AN ABSTRACT OF THE THESIS OF

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Title: SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF CALCIUM AND MAGNESIUM WITH ARSENAZO III

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A dual-pH simultaneous direct spectrophotometric method for the determination of calcium and magnesium using Arsenazo III has been developed.

An absorption spectra study was made of Arsenazo III and its magnesium, calcium, strontium, and barium complexes at various pH values. This study showed that total calcium and magnesium can be determined at 652 mμ and pH 8.4; however, only calcium is determined at 652 mμ and pH 4.1 as the pH 4.1 magnesium complex has a very low molar absorptivity. The approximate apparent molar absorptivities, in 1 $mole^{-1}$ cm^{-1}, are: calcium complex, at pH 4.1, 6.3×10^3, and at pH 8.4, 2.9×10^4; magnesium complex, at pH 4.1, less than 100, and at pH 8.4, 9.6×10^3.

The extent that the complexes followed Beer's law was 0-1.20 ppm calcium at pH 4.1, 0-0.80 ppm calcium at pH 8.4, and 0-0.60 ppm magnesium at pH 8.4. The complexes were found to be stable for

at least one day, whereas the reagent was stable for at least 85 days. Several calcium complexing anions and the alkali metal elements were found to not interfere in the determination of calcium, but aluminum (III), iron (III), copper (II), strontium (II), and barium (II) interfere at the 1:1 concentration level at pH 4.1 and/or pH 8.4.

A preliminary study was made of an alternate method to determine calcium and magnesium using a combination of EGTA and Arsenazo III. Equations were developed for the direct determination of calcium and magnesium by the dual-pH method and by the EGTA-Arsenazo III method. A series of synthetic standard solutions of calcium and magnesium were determined using both methods.

Simultaneous Spectrophotometric Determination of Calcium and Magnesium With Arsenazo III

by

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SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF CALCIUM AND MAGNESIUM WITH ARSENAZO III

INTRODUCTION

Direct spectrophotometric methods have the advantages of rapidity and applicability to trace analysis. Since 1950 several reagents have been reported in the literature for the spectrophotometric determination of either calcium or magnesium without excessive interference from the other element. Up to the present, only three methods have been reported for the simultaneous direct spectrophotometric determination of calcium and magnesium.

Eriochrome Black T has been proposed by Young, Sweet, and Baker (27) for the simultaneous direct spectrophotometric determination of calcium and magnesium in water samples. The reagent forms a colored complex with magnesium at pH 9.52, whereas calcium forms only a weakly colored complex. Both calcium and magnesium form colored complexes at pH 11.7 with absorption maxima at 520-560 mμ. This method determines the extent of complexation by measuring the decrease in absorbance of the dye absorption maximum, where the absorption spectrum of the complex has a low slope. The decrease is measured at 630 mμ for both pH 9.52 and pH 11.7 solutions. The calcium and magnesium concentrations are determined by solving two simultaneous equations. This method has the disadvantages of only moderate sensitivity, and unstable reagent and complex solutions.

Chlorophosphonazo III, an analog of Arsenazo III, was reported by Ferguson et al. (5) to be suitable for the simultaneous direct spectrophotometric determination of calcium and magnesium. The absorbance due to the total calcium and magnesium complexes is measured at pH 7.0 and 699 mμ. The calcium and magnesium complexes follow Beer's law between 0-0.40 ppm calcium or magnesium. The calcium concentration is determined at pH 2.2 and 667.5 mμ, where the complex follows Beer's law between 0-1.2 ppm calcium. At pH 2.2 the magnesium complex absorbance is almost negligible. The method has high sensitivity and both the reagent and the complexes are stable for at least one week.

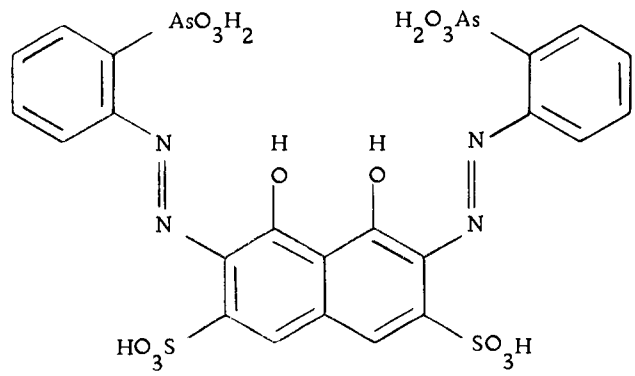
Lamkin and Williams (8) have reported the simultaneous spectrophotometric determination of calcium and magnesium in blood serum with Arsenazo I, o-(1,8-dihydroxy-3,6-disulfo-2-naphthylazo)-benzenearsonic acid, and EGTA, [ethylenebis(oxyethylenenitrilo)] tetraacetic acid. The absorbance due to the complexation of both calcium and magnesium by Arsenazo I is determined at 580 mμ and pH 9.6, against a reagent blank. One half of this original solution, Arsenazo I plus sample, is then transferred to another flask, EGTA added to complex the calcium, and the solution diluted to volume. The absorbance of the Arsenazo I complex with magnesium is measured at 580 mμ against a reagent blank prepared in the same manner, and the calcium concentration determined by difference.

The method has good sensitivity and the complexes are stable for at least one day.

The object of this investigation was to develop a method for the simultaneous direct spectrophotometric determination of calcium and magnesium with Arsenazo III. Since Arsenazo III has been shown to be superior to Arsenazo I for the determination of the rare earth elements, it was thought that it might also be a superior reagent for the determination of calcium and magnesium. The properties which make Arsenazo III the superior reagent are described in the next section.

PROPERTIES AND HISTORY OF ARSENAZO III

Arsenazo III, o-(1,8-dihydroxy-3,6-disulfononaphthalene-2,7-bisazo)-bisbenzenearsonic acid, was first successfully synthesized and studied by Savvin (21) in 1959. The first application was to the spectrophotometric determination of thorium in 4-10 N hydrochloric acid and uranium at pH 3. Ockenden (13) in 1956 reported the synthesis of "diuranol", identical in structure to Arsenazo III. He attempted to determine plutonium at pH 1 but discontinued the investigation because of difficulty in the synthesis and purification of the reagent. The formula of Arsenazo III is



The properties of Arsenazo III and its complexes have been reported by Savvin (16, 18, 21). The reagent is a dark red-black crystalline solid. It is reported to be indefinitely stable in solid and solution form in the absence of strong oxidizing and reducing agents. The solubility of Arsenazo III increases in going from acidic to basic

solutions. It is insoluble in strong acid, saturated sodium chloride, and most organic solvents. Arsenazo III is green in concentrated sulfuric acid, rose in acidic and neutral solutions, and blue in alkaline solutions.

The dissociation constants of Arsenazo III have been determined by Budesinsky (3) from changes in molar absorptivity. The dissociation constants have been assigned by Budesinsky as follows. The constants K_1, 4.7×10^{-13}, and K_2, 3.3×10^{-8}, are assigned to the dissociation of the two phenol groups. The constants K_3, 5.6×10^{-6}; K_4, 5.6×10^{-6}; K_5, 3.9×10^{-3}; and K_6, 3.9×10^{-3}, are assigned to the dissociation of the arsonic acid groups. The constants K_7, 1.0, and K_8, 353, are assigned to the dissociation of the sulfonic acid groups. Since Budesinsky determined these constants using impure Arsenazo III, there is reason to doubt the validity of these values and assignments.

Arsenazo III forms exceptionally stable inner complexes with many metal ions, exceeding the stability of similar complexes with Arsenazo I by several orders of magnitude. The effects of hydrolysis and complex forming anions are eliminated or reduced by the ability of the reagent to form complexes in very acidic solutions. On complexation, the absorption maximum of the Arsenazo III shifts from 540 mμ to two maxima, 600-610 mμ and 650-665 mμ, which are characteristic for the complexes. The longer wavelength maximum

is used for all quantitative determinations. Arsenazo III is not specific for any element, although its selectivity has been improved through the use of masking reagents, pH changes, and liquid extraction procedures.

Arsenazo III has been most extensively investigated for the spectrophotometric determination of the lanthanides, the actinides, scandium, zirconium, and hafnium. The sensitivity for the determination of these elements is 0.01-0.05 ppm. A number of reviews have been published which cover the investigation of Arsenazo III (16, 17) and of Arsenazo III and its analogs (1, 18, 20, 22). Savvin has recently written a monograph on Arsenazo III and its analogs (19).

PURIFICATION OF ARSENAZO III

The Arsenazo III used in this research was obtained from the J. T. Baker Chemical Company in the form of the disodium salt. An absorption spectrum of the commercial product at pH 5 exhibited an absorption maximum at 650 mμ, in addition to the main absorption maximum at 540 mμ. Since the calcium complex exhibits an absorption maximum at 652 mμ, the commercial product's absorption maximum at 650 mμ was thought to be due to the complexation of the calcium which is used to facilitate the diazotization step in the synthesis of Arsenazo III.

Savvin reported that Arsenazo III may be purified by repeated precipitation either in an aqueous saturated solution of sodium chloride (21) or in a solution of concentrated hydrochloric acid (18). Perez-Bustamante and Burriel-Marti (14) have reported the successful purification of Arsenazo III by passing an aqueous solution of the Arsenazo III through a Dowex 50W-X16, 50-100 mesh, acid form, cation exchange resin followed by precipitation with concentrated hydrochloric acid.

Procedure

The first attempt at purification involved following the procedure of Savvin (18) by making repeated precipitations from a

saturated aqueous solution of Arsenazo III using equal volumes of concentrated hydrochloric acid. After the final precipitation, the gelatinous precipitate was filtered, washed with acetonitrile and ethanol, and dried at 110°C. This method failed to remove the suspected impurity as indicated by the absorption maximum at 650 mμ.

The Arsenazo III was finally purified by passing a nearly saturated aqueous solution of the reagent, at pH 4-5, through a 35 cm × 2.5 cm Dowex 50W-X4, 20-50 mesh, acid form, cation exchange resin column. After the ion exchanged solution was dried at room temperature by forced air evaporation, the solid was washed with acetonitrile and dried at 105-110°C to constant weight. The purified reagent was stored in a desiccator over anhydrous calcium chloride. Air evaporation was employed in order to eliminate the need to precipitate the reagent in concentrated hydrochloric acid and to prevent the possibility of decomposition of the reagent occurring in very acidic solutions. A recorded absorption spectrum of an aqueous alkaline solution of the ion exchanged Arsenazo III showed that the absorption maximum at 650 mμ was eliminated. On the basis of this evidence the suspected calcium impurity was considered to have been removed.

The purity of the Arsenazo III prepared by the above procedure was determined by potentiometric titration with standardized

0.09855 N sodium hydroxide. A 10 ml microburet was used to deliver the titrant and the potential was measured using a glass electrode-calomel electrode combination with readout by a Heath electrometer. The volume of base delivered was determined at two inflection points by the second derivative method.

Discussion

The first inflection point, at about pH 6.4, corresponded to the titration of four hydrogen ions. The hydrogens titrated were the two sulfonic acid hydrogens and the first hydrogen from each of the two arsonic acid groups. The second inflection point, pH 8.8, corresponded to the titration of a fifth hydrogen. Perez-Bustamante and Burriel-Marti (14) disagree with the dissociation constant assignment of Budesinsky (3) and have proposed that the fifth hydrogen titrated is from one of the phenols. The mean molecular weight found for the titration of four 0.1000 gram samples was 828.7 ± 7.7 (95% confidence level) for the first inflection point and 829.5 ± 3.2 (95% confidence level) for the second inflection point. The difference between the experimentally determined molecular weight and the true formula weight is 53, which may be due to three moles of water of hydration per mole of Arsenazo III.

Warren (25, p. 12) reported finding two moles of water of hydration per mole of Arsenazo I, as determined by potentiometric

titration with base. Nemodruk and Kochetkova (12), using precipitation in concentrated hydrochloric acid and vacuum drying, found a weight differential between the purified Arsenazo III experimental molecular weight and the true formula weight equal to two moles of water. The molecular weight was determined, in this case, by a percentage analysis of the arsenic, sulfur, nitrogen, and carbon in the sample. Other authors have not reported the presence of water of hydration with Arsenazo III.

Although neither of these authors identified the position of the two moles of water of hydration, it is possible that they are associated with the two sulfonic acid groups. The third mole of water is possibly associated with the two arsonic acid groups. This view is supported by Thorne and Roberts (23, p. 732) who reported that arsenic acid, evaporated from water at temperatures lower than 100°C, crystallized as $2\ H_3AsO_4 \cdot H_2O$; and by Kalb (6), who reported that o-phenyldiarsonic acid crystallized with one mole of water.

INSTRUMENTATION AND STOCK SOLUTIONS

Instrumentation

A Beckman Model DB spectrophotometer, narrow slit program mode, was used for all transmittance measurements. A Heath EUW-20A recorder was used to record all percent transmittance data, except the pH dependence spectra study for which a Beckman 9350C recorder was used. Beckman matched 1.0 cm silica cells were used for all transmittance measurements. Percent transmittance readings were converted to absorbance manually. All pH measurements were made with a Beckman Zeromatic pH meter.

Stock Solutions

Arsenazo III. A 5.0×10^{-4} M solution was prepared by dissolving 0.4145 gram of purified trihydrate Arsenazo III in distilled water and diluting to 1000 ml.

Magnesium chloride. A 1.00×10^{-3} M solution was prepared by dissolving 0.0243 gram of Merck magnesium ribbon in 1 ml of concentrated hydrochloric acid and 10 ml of distilled water. The dissolved magnesium chloride was diluted to about 900 ml with distilled water and 20 ml of 0.50 M sodium hydroxide was added to partially neutralize the excess acid. The solution was then diluted

to 1000 ml.

Calcium chloride. A 1.00×10^{-2} M solution was prepared by dissolving 1.0009 grams of J. T. Baker 'Baker Analyzed' calcium carbonate in 4 ml of concentrated hydrochloric acid and 20 ml of distilled water. After diluting the solution to about 900 ml with distilled water, 50 ml of 0.50 M sodium hydroxide was added to partially neutralize the excess acid. The solution was then diluted to 1000 ml.

Strontium chloride. A 1.00×10^{-3} M solution was prepared by dissolving 0.1496 gram of J. T. Baker 'Baker Analyzed' strontium carbonate in a minimum amount of concentrated hydrochloric acid. The dissolved strontium chloride was then diluted to 1000 ml with distilled water.

Barium chloride. A 1.00×10^{-3} M solution was prepared by dissolving 0.2443 gram of Baker and Adamson Reagent grade barium chloride, dihydrate, in distilled water, followed by dilution to 1000 ml.

