

A SPECTROPHOTOMETRIC STUDY OF ARSENAZO  
AND ITS CALCIUM COMPLEX

by

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# A SPECTROPHOTOMETRIC STUDY OF ARSENAZO AND ITS CALCIUM COMPLEX

## INTRODUCTION

Until recently, small amounts of calcium were determined by indirect colorimetric methods based on the precipitation of calcium oxalate or phosphate.

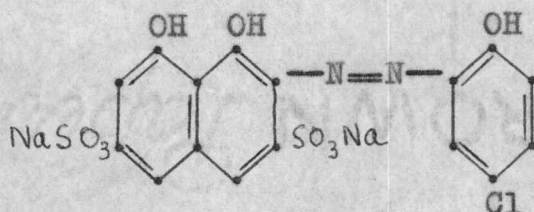
In the last few years several new methods have been developed for the colorimetric determination of calcium. One of these is a direct method with ammonium purpurate (murexide) as the colorimetric reagent. Williams and Moser (23) have developed a procedure for calcium with murexide in which concentrations of calcium up to 1.2 p.p.m. may be determined. A very attractive feature of this method is its sensitivity. On the other hand, the pH must be adjusted carefully, the reagent absorbs strongly at the optimum wavelength, the reagent is unstable, and many metals interfere.

Another reagent, 2,6-xylenolphthalein- $\alpha,\alpha'$ -bis(imino) diacetic acid, is a light rose color in weakly alkaline solution but gives a red color with calcium and other alkaline earths. A description has been given for its use in the determination of calcium by Pollard and Martin (19). The sensitivity is quite high but conditions must be carefully controlled and like murexide the

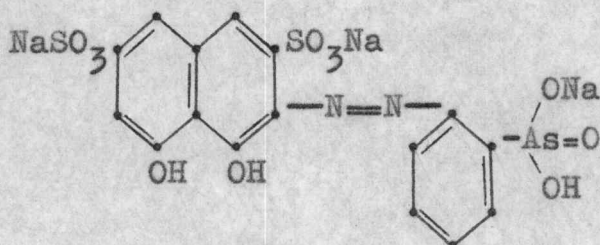


color is not stable.

Various azo dyes in basic solution have been suggested for the determination of calcium. One of these is Eriochrome Black T (1) which is also used as a reagent for magnesium. Another azo dye of the same type is 2-(2-hydroxy-5-chlorophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid, disodium salt (24).



Jones (9) has recently made a spectrophotometric investigation of the reagent 3-(2-arsonophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid, commonly referred to as arsenazo. In this investigation he studied the uranium (as  $\text{UO}_2^{+2}$ ), aluminum and calcium complexes of this reagent. He has indicated that arsenazo might be useful as a colorimetric reagent for calcium. The formula of arsenazo is



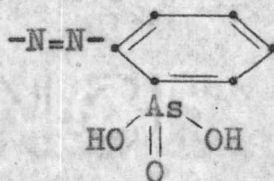
It was one of the objects of this thesis to explore the possibility of determining low level concentrations of calcium by means of the color reaction of calcium with arsenazo. Before this investigation was carried out, the absorbance characteristics of arsenazo in the ultraviolet and visible regions of the spectra were explored at various pH values ranging from 1 to 12. Also a study of the reactions between arsenazo and Zr and Hf was undertaken.

## PREVIOUS WORK WITH ARSENAZO

In its unpurified form arsenazo is a trisodium salt as is shown on page 2. It is a dark brown solid which may be prepared by coupling diazotized o-arsanilic acid with chromotropic acid (4). The trisodium salt was purified and converted into the acid form by pouring a saturated solution of the trisodium salt into an equal volume of concentrated HCl. The precipitate was filtered on a Buchner funnel, washed with acetonitrile, and allowed to air dry. The purified reagent which is orange dissolves readily in water. However, it is insoluble in most organic solvents including absolute alcohol, anhydrous ether, acetone, benzene, chloroform and carbon tetrachloride.

Arsenazo in its purified form was used for all studies presented in this thesis.

The first reports on arsenazo appeared in 1941 when Kuznetsov (11) published a paper describing the color reactions for a number of compounds containing the o-arsenophenylazo group:





Color reactions in neutral and acid solutions for arsenazo and the rare earths, Ti, Sn, Th, Cb, Ta, and U were given.

Further studies of the color reactions by Kuznetsov demonstrated the value of arsenazo in the detection of a number of elements. For example, as a reagent for the detection of rare earths arsenazo was suggested (14). The reaction is carried out at pH 7.2 using uratropine. The presence of the rare earths is indicated by the appearance of a red violet color which disappears on addition of  $\text{Na}_2\text{HPO}_4$ . A few interfering ions must be removed or masked.

Arsenazo will detect Th (12) in the presence of rare earths and other metals by the formation of a strawberry-red precipitate.

For the detection of quadrivalent vanadium (13) arsenazo is very sensitive ( $0.5 \times$  per ml). Other metals show similar violet colors but vanadium can be distinguished by the fact that the original pink of the reagent itself is restored by  $\text{H}_2\text{O}_2$ .

Beryllium (15) also may be detected in low concentrations (0.2 ug) in minerals by the red-violet color it gives in neutral solutions with arsenazo. All other interfering ions may be masked.

The past few years have seen the development of arsenazo as an analytical reagent particularly in the field of spectrophotometry. There are methods developed for aluminum, zirconium, thorium and uranium.

Kuznetsov has proposed two methods (16, 17) for aluminum, one which is very general and not applicable to any particular mineral or alloy and one based on the use of arsenazo for the determination of aluminum in chromium-nickel alloys. Both of these methods originate from the fact that at a pH 4.3 arsenazo gives a violet color with aluminum. With these procedures up to 1 p.p.m. aluminum may be determined.

A colorimetric determination of zirconium in its aluminum and magnesium alloys (18) has been proposed. Arsenazo which is orange at pH 1.6 changes to violet in the presence of zirconium and hafnium which must be determined together.

Zaikovskii and Gerhardt (25) have developed a technique for the spectrophotometric determination of thorium with arsenazo in the presence of zirconium, titanium and the rare earth elements. The interferences of these elements may be eliminated.

There have been two methods proposed for the determination of uranium with arsenazo within the last



year (6, 7). Uranium forms a stable blue complex with arsenazo with optimum absorbance at 590 m $\mu$ . The color reaction conforms to Beer's law and may be used to determine uranium in a number of alloys.

A new application for arsenazo in the analytical field has been recently developed. It is used as an indicator (5) in the titration of the rare earths, magnesium, and calcium with the chelating agent, ethylenediamine tetraacetic acid, EDTA. Rare earths are titrated at pH 5.0-6.5 and calcium or magnesium are titrated at pH 10 with arsenazo indicator.



## VARIATIONS IN ULTRAVIOLET AND VISIBLE SPECTRA OF ARSENAZO WITH pH

Since there has been no report regarding the absorption characteristics of arsenazo in the ultra-violet range of the spectrum, it was decided that such a study should be made. The absorbance peaks of most chelating compounds vary with the hydrogen ion concentration; therefore, the pH range from 1.15 to 12.35 was investigated. To record the spectra obtained at various pH values, a Beckman Model DK-1 automatic recording spectrophotometer was employed. Because of ease of use of such an instrument, the spectra were recorded from 200 to 700 mu with a matched pair of Beckman silica cells to hold sample and reference solutions.

### Instruments

#### Beckman Model DK-1 Spectrophotometer

##### Instrument settings.

Radiation sources:	Tungsten lamp (330 to 700 mu)
	Hydrogen lamp (200 to 330 mu)
Photomultiplier:	IX
Scan Time:	3 minutes
Time Constant:	0.1 second
Sensitivity:	50

#### Beckman Model H-2 pH Meter

## Reagents

### Arsenazo Solution

A 0.0011M solution of arsenazo was prepared by dissolving 0.6510 grams of purified reagent followed by dilution in a 1-liter volumetric flask.

### Buffers

Standard pH and constant ionic strength (0.1) buffer solutions were prepared according to Bates (2, p. 117) except for pH 1.15, 9.9 and 12.35 solutions. The pH 1.15 and 12.35 solutions were prepared with 0.1N HCl and 0.1N NaOH respectively. The pH 9.9 solution had the composition 0.001N NaOH-0.099N KCl.

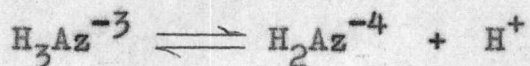
## Procedure

To each of seven different 100-ml. volumetric flasks 3 ml. of 0.0011M arsenazo was added. Then each flask was diluted to the mark with one of the seven prepared buffer solutions. Seven other solutions were prepared in the same manner with the exception that water replaced the arsenazo solution. These latter solutions were for reference purposes. The spectra of the seven solutions were then obtained with the spectrophotometer. Since spectra were recorded on chart paper with a non-linear scale from 200 to 700 mμ, they were replotted on regular graph paper.



### Discussion of Results

Table 1 and Figures 1a and 1b show how the wavelengths of maximum absorption vary over the pH range studied. There is a gradual decrease in the absorbance at 500 mu until a pH of 8.5 is reached where this absorbance is close to a minimum. The gradual decrease to this low absorption value is probably due to the removal of the second hydrogen of the arsonic acid group according to the following reaction:



According to Klygin and Pavlova (10) the pK values for the dissociation of the first and second hydrogens of the arsonic acid group of arsenazo are 2.73 and 7.5 respectively. Therefore, at a pH of 7.11 where the absorbance at 500 mu is still high, the predominant species is  $\text{H}_3\text{Az}^{-3}$  but when a pH of 8.5 is attained the ratio of  $\text{H}_3\text{Az}^{-3}$  to  $\text{H}_2\text{Az}^{-4}$  is less than one. At pH 9.9 where there is a high absorption at 520 mu, it may be surmised that most of the arsenazo is in the form  $\text{H}_2\text{Az}^{-4}$ .

