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Hydroxyazo compounds as reagents for beryllium

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HYDROXYAZO COMPOUNDS AS REAGENTS FOR
BERYLLIUM.**

**Iowa State University of Science and Technology, Ph.D., 1966
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HYDROXYAZO COMPOUNDS AS REAGENTS FOR BERYLLIUM

by

Laurie Murray Grennan

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Analytical Chemistry

Approved:

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Head of Major Department

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Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1966

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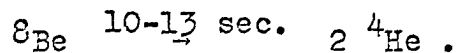
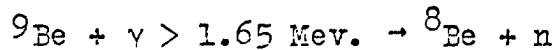
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I. INTRODUCTION

A. The Element Beryllium

Before World War II, beryllium was of limited interest to mineral technologists and its chemistry was relatively undeveloped. Since 1942, however, the applications of beryllium have increased enormously; the remarkable metallurgical properties of metallic beryllium make it likely this trend will continue. Pure beryllium is both light in weight and high in tensile strength, properties which make it a favorite in the construction of space vehicles. Beryllium copper has the properties of mild steel and does not work harden. Beryllium compounds are poisonous and thus, widespread use of beryllium chemicals is precluded.

Beryllium is a poor absorbent of X-rays and has a low cross section for neutrons. It is comparable to deuterium or graphite as a moderator or reflector in atomic piles and produces neutrons more easily than any other element when excited with γ rays or other radiation (1):



Although thirty-second in abundance of the elements in the earth's crust, beryllium is actually more prevalent than arsenic, gold, silver, and molybdenum. However, it is widely dispersed in the crust of the earth, and its recovery is

difficult. Because of its small ionic radius, strong electro-positive character and low coordination number, high concentrations of beryllium in minerals are rare. The element tends to be dissipated by weathering and sedimentation and is probably more widely distributed in rocks than has been assumed. It is estimated at 6 p.p.m. in igneous rocks and 3.5 p.p.m. in shales (2).

Beryllium tends to form its own minerals and substitution in the crystal structure of other minerals is slight, although in silicates, beryllium may replace aluminum and silicon to a small extent. The most stable beryllium minerals are beryl ($\text{Be}_3\text{Al}_2\text{Si}_6\text{O}_{18}$), helvite ($(\text{Mn}, \text{Fe}, \text{Zn})_4\text{Be}_3\text{Si}_3\text{O}_{12}\text{S}$), chrysoberyl (BeAl_2O_4), phenakite (Be_2SiO_4), and bertrandite ($\text{Be}_4\text{Si}_2\text{O}_8 \cdot \text{H}_2\text{O}$).

Beryl, which is classed as a cyclosilicate because the crystal structure contains rings of six linked SiO_4 tetrahedra, is by far the most important of the beryllium minerals. It is estimated that as high as eighty percent of beryllium in pegmatite, is in this form. The mineral contains roughly four percent beryllium and is easily mistaken for quartz or apatite. Emerald and aquamarine are transparent, gem varieties of beryl.

In the late 1940's, in a joint effort, the U.S. Geological Survey and the U.S. Atomic Energy Commission began a comprehensive survey of the beryllium resources of the U.S. (1). An attempt was made to locate and evaluate deposits of beryllium, particularly of non-pegmatite origin. The study turned up no commercial value deposits, but set certain criteria for

future prospecting. Fluorine appears to be abundant in ores containing beryllium. At present, the only economically important concentrations are found in pegmatites associated with feldspars, micas, and lithium minerals.

Pegmatites (3) are usually coarse grained, often tabular, deposits resulting from the fractional crystallization of a magma and are commonly associated with granite. Many pegmatites contain rare elements and unusual minerals. Igneous rocks also resulted from the same crystallization process but in general are much less varied in composition.

Pegmatites in the Black Hills, S.D., southwestern Africa, northeastern Brazil and parts of Madagascar are rich in beryl, while the pegmatites in southern Norway and southeastern U.S., excepting the "tin-spodumene belt" of North Carolina, are poor in beryl (4). In North Carolina the beryl and spodumene (a lithium mineral) bearing pegmatites are fine grained and a suitable flotation operation has been devised for the recovery of the beryl. In Brazil, beryl is a by product in the mining of sheet mica.

It is important to note that beryllium is a highly toxic material and can produce a variety of maladies in humans. Berylliosis (2) is considered a distinct disease; it can be identified by various clinical tests which distinguish it from other lung diseases. Apparently, individuals vary in their sensitivity to beryllium and cases have been cited of delayed reactions to exposure to the oxide dust. All beryllium

compounds and solutions must be handled with care.

Because of all these activities, there exists a strong interest in the analysis of this minor, but important element.

B. Survey of Methods for the Determination of Beryllium

At a 1953 symposium on the Analytical Chemistry of the Less Familiar Elements, Vinci (5) reviewed the state of beryllium chemistry and described the information available as "generally scattered and somewhat controversial". Sandell (6, p. 218) in Colorimetric Determination of Traces of Metals has also summarized the available methods for beryllium and pointed out inadequacies.

In the qualitative analysis scheme, beryllium falls with aluminum, iron and chromium in "Group III". It is amphoteric, the hydroxide dissolving in excess alkali to form a beryllate ion. There is considerable question as to the actual species that exist in aqueous solution. A basic beryllium ion, $(\text{BeOH})_3^{+3}$, is a possibility in as much as beryllium is known to form Be-OH-Be and Be-O-Be bridges (7, p. 168). Beryllium salts are extensively hydrolyzed and this has proven a major difficulty in alkalimetric titration procedures in which a neutral solution is required for reaction of beryllium with a reagent. The fact that beryllium salts seem to hydrolyze incompletely in the presence of pyridine has been used by Przheval'skii (8) in his study of the precipitation of beryllium with β -diketones and this may explain why color develop-

ment is more rapid in the presence of pyridine when beryllium is added to a reagent.

Any beryllium solution above pH 7 is quite likely to coat the glassware with a thin film of hydroxide which is difficult to remove unless acid is used for cleaning. Beryllium hydroxide is quite insoluble compared with hydroxides of other divalent metals. Sill (9) in his fluorometric determination of submicrogram quantities of beryllium with morin checked his procedure with a ^7Be tracer and found that the precision of the instrumental measurements far surpassed that of the chemical operations. He recommended coating glassware and pipets used for beryllium solutions with a silicone such as Beckman Desicote.

In macro methods and when high accuracy is required, beryllium hydroxide is ignited to beryllium oxide for weighing or beryllium ammonium phosphate is precipitated and ignited to beryllium pyrophosphate for weighing.

Vinci (5) lists ten reagents used for the detection or quantitative "estimation" of beryllium as of 1953:

1. p-Nitrobenzeneazoarcinol (known also as "Zenia")
2. Quinalizarin
3. Curcumin
4. Alkanin (Naphthazarin)
5. Naphthachrome Green G (Naphthachrome Azurine 2B)
6. Aluminon (Aurintricarboxylic acid)
7. Quinizarin-2-sulfonic acid

8. Morin (3,4,7,2',4'-Pentahydroxyflavone)
9. 1,4-Dihydroxyanthraquinone
10. 1-Amino-4-hydroxyanthraquinone.

The last three reagents produce fluorescence with an alkaline solution of beryllium in ultraviolet light. Vinci (5) recommends p-nitrobenzeneazoarcinol as a photometric reagent for small quantities of beryllium.

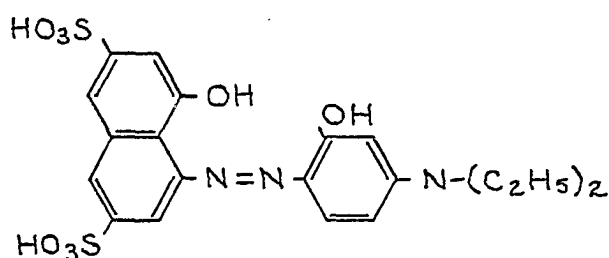
Beryllium reagents can be classed as those that form a "lake", in which the reagent is adsorbed on beryllium hydroxide, and those which form an actual compound with the beryllium. Many qualitative tests are based on lake formation, but the formation of true, soluble compounds is preferable for quantitative work.

Since 1953 several intensive studies have been made of reagents for beryllium in addition to those listed by Vinci but no reagent has been found which supercedes p-nitrobenzeneazoarcinol, the various proposed reagents suffering on the scores of availability, sensitivity, or stability.

For milligram and microgram quantities of beryllium, the colorimetric determination with p-nitrobenzeneazoarcinol is currently the most widely used reagent. This reagent was introduced by Komarowasky and Poluektoff (10) in 1934, studied by Osburn and Stross (11) in 1944, and worked out as a differential spectrophotometric procedure by White, Meyer and Manning (12) in 1956. Diehl, Cryberg, King and Melick (13)

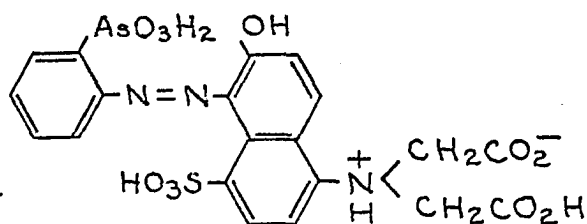
obtained data on the structure, combining ratio, formation constant, absorption spectrum, effect of pH, solubility, and stability of the reagent. Besides being somewhat unstable, the reagent does not show an intense color change on reaction with beryllium and the formation constant of the beryllium derivative is low, $10^{2.77}$. Another difficulty with the method is that a hundred fold excess of beryllium is required for reaction. Solutions of the reagent alone are not stable; this probably results from the presence in the molecule of both oxidizing and reducing groups (nitro- and phenolic groups).

Of special interest for this particular research work is a comparative study of reagents for the photometric determination of beryllium by Kuznetsov in 1955 (14), which led in 1963 (15) to the synthesis and study of two new reagents, Beryllon III and Beryllon IV.



Beryllon III

8-Hydroxynaphthalene-3,6-disulfonic acid-⟨1-azo-1⟩-2 hydroxy-4-diethylaminobenzene

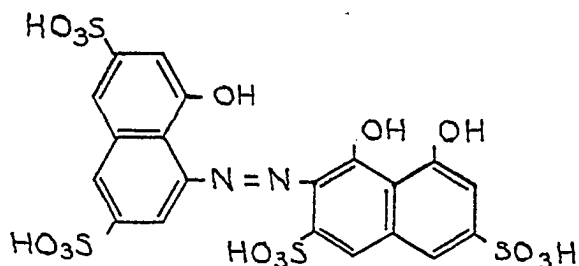


Beryllon IV

Benzene-2-arsonic acid-⟨1-azo-2⟩-1-hydroxynaphthalene-6-iminodiacetic-3-sulfonic acid

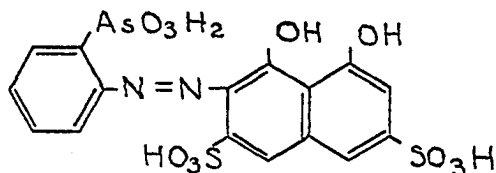
(Russian nomenclature)

These compounds appeared superior to others in a group of twenty-four dyes studied in the two papers, including Beryllon II and Arsenazo. All but two of the group are hydroxyazo compounds.



Beryllon II

8-Hydroxynaphthalene-3,6-disulfonic acid-⟨1-azo-2⟩-1,8-dihydroxynaphthalene-3,6-disulfonic acid

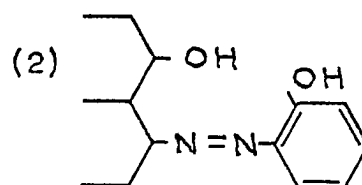
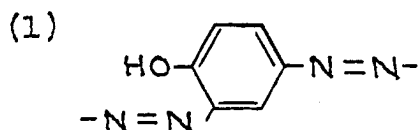


Arsenazo

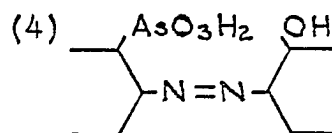
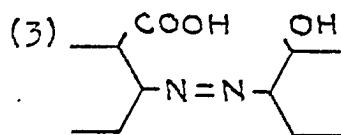
Benzene-2-arsonic acid-⟨1-azo-2⟩-1,8-dihydroxynaphthalene-3,6-disulfonic acid

(Russian nomenclature)

The following azo groupings are reported in the first paper (14) as characteristic of reagents which give color reactions with beryllium:

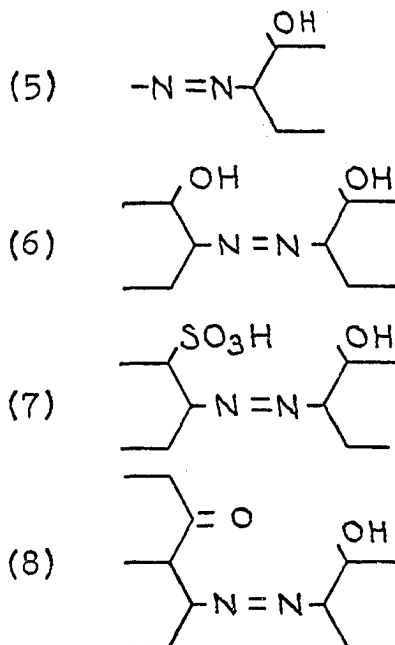


and in conjugation with other groups:



Kuznetsov states "the mechanism of the reaction of a widely used reagent for beryllium, p-nitrobenzeneazoocinol, has not yet been clarified".

The groupings:



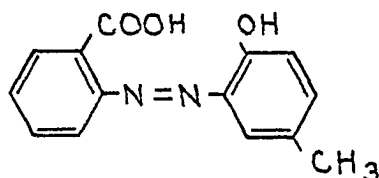
were also studied by Kuznetsov and found not to give color reactions with beryllium.

Adamovich (16) has determined five dissociation constants and six molar extinction coefficients for the various forms of Beryllon II.

In a series of twenty-six monoazo compounds prepared by Ellingboe (17), only those that contained two hydroxy groups, o- and o'- to the azo group, or one hydroxy group and one carboxy group, again o- and o'- to the azo group, were found to react with calcium and magnesium. Blair (18) later found that none of the group that reacted with calcium and magnesium

reacted with beryllium at pH 10.

However, Snavely (19) reported that beryllium did form a 1 to 1 compound with 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene, a compound investigated by Ellingboe (designated in his thesis as B-5), at pH 3. The formation constant shows beryllium to be between copper and nickel in stability of the compound formed.



B-5

Snavely reported this information in connection with a study of the relative stabilities of the metal derivatives of six o,o'-dihydroxy azo compounds and the above mentioned o-hydroxy-o'-carboxyazo compound. The formation constants were calculated from the data obtained from a potentiometric titration with alkali. The given metal and reagent were titrated with standard sodium hydroxide solution in a dioxane-water solution (See Section IIA on potentiometric titration procedure for details.). "Displaced" formation constants for each metal compound were reported and the order of stabilities was found to be $\text{Cu}^{+2} > \text{Ni}^{+2} > \text{Co}^{+2} > \text{Zn}^{+2} > \text{Pb}^{+2} > \text{Cd}^{+2} > \text{Mn}^{+2} > \text{Mg}^{+2} > \text{Ca}^{+2} > \text{Sr}^{+2} > \text{Ba}^{+2}$.

The o-hydroxy-o'-carboxyazo compound (B-5) formed less stable metal derivatives than the other azo compounds but differed in that it was the only one coordinating with beryllium, showing a "displaced" formation constant (K_{eq}) of $10^{-3.8}$. (See mathematical treatment in Section IIB for explanation of the negative value.) The smaller the numerical value of K_{eq} , the greater K_f will be since $\log K_{eq} = \log K_f - pk_{1,2}$. This approach was used where values of pk for the azo compounds were difficult to obtain and a relative formation constant was nevertheless quite useful.

Using Fisher-Herschfelder-Taylor models to show the space relationship, Snavelly suggested that the smaller beryllium ion (estimated ionic radius 0.34 \AA) fits more snugly in the o-hydroxy-o'-carboxy grouping or "hole" than in the o,o'-dihydroxy grouping. However, the exact nature of the beryllium species is not understood.

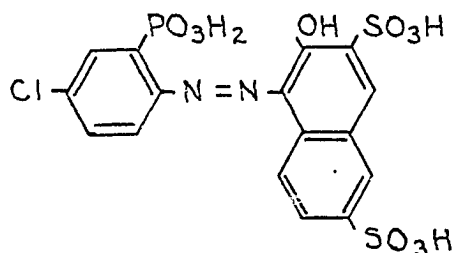
Akiyama (20) suggests identification of magnesium and beryllium with a number of azo compounds in sodium hydroxide solution. Of these, o-carboxy-phenylazothymol, 1-(4-sulfo-1-naphthylazo)-4,6-dihydroxy-2 methylbenzene, (o-carboxyphenylazo)-orcinol and chloro-chloro'-(p-diphenylenediazo)diorcinol can be used for beryllium.

Majumdar and Chatterjee (21) report determining beryllium with 2-(pyridyl-2-azo)chromotropic acid (Reagent A), with the 3-azo compound (Reagent B) and with 2-(2-carboxypyridyl-3-azo)

chromotropic acid (Reagent C). In this paper they attempt to justify a six coordinated beryllium compound and show beryllium to form a 3 to 1 compound with Reagent A, a 2 to 1 compound with Reagent B and a 2 to 3 compound with Reagent C (absorption at 580-590 m μ .).

Cabrera and West (22) determine beryllium with Fast Sulphon Black F (color index 306) using a glycine-sodium hydroxide buffer at pH 11.

Luk'yanov (23) has used chlorophosponazo R to determine beryllium photometrically at pH 7 to 12; this reagent also forms a compound with magnesium at pH 9 to 12, with calcium at pH 9 to 12 and aluminum at pH 1 to 3.

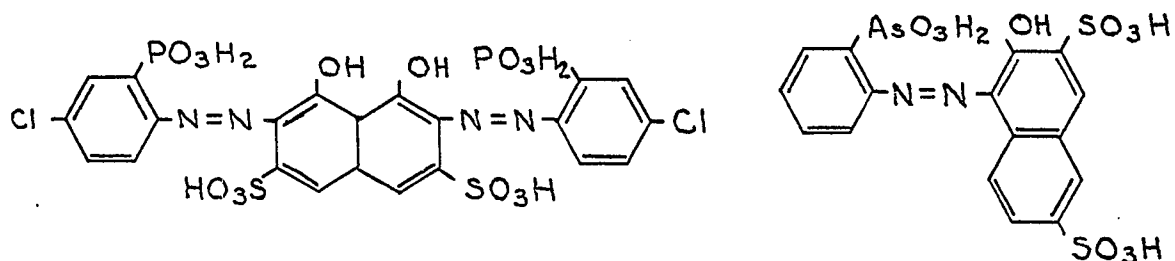


Chlorophosponazo R

Nemodruk (24) developed Chlorophosponazo III which reacts with uranium, titanium, magnesium, calcium, beryllium, zinc, thorium, scandium, and the rare earths. This work was extended by Ferguson, Richard, O'Laughlin and Banks (25) for trace amounts of calcium and magnesium after a reversed phase chromatographic separation.

Thorin (26) (the sodium salt of 2-(2'-hydroxy-3',6'-

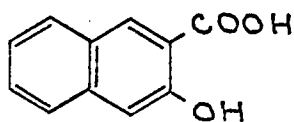
disulfo-1'-naphthylazo)benzeneearsonic acid) also reacts with beryllium at pH 12.5.



Chlorophosphonazo III

Thorin

Cherkesov (27) has studied 3-hydroxy-2-naphthoic acid as a colorimetric and fluorometric reagent for beryllium, aluminum and iron. Beryllium forms a 2 to 1 compound at pH 2.5. EDTA can be added to mask aluminum and iron and hexylamine is added to enhance fluorescence. Kirkbright (28) has extended this procedure to the fluorometric determination of submicrogram amounts of aluminum and beryllium.



3-Hydroxy-2-naphthoic acid

Hill (29) uses Eriochrome Cyanine R for the photometric determination of beryllium.

Several authors have extended the analytical work on the

beryllium-morin compound: Sill (9), who has been mentioned as studying the coating of glassware with beryllium hydroxide in trace analysis, and Fletcher (30) of the U.S. Geological Survey, who has made a fluorometric study of the beryllium-morin system. She reports three principal beryllium-morin compounds: a 1 to 1 monomer, a 1 to 1 dimer and a 1 to 2 compound. Conditional equilibrium constants were evaluated by use of a digital computer. Five morin species must be considered. Fletcher (31) had also described a vertical axis transmission type filter fluorometer for use with microgram quantities of beryllium with morin and quinizarin.

Also of interest is a photometric titration (32) method for beryllium. In this method an excess of sodium sulfosalicylate is added to the beryllium sample. Excess reagent not uniting with the beryllium is back titrated photometrically with aqueous beryllium sulfate at pH 10.6 using Arsenazo as indicator.

Sulfosalicylic acid (33) can be used for the spectrophotometric determination of beryllium at pH 10-11.

Both 2-mercaptobenzoic (34) acid and α -tropolone (35) have been shown to form compounds with beryllium and salicylic acid which is fluorescent gives a fluorescent beryllium compound.

Separation of beryllium from interfering ions does not seem to present too great a problem. A recent correspondence

(36) in Analytical Chemistry suggests an anion exchange separation of beryllium from virtually all other cations having an ionic radius greater than that of beryllium using a 90 percent isopropanol-10 percent 6N-hydrochloric acid medium eluent. Beryllium is eluted ahead of all other metal ions except the common alkali metals, ammonium ion and phosphoric acid.

Since beryllium does not react appreciably with EDTA, various metal ions can be masked in the presence of beryllium with EDTA and triethanolamine. Adamovich (37) gives the instability constant of beryllium-EDTA as 1.5×10^{-9} . Beryllium can be precipitated as the hydroxide from a 0.1 M solution in the presence of excess EDTA by 1 M ammonia to the extent of 99.7 percent.

Athavale (38) and others mask copper, zinc, aluminum, and iron with EDTA, extract the beryllium-acetylacetonate into carbon tetrachloride, decompose the beryllium compound and measure the beryllium using Thorin at 470 m μ (pH 12).

Kida (39) masks interference with potassium cyanide and EDTA, extracts the 8-hydroxyquinoline compound of beryllium with chloroform and measures the absorbance at 380 m μ .

Ostromnov and Volkov (40) describe the separation of beryllium from manganese, nickel, cobalt and zinc by precipitation with ammonium cinnamate, the precipitate being ignited and weighed as beryllium oxide.

C. Survey of Hydroxyazo Compounds as Reagents and
Review of Immediately Related Work

The study of hydroxyazo compounds as reagents for beryllium was begun as one aspect of a continuing interest on the part of Dr. Harvey Diehl and his graduate students at Iowa State University in analytical reagents for the alkaline earth metals.

Diehl and Lindstrom (41) had studied Eriochrome Black T and its calcium and magnesium derivatives in 1959 and later developed Calmagite (42) as a similar but more stable metallochromic indicator for these elements in EDTA titrations.

Following Diehl and Ellingboe's study (17) in 1960 of other hydroxyazo compounds for calcium and magnesium, which has already been discussed, Section B, Diehl and Olsen (43) developed *o,o'*-dihydroxyazobenzene as a fluorometric and spectrophotometric reagent for magnesium.

In 1963, after a study of the properties of the beryllium reagent *p*-nitrobenzeneazoarcinol by Diehl, Cryberg and coworkers (13), it was decided that an attempt should be made to alter the stereochemistry of this reagent to improve its stability and sensitivity. Work on the synthesis of new compounds was done by H. Peterson working under a National Science Foundation Teacher Research Participation summer grant. Peterson prepared compounds having large substituent groups in positions neighboring the azo group, as well as compounds

containing the fluoride, arsonic, carboxyl, and diethylamino groups para to the azo group. He also prepared compounds in which methoxy and nitro groups were located ortho to the azo group (in addition to the o-hydroxy group already present). Twenty azo compounds were synthesized the summer of 1964 and eight more at a later date.

Concurrent with the work of Peterson was the work described in this thesis on the reevaluation of the hydroxyazo compounds previously studied by Ellingboe for their possible reaction with beryllium, particular attention being paid to those with only one hydroxy group positioned ortho to the azo group.

Purification of the azo compounds in preparation for analytical evaluation proved to be more of a problem than the actual synthesis. In every case, careful purification of the initial reactants was essential and even this did not assure satisfactory final products. Preliminary evaluation in almost every case was done on crude azo compounds to determine whether a particular compound was worthy of more intensive study. In some instances impure products gave indication of reaction with beryllium which failed when the compound was purified. Fluorescence was also noted in several impure compounds which was not present in the recrystallized compound.