Buffers

The buffers used in this work and the reagents used in their preparation are listed below. Reagent grade chemicals were used in all cases except the sodium borate and tris(hydroxymethyl)aminomethane, the latter was Matheson, Coleman and Bell practical grade.

pH 3.4-6.0. Constant 0.1 μ buffers were prepared according to Bates (2, p. 121) from 0.05 M sodium formate-0.05 M potassium chloride or 0.05 M sodium acetate-0.05 M potassium chloride base stock solutions. To obtain the desired pH value, the base stock was diluted with the acid stock, 0.2 M hydrochloric acid-0.1 M potassium chloride.

pH 6.0-7.0. Buffers with pH values in this range were prepared from 0.2 M imidazole, J. T. Baker, and 0.1 M hydrochloric acid according to the instructions of Mertz and Owen (9).

pH 7.0-12.0. Buffers for this range were prepared according to Bates (2, p. 160-162) using tris(hydroxymethyl)aminomethane-hydrochloric acid, sodium borate-hydrochloric acid, sodium borate-sodium hydroxide, and potassium chloride-sodium hydroxide combinations. The only change made was to double the initial concentration of tris(hydroxymethyl)aminomethane in order to provide greater buffering capacity.

For all work requiring a final pH value of 8.4, a pH 8.5 tris(hydroxymethyl)aminomethane buffer was substituted for the pH 8.4 sodium borate buffer, which had been used previously for the pH dependence absorption spectra study and some of the combining ratio study. The sodium borate buffer was found to react with the Arsenazo III in a manner which increases the absorbance of the Arsenazo III solutions. The absorbance of the 5.0×10^{-5} M Arsenazo III blank

solutions was increased 0.02 absorbance units, as shown in Table 7. The tris(hydroxymethyl)aminomethane buffer does not appear to react with the Arsenazo III. Dilution of 10 ml of pH 8.5 tris(hydroxymethyl)-aminomethane buffer to 100 ml with distilled water resulted in a final pH value of 8.4.

ABSORPTION SPECTRA OF ARSENAZO III AND THE ALKALINE EARTH ELEMENT COMPLEXES

A study of the visible absorption spectra of the purified Arsenazo III, and its complexes with the alkaline earth elements, was undertaken at various pH values for two reasons: first, the spectra were required in order to determine the wavelengths and the pH values required for the simultaneous determination of calcium and magnesium; second, the only report of the spectra of Arsenazo III at various pH values, that of Budesinsky (3), shows the Arsenazo III spectra with a side peak at 650 mμ. Although Budesinsky did not comment on the reason for the side peak, it is probably due to the calcium impurity from the synthesis which was not removed in the purification process. The strontium and barium complexes of Arsenazo III were also studied in this portion of the work to determine if the simultaneous determination method could be extended to include either or both of these elements.

Procedure

Five solutions were prepared at each pH value consisting of Arsenazo III, Arsenazo III plus magnesium, Arsenazo III plus calcium, Arsenazo III plus strontium, and Arsenazo III plus barium. In each case the metal ion concentration was 5.0×10^{-6} M and the

Arsenazo III concentration was 3.4×10^{-5} M. A sixth volumetric flask was prepared which contained only the buffer component diluted to volume to be used as a blank.

The solutions were prepared by adding 10 ml of the buffer and 4 ml of 8.5×10^{-4} M Arsenazo III to each of the 100 ml volumetric flasks, and 5 ml of 1.0×10^{-4} M stock solutions of magnesium chloride, calcium chloride, strontium chloride, and barium chloride to their respective flasks. The flasks were then diluted to exactly 100 ml with distilled water, thoroughly mixed, and the absorption spectra recorded between 700-340 mμ against the buffer blank. The pH of each solution was measured after the spectrum was recorded.

The absorption spectra of Arsenazo III and the alkaline earth element complexes were recorded at intervals of 0.3-0.5 pH units between pH 3.4-11.1. A constant ionic strength was not maintained, although in all cases the ionic strength did not exceed 0.011. Figures 1 and 2 summarize the absorption spectra of Arsenazo III at various representative pH values.

Discussion of the Absorption Spectra of Arsenazo III

The salient points of the Arsenazo III absorption spectra at various pH values were the changes in the two absorption maxima at 540 mμ and 390 mμ. The absorbance of the 540 mμ absorption maximum increased in the intervals pH 3.4-5.8 and pH 8.7-10.3.

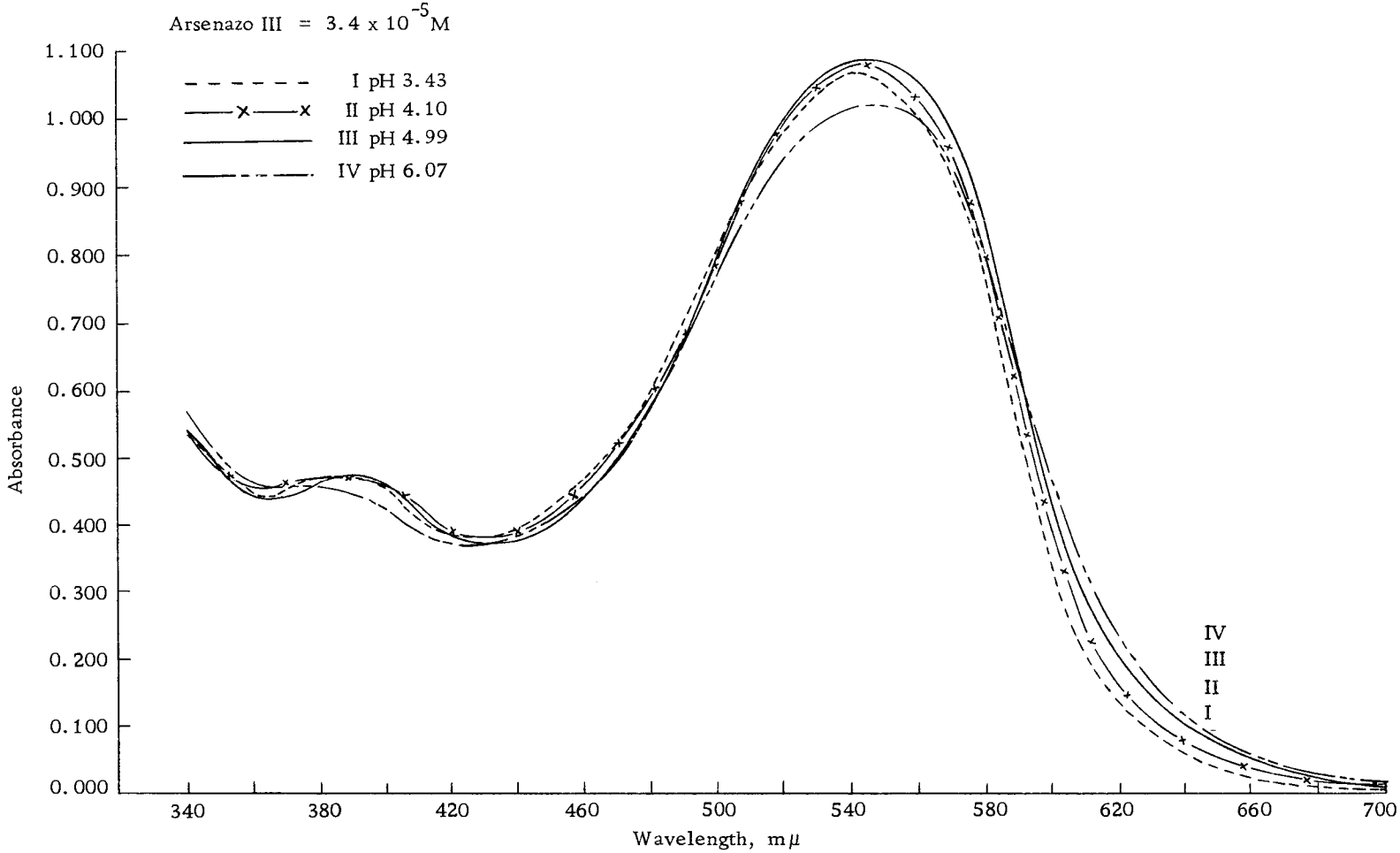


Figure 1. Absorption Spectra of Arsenazo III at Various pH Values

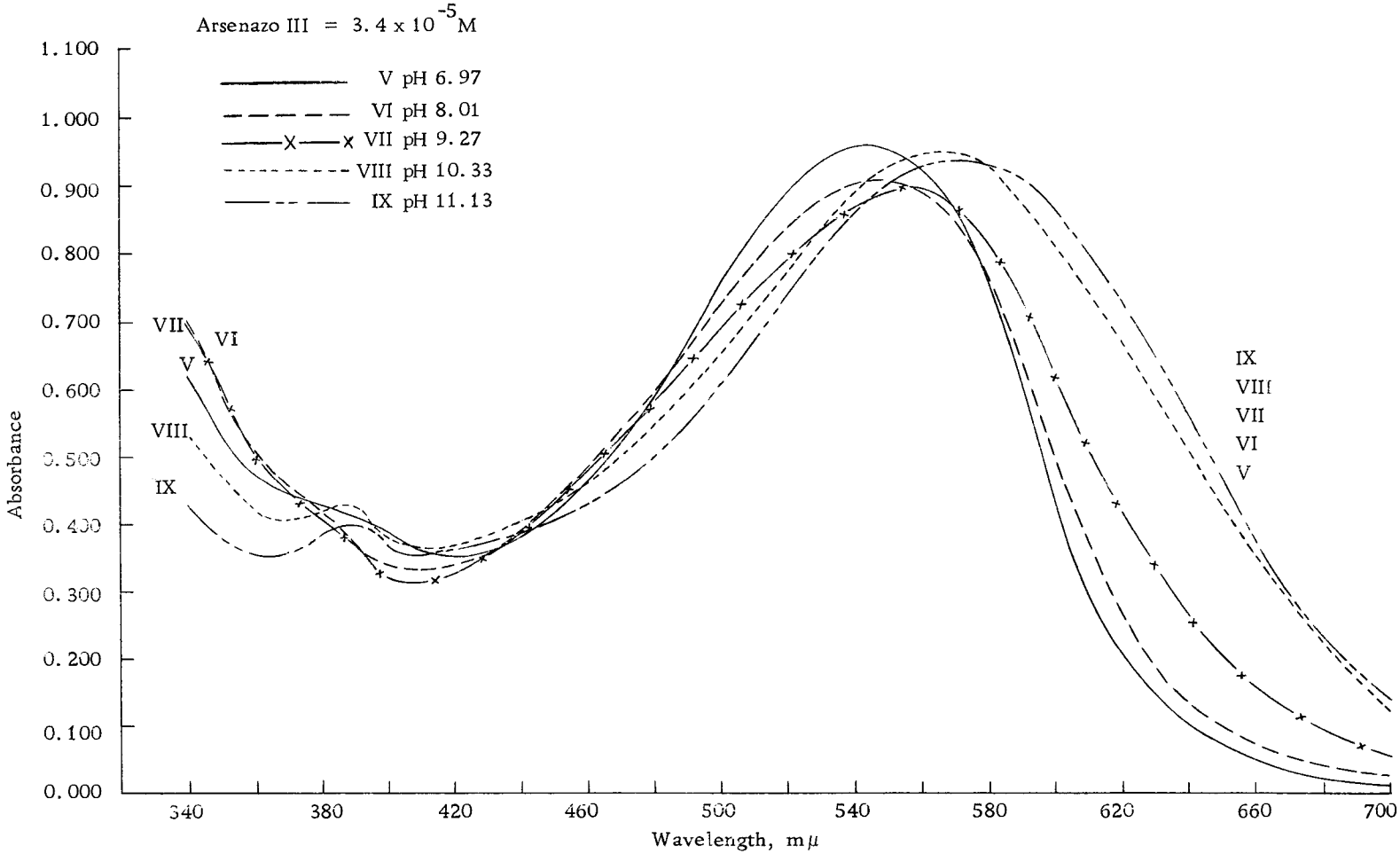


Figure 2. Absorption Spectra of Arsenazo III at Various pH Values

The increase in absorbance is thought to be due to the loss of the four arsonic acid hydrogens. In the interval pH 5.8-8.7 a decrease in the absorbance of the maximum was observed which may be related to the loss of the first phenolic hydrogen. The wavelength of the first absorption maximum also shifted with changes in pH. The absorption maximum was located at 540 mμ within the range pH 3.4-7.0, shifted to 550 mμ at pH 8.0, shifted again to 560 mμ at pH 9.3, and at pH 11.1 it was located at 570 mμ. The absorption maximum broadened significantly at pH values greater than pH 8.0.

The other significant absorption spectra change concerns the absorption maximum at 390 mμ. The absorbance of the maximum increased in the interval pH 3.4-5.8. From pH 5.8-9.3 the absorbance decreased, and the maximum almost lost its identity entirely between pH 7.0-9.3. At pH 10.3 and pH 11.1 the maximum again became prominent, although the absorbance was still low. The loss of identity may be due to an absorption maximum in the ultraviolet undergoing a bathochromic shift.