In the ultraviolet region of the spectra from pH 1.15 to 7.11 there are three peaks occurring at 227, 290-300, and 360 mu. It may be noted that between pH values 4.85 and 8.5 that the absorption maximum at 227



Table 1

## Absorption Spectra of Arsenazo

Wavelength in mμ	pH						
	1.15	2.90	4.85	7.11	8.50	9.90	12.35
600	.000	.000	.000	.000	.000	.000	.000
590	--	--	--	--	--	--	.07
560	.200	.220	.210	.230	.150	.500	.550
520	.930	.930	.940	.870	.420	.900	.900
500	1.015	.995	1.000	.925	.498		--
480	--	.860	.870	.820	.495	.670	.690
450	.400	.360	.370	.390	.450	.330	.320
410	.155	.140	.130	.140	.250	.200	.240
370	.265	.250	.220	.200	.140	.150	.150
365	.280	.265	.240	.245	.180	.170	.140
358	.285	.265	.245	.250	.190	.170	.142
351	.250	.240	.220	.245	.233	.180	.140
344	.210	.200	.190	.230	.290	.190	.140
337	.225	.170	.165	.230	.350	.230	.170
330	.160	.160	.165	.250	.410	.290	.230
323	.295	.300	.245	.375	.500	.385	.330
312	.460	.450	.445	.490	.630	.430	.370
305	.520	.510	.510	.545	.570	.510	.535
298	.550	.530	.520	.545	.655	.470	.385
290	.550	.530	.505	.510	.590	.430	.350
283	.550	.515	.490	.480	.510	.420	.385
276	.490	.470	.460	.450	.445	.450	.465
269	.385	.370	.360	.370	.390	.420	.455
262	.335	.320	.310	.340	.385	.385	.410

Table 1 - Cont.

Wavelength in mu	pH						
	1.15	2.90	4.85	7.11	8.50	9.90	12.35
253	.375	.360	.300	.400	.450	.425	.400
247	.580	.570	.575	.620	.640	.570	.470
240	.880	.860	.860	.880	.960	.850	.810
233	.982	.955	.950	.950	1.070	1.050	1.060
227	1.020	.990	.980	.970	1.020	.975	.920
218	.985	.940	.940	--	.980	.895	.795
212	1.000	.930	--	--	1.030	1.040	1.050
205			--	--	1.140	1.340	--

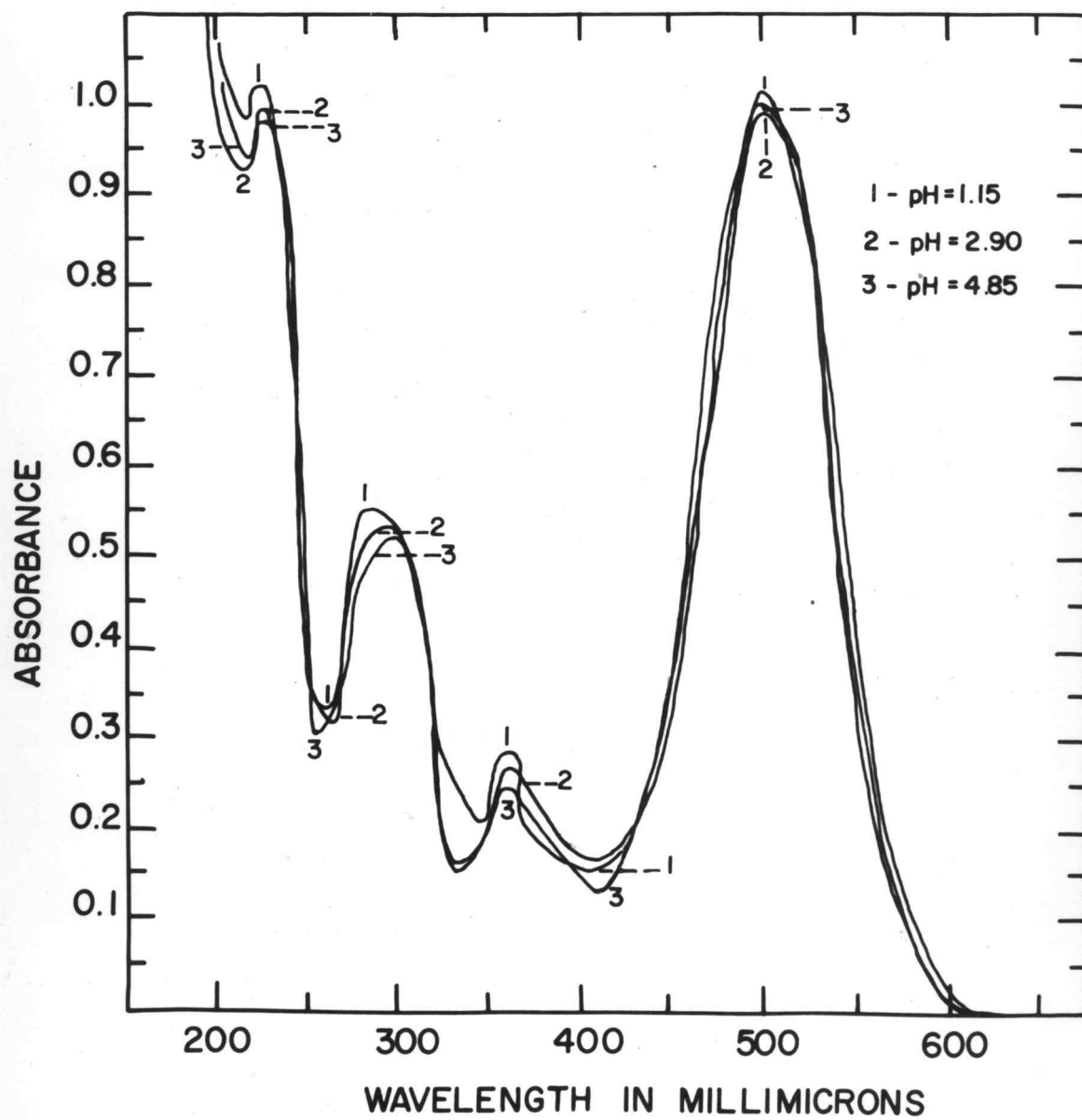


Figure 1a. Absorption Spectra of Arsenazo at Various pH Values



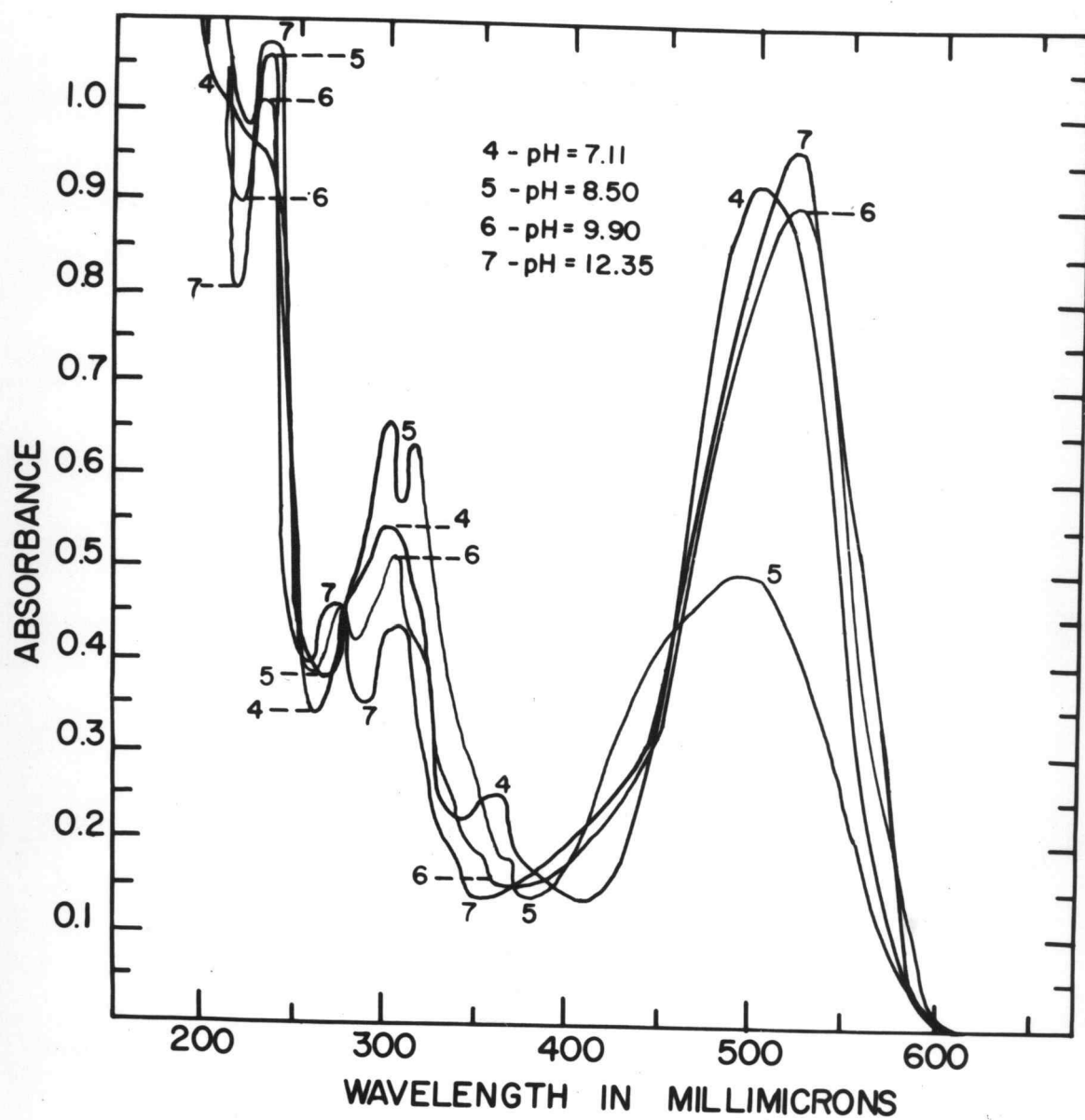


Figure 1b. Absorption Spectra of Arsenazo at Various pH Values

shifts to 233  $\mu$ . Again this shift is probably due to the removal of the second hydrogen from the arsonic acid group. Accompanying this shift is the occurrence of a new peak at 312  $\mu$ .

