

AN ABSTRACT OF THE THESIS OF

HERBERT DALE WARREN for the Ph. D. in Chemistry
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Title INVESTIGATION OF THE COMPLEXES OF 3-(2-ARSONO-
PHENYLAZO)-4, 5-DIHYDROXY-2, 7-NAPHTHALENEDISUL-
FONIC ACID, "ARSENAZO," WITH ALKALINE EARTHS

Abstract approved

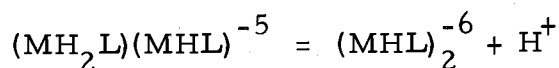
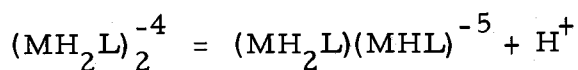
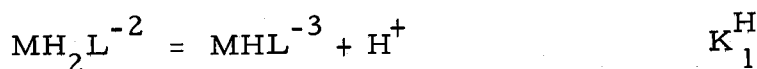
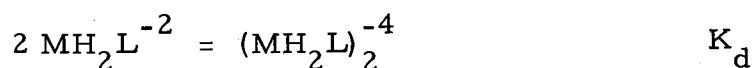
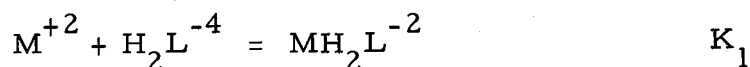
Major professor

The reactions of 3-(2-arsonophenylazo)-4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid, arsenazo, with the ions of magnesium, calcium, strontium and barium have been studied spectrophotometrically and potentiometrically.

Using Fronaeus' method of identifying corresponding solutions (Fronaeus, S. Dissertation, Lund, 1948.), the formation curve, to \bar{n} about 0.6, was determined for each cation. At higher values of \bar{n} the method failed. Suitable treatment of the formation curve showed that a 1:1 complex was formed in each case. Subsequent failure of the method was taken as evidence for the formation of polynuclear species in the system.

The equations of Irving and Rossotti (J. Chem. Soc. 1954, 2904-2910) have been elaborated to permit study by potentiometric titration of systems involving a ligand capable of ionizing protons from the complex. Curves for the titration with potassium hydroxide of

solutions containing arsenazo:central-ion ratios of 1:1 and 2:1 showed that the reaction mechanism for the systems must account for the loss of two protons from the ligand, either simultaneously with complex formation or subsequently but over the same pH range over which complex formation occurs. Five possible mechanisms were considered. Appropriate equations were cited or derived for each case, and all but one eliminated. The sequence of reactions concluded to represent the systems at hand are:



Equilibrium constants for the first three reactions were determined from the potentiometric titration data for each cation using a successive-approximation fitting of the constants to a derived equation. The values obtained at 25.0° and ionic strength 0.0364 were:

Cation	log K_1	log K_d	p K_1^H	log K_1
Magnesium	3.40 ± 0.025	2.70 ± 0.09	7.51 ± 0.014	2.96 ± 0.029
Calcium	2.90 ± 0.027	2.60 ± 0.11	7.53 ± 0.014	2.38 ± 0.029
Strontium	2.24 ± 0.062	1.40 ± 0.30	7.50 ± 0.014	2.24 ± 0.061
Barium	2.00 ± 0.066	1.70 ± 0.24	7.51 ± 0.014	1.92 ± 0.042

The last column shows the value of K_1 calculated from the equilibrium constant determined spectrophotometrically for each cation.

An assignment of the acid dissociation constants for arsenazo, determined previously, to functional groups was made by comparison with the constants for several related compounds. The acidic groups of arsenazo are believed to ionize in the order: the two sulfonic groups, the first arsono proton, the naphthoic group in the 4 position, the second arsono proton and finally the remaining naphthoic group.

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2, 7-NAPHTHALENEDISULFONIC ACID,
"ARSENAZO, " WITH ALKALINE EARTHS

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HERBERT DALE WARREN

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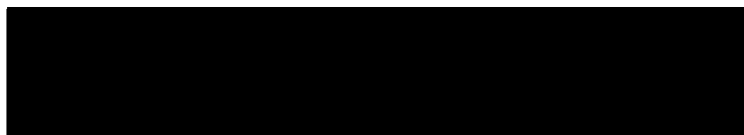
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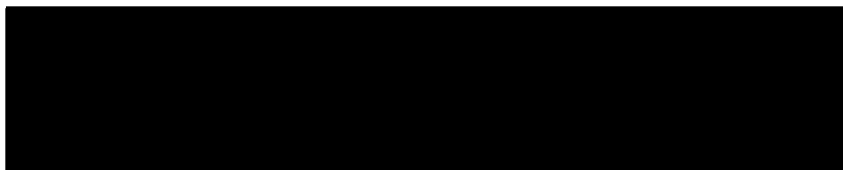
APPROVED:



Professor of Chemistry



Chairman of Department of Chemistry



Dean of Graduate School

Date thesis is presented

Sept 3, 1965

Typed by Muriel Davis

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The greatest debt of gratitude is due the author's wife Valerie, who, patiently and without complaint, has watched the author's graduate study stretch on well beyond his initial hopes. Throughout the work she has sustained him with great understanding and continuous encouragement, while showing no sign that she ever doubted its ultimate successful completion. A special debt of a different kind is owed the author's son Christopher who all his life has had to accept in curtailed form so many of the father-son activities which he richly deserves.

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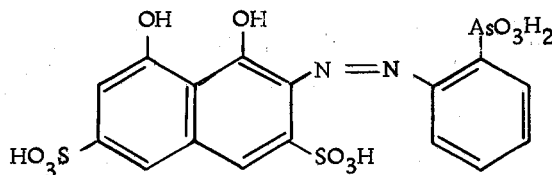
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INVESTIGATION OF THE COMPLEXES OF
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2, 7-NAPHTHALENEDISULFONIC ACID,
"ARSENAZO, " WITH ALKALINE EARTHS

I. INTRODUCTION

Arsenazo

The compound 3-(2-arsonophenylazo)-4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid was first reported by Kuznetsov (38) in 1941 in a paper describing the color reactions of a variety of cations with several compounds containing the o-hydroxy-o'-arsonophenylazo structure.



Kuznetsov gave the subject compound the trivial name "arsenazo, " a name which has been almost universally accepted. Aside from the usual variations on systematic names common for a compound of its complexity, the only other names that have been used to denote arsenazo are "neo-thoron" (or "neothorone") a name limited almost exclusively to the Japanese literature but fairly popular there, and "uranon, " which was used in only a single paper.

In the ensuing 24 years, arsenazo has been the subject of nearly 150 papers describing its chemistry, including its use as a

qualitative reagent for some 43 ions and as a reagent for the quantitative determination of 27 of these. Aside from its obvious use as a spectrophotometric reagent, it has served as an indicator for complexometric titrations and more recently as a coprecipitant for trace metals, particularly in the field of radiochemistry. The analytical uses of arsenazo have been the subject of reviews by Toei (60) and Savvin (54), so this material will not be repeated here.

The synthesis of arsenazo, as described by Kuznetsov (37) begins with the diazotization in slightly acidic solution of o-aminophenylarsonic acid with sodium nitrite. The diazo compound is poured into a cooled solution of 1,8-dihydroxy-3,6-naphthalenedisulfonic acid (chromotropic acid) containing sodium carbonate. While standing two hours diazo coupling occurs, and the product may be recovered in the form of the free acid by addition of an excess of concentrated hydrochloric acid to the solution.

It is not unexpected that arsenazo reacts with such a number and variety of cations, incorporating as it does the functional groups characteristic of the aromatic arsonic acids and of chromotropic acid, both of which are known to form complexes, or at least sparingly soluble salts. In addition it has the azo group with potential coordination sites in positions ortho to it, a characteristic shared with many of the complex-forming dyes of analytical interest. In view of the variety of possible manners in which the reagent could coordinate

with a cation it is perhaps surprising that relatively few of the papers concerning the reagent have considered this aspect of their respective systems.

Among the workers who have determined the combining mole ratio of the reagent and some cation are Przheval'skii and co-workers (52), Holcomb and Yoe (18) and Kuznetsov and Nikol'skaya (39), all of whom concluded on the basis of spectrophotometric measurements that the thorium-arsenazo complex was 1:1, and Takahashi and Miyake (58) who, working at a different wave length, observed the 2:1 (arsenazo:thorium) complex. Using similar methods Matsuyama and co-workers (44) concluded the complex of uranyl ion with arsenazo was 3:2, while Fritz and Johnson-Richard (11), Shibata and Matsumae (56) and Holcomb and Yoe (19) all observed a 1:1 mole ratio. All were using virtually the same pH and wave length. Unfortunately only an abstract of the first paper is available in English, so nothing further can be said about the controversy.

Fritz, Johnson-Richard and Lane (13) observed from a mole ratio plot of optical data that lanthanum forms a 1:1 complex with arsenazo. Matsumae (43), using the same method for indium, detected a 3:2 complex. Shibata, Ishiguro and Matsumae (55) reported a complex with two moles of arsenazo per mole of zirconium, and the same authors (21) observed a 1:1 complex for gallium.

Going one step further in the study of the complex-formation

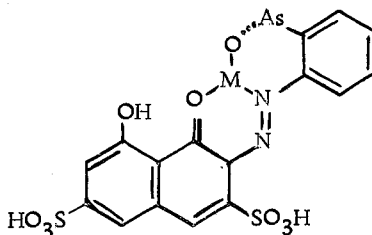
reactions of arsenazo, a number of authors have determined formation or instability constants for the complexes. Chief among these is Kuteinikov (35) who reported the mole ratio, optimum pH for complex formation, wave length of maximum absorbance and in most cases the number of hydrogen ions replaced by the cation and the formation constants for zirconium (IV), titanium (III), thorium, uranium (IV) and (VI), iron (III), beryllium, gallium, indium, scandium, aluminum, yttrium, copper, nickel and cobalt (II). Unfortunately the conditions and method used in determining the formation constants are not clearly stated. Jones (23) evaluated the formation constant for the 3:2 complex of uranium spectrophotometrically.

Klygin and Pavlova (26) showed that the thorium-arsenazo complex contained four ionizable protons, and also evaluated the formation constant of the complex from measurement of absorbance as a function of pH. Kononenko and co-workers (29) reported constants for the 2:1 complexes of hafnium and zirconium. Lane (41) and Kuteinikov and Lanskoi (36) have each used absorbance measurements to determine the equilibrium constants for the lanthanum complex. The two values cannot be meaningfully compared because the equilibrium to which Lane's constant refers is not clear from the abstract alone. The latter paper also reported constants for cerium, neodymium, praseodymium and yttrium. More recently, Tataev (59) also studied the neodymium and praseodymium systems

spectrophotometrically, but he found 3:2 complexes, so again no meaningful comparison of the constants is possible.

For the plutonium-arsenazo system, Klygin and Pavlova (27) were unable to distinguish between two possible products having a net difference of one water molecule associated with the complex, but were able to calculate an equilibrium constant for each structure. Kornilova and Nazarchuk (31) found a constant for the formation of a 1:2 complex of arsenazo with niobium. Kovalenko and Bagdasarov measured the instability constants for the arsenazo complexes of scandium (32) and dysprosium (33). A value for scandium was also calculated by Cherkesov and Alykov (7). The constants reported in all five of these papers were derived from spectrophotometric data.

No definitive studies of the structure of the arsenazo complexes have been reported. Savvin (54) proposed a structure for the complexes of divalent cations as shown below, but cited no evidence to support it.



Mikhailov (46) proposed a very similar structure for the uranyl complex of thoron, 1-(2-arsenophenylazo)-2-naphthol-3,6-disulfonic acid, but again cited no evidence to support it.

Complexes of the Alkaline Earths

The cations of the alkaline earths are known to form complexes with a variety of ligands. Consistent with their large ionic radii and small ionic potential as compared with the transition metals, the alkaline earths complex more readily with ligands containing oxygen donor groups than with those containing nitrogen donor groups only. Ammonia, for example forms only very weak complexes with the alkaline earths (57). The data summarized by Sillén and Martell (57) show that carboxylic and dicarboxylic acids, hydroxycarboxylic acids and amino acids all form reasonably stable complexes with these ions. There is a very consistent decrease in stability down the series calcium, strontium, barium, but the place of magnesium in the series varies with the class of compounds, and even within a given class. Only with the amino acids does magnesium have a consistent place at the top of the order of stability. These ions also form stable complexes with the complexones such as nitrilotriacetate ion and ethylenedinitrilotetraacetate ion, the order of decreasing stability being in each case calcium, magnesium, strontium, barium. Bailar and Busch (3, p. 176) cite, for the β -diketones, an order of decreasing stability moving down the periodic table.

The first direct statement concerning the color reaction of arsenazo with some of the alkaline earths was in a paper by Fritz,

Oliver and Pietrzyk (12) in 1958 in which the authors described the titration of magnesium and calcium with EDTA at pH 10 using arsenazo as an indicator. The end point was even sharper in 30% acetone. Jones (23), using the methods of continuous variation (22) and mole ratio (45), showed that a complex was formed with three moles of arsenazo to two of calcium. Kirshen (25), two years later, was unable to duplicate these findings, observing instead a 1:1 complex. Kirshen described a spectrophotometric method for calcium involving development of the color at pH 10 and measurement of the absorbance at 580 m μ against a reagent blank. Magnesium, strontium, and barium all form colors under these conditions. The same year, 1961, Polyak (50) described a method for the determination of calcium in magnesium alloys. A preliminary separation was made by precipitating magnesium as the hydroxide while complexing calcium with Trilon B. After boiling with hydrogen peroxide to destroy this ligand, the solution was made 0.1 N in sodium hydroxide, arsenazo added, and after 15 minutes, the absorbance measured. Korenman and Levina (30) investigated arsenazo, among other dyes, as a reagent for magnesium, but concluded that others were more selective.

Gimesi and co-workers (16) reported the suitability of arsenazo as an indicator for the complexometric titration of magnesium, thorium, cerium, lanthanum, yttrium and erbium with EDTA. Lamkin and Williams (40) recently described the determination of calcium

and magnesium in blood serum. The total concentration was determined from the absorbance at 580 m μ of a solution at pH 9.6. After sequestering the calcium with ethylene bis(oxyethylenitrilo)tetraacetic acid (EGTA) magnesium was determined, and calcium calculated by difference.

The reactions of the alkaline earths with various analogs of arsenazo has been reviewed by Savvin (54). Suffice it to say that a number of substituted arsenazo compounds have been reported to give color reactions with the alkaline earths. Compounds with carboxylate or sulfonate groups substituted for the arsono group of arsenazo also give color reactions with these ions. Mukherji and Day (48) have presented evidence for the reaction of chromotropic acid with calcium, strontium and barium. Tolmachev and Lomakina (62) have determined formation constants for the 2:1 complexes of the 2-(2-hydroxyphenylazo) derivative of chromotropic acid with the four alkaline earth cations, and Uesugi (63) has described the determination of magnesium spectrophotometrically with the p-amino analog of this compound. Datta (8) has described the color reaction of p-nitrophenylazochromotropic acid with magnesium and calcium in strongly alkaline solution.

Purpose of This Study

This investigation was undertaken with the intention of

determining the reaction or sequence of reactions which occur between arsenazo and the cations of the alkaline earths, and the equilibrium constants governing those reactions. There are several justifications for such a study. First, as cited above, there have been few papers which determined more than a single formation constant for the species formed. Arsenazo would be expected, in many cases, to retain one or more protons even when complexed but, except for the work of Kuteinikov and Lanskoi (35, 36) and Klygin and Pavlova (26, 27), this aspect of the systems has been neglected. Clarification of the reactions involved may well throw light on the structure of the complexes of arsenazo with a number of similar cations. Second, there is a decided lack of suitable methods for determining the alkaline earths in the presence of one another. Greater understanding of the quantitative aspects of their reaction with a promising reagent may lead to the development of such methods. Third, the study of a new system of complexes often leads to the elaboration of old methods, or the establishment of new ones for the study of such systems. This in itself is a desirable result.

II. GENERAL OBSERVATIONS ON ARSENAZO AND ITS ALKALINE EARTH COMPLEXES

Arsenazo is commercially available in practical grade as the trisodium salt (Eastman Organic Chemicals). In this form it is a dull, brick-red solid, soluble in water to the extent of about 5 g. per 100 ml. The free acid, a bright red-orange solid is soluble in water, and to a slight extent in acetonitrile, but insoluble in the other common organic solvents. Aqueous solutions of the salt are dull reddish-brown in color. Strongly acidic solutions are bright red. As base is added to a neutral solution the color gradually shifts to a reddish-violet, and beyond pH 10 no further change is visually detectable.

Acidic or neutral solutions of arsenazo containing a five-fold molar excess of an alkaline earth salt are not perceptibly different in color from solutions of arsenazo alone at corresponding acidities. Above pH 8 the alkaline earth solutions change to a reddish-violet, resembling the alkaline color of arsenazo solutions. The change occurs approximately two pH units lower than does the change for the reagent alone however, and even in 0.1 N sodium hydroxide the solutions containing alkaline earth ions are slightly more violet than those containing the reagent alone.

Crystals of the trisodium salt and also the free acid were examined with a polarizing microscope. Both grow in the form of flat needles. The needles show parallel extinction coupled with flash

interference figures. The slow ray parallels the long axis of the crystal. Both forms are pleochroic, appearing red when the plane of polarization is perpendicular to the long axis of the crystal and yellow when the crystal is rotated 90° . A few crystals of the free acid were observed on the Kofler hot stage. They darkened around 150° , perhaps losing water. Around 230° the crystals became opaque and by 325° had cracked severely, appearing to be charring. They did not melt below 350° , the maximum temperature attainable on the instrument.

The most widely used method of purifying arsenazo, judging from those papers where this aspect of the work is discussed, is that of Fritz and Bradford (10). A saturated solution of the trisodium salt of arsenazo is added dropwise with stirring to an equal volume of concentrated hydrochloric acid. The precipitated free acid is then filtered off, washed with acetonitrile to remove sodium chloride, and dried at 110° . This method was modified slightly for this work. After addition of the arsenazo solution to the hydrochloric acid was complete, the mixture was heated to about 90° whereupon the precipitated acid all dissolved. Upon slow cooling and standing a few hours the acid reprecipitated in needles approximately ten times as long as those obtained in the initial precipitation. The solution was chilled in an ice bath and then filtered on a Büchner funnel. The precipitate was washed several times with acetonitrile, after which

the addition of silver nitrate to an acid solution of the solid gave no indication of chloride. Solutions of the acid prepared with and without heating were spectrophotometrically identical over the range 650-225 m μ .

The purity of the acid form of arsenazo was determined by potentiometric titration with standard base of samples dried two hours at 110^o. The titrations revealed two inflection points, a very distinct one at pH 5.56 and a second, less distinct but clearly detectable one at pH 9.2, corresponding to the neutralization of the first three and the fourth protons from arsenazo. The mean molecular weight calculated from the inflection points for three runs was 586.2 ± 3.2 (95% confidence level). The molecular weight of the dihydrate of arsenazo is 584.4. Analysis of variance showed there was no significant difference between the molecular weights calculated from the first inflection point and those calculated from the second, and application of the t-test showed no significant difference between the mean molecular weight and that calculated for the dihydrate, both conclusions at the 95% confidence level.

To determine the nature of the darkening observed when crystals were heated on the hot stage, a portion of the purified arsenazo acid was dried for two hours at 150^o. The product was a lustrous, blue-black solid. There was no visual evidence of decomposition and solutions of the black solid were spectrophotometrically identical to

those of the red solid dried at 110° . Samples of the black form were titrated potentiometrically with standard base, giving the same two inflection points. The mean molecular weight was 551.8 ± 3.3 (95% confidence level). The molecular weight calculated for anhydrous arsenazo acid is 548.3. Analysis of variance showed no significant difference between molecular weights from the first and second inflection points, but the t -value was just inside the critical region, indicating the molecular weight observed was slightly higher than that expected for the anhydrous form. It should be noted however that the molecular weights for a run made within hours of removal of the sample from the drying oven were in excellent agreement with the calculated value, while those for subsequent runs made a few days later were increasingly higher, even though the solid had been stored in a closed weighing bottle over calcium chloride. On standing a few months in this condition the black solid even began to show reddish tones. All this indicates clearly that the black solid is the anhydrous form of the acid, and that it is quite hygroscopic. The dihydrate is a much more suitable weighing form, revealing no tendency to absorb water, and was used in all subsequent work.

III. SPECTROPHOTOMETRIC STUDY OF THE COMPLEXES

Choice of a Method

A number of methods based on optical properties of solutions have been advanced for the study of complex ionic species in the solutions. Among the more widely used have been the methods of continuous variations, ascribed to Job (22), the mole ratio method first advanced by Meyers and Ayres (45), and the slope ratio method of Harvey and Manning (17). Although initially proposed as methods for determining the stoichiometry of species in solution, each has been used with at least moderate success for determining formation constants working from the departure of the appropriate experimental curves from the linear relationships observed for very stable complex species. The chief limitation of the first and third methods are that they are only suitable for systems in which conditions can be adjusted so that only a single complex species will exist over a wide range of concentrations, in both excess central ion and excess ligand. In a system such as the one here discussed, two or more species may very well exist simultaneously, so neither continuous variations nor slope ratio were used in this investigation. The method of Meyers and Ayres affords additional evidence of the stoichiometry of the arsenazo complexes, as will be discussed below.

An approach which avoids this limitation is the method of corresponding solutions described by Bjerrum (6), in which the formation curve for the system is determined from knowledge of the total concentrations of ligand and central ion in pairs of "corresponding solutions," i. e., solutions with the same free ligand concentration. From this curve the stoichiometry of at least some of the species formed will be apparent, and by suitable treatment the formation constants can be evaluated. In its original form, Bjerrum's method depended, for the identification of corresponding solutions, on absorbance measurements at a wave length where only a single component of the system absorbed. This restriction prevented the use of the method for all but a small fraction of the systems of potential interest. Since that time, several alternate methods of identifying these solutions have been advanced, one particularly useful one being that of Fronaeus (14), as described by Rossotti and Rossotti (53, p. 44-46, 282-284). In this method, some property which depends only on the free ligand concentration is measured at various total ligand and total central ion concentrations. From these data, the total concentrations of two or more corresponding solutions can be determined. Extrapolation to zero central ion concentration gives the free ligand concentration. One property which depends only on free ligand concentration, as will be shown below, can be calculated from the absorbance of the solution. It is defined by the equation

$$\epsilon' = \frac{A - a_L b C_L}{b C_M} \quad (\text{III-1})$$

where A is the absorbance, a_L the molar absorptivity of the ligand, b the optical path length and C_L and C_M the total molar concentrations of the ligand and the central ion respectively. In the literature no name is given to the quantity ϵ' , and no adequately descriptive one occurs to this author. It will be seen however that it can be thought of as a weighed average of the net increase in absorptivity of the complex over that of the ligand removed from solution by complex formation.

Since absorbance is an additive property,

$$A = \sum_0^N a_n b [ML_n] + a_L b [L] \quad (\text{III-2})$$

where a_n is the molar absorptivity of the species ML_n , and brackets indicate molarity. Furthermore,

$$C_M = \sum_0^N [ML_n] \quad (\text{III-3})$$

and

$$C_L = [L] + \sum_0^N n [ML_n] \quad (\text{III-4})$$

Upon combining equations (III-2) through (III-4), and introducing the overall formation constants $\beta_n = [ML_n]/[M] [L]^n$, i. e., the overall formation constant for the species ML_n , one obtains

$$\epsilon' = \frac{\sum_0^N (a_n - na_L) [ML_n]}{\sum_0^N [ML_n]} = \frac{\sum_0^N (a_n - na_L) \beta_n [L]^n}{\sum_0^N \beta_n [L]^n} \quad (\text{III-5})$$

demonstrating that ϵ' is a function of the free ligand concentration alone.

It should be noted that, inasmuch as arsenazo has six ionizing hydrogens, with acid dissociation constants reported (28) as ranging from 0.85 to 3.4×10^{-13} , at nearly any pH the free ligand will exist as a mixture of two or more forms with different degrees of protonation. In this chapter no attempt will be made to distinguish these forms, and the symbol $[L]$ will represent the total concentration of the uncombined ligand. Similarly no attempt will be made to indicate the number of protons on the ligand in the complex species, or to show the charge on those species.

In the foregoing paragraphs the absence of an appreciable concentration of any polynuclear species has been assumed. If such species are present, the equations for A , C_M and C_L must be augmented to include the contribution of these species to the totals. If this is done, equation (III-5) will include in the numerator and denominator respectively terms of the form

$$\sum_0^N (a_{in} - n a_L) \beta_{in} [M]^{i-1} [L]^n \text{ and } \sum_0^N \beta_{in} [M]^{i-1} [L]^n \text{ where}$$

β_{in} is the overall formation constant of the species $M_i L_n$, and a_{in} is its molar absorptivity. Such a summation term would appear for each order of polymerization represented, i. e., dimer, trimer, etc. Obviously in this latter case, ϵ' will depend on both the free central ion and free ligand concentrations, and two solutions having identical values of ϵ' will not necessarily have the same free ligand concentration, i. e., not be corresponding solutions. This is the most serious limitation of Fronaeus' method; there must be no appreciable concentration of polynuclear species in the solutions, the absorbance of which is the basis of the calculation of ϵ' . The recommended manner for demonstrating the absence of polynuclear species involves measuring the absorbance at two wave lengths, associated with different absorption bands, and checking the constancy of the free ligand concentration calculated from the resulting ϵ' values. In the arsenazo-alkaline earth systems this was not feasible, so a different test was devised, as will be discussed on page 62.

In systems where only mononuclear complexes are formed, a plot of C_L against C_M for a set of corresponding solutions must give linear plots since

$$C_L = [L] + \bar{n} C_M, \quad (\text{III-6})$$

where \bar{n} , the ligand number, is defined as the average number of ligands bound to a central ion. Not only must the intercept ($C_M = 0$) give $[L]$ as noted above, but also the slope of the line must give \bar{n} . These two quantities define the formation curve, from which the stoichiometry and formation constants of the complex species are obtained.

The other two limitations of Fronaeus' method derive from the effect of error in determination of ϵ' on the values obtained for \bar{n} and $[L]$. Obviously when $C_L \cong [L]$, i.e., in solutions with a large excess of ligand over central ion, the value obtained for \bar{n} will be very sensitive to error in either of these quantities. Similarly, when $[L] \cong 0$, i.e. when the central ion is present in large excess, the value of free ligand concentration will be very sensitive to error in C_L and C_M .

Choice of a Buffer System

As remarked in the preceding section, the distribution of the free ligand as well as the complex species among forms with various degrees of protonation will depend on pH of the solution. Since the formation of complex species will almost certainly involve the release of protons to the solution, the need of working with buffered solutions is obvious. Preliminary observations indicate a pH range from 8 to 12 may be useful.

The buffer systems most frequently used over the pH range of interest are based on phosphates, glycine, or borates. Of these, the first two are both known to form alkaline earth complexes (57, p. 182-183, 378-380). Other readily available polycarboxylic and amino acids with appropriate acid dissociation constants were eliminated for the same reason. Preliminary examination of the spectra of arsenazo-sodium borate solutions, as compared to arsenazo solutions adjusted to the same pH with sodium hydroxide showed that borate reacts with arsenazo, at least between pH 8 and 10. The interaction of these species appears to be relatively weak, as the spectra do not differ greatly, and there is no visual difference between the solutions at a given pH, but wishing to avoid as far as possible any competing equilibria, the borate buffers were abandoned.

Bailar (3, p. 25, 125) observed that while triethanolamine reacts with magnesium, calcium and strontium, ammonia forms, at most, very weak complexes, and primary, secondary and tertiary aliphatic amines form complexes with stabilities decreasing in that order. Hence tables of acidic and basic dissociation constants (2, p. 124-153; 42, p. 1198-1204) were scanned for amines with pK^a values around 9 and 11. The most promising, considering solubility, number of alkyl substituents, and availability were piperazine ($pK_1^a = 5.68$, $pK_2^a = 9.82$) and diethylamine ($pK^a = 10.93$). Potentiometric titration with sodium hydroxide of an equimolar mixture of

these two amines showed a buffer region extending from pH 5.0 to 6.8, an inflection point centered around pH 7.7, and a second buffer region extending from 8.3 to beyond 12. Bates (4, p. 117) has shown that a series of buffers of constant ionic strength can be obtained, so long as the base is so weak that its contribution to the ionic strength is negligible, by mixing the hydrochloride of the base with appropriate amounts of sodium hydroxide. He also states (op. cit., p. 95) that the maximum buffer capacity to be expected of a given system is approximately equal to $0.576 C$ where C is the total molarity of the weak acid or base and its salt. Wishing to minimize the ionic strength, while keeping a reasonable buffer capacity a reasonable compromise seemed to be a series of solutions 0.0250 M in each of the amines. Such a series of buffers were prepared as described on page 25, below, for integral values of pH from 8 to 12. Since ionic strength of the series was found to be equal to $0.0500 + [\text{OH}^-]$, sufficient potassium perchlorate was added to bring the ionic strength at the lower pH's to 0.0600, as it was at pH 12 due to the amines and potassium hydroxide alone.

Absorption spectra of solutions of arsenazo in these buffers over the range 650 to 300 m μ did not differ significantly from spectra of solutions of arsenazo adjusted to the same pH with potassium hydroxide. Similarly, buffered solutions of the cations being studied showed no greater absorbance than the distilled water reference

except below 225 m μ where the amines themselves absorb.

Preparation and Standardization of Stock Solutions

Magnesium perchlorate, 0.01 M, was prepared by weighing a calculated amount of basic magnesium carbonate (Baker & Adams, CP grade, Ca .005%) into a 5% excess of 1 M perchloric acid, boiling gently for five minutes to remove carbon dioxide and diluting to one liter with recently boiled, distilled water. The solution was analyzed gravimetrically with 8-hydroxyquinoline (9, p. 174).

Calcium perchlorate, 0.01 M, was prepared in an analogous manner from calcium carbonate (Baker Analyzed) which had been dried for four hours at 550^o. After boiling, dilution was made carefully in a volumetric flask. The solution was then analyzed by precipitation of the oxalate followed by titration with standard potassium permanganate (15, p. 264-265). Agreement with the calculated molarity was good.