Discussion of the Absorption Spectra of the Alkaline Earth Element Complexes

The absorption spectra of the alkaline earth element complexes of Arsenazo III at pH 8.5 and the calcium complex at pH 4.1 are shown in Figures 3-7. Calcium and strontium produced the greatest

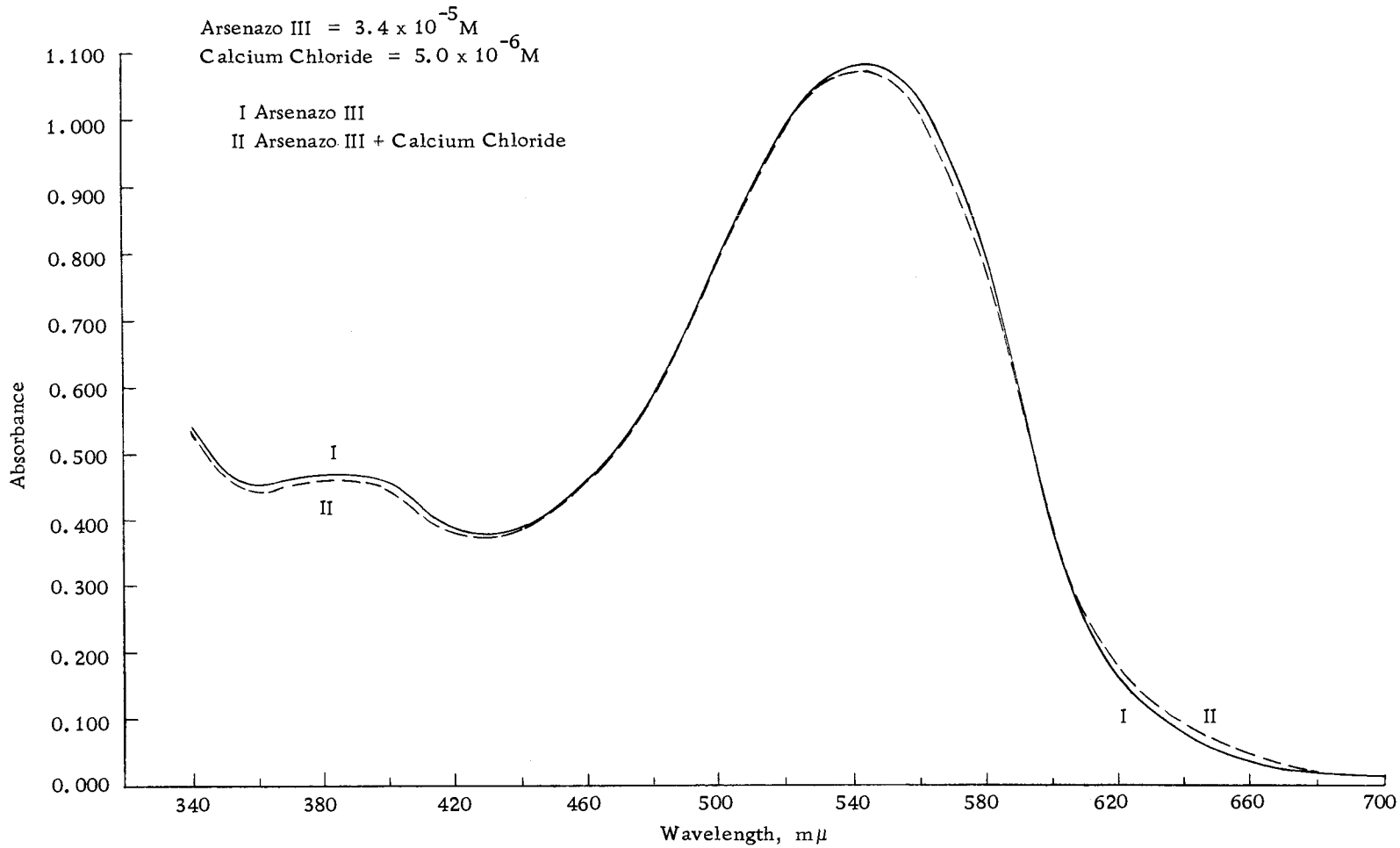


Figure 3. Absorption Spectrum of Calcium-Arsenazo III Complex at pH 4.1

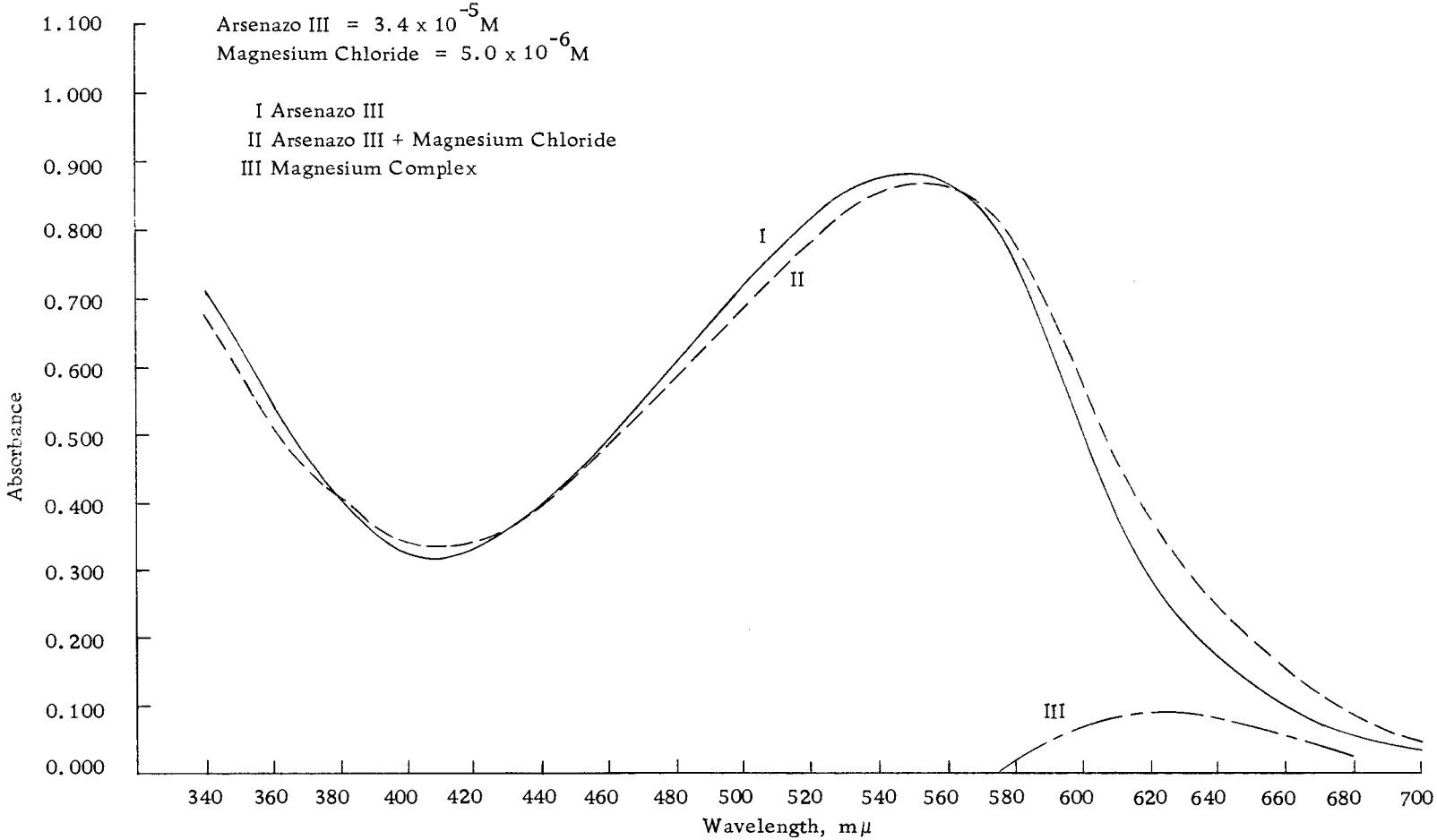


Figure 4. Absorption Spectra of Magnesium-Arsenazo III Complex at pH 8.5

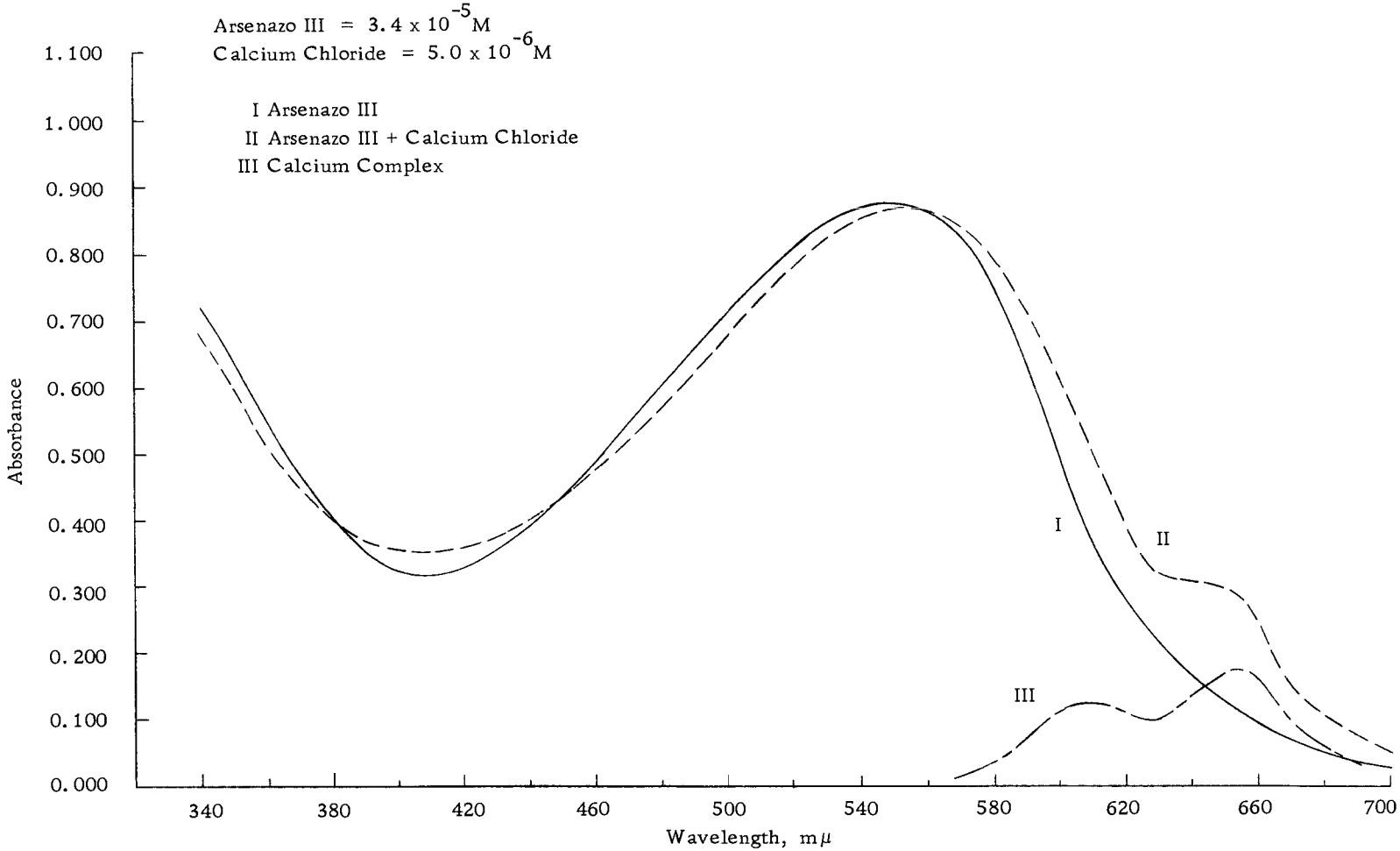


Figure 5. Absorption Spectra of Calcium-Arsenazo III Complex at pH 8.5

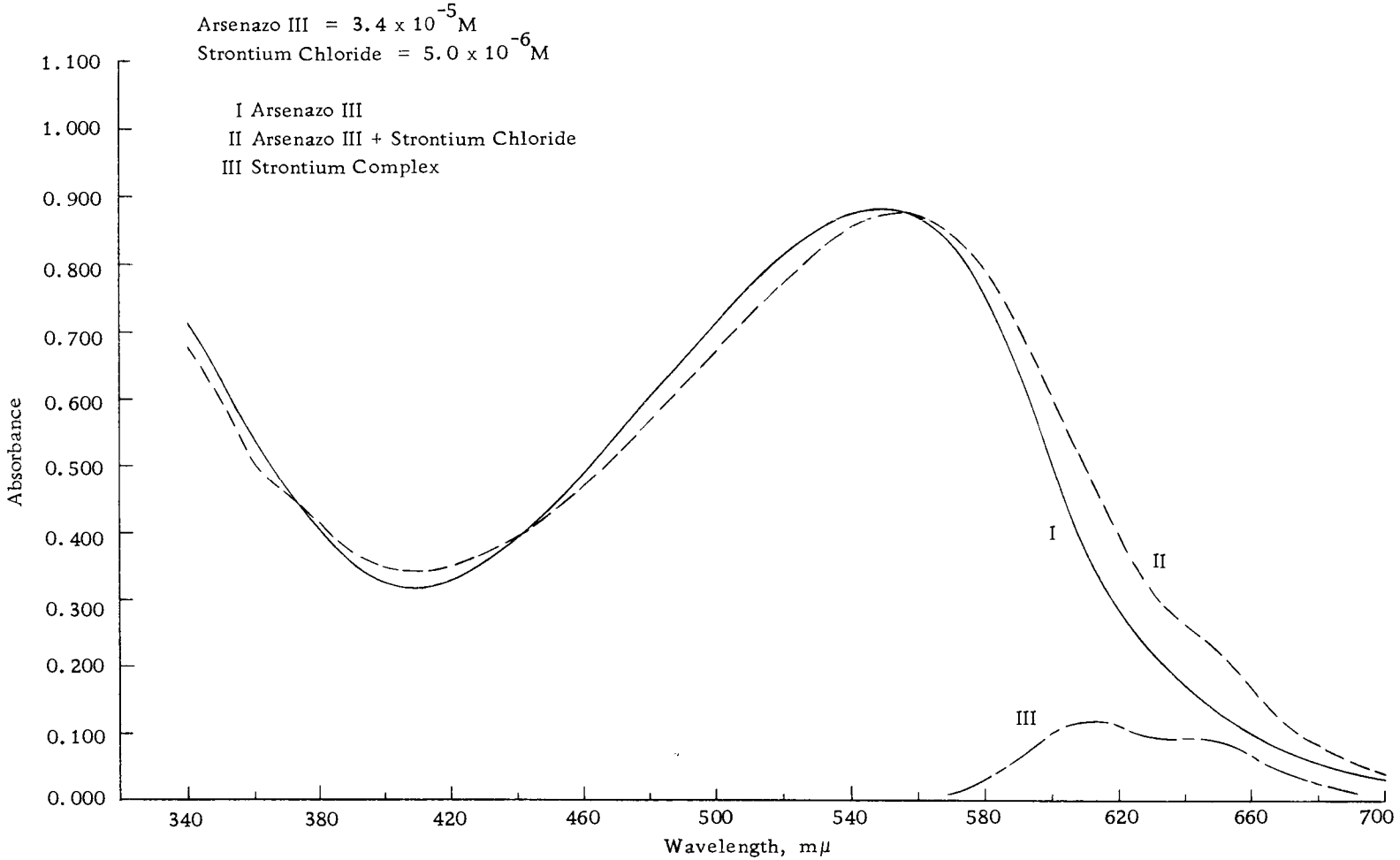


Figure 6. Absorption Spectra of Strontium-Arsenazo III Complex at pH 8.5

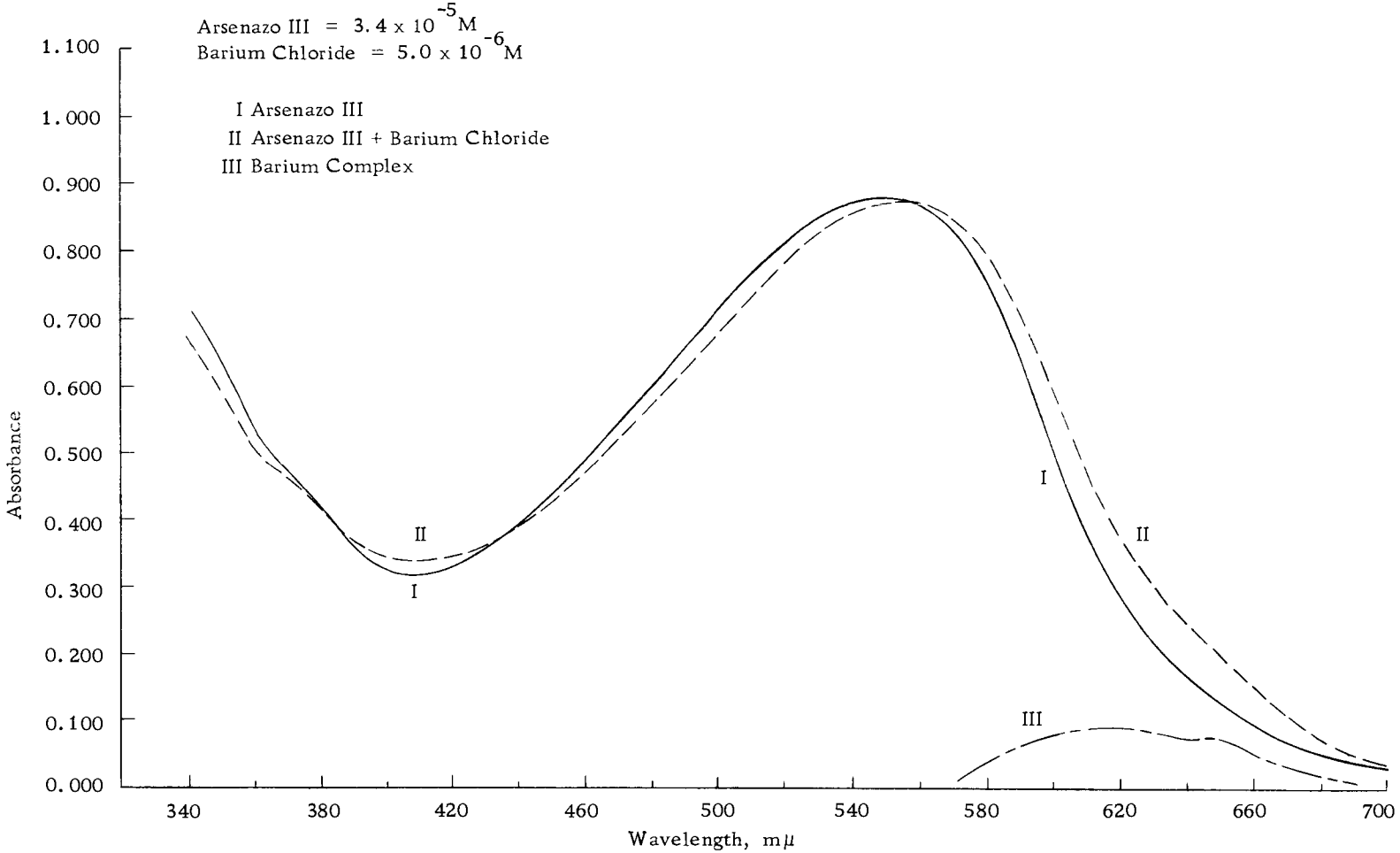


Figure 7. Absorption Spectra of Barium-Arsenazo III Complex at pH 8.5

absorbance change on complexing with Arsenazo III. The double peaked differential absorption spectrum characteristic for the complexes of Arsenazo III and its analogs was formed by the calcium, strontium, and barium complexes, but not by magnesium. The single peaked differential absorption spectrum of magnesium, not reported before in the literature, may be a result of its lower polarizing ability so that the two peaks are not resolved.

As discussed by Zollinger (28, p. 323), it is generally accepted that a phenol, with an azo group in the ortho position, will shift the hydrogen to form the quinone-hydrazonium structure under certain conditions. The double peaked differential spectra of the complexes have been attributed to the formation of the quinone-hydrazonium structure when the complex is formed. Nemodruk (11) reports that the quinone-hydrazonium structure forms in the half of the Arsenazo III molecule which does not form a complex with the metal ion. Cherkesov and Alykov (4) propose, however, that the quinone-hydrazonium structure forms in the complexed half of the Arsenazo III molecule.

The plots of absorbance against pH for the calcium and magnesium complexes at 652 mμ, for the strontium complex at 644 mμ, and for the barium complex at 640 mμ are given in Figures 8 and 9. In all cases, the metal ion concentration was 5.0×10^{-6} M and the Arsenazo III concentration was 3.4×10^{-5} M. All of the

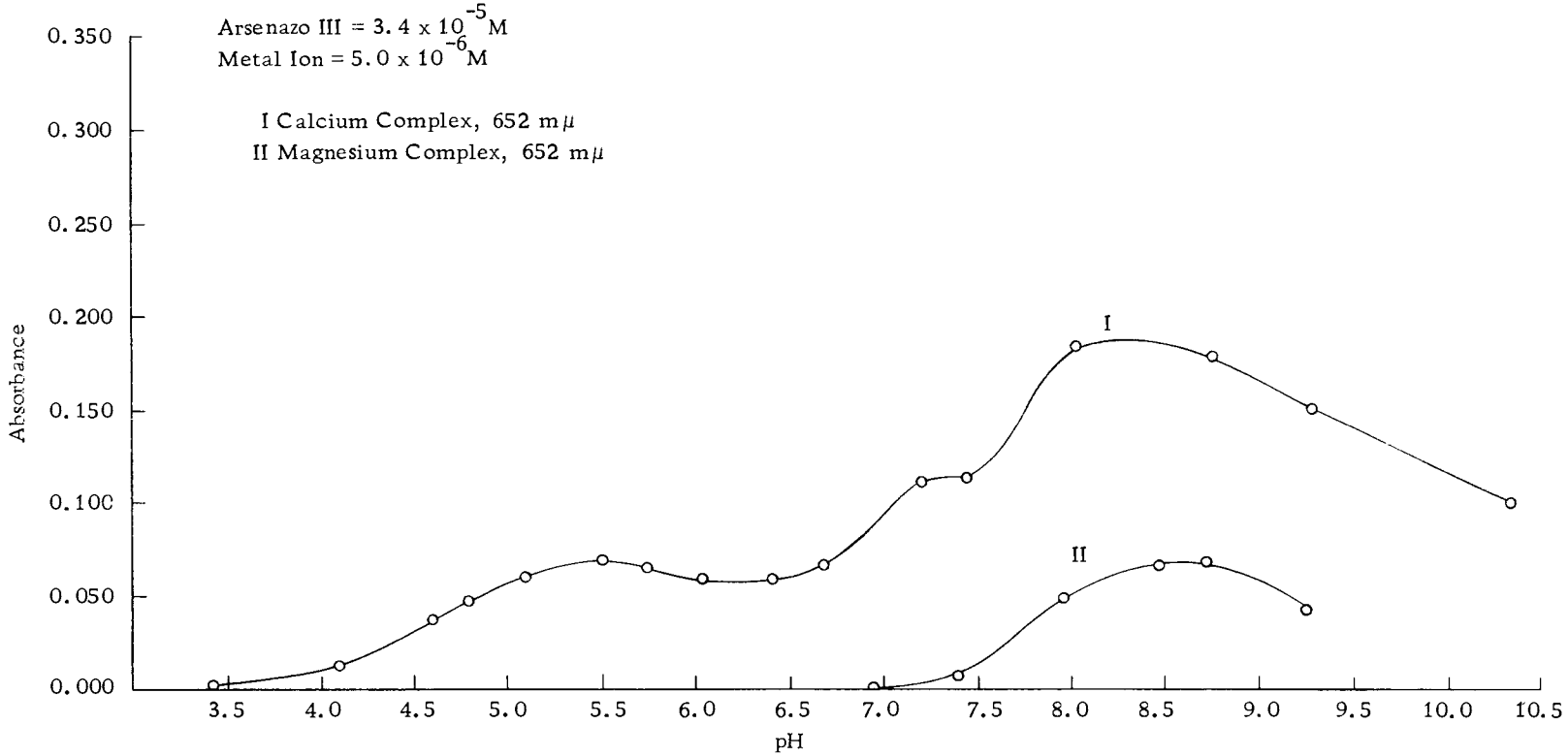


Figure 8. pH Dependence of the Calcium and Magnesium Complexes of Arsenazo III

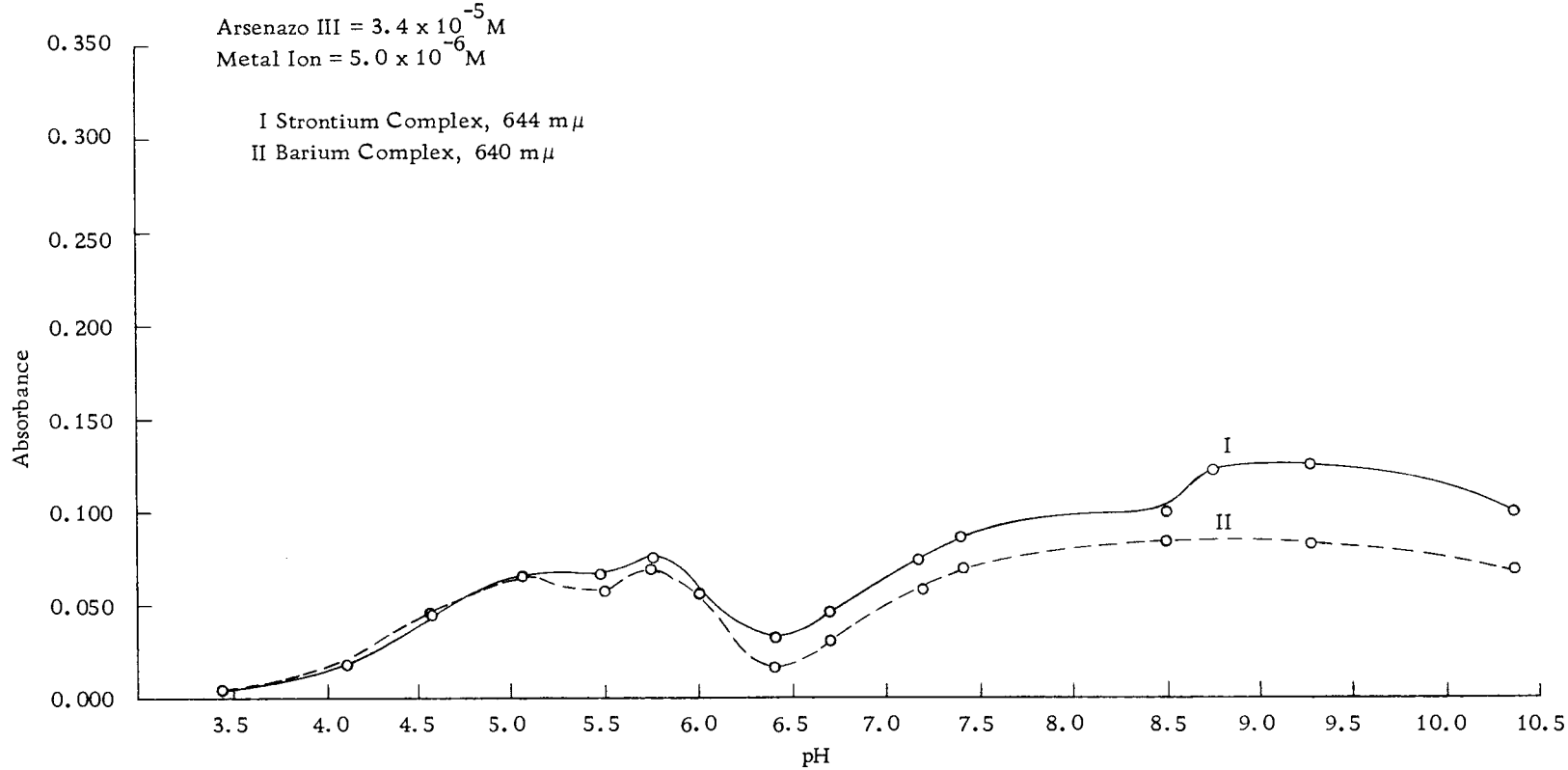


Figure 9. pH Dependence of the Strontium and Barium Complexes of Arsenazo III

alkaline earth elements, except magnesium, form violet colored complexes between pH 4.0-6.4 at the concentration studied. Between pH 8.0-9.0 deep blue complexes are formed between Arsenazo III and all four alkaline earth elements. The absorption spectra show that a dual-pH method would be feasible for magnesium and any one of the other three alkaline earth elements. Since calcium, strontium, and barium form colored complexes at all pH values studied, any of the three elements would interfere significantly in the determination of the other two elements. No combination of calcium, strontium, or barium could be determined using Arsenazo III without the solution of at least two complicated simultaneous equations.

