITIONING MEASUREMENTS

by

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Elucidation of the chemical speciation of trace metals in the natural aquatic environment will lead to a better understanding of their distribution and ecological effects. One approach which can provide useful information about the chemical reactivity of metal ions is the measurement of their activity. Phase equilibrium methods are required and liquid-liquid partition equilibria are applicable. This study

utilized model systems to demonstrate this applicability.

The partitioning of copper(II) ions as a chelate of acetylacetone was used to determine the trace activity coefficients of the copper(II) electrolyte in the $Cu(NO_3)_2$-HNO_3-KNO_3, $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ and $CuCl_2$-HCl-KCl systems over a wide range of ionic strengths (μ). By careful control of pH and acetylacetone concentration only 1-3% of the metal ion was extracted. Under these conditions the amount extracted is proportional to the activity. The concentration of the bis(Acetylacetonato) Copper(II) was determined in the organic phase by spectrophotometric and atomic absorption methods but any convenient concentration technique could be used to measure the amount extracted.

A comparison of activity measurements by liquid-liquid partitioning was made with electrochemical measurements by utilizing a copper ion selective electrode. The significantly lower activity coefficient values obtained by the electrochemical method were explained in terms of the liquid junction potential and the necessity for extrathermodynamic approaches to single ion activities.

Potential application of the liquid-liquid partitioning method to the determination of trace activity coefficients in natural aquatic systems was demonstrated by extension of the method to measurements in copper(II) amino acid solutions at $\mu \simeq 0.001$ and $\mu = 0.723$. The ionic strength adjustments in this case were made with NaCl. A significant difference in the free copper(II) ion activity was observed between solutions of copper(II) glycinate and copper(II) alaninate under identical conditions of metal and ligand concentrations, pH and ionic strength. The copper(II) activity measurements made in the presence of the amino

acids at $\mu = 0.723$ are not possible with copper ion selective electrodes due to chloride interference.

DEDICATION

This work is dedicated to my mother, Ida Marie, for her gift of perseverance; to my father, John William, for his gift of insatiable curiosity; and to my wife, Patricia Fleming, for her gift of love.

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CHAPTER I

INTRODUCTION

Recent research directed toward understanding the distribution and ecological effects of trace metals in the natural aquatic environment has led to the realization that a complete elucidation of their chemical speciation is required. Determining the analytical concentration of the particular metal of interest is no longer sufficient. This conclusion has resulted from the observed response of biological systems to trace metals in natural waters. Frequently these observations have appeared to disagree with the known chemistry of the metals. Our lack of knowledge about metal speciation is the basic cause and should be of concern since many trace metals are essential for the growth of aquatic biota but become toxic under the appropriate conditions (1).

The biological availability of metals such as iron and copper to phytoplankton has been shown to depend upon the chelating properties of naturally occurring ligands (2). Phytoplankton growth experiments by Barber (3) demonstrated significant response to small additions of both metals and chelators. These and similar observations led Gächter, Lum-Shue-Chan and Chau (4) to conclude that the free copper ion and its inorganic complexes are more toxic than are its organic complexes. They estimated that ionic copper becomes toxic to planktonic algae at concentrations of approximately 10^{-10} M. Stiff (5) and Pagenkopf,

Russo and Thurston (6) indicated that reduced copper toxicity results from bicarbonate alkalinity through formation of carbonate complexes or precipitates. Sylva (7) showed that the influence of organic complexing agents such as humic and fulvic acid cannot be overlooked.

More recently, detailed studies utilizing equilibrium calculations have provided increased evidence that copper toxicity is indeed related to the activity of the free copper ion. Sunda and Guillard (8), utilizing cultures of Thalassiosira pseudonana and Nannochlous atomus, found that both growth rate inhibition and the copper content of the cells were related to the activity of the cupric ion. The growth rates of Thalassiosira pseudonana and Nannochlous atomus were inhibited by copper activity between 3×10^{-11} to 5×10^{-9} M and 4×10^{-11} to 2×10^{-9} M, respectively. Andrew, Biesinger and Glass (9) also demonstrated that mortality rates for Daphnia magna can be correlated with the activities of Cu^{+2} and $Cu(OH)^{+}$. Negative correlation was found between toxicity and the activities of soluble copper carbonate and other complexes. Toxicity was shown to be independent of dissolved copper or total copper concentrations. These studies (6,8,9) demonstrate that equilibrium calculations can be used to interpret toxicity data if factors such as pH, alkalinity and total concentration of strong chelating agents are known.

Ideally, then, one might like to know the analytical concentration of each interactive metal ion and each ligand, all equilibrium constants, and conditions of temperature, pH and pE. This kind of information can provide chemical insight into the basis of the biological response of a given aquatic system to changes in any of the above parameters.

The role of coordination chemistry in the development of complex chemical equilibrium models which are applicable to natural aquatic systems has been well demonstrated (10-12). A computer program (13) based on these concepts has been used to calculate the equilibrium distribution for a model system of 20 metals and 31 ligands. Extensions of this program (14,15) consider redox reactions, interaction intensities, buffer capacity and pH stability in complex aqueous systems. Lerman and Childs (16) have also demonstrated the importance of kinetic relationships and interactions with sediments in the control of soluble specie concentrations. Morel and Yearsted (17) have discussed a more generalized approach which interfaces chemical, physical and biological water quality models for the purpose of predicting the results of complex interactions.

Experimentally, relatively few analytical methods have been applied to the characterization of metal ion species in natural waters. The requirements are rather restrictive. They include detection of the trace metal ions at their naturally occurring levels and differentiation of the various chemical species without significantly changing their equilibria (18,19). Considering the fact that reliable methods for the measurement of metal concentration at the parts per billion level on a routine basis are relatively new, it is not surprising that considerable difficulty has been encountered in developing analytical methodology to elucidate the chemical nature of metals in natural waters. Techniques like neutron activation analysis and atomic absorption spectrometry (AAS) are highly sensitive but only to the total metal concentration. Application of these methods to speciation studies

requires separation procedures which shift the existing equilibria. Therefore, the investigation of metal ion complexes in natural waters has centered on electrochemical methods.

Potentiometric and voltammetric methods are finding increasing use in studies of both natural and model systems. Studies utilizing ion selective electrodes (ISE) have been few due to lack of sensitivity ($\leq 10^{-7}$ M) and availability of electrodes for each ion of interest. The low sensitivity has unfortunately outweighed the ISE main advantage, that of response to the metal ion activity. That is to say, the potential of the indicator electrode, E_i, (in volts) can be written in terms of the activity of the ion to which it is responding. Difficulty does arise, however, in evaluating E_i because it depends on a knowledge of the liquid junction potential, E_j, which can vary between electrode standardization and measurement of an unknown test solution.

Stiff (20) used the copper ISE to measure the free copper concentration in polluted fresh waters as part of an analytical scheme to differentiate the forms of soluble copper. He concluded that amino acids and polypeptides may play as important a role as bicarbonate (5) in determining the free copper concentration in polluted river water and sewage-treatment plant effluent. Gardiner (21) studied the formation of cadmium complexes in model systems, river water and sewage effluent samples utilizing the cadmium ISE. Although the minimum detectable concentration of the electrode is considerably higher than the concentrations found in natural waters, Gardiner claims extrapolations can be made with reasonable certainty. He found that a

large fraction of the total cadmium is usually present as the free metal ion and that complexation is dependent on pH and the presence of humic material. Ramamoorthy and Kushner (22) developed a method of determining heavy metal (Hg, Pb, Cu, Cd) binding capacity of various molecular weight fractions utilizing ISE and ultrafiltration. These experiments offer promise for evaluating the effects of fulvic and humic substances on heavy metal binding by natural waters (23).

More recently, Buffle et al. (24) presented a method for determining the complexation properties of humic and fulvic acids in natural waters using Pb^{+2} and Cu^{+2} ISE. Through graphical and computer evaluation of the data, values were obtained for the mean molecular weight of the ligand, the stability constants of the complexes, number of ligands fixed per metal ion, and the dependence of the stability of the complexes on pH. The above method seems advantageous since useful parameters are measured with little change in the natural matrix.

Similar attempts to investigate metal-organic ligand interactions in seawater (25,26) utilizing a copper ISE have suffered from several difficulties. The most serious drawback has been a non-Nernstian response of approximately 50 mv per decade change in copper concentration. A recent study (27) has demonstrated that the response slope is dependent upon the presence of chloride ion and is independent of the background ionic strength. Likewise, difficulties have been encountered (28) in applying the copper ISE to concentrations below 10^{-7} M, where the electrode response time becomes long. In metal ion buffers this response time remains linear with ion activity to 10^{-10} - 10^{-12} M. The metal's analytical concentration must be higher, however, to

provide thermodynamic response. This means that care must be taken in interpreting electrode response in unknown solutions which do not contain the same chelating ligands as the metal ion buffer used for calibration.

Anodic stripping voltammetry (ASV) has received increased interest of late because its detection limit (10^{-9} - 10^{-10} M) for heavy metals of interest (Cu, Cd, Pb, Zn) is well within the range of naturally occurring levels. It also has the additional advantages of application to in situ measurements, high selectivity, high precision at very low concentration levels, adaptability to field studies, relatively low capital and analysis cost, as well as the advantage of electroanalytical techniques to distinguish between the free metal ion and complexed species.

ASV has been recognized as a potentially useful tool for trace characterization (29) for some time and was first successfully applied to the study of environmental samples over a decade ago (30). A comprehensive study of its utility was completed by Matson (31). He noted that inorganic metal complexes dissociate rapidly during the ASV reduction step while organic complexes formed from multidentate organic ligands dissociate slowly or not at all. The former were termed "labile" and the latter "nonlabile" complexes. Matson took advantage of this property to estimate the concentration of nonlabile ligands in solution by titration with metal ions and determination of the endpoint by monitoring the free metal concentration with ASV. Formation constants were also calculated following equilibrium potential measurements and an acid digestion of the sample. Shuman and Woodward

(32,33) have since shown that this same complexometric titration alone is sufficient to estimate the conditional formation constants.

Matson (31) also used ASV to estimate the amount of metal bound in organic complexes. The metal was displaced by addition of a competing cation (H^{+}, Ca^{+2}, Fe^{+3}) or by ultraviolet irradiation. In a subsequent study Allen, Matson and Mancy (34) were able to provide evidence of metal complexation in the waters of the Great Lakes and several rivers by analysis for Cd^{+2}, Pb^{+2} and Cu^{+2} before and after acidification to pH 2. They noted that the rate of metal release varied with sample location and concluded that slower exchange was characteristic of more stable nonlabile complexes. Bender, Matson and Jordan (35) extended the technique to secondary sewage effluents and determined the ligand concentration by titration with copper. Chau et al. (4,36-39) utilized this technique as the basis for the determination of complexing capacity in lake water and nutrient medium.

The determination of ligand concentration by this method does, however, require caution. By measuring the copper complexing capacity of several known complexing agents, it was determined (39) that the technique can measure only those complexes which bind copper with a log stability constant (log K) greater than approximately 13. A study (40) of the copper(II) glycinate system indicates that the method does not distinguish the complex from the metal ion. Hanck and Dillard (41,42) suggest that the labile or inert nature of the metal complex is a function of the electronic structure of the metal ion. They recommend use of Cr^{+3}, Fe^{+3}, or Co^{+3} rather than metals like Cu^{+2}, In^{+2} and Co^{+2}, which form labile complexes. Their method (42) for the titration of

natural ligands involves titration with Co^{+2}, oxidation of the complexes formed to inert Co^{+3} complexes with H_2O_2, followed by determination of the unreacted Co^{+2} by differential pulse polarography (DPP), after removal of the excess H_2O_2 with the enzyme catalase.

Several interesting studies have also been conducted in the field of chemical oceanography. Zirino and Healy (43-45) utilized pH-controlled differential ASV to study Zn^{+2}, Pb^{+2} and Cd^{+2} distribution and complexation in seawater. They noted that reduction of the pH from 8.3 to 5.6 by the addition of CO_2 (20% CO_2 in N_2) resulted in an increase in the peak currents of zinc and lead but not for cadmium. By applying a thermodynamic model (12) to their experimental conditions, they were able to indicate that these metals are complexed with inorganic anions in the open ocean. Bradford (46), through a laboratory study utilizing ASV, was able to confirm Zirino and Healy's conclusion (43) that soluble zinc in seawater exists mainly as $Zn(OH)_2$ ($\simeq$75%) while $ZnCO_3$ represents a small fraction of the total ($\simeq$4%).

More recently, ASV measurements have been used as part of large metal speciation schemes which are dependent upon physiochemical properties of the metal species. Guy and Chakrabarti (23,47-49) developed a scheme utilizing model systems based on ASV, dialysis and ultrafiltration. ASV (48) was used to separate the metal species into free metal ions, labile species and electroinactive or nonlabile species. Dialysis and ultrafiltration combined with AAS measurements (49) were used to differentiate between species by size fraction. Smith (50)

applied ultrafiltration and ASV to the determination of complexation capacity of various molecular weight fractions of dissolved organic matter in estuarine waters. He found that high molecular weight fractions demonstrated a significant copper complexation in fresh water and that the low molecular weight range ($\leq$ 1000) had increased complexation capacity with increasing salinity.

Direct ASV measurements, based on Matson's work (31), are the foundation of another analytical scheme (18,51) for characterization of metals in natural waters. The data collected is based on peak current (i_p) and peak potential (E_p) measurements and metal-ligand titrations. Free metal ions and labile complexes are differentiated from nonlabile metal complexes by i_p measurements; E_p measurements are used to distinguish free metal ions from complexed metal. Taken together these measurements can differentiate free metal ion and labile metal complexes. The metal-ligand titrations are used to distinguish labile from nonlabile metal complexes. The form of the titration curve depends upon the metal-ligand interactions and the rate and extent of interaction between the trace metal and the working electrode.

Batley and Florence (52-54) have developed the most extensive scheme for the classification of heavy metal species in natural waters. Seven different species of each metal (Zn, Cd, Pb, Cu) can be quantitatively evaluated using ASV in conjunction with chelating resin (Chelex - 100) separations and decomposition of organic matter by ultra violet irradiation. This scheme consists of ASV measurements of labile and total metal concentration of the 0.45 μm filtrate (irradiated and

non-irradiated) before and after passage through a chelating resin column.

Although this trace metal speciation scheme can provide more information about the chemical forms of Cu, Cd, Pb and Zn in natural water than previously described methods, there are disadvantages (54). First, the scheme requires an experienced operator. Secondly, the measurements probably disturb the equilibria. Finally, it may not be possible to relate the various classifications to the chemical species present in the original sample. However, the latter disadvantages are encountered in nearly every analytical method which has been applied to natural systems except for the use of ISE.

Experience in ASV operation appears to be critical for its application to metal speciation studies. Hume and Carter (55) have shown that proper preparation of the thin film mercury-coated graphite electrode is necessary for obtaining reproducible results in determining trace metal concentrations and expressed doubt that direct evaluation of changes in stripping curves was meaningful in the investigation of metal-ligand interactions. In addition, several researchers (47,49,56-58) have noted that surfactants of the type found in natural waters adsorb on the mercury surface and inhibit the electrode reactions. The general conclusion has been that ASV data must be evaluated with care. In a recent review (59) of voltammetric and polarographic parameters with respect to chemical speciation studies, the authors noted that correct interpretation of electroanalytical results requires not only a knowledge of mechanics, electronics and mathematics, but also the chemical properties of the species to be analyzed.

Nevertheless, ASV has provided the first detailed quantitative information on the speciation of an important, although limited,

number of trace metals. This attests to the fact that ASV is a convenient and highly useful tool for distinguishing between various species of a metal. The method has provided many answers and added new insight for future investigation.

Prior to the application of electrochemical techniques, separation methods had been used to confirm the occurrence of and measure the extent of metal ligand interactions in the natural aquatic environment. More specialized separation techniques are also being used today. In separating the constituents of interest, both their physical and/or chemical properties may be utilized. The methods which have been applied to natural systems have recently been reviewed (19) and include precipitation, solvent extraction, ion exchange, dialysis, ultrafiltration and gel permeation chromatography.

Several of these (e.g., ion exchange and liquid-liquid partitioning) are based on an equilibrium similar in nature to that which establishes an electrode potential and therefore can provide the same kind of information about metal speciation as electrochemical methods. For example, the determination of relative activity coefficients in mixed electrolytes was first demonstrated by Vanselow (60) who used the cation-exchange reactions of bentonite to investigate the barium-cadmium exchange reaction. Schubert (61), in a theoretical discussion on the applicability of synthetic ion exchangers for the determination of physiochemical properties of substances, suggested that Vanselow's method could be applied more successfully with synthetic exchangers and carrier-free radiotracers.

In a subsequent work Schubert (62) demonstrated this by reporting the activity coefficients of barium nitrate in uranyl nitrate solutions. The measurements were made over a fairly wide range of ionic strengths. He also noted that the technique might have potential for elucidating the role of trace metals in biological systems. The method has been extended to complex natural solutions such as milk (63). This more recent methodology involves equilibrating the solution of unknown metal ion activity with a strong cation exchange resin which has been previously calibrated by equilibration with standard solutions.

Recently Allen, Crosser and Brisbin (58) utilized ion exchange equilibrium techniques, with cation resin, to measure stability constants and complexation capacity of model systems composed of copper salicylate, copper glycinate or a mixture of the two. These measurements require constant conditions of temperature, pH, ionic strength, solution volume and weight of ion exchange resin. Their research has been directed toward demonstrating the significance of the stability constant of multi-ligand systems since these systems more closely approximate those observed in natural systems and should help in understanding complex environmental samples. The authors noted that ion exchange equilibrium appears to have some advantages over electrochemical techniques; for example, it can provide the stability constants and the complexation capacity of both glycine and humic acid but ASV can not provide the stability constant for humic material due to adsorption effects, nor can it provide the complexation capacity for glycine.

A similar approach has been outlined by MacCarthy (64,65) and

applied to the study of adsorption and desorption of metal ions on sediments. Following determination of the ion exchange isotherm for the metal of interest, the resin is equilibrated with the system of interest. Measurement of the metal bound by the resin allows calculation of the free metal in solution and subtraction of this value from the total dissolved metal provides the concentration of the total complexed metal. The method has potential application to simultaneous measurement of several metals and has been applied to Zn^{+2} desorption from sediments by mine drainage.

In addition to analytical techniques, some consideration should be given to the potential application of biological methods to metal speciation studies. It has been demonstrated that bio-assays are highly sensitive to low concentrations of both organic and inorganic nutrients and/or toxicants in aquatic systems. Euglena, for example, has been used to determine Vitamin B_{12} concentrations as low as 10^{-16} $liter^{-1}$ (11). Similar sensitivity has been observed for cupric ion by phytoplankton (18), daphnia (9) and bacteria (66). Sunda (66) has demonstrated correlations between the growth response of bacteria and the response of the ISE to the activity of the free cupric ion. Unfortunately, the copper ISE is not sensitive enough to allow quantitative calibration of the full response range of most organisms but, as mentioned earlier, thermodynamic calculations have been used with reasonable success to show correlation of the activity of the free cupric ion with biological growth rate and toxicity (8,9). This method can be applied to well-defined systems, such as nutrient medium, with reliable certainty. Extension to natural aquatic systems is

doubtful at present because currently available analytical methods can not totally define these systems, although average properties such as complexation capacity, which has also been measured by a biological method (67), might be helpful.

Until very recently most bio-assay experiments were conducted with little or no consideration of the equilibrium chemistry involved. Mancy and Allen (51) reported a bio-assay system for which they carefully considered the physiochemical and biological interrelationships and controlled the independent variables. The major controlling factor is the physiochemical character of the bio-assay water. When at thermodynamic equilibrium, it essentially establishes the activity of the toxic metal species which then affect metal uptake by the organism. Ultimately the rate and total metal uptake will determine the biological response. It is this response that indicates the physiological condition of the organism and in turn affects metal uptake. If one desires to understand the effect of each variable on the observed result, control of the hydrodynamics, temperature, ionic strength, light, dissolved oxygen, pH, alkalinity and chemical composition of the medium is necessary (51).

Continuous flow bio-assay offers the most promise for metal speciation study since it allows continuous addition of the desired chemically equilibrated bio-assay medium. This helps to insure constant conditions throughout the experimental period. It also prevents loss of the toxic substance through uptake, adsorption and buildup of extracellular products in studies utilizing microorganisms. The extracellular products may bind the trace metal ion or may themselves

be toxic. Analytical monitoring of the bio-assay water is still essential in order to assure that the speciation is constant and to allow accurate evaluation of the growth and/or uptake kinetics. Herbert, Elsworth and Telling (68) have presented a thorough theoretical and experimental evaluation of the continuous flow methodology for the culture of microorganisms.

Bio-assay methods do have some disadvantages. The experiments generally take days or weeks to conduct and the mode of uptake and response of most organisms is not well understood. In addition, mutations may occur as a result of exposure of the organism to the toxic substance. In comparison, analytical methods take only minutes or hours and their basis is usually well-defined, although at present their sensitivity is generally much less than biological monitors. This difficulty continues to offer the analytical chemist an outstanding challenge.

The previous discussion demonstrates the present capability of analytical and biological methods for providing useful data on metal speciation in natural aquatic systems. This information is necessary if the distribution, transport and environmental effects of trace metals is to be thoroughly understood. Electrochemical techniques have a distinct advantage over other methods since they can provide a wide range of information, such as relative activities of metal ions, oxidation states of these ions, chemical potentials of the complexed metal ions, the capacity of ligands to bind the metal ions, interaction intensities, and the total metal concentrations. The data can be gathered quickly with little contamination of the sample or

shift in the chemical equilibria. The techniques, however, are applicable to only a limited number of metals, mainly the post -transition metals Zn, Cu, Pb and Cd, although In, Sn, Sb, Hg, Th and Bi can also be studied if the solution composition allows. This means that thermodynamic data can not be gathered for many biologically important metals by electrochemical methods.

In principle, the type of information provided for metal speciation by electrochemical methods can be obtained through liquid -liquid partitioning equilibria. Two advantages of this technique are its applicability to a wide range of metals and the fact that concentration measurements can be made by a substantial number of analytical methods. These advantages are reflected in the wide use of solvent extraction methodology in trace metal analysis (69-74). Traditionally, spectrophotometric and radiometric methods have been used for measurement of the analytical concentration of the metal, but more recently neutron activation, atomic absorption, gas-liquid chromatography, x-ray fluorescence, emission spectroscopy and others have been used as well.

For measurements of the activity of the free metal ion at a given point in time, however, the amount of metal extracted must be a relatively small fraction of the total analytical concentration. Ion activity means the effective concentration of the free ion in solution as established by the presence of complexes and ion-pairs and the effect of ionic strength. The percentage of metal extracted then must reflect itself in a corresponding shift of the existing thermodynamic equilibria. The magnitude of this shift will be reflected as an error in the activity measurement. A strong advantage of an electrode

measurement is that the number of ions which are transported across the membrane to establish the equilibrium potential is infinitesimal compared to the total number of ions even in very dilute solutions. In principle, an insignificant fraction of metal can be extracted by the solvent extraction method as well if care is taken in adjusting the appropriate parameters.

Nernst (75) was the first to suggest that solvent extraction be used to investigate equilibria in solution. The advent of chelating agents and improved analytical methodology, such as radiometry, has resulted in major advances. Solvent extraction is now used widely for the determination of constants for chemical equilibria in both aqueous and organic solutions (76-78). Distribution experiments can now be made 10 to 100 times faster and with greater precision than the separatory funnel method using the automated AKUFVE apparatus (79,80). AKUFVE is the Swedish abbreviation for "apparatus for continuous measurement of distribution factors in solvent extraction."

Solvent extraction has also been recognized as a potentially useful method for the determination of the thermodynamic activity of chemical species in solution (81). This applicability is not generally recognized although it has been demonstrated. For example, the activity of water which is affected by the nature and concentration of the solutes present in solution has been measured for various electrolyte solutions by equilibration with benzene (82,83). The activity of water in these solutions was determined by measuring its concentration in the organic phase and then comparing these values to that for pure water. The activity of the organic solvent

in the aqueous phase can be determined in the same manner. In general, the activity of water has been determined by vapor pressure methods, but these require special apparatus, very accurately controlled experimental conditions and often long equilibration times.

The solvent extraction method has also been utilized in the evaluation of the activity of solutes. It has found widest application in the determination of the activity of both inorganic (79) and organic (84) nonelectrolytes. Changes in the activity of nonelectrolytes resulting from (a) increasing solute concentration when all other factors are constant, (b) salting in and salting out effects and finally (c) the addition of another molecular substance have been studied by this method. The determination of the activity of electrolytes in solution has, however, been rather limited. Only studies involving ion-pair extraction have previously been reported.

The activity of uranyl nitrate was measured by passing an organic uranyl solution through a series of aqueous solutions containing various concentrations of uranyl nitrate or uranyl nitrate and nonextractable sodium nitrate (85). At equilibrium the activity of the uranyl nitrate in the aqueous "iso-active" solutions must be the same since they are at equilibrium with the same concentration of uranyl nitrate in the organic phase. Since the activity of pure uranyl nitrate solutions was known from isopiestic measurements, the activity coefficients of the mixed nitrate solutions could be calculated. An extensive series of detailed thermodynamic studies (86-91) followed which affirmed the validity of the method.

More recently a similar methodology was used to study the self-association of methylene blue (92-97) which included the determination of mean ionic activity coefficients of methylene blue perchlorate in the presence of urea (92) and at low ionic strengths in sodium fluoride and sodium sulfate solutions (95). The "isoextraction" method serves to maintain a constant activity of a given species in the same fashion as that for iso-active solutions. In these studies potassium perchlorate was added to the aqueous phase such that the activity of the methylene blue perchlorate in the organic phase remained constant. The mean ionic activity coefficient of $MBClO_4$ in each solution was determined by assuming an activity coefficient of unity for 4×10^{-4} M methylene blue in 0.001 M HCl.

The above demonstrates the potential of liquid-liquid partitioning to provide activity coefficients and other thermodynamic information. If solvent extraction is to give information about metal speciation in a fashion similar to electrochemical methods, metal chelate extraction is the logical approach. There is a large number of organic chelating compounds which can be used to extract a substantial number of metal ions with a fair degree of specificity. Careful control of the appropriate parameters, such as pH and ligand concentration, make the method very flexible.

This study utilizes model systems to demonstrate the application of liquid-liquid partitioning equilibria to the determination of trace metal ion activity. The partitioning of copper(II) ions as a chelate of acetylacetone (AcAc) is used to determine the trace activity

coefficient of the copper(II) salt in the $Cu(NO_3)_2$-HNO_3-KNO_3, $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ and $CuCl_2$-HCl-KCl systems. A comparison of these activity coefficients is made with electrochemical measurements utilizing a copper ISE. Potential applicability of this solvent extraction method to natural water systems is also demonstrated by measuring the activity of the free copper ion in the presence of various concentrations of amino acids and nonextractable electrolytes.

CHAPTER II

THEORY

The mathematical models and related theories which define solvent extraction chemistry have been well reviewed (69-74,76,99). The direction of this chapter will be to present the theory related to the partitioning of metal chelate compounds in order to interpret the data reported in Chapter IV. It will ultimately be necessary to include activity coefficients in the now-standard equations for the purpose of relating the experimental results to the mean activity coefficients of the electrolyte of interest.

