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REAGENTS FOR DETERMINATIONS OF TRACE IMPURITIES IN WATER, PHASE II

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ABSTRACT

REAGENTS FOR DETERMINATIONS OF TRACE IMPURITIES IN WATER, PHASE II

Determinations of trace impurities, pollutants, in water are often complicated by a large number of factors which consume a great deal of time. Thus, ideal reagents--sensitive, specific, stable, water-soluble reagents--are needed for rapid, spectrophotometric determinations of trace impurities in water. A new method for the determination of nitrate in water is described which uses zinc 1-naphthol-4-sulfonate, an easily prepared, readily purified, stable, water-soluble reagent; the reagent is much better than 1-naphthol-4-sulfonic acid, for the determination of nitrate proposed earlier by another worker and subsequently found in this laboratory to be satisfactory only under very carefully controlled conditions. The same reagent, zinc 1-naphthol-4-sulfonate, also has been proposed for the rapid, specific, sensitive determination of nitrite in natural waters. Nitrite readily can be determined at ppm levels in the presence of several thousand fold excess of nitrate. Full development of water resources, and the control of pollutants returned to natural-water systems, are dependent upon methods of determining trace constituents; significant contributions toward meeting those needs have been accomplished in this work. The methods can readily be applied to such diverse systems as lakes where agricultural fertilizer run-off may be significant, to effluents from plants in the food-preparation industry, and to natural-water systems in highly mineralized areas. Effective new research areas are readily identified as an extension of the work reported here, particularly the study of systems which contain ppm-levels of nitrite in the presence of significant concentrations of nitrate.

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INTRODUCTION

The efficiency of assimilation and the long-term appreciation
of research are inherently related to the efficiency of communication
of research results, which often are best understood in the perspective
of the major research goals of the originating laboratory. The reader
extends the reported information into the realms of new areas and
untouched scientific frontiers. The perspective from which this
reported research was developed was summarized in the introduction
to the first report in this series [Illinois Water Resources Research
Center Report No. 49], which is partially quoted here.

Determinations of trace impurities in water are
complicated in many instances, as anyone who has analyzed
water samples knows, by the necessity of prior separations
for removal of interfering substances, the use of prolonged
evaporation steps to concentrate the impurities to
concentrations above minimum detection levels, the use of
non-aqueous-solvent extractions to enable use of water-
insoluble reagents, the necessity of baking to dryness for
example to allow nitrations to occur in concentrated acids,
the careful regulation of time to control the decomposition
of unstable chemical substances in the system, and by many
other factors. The long-term objective of this research is
to develop ideal reagents; sensitive, specific, stable, water-

soluble reagents for rapid, spectrophotometric determinations of trace impurities in water.

The medium-term objective of the research is to study water-soluble, sulfonated *ortho*hydroxynitroso aromatic compounds, particularly sulfonated nitrosonaphthols.

It is believed that this typical group of chelating agents is one among which one or more such ideal reagents may be identified. The well known nitroso-R-salt is one example of this type of reagent. All these compounds react with Fe(II), Co(II), Ni(II), and Cu(II) and probably Pd(II), Pt(II), and Mn(II) in relatively dilute, aqueous solutions to form colored complexes. But as the substituents on the organic reagents are varied the complexes (1) have different stabilities in terms of formation constants, pH, light sensitivity, and temperature; (2) form at various concentrations; (3) vary in intensity of color; (4) have different oxidation states of the metal atom present; and (5) are formed with various degrees of specificity. It is desired to relate these variations to the structures of the ligand molecules, and in so doing relate the specificity of the chelating functional group for a particular metal ion to the structure of the ligand molecule.

The structural correlations will then be extended to other systems, particularly to those exhibiting steric effects in chelation. Similarly, the development of more highly colored complexes than those presently known should be possible. The preparation and study of such compounds should

lead to more sensitive, more specific, water-soluble reagents

for the determination of trace impurities in the aqueous

environment.

Several short-term problems were first studied

believing that they would contribute several new determinations

for trace impurities in water while laying the groundwork for

the medium-term and long-term objectives discussed above.

Short-term studies which preceded those reported in this

document include: The Spectrophotometric Determination of Nitrate in

Water Using 2-Nitroso-1-naphthol-4-sulfonic Acid; Sodium 2-Nitroso-1-

naphthol-5-sulfonate as a Reagent for Cobalt in Water; The Values of

Some Thermodynamic Functions of Naphthol Sulfonic Acid Derivatives;

and, Feasibility of Gas-Chromatographic Separation and Determination

of Derivatives of Naphtholsulfonic Acids.

The reader's attention is called to Chapters 1 and 2 of this

report where detailed presentations of more recent achievements are

presented, as are summaries of the research procedures used and the

specific results and conclusions that were reached.

Similarly, the efficiency of assimilation and the long-range

effectiveness of research are related inherently to the effectiveness

of communication of the research results, not only to professional

peers, but to professionals in other scientific disciplines and to

the educated, reading public. In an effort to facilitate effective

communication, Chapter 3 was prepared to declare, in non-scientists'

language and terms, the findings of the research herein described.

Chapter 1

THE SPECTROPHOTOMETRIC DETERMINATION OF NITRATE IN
WATER USING ZINC 1-NAPHTHOL-4-SULFONATE AS A REAGENT

Richard J. Antepenko and Albert L. Caskey

I. INTRODUCTION

The nitrate ion exists in most surface waters and ground waters in
trace quantities and is recognized as a major contributor to pollution
in natural waters. Common sources of nitrate pollution include
various industrial operations and the manufacture and use of fertilizers.
Kurty (1) reported that in ground waters the major polluting species
which results from the use of fertilizers is nitrate. Two other forms
of nitrogen commonly used in agriculture are ammonia and urea, but no
matter what forms originally may be present, reactions within the soil
usually cause their conversion to nitrate. Since the nitrate ion is a
species loosely held within the soil, it passes readily into ground
waters.

High nitrate concentrations in water may indicate contamination by
organic matter. Nitrate is the final product of aerobic decomposition
of organic matter containing nitrogen and its presence in water may

indicate the final stages of biological oxidation. Consumption by man or animal of water that contains high levels of nitrate may lead to sickness and in many cases death. The toxic effects of high nitrate levels in drinking water as well as suitable limits for the presence of this constituent have been reported (2,3).

The study of effects of nitrates on biological systems require reliable methods for the determination of nitrates in water. This research focuses on the development of an accurate, precise and reasonably specific method for the quantitative determination of nitrate in water.

II. HISTORICAL

The recognition of nitrate as a water-polluting species and studies
on the toxic effects of nitrate in drinking water have led to the
development of analytical methods for its quantitative determination.
As a result, numerous methods for the determination of nitrate in
water have been reported in the literature. The most widely used
method today, the phenoldisulfonic acid method, was first proposed by
Springle (4) in 1863. In the presently accepted procedure, nitrate, on
treatment with phenoldisulfonic acid in a concentrated sulfuric acid
medium which contains fuming sulfuric acid, forms a yellow-colored
compound, the color of which is intensified in basic media. The
concentration of nitrate is estimated either by colorimetric comparisons
against standard solutions or by spectrophotometrically measuring the
absorbance of the solutions and comparing with a similarly obtained
calibration curve.

In 1941, Murty and Gopalarao (5) reported a colorimetric method
for the determination of nitrate using α-naphtholsulfonic acid. The
reagent reacted with a small amount of nitrate and formed a species
that developed an intense-yellow color in basic solution. The procedure
was similar to the one used in the phenoldisulfonic acid method. A
sample of water containing nitrate was evaporated to dryness on a steam
bath and the residue allowed to cool. Two ml of α-naphtholsulfonic
acid solution was mixed thoroughly with the nitrate residue. After
15 minutes the reaction solution was diluted and neutralized with 8 ml
of 10 $\underline{N}$ potassium or sodium hydroxide and finally diluted to 250 ml.
The developed color was compared with those of similarly prepared

nitrate standards using a Duboscq colorimeter.

Osmo Makitie (6), in 1963, reported a spectrophotometric method for the determination of nitrate in water as a nitronaphtholsulfonate. 1-Naphthol-4-sulfonic acid, the reagent, which was also the major species in the reagent solution employed by Murty and Gopalarao (5), was said to react with nitrate in a dilute sulfuric acid medium to form the *ortho*-nitronaphtholsulfonic acid. The system was reported to follow a nitration procedure similar to that of the phenoldisulfonic acid method. The paste, containing nitrate and obtained after evaporation of the sample, was treated with the reagent in 2.5 $\underline{M}$ sulfuric acid at room conditions; the slurry was made basic and the concentration of nitrate was estimated by measuring the absorbance of the neutralized nitration product, said to be sodium 2-nitro-1-naphthol-4-sulfonate, at 435 nm ($\varepsilon = 5.3 \times 10^3$).

Abercrombie and Caskey (7) prepared 2-nitro-1-naphthol-4-sulfonic acid by two independent methods. First, 2-nitroso-1-naphthol-4-sulfonic acid was reacted with equal molar or less nitrate in dilute acid to form the product. In the second method of synthesis, 2-nitroso-1-naphthol-4-sulfonic acid was oxidized by hydrogen peroxide in a trifluoroacetic acid medium to form the desired species. The ultraviolet spectrum of the synthesized 2-nitro-1-naphthol-4-sulfonic acid in basic medium, yielded an absorbance maximum at 431 nm ($\varepsilon = 10 \times 10^3$). A comparison of the spectral data for 2-nitro-1-naphthol-4-sulfonic acid reported by Makitie with spectral data obtained by Abercrombie and Caskey revealed major discrepancies and presented the need for an examination of the nitration system reported by Makitie and the simultaneous evaluation of the method he proposed.

In an earlier study (8,9), the procedure Makitie proposed for the determination of nitrate with 1-naphthol-4-sulfonic acid was shown to be faulty. The nitration of 1-naphthol-4-sulfonic acid could not be duplicated using the reported conditions, 2.5 $\underline{M}$ sulfuric acid at room temperature, or even at temperatures close to 100 °C. The nitration was found to require a 7 $\underline{M}$ or more-concentrated sulfuric acid medium as well as heating. The earlier study indicated that under optimum conditions, the nitration of 1-naphthol-4-sulfonic acid may be adaptable to a spectrophotometric method for the determination of nitrate, and thus formed the basis for the work reported here. A detailed study of the system is reported here with respect to the effects of temperature, reaction time and sulfuric acid concentration. Also presented are the effects of foreign ions and a suggested procedure for the use of 1-naphthol-4-sulfonic acid for the determination of nitrate in natural waters.

III. EXPERIMENTAL

A. Reagents

Reagent-grade chemicals meeting American Chemical Society specifications were used whenever they were commercially available. All other chemicals were of the highest purity available.

Standard solutions of nitrate were prepared by weight from reagent-grade sodium nitrate obtained from Matheson, Coleman and Bell.

Sodium 1-naphthol-4-sulfonate, practical grade obtained from Eastman Kodak, was purified as the p-toluidinium salt. Sodium 1-naphthol-4-sulfonate, 80 g (0.33 mole), was dissolved in approximately 800 ml of hot 10 % hydrochloric acid, and to this solution was added an equal-molar quantity of p-toluidinium chloride, 47 g (0.33 mole). The highly colored solution was treated with 5 g of degassed, Darco G-60, activated carbon, filtered and cooled at 5 °C. After two recrystallizations in dilute (1:5 v/v) hydrochloric acid, the p-toluidinium 1-naphthol-4-sulfonate was dissolved in a minimum amount of deionized water, approximately 1 liter, and the pH of the resulting solution was adjusted to 9 with 3 $\underline{M}$ sodium hydroxide. The basic solution was extracted several times with diethyl ether to remove the p-toluidine, adjusted to pH 5 with hydrochloric acid (1:1 v/v), and then evaporated to dryness under reduced pressure. The light-brown colored sodium 1-naphthol-4-sulfonate obtained was placed in a 1-liter beaker and extracted with 5, 300-ml portions of hot, 100 % ethanol. The solution obtained by combining the ethanol extracts was treated with degassed, Darco G-60, activated carbon and evaporated to dryness under reduced pressure.

Zinc 1-naphthol-4-sulfonate was prepared using a procedure similar to the one reported by Knusli (10). Purified sodium 1-naphthol-4-sulfonate, 40 g (0.16 mole), was dissolved in 120 ml of hot, deionized water. To this solution was added a hot, aqueous solution of zinc chloride, 50 g (0.37 mole), dissolved in a minimum amount of hot water. Upon slow cooling at room temperature, large rhombic crystals formed which were collected on a sintered-glass funnel. After 3 recrystallizations from water, the zinc 1-naphthol-4-sulfonate was air dried for 24 hours at room temperature.

B. Equipment

A Beckman, Model DB-G (1403), recording spectrophotometer equipped with 1.0-cm, matched, silica absorption cells was used for absorbance measurements.

A Fisher Isotemp Oven, Junior Model (Model No. 101), was used for drying solutions in volumetric flasks.

A constant-temperature bath fitted with a Sargent Thermonitor unit (Catalog No. S-82052) and Sargent Heater and Circulator unit (Model No. N51-12) was used for constant-temperature measurements.

C. Procedures

A general procedure was employed in all studies with only slight modifications utilized when necessary. Samples containing nitrate were prepared by placing aliquots of a sodium nitrate solution into either 50-ml or 100-ml volumetric flasks. The flasks containing the solutions were placed in an oven varying in temperature from 130-140 °C, evaporated to dryness, and cooled to room temperature. The nitration

reaction was initiated for each nitrate solution separately and employed the following order for the addition of reactants. An aliquot, 9.0 ml, of sulfuric acid solution was added, the flask was shaken vigorously to dissolve the nitrate residue, and placed in the constant temperature bath at a pre-set temperature. After approximately one minute in the bath, a 1.00-ml aliquot of zinc 1-naphthol-4-sulfonate solution was added, the flask was shaken and replaced in the bath for a specified reaction time. Except during the addition of reagents, the flasks were stoppered at all times. The reaction-time intervals were the elapsed times from the addition of the last reactant solutions to the moment the reaction mixtures were quenched by dilution with ice-cold deionized water. All solutions were allowed to equilibrate to room temperature before being diluted to volume. All solutions were thoroughly shaken before their spectra were recorded.

In studies where solutions of sodium nitrate were added to concentrated sulfuric acid solutions, the flasks containing the sulfuric acid were first cooled in an ice bath. This procedure was also followed in studies where water was added to concentrated sulfuric acid.

D. Proposed Procedure for the Determination of Nitrate in Natural Waters

1. Special Stock Solutions

a. Zinc 1-Naphthol-4-Sulfonate Reagent

Dissolve 1.330 g zinc 1-naphthol-4-sulfonate and dilute to 100.0 ml with deionized water to prepare a 2.000×10^{-2} M zinc 1-naphthol-4-sulfonate solution.

b. Stock Nitrate Solution

Dissolve 0.6071 g anhydrous sodium nitrate and dilute to 1000 ml with deionized water. This solution contains 100 mg N/ml.

c. Standard Nitrate Solution

Carefully dilute 10.00 ml of the stock nitrate solution to 100.0 ml with deionized water; 1.00 ml = 10.00 x 10^{-3} mg N (10.00 μg N/ml).

d. Standard Silver Solution

Dissolve 2.20 g silver sulfate in deionized water and dilute to 1000 ml; 1.00 ml is equivalent to 0.50 mg Cl.

e. Potassium Chromate Indicator Solution

Dissolve 5.0 g potassium chromate in a small amount of deionized water. Add silver sulfate solution until a red precipitate is formed. Allow the solution to stand for 12 hours, filter, and dilute to 200 ml with deionized water.

f. Potassium Permanganate Solution, 0.1 $\underline{N}$

Dissolve 0.316 g potassium permanganate in deionized water and dilute to 100 ml.

g. Disodium Hydrogen Phosphate Solution, 1 %

Dissolve 1.0 g disodium hydrogen phosphate in deionized water and dilute to 100 ml.

h. Aluminum Hydroxide Floc

Dissolve 125 g potassium or ammonium alum in 1-liter deionized
water. Warm to 60 °C and add 55-ml ammonium hydroxide slowly with
stirring. Allow the mixture to stand for about 1 hour and wash
the precipitate by successive additions, thorough stirring, and
decantations of 1-liter portions of deionized water, until the
supernatant liquid is free from ammonia, chloride, nitrate and nitrite.

i. Sulfuric Acid Solution, 1 $\underline{N}$

Add 28-ml concentrated sulfuric acid to 750-ml deionized water
and after cooling, dilute to 1 liter.

j. Sodium Hydroxide Solution, 1 $\underline{N}$

Dissolve 40 g sodium hydroxide and dilute to 1 liter with deionized
water.

k. Standard Sodium Hydroxide Solution, 0.1 $\underline{N}$

Dilute 100 ml of the 1 $\underline{N}$ sodium hydroxide solution to 1 liter with
deionized water. Standardize against primary-standard potassium
hydrogen phthalate using phenolphthalein indicator and calculate the
normality to 4 significant digits.

l. Sulfuric Acid Reaction Solution, 12.2 $\underline{M}$

Add slowly with stirring, 680 ml of cold, concentrated sulfuric
acid to 320 ml of cold, deionized water in a 1-liter volumetric flask.
Cool the solution and dilute to volume with deionized water.

The sulfuric acid concentration must be within the range 12.0 to 12.4 $\underline{M}$. Concentration check: dilute a 25.00-ml aliquot to 250.0 ml with deionized water. Titrate a 2.00-ml aliquot of the diluent with the standard, 0.1 $\underline{N}$, sodium hydroxide solution using phenolphthalein indicator. A sulfuric acid concentration outside the range of 12.0 to 12.4 $\underline{M}$, must be adjusted with either sulfuric acid or deionized water and the adjustment followed by a repeat concentration check.

2. Pretreatment of Water Samples

The necessity for employing the following water pretreatment steps and the order in which they are presented are dependent upon the nature of the individual water sample.

a. Color Removal

Decolorize a 50.0-ml water sample by adding 2 ml of aluminum hydroxide floc and stir for 5 minutes. Allow the precipitate to settle for a few minutes before filtering through a 60° funnel fitted with rapid, filter paper. Rinse the beaker with several small portions of deionized water and filter, combining all filtrates.

b. Nitrite Conversion

Add 0.5 ml of 1 $\underline{N}$ sulfuric acid to a clear, 50.0-ml water sample. Add dropwise while stirring, 0.1 $\underline{N}$ potassium permanganate until a faint pink color persists and allow the sample to stand for 15 minutes. (A faint pink color persists for at least 15 minutes when sufficient potassium permanganate solution is used for the complete conversion of nitrite to nitrate.) At the end of the nitrate determination, make the

proper deduction for the nitrite concentration as determined by the method described in "Nitrogen (Nitrite)" in "Standard Methods for the Examination of Water and Wastewater" (12). Adjust the pH of the solution to 7 with 1 $\underline{N}$ sodium hydroxide.

If the water sample does not contain chloride, proceed with step a, "Color Removal", for the removal of insoluble manganese dioxide.

c. Chloride Removal

If chloride is present in the water, adjust a separate 50.0-ml sample to within pH 7 to 10 with either 1 $\underline{N}$ sulfuric acid or 1 $\underline{N}$ sodium hydroxide. Add 1.0 ml of potassium chromate indicator solution and titrate with the silver sulfate solution to a pinkish-yellow endpoint. Repeat the titration on a blank sample consisting of 1.0 ml of potassium chromate indicator solution diluted to 50 ml with deionized water. The volume of silver sulfate solution added to a 50.0-ml water sample, which is equivalent to the amount of chloride in the sample, is equal to the volume of silver sulfate solution used for the water sample titration minus the volume used for the titration blank. To water samples containing only a trace of chloride, add 0.5 ml of standard silver sulfate solution. Add the appropriate volume of standard silver sulfate.

Treat the water sample as described in step a, "Color Removal", for the removal of the silver chloride precipitate.

d. Evaporation

Neutralize the clarified sample to pH 7, add 1.0 ml of 1.0 % disodium hydrogen phosphate, and evaporate to about 10 ml over a steam

cone or hot plate. Do not evaporate to dryness. Transfer the solution to a 50-ml volumetric flask. Rinse the beaker with small volumes of deionized water and add the rinsings to the volumetric flask. Evaporate the water sample to dryness in an air-circulating oven at 130-140 °C. Allow the flask and residue to cool before proceeding with the nitration reaction.

3. Treatment of Water Sample and Standard Solutions for Absorbance Measurements

Prepare a series of standard solutions by placing 0.00, 1.00, 2.00, 3.00, 5.00, 7.00, and 10.00 ml of the standard nitrate solution in 50-ml volumetric flasks. Treat the standards as described in step d, "Evaporation".