The absorption spectra of the complexes suggest that the total magnesium and calcium could be determined within the range pH 8.3-8.5. Calcium could then be determined in the range pH 3.5-6.5, where the magnesium complex has only a minimal absorbance. The latter proposal was confirmed by adding a 20-fold excess of magnesium chloride to Arsenazo III at several pH values within this pH range. A molar absorptivity of less than 100 l $mole^{-1}$ cm^{-1} was found at pH 4.1, which means that the interference of the magnesium complex would be negligible in most cases.

COMBINING RATIOS OF THE ARSENAZO III COMPLEXES OF CALCIUM AND MAGNESIUM

The determination of the combining ratios for the calcium and magnesium complexes of Arsenazo III is essential for the complete study of the proposed direct simultaneous spectrophotometric method. Savvin (17) has reported that cations having a plus two charge form only 1:1 complexes with Arsenazo III, although it is doubtful that he studied the complexes formed in alkaline solutions. The continuous variations methods of Job, as extended by Vosburg and Cooper (24), and the molar-ratio method of Yoe and Jones (26), were used to determine the combining ratios of the three complexes studied. The absorbance measurements were usually made at several wavelengths for both methods in order to determine all possible complexes at the concentrations used.

In the continuous variations method, the mole ratios of the metal ion and the reagent are continuously varied while holding the total concentration constant. If a single complex is formed, the maximum absorbance is found at the concentration ratio of metal ion to reagent which duplicates the ratio of metal ion to reagent in the complex.

The molar-ratio method is useful for the determination of the ratio of metal ion to reagent for complexes of high stability. Either

the reagent or the metal ion concentration is held constant while the other constituent's concentration is varied and the absorbance measured for each ratio. The combining ratio is found by extrapolation of the two linear portions of the curve to an intersection.

Procedure

The solutions for the continuous variations method were prepared in 100 ml volumetric flasks. Ten ml of buffer, followed by 10 - X ml of 5.0×10^{-4} M Arsenazo III and X ml of 5.0×10^{-4} M calcium chloride or magnesium chloride, was added to each flask. The buffer used for the pH 4.1 solutions was sodium formate, whereas tris(hydroxymethyl)aminomethane buffer was used for the pH 8.4 solutions. The flasks were diluted to volume with distilled water, mixed, and the absorption spectra recorded from 700-500 mμ using a blank prepared from buffer only. An exception to this procedure was made for the pH 4.1 calcium complex solutions for which the absorbance was measured only at 652 mμ. A manual subtraction was made for the absorbance due to the Arsenazo III in each flask. The data for the determination of the combining ratio by the continuous variations method are given in Tables 1-3, and Figures 10-12.