Strontium perchlorate, 0.01 M, was prepared in an analogous manner from strontium carbonate (Baker Analyzed, Reagent) which had been dried for four hours at 550^o. After boiling, dilution was made using a calibrated volumetric flask. Molarity was calculated from the manufacturers analysis, 99.0%. This analysis was later confirmed by a gravimetric determination of strontium as the sulfate (15, p. 996-997).

Barium chloride, 0.01 M, was prepared by dissolving a weighed quantity of barium chloride dihydrate (J. T. Baker Analyzed) in recently boiled water and diluting in a volumetric flask. The solution was analyzed gravimetrically as barium sulfate (15, p. 148).

Arsenazo, 2×10^{-3} M, was prepared by careful weighing of calculated quantities of the free acid dihydrate, dried two hours at 110° , dissolving in a volume of water about 80% of the final volume, adjusting the pH of the solution to 9.0 or 10.0 as required with 0.2 M KOH, and transferring quantitatively to a volumetric flask for final dilution. The adjustment of pH was done as quickly as possible to minimize pick-up of carbon dioxide from the atmosphere. The acid had been prepared and purified as described in Chapter II.

Potassium hydroxide, 0.2 M, was prepared by dissolving potassium hydroxide (Baker and Adams, Reagent, Special Grade, maximum potassium carbonate 0.5%) from a freshly opened bottle in freshly boiled distilled water. To remove carbonate, the solution was passed over a column of Dowex-2 (hydroxide form) and collected in a stoppered flask protected with a soda-lime tube.

Diethylamine-piperazine hydroperchlorates, 0.250 M in each, was prepared by measuring a calculated amount of the purified amines into water and diluting to volume. An aliquot was titrated potentiometrically with standard acid to confirm the concentrations. The solution was passed over a column of Dowex-2 (hydroxide form)

to remove carbonate and caught in a closed flask. The solution was then quickly neutralized to pH 7.7 with perchloric acid and diluted to the desired final volume. When this stock was to be stored more than a few days, the bottle was flushed with nitrogen to retard oxidation.

The diethylamine (Matheson, Coleman and Bell, reagent grade) was distilled at a pressure of 769 mm., the fraction boiling 55-57° being collected for use in the buffers. The label showed a boiling range 55-56°.

Piperazine hexahydrate (DuPont) was distilled at 754 mm., a small amount of excess water having been added. The solid dissolved or melted, then boiled continuously from 100° to 125°, then the boiling point rose rapidly to above 150°, where only a slight, yellow residue remained. Fractions taken at 125-145° and 145-150° solidified immediately, giving a white waxy product, m.p. 44-45.5°. Lange (42, p. 502-503) gives for piperazine hexahydrate m.p. 44°, b.p. 125-130°. These fractions were used for preparation of the amine buffers.

Perchloric acid, 1 M, 0.1 M, were prepared by diluting 70% perchloric acid (Baker and Adams, Reagent) with boiled distilled water. The solutions were standardized by potentiometric titration of dried sodium carbonate (Baker and Adams, Reagent).

Standardizing buffers, pH 7.00 and 9.20 were prepared by

ten-fold dilution of Beckman pH 7.00 concentrate and by dissolving the proper amount of sodium tetraborate decahydrate (Baker and Adams, Reagent) in distilled water, as described by Bates (4, p. 118-121).

Amine buffers, at integral pH values from 8 to 12, were prepared by diluting 50.0 ml. of the amine hydroperchlorate stock and a volume of perchloric acid calculated to give, after neutralization, the desired ionic strength to about 425 ml., adding potassium hydroxide to attain the desired pH as indicated in Table 1, transferring to a volumetric flask and diluting to final volume. The pH to which each buffer was adjusted was chosen so that after dilution of the buffer with other reagents in solution makeup the resulting pH would be integral, within experimental error.

Since this buffer system has not been discussed in the literature before, the buffer capacity and dilution value of each of the buffers were measured. To determine the former 1.000 M sodium hydroxide or perchloric acid was added to a 25.0 ml. aliquot of the buffer until a pH change of about 0.5 was produced. The volume of acid or base used was read from the buret and the capacity calculated from the relationship

$$\beta = \frac{(1.000) (V)}{(25.0) (\Delta \text{pH})} \quad .$$

Buffer capacities are shown in Table 1, below.

The usual dilution value found for a buffer is $\Delta\text{pH}_{\frac{1}{2}}$, the change in pH when an aliquot of buffer is diluted to twice its initial volume. In this work however, three volumes of buffer will be diluted with two volumes of stocks and water, so the quantity $\Delta\text{pH}_{0.6}$ was determined instead, by diluting 15 ml. aliquots of buffer with 10 ml. of distilled water. Results are shown in Table 1.

Table 1. Characteristics of buffers with constant ionic strength 0.0600, prepared from an equimolar mixture of diethylamine and piperazine

Final pH	Buffer Capacity (β)	Dilution Value	Minimum Permissible pH for Stock Solutions
8.05	0.0022	-0.05	6.0
9.00	0.0086	0.00	4.0
10.02	0.020	-0.02	4.0
11.02	0.019	-0.02	10.9
12.10	0.024	-0.10	12.0

The final column in Table 1 derives from a calculation, based on the observed buffer capacities, of the minimum pH the stock solutions may have and still produce a change of pH less than 0.01 when 10 ml. of the stock is mixed with 15 ml. of the buffer.

The above stock solutions were diluted as necessary for working solutions of the concentration needed. Dilutions were done with calibrated glassware.

Apparatus

All spectra were taken with a Beckman Model DB Recording

Spectrophotometer. A matched pair of silica cells with path length 1.001 cm, was used. In the wavelength region 650 to 320 m μ , the light source was a tungsten lamp. In the region 320 to 220 m μ , a hydrogen lamp was used. The medium slit program was used throughout. The reference solution was either distilled water or a solution of arsenazo of the same concentration and in the same buffer as the sample.

Absorbance measurements at a fixed wave length were taken with a Beckman Model B Spectrophotometer. The pair of 1 cm. cells described above was used for most of the measurements, but in addition, for solutions of relatively low absorbance, a matched pair of cylindrical silica cells with path length 5.005 cm, was used. All measurements were taken at 570 m μ , so a tungsten source was adequate. The reference solution was distilled water. Zero absorbance was set with sensitivity two, requiring a slit width of 0.23 mm. for an effective band width of 5.7 m μ . When absorbance exceeded 1.5, the scale zero was reset on sensitivity one, with a 0.40 mm. slit, and a band width of 9.8 m μ .

In the spectrophotometric titrations, a calibrated 10 ml. microburet was used. In the portion used, the volume correction was greater than -0.002 and less than 0.003 ml., so was not applied.

Absorption Spectra

Absorption spectra were taken for a series of solutions each 4×10^{-5} M in arsenazo, and each containing 15 ml. of the appropriate buffer diluted to 25 ml.

In some preliminary work designed to determine whether the particular base used in making the solutions alkaline and used in preparing the buffers was critical, spectra were taken for solutions of arsenazo adjusted to pH 12.0 and 13.0 with tetramethylammonium hydroxide, and containing also a five-fold molar excess, over the arsenazo present, of the chlorides of lithium, sodium, potassium, rubidium and cesium. Over the range 650 to 220 m μ , all these spectra were identical to each other and to the spectrum of the solution containing no additional cation other than the tetramethylammonium ion. One can conclude then that if any of the alkali metal cations interact with arsenazo it is either very weakly, or in such a manner as to have no effect on the spectrum. Potassium hydroxide was chosen as the base for further work in order to avoid the necessity of applying sodium ion corrections at higher pH.

Figure 1 shows the spectra of arsenazo at various pH values. As can be seen, there is no drastic change in the shape of the spectrum, there being a gradual shift of the maximum from 500 to 525 m μ , and an approximately two-fold increase in absorbance around 575 m μ

over this pH range. Changes in other regions of the spectra are even less pronounced.

Spectra of solutions containing a five-fold molar excess of one of the cations under study were also recorded. For a given cation, a shift similar to that observed for the free ligand takes place. Spectra taken with an arsenazo solution as reference indicate a divergence of the spectrum of the complex from that of the ligand which increases with pH to about 10, then changes very little from 10 to 12. At all pH's observed, the magnitude of this divergence increases up the series barium, strontium, calcium, magnesium, suggesting that the magnesium-arsenazo complex is the most stable, being formed at lower pH and having the most pronounced effect on the spectrum, i. e., on the energy levels of the ligand. Figure 2 shows the spectra obtained at pH 10 for the free ligand, and for solutions containing an excess of each of the cations.

On the basis of these spectra one or more wave lengths and pH values had to be chosen for use in determining corresponding solutions. At best, these systems do not afford more than about a two-fold increase in absorbance in going from the free ligand to the complex, and this only around 575 m μ . At the wave length of next greatest difference, around 465 m μ , the maximum change expected is only about 60% of the absorbance of the ligand. This is on the other side of the same band as the 575 m μ maximum, so it could hardly be

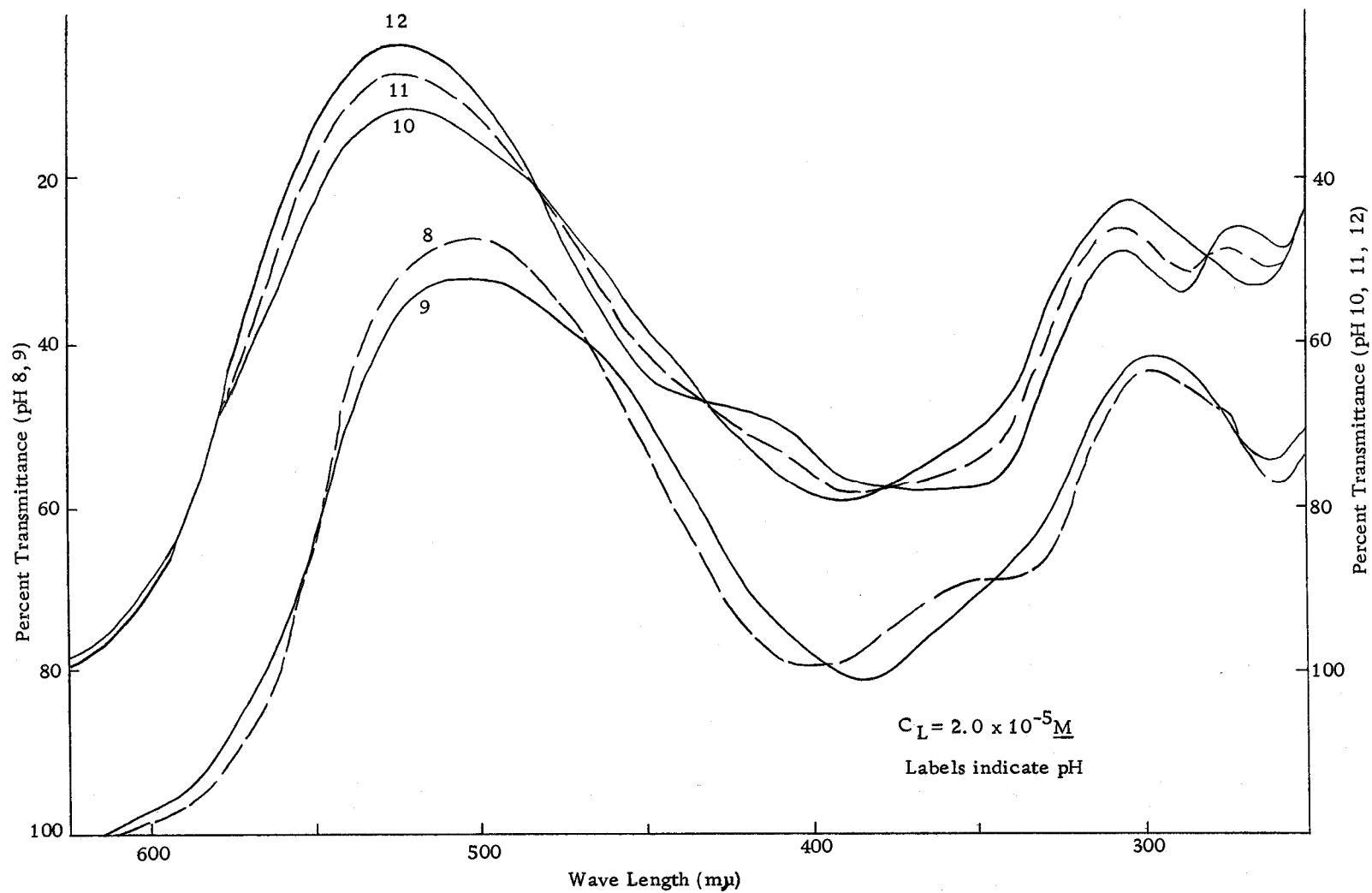


Figure 1. Absorption Spectra of Arsenazo

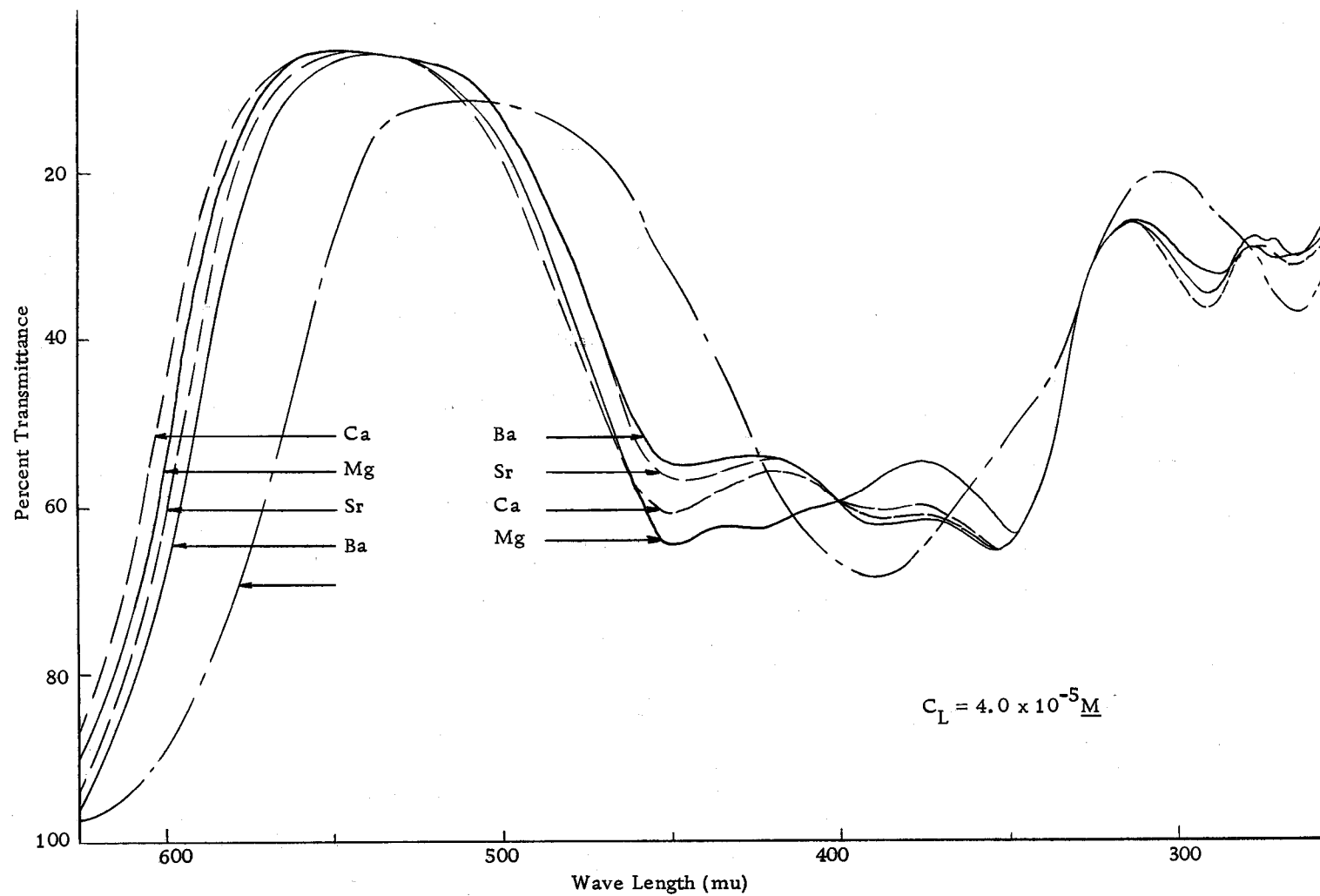


Figure 2. Absorption Spectra of the Arsenazo-Alkaline Earth Complexes, pH 10.00

expected to afford much additional information over that available from measurements at only a single wave length. At still lower wave lengths, around 375 mμ, another region of divergence exists, but here the change in absorbance is still smaller, about 30% of the absorbance of the ligand. For these reasons, measurements for corresponding solutions were taken at only a single wave length, near the wave length of greatest divergence.

In choosing a wave length for further work, not only the absolute difference between the absorbances of the two species but also the value of this difference relative to the absorbance of the ligand at that wave length is important. For all four of the cations, at pH 10, the maximum difference occurs at 560 mμ. The greatest relative difference occurs however at or near 570 mμ. This latter figure was used for all further spectrophotometric work.

A pH for further work was chosen with consideration not only for its effect on the change of absorbance, but also with a desire to avoid the additional complication of having to consider the hydrolysis of the cations. If the equilibrium constant for the reaction $M^{+2} + OH^{-} = MOH^{+}$ is known, the fraction of free cation in the hydrolyzed form is given by:

$$\frac{[MOH]}{[M]} = K[OH] \quad .$$

Sillén and Martell (57, p. 42) give values for these constants as

shown in Table 2. Also shown in the table are the maximum pH values for which the fraction of free cation in the hydrolyzed form is less than 1%, based on the highest value reported for the hydrolysis constant.

Table 2. Hydrolysis constants for the alkaline earth cations, and the maximum pH which can be used for each while avoiding significant hydrolysis

Cation	pK	pH
Mg	-2.58	9.4
Ca	-0.64 to -1.51	10.5
Sr	-0.23 to -0.96	11.0
Ba	-0.37 to -0.85	11.1

Considering these criteria, pH 10 was chosen for titrations of calcium, strontium, and barium, and pH 9 for magnesium.

Experimental Procedure

For calcium, strontium and barium, working solutions $9.00 \times 10^{-5} \text{ M}$ and $2.00 \times 10^{-4} \text{ M}$ were prepared by suitable dilution of the stock solutions described on page 22. For magnesium, solutions $1.25 \times 10^{-4} \text{ M}$ and $5.00 \times 10^{-4} \text{ M}$ were used. Solutions for titration were prepared by mixing in a 125 ml, Erlenmeyer flask 30 ml, of the amine buffer with an appropriate volume of one of the working solutions and sufficient water to make the volume to 50.0 ml. The resulting solution had an ionic strength of 0.0364. The solution

was then titrated with the 2.00×10^{-3} M arsenazo solution of the same pH, using increments of 0.20 ml. to a total added volume of 4.00 ml. After each addition of titrant, a portion of the solution was used to rinse the spectrophotometer cell, returned to the flask, the cell then filled, the absorbance measured, and the portion returned to the flask before the addition of the next increment. The error in reading the microburet is believed to be no greater than ± 0.001 ml., although in the tables of data, volumes are shown to only the second decimal place. Because absorbance readings were being taken on the side of an absorption band, where the readings observed would be particularly sensitive to any error in setting the wavelength scale, all measurements at pH 10 were taken on the same instrument over a period of a few days during which time the wavelength setting was left unchanged. The readings at pH 9 were taken at a later date, on another instrument, although again during the series of measurements the wave length was unchanged. No attempt was made to control the temperature of the solutions or of the cell compartment, but it was noted that the ambient temperature remained at $24-25^{\circ}$ throughout the titrations, and all solutions had been at that temperature for some hours before the titrations were begun. Preliminary work indicated that color development was very rapid in the systems, there being no change in absorbance on standing from 30 seconds to 12 hours.

For each cation, titrations were done at four different concentrations, in each case adding an excess of ligand that varied from 2.86-fold for the most concentrated to 22.2-fold for the most dilute solution. In order to obtain an accurate value for the molar absorptivity of the ligand under these conditions of ionic strength and pH, solutions containing no alkaline earth cation were titrated in the same manner. These data are presented in Table 3. The data for the titrations of the solutions containing cations are shown in Tables 4, 5, 6 and 7.

Calculations and Discussion

During the course of a titration, the volume increased from 50.0 to 54.0 ml. To correct for this dilution, each absorbance, after application of a cell correction, was multiplied by the factor $(50 + v)/50$, where v is the volume of titrant added. The corrected absorbances are shown in Tables 3, 8, 10, 12 and 14, and are plotted, as ordinate against the total ligand concentration as abscissa, in Figures 3, 6, 8 and 10, for magnesium, calcium, strontium and barium, respectively.

In order to calculate the function ϵ' , as defined by equation (III-1), a value of the molar absorptivity of arsenazo at each pH was needed. A plot of the corrected absorbances shown in Table 3 against concentration of arsenazo showed that Beer's law is obeyed over this

Table 3. Spectrophotometric titration of buffered solutions with arsenazo

Volume of Titrant (ml)	Absorbance		Corrected Absorbance	
	pH 9.0	pH 10.0	pH 9.0	pH 10.0
0.20	0.052	0.221*	0.0522	0.0444
0.40	0.103	0.440*	0.1038	0.0887
0.60	0.155	0.658*	0.1569	0.1332
0.80	0.205	0.874*	0.2083	0.1776
1.00	0.254	0.218	0.2591	0.2213
1.20	0.305	0.261	0.3125	0.2662
1.40	0.353	0.304	0.3629	0.3115
1.60	0.405	0.344	0.4179	0.3540
1.80	0.452	0.387	0.4683	0.3999
2.00	0.500	0.427	0.5200	0.4431
2.20	0.549	0.470	0.5732	0.4896
2.40	0.597	0.510	0.6257	0.5335
2.60	0.643	0.549	0.6764	0.5765
2.80	0.690	0.590	0.7286	0.6219
3.00	0.736	0.623	0.7801	0.6593
3.20	0.782	0.669	0.8320	0.7106
3.40	0.832	0.708	0.8885	0.7550
3.60	0.875	0.746	0.9380	0.7986
3.80	0.919	0.786	0.9889	0.8446
4.00	0.965	0.822	1.0422	0.8878

Path length(cm) 1.000

1.001

Cell correction 0.000

-0.001

Wave length 570 mu

* b = 5.005 cm. cell correction 0.000

pH	Slope (1.mole ⁻¹)	^a L(1.mole ⁻¹ cm. ⁻¹)
9.0	5210	5210
10.0	4440	4436

Table 4. Spectrophotometric titration of magnesium solutions with arsenazo

Volume of Titrant (ml)	Absorbance for C_M^O Shown			
	$1.25 \times 10^{-5} \underline{M}$	$2.75 \times 10^{-5} \underline{M}$	$4.25 \times 10^{-5} \underline{M}$	$7.00 \times 10^{-5} \underline{M}$
0.20	0.099	0.136	0.167	0.176
0.40	0.187	0.257	0.327	0.369
0.60	0.267	0.370	0.466	0.554
0.80	0.341	0.465	0.586	0.714
1.00	0.401	0.542	0.685	0.867
1.20	0.463	0.620	0.783	0.996
1.40	0.523	0.689	0.866	1.118
1.60	0.572	0.758	0.940	1.210
1.80	0.628	0.819	1.006	1.305
2.00	0.675	0.878	1.075	1.386
2.20	0.728	0.931	1.131	1.457
2.40	0.781	0.990	1.186	1.518
2.60	0.830	1.044	1.241	1.592
2.80	0.880	1.096	1.290	1.638
3.00	0.924	1.146	1.344	1.693
3.20	0.967	1.191	1.398	1.749
3.40	1.015	1.238	1.441	1.802
3.60	1.061	1.290	1.489	1.846
3.80	1.101	1.331	1.515	1.896
4.00	1.143	1.380	1.560	1.940
Cell correction	0.000	0.000	0.000	0.000
Path length 1.000 cm, Wave length 570 mμ pH 9.00				

Table 5. Spectrophotometric titration of calcium solutions with arsenazo

Volume of Titrant (ml)	Absorbance for C_M^0 Shown			
	$9.0 \times 10^{-6} M$	$1.90 \times 10^{-5} M$	$3.40 \times 10^{-5} M$	$5.40 \times 10^{-5} M$
0.20	0.532*	0.741*	0.190	0.212
0.40	0.911*	0.268	0.344	0.401
0.60	1.225*	0.362	0.476	0.568
0.80	0.306	0.431	0.583	0.721
1.00	0.354	0.493	0.674	0.851
1.20	0.406	0.550	0.745	0.965
1.40	0.450	0.603	0.812	1.055
1.60	0.493	0.656	0.870	1.195
1.80	0.543	0.704	0.924	1.204
2.00	0.581	0.751	0.975	1.268
2.20	0.625	0.791	1.016	1.324
2.40	0.665	0.835	1.064	1.376
2.60	0.709	0.880	1.110	1.427
2.80	0.745	0.918	1.150	1.475
3.00	0.786	0.961	1.193	1.516
3.20	0.827	0.996	1.234	1.555
3.40	0.862	1.035	1.269	1.600
3.60	0.902	1.077	1.308	1.643
3.80	0.939	1.114	1.343	1.680
4.00	0.979	1.156	1.380	1.719
Cell correction	-0.001	0.001	-0.001	-0.001
Path length 1.001 cm. Wave length 570 mμ pH 10.00		* Path length 5.005 cm. Cell correction 0.000		

Table 6. Spectrophotometric titration of strontium solutions with arsenazo

Volume of Titrant (ml)	Absorbance for C_M^0 Shown			
	$9.0 \times 10^{-6} \underline{M}$	$1.90 \times 10^{-5} \underline{M}$	$3.40 \times 10^{-5} \underline{M}$	$5.40 \times 10^{-5} \underline{M}$
0.20	0.358*	0.450*	0.552*	0.124
0.40	0.671*	0.840*	1.021*	0.242
0.60	0.951*	1.193*	0.299	0.356
0.80	1.215*	0.310	0.377	0.449
1.00	0.294	0.368	0.449	0.536
1.20	0.342	0.423	0.513	0.620
1.40	0.390	0.475	0.578	0.700
1.60	0.436	0.528	0.637	0.770
1.80	0.481	0.576	0.696	0.838
2.00	0.526	0.625	0.750	0.905
2.20	0.567	0.676	0.807	0.960
2.40	0.610	0.719	0.854	1.016
2.60	0.651	0.763	0.903	1.073
2.80	0.691	0.807	0.950	1.125
3.00	0.731	--	0.994	1.176
3.20	0.770	0.896	1.040	1.222
3.40	0.814	0.932	1.084	1.270
3.60	0.851	0.978	1.128	1.313
3.80	0.893	1.021	1.170	1.358
4.00	0.933	1.055	1.212	1.402
Cell correction	0.000	-0.001	-0.001	-0.001
Path length 1.001 cm. Wave length 570 m μ pH 10.00			* Path length 5.005 cm. Cell correction -0.001	

Table 7. Spectrophotometric titration of barium solutions
with arsenazo

Volume of Titrant (ml)	Absorbance for C_M^O Shown			
	$9.0 \times 10^{-6} M$	$1.90 \times 10^{-5} M$	$3.40 \times 10^{-5} M$	$5.40 \times 10^{-5} M$
0.20	0.306*	0.381*	0.460*	0.105
0.40	0.580*	0.704*	0.859*	0.203
0.60	0.838*	1.015*	1.218*	0.287
0.80	1.088*	0.261	0.316	0.371
1.00	0.267	0.315	0.383	0.450
1.20	0.314	0.368	0.442	0.516
1.40	0.362	0.418	0.496	0.585
1.60	0.406	0.468	0.551	0.651
1.80	0.451	0.516	0.603	0.712
2.00	0.494	0.562	0.659	0.770
2.20	0.537	0.607	0.709	0.826
2.40	0.578	0.651	0.752	0.882
2.60	0.620	0.695	0.803	0.939
2.80	0.662	0.738	0.849	0.984
3.00	0.701	0.779	0.893	1.035
3.20	0.741	0.820	0.939	1.083
3.40	0.782	0.863	0.982	1.129
3.60	0.821	0.901	1.023	1.176
3.80	0.860	0.942	1.062	1.215
4.00	0.899	0.980	1.101	1.258
Cell correction	-0.001	0.000	-0.001	-0.001
Path length 1.001 cm. Wave length 570 mμ pH 10.00		* Path length 5.005 cm. Cell correction 0.000		

concentration range. A least squares fit of the data was done, the resulting slope being the molar absorptivity. These values are shown in Table 3.

From the corrected absorbances and these values of a_L , the quantity ϵ' was calculated from equation (III-1). The results are given in Tables 8, 10, 12 and 14. Although absorbances were read to only the third decimal place, corrected absorbances, the absorbance of the ligand alone, and the difference between these quantities were calculated to four significant figures to avoid the accumulation of rounding-off errors in the ϵ' values.

To determine pairs of concentrations, C_L and C_M , for corresponding solutions, ϵ' for each C_M was carefully plotted vs. C_L on a large graph and the intersection of horizontal lines defining constant ϵ' with the experimental curves read with the aid of a four-power magnifier. The uncertainty in values of $C_L \times 10^5$ obtained by this process is estimated to be ± 0.02 at the smaller levels of ϵ' , where the slope of the curves was relatively steep, increasing to an estimated ± 0.10 toward the highest values of ϵ' used. Since the values of $C_L \times 10^5$ increased from around 1 to around 10 over this same interval, the relative uncertainty of the values remained about 1-2% throughout. The curves of ϵ' vs. C_L for the four cations are shown in Figures 4, 7, 9 and 11.

For each level of ϵ' used, C_L was plotted vs. C_M . The

resulting family of curves, for each cation, were linear for the lower levels of ϵ' , becoming concave downward at higher levels, i. e., at higher concentrations of the complex species. The plots for magnesium, typical of the others, is shown in Figure 5. The slope and intercept, at C_M equals zero, were determined for each line, until the departure from linearity became greater than could reasonably be attributed to experimental error. These quantities, representing the ligand number and free ligand concentration respectively (see equation (III-6)), are presented in Tables 9, 11, 13 and 15.