One other shift is apparent from Figure 1b in the ultraviolet region. This displacement takes place between pH 8.5 and 9.9. Peaks at 312 and 298 are displaced to the shorter wavelengths 305 and 276  $\mu$ .

Finally it can be seen that the peak at 360  $\mu$  gradually decreases in absorbance and disappears almost completely at pH 9.9.

## REACTIONS OF ARSENAZO WITH ZIRCONIUM AND HAFNIUM

### Color Reaction

In a few preliminary experiments with arsenazo it was noted that zirconium and hafnium gave somewhat different colors when reacted with arsenazo in very acidic solutions. Since at the present time there is no known reagent which can distinguish between these elements by spectrophotometric means it was decided to study this color difference further.

### Procedure

Standard  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  and  $\text{HfOCl}_2 \cdot 8\text{H}_2\text{O}$  solutions,  $1.68 \times 10^{-3}$  and  $0.47 \times 10^{-3}\text{M}$  respectively, were prepared. Enough zirconium solution was added to 3 ml. of 0.0011M arsenazo solution in a 100-ml. volumetric flask to give a three to one mole ratio of metal ion to reagent. A hafnium solution with the reagent was prepared in the same manner. In both cases the arsenazo was diluted with 3N HCl prior to the addition of either the hafnium or zirconium after which solutions were diluted to volume with the 3N HCl.

The spectrum of each solution was recorded with the Beckman DK-1 Spectrophotometer. These spectra are



shown in Table 2 and Figure 2. The spectra are seen to be almost identical, peaks occurring principally at 580 mμ in the visible region and at 323 mμ in the ultra-violet region. Although both complexes give similar spectra, the solutions exhibited a definite difference in color. Zirconium formed a violet solution whereas hafnium gave a reddish-violet color.

After standing for an hour a very fine precipitate was noticed in the zirconium but not in the hafnium solution. After two hours had elapsed a precipitate was also visible in the hafnium solution and on standing over night both solutions had settled leaving clear supernatant liquids. The cause of the initial color difference between the zirconium and hafnium complexes with arsenazo may be due to the rates of formation of the precipitates.

#### Quantitative Reaction in the Determination of Zirconium

As stated above, after the precipitates settled there was no color left in the supernatant liquid indicating all the arsenazo had been precipitated by the excess zirconium or hafnium. It was thought if arsenazo were added in excess to a zirconium or hafnium containing solution that these elements would possibly be

Table 2

Absorption Spectra of Zirconium-Arsenazo  
and Hafnium-Arsenazo Complexes

<u>Wavelength</u> <u>in mμ</u>	<u>Absorbance values of Complexes</u>	
	<u>ZrAz</u>	<u>HfAz</u>
700	0.135	0.135
620	0.320	0.320
580	0.405	0.405
520	0.310	0.310
480	0.160	0.160
450	0.200	0.200
410	0.280	0.270
370	0.270	0.290
365	0.261	0.262
358	0.269	0.270
351	0.330	0.330
344	0.420	0.400
337	0.460	0.440
330	0.490	0.470
323	0.520	0.500
312	0.500	0.490
305	0.480	0.480
298	0.460	0.460
290	0.430	0.440
283	0.420	0.430
276	0.430	0.440
269	0.480	0.490
253	0.630	0.600
247	0.700	0.670
240	0.730	0.710
233	0.740	0.720
227	0.780	0.740
218	0.890	0.830

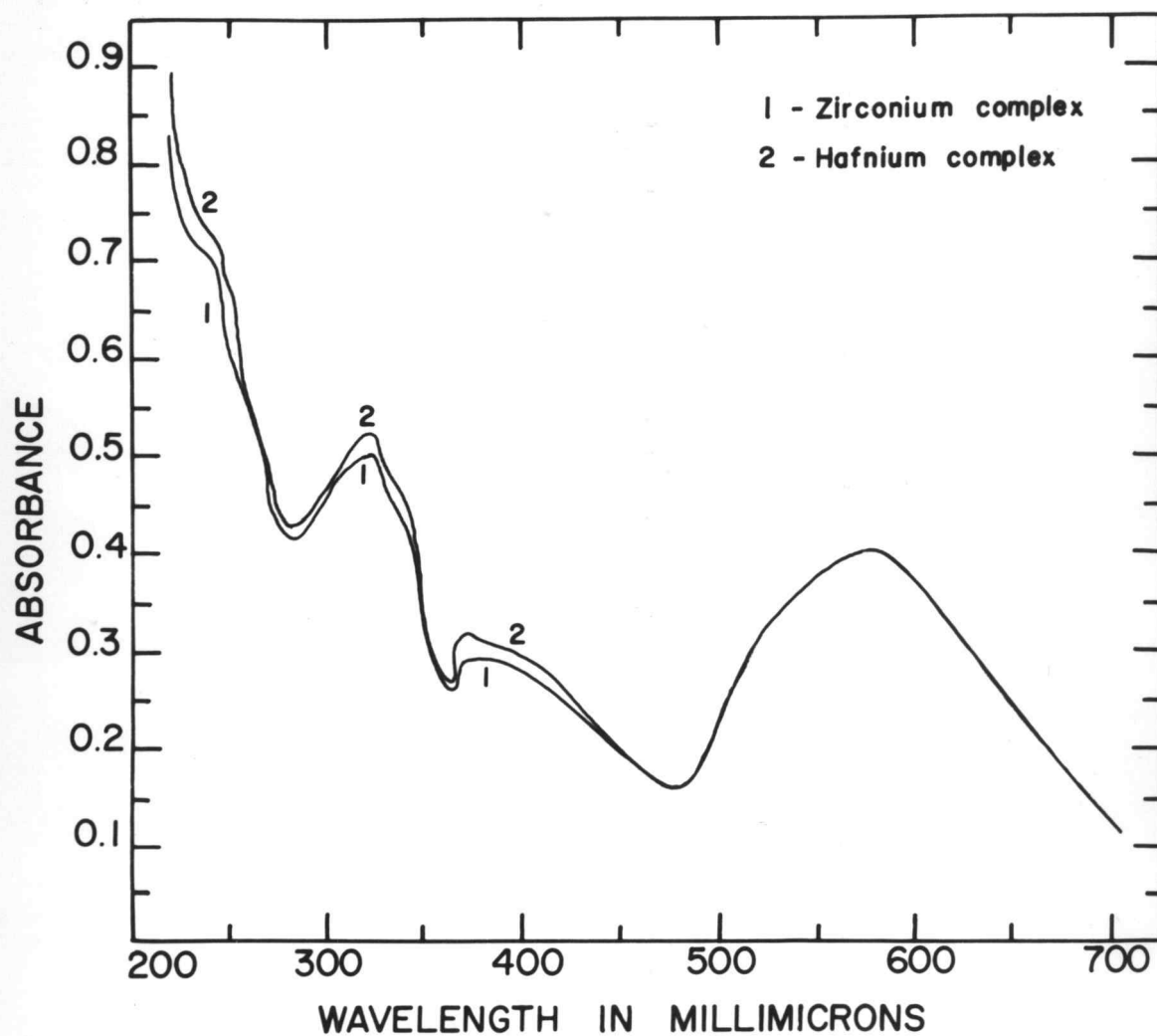


Figure 2. Absorbance of Zirconium and Hafnium Complexes with Arsenazo



precipitated quantitatively. This assumption was based on previous work (3, 20) in which compounds containing the arsonic acid group such as methylarsonic acid and p-hydroxyphenylarsonic acid have been employed in the gravimetric determination of zirconium. When this type of reagent is used, the precipitate is generally ignited to the metallic oxide,  $\text{ZrO}_2$ . With this in mind a study was carried out to see if arsenazo reacts quantitatively with zirconium.

### Reagents

#### Zirconium Solution

A zirconium solution was prepared by dissolving 1.310 grams of  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  in 3N HCl and diluting to approximately 500 ml. with the HCl. The solution was standardized against cupferron. Samples of exactly 50 ml. were taken and to these cupferron was added. The precipitates formed were filtered on Whatman No. 42 filter paper, ignited at  $900^\circ\text{C}$  for one hour, cooled and then weighed. In two runs, values of 53.5 and 52.9 mg./50 ml. were obtained.

#### Arsenazo

A 100-ml. solution of 0.01M arsenazo was prepared with 0.58 gram of arsenazo.

### Procedure

Fifty-ml. samples of previously standardized zirconium solution were pipetted into 250-ml. beakers and diluted with about 50 ml. of distilled water. After the solutions were heated to about 80°C on the hot plate, approximately 50 ml. of arsenazo were added. A precipitate which is very dark and fine forms immediately. The samples were digested for an hour and the precipitates allowed to settle for two hours. After settling, the precipitates were filtered through Whatman No. 42 filter paper. Filtering required about three hours since the very fine precipitate clogged the filter paper very shortly after the filtration was begun. Suction was attempted on previous runs but the precipitate would pass through the filter paper so this was abandoned. Because of the clogged condition washing of the precipitate was not feasible. Furthermore, it was thought that the elements carbon, hydrogen, and arsenic which occur in the excess arsenazo would be driven off as the oxides.

Next, the filter papers were folded and placed in weighed crucibles. These were placed in an oven at 120°C and dried for a few hours after which the filter papers were charred and the crucibles placed in a muffle furnace for one hour at 900°C. The weight of the  $ZrO_2$



precipitate is determined by difference between the initial and final weights of the crucible. For two runs values of 54.9 and 54.6 mg./50 ml. were found, the average being 54.7 mg./50 ml.

### Discussion of Results

The arsenazo value is 1.2 mg./50 ml. higher than the highest value found with cupferron. There is one factor which is most likely responsible for this error. In the ignition procedure all the arsenic was not removed. Simpson and Chandlee (20) have found that when the precipitate formed between zirconium and p-hydroxyphenylarsonic acid is first heated in a hydrogen stream and then ignited in air this small error due to unremoved arsenic is eliminated. The arsenic is removed as arsine,  $\text{AsH}_3$ .