The solutions for the molar-ratio method were also prepared in 100 ml volumetric flasks to which 10 ml of buffer and 10 ml of

Table 1. Combining Ratio of Calcium-Arsenazo III Complex at pH 4.1 by the Continuous Variations Method

Mole Fraction Arsenazo III	A corr. 652 mμ
0.0	0.000
0.1	0.023
0.2	0.042
0.3	0.060
0.4	0.074
0.5	0.081
0.6	0.080
0.7	0.066
0.8	0.047
0.9	0.020
1.0	0.000

Table 2. Combining Ratio of Calcium-Arsenazo III Complex at pH 8.4 by the Continuous Variations Method

Mole Fraction Arsenazo III	A corr. 652 mμ	A corr. 604 mμ	A corr. 540 mμ	A corr. 500 mμ
0.0	0.000	0.000	0.000	0.000
0.1	0.087	0.088	-0.027	-0.040
0.2	0.198	0.180	-0.051	-0.074
0.3	0.311	0.273	-0.073	-0.100
0.4	0.452	0.367	-0.094	-0.122
0.5	0.505	0.426	-0.120	-0.154
0.6	0.538	0.429	-0.119	-0.151
0.7	0.428	0.330	-0.092	-0.115
0.8	0.288	0.222	-0.054	-0.074
0.9	0.143	0.085	-0.022	-0.019
1.0	0.000	0.000	0.000	0.000

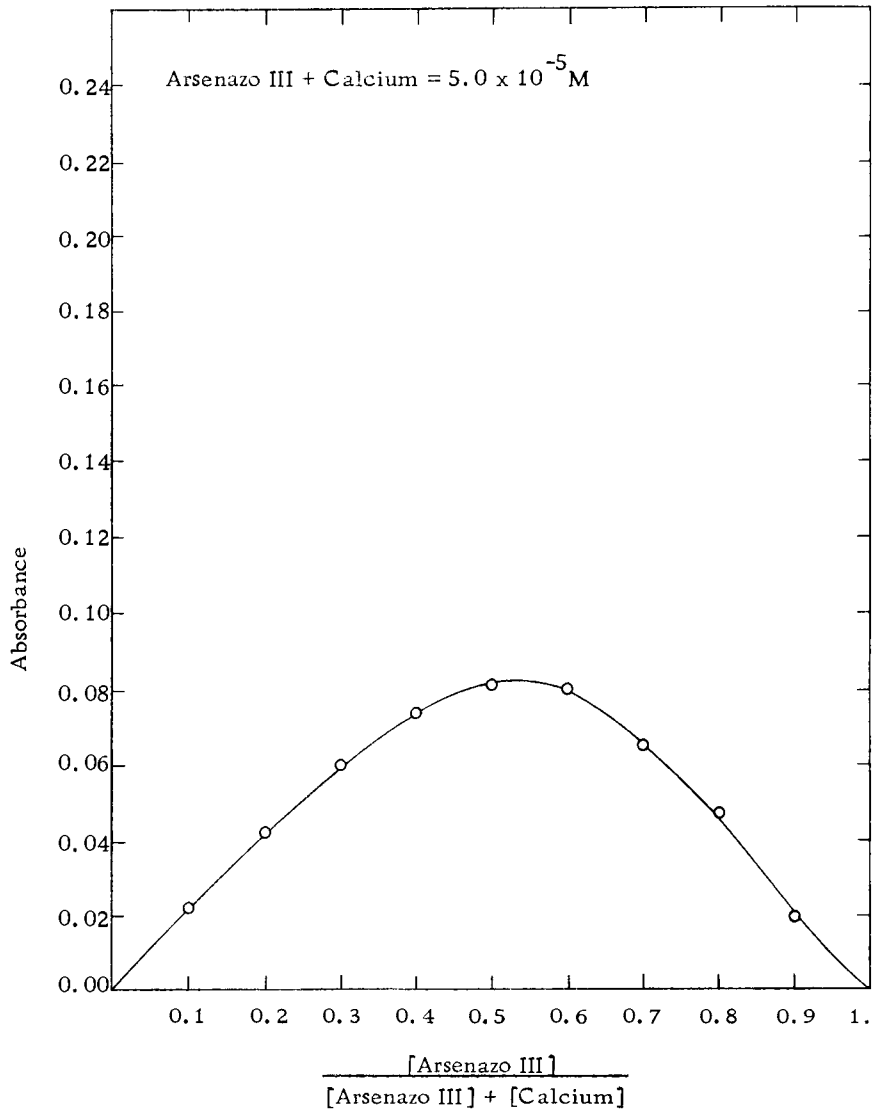


Figure 10. Continuous Variations Method for the Calcium-Arsenazo III Complex at pH 4.1 and 652 mμ.

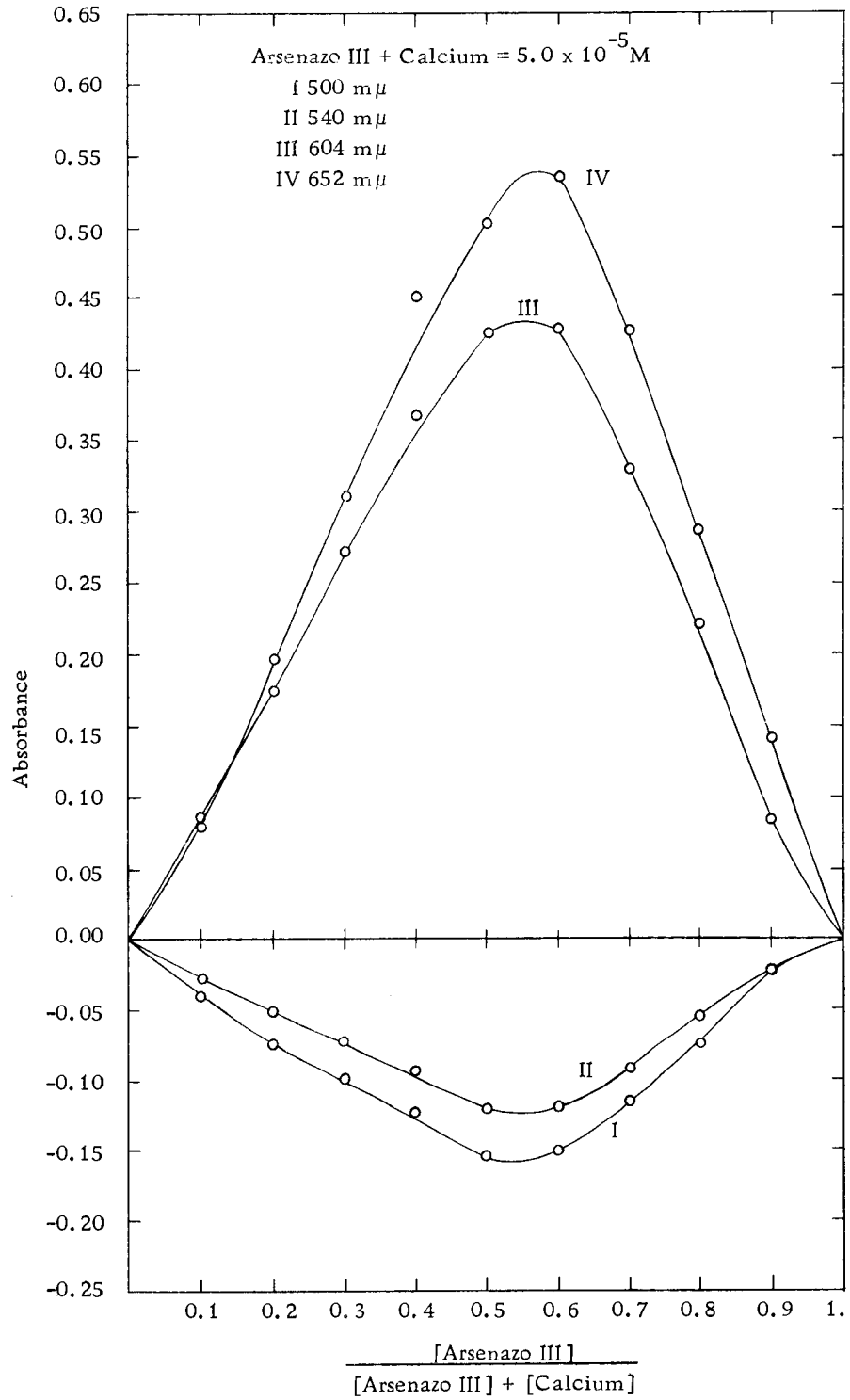


Figure 11. Continuous Variations Method for the Calcium-Arsenazo III Complex at pH 8.4

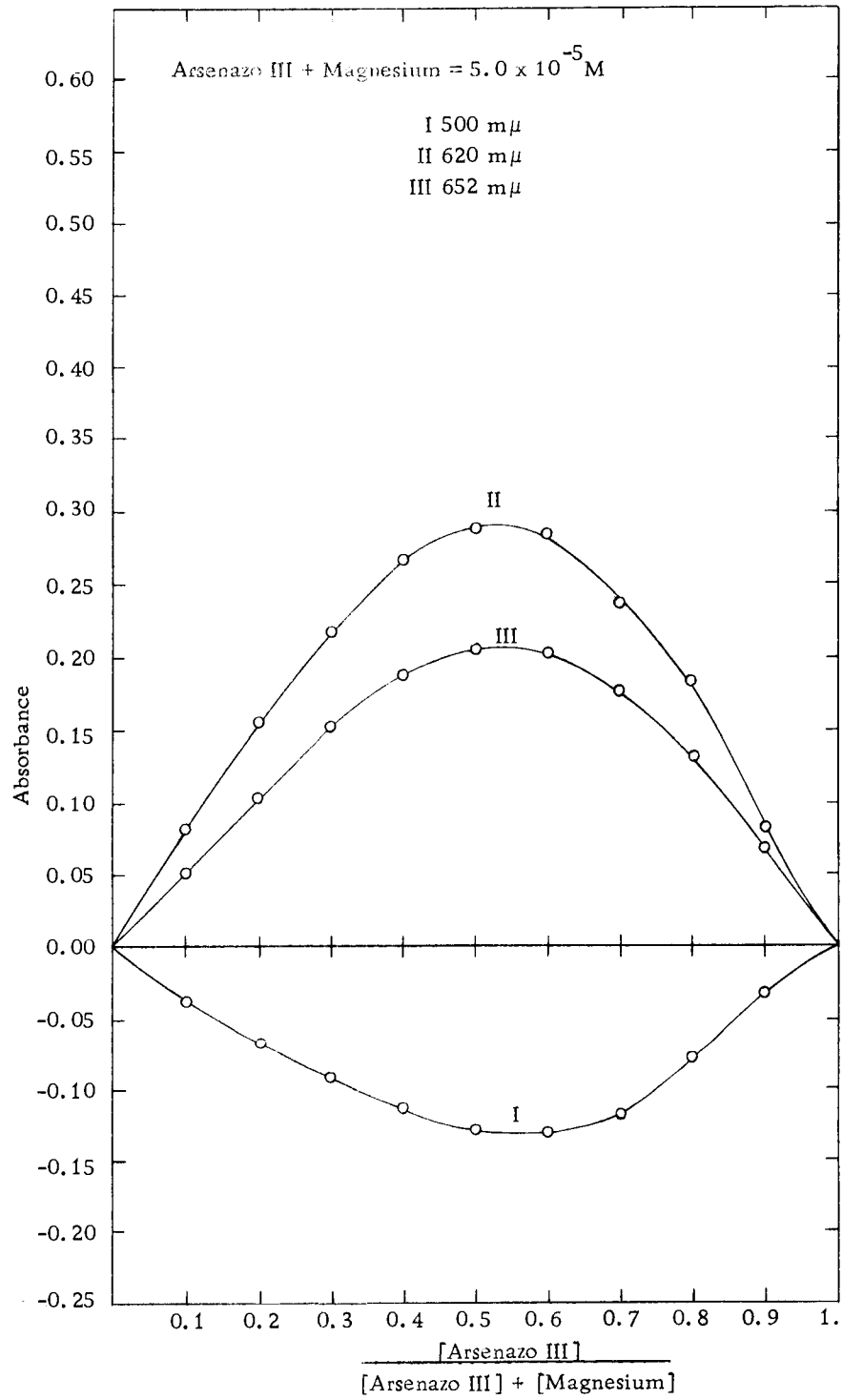


Figure 12. Continuous Variations Method for the Magnesium-Arsenazo III Complex at pH 8.4.

5.0×10^{-4} M Arsenazo III were added. Various volumes of 1.0×10^{-3} M or 1.0×10^{-4} M calcium chloride or magnesium chloride were added to each flask. The flasks were diluted to volume, mixed, and the absorption spectrum of each solution recorded from 700-600 mμ against a buffer blank. The absorbance due to the Arsenazo III was subtracted manually. In two cases, the differential absorption spectra of the complex were determined for Arsenazo III concentrations of 5.0×10^{-5} M and 2.5×10^{-5} M, and various concentrations of calcium chloride, by recording the spectra against an Arsenazo III blank. The data obtained by the molar-ratio method for the pH 8.4 complexes are given in Tables 4 and 5, and Figures 13 and 14.