Distribution Constant

The distribution of a molecular solute between two immiscible liquids was first described thermodynamically by Nernst (75). It is the basic law of solvent extraction chemistry and has been so recognized for some time (98). For a molecular solute, A, at equilibrium between water and an organic phase, the distribution law can be described by

$$A\ (\text{aqueous}) = A\ (\text{organic})$$

$$K_D = \frac{a_{A,o}}{a_A} \tag{1}$$

where K_D is the distribution constant and a_A is the chemical activity of species A. The subscript "o" represents the organic phase and

"aq" which generally denotes water is omitted.

The distribution constant can be derived thermodynamically from the chemical potential

$$\mu_A = \mu_A^o + RT \ln a_A \tag{2}$$

where R is the universal gas constant, T is the absolute temperature, a_A is the activity of the given solute and μ_A^o is the chemical potential at $a_A = 1$. At equilibrium the chemical potential is the same in each phase

$$\mu_A = \mu_{A,o} \tag{3}$$

and

$$\frac{\mu_A^o - \mu^o_{A,o}}{RT} = \ln \frac{a_{A,o}}{a_A} \tag{4}$$

The thermodynamic distribution constant K_D can be expressed by use of activity coefficients

$$K_D = \frac{\gamma_o [A]_o}{\gamma [A]} = \frac{\gamma_o}{\gamma} K_D' \tag{5}$$

since

$$a_A = C_A \gamma_A \tag{6}$$

K_D is generally obtained by extrapolating the experimental values of the stoichiometric distribution ratio, K_D', at varous ionic strengths to the limiting value at zero ionic strength.

Distribution Ratio

A solute may exist as various chemical species. Under such conditions it is more useful to consider the distribution of the total analytical concentration of the solute between phases. This can be accomplished by using a broadly defined concentration ratio, commonly called the distribution ratio, D, defined as

$$D = \frac{C_{A,o}}{C_A} \tag{7}$$

This ratio is dependent upon experimental conditions, such as pH, etc.

Extraction of Chelate Compounds

Distribution of the Chelate-forming Reagent and the Associated Equilibria. In a chelate extraction system the metal ion is commonly coordinated with a weak organic acid, many of which are monoprotic and bidentate, to form a neutral complex. The following discussion will be limited to this type of ligand.

The distribution of the molecular form of the reagent is defined by equation (1) which can be written more specifically as

$$HL\ (aqueous) = HL\ (organic)$$

$$K_{D,HL} = \frac{a_{HL,o}}{a_{HL}} \tag{8}$$

and must be distinguished from its distribution ratio which is pH dependent.

The chelating reagent, which is generally dissolved in the organic phase prior to equilibration, will undergo dissociation in

the aqueous phase during distribution, which is represented by

$$HL = H^+ + L^-$$

$$K_{a,HL} = \frac{a_{H^+}a_{L^-}}{a_{HL}} \tag{9}$$

Distribution of a Metal Chelate Compound and the Associated Equilibria. The distribution constant for the metal chelate is defined as

$$ML_n \text{ (aqueous)} = ML_n \text{ (organic)}$$

$$K_{D,ML_n} = \frac{a_{ML_n,o}}{a_{ML_n}} \tag{10}$$

and the distribution ratio can take the following form

$$D = \frac{C_{M,o}}{C_M} = \frac{[ML_n]_o}{[ML_n] + [M^{n+}]} = \frac{K'_{D,ML_n}}{1 + [M^{n+}]/[ML_n]} \tag{11}$$

Equation (11) assumes that ML_n is the only form of the metal in the organic phase and that the system is in the pH range where lower reagent complexes, hydrolysis and competing complexes are not formed in the aqueous phase.

If equations (8), (9), (10) and the overall formation constant of the complex

$$M^{n+} + nL^- = ML_n$$

$$\beta_{n,L} = \frac{a_{ML_n}}{a_{M^{n+}} a_{L^-}^n} \tag{12}$$

are considered, then

$$\frac{[M_n^{+2}]}{[ML_n]} = \frac{[H^+]^n}{\beta'_{n,L} K'^n_{a,HL} [HL]^n} = \frac{[H^+]^n K'^n_{D,ML_n}}{\beta'_{n,L} K'^n_a [HL]_o^n} \tag{13}$$

and the distribution ratio is

$$D = \frac{K'_{D,ML_n}}{1 + [H^+]^n K'^n_{D,HL} / \beta'_{n,L} K'_{a,HL}{}^n [HL]_o^n} . \tag{14}$$

Equation (14) is important because it describes the distribution ratio of the metal in terms of the properties of the solvent and metal chelate system through the given thermodynamic constants and the experimental variables $[H^+]$ and $[HL]_o$.

Under certain experimental conditions equation (14) takes simpler forms. For example, when only a small portion of the metal in the aqueous phase is present as the chelate, $[M^{n+}] >> [ML_n]$, then D can be described by

$$D = \left[\frac{K'_{a,HL} [HL]_o}{K'_{D,HL} [H^+]} \right]^n K'_{D,ML_n} \beta'_{n,L} \tag{15}$$

This is a valid approximation if the complex has a greater affinity for the organic phase and a small fraction of the metal is extracted.

When a large portion of the metal is extracted or if most of the metal in the aqueous phase is present as the chelate, $[M^{n+}]/[ML_n] << 1$, then

$$D \simeq K'_{D,ML_n} \tag{16}$$

Equation (15) has also been derived and verified experimentally (100) by considering the overall extraction process as one chemical reaction, where the equilibrium constant for this reaction is termed the extraction constant K_{ex} for the reaction

$$M^{n+} + nHL_o = ML_{n,o} + nH^+$$

$$K'_{ex} = \frac{[ML_n]_o[H^+]^n}{[M^{n+}][HL]_o^n} \tag{17}$$

By applying equations (8), (9), (10) and (12) one can arrive at the following

$$K'_{ex} = \left[\frac{K'_{a,HL}}{K'_{D,HL}}\right]^n K'_{D,ML_n}\beta'_{n,L} \tag{18}$$

from which

$$D = K'_{ex}\left[\frac{[HL]_o}{[H^+]}\right]^n = \left[\frac{K'_{a,HL}[HL]_o}{K'_{D,HL}[H^+]}\right]^n K'_{D,ML_n}\beta'_{n,ML_n} \tag{19}$$

and is the same as equation (15).

The above equation is often expressed in logarithmic form

$$\log D = \log K'_{ex} + n\ pH + n \log [HL]_o \qquad (20)$$

and

$$\log D = n \log \frac{K'_a}{K'_{D,HL}} + \log K'_{D,ML_n} \beta'_n + n\ pH + n \log [HL]_o \qquad (21)$$

which are useful for characterizing solvent extraction systems (73). According to equation (20), when the equilibrium concentration of the complexing agent and the metal is constant, the extract is pH dependent. Experimental plots of log D *vs.* pH with constant metal and chelating agent concentrations have a slope of n at low pH, zero at mid-range and a negative slope at high pH. In the low pH region the slope will deviate from n in a positive direction if the reagent undergoes hydrolysis, complex formation with some other competing agent or reacts with the complexing reagent itself to form lower complexes.

At constant pH and constant analytical metal concentration, a plot of log D *vs.* log $[HL]_o$ will normally give a straight line of slope n. Deviations from n will be observed if reagent molecules associate with the metal complex in the organic phase or if the reagent molecules and/or anions associate with the metal ion in the aqueous phase.

A plot of log D *vs.* log[M] at constant pH and $[HL]_o$ can also be helpful in interpreting extraction systems (101). Since chelate compounds generally exist as mononuclear species, log D is independent of the metal concentration if this concentration is not too high. Above a certain concentration the distribution ratio will decrease as a result of several causes including a decrease in the equilibrium

concentration of the chelating agent, exceeding the solubility limit of the metal chelate in the organic phase and polynuclear formation in the aqueous phase. If the degree of polynuclear formation is the same in both phases, the slope of log D vs. $\log[M^{n+}]$ is zero. Polynuclear formation in the organic phase will cause a positive deviation.

In general, solvent extraction studies are conducted in the 10^{-3} - 10^{-7} M range depending upon the chemistry of the metal (73). Studies have been made at metal concentrations as low as 10^{-10} M utilizing β-diketones in benzene (71). At these low metal ion concentrations difficulties result from adsorption effects, complexation by trace organic impurities and changes in valence state due to oxidizing or reducing agents.

For analytical applications plots of log D vs. pH, $[HL]_o$ or $[M^{n+}]$ are of limited utility. It is more useful to know the fraction of metal transferred from the aqueous into the organic phase. This is generally accomplished by calculating the percentage of extraction, E, which is given in terms of concentration and volume as

$$E + \frac{C_{M,o}V_o}{C_{M,o}V_o + C_MV} 100 \qquad (22)$$

where V_o is the volume of the organic phase and V is the volume of the aqueous phase. Dividing through equation (22) by C_MV_o and applying equation (7) results in

$$E = \frac{D}{D + V/V_o} 100. \qquad (23)$$

The above can be used to solve for D in terms of E which gives

$$D = \frac{E}{(100 - E)} \frac{V}{V_o} . \tag{24}$$

or by taking logarithms,

$$\log D = \log E - \log(100 - E) + \log V/V_o . \tag{25}$$

By equating equations (20) and (25), equation (26) is generated.

$$\log K'_{ex} + n\ pH + n \log[HL]_o = \log \frac{E}{100 - E} \frac{V}{V_o} \tag{26}$$

By plotting E *vs.* pH under conditions of constant $[HL]_o$ one obtains a sigmoidal curve. Its position on the x-axis is dependent on the value of K'_{ex} and the ratio V/V_o.

Another useful quantity is that of $pH_{\frac{1}{2}}$ (102). It is simply the pH at which the concentration of metal is equal in both phases. If $V = V_o$, then $pH_{\frac{1}{2}}$ is the pH at which half the metal ion has been extracted. Under these conditions log D = 0 and equations (20) and (21) take the following forms

$$pH_{\frac{1}{2}} = -\frac{1}{n} \log K'_{ex} - \log[HL]_o \tag{27}$$

$$pH_{\frac{1}{2}} = pK'_a + \log K'_{D,HL} - \frac{1}{n} \log[\beta'_{n,L} K'_{D,ML_n}] - \log[HL]_o \tag{28}$$

Equation (27) is used to evaluate K'_{ex} and equation (28) is useful for evaluating chelating agents and solvents which will minimize

$pH_{\frac{1}{2}}$ and therefore optimize the extraction of a given metal ion (73). In addition $pH_{\frac{1}{2}}$ values allow rapid comparison of the extractability of various metal ions by a single reagent.

Competing Complexation. The distribution of metal chelate compounds is highly dependent on competing complexation reactions. In the aqueous phase these reactions include the formation of lower complexes with the chelating agent itself, hydrolysis, complex formation with additional ligands and polymerization. All of these serve to decrease the distribution ratio by binding the free metal ion. In addition, adducts may be present in the organic phase as a result of foreign ligands and solvent molecules replacing water of hydration or in some cases by binding to the chelating agent. These species are more soluble in the organic phase than the hydrated neutral metal chelate and the distribution ratio usually increases.

Ignoring polynuclear species which are relatively unimportant in chelate extraction, the distribution ratio can be given by

$$D = \frac{[ML_n]_o + \Sigma_m[ML_n \cdot B_m]_o}{[M^{n+}] + \Sigma_j[ML_j] + \Sigma_k[M(OH)_k] + \Sigma_l[MX]_l} \qquad (29)$$

where charges have been omitted and

$\Sigma_j[ML_j]$ = sum of the concentrations of the metal complexes with the chelating agent;

$\Sigma_k[M(OH)_k]$ = sum of the concentrations of all hydroxyl complexes;

$\Sigma_l[MX]_l$ = sum of the concentrations of all additional metal complexes

$\Sigma_m[ML_n \cdot B_m]_o$ = sum of the concentrations of all metal complexes in the organic phase with an adduct B.

Equation (29) can generally be simplified because some of the species will be absent under a given set of extraction conditions.

Trace Mean Activity Coefficients from Metal Chelate Extraction. By utilizing equation (29) and making appropriate assumptions, one can arrive at a relationship between D and the trace mean activity coefficient, $\gamma_{\pm}^{tr}$, of the trace metal electrolyte; "tr" is omitted in the remaining discussion. The concept of "trace" activity coefficients is used to describe the influence of one electrolyte on the activity coefficient of a second which is present in a "vanishingly small" concentration in a mixed electrolyte system (103).

For the purpose of this discussion the following assumptions have been made: (a) a small fraction of the trace metal is extracted; (b) the ultimate complex ML_n is the only metal ion species present in the organic phase; (c) no polymerization occurs in either phase; (d) the extraction is completed under conditions where hydrolysis of the metal ion is negligible; (e) the effect of the organic solvent on the activity of the aqueous species is negligible; (f) the activity coefficients of the chelating reagent and metal complex in the organic phase are constant with small changes in concentration and are unaffected by changes in the aqueous phase; and (g) the temperature is constant.

Equation (29) is then given as

$$D = \frac{[ML_n]_o}{[M^{n+}] + \Sigma_j[ML_j] + \Sigma_l[MX]_l} \qquad (30)$$

If one applies the above equation to the distribution of a divalent metal and makes the appropriate substitutions and rearrangements using equations (8), (9) and (12), then

$$D = \frac{[ML_2]_o}{[ML_2][\psi/\gamma_M\gamma_A^2 + \theta} \tag{31}$$

where

$$\psi = \frac{K_{D,HL}^2[H^+]^2\gamma_{HA}^2\gamma_{ML_2}}{\beta_{n,L}K_{a,HL}^2[HL]_o^2\gamma_{o,HL}^2} \tag{32}$$

and

$$\theta = \frac{K_{D,HL}[H^+]\gamma_{HA}\gamma_{ML_2}}{K_{a,HL}K_{2,L}[HL]_o\gamma_{o,HL}\gamma_{MLA}} + 1 + \frac{K_{D,HL}^2K_{1,X}K_{a,HX}[H^+][HX]\gamma_{HA}\gamma_{HX}\gamma_{ML_2}}{\beta_{n,L}K_{a,HL}^2[HL]_o^2\gamma_{o,HL}^2\gamma_{MXA}}$$

$$+ \frac{K_{D,HL}^2\beta_{n,X}K_{a,HX}^2[HX]^2\gamma_{HX}^2\gamma_{ML_2}}{\beta_{n,L}K_{a,HL}^2[HL]_o^2\gamma_{o,HL}^2\gamma_{MX_2}} \tag{33}$$

In these expressions, the equilibrium constants $K_{2,L}$, $K_{1,X}$ and $\beta_{2,X}$ refer, respectively, to the reactions

$$ML^+ + L^- = ML_2$$

$$M^{+2} + X^- = MX^{+(2-m)}$$

$$M^{+2} + 2X^{-m} = MX_2^{+2(1-m)}$$

By taking X^{-m} as an anion of a weak acid, protonation equilibria are introduced into equation (33).

Substitution of K_{D,ML_2}, equation (10), into equation (31) provides the final equation which relates the measured values of D with all experimental variables, including the activity coefficient of the trace electrolyte MA_2

$$D = \frac{K_{D,ML_2}\gamma_{ML_2}}{\gamma_{o,ML_2}(\theta + \psi/\gamma_M\gamma_A^2)} \tag{34}$$

While several approaches may be used, the one requiring minimum a priori information is to measure D as a function of ionic strength at constant concentration of hydrogen ions such that only a few percent of the total metal ion is extracted. In these experiments there is no competing ligand, X, so that $\theta = 1$ and $\psi >> \theta$. Noting that the extracted species is unchanged, the ratio $\gamma_{ML_2}/\gamma_{o,ML_2}$ can be assumed to be equal to 1 and therefore D is dependent only on $\gamma_M\gamma_A^2$. A plot of log D vs. square root of ionic strength will have an intercept, log D_r, at zero ionic strength which is equal to log $K_{D,ML_2}/\psi$. With this intercept activity coefficients are calculated directly from the simplified, rearranged form of equation (34)

$$\gamma_M\gamma_A^2 = \frac{\psi D}{K_{D,ML_n}} \tag{35}$$

Secondary corrections can be made by adjusting the equilibrium concentrations of the chelating reagent in the organic phase $[HL]_o$

as well as the acid concentration in the aqueous phase [HA] for each distribution ratio.

Conventionally, mean ionic activity coefficients, $\gamma_{\pm}$, are reported, these are obtained from the general expression

$$\gamma_{\pm} = (\gamma_M^m \gamma_A^n)^{1/m+n} \tag{36}$$

written for an electrolyte M_mA_n. In the systems of interest here, these are trace mean ionic activity coefficients, or more simply, trace activity coefficient, $\gamma_{\pm}^{tr}$. In its present form equation (36) represents the trace activity coefficient for the molal concentration scale but $\gamma_{\pm}^{tr}$ is generally represented by $y_{\pm}^{tr}$ and $f_{\pm}^{tr}$ for the molar and mole fraction scale, respectively.

Once the relative activity of one or more metal ions is established for standard solutions, such as metal ion buffers, then the activity of these ions in an unknown solution can be related to these standards. This can be accomplished by the above method or by pre-equilibrating an organic phase containing an appropriate chelating agent with a standard metal ion buffer. This pre-equilibration must be designed to prevent analytical extraction of the metal in the unknown solution. The change in metal chelate concentration in the organic phase will be proportional to the difference in metal ion activity in the sample and standard.

CHAPTER III

EXPERIMENTAL

Apparatus and Instrumentation

Extraction System. All samples were equilibrated in 125 ml Pyrex separatory funnels. The funnels were cleaned with alcoholic potassium hydroxide and then silanized with dichlorodimethylsilane as necessary. This latter treatment facilitated rapid phase separation without centrifugation. All equilibrations were performed on a Burrell Wrist-Action shaker at a setting of 5 unless otherwise indicated.

Distillation Systems. A glass still equipped with a 25.4 cm Vigreux distilling column was used to purify the extracting reagent. One solvent (cyclohexane) was distilled through a 12 mm i.d. x 900 mm distilling column packed with 3.2 mm Pyrex helices.

Potentiometric Measurements. Measurements of pH were made with a combination electrode and readout from a Photovolt Digicord Model 111 pH meter or a Chemtrix pIon Type 50 meter. The electrode was inserted directly into the separatory funnel to obtain the pH.

Potentiometric measurements of copper(II) activities were made with either the Chemtrix meter or a digital volt meter and a copper ISE.

Gas-Liquid Chromatography (GLC). Analysis by GLC was conducted utilizing a Carle Model 8000 equipped with a thermal conductivity detector (TCD) and a Hewlett-Packard Model 5750B equipped with both

flame ionization (FID) and electron capture (ECD) detection.

The instrument, column and conditions of GLC are listed in the appropriate sections.

Ultraviolet-Visible Spectra. A Cary Model 14 recording spectrophotometer was used to obtain all UV-visible spectra. The cells used were 1 or 5 cm path length quartz cells.

Atomic Absorption Spectrophotometry (AAS). AAS measurements were made on a Perkin-Elmer Model 305B atomic absorption spectrophotometer. The 305B is equipped with the 303-0240 burner control box for fuel and oxidizer control, the 303-0358 nebulizer assembly and a 303-0418 single 4-inch slot burner.

Calculations. All extensive calculations were completed on a Tektronix Model 31 porgrammable calculator equipped with a Model 4661 digital x-y plotter.

Linear regression fit of data to higher order equations, when necessary, was completed using the Poly 1 program run on the Harris System 200 Model 220.

Some equilibrium calculations were verified by the MINEQL program (104) run on the Harris System 200 Model 220 computer.

Where required, standard statistical methods were applied to the data. In such cases the results are reported as the mean value plus and minus the standard deviation.

Reagents

Analytical reagent grade chemicals were used unless otherwise stated. All aqueous solutions were prepared from distilled water

and stored in one liter linear polyethylene bottles. Working solutions were prepared by addition of an aliquot of the stock solution by pipette or burette and/or a weighed amount of the required reagent to volumetric flasks followed by dilution to volume by the required solvent.

Acetylacetone (2,4-pentanedione). Acetylacetone (MCB, commercial grade) - $CH_3COCH_2COCH_3$ was found to contain approximately 10% acetic acid by GLC analysis. The impure reagent was equilibrated successively with an equal volume of 5% sodium bicarbonate solution and distilled water. The AcAc was dried over calcium sulfate and then distilled. The fraction boiling between 139-140°C (760 mm of mercury) was collected. The boiling point agreed well with the literature (105).

The purity of the distillation product was checked by GLC. A single peak was obtained indicating that only one species was present. The column and conditions are given in Table I.

TABLE I

GLC CONDITIONS FOR ACETYLACETONE

Instrument	Carle Model 8000
Column	6.0 ft. x 1/8 inch i.d. Stainless Steel
Packing	8% GE SF 96 on Anakrom ABS (90-100 mesh)
Column and Injection Port Temperature	125°C
Detector and Temperature	TC; 125°C
Carrier Gas	Helium; 25 ml/min

Aqueous and chloroform solutions were prepared by weighing the AcAc and diluting to volume.

Alanine (dl-α-aminopropionic acid). Alanine (Sigma; Sigma Grade) - $C_3H_7NO_2$ was used without further purification.

Bis(Acetylacetonato) Copper(II). Bis(Acetylacetonato Copper(II) - $Cu(CH_3COCHCOCH_3)_2$ was synthesized by equilibrating 100 ml of 0.05 M $Cu(NO_3)_2$ with 200 ml of 0.100 M AcAc in chloroform for one hour. The pH of the aqueous phase was adjusted to 4.9 with acetate buffer. Following phase separation the organic phase was evaporated to dryness. The complex was recrystallized twice from benzene and dried at 120°C for one hour. The light blue, needle-like crystals decomposed at 236°C.

A stock solution (0.0100 M) was prepared by dissolving the complex in chloroform.

Chloroform. Chloroform - $CHCl_3$ (Mallinckrodt, SpectrAR and Analytical Reagent) was used without further purification.

Cyclohexane. Cyclohexane (Mallinckrodt, Analytical Reagent) - C_6H_{12} was fractionally distilled. The fraction (bp 81°C) which produced only one peak by ECD was collected. The column and conditions are given in Table II.

Cupric Chloride. Cupric Chloride (Mallinckrodt, Analytical Reagent) - $CuCl_2 \cdot 2H_2O$ was used to prepare an approximately 0.05 M solution. It was standardized by a volumetric titration (106) with ethylenediaminetetraacetic acid (EDTA) which was modified by utilizing potentiometric end point detection. The final concentration was $5.033 \pm 0.002 \times 10^{-2}$ M.

Cupric Nitrate. Cupric nitrate (Mallinckrodt, Analytical

TABLE II

GLC CONDITIONS FOR CYCLOHEXANE

Instrument	Hewlett-Packard Model 5750B
Column	6.0 ft. x 6 mm i.d. glass
Packing	5% SE 30 on Chromosorb W HP(80-100 mesh)
Injection Port Temperature	100°C
Column Temperature	60°C
Detector and Temperature	ECD: 155°C
Carrier Gas	5% methane/95% argon; 50 ml/min

Reagent) - $Cu(NO_3)_2 \cdot 3H_2O$ was used to prepare an approximately 0.05 M solution. It was standardized in the same manner as the cupric chloride solution. The final concentration was $5.228 \pm 0.001 \times 10^{-2}$ M.

Dichlorodimethysilane. Dichlorodimethysilane (MCB) - $C_2H_6SiCl_2$ was used without further purification.

Disodiumethylenediaminetetraacetic Acid. Disodiumethylenediaminetetraacetic acid (Mallinckrodt, Analytical Reagent) - $C_{10}H_{12}O_8N_2Na_2 \cdot 2H_2O$ was used to prepare approximately 0.10 M and 0.010 M solutions which were standardized by volumetric titration using primary - standard copper metal (106). The final concentrations were $1.011 \pm .001 \times 10^{-1}$ M and $1.010 \pm .001 \times 10^{-2}$ M.

Glycine (aminoacetic acid). Glycine (MCB) - $C_2H_5NO_2$ - was used without further purification.

Hydrochloric Acid. Hydrochloric acid (Allied Chemical) - 37.5% HCl was used to prepare an approximately 0.20 M stock solution. It was standarized by volumetric titration using primary-standard sodium carbonate (107). The final concentration was $2.013 \pm .004 \times 10^{-1}$ M.

Methylene Blue Chloride (3,9-Bisdimethylaminophenazothionium chloride. Methylene blue chloride (MBCl; Allied Chemical) - $C_{16}H_{18}ClN_3S$ - was recrystallized three times from absolute ethanol and dried overnight at 120°C. Stock solutions of 1.00×10^{-3} and 1.00×10^{-5} M MBCl were prepared by dissolving the solid in 1×10^{-3} M HCL.

Methylene Blue Perchlorate (3,9-Bisdimethylaminophenazothionium perchlorate). Methylene blue perchlorate ($MBClO_4$) - $C_{16}H_{18}O_4ClN_3S$ - was prepared by precipitation from MBCl with sodium perchlorate (93). The brilliant purple precipitate was washed with a minimum of water to prevent hydrolysis (108). The product was recrystallized from absolute ethanol and dried overnight at 120°C. A stock solution (1.00×10^{-5} M) was prepared by dissolving the product in chloroform.

Nitric Acid. Nitric acid (Mallinckrodt, Analytical Reagent) - 70% HNO_3 - was used to prepare an approximately 0.20 M stock solution which was standardized in the same manner as the HCl stock solution. The final concentration was $2.023 \pm .002 \times 10^{-1}$ M.

Potassium Chloride. Potassium chloride (Baker and Adamson, Reagent Special) - KCl - was dried for 2 hours at 120°C before use.

Potassium Nitrate. Potassium nitrate (Baker, Purified) - KNO_3 - was dried for 2 hours at 120°C before use.

Perchloric Acid. Perchloric acid (Mallinckrodt, Analytical Reagent) - 70% $HClO_4$ - was used to prepare approximately 0.20 M and 4.0 M stock solutions. The solutions were standardized in the same manner as the HCL stock solution. The final concentrations were $2.040 \pm .001 \times 10^{-1}$ M and $4.070 \pm .002$ M.

Sodium Chloride. Sodium chloride (Mallinckrodt, Analytical Reagent) - NaCl - was dried for 2 hours at 120°C before use.

Sodium Hydroxide. Sodium hydroxide (Mallinckrodt, Analytical Reagent) - NaOH - was used to prepare approximately 0.20 M and 4.0 M stock solutions. The solutions were standardized by volumetric titration using primary - standard potassium hydrogen phthalate (107). The final concentrations were $1.971 \pm .002 \times 10^{-1}$ M and $3.758 \pm .003$ M.

Sodium Perchlorate. Sodium perchlorate - $NaClO_4$ - was prepared by potentiometric titration of a known volume of standard NaOH with standard $HClO_4$. The final concentration was 1.95 M.

Procedures

Extractions. Unless otherwise stated, the following general methodology was used. Equal volumes of the aqueous and organic phase were equilibrated by shaking for 1 hour at room temperature. The pH was measured prior to phase separation and was assumed to be the equilibrium value. In several studies the ionic strength (μ) was adjusted by the addition of $NaClO_4$, KNO_3, KCl or NaCl, and the pH was held constant with the acid of the common ion.