The nitrate reaction is initiated for each pretreated water sample and standard independently and employs the following order for the addition of reactants. Add 9.00 ml of the sulfuric acid reaction solution to a cooled flask. Stopper, shake vigorously to dissolve the nitrate residue and place the flask in a constant temperature bath at 50 °C. After a one-minute heating period, remove the flask and add 1.00 ml of zinc 1-naphthol-4-sulfonate solution. Stopper, shake well, and replace the flask in the bath. Remove the flask after 30 minutes and quench the reaction by adding about 25 ml of cold deionized water. Allow the flask to equilibrate to room temperature and dilute to volume.

Measure the absorbances at 384 nm. Use a blank consisting of 9.00 ml of sulfuric acid reaction solution diluted to 50.0 ml.

Construct a working graph by plotting absorbance *versus* micrograms/50 ml solution of nitrogen. A linear working graph should be obtained. Read the micrograms of nitrogen from the graph for each sample.

IV. RESULTS AND DISCUSSION

Zinc 1-naphthol-4-sulfonate, the analytical reagent, was easily prepared, and its purification by repeated recrystallizations from water was rapid yielding a stable, white, crystalline material. The reagent was characterized as having 8 1/2 water molecules of hydration per zinc 1-naphthol-4-sulfonate molecule. Friedlander and Taussig (11) prepared zinc 1-naphthol-4-sulfonate and reported it to be the 8 hydrate. A loss in water study conducted on the air-dried material in a vacuum desiccator over anhydrous magnesium perchlorate gave an average experimental weight loss of 22.77 %, which compared well with the theoretical value of 23.02 % for the 8 1/2 hydrate. Titration with EDTA of both the hydrated and the anhydrous zinc 1-naphthol-4-sulfonate yielded 9.64 % and 12.76 % zinc, respectively. These values were in good agreement with the theoretcal values of 9.83 % and 12.77 %, respectively.

The ultraviolet spectra of zinc 1-naphthol-4-sulfonate were recorded; in acidic medium it has absorbance maxima at 227 nm ($\varepsilon = 8.0 \times 10^4$) and at 297 nm ($\varepsilon = 1.54 \times 10^4$), and in basic medium it has absorbance maxima at 248 nm ($\varepsilon = 5.13 \times 10^4$) and at 330 nm ($\varepsilon = 2.05 \times 10^4$). Therefore, in using zinc 1-naphthol-4-sulfonate as a spectrophotometric reagent for the determination of nitrate, one can estimate the concentration of nitrate indirectly by following one of two absorption bands for the reagent in either acidic media or basic media.

The infrared spectrum of zinc 1-naphthol-4-sulfonate, prepared as a potassium bromide pellet, is presented in Figure 1. The spectrum contained the strong sulfonic acid absorption bands between 1260-1150 cm^{-1}
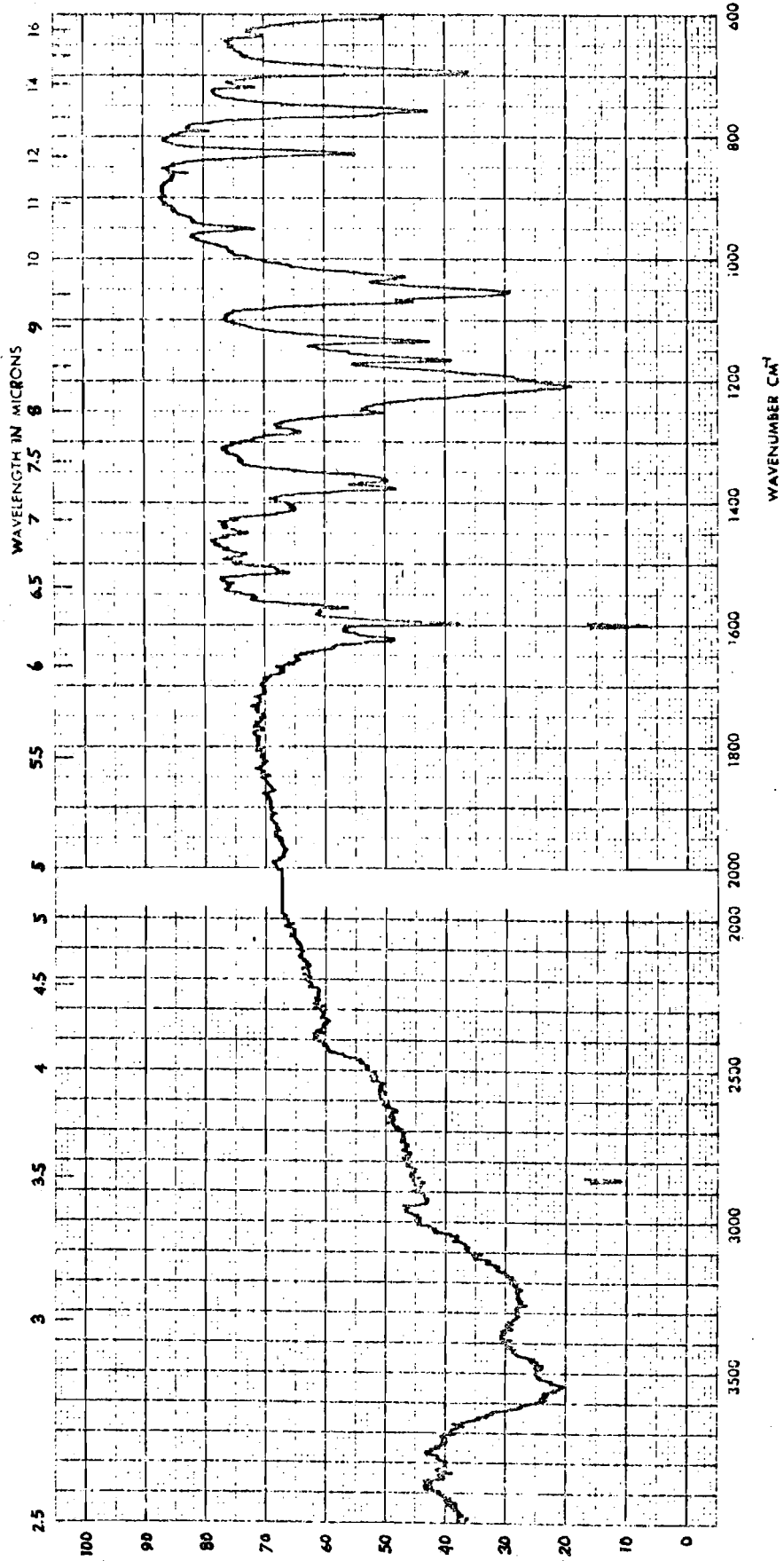
Figure 1. Infrared spectrum of zinc 1-naphthol-4-sulfonate: potassium bromide pellet

for asymmetric-stretching vibrations and between 1080-1100 cm^{-1} for symmetric-stretching vibrations of the $-SO_2$-functional group. Also noted were the bands between 1500-1650 cm^{-1}, which result from the naphthyl-ring system.

A. Time and Temperature Effects

The effect of sulfuric acid concentration on the nitration reaction was studied at several temperatures, and the results are graphically presented in Figure 2. The objective of this experiment was to determine a workable sulfuric acid reaction concentration that facilitated the nitration reaction. The formation of 2-nitro-1-naphthol-4-sulfonic acid from the reaction of 4.245×10^{-4} $\underline{M}$ sodium nitrate with 5.510×10^{-4} $\underline{M}$ zinc 1-naphthol-4-sulfonate was observed in varied sulfuric acid concentrations ranging from 5.4 to 14.4 $\underline{M}$. A 1-hour reaction time and reaction temperatures of 29, 40, 50 and 60 °C were used.

The nitration reaction was followed by observing the increase in absorbance at 386 nm, a wavelength maximum for the product in acidic medium. The reagent, 1-naphthol-4-sulfonic acid, does not absorb at this wavelength. The data shows that for reaction temperatures of 29, 40, 50 and 60 °C, the maximum absorbance from the product was obtained for solutions that corresponded to a 10.8 $\underline{M}$ sulfuric acid reaction medium. At this sulfuric acid reaction concentration and at all reaction temperatures, with the exception of the 60 °C reaction temperature, the absorbance values at 386 nm were slightly below the theoretical absorbance of 0.224 that was calculated for a 4.245×10^{-5} $\underline{M}$ 2-nitro-1-naphthol-4-sulfonic acid solution.

Using the sulfuric acid reaction concentration of approximately
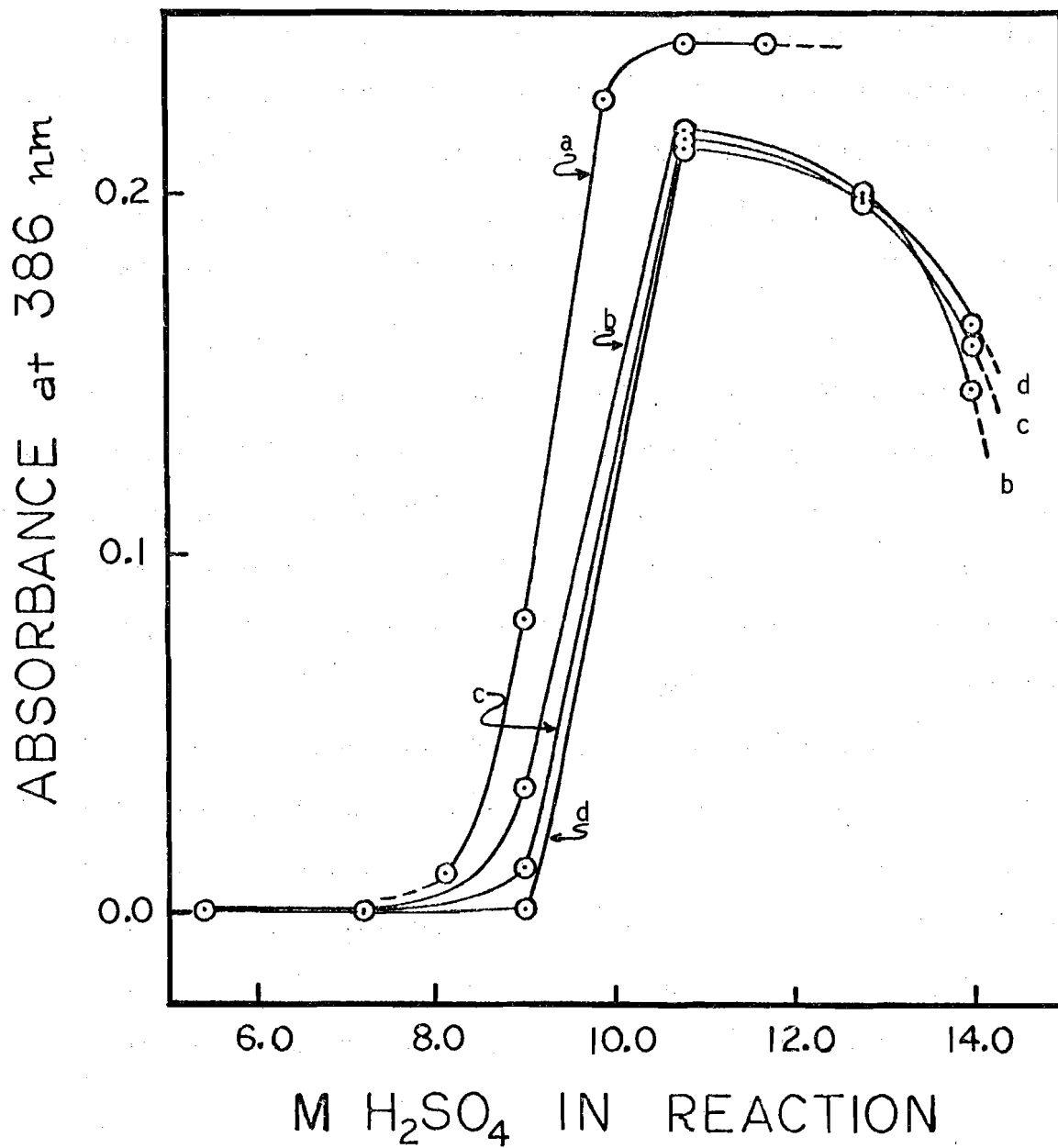
Figure 2. Formation and stability of 2-nitro-1-naphthol-4-sulfonic acid as a function of sulfuric acid concentration at various temperatures: 5.510 x 10^{-5} $\underline{M}$ reagent, 4.245 x 10^{-5} $\underline{M}$ $NaNO_3$, time of 1 hour, and temperatures of

(a) 60 °C,
(b) 50 °C,
(c) 40 °C, and
(d) 29 °C

10.8 $\underline{M}$, the reaction time required for the quantitative nitration of 1-naphthol-4-sulfonic acid was studied at several reaction temperatures. The progress of the nitration reaction was followed by observing the formation of 2-nitro-1-naphthol-4-sulfonic acid from the reaction of 4.245×10^{-4} $\underline{M}$ sodium nitrate with 5.510×10^{-4} $\underline{M}$ zinc 1-naphthol-4-sulfonate.

The pertinent results, which have been presented in Table 1 as well as graphically in Figure 3, show that for a constant reaction time, an increase in the reaction temperature produced an increase in the formation of 2-nitro-1-naphthol-4-sulfonic acid. A similar increase in product formation was obtained from an increase in the reaction time for a specific reaction temperature.

The optimum nitration conditions of time and temperature should facilitate the quantitative formation of product. Also, the concentration of 2-nitro-1-naphthol-4-sulfonic acid present in the diluted reaction mixture resulting from the quantitative reaction of nitrate with 1-naphthol-4-sulfonate, should give an absorbance of 0.224 at 386 nm. Therefore, a comparison of the experimentally obtained data with this theoretical absorbance value provided, in part, the basis for choosing the best reaction time and temperature.

For reaction temperatures of 40, 45, and 50 °C, the formation of 2-nitro-1-naphthol-4-sulfonic acid reached a maximum with time. Increasing the reaction time up to one hour, for any one of these temperature studies, produced a negligible effect on the stability of the product.

At a reaction temperature of 50 °C, the greatest amount of product was formed, because at this temperature, the absorbance values were

Table 1.   The Formation of 2-Nitro-1-Naphthol-4-Sulfonic Acid in 10.88 $\underline{M}$
Sulfuric Acid as a Function of Time at Various Temperatures

| Reaction Time (min.)	Absorbance at 386 nm for Reaction Temperature of:						
	30 °C	40 °C	45 °C	50 °C	60 °C	70 °C	80 °C
5.0	-----	-----	-----	-----	0.187	0.216	0.226
10.0	-----	0.142	0.174	0.195	0.215	0.228	0.234
15.0	-----	-----	-----	-----	-----	0.233	0.243
20.0	0.135	0.193	0.209	0.218	0.222	0.238	0.278
30.0	0.165	0.210	0.218	0.225	0.226	0.246	0.260
40.0	0.182	0.218	0.223	0.225	0.231	0.247	0.273
50.0	0.193	0.221	0.224	0.227	0.234	0.257	0.272
60.0	0.200	0.222	0.224	0.226	-----	-----	-----
70.0	0.206	-----	-----	-----	-----	-----	-----
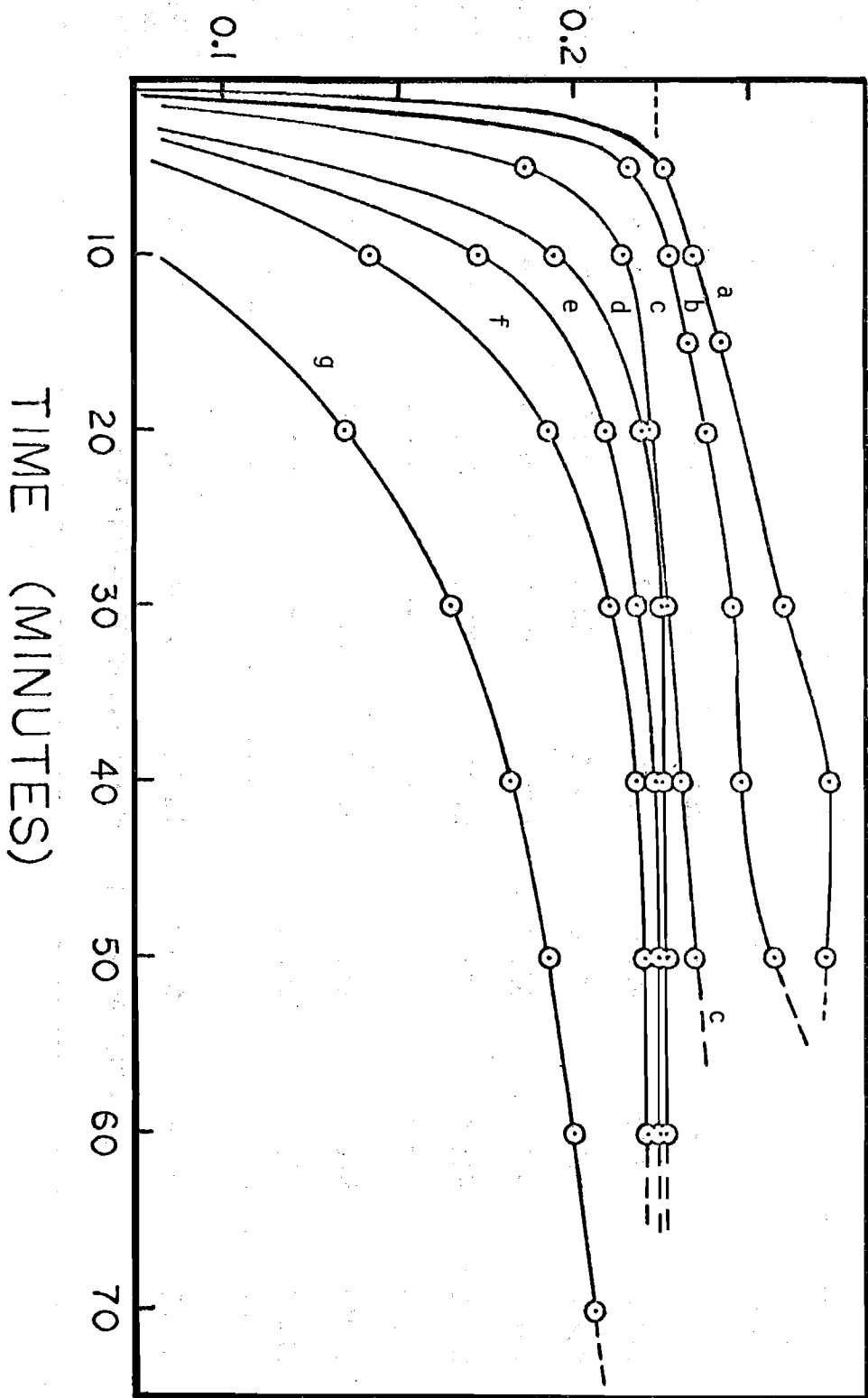
Figure 3. Formation and stability of 2-nitro-1-naphthol-4-sulfonic acid as a function of time at various temperatures: 5.511 x 10-5 M reagent, 4.245 x 10-5 M NaNO3, 10.88 M H2SO4; and temperatures of (a) 80 °C, (b) 70 °C, (c) 60 °C, (d) 50 °C, (e) 45 °C, (f) 40 °C, and (g) 30 °C

closer to the theoretical value. Also at a 50 °C temperature, a
minimum reaction time of approximately 30 ± 5 minutes was required for
the quantitative formation of 2-nitro-1-naphthol-4-sulfonic acid. The
50 °C reaction temperature and 30 minute reaction time were chosen as the
optimum temperature and time variables for the nitration of 1-naphthol-
4-sulfonic acid in a 10.88 M sulfuric acid reaction medium.

B. Optimum Sulfuric Acid Concentration

The effect of sulfuric acid concentration on the nitration reaction
was studied using the optimum reaction temperature and time. The
formation of 2-nitro-1-naphthol-4-sulfonic acid from the reaction of
4.245×10^{-4} M sodium nitrate with 5.511×10^{-4} M zinc 1-naphthol-
4-sulfonate was studied in a reaction medium ranging from 5.4 to
14.4 M sulfuric acid, at 50 °C for 30 minutes. The pertinent data
have been presented in Table 2 and Figure 4.