The maximum ligand number which could be determined by this method was 0.614, 0.670, 0.571 and 0.588, for the four cations in the usual order. These suggest the formation of the complexes with no more than one ligand per cation. If this were the case, the pertinent equilibrium, at least for the first step of the complex formation, would be



where, as mentioned in Section III, page 17, protons and ionic charges are neglected. For a system of this type, the ligand number is given by

$$\bar{n} = \frac{[ML]}{[M] + [ML]} \quad \text{and} \quad 1 - \bar{n} = \frac{[M]}{[M] + [ML]} \quad (\text{III-8})$$

Solving these two equations for $[ML]$ and $[M]$ respectively,

substituting into equation (III-7) and taking logarithms of both sides of the equation yields

$$\log \left(\frac{\bar{n}}{1 - \bar{n}} \right) = \log K_1^* + \log [L] \quad (\text{III-9})$$

indicating that a plot of $\log \left(\frac{\bar{n}}{1 - \bar{n}} \right)$ vs. $\log [L]$ should yield a straight line of unit slope, with intercept, $\log \left(\frac{\bar{n}}{1 - \bar{n}} \right) = 0$, equal to the desired equilibrium constant.

These quantities were calculated from the ligand number and free ligand concentrations found earlier, and are shown in Tables 9, 11, 13 and 15 for the four cations, as are the slopes and intercepts for the resulting plots. As is customary in such calculations, points with ligand number less than 0.1 or greater than 0.9 were ignored. If \bar{n} is too large or too small, the value of $\frac{\bar{n}}{1 - \bar{n}}$ becomes too sensitive to error in determining the ligand number. The plots are shown in Figure 12. The slopes all lie reasonably close to unity, so a conclusion that a complex containing one mole of the ligand to one of the cation is justified.

In order to evaluate the uncertainty in the values of K_1^* , a separate value was calculated for each experimental point, using equation (III-9). The standard deviation for each set, and thence a confidence interval at the 95% confidence level was calculated. These confidence intervals are shown as uncertainties in Tables 9, 11, 13 and 15.

Table 8. Corrected absorbances and ϵ' for magnesium-arsenazo solutions

Initial Total Central Ion Concentration (molar)							
1.25×10^{-5}		2.75×10^{-5}		4.25×10^{-5}		7.00×10^{-5}	
A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$
0.0994	3.78	0.1365	3.07	0.1576	2.48	0.1767	1.78
0.1885	6.74	0.2591	5.63	0.3155	4.97	0.3719	3.82
0.2702	9.11	0.3744	7.93	0.4584	7.11	0.5606	5.78
0.3465	11.05	0.4724	9.00	0.5862	8.89	0.7254	7.39
0.4090	11.88	0.5651	11.08	0.6987	10.31	0.8843	8.91
0.4741	12.92	0.6482	12.20	0.8018	11.55	1.0199	10.10
0.5376	13.83	0.7216	12.98	0.8902	12.36	1.1493	11.21
0.5903	13.88	0.7884	13.51	0.9701	13.02	1.2488	11.89
0.6506	14.54	0.8536	13.99	1.0422	13.49	1.3520	12.62
0.7051	14.73	0.9183	14.45	1.1180	14.05	1.4415	13.15
0.7600		0.9720	14.51	1.1808	14.30	1.5211	13.54
0.8184		1.0375	14.99	1.2429	14.53	1.5909	13.94
0.8731		1.0983	15.31	1.3056	14.78	1.6748	14.25
0.9292		1.1573	15.56	1.3623	14.89	1.7297	14.29
0.9794		1.2147	15.75	1.4247	15.13	1.7947	14.47
1.0289		1.2674	15.77	1.4875	15.39	1.8608	14.67
1.0840		1.3222	15.87	1.5390	15.37	1.9246	14.84
1.1375		1.3830	16.19	1.5961	15.49	1.9789	14.87
1.1848		1.4322	16.08	1.6301	15.30	2.0400	15.00
1.2345		1.4903	16.30	1.6849	15.13	2.0951	15.04

 ϵ' in $\text{l. mole}^{-1} \text{cm}^{-1}$

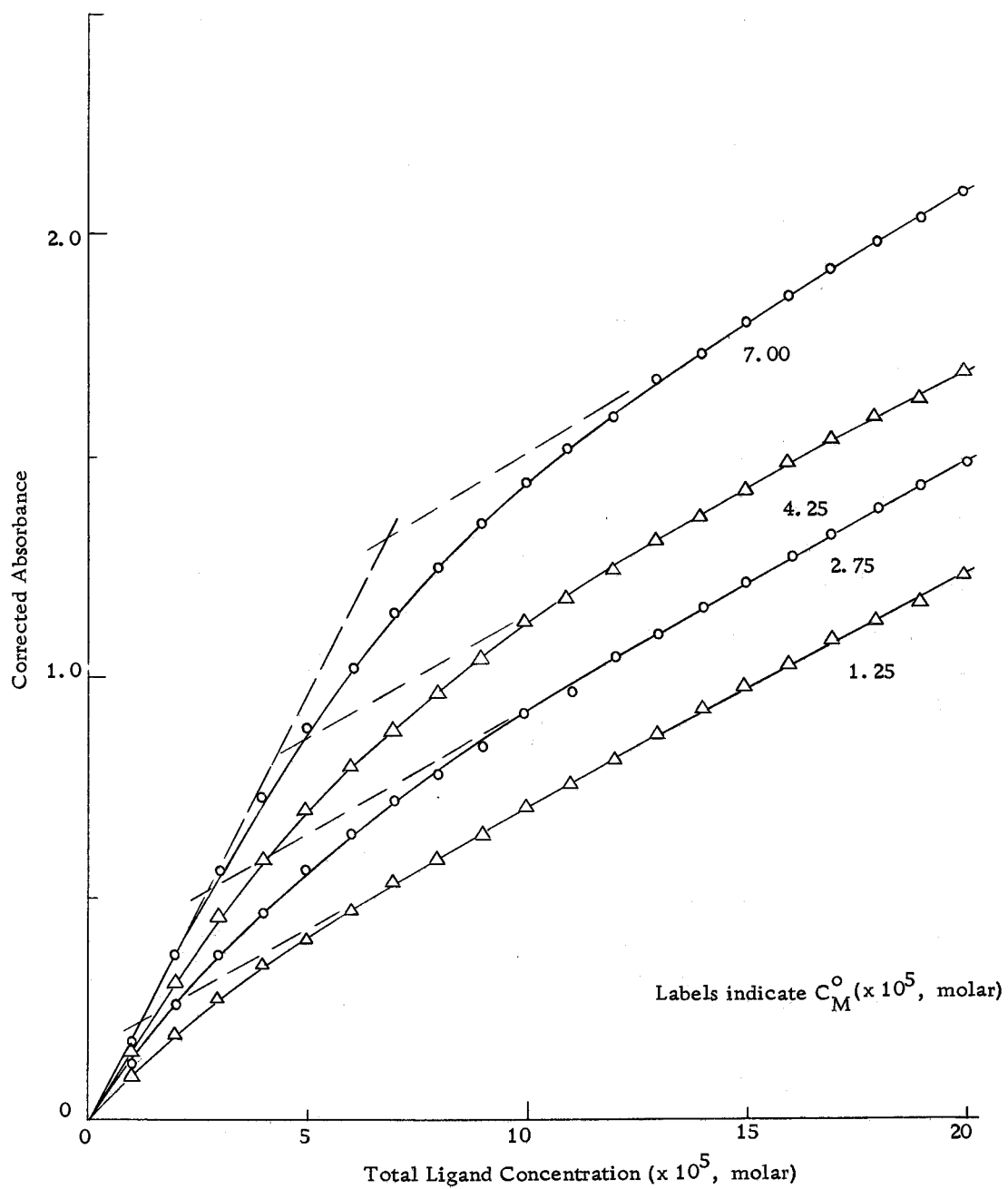


Figure 3. Spectrophotometric Titration of Magnesium with Arsenazo, pH 9.00

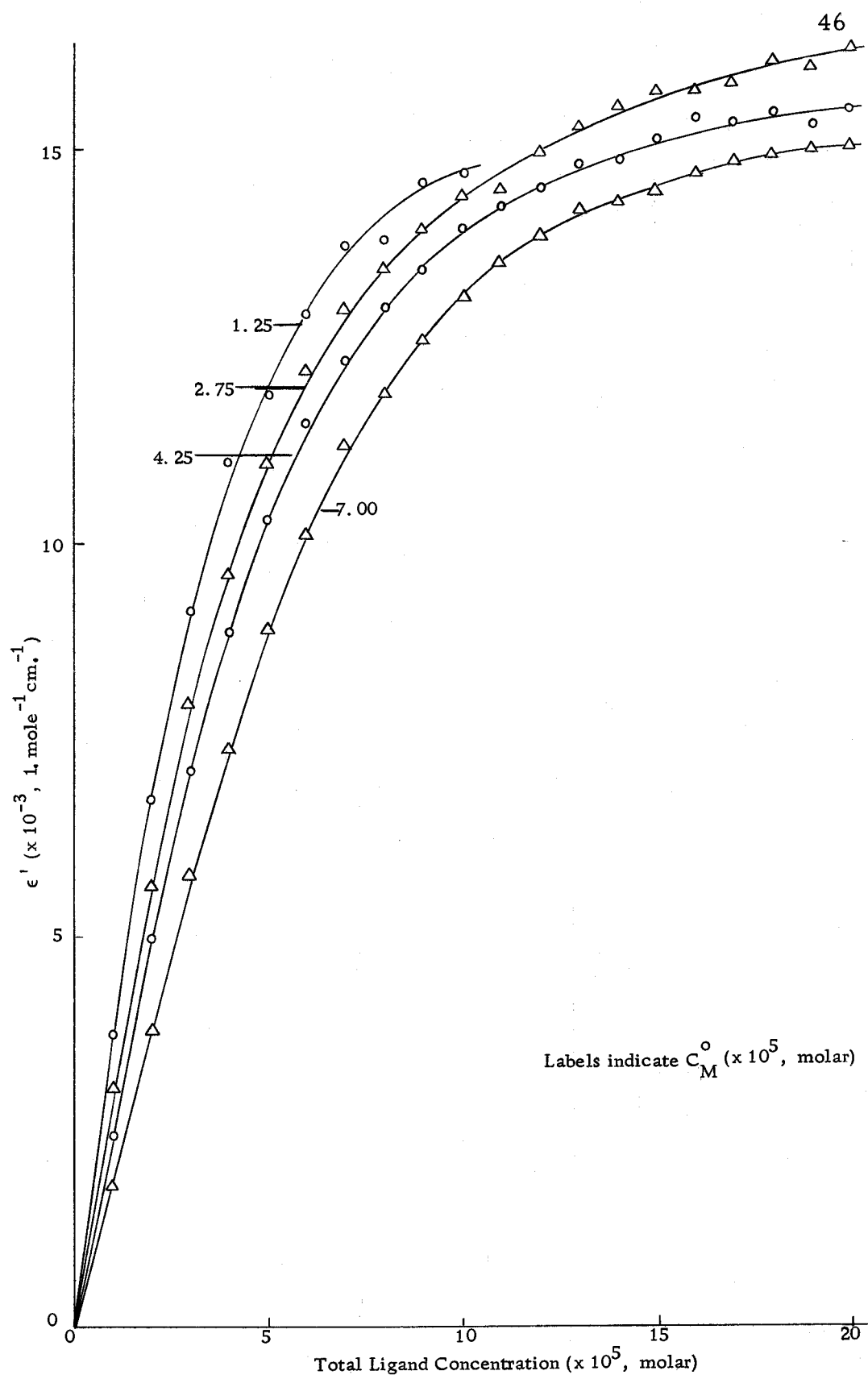


Figure 4. ϵ' as a Function of Total Ligand Concentration, Magnesium, pH 9.00

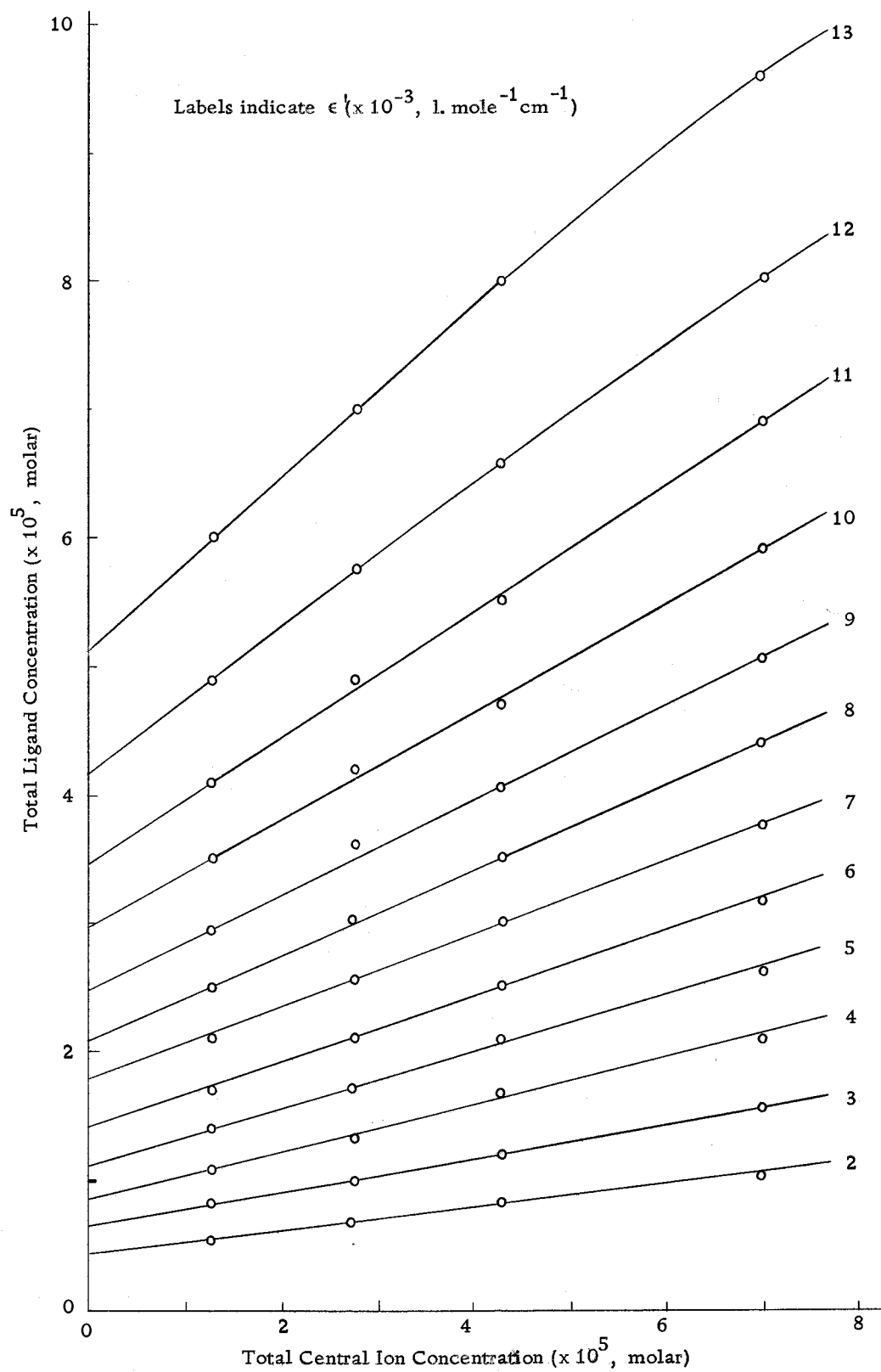


Figure 5. Composition of Corresponding Solutions, Magnesium

Table 9. Corresponding solutions and calculation of K_1^* for magnesium

$\epsilon' \times 10^{-3}$ (l.mole ⁻¹ cm ⁻¹)	$C_L \times 10^5$ (molar)				$[L] \times 10^5$ (molar)	\bar{n}	$\log \left(\frac{\bar{n}}{1-\bar{n}} \right)$	$\log [L]$	$\log K_1^*$
	1.20	2.75	4.25	7.00					
2.0	0.53	0.64	0.75	1.04	0.410	0.0857	-1.028	-5.387	4.359
3.0	0.80	0.98	1.12	1.55	0.625	0.128	-0.834	-5.204	4.370
4.0	1.08	1.32	1.50	2.07	0.840	0.174	-0.675	-5.076	4.401
5.0	1.38	1.68	1.91	2.61	1.115	0.214	-0.566	-4.952	4.386
6.0	1.71	2.09	2.35	3.16	1.380	0.254	-0.467	-4.860	4.393
7.0	2.06	2.53	2.81	3.76	1.705	0.295	-0.379	-4.768	4.389
8.0	2.47	3.03	3.34	4.39	2.100	0.331	-0.306	-4.678	4.372
9.0	2.95	3.57	3.96	5.10	2.530	0.374	-0.223	-4.597	4.374
10.0	3.49	4.19	4.68	5.93	2.945	0.439	-0.106	-4.531	4.425
11.0	4.13	4.89	5.52	6.86	3.500	0.493	-0.012	-4.456	4.444
12.0	4.94	5.74	6.55	8.03	4.250	0.537	0.064	-4.373	4.437
13.0	6.05	7.02	7.96	9.63	5.300	0.614	0.202	-4.276	4.478
Slope = 1.069 Intercept = 4.424									Mean 4.402 ± 0.023 (95% C.L.)

Table 10. Corrected absorbances and ϵ' for calcium-arsenazo solutions

Initial Total Central Ion Concentration (molar)							
9.0×10^{-6}		1.90×10^{-5}		3.40×10^{-5}		5.40×10^{-5}	
A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$
0.1068	6.93	0.1488	5.49	0.1898	4.27	0.2118	3.10
0.1837	10.53	0.2692	9.48	0.3457	7.55	0.4032	5.82
0.2479	12.73	0.3653	12.20	0.4807	10.21	0.5738	8.15
0.3099	14.68	0.4389	13.74	0.5913	12.16	0.7315	10.25
0.3601	15.33	0.5038	14.82	0.6865	13.65	0.8670	11.93
0.4147	16.46	0.5642	15.63	0.7619	14.56	0.9871	13.33
0.4616	16.74	0.6209	16.30	0.8337	15.36	1.0835	14.30
0.5077	16.93	0.6780	16.97	0.8968	15.91	1.1703	15.26
0.5615	17.97	0.7304	17.39	0.9562	16.35	1.2463	15.66
0.6032	17.67	0.7800	17.67	1.0130	16.72	1.3177	16.16
0.6515		0.8268	17.79	1.0597	16.79	1.3812	16.52
0.6959		0.8761	18.05	1.1140	17.08	1.4420	16.82
0.7448		0.9247	18.27	1.1667	17.32	1.5002	17.07
0.7857		0.9704	18.33	1.2133	17.39	1.5565	17.29
0.8321		1.0176	18.48	1.2635	17.55	1.6059	17.39
0.8789		1.0587	18.31	1.3119	17.67	1.6535	17.45
0.9195		1.1043	18.37	1.3542	17.61	1.7077	17.63
0.9659		1.1535	18.62	1.4011	17.68	1.7602	17.78
1.0093		1.1976	18.61	1.4440	17.64	1.8066	17.81
1.0562		1.2474	18.89	1.4893	17.66	1.8554	17.90

ϵ' in $1. \text{ mole}^{-1} \text{ cm.}^{-1}$

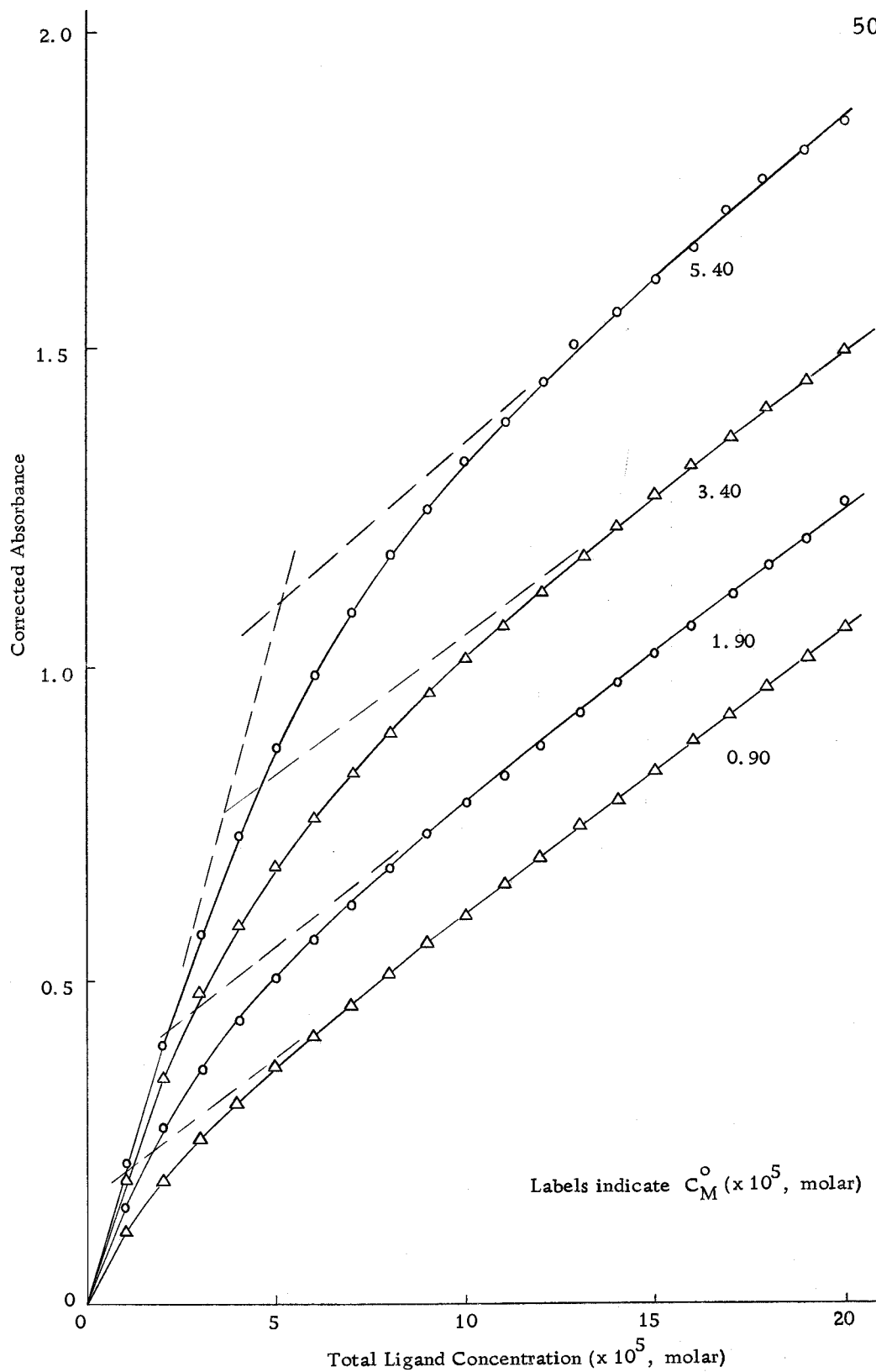


Figure 6. Spectrophotometric Titration of Calcium with Arsenazo, pH 10.00

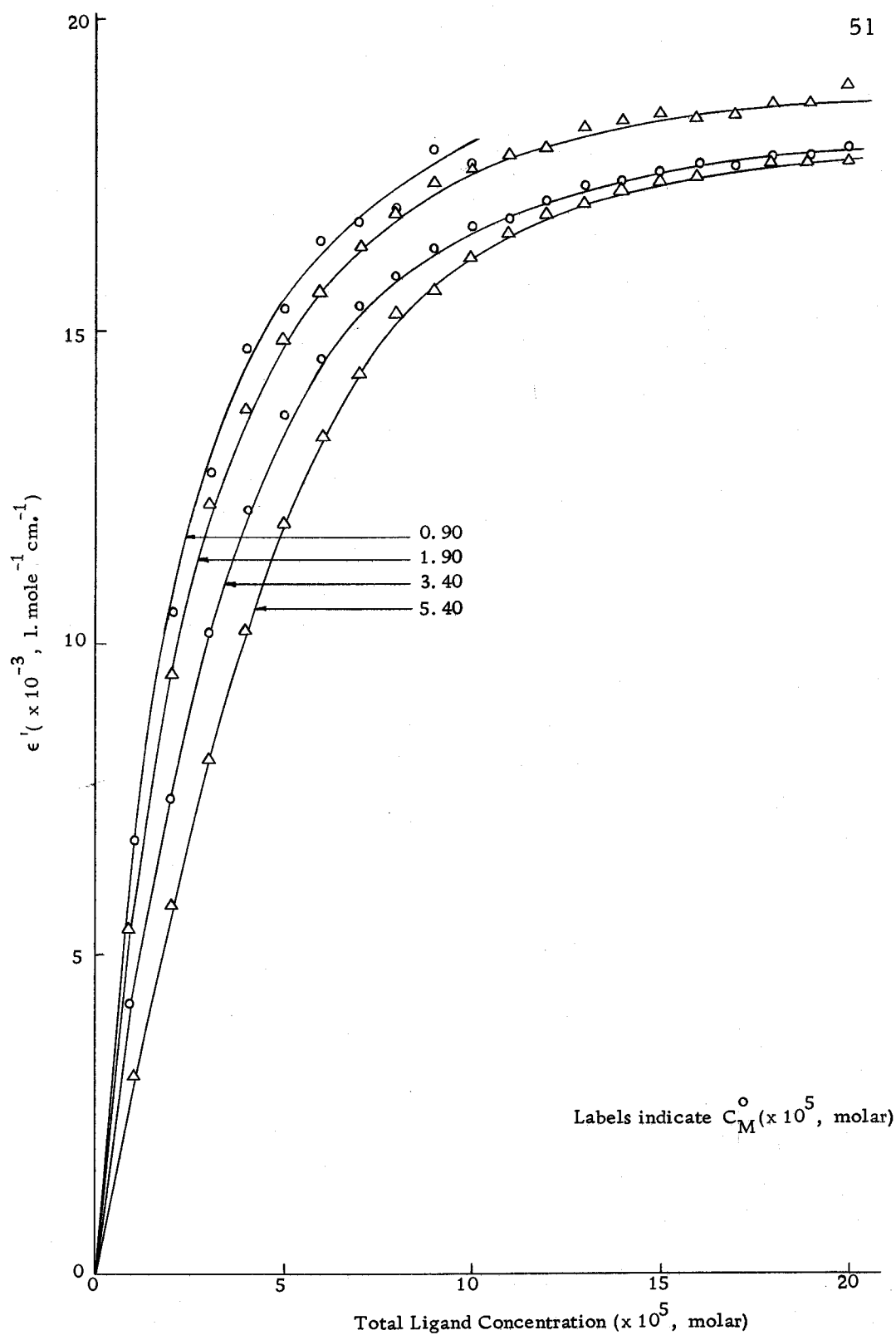


Figure 7. ϵ' as a Function of Total Ligand Concentration, Calcium, pH 10.00

Table 11. Corresponding solutions and calculation of K_1^* for calcium

$\epsilon' \times 10^{-3}$ (l. mole ⁻¹ cm. ⁻¹)	$C_L \times 10^5$ (molar)				$[L] \times 10^5$ (molar)	\bar{n}	$\log \left(\frac{\bar{n}}{1-\bar{n}} \right)$	$\log [L]$	$\log K_1^*$
	0.90	1.90	3.40	5.40					
3.0	0.39	0.53	0.68	1.00	0.270	0.134	-0.811	-5.569	4.778
4.0	0.54	0.70	0.93	1.36	0.370	0.180	-0.657	-5.432	4.758
5.0	0.69	0.89	1.17	1.70	0.495	0.218	-0.555	-5.305	4.775
6.0	0.85	1.08	1.44	2.06	0.585	0.266	-0.441	-5.233	4.750
7.0	1.03	1.31	1.75	2.45	0.725	0.313	-0.341	-5.140	4.792
8.0	1.24	1.55	2.08	2.87	0.875	0.358	-0.254	-5.058	4.804
9.0	1.47	1.82	2.47	3.34	1.035	0.422	-0.136	-4.985	4.849
10.1	1.76	2.14	2.93	3.86	1.275	0.476	-0.042	-4.894	4.852
11.0	2.09	2.52	3.37	4.44	1.580	0.526	0.045	-4.801	4.846
12.0	2.51	2.96	3.91	5.07	1.970	0.570	0.122	-4.706	4.828
13.0	3.00	3.51	4.52	5.80	2.380	0.604	0.184	-4.623	4.807
14.0	3.62	4.23	5.30	6.65	3.010	0.670	0.307	-4.521	4.828
Slope = 1.082 Intercept = 4.823									Mean 4.806 ± 0.023 (95% C. L.)