Extraction of Copper(II) as a Function of Equilibration Time at pH = 5.36 and pH = 1.75. Copper(II) solutions of 2.09×10^{-3} M were

buffered with acetate and those of 5.23×10^{-3} M ($\mu = 0.1$) were buffered with HNO_3 and equilibrated with AcAc (0.100 M) in chloroform (25 ml) at 22 ± 1°C for prescribed periods of time. The phases were separated as soon as possible after equilibration and then the pH of the aqueous phase was measured. The final pH for each extraction sample was 5.36 ± .02 and 1.75 ± .02. The absorbance of $Cu(AcAc)_2$ was measured spectrophotometrically.

(Copper(II) Extraction as a Function of pH. Copper(II) solutions (5.03×10^{-3} M) and AcAc (0.100 M) in chloroform (25 ml) were equilibrated at 23 ± 1°C. The pH of the aqueous phase was adjusted by the addition of microliter volumes of 0.2 M HCl or 0.2 M NaOH before equilibration. The absorbance of $Cu(AcAc)_2$ was measured spectrophotometrically.

Distribution of Acetylacetone as a Function of Ionic Strength. Chloroform solutions of AcAc (0.100 M) were equilibrated in triplicate at 21 ± 1°C with aqueous solutions (20 ml) of increasing μ (0.036 - 1.00) and constant pH (1.75 ± .02). Following equilibration a 10 ml aliquot of the aqueous phase was diluted to 100 ml with water. The absorbance of AcAc was measured spectrophotometrically.

Copper(II) Distribution as a Function of Ionic Strength. Copper(II) solutions (5.03×10^{-3} M and/or 5.23×10^{-3} M) of various μ and constant pH (1.75 ± .02) were equilibrated in quintuplicate with AcAc (0.100 or 0.0100 M) in chloroform (25 ml). The absorbance of $Cu(AcAc)_2$ was measured spectrophotometrically.

Distribution of Copper(II) as a Function of Total Copper(II). Copper(II) solutions (25 ml) of increasing concentration (5.03×10^{-4} -

6.28×10^{-3} M), held at constant μ (0.200) and pH (1.75 ± .02), were equilibrated in quintuplicate with AcAc (0.100 M) in chloroform at 22 ± 1°C. The absorbance of $Cu(AcAc)_2$ was measured spectrophotometrically.

The chloroform phases which were equilibrated with the copper(II) solutions, in which μ and pH were controlled by $NaClO_4$ and $HClO_4$, were also analyzed by AAS. The organic phase (20 ml) was stripped of the complex by back extraction into 0.2 M HNO_3 and analysis was performed on the aqueous phase.

Test For Perchlorate Ion-Pair Formation. The method of Mukerjee and Ghosh (93) was used to test for the presence of perchlorate ion in the chloroform phase. Aliquots (5 ml) of the chloroform phase containing $Cu(AcAc)_2$ extracted from copper(II) solutions with μ = 0.200 and pH = 1.75 ± .02 from the nitrate and perchlorate systems were analyzed by the standard addition method. The organic phase was equilibrated with a series of aqueous phases containing MBCl (1.00×10^{-3} M) and HCl^- (1.0×10^{-3} M) to which had been added increasing amounts of $HClO_4$ (1.00×10^{-5} - 3.94×10^{-5} M). The absorbance of the chloroform phase was measured.

Solubility of Chloroform as a Function of Ionic Strength. Copper(II) solutions (5.03×10^{-3} M) of various μ (0.035 - 0.600) and constant pH (1.75 ± .02) were equilibrated in triplicate with 25 ml of chloroform at 22 ± 1°C. The phases were allowed to separate before a 10 ml aliquot of the aqueous phase was taken by pipette. This sample was then equilibrated for 1 hour with 100 ml of cyclohexane. A 1 ml aliquot of this phase was diluted to 100 ml with cyclohexane and

analyzed by GLC against a standard curve under the conditions listed in Table II.

The density of the original aqueous phase was also determined following equilibrium by weighing a 10.00 ml aliquot.

Effect of Equilibration Time on the Extraction of Copper(II) in the Presence of Amino Acids. Copper(II) solutions (1.50×10^{-4} M) containing glycine (5.00×10^{-3} M) with $\mu = 0.723$ and $\mu \simeq 0.001$ were equilibrated at 22 ± 1°C with 20 ml of AcAc (1.00×10^{-3} M) in chloroform for prescribed periods of time at shaker settings of 5 and 10, respectively. Phase separation was completed as soon as possible after shaking, followed by pH determination. The final pH for each extraction was 4.07 ± .04 at $\mu \simeq 0.001$ and 4.20 ± .02 at $\mu = 0.723$. The ionic strength at 0.723 was adjusted with NaCl. The chloroform phase was analyzed for copper by AAS.

Distribution of Copper(II) in the Presence of Amino Acids at pH = 4.0. Solutions of copper(II) and amino acids (1.00×10^{-3} - 1.00×10^{-2} M in glycine or alanine) at $\mu \simeq 0.001$ and $\mu = 0.723$ were equilibrated in triplicate with AcAc (1.00×10^{-3} M) in chloroform (20 ml) at 22 ± 1°C and a shaker setting of 10. The pH was adjusted to 4.00 ± .02 with appropriate additions of acid or base. The organic phase was analyzed for copper by AAS.

The copper-amino acid stock solutions were preserved by the addition of chloroform.

Activity Measurements by Copper Ion Selective Electrode. 50 ml aliquots of a copper(II) solution (5.23×10^{-3} M) at pH = 4.00 were titrated with a colution of copper(II) of identical concentration

and pH but which was also 1 M in either KNO_3 or $NaClO_4$. The titration was carried out so that the potential measurements could be made at any desired ionic strength.

The outer portion of a double junction reference electrode was filled with $NaNO_3$ to prevent precipitation of $KClO_4$.

Analysis

The absorbance of $Cu(AcAc)_2$ was measured in the chloroform phase at 385 nm. The molar absorptivity (ε) was found to be $3.14 \pm .02 \times 10^2$ L mole^{-1} cm^{-1} by measuring a range of standards (5.00×10^{-4} - 2.50×10^{-3} M). Beer's law was obeyed over the concentration range studied (Figure 1). All $Cu(AcAc)_2$ absorbance measurements were made in 5 cm pathlength cells, except for the standards and the equilibration samples at pH 5.36 which were measured in 1 cm cells.

The absorbance of AcAc was measured in 2.0×10^{-3} M acid solutions at 272 nm. The measurement of standards (1.00×10^{-4} - 5.00×10^{-4} M) showed $\varepsilon_{max} = 1.81 \pm .01 \times 10^3$ L mole^{-1} cm^{-1} and Beer's law was obeyed (Figure 2). In all cases 1 cm pathlength cells were used.

The absorbance of $MBClO_4$ was measured in chloroform at 650 nm in 1 cm cells. The extraction of MBCl and $MBClO_4$ is additive. $MBClO_4$ has a high partition coefficient in favor of chloroform. The extractability of MBCl in 1.0×10^{-3} M HCl is much smaller and was corrected from blank experiments (93).

Samples analyzed by AAS were compared to standards prepared in the same manner as, or with approximately the same concentrations of

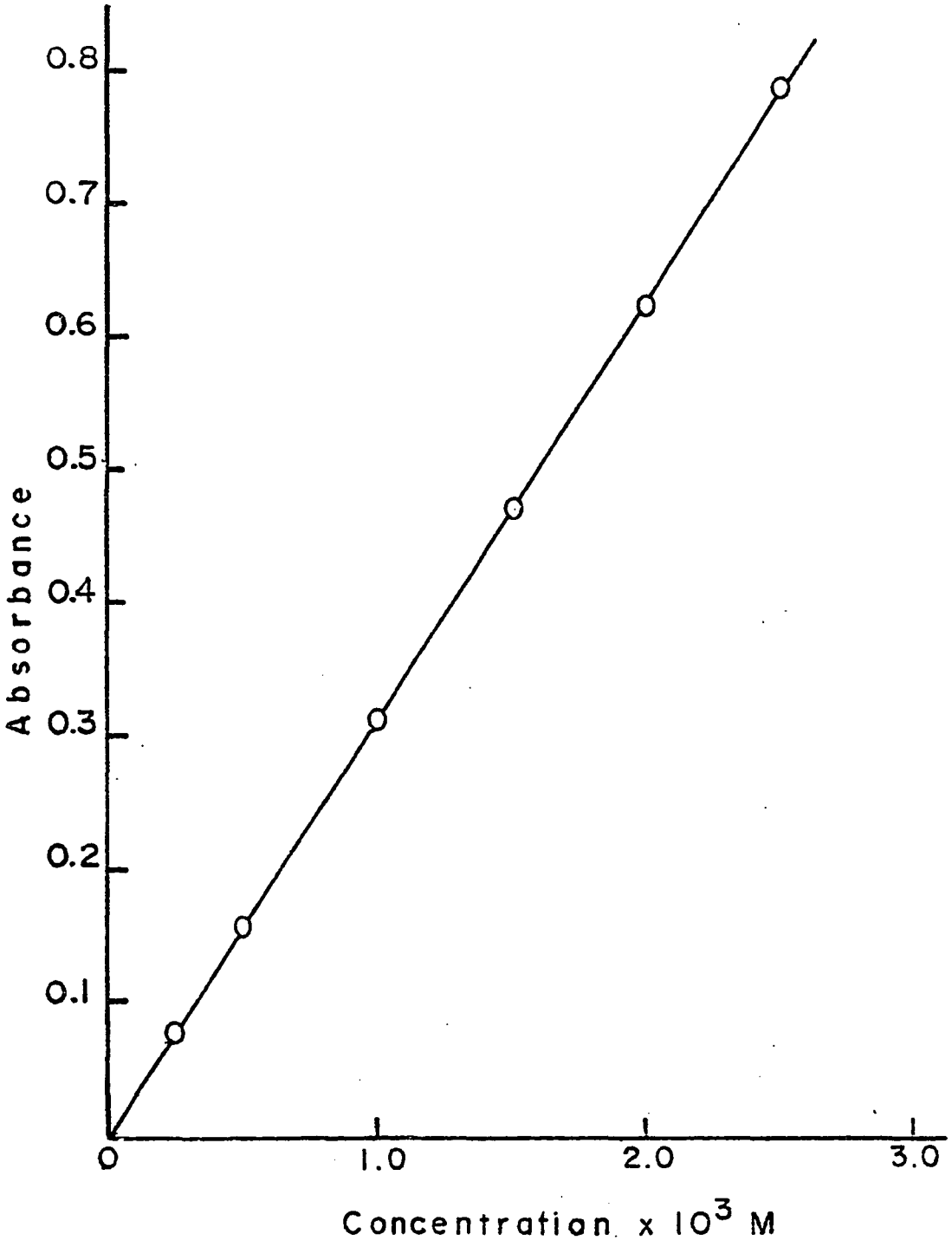

Figure 1. Absorbance of bis(Acetylacetonato) copper(II) in $CHCl_3$ at 385 nm vs. concentration.

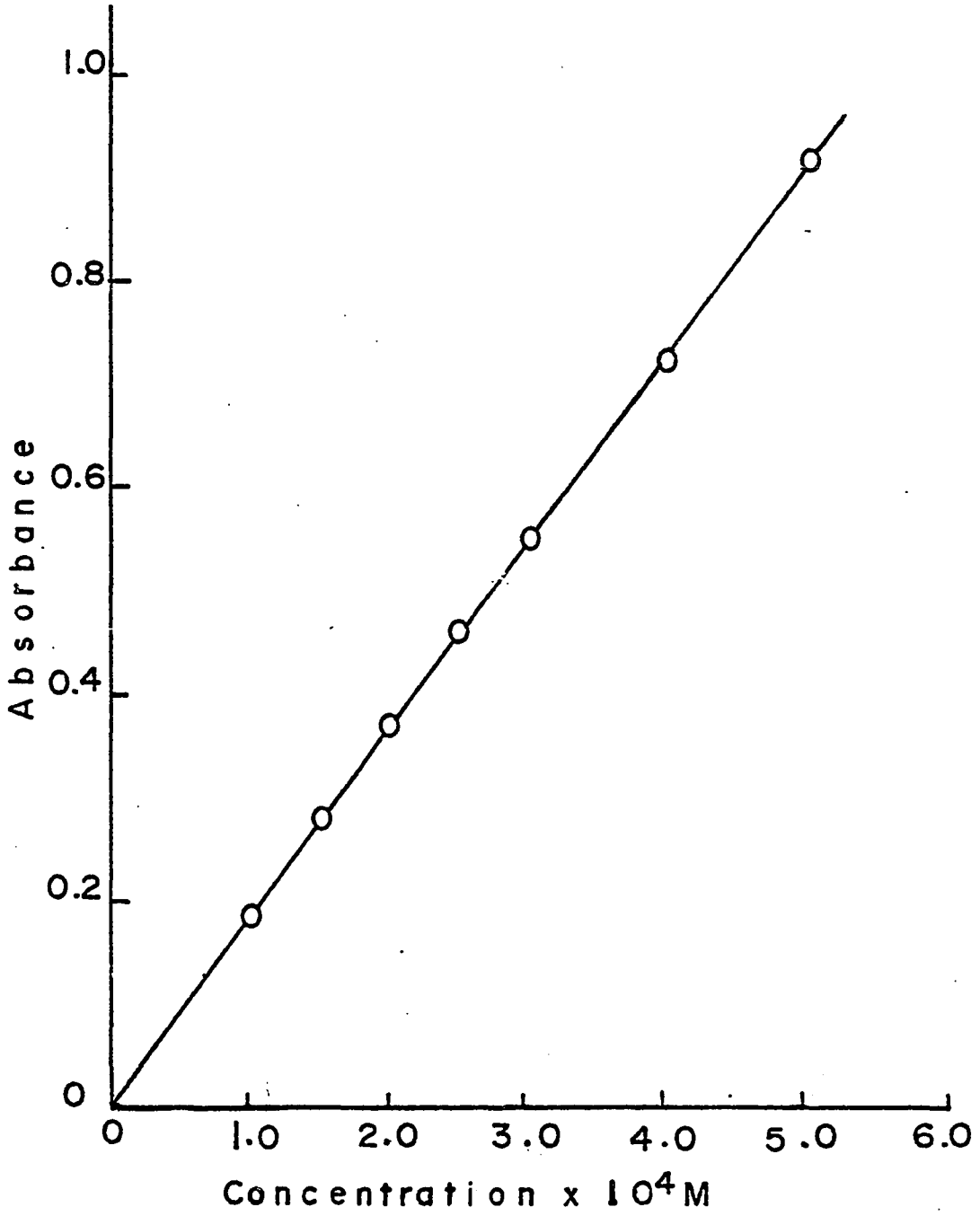

Figure 2. Absorbance of acetylacetone in 0.002 M HCl at 272 nm vs. concentration.

reagents as contained in the samples to assure uniform matrix effects. The standard and samples were aspirated into an air/acetylene flame.

CHAPTER IV

RESULTS

Extraction of Copper(II) as a Function of Equilibration Time at pH = 5.36 and pH - 1.75

The rate at which equilibrium is attained for the extraction of copper(II) as $Cu(AcAc)_2$ into chloroform was measured at constant values of pH and AcAc concentration (Figure 3). The percent extraction was calculated using equations (7) and (23). The extraction equilibrium is reached in approximately 10 minutes at pH = 5.36 and 30 minutes at pH = 1.75 (Figures 4 and 5). Similar results were observed for the extraction of copper(II) by 0.100 AcAc in benzene (109).

Copper(II) Extraction as a Function of pH

The effect of pH on the extraction of copper(II) as $Cu(AcAc)_2$ was measured in the pH range 1.7 - 6.0 at constant AcAc concentration (Figure 6). The percent extraction and log D were then calculated from this data (Figures 7 and 8). The $pH_{\frac{1}{2}}$ (2.58) and n (2.00 ± .04) were determined from Figure 8 and these values along with equation (20) provided the value of log K_{ex} (-3.16). The plots of % Extraction and log D *vs.* pH obey the theoretical equations (20) and (23) which were used to generate the curves through the points. In Figure 8 log D approaches 2.52 in the pH independent region in good agreement with

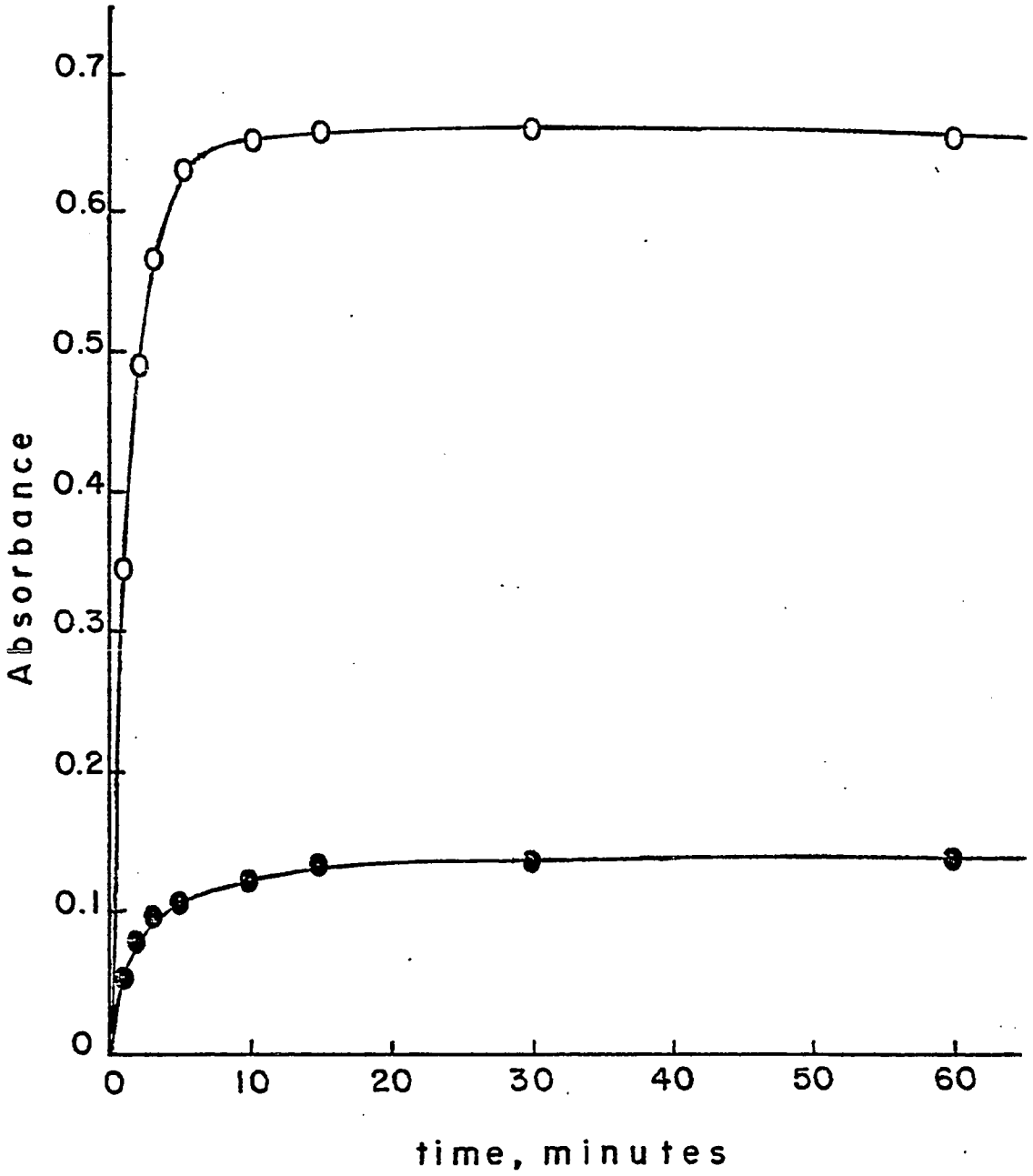

Figure 3. Absorbance vs. equilibration time for $Cu(AcAc)_2$ at pH 5.36 -O- and pH 1.75 -●-.

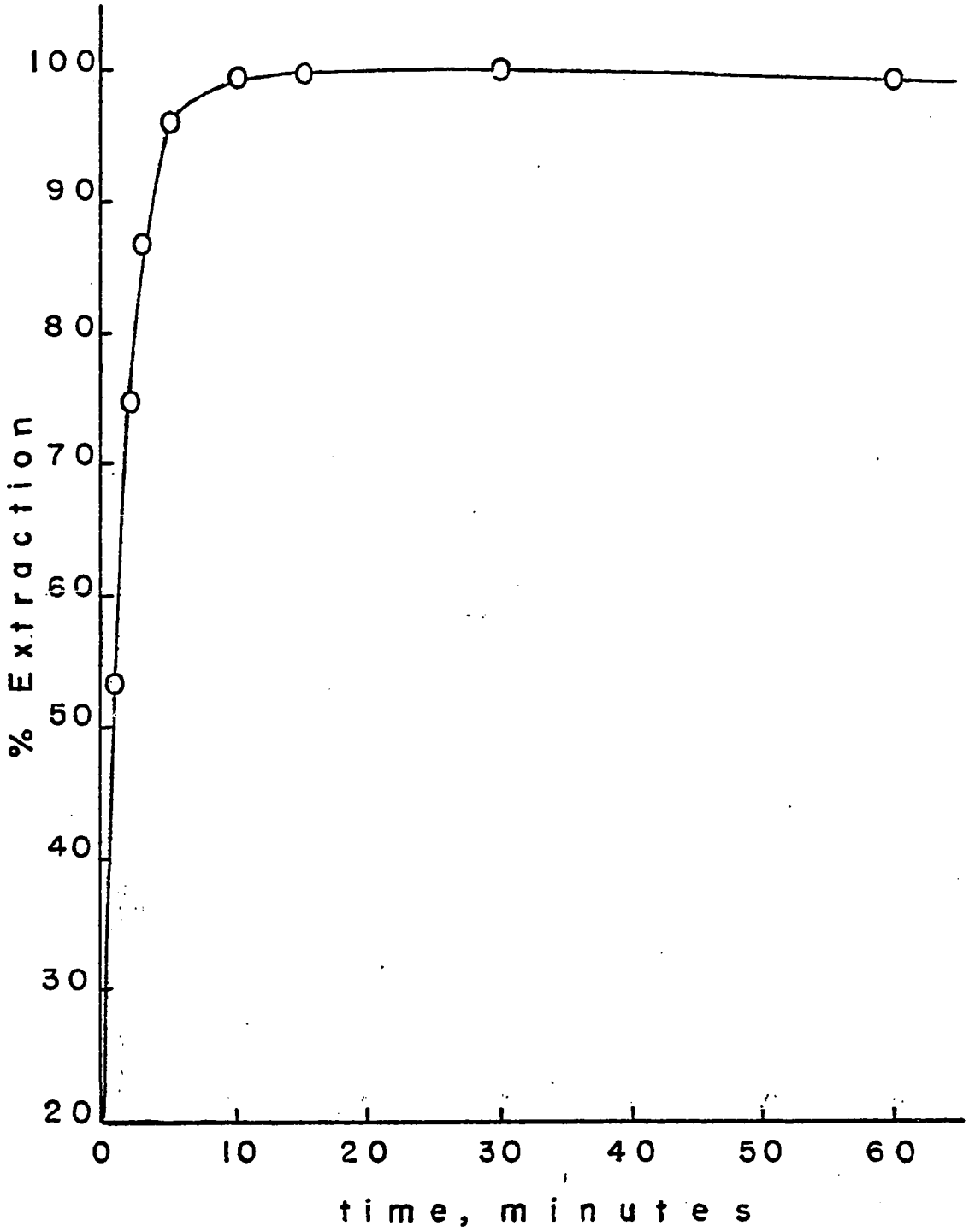

Figure 4. % Extraction vs. equilibration time for $Cu(AcAc)_2$ at pH 5.36.

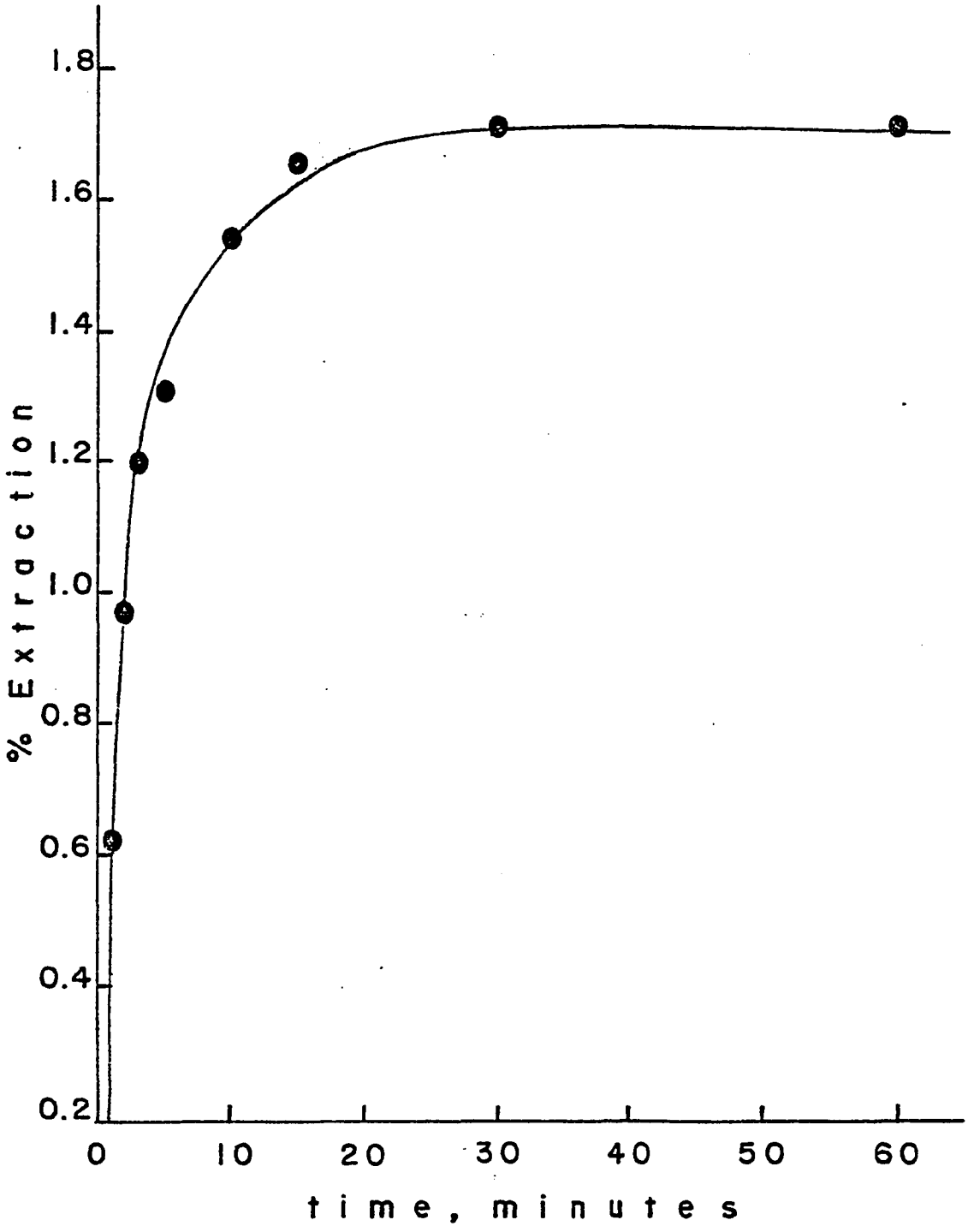

Figure 5. % Extraction vs. equilibration time for $Cu(AcAc)_2$ at pH 1.75 and μ = 0.10.