The absorbance at 386 nm, an absorbance maximum for the product,
increased to a maximum for reactions run in 10.88 M sulfuric acid
and remained a constant value up to 11.25 M sulfuric acid. The
results indicated that the optimum range of acid concentration
facilitating the formation of 2-nitro-1-naphthol-4-sulfonic acid
was 11.00 ± 0.20 M. Within this range of acid concentration, the
average absorbance value of 0.456 compared well with the theoretical
absorbance value of 0.448, which was calculated for an 8.490 ×
10^{-5} M 2-nitro-1-naphthol-4-sulfonic acid solution, and indicated
that the nitration reaction proceeded quantitatively. Similarly,
for a sulfuric acid medium of 11.25 M or above, the observed
absorbance values of the diluted reaction mixtures were less than

Table 2.   The Effect of Sulfuric Acid Concentration on the
Formation and the Stability of 2-Nitro-1-Naphthol-
4-Sulfonic Acid at 50 °C for 30 Minutes

Water (ml)	H_2SO_4 18 M (ml)	Conc.[a] H_2SO_4 M	A @ 386 nm	A @ 326 nm
5.00	3.00	5.40	0.000	0.240
4.00	4.00	7.20	0.000	0.255
3.50	4.50	8.10	0.003	0.260
3.00	5.00	9.00	0.036	0.288
2.75	5.25	9.45	0.112	0.340
2.50	5.50	9.90	0.275	0.405
2.25	5.75	10.40	0.434	0.501
2.00	6.00	10.80	0.457	0.537
1.75	6.25	11.30	0.455	0.564
1.50	6.50	11.70	0.446	0.620
1.25	6.75	12.20	0.439	0.703
1.00	7.00	12.60	0.434	0.782
0.50	7.50	13.50	0.416	0.856
0.00	8.00	14.40	0.340	0.809

[a]This is the sulfuric acid concentration in the reaction mixture,
after the addition of 1.00 ml of nitrate solution and 1.00 ml of
reagent solution, for a 10.0 ml total reaction volume.
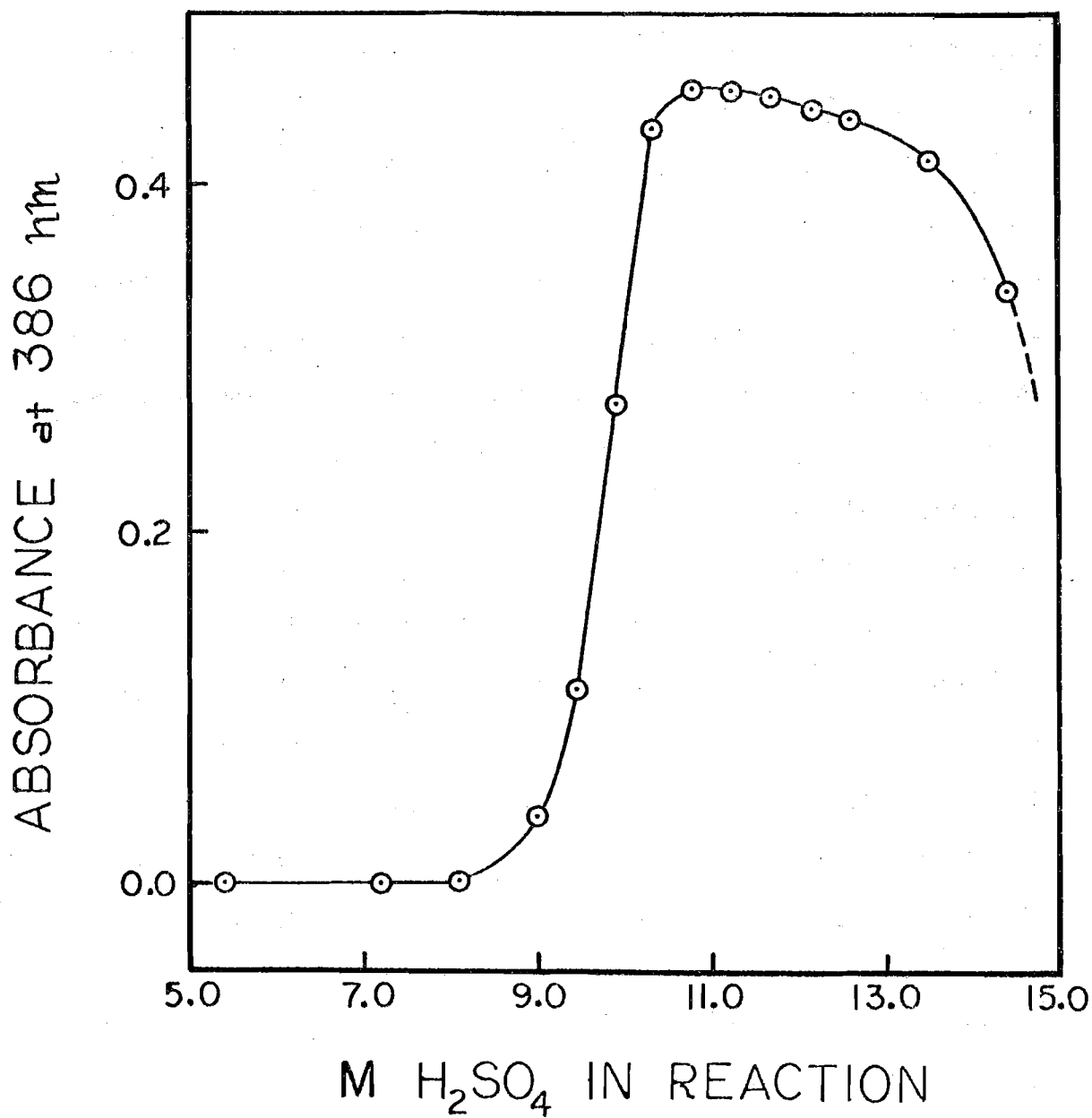
Figure 4. Formation of 2-nitro-1-naphthol-4-sulfonic acid as a function of sulfuric acid concentration: 1.102×10^{-4} $\underline{M}$ reagent, 8.490×10^{-5} $\underline{M}$ $NaNO_3$, temperature of 50 °C, and time of 30 minutes

those expected for quantitative reaction. For sulfuric acid reaction media of 12.6 $\underline{M}$ and higher, the observed absorbance values were even smaller; this was reasoned to be due to the loss of nitrogen oxides or the decomposition of 2-nitro-1-naphthol-4-sulfonic acid. Therefore, the maximum formation of 2-nitro-1-naphthol-4-sulfonic acid required an 11.00 ± 0.20 $\underline{M}$ sulfuric acid concentration in the reaction medium. Concentrations above or below this value resulted in either a decrease in the amount of product from the degradation of either nitric acid, the analytical reagent, or the product, or the incomplete formation of product, respectively.

Included in Table 2 are the values recorded from the absorbance at 326 nm. The increase in absorbance at this wavelength with an increase in sulfuric acid concentration, indicated the formation of a second product. Neither zinc 1-naphthol-4-sulfonate nor 2-nitro-1-naphthol-4-sulfonic acid have absorbance maxima at 326 nm. A similar absorbance maximum was noted in the spectra from a study of the acid decomposition of zinc 1-naphthol-4-sulfonate as a function of temperature, as well as from the study on the effect of sulfuric acid concentration.

C. Stability of Zinc 1-Naphthol-4-Sulfonate in Sulfuric Acid

The stability of zinc 1-naphthol-4-sulfonate in a sulfuric acid medium was studied as a function of reaction temperature, and the pertinent data are presented in Table 3 and Figure 5. The reaction mixture was 5.510 x 10^{-4} $\underline{M}$ zinc 1-naphthol-4-sulfonate and 10.88 $\underline{M}$ sulfuric acid. The reaction time was held constant at 1 hour.

As the reaction temperature was increased from the initial

Table 3. The Effect of Temperature on the Acid Decomposition
of Zinc 1-Naphthol-4-Sulfonate in 10.88 $\underline{M}$ Sulfuric
Acid for One Hour

Solution Number	Temperature °C ± 0.1	A @ 297 nm	Average A @ 297 nm
133	25	0.868	-----
134	25	0.867	0.868
127	30	0.858	-----
128	30	0.864	0.861
135	35	0.867	-----
136	35	0.865	0.866
131	40	0.865	-----
132	40	0.863	0.864
129	45	0.855	-----
130	45	0.850	0.853
140	50	0.854	-----
141	50	0.855	0.855
401	55	0.833	-----
402	55	0.831	0.832
149	60	0.805	-----
150	60	0.805	-----
151	60	0.804	0.805
152	70	0.722	-----
153	70	0.723	-----
154	70	0.722	0.722
419	80	0.661	0.661
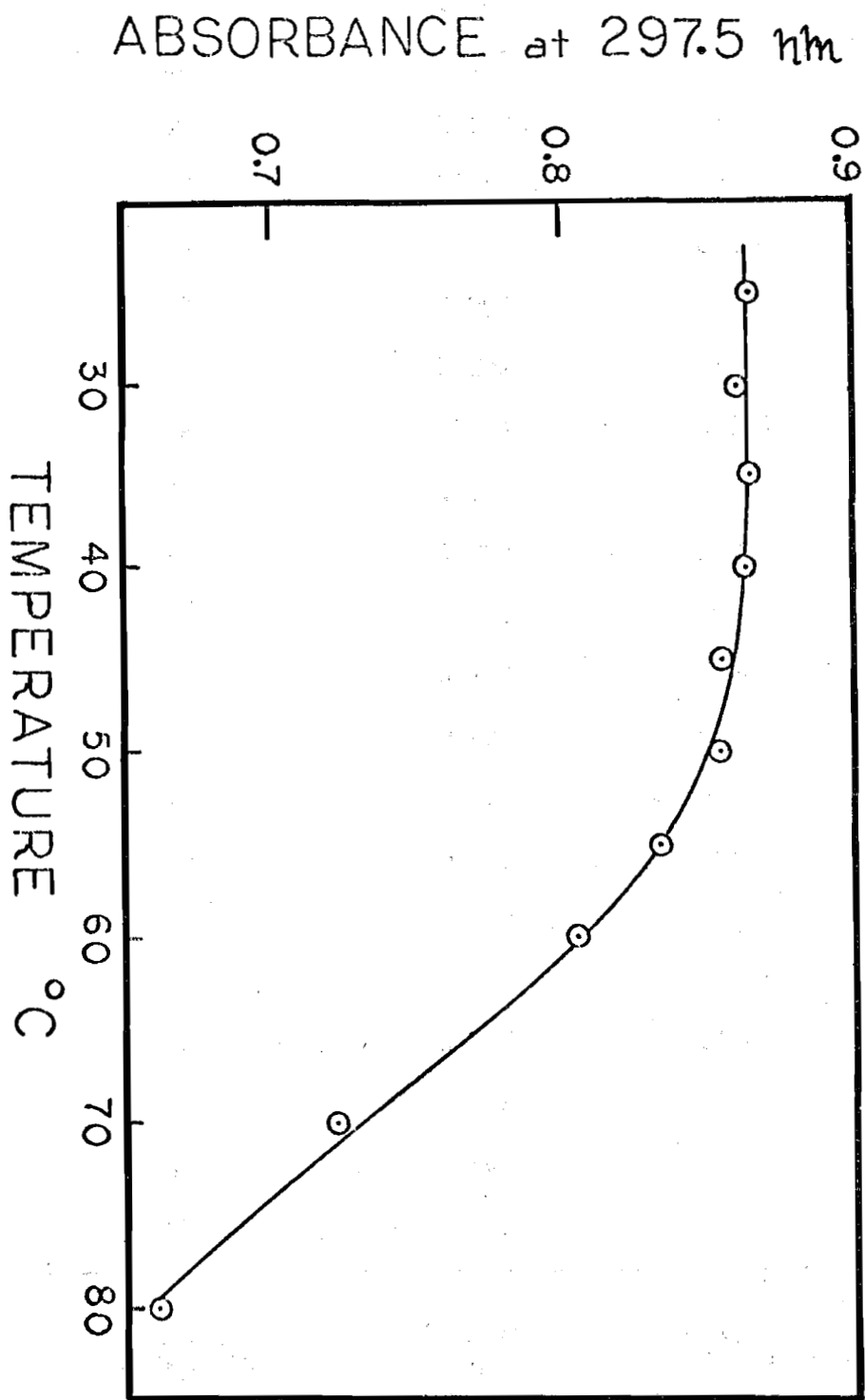
Figure 5. Stability of zinc 1-naphthol-4-sulfonate as a function of temperature: 10.88 $\underline{M}$ H_2SO_4 and 5.5 $\times$ 10⁻⁵ $\underline{M}$ reagent

value of 25 °C, the reagent absorbance maximum at 297 nm remained nearly constant until the reaction temperature exceeded 50 °C. Since the reaction time was 1 hour for all studies, the nearly constant absorbance indicated that the reagent was stable at 50 °C or lower temperatures for 1 hour. Increasing the temperature above 50 °C produced a significant decrease in the absorbance and indicated that a significant amount of zinc 1-naphthol-4-sulfonate was decomposing.

The stability of zinc 1-naphthol-4-sulfonate was studied in a 10.88 $\underline{M}$ sulfuric acid reaction medium at 50 °C as a function of reaction time. The pertinent data from this study is presented in Table 4 and Figure 6. The reagent stability as a function of reaction time was good, with only a small amount of degradation observed after a 30 minute interval and a less than 9 % loss observed after 5 hours.

D. Optimum Reagent Concentration

A mole-ratio study was conducted to determine the optimum ratio of nitrate to zinc 1-naphthol-4-sulfonate for use in the nitration system. The sodium nitrate, which concentration ranged from 1.00 - 10.0 x 10^{-4} $\underline{M}$, was reacted with 4.00 x 10^{-4} $\underline{M}$ zinc 1-naphthol-4-sulfonate in 10.95 $\underline{M}$ sulfuric acid at 50 °C for 30 minutes. The pertinent data are presented in Table 5. The experiment was conducted in duplicate and the average values are presented graphically in Figure 7. The quantitative formation of 2-nitro-1-naphthol-4-sulfonic acid was observed through a mole ratio, sodium nitrate to zinc 1-naphthol-4-sulfonate ratio of 1.0; Beer's law was obeyed. In situations where this mole ratio must be exceeded, nearly quantitative results would be obtained through a mole ratio of 1.75. Beyond a mole ratio of 1.0

Table 4. The Effect of Time on the Acid Decomposition
of Zinc 1-Naphthol-4-Sulfonate in 10.88 M
Sulfuric Acid at 50 °C

Solution Number	Time		Absorbance @ 297 nm
	Minutes	Hours	
137	15	0.25	0.872
138	30	0.50	0.864
139	45	0.75	0.857
140	60	1.00	0.854
141	60	1.00	0.855
142	90	1.50	0.842
143	120	2.00	0.837
144	150	2.50	0.830
145	180	3.00	0.825
146	210	3.50	0.817
147	240	4.00	0.804
148	300	5.00	0.796

ABSORBANCE at 297.5 nm
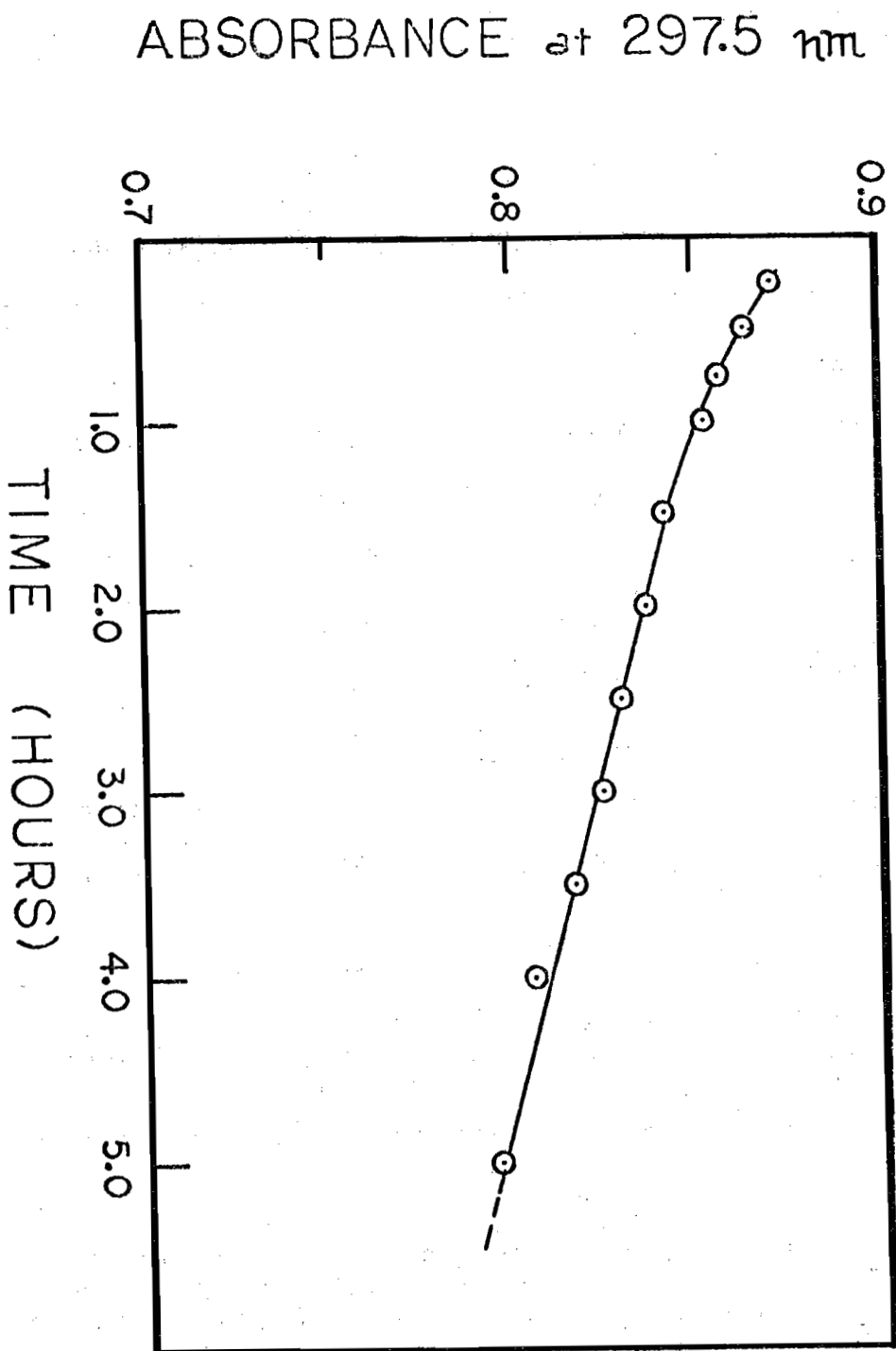
Figure 6. Effect of time on the acid decomposition of zinc 1-naphthol-4-sulfonate: 5.511 × 10⁻⁵ M reagent, 10.88 M H₂SO₄, and temperature of 50 °C

Table 5. Mole Ratio Study. The Effect of Sodium Nitrate
Concentrations on the Formation of 2-Nitro-1-
Naphthol-4-Sulfonic Acid in 10.95 $\underline{M}$ Sulfuric Acid

Mmoles NaNO$_3$	Mmoles ZnR$_2$	Mole Ratio[a] NO$_3$/ZnR$_2$	Run 1 A@ 386 nm	Run 2 A@ 386 nm
1.00	4.00	0.25	0.105	0.105
2.00	4.00	0.50	0.209	0.207
3.00	4.00	0.75	0.306	0.307
4.00	4.00	1.00	0.400	0.402
5.00	4.00	1.25	0.489	0.469
6.00	4.00	1.50	0.577	0.581
7.00	4.00	1.75	0.705	0.705
8.00	4.00	2.00	0.664	0.663
9.00	4.00	2.25	0.650	0.647
10.00	4.00	2.50	0.624	0.617

[a]Mole ratio, sodium nitrate to zinc 1-naphthol-4-sulfonate
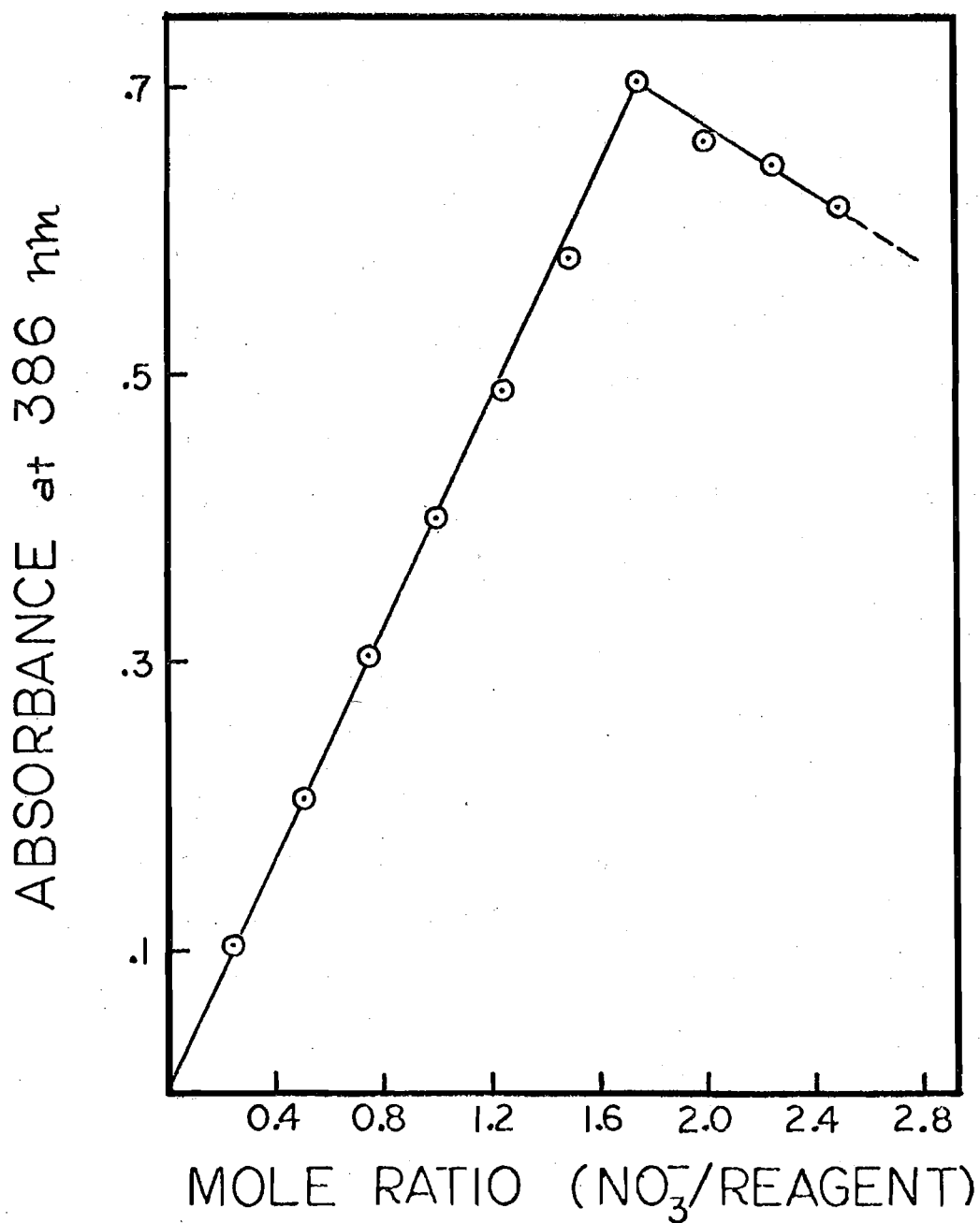
Figure 7. Mole-ratio plot of the reaction of zinc 1-naphthol-4-sulfonate with nitrate: 8.000×10^{-5} $\underline{M}$ reagent, 2.000×10^{-5} to 2.000×10^{-4} $\underline{M}$ $NaNO_3$, 10.95 $\underline{M}$ H_2SO_4, temperature of 50 °C, and time of 30 minutes

and up to a mole ratio of 1.75, the absorbances of the diluted reaction mixtures were less than those expected for quantitative reaction.