Table 3. Combining Ratio of Magnesium-Arsenazo III Complex at pH 8.4 by the Continuous Variations Method

Mole Fraction Arsenazo III	A corr. 652 mμ	A corr. 620 mμ	A corr. 500 mμ
0.0	0.000	0.000	0.000
0.1	0.050	0.082	-0.038
0.2	0.103	0.184	-0.066
0.3	0.150	0.238	-0.091
0.4	0.188	0.285	-0.113
0.5	0.205	0.288	-0.129
0.6	0.203	0.266	-0.131
0.7	0.176	0.218	-0.118
0.8	0.132	0.155	-0.079
0.9	0.066	0.080	-0.033
1.0	0.000	0.000	0.000

Table 4. Combining Ratio of Calcium-Arsenazo III Complex at pH 8.4 by the Molar-Ratio Method

Arsenazo III	Calcium Chloride	$A_{complex}$ 652 mμ	$A_{complex}$ 604 mμ
5.0×10^{-5} M	5.0×10^{-6} M	0.149	0.118
	1.0×10^{-5} M	0.294	0.254
	1.5×10^{-5} M	0.439	0.352
	2.0×10^{-5} M	0.580	0.466
	2.5×10^{-5} M	0.719	0.587
	3.0×10^{-5} M	0.845	0.695
	5.0×10^{-5} M	1.143	0.959
	1.0×10^{-4} M	1.194	1.022
	2.0×10^{-4} M	1.168	1.036
	3.0×10^{-4} M	1.161	1.046
2.5×10^{-5} M	2.0×10^{-6} M	0.049	0.023
	5.0×10^{-6} M	0.128	0.057
	7.0×10^{-6} M	0.184	0.098
	1.0×10^{-4} M	0.548	0.406
	2.0×10^{-4} M	0.509	0.407
	5.0×10^{-4} M	0.548	0.413

Table 5. Combining Ratio of Magnesium-Arsenazo III Complex at pH 8.4 by the Molar-Ratio Method

Arsenazo III	Magnesium Chloride	$A_{complex}$ 652 mμ	$A_{complex}$ 620 mμ
5.0×10^{-5} M	5.0×10^{-6} M	0.044	0.068
	1.0×10^{-5} M	0.098	0.170
	2.0×10^{-5} M	0.209	0.328
	4.0×10^{-5} M	0.388	0.576
	5.0×10^{-5} M	0.435	0.649
	1.0×10^{-4} M	0.539	0.820
	2.0×10^{-4} M	0.576	0.916
	5.0×10^{-4} M	0.583	0.959

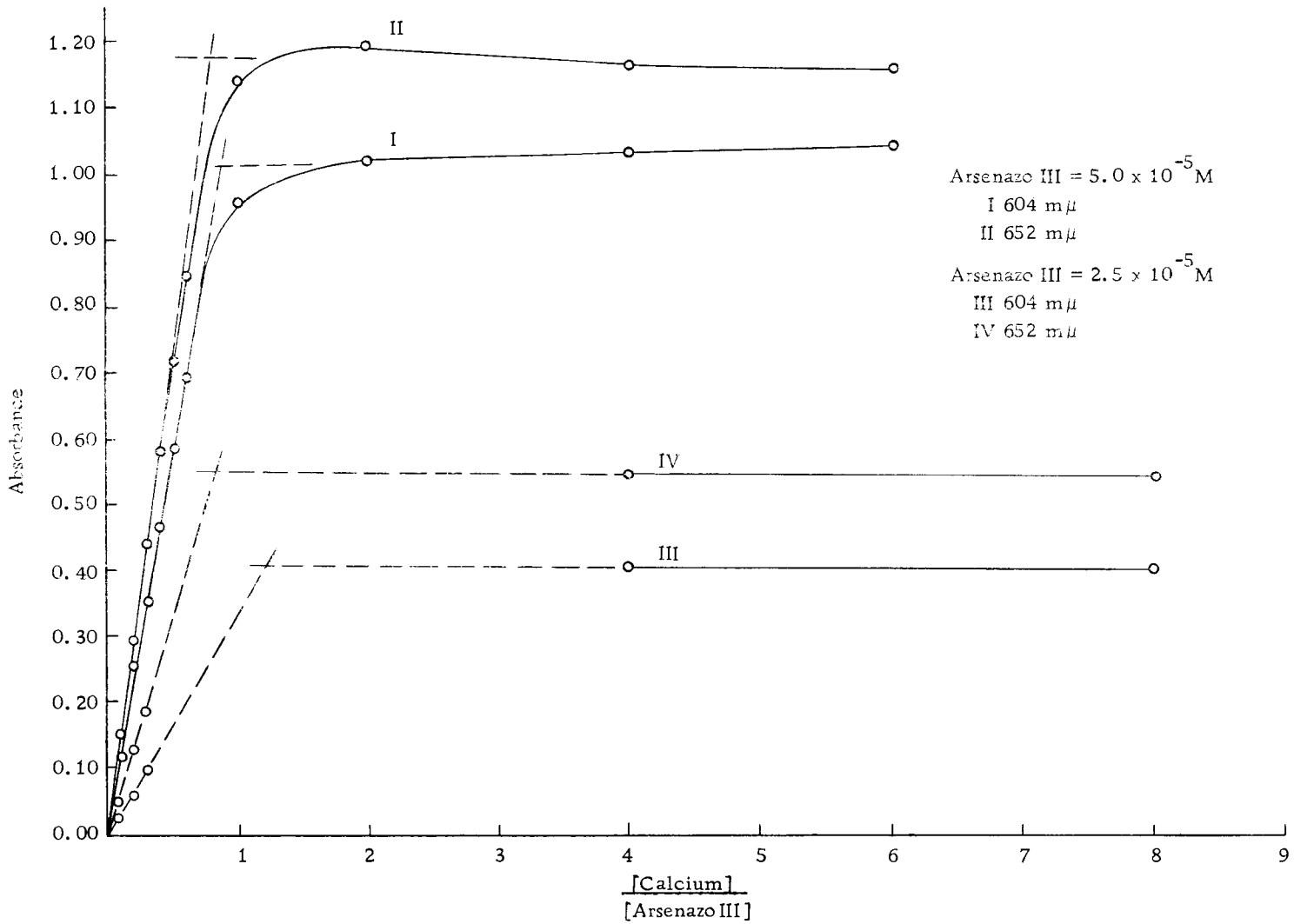


Figure 13. Molar-Ratio Method for the Calcium-Arsenazo III Complex at pH 8.4

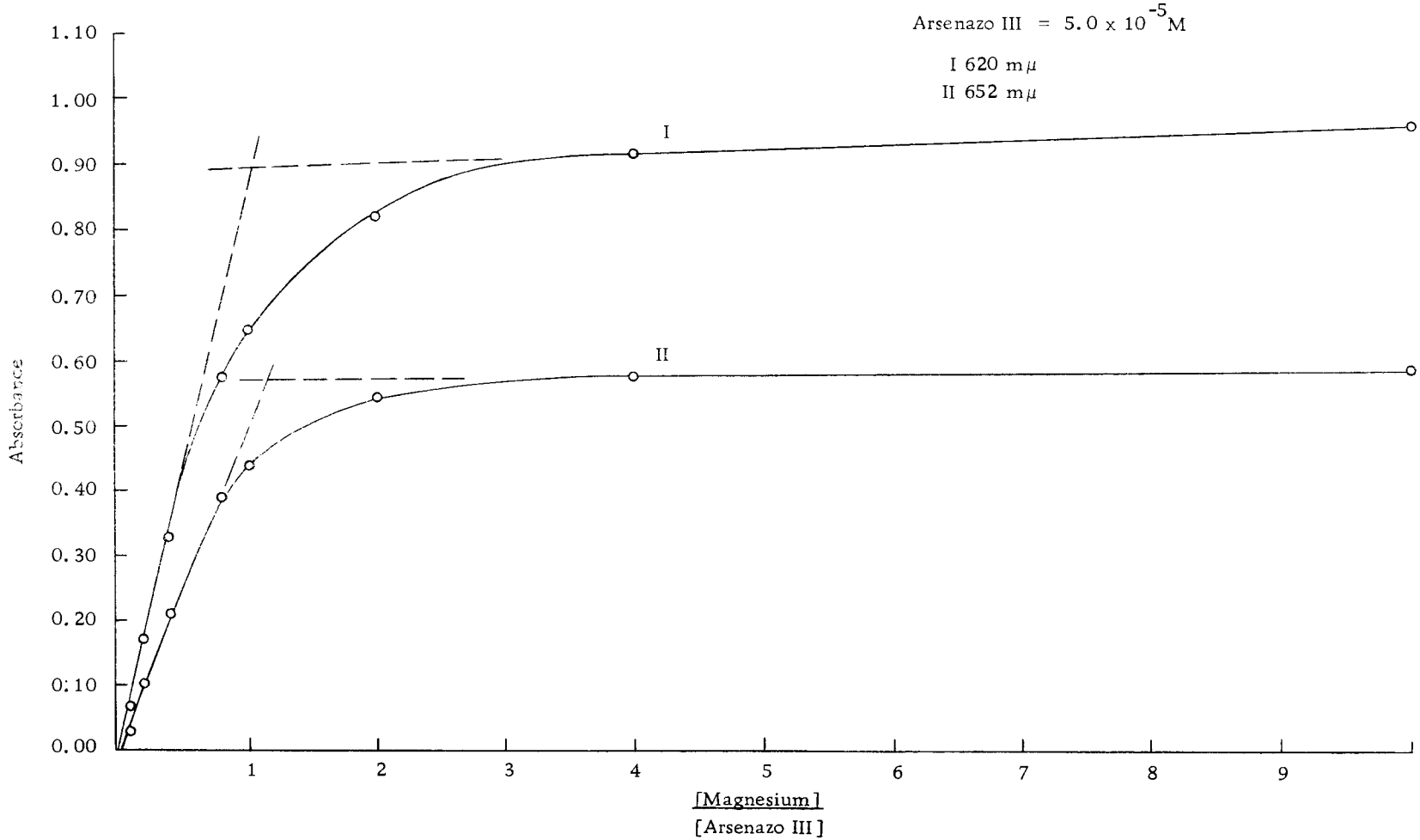


Figure 14. Molar-Ratio Method for the Magnesium-Arsenazo III Complex at pH 8.4

The data presented for the calcium and magnesium complexes at pH 8.4 in this report are representative of the data obtained for all wavelengths in the range 500-700 mμ. The wavelengths of 500 mμ, 604 mμ, and 652 mμ represent maxima in the differential absorption spectrum of the calcium complex. The wavelengths 500 mμ and 620 mμ represent maxima in the differential absorption spectrum of the magnesium complex.

Discussion

As noted in the introduction, the complexes formed between the plus two charged cations and Arsenazo III usually have a combining ratio of 1:1. As shown in Figure 10 and Table 1, the continuous variations study of the calcium complex of Arsenazo III at pH 4.1 gave a combining ratio of nearly 1:1, the absorption maximum occurring between 0.5-0.6 mole fraction Arsenazo III. The continuous variations method also yielded a nearly 1:1 complex for the pH 8.4 calcium complex and for the pH 8.4 magnesium complex, as shown in Figures 11 and 12 respectively. For both the calcium and magnesium complexes, the absorption maximum for the continuous variations data occurred between 0.5-0.6 mole fraction Arsenazo III.

The molar-ratio method as applied to the pH 8.4 calcium complex gave a combining ratio of slightly greater than 1:1,

Arsenazo III to calcium, at both 604 mμ and 652 mμ, when holding the Arsenazo III at 5.0×10^{-5} M. When holding the Arsenazo III at 2.5×10^{-5} M, the pH 8.4 calcium complex combining ratio was determined to be slightly less than 1:1 at 604 mμ and slightly greater than 1:1 at 652 mμ. The data for the molar-ratio determination of the calcium complex are given in Figure 13 and Table 4. The pH 8.4 magnesium complex combining ratio, as determined by the molar-ratio method, was 1:1. The data for this complex are given in Figure 14 and Table 5.

Several explanations are available for why the two methods for determining the combining ratio gave results which deviate slightly from a 1:1 ratio. The deviation may be due to the association of uncomplexed Arsenazo III molecules with complexed Arsenazo III molecules. Zollinger (28, p. 327) has reported that an association of azo dye molecules in alkaline solution is revealed by a decrease in the wavelength of the dye's absorption maximum; and the author has found that the absorption maximum of Arsenazo III at pH 8.4 shifts from 564 mμ at 1.0×10^{-5} M to 540 mμ at 5.0×10^{-5} M. The deviation may also be due to the formation of two or more complexes as indicated by the data in Figure 13 for the pH 8.4 calcium complex at two concentrations and two wavelengths. These data may indicate that the two absorption maxima of the complex are independent functions under certain conditions.

APPLICATION OF BEER'S LAW

The applicability of Beer's law to the calcium and magnesium complexes is the problem of this study. The conditions for the simultaneous determination of calcium and magnesium with Arsenazo III have been developed from the previous study of the pH dependence of the absorption spectra of the reagent and complexes. The absorbance for both the calcium and magnesium complexes is to be measured at 652 mμ. The complexes are to be formed at pH 4.1 and pH 8.4.

Procedure

For the Beer's law determination of calcium at pH 4.1, the following solutions were prepared. To 100 ml volumetric flasks were added 10 ml of pH 4.1 buffer, 10 ml of 5.0×10^{-4} M Arsenazo III, and then various concentrations of 1.00×10^{-4} M calcium chloride. After diluting to volume with distilled water, the absorbance of the solutions were measured at 652 mμ against a blank prepared from the buffer. A manual correction was made for the absorbance due to Arsenazo III. The only reason for not using an Arsenazo III blank was that the same solutions were to be used for the complex stability studies, for which it was desirable to measure the absorption spectra against the buffer blank.

The applicability of Beer's law to both the calcium and magnesium complexes at pH 8.4 was also studied. The same preparation and measurement procedure was followed, as for the pH 4.1 studies, except for the substitution of tris(hydroxymethyl)-aminomethane buffer for the sodium formate buffer. The data obtained for the Beer's law study are given in Table 6.