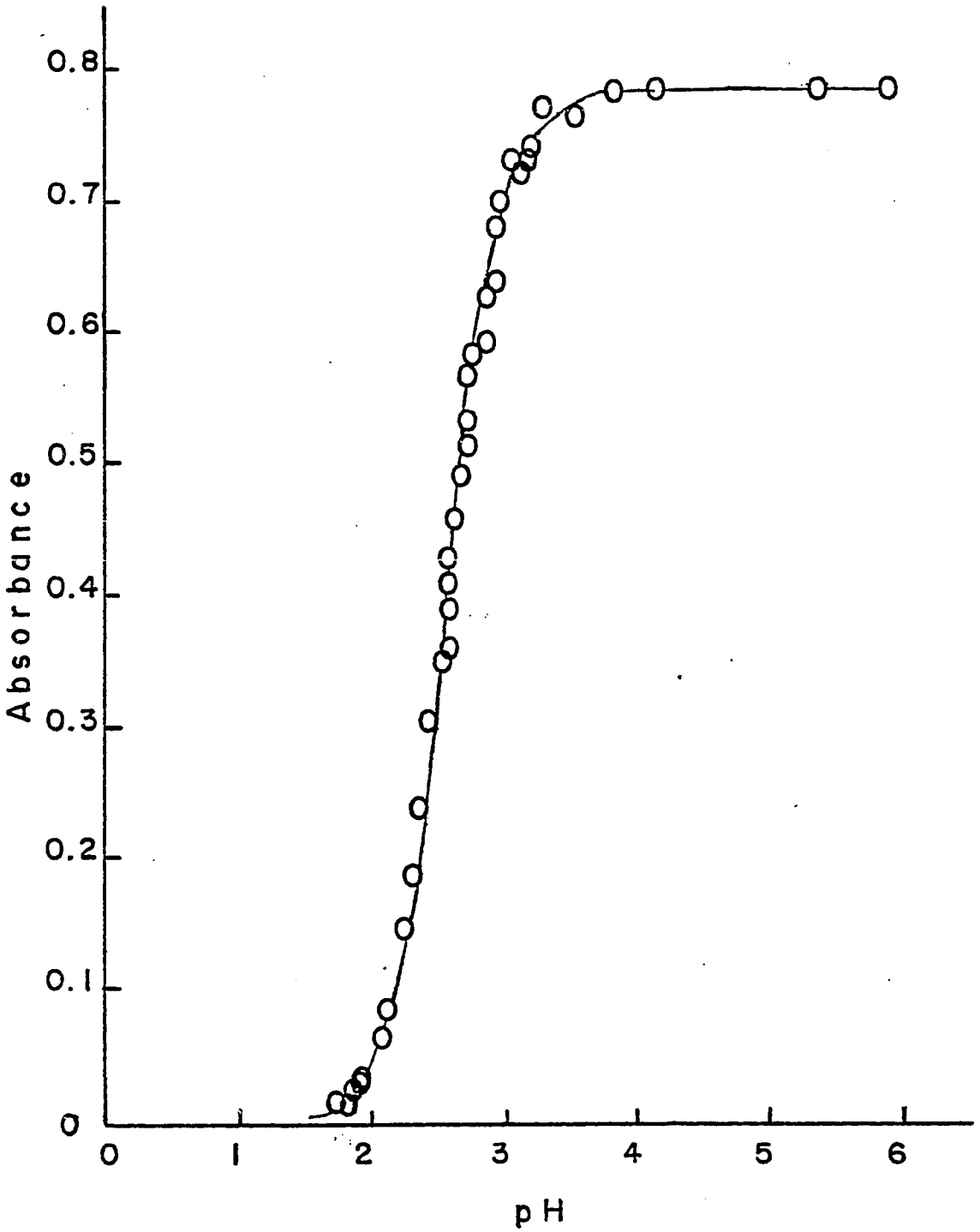

Figure 6. Absorbance of $Cu(AcAc)_2$ in $CHCl_3$ at 385 nm vs. pH.

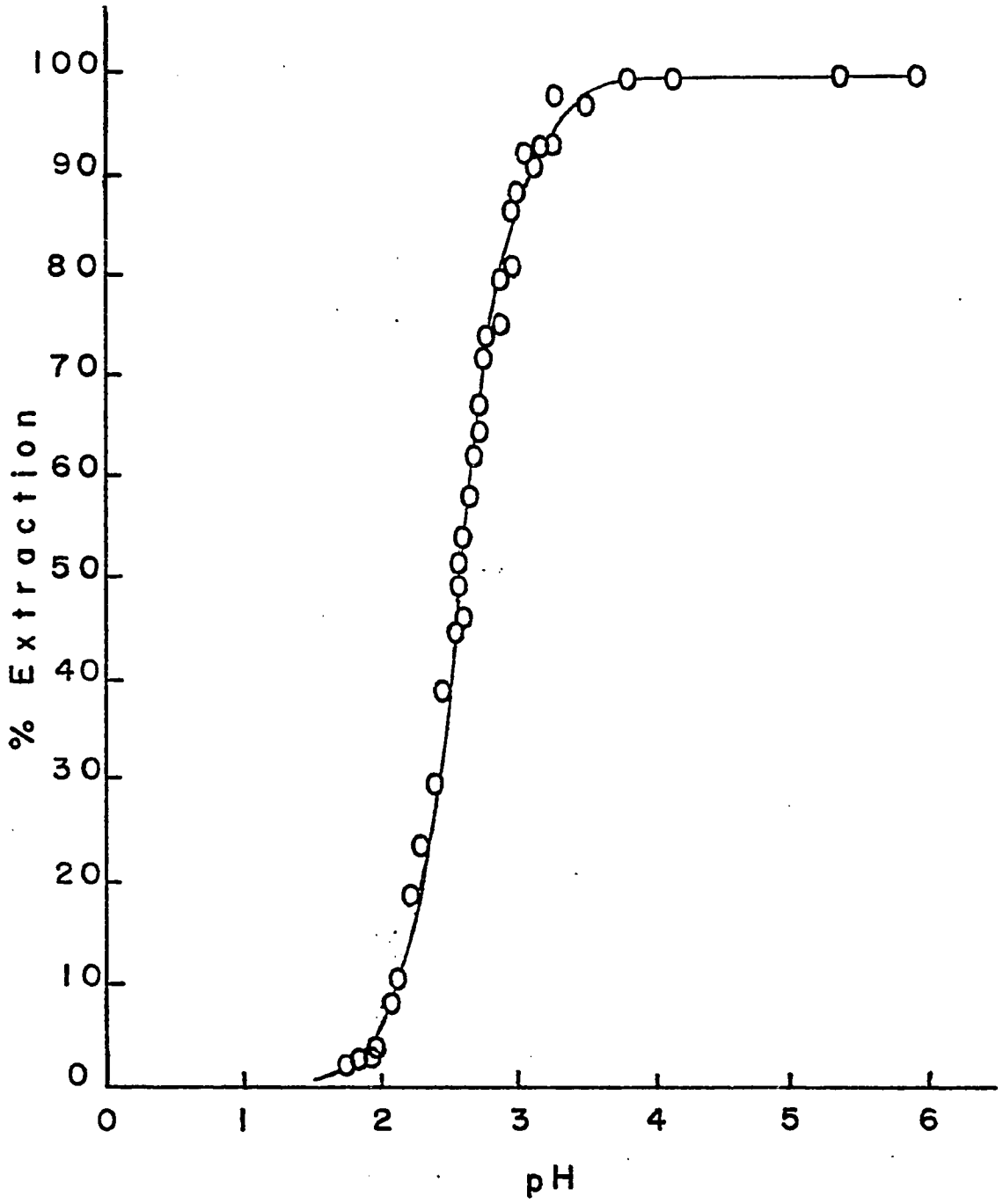

Figure 7. % Extraction of copper(II) by 0.10 M acetylacetone in $CHCl_3$ vs. pH.

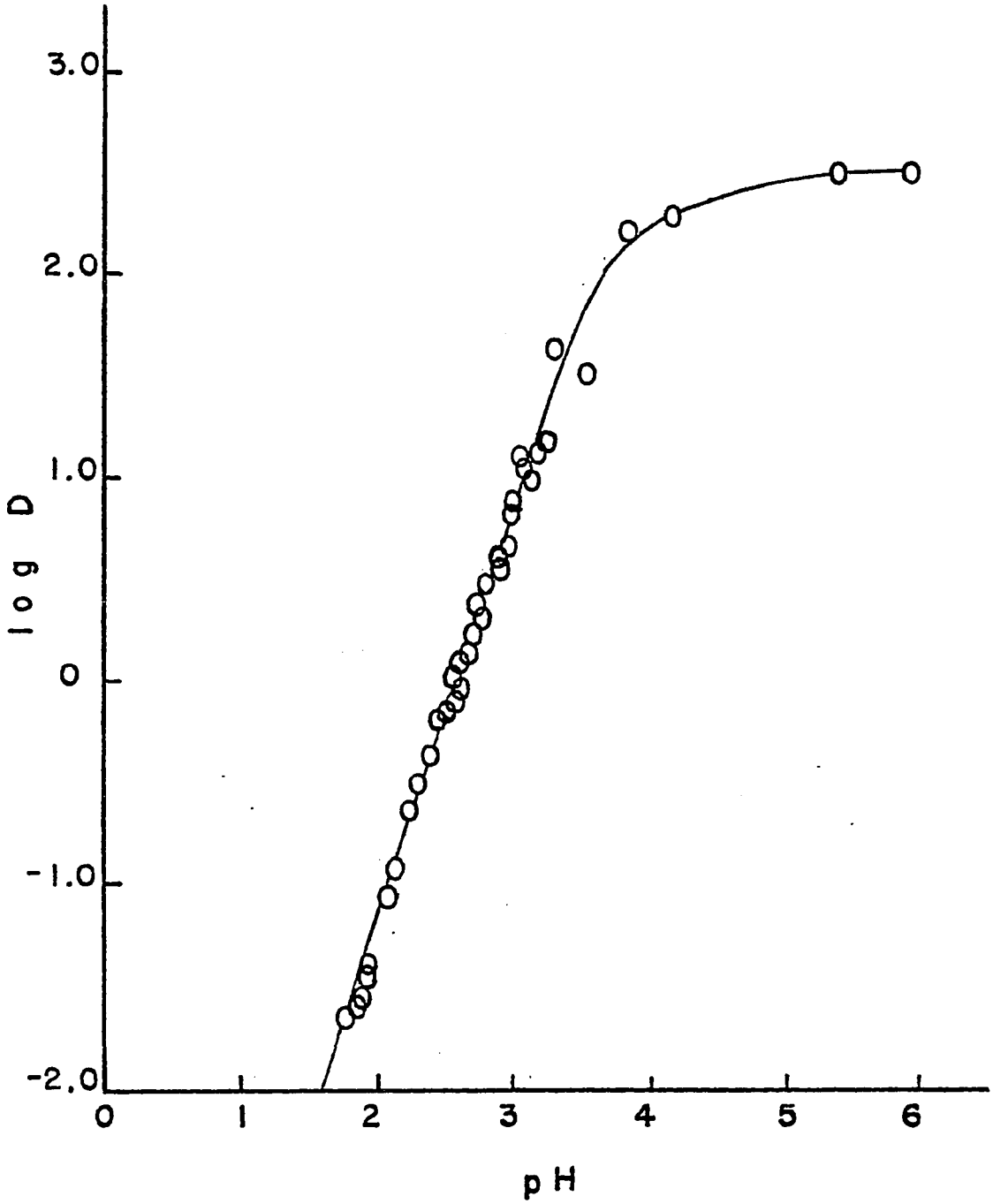

Figure 8. log D vs. pH.

the literature (78).

Distribution of Acetylacetone as a Function of Ionic Strength

The absorbance of AcAc was measured as a function of μ at constant pH (1.75) using four different electrolytes (Figure 9). The partition constant and its logarithm (Figure 10) were calculated from this data using equations (7) and (16). Log K_D at μ = 0 was obtained by extrapolation using linear regression for the KNO_3 and $NaClO_4$ systems and by taking the tangent for the KCl and NaCl systems. The average value of K_D is 25.4 ± 1.0 in good agreement with the literature (71,110-113).

Distribution of Copper(II) as a Function of Ionic Strength

The absorbance of $Cu(AcAc)_2$ was measured in the chloroform phase as a function of μ at constant pH (1.75) for three different systems: $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$, $Cu(NO_3)_2$-HNO_3-KNO_3 and $CuCl_2$-HCl-KCl (Figure 11). The distribution ratio (D) and -log D were calculated using equation (7) (Figures 12 and 13). -Log D at μ = 0 was estimated by regression fit of the data to a third order equation. The average value of -log D was found to be 1.50 ± .01 and was used as the reference value of μ = 0 for the calculation of the mean ionic trace activity coefficients $y_{\pm}^{tr}$ and -log $y_{\pm}^{tr}$ (Figures 14 and 15). The activity coefficients were calculated from equation (35). The values of the thermodynamic constants used are given in Table III.

Distribution of Copper(II) as a Function of Total Copper(II)

The concentration of $Cu(AcAc)_2$ was measured in the chloroform phase as a function of the total copper concentration at constant pH

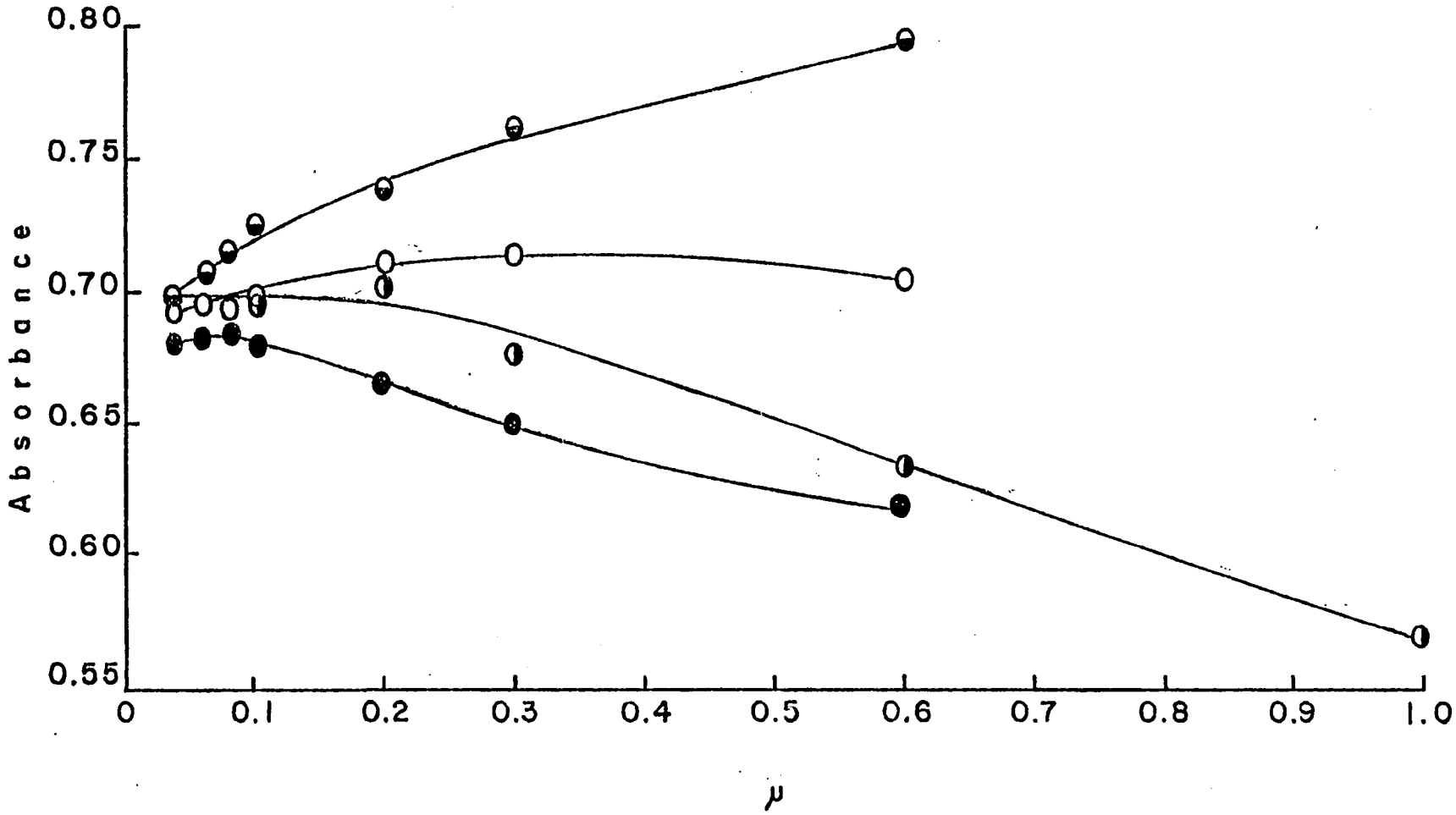

Figure 9. Absorbance vs. μ for acetylacetone; μ adjusted by the addition of KNO_3 -O-, KCl -●-, $NaClO_4$ -Θ- and NaCl -◑-.

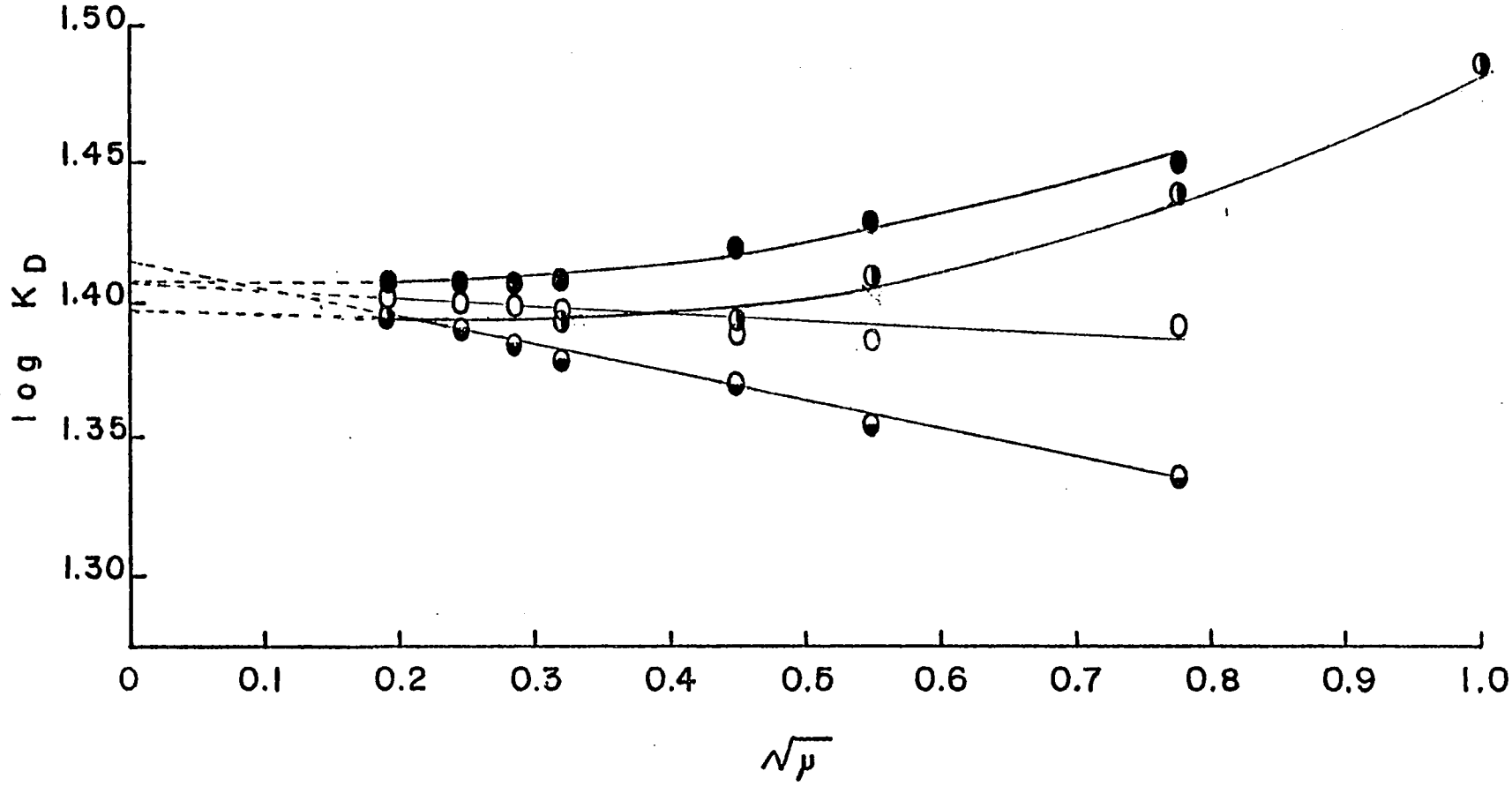

Figure 10. log K_D vs. $\mu^{\frac{1}{2}}$ for AcAc; μ adjusted by addition of KNO_3 -O-, KCl -●-, $NaClO_4$ -●- and NaCl -●-.

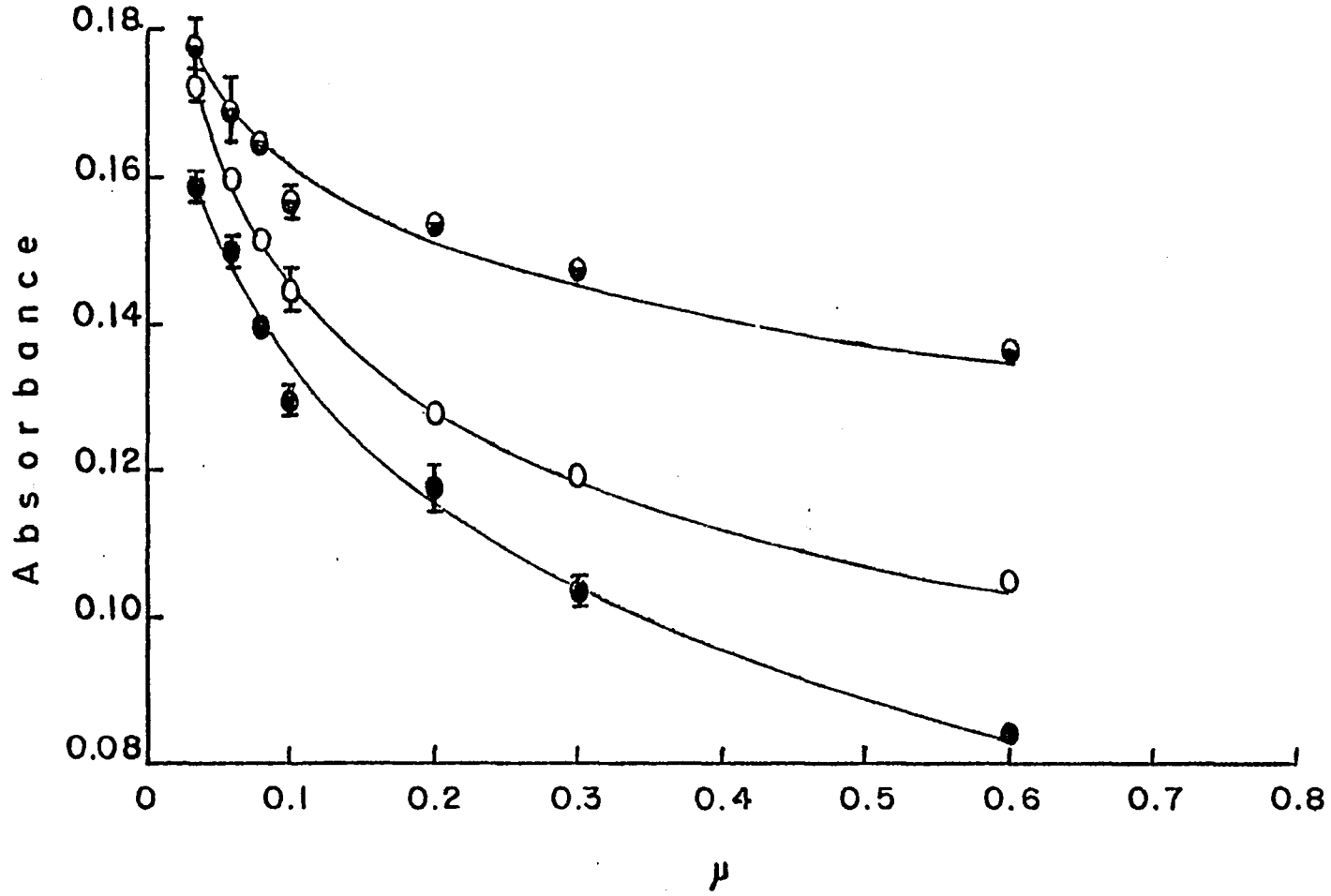

Figure 11. Absorbance of $Cu(AcAc)_2$ vs. μ for the $Cu(NO_3)_2$-HNO_3-KNO_3 -O-, $CuCl_2$-HCl-KCl -●- and $Cu(NO_3)_2$-$NaClO_4$-$HClO_4$ -Θ- systems.