At a mole ratio of 1.75 through 2.50, the normal yellowish-green color of the nitration mixture was not observed, but a deep-red color developed. The ultraviolet-visible spectra from these solutions differed from the usual nitration product spectrum. The shift in the position of wavelength maximum indicated the formation of some 2,4-dinitro-1-naphthol. In an earlier study (8,9), 2,4-dinitro-1-naphthol was a confirmed product of the nitration system when a mole ratio of one or greater, sodium nitrate to 1-naphthol-4-sulfonic acid, was reacted.

E. Precision Study

The reproducibility in the formation of 2-nitro-1-naphthol-4-sulfonic acid was studied under the optimum nitration conditions of 10.95 $\underline{M}$ sulfuric acid at 50 °C for 30 minutes. The precision of this method was determined so that an actual interference, such as the presence of an interfering foreign ion, could be distinguished from normal variations inherent in the spectrophotometric procedure. Nitrate samples containing 4.00-ml aliquots of a 1.000×10^{-3} $\underline{M}$ sodium nitrate solution were reacted with 1.00 ml of 2.000×10^{-2} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution. The mole ratio sodium nitrate to reagent was 0.2. The absorbances of the diluted reaction mixtures were measured at 384 nm.

The precision of the method was quite good, as shown by the data presented in Table 6. The data show the precision that was obtained from the analysis of 10 nitrate samples, solutions numbered

Table 6. Precision Data

Solution Number	A @ 384 nm	Solution Number	A @ 384 nm	Solution Number	A @ 384 nm
241	0.450	259	0.452	434	0.454
242	0.454	260	0.452	435	0.458
243	0.453	266	0.453	459	0.454
244	0.452	267	0.455	468	0.457
245	0.452	331	0.454	481	0.452
246	0.452	375	0.451	482	0.454
247	0.452	376	0.452	501	0.455
248	0.457	377	0.454	502	0.458
249	0.452	378	0.458		
250	0.453	388	0.458		

	Optimum Results[a]	Long-Range Results[b]
Mean Absorbance:	0.453	0.454
Average Deviation:	0.0013	0.0018
Standard Deviation:	0.0019	0.0024
Relative Standard Deviation:	0.41 %	0.53 %

[a]Solutions number 241-250 were prepared using one reagent solution and one nitrate solution. The precision data calculated from their absorbance values have been listed under optimum results.

[b]Solutions number 259-502 were prepared using three different reagent solutions and 3 different nitrate solutions over a 3-month period. The precision data calculated using the absorbance values of all solutions have been listed under the long-range results.

241-250, that were analyzed in a single day and presented as the "Optimum Results", as well as the precision that was obtained from nitrate samples which were run as standards for the studies in this research and collected over a 3-month period and listed as the "Long Range Results". The long-range precision of 0.53 %, relative standard deviation, which also employed several reaction solutions prepared over the 3-month period, differed only slightly from the precision of 0.41 %, relative standard deviation, obtained on a single-day's run. The data indicated that the relative standard deviation of this method for the determination of nitrate under ideal conditions was lower in magnitude than the error usually encountered with the instrumentation employed.

F. Interference Study

The effect on the formation of 2-nitro-1-naphthol-4-sulfonic acid of several foreign ions that may be present as impurities in natural waters was studied under optimum experimental conditions, as described in the precision experiments and the results have been presented in Table 7. A particular foreign ion was considered to interfere with the nitration reaction if the absorbance of the diluted reaction mixture that contained this species fell outside the range of ± 3 standard deviations of the average value for standard solutions run without the foreign ion. From the long-range results of the precision study, which consisted of the standards that were run with the foreign-ion interference study, an average absorbance at 384 nm of 0.454 was obtained with a standard deviation of ± 0.0024. Therefore, a species was considered to interfere with the nitration system if the absorbance of the diluted reaction mixture that contained the species

Table 7. The Effect of Foreign Ions

Foreign Ion Added (ml)	Substance Added	Conc.[a] (ppm)	A @ 384 nm	Deviation
1.00	NaNO$_2$	20.0	2.355	+
1.00	NaF	20.0	0.456	No
10.00	NaCl	20.0	0.480	+
1.00	NaBr	20.0	0.406	-
1.00	NaI	20.0	0.402	-
1.00	NaClO$_2$	20.0	0.685	+
1.00	NaClO$_3$	20.0	0.762	+
1.00	NaClO$_4$	20.0	0.456	No
1.00	NaBrO$_3$	20.0	0.845	+
1.00	KIO$_3$	20.0	0.738	+
1.00	KIO$_4$	20.0	0.741	+
1.00	FeSO$_4 \cdot 7H_2O$	20.0	0.092	-
1.00	MgSO$_4 \cdot 7H_2O$	200	0.430	-
1.00	MnSO$_4 \cdot H_2O$	20.0	0.449	No
1.00	NiSO$_4 \cdot 6H_2O$	20.0	0.324	-
1.00	CoSO$_4 \cdot 7H_2O$	20.0	0.419	-
1.00	(NH$_4$)$_2$SO$_4$	20.0	0.407	-
1.00	PbSO$_4$	--b	0.456	No
1.00	CuSO$_4$	--b	0.460	No
1.00	CaSO$_4$	--b	0.460	No
5.00	CaSO$_4$	--b	0.459	No
1.00	KMnO$_4$	20.0	0.970	+

Table 7. The Effect of Foreign Ions
(Continued)

Foreign Ion Added (ml)	Substance Added	Conc.[a] (ppm)	A @ 384 nm	Deviation
1.00	$NaHCO_3$	1000	0.446	No
1.00	$K_2S_2O_8$	20.0	0.060	-
1.00	$K_2Cr_2O_7$	20.0	0.140	-
1.00	$Na_2S_2O_3 \cdot 5H_2O$	20.0	0.259	-
1.00	Na_2SO_3	20.0	0.449	No
1.00	$Na_2C_2O_4$	20.0	0.456	No
1.00	$NaH_2PO_4 \cdot H_2O$	20.0	0.457	No
1.00	$Na_2MoO_4 \cdot 2H_2O$	20.0	0.460	No
1.00	$Na\ C_2H_3O_2 \cdot 3H_2O$	20.0	0.457	No
1.00	$Na_4P_2O_7 \cdot 10H_2O$	20.0	0.452	No
1.00	$Na_2B_4O_7 \cdot 10H_2O$	20.0	0.277	-
1.00	$Na_2EDTA \cdot 2H_2O$	200	0.455	No
1.00	Ag_2SO_4	25.0	0.454	No
solid[c]	$HgSO_4$	200	0.454	No
solid[c]	$Fe_2(SO_4)_3 \cdot 7H_2O$	200	0.621	+
0.10	H_2NSO_2OH	100	0.430	-
0.10	Sb^{+++}	100	0.461	No
1.00	Sb^{+++}	1000	0.461	No
1.0	$CO(NH_2)_2$	200	0.455	No

[a]This is the ppm concentration of substance calculated in a 50-ml water sample.

[b]This is a saturated solution. [c]Approximately 10 mg of solid was added.

fell outside the absorbance range of 0.454 ± 0.007. In Table 7, a plus sign (+), No, or negative sign (-) under the column headed "Deviation", referred to whether the added foreign ion interfered by increasing the absorbance, did not interfere, or interfered by decreasing the absorbance, respectively. The more common ions usually found in natural waters that interfered in the nitration system included nitrite, chloride, iron, and magnesium. As expected, most oxidizing agents and reducing agents interfered with the nitration system.

Chloride ion, a species found in natural waters at various levels as well as one that is usually a major offender with the majority of methods based upon a nitration system, and silver ion, a species commonly used for the removal of chloride, were examined in detail, and interference thresholds were determined for these by varying their concentrations in a series of nitrate samples. The interference threshold data for chloride and silver are presented in Tables 8 and 9, respectively.

The interference threshold for chloride ion was found to lie between 1.0 and 2.0 ppm. In using the phenoldisulfonic acid method for the determination of nitrate, the American Water Works Association (12) described that even a small concentration of chloride resulted in nitrate loss and that the chloride content should be reduced below 10 ppm. For silver ion, the interference threshold was found to lie between 175 and 200 ppm as indicated in Table 9. The relatively high concentration of silver ion over chloride ion that was compatable with this nitration system, allowed the addition of a known amount of silver solution to a water sample containing a small amount of chloride as described in the proposed procedure for the determination of nitrate in water.

Table 8. Chloride Interference Threshold Data

Cl⁻ (mg)	Concentration[a] (ppm)	A @ 384 nm
0	0	0.453
0	0	0.455
0.001	0.02	0.454
0.005	0.10	0.455
0.010	0.20	0.453
0.025	0.50	0.453
0.050	1.0	0.460
0.10	2.0	0.438
0.50	10.0	0.522
1.00	20.0	0.480
1.50	30.0	0.489

[a]The ppm concentration of chloride ion was calculated from the mg chloride in a 50-ml water sample.

Table 9. Silver Ion Interference Threshold Data

Ag_2SO_4 Soln. Added[a] (ml)	Silver Ion Concentration[b] (ppm)	A @ 384 nm
-----	-----	0.453
-----	-----	0.454
1.00	25.0	0.454
2.00	50.0	0.451
3.00	75.0	0.449
4.00	100	0.446
5.00	125	0.449
6.00	150	0.447
7.00	175	0.446
8.00	200	0.441

[a]This solution contained 1.25 mg silver-ion/ml.

[b]The ppm concentration of silver ion was calculated from the mg silver ion in a 50-ml water sample.

G. Beer's Law

The effect of varied nitrate concentrations on the nitration system was studied in 10.95 $\underline{M}$ sulfuric acid at 50 °C. The objective was to determine the working range of nitrate nitrogen that could be determined by the reaction with zinc 1-naphthol-4-sulfonate. Since 2-nitro-1-naphthol-4-sulfonic acid, the product of the nitration system, shows wavelength maxima at 384 nm in acidic medium and at 432 nm in basic medium (13), Beer's-law studies were conducted at both wavelengths. The Beer's-law curves for the nitration system in acidic medium and in basic medium are shown in Figures 8 and 9, respectively.

In acidic medium, nitrate samples that contained from 0.28 to 2.8 ppm nitrate nitrogen, were reacted with a 2.00 x 10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, so that the maximum mole ratio sodium nitrate to reagent was 0.5. The absorbances of the diluted reaction mixtures, which contained 1.10 $\underline{M}$ sulfuric acid, were measured at 384 nm. Beer's law was obeyed over the entire nitrate-nitrogen range of 0.28 to 2.8 ppm. This linearity of the working curve through the absorbance range of 0 to 1.14 indicated that a working curve could be obtained from a minimum number of nitrate standards and still achieve a high degree of accuracy.

For the Beer's-law study of the nitration system in basic medium, the general reaction procedure was employed except that after the reaction was quenched with ice-cold water, 25 ml of 12 $\underline{M}$ sodium hydroxide were slowly added. The flasks were equilibrated to room temperature and diluted to volume, and the absorbances of the solutions were measured at 442 nm. The diluted reaction mixtures in 100-ml
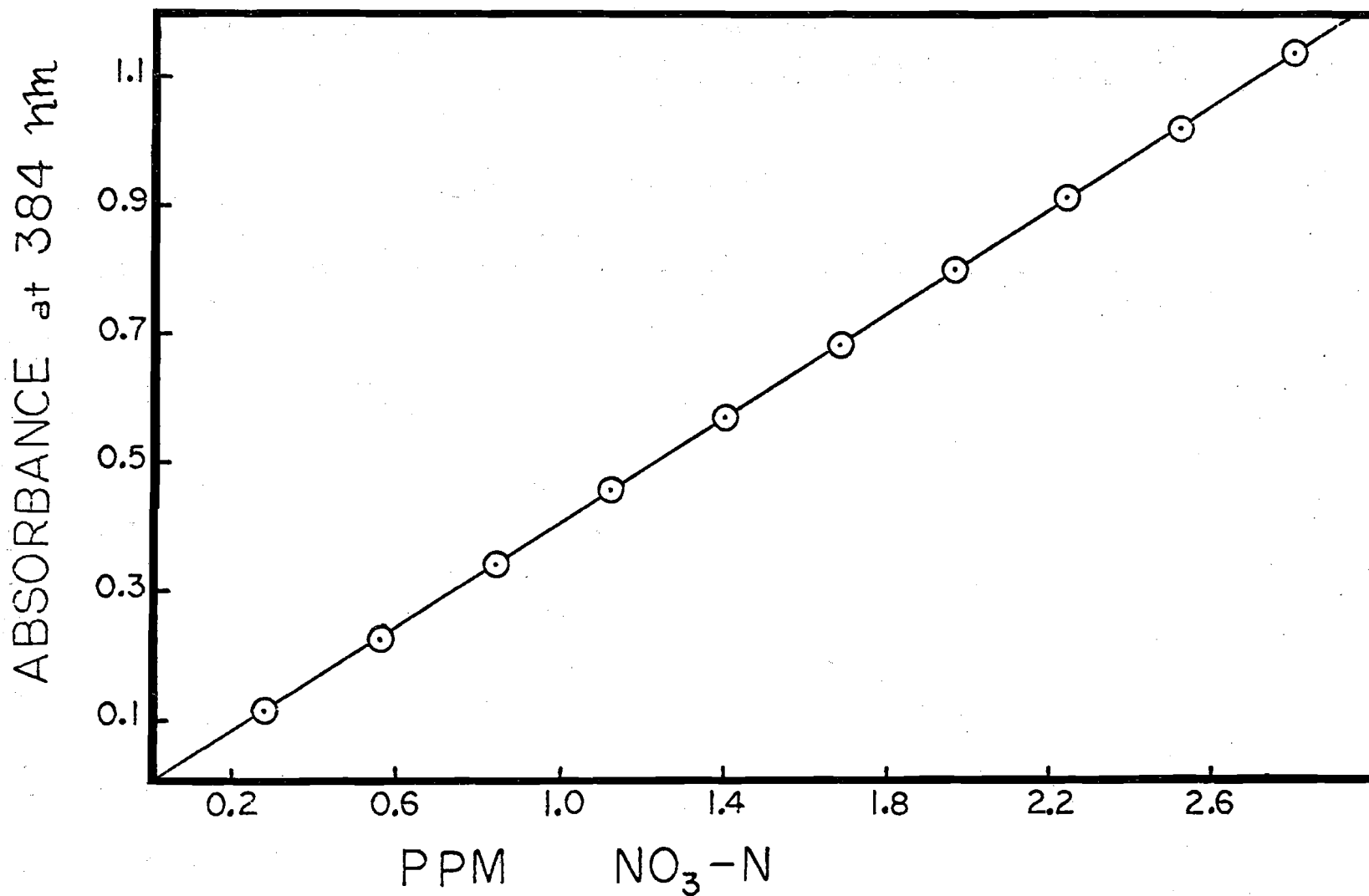
Figure 8. Beer's-law plot of 2-nitro-1-naphthol-4-sulfonic acid as a function of nitrate-nitrogen concentration: 4.000 x 10^{-4} $\underline{M}$ reagent, 2.000 x 10^{-5} to 2.000 x 10^{-4} $\underline{M}$ $NaNO_3$, 10.95 $\underline{M}$ H_2SO_4, temperature of 50 °C, and time of 30 minutes
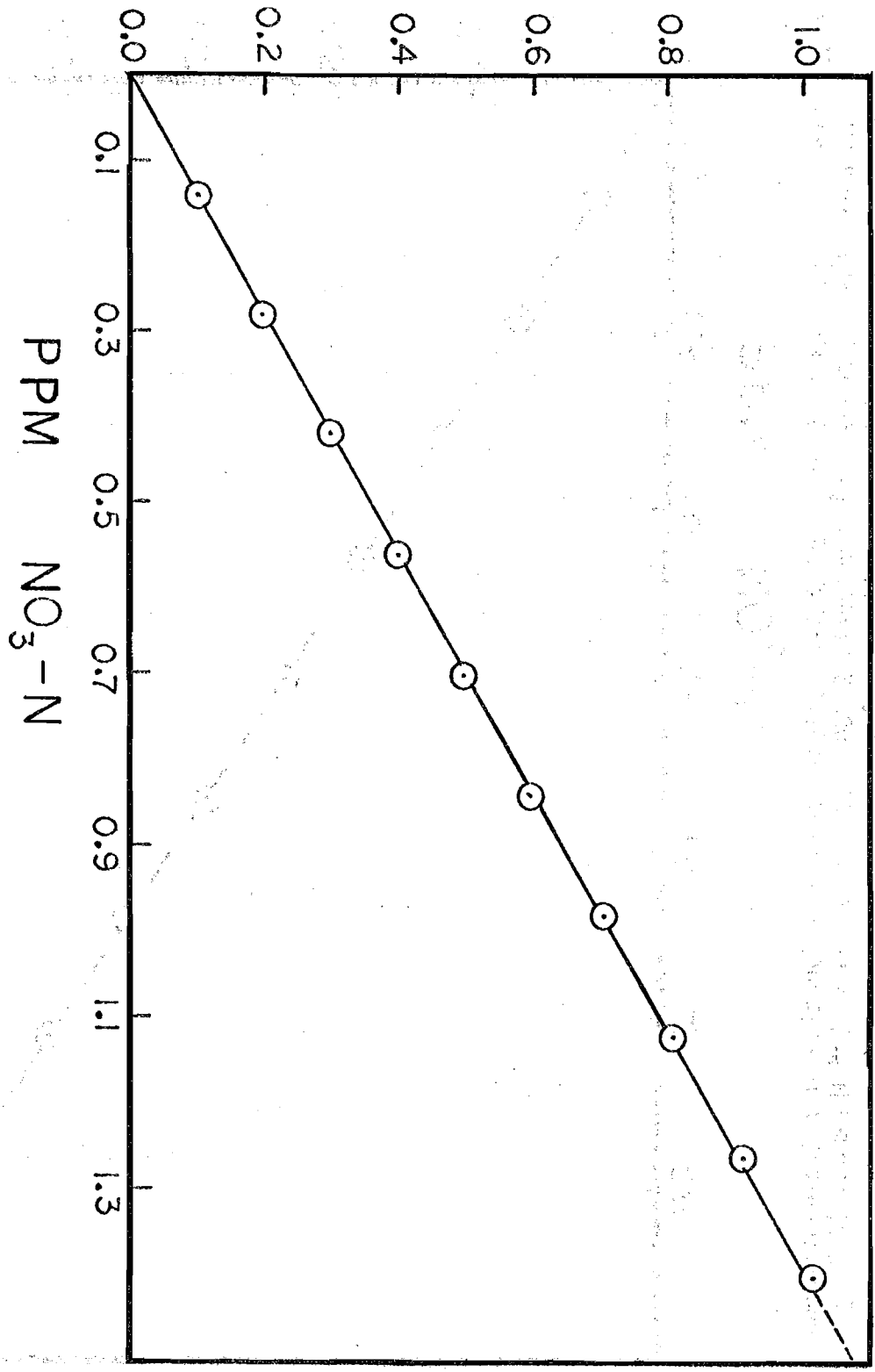
Figure 9. Beer's-law plot of disodium 2-nitro-1-naphtholate-4-sulfonate as a function of nitrate-nitrogen concentration: 1.000 x 10-5 to 1.000 x 10-4 $\underline{M}$ NaNO$_3$, 2.000 x 10-4 $\underline{M}$ reagent, 10.95 $\underline{M}$ H$_2$SO$_4$, temperature of 50.0 °C, and time of 30 minutes.

volumetric flasks contained 1.9 $\underline{M}$ sodium hydroxide. Beer's law was obeyed over the nitrate-nitrogen range of 0.14 to 1.4 ppm. The working curve was linear over the entire absorbance range of 0 to 1.02. A significant advantage in using the 442 nm wavelength maximum for the reaction product in basic medium, was the approximate twofold increase in sensitivity that resulted. An interesting phenomena that could not be explained was the shift in an absorbance maximum for the 2-nitro-1-naphtholate-4-sulfonate dianion from the reported 432 nm (7) to the experimentally observed wavelength maximum at 442 nm. Possibly, this shift may have resulted from solution effects produced on the product by the diluted reaction mixture.