The study made seems to indicate a quantitative reaction between zirconium and arsenazo, but the difficulty encountered in filtration and washing would appear to make this an impractical method for zirconium.



## INVESTIGATION OF CALCIUM-ARSENAZO COMPLEX

A number of studies of the complex formed between calcium and arsenazo have been carried out. The purpose behind these studies was to investigate the possibility of using arsenazo for the determination of calcium in low level concentrations. It was also intended that from the following investigations the optimum conditions for calcium determinations with arsenazo could be found.

### Reagents

#### Arsenazo Solution

One liter of 0.001M arsenazo was prepared with 0.5843 gram of the purified compound. This was dissolved and diluted with distilled water in a volumetric flask.

#### CaCl<sub>2</sub> Solution

One liter of 0.001M CaCl<sub>2</sub> solution was prepared by dissolving 0.1001 gram of Baker's Analyzed CaCO<sub>3</sub> in 5 ml. of concentrated HCl and then diluting this to exactly one liter. Since in further investigations it was necessary to know the acidity of this solution a 50-ml. aliquot was titrated to a bromthymol blue endpoint with 0.1N NaOH.

### Spectra of Arsenazo and its Calcium Complex at Varying pH

A study was made on the comparative absorbance properties of arsenazo and its calcium complex at several pH values in the critical absorbance region. The pH values chosen were 9.4, 10.0 and 11.0. The purpose of this investigation was to aid in determining the pH and wavelength best suited for an eventual Beer's law plot of calcium concentration versus absorbance.

#### Instruments

Beckman Model B Spectrophotometer

Beckman Model H-2 pH meter

#### Procedure

At each of the pH values mentioned above 100-ml. solutions of arsenazo and of the calcium-arsenazo complex were prepared as well as a solution containing only the buffer components. This latter solution served as the blank. The calcium-arsenazo solutions contained excess calcium ion to insure complete complexation of arsenazo. Solutions were prepared as follows:

#### Arsenazo Solutions

These were prepared by first adding proper amounts of buffer components (22, p. 470) to 100-ml. volumetric



flasks. For pH 9.4 and 10.0 a mixture of  $H_3BO_3$ , NaOH, and KCl was employed while 0.1N NaOH was used to prepare pH 11.0 solutions. This was followed with the addition of 3 ml. of 0.001M arsenazo after which solutions were diluted to volume with distilled water.

#### Calcium-arsenazo Solutions

The complex containing solutions were prepared as the arsenazo solutions except in this case 25 ml. of 0.001M  $CaCl_2$  was added followed by enough 0.1M NaOH to neutralize excess acid in the  $CaCl_2$  solution.

#### Blanks

The blanks were made up by adding buffer components to 100-ml. volumetric flasks and then diluting with distilled water.

### Discussion of Results

The spectra of arsenazo and its calcium complex were found with the Beckman Model B Spectrophotometer. These spectra are shown in Table 3 and Figures 3, 4 and 5. Arsenazo is seen to absorb quite strongly at the wavelengths of optimum absorption of the complex. Since the maxima absorbances of the complex and arsenazo are so close together, a wavelength on the slope of the complex curve should be chosen for an eventual Beer's law plot, thereby reducing the absorbance contribution of



Table 3

## Absorbance of Arsenazo and its Calcium Complex

Wavelength, mu	pH 9.4		pH 10		pH 11	
	Az	CaAz	Az	CaAz	Az	CaAz
400	.127	.140	.127	.136	.120	.151
405			.140			
410	.155	.160	.155	.156	.142	.164
420	.195	.175	.185	.164		
425					.200	.159
430	.245	.185	.224	.166	.250	.154
440	.300	.200	.268	.168		.146
450	.355	.218	.335	.180	.362	.137
460	.415	.255	.395	.220	.450	.172
470	.458	.320	.455	.285	.530	.240
480	.490	.382	.515	.363	.590	.340
490	.520	.460	.562	.450	.660	.438
495	.530		.590			
500	.540	.550	.610	.560	.700	.570
505	.530					
510	.526	.620	.620	.660	.700	.699
515	.515		.625		.705	
520	.508	.680	.622	.750	.710	.800
525		.710	.610		.690	
530	.450	.718	.580	.790	.630	.880
535		.710		.800		.890
540	.336	.700	.465	.800	.485	.900
545				.785		.900
550	.230	.640	.340	.760	.320	.880

Table 3 - Cont.

Wavelength, mμ	pH 9.4		pH 10		pH 11	
	Az	CaAz	Az	CaAz	Az	CaAz
560	.133	.555	.220	.680	.186	.800
570	.082	.438	.140	.545	.110	.650
580	.046	.310	.083	.370	.060	.468
590	.016	.190	.044	.238	.033	.283
600	.005	.093	.018	.125	.012	.148
610	.000	.041	.005	.060	.000	.075
620		.013	.000	.024		.025
630		.000		.000		.005
640						.000