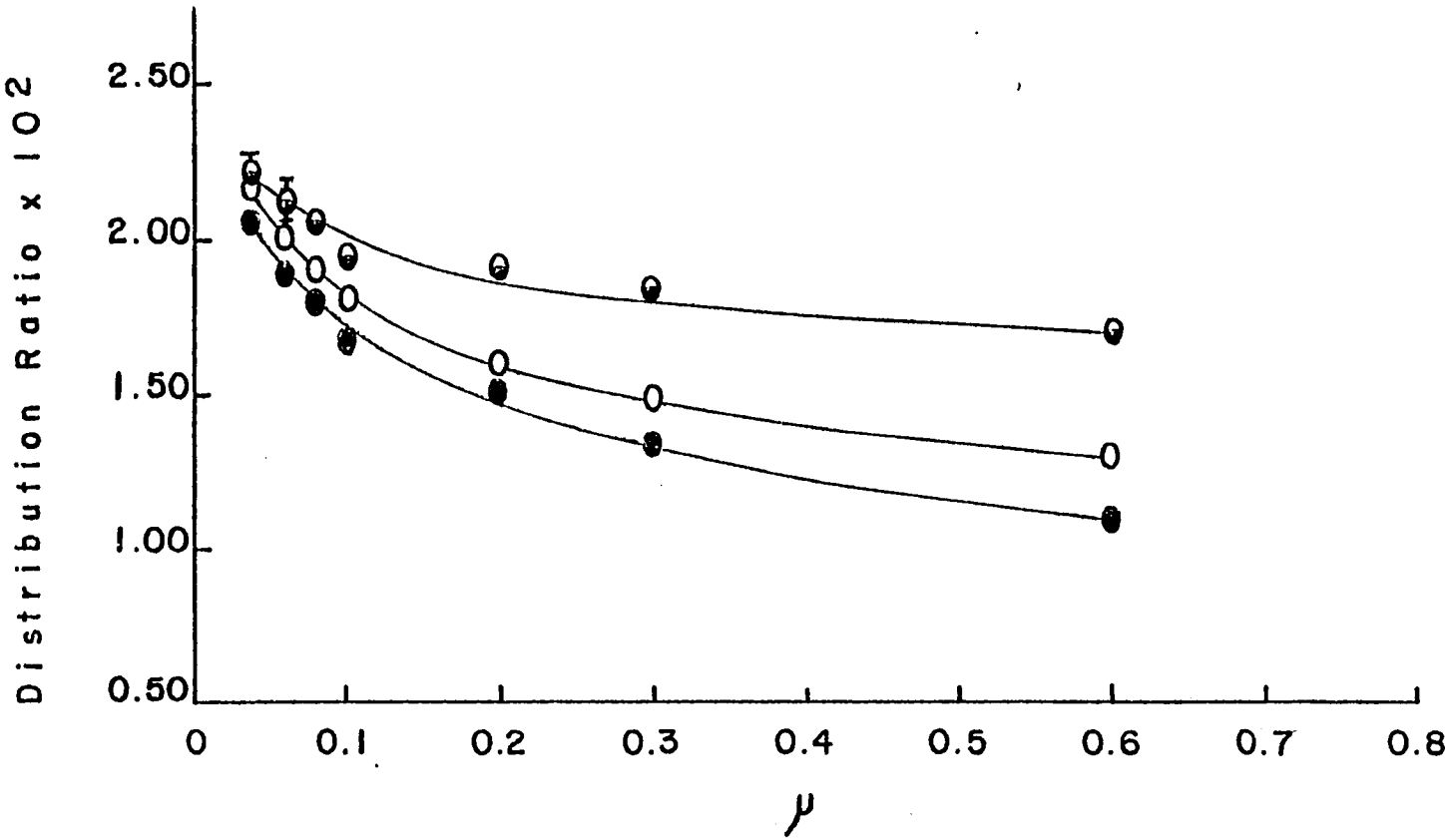

Figure 12. Distribution ratio (D) vs. μ for $Cu(NO_3)_2$-HNO_3-KNO_3 -O-, $CuCl_2$-HCl-KCl -●- and $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ -Θ- systems.

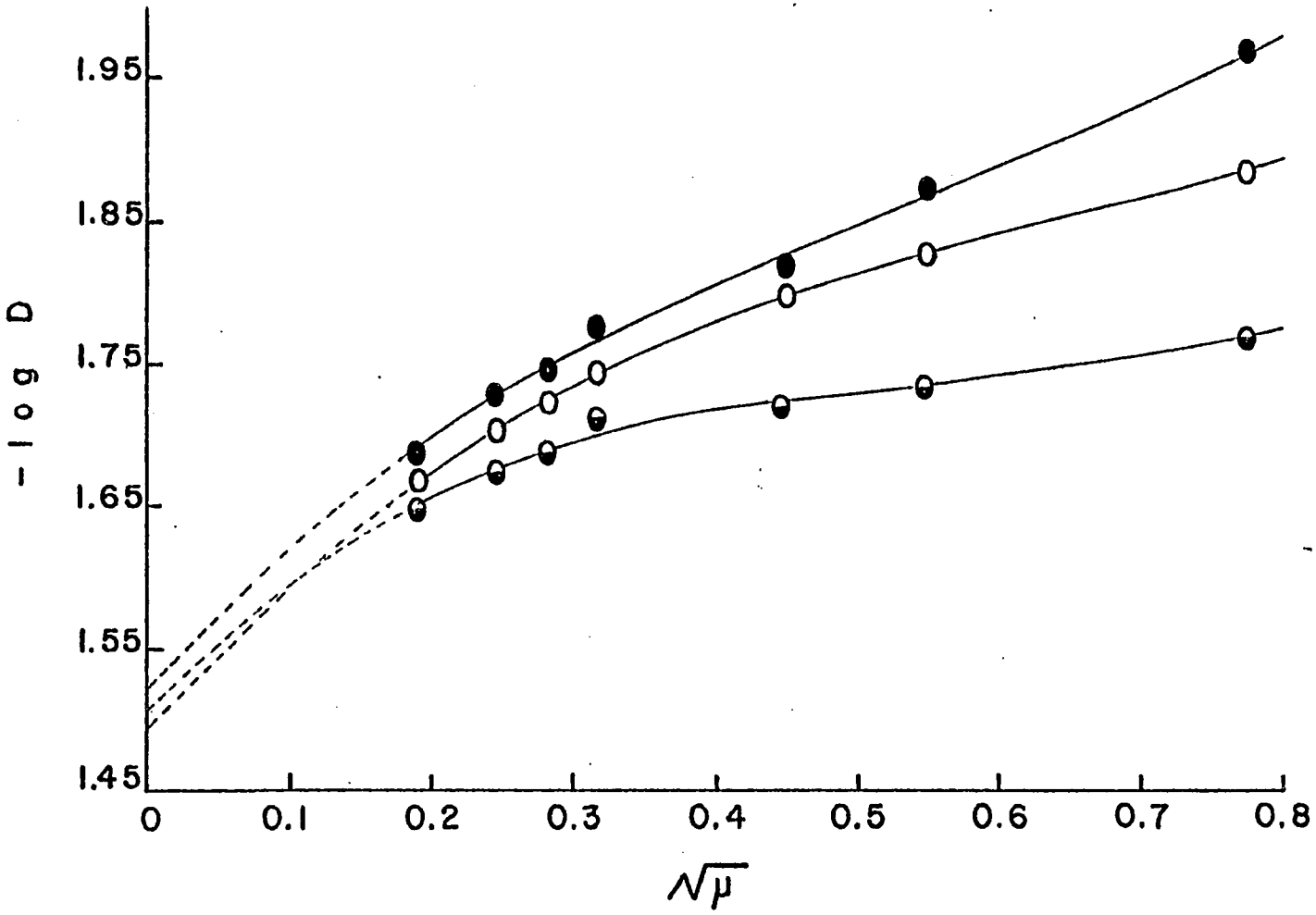

Figure 13. -log D for $Cu(AcAc)_2$ vs. $\mu^{\frac{1}{2}}$ for $Cu(NO_3)_2$-HNO_3-KNO_3 -O-, $CuCl_2$-HCl-KCl -●- and $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ -Θ- systems.

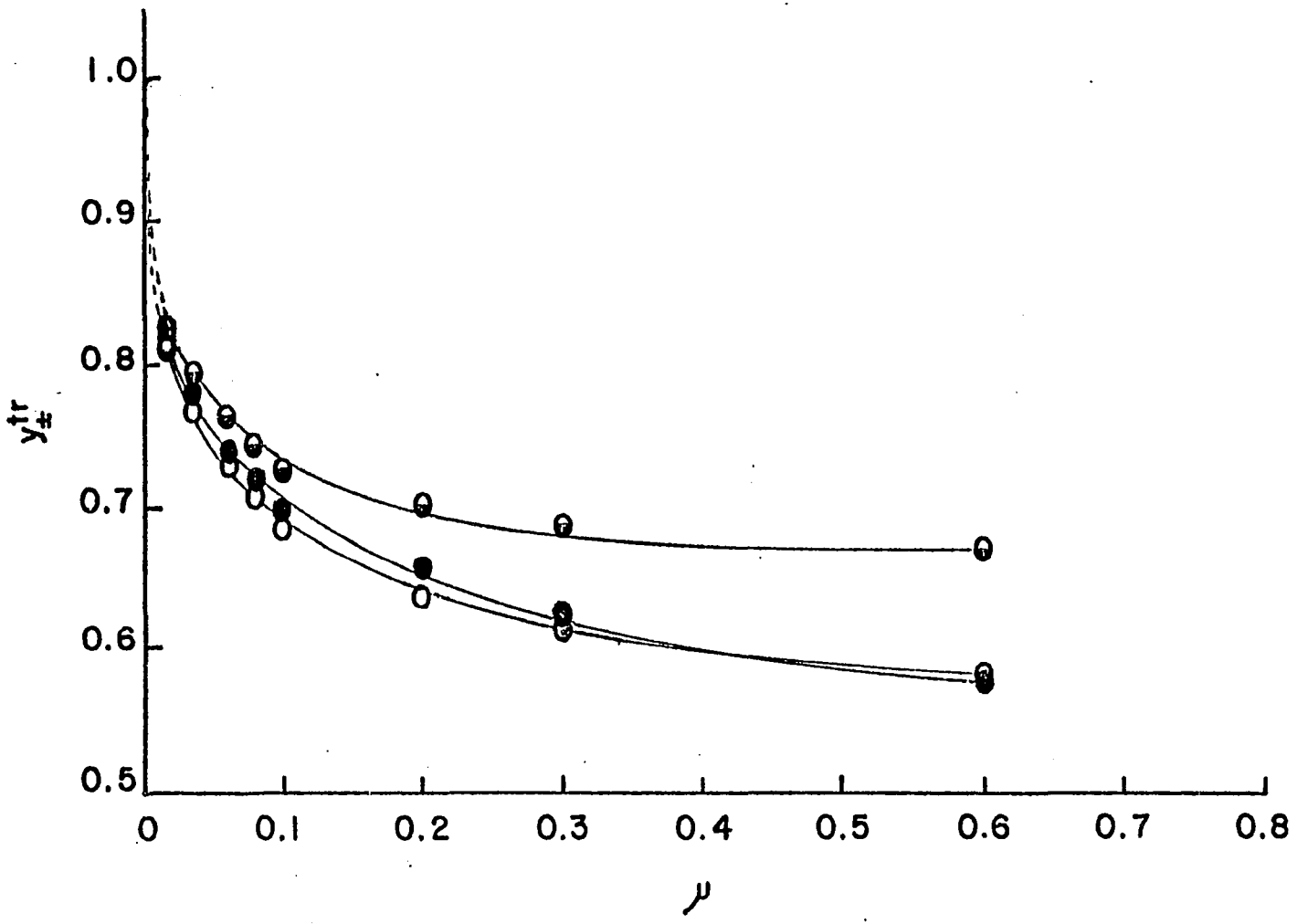

Figure 14. $y_{\pm}^{tr}$ vs. μ for the $Cu(NO_3)_2$-KNO_3-HNO_3 -O-, $CuCl_2$-KCl-HCl -●-, $Cu(NO_3)_2$-$NaClO_4$-$HClO_4$ -Θ- systems.

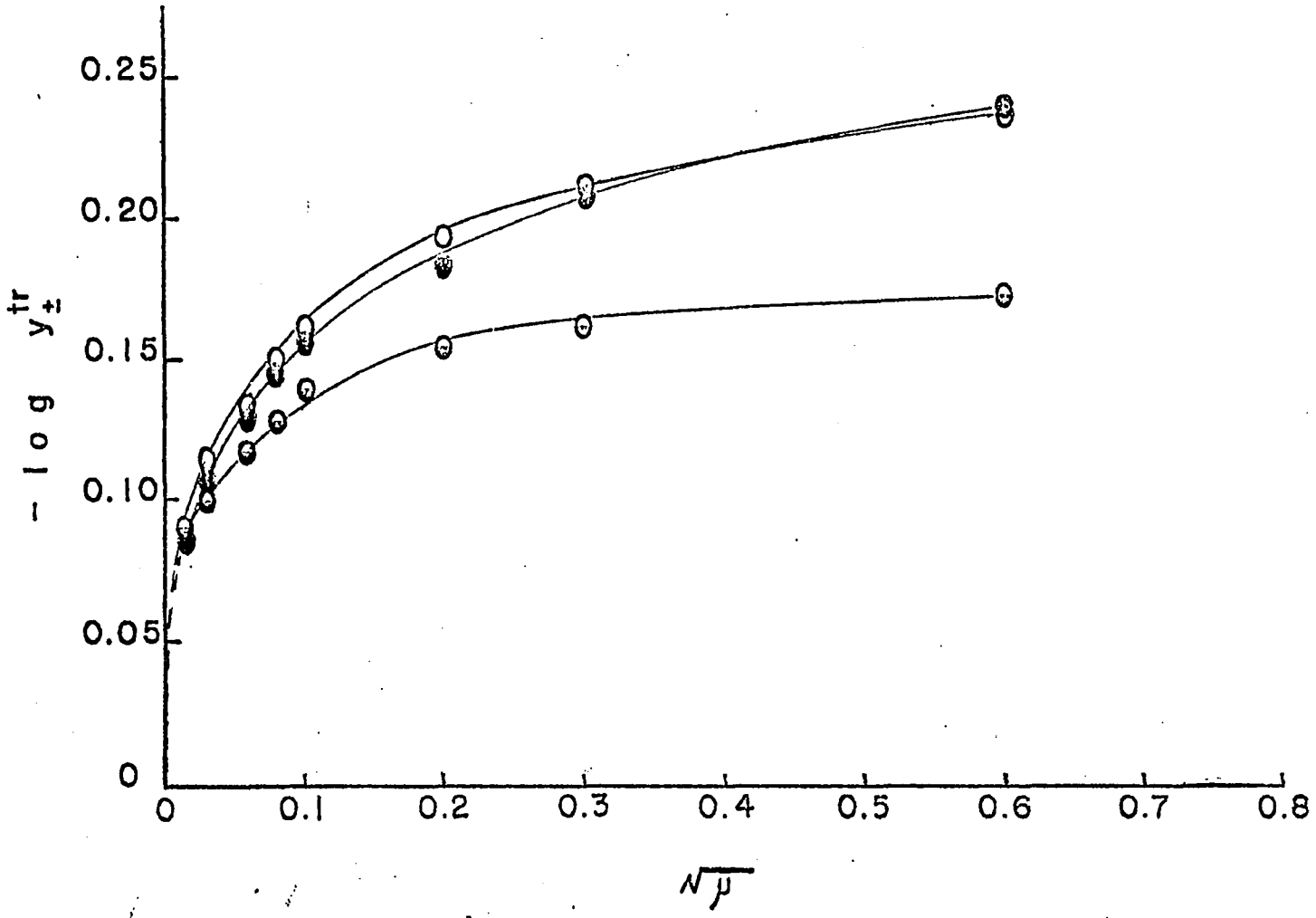

Figure 15. -log $y_{\pm}^{tr}$ vs. $\mu^{\frac{1}{2}}$ for the $Cu(NO_3)_2$-KNO_3-HNO_3 -O-, $CuCl_2$-HCl-KCl -●- and $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ -Θ- systems.

TABLE III

THERMODYNAMIC CONSTANTS USED TO CALCULATE MEAN TRACE ACTIVITY COEFFICIENTS

Constant	log K
$K_{1,HL}$	8.30[a]
$K_{2,HL}$	6.80[a]
$K_{a,HL}$	-8.90[a]
$K_{D,HL}$	1.40[b]
K_{D,ML_2}	2.54[c]
D_r	-1.50[b]

[a]Taken from Sillen and Martell (114)
[b]This work
[c]Taken from Allard, Johnson and Rydberg (78)

(1.75) and μ(0.20) for the $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$, $Cu(NO_3)_2$-HNO_3-KNO_3 and $CuCl_2$-HCl-KCl systems (Figures 16 and 17). The equilibrium concentration of $Cu(AcAc)_2$ for the $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system was also measured by AAS (Figures 18 and 19). The result obtained by AAS and spectrophotometry are comparable (Figure 20). Good agreement was found between the two methods by regression analysis m = 1.01 ± .01, b = -2.35 ± 1.09 x 10^{-6} M.

Test for Perchlorate Ion-Pair Formation

The absorbance of $MBClO_4$ in the chloroform phase was measured as a function of ClO_4^- added to the aqueous phase which was 1.00 x

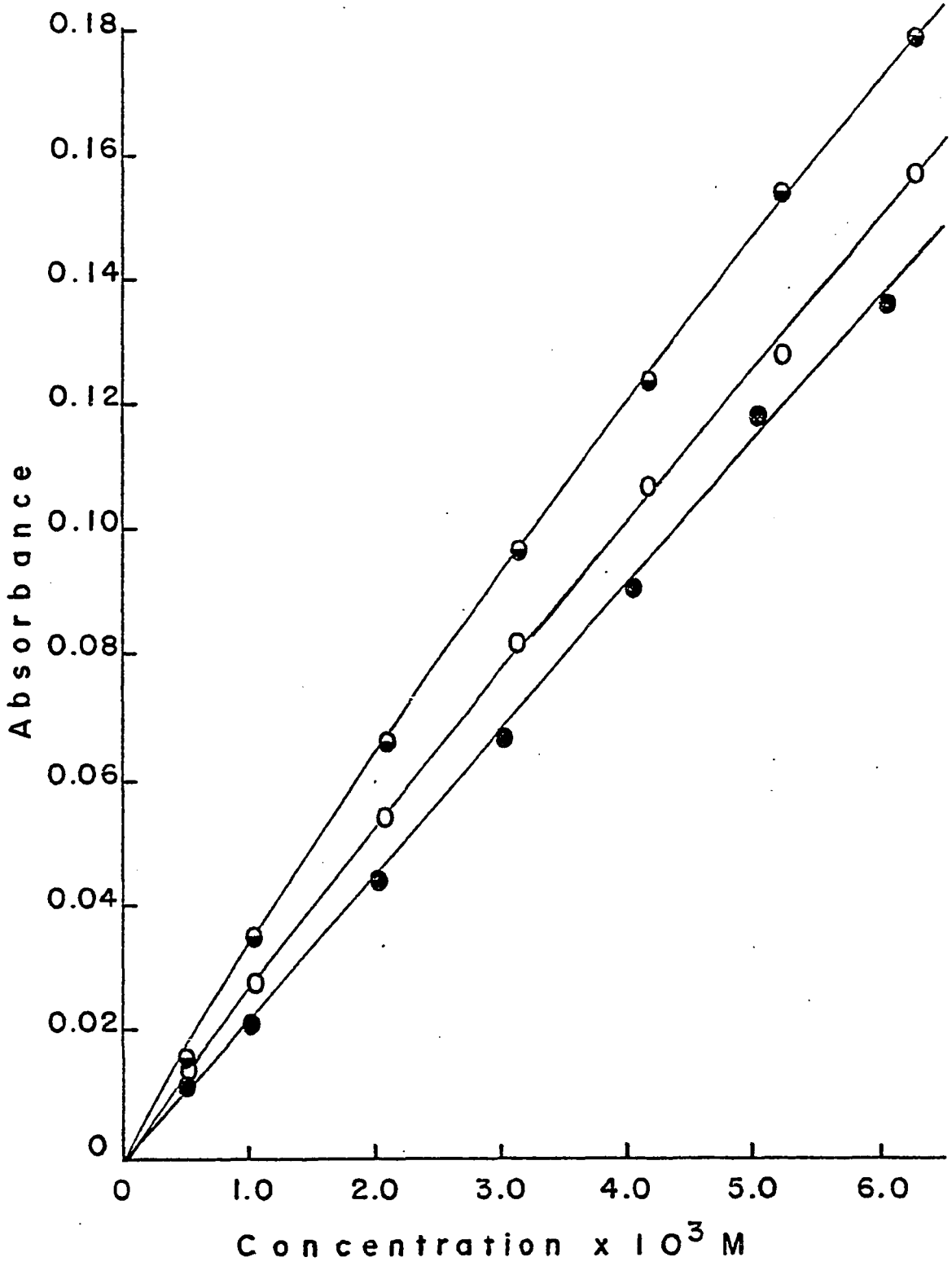

Figure 16. Absorbance vs. total copper(II). -O- $Cu(NO_3)_2$-HNO_3-KNO_3, -●- $CuCl_2$-HCl-KCl and -Θ- $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ systems at $\mu = 0.2$.

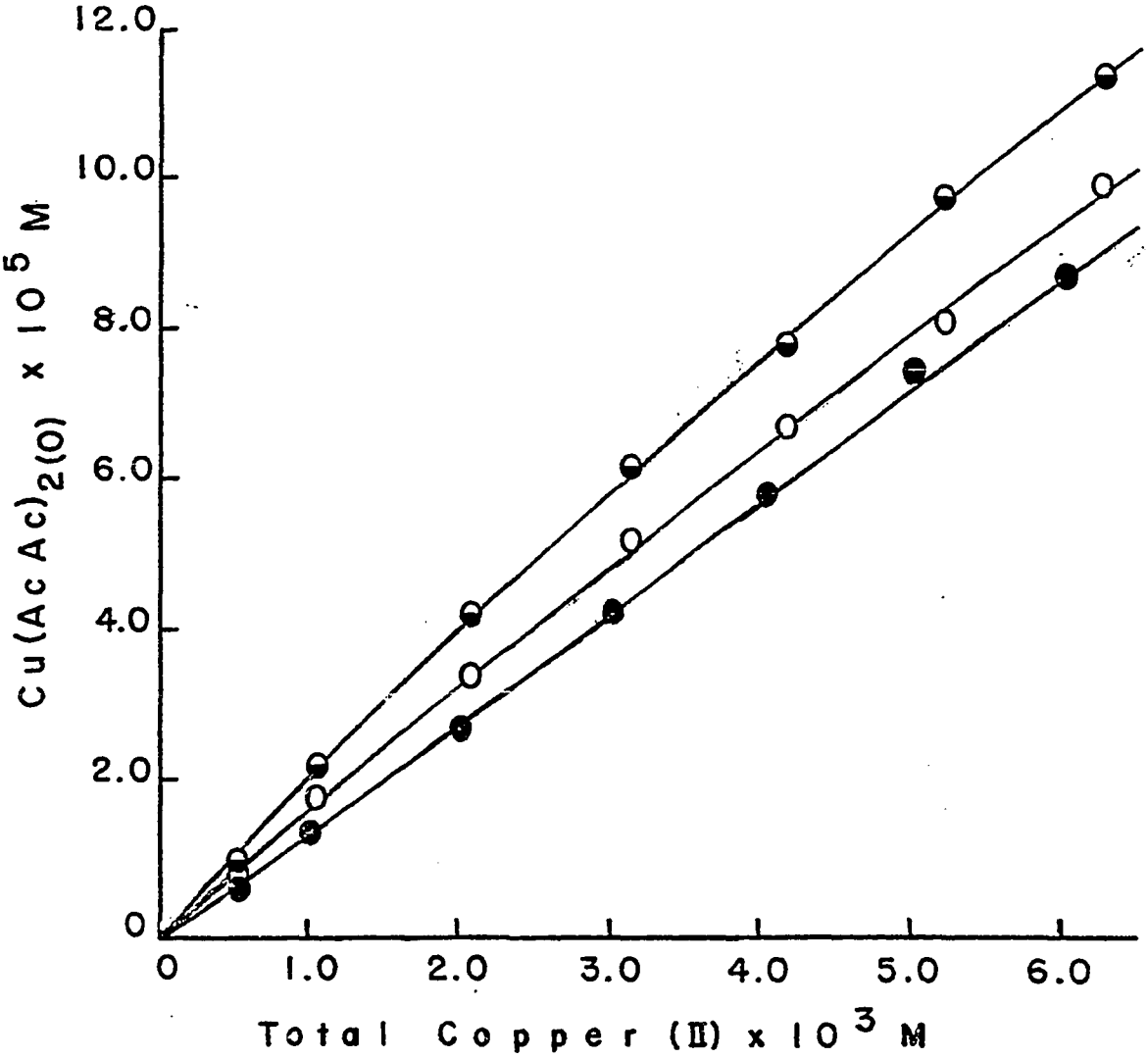

Figure 17. $Cu(AcAc)_{2(o)}$ vs. total copper(II). -O- $Cu(NO_3)_2$-HNO_3-KNO_3, -●- $CuCl_2$-HCl-KCl and -Θ- $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ systems.

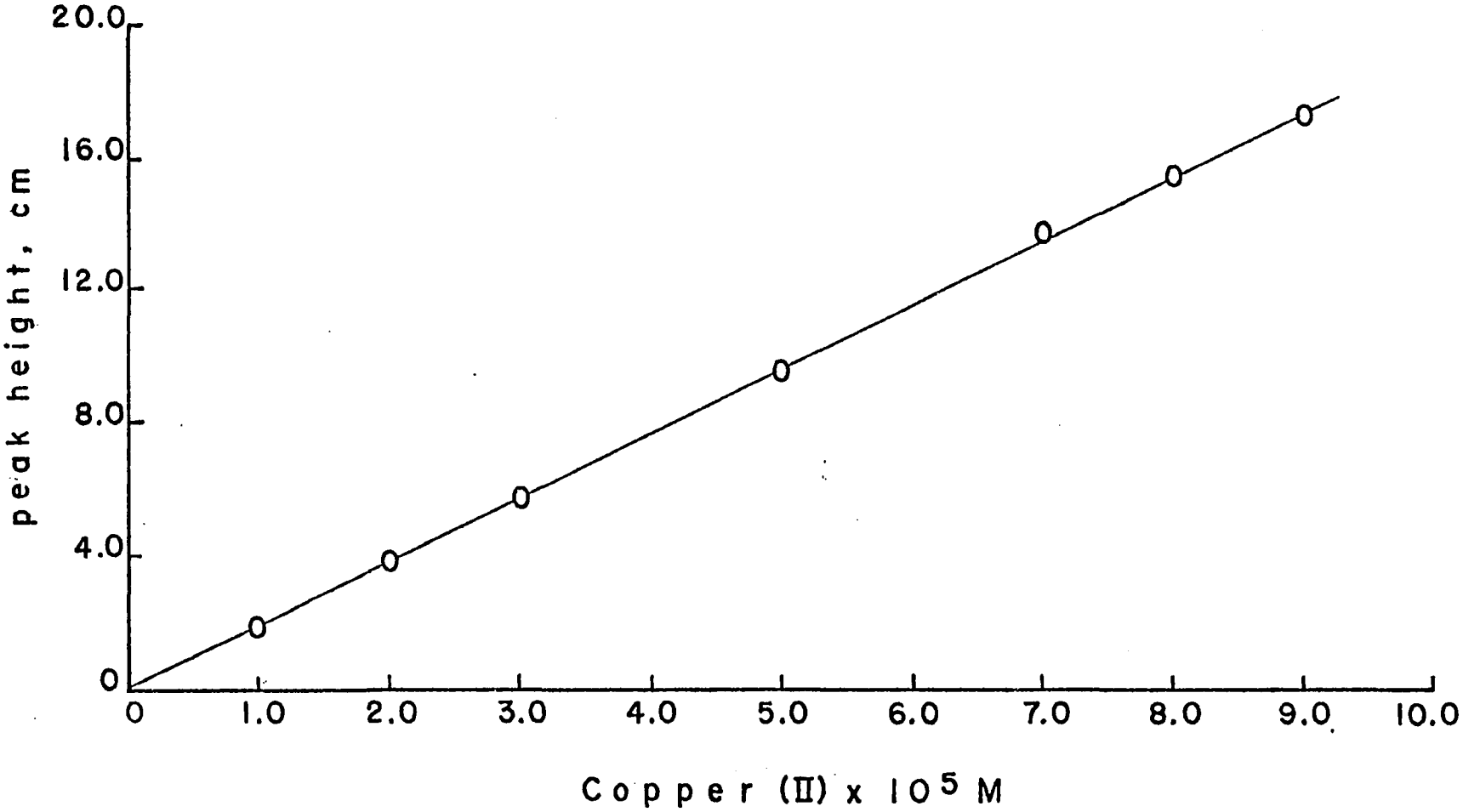

Figure 18. Standard curve for analysis of copper by AAS.