Peterson had obtained the spectra of his first twenty azo compounds in solutions of pH 12.8 containing a 100-fold excess

of beryllium. The six compounds which showed some difference in absorbance between the azo compound and the azo compound plus beryllium were studied at pH 7 and pH 9, using Clark and Lubs buffers and adding potassium chloride to maintain constant ionic strength. A Beckman DU spectrophotometer was used.

Ellingboe compounds A-3, A-4, A-6, A-7, A-14 and A-15 were also examined at various pH levels. None of the Peterson and Ellingboe compounds gave indication of significant reaction toward beryllium and attention was then directed to two azo reagents, 1-(2'-hydroxy-4'-diethylamino-1'-benzeneazo)-3,6-disulfo-8-hydroxynaphthalene (Beryllon III) and 1-(1'-hydroxy-3'-sulfo-6'-iminodiocetic-2'-naphthylazo)benzene-2-arsonic acid (Beryllon IV), described by the Russian workers.

Work on 1-(2-carboxybenzenazo)-2-hydroxy-5-methyl benzene (B-5), already shown to react with beryllium, and on o-carboxybenzeneazoorcinol (P-15) and p-hydroxybenzeneazoorcinol (P-17), typical of the stereochemically altered azo compounds studied in showing some reaction with beryllium, is also included here.

In work during the summer of 1965, Peterson determined the purity of the azo compounds he had synthesized, using for this purpose the Knecht method (44, p. 78), for the determination of the azo group. An excess of titanous chloride is added to a sulfuric acid solution of the azo compound, an atmosphere of nitrogen being maintained, and the unused titanous chloride back titrated with standard ferric sulfate. This method was also applied to the purified reagents, Beryllon III, Beryllon IV, B-5, P-15 and P-17 by Mr. Peterson.

II. POTENTIOMETRIC TITRATION PROCEDURE FOR THE DETERMINATION OF FORMATION CONSTANTS

A. Experimental Procedure

The technique followed was essentially that used by Snavely (45,46), a potentiometric titration with alkali of the azo compound dissolved in a water-dioxane mixture, this solvent having been earlier used by Calvin and Wilson (47). The method depends on having all the species of one phase, so that at any point, if the concentration of one species is known, the concentration of the other species may be calculated. The mixed solvent, although necessary to hold in solution some of the azo compounds studied in this work, complicates the calculation of true thermodynamic data. Van Uitert and Haas (48) and later Block and McIntyre (49) worked out correction procedures. Goldberg (50) further clarified the calculations by including activity coefficients. All calculations are based on the original work of Bjerrum (51). In a procedure used by Chaberek and Martell (52) provision was made for the separate titration of the reagent (in this case the azo compound) and calculation of the acid dissociation constants of the reagent from titration data.

A solution containing a known concentration of metal salt, chelating agent and a mineral acid (all in 75 percent dioxane solution) was titrated with standard sodium hydroxide potentiometrically using a Beckman Zeromatic pH meter with saturated

calomel reference electrode (Beckman No. 39170) and glass, high alkalinity, indicating electrode (Beckman No. 41260). Solutions were brought to $30^{\circ} \pm 0.1^{\circ}$ in a constant temperature bath. Because of the possible oxidation of phenolic compounds at high pH by oxygen, the titrations were carried out in an atmosphere of nitrogen. The nitrogen used was first bubbled through a solution consisting of 75 percent dioxane and 25 percent water to saturate the nitrogen with vapor and thus reduce evaporation in the titration flask. The titration flask provided three openings, for the electrodes and for the buret, and also a side-tube inlet for the introduction of the gas. The stream of nitrogen gas served to stir the solution.

A 10 ml. buret with 0.05 ml. divisions was used. It was provided at the top with a carbon dioxide absorption tube. Titrations were performed slowly, $1\frac{1}{2}$ to $2\frac{1}{2}$ hours being required. The potential was measured after each addition of sodium hydroxide until an equilibrium value was obtained. The mineral acid present provided a constant ionic strength.

It was necessary to calibrate the pH meter for the dioxane-water medium by titrating a known aqueous solution of nitric acid with dioxane, and from this to work out a correction factor. Van Uitert and Haas (48) showed that a glass-s.c.e. cell measures the hydrogen ion activity in dioxane and developed an empirical calibration which holds over the range pH 2 to 11 at given salt concentrations. In the present work a correction factor of 0.40, added to each pH reading, was used.

Water soluble azo compounds (Beryllon III) were titrated in a water medium.

1. Reagents

a. Dioxane Preliminary work indicated that reagent grade, stabilized dioxane contained a small amount of acetic acid which confused the experiments made with it; it was necessary, therefore, to carefully purify the dioxane used and this was done by the method described by Weissberger (53, p. 140). Two liters of technical-grade dioxane was refluxed with 27 ml. of concentrated hydrochloric acid and 200 ml. of water for twelve hours under an atmosphere of nitrogen. Pellets of potassium hydroxide were then added to the mixture until two layers formed. The dioxane was decanted and the addition of potassium hydroxide repeated. The resulting dioxane was refluxed over sodium for another twelve hours under an atmosphere of nitrogen gas. The dioxane was then distilled through a fractionating column, the first 300 ml. of the distillate, boiling below 101° , being discarded. The distillate boiling at 101° was collected and stored over aluminum oxide in a brown bottle. This dioxane was filtered before use.

b. Beryllium solution (0.05515N) Electrolytically refined beryllium flakes, 99.45 percent pure (Lithium Corporation of America), were dissolved in an excess of concentrated hydrochloric acid, 1.0 g. of beryllium requiring about 30 ml. of acid. The solution was diluted to one liter. The pH was

found to be 3.5. A 50-ml. aliquot was diluted to 90 ml., the pH adjusted to 2 with acid, and made up to a final volume of 100 ml. When a 4 ml. aliquot of the solution adjusted to pH 2 was added to the titration mixture of dioxane, water, azo compound and nitric acid, the pH value was essentially unaffected by the addition. Snavely had apparently used a beryllium nitrate solution without pH adjustment.

c. Sodium hydroxide (1.129N) This was prepared from a 50 percent sodium hydroxide solution which had stood 24 hours to allow any carbonate to settle out. Freshly boiled deionized water was used. The base was standardized against dried reagent grade potassium acid phthalate.

d. Nitric acid (0.7550N) This was prepared from concentrated nitric acid and standardized against the sodium hydroxide.

B. Mathematical Treatment

The potentiometric titration data collected (see Section III C) for the determination of the formation constants of the beryllium-azo compounds being studied, using the procedure described in Section II A, was treated mathematically according to the general theoretical considerations used by Bjerrum for metal-amine complexes. Modifications by Snavely, Chaberek and Martell form the basis of the treatment used here.

The pH readings taken were corrected for the mixed solvent and titration curves (pH versus ml. of sodium hydroxide)

were drawn for the azo compound in nitric acid and the azo compound plus beryllium in nitric acid. An additional buffer zone was observed in the titration curve of the azo compound-beryllium mixture if compound formation was taking place. The position of the buffer zone depended on the stability of the compound formed as well as the values of the acid dissociation constants of the acid groups of the azo compound. For 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene with acid dissociation constants, $pK_1 = 6.8$ and $pK_2 = 11.4$, the buffer zone occurred at pH 3.

A complete mathematical analysis of the beryllium-azo compound curve can not be made unless the acid dissociation constants of the azo compound are known. These values can be calculated from data obtained in the titration of the reagent alone. If values of the acid dissociation constants are not greatly different or are very high, they may be difficult to calculate.

Snavelly has calculated so called "displaced" formation constants where the acid dissociation constants of the azo compound could only be estimated. This method of treating the data is also discussed because two values had been reported earlier for 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5) as a "displaced" formation constant.

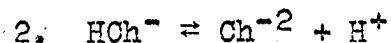
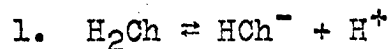
The purpose of this mathematical treatment is to furnish a basis for comparing the relative stability of the beryllium

compounds of five different azo compounds at a set ratio of metal to azo compound. Formation constants were calculated in this work, but it should be noted that these are not true thermodynamic constants because concentrations rather than activities have been used. In the titration procedure, for the five compounds studied, the formation function seemed to be highly dependent on the concentration of the beryllium solution, its initial pH value, and perhaps even its previous history. Values obtained, therefore, should properly be labelled conditional constants.

Chaberek and Martell (52) include a calculation of the acid dissociation constants necessary for subsequent calculation of the formation constants. The symbols used here conform to the more generalized Snavely treatment. One of the five azo compounds studied, p-hydroxybenzeneazoarcinol, has only one dissociable proton that is involved in the compound formation with beryllium; modification of the equilibrium expressions is necessary in this case and will be shown here under the discussion of Snavely's "displaced" formation constant.

1. Acid dissociation constants

The constants are obtained from the titration data of the azo compound alone. For a diprotic chelating agent or weak acid, the two dissociation steps can be expressed as:

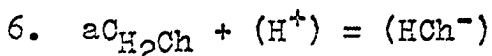
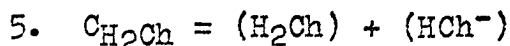


From these expressions, where there is a large difference between k_1 and k_2 , the acid dissociation constants are:

$$3. \quad k_1 = \frac{(\text{H}^+)(\text{HCh}^-)}{(\text{H}_2\text{Ch})}$$

$$4. \quad k_2 = \frac{(\text{H}^+)(\text{Ch}^{-2})}{(\text{HCh}^-)}$$

For the low buffer region (at pH values about equal to the value of pk_1), the total concentration of the azo compound ($\text{C}_{\text{H}_2\text{Ch}}$) is the sum of the concentrations of all species present and can be determined from the titration data by using the number of moles of base added, a , at a given pH (after subtracting from a the number of moles of mineral acid added at the start of the titration for purposes of maintaining constant ionic strength).



By substituting 6 into 3, the k_1 can be calculated according to:

$$7. \quad k_1 = \frac{(\text{H}^+) \left[a\text{C}_{\text{H}_2\text{Ch}} + (\text{H}^+) \right]}{\text{C}_{\text{H}_2\text{Ch}} - \left[a\text{C}_{\text{H}_2\text{Ch}} + (\text{H}^+) \right]}$$

In the high buffer region (at pH values close to values of pk_2), the concentration of H_2Ch species can be neglected,

so the total concentration (C_{H_2Ch}) is:

$$8. C_{H_2Ch} = (HCh^-) + (Ch^{-2}).$$

From the number of moles of base added at a given pH in this region, $a-1$, and assuming one mole of base was used up to the first end point:

$$9. (a-1)C_{H_2Ch} - (OH^-) = (Ch^{-2}).$$

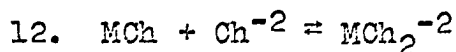
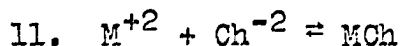
Substituting 9 into 4, the form used for calculating k_2 becomes:

$$10. k_2 = \frac{(H^+) \left[(a-1) C_{H_2Ch} - (OH^-) \right]}{C_{H_2Ch} - \left[(a-1) C_{H_2Ch} - (OH^-) \right]}$$

2. The equilibria involved in compound formation

The treatment of the titration curves obtained with solutions of azo compound plus beryllium makes use of the term \bar{n} which is defined as the ratio of the concentration of the ligand bound to the metal to the total concentration of the metal ion present. A graph of \bar{n} against pCh^{-2} will give the formation curve. The following mathematical expressions must be considered to calculate pCh^{-2} and \bar{n} .

The equilibrium expressions for a chelating agent having two replaceable hydrogen atoms, H_2Ch , reacting with a metal ion, M^{+2} , and the resulting formation constants K_1 and K_2 , are:



$$13. K_1 = \frac{(MCh)}{(M^{+2}) (Ch^{-2})}$$

$$14. \quad K_2 = \frac{(MCh^{-2})}{(MCh)(Ch^{-2})}$$

The total concentration (C_{H_2Ch}) of the chelating agent is the sum of all the species present:

$$15. \quad C_{H_2Ch} = (H_2Ch) + (HCh^{-1}) + (Ch^{-2}) + (MCh) + (MCh_2^{-2}).$$

The total concentration of the metal ions (C_{M+2}) is also the sum of all species present:

$$16. \quad C_{M+2} = (M^{+2}) + (MCh) + (MCh_2^{-2}).$$

Moles of base, a , added at a given pH indicate the possible formation of the given species (the coefficients 2 and 4 corresponding to the moles of H^+ liberated to form that species) and the following equality is valid:

$$17. \quad aC_{H_2Ch} + (H^+) - (OH^-) = (HCh^-) + 2(Ch^{-2}) + 2(MCh) + 4(MCh_2^{-2}).$$

By subtracting 17 from 15 and eliminating (H_2Ch) and (HCh^-) using Equations 3 and 4, the resulting expression can be used for direct calculation of (Ch^{-2}):

$$18. \quad (Ch^{-2}) = \frac{(2-a) [C_{H_2Ch} - (H^+) + (OH^-)]}{\frac{2(H^+)^2}{k_1 k_2} + \frac{(H^+)}{k_2}}.$$

\bar{n} , as defined above, is the ratio of the bound ligand to the total metal ion concentration:

$$19. \quad \bar{n} = \frac{(MCh) + 2(MCh_2^{-2})}{C_{M+2}}.$$

Then, by using 15 and 3 and 4:

$$20. \quad \bar{n} = \frac{1}{C_{M+2}} \left(C_{H_2Ch} - \left[\frac{(H^+)^2}{k_1 k_2} + \frac{(H^+)}{k_2} + 1 \right] (Ch^{-2}) \right).$$

Values calculated for \bar{n} , 20, and (Ch^{-2}) , 18, (after converting (Ch^{-2}) to pCh^{-2}) at a number of points on the titration curve are used to graph the formation curve for the metal compound. On this curve, at values of $\bar{n} = 0.5, 1.5$ and 2.5 respectively, the pCh^{-2} value is the logarithm of K_f for a 1 to 1, 1 to 2 or 1 to 3 compound. The \bar{n} curve will level off at $\bar{n} = 1, 2$ or 3 depending on whether a 1 to 1, 1 to 2 or 1 to 3 compound is indicated.

K_f can also be calculated, instead of being read from the graph, by combining 13 and 15, but this method was not used.

3. "Displaced" formation constants

Snavelly has plotted \bar{n} against values of $-C$, instead of values of pCh^{-2} , to obtain a "displaced" formation constant. Using the same basic equations for k_1, k_2, K_1 and K_2 , but combining these expressions in a different manner, this author takes care of the problem which arises when acid dissociation constants of the azo compound are difficult to obtain (especially k_2).

If \bar{n}_{Ch} is defined as the mean number of protons bound to the non-coordinated chelate ion, then:

$$21. \quad \bar{n}_{Ch} = \frac{(HCh^-) + (H_2Ch)}{(Ch^{-2}) + (HCh^-) + (H_2Ch)}$$

By substituting Equations 3 and 4 into 21:

$$22. \quad \bar{n}_{\text{Ch}} = \frac{k_1(\text{H}^+) + 2(\text{H}^+)^2}{k_1k_2 + k_1(\text{H}^+) + (\text{H}^+)^2}$$

For the monoprotic case, this value is simply:

$$23. \quad \bar{n}_{\text{Ch}} = \frac{(\text{H}^+)}{k_1 + (\text{H}^+)}$$

This value (\bar{n}_{Ch}) is usually 1 for the monoprotic case and 2 for the diprotic case (at low pH values) and as a result k_2 can be estimated without having too much effect on \bar{n}_{Ch} .

Snaveley calculated C_{H^+} or the total concentration of titratable protons present from the total concentration of other species present at a given pH:

$$24. \quad C_{\text{H}^+} = 2C_{\text{H}_2\text{Ch}} + C_{\text{HNO}_3} - C_{\text{NaOH}} \quad (\text{diprotic case})$$

or

$$25. \quad C_{\text{H}^+} = C_{\text{HCh}} + C_{\text{HNO}_3} - C_{\text{NaOH}} \quad (\text{monoprotic case}).$$

The total concentration of free chelating agent $(\text{H}_2\text{Ch})_f$ is obtained by subtracting the (H^+) at a given point on the titration curve from the total titratable protons (C_{H^+}) and dividing by the mean number of protons bound to the non-coordinated chelate ion at that pH or $C_{\text{H}^+} - (\text{H}^+)/\bar{n}_{\text{Ch}}$. The total concentration of free chelating agent is now used in an alternate (but identical) definition of \bar{n} :

$$26. \quad \bar{n} = \frac{C_{\text{H}_2\text{Ch}} - (\text{H}_2\text{Ch})_f}{C_{\text{M}+2}}$$

or

$$27. \quad \bar{n} = \frac{C_{H_2Ch} - \frac{C_{H^+} - (H^+) + (OH^-)}{\bar{n}_{Ch}}}{C_{M+2}}$$

At high pH values the (H^+) is negligible but now (OH^-) should be incorporated into the calculation above.

The $-C$ value corresponding to a given \bar{n} for one point on the beryllium-azo compound titration curve is obtained by converting the concentration of the azo compound at that point to a pH_2Ch value and subtracting two times the pH (since $2H^+$ can be liberated by each H_2Ch molecule):

$$28. \quad -C = pH_2Ch - 2pH \quad (\text{diprotic case})$$

or

$$29. \quad -C = pHCh - pH \quad (\text{monoprotic case})$$

The $-C$ values plotted against \bar{n} values yield a formation curve which is shifted along the pCh^{-2} axis, but is identical in form to the curves shown in Section III C.

The relationship between $-C$ and pCh^{-2} is shown by:

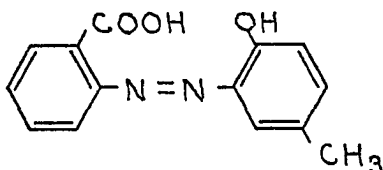
$$30. \quad pCh^{-2} = -C + pk_1 + pk_2 \quad (\text{diprotic})$$

$$31. \quad pCh^{-1} = -C + pk_1 \quad (\text{monoprotic})$$

It should be pointed out that by substituting exact pk_1 and pk_2 values in 22 and 30, this alternate calculation gives identical results with the Chaberek and Martell treatment.

III. EXPERIMENTAL WORK

A. Preparation and Purification of Azo Compounds

1. 1-(2'-Carboxybenzeneazo)-2-hydroxy-5-methylbenzene

Crude material (500 g.) left by Ellingboe (54) (designated Ellingboe B-5) was purified. The compound had been synthesized from anthranilic acid and p-cresol by the diazonium reaction, the anthranilic acid (Eastman) having been purified by recrystallization, the p-cresol by vacuum distillation.

a. Purification and analysis The crude compound was dissolved in sodium hydroxide, filtered to remove all base-insoluble impurities, and reprecipitated by acidifying. The product was washed with water, dried and recrystallized from hot ethanol with Norite and finally vacuum dried at 80°. The material decomposed at 185° in agreement with Snavely: 197-200° (46), Ellingboe: 193°, and Drew and Lindquist: 186° (55).

When the purified compound was sent for elemental analysis (Huffman), the value for nitrogen was low and it was suspected that trace amounts of the sodium salt remained. This was confirmed by ignition of a sample of the azo compound in a platinum crucible which showed 1.7 percent sodium calculated on the basis that the residue consisted of sodium carbonate.

When subjected to thin layer chromatography (in 10 percent methanol-90 percent chloroform) two bands were observed which moved at approximately the same speed. The lesser band was undoubtedly traces of the sodium salt.

Meanwhile a second sample of the identical material had been analyzed by Galbraith and found more nearly in agreement with the calculated values. This suggested the material was not entirely homogeneous with respect to traces of the sodium salt.

Table 1. Elemental analysis of B-5

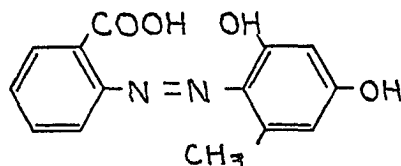
	Calculated for $C_{14}H_{12}N_2O_3$ percent	Found (Huffman Laboratories, Inc.) percent	Found (Galbraith Laboratories, Inc.) percent
Carbon	65.72	65.19	65.28
Hydrogen	4.72	4.93	4.86
Nitrogen	10.93	9.51	10.99

In order to remove the last traces of sodium, the above compound was recrystallized from ethanol after thorough washing with 1:1 hydrochloric acid and showed a decomposition point of 194°.

An azo group analysis with titanous chloride on the re-washed material showed an equivalent weight of 63.9, corresponding to a molecular weight of 255.6 (four electron reduc-

tion); calculated for $C_{14}H_{12}N_2O_3$: 256.

2. O-Carboxybenzeneazoorcinol



a. Reagents

(1) Anthranilic acid (O-aminobenzoic acid).

Commercial anthranilic acid was recrystallized from hot water with Norite; m.p.: 142-146°, reported: 145° (56).

(2) Orcinol Orcinol was used as obtained in the anhydrous form from the Eastern Chemical Corp., Newark, N.J.; m.p.: 107-108°, reported: 107-108° (56).

b. Preparation Recrystallized anthranilic acid (0.05 mole) was suspended in 25 ml. of water and 0.05 mole of sodium nitrite in a small amount of water was added with stirring. The smooth slurry was poured into 50 g. of ice containing 25 ml. of concentrated hydrochloric acid in an ice-salt bath. After ten minutes, the resulting mixture was filtered and urea added to the filtrate to remove traces of nitrite.

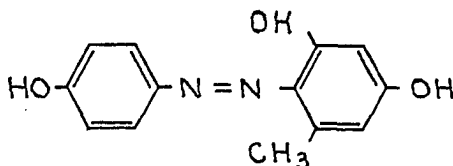
The filtrate was added rapidly with mechanical shaking to 0.05 moles of orcinol dissolved in a cold alkaline solution. The mixture was made alkaline with sodium hydroxide, heated and filtered while hot. The filtrate was acidified with

hydrochloric acid, diluted to one liter, boiled, and filtered while hot. The orange precipitate was washed well with hot water, air dried, and then vacuum dried at 80° for two hours.

c. Purification The crude product was redissolved in sodium hydroxide, Norite was added, the solution filtered, and the azo compound reprecipitated by the addition of sufficient hydrochloric acid to bring the pH to 1.5. The material was filtered, washed well with water, and again vacuum dried.

The material was then recrystallized from ethanol, leaving some alcohol-insoluble material. The alcohol-soluble portion yielded a fine, red powder which decomposed at 247-249°. The equivalent weight (by titanous chloride method) of this material was 68.3 which corresponds to a molecular weight of 273 (four electron reduction) calculated for $C_{14}H_{12}O_4N_2:272$

3. p-Hydroxybenzeneazoorcinol



a. Reagents

(1) p-Aminophenol p-Aminophenol (Eastman) was recrystallized from hot water; m.p.: 182°, reported: decomposes 184° (56).

(2) Orcinol Commercial anhydrous orcinol, Eastern Chemical Corp, Newark, N.J. was used directly. m.p. 107-108°, reported: 107-108° (56).

b. Preparation Recrystallized p-aminophenol (0.25 mole) was suspended in 25 ml. of water and added to a slurry of sodium nitrite (0.05 mole). This mixture was poured into 50 g. of ice containing 25 ml. of concentrated hydrochloric acid and stirred vigorously for ten minutes in a salt-ice bath.

The mixture was filtered, treated with a small amount of urea to destroy any excess nitrite and then poured into 0.25 moles of orcinol which had been dissolved in 25 ml. of 1 N sodium hydroxide. A saturated solution of sodium acetate was added with stirring until a color change occurred. The mixture was allowed to stand for two hours and then concentrated hydrochloric acid was added dropwise until precipitation occurred. The red precipitate was filtered off the next day, redissolved in sodium hydroxide, heated and the solution filtered while hot. The solution was acidified with hydrochloric acid causing the precipitation of an orange-red material. The mixture was diluted to one liter and boiled, the precipitate becoming yellow-orange in color. The precipitate was filtered, washed with hot water, and dried in a vacuum oven at 80° for two hours.

The compound was recrystallized from hot ethanol with Norite. The final product consisted of red crystals which decomposed at 214-215°. Equivalent weight (titanous chloride

(2) Orcinol Commercial anhydrous orcinol, Eastern Chemical Corp, Newark, N.J. was used directly; m.p. 107-108°, reported: 107-108° (56).

b. Preparation Recrystallized p-aminophenol (0.05 mole) was suspended in 25 ml. of water and added to a slurry of sodium nitrite (0.05 mole). This mixture was poured into 50 g. of ice containing 25 ml. of concentrated hydrochloric acid and stirred vigorously for ten minutes in a salt-ice bath.