Table 12. Corrected absorbances and ϵ' for strontium-arsenazo solutions

Initial Total Central Ion Concentration (molar)							
9.0×10^{-6}		1.90×10^{-5}		3.40×10^{-5}		5.40×10^{-5}	
A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$
0.0717	3.03	0.0904	2.42	0.1108	1.95	0.1235	1.46
0.1351	5.14	0.1693	4.23	0.2058	3.44	0.2429	2.85
0.1923	6.56	0.2415	5.69	0.3016	4.95	0.3593	4.18
0.2467	7.67	0.3139	7.17	0.3820	6.01	0.4552	5.14
0.2999	8.65	0.3743	8.01	0.4570	6.90	0.5457	5.98
0.3502	9.30	0.4321	8.71	0.5243	7.58	0.6339	6.80
0.4009	10.00	0.4873	9.28	0.5932	8.30	0.7186	7.54
0.4500	10.52	0.5439	9.92	0.6564	8.85	0.7936	8.11
0.4983	10.96	0.5957	10.31	0.7200	9.41	0.8671	8.65
0.5470	11.43	0.6490	10.78	0.7790	9.84	0.9402	9.18
0.5930		0.7047	11.37	0.8415	10.38	1.0012	9.49
0.6393		0.7525	11.55	0.8939	10.61	1.0637	9.82
0.6848		0.8016	11.79	0.9489	10.92	1.1277	10.18
0.7297		0.8511	12.06	1.0021	11.18	1.1869	10.46
0.7749		---	--	1.0526	11.36	1.2455	10.72
0.8193		0.9523	12.71	1.1055	11.61	1.2991	10.89
0.8694		0.9943	12.59	1.1566	11.80	1.3553	11.11
0.9123		1.0473	13.04	1.2092	12.04	1.4065	11.23
0.9509		1.0975	13.34	1.2578	12.17	1.4601	11.40
1.0075		1.1383	13.16	1.3079	12.34	1.5131	11.56

ϵ' in l. mole⁻¹ cm.⁻¹

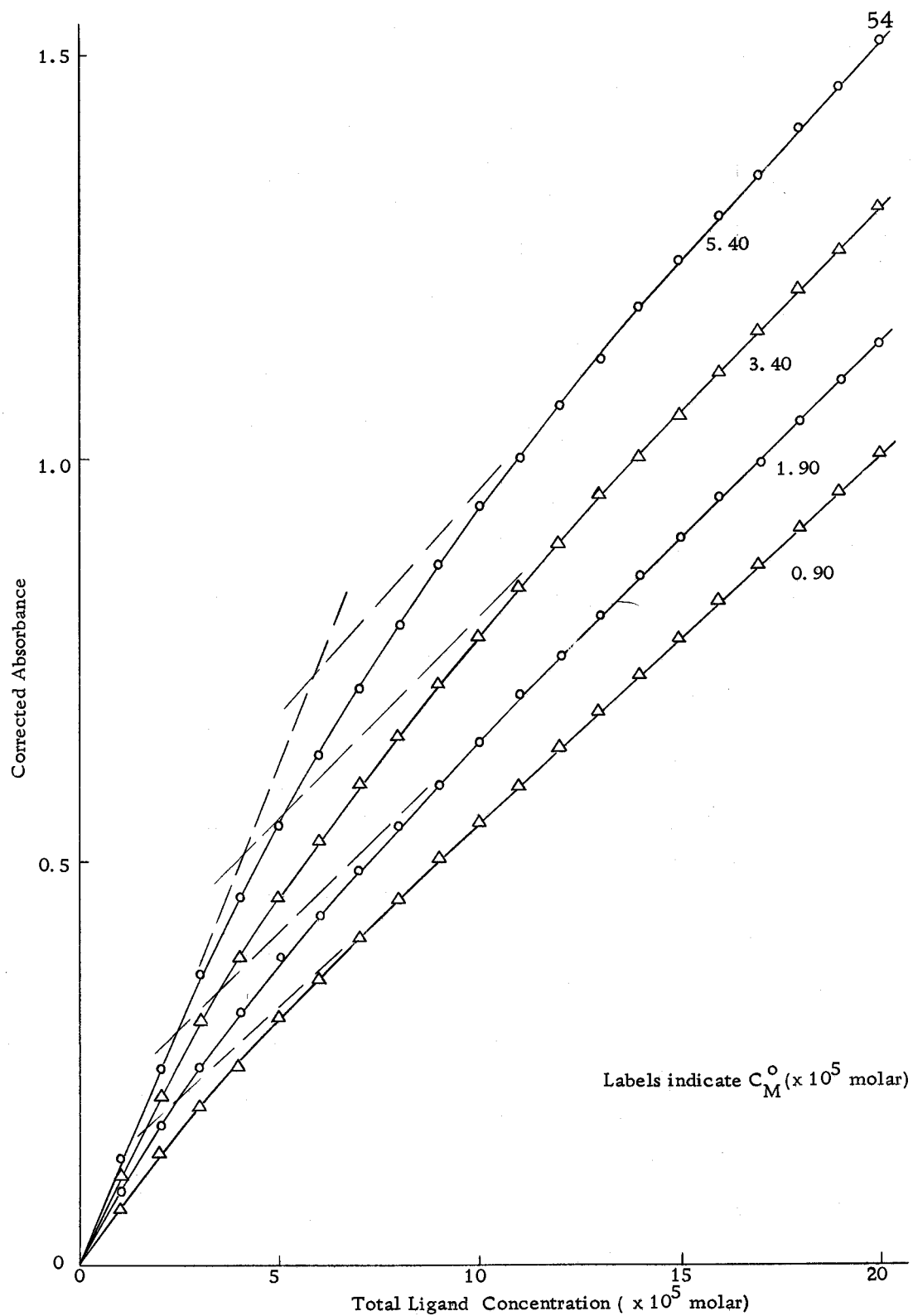


Figure 8. Spectrophotometric Titration of Strontium with Arsenazo, pH 10.00

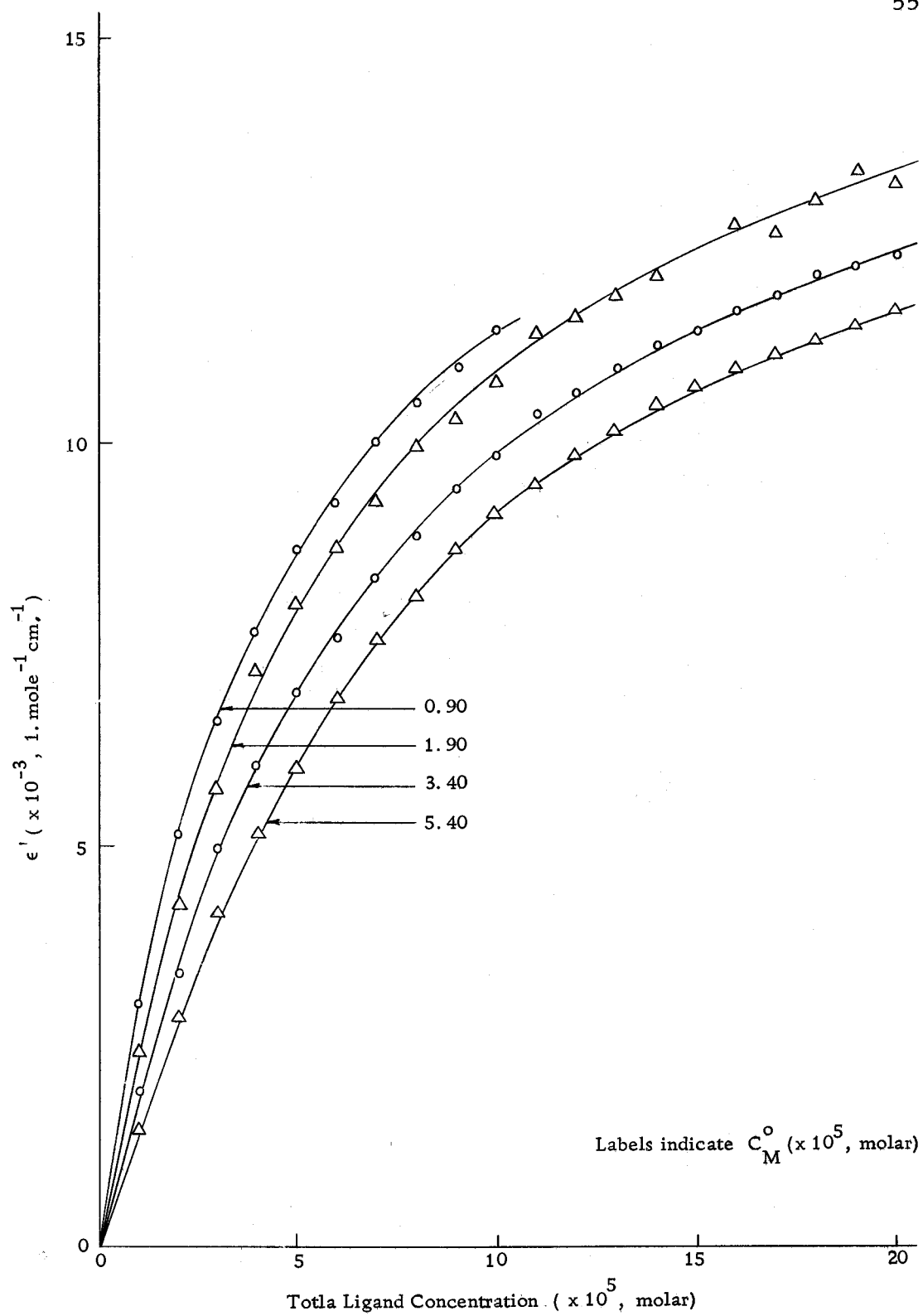


Figure 9. ϵ' as a Function of Total Ligand Concentration, Strontium, pH 10.00

Table 13. Corresponding solutions and calculation of K_1^* for strontium

$\epsilon' \times 10^{-3}$ (1.mole ⁻¹ cm. ⁻¹)	$C_L \times 10^5$ (molar)				$[L] \times 10^5$ (mclar)	\bar{n}	$\log \left(\frac{\bar{n}}{1-\bar{n}} \right)$	$\log [L]$	$\log K_1^*$
	0.90	1.90	3.40	5.40					
1.5	0.46	0.59	0.78	1.02	0.360	0.121	-0.860	-5.760	4.900
2.0	0.64	0.80	1.06	1.37	0.490	0.164	-0.705	-5.444	4.739
2.5	0.82	1.03	1.35	1.74	0.630	0.204	-0.590	-5.310	4.720
3.0	1.00	1.26	1.65	2.12	0.785	0.246	-0.486	-5.201	4.715
3.5	1.21	1.54	1.97	2.52	1.005	0.280	-0.411	-5.105	4.694
4.0	1.44	1.82	2.31	2.94	1.190	0.324	-0.319	-4.998	4.679
4.5	1.67	2.12	2.67	3.40	1.435	0.363	-0.245	-4.924	4.679
5.0	1.94	2.45	3.06	3.87	1.685	0.401	-0.175	-4.843	4.668
5.5	2.24	2.82	3.49	4.40	1.960	0.449	-0.089	-4.773	4.684
6.0	2.56	3.21	3.99	5.00	2.245	0.506	0.011	-4.648	4.659
6.5	2.93	3.62	4.51	5.59	2.530	0.571	0.125	-4.403	4.528
Slope = 1.019 Intercept = 4.602									Mean 4.696 ± 0.058 (95% C. L.)

Table 14. Corrected absorbances and ϵ' for barium-arsenazo solutions

Initial Total Central Ion Concentration (molar)							
9.0×10^{-6}		1.90×10^{-5}		3.40×10^{-5}		5.40×10^{-5}	
A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$	A_{corr}	$\epsilon' \times 10^{-3}$
0.0614	1.89	0.0765	1.69	0.0924	1.41	0.1044	1.11
0.1169	3.12	0.1419	2.79	0.1732	2.48	0.2036	2.12
0.1696	4.04	0.2054	3.80	0.2465	3.33	0.2894	2.89
0.2211	4.83	0.2652	4.61	0.3200	4.18	0.3759	3.67
0.2713	5.47	0.3213	5.22	0.3896	4.92	0.4580	4.37
0.3205	6.00	0.3768	5.80	0.4516	5.44	0.5274	4.83
0.3711	6.69	0.4297	6.25	0.5089	5.82	0.6004	5.36
0.4180	6.97	0.4830	6.72	0.5676	6.24	0.6708	5.84
0.4662	7.39	0.5346	7.10	0.6237	6.58	0.7366	6.23
0.5127	7.63	0.5845	7.39	0.6843	7.06	0.7998	6.58
0.5596		0.6337	7.64	0.7392	7.37	0.8613	6.90
0.6047		0.6822	7.86	0.7870	7.47	0.9233	7.22
0.6512		0.7311	8.09	0.8437	7.83	0.9868	7.58
0.6980		0.7793	8.29	0.8955	8.04	1.0380	7.70
0.7420		0.8257	8.39	0.9455	8.21	1.0960	7.95
0.7874		0.8725	8.52	0.9980	8.45	1.1512	8.15
0.8341		0.9216	8.76	1.0477	8.60	1.2047	8.32
0.8790		0.9659	8.76	1.0956	8.71	1.2596	8.52
0.9243		1.0136	8.93	1.1416	8.75	1.3063	8.56
0.9698		1.0584	8.95	1.1880	8.81	1.3576	8.69

ϵ' in l. mole⁻¹ cm.⁻¹

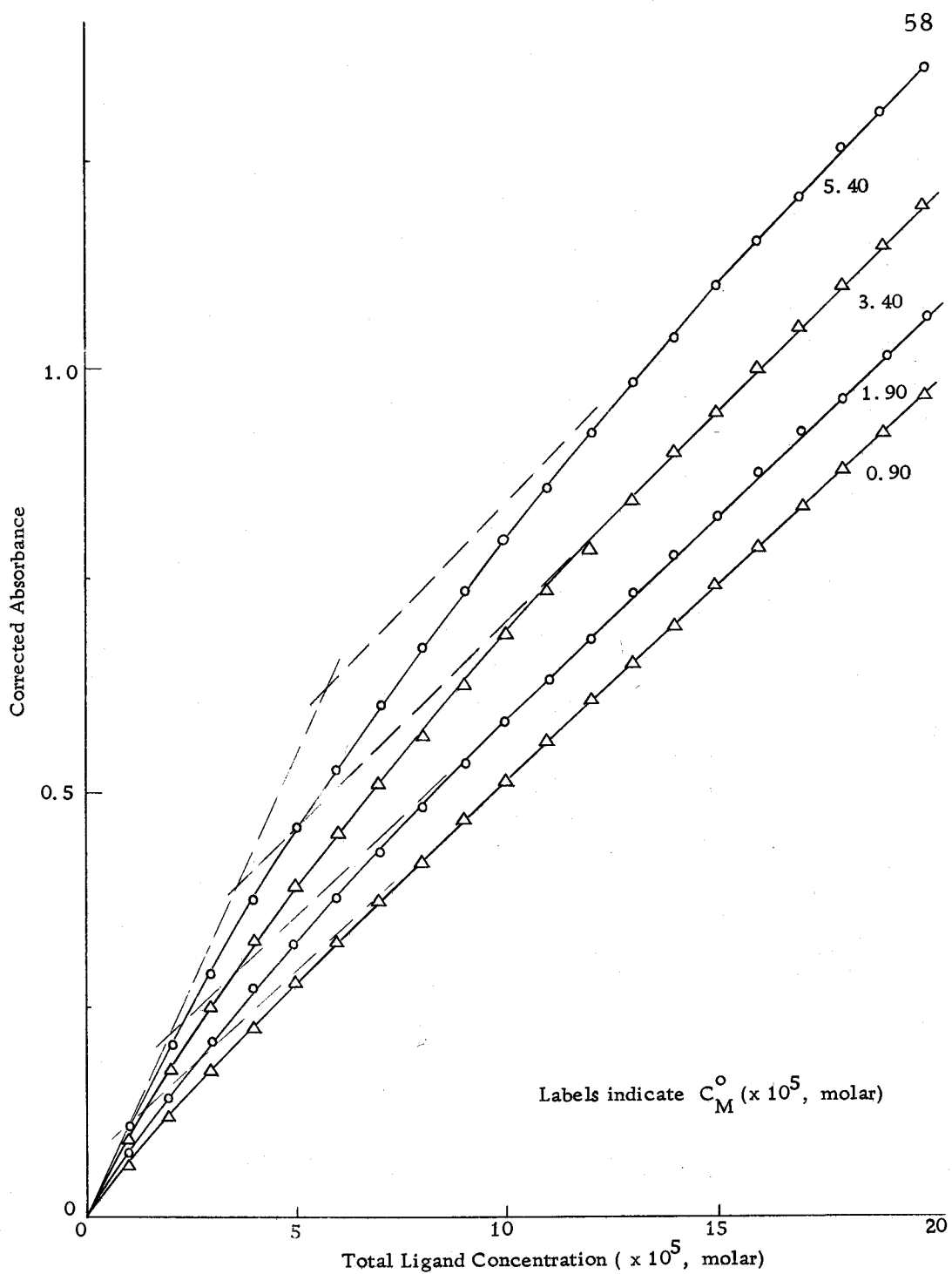


Figure 10. Spectrophotometric Titration of Barium with Arsenazo, pH 10.00

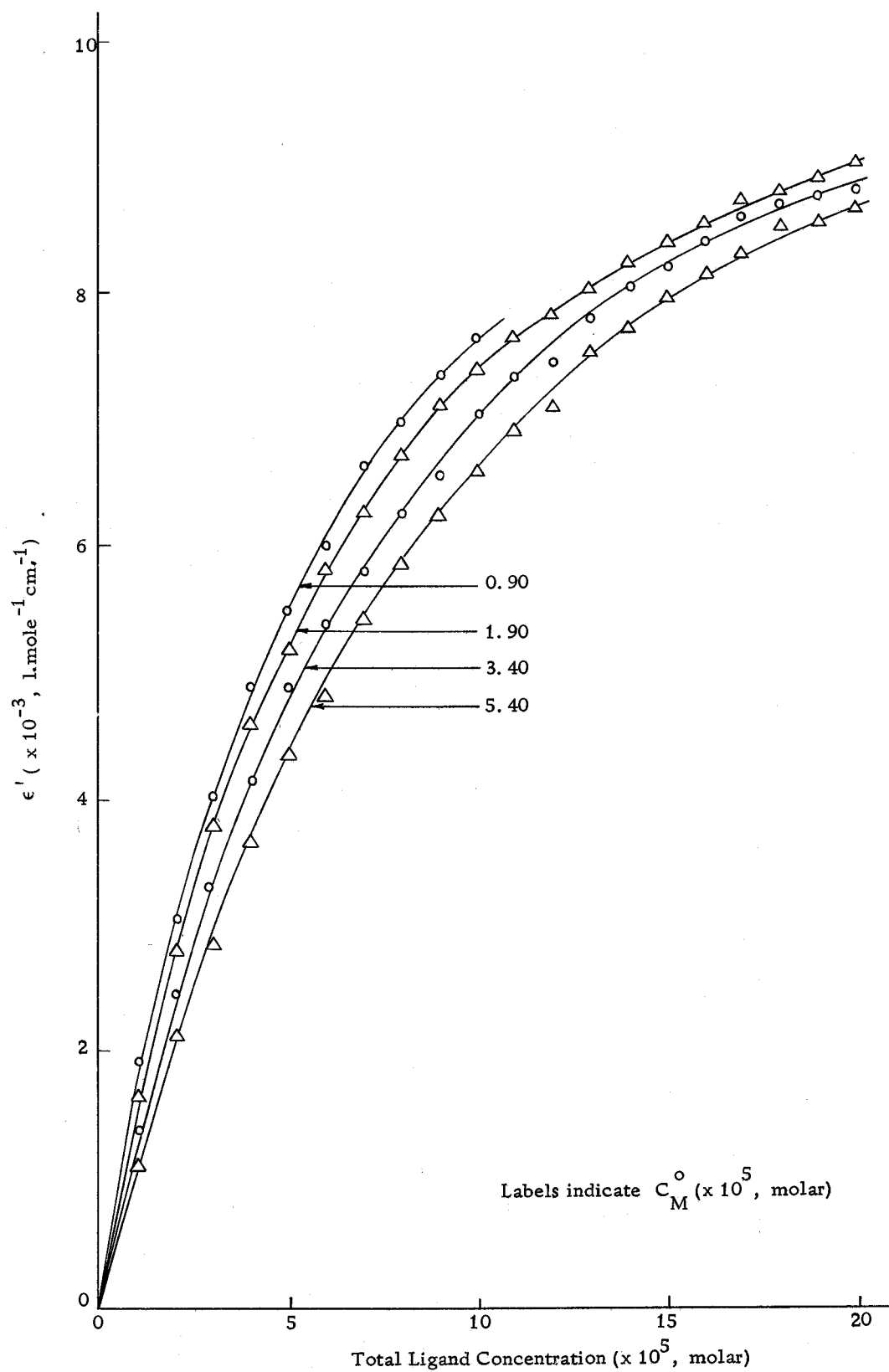


Figure 11. ϵ' as a Function of Total Ligand Concentration, Barium, pH 10.00

Table 15. Corresponding solutions and calculations of K_1^* for barium

$\epsilon' \times 10^{-3}$ (1.mole ⁻¹ cm ⁻¹)	$C_L \times 10^5$ (molar)				$[L] \times 10^5$ (molar)	\bar{n}	$\log \left(\frac{\bar{n}}{1-\bar{n}} \right)$	$\log [L]$	$\log K_1^*$
	0.90	1.90	3.40	5.40					
1.0	0.48	0.59	0.66	0.83	0.320	0.097	-0.968	-5.495	4.527
1.5	0.78	0.92	1.08	1.32	0.630	0.129	-0.831	-5.201	4.370
2.0	1.12	1.29	1.54	1.86	0.925	0.178	-0.665	-5.034	4.369
2.5	1.48	1.71	2.04	2.45	1.320	0.213	-0.567	-4.879	4.312
3.0	1.92	2.18	2.58	3.09	1.700	0.256	-0.463	-4.770	4.307
3.5	2.44	2.68	3.18	3.80	2.040	0.332	-0.303	-4.690	4.387
4.0	2.98	3.23	3.82	4.52	2.575	0.356	-0.257	-4.590	4.333
4.5	3.56	3.87	4.53	5.31	3.175	0.393	-0.189	-4.498	4.309
5.0	4.24	4.58	5.33	6.21	3.780	0.446	-0.093	-4.422	4.329
5.5	5.03	5.40	6.21	7.21	4.470	0.509	0.015	-4.350	4.365
6.0	5.84	6.38	7.23	8.31	5.350	0.546	0.081	-4.272	4.353
6.5	6.83	7.48	8.36	9.54	6.350	0.588	0.154	-4.197	4.351
Slope = 0.989 Intercept = 4.336									Mean 4.359 ± 0.038 (95% C. L.)

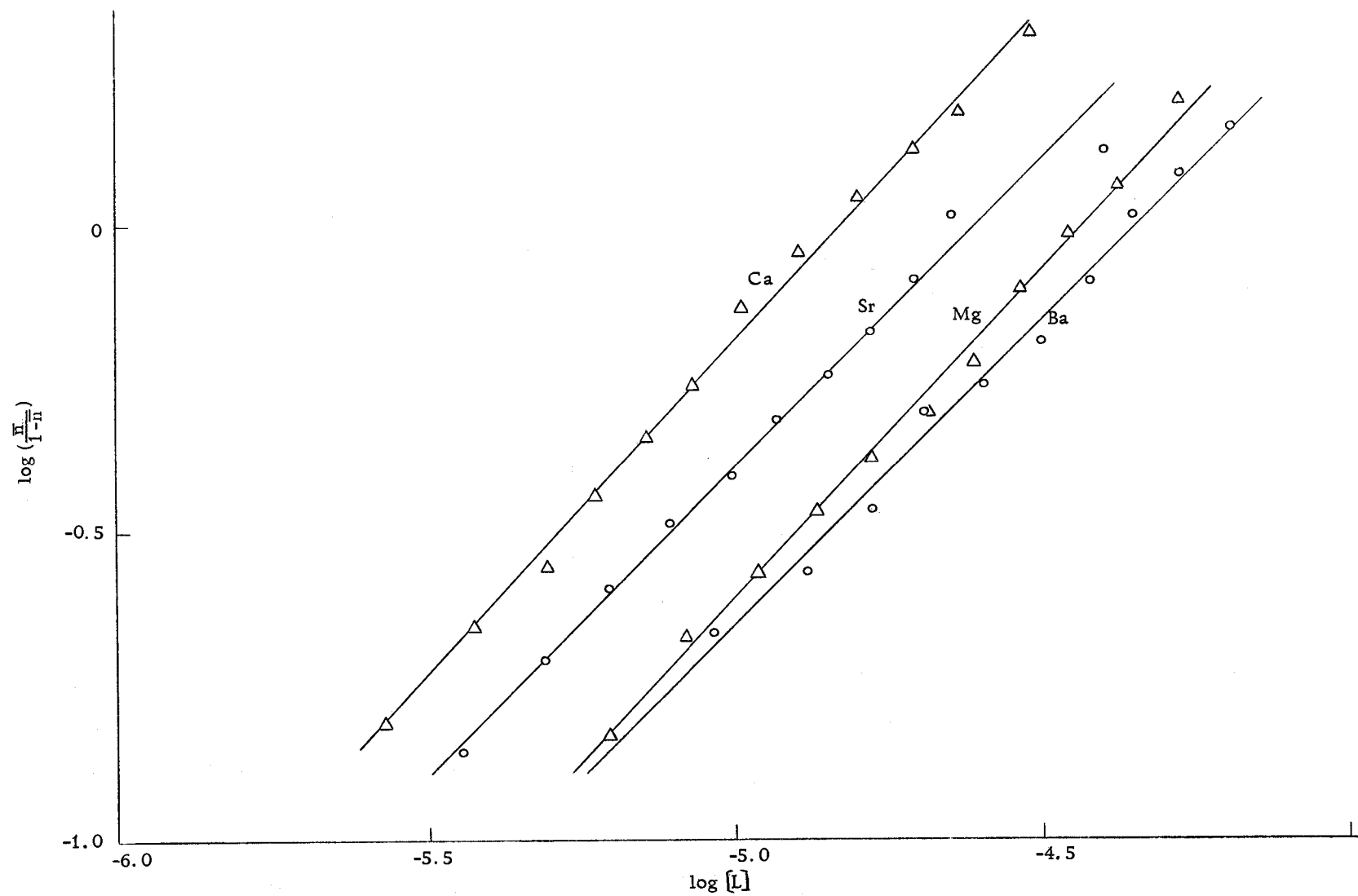


Figure 12. Graphical Determination of K_1^*

Ahrland (1) states that a linear relationship between C_L and C_M at constant ϵ' is a necessary but not sufficient condition to prove the absence of polynuclear species in a system, and that this absence must be proved by some other means. In order for ϵ' to be independent of the free central ion concentration and for the linear relationship to exist, the partial derivative of ϵ' with respect to $[M]$ must be identically equal to zero. If ϵ' is of the form given in equation (III-5), this condition is obviously met. For the simplest case involving other than mononuclear species, that in which only mononuclear and binuclear species are present, equation (III-5) must be augmented to the form

$$\epsilon' = \frac{\sum_0^N (a_n - na_L) \beta_n [L]^n + \sum_0^J (a_{2j} - ja_L) \beta_{2j} [M] [L]^j}{\sum_0^N \beta_n [L]^n + \sum_0^J \beta_{2j} [M] [L]^j} \quad (\text{III-10})$$

where j represents the number of ligands associated with two central ions in the complex M_2L_j , and J is the maximum value j can have for the system.

Then

$$\frac{\partial \epsilon'}{\partial [M]} = \frac{\left(\sum_0^N \beta_n [L]^n + \sum_0^J \beta_{2j} [M] [L]^n \right) \left(\sum_0^J (a_{2j} - jn) \beta_{2j} [L]^n \right)}{\left(\sum_0^N \beta_n [L]^n + \sum_0^J \beta_{2j} [M] [L]^n \right)^2} \quad (\text{III-11})$$

$$- \frac{\left(\sum_0^N (a_n - na_L) \beta_n [L]^n + \sum_0^J (a_{2j} - ja_L) \beta_{2j} [M] [L]^n \right) \left(\sum_0^J \beta_{2j} [L]^n \right)}{\left(\sum_0^N \beta_n [L]^n + \sum_0^J \beta_{2j} [M] [L]^n \right)^2}$$

The denominator of the fraction is always positive, so the derivative can be zero only when the numerator is. Setting the numerator equal to zero and collecting terms yields

$$\sum_0^N \beta_n [L]^n \sum_0^J (a_{2j} - ja_L) \beta_{2j} [L]^j - \sum_0^J \beta_{2j} [L]^j \sum (a_n - na_L) \beta_n [L]^n = 0 \quad (\text{III-12})$$

This equation expands, for any N and J into a power series in $[L]$, the coefficients of which contain only the formation constants β_n and β_{2j} and the molar absorptivities a_L , a_n and a_{2j} . In order for such a series to be identically zero, the coefficient of each power of the variable must also equal zero. This result modifies Ahrlund's contention somewhat. If a linear relationship between C_L and C_M exists throughout the range of ϵ' , a further test for the absence of

polynuclear species must be made. If, however, as in the present case such a linear relationship exists for relatively low concentrations of complex species but ceases to exist at higher concentrations, the only conclusion to be drawn is that polynuclear species are at first absent, but at higher concentrations one or more of them attains a significant concentration.

A similar treatment for a system containing trinuclear species does not work out as neatly. This case yields, as the numerator of the partial derivative, a quadratic in $[M]$, the coefficients of which are summations which would expand into power series in $[L]$. The argument still holds however, because in order for the quadratic to be identically zero the coefficient of each power of $[M]$ must be identically zero and this requirement again demands that the coefficient of each power of $[L]$ be identically zero, as before. The expansion of equation (III-10) further to account for still higher degrees of polymerization was not attempted but there is no apparent reason to believe the ultimate conclusion would be different, whatever the degree.

The data presented in Figures 3, 6, 8 and 10 are just that which is required for the mole ratio method of determining the stoichiometry of the complex species formed. In these systems however, the plots of absorbance vs. total ligand concentration (which is proportional, for each curve, to the mole ratio of ligand to central ion) are

so gradually curved that, with the possible exception of the curves corresponding to the highest central ion concentration, it would be difficult to draw line segments approximating the curves, the intersection of such segments giving the composition of the complex species present. This problem can be resolved by considering the initial slope expected of such a curve. At the beginning of a titration, when the central ion is present in large excess, any ligand added should be virtually completely complexed in the form having the lowest ligand to metal ratio. No polynuclear species would be expected because of the low concentration of complex present. Under these circumstances,

$$A = a_L b[L] + a_1 b[ML] \approx a_1 b C_L \quad (\text{III-13})$$

and

$$\frac{dA}{dC_L} = a_1 b \quad (\text{III-14})$$

independent of the total central ion concentration, so long as it is high enough to cause "complete" complexation of the ligand. If this condition is met at the concentrations used in these titrations, the absorbance of the first point on each curve should be the same. Examination of Figures 3, 6, 8 and 10 shows this not to be the case, there being a regular decrease in absorbance with decreasing C_M . At best the condition will be met for the curve with highest C_M . To determine whether it is met, the initial slope, as determined by the

first point and the origin, for the highest curve for each cation was extended. The final slope of each curve was extended to intersect this line, and the value of C_L for the intersection read from the graph. The ratio of this C_L to C_M for the curve gives the mole ratio of the complex formed. The results are summarized in Table 16.