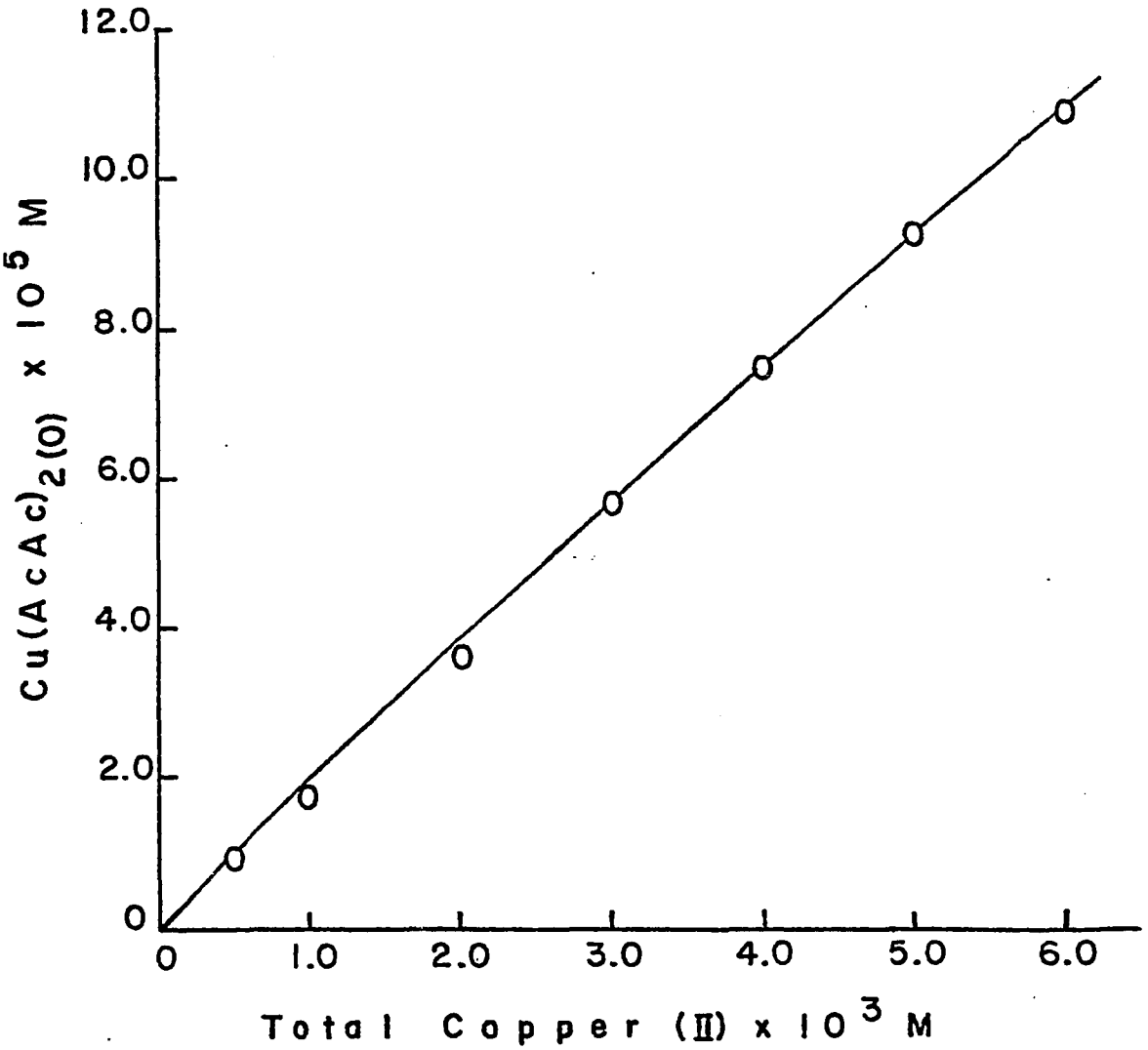

Figure 19. $Cu(AcAc)_{2(o)}$ vs. total copper(II). $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system analyzed by AAS.

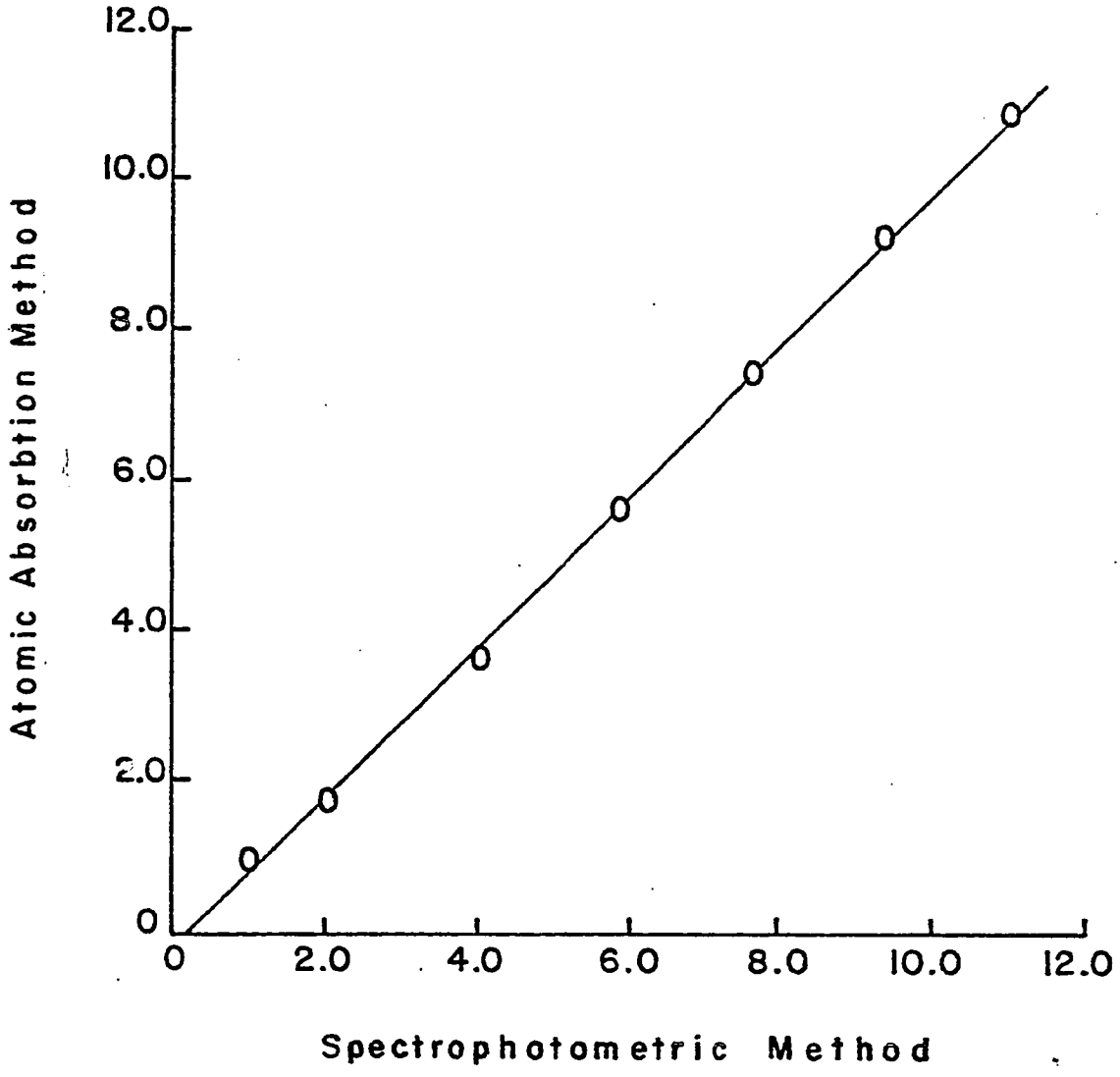

Figure 20. Comparison between atomic absorption and spectrophotometric analysis of the chloroform equilibrium phase from the $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system.

10^{-3} M in both MBCl and HCl (Figure 21). The experimental curve is linear and passed through the blank indicating that the extraction of MBCl and $MBClO_4$ is additive. Blank corrections were determined for each experiment since some scatter and systematic difference can occur between sotck solutions (93). The detection limit for ClO_4^- from the given measurements, calculated by linear regression, is 1.32×10^{-6} M at the 95% confidence level (115).

The chloroform phase containing $9.81 \pm .13 \times 10^{-5}$ M $Cu(AcAc)_2$ extracted from the $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system was treated as previously described. The value of the blank was subtracted from each measurement and the results are plotted in Figure 22. The intercept at y = 0 is $9.3 \pm 2.8 \times 10^{-7}$ M ClO_4^-.

Solubility of Chloroform as a Function of Ionic Strength

The solubility of chloroform was measured as a function of μ (Figures 23-25). The solubility at $\mu = 0$ is $6.90 \pm .63 \times 10^{-2}$ M or $8.30 \pm .75$ g/kg as determined by linear regression. The results are in good agreement with the literature (116) with the exception of those determined by the AKUFVE (117,118).

Effect of Equilibration Time on the Extraction of Copper(II) in the Presence of Amino Acids

The rate at which equilibrium is attained for the extraction of copper(II) as $Cu(AcAc)_2$ in the presence of glycine was measured at constant values of pH and AcAc concentration. Solutions with $\mu = 0.723$ were shaken at a setting of 5 (Figure 26) and those with $\mu \simeq 0.001$ were shaken at a setting of 10 (Figure 27). At the higher

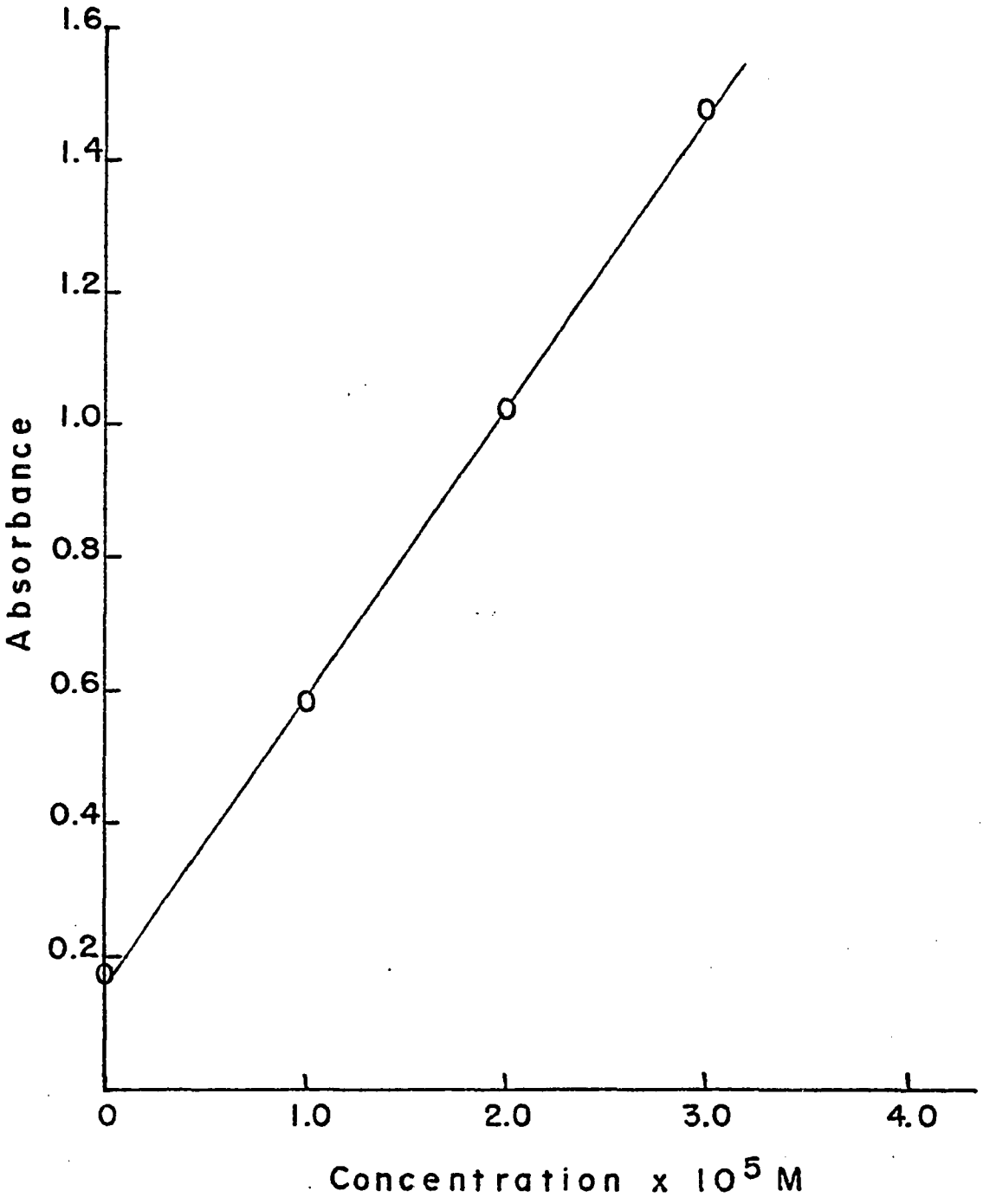

Figure 21. Absorbance vs. concentration of perchlorate ion.

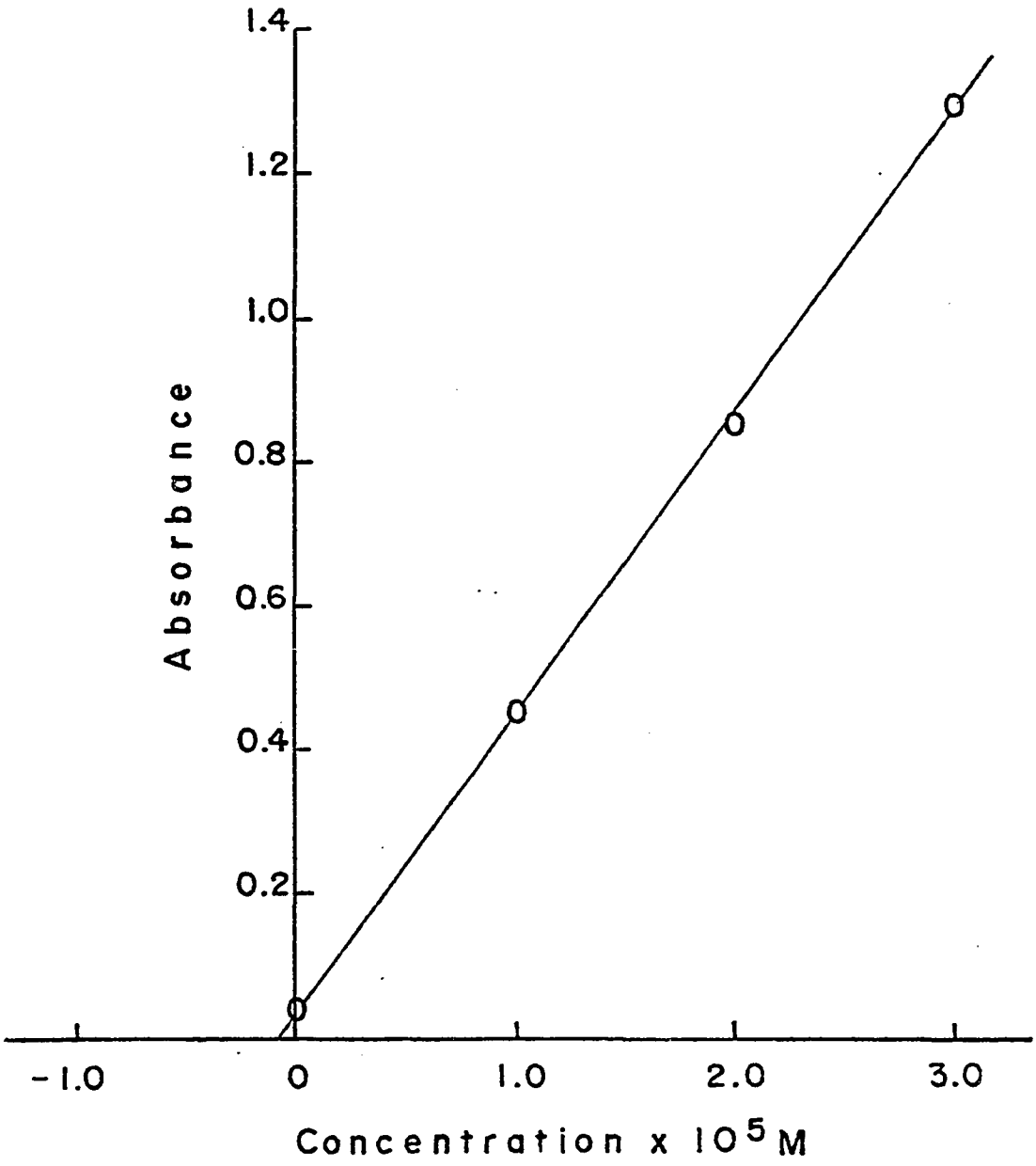

Figure 22. Absorbance vs. $[ClO_4^-]$. Analysis of the organic phase from the $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system by the standard addition method.

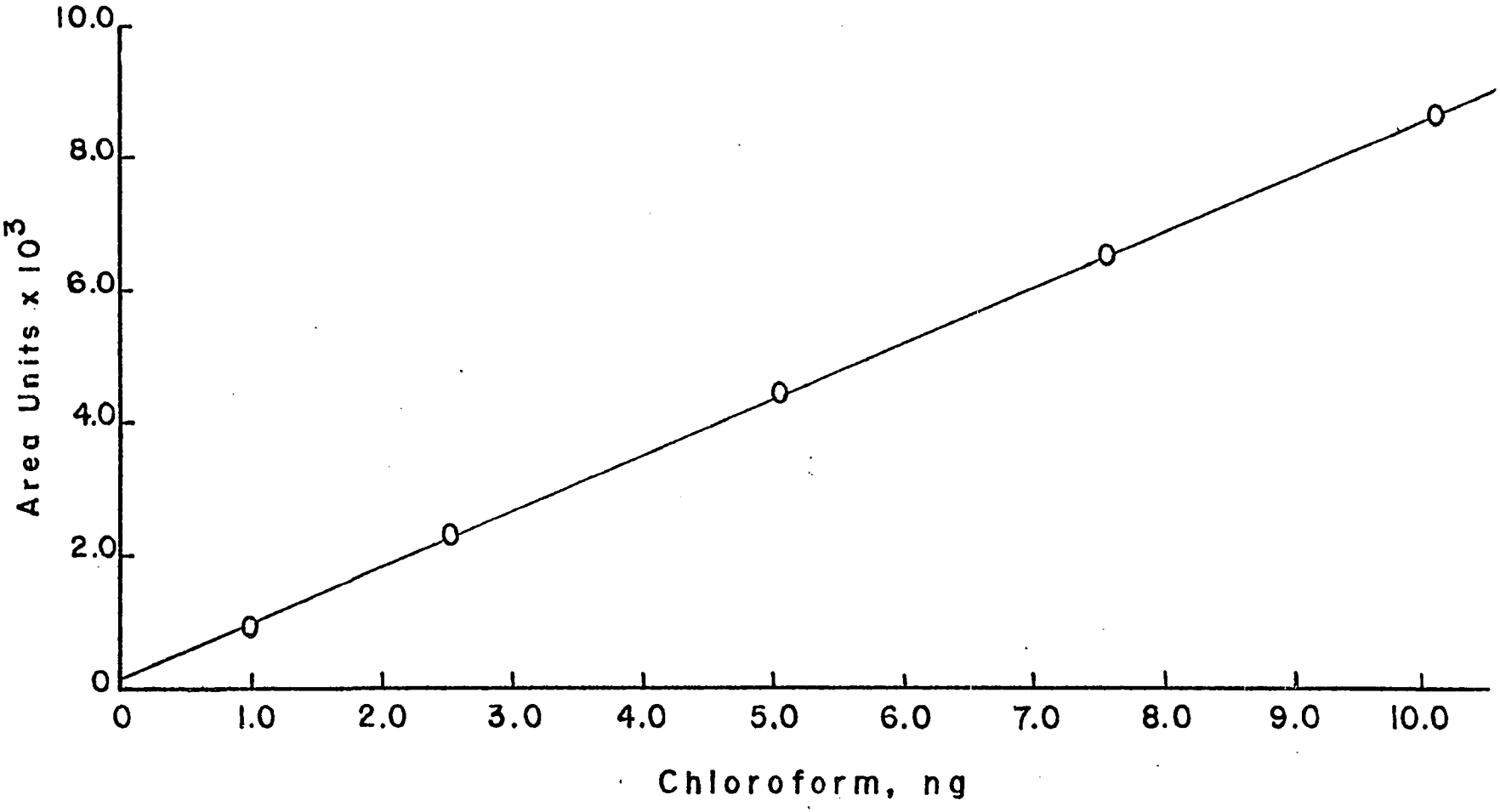

Figure 23. Typical standard curve for the analysis of chloroform by ECD.

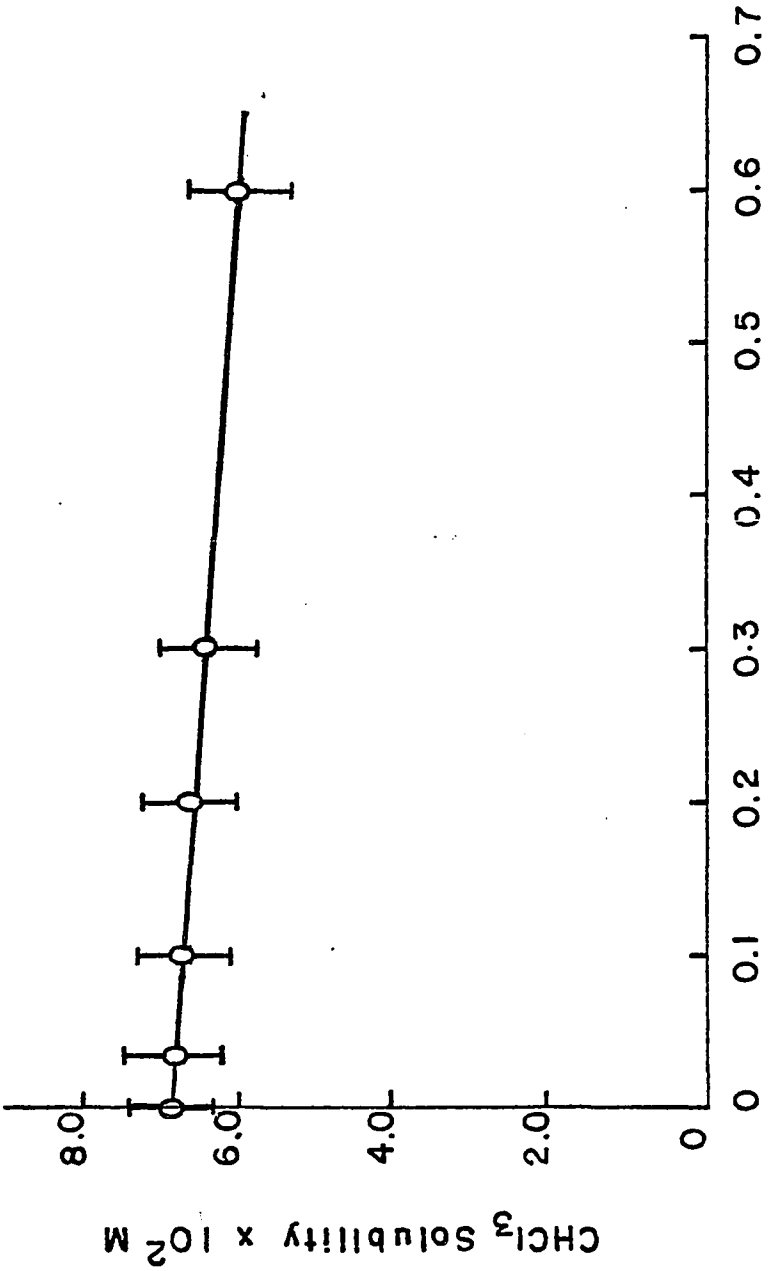

Figure 24. Solubility of chloroform vs. μ.

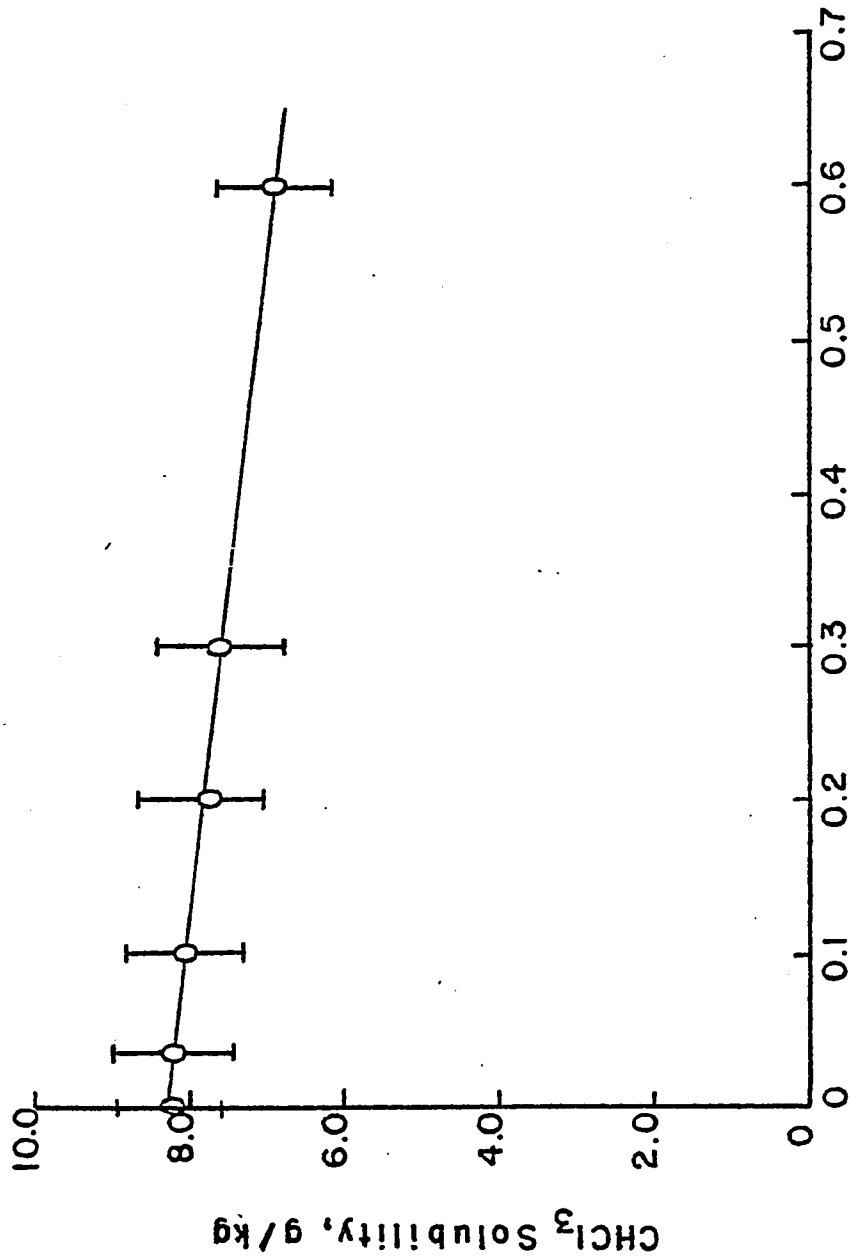

Figure 25. Solubility of chloroform vs. μ.