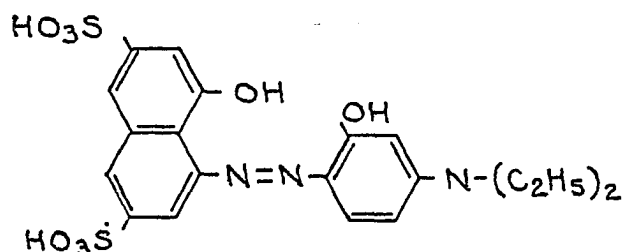
The mixture was filtered, treated with a small amount of urea to destroy any excess nitrite and then poured into 0.05 moles of orcinol which had been dissolved in 25 ml. of 1 M sodium hydroxide. A saturated solution of sodium acetate was added with stirring until a color change occurred. The mixture was allowed to stand for two hours and then concentrated hydrochloric acid was added dropwise until precipitation occurred. The red precipitate was filtered off the next day, redissolved in sodium hydroxide, heated and the solution filtered while hot. The solution was acidified with hydrochloric acid causing the precipitation of an orange-red material. The mixture was diluted to one liter and boiled, the precipitate becoming yellow-orange in color. The precipitate was filtered, washed with hot water, and dried in a vacuum oven at 80° for two hours.

The compound was recrystallized from hot ethanol with Norite. The final product consisted of red crystals which decomposed at 214-215°. Equivalent weight (titanous chloride

method) found 58.8 corresponding to a molecular weight of 235 (four electron reduction); calculated for $C_{13}H_{12}N_2O_3$: 244.

c. Properties p-Hydroxybenzeneazoorcinol functions as an acid-base indicator changing from yellow-orange to red above pH 10 ($pk_1 = 9.6$ from titration data).

4. 1-(2'-Hydroxy-4'-diethylamino-1'-benzeneazo)-3,6-disulfo-8-hydroxynaphthalene (Beryllon III)



In this synthesis, diazotized H-acid is coupled with m-diethylaminophenol. The method of preparation used was essentially that recommended by Kuznetsov (15) but with additional steps to purify the intermediates.

a. Purification of reagents

(1) H-acid Fifty g. of Eastman practical grade H acid (1-naphthol-3,6-disulfonic acid-8-amino-monosodium salt. H_2O) was treated with 1 N sodium hydroxide until it just dissolved. The solution was heated to boiling with 0.5 g. of Norite and filtered while hot. The filtrate was poured into one liter of deionized water which had been adjusted to pH 3 with hydrochloric acid. A pale green material precipitated.

This was filtered, washed with ethanol, and dried overnight in a vacuum oven at 50°. Neutralization equivalent found: 383.6; calculated for disodium salt $C_{10}H_7O_7NS_2Na_2 \cdot H_2O$: 381.3. Yield: 37 g.

H acid was also recrystallized from a hot, saturated solution of sodium bisulfite. A cream-colored, crystalline material resulted which was used in later preparations of Beryllon III (see comments under diazotization and coupling).

(2) m-Diethylaminophenol Twenty-five g. of Eastman practical grade m-diethylaminophenol was dissolved in ethanol. The solution was heated with 0.4 g. of Norite and then filtered. Three volumes of water was added to the filtrate and the mixture cooled in ice. On stirring, crystallization occurred. The precipitate was filtered, washed thoroughly with deionized water and dried in a vacuum oven with no heat. The product was a purple powder melting at 72-75°; reported: 78° (56).

b. Diazotization and Coupling Purified H acid, 0.075 moles, 29 g., was suspended in 200 ml. of 0.75 N hydrochloric acid and the mixture cooled to approximately 0°. A solution of 5.3 g. of sodium nitrite in 50 ml. of water was gradually added to the cold H acid over a period of two hours, the solution being stirred mechanically. The resulting bright yellow mass was checked with starch-iodide paper for the presence of excess nitrite. After two hours, excess nitrite being present, sulfamic acid crystals were added until the starch-iodide test

failed. The bright yellow intermediate was filtered through a large sintered glass funnel without washing.

The filtered diazonium salt was suspended in 50 ml. of water, placed in an ice bath and 12.4 g. of purified m-diethylaminophenol (0.075 mole) in 20 ml. of ethanol was added with stirring. After two hours the dark orange-red solution was filtered to remove unreacted material and treated with about 13 g. of sodium chloride. A black material precipitated which was filtered off the following day and washed with two 100-ml. portions of a solution containing 150 g. of sodium chloride and 50 ml. of concentrated hydrochloric acid per liter. This crude product was dried in a vacuum at room temperature. The yield was 25.8 g. of black powder.

Best results in this preparation were obtained when the amounts of water and ethanol was close to the amount specified because the final salting out procedure could only be accomplished with a minimum of sodium chloride if the final volume was approximately 70 ml. Also, it was observed that the diazonium salt tends to hydrolyze with excess water.

When the same procedure was followed on a smaller scale using 0.0075 mole quantities and the H acid recrystallized from a saturated sodium bisulfite solution, the yield of product was better.

The crude compound was quite satisfactory for the colorimetric determination of beryllium. A color change from dark

orange to raspberry red between pH 6 and 8 was obtained on the addition of the metal ion.

c. Purification A column of Amberlite IR 120, a strong acid, cation exchange resin was prepared, the column being 50 cm. long and 1.9 cm. in diameter. The resin was placed in the hydrogen form by treatment with 6 N hydrochloric acid. The column was backwashed with deionized water to free it of all air bubbles and the washing was continued until no chloride test was obtained on the eluate.

To a solution of 3 g. of Beryllon III in 500 ml. of water was added 0.5 g. of Norite. The mixture was heated to boiling and filtered while hot. The filtrate was cooled, diluted to two liters and passed through the resin column at a flow rate of 5 to 8 ml. per minute. The eluate was evaporated to dryness by vacuum distillation in a "roto-vac" apparatus. The residue was dissolved in a minimum of ethanol, filtered to remove any alcohol insoluble impurities, and treated with five volumes of acetone with stirring. The brown-red flocculent precipitate which appeared was filtered and dried overnight at 80° in a vacuum oven. The final product consisted of black-green, lustrous crystals.

d. Properties and composition The black-green, lustrous crystals of Beryllon III, prepared as just described, decomposed without melting when heated over 200°.

No residue remained on ignition of the material in a platinum crucible.

A sample of material weighing 0.1060 g. was titrated potentiometrically with sodium hydroxide. Three end-points were observed, Figure 1, the volumes to the points marked a, b and c, and the corresponding values of pH being, respectively: 2.18 ml., 3.0; 4.37 ml., 6.8; 6.55 ml., 9.6. The equivalent weights found thus are respectively: 585, 291 and 194, in good agreement with the values for 1, 2 and 3 replaceable hydrogen atoms respectively in a pentahydrate: $C_{20}H_{21}S_2O_8 \cdot 5H_2O$ for which the molecular weight is 585, and the equivalent weights respectively, 585, 292 and 195. A second titration with 0.1194 g. of material required 2.47, 4.93 and 7.40 ml. of 0.08304 N sodium hydroxide to replace the three hydrogen atoms yielding equivalent weights of 584, 292 and 194 at each break.

A thermogravimetric analysis indicated the presence of five molecules of water of hydration, gradual decomposition after 200° , and a negligible residue.

Titration of the azo group (titanous chloride method) showed an equivalent weight of 146; calculated for $C_{20}H_{21}S_2O_8 \cdot 5H_2O$, $585/4$ or 146 (four electron reduction).

Material dried in a vacuum at 80° was subjected to elemental analysis, Table 2.

e. Discussion The problem of purifying Beryllon III was two-fold: removal of contaminants, including the sodium chloride used in the salting out procedure, and the conversion of the salt of the azo compound to the acid form. The

Table 2. Elemental analysis of Beryllon III

	Calculated for $C_{20}H_{21}S_2O_8N_3$ $\cdot 5H_2O$ per cent	Calculated for $C_{20}H_{21}S_2O_8N_3$ $\cdot 4H_2O$ per cent	Found (Galbraith Laboratories, Inc.) per cent
Carbon	41.03	42.33	42.67
Hydrogen	5.30	5.12	5.23
Nitrogen	7.18	7.41	6.98
Sulfur	10.94	11.29	11.52

solubility in water is too great to permit recrystallization from water. Recrystallization from alcohol was unsuccessful. Extraction of the crude compound with alcohol in a Soxhlet extractor yielded a red powder when the alcohol was removed by evaporation from the extract. Prolonged heating apparently caused some detrimental change or polymerization. The final material did, however, give a color reaction with beryllium.

Chromatography on alkaline aluminum oxide was also not satisfactory. An alcohol solution of the crude compound yielded two distinct bands on a column of alkaline aluminum oxide. When eluted with 50 percent alcohol solution, three bands: pink, purple and black, moved out ahead of the two major bands. Elution with water removed these three bands from the column completely but separation of the bands from each other was poor. Elution with a 20 percent ammonia-80

percent ethanol solution removed a fourth fraction and 0.1 N sodium hydroxide completely cleared the column of the gray band that remained. This method of separation was rejected as unsatisfactory because the bands were not well defined.

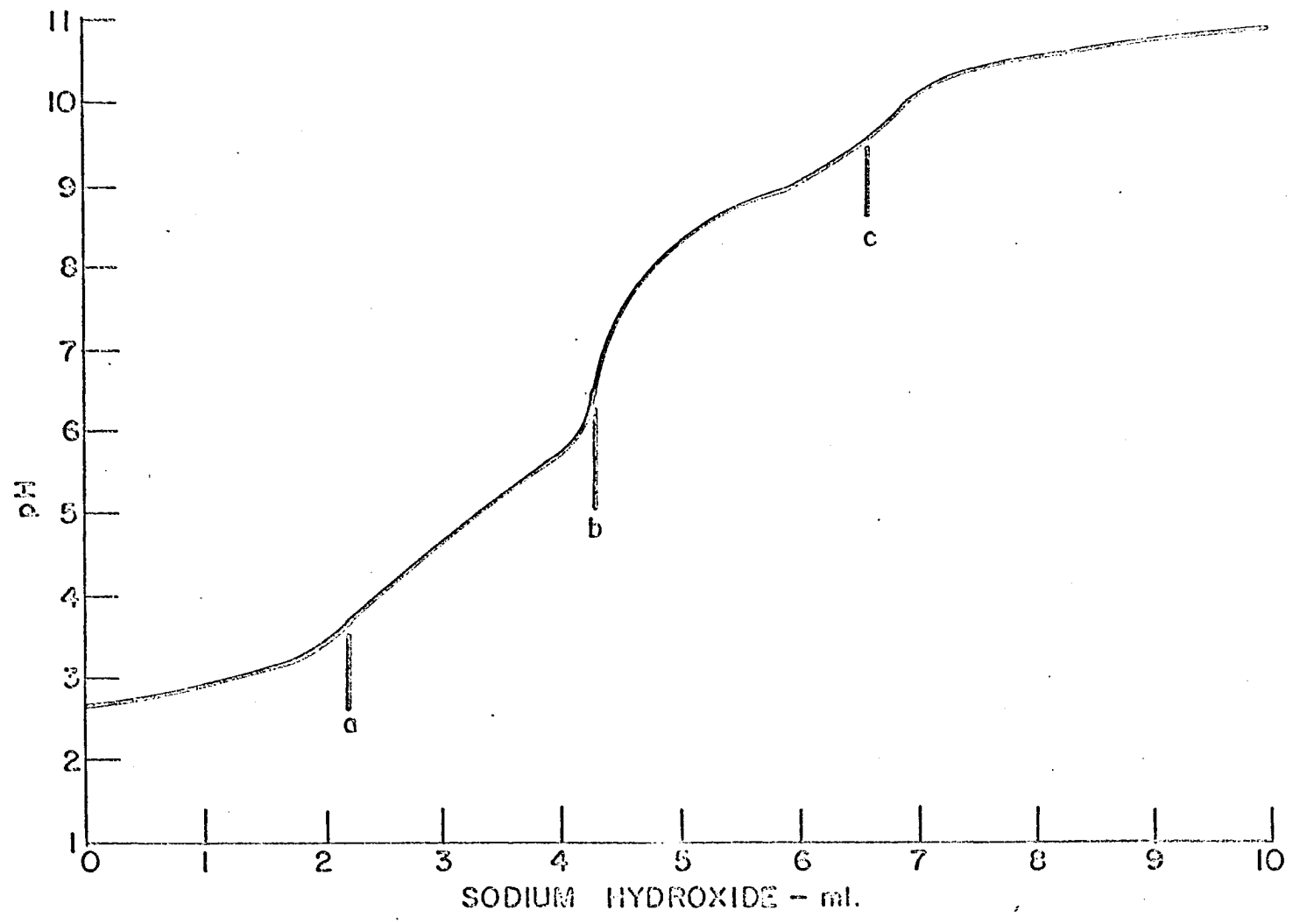
The purification procedure finally adopted employed passage of the crude Beryllon III through a strong acid, cation exchange resin, a procedure used earlier by Richardson (57) for the purification of azo dyes bearing sulfonic groups. This operation produces the free acid of the azo compound and also the acid of any inorganic anions present. In the Richardson procedure the eluate from the cation exchange resin was then passed through an anion exchange resin to remove the inorganic acid, anions greater than 30 Å diameter not being absorbed by the resin. Beryllon III, however, was absorbed by the anion exchange resin tried and the second part of the Richardson procedure was not useful.

Following evaporation of the water of the eluate from the cation exchange resin and dissolution of the residue of Beryllon III in alcohol, the Beryllon III was precipitated by the addition of ten volumes of ether. A gummy mass formed which made the manipulations difficult. Precipitation with acetone was somewhat more satisfactory.

The final product obtained was of satisfactory purity as indicated by the values found for the neutralization equivalents, the titanous chloride reduction equivalent, and the elemental analysis. The material was satisfactory for the

Figure 1. Titration of Beryllon III with sodium hydroxide

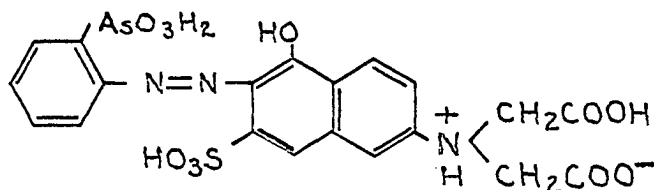
Beryllon III purified by cation exchange resin method.
Weight of Beryllon III titrated: 0.1060 g. Concentration of sodium hydroxide: 0.08304 N. Neutralization equivalents found: 585 (point a); 219 (point b); 194 (point c); calculated for $C_{20}H_{21}S_2O_8 \cdot 5H_2O$: 585, 292, and 195 for one, two and three replaceable hydrogen atoms, respectively.



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determination of beryllium and for the measurements of the physical and chemical properties of the compound and its beryllium derivative.

5. 1-(1'-Hydroxy-3'-sulfo-6'-iminodiacetic-2'-naphthylazo) benzene-2-arsonic acid (Beryllon IV)



Beryllon IV is obtained by coupling diazotized *o*-aminophenylarsonic acid with 1-hydroxyanthralene-6-iminodiacetic-3-sulfonic acid. The procedure followed in this work was essentially that proposed by Kuznetsov (15) but with particular attention to the purification of the intermediates and the final product.

a. Purification of reagents

(1) *o*-Aminophenylarsonic acid *o*-Aminophenyl-
acid was obtained in pure form from Fluka, A. G., Buchs, S. G., Switzerland. It may be prepared from 2-nitrobenzenearsonic acid by the procedure reported by Jacobs (58).

(2) J acid 2-Amino-5-naphthol-7-sulfonic acid-
monosodium salt was obtained in practical grade from Eastman. It was purified by dissolving it in sodium hydroxide and heat-

ing with Norite. After filtering, it was precipitated with hydrochloric acid by pouring the basic solution into a liter of distilled water adjusted to pH 3. When filtered and dried the material showed an equivalent weight of 263.5 (monosodium salt) by alkalimetric titration in 33 percent dioxane; calculated for $C_{10}H_9N_2O_4SNa$, molecular weight, 262.

(3) Monochloroacetic acid Baker analyzed reagent, 99.5 percent pure, was used.

b. Preparation of 1-Hydroxy-naphthalene-6-iminodiacetic-3-sulfonic acid J. Acid, 49 g. (0.2 mole), was suspended in 150 ml. of deionized water and the mixture was heated to 80°. Approximately 30 ml. of 20 percent sodium hydroxide was added to bring the pH to 8. A solution of 56.5 g. of monochloroacetic acid in 100 ml. of water was treated with 24 g. of sodium hydroxide, producing 0.6 mole of sodium chloroacetate. This solution was added to the suspension of the J acid and the mixture heated to 80-90° for two hours. The hydrogen ion liberated as reaction occurred was neutralized at intervals to keep the pH at 8-9 by the addition of small portions of sodium hydroxide, for a total of approximately 80 ml. of 20 percent solution.

The solution was cooled and filtered. The dark brown filtrate was acidified to pH 3 with hydrochloric acid and evaporated under vacuum to a volume of 200 ml. The black precipitate which separated was filtered off and recrystallized

from 70 ml. of water. The light brown material was vacuum dried without heat. When titrated with sodium hydroxide, it showed an equivalent weight of 431.3; molecular weight calculated for $C_{14}H_{11}O_8SNNa_2 \cdot 2H_2O$: 435.

A small sample was ignited at a red heat in a platinum crucible. Assuming the residue to be sodium carbonate, the residue indicated a sodium content of 10.71 percent, corresponding to two sodium atoms per molecule.

c. Preparation of Beryllon IV To a solution of 11 g. of O-aminophenylarsonic acid (0.05 mole) in 35 ml. of 1.5 M hydrochloric acid which had been cooled to 0° , was added 3.5 g. of sodium nitrite in 10 ml. of water, gradually with stirring. After one to two hours the mixture was checked with starch-iodide paper for the presence of excess nitrite, and a few crystals of sulfamic acid were then added until the test failed. The intermediate, 1-hydroxy-naphthalene-6-iminodiacetic-3-sulfonic acid, 20 g. and anhydrous sodium carbonate, 15 g., were dissolved in 130 ml. of water and the mixture cooled in ice. The suspended diazonium compound, without filtering, was added gradually to the intermediate. After two hours the dark red solution was filtered and the filtrate acidified with concentrated hydrochloric acid to pH 3. The red brown precipitate which formed was filtered off and dried in a vacuum without heating. The yield was 13.8 g. of brown-red powder.

This crude Beryllon IV was recrystallized in the following manner: 3 g. was suspended in 70 ml. of water and 0.1 N sodium hydroxide was added dropwise with stirring until the material just dissolved. The solution was filtered to remove any base-insoluble impurities and poured rapidly with vigorous stirring into 70 ml. of concentrated hydrochloric acid. The mixture was immediately immersed in an ice bath and stirring continued until precipitation occurred. After one half hours, the dark red product was filtered off, washed with 50 ml. of acetonitrile and dried at 110° for two hours.

The material was quite hygroscopic. It showed no definite melting point but gradual decomposition began at approximately 140° accompanied by a color change from red to brown.

d. Analysis Analytical work was done on three portions of Beryllon IV prepared as just described but subjected to variations in the purification step:

Lot 1. Precipitated at pH 1.5 and actually a mixture of mono- and di-sodium salts; dried in a vacuum.

Lot 2. Lot 1 dissolved in alkali and poured into concentrated hydrochloric acid; dried in vacuum at 80° . Free acid monohydrate.

Lot 3. Prepared as described above, final precipitation being made from concentrated hydrochloric acid; dried in vacuum at 110° . Free acid, anhydrous.

Equivalent weight by neutralization. Lot 3. A sample

weighing 0.0934 g. was titrated potentiometrically using the usual glass electrode assembly with 0.08304 N sodium hydroxide. Equivalent weight found: 193; calculated for $C_{20}H_{18}N_3O_{11}AsS$: $583/3$ or 194 (three replaceable hydrogen atoms). Only one break found in titration curve; see Figure 2.

Lot 2. Determined by titrating a 0.0877 g.-sample with 0.08304 N sodium hydroxide. Equivalent weight found: 199; calculated for $C_{20}H_{18}N_3O_{11}AsS \cdot H_2O$: $601/3$ or 200.

Equivalent weight by titanous chloride reduction (four-electron reduction). Found on Lot 2 which had been exposed to moist air subsequent to vacuum drying: 151; calculated for $C_{20}H_{18}N_3O_{11}AsS \cdot H_2O$: $601/4$ or 150.

Elemental analysis. Lot 1. This material was subjected to the usual micro-combustion analysis for carbon, hydrogen, nitrogen, sulfur and arsenic; Table 3. The determinations of sodium and potassium were made by the flame photometer method using a Baird Atomic Clinical Flame Photometer (Model KY). Calibration curves were prepared using standard sodium chloride and potassium chloride solutions. The sample of Beryllon IV was first wet ashed with perchloric acid.

e. Discussion The method used here for the final purification of Beryllon IV, precipitation from concentrated hydrochloric acid, is apparently the only way to obtain the acid form of the material. A similar procedure had been reported previously for the purification of Arsenazo (59).

Figure 2. Titration of Beryllon IV with sodium hydroxide

Beryllon IV purified by precipitation from concentrated hydrochloric acid. Weight of Beryllon IV (acid form, Lot 3) titrated: 0.0934 g. Concentration of sodium hydroxide: 0.08304N. Neutralization equivalent found: 193; calculated for $C_{20}H_{18}N_3O_{11}AsS$: $583/3$ or 194.

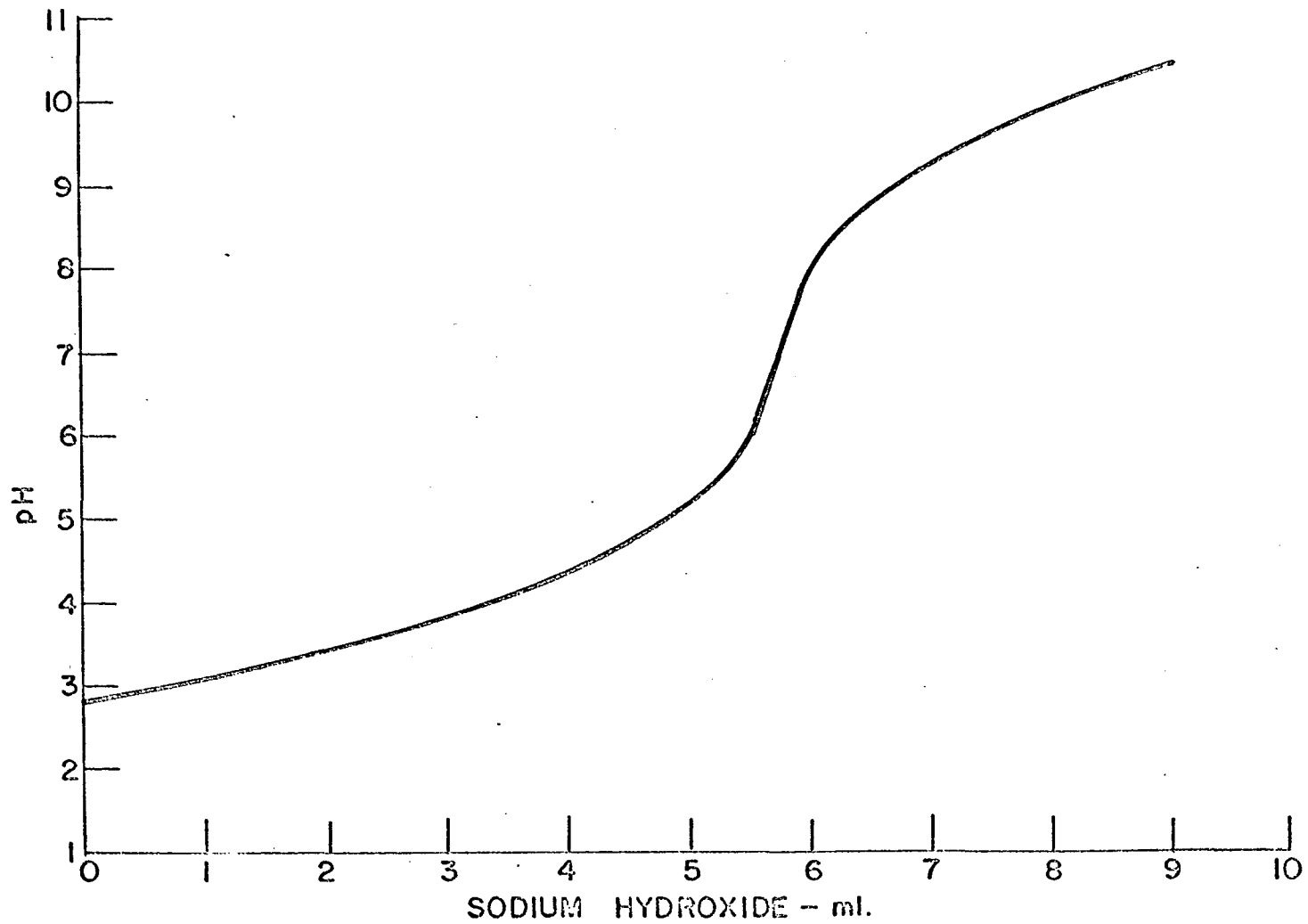


Table 3. Elemental analysis of Beryllon IV-sodium salt
(Lot 1)

	Calculated for $C_{20}H_{16.5}AsO_{11}N_3S \cdot$ $1.5 Na \cdot 3H_2O$ percent	Found percent
Carbon	35.82	36.54 ^a
Hydrogen	3.36	3.17 ^a
Nitrogen	6.27	6.28 ^a
Sulfur	4.78	5.06 ^a
Arsenic	11.19	11.37 ^a
Sodium	5.14	5.24 ^b
Potassium		0.16 ^b

^aGalbraith Laboratories, Inc.