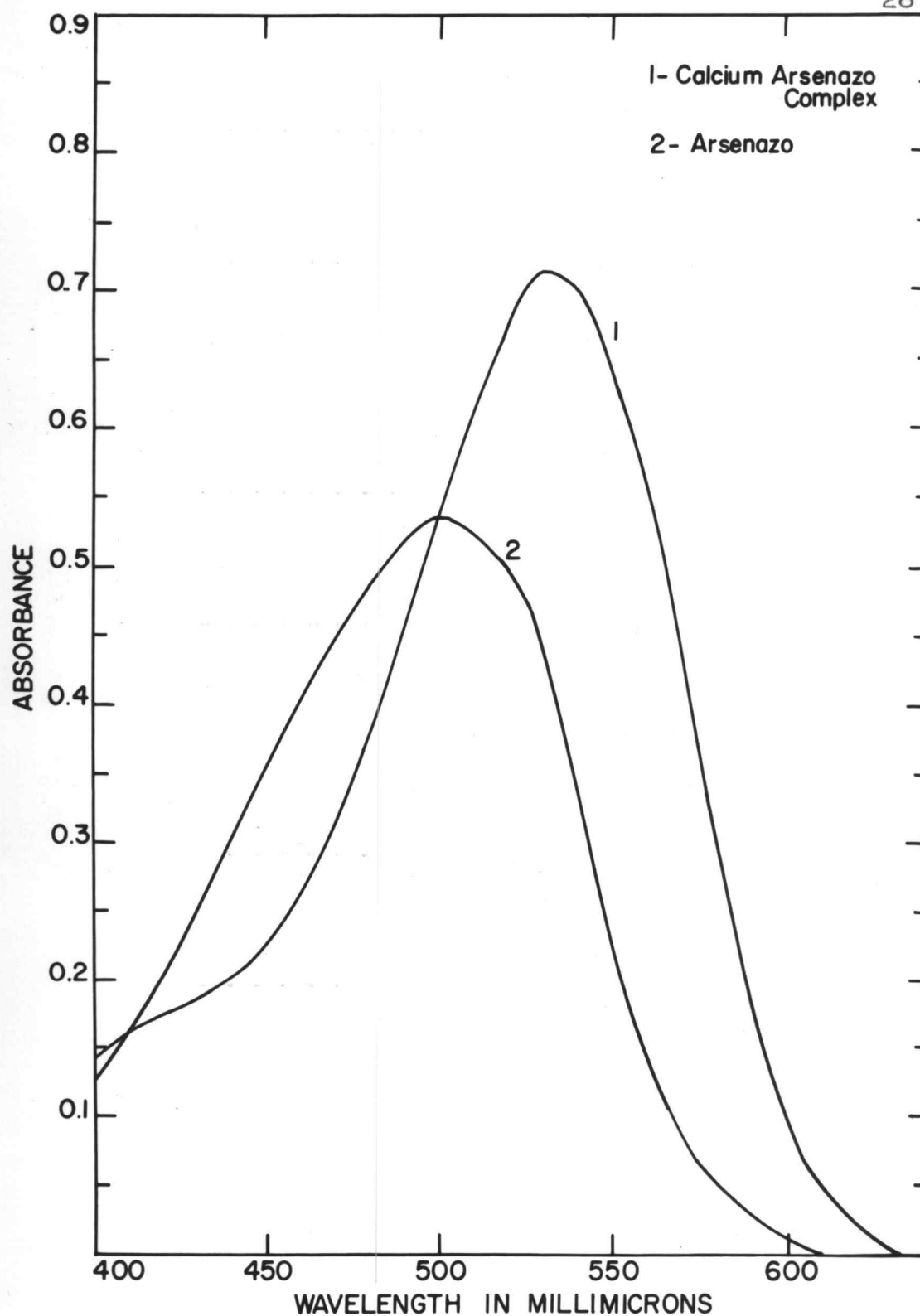


Figure 3. Absorption Spectra of Arsenazo with and without Calcium at pH 9.4



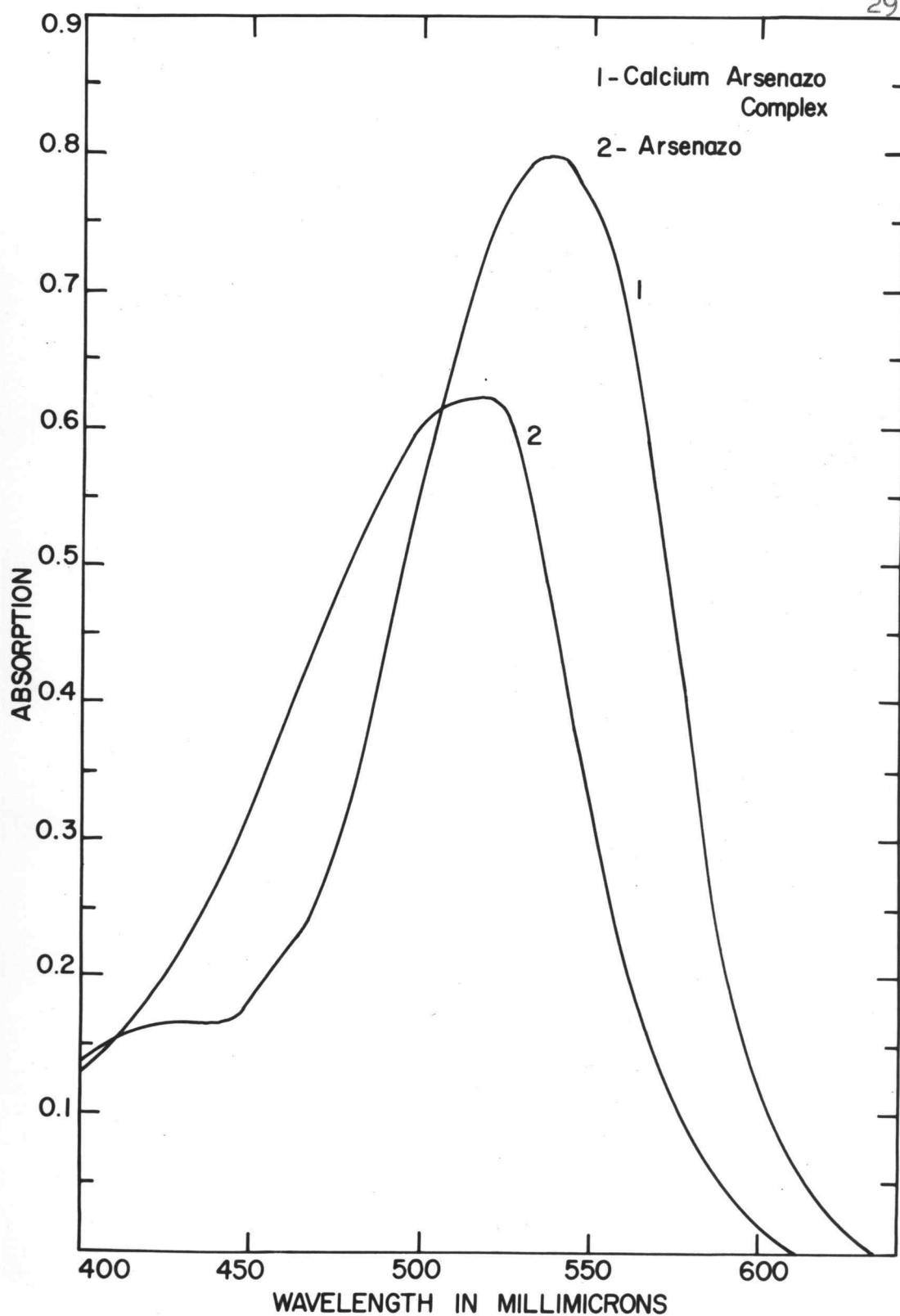


Figure 4. Absorption Spectra of Arsenazo without and with Calcium at pH 10

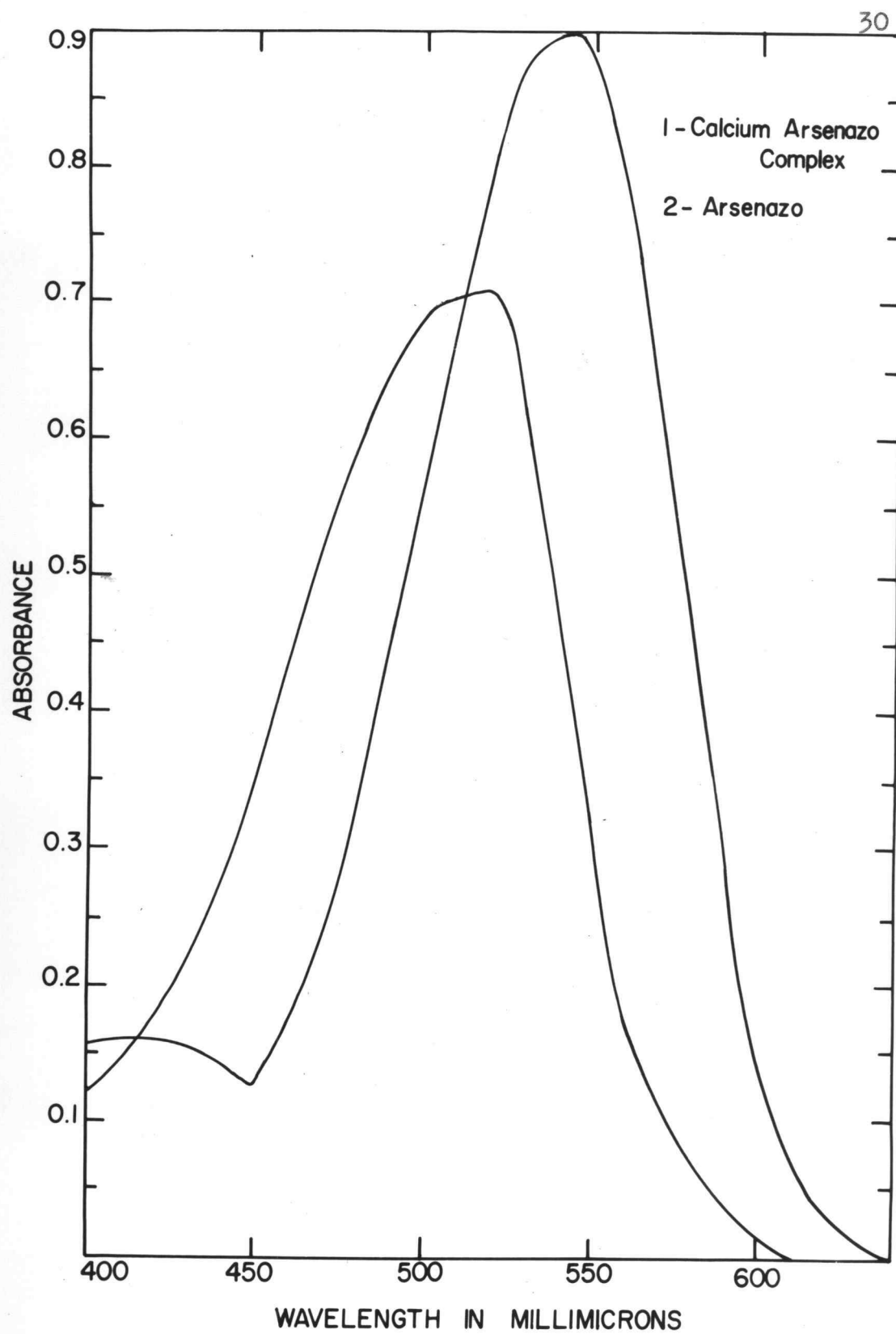


Figure 5. Absorption Spectra of Arsenazo with and without Calcium at pH 11

uncomplexed arsenazo. A wavelength of 580 mμ was tentatively chosen since at this wavelength the arsenazo absorbance is low while that of the calcium complex is still fairly high. As to what pH should be chosen it would seem to be a matter of convenience and a buffered solution would certainly be preferred. Therefore a pH 10.0 was tentatively chosen. Further studies of the complex seemed to substantiate this choice of conditions.

#### Effect of pH on Complex Formation

It was desirous to know in what pH range the calcium complex would form. In order to carry out such a study a calcium-arsenazo solution was prepared at pH 11.0 as related on page 25.

#### Procedure

The 100-ml. solution of complex was transferred to a 250-ml. beaker in which were placed the electrodes from a Beckman Zeromatic pH meter. A 10-ml. buret containing 0.1N HCl was positioned directly over the beaker. Small increments of 0.1N HCl were added to the solution and the pH was read. Then a few milliliters were transferred to a 1-cm. absorption cell and the absorbance read versus distilled water with the Model B at 580 mμ. In this manner a number of points were found for a pH versus



absorbance curve which is shown in Figure 6. The data are listed in Table 4.

### Discussion of Results

The complex starts to form at a pH 7.5 and eventually reaches a maximum at about pH 11. Where the curve is more horizontal, the complex is less pH sensitive; therefore, a pH between 9.75 and 11 would appear to be the logical choice for a Beer's law plot. This evidence supports the choice of pH 10 made previously. Although a slight pH change at 11 has even less effect on values of absorbance it would be more difficult to work at this pH since no buffers are readily available.

### Composition of Complex

Since conflicting data on the composition of arsenazo complexes have been reported it was decided that the composition of the calcium complex should be investigated. This further investigation should be by Job's method of continuous variation (8). In this method the mole ratio,  $\text{Ca}^{+2}/\text{Ca}^{+2} + \text{Az}$ , where  $\text{Ca}^{+2}$  and Az represent molar concentrations of these ions, is continuously varied in a series of solutions with total concentration of calcium and arsenazo held constant. The concentration, and therefore the absorbance of the