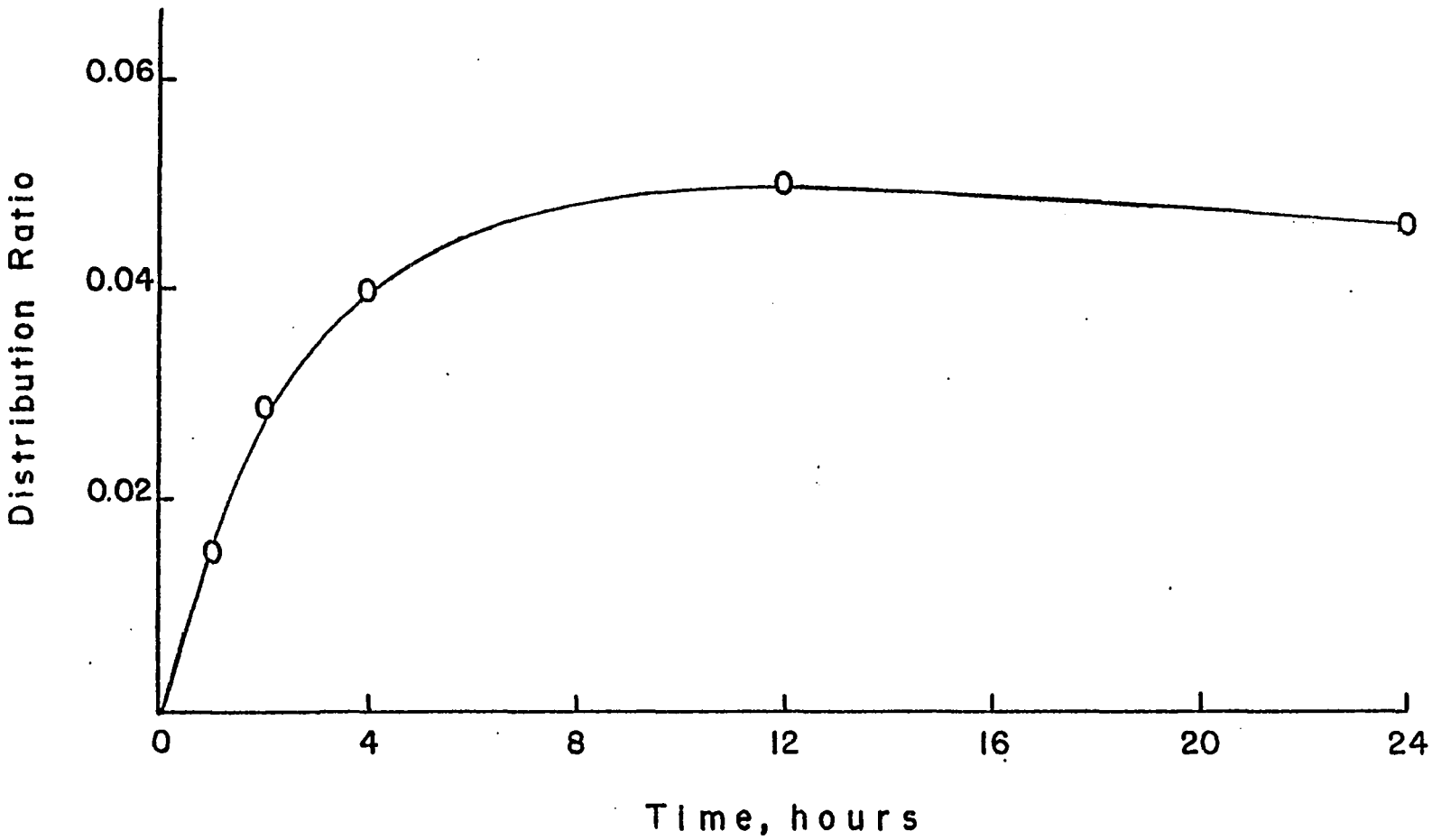

Figure 26. Distribution ratio vs. time for copper(II). Cu(II)-glycine system $\mu = 0.723$ and shaker setting at 5.

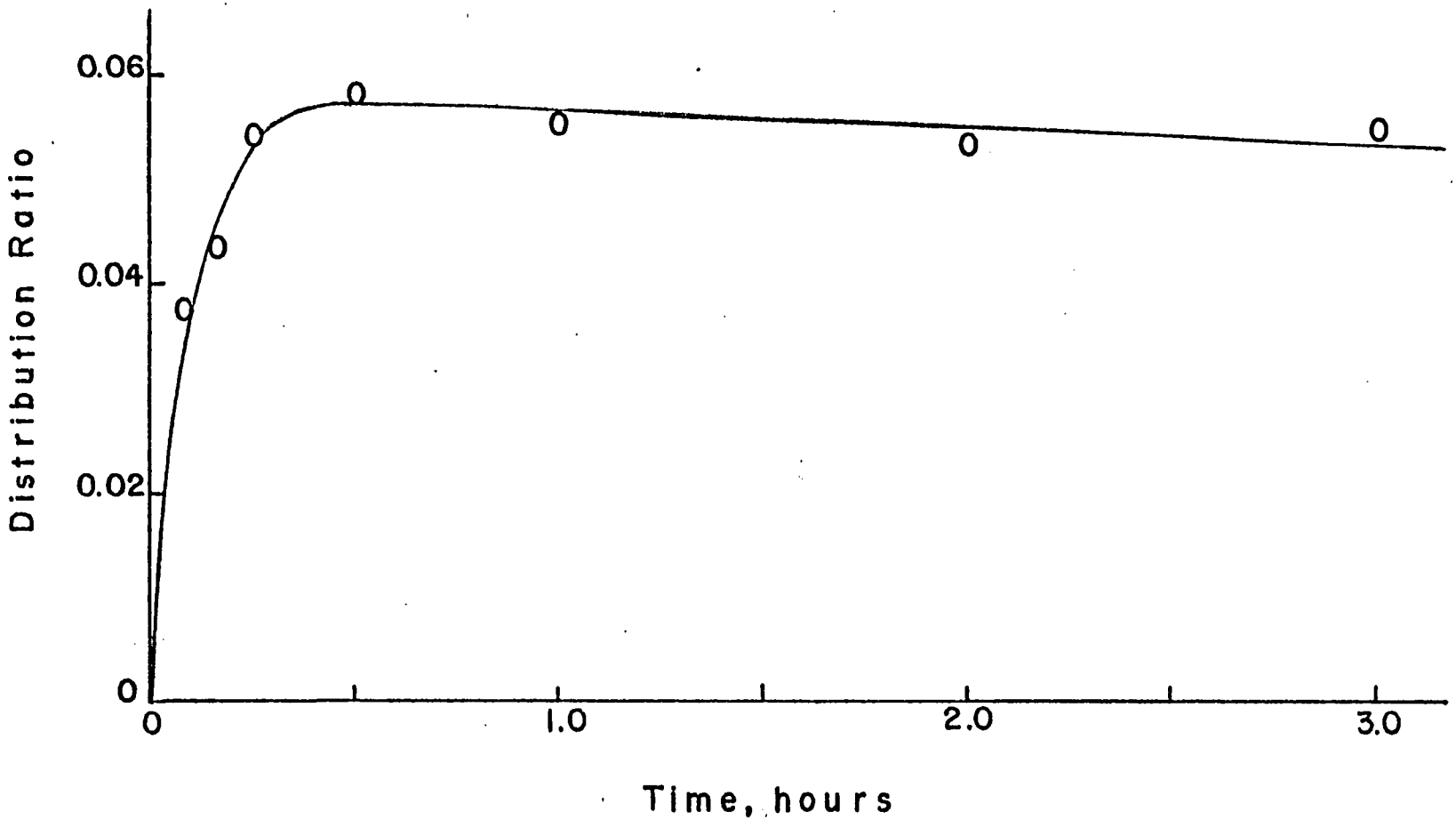

Figure 27. Distribution ratio vs. time for copper(II). Cu(II)-glycine system. $\mu \simeq 0.001$ and shaker setting at 10.

shaking rate equilibrium was attained in about 30 minutes while at the reduced rate equilibrium was not reached for 12 hours.

Distribution of Copper(II) as a Function of Amino Acid Concentration

The concentration of $Cu(AcAc)_2$ in the chloroform phase was measured by AAS as a function of glycine and alanine concentration (Figures 28 and 29). One series of measurements for each amino acid were made at $\mu = 0.723$ and a second without ionic strength adjustment at $\mu \approx 0.001$. The distribution ratio was calculated from equation (8) and plotted as -log D *vs.* amino acid concentration (Figures 30 and 31).

Activity Measurements by Copper(II) Ion Selective Electrode

The response of the copper(II) ISE used in this study was determined as a function of pCu (Figure 32). The linear portion of this curve has a near Nernstian slope of 27.2 ± 0.1 mv. Figure 33 presents the potential measurements for the $Cu(NO_3)_2$-HNO_3-KNO_3 and $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ systems as a function of $\mu^{\frac{1}{2}}$. The curves were fit to a power regression which provided intercept values of 290.9 and 290.0 mv for the nitrate and perchlorate systems, respectively. These limiting values were applied to the standard electrochemical equations to determine the single ion activity coefficient, $\gamma_{M^{+2}}$, of Cu^{+2}. The Garrels (119) assumption which takes the following form

$$\gamma_{M^{+2}} = [\gamma_{\pm MA_2}]^3 / \gamma^{o}_{\pm MA}]^2 \qquad (37)$$

was used to estimate the trace activity coefficients for the two systems (Figure 34). $\gamma^{o}_{\pm MA}$ is the mean activity coefficient of the adjusting electrolyte in its own solution at the same ionic strength.

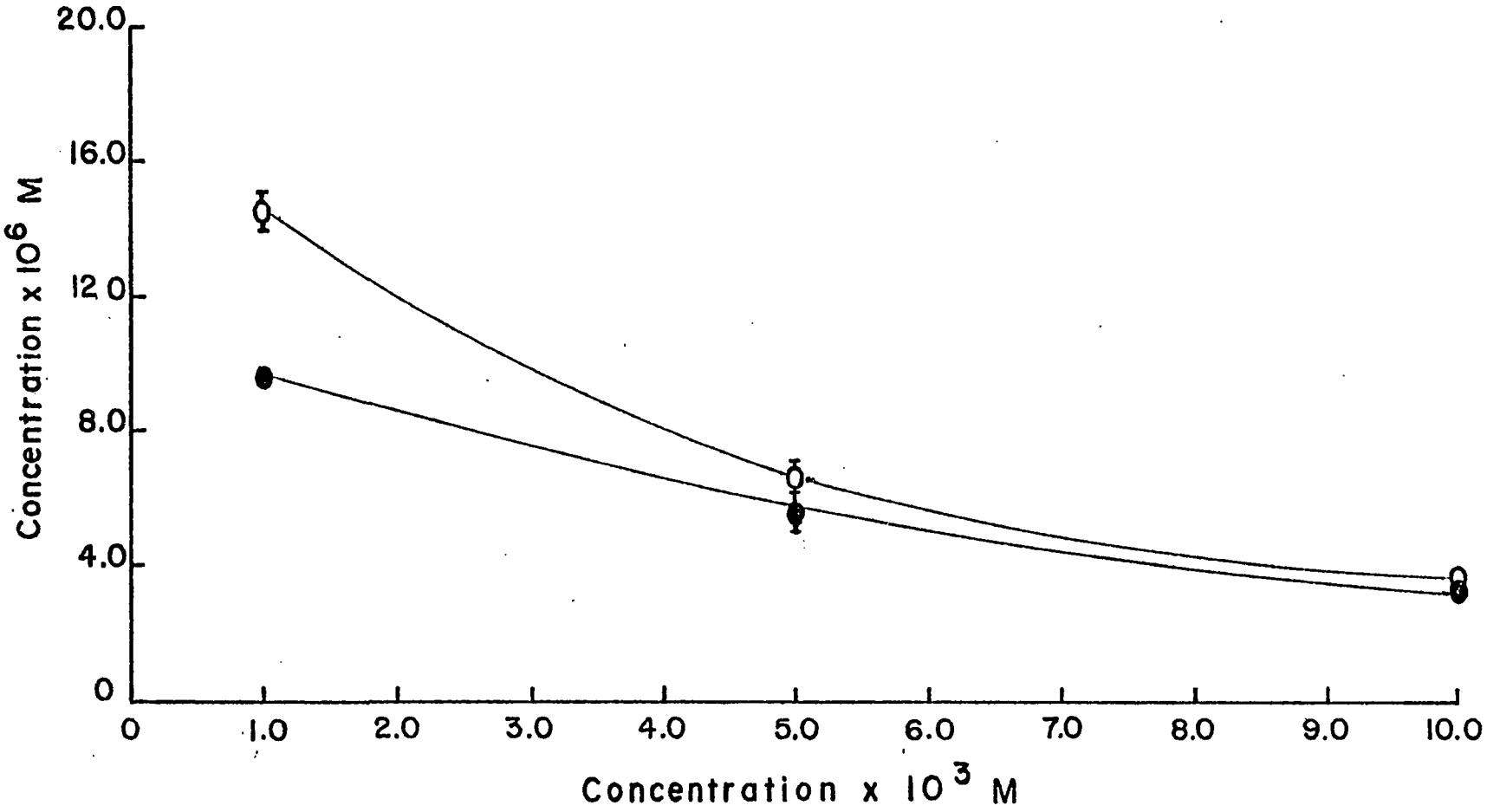

Figure 28. $[Cu(AcAc)_2]_o$ vs. [glycine]. -O- $\mu \simeq 0.001$, -●- $\mu = 0.723$.

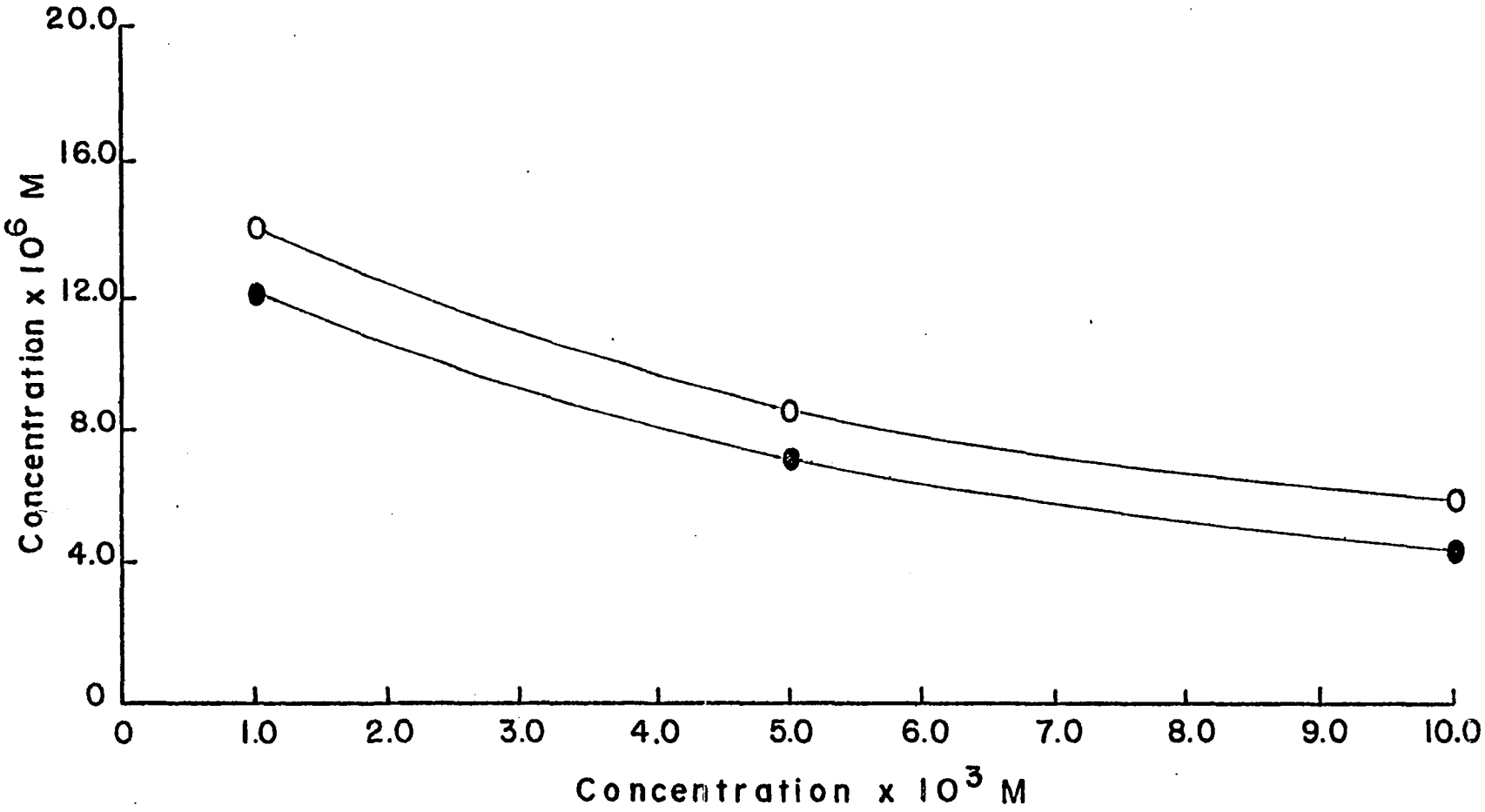

Figure 29. $[Cu(AcAc)_2]_o$ vs. [alanine]. -0- $\mu \simeq 0.001$, -●- $\mu = 0.723$.

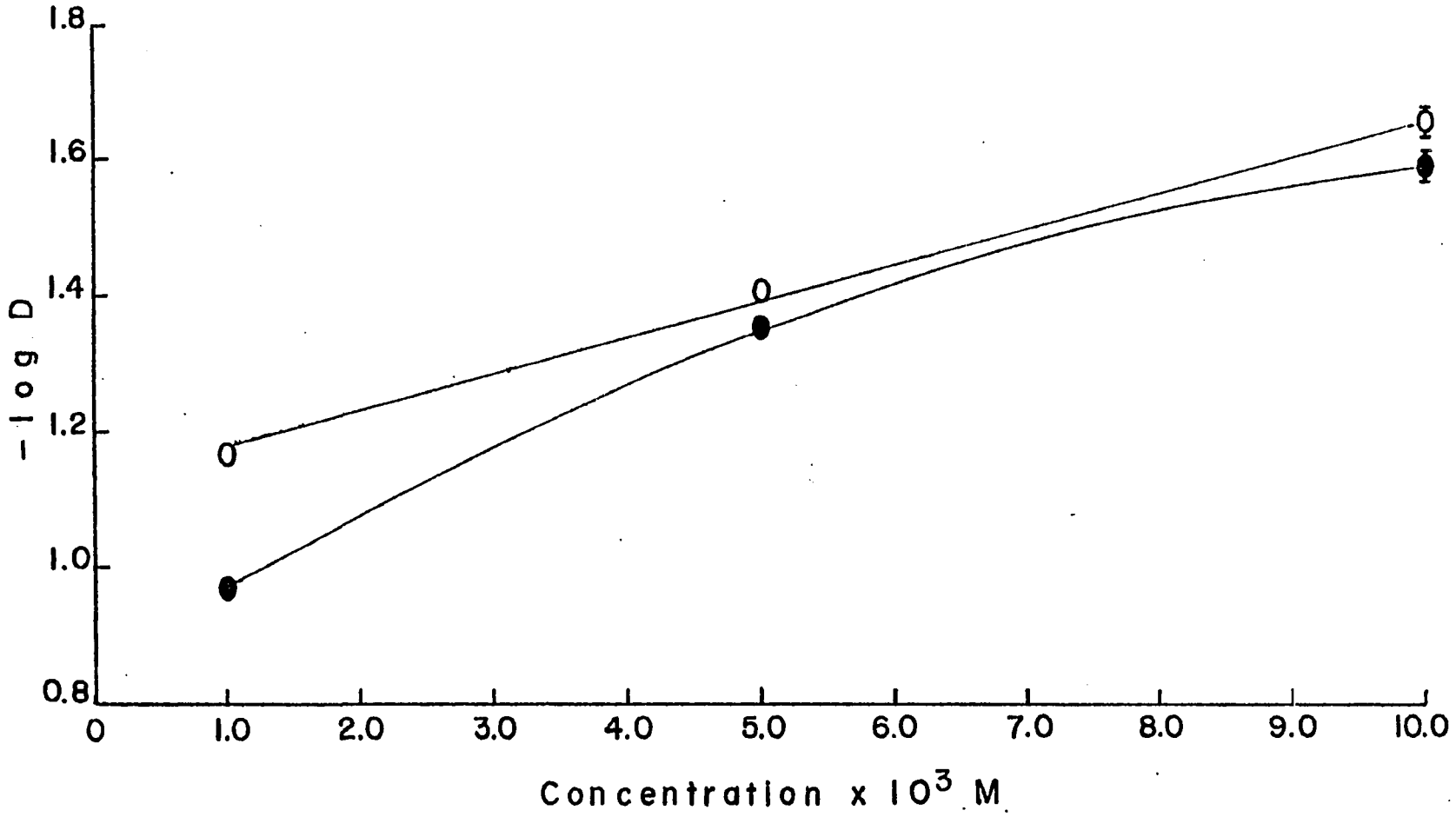

Figure 30. -log D vs. [glycine]. -O- $\mu \simeq 0.001$, -●- $\mu = 0.723$.

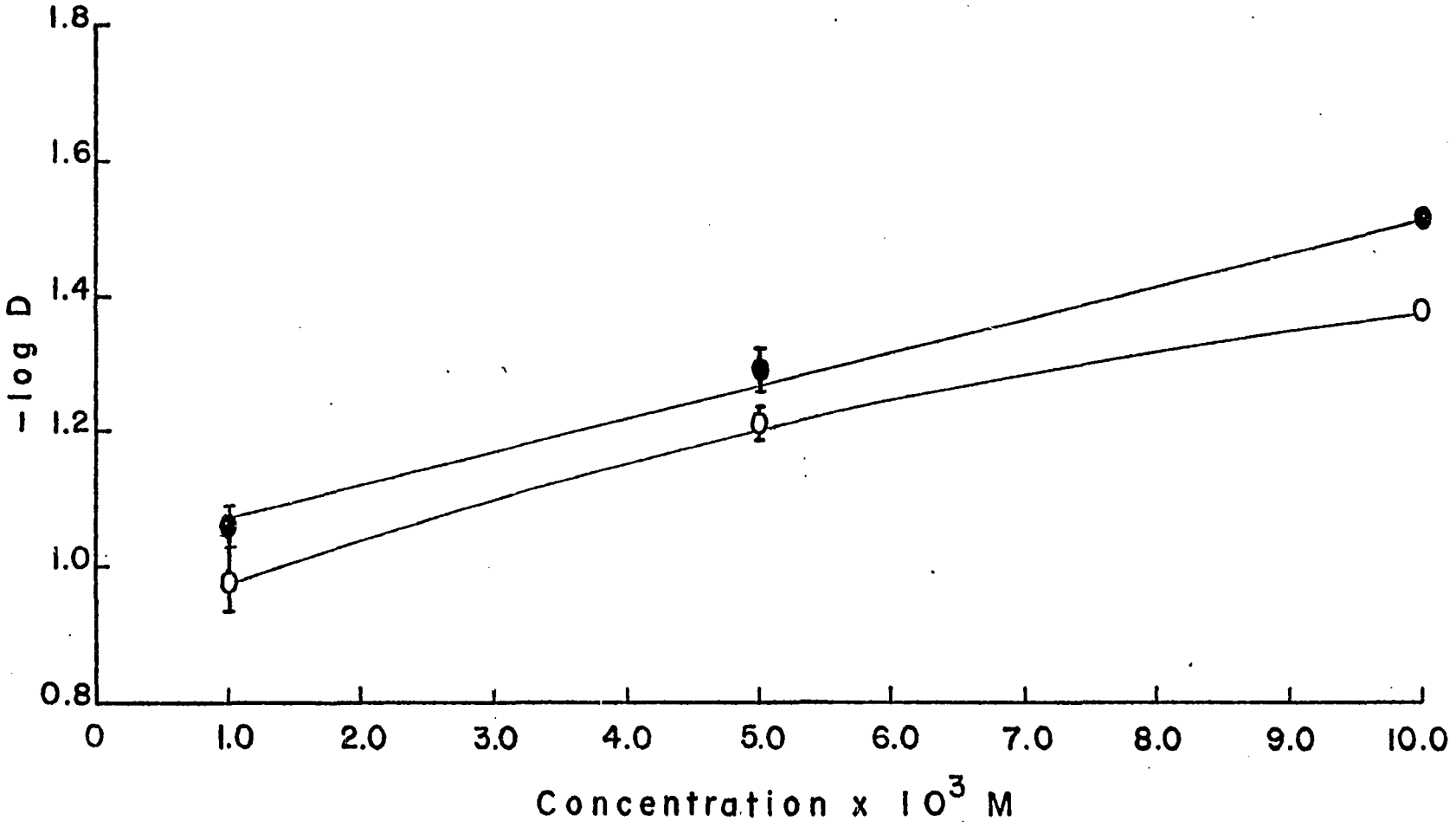

Figure 31. -log D vs. [alanine]. -O- $\mu \simeq 0.001$, -●- $\mu = 0.723$.