^bL. M. Grennan by flame photometer method.

The sodium salt of Beryllon IV, obtained by precipitation at pH 3, is satisfactory for the colorimetric determination of beryllium but the free acid was desired in this work, for the determination of the various physical and chemical properties of the reagent and its beryllium derivative.

Beryllon IV is obtained as a monohydrate from concentrated hydrochloric acid. This molecule of water is lost on heating in a vacuum at 110° but is quickly regained on exposure to moist air. The equivalent weights found indicated satisfactory purity. The elemental analysis, although obtained on the mix-

ture of sodium salts, is satisfactory proof of the composition; Table 3. The low value for carbon is undoubtedly caused by the retention of carbon as sodium carbonate as a residue in the combustion

$$(\text{Per cent sodium} + \text{per cent potassium}) \frac{\text{At. wt. C}}{\text{Mol. wt. Na}_2\text{CO}_3} =$$

per cent carbon retained

$$(5.24 + 0.16) \frac{12}{86} = 0.75 \text{ per cent carbon retained}$$

almost exactly the difference between the carbon found and the calculated value.

When precipitated from slightly acid solution, Beryllon IV was obtained in red and orange forms. The infrared absorption spectra of these materials were essentially identical and the materials proved to differ only in the content of sodium. The free acid is red and the color shifts to orange with increasing sodium content. Mixtures of the sodium salts and the free acid showed absorption maxima at 485 m μ and in the presence of excess beryllium showed an absorption maxima at 528 m μ .

B. Absorption Spectra of Azo Compounds and Beryllium Derivatives

Figures 3 through 7 show the absorption spectra of the five azo compounds studied and their beryllium derivatives.

A Cary Model 15 recording spectrophotometer was used with quartz cells of 1 cm. light path.

Figure 3. Absorption spectra of 1-(2-carboxybenzeneazo)-2-hydroxy-5 methylbenzene (B-5) at pH 7.2

A. B-5: $3.83 \times 10^{-5} \text{M}$. Absorption maximum at 325 m μ .

B. B-5 plus beryllium: 3.83×10^{-5} in B-5 and $4.68 \times 10^{-4} \text{M}$ in beryllium. Absorption maxima at 345 m μ and 470 m μ .

Cary Model 15 Spectrophotometer. 1 cm. cell.

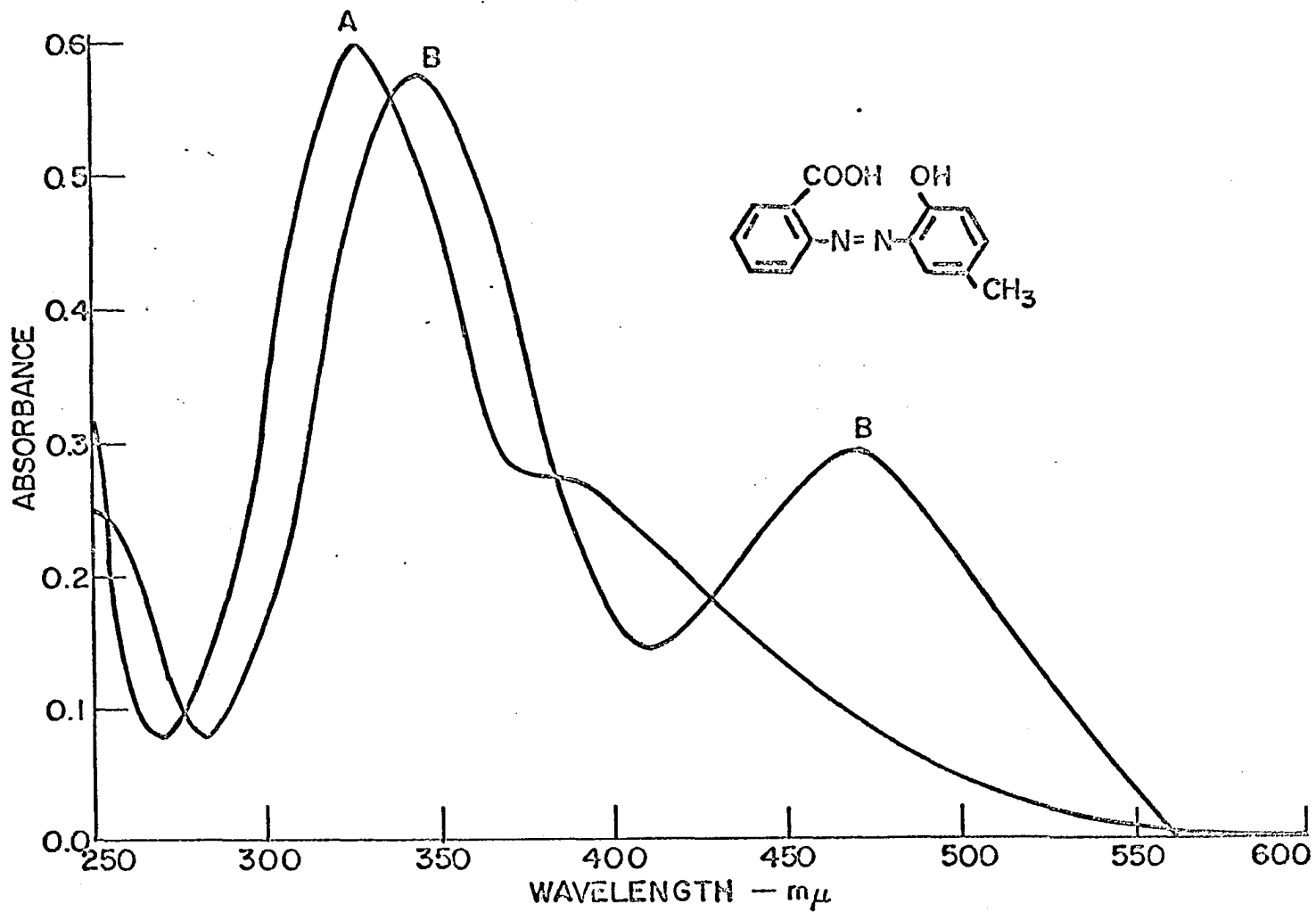


Figure 4. Absorption spectra of o-carboxybenzeneazoarcinol (P-15) at pH 7.0

A. P-15: $2.83 \times 10^{-5} \text{M}$. Absorption maximum at 425 m μ .

B. P-15 plus beryllium: $2.83 \times 10^{-5} \text{M}$ in P-15 and $2.34 \times 10^{-4} \text{M}$ in beryllium. Absorption maximum at 465 m μ .

Cary Model 15 Spectrophotometer. 1 cm. cell.

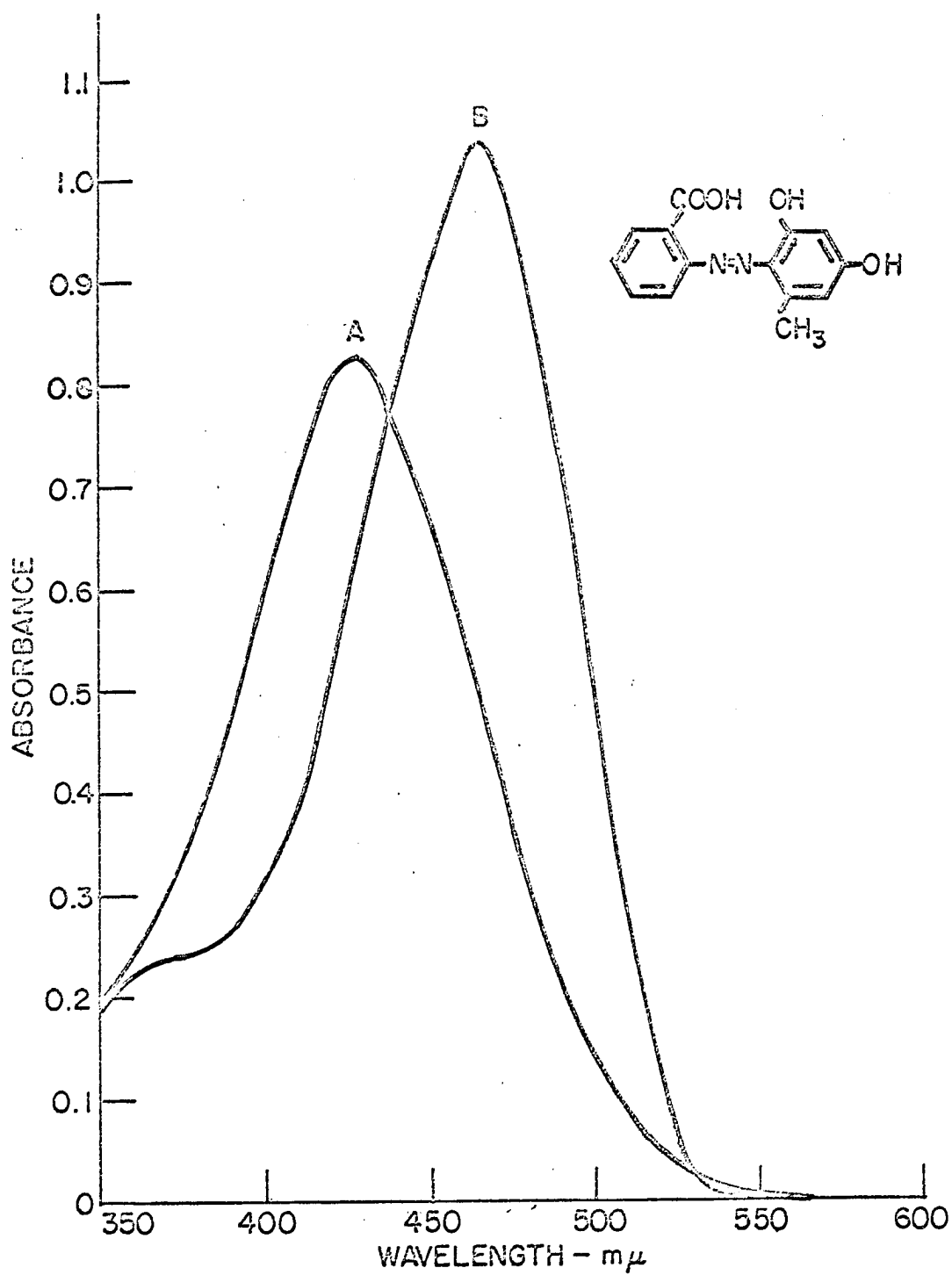


Figure 5. Absorption spectra of p-hydroxybenzeneazoarcinol (P-17) at pH 9.2

A. P-17: $3.5 \times 10^{-5} \text{M}$. Absorption maximum at 465 m μ .

B. P-17 plus beryllium: $3.5 \times 10^{-5} \text{M}$ in P-17 and $1.1 \times 10^{-5} \text{M}$ in beryllium. Absorption maximum at 465 m μ .

Cary Model 15 Spectrophotometer. 1 cm. cell.

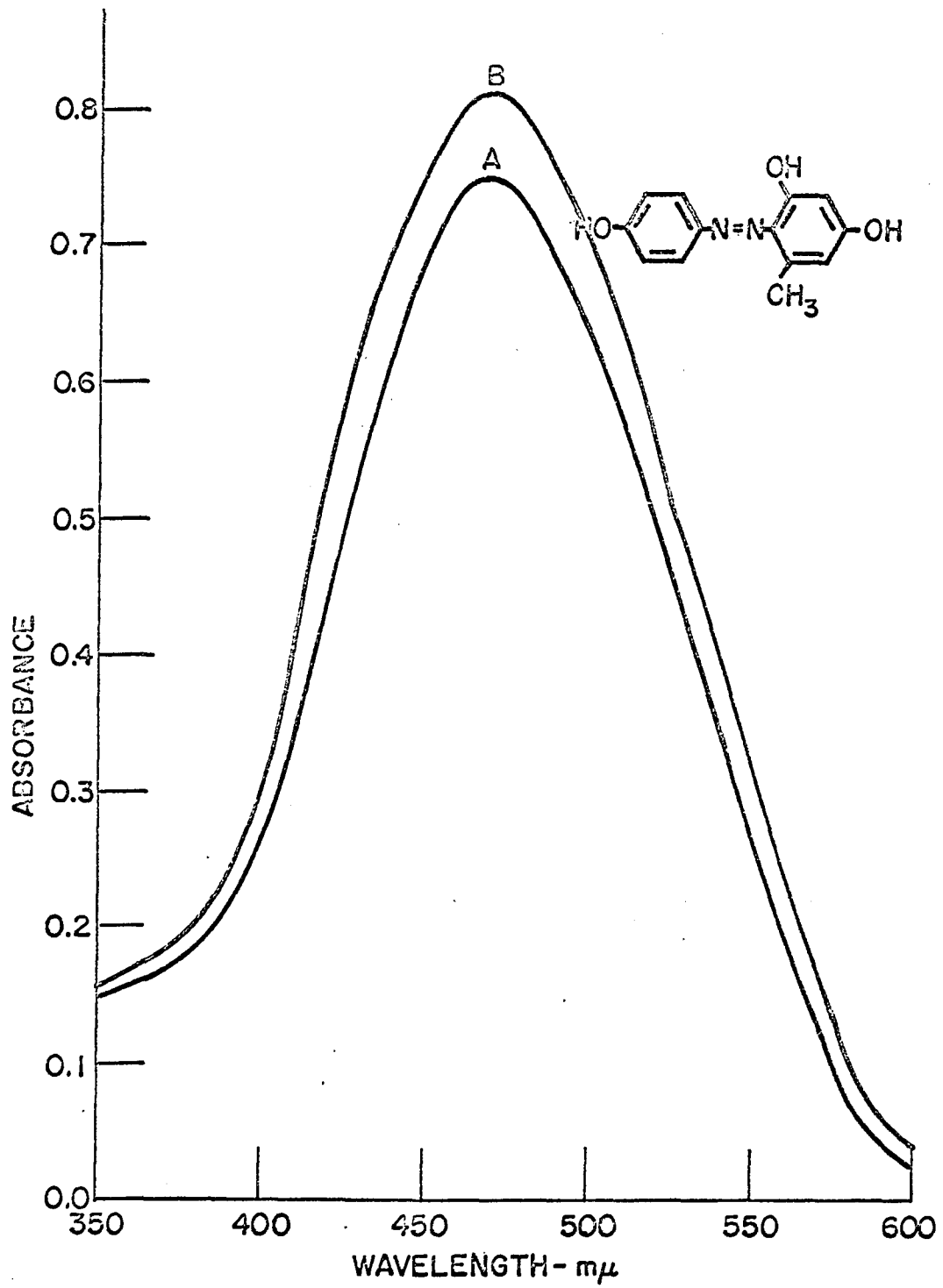


Figure 6. Absorption spectra of Beryllon III and Beryllon III plus beryllium at pH 7.2

A. Beryllon III: $2.18 \times 10^{-5} \text{M}$. Absorption maximum: 490 m μ .

B. Beryllon III plus beryllium: $2.18 \times 10^{-5} \text{M}$ in Beryllon III and $2.34 \times 10^{-4} \text{M}$ in beryllium. Absorption maximum: 525 m μ .

Cary Model 15 Spectrophotometer. 1 cm. cell.

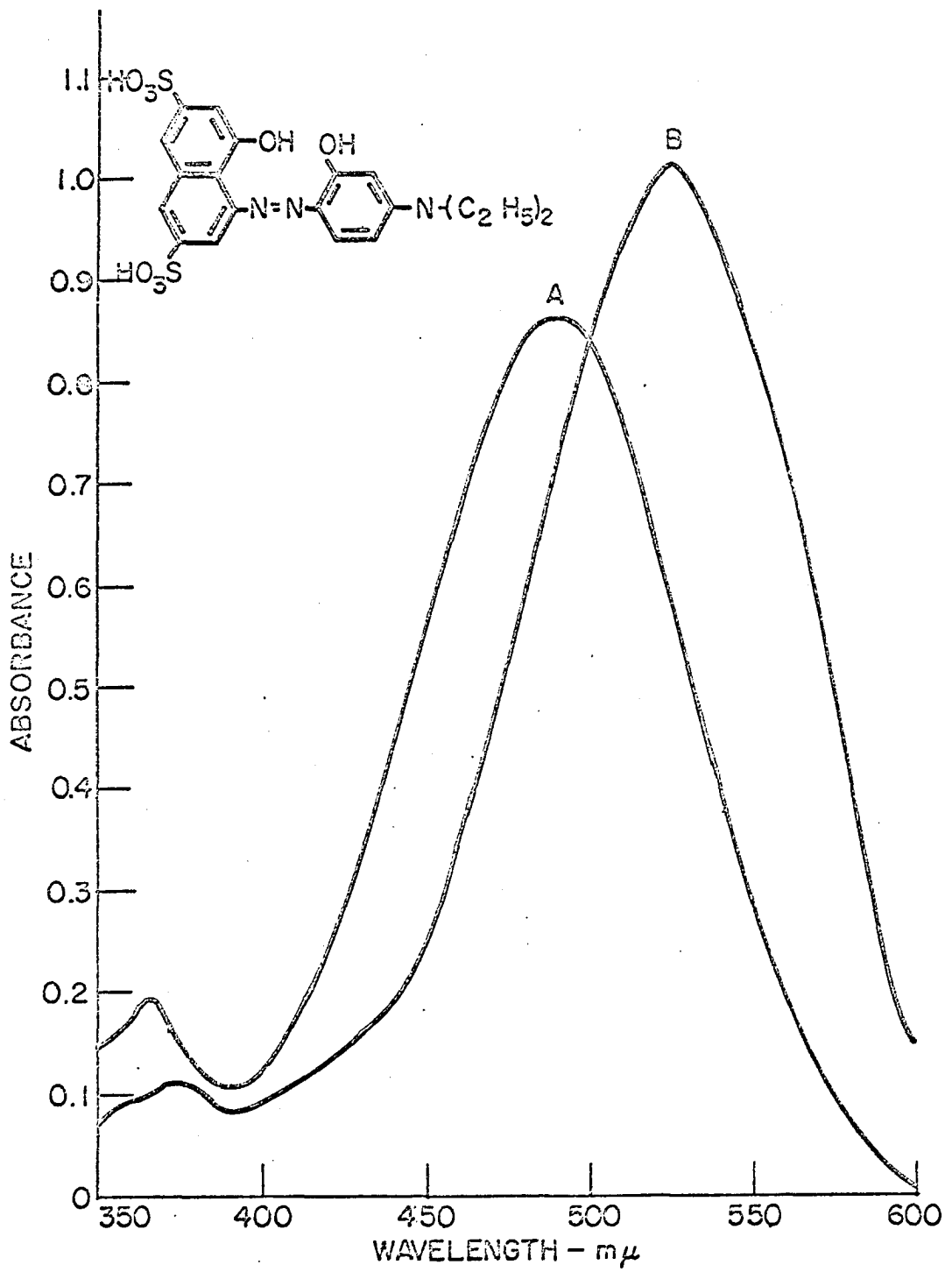
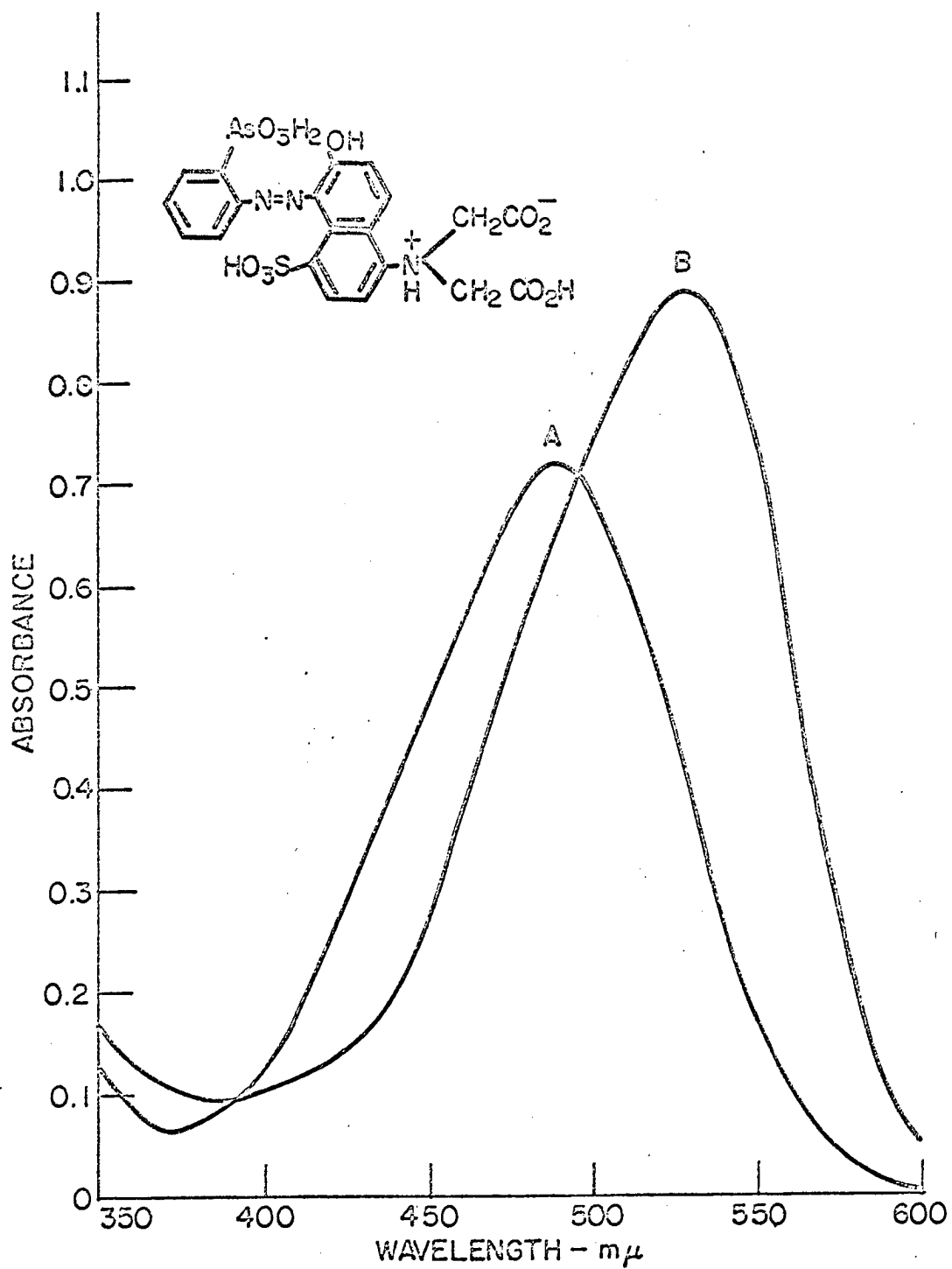


Figure 7. Absorption spectra of Beryllon IV and Beryllon IV plus beryllium at pH 7.2

A. Beryllon IV: $2.1 \times 10^{-5} \text{M}$. Absorption maximum at 485 m μ .

B. Beryllon IV plus beryllium: $2.1 \times 10^{-5} \text{M}$ in Beryllon IV and $2.34 \times 10^{-4} \text{M}$ in beryllium. Absorption maximum at 528 m μ .

Cary Model 15 Spectrophotometer. 1 cm. cell.



The pH selected for the solutions of each azo compound and its beryllium derivative lies within the range of pH of maximum absorption for the given compound. Hexamethylene-tetramine (25 percent) was used to buffer at pH 7 and a sodium hydroxide-boric acid buffer was used at pH 9. The beryllium derivatives of Beryllon III and Beryllon IV were found to show a narrower pH range of maximum absorption using phosphate-borate buffers. A constant ionic strength was maintained by making the solutions 0.1M in potassium chloride.