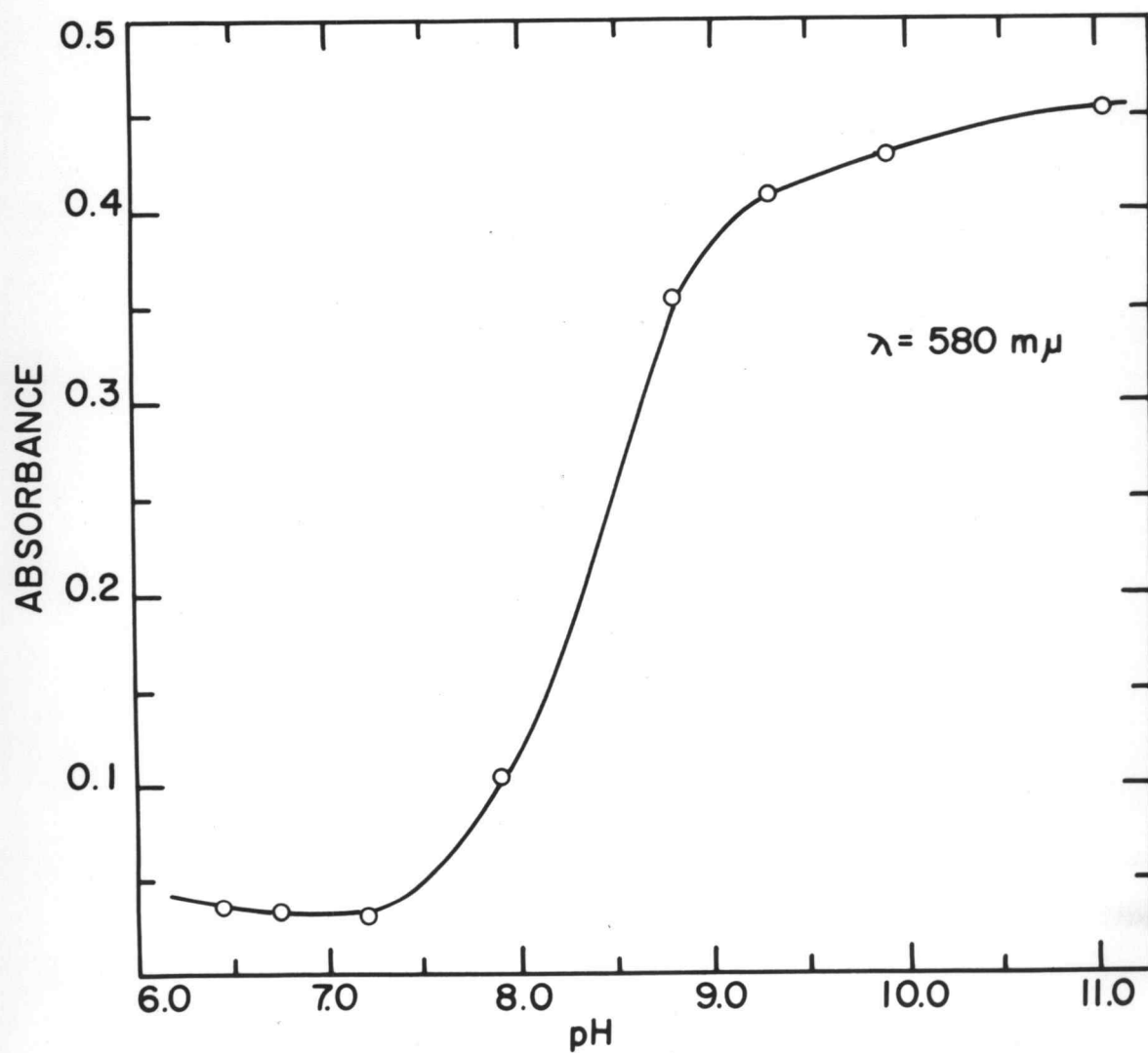


Figure 6. Effect of pH on Absorbance of Calcium  
Arsenazo Complex

Table 4

## Effect of pH on Complex Formation

<u>pH</u>	<u>Absorbance</u>
11.00	0.453
9.90	0.430
9.30	0.410
8.80	0.355
7.90	0.105
7.20	0.032
6.75	0.035
6.45	0.036

Table 5

Composition of Calcium-Arsenazo Complex  
by Continuous Variation Method

Solution No.	ml. Arsenazo	ml. Calcium	A Obs.	A Corr.	$\frac{Ca^{+2}}{Ca^{+2} + Az}$
0	5.0	0.0	0.124	0.000	0.0
1	4.5	0.5	0.151	0.039	0.1
2	4.0	1.0	0.176	0.081	0.2
3	3.5	1.5	0.191	0.104	0.3
4	3.0	2.0	0.194	0.119	0.4
5	2.5	2.5	0.186	0.124	0.5
6	2.0	3.0	0.161	0.111	0.6
7	1.5	3.5	0.126	0.088	0.7
8	1.0	4.0	0.091	0.066	0.8
9	0.5	4.5	0.047	0.035	0.9
10	0.0	5.0	0.000	0.000	1.0



chelate, is greatest when calcium and arsenazo are in the same ratio in which they exist in the chelate.

### Procedure

Solutions were prepared in 100-ml. volumetric flasks at pH 10. The  $\text{CaCl}_2$  and arsenazo reagent, both 0.001M, were combined in continuously varying amounts with total molar concentration kept at  $5 \times 10^{-5}\text{M}$ . This was done by adding 0 to 5 ml.  $\text{CaCl}_2$ , in steps of 0.5 ml., to each of eleven different volumetric flasks. The arsenazo was then added to each so that the total volume of arsenazo plus calcium solution equaled 5.0 ml. The absorbance for each solution was measured at 580 m $\mu$ . Since arsenazo absorbs at this wavelength, corrections for its absorbance must be made. This is done by subtracting from the observed absorbance the absorbance that would have occurred had no chelation taken place. This difference was then plotted against composition in Figure 7. Data obtained are shown in Table 5.

### Discussion of Results

It may be seen from the plot that the absorbance is greatest at  $\text{Ca}^{+2}/\text{Ca}^{+2} + \text{Az}$  approximately equal to 0.5. This indicates a 1:1 complex,  $\text{CaAz}$ . These results are

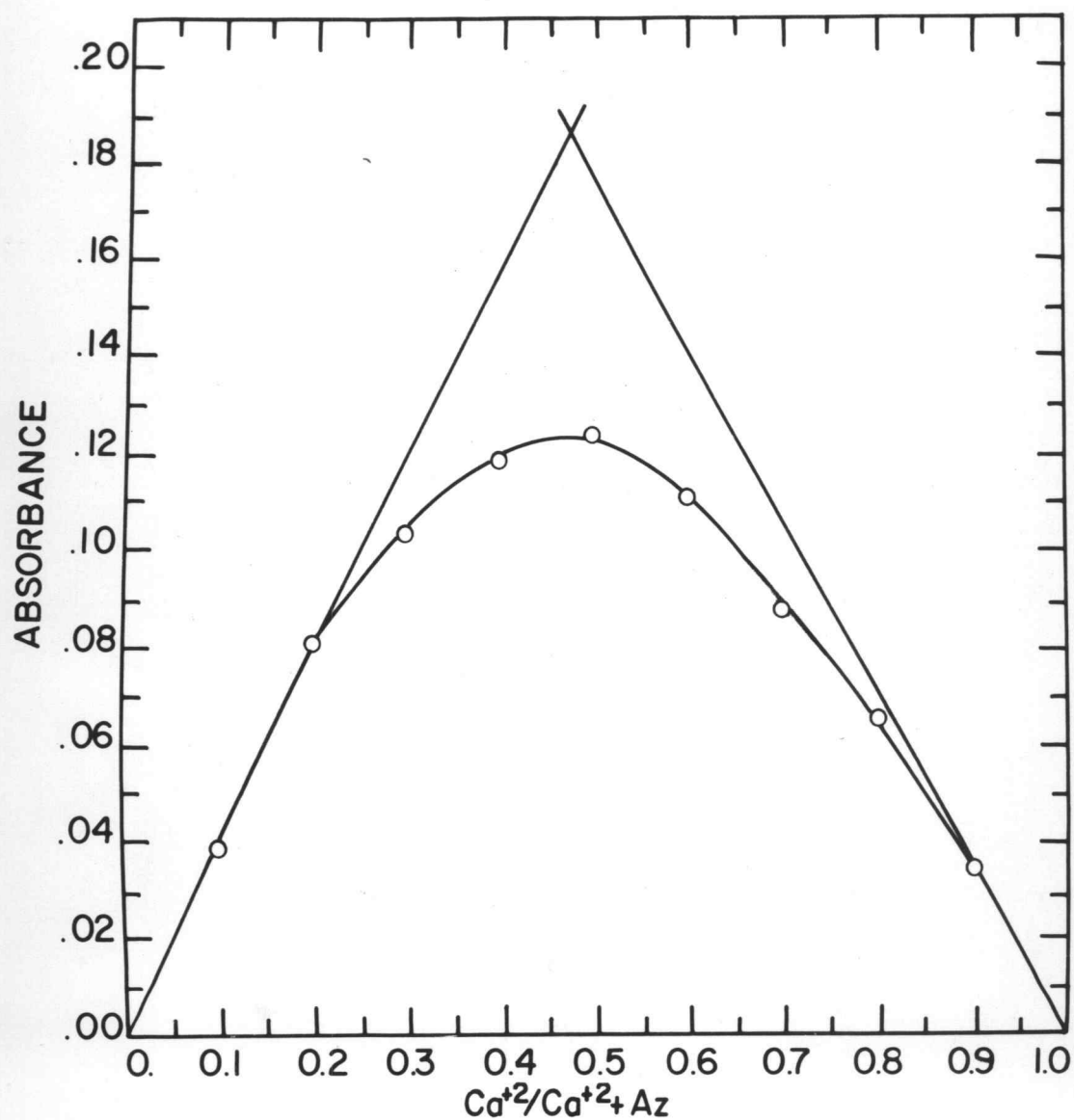


Figure 7. Job's Continuous Variation Method for Calcium Arsenazo Complex

pH 10

580 m $\mu$





calcium if there were no dissociation and if the formula for the complex is in reality  $\text{CaAz}$ . After arsenazo was added, 0.001M  $\text{CaCl}_2$  solution was transferred to each of the volumetric flasks in varying amounts so that the calcium concentration ranged from 0.2 up to 4.0 p.p.m. Enough 0.1N NaOH solution also had to be added to each solution to neutralize the acidic 0.001M  $\text{CaCl}_2$  used. After solutions were diluted and mixed, absorbances were determined versus an arsenazo blank at 580 m $\mu$  with the Beckman Model B Spectrophotometer.

### Discussion of Results

Figure 8 shows the plot of absorbance versus calcium concentration at 580 m $\mu$ . The straight line portion of the curve is seen to extend through about 1.9 p.p.m. calcium. After this point the curve bends off slightly up to 4.0 p.p.m. Therefore, the most useful part of this curve lies between 0.0 and 1.9 p.p.m. calcium.