Table 16. Summary of mole ratio data

Cation	$C_M \times 10^5$ (molar)	$C_L \times 10^5$ (molar)	C_L/C_M
Magnesium	1.25	1.26	1.01
	2.75	2.60	0.95
	4.25	4.26	1.00
	7.00	7.31	1.05
Calcium	0.90	0.92	1.02
	1.90	1.91	1.01
	3.40	3.54	1.04
	5.40	5.36	0.99
Strontium	0.90	0.99	1.10
	1.90	2.10	1.10
	3.40	3.72	1.09
	5.40	5.80	1.07
Barium	0.90	0.84	0.94
	1.90	1.89	1.00
	3.40	3.55	1.04
	5.40	5.63	1.04

The fact that for each cation a nearly constant mole ratio is observed indicates that the highest concentration of central ion is sufficient to meet the requirement of complete complexation of the ligand. The fact that this mole ratio is near unity in each case is

strong evidence that no species higher than 1:1 are formed even in a large excess of ligand. The nonlinearity of the C_L vs. C_M plots (Figure 5) indicates some degree of polymerization is occurring. To clarify the degree of polymerization as well as to determine the number of protons on the coordinated ligand, these systems were also studied by means of potentiometric titrations.

IV. POTENTIOMETRIC STUDY OF THE COMPLEXES

Theory of the Method

A large number of ligands are also weak acids, e. g. , amino acids, carboxylic acids and phenolic compounds, or weak bases, such as amines. Hence the formation of complexes of these ligands can be regarded as the competition between protons and other cations for the coordination sites of the ligand. This fact permits the determination of the extent of complex formation by measurement of the hydrogen ion concentration of solutions of known total central ion and total ligand concentrations. Uses which have been made of such measurements have been summarized by Rossotti and Rossotti (53, p. 150-165) and by Albert and Serjeant (2, p. 154-167).

In the approaches most often used, the hydrogen ion activity is measured, and this quantity converted in some manner to a concentration. Often the conversion is made by assuming an activity coefficient of unity. A superior method is that advanced by Irving and Rossotti (20) in which the hydrogen ion concentration needs not be evaluated, the readings of a pH meter being used simply to identify pairs of solutions having the same (unknown) hydrogen ion concentration. The work described in this chapter is based on an elaboration of this method.

Using the approach of Irving and Rossotti, the following solutions are titrated with standard base: (a) the solvent alone, (b) a solution of the ligand and (c) a solution of the ligand and central ion. Each of these solutions may also contain a strong acid and an inert salt to provide the desired initial pH and ionic strength. Writing a charge balance equation for any point on the curve for solution (a) yields

$$[H] = E + [OH] - Na \quad (IV-1)$$

where E represents the concentration of strong acid present initially diminished by dilution with titrant, Na is the concentration of strong base added up to that point on the curve and $[OH]$ accounts for hydrogen ion formed by ionization of the solvent or hydrolysis of any of the anions present. In this and subsequent equations ionic charges are omitted for the sake of clarity. A similar equation describing points on curve (b) is

$$[H]' = E' + [OH]' - Na' + yC_L' - \bar{\pi}_A' C_L' \quad (IV-2)$$

where the primes indicate that this refers to a different solution, y is the number of ionizable hydrogens per ligand initially and $\bar{\pi}_A$ is defined as the ratio of the total concentration of ionizable hydrogen bound to free ligand to the total concentration of free ligand; i. e., the average number of protons per uncomplexed ligand. Finally, for

points on curve (c)

$$[H]'' = E'' + [OH]'' - Na'' + yC_L'' - \bar{n}_A'' C_L'' + (\bar{n}_A'' - \bar{n}_H'') \bar{n}'' C_M'' \quad (IV-3)$$

where \bar{n}_H is defined as the ratio of the total concentration of ionizable hydrogen bound to the complex species to the total concentration of ligand in the complex species; i. e., the average number of protons per ligand in the complex species. In their paper Irving and Rossotti (20) considered only those ligands which lose no further protons after forming the complex, such as ethylenediamine, glycine or oxalic acid, so \bar{n}_H was identically equal to zero, or at least was constant so that y could be given a value to reflect only the number of protons lost during complex formation. The present treatment will lead to a more general equation, applicable also to larger, polyfunctional ligands having weakly acidic sites which are not occupied by the coordinated central ion, and hence can ionize after the complex is formed. Obviously if the concentration of hydrogen ion and of hydroxide ion were evaluated, \bar{n}_A and \bar{n} could be calculated directly from equations (IV-2) and (IV-3), provided \bar{n}_H could be evaluated, e. g. by working at a low enough pH that further ionization of the complexed ligand did not occur, or by determining the constant governing its ionization. Calculation of concentrations from activities is often difficult in solutions of practical interest however, so in the present approach this problem will be eliminated. Consider a

solution (a) and a solution (b) which give identical pH-meter readings. For them $H = H'$, where H indicates the activity of the hydrogen ion, and if, further, the two solutions have the same ionic strength, then $[H] = [H]'$ and $[OH] = [OH]'$. By making the initial concentrations of strong acid and the initial volumes equal, $E^o = E^{o'}$, $V^o = V^{o'}$, equations (IV-1) and (IV-2) can be combined to give

$$(E - E') - (Na - Na') = (y - \bar{n}'_A) C'_L \quad (IV-4)$$

Also,

$$E = E^o \left(\frac{V^o}{V^o + v} \right), \quad Na = \frac{Nv}{V^o + v}, \quad C'_L = C^o_L \left(\frac{V^o}{V^o + v'} \right) \quad (IV-5)$$

where N is the normality of the strong base used as titrant, v and v' are the volumes of titrant added to solutions (a) and (b) respectively, and C^o_L is the initial concentration of the ligand. Similar equations can be written for E' and Na' . Substituting equations (IV-5) into (IV-4) and rearranging terms gives

$$\bar{n}_A = y - \left(\frac{E^o + N}{C^o_L} \right) \left(\frac{v' - v}{V^o + v} \right) \quad (IV-6)$$

an equation involving only experimentally determined quantities.

Now consider a solution (b) and a solution (c), again with identical pH-meter readings and the same ionic strength. The equalities used in deriving equation (IV-6) will still exist. Furthermore, the quantity \bar{n}_A can be expressed

$$\bar{n}_A = \frac{\sum_0^M i [H_i L]}{\sum_0^M [H_i L]} = \frac{\sum_0^M i \beta_i^H H^i [L^{-M}]}{\sum_0^M \beta_i^H H^i [L^{-M}]} = \frac{\sum_0^M i \beta_i^H H^i}{\sum_0^M \beta_i^H H^i}$$

where β_i^H is the overall stability constant of $H_i L$, M is the number of ionizable protons in the free acid, and L^{-M} represents the fully ionized form of the ligand, so obviously \bar{n}_A is a function of hydrogen ion activity alone, and $\bar{n}_A' = \bar{n}_A''$. Making E^0 , C_L^0 and V^0 equal for the two solutions and combining equations (IV-2) and (IV-3) gives first

$$(E' - E'') - (Na' - Na'') = (y - \bar{n}_A') (C_L'' - C_L') + (\bar{n}_A' - \bar{n}_H'') \bar{n}_H'' C_M'' \quad (IV-7)$$

and after substitutions of the type used above and rearrangement,

$$\bar{n} = \left\{ \frac{E^0 + N + (y - n_A') C_L^0}{(\bar{n}_A' - \bar{n}_H'') C_M^0} \right\} \left(\frac{v'' - v'}{V^0 + v'} \right) \quad (IV-8)$$

another equation involving only experimentally determined quantities.

In the work described below, equation (IV-6) was used to relate \bar{n}_A to pH-meter readings and ultimately to relate the concentrations of the various protonated ligand species to these readings. Then equation (IV-8) served to define \bar{n} as a function of the meter readings, \bar{n}_A and \bar{n}_H . The last quantity was defined by a variety of functions, depending on the set of equilibria under consideration.

In this section identification of the pH-meter reading as "pH" has been carefully avoided, as the approach described and equations derived are equally valid for mixed or even some non-aqueous solvents where the usual pH scale is meaningless. All work described herein however was done in aqueous solution, so in subsequent sections this distinction will no longer be made.

Preparation and Standardization of Stock Solutions

1. Solutions of magnesium perchlorate, calcium perchlorate, strontium perchlorate and barium chloride were prepared and standardized as described on page 22 .

2. Potassium hydroxide, 0.15 M, was prepared by dissolving the reagent grade solid described on page 23 in recently boiled, distilled water. The solution was passed successively over columns of Dowex-50W (potassium form) and Dowex-2 (hydroxide form) into a polyethylene bottle protected with a soda lime tube. The solution was standardized by titration of dried potassium acid phthalate (Baker and Adams, Reagent) to a phenolphthalein end point.

3. Arsenazo, tripotassium salt, 0.03 M, was prepared by weighing the calculated quantity of the free acid dihydrate into a volumetric flask, adding a quantity of standard potassium hydroxide calculated to neutralize three of the hydrogen ions, and diluting to volume. The solution was standardized with respect to both arsenazo

and potassium by titrating potentiometrically with standard base and back titrating with standard acid.

4. Standardizing buffer, pH 10.80, to supplement the pH 7.00 and 9.20 buffers described on page 24 , was prepared from sodium bicarbonate (Baker and Adams, Reagent) and standard sodium hydroxide solution, following the directions given by Bates and Bower (5). The sodium ion concentration of the buffer was calculated and, from the nomogram accompanying the glass electrode used in the apparatus, a sodium ion correction of -0.02 was determined, giving the buffer an apparent pH of 10.78.

Apparatus

The titration vessel was prepared by removing the flare and pouring lip from a 250 ml. beaker. A #13 rubber stopper was cut in half along a circular cross section and the top portion bored to accommodate in the center a glass stirring rod with teflon blade fitted through a rubber seal (Kontes Kim-Kit Stirrer, K-30390). This was driven by an electric stirring motor. To one side, holes were bored to admit a glass electrode (Beckman General Purpose, 41262) and a reference electrode (Beckman, Fiber Junction Saturated Calomel, 39970). On the side opposite from the electrodes, a hole admitted the tip of a calibrated 10 ml. microburet, the end of which was just submerged at the beginning of a titration. Air leakage between

the stopper and the beaker was prevented by placing a rubber band 3/4" wide around the beaker and stopper covering their junction. The apparatus was submerged to within 1" of the top of the beaker in a Sargent thermostatic water bath with mercurial regulator (S-84805) which maintained the temperature at $25.00 \pm 0.05^{\circ}$.

pH measurements were taken with a Beckman Zeromatic pH meter, modified as described by Keegan and Matsuyama (24). The scale was expanded by connecting a decade resistance box across the thermocompensator terminals of the meter and setting the box for a resistance of 2072 ohms, calculated to provide a two-fold expansion of the scale. The asymmetry potential was set to make the left end of the scale correspond to pH 6.00, the right end then corresponding to pH 13.00. This arrangement permitted reading the scale with an uncertainty of ± 0.005 pH unit.

Experimental Procedure

A 10 ml. aliquot of the arsenazo stock was pipetted into the titration vessel, the required volume of cation stock solution added from a buret, and the solution brought to a total volume of 100 ml. with water from a pipet and a buret. The vessel was closed and placed in the water bath for 20 minutes to attain the equilibrium temperature. Then potassium hydroxide was added in 0.200 ml. increments, the pH being recorded after each addition. A stable pH was reached within

one to two minutes after each addition. Titration was continued to a total volume of 5.800 ml, corresponding to just over three moles of base per mole of arsenazo.

Titration were made with no cation stock added to determine the curve for arsenazo alone, and with such volumes of stock as would give mole ratios of arsenazo to central ion of 1:1 and 2:1. Also, two titrations were made of a solution containing only potassium perchlorate (0.0350 M) to provide the curve (a) needed for the calculation of \bar{n}_A . The choice of this particular concentration will be justified in the following section.

Before each titration the pH meter was set by means of the asymmetry potential control and if necessary minor changes of the setting of the decade resistance box to give correct readings from the pH 7.00 and 10.80 buffers. A reading was then taken for the pH 9.20 buffer. The correct reading was obtained in every case. After the titration, these readings were checked again. Agreement at the lower two pH values was excellent. The reading for 10.80 was always within ± 0.03 units. The buffers were brought to 25.00° before readings were made. In no case was a change of more than ± 15 ohms from the theoretical resistance box setting required for balance of the meter.

Data and Calculations for the Titration of Arsenazo

The data obtained by titration of a 0.0350 M potassium perchlorate solution, prepared by weighing the dried, reagent grade salt and dissolving in 100 ml. of water, and of an aliquot of the arsenazo stock are shown in Tables 17 and 18. The volumes shown are the volumes delivered, corrected for the initial buret reading and the buret error. Since the reading for each increment was made to coincide with a mark on the buret scale, the error associated with the buret reading is believed to be no greater than 0.001 ml. The titration data for arsenazo shows, as expected, only a single inflection point corresponding to neutralization of the fourth proton, as indicated in Table 18.

In order to obtain the volume difference ($v' - v$) needed for the calculation of \bar{n}_A from equation (IV-6), the pH values observed were plotted vs. volume of titrant on a large sheet of graph paper, and the volumes v and v' at pH intervals of 0.1 were read with the aid of a four-power magnifier. These volumes are considered in error by no more than ± 0.002 ml. for pH below about 10.8 and no more than ± 0.004 ml. above that value where the slope is diminished. The values for v and v' determined in this manner are shown in Table 19, along with the values of \bar{n}_A calculated from them. Standardization of the stock arsenazo solution showed it to be 0.02976 M

and to be 0.08848 M in potassium giving, in the solutions titrated,

$$C_L^O = 0.002971 \text{ M and } y = 2.973.$$

As a check on the values of \bar{n}_A found by titration of the stock solution, and particularly to define more clearly the value of \bar{n}_A below pH 7.5 and above 10.5, two samples of the free acid were dissolved in 100 ml. of water and titrated with standard potassium hydroxide. Since no comparison volumes (v) were available for calculation of \bar{n}_A in the manner used above, this quantity was calculated using a combination of equations (2) and (6) from the paper of Irving and Rossotti (20) with \bar{n} and E equal to zero:

$$\bar{n}_A = \frac{yC_L - Na + [OH] - [H]}{C_L} \quad (IV-9)$$

No attempt was made to calculate the activity coefficients of the hydrogen and hydroxide ions, the concentration of the former being taken as the antilogarithm of the pH meter reading and the latter being calculated from the ion product constant of water at 25°. For this reason, and because of the relative values of the terms added and subtracted in the calculation of \bar{n}_A , these values are considered more uncertain than those derived from equation (IV-6). Nevertheless, they agree reasonably well with the former values and so were incorporated into the \bar{n}_A vs. pH curve shown in Figure 13. Also, they were used in the calculation of the ionization constants of arsenazo described below. The data for these titrations and the \bar{n}_A

Table 17. Potentiometric titration of sodium perchlorate solution

Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH
0.000	6.95	1.195	11.24
0.100	10.09	1.394	11.30
0.199	10.45	1.594	11.36
0.398	10.75	1.793	11.41
0.598	10.92	1.992	11.45
0.797	11.06	2.192	11.50
0.996	11.15		

Table 18. Potentiometric titration of arsenazo solutions

Stock solution		Acid, Run 1		Acid, Run 2	
Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH
0.000	7.05	5.791	7.31	5.392	4.98
0.095	7.30	5.990	7.60	5.442	5.50
0.194	7.45	6.190	7.78	5.491	6.16
0.398	7.70	6.390	7.99	5.541	6.58
0.593	7.78	6.590	8.18	5.591	6.79
0.802	8.06	6.790	8.35	5.791	7.33
0.991	8.23	6.990	8.55	5.990	7.63
1.190	8.43	7.191	8.80	6.190	7.83
1.389	8.63	7.391	9.10	6.390	8.01
1.589	8.90	7.591	9.46	6.590	8.19
1.788	9.25	7.791	9.80	6.790	8.36
1.997	9.65	7.991	10.05	6.990	8.56
2.187	9.94	8.192	10.25	7.191	8.81
2.388	10.15	8.394	10.40	7.391	9.11
2.588	10.32	8.595	10.52	7.591	9.46
2.794	10.46	8.797	10.63	7.791	9.80
2.994	10.57	8.998	10.73	7.991	10.06
3.189	10.68	9.200	10.81	8.192	10.25
3.400	10.77	9.399	10.89	8.394	10.40
3.590	10.83	9.598	10.96	8.595	10.53
3.791	10.90	9.798	11.03	8.797	10.65
3.991	10.97	9.997	11.10	8.998	10.73
4.190	11.04	10.196	11.15	9.200	10.82
4.390	11.10	10.395	11.20	9.399	10.90
4.589	11.15	10.594	11.25	9.598	10.98
4.789	11.20	10.794	11.30	9.798	11.05
4.988	11.25	10.993	11.34	9.997	11.10
5.187	11.30	11.192	11.38	10.196	11.15
5.397	11.33	11.392	11.41	10.395	11.21
5.586	11.36	11.593	11.45	10.594	11.26
5.786	11.40	11.793	11.48	10.794	11.30
		11.994	11.50	10.993	11.35
Sample weight (mg)		175.2		175.3	

Table 19. Calculation of \bar{n}_A from titration of arsenazo solutions

Stock solution				Run 1		Run 2	
pH	v'(ml)	v(ml)	\bar{n}_A	pH	\bar{n}_A	pH	\bar{n}_A
7.20	0.050	0.005	2.949	7.31	2.931	4.98	3.141
7.30	0.095	0.008	2.927	7.60	2.825	5.50	3.117
7.40	0.150	0.011	2.899	7.78	2.720	6.16	3.092
7.50	0.215	0.014	2.866	7.99	2.614	6.58	3.065
7.60	0.280	0.017	2.833	8.18	2.508	6.79	3.039
7.70	0.375	0.020	2.784	8.35	2.402	7.33	2.933
7.80	0.490	0.023	2.725	8.55	2.297	7.63	2.828
7.90	0.615	0.026	2.660	8.80	2.191	7.83	2.722
8.00	0.745	0.029	2.592	9.10	2.087	8.01	2.616
8.10	0.860	0.032	2.533	9.48	1.987	8.19	2.510
8.20	0.970	0.035	2.476	9.80	1.894	8.36	2.405
8.30	1.075	0.038	2.421	10.05	1.805	8.56	2.299
8.40	1.180	0.041	2.367	10.25	1.723	8.81	2.194
8.50	1.280	0.044	2.316	10.40	1.642	9.11	2.090
8.60	1.370	0.046	2.269	10.52	1.565	9.46	1.990
8.70	1.445	0.049	2.231	10.63	1.493	9.80	1.897
8.80	1.520	0.052	2.192	10.73	1.427	10.06	1.809
8.90	1.585	0.055	2.159	10.81	1.360	10.25	1.726
9.00	1.655	0.058	2.124	10.89	1.303	10.40	1.646
9.10	1.715	0.061	2.093	10.96	1.248	10.53	1.571
9.20	1.765	0.064	2.069	11.03	1.202	10.65	1.504
9.30	1.815	0.067	2.044	11.10	1.166	10.73	1.431
9.40	1.865	0.070	2.019	11.15	1.118	10.82	1.369
9.50	1.915	0.073	1.994	11.20	1.077	10.90	1.313
9.60	1.965	0.075	1.968	11.25	1.044	10.98	1.268
9.70	2.020	0.078	1.941	11.30	1.020	11.05	1.224
9.80	2.085	0.081	1.908	11.34	0.988	11.10	1.170
9.90	2.160	0.084	1.869	11.38	0.962	11.15	1.122
10.00	2.235	0.087	1.831	11.41	0.922	11.21	1.095
10.10	2.325	0.090	1.785	11.45	0.910	11.26	1.064
10.20	2.430	0.112	1.741	11.48	0.882	11.30	1.024
10.30	2.555	0.140	1.690	11.50	0.830	11.35	1.011
10.40	2.690	0.175	1.637			11.38	0.966
10.50	2.850	0.220	1.577			11.42	0.948
10.60	3.040	0.275	1.506				
10.70	3.250	0.345	1.433				
10.80	3.500	0.440	1.352				
10.90	3.775	0.570	1.277				
11.00	4.050	0.715	1.211				

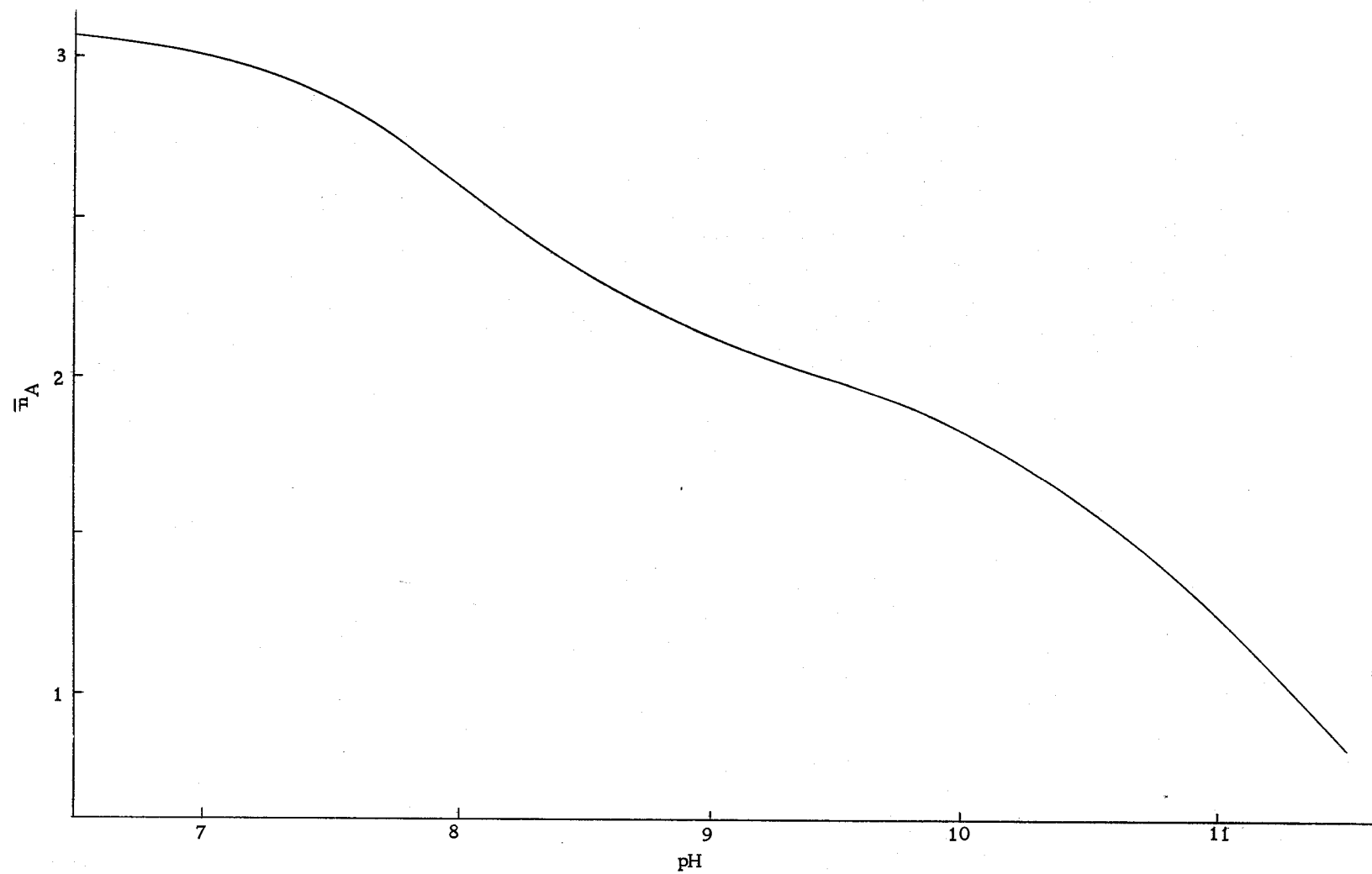


Figure 13. Average Number of Protons on a Free Arsenazo Ion as a Function of pH

values calculated from them are given in Table 19.

For a portion of this work to be described in a later section, it was necessary to relate the concentrations of the various protonated species of the free ligand to the pH meter readings. To this end, practical constants for the last three steps of the acid dissociation of arsenazo were determined. These constants, sometimes called Brønsted dissociation constants, involving as they do the activity of the hydrogen ion and the molarity of various arsenazo species will only be valid for the temperature and ionic strength for which they are determined.

Approximate values of the acid dissociation constants, K_i^a can be read from Figure 13, the pH at $\bar{n}_A = 6.5 - i$ being approximately equal to pK_i^a for integral i . This gives values of 8.2 and 10.6 for pK_4^a and pK_5^a respectively. Since the inflection point at \bar{n}_A is relatively well defined, for \bar{n}_A less than about 2.8 the concentration of the species H_4L^{-2} should be negligible relative to the total concentration of all arsenazo species. Also, from the approximate value of pK_5^a it follows that for a pH below about 8.6 the concentration of HL^{-5} is also negligible. Therefore,

$$[H_3L] + [H_2L] = C_L \quad (IV-10)$$

and

$$3[H_3L] + 2[H_2L] = \bar{n}_A C_L \quad (IV-11)$$

Combining these equations yields

$$\frac{[H_2L]}{[H_3L]} = \frac{3 - \bar{n}_A}{\bar{n}_A - 2} \quad (IV-12)$$

and since

$$pH = pK_4^a + \log \left(\frac{[H_2L]}{[H_3L]} \right) = pK_4^a + \log \left(\frac{3 - \bar{n}_A}{\bar{n}_A - 2} \right) \quad (IV-13)$$

a plot of $\log \left(\frac{3 - \bar{n}_A}{\bar{n}_A - 2} \right)$ vs. pH should give a straight line of unit slope with intercept (where the log term is zero) equal to pK_4^a . Curvature at either end of the range would indicate the assumptions concerning the concentrations of other species are invalid. In addition one can calculate, for each experimental point, a value of pK_4^a and from the mean and standard deviation of these values, calculate a confidence interval for the constant. These calculations were done, the results being shown in Table 20 and Figure 14.

Examination of Figure 13, gives little hope that there will be any region where only the two species H_2L^{-4} and HL^{-5} will exist in appreciable concentration. Therefore, a more sophisticated method must be used for the calculation of the remaining two dissociation constants. From the value of K_4^a found above, it can be shown that above pH 10.2 there will be no appreciable concentration of H_3L^{-3} . It follows then from the definition of \bar{n}_A that

Table 20. Calculation of K_4^a from titration data

	pH	$\log\left(\frac{3-n_A}{n_A-2}\right)$	pK_4^a	$K_4^a \times 10^9$
Stock Solution	7.80	-0.421	8.221	6.012
	7.90	-0.288	8.188	6.486
	8.00	-0.162	8.162	6.887
	8.10	-0.054	8.157	6.966
	8.20	0.042	8.158	6.951
	8.30	0.138	8.162	6.887
	8.40	0.237	8.163	6.871
	8.50	0.336	8.164	6.855
	8.60	0.434	8.166	6.823
Run 1	7.78	-0.410	8.190	6.457
	7.99	-0.202	8.192	6.427
	8.18	-0.014	8.194	6.397
	8.35	0.172	8.178	6.637
	8.55	0.374	8.176	6.668
	8.80	0.627	8.173	6.714
Run 2	7.83	-0.414	8.244	5.702
	8.01	-0.205	8.215	6.095
	8.19	-0.017	8.207	6.209
	8.36	0.167	8.193	6.412
	8.56	0.370	8.190	6.457
	8.81	0.618	8.192	6.427
Mean $K_4^a = 6.540 \times 10^{-9}$				
± 0.154 (95% C. L.)				

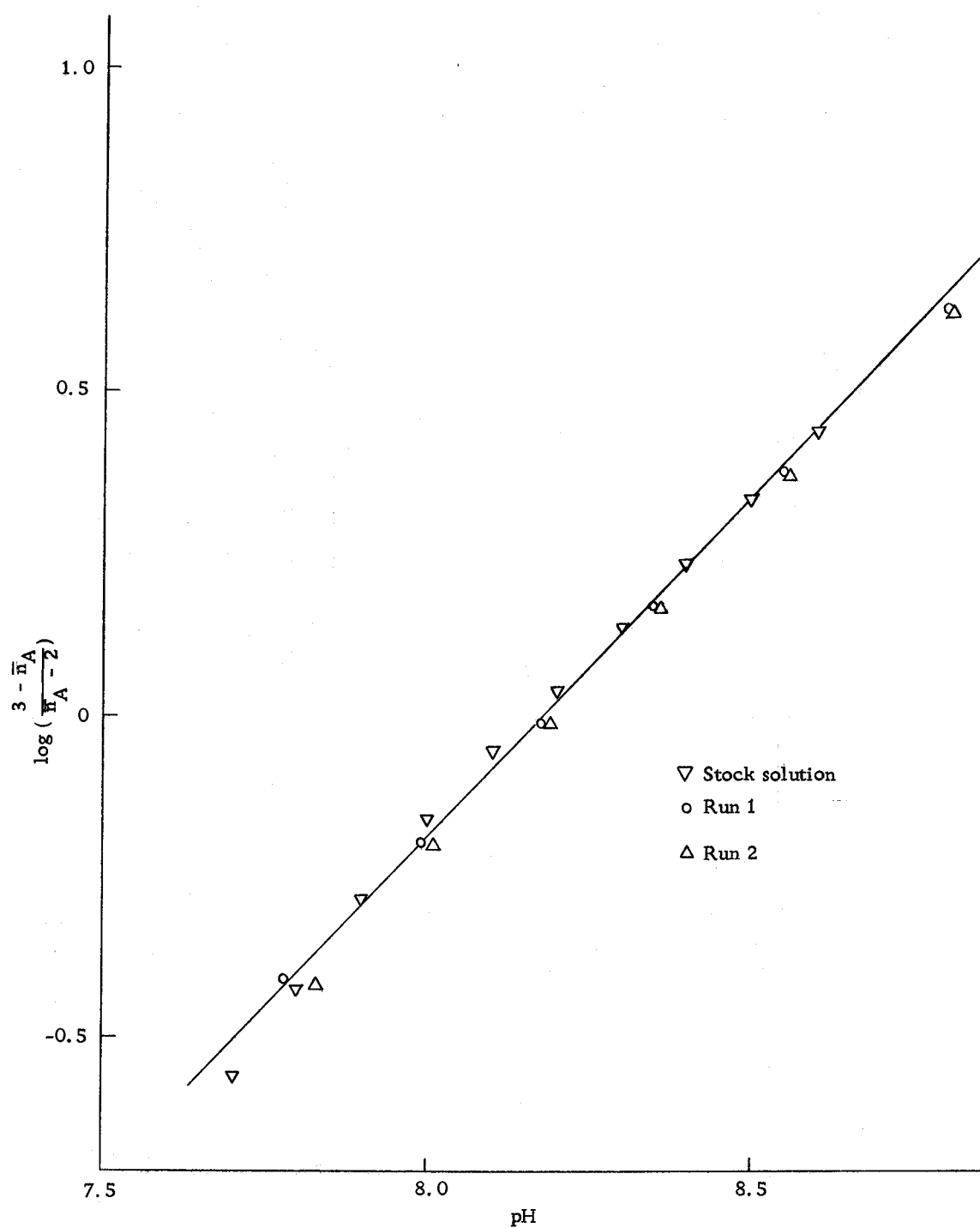


Figure 14. Graphical Determination of K_4^a

$$\bar{n}_A = \frac{2[H_2L] + [HL]}{[H_2L] + [HL] + [L]} = \frac{2H^2 + K_5^a H}{H^2 + K_5^a H + K_5^a K_6^a} \quad (\text{IV-14})$$

where H is the hydrogen ion activity. Equation (IV-14) can be rewritten

$$(\bar{n}_A - 2)H^2 + (\bar{n}_A - 1)HK_5^a + \bar{n}_A K_5^a K_6^a = 0 \quad (\text{IV-15})$$

or written in a form amenable to a linear least squares treatment

$$\left(\frac{2 - \bar{n}_A}{\bar{n}_A} \right) H^2 = \left(\frac{\bar{n}_A - 1}{\bar{n}_A} \right) HK_5^a + K_5^a K_6^a \quad (\text{IV-16})$$

The functions of \bar{n}_A and H necessary to determine the two dissociation constants are shown in Table 21. The usual linear least squares treatment gave values of K_5^a and the product $K_5^a K_6^a$ as also shown in Table 21. The uncertainties in these two quantities were determined by using the best value of one to calculate individual values of the other, determining the standard deviation of these individual values and thence calculating a confidence interval for the quantity. The constant K_6^a was then evaluated as the quotient of the two quantities determined by the least squares treatment, and its confidence interval taken as the root-mean-square of the other two confidence intervals. The final values of the K_5^a and K_6^a and the confidence interval of each are given in Table 21.