The spectra of the derivatives were all run with an excess of beryllium to assure maximum compound formation. Examination of each set of spectra gave indication as to whether the color difference between the azo compound and its beryllium derivative was sufficient to merit further study of the azo compound as a colorimetric reagent for beryllium.

C. Titration Data and Curves from Potentiometric Titration Procedure

Each azo compound studied was titrated potentiometrically alone and with beryllium added. Figures 8 through 17 show the titration curves and the formation curves obtained. All titrations were performed in a constant temperature bath ($30^{\circ} \pm 0.1^{\circ}$) using 1.129 N sodium hydroxide.

Neutralization equivalents obtained from the titrations of the azo compounds without beryllium present are approximate, owing to the high concentration of base used, but serve as a

check on the purity of the dioxane. In every case, the purity of the azo compound had been ascertained by other means. Acid dissociation constants obtained for the azo compounds are reported without correction for ionic strength, but with solutions 0.018 M in nitric acid, the correction would be less than 0.10 of a pH unit.

Representative pH values at various points on the beryllium-azo compound curves are listed in Tables 4 through 8, as well as the resulting \bar{n} , $-C$, and pCh^{-2} values calculated from these titrations for each compound studied. The formation curves, in each case, are obtained from the calculated values. (See Section II B for mathematical treatment).

In the case of 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5) for which a "displaced" formation constant had already been reported by Snavely, considerable preliminary investigation of the potentiometric titration method as applied to beryllium-azo compounds was necessary before satisfactory conditions were achieved and the reported "displaced" formation constant was confirmed. It was learned that the beryllium solution must be added to the titration mixture in an acid state (pH 2) or incomplete compound formation results. This can be more readily explained by referring to Figure 9 in which the formation curve levels off at $\bar{n} = 1$, indicating a one to one compound being formed. When the beryllium solution was adjusted to pH 5 (which was the highest value possible without

precipitation of beryllium hydroxide), a maximum \bar{n} value of 0.25 was obtained; when adjusted to pH 3.5, a maximum \bar{n} value of 0.60 was obtained, in both cases indicating incomplete compound formation. It was also learned that commercial, "stabilized", reagent grade dioxane was unsatisfactory as a titration medium, due to small traces of acetic acid present.

1. 1-(2'-Carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5)

(See Figures 8 and 9)

The titration vessel contained 0.6828 mM of B-5, 1.8875 mM of nitric acid, 75 ml. of purified dioxane and water up to a 100 ml. volume for the titration shown on Curve A of Figure 8. For Curve B, 0.2206 mM of beryllium was added to the same constituents before the solution was made up to a final volume of 100 ml. with water.

a. Titration of B-5 From the titration (Curve A, Figure 8) of B-5 alone, the following calculations were made: neutralization equivalent found: 258; theoretical value: 256; $\text{p}K_1$: 6.90. Reported by Snavely (46), $\text{p}K_1$: 6.83.

b. Titration of B-5 plus beryllium Table 4 shows representative pH values and calculations made from Curve B, Figure 8. These values were used to obtain the formation curve, Figure 9. The "displaced" formation constant ($-\text{p}K_{\text{eq}}$) was read from the formation curve as 4.03 and the logarithm of K_f as 14.20 at $\bar{n} = 0.50$. The curve levels off at $\bar{n} = 1$, indicating a one to one compound between beryllium and 1-(2'-carboxybenzene-azo)-2-hydroxy-5-methylbenzene.

Table 4. Representative titration values and calculations from titration of B-5 plus beryllium using 1.129 N sodium hydroxide

ml. NaOH	pH reading	pH reading corrected (+.40)	\bar{n}	-C	pCh^{-2}
0	1.42	1.82			
1.40	2.10	2.50	0.18	2.83	15.40
1.70	2.52	2.92	0.34	3.67	14.56
1.80	2.70	3.10	0.50	4.03	14.20
1.90	2.88	3.28	0.67	4.39	13.84
2.02	3.15	3.55	0.97	4.83	13.40
2.05	3.28	3.68	1.02	5.19	13.04
2.10	3.79	4.19	1.10	6.21	12.02
2.15	5.22	5.62	1.10	9.07	9.16

c. Discussion The pk_1 value (6.90) agrees reasonably well with the value 6.83 reported by Snavely. The pk_2 value for B-5 (11.4), used in the calculations, was reported by Ellingboe (17) using a spectrophotometric procedure. The displaced formation constant -C or $-pK_{eq}$ of 4.03 is in agreement with 3.88 reported by Snavely.

2. o-Carboxybenzeneazoorcinol (P-15) (See Figures 10 and 11)

The titration vessel contained 0.7367 mM of P-15, 1.8875 mM of nitric acid, 75 ml. of purified dioxane and water up to a 100 ml. volume for the titration shown on Curve A, Figure 10. For Curve B, 0.2206 mM of beryllium was added to the same constituents before the solution was made up to a final volume of 100 ml. with water.

a. Titration of P-15 From the titration (Curve A, Figure 10) of P-15 alone, the following calculations were made:

Figure 8. Potentiometric titration of 1-(2-carboxybenzene-azo)-2-hydroxy-5-methylbenzene in 75 percent dioxane

A. Compound alone

B. Compound plus beryllium

Sodium hydroxide: 1.129 N

Compound: 0.6828 mM

Beryllium: 0.2206 mM

Initial volume: 100 ml.

μ : 0.019

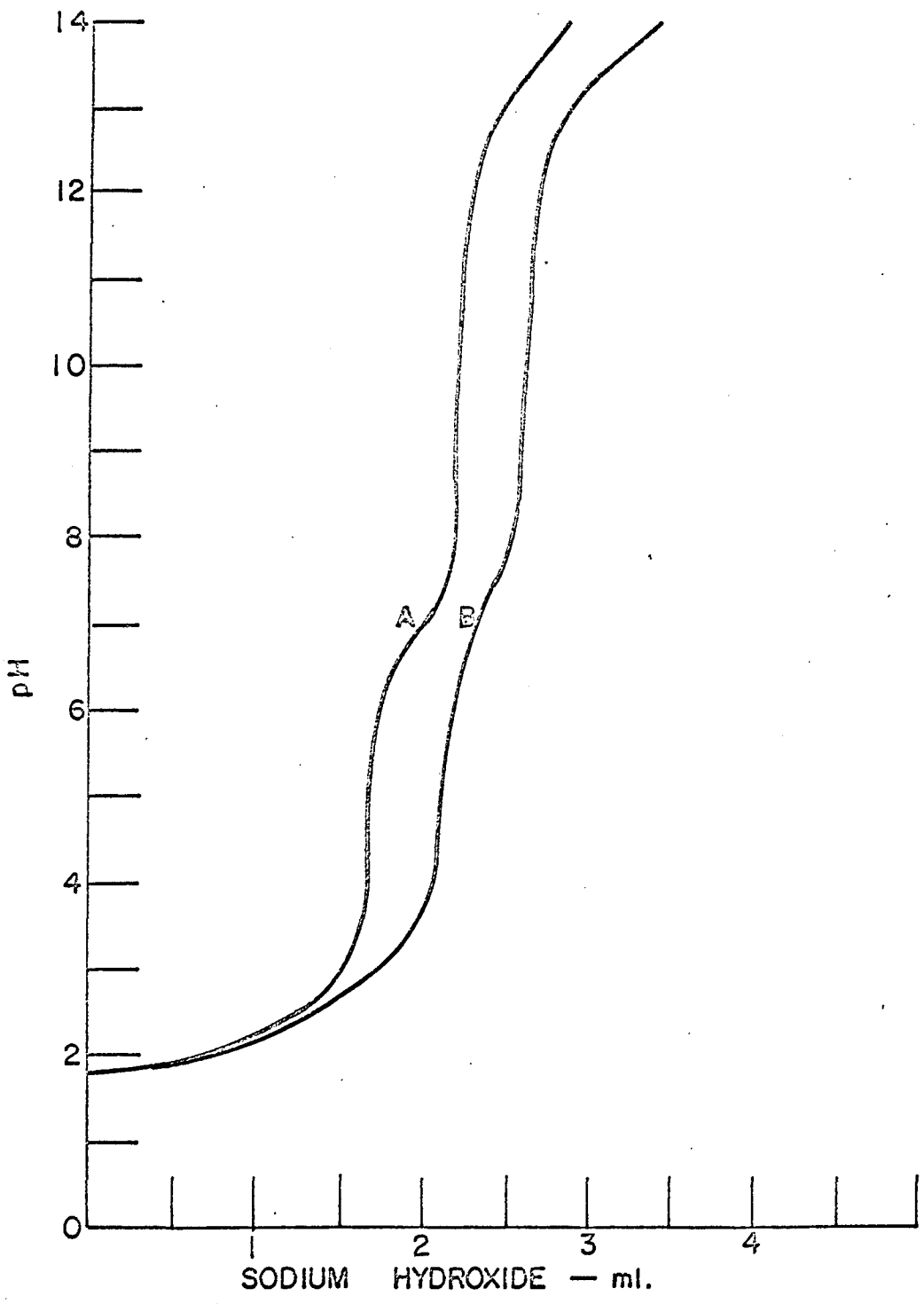
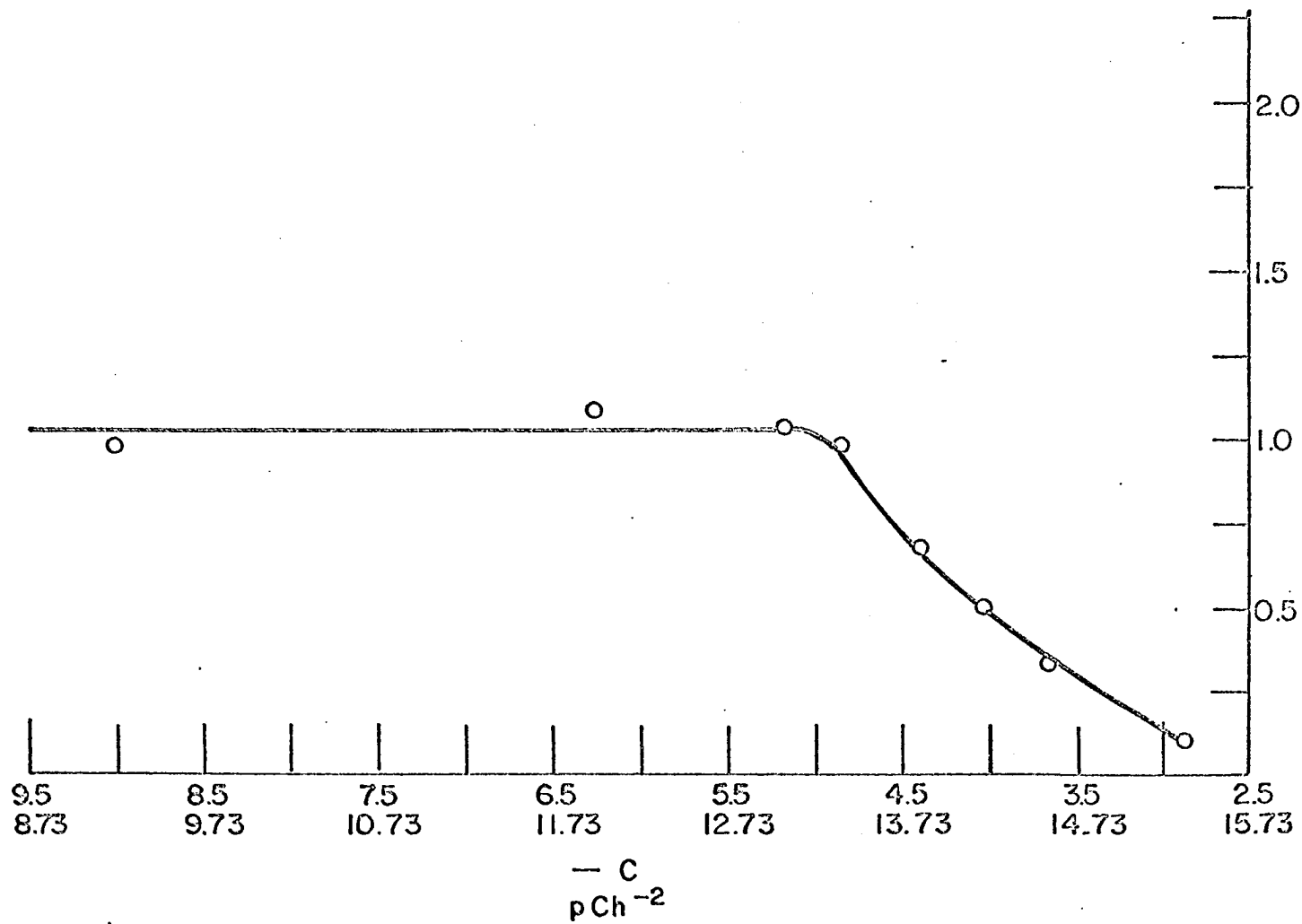


Figure 9. Formation curve for 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene with beryllium
1 to 1 compound
Logarithm of K_f : 14.20 (\bar{n} : 0.5)



Neutralization equivalent found: 138; theoretical value: 136;
 pK_1 : 6.65; pK_2 : 8.60.

b. Titration of P-15 plus beryllium Table 5 shows representative pH values and calculations made from Curve B, Figure 10. These values were used to obtain the formation curve, Figure 11. The "displaced" formation constant ($-pK_{eq}$) was read from the curve as 3.90 and the logarithm of the K_f as 11.35 at $\bar{n} = 0.50$. The curve levels off at $\bar{n} = 1$, indicating a one to one compound between beryllium and o-carboxybenzeneazoorcinol.

Table 5. Representative titration values and calculations from titration of P-15 plus beryllium using 1.129 N sodium hydroxide

ml. NaOH	pH reading	pH reading corrected (+.40)	\bar{n}	-C	pCh^{-2}
0	1.45	1.85			
1.60	2.42	2.82	0.18	3.50	11.75
1.80	2.65	3.05	0.53	3.96	11.29
1.90	2.82	3.22	0.72	4.30	10.95
2.00	3.05	3.45	0.93	4.76	10.49
2.05	3.22	3.62	1.00	5.10	10.15
2.10	3.92	4.32	1.10	6.50	8.75
2.15	5.05	5.45	1.10	8.76	6.49

c. Discussion The values of the acid dissociation constants and the formation constant with beryllium for o-carboxybenzeneazoorcinol have not been previously reported.

Figure 10. Potentiometric titration of o-carboxybenzeneazo-
orcinol in 75 percent dioxane

A. Compound alone

B. Compound plus beryllium

Sodium hydroxide: 1.129 N

Compound: 0.7367 mM

Beryllium: 0.2206 mM

Initial volume: 100 ml.

μ : 0.019

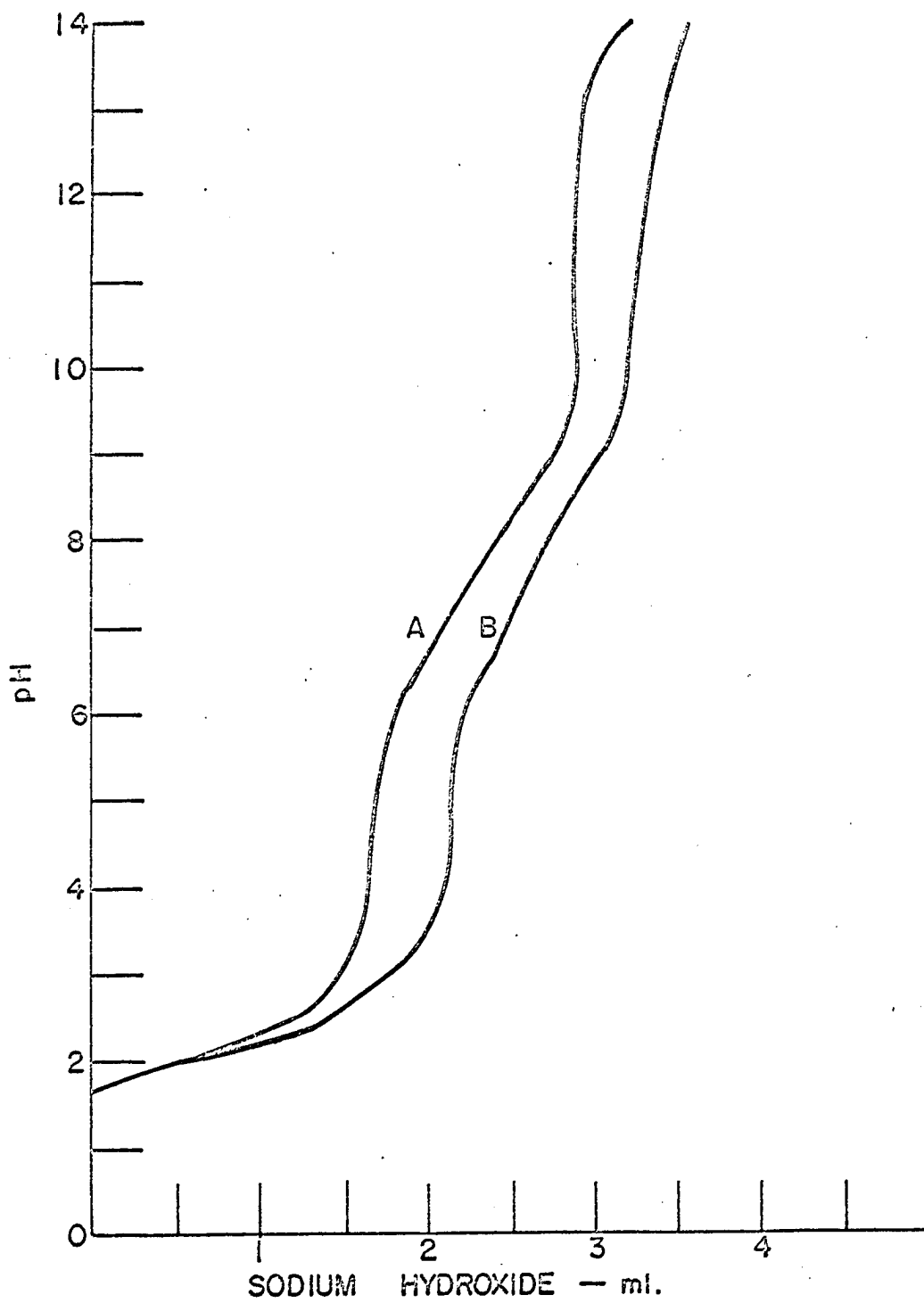
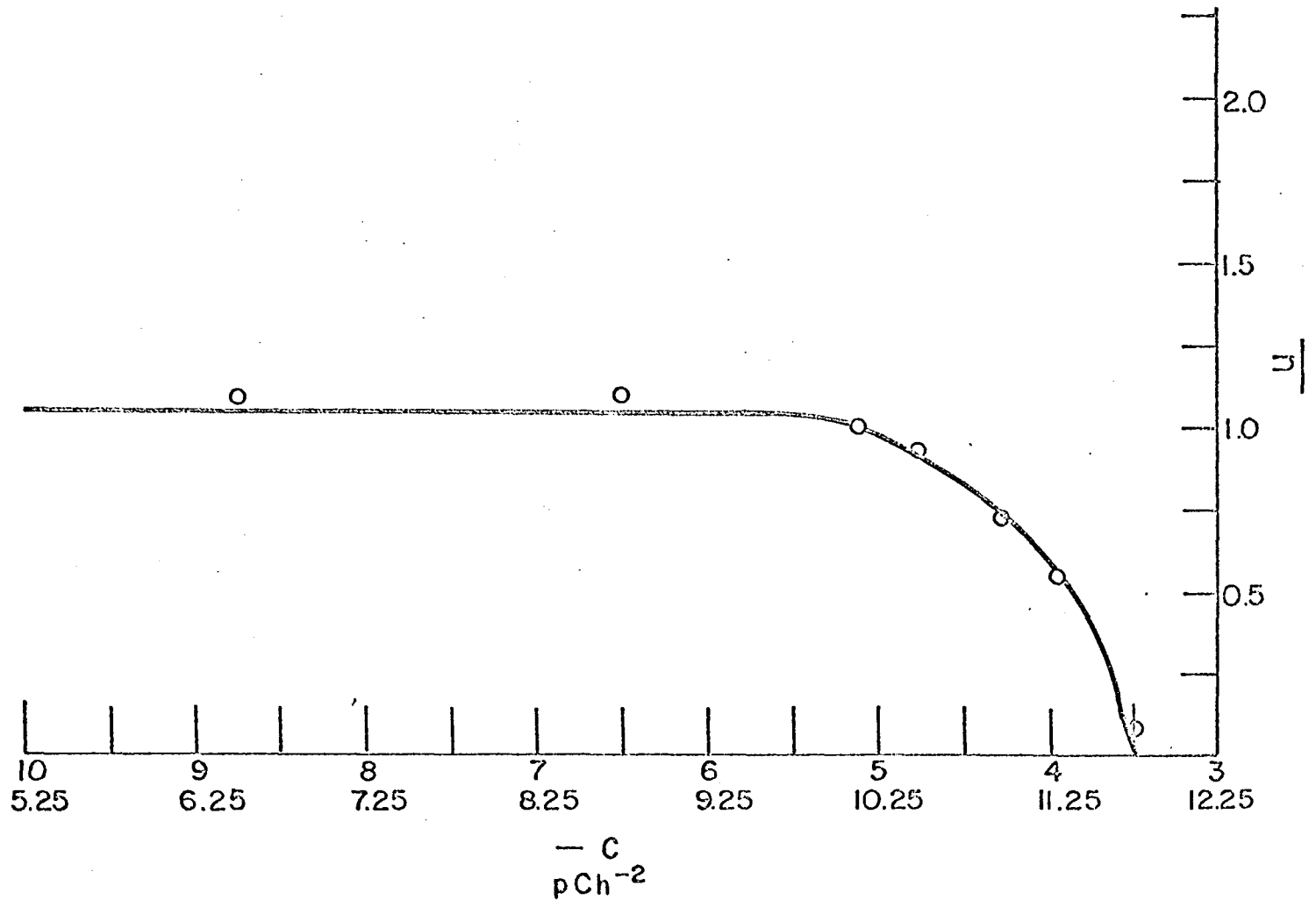


Figure 11. Formation curve for o-carboxybenzeneazoarcinol with beryllium

1 to 1 compound

Logarithm of K_f : 11.35 ($\bar{n} = 0.5$)



3. p-Hydroxybenzeneazoarcinol (P-17) (See Figures 12 and 13)

The titration vessel contained 0.6324 mM of P-17, 1.8875 mM nitric acid, 75 ml. of purified dioxane and water up to a 100 ml. volume for the titration shown on Curve A, Figure 12. For Curve B, 0.2206 mM of beryllium was added to the same constituents before the solution was made up to a final volume of 100 ml. with water.

a. Titration of P-17 From the titration data (Curve A, Figure 12) of P-17 alone, the following calculations were made: neutralization equivalent found: 127; theoretical value: 122; pK_1 : 9.60.

b. Titration of P-17 plus beryllium Table 6 shows representative pH values and calculations made from Curve B, Figure 12. These values were used to obtain the formation curve, Figure 13. The "displaced" formation constant ($-pK_{eq}$) was read from the curve as 3.60 and the logarithm of K_f as 6.00 at $\bar{n} = 1.50$. The curve levels off at $\bar{n} = 2$, indicating a two to one compound is formed between p-hydroxybenzeneazoarcinol and beryllium.

c. Discussion The value of the first acid dissociation constant and the formation constant with beryllium for p-hydroxybenzeneazoarcinol have not previously been reported. The fact that the formation curve levels off at $\bar{n} = 2.0$ and then begins to rise again would indicate the possibility of a mixture of several species.

Figure 12. Potentiometric titration of p-hydroxybenzeneazo-
orcinol in 75 percent dioxane

A. Compound alone

B. Compound plus beryllium

Sodium hydroxide: 1.129 N

Compound: 0.6324 mM

Beryllium: 0.2206 mM

Initial volume: 100 ml.