Another plot, Figure 9, was prepared a short time later. The solutions of varying calcium concentration were prepared as above except that only 10 ml. of arsenazo was added. A straight line plot was found to extend to 1.9 p.p.m. calcium. Since 10 ml. of arsenazo was sufficient to maintain the straight line portion of the curve up to 1.9 p.p.m. calcium, this quantity was used

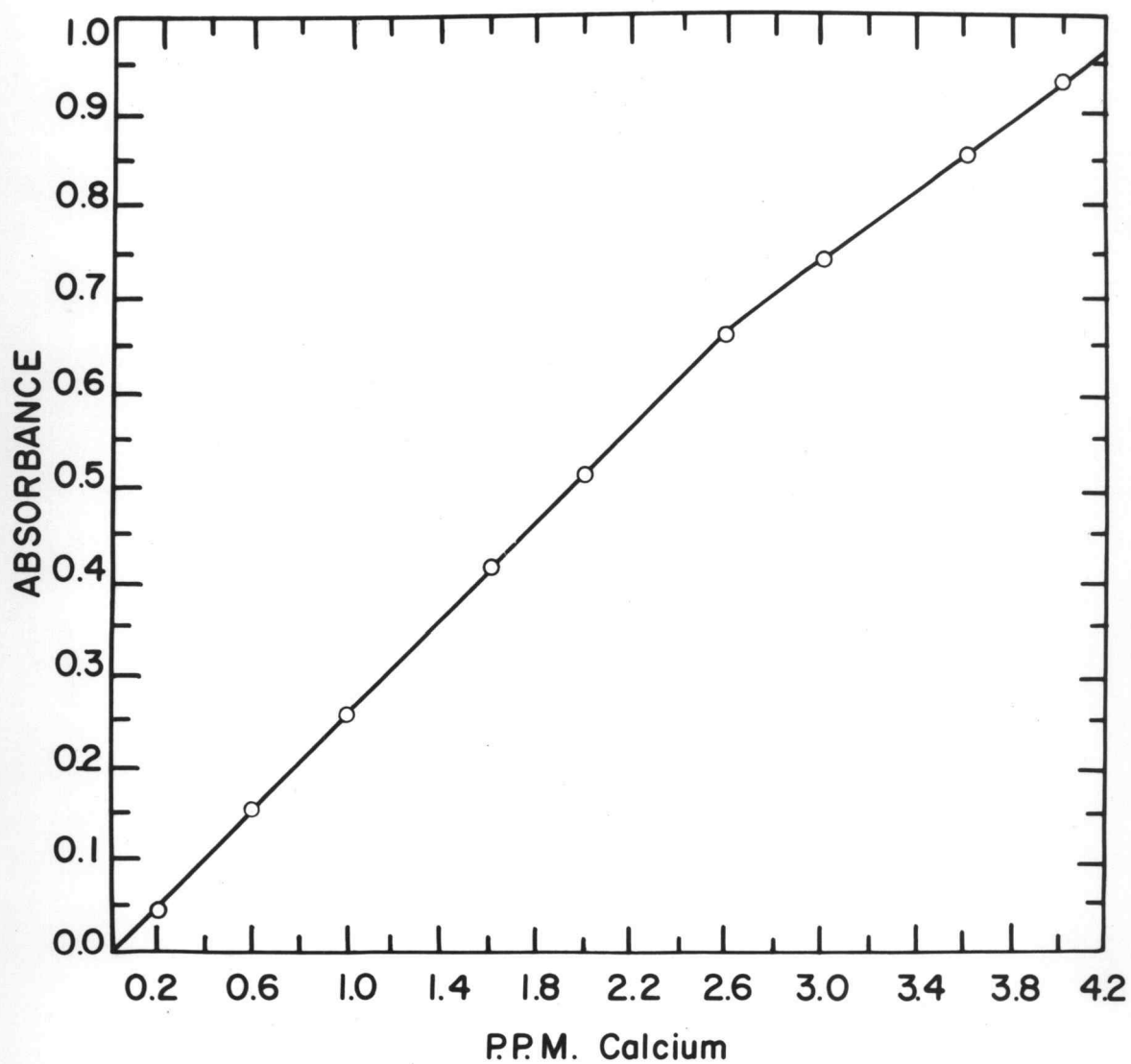


Figure 8. Beer's Law Plot of Calcium Concentration versus Absorbance

pH 10

580 m $\mu$

15 ml. Arsenazo

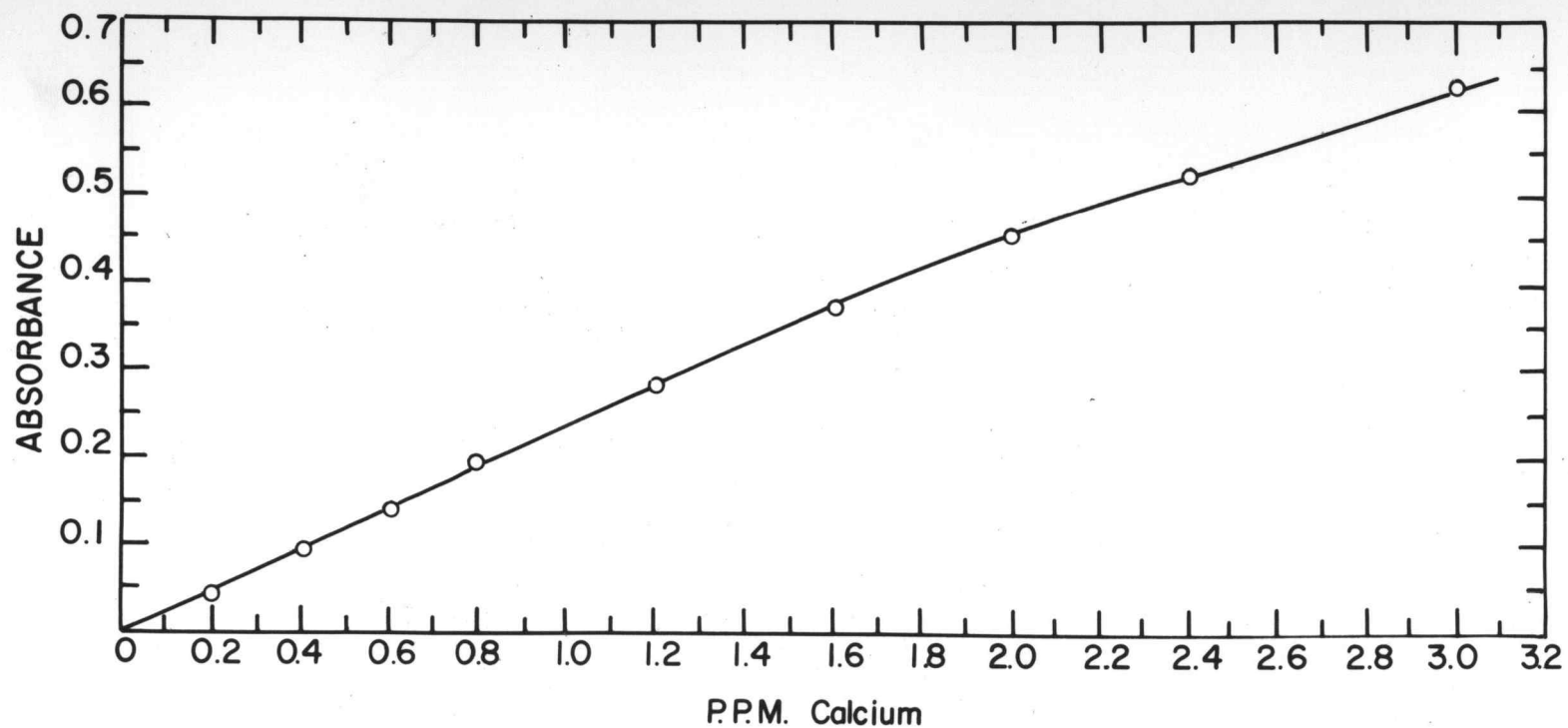


Figure 9. Beer's Law Plot of Calcium Concentration versus Absorbance  
pH 10                      580 m $\mu$                       10 ml. Arsenazo



Table 6

## Beer's Law Plot

15 ml. Arsenazo

580 mu

pH 10

ml. $\text{CaCl}_2$	p.p.m. $\text{Ca}^{+2}$	A
0.00	0.0	0.000
0.50	0.2	0.048
1.50	0.6	0.156
2.50	1.0	0.256
4.00	1.6	0.416
5.00	2.0	0.511
6.50	2.6	0.661
7.50	3.0	0.746
9.00	3.6	0.856
10.00	4.0	0.936

Table 7

## Beer's Law Plot

10 ml. Arsenazo

580 mu

pH 10

ml. $\text{CaCl}_2$	p.p.m. $\text{Ca}^{+2}$	A
0.00	0.0	0.000
0.50	0.02	0.048
1.00	0.4	0.096
1.50	0.6	0.141
2.00	0.8	0.194
3.00	1.2	0.283
4.00	1.6	0.371
5.00	2.0	0.456
6.00	2.4	0.526
7.50	3.0	0.626

hereafter.

### Stability of the Complex

The stability of the calcium-arsenazo complex was determined by making periodic checks on the absorption of the pH 11 solution which was used initially to determine the spectra of the complex (page 25). These checks were run over a period of about a month at a few different wavelengths. During this time there was a very slight decrease in the absorbance of the complex as may be seen in Table 8, but the complex may be considered to be quite stable over a month period. It should be pointed out that the two other reagents for calcium which were referred to in the introduction formed unstable complexes; therefore, in this respect arsenazo would be much more convenient to use as an analytical reagent for calcium.

### Interferences of a few Common Cations

Another factor which helps evaluate the usefulness of a reagent for spectrophotometric determinations is a knowledge of the effects of diverse ions on the absorbance. In the following investigation the effects of a few cations which would most likely occur with calcium were studied. The alkali metals were not investigated since the calcium-arsenazo solutions contain high concentrations



Table 8

## Stability of Calcium-Arsenazo Complex

<u>Time</u> (days)	450 mu	<u>Absorbance</u>		580 mu
		540 mu	570 mu	
0	0.137	0.900	0.650	0.468
8	0.137	0.900	0.650	0.458
23	0.150	0.900	0.640	0.455



of sodium and potassium contributed by the buffer components.