As the investigation developed, it became apparent that the

Table 21.. Calculation of K_5^a and K_6^a from titration data

	pH	$\left(\frac{2-\bar{n}_A}{\bar{n}_A}\right) H^2 \times 10^{22}$	$\left(\frac{\bar{n}_A-1}{\bar{n}_A}\right) H \times 10^{11}$	
Stock	10.40	3.504	1.549	
Solution	10.50	2.682	1.156	
	10.60	2.070	0.843	
	10.70	1.575	0.601	
	10.80	1.203	0.411	
	10.90	0.895	0.273	
	11.00	0.652	0.174	$K_5 = (2.00 \pm .061) \times 10^{-11}$
	11.10	0.489	0.090	
	11.20	0.359	0.031	$K_5 K_6 = (2.70 \pm .21) \times 10^{-23}$
	11.30	0.276	-0.025	
	11.40	0.212	-0.067	$K_6 = (1.35 \pm .11) \times 10^{-12}$
Run 1	10.25	5.083	2.360	(95% C. L.)
	10.40	3.456	1.557	
	10.52	2.535	1.090	
	10.63	1.866	0.774	
	10.73	1.392	0.557	
	10.81	1.129	0.910	
	10.89	0.887	0.300	
	10.96	0.724	0.218	
	11.03	0.578	0.157	
	11.10	0.451	0.113	
	11.15	0.395	0.075	
Run 2	10.25	5.020	2.365	
	10.40	3.409	1.562	
	10.53	2.378	1.073	
	10.65	1.653	0.750	
	10.73	1.379	0.561	
	10.82	1.056	0.408	
	10.90	0.829	0.300	
	10.98	0.633	0.221	
	11.05	0.504	0.163	
	11.10	0.448	0.115	
	11.15	0.392	0.077	

species H_2L^{-4} and HL^{-5} both would have to be considered as potentially the coordinating form of the ligand. Expressions for the mole fraction of the free ligand in each of these two forms, as a function of pH were derived. By definition

$$X_{H_2L} = \frac{[H_2L]}{[H_3L] + [H_2L] + [HL] + [L]} \quad (IV-17)$$

Taking the reciprocal of the mole fraction gives a sum of ratios of the concentrations of the various species of the ligand.

$$\frac{1}{X_{H_2L}} = \frac{[H_3L]}{[H_2L]} + 1 + \frac{[HL]}{[H_2L]} + \frac{[L]}{[H_2L]} \quad (IV-18)$$

Recalling that the practical constants determined above refer to equilibrium expressions in hydrogen ion activity but ligand concentrations, the expressions

$$\frac{[H_2L] H}{[H_3L]} = K_4^a, \quad \frac{[HL] H}{[H_2L]} = K_5^a, \quad \frac{[L] H}{[HL]} = K_6^a \quad (IV-19)$$

can be written. Substituting equations (IV-19) into (IV-18) gives the desired expression, a function of constant terms and hydrogen ion activity only.

$$\frac{1}{X_{H_2L}} = \frac{H}{K_4^a} + 1 + \frac{K_5^a}{H} + \frac{K_5^a K_6^a}{H^2} \quad (IV-20)$$

A similar treatment, beginning with the defining expression of the

mole ratio of the species HL^{-5} gives the end result

$$\frac{1}{X_{HL}} = \frac{H^2}{K_4^a K_5^a} + \frac{H}{K_5^a} + 1 + \frac{K_6^a}{H} \quad (IV-21)$$

Whenever in subsequent calculations mole fractions of the ligand species were required, they were calculated from these equations.

Several matters related to the ionic strength of the solutions titrated in this part of the investigation should be discussed at this point. Opinion is divided regarding the desirability of using a large excess of an inert salt to hold constant the ionic strength, and hence the activity coefficients of the ionic species in solution (e. g., 53, p. 17-27; 2, p. 57, 157). In this work the presence of a relatively large concentration of an inert electrolyte was avoided because, no extrapolation to zero ionic strength being anticipated, an ionic strength as low as practicable was desirable to minimize departure of the calculated values of the equilibrium constants from the values of the corresponding thermodynamic constants. The particular concentrations of ligand and central ion used were chosen so as to coincide as nearly as possible with the ionic strength of the buffered solutions used in the spectrophotometric measurements, 0.0364, while still providing data for concentrations 20 to 40 times as great as any used in the former series of solutions. Maintaining comparable ionic strengths between the two series of measurements permitted meaningful comparison of the constants derived by the two methods.

In the absence of a bulk electrolyte the ionic strength of a solution of an acid will change as the acid is neutralized. The change may be even greater if complex ion formation leads to the existence of ionic species with relatively large charges. Since the method used in evaluating the titration data assumes identical ionic strengths, at least for solutions with the same pH, the effect on the calculated quantities of a difference in the ionic strengths of two solutions being compared had to be determined.

Given two solutions with the same pH but different ionic strengths, $H_1 = H_2$, but since the activity coefficients are different, $[H]_1 \neq [H]_2$ and $[OH]_1 \neq [OH]_2$. These inequalities lead to an error in the value of \bar{n}_A or \bar{n} calculated for this pH. The error may be considered as an error in the term $(v_2 - v_1)$ since at some other volume v_1 (and other pH_1) the two hydrogen ion concentrations will be equal, and the two hydroxide concentrations very nearly so. Let pH_2 represent the pH at which \bar{n}_A or \bar{n} is to be evaluated and pH_1 the pH of the comparison solutions having the same hydrogen concentration. Then $H_1/f_1^+ = H_2/f_2^+$, and $\Delta pH = pH_2 - pH_1 = \log H_1 - \log H_2 = \log f_2^+ - \log f_1^+$. The error in the volume difference can be calculated by dividing ΔpH by the slope of titration curve 1 at pH_1 .

To determine the effect of an error, Δ , in the volume difference $(v' - v)$ on the value of \bar{n}_A calculated for that difference, substitute the quantity $(v' - v + \Delta)$ into equation (IV-6) and subtract from the

result equation (IV-6) in its present form. After simplification the result is

$$\Delta \bar{n}_A = \frac{N}{C_L^0 (V^0 + v)} \Delta \quad (\text{IV-22})$$

In order to determine the magnitude of the errors to be expected in the solutions titrated, the ionic strengths of the potassium perchlorate solution and the arsenazo solution were calculated for pH 7.3, 9.3, 10.5 and 11.3. These values correspond closely to the initial solutions, the inflection point for the arsenazo curve, and points 0.5 and 1.75 equivalents of base beyond the inflection point respectively. These points were chosen to cover the entire pH range of interest, yet to give easily estimated concentrations of the various ionic species. Values of $\log f^+$ were calculated for the solutions using a form of the Debye-Hückel equation, with the constants given by Moore (47, p. 464).

$$\log f^+ = - \frac{0.509 \sqrt{\mu}}{1 + 3.0 \sqrt{\mu}} \quad (\text{IV-23})$$

Values of $\frac{dpH}{dv}$ and \bar{n}_A were estimated from Table 17 and Figure 13 respectively, and first Δ , then $\Delta \bar{n}_A$, and finally the relative error in \bar{n}_A were calculated. The results are shown in Table 22A. Obviously the error is completely negligible up to pH 10.5, and even at the upper end of the pH range the error is not intolerable.

In assessing the effect of ionic strength changes on the calculated values of \bar{n} consideration must be given both to the effect of

an error in the volume difference ($v'' - v'$), represented by the symbol Δ' , and also to any effect the error $\Delta \bar{n}_A$ may have. To determine the former effect, substitute for $(v'' - v')$ in equation (IV-8) the quantity $(v'' - v' + \Delta')$ and then subtract from the result equation (IV-8) in its present form. After combination of terms and simplification the result is

$$\Delta \bar{n} = \frac{N + (y - \bar{n}'_A) C_L^O}{(n'_A - \bar{n}_H) C_M^O (V^O + v')} \Delta' \quad (\text{IV-24})$$

The magnitude of the errors was estimated by calculating ionic strengths of the 1:1 and 2:1 solutions at pH 7.3, 9.3, and 11.3. Complete dimerization was assumed giving the maximum possible ionic strength. Greater differences from the corresponding values of the arsenazo solutions were observed for the 1:1 solution, so only that set of values was used in further calculations. Also, only the data from the titration of the barium-arsenazo solution were used as barium, forming the weakest complex, has the smallest values of $(v'' - v')$, so is most susceptible to errors, Δ' . Values of $\log f^+$ and ΔpH were calculated as before, those of $\frac{d\text{pH}}{dv'}$ and \bar{n}_A were estimated from Table 18 and Figure 13 respectively, and values of \bar{n} and \bar{n}_H were inferred from the shape of the barium-arsenazo titration curve. The results of these calculations are shown in Table 22B. Again, the relative error introduced by variation of ionic

strength is negligible.

The importance of the second source of error in \bar{n} , the carry-over of error in \bar{n}_A can be examined by substituting $(\bar{n}_A + \Delta \bar{n}_A)$ for \bar{n}_A in equation (IV-8) and subtracting from the result the present form of equation (IV-8). After simplification it is found that

$$\Delta \bar{n} = \frac{(v'' - v')}{C_M^0 (V^0 + v')} \left\{ \frac{N + (y - \bar{n}_H) C_L^0}{(\bar{n}_A - \bar{n}_H)^2 + \Delta \bar{n}_A (\bar{n}_A - \bar{n}_H)} \right\} (-\Delta \bar{n}_A) \quad (\text{IV-25})$$

The magnitude of the error in \bar{n} arising from error in \bar{n}_A was evaluated by again using $(v'' - v')$ from the barium data, \bar{n}_A from Figure 13, estimated values of \bar{n} and \bar{n}_H , and the values of $\Delta \bar{n}_A$ found in Table 22A. The results of the calculation are given in Table 22C. Again, the relative errors are negligible.

One last aspect of the ionic strength problem should be considered; the effect that change of this quantity during a given titration has on the constancy of the stoichiometric equilibrium constants being evaluated. In view of the large charges present on some of the ionic species and the fact that appropriate values of the size parameter for the various arsenazo and complex species are unknown, calculation of activity coefficients from the Debye-Hückel equation seems impracticable. During a titration, ionic strength increases from 0.018 to 0.056 for arsenazo alone, 0.024 to 0.053 for the 1:1

Table 22. Analysis of errors due to variation of ionic strength during titrations

A. Effect on \bar{n}_A of an error in $(v' - v)$

pH	$\log f_2^+$	$\log f_1^+$	ΔpH	$\frac{d\text{pH}}{dv}$	Δ	$\Delta\bar{n}_A$	\bar{n}_A	Relative Error (%)
7.3	-0.0485	-0.0610	0.0125	37.2	0.00034	0.00018	2.94	0.0061
9.3	-0.0578	-0.0610	0.0032	37.0	0.00009	0.00005	2.04	0.0025
10.5	-0.0616	-0.0611	-0.0005	2.14	-0.00023	-0.00012	1.59	-0.0076
11.3	-0.0705	-0.0619	-0.0086	0.30	-0.0286	-0.015	0.95	-1.58

B. Effect on \bar{n} of an error in $(v'' - v')$

pH	$\log f_2^+$	$\log f_1^+$	ΔpH	$\frac{d\text{pH}}{dv'}$	Δ'	$\Delta\bar{n}$	\bar{n}	Relative Error (%)
7.3	-0.0539	-0.0485	-0.0054	1.85	-0.0029	0.00053	ca. 0	-
9.3	-0.0600	-0.0578	-0.0032	1.34	-0.0016	0.00082	1.0	0.082
11.3	-0.0694	-0.0705	0.0011	0.238	0.0046	-0.0046	1.0	-0.46

C. Effect on \bar{n} of an error in \bar{n}_A

pH	$v'' - v'$	\bar{n}_A	\bar{n}_H	$\Delta\bar{n}_A$	$\Delta\bar{n}$	\bar{n}	Relative Error (%)
7.3	0.278	2.94	2.0	0.00018	-0.00003	ca. 0	--
9.3	1.491	2.04	1.0	0.00005	-0.00004	1.0	0.004
11.3	0.202	1.02	0.13	-0.015	0.0020	1.0	0.20

solutions, and 0.021 to 0.045 for the 2:1 solutions. Intuitively such changes would not be expected to alter significantly the values of the equilibrium constants which involve only ionic species in both numerator and denominator. The only obvious test for this assumption is the absence of any pronounced trend in the values of the constants calculated for the individual experimental points.

Data and Calculations for the Titration of Arsenazo Plus Central Ions

The data obtained by titration with potassium hydroxide of a solution 0.002971 M in arsenazo and 0.003000 M in the alkaline earth ion and a second solution with the same C_L^O but with C_M^O equal to 0.001500 M are given in Tables 23, 24, 25, and 26 for magnesium, calcium, strontium and barium respectively. The volumes shown are the volumes delivered, corrected for the initial buret reading and the buret error.

For each curve a single inflection point was observed. The volume of titrant used at the inflection point was calculated by interpolation of the second derivative of the curve, calculated by divided differences, to a zero value. The pH corresponding to that volume was calculated by linear interpolation within the interval containing the equivalent volume. The total moles of base added per mole of arsenazo, including that added in the preparation of the stock solution,

Table 23. Potentiometric titration of magnesium-arsenazo solutions

1:1		2:1	
Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH
0.000	6.38	0.000	6.57
0.181	6.64	0.190	6.85
0.380	6.81	0.389	7.04
0.580	6.94	0.589	7.18
0.779	7.05	0.798	7.31
0.978	7.14	0.987	7.42
1.177	7.22	1.186	7.54
1.385	7.30	1.390	7.65
1.576	7.39	1.585	7.77
1.775	7.46	1.784	7.90
1.974	7.55	1.983	8.07
2.174	7.65	2.183	8.27
2.375	7.74	2.384	8.53
2.575	7.85	2.584	8.90
2.776	7.98	2.785	9.39
2.976	8.13	2.985	9.87
3.176	8.35	3.185	10.21
3.397	8.65	3.386	10.43
3.577	9.15	3.586	10.60
3.778	9.79	3.787	10.74
3.978	10.315	3.987	10.85
4.177	10.62	4.186	10.95
4.377	10.80	4.386	11.04
4.576	10.95	4.585	11.11
4.781	11.04	4.785	11.18
4.975	11.13	4.994	11.24
5.284	11.20	5.183	11.30
5.374	11.25	5.383	11.34
5.573	11.30	5.582	11.38
5.773	11.35	5.782	11.43

Table 24. Potentiometric titration of calcium-arsenazo solutions

1:1		2:1	
Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH
0.020	6.64	0.005	6.75
0.199	6.85	0.189	7.01
0.398	7.03	0.388	7.20
0.598	7.15	0.588	7.35
0.797	7.26	0.787	7.46 ₅
1.006	7.36	0.986	7.59
1.195	7.44	1.185	7.70
1.394	7.53	1.384	7.80
1.594	7.62	1.584	7.92
1.793	7.70	1.783	8.05
1.992	7.79	1.982	8.20
2.192	7.88 ₅	2.182	8.37
2.393	7.99	2.383	8.60
2.598	8.10	2.593	8.94
2.799	8.25	2.789	9.38
2.994	8.42	2.984	9.81
3.194	8.62 ₅	3.184	10.15
3.395	8.93	3.385	10.37
3.595	9.35	3.585	10.55
3.796	9.89	3.786	10.70
3.996	10.32	3.986	10.80
4.195	10.61	4.195	10.90
4.400	10.80	4.385	10.99
4.594	10.93	4.584	11.07
4.804	11.04	4.784	11.14
4.993	11.13	4.983	11.20
5.192	11.20	5.182	11.25
5.392	11.25	5.382	11.30
5.591	11.31	5.581	11.35
5.791	11.36	5.791	11.39

Table 25. Potentiometric titration of strontium-arsenazo solutions

1:1		2:1	
Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH
0.015	6.80	0.000	6.87
0.194	7.08	0.199	7.20
0.393	7.29	0.398	7.40
0.603	7.44	0.598	7.56
0.792	7.55	0.797	7.70
0.991	7.66	0.996	7.81 ₅
1.190	7.76	1.195	7.93
1.399	7.85	1.394	8.04
1.589	7.95	1.594	8.16
1.793	8.05	1.793	8.29
1.987	8.14	1.992	8.43
2.187	8.25	2.192	8.60
2.388	8.36	2.393	8.80
2.588	8.49	2.593	9.07
2.798	8.63	2.799	9.44
2.989	8.80	2.994	9.84
3.189	9.02	3.194	10.16
3.400	9.30	3.395	10.40
3.590	9.65	3.595	10.57
3.791	10.06	3.796	10.72
3.991	10.43	3.996	10.83
4.190	10.68	4.195	10.93
4.400	10.85	4.405	11.02
4.589	10.98	4.604	11.10
4.789	11.10	4.794	11.16 ₅
4.988	11.17	4.993	11.23
5.187	11.24	5.192	11.28
5.392	11.30	5.392	11.33
5.586	11.35	5.591	11.37
5.786	11.40	5.791	11.41

Table 26. Potentiometric titration of barium-arsenazo solutions

1:1		2:1	
Volume of Titrant (ml)	pH	Volume of Titrant (ml)	pH
0.020	6.84	0.000	6.92
0.159	7.07	0.199	7.25
0.388	7.33	0.398	7.46 ₅
0.588	7.48	0.598	7.63
0.787	7.61 ₅	0.797	7.76
0.991	7.73 ₅	0.996	7.88
1.185	7.85	1.195	8.00
1.384	7.94	1.394	8.12
1.584	8.03	1.594	8.25
1.783	8.13	1.793	8.37
1.982	8.24	1.992	8.53
2.182	8.34	2.192	8.69
2.383	8.46	2.393	8.90
2.588	8.60	2.593	9.18
2.784	8.74	2.794	9.52
2.984	8.92	2.994	9.90
3.184	9.13	3.194	10.21
3.385	9.40	3.395	10.42
3.585	9.72	3.595	10.60
3.786	10.10	3.796	10.73
3.986	10.44	3.996	10.85
4.186	10.69	4.200	10.96
4.385	10.85	4.395	11.02
4.584	10.99	4.594	11.10
4.784	11.10	4.794	11.18
4.983	11.17	4.993	11.25
5.182	11.25	5.192	11.30
5.392	11.30	5.392	11.34
5.581	11.36	5.591	11.38 ₅
5.781	11.41	5.791	11.42

was also calculated. This ratio, r , and the pH at the equivalence point are given in Table 27. In each case, the 1:1 mixture of arsenazo and central ion has reacted, by the time the inflection point is reached, with two equivalents of base. The 2:1 mixture has reacted with 1.5 equivalents of base. The results for the 1:1 solutions are in excellent agreement. For the 2:1 solutions strontium and barium appear to have reacted with a little more than 1.5 equivalents of base, 1.553 and 1.552 being observed respectively. There is no obvious explanation for this behavior, although the discrepancy seems too large to be charged solely to experimental error.

The ratios, r , observed can be explained by the formation of a 1:1 complex either with the simultaneous ionization of two protons from the ligand or with the release of one proton upon complex formation followed by the ionization of a second proton from the complex, this latter ionization being governed by an equilibrium constant of such magnitude that the complex formation and the subsequent ionization occur over the same pH range. This mechanism also explains the r observed for the 2:1 solution, as the complexation would cause the release of two protons from half the arsenazo present, while the second half of the ligand, uncomplexed, would lose one proton, as the free ligand has been shown to do over this same pH range. This would give a value of r equal to 1.5. No evidence is seen for the formation of a 2:1 complex, in agreement with the spectrophotometric

data. One other mechanism which could explain the neutralization of two equivalents of base by the 1:1 solution is the formation of a complex species MH_3L^- followed by ionization of two protons from the complex, with acid dissociation constants sufficiently closely spaced that only a single inflection point is observed. This mechanism is thought highly unlikely, as the absorption spectra discussed in Chapter III indicated little complex formation even at pH 8, whereas the acid dissociation constants for arsenazo determined by Klygin and Pavlova (28) indicate that H_3L^{-3} is the major arsenazo species at a pH as low as 3. If this were the reacting species, complex formation should begin at a much lower pH. Further consideration was not given this mechanism.

Table 27. Summary of mole ratios of base to arsenazo. pH at inflection points

Cation	L:M	r	pH
None	-	4.013	9.37
Mg	1:1	4.998	9.34
	2:1	4.505	9.35
Ca	1:1	4.996	9.62
	2:1	4.504	9.35
Sr	1:1	5.001	9.90
	2:1	4.553	9.58
Ba	1:1	4.999	9.95
	2:1	4.552	9.67

Calculations from the titration for the arsenazo-cation solutions were made using equation (IV-8), with E^0 equal to zero. For each

point, values of v' and \bar{n}_A were read from large plots of the titration curve for arsenazo and the curve relating \bar{n}_A to pH, using a magnifier. These values are uncertain to no more than ± 0.002 and ± 0.001 ml, respectively. Of the terms in the right side of equation (IV-8), only \bar{n}_H could not be definitely evaluated at this point. The appropriate values of it can be determined only in terms of the mechanism under consideration. Therefore equation (IV-8) was rewritten in the form

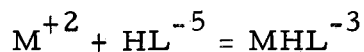
$$\bar{n} = \frac{D}{\bar{n}_A - \bar{n}_H} \quad (\text{IV-25})$$

where all the experimental quantities are lumped into the term D .

The quantities \bar{n}_A and D for the four cations, with the associated pH values are tabulated in Table 28.

Mechanism 1

The simplest of the mechanisms discussed above can be represented by the equation



For this mechanism \bar{n}_H is unity for all points sufficiently below the inflection point that further ionization to ML^{-4} may be neglected.

As shown on page 43, for a reaction of this form the equation

$$\log \left(\frac{\bar{n}}{1 - \bar{n}} \right) = \log K_1 + \log [HL] \quad (\text{IV-26})$$

Table 28. Data for calculations of \bar{n} and \bar{n}_A for magnesium, calcium, strontium and barium

	Magnesium			Calcium			Strontium			Barium		
	pH	\bar{n}_A	D	pH	\bar{n}_A	D	pH	\bar{n}_A	D	pH	\bar{n}_A	D
1:1	7.05	2.997	0.4197	7.03	3.007	0.2223	7.08	2.992	0.1064	7.07	2.995	0.0890
	7.14	2.981	0.5069	7.15	2.978	0.3035	7.29	2.945	0.1607	7.33	2.932	0.1464
	7.22	2.964	0.5936	7.26	2.953	0.3820	7.44	2.895	0.2207	7.48	2.879	0.1981
	7.30	2.942	0.6817	7.36	2.923	0.4624	7.55	2.850	0.2766	7.61 ₅	2.821	0.2461
	7.39	2.913	0.7511	7.44	2.894	0.5330	7.66	2.799	0.3325	7.73	2.764	0.2966
	7.46	2.886	0.8316	7.53	2.857	0.6022	7.76	2.749	0.3869	7.85	2.699	0.3345
	7.55	2.850	0.8988	7.62	2.818	0.6698	7.85	2.698	0.4462	7.94	2.643	0.3841
	7.65	2.804	0.9609	7.70	2.779	0.7350	7.95	2.637	0.4847	8.03	2.586	0.4320
	7.74	2.758	1.0211	7.79	2.732	0.7921	8.05	2.573	0.5295	8.13	2.525	0.4755
	7.85	2.698	1.0660	7.88 ₅	2.678	0.8430	8.14	2.519	0.5819	8.24	2.460	0.5181
	7.98	2.617	1.0919	7.99	2.612	0.8836	8.25	2.454	0.6187	8.34	2.403	0.5654
	8.13	2.525	1.1038	8.10	2.542	0.9242	8.36	2.393	0.6625	8.46	2.343	0.6065
	8.35	2.400	1.0828	8.25	2.432	0.9421	8.49	2.327	0.6979	8.60	2.277	0.6459
				8.42	2.363	0.9490	8.63	2.264	0.7368	8.74	2.218	0.6881
				8.62 ₅	2.266	0.9535						
2:1	7.04	3.000	0.4309	7.01	3.006	0.2329	7.20	2.967	0.1655	7.25	2.956	0.1380
	7.18	2.972	0.5848	7.20	2.967	0.3645	7.40	2.909	0.2571	7.46 ₅	2.884	0.2055
	7.31	2.938	0.7553	7.35	2.926	0.4909	7.56	2.845	0.3414	7.63	2.813	0.2804
	7.42	2.902	0.8626	7.46 ₅	2.885	0.6165	7.70	2.780	0.4204	7.76	2.749	0.3594
	7.54	2.853	0.9782	7.59	2.832	0.7225	7.81 ₅	2.718	0.5101	7.88	2.681	0.4300
	7.65	2.803	1.0955	7.70	2.779	0.8294	7.93	2.650	0.5807	8.00	2.605	0.4952
	7.77	2.743	1.1772	7.80	2.726	0.9353	8.04	2.580	0.6555	8.12	2.531	0.5541
	7.90	2.669	1.2392	7.92	2.656	1.0030	8.16	2.507	0.7168	8.25	2.453	0.6152
	8.07	2.560	1.2360	8.05	2.573	1.0464	8.29	2.413	0.7764	8.37	2.387	0.6856
	8.27	2.442	1.2129	8.20	2.482	1.0823	8.43	2.357	0.8340	8.53	2.308	0.7292
				8.37	2.386	1.0955						

must be valid, i. e. a plot of $\log \left(\frac{\bar{n}}{1 - \bar{n}} \right)$ vs. $\log [HL]$ must be linear with unit slope. To test this mechanism \bar{n} was calculated from equation (IV-25), assigning the value of unity to \bar{n}_H . The concentration of the species HL^{-5} was calculated as

$$[HL] = X_{HL} (C_L^0 - \bar{n} C_M^0) \left(\frac{100}{100 + v'} \right) \quad (IV-27)$$

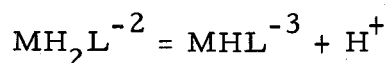
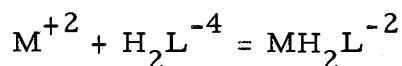
Plots of the required functions were linear in the lower pH range, but the slopes were all around 0.65. Each curve had an intercept, $\log \left(\frac{\bar{n}}{1 - \bar{n}} \right) = 0$, within the linear portion. The slopes, intercepts, and maximum pH where linearity was observed are shown in Table 29. There is no apparent explanation for a slope of other than unity for such a plot, except that mechanism 1 is not the proper one to describe complex formation in these systems. It would have been surprising of course if equation (IV-26) had been valid over the entire range up to the inflection point, in view of the spectrophotometric evidence for the formation of other than mononuclear species, but it could have held at relatively lower complex ion concentrations.