The reaction of zinc 1-naphthol-4-sulfonate with nitrate was studied in detail, and the optimum reaction parameters of temperature, time, sulfuric acid concentration and nitrate concentration are summarized in Table 10. The optimum nitration conditions were a 0.5 mole ratio or less of nitrate nitrogen to zinc 1-naphthol-4-sulfonate, in an 11.0 $\pm$ 0.2 $\underline{M}$ sulfuric acid medium at a reaction temperature of 50 °C for 30 minutes.

The proposed method may be applicable to water samples containing nitrate-nitrogen concentrations at either of two levels determined by the final pH of the diluted reaction mixtures. Dilution of the sulfuric acid reaction mixtures with water, followed by the direct absorbance measurements at 384 nm, is applicable to water samples containing from 0.028 to 2.8 ppm nitrate nitrogen. Neutralization of the reaction mixtures with alkali, subsequent dilution with water, and measurement of the absorbances at 442 nm, allows the determination of nitrate levels in water ranging from 0.014 to 1.4 ppm nitrate nitrogen. This latter procedure provides an approximate two-fold increase in sensitivity.

Table 10. The Optimum Reaction Parameters for the Quantitative
Formation of 2-Nitro-1-Naphthol-4-Sulfonic Acid

Temperature	50 °C
Time	$\geq$ 30 minutes
H_2SO_4 Concentration	10.8-11.2 $\underline{M}$
Ratio: (NO_3^-)/(Reagent)	$\leq$ 0.5
Nitrate-N Concentration[a]	
384 nm (Acidic Media)	1.4-140 µg/50 ml Sample
	(0.028-2.8 ppm NO_3^--N)
422 nm (Basic Media)	1.4-140 µg/100 ml Sample
	(0.014-1.4 ppm NO_3^--N)

[a]The lower limit was estimated as the nitrate nitrogen
concentration in either acidic or basic media that produced
an absorbance of 0.01 on the respective Beer's-law graphs
presented in Figures 8 and 9, respectively.

H. Natural-Water Samples

Before a natural-water sample was tested for nitrate concentration by the proposed method, it was necessary to first determine the recovery of nitrate in standard-nitrate samples. Following the pretreatment steps for the removal of color, nitrite, and chloride, nitrate nitrogen in standard samples was determined by the proposed method. Several standards were not subjected to water pretreatment steps, but were subjected only to the spectro-photometric procedure for nitrate determination. The pertinent results from this study are presented in Table 11.

The results from the determination of nitrate in standard nitrate samples, with and without water pretreatment steps, would provide data for an evaluation of the proposed method. The data would clearly indicate losses in nitrate recovery resulting from the sample pretreatment steps as well as nitrate losses that result from the proposed spectrophotometric procedure. Also, the calculation of statistical data for the two sets of samples would provide an estimate of the accuracy and precision of the proposed method.

The data obtained from the determination of nitrate in untreated standards (standards that were not subjected to water pretreatment steps) gave an average absorbance of 0.451, which corresponded to 1.11 ppm nitrate nitrogen. A comparision of the experimentally found average absorbance value with the theoretically calculated absorbance of 0.448, gave a 101 % average recovery of nitrate from the determination of 6 samples. This high recovery

Table 11. Results on Standard Nitrate Samples with and without Water
Pretreatment Steps Using the Proposed Spectrophotometric Procedure[a,b]

	Untreated Standards			Treated Standards		
	A @ 384 nm	NO_3^- ppm		A @ 384 nm	NO_3^- ppm	Recovery %
	0.448	1.10		0.213	0.53	47
	0.452	1.11		0.198	0.50	44
	0.451	1.11		0.270	0.67	60
	0.447	1.10		0.327	0.81	73
	0.455	1.12		0.142	0.36	31
	0.454	1.12		0.188	0.47	42
	0.449	1.10		0.234	0.58	52
	0.454	1.12				
Average	0.451	1.11		0.225	0.56	50
Std. Dev.	0.003			0.060		

[a]Each standard nitrate sample contained 0.340 mg sodium nitrate and was prepared from a 4.00 ml aliquot of 1.00×10^{-3} $\underline{M}$ sodium nitrate solution.

[b]Treated standards were subjected to the water pretreatment procedure for the removal of color, nitrite, and chloride, while untreated standards were not.

of known concentration of nitrate indicated that the proposed spectrophotometric procedure was very accurate. Also, high precision was achieved with the proposed method, as indicated by the low standard deviation of 0.003.

The determination of nitrate in treated standards (standard nitrate samples that were subjected to the water pretreatment steps for the removal of color, nitrite, and chloride) yielded an average absorbance of 0.225, which corresponds to 0.56 ppm nitrate nitrogen. A comparison of this data with the data from the untreated standards gave nitrate recoveries ranging from 31 % to 73 %, with an average nitrate recovery of 50 %. The low recoveries of nitrate were accompanied by low precision, as shown by the large standard deviation of 0.060 that was calculated from the data.

The low accuracy and precision that were obtained for the determination of nitrate in treated standards resulted from nitrate losses that occurred during the water pretreatment steps. The pretreatment steps were similar to those employed in the phenol-disulfonic acid method for the determination of nitrate in water (12). When considering the chemistry associated with the pretreatment procedures, several steps may perhaps be responsible for nitrate losses at the ppm levels. In the step for the oxidation of nitrite to nitrate, acidification with sulfuric scid may produce nitrate losses through the evolution of nitric acid. In this same step and during removal of color by flocculation with aluminum hydroxide, neutralization of the acidic solution precipitates hydrous manganese dioxide and hydrous aluminum oxide as well as metals that may be present in some natural waters in high concentrations, and thus

may produce nitrate losses through coprecipitation. In the sample evaporation step, absorbed carbon dioxide may lower the solution pH to produce loss of nitrate as nitric acid.

Using the proposed method, the nitrate concentration was determined in a natural-water sample obtained from the Carbondale City Reservoir, Carbondale, Illinois. Aliquots of the water sample, both "spiked" with a known amount of nitrate, and unspiked, were subjected to the water pretreatment steps for the removal of color, nitrite, and chloride. The low chloride concentration found in the natural water, allowed the removal of this contaminant by the addition of a tolerable excess of silver sulfate solution. The pertinent data from the determination of nitrate in the natural-water sample are presented in Table 12.

The nitrate determinations on the natural water and "spiked" water were repeated 6 times in order to have sufficient data for the calculation of meaningful, statistical data. The standard deviation of 0.009 calculated from the nitrate concentrations found in the "unspiked" water samples indicated that an acceptable level of precision was achieved by the proposed method. The larger standard deviation of 0.032 calculated for the "spiked" water samples indicated that lower precision was achieved at the higher nitrate level.

The experimental recovery of the nitrate added to the "spiked" water samples ranged from 70 to 90 %, with an average recovery of 80 % for six results. The 0.340 mg of sodium nitrate that was added to the "spiked" water samples was equivalent to the weight of sodium nitrate in the standards, whose data are presented in Table 11. Therefore, for a 100 % recovery of the "spiked" nitrate over the nitrate content in the water sample, a difference in absorbance of 0.451 between the

Table 12. Determination of Nitrate in a Natural-Water Sample

	Water Samples			"Spiked" Water Samples[a]		
	A @ 384 nm	NO_3^- ppm		A @ 384 nm	NO_3^- ppm	Recovery[b] %
	0.126	0.32		0.482	1.18	83
	0.103	0.26		0.438	1.08	73
	0.102	0.26		0.514	1.27	90
	0.110	0.28		0.477	1.17	81
	0.108	0.27		0.484	1.19	83
	0.110	0.28		0.427	1.05	70
Average	0.110	0.28		0.470	1.15	80
Std. dev.:	0.009			0.032		

[a]The "spike" consisted of a 4.00 ml aliquot of 1.00×10^{-3} M sodium nitrate solution, the same amount of nitrate in standards in Table 11.

[b]The recovery of "spiked" nitrate in the water samples was based upon the recovery of nitrate in standards not subjected to water pretreatment steps. A 0.451 absorbance for the spiked water sample higher than the 0.110 average absorbance for the water sample would indicate 100 % recovery.

"spiked" water sample and the "unspiked" water sample should have been observed. This difference in absorbance would be quivalent to 1.11 ppm nitrate nitrogen. Also, an absorbance of 0.561 for the spiked water sample would indicate 100 % recovery.

The large range in recovery values is not uncommon for methods presently in use for the determination of nitrate in natural waters. Greenberg *et al.* (14) studied the accuracy and precision of the phenoldisulfonic acid method and reported an average range of recoveries varying from 92.1 % to 115.8 %. For evaluation purposes, Abercrombie and Caskey (9) employed the phenoldisulfonic acid method for the determination of nitrate in natural waters and obtained nitrate recoveries ranging from 45 % to 115 %.

In order to evaluate either the precision or the accuracy of the proposed spectrophotometric method for the determination of nitrate, a careful evaluation of the data in Tables 11 and 12 must be made. Also, the sample pretreatment procedure must be deleted from the proposed spectrophotometric procedure before the latter is evaluated. The sample pretreatment procedure was similar to the procedure employed in the phenoldisulfonic acid method and accepted by the American Water Works Association (12). In evaluating the data presented in Tables 11 and 12 for the recoveries of nitrate found in standards and water samples, the lower precision and accuracy appeared to result from nitrate losses within the pretreatment procedures. In Table 11 good precision and accuracy were obtained with untreated nitrate standards, while lower results were obtained for nitrate standards that were carried through the pretreatment steps. An improvement in the pretreatment steps would produce an equivalent improvement in the

accuracy and precision of the proposed method, as well as the currently
employed phenoldisulfonic acid method.

V. CONCLUSION

Zinc 1-naphthol-4-sulfonate, prepared from practical-grade sodium 1-naphthol-4-sulfonate, was purified, isolated, and characterized as the eight and one-half hydrate, which yielded a molecular weight of 664.95.

The chemistry of the reaction of sodium nitrate with zinc 1-naphthol-4-sulfonate in a sulfuric acid medium was extensively studied and the optimum nitration conditions were determined. The quantitative formation of 2-nitro-1-naphthol-4-sulfonic acid required an 11.0 $\pm$ 0.2 $\underline{M}$ sulfuric acid reaction medium at 40 °, 45 °, and 50 °C for reaction times of 50, 40, and 30 minutes, respectively. The 50 °C reaction temperature and 30 minute reaction time were chosen as optimum conditions for these variables.

The stability of zinc 1-naphthol-4-sulfonate in 11.0 $\underline{M}$ sulfuric acid as a function of time and temperature was excellent.

The effects of foreign ions on the formation of 2-nitro-1-naphthol-4-sulfonic acid were studied and the more common species causing interference were chloride, fluoride, nitrite, iron and manganese. Interference threshold limits were found for chloride and silver.

Using the optimum, experimentally-found reaction conditions, zinc 1-naphthol-4-sulfonate was proposed as a spectrophometric reagent for the determination of nitrate in natural waters. The proposed method utilized the same water pretreatment procedure given for the phenoldisulfonic acid method for nitrate determination as described by the American Public Health Association in "Standard Methods for

the Examination of Water and Wastewater". The system conformed to Beer's law from 1.4 to 140 µg nitrate-N/50-ml water sample for absorbance measurements at 384 nm, an absorbance maximum for the nitration product in an acidic medium. By using the 442 nm absorption maximum for the nitration product in a basic medium, Beer's law was conformed from 1.4 to 140 µg nitrate-N/100-ml water sample.

The proposed spectrophotometric procedure was shown to be highly accurate and precise for nitrate recovery in standard, nitrate samples, giving an average nitrate recovery of 101 % with a standard deviation of 0.003 for 6 determinations. Utilization of a water pretreatment procedure currently employed in the phenoldisulfonic acid method for the removal of color, nitrite, and chloride in natural water samples decreased the accuracy and precision for nitrate recovery.

Nitrate was determined in natural-water samples using the proposed spectrophotometric method. The observed precision was only slightly poorer than that observed for standards, but the observed recovery of nitrate was comparable to that of the phenoldisulfonic acid method.

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Chapter 2

ZINC 1-NAPHTHOL-4-SULFONATE AS A REAGENT FOR THE
SPECTROPHOTOMETRIC DETERMINATION OF NITRITE IN WATER

Jean C. Lewis Swan and Albert L. Caskey

I. INTRODUCTION

Nitrogen, like all other elements involved in life processes, is
cycled through a highly ordered pattern. There are many possible paths
a single nitrogen atom may follow around the cycle, but it is subject
to physical and chemical laws. The object of scientific thought and
research is to discover this true order, and to define laws. The
realization that the universe is not randomly chaotic, but the orderly
and consistent creation of a reasonable God, provides a basis for
efforts to discover--via reason, including the scientific method--
truths in the universe.

The scope of human observation and evaluation of the universe is
limited by the resolution of our instruments and by the finiteness of
our minds, but our very real ability to conceive of infinity suggests
that there is no end to the order of our environment, as well as no

possibility of our minds exhaustively understanding it.

All the cycles of life processes, whether they be of elements, water or of seasons and forces in and around the earth, may be pictured as an elaborate web--paths of matter directed by predetermined order.

Nitrogen is essential to life in that it is required for the biosynthesis of proteins, nucleic acids and vitamins. While there is an abundance of nitrogen, N_2, in the atmosphere, nitrogen in this form is relatively chemically inert and most organisms must assimilate nitrogen in combined form. Ammonia, nitrite ion amd nitrate ion are present in low concentrations in soils and natural waters, so their supply must be replenished as it is used. Nitrogen is cycled through living organisms via nutritional interdependence.

Green plants assimilate nitrogen as nitrate from the soil and reduce it to ammonia, and while the reduction is not energy yielding, it puts the nitrogen in a form useful for biosynthesis. Amino acids which are then synthesized are mostly for protein synthesis since plants grow continuously, but they are also used for oxidative catabolism.

In addition, green plants may assimilate nitrogen in the form of ammonia which is provided by nitrogen-fixing bacteria. These microorganisms have the ability to reduce atmospheric molecular nitrogen (N_2), though not in an energy yielding reaction, and incorporate the relatively inert N_2 into the life cycle. Nitrogen is also lost from life processes to the atmosphere as N_2 as the result of denitrification by other bacteria which use nitrate as their terminal electron-acceptor.

When animals ingest plant protein, it is hydrolized and then is either reassembled as animal protein, or is catabolized for energy and ultimately excreted as urea, ammonia or uric acid, depending on the animal. Upon death of the animal, deamination of amino acids during decomposition releases ammonia. During this process of ammonification in animals, the nitrogen remains largely in its reduced form.

Most microorganisms can use ammonia as their only or main nitrogen source incorporating it by amination, with no reduction steps necessary. Also, nitrification is performed by two groups of obligately aerobic chemolithotrophic bacteria. Bacteria of the genus *Nitrosomonas* oxidize ammonia to nitrite, and bacteria of the genus *Nitrobacter* oxidize nitrite to nitrate, both for the purpose of obtaining energy. Thus the nitrate supply for plants is replenished.

An abundance of nitrate in the soil enhances plant growth, and so nitrogen-containing fertilizers are applied to croplands. The nitrogen may be applied in the form of nitrate or ammonia. In the latter case, it is oxidized by the soil bacteria (above) prior to assimilation by the plant. However, since nitrates are water soluble, they are easily leached, and erosion of fertilized soil may lead to their accumulation in ponds. Algae and fungi growing in the ponds assimilate nitrate, reducing it to ammonia for amination. In such a reducing atmosphere, nitrite is short-lived.

In still, quiet water that is rich in fertilizer runoff, algae will grow abnormally fast, until without enough natural feeders present, the surface of the water becomes covered with algae. This blocks sunlight to water below, causing the algae in the lower layers to die. The pond is then no longer a reducing medium and

nitrite may accumulate.

An accurate method for the determination of nitrite would help to indicate nitrite pollution in waters affected by fertilizer runoff. Nitrite itself is dangerous in that one of its decomposition products, nitric oxide, is able to complex irreversibly with the hemoglobin molecule in the place of oxygen. Nitrite is also known to react with secondary amines in acid (such as in the digestive tract) to form carcinogenic nitrosamines. These are facts which make it desirable to have knowledge concerning nitrite concentrations in the aqueous environment.

However, many threads--not facts--of other evidences also exist. The tentative conclusion of recent research which studied deaths from cancer in the town of Worksop, Nottinghamshire, England, and which was reported in the *British Journal of Cancer*, indicated drinking water containing unusually high concentrations of nitrate may increase the incidence of cancer of the stomach. Comparisions were made with nearby towns where much lower concentrations of nitrate were observed, and it was shown that stomach cancer was the cause of 27 percent more fatalities in Worksop. Worksop's water contained 90 mg nitrate per liter, which was believed to come from treated sewage. Was the real culprit nitrite, and not nitrate? To study that question, a method is needed which determines nitrite in the presence of relatively large concentrations of nitrate.

II. HISTORICAL

Witt and Kaufmann (1), in 1891, first described the synthesis of 2-nitroso-1-naphthol-4-sulfonic acid from sodium 1-naphthol-4-sulfonate and sodium nitrite. The sodium 1-naphthol-4-sulfonate was dissolved in water, and the resulting solution was acidified with hydrochloric acid and subsequently was well cooled. To this cold solution, a concentrated solution of sodium nitrite was added dropwise. The crystalline product was suction filtered, dissolved in hot water and reprecipitated by addition of hydrochloric acid. The large yellow-brown crystals of the product, the free acid (not the sodium salt), were obtained in near quantitative yield.

Recent work in this laboratory has shown that several recrystallizations and air drying yield the tetrahydrate. Drying to constant weight at 115 °C gives the one-half hydrate, a very hygroscopic and thermally unstable material. The anhydrous acid may be prepared over anhydrous magnesium perchlorate in vacuum at room temperature. The water-free compound is very soluble in aqueous alcohol, giving a strongly acidic solution, and considerably less soluble in water and aqueous hydrochloric acid.

It seems reasonable to expect the synthesis of 2-nitroso-1-naphthol-4-sulfonic acid to follow a classical mechanism. The reaction probably involves aromatic-ring nitrosation in which the nitrosonium ion, NO^+, attacks the 1-naphthol-4-sulfonate ion by electrophilic aromatic substitution, displacing H^+. The hydroxy group is a strongly activating, *ortho-para* director while the sulfonic acid group is a deactivating, *meta* director. The nitrosonium ion therefore displaces the H^+ at the number-2 position.