μ : 0.019

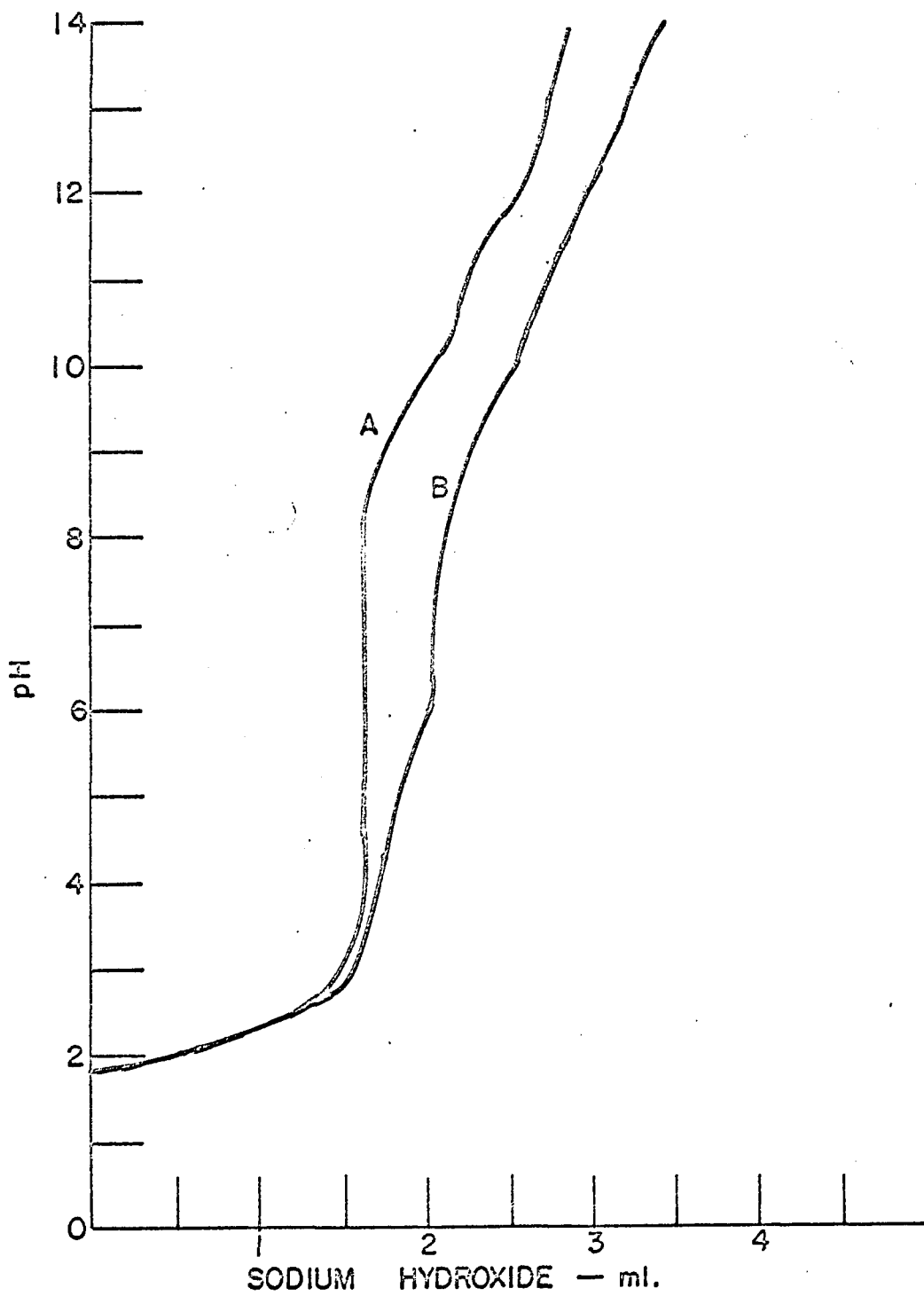


Figure 13. Formation curve for p-hydroxybenzeneazoarcinol
with beryllium

1 to 2 compound

Logarithm of K_f : 6.00 ($\bar{n} = 1.5$)

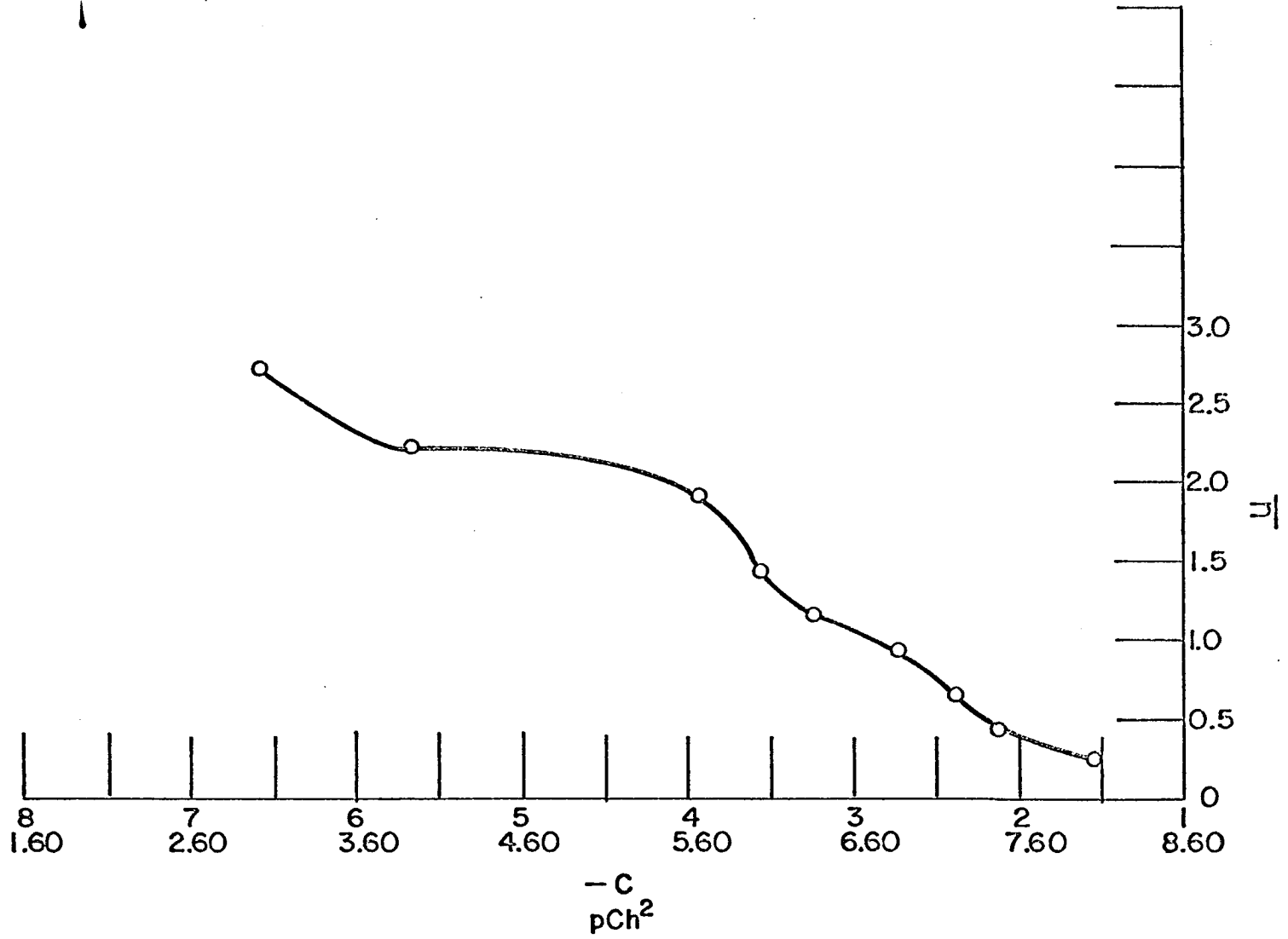


Table 6. Representative titration values and calculations from titration of P-17 plus beryllium using 1.129 N sodium hydroxide

ml. NaOH	pH reading	pH reading corrected (+0.40)	\bar{n}	-C	pCh^{-2}
0	1.48	1.88			
1.70	3.32	3.72	0.23	1.51	8.09
1.75	3.92	4.32	0.42	2.11	7.49
1.80	4.15	4.55	0.66	2.34	7.26
1.85	4.52	4.92	0.91	2.71	6.91
1.90	5.08	5.48	1.17	3.27	6.33
1.95	5.38	5.78	1.43	3.57	6.03
2.00	5.48	5.88	1.68	3.67	5.93
2.05	5.72	6.12	1.90	3.91	5.69
2.10	7.49	7.88	2.20	5.67	3.93
2.15	8.32	8.72	2.70	6.51	3.09

4. Beryllon III (See Figures 14 and 15)

The titration vessel contained 0.7010 mM of Beryllon III, 1.24 ml. of 1.129 N sodium hydroxide (to neutralize the sulfonic acid groups), 1.8875 mM of nitric acid and water up to a 100 ml. volume for the titration shown on Curve A, Figure 14. For Curve B, 0.2206 mM of beryllium was added to the same constituents before the solution was made up to a final volume of 100 ml. with water. In each case the nitric acid was added just before the solution was ready for final dilution.

a. Titration of Beryllon III From the titration data (Curve A, Figure 14) of Beryllon III alone, the following calculations were made: Neutralization equivalent found: 575; theoretical value: 585; pk_3 : 8.85; pk_4 : 11.17.

b. Titration of Beryllon III plus beryllium Table 7

shows representative pH values and calculations made from Curve B, Figure 14. These values were used to obtain the formation curve, Figure 15. The "displaced" formation constant ($-pK_{eq}$) was read from the curve as 7.12 and the logarithm of the K_f as 12.9 at $\bar{n} = 1.50$. The curve levels off at $\bar{n} = 2.0$, indicating a two to one compound between Beryllon III and beryllium.

Table 7. Representative titration values and calculations from titration of Beryllon III plus beryllium using 1.129 N sodium hydroxide

ml. NaOH	pH	\bar{n}	-C	pCh^{-2}
1.75	4.08	0.23	5.99	14.03
1.90	4.22	0.62	6.27	13.75
2.00	4.26	0.86	6.39	13.63
2.10	4.42	1.13	6.65	13.37
2.20	4.58	1.40	6.99	13.03
2.35	5.09	1.70	8.01	12.01
2.40	5.32	1.90	8.47	11.55
2.50	6.02	2.10	9.87	10.15
2.55	6.72	2.20	11.27	8.75
2.60	7.32	2.40	12.47	7.55
2.65	7.82	2.50	13.47	6.55
2.70	8.08	2.50	13.89	6.13

c. Discussion No acid dissociation constants have previously been reported for Beryllon III. In the titration with beryllium added, the acid groups which are neutralized before pH 7 and are not involved in the union with beryllium make interpretation of the titration curve difficult. By adding sufficient base to neutralize the two sulfonic acid

Figure 14. Potentiometric titration of Beryllon III

A. Compound alone

B. Compound plus beryllium

Sodium hydroxide: 1.129 N

Compound: 0.7010 mM

Beryllium: 0.2206 mM

Initial volume: 100 ml.

μ : 0.019

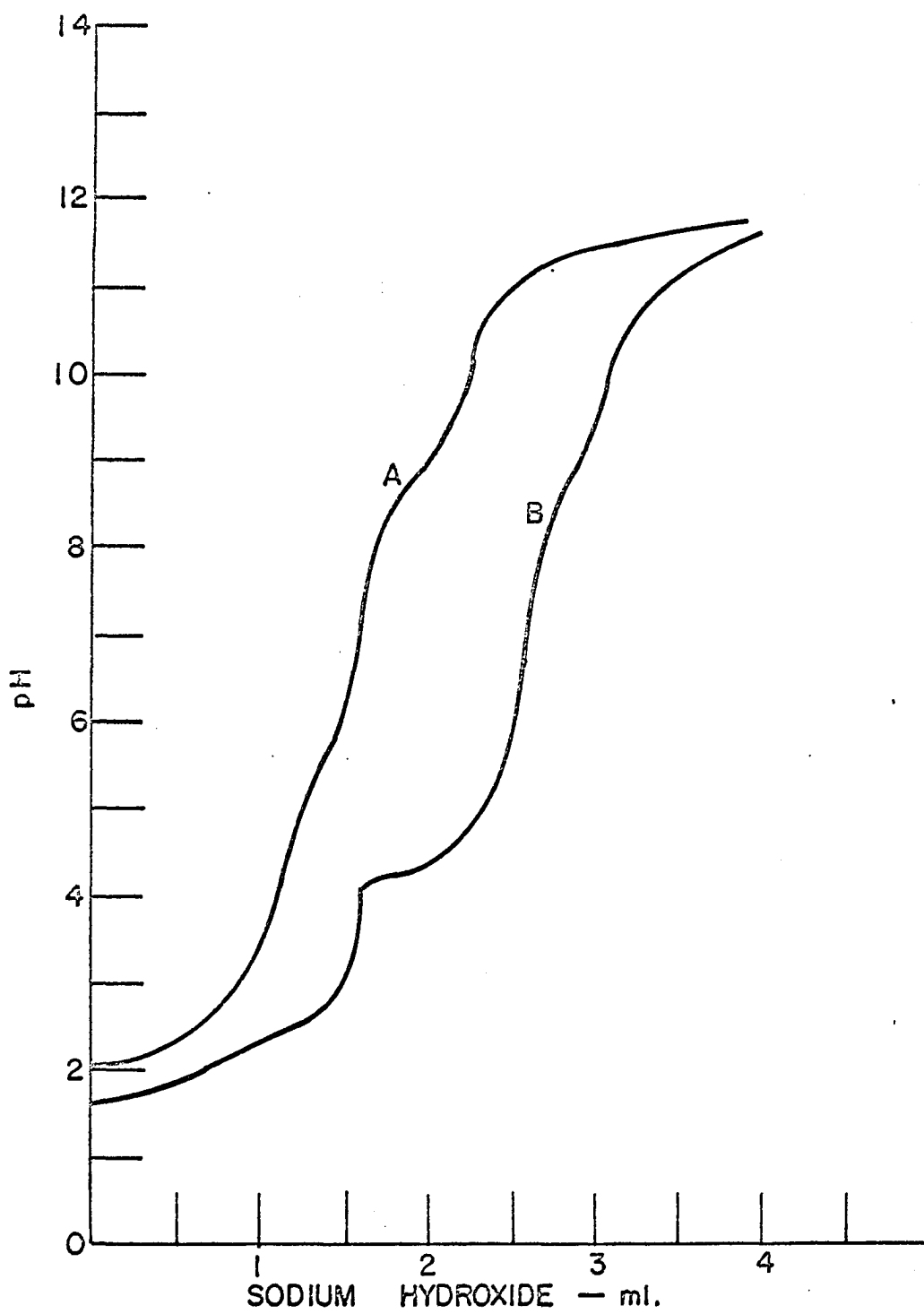
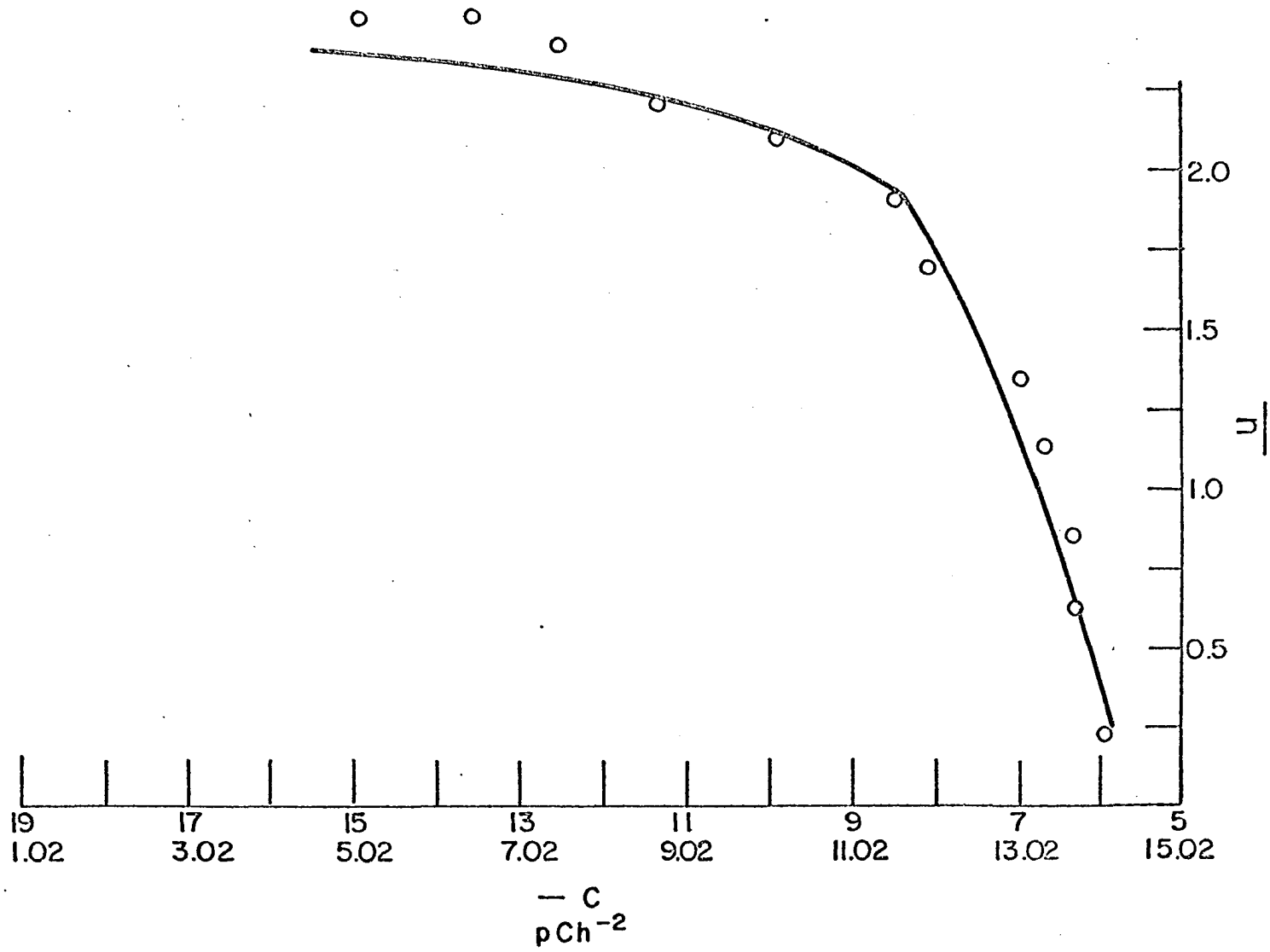


Figure 15. Formation curve for Beryllon III with beryllium
1 to 2 compound
Logarithm of K_f : 12.9 ($\bar{n} = 1.5$)



groups on the Beryllon III before introducing the beryllium solution and making up to the final volume, this problem was resolved. However, Curve B (with beryllium) can not be compared point for point with the curve of the azo compound alone, since nitric acid added to maintain constant ionic strength may convert the azo compound to the acid form when no beryllium is present. The pair of curves are shown together since the titration figures of the azo compound were used to calculate pK values. The later spectrophotometric titration of Beryllon III with beryllium (Section III D) confirmed the two to one compound indicated here.

5. Beryllon IV (See Figures 16 and 17)

The titration vessel contained 0.6763 mM of Beryllon IV, 1.20 ml. of 1.129 N sodium hydroxide (to neutralize the sulfonic acid group and the zwitter ion on the iminodiacetic acid group), 2 mM of potassium chloride and water up to a 100 ml. volume for the titration shown on Curve A, Figure 16. For Curve B, 0.2206 mM of beryllium was added to the same constituents (after introduction of the base).

a. Titration of Beryllon III From the titration data (Curve A, Figure 16) of Beryllon IV alone, the following calculations were made: Neutralization equivalent found: 583; theoretical value: 583; pK_3 : 8.74; pK_4 : 11.27.

b. Titration of Beryllon IV plus beryllium Table 8 shows representative pH values and calculations made from Curve

B, Figure 16. These values were used to obtain the formation curve, Figure 17. The "displaced" formation constant ($-pK_{eq}$) was read from the curve as 8.59 and the logarithm of the K_f as 11.42 at $\bar{n} = 0.50$. The curve levels off at $\bar{n} = 1.0$, indicating a one to one compound between beryllium and Beryllon IV.

Table 8. Representative titration values and calculations from titration of Beryllon IV plus beryllium using 1.129 N sodium hydroxide

ml. NaOH	pH	\bar{n}	-C	pCh ⁻²
0	3.62			
0.65	4.99	0.09	7.81	12.20
0.70	5.08	0.28	7.99	12.02
0.80	5.38	0.50	8.59	11.42
0.90	5.72	0.77	9.27	10.74
1.00	6.38	1.00	10.59	9.42
1.10	7.75	1.09	13.35	6.68

c. Discussion No acid dissociation constants have previously been reported for Beryllon IV. Here, again, as in the case of Beryllon III, the acid groups which are neutralized before pH 7 and are not involved in the union with beryllium make interpretation of the titration curve difficult. These groups were neutralized with an equivalent amount of base before introducing the beryllium. However, this time potassium chloride was used to maintain a constant ionic strength ($\mu = 0.02$) instead of nitric acid. In this case the buffer zone indicating compound formation was not well defined, suggesting that compound formation takes place more readily when the

Figure 16. Potentiometric titration of Beryllon IV

A. Compound alone

B. Compound plus beryllium

Sodium hydroxide: 1.129 N

Compound: 0.6763 mM

Beryllium: 0.2206 mM

Initial volume: 100 ml.