Table 29. Slopes, intercepts and upper limits of pH for the linear region for mechanism 1 (from equation (IV-26))

Cation	Composition	Slope	Intercept	Upper limit of pH
Mg	1:1	0.666	6.65	9.15
	2:1	0.715	6.65	8.07
Ca	1:1	0.673	6.11	8.93
	2:1	0.678	6.15	8.94
Sr	1:1	0.649	5.32	9.02
	2:1	0.639	5.34	9.07
Ba	1:1	0.683	5.10	9.13
	2:1	0.680	5.15	9.18

Mechanism 2

The second of the mechanisms mentioned above, in order of increasing complexity, can be represented by the equations



Further ionization of MHL^{-3} may be neglected provided only points well below the inflection point are used. For such a system

$$\bar{n} = \frac{[MH_2L] + [MHL]}{[M] + [MH_2L] + [MHL]} \quad \bar{n}_H = \frac{2[MH_2L] + [MHL]}{[MH_2L] + [MHL]} \quad (IV-28)$$

The relevant equilibrium constant expressions are

$$K_1 = \frac{[MH_2L]}{[M][H_2L]} \quad K_1^H = \frac{[MHL]H}{[MH_2L]} \quad (IV-29)$$

(K_1^H is a practical constant, containing both an activity and concentrations.) Substituting equations (IV-29) into (IV-28) gives

$$\bar{n} = \frac{K_1[M][H_2L] + K_1\left(\frac{K_1^H}{H}\right)[M][H_2L]}{[M] + K_1[M][H_2L] + K_1\left(\frac{K_1^H}{H}\right)[M][H_2L]} \quad (IV-30)$$

and after rearrangements of terms

$$\frac{\bar{n}}{1 - \bar{n}} = K_1[H_2L] \left(1 + \frac{K_1^H}{H}\right) \quad (IV-31)$$

A plot of $\log \left(\frac{\bar{n}}{1-\bar{n}} \right)$ vs. $\log [H_2L] + \log \left(1 + \frac{K_1^H}{H} \right)$ should give a line with unit slope and intercept equal to $-\log K_1$. Similar treatment of the equation for \bar{n}_H yields

$$\bar{n}_H = 1 + \frac{K_1 \left(\frac{K_1^H}{H} \right) [M] [H_2L]}{K_1 [M] [H_2L] + K_1 \left(\frac{K_1^H}{H} \right) [M] [H_2L]} = 1 + \frac{1}{\left(1 + \frac{K_1^H}{H} \right)} \quad (IV-32)$$

To test this mechanism, several values of K_1^H were chosen and for each a set of values of \bar{n}_H calculated. (K_1^H must be of the order of magnitude of 10^{-8} . If it were too much larger than K_4^a a second inflection point might be evident. A value of 1×10^{-8} leads to values of \bar{n} greater than unity for the 1:1 solutions which is clearly impossible.) For each value of K_1^H , \bar{n} values were calculated from equation (IV-25), and concentrations of H_2L^{-4} calculated from an equation analogous to (IV-27). A plot of the functions of the logarithmic form of equation (IV-31) for the magnesium 2:1 data is shown in Figure 15. The curves for the 1:1 data were similar, being somewhat more curved and displaced toward higher values of the ordinate. It can be seen that while the curves are approaching an initial slope of unity with increasing K_1^H they are departing further from linearity. Apparently this mechanism cannot represent the whole reaction sequence and, for reasons to be discussed in terms of the equations derived for mechanism 4, it cannot even give reliable data

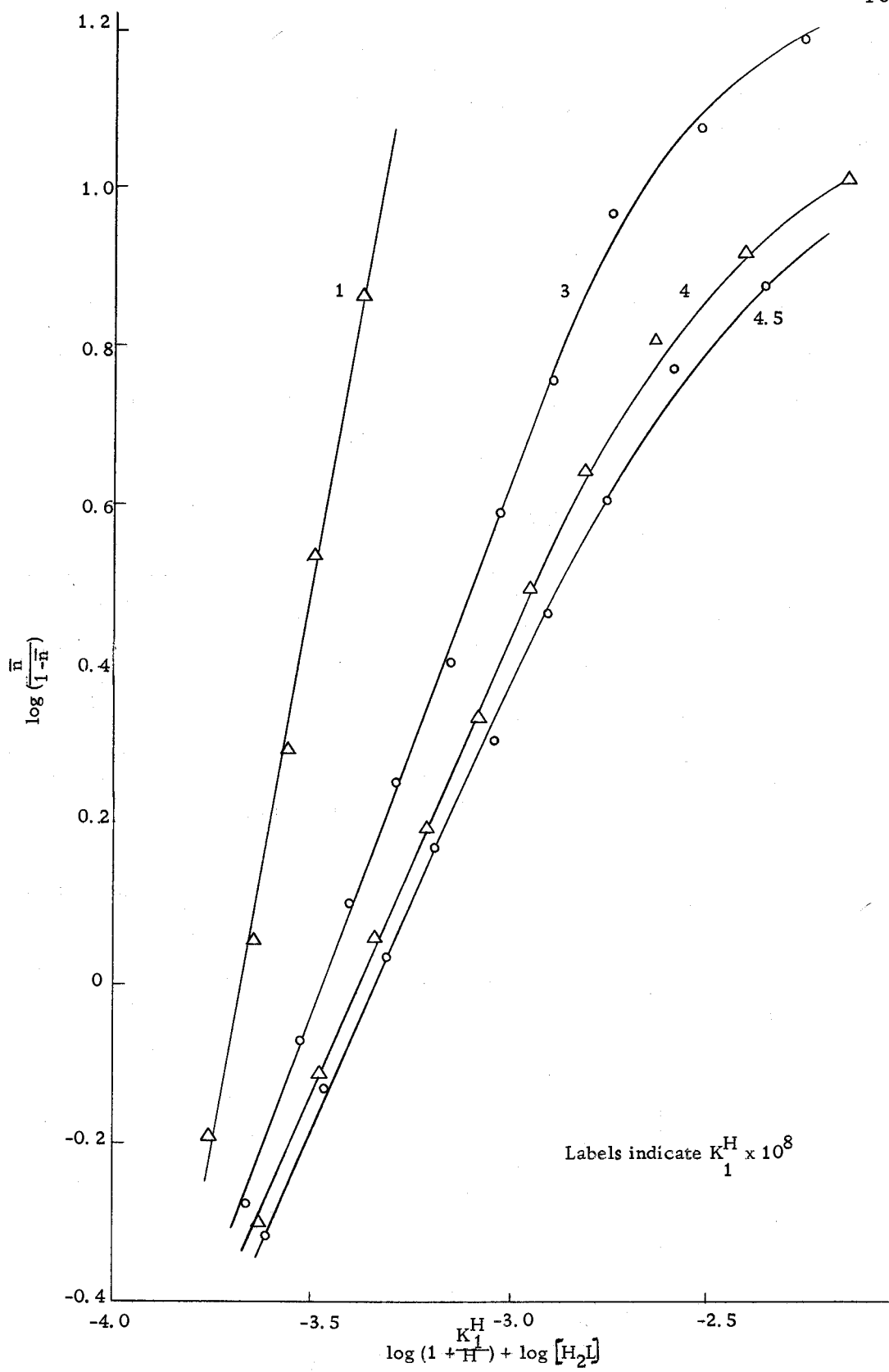
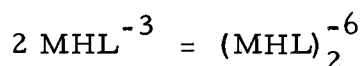
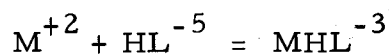


Figure 15. Test of Suitability of Mechanism 2

as a limiting case in the lower pH region. As mechanism 2 can be regarded as a special case of mechanism 4, with one of the equilibrium constants of that latter mechanism being zero, calculations for the other cations were not pursued.

Mechanism 3

This is the simplest of the possible mechanism involving the formation of polynuclear species. It may be represented by the equations



For this system the equilibrium constant expressions are

$$K_1 = \frac{[MHL]}{[M][HL]} \quad K_d = \frac{[(MHL)_2]}{[MHL]^2} \quad (IV-33)$$

From the definition,

$$\bar{n} = \frac{[MHL] + 2[(MHL)_2]}{[M] + [MHL] + 2[(MHL)_2]} \quad (IV-34)$$

and \bar{n}_H is unity. Substituting equations (IV-33) into (IV-34) yields

$$\bar{n} = \frac{K_1[M][HL] + 2K_d(K_1)^2[M]^2[HL]^2}{[M] + K_1[M][HL] + 2K_d(K_1)^2[M]^2[HL]^2} \quad (IV-35)$$

During simplification of this equation the free central ion concentration does not cancel out of all terms as it has previously. Another

expression for this quantity is needed. As long as the only complex species formed contain equimolar amounts of cation and ligand,

$$\bar{n} = \frac{\sum_0^Q i[M_i A_i]}{[M] + \sum_0^Q i[M_i A_i]} \quad 1 - \bar{n} = \frac{[M]}{[M] + \sum_0^Q i[M_i A_i]} = \frac{[M]}{C_M} \quad (\text{IV-36})$$

where Q is the maximum degree of polymerization of the 1:1 complex. Simplifying equation (IV-35) and substituting the final form of equation (IV-36) yields

$$\bar{n} = K_1 [HL] (1 - \bar{n}) + 2K_d (K_1)^2 (1 - \bar{n})^2 (H)^2 C_M \quad (\text{IV-37})$$

which can be rewritten

$$\frac{1}{[HL]} \left(\frac{\bar{n}}{1 - \bar{n}} \right) = K_1 + 2K_d (K_1)^2 (1 - \bar{n}) C_M [HL]. \quad (\text{IV-38})$$

this latter equation being of a linear form, with intercept K_1 and slope $2K_d (K_1)^2$.

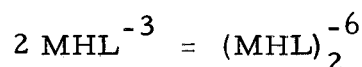
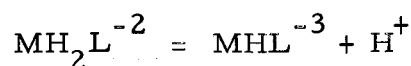
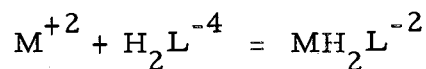
To test this mechanism, \bar{n} was calculated from equation (IV-25) and $[HL]$ from equation (IV-27). The functions $\frac{1}{[HL]} \left(\frac{\bar{n}}{1 - \bar{n}} \right)$ and $(1 - \bar{n}) C_M [HL]$ for the four cations are shown in Table 30. In each case the slope is obviously negative, leading to a negative and hence meaningless value for the dimerization constant K_d . This mechanism must not be the correct one.

Table 30. Summary of functions X and Y calculated to test the suitability of mechanism 3

	Magnesium		Calcium		Strontium		Barium	
	$X \times 10^{11}$	$Y \times 10^{-5}$	$X \times 10^{11}$	$Y \times 10^{-5}$	$X \times 10^{11}$	$Y \times 10^{-5}$	$X \times 10^{11}$	$Y \times 10^{-5}$
1:1	8.289	77.18	9.647	33.54	13.88	11.52	13.69	9.756
	11.005	70.34	15.21	30.08	32.21	7.668	39.92	5.673
	13.57	67.07	21.83	26.65	58.99	5.892	71.84	4.376
	16.21	64.95	30.43	23.47	87.75	5.070	117.7	3.418
	21.38	54.92	37.87	22.02	124.2	4.421	175.0	2.856
	24.72	53.16	49.08	19.54	171.1	3.831	261.0	2.236
	30.57	47.25	61.75	17.62	218.1	3.565	327.6	2.111
	37.34	42.26	73.77	16.51	286.8	3.047	409.3	1.965
	43.01	39.93	93.96	14.31	364.0	2.725	521.2	1.763
	51.29	36.10	110.8	13.31	435.3	2.589	658.0	1.586
	62.91	31.59	132.4	12.13	533.3	2.342	783.0	1.511
	76.83	27.67	154.4	11.35	640.6	2.176	968.3	1.367
	106.0	21.40	195.2	9.698	779.5	1.972	1175	1.258
	174.7	16.76	255.1	7.950	923.3	1.843	1361	1.212
	181.4	14.42	313.4	6.985	1077	1.751	1628	1.129
			378.8	6.278	1174	1.783	1757	1.166
			322.2	8.073	1152	2.019	1717	1.327
					835.9	3.066	1036	2.465
					680.0	41.37	160.8	17.34
2:1	4.532	72.58	4.804	35.35	11.40	11.05	14.91	7.084
	7.043	63.77	9.667	28.65	25.79	7.804	35.01	4.657
	10.76	53.13	16.43	23.13	47.27	5.834	66.15	3.484
	13.97	48.74	23.47	20.74	75.09	4.681	100.6	3.038
	19.51	40.48	34.19	17.13	104.4	4.223	149.1	2.548
	24.43	37.13	44.50	15.54	142.1	3.671	199.8	2.289
	30.46	33.01	55.11	14.55	185.1	3.316	278.7	1.922
	38.00	29.00	71.67	12.48	244.9	2.868	371.7	1.682
	54.03	21.73	94.01	10.43	315.3	2.536	470.3	1.549
	77.26	16.12	117.0	9.178	392.2	2.305	650.9	1.260
	133.1	9.683	154.1	7.533	505.8	1.989	777.7	1.212
	263.9	5.059	220.1	5.609	630.7	1.786	973.2	1.098
	406.4	3.442	364.8	3.592	732.9	1.706	1220	0.985
					572.5	2.422	1078	1.248

Mechanism 4

This mechanism may be represented by the equations



with the equilibrium constant expressions

$$K_1 = \frac{[MH_2L]}{[M][H_2L]} \quad K_1^H = \frac{[MHL]H}{[MH_2L]} \quad K_1 = \frac{[(MHL)_2]}{[MHL]^2} \quad (IV-39)$$

From the definition

$$\bar{n} = \frac{[MH_2L] + [MHL] + 2[(MHL)_2]}{[M] + [MH_2L] + [MHL] + 2[(MHL)_2]} \quad (IV-40)$$

Substituting equations (IV-39) into (IV-40) yields

$$\bar{n} = \frac{K_1[M][H_2L] + K_1\left(\frac{K_1^H}{H}\right)[M][H_2L] + 2K_d\left(K_1\frac{K_1^H}{H}\right)^2[M]^2[H_2L]^2}{[M] + K_1[M][H_2L] + K_1\left(\frac{K_1^H}{H}\right)[M][H_2L] + 2K_d\left(K_1\frac{K_1^H}{H}\right)^2[M]^2[H_2L]^2}$$

Simplifying and substituting (IV-36) into the result gives

$$\bar{n} = (1 - n) K_1 [H_2L] \left\{ 1 + \left(\frac{K_1^H}{H} \right) + 2K_d K_1 \left(\frac{K_1^H}{H} \right)^2 [H_2L] (1 - \bar{n}) C_M \right\} \quad (IV-41)$$

For \bar{n}_H , from its definition,

$$\bar{n}_H = \frac{2[\text{MH}_2\text{L}] + [\text{MHL}] + 2[(\text{MHL})_2]}{[\text{MH}_2\text{L}] + [\text{MHL}] + 2[(\text{MHL})_2]} \quad (\text{IV-42})$$

Substituting equations (IV-39) and simplifying gives

$$\begin{aligned} \bar{n}_H &= 1 + \frac{K_1[\text{M}][\text{H}_2\text{L}]}{K_1[\text{M}][\text{H}_2\text{L}] + K_1 \left(\frac{K_1^H}{H} \right) [\text{M}][\text{H}_2\text{L}] + 2K_d \left(K_1 \frac{K_1^H}{H} \right)^2 [\text{M}]^2 [\text{H}_2\text{L}]^2} \\ &= 1 + \frac{1}{1 + \frac{K_1^H}{H} \left\{ 1 + 2K_d K_1 \left(\frac{K_1^H}{H} \right) [\text{H}_2\text{L}] (1 - \bar{n}) C_M \right\}} \end{aligned}$$

It can be seen that if K_d , the dimerization constant, equals zero, that is, if no dimerization occurs, equations (IV-41) and (IV-43) reduce to equations (IV-31) and (IV-32) respectively, these being the equations which were derived for mechanism 2. Examination of the curves in Figure 15 show that an initial linear portion of approximately unit slope can be obtained for $K_1^H = 4.5 \times 10^{-8}$. The intercept for this curve implies a value of K_1 around 1.6×10^3 . Calculation of the last term in the denominator of equation (IV-43) shows that for the first experimental point, for a value of K_d of 200, use of equation (IV-32) would lead to an error of 1.3% in the value of $\bar{n}/(1-\bar{n})$. Similarly the use of the logarithmic form of equation (IV-31) would lead to an error of -0.013 in the ordinate. The direction of

these two errors is such that the point on Figure 15 would lie below and to the right of its correct position, leading to a slope and intercept both too small. The magnitude of these errors increases rapidly with increasing pH and K_d . Even with a small dimerization constant one could not expect this treatment to lead to useful values of K_1 and K_1^H , using that mechanism as a limiting case.

The solution of equations (IV-41) and (IV-43) is not simple. It has been shown that \bar{n} is always dependent on \bar{n}_H . For this mechanism \bar{n}_H is a function of \bar{n} as well. One possibility involves the exact solution of simultaneous equations. Equations (IV-25), (IV-41), and (IV-43) provide, for each experimental point, a set of three equations in five unknowns. Of the unknowns, three are constant while the other two, \bar{n} and \bar{n}_H , vary from point to point. Therefore m experimental points would provide $3m$ equations in $2m + 3$ unknowns. Obviously then consideration of three experimental points would provide nine equations in nine unknowns. Theoretically such sets could be solved exactly for the three equilibrium constants. Practical methods for the solution sets of non-linear simultaneous equations are lacking however so this approach was not pursued.

The first approach to the solution of these equations to which much effort was devoted began with the rewriting of equation (IV-41) in a linear form

$$\left(\frac{\bar{n}}{1-\bar{n}}\right) \left(\frac{H}{K_1^H + H}\right) \left(\frac{1}{X_{H_2L}(C_L - \bar{n}C_M)}\right) =$$

$$K_1 + 2K_d(K_1)^2 \left(\frac{K_1^H}{H}\right)^2 \left(\frac{H}{K_1^H + H}\right) X_{H_2L} (C_L - \bar{n}C_M) (1 - \bar{n}) C_M$$

(IV-44)

This equation contains two functions Y and X containing only known terms, \bar{n} and K_1^H . It is of the form $Y = K_1 + 2K_d(K_1)^2 X$, where the identity of Y and X is readily apparent by comparison with the preceding equation. A plot of Y vs. X should, for a properly chosen value of K_1^H , give a linear plot with slope $2K_d(K_1)^2$ and intercept K_1 . In order to solve the equation, a program was written for an IBM 1620 computer which would, for selected values of K_1^H , K_1 and K_d , compute from equation (IV-43) a value of \bar{n}_H for each experimental point based on the initial assumption $\bar{n} = 0$. This value of \bar{n}_H was then used in equation (IV-25) to compute an \bar{n} value more accurate than the value initially assumed. The new value of \bar{n} was then used to calculate a second value of \bar{n}_H . This iteration was continued until successive values of \bar{n} differed by no more than 0.001, a process which usually took three iterations, and in no case required more than six. The final \bar{n} was then used, along with the assumed value of K_1^H to calculate Y and X for the experimental point, and the whole process repeated for each point. It was thought

that from the slope and intercept of Y vs. X values of K_d and K_1 better than those initially assumed could be obtained. It was found however that the resulting Y vs. X plots curved so sharply that a meaningful straight line could not be drawn. When the computer fitted the best straight line (in the least squares sense) to the data, values of K_1 and K_d were obtained, but when an attempt was made to iterate through the entire process using these new values of the two constants, either the slope or the intercept of the best fit through the (X, Y) points became negative after one to three iterations. When it was seen that this procedure would not give convergence to constant values of K_1 and K_d , even with a non-linear Y vs. X plot, this approach was abandoned.

The second approach to solution of the equations for the three desired equilibrium constants also involved the use of the computer. Equation (IV-41) can be written in a form analogous to those used in relaxation methods for solution of equations

$$R = (1-\bar{n})(C_L - \bar{n}C_M) X_{H_2L} K_1 \left\{ 1 + \frac{K_1^H}{H} + \right. \\ \left. + 2K_d K_1 \left(\frac{K_1^H}{H} \right)^2 (C_L - \bar{n}C_M) X_{H_2L} (1-\bar{n})C_M \right\} - \bar{n} \quad (IV-45)$$

This equation will give for each experimental point, a residue R which is dependent on the values of the three equilibrium constants used in its calculation. In the absence of experimental error, for the

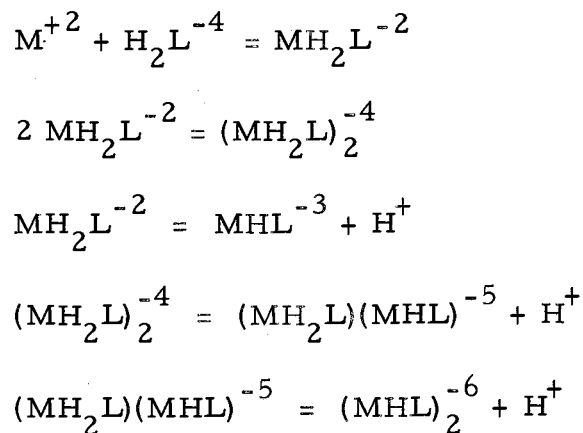
correct values of K_1^H , K_1 and K_d all these residues would equal zero. The plan then was to find a set of three constants which would reduce the residues to a minimum. Attainment of residues no greater than a few hundredths, particularly if the signs of the residues alternated, could be taken as evidence both of the correct values of the constants and of the correct mechanism for the system. To this end, a program was written which would calculate for each point a value of \bar{n} by iteration as described in the preceding paragraph and then, using the assumed trial values for the three constants and this value of \bar{n} , calculate the residue for that point. Proceeding in this manner, residues were calculated for an array of values of the three constants, inspection of the residues guiding choice of further values of the constants to try.

Using the calcium data for the 1:1 solution, residues were calculated for $K_1^H \times 10^8 = 2.0, 2.6, 2.8, 3.0, 3.2$ and 3.4 , for each of these covering the intervals $200 \leq K_1 \leq 800$, $200 \leq K_d \leq 1000$ with increments of each K of 200. Similarly the magnesium 1:1 data were calculated for $K_1^H = 1.5, 2.0, 2.5$ and 3.0 over the intervals $250 \leq K_1 \leq 2000$, $250 \leq K_d \leq 750$ with increments of 250. From these residues it became apparent that the curve calculated from equation (IV-41) could not be made to fit the experimental points very closely. The trial values of K_1 and K_d were sufficiently extensive that the residues passed from all negative to all positive, but in nearly all

cases it was apparent that the calculated curve had too great a slope, and in no case could a reasonably close fit be had for more than about the first eight points, and even for these, the fit could not be considered satisfactory. At this point mechanism 4 was tentatively abandoned and a fifth set of reactions tried.

Mechanism 5

This mechanism differs from the preceding one in the order in which ionization of a proton from the complex and dimerization occur. It can be represented by the equations



with equilibrium constant expressions

$$\begin{aligned}K_1 &= \frac{[MH_2L]}{[M][H_2L]} & K_d &= \frac{[(MH_2L)_2]}{[MH_2L]^2} & K_1^H &= \frac{[MHL] H}{[MH_2L]} \\K_2^H &= \frac{[(MH_2L)(MHL)] H}{[(MH_2L)_2]} & K_3^H &= \frac{[(MHL)_2] H}{[(MH_2L)(MHL)]}\end{aligned} \quad (IV-46)$$

From the definition,

$$\bar{n} = \frac{[\text{MH}_2\text{L}] + [\text{MHL}] + 2[(\text{MH}_2\text{L})_2] + 2[(\text{MH}_2\text{L})(\text{MHL})] + 2[(\text{MHL})_2]}{[\text{M}] + [\text{MH}_2\text{L}] + [\text{MHL}] + 2[(\text{MH}_2\text{L})_2] + 2[(\text{MH}_2\text{L})(\text{MHL})] + 2[(\text{MHL})_2]} \quad (\text{IV-47})$$

Substituting equations (IV-46) into (IV-47) yields

$$\bar{n} = \frac{K_1[\text{M}][\text{H}_2\text{L}] + K_1 \left(\frac{K_1^{\text{H}}}{\text{H}} \right) [\text{M}][\text{H}_2\text{L}] + 2K_d(K_1)^2[\text{M}]^2[\text{H}_2\text{L}]^2 \left\{ 1 + \frac{K_2^{\text{H}}}{\text{H}} + \frac{K_2^{\text{H}}K_3^{\text{H}}}{\text{H}^2} \right\}}{[\text{M}] + K_1[\text{M}][\text{H}_2\text{L}] + K_1 \left(\frac{K_1^{\text{H}}}{\text{H}} \right) [\text{M}][\text{H}_2\text{L}] + 2K_d(K_1)^2[\text{M}]^2[\text{H}_2\text{L}]^2 \left\{ 1 + \frac{K_2^{\text{H}}}{\text{H}} + \frac{K_2^{\text{H}}K_3^{\text{H}}}{\text{H}^2} \right\}} \quad (\text{IV-48})$$

Simplifying and substituting (IV-36) into the result gives

$$\bar{n} = (1 - \bar{n})K_1[\text{H}_2\text{L}] \left\{ 1 + \left(\frac{K_1^{\text{H}}}{\text{H}} \right) + 2K_dK_1(1 - \bar{n})[\text{H}_2\text{L}] C_M \left(1 + \frac{K_2^{\text{H}}}{\text{H}} + \frac{K_2^{\text{H}}K_3^{\text{H}}}{\text{H}^2} \right) \right\} \quad (\text{IV-49})$$

For \bar{n}_{H^-} , from the definition,

$$\bar{n}_{\text{H}^-} = \frac{2[\text{MH}_2\text{L}] + [\text{MHL}] + 4[(\text{MH}_2\text{L})_2] + 3[(\text{MH}_2\text{L})(\text{MHL})] + 2[(\text{MHL})_2]}{[\text{MH}_2\text{L}] + [\text{MHL}] + 2[(\text{MH}_2\text{L})_2] + 2[(\text{MH}_2\text{L})(\text{MHL})] + 2[(\text{MHL})_2]} \quad (\text{IV-50})$$

Substituting equations (IV-46) and simplifying gives

$$\bar{n}_{\text{H}^-} = 1 + \frac{1 + K_dK_1[\text{H}_2\text{L}](1 - \bar{n})C_M \left(2 + \frac{K_2^{\text{H}}}{\text{H}} \right)}{1 + \left(\frac{K_1^{\text{H}}}{\text{H}} \right) + 2K_dK_1[\text{H}_2\text{L}](1 - \bar{n})C_M \left(1 + \frac{K_2^{\text{H}}}{\text{H}} + \frac{K_2^{\text{H}}K_3^{\text{H}}}{\text{H}^2} \right)} \quad (\text{IV-51})$$

Again, if $K_d = 0$, these equations for \bar{n} and \bar{n}_H reduce to those derived for mechanism 2. The remarks made under consideration of the previous mechanism about the unsuitability of mechanism 2 as a limiting case are applicable here as well. In fact, the errors arising from the use of the equations for mechanism 2 to investigate this system are even larger than those encountered under mechanism 4.

Equation (IV-49) could be written in a linear form as was done for equation (IV-41) of mechanism 4. In view of the lack of success of this approach for mechanism 4, it was not pursued here. Instead, (IV-49) was written in a residual form

$$R = (1 - \bar{n})(C_L - \bar{n}C_M)X_{H_2}L K_1 \left\{ 1 + \frac{K_1^H}{H} \right\} + 2K_d K_1 \left(1 + \frac{K_2^H}{H} + \frac{K_2^H K_3^H}{H^2} \right) (C_L - \bar{n}C_M) X_{H_2} L^{(1-\bar{n})} C_M \bar{n} \quad (IV-52)$$

The second program prepared for mechanism 4 was modified to account for the changes in the equations for \bar{n} and \bar{n}_H , and the residue calculated for each experimental point over an array of values of the three constants as before. To simplify calculations, it was assumed that K_1^H , K_2^H and K_3^H are all equal. This should be reasonable, as each refers to the ionization of a proton from the same functional group on the complexed ligand.

Perusal of the residues calculated from equation (IV-52) showed that this mechanism was capable of producing curve which approximate much more closely the experimental points. Even so, for each of the four cations, and for nearly every combination of the three equilibrium constants the residues for the last few experimental points rose relatively sharply. Three possible explanations for this phenomenon will be advanced below.

In order to determine the optimum values of the three constants some criterion of goodness of fit of the calculated curves to the experimental data was needed. The criterion most commonly used in such cases, the root-mean-square error was not used because of the range of possible values of the residues. Since the quantity $R + \bar{n}$, from equation (IV-52) can never be negative for physically meaningful values of \bar{n} , R can never be smaller than $-\bar{n}$, while in the positive direction it can increase virtually without limit. It was felt that in the accumulation of sums of squares of the residues, values of the constants which lead to positive residues would tend to be unduly penalized. In order to avoid this, the mean of the absolute values of the residues was taken as the criterion of fit.

Even after a criterion of fit has been chosen, interpretation of the results must remain somewhat subjective. It is known that equation (IV-52) will become invalid at some pH, i. e., where the concentrations of species with n_H equal zero become significant. In

choosing the part of the titration data to be used in these calculations, an attempt was made to eliminate those points so near the inflection point that these species are likely to be present, but this choice was of necessity arbitrary. Hence it becomes necessary in evaluating the fit of the curve to decide whether a given positive residue is large because of unsuitability of the constants or because of invalidity of the equation used. The criterion used to decide how many residues to use in calculating the mean was based on an appraisal of the trend shown by the residues for a given set of constants. Starting with the lowest pH, all residues were retained until one was reached which exceeded either the absolute value of the smallest residue of that set, or 0.100, whichever was larger.

Table 31 shows the results of these calculations for the 1:1 data, including the values used for K_1^H , the values of K_1 and K_d producing a minimum mean absolute residue, the value of that mean, and the range of values of K_1 and K_d used for that level of K_1^H . The last quantity is indicated by the individual values used. In each case, they were used in all possible combinations.

Table 32 shows the quantities used to estimate the uncertainties in the values found for the constants. Again, this value is estimated subjectively from a comparison of the minimum value of the mean absolute residue with the values observed for the closest surrounding points. The calculations for the 2:1 data were not so complete as

those for the 1:1 sets. Rather it was restricted to calculation of residues for K_1^H equal to the best value as indicated by the 1:1 data, plus values 0.1×10^{-8} higher and lower than this value. For each K_1^H only those values of K_1 and K_d immediately surrounding the corresponding values found from the previous calculations were used. The results of these calculations are also shown in Table 32.

Several remarks should be made regarding the termination of calculations at this point. Residues could have been calculated for other values of the constants near the best values found to this stage in order to define the best values more closely. This was not done because of limitations on the available computer time. Furthermore, even at these intervals the differences between adjacent values of the mean absolute residue are comparable to the least value obtained for that mean, indicating that further refinements would probably be unjustified because of experimental error.

Even for the optimum values of the constants, the curves described approximate the experimental data for only part of the points before the inflection point. An attempt to extend the calculations beyond, or perhaps even much closer to, the inflection point would have required the augmentation of equations (IV-47) through (IV-51) to include the concentrations of species with n_H less than unity. This would require the introduction of three more acid dissociation constants, resulting in equations that were much more

Table 31. Minimum mean absolute residues and related data for mechanism 5.