1-Naphthol-4-sulfonate Ion

$$NO_2^- + H^+ \rightleftharpoons HONO \xrightarrow[H^+]{} [H_2ONO^+] \longrightarrow H_2O + NO^+$$
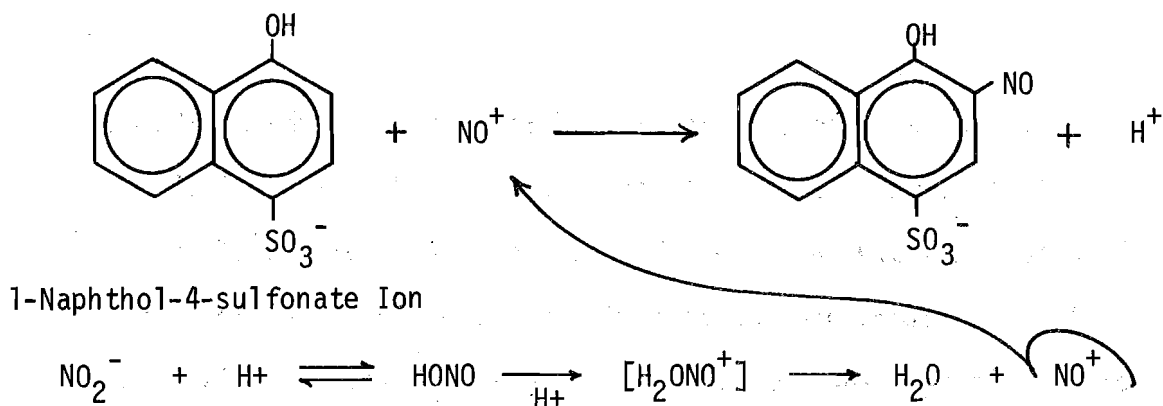
The red-colored, water-soluble complex which is formed between 2-nitroso-1-naphthol-4-sulfonic acid and cobalt(II), was investigated by Wise and Brandt (2) for use as the basis for a spectrophotometric method to quantitatively determine cobalt. They reported that the reagent reacts with cobalt(II) in aqueous solution at a pH of 6.0 - 10.0 to give constant absorbance readings at 525 nm, an absorption maximum for the complex. A pH of 7.0 was chosen by Wise and Brandt for their work, because at a pH of 7.0 the absorbance of the complex at 525 nm is at a maximum, and the absorbance of the reagent, which is in excess, is at a minimum. Sodium hydroxide was used for neutralization since ammonium hydroxide interferes by forming complexes between ammonia and cobalt(II).

The system is stable with respect to time (at least three months), to the temperature of the reaction (10 - 95 °C), and to the temperature of the reactants, provided the temperature of the system at the time of absorbance measurement is constant.

The ratio of reagent to cobalt concentration should be such that enough reagent (ligand) is present to complex all of the cobalt. Higher ratios at a pH of 7, will not significantly increase the absorbance at 525 nm. A ligand to cobalt ratio of 4.8 was found by

Wise and Brandt to be sufficient in the determination of cobalt.

Due to the completeness of the complex formation, its water solubility and stability, and due to the ease of preparation of 2-nitroso-1-naphthol-4-sulfonic acid, it seemed profitable to investigate the conditions necessary to quantitatively nitrosolate the 1-naphthol-4-sulfonate for the purpose of developing a spectrophoto-metric method for the determination of nitrite in natural waters.

If the nitrosolation can be forced to completion by excess 1-naphthol-4-sulfonate ion, and if the complex formation then can be forced to completion by excess cobalt(II), the development of such a method is possible.

III. EXPERIMENTAL

A. Reagents

Reagent-grade chemicals meeting American Chemical Society specifications were used whenever they were commercially available. A list describing the commercial chemicals used has been presented in Table 1.

All water used was distilled water, and was further purified by monobed ion-exchange resin.

The zinc 1-naphthol-4-sulfonate was prepared by the procedure of Antepenko (3). Purified sodium 1-naphthol-4-sulfonate, 40 g, was dissolved in 120 ml of hot, deionized water. Zinc chloride, 50 g, was dissolved in a minimum amount of hot water and this solution was added to the sodium 1-naphthol-4-sulfonate solution. The large crystals which formed upon slow cooling, were collected on a sintered-glass funnel, recrystallized three times from water and air dried 24 hours at room temperature.

The 2-nitroso-1-naphthol-4-sulfonic acid was prepared using a modification of the procedure of Witt and Kaufmann (1). The material was recrystallized from dilute hydrochloric acid, and the solutions were decolorized with activated charcoal in the recrystallization process.

A stock solution of 4.90×10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate was prepared by rinsing 0.9800 g of this substance into a 500-ml volumetric flask and diluting to volume with distilled water.

Table 1. Chemicals Used and Chemical Suppliers

Compound	Formula	Vendor*	Vendors Catalog Number
Hydrochloric Acid	HCl	1	1090
Sodium Nitrite, crystals	$NaNO_2$	2	SX665 CB735
Sodium Hydroxide, pellets	NaOH	2	SX590 CB724
Cobalt(II) sulfate (Iron and Nickel Free)	$CoSO_4 \cdot 7\ H_2O$	3	
Sodium Nitrate, crystals	$NaNO_3$	2	CB733 SX655
Citric acid, monohydrate granular	$C_6H_8O_7 \cdot H_2O$	2	CX 1725
disodium hydrogen phosphate	Na_2HPO_4	1	2313
hydrogen peroxide 30 % solution	H_2O_2 (30 %)	2	HX640 CB920

* 1 - Baker and Adamson

2 - Matheson, Coleman and Bell

3 - G. F. Smith Chemical Company

A stock solution of 6.608×10^{-3} $\underline{M}$ 2-nitroso-1-naphthol-4-sulfonic acid was prepared by rinsing 0.5739 g of this substance into a 250-ml volumetric flask and diluting to volume with distilled water.

A stock solution of 1.000×10^{-2} $\underline{M}$ cobalt(II) sulfate solution was made by dissolving 2.8114 g of cobalt(II) sulfate heptahydrate in distilled water and diluting to volume with same in a 1000-ml volumetric flask.

A stock solution of 8.00×10^{-4} $\underline{M}$ cobalt(II) sulfate was prepared by diluting 40.00 ml of the 1.000×10^{-2} cobalt(II) sulfate stock solution to 500.0 ml with distilled water.

A stock solution of 4.00×10^{-4} $\underline{M}$ cobalt(II) sulfate was prepared by diluting 20.00 ml of the 1.000×10^{-2} $\underline{M}$ cobalt(II) sulfate stock solution to 500.0 ml with distilled water.

A stock solution fo 2.00×10^{-4} $\underline{M}$ cobalt(II) sulfate was prepared by diluting 10.00 ml of the 1.000×10^{-2} $\underline{M}$ cobalt(II) sulfate stock solution to 500.0 ml with distilled water.

A stock solution of 0.4962 $\underline{M}$ sodium nitrite was prepared by rinsing 17.1189 g of this substance (dried 2.5 hours at 105 °C and cooled) into a 500-ml volumetric flask and diluting to volume with distilled water.

A stock solution of 6.947×10^{-3} $\underline{M}$ sodium nitrite was prepared by diluting 7.00 ml of the 0.4962 $\underline{M}$ sodium nitrite stock solution to 500.0 ml with distilled water.

A stock solution of 6.668×10^{-4} $\underline{M}$ sodium nitrite was prepared by diluting 24.00 ml of the 6.947×10^{-3} $\underline{M}$ sodium nitrite stock solution to 250.0 ml with distilled water.

A stock solution of 6.668×10^{-5} $\underline{M}$ sodium nitrite was prepared by diluting 10.00 ml of the 6.668×10^{-4} $\underline{M}$ sodium nitrite stock solution to 100.0 ml with distilled water.

A stock solution of 0.1613 $\underline{M}$ sodium nitrate was prepared by rinsing 1.3707 g of this substance, which had been dried for four hours at 110 °C and then cooled, into a 100-ml volumetric flask and diluting to volume with distilled water.

A stock solution of 1.613×10^{-2} $\underline{M}$ sodium nitrate solution was prepared by diluting 10.00 ml of the 0.1613 $\underline{M}$ sodium nitrate stock solution to 100.0 ml with distilled water.

A stock solution of 1.0 $\underline{M}$ citric acid was prepared by rinsing 21.0119 g of this substance into a 100-ml volumetric flask and diluting to volume with distilled water.

A stock solution of 0.400 $\underline{M}$ disodium hydrogen phosphate solution was prepared by rinsing 28.3985 g of this substance into a 500-ml volumetric flask and diluting to volume with distilled water.

A stock solution of 0.127 $\underline{M}$ citric acid and 0.0375 $\underline{M}$ disodium hydrogen phosphate was prepared by diluting 12.70 ml of the 1.0 $\underline{M}$ citric acid solution and 9.365 ml of the 0.400 $\underline{M}$ disodium hydrogen phosphate solution to 100.0 ml with distilled water.

B. Equipment

A Beckman Expandomatic SS-2 pH meter (model 76) or a Beckman Research pH meter (model 1019) with a fiber-type saturated-calomel electrode and a glass electrode were used for all pH measurements.

Beckman DB (Cat #1401) and Beckman DK-1A (Cat #96000Y) recording spectrophotometers were used with 1.00-cm, matched, silica

absorption cells or with 10.00-cm, matched, quartz absorption cells.
Absorbance measurements were made at a wavelength of 525 nm with a
chart range of 0-1.0, using a distilled-water blank unless otherwise
indicated.

All glassware was washed with Alconox in hot water and rinsed
six times with distilled water. All volumetric glassware used was
borosilicate and was NBS Class A quality.

C. General Procedure

All work was based on the same procedure, with modifications
occurring when necessary. Accurately measured volumes of sodium
nitrite solution and of reagent solution, zinc 1-naphthol-4-
sulfonate, were pipetted into a 200-ml beaker, diluted to 40 ml
with water, and acidified to pH 2.4 with 2.00 ml 0.098 $\underline{N}$ HCl.
After 15 to 30 minutes for nitrosolation at room temperature, a
volume of cobalt(II) sulfate solution was added and the solution
neutralized to pH 7.0 with dilute sodium hydroxide solution. It
was then transferred to a 100-ml volumetric flask and diluted to
volume with water. The absorbance was measured at 525 nm versus a
distilled-water blank, using 1-cm cells.

D. Recommended Procedure

Quantitatively transfer a water sample (50 to 1000 µg nitrite)
to a 200-ml beaker. Add 20 ml of 5 x 10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate
and adjust to pH 2.4 (add 1.00 ml 0.10 $\underline{N}$ hydrochloric acid for each
20 ml of solution). Allow the solution to stand at least 30 minutes at
room temperature, add 20 ml 8.00 x 10^{-4} $\underline{M}$ cobalt(II) sulfate, add 2.00 ml

buffer solution (0.127 M citric acid and 0.0375 M disodium hydrogen phosphate), and adjust the solution to pH 7 with dilute sodium hydroxide and a pH meter. Transfer the solution to a 100-ml volumetric flask and dilute to volume with distilled water. Allow at least 30 minutes to pass after neutralization to pH 7, then measure the absorbance at 525 nm versus a distilled water blank, using 1-cm, matched cells.

Prepare a calibration plot by the use of standard sodium nitrite solutions prepared from reagent-grade sodium nitrite dried at 110 °C for two hours. Prepare daily very dilute sodium nitrite solutions, less than 1.0×10^{-4} M.

Use more dilute reagent solutions and longer cells with samples which contain less than 0.5 ppm nitrite. Quantitatively transfer a water sample (2 to 50 µg nitrite) to a 200-ml beaker. Follow the above-written procedure, but use 2×10^{-3} M zinc 1-naphthol-4-sulfonate, 4×10^{-4} M cobalt(II) sulfate, and 10-cm, matched cells.

IV. RESULTS AND DISCUSSION

The first problem one faces in the examination of a chemical system, is the availability of pure chemicals. Sodium 1-naphthol-4-sulfonate is available commercially, but is available only in a rather impure state. One would never guess by looking at the commercial material that sodium 1-naphthol-4-sulfonate is a white solid when pure. The reason for the lack of purity is the lack of a good method for its purification. Anhydrous sodium 1-naphthol-4-sulfonate is soluble in absolute ethanol, as are several other sodium salts of organic sulfonic acids; solid-liquid continuous-extraction techniques permit the removal of the desired material from highly colored impurities, but do not permit its separation from isomeric naphtholsulfonic acids.

Hence, following preliminary purification by ethanol extraction, the sodium salt was converted into the zinc salt, which lends itself to recrystallization from aqueous solution (3). White, crystalline, highly pure zinc 1-naphthol-4-sulfonate was used as the source of 1-naphthol-4-sulfonate ions in this work.

A. Effect of pH on Nitrosolation

Protonation of nitrous acid, HONO, and subsequent formation of the electrophile, NO^+, do not occur in neutral media, but they do occur in acidic media. However, in very acidic media nitrous acid decomposes and forms nitric oxide, NO, which is then lost to the atmosphere and rapidly air oxidized to nitrogen dioxide, NO_2, red-brown gas. Thus, it is essential to determine the optimum pH at which nitrosolation quantitatively occurs. A series of solutions

was prepared following the general procedure except that the pH to
which each was adjusted for nitrosolation was varied and the period
of nitrosolation was untimed; 20.00 ml of 4.90 x 10^{-3} $\underline{M}$ zinc
1-naphthol-4-sulfonate solution, 10.00 ml of 6.668 x 10^{-4} $\underline{M}$ sodium
nitrite solution, and 20.00 ml of 2.00 x 10^{-4} $\underline{M}$ cobalt(II) sulfate
solution were used.

The data, reported in Table 2, indicate that maximum absorbance
values were observed in the solutions which were nitrosolated in
the range of pH 2.2 to 2.6. A pH of 2.4 was chosen for routine work.

When the concentrations and volumes of reagent and sodium
nitrite are approximately what they were in this experiment, and are
diluted to 40 ml, addition of 2.00 ml 0.098 $\underline{N}$ HCl acidifies the solution
to pH 2.4, and use of a pH meter is not necessary.

B.   Effect of Time on Nitrosolation

2-Nitroso-1-naphthol-4-sulfonic acid forms almost instantly at
2 °C when a solution of sodium 1-naphthol-4-sulfonate, acidified with
considerable excess hydrochloric acid, is treated with an excess of
a solution of sodium nitrite. Formation of the electrophile, NO^+,
is favored in such a solution and the reaction proceeds rapidly. The
reaction may not proceed rapidly to completion when 1-naphthol-4-
sulfonate ion is in excess.

Hence, the optimum amount of time required for nitrosolation to
quantitatively occur was determined in the presence of excess 1-naphthol-
4-sulfonate ion. A series of solutions was prepared following the
general procedure, except that the period of time allowed for
nitrosolation, that is, the time elapsed between the addition of HCl

Table 2. Effect of pH on Nitrosolation

Solution Number	pH	A @ 525 nm
299	0.0	0.260
300	0.5	0.268
301	1.0	0.273
302	1.5	0.281
303	2.0	0.284
304	2.2	0.285
305	2.4	0.285
306	2.5	0.284
307	2.6	0.285
308	2.8	0.282
309	3.0	0.251
310	3.5	0.066
311	4.0	0.010
312	4.5	0.003
313	5.0	0.003
314[a]	2.5	0.003

[a]Reagent blank.

and the addition of cobalt(II) sulfate solution and neutralization, was varied. Dilute solutions and 10-cm absorption cells were used in order to detect small differences in absorbance; 20.00 ml of 9.882×10^{-4} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, 15.00 ml of 6.668×10^{-5} $\underline{M}$ sodium nitrite solution, and 20.00 ml of 2.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used.

Maximum absorbance values were observed in the solutions which were allowed to nitrosolate for 15 to 30 minutes (Table 3). The reaction was not complete in 10 minutes. A time period of at least 15 minutes was allowed for nitrosolation in all subsequent work.

C. Effect of Temperature on Nitrosolation

The reaction of sodium nitrite with sodium 1-naphthol-4-sulfonate is classically effected at a temperature near 0 °C to minimize the decomposition of nitrous acid, the companion evolution of gas, and the simultaneous formation of foam on the top of the reaction medium. One must consider whether significant decomposition occurs in the system, as used here, at temperatures higher than 0 °C.

Hence, the most practical working temperature was determined at which nitrosolation of zinc 1-naphthol-4-sulfonate quantitatively occurs. A series of solutions was prepared following the general procedure except that the temperature at which they were nitrosolated was varied, and all solutions subsequently were acidified to pH 1 for 10 minutes to maximize any decomposition of nitrous acid; 20.00 ml of 4.918×10^{-3} $\underline{M}$ or 4.173×10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, 10.00 ml of 6.668×10^{-4} $\underline{M}$ sodium nitrite solution, and 20.00 ml of 2.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used.

Table 3. Time Period Needed to Effect Nitrosolation
at 19 °C and pH 2.4

Solution Number	Time Period of Nitrosolation (minutes)	A @ 525 nm
390	0	0.041
391	2.5	0.089
392	5.0	0.155
393	10	0.200
394	15	0.219
395	20	0.213
397	20	0.207
398	25	0.220
399	30	0.221
400	40	0.214

The reaction proceeded satisfactorily at all temperatures studied (Table 4), both above and below room temperature. Laboratory temperature (essentially 20 °C, since the laboratory was air-conditioned; the temperature varied as high as $\sim$ 28 °C) was chosen for nitrosolation in all further work to minimize the total time needed.

D. pH at which Zinc Hydroxide Forms

A precipitate, which caused interference in several solutions, was found to be formed when solutions were made over-alkaline during the neutralization process. In order to determine the pH at which this precipitate forms, a series of solutions was prepared following the general procedure except that in the neutralization process, the pH was increased slowly and carefully until the precipitate formed. The pH was then determined on a pH meter, but absorbance was not measured; 20.00 ml of 4.90 x 10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, 10.00 ml of 6.668 x 10^{-4} $\underline{M}$ sodium nitrite solution, and 20.00 ml of 2.00 x 10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used.

The average pH at which the precipitate formed was 7.97, as is indicated in Table 5, so care was exercised in subsequent experiments to avoid increasing the pH above 7.3 in the neutralization process.

The precipitate was identified as zinc hydroxide. It was soluble in $\sim$1 $\underline{M}$ hydrochloric acid and in concentrated sodium hydroxide. It contained zinc, as shown by the formation from it of white zinc sulfide.

In solutions prepared subsequent to this experiment, the precipitate was occasionally found, though effort had been made to not increase the pH above 7.3 in the neutralization process. After

Table 4. Effect of Temperature on Nitrosolation

Solution Number	Temperature (°C)	A @ 525 nm
265[a]	---	0.005
257	0-4	0.270
267	10	0.271
268	20	0.274
269	30	0.273

[a]Reagent blank.

Table 5. pH at which Zinc Hydroxide Forms

Solution Number	pH
315	8.02
316	7.98
317	7.96
318	7.97
319	7.92
320	7.98
Mean	7.97

the absorbance measurement of one solution containing the precipitate was made, the pH was measured and found to be 7.95.

E. Effect of Excess Zinc 1-Naphthol-4-sulfonate

The classically known common-ion effect declares that a chemical reaction can be forced nearer to completion by the use of excess quantities of one of the reactants. Excess zinc 1-naphthol-4-sulfonate should facilitate quantitative reaction of nitrite ion.

Therefore, the optimum ratio of reagent concentration to nitrite concentration was determined. A series of solutions was prepared as in the general procedure except that the amount of reagent used was varied; 5.00 ml of 6.600×10^{-4} $\underline{M}$ sodium nitrite solution, 1.00 to 20.00 ml of 1.889×10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, and 20.00 or 25.00 ml of 8.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used.

As the mole ratio of reagent, R^{-}, to nitrite increased, the absorbance first rapidly increased and subsequently reached a maximum value (Table 6). Maximum absorbance values were found for the solutions whose mole ratio of reagent to nitrite was 5.7 or higher. The highest ratio studied was 11.45. The mole ratio of cobalt(II) to nitrite was a minimum of 4.85; higher ratios did not affect the data.

The mole ratio of reagent to nitrite was selected to be 9 - 10 for routine work.

F. Time Dependence of Complex Formation

A gradual development of the color of the cobalt complex had been observed in some solutions in which complexation was occurring in the

Table 6. Effect of Excess Zinc 1-Naphthol-4-sulfonate

Solution Number	1.889×10^{-3} M Reagent (ml)	6.600×10^{-4} M NaNO$_2$ (ml)	8.00×10^{-4} M CoSO$_4$ (ml)	A @ 525 nm	Mole Ratio	
					$\frac{R^-}{NO_2^-}$	$\frac{Co^{++}}{NO_2^-}$
501	1.00	5.00	20.00	0.049	0.5725	4.85
502	2.00	5.00	20.00	0.083	1.145	4.85
503	3.00	5.00	20.00	0.109	1.7175	4.85
494	4.00	5.00	25.00	0.117	2.29	6.06
504	5.00	5.00	20.00	0.139	2.863	4.85
495	6.00	5.00	25.00	0.138	3.435	6.06
496	8.00	5.00	25.00	0.146	4.58	6.06
505	10.00	5.00	20.00	0.148	5.725	4.85
497	12.00	5.00	25.00	0.152	6.87	6.06
498	15.00	5.00	25.00	0.150	8.588	6.06
499	16.00	5.00	25.00	0.150	9.16	6.06
500	18.00	5.00	25.00	0.145	10.305	6.06
506	20.00	5.00	20.00	0.150	11.45	4.85

presence of excess cobalt. Therefore, it was essential to investigate
the effect of time on complexation in the presence of excess cobalt.
This effect was somewhat surprising, inasmuch as Wise and Brandt (2)
reported the effectively instantaneous formation of the complex in the
presence of excess ligand.