μ : 0.02

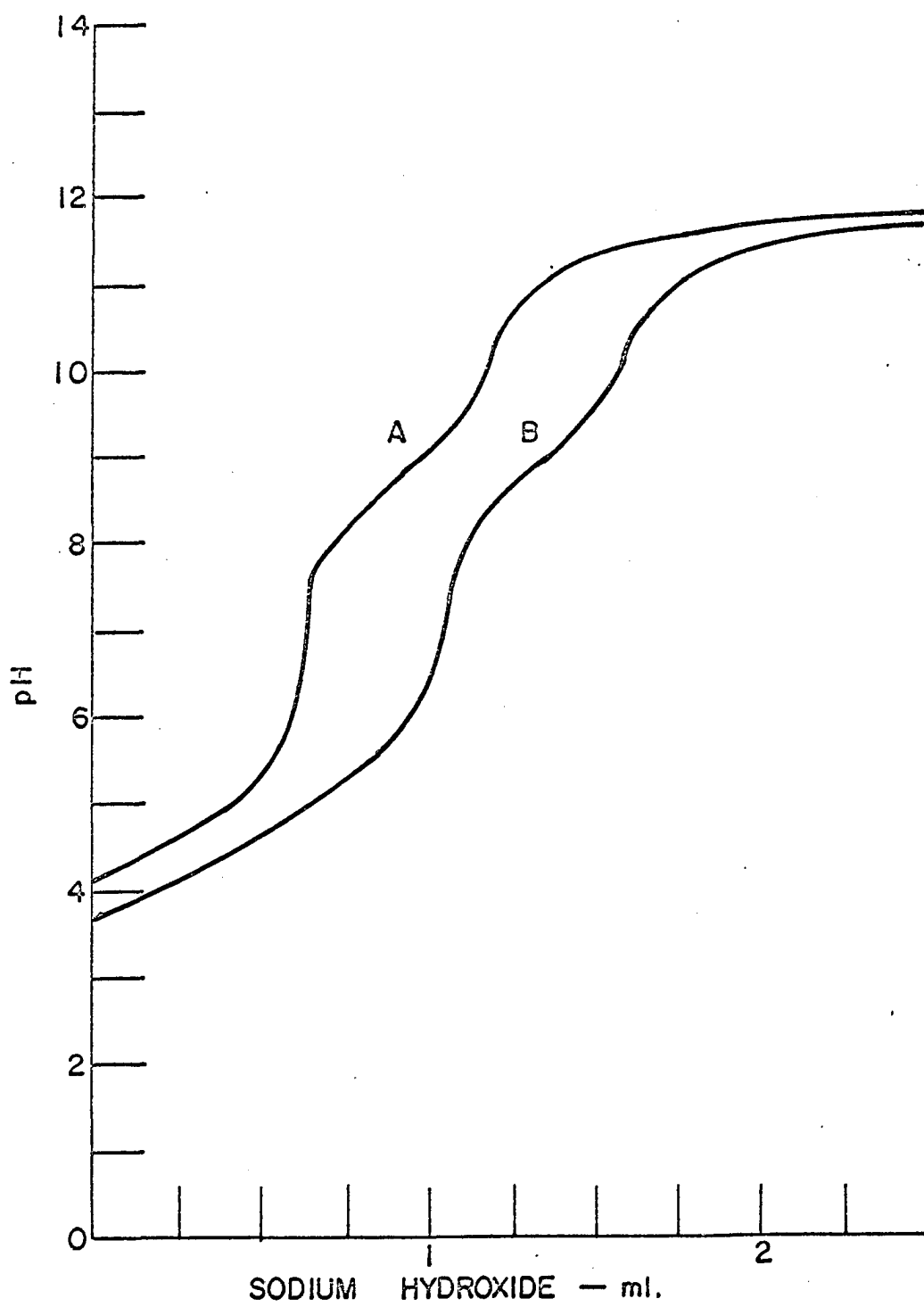
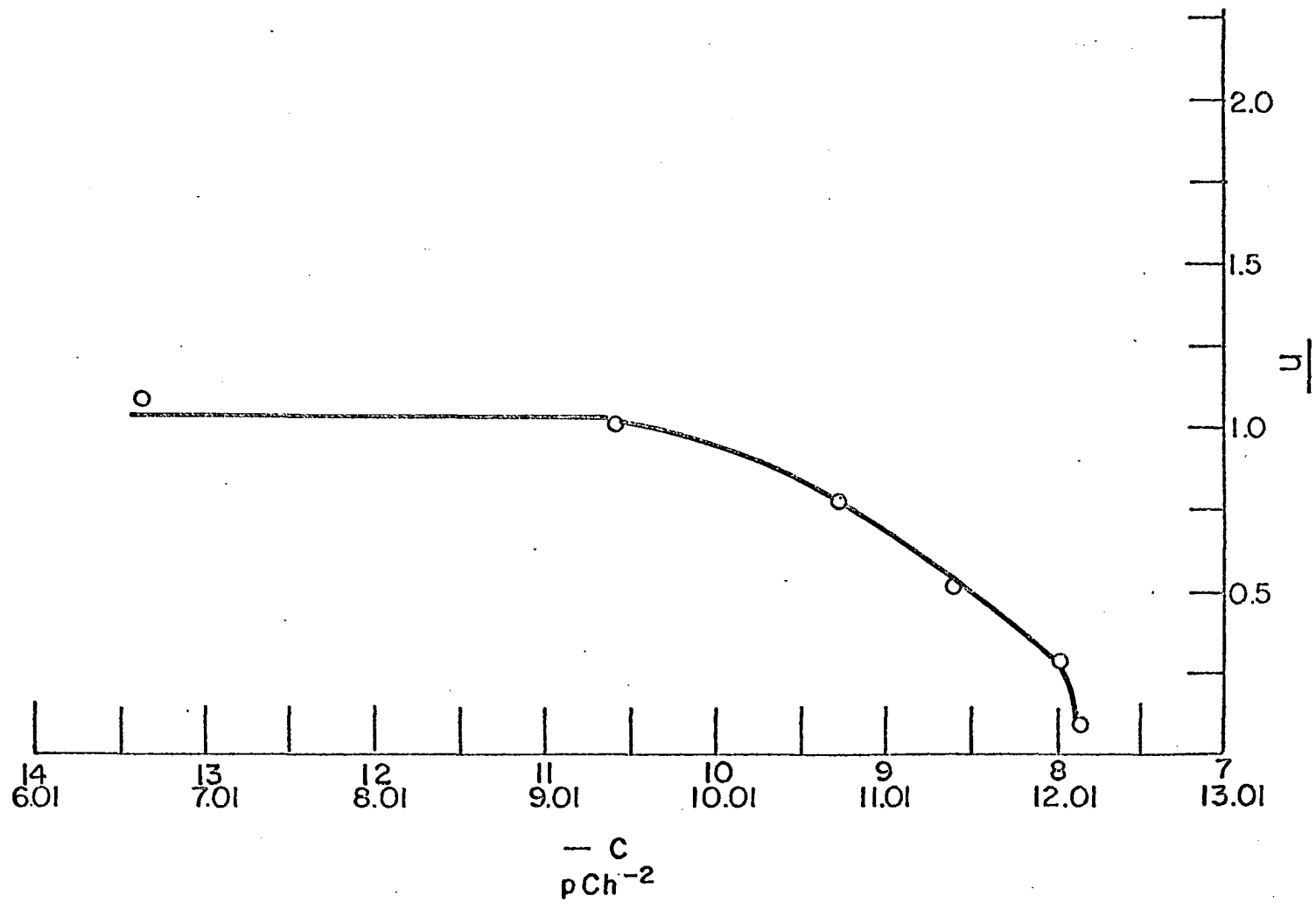


Figure 17. Formation curve for Beryllon IV with beryllium
1 to 1 compound
Logarithm of K_f : 11.42 ($\bar{n} = 0.5$)



titration is begun at a lower pH.

The addition of base also made possible the titration of Beryllon IV in water medium, since the free acid is insoluble in water.

D. The Use of Beryllon III and Beryllon IV
for the Determination of Beryllium

Purified preparations of Beryllon III and Beryllon IV having been made, as described in the preceding Section A, and with the knowledge of the absorption spectra of these reagents and their beryllium derivatives, it became possible to examine in detail the various factors involved in using these reagents for the spectrophotometric determination of beryllium. Of the various factors, pH, the effects of interfering elements, conformity of Beer's law, and the molar extinction coefficients of the beryllium derivatives are particularly important.

In the following work, all spectrophotometric measurements were made with a Beckman Model DU spectrophotometer, with the exception of the spectrophotometric titrations which were made on a Beckman Model B spectrophotometer which had been modified with a dark box containing a fitting for a 150 ml. "tall" beaker, a water driven stirrer, and a small opening for a 10 ml. buret. The entire assembly was covered with a black cloth to exclude light. Spectrophotometric titrations were performed slowly, at least one hour being taken.

1. Effect of pH on absorbance

In Figures 18 and 19 are shown the effect of pH on the absorbance of the beryllium derivatives of Beryllon III and Beryllon IV. Clark and Lubs buffers were used to adjust the pH. The pH range covered was 3.0 to 10.0. The absorbance was measured after the solutions had been allowed to stand for ten minutes. Each solution contained 5 ml. of 0.02 percent azo compound, 2.0 ml. of 1.1×10^{-4} M beryllium and 18.0 ml. of the given buffer. The pH was checked immediately after the measurement of the absorbance. A blank for each contained 2.0 ml. of water instead of beryllium solution.

2. Effect of Interferences

The effect of the addition of calcium and magnesium salts and potassium chloride on the absorbance (measured against a blank) is tabulated in Table 9. Each solution contained 5 ml. of 0.02 percent azo compound, 1 ml. of 25 percent urotroline (hexamethylenetetramine) and 2 ml. of 1.1×10^{-4} M beryllium (1.98 μ g.) and was made up to a 25 ml. volume after the addition of an amount of solid salt equivalent to the specified quantity of calcium, magnesium, or potassium chloride.

The difference in absorbance of solution and blank is indicated and the percent relative error recorded.

3. Conformity to Beer's law and molar extinction coefficients

In Figure 20 is shown a Beer's law plot of the beryllium derivative of Beryllon III. The solutions measured were pre-

Figure 18. pH effect on absorbance of beryllium derivative of Beryllon III

Composition of solutions: 5 ml. of 0.02% Beryllon III, 2.0 ml. of 1.1×10^{-4} M beryllium, made up to 25 ml. with given buffer. Absorbance measured at 525 m μ on Beckman DU spectrophotometer using a blank solution containing no beryllium in reference cell.

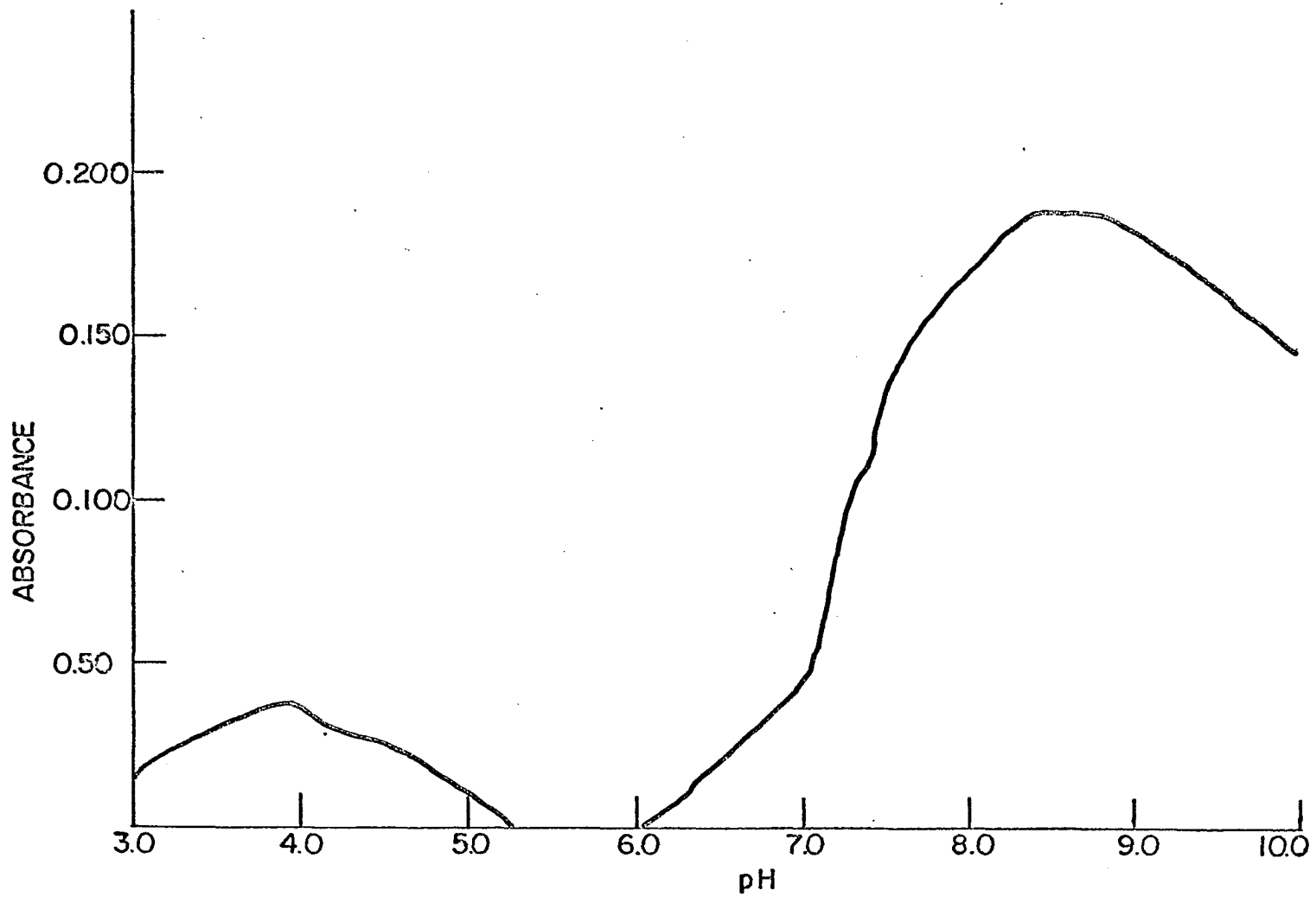


Figure 19. pH effect of absorbance of beryllium derivative
of Beryllon IV

Composition of solutions: 5 ml. of 0.02% Beryllon IV,
2.0 ml. of 1.1×10^{-4} M beryllium, made up to 25 ml.
with given buffer. Absorbance measured at 528 $m\mu$ on
Beckman DU spectrophotometer using a blank solution
containing no beryllium in reference cell.

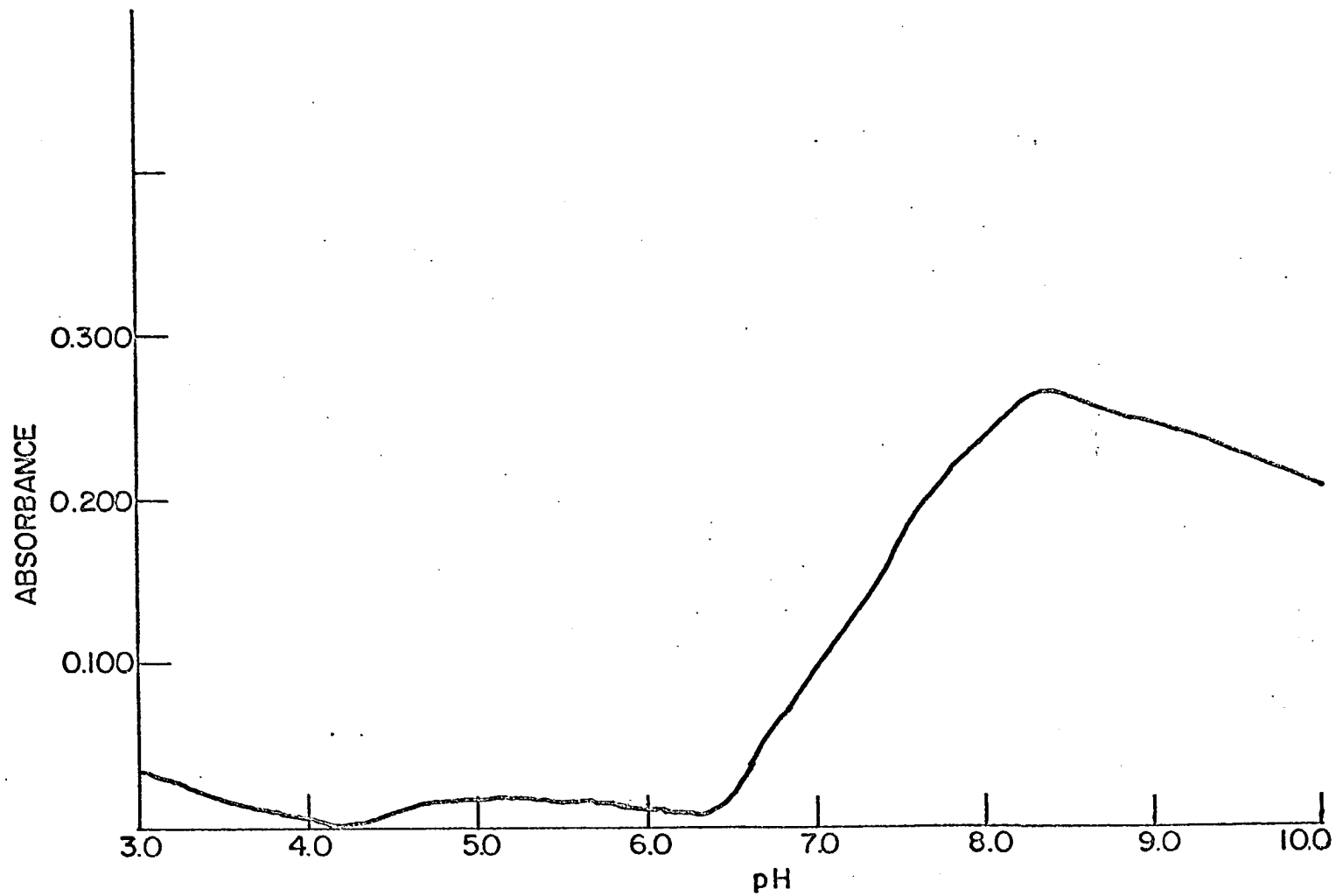
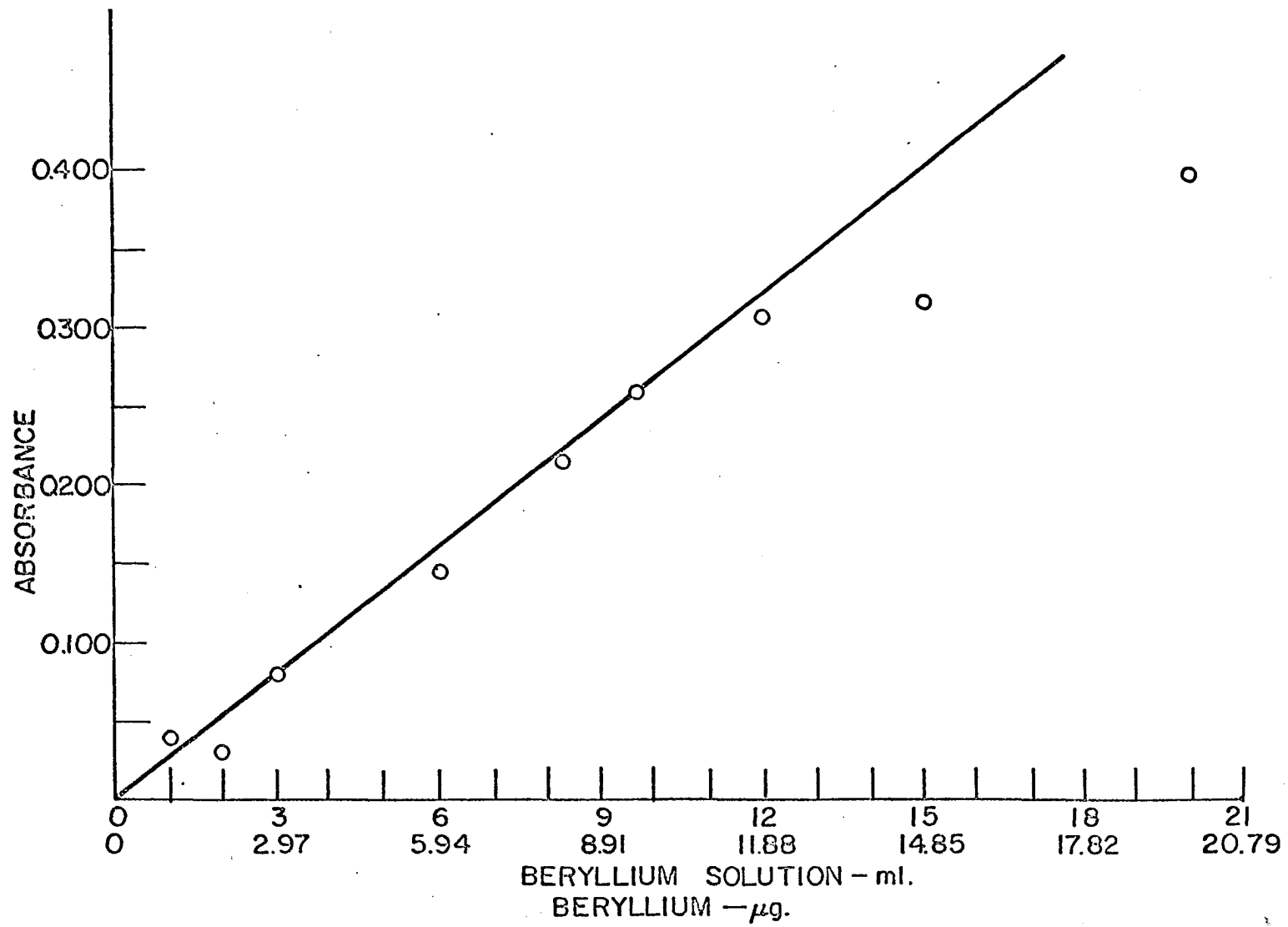


Table 9. Effect of addition of calcium and magnesium salts, and potassium chloride on absorbance of azo compounds at pH 8 in presence of 1.98 ug of beryllium

	No addition	Ca added 1 mg.	Relative error %	Mg added 1 mg.	Relative error %	KCl added 18.5 mg.	Relative error %
Beryllon III	0.255	0.200	-22	0.222	-13	0.195	-24
Beryllon IV	0.244	0.405	+66	0.333	+36	0.249	+ 2

Figure 20. Beer's law plot for Beryllon III plus beryllium at pH 8

Composition of solutions: 5 ml. of 25% urotropine, 20 ml. of 0.02% Beryllon III, 0 to 19.8 μ g of beryllium in final volume of 100 ml. Absorbance measured at 525 $m\mu$ on Beckman DU spectrophotometer.



pared by mixing 20 ml. of a 0.02 percent solution of the azo compound with 5 ml. of 25 percent urotropine to bring the pH to 8.0 and amounts of 1.1×10^{-4} M beryllium solution varying from 0 to 19.8 $\mu\text{g.}$ of beryllium in a 100-ml. volume. A straight line was obtained for points from 0 to 12 $\mu\text{g.}$ of beryllium per 100 ml. The color developed (without heating) in 10 minutes and was stable beyond 24 hours.

A series of points substituted in the expression $A = abc$ yielded an average molar extinction coefficient value (a) at 525 $m\mu$ of 23,200 ($b = 1 \text{ cm.}$).

Figure 21 shows a similar plot for Beryllon IV using 20 ml. of 0.02 percent azo compound, 5 ml. of 25 percent urotropine (pH 7.3) and amounts of 1.1×10^{-4} M beryllium varying from 0 to 19.8 $\mu\text{g.}$ per 100 ml. Here Beer's law held over the entire range. The color developed in 10 minutes and was stable up to 96 hours.

Individual points substituted in the expression $A = abc$ gave an average molar extinction coefficient (a) of 29,900 for Beryllon IV at 528 $m\mu$ ($b = 1 \text{ cm.}$).

4. Combining ratios from spectrophotometric titrations

A spectrophotometric titration (at 525 $m\mu$) of 1.4×10^{-6} moles of Beryllon III (in 100 ml. volume) with 1.1×10^{-4} M beryllium is shown in Figure 22. The extrapolated end point at 6.49 ml. of beryllium corresponds to 0.701×10^{-6} moles of beryllium and is evidence of a 2 to 1 compound. The solution

Figure 21. Beer's law plot for Beryllon IV plus beryllium at pH 7.3

Composition of solutions: 5 ml. of 25% urotropine, 20 ml. of 0.02% Beryllon IV, 0 to 19.8 μ g. of beryllium in a final volume of 100 ml. Absorbance measured at 528 $m\mu$ using a Beckman DU spectrophotometer.