$K_1^H \times 10^8$	K_1	K_d	Mean Absolute Residue	Number of Points Used	Region Covered
<u>For 1:1 Data:</u>					
<u>Magnesium</u>					
2.7	2500	750	0.1022	11	K_1 : 2000, 2250, 2500, 2750, 3000 K_d : 250, 500, 750, 1000
2.8	2250	1000	0.0751	10	K_1 : 2000, 2250, 2500, 2750 K_d : 250, 500, 750, 1000, 1250
2.9	2000	1250	0.0681	10	K_1 : 1750, 2000, 2500, 2750 K_d : 500, 750, 1000, 1250, 1500
3.0	2250	750	0.0624	10	K_1 : 2000, 2250, 2500, 2750, 3000 K_d : 250, 500, 750, 1000
3.1	2500	500	0.0569	10	K_1 : 2000, 2250, 2500, 2750, 3000 K_d : 250, 500, 750, 1000
3.2	2000	1000	0.0591	9	K_1 : 1750, 2000, 2500, 2750 K_d : 500, 750, 1000, 1250, 1500
<u>Calcium</u>					
2.8	800	400	0.0666	11	K_1 : 200, 400, 600, 800, 1000 K_d : 200, 400, 600, 800
2.9	800	400	0.0525	10	K_1 : 200, 400, 600, 800, 1000 K_d : 200, 400, 600, 800
3.0	800	400	0.0370	10	K_1 : 200, 400, 600, 800, 1000 K_d : 200, 400, 600, 800
3.1	800	400	0.0528	10	K_1 : 200, 400, 600, 800, 1000 K_d : 200, 400, 600, 800
<u>Strontium</u>					
2.9	175	50	0.0606	12	K_1 : 100, 125, 150, 175, 200 K_d : 0, 25, 50, 75, 100
3.0	175	25	0.0577	12	K_1 : 100, 125, 150, 175, 200 K_d : 0, 25, 50, 75, 100
3.1	175	25	0.0534	12	K_1 : 100, 125, 150, 175, 200 K_d : 0, 25, 50, 75, 100

Table 31. (continued)

$K_1^H \times 10^8$	K_1	K_d	Mean Absolute Residue	Number of Points Used	Region Covered
<u>Strontium</u> (continued)					
3.2	175	25	0.0534	12	K_1 : 100, 125, 150, 175, 200 K_d : 0, 25, 50, 75, 100
3.3	175	25	0.0560	12	K_1 : 100, 125, 150, 175, 200 K_d : 0, 25, 50, 75, 100
<u>Barium</u>					
2.9	100	100	0.0621	12	K_1 : 75, 100, 125, 150 K_d : 50, 100, 150, 200
3.0	100	75	0.0598	12	K_1 : 75, 100, 125, 150 K_d : 50, 100, 150, 200
3.1	100	50	0.0574	12	K_1 : 75, 100, 125, 150 K_d : 25, 50, 75, 100
3.2	100	50	0.0585	12	K_1 : 75, 100, 125, 150 K_d : 25, 50, 75, 100
<u>For 2:1 Data:</u>					
<u>Magnesium</u>					
3.0	2250	750	0.0631	7	
3.1	2500	500	0.0560	7	
3.2	2000	750	0.0595	7	
<u>Calcium</u>					
2.9	800	400	0.0525	7	
3.0	800	400	0.0380	7	
3.1	800	400	0.0533	7	
<u>Strontium</u>					
3.1	175	25	0.0559	9	
3.2	175	25	0.0541	9	
3.3	175	25	0.0566	9	
<u>Barium</u>					
3.0	100	50	0.0602	9	
3.1	100	50	0.0580	9	
3.2	100	50	0.0596	9	

Table 32. Estimation of uncertainties of constants for mechanism 5.
(Values for the 2:1 data are in parentheses)

Magnesium

K_1	K_d		
	250	500	750
2750		0.1032 (0.1026)	
2500	0.1244 (0.1223)	0.0569 (0.0560)	0.1474 (0.1465)
2250		0.0866 (0.0892)	

$$K_1^H = (3.1 \pm 0.1) \times 10^{-8}$$

$$K_1 = 2500 \pm 150$$

$$K_d = 500 \pm 100$$

Calcium

K_1	K_d		
	200	400	600
1000		0.1527 (0.1544)	
800	0.0952 (0.0998)	0.0370 (0.0380)	0.0503 (0.0512)
600		0.1488 (0.1493)	

$$K_1^H = (3.0 \pm 0.1) \times 10^{-8}$$

$$K_1 = 800 \pm 50$$

$$K_d = 400 \pm 100$$

Strontium

K_1	K_d		
	0	25	50
200		0.0698 (0.0701)	
175	0.0593 (0.0590)	0.0534 (0.0541)	0.0581 (0.0586)
150		0.0687 (0.0684)	

$$K_1^H = (3.2 \pm 0.1) \times 10^{-8}$$

$$K_1 = 175 \pm 25$$

$$K_d = 25 \pm 15$$

Barium

K_1	K_d		
	25	50	75
125		0.0538 (0.0661)	
100	0.0596 (0.0603)	0.0574 (0.0580)	0.0589 (0.0592)
75		0.1049 (0.1053)	

$$K_1^H = (3.1 \pm 0.1) \times 10^{-8}$$

$$K_1 = 100 \pm 15$$

$$K_d = 50 \pm 25$$

complicated, and virtually impossible to solve, even approximately.

No attempt was made to improve the fit of the curve to the points of higher pH but still below the inflection point. As mentioned earlier, this discrepancy could arise from three different causes. First, the approximation of setting the three acid dissociation constants for the complex species equal may not be justified. Because the total charge on the dimer would be greater than that on the monomer at a given stage of ionization, K_2^H and K_3^H may be smaller than K_1^H . Neglect of this fact would very likely cause a deviation of the calculated curve in the observed direction. No attempt was made to evaluate these constants for reasons cited on page 122, and because of the increased complexity of the program necessary to handle the computations for this more difficult case.

A second, simpler explanation of the failure to fit the points at higher pH lies in the appearance, at a lower pH than anticipated, of appreciable concentrations of species with n_H less than unity. This could be pursued in only two manners, by disregarding the latter points, as was done, or by resorting to the formidable equations mentioned above which take into account the presence of these species.

The third explanation is that at higher pH values the equations of this mechanism are no longer adequate to describe the system because of the formation of higher order polymeric forms. The equations defining \bar{n} and \bar{n}_H for such a system would involve one more

polymerization constant and at least three more acid dissociation constants and would be extremely difficult to solve. It should be remarked however that while a simple and entirely reasonable structure can be drawn for the 1:1 dimer (cf. Chapter V) it is difficult to propose a structure for any higher polymers. It is not felt this possibility can be profitably pursued.

V. CONCLUSIONS

Comparison of the Formation Constants Derived by Two Methods

The values for the formation constant for the 1:1 species cannot be compared directly. The constant derived spectrophotometrically governs the equilibrium constant expression

$$K_1^* = \frac{[ML]}{[M][L]}$$

where $[L]$ refers to the total concentration of free ligand and $[ML]$ refers to the total concentration of the monomeric species, regardless of the extent of protonation. The constant determined potentiometrically refers to the expression

$$K_1 = \frac{[MH_2L]}{[M][H_2L]}$$

where the degree of protonation is now considered.

Apparently the two constants are related by

$$\log K_1 = \log K_1^* + \log X_{MH_2L} - \log X_{H_2L} \quad (V-1)$$

where the symbol X_i represents the mole fraction of that form. An expression has been derived for the mole fraction of H_2L^{-4} , equation (IV-20). For the mole fraction of the complex in the form MH_2L^{-2} ,

$$X_{\text{MH}_2\text{L}} = \frac{[\text{MH}_2\text{L}]}{[\text{MH}_2\text{L}] + [\text{MHL}] + [\text{ML}]} = \frac{1}{1 + \frac{K_1^{\text{H}}}{\text{H}} + \frac{K_1^{\text{H}} K_4^{\text{H}}}{\text{H}^2}} \quad (\text{V-2})$$

where K_4^{H} is constant governing the acid dissociation of the species MHL^{-3} . The concentrations of dimeric species have been neglected in equation (V-2), a simplification that is justified by the treatment on which the evaluation K_1^* is based. An approximate value of K_4^{H} for each cation can be obtained from the pH of the 1:1 solution when 2.5 moles of base have been added per mole of arsenazo, i. e., when the volume of titrant is 4.80 ml. The comparison of the constants from the two sources is shown in Table 33.

The Acid Dissociation Constants of Arsenazo

Several workers have determined the acid dissociation constants of arsenazo, as shown in Table 34. Jones (23) and Kuteinikov and Lanskoï (36) have each made assignments of the constants to the several functional groups in the molecule, agreeing that the two sulfonate groups will ionize most strongly, followed by the two protons from the arsono group, and ionizing most weakly, the two naphthoic hydrogens. Neither gives a reason for this assignment, although at first it appears reasonable. A more definite assignment of the order in which protons are lost from the ligand would aid in advancing a hypothetical structure for the alkaline earth complexes. To this end,

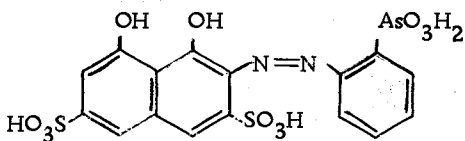
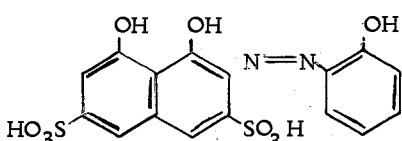
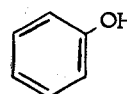
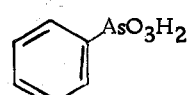
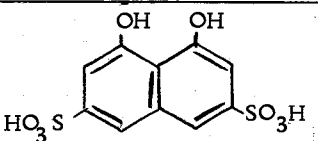
Table 33. Comparison of K_1 determined potentiometrically and K_1 calculated from K_1^* and summary of K_d and K_1^H

Cation	$\log K_1^*$	$\log X_{MH_2L}$	$\log X_{H_2L}$	Spectrophotometric $\log K_1$	Potentiometric $\log K_1$	$\log K_d$	pK_i^H
Mg	4.402 ± 0.023	-1.509	-0.071	2.964 ± 0.029	3.398 ± 0.025	2.70 ± 0.09	7.51 ± 0.014
Ca	4.806 ± 0.023	-2.516	-0.087	2.377 ± 0.029	2.903 ± 0.027	2.60 ± 0.11	7.53 ± 0.014
Sr	4.696 ± 0.058	-2.540	-0.087	2.243 ± 0.061	2.243 ± 0.062	1.40 ± 0.30	7.50 ± 0.014
Ba	4.359 ± 0.038	-2.526	-0.087	1.920 ± 0.042	2.000 ± 0.066	1.70 ± 0.24	7.51 ± 0.014

the acid dissociation constants of other, related compounds as reported in the literature are also shown in Table 34.

From the statement concerning structure VI there can be no reasonable doubt that for arsenazo, K_1^a and K_2^a refer to the ionization of the two sulfonate protons, as has been stated before. Comparison of the three weaker dissociations for I and II shows a striking similarity between the first and third in each case. Since the two weakly ionizing groups these two structures have in common are the naphthoic groups, this strongly suggests assignment of K_4^a and K_6^a of arsenazo to these two groups. Further support of this assignment can be had from a comparison of the constants for III and II, which indicate that K_4^a of structure II is associated with the phenolic group. This would leave K_3^a and K_5^a to govern the ionization of the arsono protons. Agreement between the first constant of IV and K_3^a of arsenazo is quite satisfactory. K_5^a for I is somewhat smaller than this comparison would suggest, but it is difficult to assess the effect that prior ionization of groups on the naphthalene nucleus would have on the ionization of the second arsono proton. As a final item of support for an assignment of constants to functional groups, the constants of V indicate that one of the naphthoic groups should be the weakest group in arsenazo. The value of K_3 for V is much closer to K_4 for I than it is to K_5 , the constant which has previously been associated with ionization of the first naphthoic group.

Table 34. Acid dissociation constants for selected compounds structurally related to arsenazo

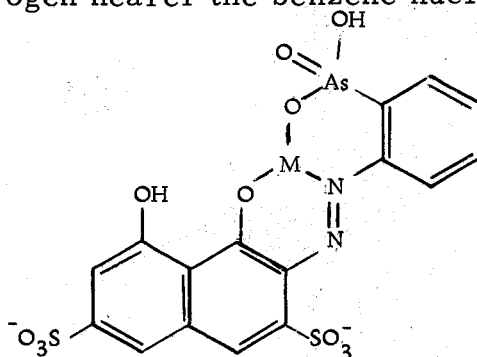
Compound	pK_1^a	pK_2^a	pK_3^a	pK_4^a	pK_5^a	pK_6^a	Reference
<p>I</p>  <p>Arsenazo</p>	0.07	1.2	2.7	7.9	10.5	12.4	(28)
	0.00	1.3	3.5	7.5	9.3	12.5	(34)
	0.07	1.2	2.7	7.7	10.5	-	(49),calculated from data of (28)
	--	--	5.2	7.5	10.2	>12	(23)
<p>II</p> 	--	--	7.56	9.5	12.4		(61)
<p>III</p> 	9.98						(2, p. 130)
<p>IV</p> 	3.47	8.48					(51)
<p>V</p> 	--	--	5.53	>12.7			(64)
VI Aryl sulfonic acids	ca0.6						(2, p. 131)

The relative strengths of the two naphthoic groups may be enlightened by the statement of Albert and Serjeant (2, p. 126, 131) to the effect that almost any substituent ortho to a naphthoic group will strengthen the acidity of that group.

On the basis of the comparisons given above, it is concluded that the two most acidic protons of arsenazo are those on the sulfono groups. These are followed in order by the first arsono proton, the proton from the 4-naphthoic group, the second arsono proton, and finally the 5-naphthoic proton.

Proposal of a Structure of the Complexes

It has been shown that the combining form of arsenazo in its reactions with the alkaline earth ions is H_2L^{-4} . On the basis of the assignment of dissociation constants to functional groups, made above, this shows that in the 1:1 monomer the cations is very likely bound to an arsono oxygen, to the naphthoic oxygen in the 4-position, and although it can not be demonstrated by the data presented, very likely to the azo nitrogen nearer the benzene nucleus.



It seems plausible that two such groups could come together, the oxygen of the arsono group of one bonding to the cation of the other. This would give each cation a coordination number of four. While the alkaline earths do not have coordination numbers so clearly characteristic of them as do the transition metals, values of four and six are suggested by some of their known complexes. The ring containing the two cations, two arsenics, and four oxygens would be eight-membered, but it would undoubtedly be non-planar, so this is permissible.

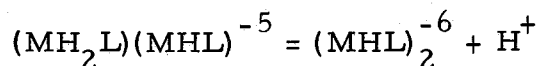
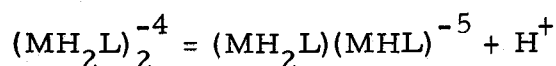
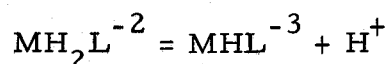
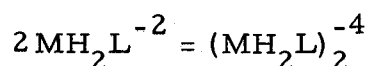
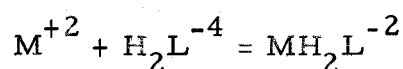
VI. SUMMARY

The reactions of the 3-(2-arsenophenylazo)-4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid, arsenazo, with the ions of magnesium, calcium, strontium and barium have been studied spectrophotometrically and potentiometrically.

Using Fronaeus' method of identifying corresponding solutions (14), the formation curve, to \bar{n} about 0.6 was determined for each cation. At higher values of \bar{n} the method failed. Suitable treatment of the formation curve showed that a 1:1 complex was formed in each case. The subsequent failure of the method was taken as evidence for the formation of polynuclear species in the system. The values of the formation constants for the 1:1 species are shown in Table 33 as K_1^* . Equations have been derived to show an alternative to Ahrlund's method of proof of the absence of polynuclear species. This alternative will be applicable to some other systems, but is not universally suitable.

The equations of Irving and Rossotti (20) have been elaborated to permit the study by potentiometric titration of systems involving a ligand capable of ionizing protons from the complex. Using these modified equations the arsenazo-alkaline earth systems were studied. Curves for the titration of solutions with arsenazo:central ion ratios of 1:1 and 2:1 showed that the reaction mechanism for the

systems must account for the loss of two protons from the ligand, either simultaneously with complex formation or subsequently but over the same pH range over which complex formation occurs. Five possible mechanism were considered. Appropriate equations were cited or derived for each case, and all but one eliminated. The sequence of reactions proposed to represent the system at hand are:



Equilibrium constants were determined for the first three reactions for each cation, using a successive-approximation fitting of the constants to a derived equation. The results are shown in Table 33 as K_1 , K_d and K_1^H respectively. The values of K_1 decrease from magnesium to barium, paralleling the order observed for amino acids, which also involve bonding of both oxygen and nitrogen to the cation. The same order is indicated for the values of K_d . The values obtained for strontium and barium are in the reverse order, but they are sufficiently close together and have large enough uncertainties that the order strontium, barium is not disproved.

A comparison was made of K_1 determined potentiometrically with the value calculated from the spectrophotometric value of K_1^* . The results, shown in Table 33, show good agreement for strontium and barium, and reasonable agreement for the other two cations, although not as good as would be expected from the confidence intervals.

BIBLIOGRAPHY

1. Ahrland, Sten. On the complex chemistry of the uranyl ion. II. The complexity of uranyl monochloroacetate, a comparative potentiometric and extinctionometric investigation. *Acta Chemica Scandinavica* 3:783-808. 1949.
2. Albert, Adrien and E. P. Serjeant. Ionization constants of acids and bases; a laboratory manual. London, Methuen, 1962. 172 p.
3. Bailar, John C., Jr. and Daryle H. Busch (eds.) The chemistry of the coordination compounds. New York, Reinhold, 1956. 834 p. (American Chemical Society. Monograph series no. 131)
4. Bates, Roger G. Electrometric pH determination. New York, Wiley, 1954. 331 p.
5. Bates, Roger G. and Vincent E. Bower. Alkaline solutions for pH control. *Analytical Chemistry* 28:1322-1324. 1956.
6. Bjerrum, Jannik. Metal ammine formation in aqueous solution. Copenhagen, P. Haase and Son, 1941. 298 p.
7. Cherkesov, A. I. and N. M. Alykov. Spectrophotometric study of organic reagents for scandium. *Zhurnal Analiticheskoi Khimii* 19:1067-1072. 1964. (Abstracted in *Chemical Abstracts* 61:15345b. 1964.)
8. Datta, Sachindra Kumar. Analytical uses of some azo dyes of chromotropic acid: volumetric, gravimetric, and spectrophotometric determinations. *Die Naturwissenschaften* 43:250-251. 1956.
9. Flagg, John F. Organic reagents used in gravimetric and volumetric analysis. vol. 4. New York, Interscience, 1948. 300 p.
10. Fritz, James S. and Evelin Carlston Bradford. Detection of thorium and uranium. *Analytical Chemistry* 30:1021-1022. 1958.
11. Fritz, James S. and Marlene Johnson-Richard. Colorimetric uranium determination with arsenazo. *Analytica Chimica Acta* 20:164-171. 1959.

12. Fritz, James S., Richard T. Oliver and Donald J. Pietrzyk. Chelometric titrations using an azoarsonic acid indicator. *Analytical Chemistry* 30:1111-1114. 1958.
13. Fritz, James S., Marlene Johnson-Richard and William J. Lane. Spectrophotometric determination of rare earths. *Analytical Chemistry* 30:1776-1779. 1958.
14. Fronaeus, Sture. *Komplexsystem hos koppar*. Lund, Gleerupska Universitets-Bokhandeln, 1948. 139 p.
15. Furman, N. Howell (ed.) *Standard methods of chemical analysis*. 6th ed. vol. 1. Princeton, Van Nostrand, 1962. 1401 p.
16. Gimesi, O., Gy. Rady and L. Erdey. Arsonic acid indicators in chelatometry. *Periodica Polytechnica* 6(1):15-20. 1962.
17. Harvey, Aubrey E., Jr. and Delmer L. Manning. Spectrophotometric methods of establishing empirical formulas of colored complexes in solution. *Journal of the American Chemical Society* 72:4488-4493. 1950.
18. Holcomb, H. Perry, and John H. Yoe. Spectrophotometric determination of uranium and thorium with arsenazo. *Microchemical Journal* 4:463-480. 1960.
19. _____. Spectrophotometric determination of uranium with 3-(2-arsenophenylazo)-4,5-dihydroxy-2,7-naphthalene-disulfonic acid (trisodium salt). *Analytical Chemistry* 32:612-617. 1960.
20. Irving, H. M., and H. S. Rossotti. The calculation of formation curves of metal complexes from pH titration curves in mixed solvents. *Journal of the Chemical Society*, 1954, p. 2904-2910.
21. Ishiguro, Yoshio, Shozo Shibata and Teiichi Matsumae. The color reaction of gallium with neothoron. *Nagoya Kogyo Gijutsu Shikensho Hokoku* 10:809-812. 1961. (Abstracted in *Chemical Abstracts* 56:7988a. 1962)
22. Job, P. Recherches sur la formation de complexes minéraux en solution et sur leur stabilité. *Annales de Chimie (Paris)*, ser. 10, 9:113-134. 1928.

23. Jones, Carl Trainer. A spectrophotometric investigation of arsenazo, 3-(2-arsonophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid and its uranium, aluminum and calcium complexes. Ph.D. thesis. Corvallis, Oregon State College, 1959. 62 numb. leaves.
24. Keegan, Jack J., and George Matsuyama. Increasing the sensitivity of the Beckman Zeromatic pH Meter. *Analytical Chemistry* 33:1292-1293. 1961.
25. Kirshen, Norman Alan. Spectrophotometric study of arsenazo and its calcium complex. Master's thesis. Corvallis, Oregon State University, 1961. 55 numb. leaves.
26. Klygin, A. E. and V. K. Pavlova. Spectrophotometric study of complex formation by thorium with benzene-2-(arsonic acid)-1,2'-azo-1',8'-digydroxynaphthalene-3',6'-disulfonic acid. *Russian Journal of Inorganic Chemistry* 5:734-737. 1960. (Translated from *Zhurnal Neorganicheskoi Khimii*)
27. _____. Spectrophotometric study of plutonium (IV) complex formation with arsenazo. *Russian Journal of Inorganic Chemistry* 6:536-539. 1961. (Translated from *Zhurnal Neorganicheskoi Khimii*)
28. _____. A study of the dissociation of benzene-2-arsonic acid-(1-azo-2)-1,8-dihydroxynaphthalene-3,6-disulfonic acid. *Journal of Analytical Chemistry of the USSR* 14: 180-186. 1959. (Translated from *Zhurnal Analiticheskoi Khimii*)
29. Kononenko, L. I., R. S. Lauer and N. S. Poluektov. Colorimetric determination of hafnium oxide in mixtures with zirconium oxide. *Ukrainskii Khimicheskii Zhurnal* 25:633-638. 1959. (Abstracted in *Chemical Abstracts* 54:9603c. 1960)
30. Korenman, I. M. and E. I. Levina. Azo dyes - derivatives of chromotropic and salicylic acids as reagents for magnesium. *Uchenye Zapiski, Gor'kovskii Gosudarstvennyi Universitet imeni N. I. Lobachevskogo, Seriya Khimicheskaya*, 1958, no. 32, p. 149-160. (Abstracted in *Chemical Abstracts* 54:5339li. 1960).

31. Kornilova, V. I. and T. N. Nazarchuk. Spectrophotometric investigation of formation of niobium compounds with arsenazo reagent. *Ukrainskii Khimicheskii Zhurnal* 29:330-335. 1963. (Abstracted in *Chemical Abstracts* 59:2372h. 1963)
32. Kovalenko, P. N. and K. N. Bagdasarov. Determination of pH at the beginning of dissolution and precipitation of scandium hydroxide with the aid of a photolorimeter. *Izvestiya Vysshikh Uchebnykh Zavedenii Khimiya i Khimicheskaya Tekhnologiya* 6:546-552. 1963. (Abstracted in *Chemical Abstracts* 60:8633c. 1964)
33. _____. Spectrophotometric pH determination at beginning of solution and dissolution of dysprosium hydroxide. *Zhurnal Prikladnoi Khimii* 37:735-741. 1964. (Abstracted in *Chemical Abstracts* 61:1247h. 1964)
34. Kuteinikov, A. F. The stability of complex arsenazo derivatives. *Industrial Laboratory (USSR)* 28:1255-1258. 1962. (Translated from *Zavodskaya Laboratoriya*)
35. _____. Use of arsenazo reagent for the determination of rare elements. *Industrial Laboratory (USSR)* 24:1171-1173. 1958. (Translated from *Zavodskaya Laboratoriya*)
36. Kuteinikov, A. F. and G. A. Lanskoi. A rapid photometric method for the determination of total rare earths. *Journal of Analytical Chemistry of the USSR* 14:761-765. 1959. (Translated from *Zhurnal Analiticheskoi Khimii*)
37. Kuznetsov, V. I. A color reaction for the rare earth elements. *Journal of Analytical Chemistry of the USSR* 7:255-262. 1952. (Translated from *Zhurnal Analiticheskoi Khimii*)
38. _____. Color reactions for thorium, uranium and other elements. *Comptes Rendus (Doklady) de l'Academie des Sciences de l'U.R.S.S.* 31:898-900. 1941.
39. Kuznetsov, V. I. and I. V. Nikol'skaya. Photometric determination of small amounts of thorium with arsenazo. *Journal of Analytical Chemistry of the USSR* 15:341-347. 1960. (Translated from *Zhurnal Analiticheskoi Khimii*)

40. Lamkin, Ervin G. and Max B. Williams. Spectrophotometric determination of calcium and magnesium in blood serum with arsenazo and EGTA. *Analytical Chemistry* 37:1029-1031. 1965.
41. Lane, William James. Metal-indicator systems in (ethylene-dinitrilo) tetraacetic acid. Ph.D. thesis. Ames, Iowa State College, 1957. 118 numb. leaves. (Abstracted in *Dissertation Abstracts* 18:773. 1958)
42. Lange, Norbert Adolph (ed.) *Handbook of chemistry*. 9th ed. Sandusky, Handbook Publishers, 1956. 1969 p.
43. Matsumae, Teiichi. Determination of indium. III. Spectrophotometric determination of indium with neo-thoron. *Bunseki Kagaku* 8:167-170. 1959. (Abstracted in *Chemical Abstracts* 55:26851f. 1961)
44. Matsuyama, Hideo, Tadashi Hara and Kyutaro Koyama. Spectrophotometric determination of uranium with 2-(o-arsonophenyl-azo)-chromotropic acid (neo-thoron). *Nippon Kagaku Zasshi* 79:958-963. 1958. (Abstracted in *Chemical Abstracts* 53:128i. 1959)
45. Meyer, Albert S., Jr. and Gilbert H. Ayres. The mole ratio method for spectrophotometric determination of complexes in solution. *Journal of the American Chemical Society* 79:49-53. 1957.
46. Mikhailov, V. A. Study of thoron and its reactions with uranium (VI). *Journal of Analytical Chemistry of the USSR* 16:145-149. 1961. (Translated from *Zhurnal Analiticheskoi Khimii*)
47. Moore, Walter J. *Physical chemistry*. 2d ed. Englewood Cliffs, N.J., Prentice-Hall, 1955. 633 p.
48. Mukherji, Anil K. and Arun K. Dey. Determination of uranium using chromotropic acid as a colorimetric reagent. *Journal of the Indian Chemical Society* 35:113-118. 1958.
49. Nazarenko, V. A. and E. A. Biryuk. Arsenazo I as a reagent for the photometric determination of scandium. *Ukrainskii Khimicheskii Zhurnal* 29:198-204. 1963. (Abstracted in *Chemical Abstracts* 59:3291g. 1963)

50. Polyak, L. Ya. Photometric determination of calcium in magnesium alloys using arsenazo reagent. Industrial Laboratory (USSR) 27:809-813. 1961. (Translated from Zavodskaya Laboratoriya)
51. Pressman, David and David H. Brown. Ionization constants of several substituted phenylarsonic acids. Journal of the American Chemical Society 65:540-543. 1943.
52. Przheval'skii, E. S., A. P. Golovina and A. F. Kuteinikov. Colorimetric determination of thorium with the use of some azo-compounds. Vestnik Moskovskogo Universiteta, Seriya Matematiki, Mekhaniki, Astronomii, Fiziki, Khimii 13(6):99-104. 1958. (Abstracted in Chemical Abstracts 53:15866b. 1959)
53. Rossotti, Francis J. C. and Hazel Rossotti. The determination of stability constants and other equilibrium constants in solution. New York, McGraw-Hill, 1961. 425 p.
54. Savvin, S. B. Use of reagents of the arsenazo-thoron group in analytical chemistry. Russian Chemical Reviews 32:93-107. 1963. (Translated from Uspekhi Khimii)
55. Shibata, Shozo, Yoshio Ishiguro and Teiichi Matsumae. Spectrophotometric determination of zirconium in magnesium-base alloys. Analytica Chimica Acta 23:384-387. 1960.
56. Shibata, Shozo and Teiichi Matsumae. Spectrophotometric determination of uranium with neo-thorone. Bulletin of the Chemical Society of Japan 32:279-282. 1959.
57. Sillén, Lars Gunnar and Arthur E. Martell. Stability constants of metal-ion complexes. 2d ed. London, The Chemical Society, 1964. 754 p. (Special Publication no. 17)
58. Takahashi, Takeo and Shingo Miyake. Spectrophotometric determination of thorium with neothorone. Talanta 3:155-159. 1959.
59. Tataev, O.A. Conditions for photocalorimetric determination of praseodymium and neodymium using arsenazo. Materialy Chetvertoi Nauchnyi Konferentskii Aspirantov (Rostov-on-Don: Rostov Universitet) Sbornik, 1962, p. 133-135. (Abstracted in Chemical Abstracts 60:15133c. 1964)

60. Toei, Kyoji. Neo-torin. *Kagaku* (Kyôto) 14:996-1002. 1959.
(Abstracted in *Chemical Abstracts* 54:18162f. 1960)
61. Tolmachev, V. N. and G. G. Lomakina. Spectrophotometric determination of the dissociation constants of sodium 1,8-dihydroxy-2-(2-hydroxyphenylazo)-3,6-naphthalenedisulfonate. *Zhurnal Fizicheskoi Khimii* 31:1027-1032. 1957. (Abstracted in *Chemical Abstracts* 52:50g. 1958)
62. _____. Spectrophotometric study of the reaction between sodium 1,8-dihydroxy-2-(2-hydroxyazobenzene)naphthalene-3,6-disulfonate and cadmium and mercuric ions. *Russian Journal of Physical Chemistry* 34:298-300. 1960.
(Translated from *Zhurnal Fizicheskoi Khimii*)
63. Uesugi, Katsuya. New spectrophotometric method for the determination of magnesium with sodium *p*-aminophenylazo-1,8-dihydroxy-3,6-naphthalenedisulfonate. *Bulletin of the Chemical Society of Japan* 38:337-338. 1965.
64. Zollinger, Hch. and W. Büchler. Zur Kenntnis der Kupplungsreaktion. III. Säurestärke and Kupplungsmechanismus von Dioxy-naphtalin-sulfosäuren. *Helvetica Chimica Acta* 34:591-599. 1951.