In order to study the completeness of the complex formation alone,
without possible interference of the nitrosolation reaction, the latter
reaction was omitted in this study. A series of solutions was prepared
following the general procedure except that 2-nitroso-1-naphthol-4-
sulfonic acid was used directly, instead of zinc 1-naphthol-4-sulfonate
and sodium nitrite; 15.00 ml of 6.608×10^{-4} $\underline{M}$ 2-nitroso-1-naphthol-4-
sulfonic acid (0.00991 mmole) and 20.00 ml of 8.00×10^{-4} $\underline{M}$ cobalt
sulfate (0.01600 mmole) were used. The prepared solutions were adjusted
to pH 10 for complexation, since there was no danger of zinc hydroxide
formation, and since effectively all of the ligand was not protonated.
All species were known to be soluble under the experimental conditions.
The complexation was quenched by acidification to permit precise
measurement of the time-interval; the complexation was halted by
acidification to pH 1.6 with hydrochloric acid. Absorbance was measured
on the acidified solutions.

There was a significant increase in absorbance accompanying an
increase in complexation time (Table 7). During the first minute, color
development progressed rapidly, but was only about 85 % complete.
Approximately 30 minutes were required to effect complete complexation,
shown by relatively stable absorbance values. After color development was
complete, as much as 6 hours complexation time did not result in increased
absorbance, but the absorbance increased after 18 hours by about 7 %,

Table 7. Time Required to Effect Formation of the Cobalt
Complex in the Presence of Excess Cobalt

Solution Number	Time	A @ 525 nm	Solution Number	Time	A @ 525 nm
Blank	----	0.000	588	10 min	0.400
567	5 sec	0.167	589	15 min	0.432
568	10 sec	0.271	590	20 min	0.417
569	15 sec	0.047	591	30 min	0.440
570	15 sec	0.140	592	45 min	0.422
571	20 sec	0.321	593	60 min	0.452
572	25 sec	0.328	594	90 min	0.457
573	30 sec	0.340	596	2 hrs	0.419
574	40 sec	0.350	597	3 hrs	0.435
575	50 sec	0.367	598	4 hrs	0.450
576	60 sec	0.387	599	5 hrs	0.448
Blank	----	0.000	600	6 hrs	0.443
583	1.0 min	0.363	604	18 hrs	0.483
584	1.5 min	0.381	601	24 hrs	0.468
585	2.0 min	0.357	602	48 hrs	0.478
586	3.0 min	0.370	603	72 hrs	0.480
587	5.0 min	0.394			

which is approximately 3 times the relative standard deviation of the proposed method and is therefore a significant increase.

The stability of the complex at pH 1.6 perhaps is indicative of the presence of cobalt(III) or a very stable cobalt(II) complex. The increase in color intensity after 6 hours may be indicative of the oxidation, by atmospheric oxygen, of cobalt(II) to cobalt(III) or of a very slow reaction during the final stages of complexation.

Did the system act differently in the presence of excess cobalt than it did in the presence of excess ligand? To confirm that the solutions and reagents were not abnormal, the study was repeated, but excess ligand was used; 15.00 ml of 6.608×10^{-3} $\underline{M}$ 2-nitroso-1-naphthol-4-sulfonic acid (0.0991 mmole) and 4.00 ml of 8.00×10^{-4} $\underline{M}$ cobalt sulfate (0.00320 mmole) were used. The procedure of Wise and Brandt (2) was followed, including complexation at pH 7.0 and measurement of absorbance at pH 7.0 against a reagent blank in 1-cm cells.

Thus the quantity of cobalt sulfate, 0.00320 mmole, limited the quantity of complex which could be formed in the latter study, while the quantity of 2-nitroso-1-naphthol-4-sulfonic acid, 0.00991 mmole, limited the quantity of complex which could be formed in the former study. Also, since the complex contains three ligands per cobalt, the ratio of limiting absorbance values in the two studies was

$$\frac{0.00991}{(3)(0.00320)} = 1.03,$$

the limiting absorbance in the former study being the highest. The data in the latter study, Table 8, was in harmony with this consideration.

The mean, limiting absorbance (at 20 hours) was 0.445 in the latter study, and therefore the limiting absorbance in the former study was

Table 8. Time Required to Effect Formation of the Cobalt
Complex in the Presence of Excess Reagent

Time After Complexation	Absorbance at 525 nm					
	Solution No. 538	Solution No. 540	Solution No. 541	Solution No. 542	Solution No. 543	Solution No. 544
2 min	0.438	0.431	0.433	0.438	0.438	0.449
4 min	0.441	0.437	0.440	0.442	0.441	0.455
6 min	0.443	0.439	0.443	0.444	0.444	0.458
8 min	0.444	0.440	0.445	0.446	0.446	0.459
10 min	0.445	0.440	0.447	0.448	0.448	0.460
21 min	0.447	0.440	0.448	0.449	0.447	0.460
65 min	0.449	0.447	0.448	0.443	0.440	0.460
170 min	0.452			0.445		
20 hrs	0.445	0.443	0.448	0.438	0.440	0.458

calculated to be (0.445) x (1.03) = 0.458

In the latter study, after the first-two minutes of complexation, the average absorbance increased 0.0075 during the next 20 hours while absorbance values in the previous study, using excess cobalt(II) sulfate, increased 0.111 in approximately the same time span. Complexation is more rapid and complete when reagent is in excess of cobalt(II) sulfate, but the system must be used with cobalt(II) sulfate in excess for the determination of nitrite in water.

It seems most reasonable to allow at least 30 minutes for complexation to occur in the presence of excess cobalt(II) sulfate before absorbance measurements are made.

Thus, while it apparently has been shown that the absorbance of the wavelength maximum of the cobalt complex increased with time in studies where acidification was used to stop the complexation process, one must consider if the acidification step was an interference.

Solutions were prepared following the general procedure except that 2-nitroso-1-naphthol-4-sulfonic acid was used in order to eliminate the nitrosolation step; 15.00 ml of 6.608 X 10^{-4} $\underline{M}$ 2-nitroso-1-naphthol-4-sulfonic acid solution (0.00991 mmole) and 20.00 ml of 8.00 X 10^{-4} $\underline{M}$ cobalt(II) sulfate solution (0.01600 mmole) were used.

After 3 - 15 hours of complexation at pH 7 absorbance was measured. The remaining solution was acidified with 6 drops of concentrated hydrochloric acid, and the absorbance was measured again.

The observed absorbance values were smaller after acidification, Table 9, which was expected if any labile ligand is protonated, but the trends were parallel. Acidification did not affect the relative values of absorbance.

G. Long-Term Study of Reactions

Recognizing the fact that the absorbance of the wavelength maximum of the cobalt complex increased with time, a long-term study of the possible reactions involved was performed. Although pH 7 was optimum for complexation, it was known to not be optimum for nitrosolation, but the latter reaction may occur slowly. Hence, large volumes of three solutions, 500 ml, were made by mixing the appropriate stock solutions at a constant pH of 7, and diluting to volume with water in 500-ml volumetric flasks. Subsequently, the pH was measured to ensure that it was 7.0 ± 0.01.

Two solutions were prepared such that 100.00 ml of 4.749×10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, 75.00 ml of 6.600×10^{-4} $\underline{M}$ sodium nitrite solution, and 100.00 ml of 8.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used in each, but in addition 7.50 ml of 6.608×10^{-4} $\underline{M}$ 2-nitroso-1-naphthol-4-sulfonic acid solution was added to one of them. Then, only 7.50 ml of 6.608×10^{-4} $\underline{M}$ 2-nitroso-1-naphthol-4-sulfonic acid solution and 100.00 ml of 8.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used to prepare the third solution.

The data, recorded in Table 10, indicated that at pH 7 both the nitrosolation and the complexation reactions continue slowly, the less striking, almost insignificant, increase in absorbance being observed when only complexation was taking place. Nitrosolation occurred

Table 9. Stability of Complex in Acid

Solution Number	Number of Hours at pH 7	A @ 525 nm	
		Before acidification	After acidification
612	3	0.483	0.460
613	6	0.487	0.460
614	9	0.485	0.456
615	12	0.490	0.463
616	15	0.490	0.460
Blank		0.000	0.000

Table 10. Long-Term Study of Reactions at pH 7

Solution Number	4.749×10^{-3} M zinc Reagent (ml)	6.600×10^{-4} M sodium nitrite (ml)	8.00×10^{-4} M cobalt sulfate (ml)	6.608×10^{-4} M Nitroso Reagent (ml)	A @ 525 nm					
					1st day	8th day	51st day	111th day	224th day	
Blank	----	----	----	----	0.000	0.000	0.000	0.005	0.000	
578	100.00	75.00	100.00	----	0.000	0.002	0.017	0.047	0.040	
579	100.00	75.00	100.00	7.50	0.043	0.052	0.060	0.080	0.076	
580	----	----	100.00	7.50	0.0405	0.051	0.054	0.057	0.053	
Blank	----	----	----	----	-0.001	0.001	0.000	0.004	0.000	

slowly but steadily at pH 7 and required several months for completion.

Small changes in the nature of the complexation occurred during the first-eight days, but thereafter the complex was stable for seven months.

The nitrosolation at pH 7 was very dependent on the concentration of excess cobalt(II) sulfate, and was not complete after seven months when added 2-nitroso-1-naphthol-4-sulfonic acid competed for available cobalt(II) ion.

Most probably, the changes in the solutions of the cobalt complex are therefore due to the slow transformation of cobalt(II) to cobalt(III) via solution absorption of atmospheric oxygen and subsequent oxidation.

H. Stability of Very Dilute Sodium Nitrite Solutions

Significant, random variations in data occurred from time to time which were not readily explicable except in terms of solution instability. The complex has been demonstrated to be stable, and therefore it seemed most reasonable for the instability to be due to solutions of sodium nitrite.

In order to observe variations in absorbance values as a result of the age of sodium nitrite solutions used or of the ages of solutions of the complex, a series of solutions was prepared following the general procedure, except that volumes and concentrations of many component solutions, and the ages of the sodium nitrite and of the solutions of the complex were varied.

The ages of the 6.600×10^{-4} $\underline{M}$ sodium nitrite solutions seemed not to affect the absorbance of the solutions after 2 hours complexation (Table 11). However, more significant differences in absorbance

Table 11. Effect of Age of Dilute Sodium Nitrite Solutions

Solution Number	6.600×10^{-4} M Sodium Nitrite (ml)	Age of Sodium Nitrite Solution	8.00×10^{-4} M Cobalt Sulfate (ml)	1.8894×10^{-3} M Zinc Reagent (ml)	Time	A @ 525 nm 1-cm cells	10-cm cells
466	5.00	10 min	25.00	10.00	5 min	0.130	
					2 hrs	0.144	
462	5.00	4 days	25.00	10.00	5 min	0.138	
					2 hrs	0.146	
481	5.00	7 days	25.00	10.00	5 min	0.132	
					2 hrs	0.149	
					18 hrs	0.149	
					24 hrs	0.151	

Solution Number	6.668×10^{-5} M Sodium Nitrite (ml)	Age of Sodium Nitrite Solution	4.00×10^{-4} M Cobalt Sulfate (ml)	1.8894×10^{-3} M Zinc Reagent (ml)	Time	A @ 525 nm 1-cm cells	10-cm cells
467	10.00	10 min	20.00	20.00	5 min	0.024	
					2 hrs	0.034	0.355
463	10.00	6 days	20.00	20.00	5 min	0.016	
					2 hrs	0.028	0.268
482	10.00	7 days	20.00	20.00	5 min	0.020	
					2 hrs	0.035	
					18 hrs	0.030	
					24 hrs	0.035	

values were observed in solutions prepared from 6.668×10^{-5} $\underline{M}$
sodium nitrite, indicating slight decomposition of the more dilute
solution over a period of six days in some instances. Sometimes a
solution was stable for 7 days (solution 482).

Dilute solutions of sodium nitrite need to be prepared freshly
each day they are used.

I. Conformance to Beer's Law

One of the ultimate criteria a chemical system should meet is
the conformance to Beer's law. It has been shown that nitrite ion
reacts at pH 2.4 with excess zinc 1-naphthol-4-sulfonate to form the
latter's 2-nitroso derivative, which serves as a ligand to complex
with excess cobalt(II) at pH 7.0 to yield a highly colored cobalt
complex. To verify that the system obeys Beer's law with respect to
nitrite, two series of solutions were prepared containing varying
amounts of sodium nitrite following the recommended procedure; 20.00 ml
of 4.952×10^{-3} $\underline{M}$ zinc 1-naphthol-4-sulfonate solution, 0.00 to
20.00 ml of 6.668×10^{-4} $\underline{M}$ sodium nitrite solution and 20.00 ml of
8.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were used in making solutions
numbered 334-341, and 20.00 ml of 1.9764×10^{-3} $\underline{M}$ zinc 1-naphthol-4-
sulfonate solution, 0.00 to 20.00 ml of 6.668×10^{-5} $\underline{M}$ sodium nitrite
solution and 20.00 ml of 4.00×10^{-4} $\underline{M}$ cobalt(II) sulfate solution were
used in preparing solutions numbered 404-408, 410 and 411.

The data, Table 12, clearly demonstrated that the system obeyed
Beer's law in the 0.3 to 9 ppm nitrate concentration range when
1.00-cm cells were used. Use of 10.00-cm cells permitted extension of
the working range down to 0.02 ppm nitrite, as shown in Figures 1 and 2.

Table 12. Beer's Law Data

Solution Number	6.668×10^{-4} M Sodium Nitrite (ml)	6.668×10^{-5} M Sodium Nitrite (ml)	ppm NO_2^-	A @ 525 nm	
				1-cm[a] cells	10-cm[b] cells
334	0.00	----	0.000	0.005	----
335	1.00	----	0.307	0.034	----
336	3.00	----	0.920	0.097	----
337	5.00	----	1.534	0.149	----
338	7.00	----	2.147	0.200	----
339	10.00	----	3.068	0.282	----
340	15.00	----	4.601	0.422	----
341	20.00	----	6.135	0.559	----
Blank	----	----	0.000	----	0.005
404	----	1.00	0.031	----	0.024
405	----	3.00	0.092	----	0.055
406	----	5.00	0.153	----	0.090
407	----	7.00	0.215	----	0.126
408	----	10.00	0.307	----	0.178
410	----	15.00	0.460	----	0.263
411	----	20.00	0.613	----	0.333

[a]Beckman DB Spectrophotometer

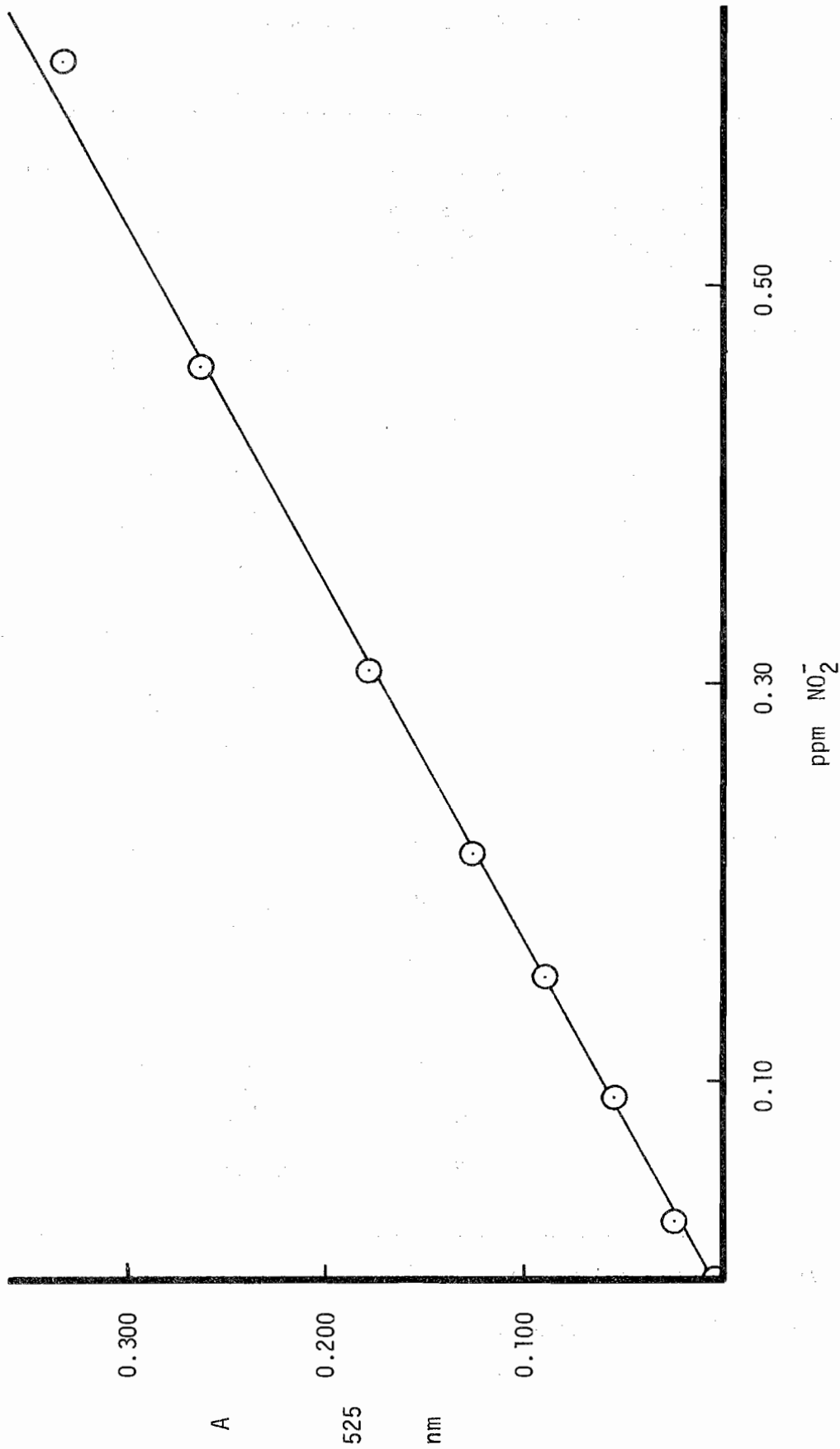[b]Beckman DK-1A Spectrophotometer

Figure 1.   Beer's Law Plot of Complex at Low Concentrations
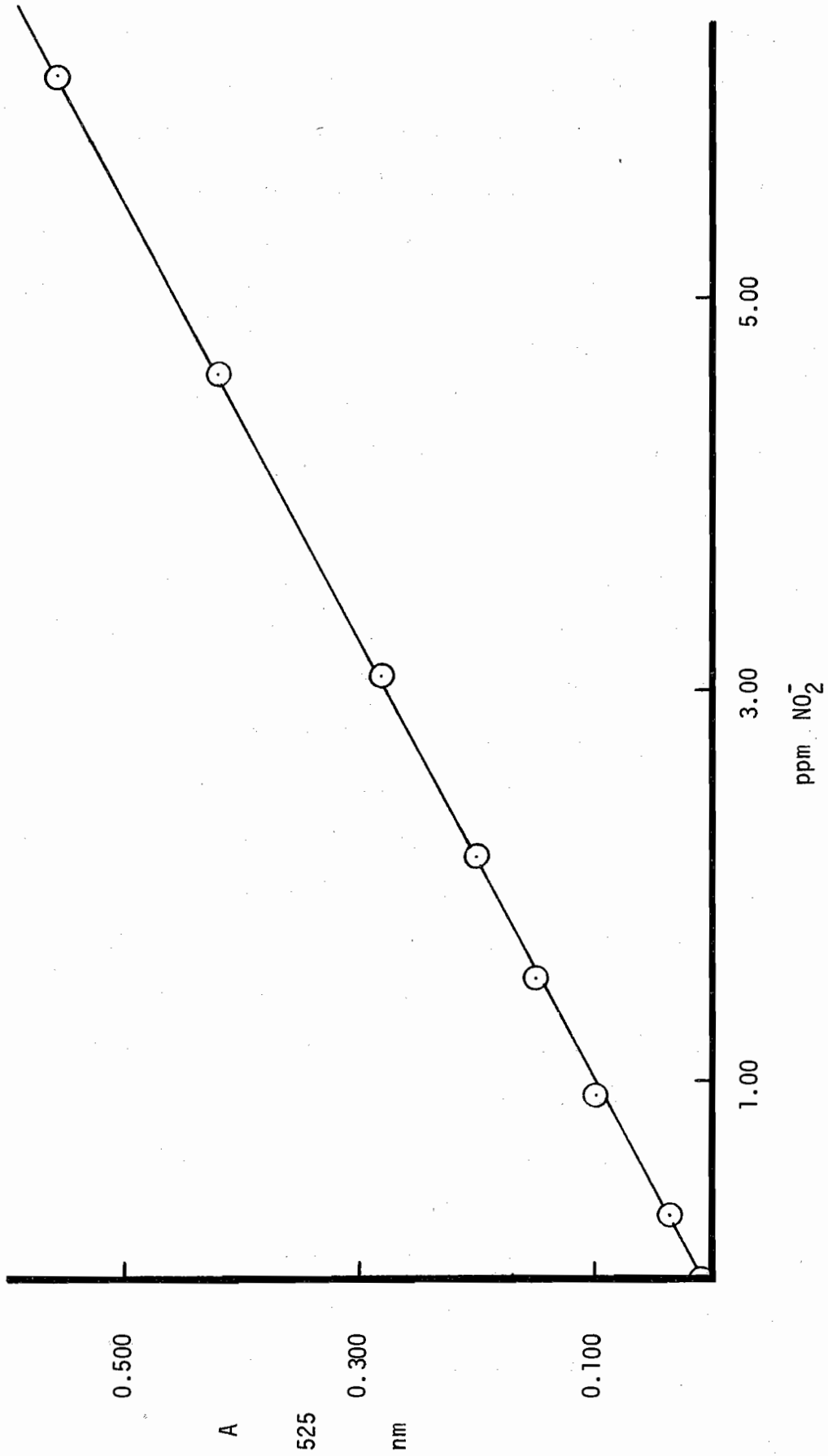
Figure 2. Beer's Law Plot of Cobalt Complex at High Concentrations

Unexpected, but readily duplicated deviations toward low values in the observed slopes occurred from one Beer's-law study to another. The data in Table 13 illustrates this effect; the difference between the mean absorbance value of solutions 468-472 and 475-480 and the mean absorbance value of solutions 510-519 is statistically significant at the 99 % confidence level. The source of the systematic error was soon recognized to be impure reagent, zinc 1-naphthol-4-sulfonate. Three recrystallizations of the reagent eliminated the deviations and yielded maximum slopes for Beer's-law plots.