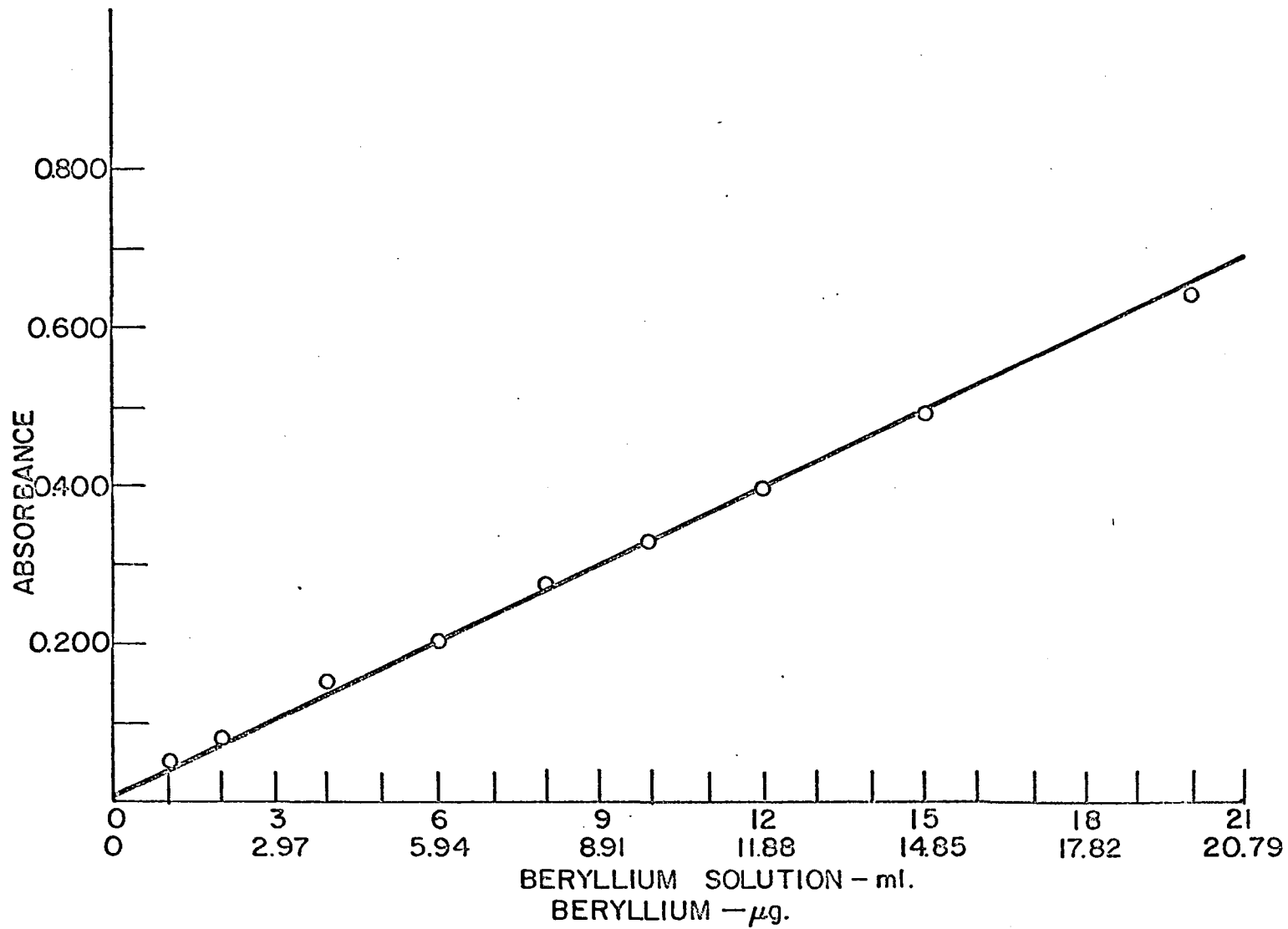
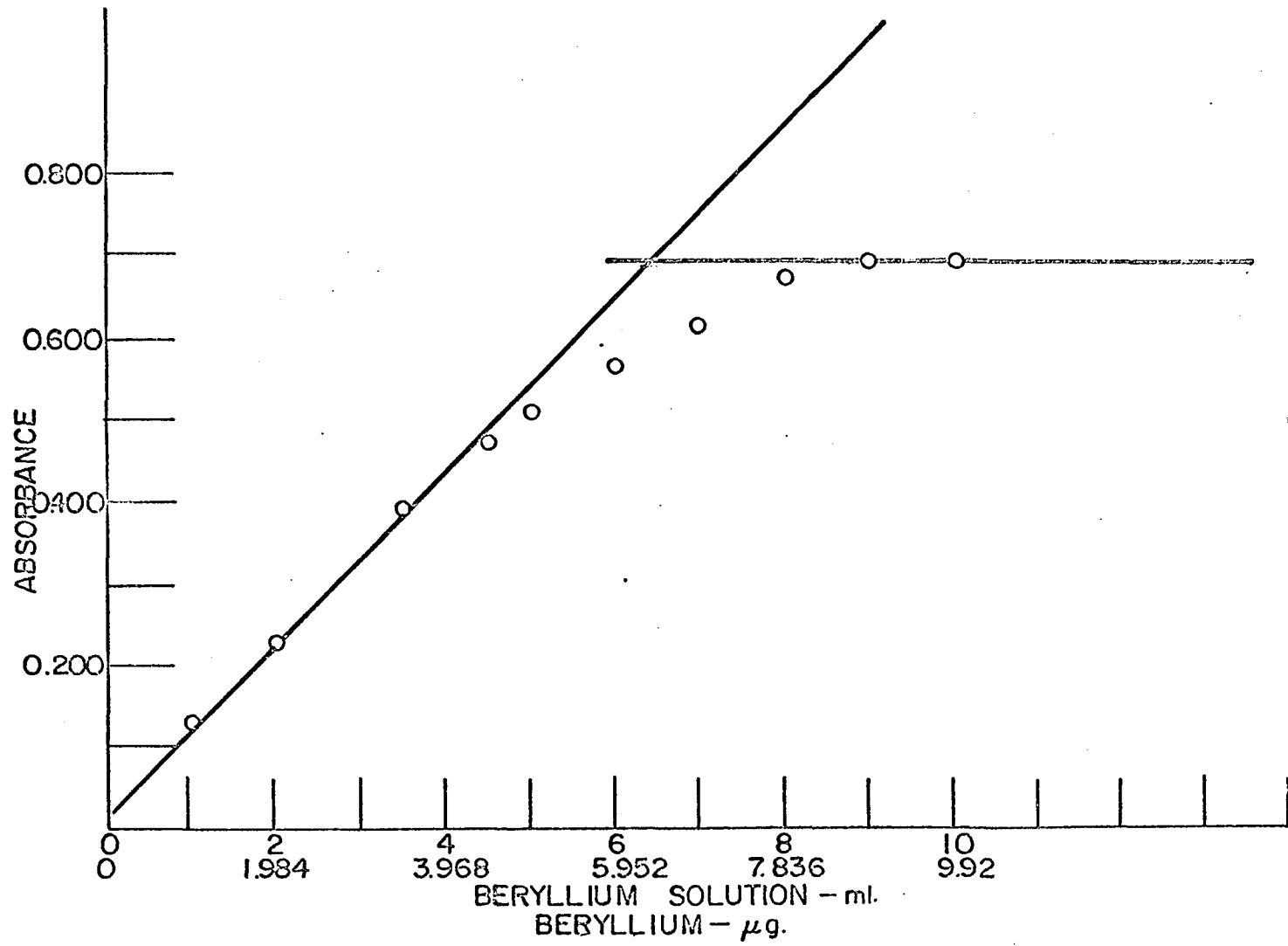


Figure 22. Spectrophotometric titration of Beryllon III with beryllium at pH 7.2

Composition of solution: 1.4×10^{-6} moles of Beryllon III, buffered with 20 ml. of 25% urotropine, ionic strength (μ) = 0.1 in KCl, in initial volume of 100 ml.

Point of intersection corresponds to 0.701×10^{-6} moles of beryllium (6.5 ml. of 1.1×10^{-4} M solution) reacting with 1.4×10^{-6} moles of Beryllon III, a ratio of 1.0 to 2.0.



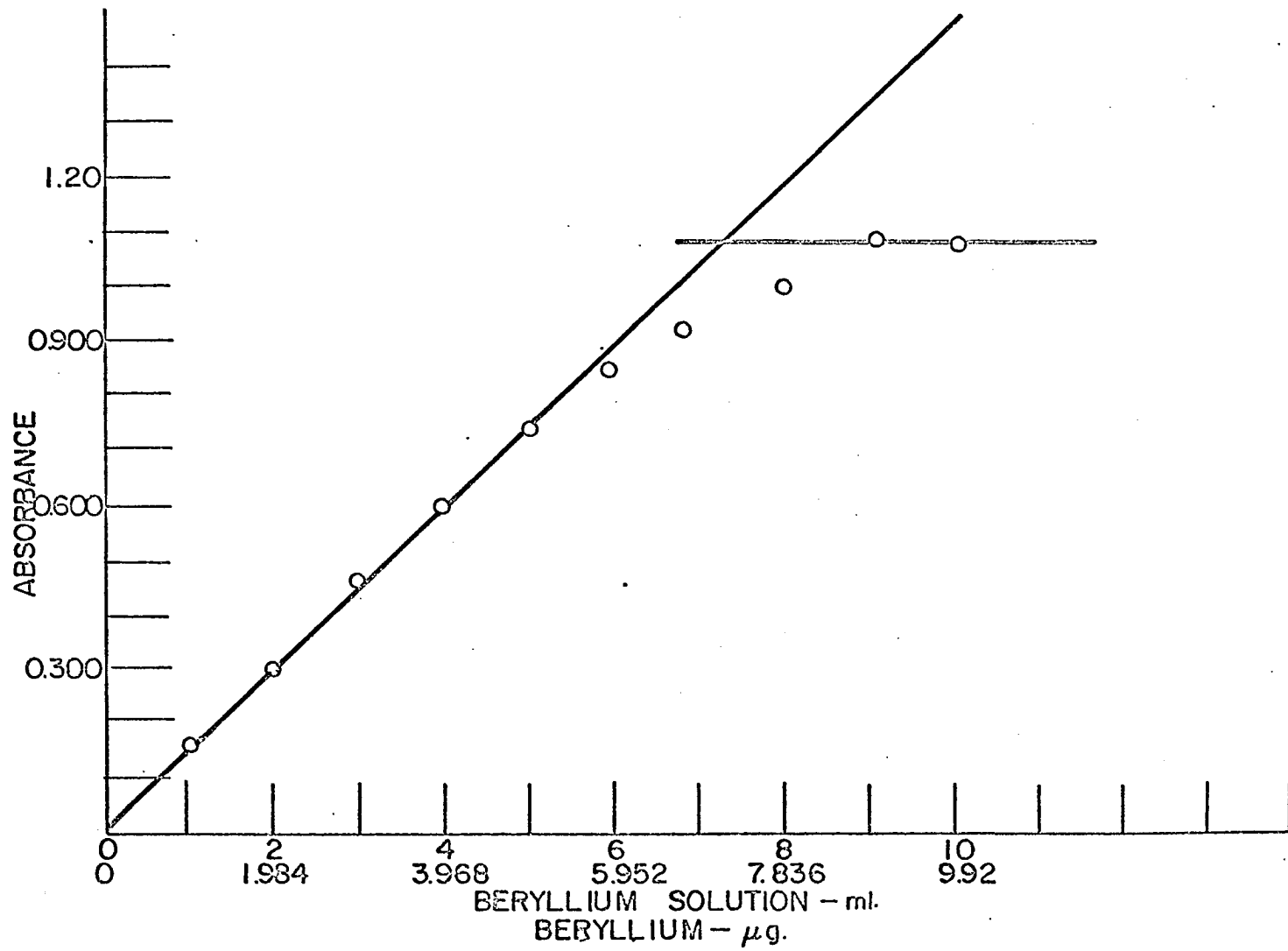
was buffered to pH 7.2 with 20 ml. of 25 percent urotropine and was 0.1 M in KCl.

Figure 23 shows a similar titration (at 528 m μ) of 0.908×10^{-6} moles of Beryllon IV (in 100 ml. volume). Using 1.1×10^{-4} M beryllium solution, a ratio of 1 mole of azo compound to 0.91 moles of beryllium is indicated. The solution was buffered to pH 7.2 with 20 ml. of 25 percent urotropine and was 0.1 M in KCl. Here a slight residual fluorescence of the azo compound which was also noted in the beryllium derivative may have affected the absorbance readings.

Figure 23. Spectrophotometric titration of Beryllon IV with beryllium at pH 7.2

Composition of solution: 0.908×10^{-6} moles of Beryllon IV, buffered with 20ml. of 25% urotropine, ionic strength (μ) = 0.1 in KCl, in initial volume of 100 ml.

Point of intersection corresponds to 0.825×10^{-6} moles of beryllium (7.5 ml. of 1.1×10^{-4} M solution) reacting with 0.908×10^{-6} moles of Beryllon IV, a ratio of 0.91 to 1.0.



IV. RESULTS AND CONCLUSIONS

Purification of the five hydroxyazo compounds synthesized for this study proved time consuming and difficult. Particular attention was paid to the purity of the intermediates as an aid in assuring a final product of high purity. For each compound the composition of the final product was confirmed by elemental analysis or azo group analysis (titanous chloride method) or both. A discussion of the purification procedure is given in Section III A for each compound. Of particular note is the use made of a cation exchange resin for the separation of Beryllon III from the considerable amount of sodium chloride with which it is contaminated when first isolated and of the necessity of making the final precipitation of Beryllon IV from concentrated hydrochloric acid to free it of sodium.

In the potentiometric titration used to measure the acid dissociation constants and formation constants, a dioxane-water mixture was used as solvent for those compounds not soluble in water. Commercial, "stabilized" dioxane proved unsatisfactory and careful purification of the dioxane proved essential.

The values obtained for the acid dissociation constants are given in Table 10 and for the combining ratios and formation constants toward beryllium in Table 11.

The value obtained for the first acid dissociation constant of 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5) differs only slightly from the value obtained earlier by

Table 10. Acid dissociation constants of the hydroxyazo compounds studied

Compound	pk ₁	pk ₂	pk ₃	pk ₄
1-(2'-Carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5)	6.90 ^a	-- ^b		
o-Carboxybenzeneazordinol (P-15)	6.65	8.60		
p-Hydroxybenzeneazorcinol (P-17)	9.60			
1'-(2'-Hydroxy-4'-diethylamino-1-benzeneazo)-3,6-disulfo-8-hydroxynaphthalene (Beryllon III)	-- ^c	-- ^c	8.85	11.17
1-(1'-Hydroxy-3'-disulfo-6'-iminodiacetic-2'-naphthylazo)benzene-2-arsonic acid (Beryllon IV)	-- ^d	-- ^d	-- ^d	8.74
				pk ₅
				11.27

^aReported by Snavely (46): 6.83.

^bReported by Ellingboe (17): 11.4.

^cFirst and second acid dissociation constants not measured.

^dFirst, second and third acid dissociation constants not measured.

Table 11. Combining ratio and formation constant with beryllium of the azo compounds studied

Compound	Combining ratio	Log formation constant K_f
1-(2'-Carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5)	1:1	14.20
o-Carboxybenzeneazoorcinol (P-15)	1:1	11.35
p-Hydroxybenzeneazoorcinol (P-17)	2:1	6.00
1'-(2'-Hydroxy-4'-diethylamino-1-benzeneazo)-3,6-disulfo-8-hydroxynaphthalene (Beryllon III)	2:1	12.90
1-(1'-Hydroxy-3'-disulfo-6'iminodiacetic-2'-naphthylazo)benzene-2-arsonic acid (Beryllon IV)	1:1	11.42

Snavely. Values for the other azo compounds have not been previously reported. Only the acid dissociation constants were measured of those acid groups which are involved in the formation of the beryllium derivatives, although some preliminary values for the first, large acid dissociation constants can be obtained from the titrations with sodium hydroxide made during synthesis to establish purity.

Thus with Beryllon III, the first two acid dissociation constants, judging from the mid-points of the titration curve of Figure 1, are 3.0 and 4.8. One of these is undoubtedly the ionization of one of the sulfonic acid groups, the other the ammonium ion produced by the neutralization of the diethylamino group by the other sulfonic acid group (formation of a zwitter ion). Similar values were obtained by Blair and Diehl (60) for the acid dissociation constants of 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid.

The titration of Beryllon IV, Figure 2, indicates three replaceable hydrogen atoms to be present, all fairly strong acids, for the curve shows only one break, at pH 7.0. Of the three acid groups titrated, the first is undoubtedly that from the sulfonic acid group and the remaining two, one each, from the arsonic acid group and the iminodiacetic acid group, the latter being present as a zwitter ion and presenting only one moderately strong replaceable hydrogen atom.

An arsonic acid group ortho to an azo group is present in

the compound Thorin (2-(2-hydroxy-3,6-disulfo-1-naphthylazo) benzene arsonic acid) and the acid dissociation constants of this material have been measured (6i); the constants for the two replaceable hydrogen atoms of this arsonic acid are $pK_3 = 3.70$ and $pK_4 = 8.33$. By analogy, one replaceable hydrogen atom of the arsonic acid group of Beryllon IV should be titrated below pH 7. The acid dissociation constant of carboxylic acid groups is increased by the positive charge on a neighboring zwitter ion ammonium nitrogen atom, the acid dissociation constant of iminodiacetic acid being $pK_1 = 2.98$. The first replaceable hydrogen atom of the arsonic acid of Beryllon IV and that of the iminodiacetic acid are thus expected to dissociate to about the same extent and thus also at pH values in agreement with the titration curve found (Figure 2).

The acid dissociation constants reported in Table 10 were those obtained by titration with alkali, in water-dioxane mixtures as solvent when necessary, and the treatment of the data given in Section II B.

In the determination of combining ratios and formation constants by potentiometric titration with alkali, a phenomenon peculiar to beryllium was observed. The pH of the beryllium solution must be brought to about 2 to render the beryllium available for compound formation with the azo compound. It is believed that stable hydroxy compounds of beryllium are

formed on standing at higher pH values and that such beryllium species do not react readily with the azo compounds.

In the titrations of Beryllon III and Beryllon IV the strongly acid groups, the sulfonic, arsonic and iminodiacetic acid groups, were neutralized before the addition of beryllium.

Unexpectedly, two of the five azo compounds studied formed two to one compounds with beryllium rather than the one to one compounds always observed previously. The fact that a two to one compound is formed between p-hydroxybenzeneazoocinol (P-17) and beryllium might be explained by the absence of a second ortho group, leaving space about the beryllium atom for the second molecule to enter. This argument obviously fails with Beryllon III, however, for in this compound hydroxyl groups are present on both sides of the azo group.

The combining ratios of Beryllon III and of Beryllon IV with beryllium were further confirmed by spectrophotometric titrations of the azo compounds with a solution of beryllium, Figures 22 and 23. The ratios found were 2.0 to 1.0 for Beryllon III and 0.91 to 1.0 for Beryllon IV; a slight fluorescence was observed in the beryllium-Beryllon IV solutions which may explain why the ratio found was not 1.0.

Of the five azo compounds, the formation constants toward beryllium of four are sufficiently great to make the compounds of interest as reagents for beryllium.

The absorption spectra of the five azo compounds and their

beryllium derivatives indicated that o-carboxybenzeneazoorcinol (P-15), Beryllon III and Beryllon IV have the most promise as analytical reagents for beryllium. No maximum occurs in the visible absorption spectrum of 1-(2'-carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5) with addition of beryllium until an excess of the latter has been added. There is no change in the wavelength of maximum absorption of p-hydroxybenzeneazoorcinol (P-17) on the addition of beryllium but an increase in absorbance does occur. The shift in the wavelength of maximum absorption occurring when beryllium is added to o-carboxybenzeneazoorcinol cannot be detected by eye, but the shifts with both Beryllon III and Beryllon IV result in visible color changes from orange to raspberry.

The study of the variation with pH of the absorbance of the beryllium-Beryllon III and beryllium-Beryllon IV systems showed a quite sharp dependence on pH, the maximum absorbance being at 8.5 and 8.3 respectively. The maximum in absorbance in both cases is just sufficiently broad that satisfactory adjustment of pH can be made without difficulty, using suitable buffers. The findings here are in sharp contrast to the report of Kuznetnov that maximum absorbance was obtained with either reagent over the pH range 5.8 to 8.3. The present work was done with Clark and Lubs buffers, the Russian work with hexamethylenetetramine. It is possible that the phosphate or borate in the Clark and Lubs buffers may be inhibiting the

color reaction in some way but a more likely explanation of the discrepancy is that the highly purified azo compounds used in this study gave a cleaner result and perhaps that the precision in the adjustment of the pH with the Clark and Lubs buffers was better.

The beryllium-Beryllon III system conforms to Beer's law over the range 0 to 12 μ g. of beryllium per 100 ml.; for beryllium-Beryllon IV, the range is 0 to 19.8 μ g. of beryllium per 100 ml. The molar extinction coefficients at the wavelength of maximum absorption are 23,200 (at 525 m μ) and 29,900 (at 528 m μ), respectively. The value for Beryllon III is somewhat higher than the value 19,200 recently reported by Pakalns and Flynn (62), this value apparently having been made on unpurified material.

It was found that both calcium and magnesium interfere in the spectrophotometric determination of beryllium using Beryllon III and Beryllon IV. Fortunately the interference of both can be obviated by the addition of ethylenediamine-tetraacetate. Potassium chloride also interferes in the reaction of beryllium with Beryllon III.

Although its synthesis is somewhat more complex than that of Beryllon III, Beryllon IV has the advantage of being less difficult to purify. Its larger molar extinction coefficient and its wider range of adherence to Beer's law would appear to give Beryllon IV a distinct advantage over Beryllon III as a spectrophotometric reagent for beryllium.

V. SUMMARY

Five hydroxyazo compounds have been prepared, purified and studied as possible spectrophotometric reagents for beryllium:

1-(2'-Carboxybenzeneazo)-2-hydroxy-5-methylbenzene
(designated B-5)

o-Carboxybenzeneazoorcinol (designated P-15)

o-Hydroxybenzeneazoorcinol (designated P-17)

1'-(2'-Hydroxy-4'-diethylamino-1'-benzeneazo)-3,6-disulfo-8-hydroxynaphthalene (designated Beryllon III)

1-(1'-Hydroxy-3'-disulfo-6'-iminodiacetic-2'-naphthylazo)benzene-2-arsonic acid (designated Beryllon IV).

For each of the compounds, the acid dissociation constants and formation constant of the beryllium derivative have been measured by potentiometric titration with alkali using a water-dioxane mixture as solvent. Absorption spectra of the azo compounds and the beryllium derivatives have been obtained. For Beryllon III and Beryllon IV, sufficient information relative to molar extinction coefficients, effect of pH on absorbance, and the interfering effects of calcium and magnesium has been obtained to evaluate these two azo compounds as spectrophotometric reagents for beryllium.

1-(2'-Carboxybenzeneazo)-2-hydroxy-5-methylbenzene (B-5) was prepared from purified starting materials. Thorough washing with 1 to 1 hydrochloric acid was required to rid the purified compound of all traces of the sodium salt. Elemental analysis and titration with titanous chloride confirmed its

purity. Only the acid dissociation constant of the carboxylic acid group could be obtained by titration with alkali: $pK_1 = 6.90$ (6.83 reported by Snavely). The acid dissociation constant of the phenolic group, $pK_2 = 11.4$, had been obtained by Ellingboe using a spectrophotometric procedure.

B-5, previously studied by Ellingboe for its reaction with calcium and magnesium, was shown by Blair not to react with beryllium at pH 10, where the calcium and magnesium derivatives are formed. However, Snavely reported a one to one compound between B-5 and beryllium occurring near pH 3 and gave the value of the "displaced" formation constant as $10^{-3.88}$. The "displaced" formation constant was confirmed by this work and also reported as a formation constant, the logarithm of the constant being 14.2.

o-Carboxybenzeneazoarcinol (P-15), prepared from purified reagents was shown to be of sufficient purity by titration with titanous chloride. The dissociation constants of the carboxylic acid group and the phenolic group, ortho to the azo bridge, were found by titration to be respectively: $pK_1, 6.65$ and $pK_2, 8.60$. P-15 forms a one to one compound with beryllium and the logarithm of the formation constant is 11.35.

p-Hydroxybenzeneazoarcinol (P-17) was synthesized from purified starting materials and its purity confirmed by titration with titanous chloride. The acid dissociation constant of the phenolic group (pK_1), ortho to the azo bridge, was

determined by titration to be 9.60. The values for the second and third phenolic groups were too high to determine by titration. This azo compound formed a two to one compound with beryllium, the logarithm of the formation constant being 6.00.

The Russian reagent for beryllium, Beryllon III, (1-(2'-hydroxy-4'-diethylamino-1'-benzeneazo)-3,6-disulfo-8-hydroxynaphthalene) was prepared; particular attention was paid to the purification of the intermediates used in the synthesis and a new method of isolation and purification of the product was devised. The free acid was obtained by passing the material through a strong acid, cation exchange resin. The composition of the material as a monohydrate was established by the determination of the neutralization equivalent, the titanous chloride reduction equivalent, and by elemental analysis. By titration it was found that Beryllon III acts as a quadribasic acid, the replaceable hydrogen atoms being respectively strong, moderately strong, weak, and very weak; the strong acid is undoubtedly one of the sulfonic acid groups present and the moderately strong acid, the (zwitter) ammonium ion associated with the second sulfonic acid group. The two phenolic groups are neutralized at higher pH, the acid dissociation constants having the values $pK_3 = 8.85$ and $pK_4 = 11.17$. Beryllon III unites with beryllium in the ratio of two molecules of the azo compound to one atom of beryllium as determined by the potentiometric method and by the spectrophotometric method. The

logarithm of the formation constant is 12.9. Light absorption of the beryllium-Beryllon III system reaches a maximum at pH 8.5. The system conforms to Beer's law over the range 0 to 12 μ g. of beryllium per 100 ml., the molar extinction coefficient being 23,200 at 525 m μ , the wave length of maximum absorption.

A second Russian reagent for beryllium, Beryllon IV, (1-(1'-hydroxy-3'-sulfo-6'-iminodiacetic-2'-naphthylazo) benzene-2-arsonic acid) was synthesized. Particular attention was paid to the purification of the intermediates used in the synthesis and a new method of isolation and purification of the final product was devised. When precipitated from acid solution, Beryllon IV is obtained as a mixture of mono- and di-sodium salts; when precipitated from concentrated hydrochloric acid the free acid is obtained as the monohydrate. Values obtained on analysis for the elements present, for the neutralization equivalent, and for the titanous chloride reduction equivalent agreed well with the calculated values. Of the six replaceable hydrogen atoms in Beryllon IV, the first three are titrated together below pH 7.0, the fourth is a weak acid, $pK_4 = 8.75$, and the fifth and sixth very weak acids, $pK_5 = 11.27$. Of the three strongly dissociated protons, one probably comes from the sulfonic acid group, another from the arsonic acid group, and the third from the free carboxyl groups of the (zwitter ion) iminodiacetic acid group. Beryl-

lon IV combines with beryllium in the ratio of one to one as determined by the potentiometric method and by spectrophotometric titration. The logarithm of the formation constant was found to be 11.42. Light absorption of the beryllium-Beryllon IV system reaches a maximum at pH 8.3. The system conforms to Beer's law over the range 0 to 19.8 μg . beryllium per 100 ml., the molar extinction coefficient being 29,900 at 528 $\text{m}\mu$, the wave length of maximum absorption.

Calcium and magnesium interfere in the spectrophotometric determination of beryllium using either Beryllon III or Beryllon IV; the interference can be obviated by masking the calcium and magnesium with ethylenediaminetetraacetate.

Of the two reagents, Beryllon IV has some advantage in having the higher molar extinction coefficient, and a wider range of adherence to Beer's law.

VI. LITERATURE CITED

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