Table 6. Beer's Law Data for Dual-pH Method

pH 4.1 Solution

Calcium ppm	$A_{complex}$ 652 mμ
0.08	0.016
0.40	0.061
0.80	0.128
1.20	0.196
2.00	0.310

pH 8.4 Solution

Calcium ppm	$A_{complex}$ 652 mμ	Magnesium ppm	$A_{complex}$ 652 mμ
0.20	0.149	0.05	0.024
0.40	0.294	0.12	0.044
0.60	0.439	0.24	0.098
0.80	0.580	0.48	0.209
1.00	0.719	0.97	0.388
1.20	0.845	1.22	0.435
2.00	1.143	2.43	0.539

Discussion

Figure 15 includes a plot of absorbance versus concentration (ppm) for the Arsenazo III complex of calcium at pH 4.1, of the

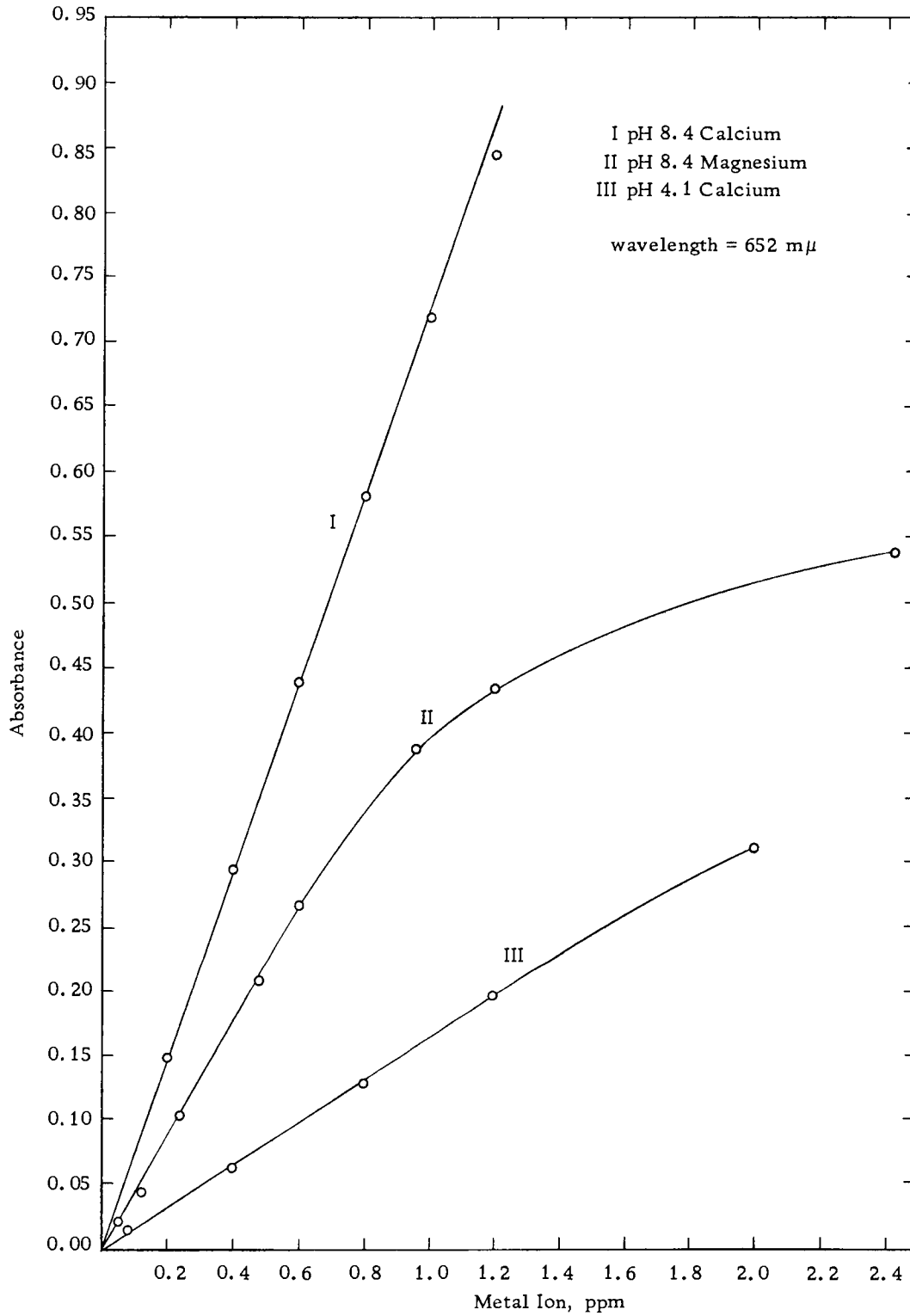


Figure 15. Beer's Law Plots for Arsenazo III Complexes of Calcium at pH 4.1 and pH 8.4, and Magnesium at pH 8.4.

calcium complex at pH 8.4, and of the magnesium complex at pH 8.4. For calcium at pH 4.1, the straight line portion of the curve extends from 0-1.20 ppm. At pH 8.4 the straight line portion of the curve extends from 0-0.80 ppm calcium and from 0-0.60 ppm magnesium.

Due to the relatively high absorbance of Arsenazo III at 652 mμ, an effort to extend the Beer's law compliance range by increasing the Arsenazo III concentration was not attempted. Increasing the Arsenazo III concentration would have required the use of wider spectrophotometer slits and would have resulted in only a slight extension of the Beer's law region at best. The final concentration of 5.0×10^{-5} M Arsenazo III is sufficient to complex 2.0 ppm calcium or 1.2 ppm magnesium, if the combining ratio of Arsenazo III and the alkaline earth elements is 1:1.

Additional sensitivity for the calcium complex could have been obtained by measuring the absorbance at pH 5.1-5.5; however, magnesium also forms a weakly colored complex in this pH range with a molar absorptivity of approximately 800 l $mole^{-1}$ cm^{-1}. Using pH 5.1-5.5 and pH 8.4 solutions to determine the calcium and magnesium would require the solving of two simultaneous equations, each having two unknowns. The sensitivity for magnesium at pH 8.4 would be increased if the absorbance were measured at 620 mμ; however, the absorbance versus wavelength slope of the Arsenazo III is

greater at this wavelength than at 652 mμ.

The approximate apparent molar absorptivities for the two complexes of interest were calculated from the Beer's law data at 652 mμ. At pH 4.1 the apparent molar absorptivity is about 6.3×10^3 l mole^{-1} cm^{-1} for the calcium complex, and less than 100 l mole^{-1} cm^{-1} for the magnesium complex. At pH 8.4 the apparent molar absorptivity for the calcium complex is about 2.9×10^4 l mole^{-1} cm^{-1}, and for the magnesium complex it is about 9.6×10^3 l mole^{-1} cm^{-1}.

REAGENT AND COMPLEX STABILITY

The stability of the calcium complex of Arsenazo III at pH 4.1 and pH 8.4, and the magnesium complex of Arsenazo III at pH 8.4 was studied over a period of several days. The samples were kept in glass stoppered pyrex bottles and divided into two sets. One set was stored in total darkness and the other set was exposed to sunlight and the fluorescent room lights. A study was also made of the stability of the Arsenazo III blank solutions during this same period.

Procedure

All solutions contained 5.0×10^{-5} M Arsenazo III and various concentrations of calcium or magnesium. The pH 4.1 solutions contained 2.0×10^{-5} M calcium chloride, the pH 8.4 calcium solutions contained 1.0×10^{-5} M calcium chloride, and the pH 8.4 magnesium solutions contained 1.0×10^{-5} M magnesium chloride. The buffers used were sodium formate at pH 4.1, sodium borate for the calcium solutions at pH 8.4, and tris(hydroxymethyl)aminomethane for the pH 8.4 magnesium solutions. Except for the calcium complex at pH 4.1, all absorbance measurements were made from recorded absorption spectra. The data for this study are presented in Table 7.

Discussion

The complex forms immediately upon addition of the alkaline earth element to the Arsenazo III in aqueous solution. The calcium and magnesium complexes are stable for at least one day, and the absorbance of the complex plus Arsenazo III solutions increases only 0.01 absorbance units in the three day period following mixing. The Arsenazo III blank prepared using sodium borate buffer was stable for at least 85 days. Comparison of the samples of each alkaline earth element complex which were left in the light with those samples kept in the dark showed no differences in complex decay rate.

The small differences in the pH 8.4 data from day to day can be attributed to the inability to exactly duplicate the same wavelength and slit settings for each measurement. For that reason, the minimum significant absorbance change is considered to be ±0.005. The difference in the reagent absorbance for the pH 8.4 calcium and magnesium complexes is due to the use of sodium borate buffer with the calcium solution. This buffer is thought to form a weak complex with Arsenazo III.

Table 7. Reagent and Complex Stability

Time After Mixing	A at 680 mμ		A at 652 mμ		A at 620 mμ	
	Arsenazo III	Cation + Arsenazo III	Arsenazo III	Cation + Arsenazo III	Arsenazo III	Cation + Arsenazo III
Calcium, pH 4.1, Sodium Formate Buffer						
1/2 hour			0.072	0.188		
1 hour			0.061	0.190		
3 hour			0.061	0.189		
1 day			0.076	0.196		
3 day			0.077	0.203		
Calcium, pH 8.4, Sodium Borate Buffer						
1/2 hour	0.081	0.180	0.189	0.451	0.457	0.590
3 1/2 hour	0.086	0.178	0.201	0.451	0.470	0.588
1 day	0.085	0.180	0.199	0.452	0.467	0.588
3 day	0.081	0.186	0.194	0.465	0.462	0.602
Magnesium, pH 8.4, Tris(hydroxymethyl)aminomethane Buffer						
1 hour	0.075	0.119	0.172	0.269	0.428	0.535
4 hour	0.074	0.118	0.171	0.269	0.427	0.536
3 day	0.078	0.124	0.180	0.283	0.432	0.545
9 day	0.078	0.119	0.166	0.268	0.388	0.533

INTERFERING IONS

In order to fully evaluate a method for the spectrophotometric determination of calcium and magnesium, it is important to determine the effect of diverse ions on the absorbance of the complex. Several cations and anions were selected which commonly interfere with the determination of calcium and magnesium. The criterion selected for designating an ion as interfering is that concentration of ion which causes a change of ± 0.01 absorbance unit in the absorbance of a calcium complex with Arsenazo III. A change of ±0.01 absorbance unit is equivalent to an error of ± 0.061 ppm calcium at pH 4.1, ± 0.014 ppm calcium at pH 8.4, and ± 0.022 ppm magnesium at pH 8.4.

Procedure

The interfering ions were prepared as 1000 ppm and 250 ppm stock solutions. The cations were prepared from either chloride or nitrate salts. The anions were prepared from either sodium or potassium salts. The cations and anions studied, and the salt from which each was prepared, are listed in Table 8.

The solutions were prepared by adding 5 ml of either pH 4.1 sodium formate buffer or pH 8.5 tris(hydroxymethyl)aminomethane buffer to each of five 50 ml volumetric flasks. Five ml of 5.0×10^{-5} M Arsenazo III, followed by 10 ml of 1.25×10^{-4} M calcium

Table 8. Permissable Concentrations of Interfering Ions

Ion	Salt	pH 4.1 ppm Ion for ±0.01 A Error	pH 4.1 ΔA	pH 8.4 ppm Ion for ± 0.01 A Error	pH 8.4 ΔA
Li^{+}	LiCl	20 ppm	decr.	5 ppm	incr.
Na^{+}	NaCl	100 ppm	"	10 ppm	"
K^{+}	KCl	100 ppm	"	20 ppm	"
NH_4^{+}	NH_4Cl	40 ppm	"	20 ppm	"
Cu^{2+}	$CuCl_2 \cdot 2\ H_2O$	< 5 ppm	incr.	0.5 ppm	"
Zn^{2+}	$Zn(NO_3)_2 \cdot 6\ H_2O$	40 ppm	decr.	1 ppm	"
Al^{3+}	$Al(NO_3)_3 \cdot 9\ H_2O$	< 1 ppm	incr.	< 0.5 ppm	"
Fe^{3+}	$Fe(NO_3)_3 \cdot 9\ H_2O$	1 ppm	"	10 ppm	"
SO_4^{2-}	Na_2SO_4 (anhyd.)	40 ppm	decr.	50 ppm	"
PO_4^{3-}	KH_2PO_4	200 ppm	"	5 ppm	"
CO_3^{2-}	Na_2CO_3 (anhyd.)	5 ppm	incr.	50 ppm	"
$citrate^{3-}$	$Na_3C_6H_5O_7 \cdot 2\ H_2O$	20 ppm	"	10 ppm	decr.
$tartrate^{2-}$	$Na_2C_4H_4O_6 \cdot 2\ H_2O$	40 ppm	"	20 ppm	"
$oxalate^{2-}$	$Na_2C_2O_4$	40 ppm	"	5 ppm	incr.

chloride, pH 4.1, or 10 ml of 6.25×10^{-5} M calcium chloride, for pH 8.4 solutions, was added to each flask. After dilution to volume, these amounts of calcium chloride equaled concentrations of 1.00 ppm calcium in pH 4.1 solutions and 0.50 ppm calcium in the pH 8.4 solutions. To the series of five flasks prepared as above were added sufficient amounts of interfering ion to yield final concentrations after dilution of 0, 1, 5, 10, and 20 ppm of the ion. The flasks were diluted to volume, mixed, and the absorbance measured at 652 mμ against a blank containing only buffer components.

For the case in which 1 ppm of the interfering ion at pH 8.4 produced an absorbance change greater than ±0.01, an additional solution was prepared which contained only 0.5 ppm of the ion and the absorbance measured in the same manner. For the case in which 20 ppm of the interfering ion was not sufficient to cause a change of ±0.01 absorbance unit, additional solutions were prepared (40, 100, and 200 ppm of the ion at pH 4.1; and 50 and 100 ppm of the ion at pH 8.4) and the absorbance measured as above. A concentration of 200 ppm of the ion was sufficient to yield an absorbance change equal to ±0.01 for all ions studied.

Discussion

From Table 8, it is seen that the cations interfering most at pH 8.4 are aluminum (III), copper (II), and zinc (II). At pH 4.1 only

aluminum (III) and iron (III) are strong interferences. With the exception of iron (III), the determination of calcium at pH 4.1 is less sensitive to the effects of interfering cations than at pH 8.4. At neither pH do the alkali metal elements interfere seriously.

Only sulfate, of the six anions studied, interfered more at pH 4.1 than at pH 8.4. Carbonate interfered at the 5 ppm level at pH 4.1, but this is probably due to an impurity in the sodium carbonate stock solution used for the pH 4.1 and pH 8.4 interference studies. Of the anions studied, only citrate, at 10 ppm, interferes at less than the 20 ppm level at either pH value.

The effect of interfering ions on the magnesium complex was not studied. It was assumed that the effect of the interfering ions on the magnesium complex would approximate the effect found for the calcium complex. The effect of strontium and barium on the calcium complex was not measured in the interference studies. An extrapolation of the results obtained in the pH dependence study of the absorption spectra of the alkaline earth element complexes projects the following results. About 1 ppm of strontium or barium would cause an absorbance change of ± 0.01 absorbance units at pH 4.1. Less than 0.5 ppm strontium or 1 ppm barium would give an absorbance change of ± 0.01 absorbance units at pH 8.4.

EGTA-ARSENAZO III METHOD

As noted in the introduction, Lamkin and Williams (8) have developed a simultaneous spectrophotometric method for the determination of calcium and magnesium with a combination of Arsenazo I and EGTA, [ethylenebis(oxyethylenenitrilo)] tetraacetic acid. A preliminary study was made of a similar method with Arsenazo III, with the idea of presenting an alternative method to the dual pH method proposed in this report.

Procedure

Five ml of a synthetic solution of calcium chloride and magnesium chloride was pipetted into a 50 ml volumetric flask, followed by 5 ml of pH 8.5 tris(hydroxymethyl)aminomethane buffer and 5 ml of 5.0×10^{-4} M Arsenazo III. After diluting to volume with distilled water and mixing, the absorbance due to the total calcium and magnesium in the sample was measured at 652 mμ against a blank prepared in the same manner from Arsenazo III and buffer. Twenty-five ml of the alkaline earth elements plus Arsenazo III solution was then pipetted into a third 50 ml flask, 2 ml of 1.0×10^{-3} M EGTA (K and K Laboratories) and 3 ml of pH 8.5 tris(hydroxymethyl)aminomethane added, and the solution diluted to volume with distilled water. After mixing, the absorbance of the sequestered

sample was measured, at 652 mμ in 1.0 cm cells, against a blank prepared from the previous blank in the same manner as for the sample solution. The data for the EGTA sequestered magnesium-Arsenazo III complex are given in Table 9.