One or more of the alkaline earths -- strontium, barium and magnesium -- are usually found occurring with calcium so that the effects of these ions were studied. A few other common cations which might occur with calcium are iron, aluminum, zinc and copper. These cations were also investigated.

### Reagents

A 250-ml. solution of each of the following reagents was prepared, the weight used giving a cation concentration of 100 p.p.m.

#### Alkaline Earths

$\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	0.076 gram
$\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$	0.0445 gram
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.209 gram

#### Others

$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$	0.181 gram
$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.114 gram
$\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$	0.348 gram
$\text{CuCl}_2$	0.053 gram

### Procedure

For each of the cations to be investigated, four 100-ml. volumetric flasks were prepared by adding to them

pH 10 buffer components, 10 ml. of 0.001M arsenazo, and 4.0 ml. of 0.001M  $\text{CaCl}_2$  solution. This amount of  $\text{CaCl}_2$  solution gives a final concentration of 1.6 p.p.m. calcium. The diverse cation was then added to each of the four prepared flasks in concentrations of 1, 5, 10 and 20 p.p.m. A standard 1.6 p.p.m. calcium solution with no diverse cations was also prepared.

The absorbances of all the solutions were then read at 580 m $\mu$  versus distilled water and compared with the standard. The data are shown in Table 9. The concentrations of interfering cations which cause a  $\pm 5\%$  error based on 1.6 p.p.m. calcium concentration are listed in the table also.

### Discussion of Results

From Table 9 it may be seen that magnesium interferes to a very great extent even when it is present in small concentrations. Strontium and barium do not interfere so greatly but still could be present in no more than 1 and 5 p.p.m. respectively.

Among the other cations studied, zinc is the most interfering. It could not be present in even very small amounts if calcium were to be determined with arsenazo. The limiting concentrations of iron (III) is that at which it starts to precipitate as the hydrous oxide.



Table 9

## Effects of Diverse Cations

Cation	Absorbance				p.p.m. for 5% error
	1 p.p.m.	5 p.p.m.	10 p.p.m.	20 p.p.m.	
Sr <sup>+2</sup>	0.640	0.740	0.810		1
Ba <sup>+2</sup>	0.610	0.650	0.700	0.750	5
Mg <sup>+2</sup>	0.950	1.350			1
Fe <sup>+3</sup>	0.610	0.600	0.590	0.560	10
Zn <sup>+2</sup>	0.490	0.300	0.350		1
Al <sup>+3</sup>	0.600	0.590	0.580	0.550	10
Cu <sup>+2</sup>	0.640	0.770	0.980		1

Standard: A = 0.610

580 mu

pH 10



Precipitates were noted in iron concentrations of 10 and 20 p.p.m. Copper (II) ion may be present only in concentrations of about 1 p.p.m. and aluminum which complexes with arsenazo at pH 4-5 may be present up to 10 p.p.m.

#### EDTA and Arsenazo Analyses of Unknown Calcium Solution

It was thought that it would be of interest to compare the results of two different methods for the determination of calcium in an unknown water sample. One method of analysis was by titration of calcium with ethylenediaminetetraacetic acid, disodium salt (EDTA). This is a very common method used at the present time for calcium and magnesium determinations. The other method used was a spectrophotometric method with arsenazo.

#### Procedure I

An unknown water sample containing approximately 18 p.p.m. calcium, 5 p.p.m. iron (III), and 0.05 p.p.m. aluminum was obtained from the Commercial Methods of Analysis laboratory. This solution was first analyzed by titration with EDTA (21, p. 137-138).

Three 50-ml. samples of the unknown were transferred to 250-ml. beakers. These were buffered at pH 10

after which 7 to 8 drops of the indicator, Eriochrome Black T, were added. Each solution was then diluted with 30 ml. distilled water and titrated with standard sodium versenate (0.364 mg. Ca/ml.) from a wine-red to clear blue solution. A few milliliters of KCN solution were needed to prevent interference of iron. Exactly 3.28 ml. of versenate were needed for each titration. The concentrations of solutions were found to be 16.7 p.p.m. calcium.

#### Procedure II

The unknown calcium solution was then determined with arsenazo. A 25-ml. aliquot of unknown was titrated to a bromthymol blue endpoint with 0.1N NaOH to determine the acidity of the calcium solution. Next, three 100-ml. volumetric flasks were prepared as usual with pH 10 buffer components and 10.0 ml. of arsenazo. Now 3, 5, and 7-ml. samples of unknown were titrated into the three flasks with a 5-ml. buret. Enough 0.1N NaOH to neutralize the volumes of unknown used was also added. After the solutions were diluted and mixed, the absorbances of the three samples were read at 580 mμ on the Beckman Model B Spectrophotometer. The concentrations of calcium were read from Figure 10 which is an expanded version of

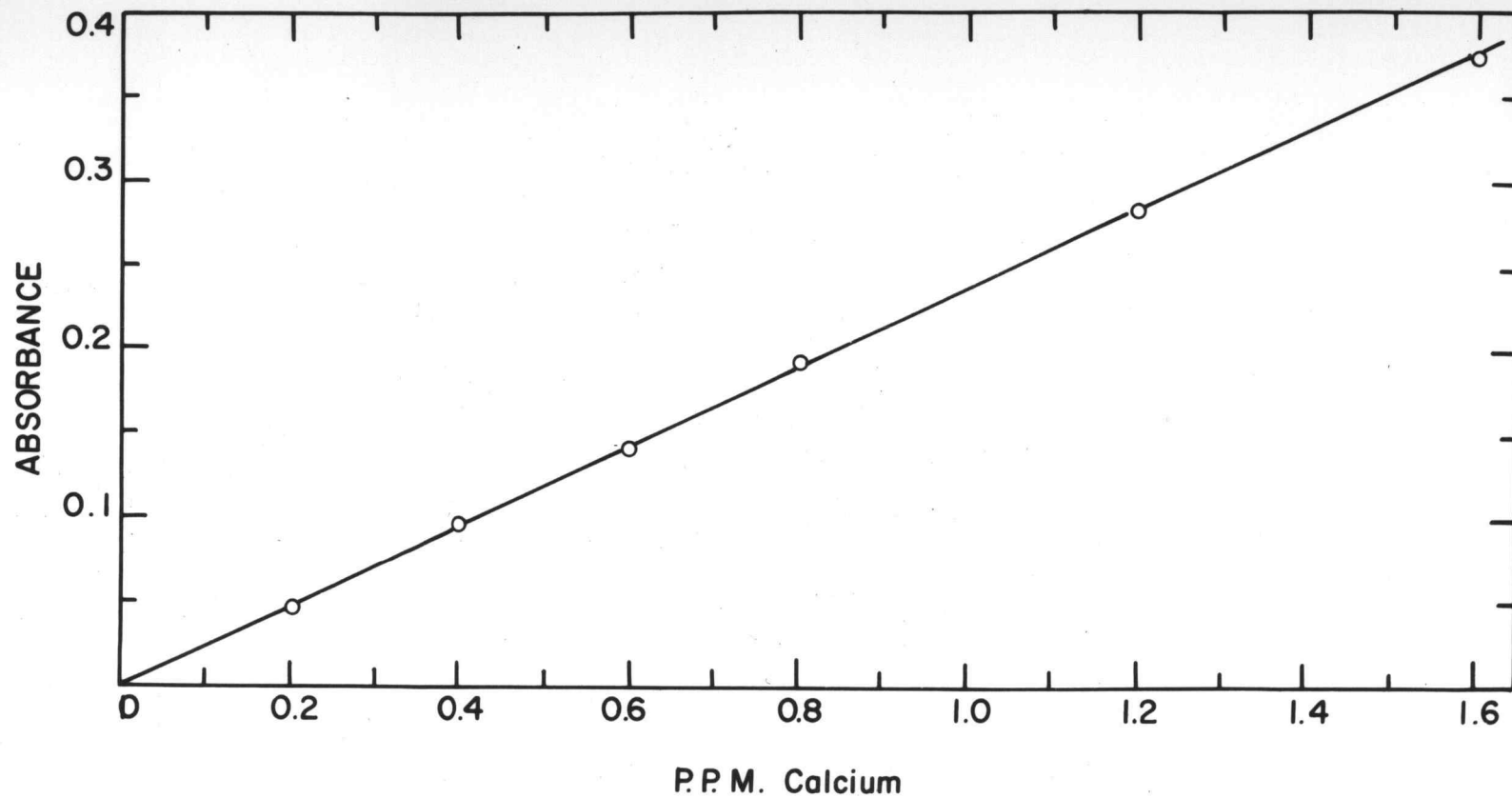


Figure 10. Expanded form of Figure 9. from 0-1.6 ppm Calcium



Figure 9 from 0.0 to 1.6 p.p.m. calcium.

After correcting the results for dilution, values of 16.8, 16.8, and 17.2 p.p.m. were calculated. This gives an average value of 16.9 p.p.m. which is in close agreement with the value obtained by EDTA titration.

## SUMMARY

The absorption characteristics of arsenazo, 3-(2-arsenophenylazo)-4,5-dihydroxy-2,7-naphthalene disulfonic acid, have been studied in the visible and ultraviolet region of the spectrum over a wide pH range. The shifts of certain wavelength maxima indicate the removal of hydrogen atoms from the molecule.

The color reactions of arsenazo with hafnium and zirconium have also been investigated. Although there exists a definite color difference to the naked eye, the spectra of the two complexes are very similar.

Arsenazo is found to react quantitatively with zirconium but because of the difficulty in the filtration it is not considered to be particularly useful analytically in a gravimetric procedure.

An investigation of the properties of the calcium-arsenazo complex has been carried out in order to determine the optimum conditions for the determination of calcium with this dye. Concentrations of calcium up to 1.9 p.p.m. may be determined at a pH of 10 and a wavelength of 580 mμ. The complex formed is very sensitive and has been shown to be stable for at least a 28-day period. The results obtained in a calcium method with arsenazo are in very close agreement with those obtained

with an EDTA titration.

The effects of various concentrations of barium, strontium, magnesium, zinc, aluminum, iron, and copper on the method were also investigated.



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