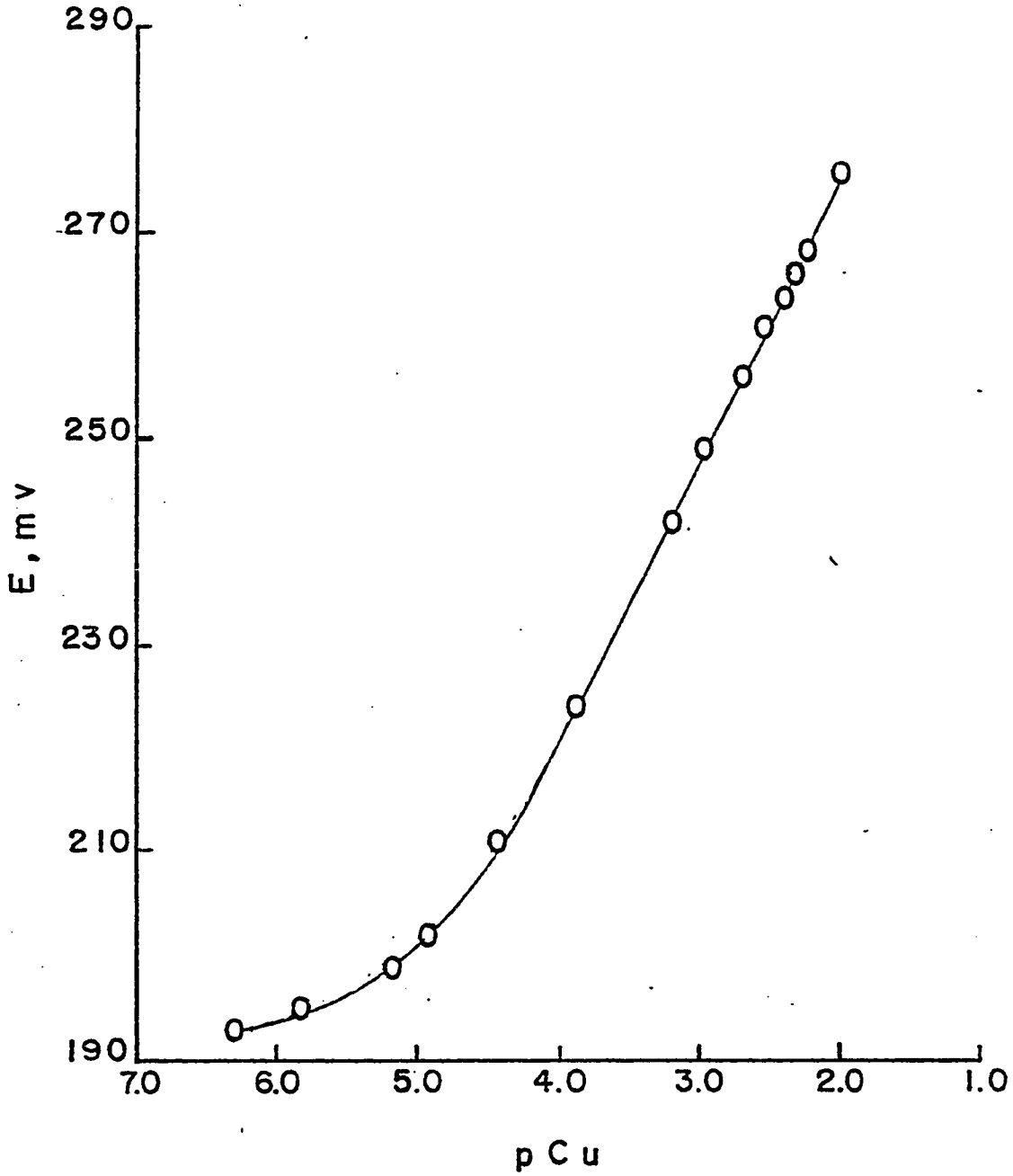

Figure 32. E,mv vs. pCu response curve for copper ISE.

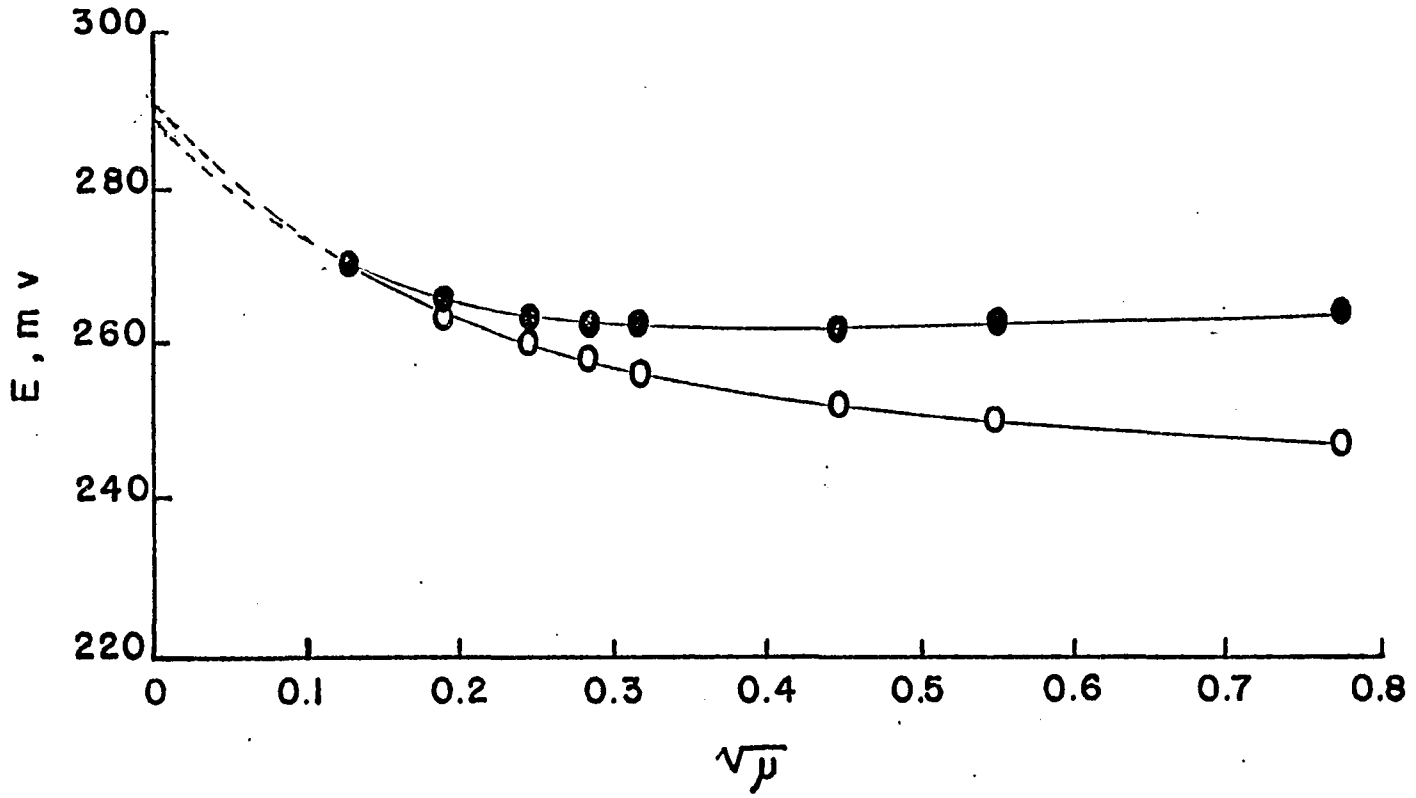

Figure 33. E,mv vs. $\mu^{\frac{1}{2}}$. -O- $Cu(NO_3)_2$-HNO_3-KNO_3 system, -●- $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system.

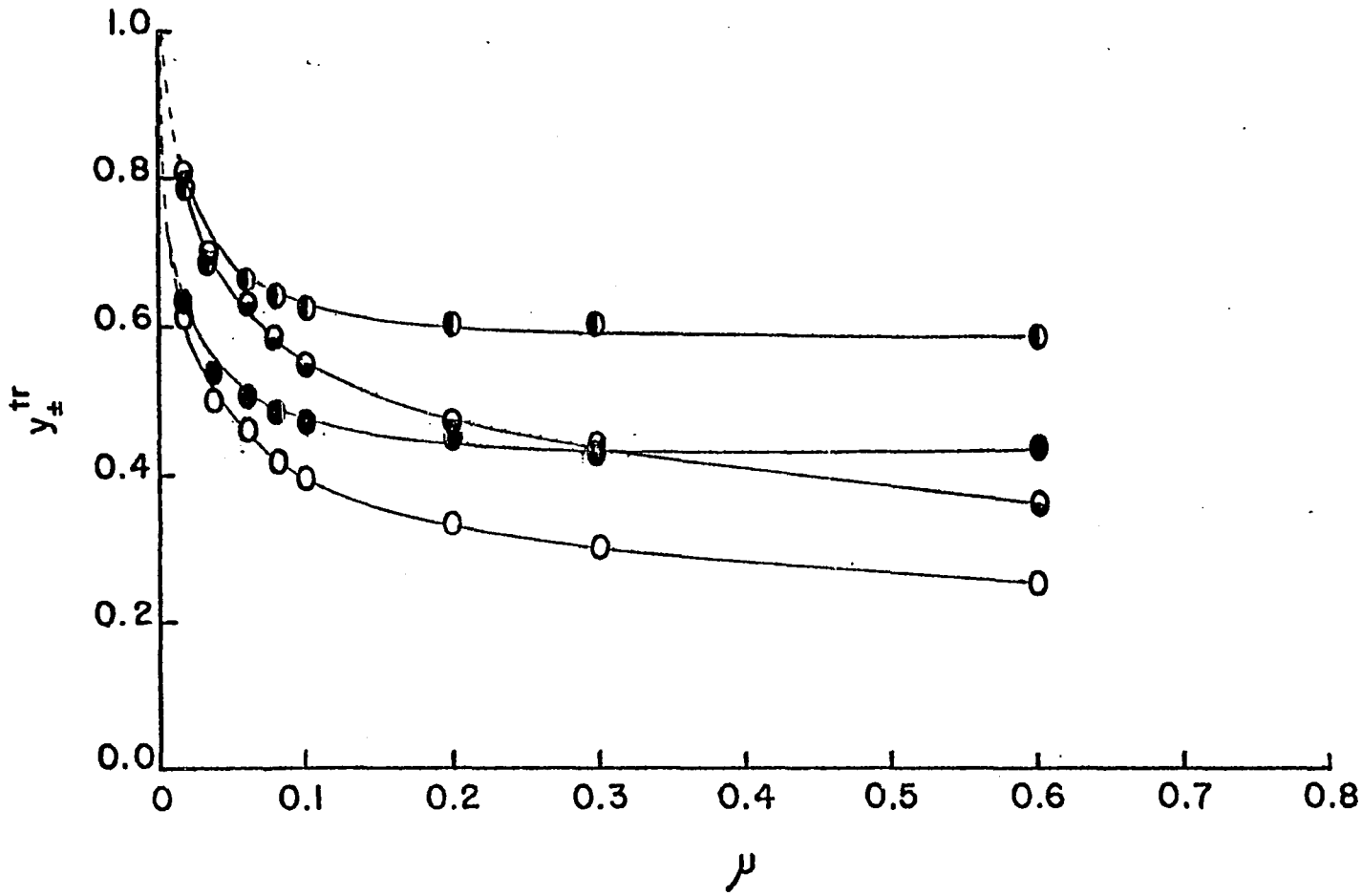

Figure 34. $y_{\pm}^{tr}$ vs. μ for the -O- $Cu(NO_3)_2$-HNO_3-KNO_3 system and the -●- $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system. Trace activity coefficients corrected for E_j -Θ- $Cu(NO_3)_2$-HNO_3-KNO_3 system and -Θ- $Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ system.

Activity coefficients for pure KNO_3 and $NaClO_4$ solutions were taken from Robinson and Stokes (81), who provide the values above $\mu = 0.1$. The activity coefficient values between $\mu = 0.0$ and 0.1 were estimated from the following equation

$$\log \gamma_{\pm} = - \frac{A/z_1 z_2/\mu^{\frac{1}{2}}}{1 + Ba\mu^{\frac{1}{2}}} + b\mu \qquad (38)$$

where A = .5115 and B = .3291 at 25°C. Parameters a and b were adjusted to give the best fit to the experimental points.

Estimates of the liquid junction potential were made using the Henderson equation

$$E_j = \frac{RT}{F} \frac{\Sigma(\lambda_i/z_i)(c_i'' - c_i')}{\Sigma\lambda_i^o (c_i'' - c_i')} \ln \frac{\Sigma c_i' \lambda_i^o}{\Sigma c_i'' \lambda_i^o} \qquad (39)$$

where c' and c" are the ion concentrations in the two boundary solutions and λ_i^o is the limiting equivalent ionic conductance. The liquid junction potentials were calculated relative to saturated KCl as demonstrated by Bates (120). The results are presented in Table II. The limiting ionic conductivities were taken from Robinson and Stokes (81).

The calculated values of E_j were used to adjust the cell potentials presented in Figure 33. The adjusted potentials were used to recalculate the trace activity coefficients which are shown in Figure 34 along with the original values.

TABLE IV

LIQUID-JUNCTION POTENTIALS, IN MILLIVOLTS AT 25°C
COMPUTED FROM LIMITING IONIC MOBILITIES
BY THE HENDERSON EQUATION[a]

Junction: Cu(II) Soln || KCl (Satd.)		
μ	E_j, nitrate system	E_j, perchlorate system
0.016	4.6	4.6
0.036	2.6	2.9
0.060	2.2	2.2
0.080	2.1	1.9
0.100	1.9	1.8
0.200	1.6	1.2
0.300	1.5	0.1
0.600	1.2	0.0

[a]Positive E_j signifies a boundary of polarity -||+

CHAPTER V

DISCUSSION

The following discussion should provide the reader with (a) a view of the experimental parameters that required control and/or monitoring to assure precise and accurate determination of trace activity coefficients from liquid-liquid partitioning of metal chelate compounds, (b) an indication of how these parameters might be used to advantage or may present difficulties in future applications, (c) a demonstration of the thermodynamic significance of these trace activity coefficients through comparison with electrochemical measurements, and finally (d) a model for environmental application.

Experimental Variables and Their Effects on the Distribution of Bis(Acetylacetonato) Copper(II)

Extraction Kinetics. The rate at which equilibrium is attained in the $Cu(AcAc)_2$ system (Figures 4, 5, 26, 27) was investigated strictly to assure that equilibrium was attained under the conditions of the study. Figures 4 and 5 show that the equilibrium is attained in a few minutes with a 0.10 M AcAc concentration in the organic phase and that the rate at which equilibrium is reached is slightly slower as the pH is decreased (Figure 5). The dependence of extraction rate for metal chelate compounds on pH has been well documented and the rate generally increases with increasing pH (71).

Figure 26 shows a considerable decrease in the extraction rate with a 0.001 M AcAc in the presence of 5.00×10^{-3} M glycine. The extraction rate was improved by increasing the shaking rate (Figure 27). It was beyond the scope of this study to investigate the dependence of the extraction kinetics of $Cu(AcAc)_2$ on variables such as reagent concentration, temperature, shaking rate, etc., and as a result it is not possible to conclude if the decrease in extraction rate is due to the decrease in AcAc concentration and/or the presence of the competing glycine complex. It has been demonstrated for a few chelate extraction systems that the extraction rate decreases with decreasing reagent concentration (121-123) and with the presence of strong masking agents (competing complexing agents) such as EDTA (123,124). The number of reported kinetic studies is small although the information which they can provide is of practical and theoretical interest.

pH. The dependence of the $Cu(AcAc)_2$ extraction on pH in the present system (Figures 7 and 8) is the classic one described by equation (15). Figure 7 shows that the percent extraction increases rapidly between pH 2 and 3 and is essentially quantitative above pH 4 with a single extraction of equal volumes. Most important, however with regard to the determination of trace activity coefficients is the region below pH 2. Between pH = 1.5 and pH = 2.0 the percent extraction is reduced to 1-5% of the analytical concentration of the metal. The advantage of extracting a small fraction of the total metal is that it leaves the initial equilibrium in the aqueous phase relatively unchanged.

One would not want to change the pH of a natural sample. This would offer a more challenging situation, namely, the extraction of a small fraction of metal at a relatively high pH. Possible solutions might include the use of low reagent concentrations, reagents with higher pK_a's, or perhaps exchange reactions with neutral metal complexes of alkaline earth metals.

Salt Effects. The effect of salts on the distribution of acetylacetone has been found to be very significant above an ionic strength of 0.1. Figure 10 shows the effect of the various electrolytes used in this study. The apparent distribution constant of acetylacetone increases in NaCl and KCl but decreases in $NaClO_4$ and KNO_3. Obviously the effect depends upon the identity of the salt. The magnitude of the observed change in the distribution of acetylacetone was taken into consideration in the calculation of the trace activity coefficients by correcting equation (40) for $[AcAc]_o$.

In a recent study Yoshemura and Suzuki (125) showed that the apparent change in the distribution ratio of benzoylacetone with electrolyte is caused by change of the activity coefficients of the respective tautomers in the aqueous phase. In addition to the above study there have been only a limited number of investigations (111,112) where the influence of electrolytes on the distribution of the chelating agent have been studied. This kind of data is important in understanding the influence of salt effects on the extraction of metal chelate compounds.

Obviously these salt effects will require consideration in application of the method to samples of higher ionic strength such as

seawater and estuarine waters. Fresh water samples would have an ionic strength of less than 0.1 and therefore no correction would be required.

Adduct and Ion-pair Formation. The distribution ratio of a metal chelate can be strongly increased by the formation of adducts and/or ion-pairs. For this reason the $Cu(AcAc)_2$ concentration in the organic phase was monitored as a function of the total copper concentration in the aqueous phase (Figure 17). The experimental results for the nitrate and chloride systems are essentially linear but the perchlorate system shows slight curvature. This deviation from linearity is in a negative direction which is contrary to what is expected for adduct or ion-pair formation. Despite the indication that no adduct and/or ion-pair formation was occurring, several experiments were performed for confirmation.

The extraction of the series of perchlorate samples was repeated and the analysis for copper in the organic phase was made by AAS (Figure 19). Figure 20 shows that the results of the analysis by the spectrophotometric method and AAS are the same within experimental error. Since AAS is dependent on absorbance of the metal atom, the experiment lends evidence that ion-pair and adduct formation are not occurring. The slightly negative curvature found by both of these analytical methods can not be explained.

Finally, the organic phase from a perchlorate sample was analyzed for the presence of perchlorate ion. Figure 22 shows that within the experimental error of the method perchlorate ion was present at a concentration of less than $\sim 1 \times 10^{-6}$ M, or less than 1% of the total $Cu(AcAc)_2$ concentration in the organic phase.

Solvent Effects. Chloroform was the only solvent used throughout the study and the main question of concern was the effect of its solubility on the electrolyte activities in the aqueous phase. Figures 24 and 25 show the solubility of chloroform as a function of ionic strength. The solubility is 6.90×10^{-2} M at $\mu = 0$ and decreases with increasing ionic strength.

McKay (90) has shown by experimental measurement and thermodynamic calculation that the changes in the activity of the electrolyte and of water are approximately equal to twice the percent by weight of the solubility of the organic solvent. Chloroform will, therefore, cause less than a 2% deviation which is within the experimental error of most analytical methods. McKay found that except for oxygen containing solvents, the effect of most solvents was negligible.

There are many other properties of organic solvents which should be considered before application in any extraction system. They have been discussed thoroughly elsewhere (71). One consideration which should be mentioned, however, is the potential solubility of naturally occurring metal complexes in the organic phase. Such an occurrence could be monitored by varying the reagent concentration or by simply running a blank depending upon the nature of the detection method and its sensitivity.

Determination of Trace Activity Coefficients

The trace activity coefficients of copper(II) electrolytes determined by liquid-liquid partitioning (Figure 14) and potentiometric measurements (Figure 34) show some disparity. This is not unexpected

if one considers the nature of the methods used. Liquid-liquid partitioning obeys the law of mass action and like an electrochemical cell without liquid junction is thermodynamically well-defined. Cells with liquid junction, such as one which utilizes a saturated calomel reference electrode with a saturated KCl salt bridge, do not reach true equilibrium. This is a result of the constant mixing which takes place at the liquid junction and which establishes a diffusion potential. According to Butler (126), E_j may vary by 5 mv with quality commercial reference electrodes resulting in possible uncertainty in $\gamma_{M^{n+}}$ of 50%. The results of this study are essentially in agreement with Butler's analysis.

The potential developed across a liquid junction can not be determined in a practical sense because it is dependent on the single ion activities which can not be determined thermodynamically. Attempts at extrathermodynamic approaches for dealing with the problem of single ion activities have met with limited success (127). An estimate of E_j can be made through the Henderson equation (equation 39) which assumes a continuously mixing junction, that the activity of each ionic species is equal to its concentration and that the transference number, t_i, of each ion is constant in the concentration range studied (128). Adjustment of the electrochemically determined trace activity coefficients (Figure 34) by correcting for E_j with estimated values (Table IV) demonstrates that the effect of the junction potential is to provide lower trace activity coefficients. Unfortunately the magnitude of the correction is dubious at best.

Table V shows that at each ionic strength where available

TABLE V

TRACE ACTIVITY COEFFICIENTS OF COPPER(II) ELECTROLYTES IN ACIDIC SALT SOLUTIONS[a]

$Cu(NO_3)_2$-HNO_3-KNO_3 System

μ	$\gamma^o_{HNO_3}$	$\gamma^o_{KNO_3}$	$\gamma^o_{Cu(NO_3)_2}$	$y^{tr}_{Cu(NO_3)_2}$
0.10	0.791	0.739		0.689
0.20	0.754	0.663		0.639
0.30	0.735	0.614	0.512	0.615
0.60	0.717	0.519	0.461	0.580

$Cu(NO_3)_2$-$HClO_4$-$NaClO_4$ System

μ	$\gamma^o_{HClO_4}$	$\gamma^o_{NaClO_4}$	$\gamma^o_{Cu(NO_3)_2}$	$y^{tr}_{Cu(NO_3)_2}$
0.10	0.803	0.775		0.726
0.20	0.778	0.729		0.702
0.30	0.768	0.701	0.512	0.688
0.60	0.776	0.656	0.461	0.671

$CuCl_2$-HCl-KCl

μ	γ^o_{HCl}	γ^o_{KCl}	$\gamma^o_{CuCl_2}$	$y^{tr}_{CuCl_2}$
0.10	0.796	0.770		0.697
0.20	0.767	0.718		0.656
0.30	0.756	0.688	0.510	0.619
0.60	0.763	0.637	0.457	0.578

[a]Values of γ^o for all electrolytes were taken from Robinson and Stokes (81).

experimental data allows comparison the trace activity coefficient of the copper(II) salt is higher in the acidic salt solution than it is in its own solution. The same effect has been observed by Schubert (62) in the study of the $Ba(NO_3)_2$-HNO_3-$UO_2(NO_3)_2$ system and by Harned for strong acids (103). The data in Table V shows that the values of $y^{tr}_{Cu(NO_3)_2}$ in the presence of $HClO_4$ and $NaClO_4$ are higher than those of both $y^{tr}_{CuCl_2}$ in the presence of HCl and KCl and $y^{tr}_{Cu(NO_3)_2}$ in the presence of HNO_3-KNO_3. The values of $y^{tr}_{CuCl_2}$ and $y^{tr}_{Cu(NO_3)_2}$ are the same within experimental error.

A limited mathematical description of the three systems is presented in Table VI. The trace activity coefficients were calculated at μ = 0.3 and 0.6 where experimental values of the osmotic and activity coefficients were available for all species. The calculations were made using the equation of Reilly, Wood and Robinson (129) for the case of a mixture of three cations and one anion (MA_2, NA, LA). Only the first four terms of equation (40) were used since values for the interaction parameter (g) are not available for the association of copper(II) with the elctrolytes used in this study. Unfortunately the properties of many common ion mixtures, which would allow calculation of (g) for transition metals are not known (131). Where equation (40) has been applied to well-defined systems such as HCl-CsCl-$BaCl_2$, the calculated values agree with the experimentally reported measurements within experimental error.

Trace Activity Coefficients in the Presence of Amino Acids

Application of the liquid-liquid partitioning method for the

TABLE VI

CALCULATED TRACE ACTIVITY COEFFICIENTS OF COPPER(II) ELECTROLYTES IN ACIDIC SOLUTIONS[a,b]

$Cu(NO_3)_2$-HNO_3-KNO_3

μ	$\gamma^{tr}_{Cu(NO_3)_2}$ (calc.)	$y^{tr}_{Cu(NO_3)_2}$ (exp.)	% Difference
0.30	0.473	0.615	23.1
0.60	0.403	0.580	30.5

$Cu(NO_3)_2$-$HClO_4$-$NaClO_4$

μ	$\gamma^{tr}_{Cu(NO_3)_2}$ (calc.)	$y^{tr}_{Cu(NO_3)_2}$ (exp.)	% Difference
0.30	0.488	0.688	29.1
0.60	0.427	0.671	36.4

$CuCl_2$-HCl-KCl

μ	$\gamma^{tr}_{CuCl_2}$ (calc.)	$y^{tr}_{CuCl_2}$ (exp.)	% Difference
0.30	0.488	0.619	21.2
0.60	0.419	0.578	27.5

[a]Values of $\gamma^{tr}_{\pm}$ (calc.) were estimated from the following equation

$$\ln \gamma_{\pm}^{MA_2} = \ln \gamma^{o}_{MA} + (1 - \frac{EN}{\mu})(1 - \phi^{o}NA) - \frac{EL}{\mu}(1 - \phi^{o}LA)$$

$$- \frac{4}{3}\frac{EM}{\mu}(1 - \phi^{o}_{MA}) + 2E^{L}[g_{N,L}A + E^{N}\frac{\partial}{\partial\mu}(g_{N,L}A)] \qquad (40)$$

$$+ \frac{4}{3}E^{M}[g_{N,M}A + E^{N}\frac{\partial}{\partial\mu}(g_{N,M}A) + \frac{4}{3}E^{L}E^{M}\frac{\partial}{\partial\mu}(g_{L,M}A)$$

taken from Reilly, Wood and Robinson (129) where (ϕ^{o} and γ^{o}) are the osmotic and activity coefficient of the pure electrolyte at a particular ionic strength (μ), (g) is an adjustment

parameter for interactions between pairs of electrolytes and (E) is the concentration scale in equivalence per kilogram.

[b]All values of γ^{o} and ϕ^{o} were taken from Robinson and Stokes (81).

determination of trace activity coefficients was extended to an aqueous model system to demonstrate the method's potential for extension to natural aquatic systems. The systems investigated were those of copper(II)-glycine (Figures 28 and 30) and copper(II)-alanine (Figures 29 and 31). Table VII shows the trace activity coefficients estimated for these systems at $\mu \simeq 0.001$ and $\mu = 0.723$.

The results show that there is a significant difference in activity between the glycine and alanine systems over the range of amino acid concentrations and ionic strengths studied. The measurements at $\mu \simeq 0.001$ show a non-linear relationship for both systems and this probably results from the uncontrolled ionic strength. The deviation is particularly strong at the lowest glycine concentration.

Several conclusions can be drawn concerning this application of the liquid-liquid partitioning method. It has several advantages over current electrochemical methods. It can be used to determine copper(II) activity in the presence of very high chloride concentration which is not possible with the copper ISE (27) and it should provide the complexation capacity for glycine which ASV can not (58). It should be stated, however, that the method is time consuming and tedious relative to electrochemical methods. In addition, sample contamination can be a problem if care is not taken to assure reagent and solvent purity. These problems should be solvable with further study.

TABLE VII

TRACE ACTIVITY COEFFICIENTS OF COPPER(II) CHLORIDE IN THE PRESENCE OF GLYCINE AND ALANINE[a]

μ = 0.001	
[Glycine] x 10^2 M	$y^{tr}_{CuCl_2}$
0.1	0.912 ± .035
0.5	0.677 ± .028
1.0	0.563 ± .023
[Alanine] x 10^2 M	
0.1	0.904 ± .097
0.5	0.760 ± .030
1.0	0.667 ± .024
μ = 0.723	
[Glycine] x 10^2 M	
0.1	0.737 ± .023
0.5	0.616 ± .036
1.0	0.503 ± .020
[Alanine] x 10^2 M	
0.1	0.802 ± .050
0.5	0.674 ± .045
1.0	0.567 ± .032

[a]Calculated from the following equation

$$y_{CuCl_2} = \frac{D\,\gamma_r\,[HL]^2_{o,r}[H^+]^2\gamma^2_{HA}}{D_r[HL]^2_o[H^+]^2_r\gamma^2_{HA,r}} \qquad (41)$$

The most important feature of the method is its potential for application to the determination of the activity of trace metals in environmental samples. Since the biological and chemical activity of trace metals is determined by their speciation, the determination of activity is of real importance. For example, the calculation of the interaction of a trace metal ion with a complexing agent requires a knowledge of the activity of the free metal ion (12). It is this kind of information that will ultimately result in a better understanding of the role of metals in the aquatic environment and their long range effects on biological systems.

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