Table 9. Beer's Law Data for Magnesium-Arsenazo III Complex in EGTA Sequestered Solutions

Magnesium, ppm First Solution	Magnesium, ppm EGTA Solution	$A_{complex}$ 652 mμ
0.19	0.095	0.017
0.49	0.243	0.046
0.97	0.486	0.088
1.46	0.730	0.120
1.95	0.973	0.148
2.43	1.216	0.168
2.92	1.459	0.190

Discussion

The absorbance of the sequestered solution is due to the magnesium complex of Arsenazo III, the calcium having been sequestered by the EGTA. The magnesium portion of the total calcium and magnesium concentration as measured at pH 8.4 in the first flask was determined from the EGTA-Arsenazo III solution calibration curve shown in Figure 16. This calibration curve was prepared by the above procedure from samples which contained only magnesium chloride and, as plotted, reads out directly the magnesium concentration in the first flask. The absorbance due to the same concentration of

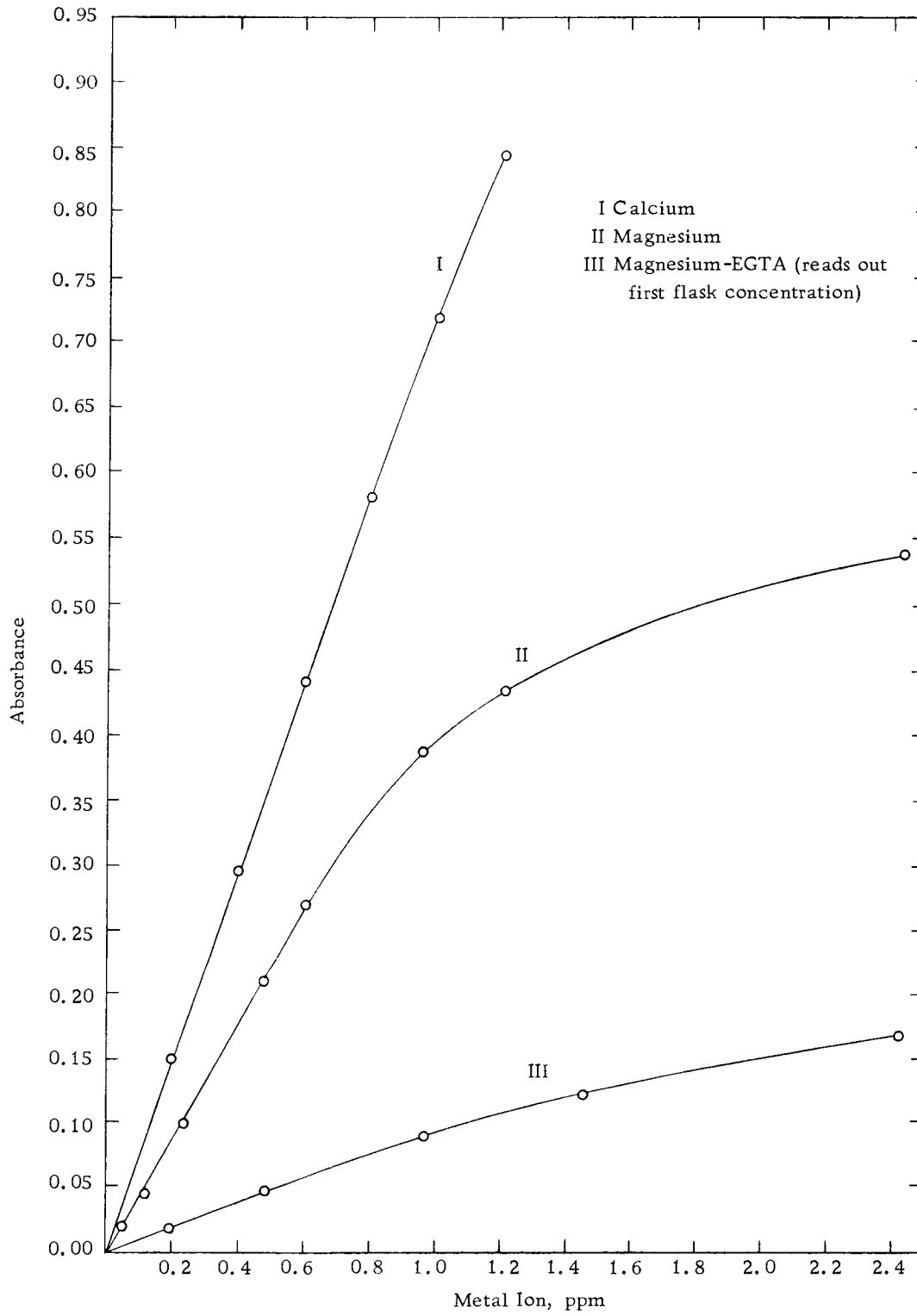


Figure 16. Beer's Law Plots for Arsenazo III Complexes of Calcium and Magnesium at pH 8.4 and 652 mμ.

magnesium in the pH 8.4 Arsenazo III solution is projected from the Beer's law magnesium calibration curve which was determined earlier. This value is subtracted from the total absorbance found for the calcium and magnesium at pH 8.4 to determine the absorbance due to the calcium complex. The calcium concentration is then determined from the Beer's law calibration curve for the pH 8.4 calcium complex. The Beer's law calibration curves for the magnesium and calcium complexes have been replotted in Figure 16 along with the EGTA-Arsenazo III-magnesium calibration curve.

The EGTA sequestered solution obeys Beer's law between 0-0.50 ppm magnesium. The results of the determination of the three synthetic mixtures of calcium chloride and magnesium chloride at different concentration ratios, listed in Table 10, are shown in Table 11. (See pages 58 and 59)

The results of this preliminary investigation show that only with the use of 4-10 cm spectrophotometer cells for measuring the absorbance of the EGTA sequestered solutions would the EGTA-Arsenazo III method approach the sensitivity for magnesium of the EGTA-Arsenazo I method. It should be noted that the EGTA-Arsenazo I method used 4.0 cm cells to measure the absorbance of the EGTA sequestered solutions. The absorbance of the EGTA sequestered Arsenazo III-magnesium solution is about half that for a similar EGTA sequestered Arsenazo I-magnesium solution, taking into account the

difference in cell length. This is probably indicative of a lower formation constant for the Arsenazo III-magnesium complex than for the Arsenazo I-magnesium complex. Thus, the greater sensitivity of the Arsenazo III method for calcium, in comparison to the Arsenazo I method, is negated by the lower sensitivity for magnesium when using the EGTA sequestration method.

The EGTA-Arsenazo III-magnesium solutions appear to be temperature sensitive. On standing in the unthermostatted cell compartment of the spectrophotometer, the absorbance of the Arsenazo III complex decreases. The absorbance change is not due to a time dependent complex decay since refilling the cell with the same solution, which was exposed to room light and kept at room temperature, produces the same initial absorbance, and the same decrease as time passes, as before. Since the original solution did not decrease in absorbance when exposed to room light, the absorbance change is probably not due to the effect of light.

DETERMINATION OF SYNTHETIC SOLUTIONS OF CALCIUM CHLORIDE AND MAGNESIUM CHLORIDE

Three synthetic mixtures were prepared from stock solutions of magnesium chloride and calcium chloride to test the accuracy of the simultaneous direct spectrophotometric method proposed in this report. The solution concentrations, and the concentrations of calcium and magnesium ions following a 1:10 dilution, are listed in Table 10.

Table 10. Synthetic Solutions of Calcium Chloride and Magnesium Chloride

Synthetic Solution	Synthetic Solution Concentration	Concentration After Diluting 1 to 10
A	2.00×10^{-4} M Ca^{2+}	0.80 ppm Ca^{2+}
	8.00×10^{-5} M Mg^{2+}	0.20 ppm Mg^{2+}
B	1.25×10^{-4} M Ca^{2+}	0.50 ppm Ca^{2+}
	2.00×10^{-4} M Mg^{2+}	0.49 ppm Mg^{2+}
C	5.00×10^{-5} M Ca^{2+}	0.20 ppm Ca^{2+}
	3.20×10^{-4} M Mg^{2+}	0.78 ppm Mg^{2+}

The synthetic solutions were determined by both the dual-pH method and the EGTA-Arsenazo III method using the procedures which have been previously discussed for each method. All determinations were made at 652 mμ and an Arsenazo III blank was used in each case. Three aliquots, diluted 1 to 10, of each synthetic solutions were determined. The results of the determination of the synthetic solutions by both methods are given in Table 11.

Table 11. Simultaneous Spectrophotometric Determination of Synthetic Calcium and Magnesium Solutions

Standard Solution	Given	Found: Dual-pH Method	Found: Arsenazo III+ EGTA Method
A	0.80 ppm Ca^{2+}	0.81 ppm	0.81 ppm
	0.80 ppm Ca^{2+}	0.82 ppm	0.80 ppm
	0.80 ppm Ca^{2+}	0.80 ppm	0.82 ppm
		0.80 ± 0.01	0.81 ± 0.01
	0.20 ppm Mg^{2+}	0.20 ppm	0.22 ppm
	0.20 ppm Mg^{2+}	0.19 ppm	0.22 ppm
	0.20 ppm Mg^{2+}	0.24 ppm	0.22 ppm
		0.21 ± 0.01	0.22± 0.00
B	0.50 ppm Ca^{2+}	0.48 ppm	0.51 ppm
	0.50 ppm Ca^{2+}	0.48 ppm	0.49 ppm
	0.50 ppm Ca^{2+}	0.51 ppm	0.53 ppm
		0.49 ± 0.01	0.49 ± 0.01
	0.49 ppm Mg^{2+}	0.52 ppm	0.49 ppm
	0.49 ppm Mg^{2+}	0.52 ppm	0.51 ppm
	0.49 ppm Mg^{2+}	0.48 ppm	0.53 ppm
		0.51 ± 0.02	0.51 ± 0.01
C	0.20 ppm Ca^{2+}	0.17 ppm	0.16 ppm
	0.20 ppm Ca^{2+}	0.17 ppm	0.16 ppm
	0.20 ppm Ca^{2+}	0.17 ppm	0.16 ppm
		0.17 ± 0.00	0.16 ± 0.00
	0.78 ppm Mg^{2+}	0.78 ppm	0.81 ppm
	0.78 ppm Mg^{2+}	0.81 ppm	0.82 ppm
	0.78 ppm Mg^{2+}	0.82 ppm	0.81 ppm
		0.80 ± 0.02	0.81 ± 0.00

Equations for the Dual-pH Method

To facilitate the determination of the concentration of calcium or magnesium in a sample, the straight line portion of the Beer's law curves for the pH 4.1 and pH 8.4 complexes have been reduced to constants. The concentrations of calcium and magnesium used in the computations are the concentration of the sample after mixing with Arsenazo III and diluting to volume. The factor A is the absorbance due to the calcium and/or magnesium complexes at the pH noted in the subscript. The sensitivity factor K is the inverse of the Beer's law curve slope for each complex.

$$K_1 = \frac{Ca^{2+}}{A_{4.1}} = \frac{6.12 \text{ ppm}}{1.000} = \frac{0.305 \text{ meq/l}}{1.000}$$

$$K_2 = \frac{Ca^{2+}}{A_{Ca8.4}} = \frac{1.46 \text{ ppm}}{1.000} = \frac{0.0679 \text{ meq/l}}{1.000}$$

$$K_3 = \frac{Mg^{2+}}{A_{Mg8.4}} = \frac{2.24 \text{ ppm}}{1.000} = \frac{0.184 \text{ meq/l}}{1.000}$$

The incorporation of these constants into two equations for the direct determination of calcium and magnesium in the diluted solutions yields the following equations.

1) calcium concentration $= K_1 \times A_{4.1}$

2) magnesium concentration $= K_3(A_{8.4} - \frac{K_1}{K_2} \times A_{4.1})$

$= K_3(A_{8.4} - 4.50 \times A_{4.1})$

Equations for the EGTA-Arsenazo III Method

Similar equations were developed for the EGTA-Arsenazo III method even though only a preliminary study was made of this method. The symbols are the same as before, and the concentrations are those of the diluted sample solutions.

$$K_4 = \frac{Mg^{2+}}{A_{Mg\text{-}EGTA}} = \frac{10.8 \text{ ppm}}{1.000} = \frac{0.889 \text{ meq/l}}{1.000}$$

$$K_5 = \frac{Ca^{2+}}{A_{Ca8.4}} = \frac{1.46 \text{ ppm}}{1.000} = \frac{0.0679 \text{ meq/l}}{1.000}$$

$$K_6 = \frac{Mg^{2+}}{A_{Mg8.4}} = \frac{2.24 \text{ ppm}}{1.000} = \frac{0.184 \text{ meq/l}}{1.000}$$

Note that the constants K_5 and K_6 are identical to K_2 and K_3 since they are derived from the same curves which are used in both methods. The incorporation of these three constants into two equations for the direct determination of calcium and magnesium yields the following equations.

3) magnesium concentration $= K_4 \times A_{Mg\text{-}EGTA}$

4) calcium concentration $= K_5(A_{8.4} - \frac{K_4}{K_6} \times A_{Mg\text{-}EGTA})$

$= K_5(A_{8.4} - 4.83 \times A_{Mg\text{-}EGTA})$

Discussion

The results of the determination of the synthetic solutions show

the possibilities of the two methods proposed in this report for the direct simultaneous spectrophotometric determination of calcium and magnesium. The relative error in all cases except the 0.20 ppm calcium, as determined by both methods, and the 0.20 ppm magnesium determined by the EGTA-Arsenazo III method, is within ± 5 percent. There is no significant difference in the concentrations as determined by either method.

Both methods are limited by one step of the two step absorbance measurements. In the dual-pH method the limiting step is the pH 4.1 calcium complex absorbance; whereas, for the EGTA-Arsenazo III method, the limiting step is the absorbance of the magnesium complex following the EGTA sequestration. The sensitivity of either limiting step would be improved by the use of 4 cm or longer spectrophotometer cells. The use of the longer cells would cause the sensitivity of the limiting steps to increase, so as to more closely approximate the sensitivity of the Arsenazo III complex of magnesium or calcium at pH 8.4. The use of the longer cells in the dual-pH method would, however, require the solving of two simultaneous equations having two unknowns each, since the magnesium complex would be a significant interference at pH 4.1.

SUMMARY

A dual-pH simultaneous direct spectrophotometric method for the determination of calcium and magnesium using Arsenazo III has been developed in this investigation. A procedure was developed to purify the commercial Arsenazo III used in this work. The method has good sensitivity, the reagent and complexes have good stability, and the complexes are relatively free from the effects of interfering ions.

Future work on the Arsenazo III-alkaline earth element system should consist of the following: determination and assignation of the dissociation constants of the pure Arsenazo III; determination of the formation constants of the alkaline earth element complexes; and application of the proposed dual-pH method to a practical situation, such as the determination of calcium and magnesium in blood serum, for which the method is immanently suited.

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