J. Precision Study

The precision of the method was determined prior to interference studies so actual interferences could be distinguished from random variations; 96 solutions were prepared, using the recommended procedure, and measured in sets of about 10, on 10 different days spread over several months. Reagent solution concentrations varied from 4.583 - 4.966 x 10^{-3} $\underline{M}$, and the reagent and cobalt(II) sulfate solutions were not always accurately measured because when these solutions were measured using a graduated cylinder, precision was maintained; accurately measured 15.00-ml volumes of 6.600 x 10^{-4} $\underline{M}$ sodium nitrite were used. All absorbance values were adjusted by subtracting the absorbance readings for the respective blanks.

The 96 trials reported in Table 13 were collected over a six-month period. Not only were different stock solutions utilized, but different spectrophotometers were used. The last 85 trials were used in this study, for which the mean absorbance was 0.438.

The sample standard deviation, ± 0.0012, betters the specifications,

93

Table 13. Precision Data

Absorbance at 525 nm

Solution Number									
468-472 [a,b] 475-480	0.420	0.423	0.411	0.425	0.424	0.422	0.420	0.421	0.421	0.422
510-519 [c]	0.433	0.429	0.436	0.434	0.432	0.432	0.437	0.435	0.435	
550-559	0.437	0.436	0.438	0.438	0.439	0.438	0.438	0.436	0.437	
711-720	0.437	0.437	0.437	0.436	0.438	0.436	0.435	0.437	0.437	
721-730	0.439	0.438	0.438	0.438	0.438	0.439	0.437	0.438	0.438	
732-741	0.438	0.438	0.436	0.437	0.437	0.437	0.436	0.437	0.436	
905-910	0.441	0.440	0.440	0.439	0.440	0.441				
913-921	0.439	0.438	0.439	0.439	0.439	0.441	0.441	0.441		
927-936	0.442	0.443	0.443	0.443	0.442	0.442	0.441	0.441	0.441	
940-949	0.442	0.441	0.441	0.440	0.439	0.442	0.441	0.439	0.439	

[a] Mean absorbance all values is 0.436.
[b] Mean absorbance all values except from solutions 468-472 and 475-480 is 0.438; standard deviation = 0.0012
[c] The difference between the mean absorbance value of solutions 468-472 and 475-480 and the mean absorbance value of solutions 510-519 is significant at the 99 % confidence level ($\pm t = 5.24$).

$\pm$ 0.004, declared by Beckman Instrument Company for the Beckman DB
spectrophotometer. Some of the measurements were made on a Beckman
DK-1A spectrophotometer. It is apparent that the system will be
limited by the precision of the spectrophotometer.

K.   Interference Studies

One quantitative definition, though empirical, of interference
utilizes a multiple of the sample standard deviation of a single
measurement. Since only 0.15 % of single measurements which are
affected by random errors are expected to lie beyond three (3) sample
standard deviations of the sample mean, such a deviation was chosen
to define deviations caused by interferences in this study. Thus an
interference was indicated by an absorbance value of a solution prepared
identical to those in the precision study and which appeared outside
the range, 0.438 $\pm$ 3(0.004), delimited by the spectrophotometer.

Inasmuch as the reaction was conducted in a hydrochloric acid
medium, and functioned identically when perchloric acid was substituted
for hydrochloric acid, chloride ion does not interfere, even when
present in a million-fold excess over nitrite ion.

The nitrosolation reaction regularly was consummated in the presence
of 175 ppm chloride, a 3500-fold excess of chloride at the low limit
of detection, 0.02 ppm nitrite, but much larger concentrations of
chloride had no effect. Even when the nitrosolation was consummated in
1.1 $\underline{M}$ hydrochloric acid at 0 °C, to prohibit volatilization of nitrous
acid, a 78 x 10^4 fold excess of chloride at the low limit of detection,
no interference of chloride was noted.

The high limit of detection was delimited by instrumental

aspects effecting deviations from Beer's law.

1. Oxidation Interference

The possibility of interference by oxidation of the ligand,
2-nitroso-1-naphthol-4-sulfonic acid, to 2-nitro-1-naphthol-4-sulfonic
acid was investigated by observing the effect of adding H_2O_2 to
complexed solutions.

Solutions were prepared following the general procedure except
that 2-nitroso-1-naphthol-4-sulfonic acid was used in order to eliminate
the nitrosolation step; 15.00 ml of 6.608 x 10^{-4} $\underline{M}$ 2-nitroso-1-naphthol-
4-sulfonic acid (0.00991 mmole) and 20.00 ml of 8.00 x 10^{-4} $\underline{M}$ cobalt(II)
sulfate (0.0160 mmole) were used. Also, before the final dilution,
0, 1 or 10 drops of 30 % H_2O_2 were added to each solution. Absorbance
was read immediately, and again after 20 hours.

The results, shown in Table 14, indicate that addition of 1 or 10
drops of 30 % H_2O_2 to 90 ml of a solution of the complex had
insignificant immediate effect, but after 20 hours it decomposed the
complex considerably. Whereas the control solution showed the expected
increase in absorbance over time, the absorbance of the solution to
which was added 1 drop of 30 % H_2O_2 decreased from 0.481 to 0.451 and
the absorbance of the solution to which was added 10 drops of 30 %
H_2O_2 decreased from 0.479 to 0.416 over a 20-hour period.

The presence of strong oxidizing species in any of the solutions
used would be a source of possible interference in this system. On
the other hand, nitrite ion would not exist in samples which contain
such strong oxidizing agents.

Table 14. Oxidation Interference

Solution Number	Number of drops of 30 % H_2O_2	A @ 525 nm Immediately	A @ 525 nm after 20 hrs
Blank		0.000	0.000
528	0	0.481	0.490
526	1	0.481	0.451
527	10	0.479	0.416
Blank		0.000	0.000

2. Nitrate

Nitrate, in acidic solution, is known to oxidize 2-nitroso-1-naphthol-4-sulfonic acid to 2-nitro-1-naphthol-4-sulfonic acid and to cause formation of this same product by nitration of 1-naphthol-4-sulfonate salts. Both the nitration and oxidation reactions occur in highly acidic solutions, and do not significantly occur in mildly acidic solutions. Hopefully, these two reactions will not compete with the nitrosolation reaction of nitrite ion at pH 2.4.

Therefore, the possible interference of nitrate was considered. A series of solutions was prepared using the recommended procedure. Various amounts of sodium nitrate solution were added along with the sodium nitrite; 15.00 ml of 6.600×10^{-4} $\underline{M}$ sodium nitrite solution was used. In concentrated solutions, a pH meter was used to ensure correct adjustment of pH.

Interference of nitrate did not occur significantly until the nitrate concentration exceeded 1000 ppm in the solution on which absorbance measurements were made, as shown in Table 15 and Figures 3 and 4. The reported absorbance values are corrected for the respective blanks and for the respective controls, which always were run simultaneously with the experimental solutions. The reported values were confirmed and reconfirmed.

The study was made using several different preparations of reagent, zinc 1-naphthol-4-sulfonate, some of which were not pure. Control solutions were therefore essential. As noted earlier, deviations caused by impure reagent could be reproduced easily and accurately.

Table 15. Nitrate Interference

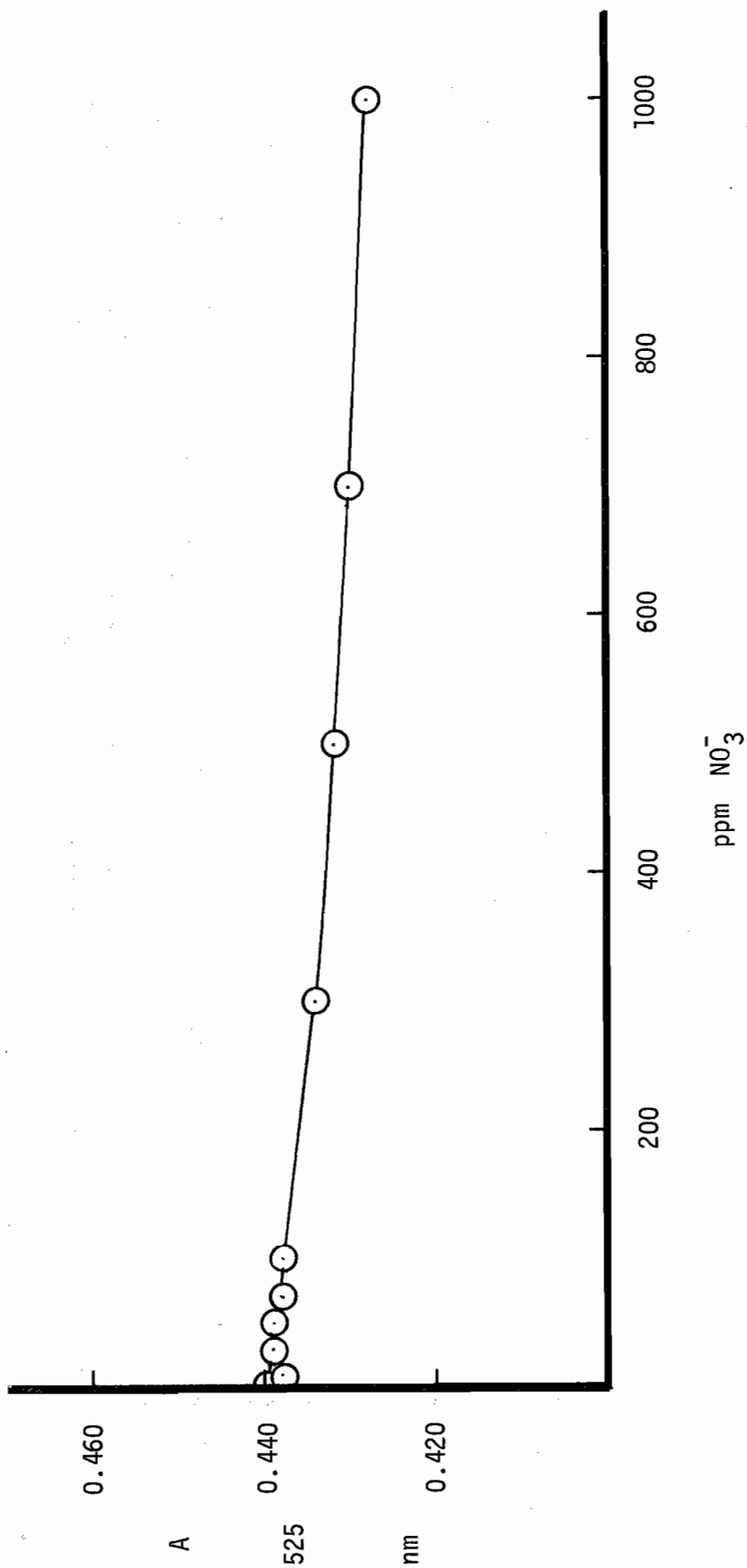| Solution Number	Sodium Nitrate Solution (ml)					ppm NO_3^-	A @ 525 nm
	0.001613 M	0.01613 M	0.1613 M	1.1613 M	8.065 M		
748						0.0	0.439
749						0.0	0.440
750						0.0	0.439
751	1.00					1.0	0.439
752	3.00					3.0	0.438
753	5.00					5.0	0.439
754	7.00					7.0	0.438
755	10.00					10.0	0.438
760		1.00				10.0	0.438
761		3.00				30.	0.439
762		5.00				50.	0.439
763		7.00				70.	0.438
764		10.00				100.	0.438
780			1.00			100.	0.437
781			3.00			300.	0.434
782			5.00			500.	0.432
783			7.00			700.	0.430
784			10.00			1000	0.428
968				1.00		1000	0.431
969				3.00		3000	0.422
970				5.00		5000	0.416
971				7.00		7000	0.413
972				10.00		10000	0.411
979					2.00	10000	0.415
980					6.00	30000	0.410
981					10.00	50000	0.410
982					15.00	75000	0.409
983					20.00	100000	0.407

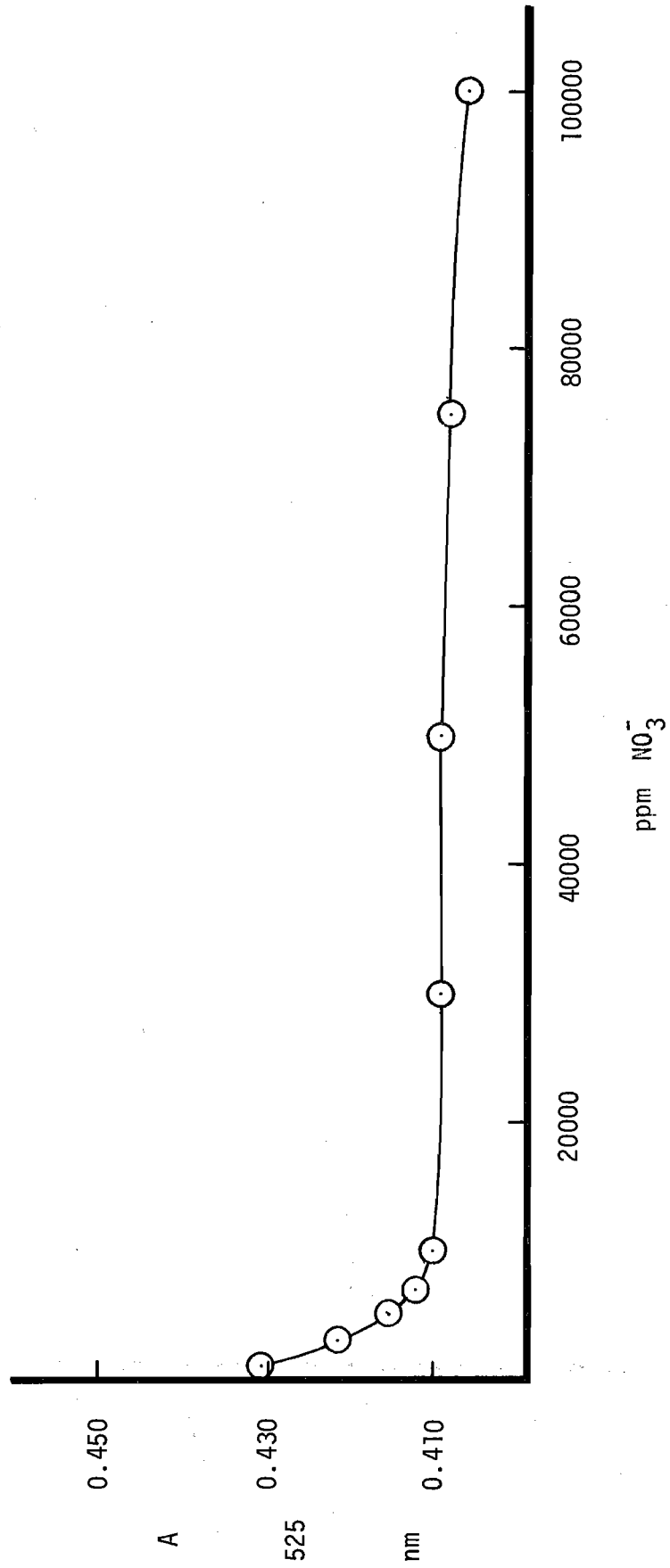Figure 3. Nitrate Interference at Low Concentrations

Figure 4.   Nitrate Interference at High Concentrations

Interference was defined as absorbance values that fell outside the range of three sample standard deviations of the sample mean, 0.438 $\pm$ 0.012. Even in the presence of 3000 ppm nitrate, interference caused a deviation of only 4.0 sample standard deviations.

The 8.065 $\underline{M}$ sodium nitrate solution was super saturated; crystals were found in it after the study was completed. Hence, the ppm nitrate reported for solutions numbered 979 to 983 may be slightly in error. Nevertheless, even the most concentrated solution of nitrate ion, caused less than a 10 % error in the observed nitrite concentration.

L. Buffer System

Adjustment to a neutral pH with a pH meter consumes much time and is awkward when only strong acids and strong bases are involved, because neither of the latter substances function as a buffer in the neutral pH region. In this system, complexation of cobalt(II) optimally occurs at pH 7, but the system does not inherently contain a buffer effective at that pH.

Hence, a buffer system was sought in order to facilitate the adjustment of pH during the chelation step. Ammonia, amines, amino acids, and amine carboxylic acids (EDTA, NTA) would complex cobalt(II) and perhaps form highly colored species, and therefore were not considered for use in a buffer system.

Potassium sodium tartrate and zinc acetate were found to be unsatisfactory. Sodium acetate and hydrogen potassium phthalate were tried and found to be somewhat satisfactory, but did not have a good buffer capacity at concentrations where they did not interfere.

Potassium sodium tartrate, sodium acetate, hydrogen potassium

phthalate, and sodium citrate all significantly increased the pH at which zinc hydroxide precipitated; for 0.003 $\underline{M}$ solutions, zinc hydroxide precipitation occurred at pH 12.6, 11.8, 11.8, and 12.3, respectively. However, potassium sodium tartrate resulted in the formation of an intensely yellow-colored solution at high pH and therefore was not considered further. Since sodium citrate retarded zinc hydroxide precipitation below pH 12.3, it was selected for further study.

Inasmuch as sodium citrate has been used classically in the well known McIlvain buffer system, which utilizes both citrate and phosphate, it was used in conjunction with phosphate in this work. A series of solutions was prepared following the recommended procedure, except various volumes of the buffer solution were added.

When 2.00 ml of the buffer solution was used, Table 16, excellent buffer capacity was exhibited at pH 7, zinc hydroxide did not precipitate during adjustment of the pH, and no deviation of the absorbance from the expected value was observed.

Thus a number of difficulties were eliminated, and it was decided that a citrate-phosphate buffer system would be incorporated into the recommended procedure.

Table 16. Effect of Buffer

Solution Number	Buffer[a] Solution (ml)	A @ 525 nm
Blank	0.00	0.004
697	0.00	0.440
698	0.00	0.441
699	0.00	0.441
700	2.00	0.442
701	2.00	0.442
702	2.00	0.440
703	3.00	0.435
704	3.00	0.435
705	3.00	0.431

[a]Citric acid, 0.127 $\underline{M}$, and disodium hydrogen phosphate, 0.0375 $\underline{M}$.

V. CONCLUSIONS

A simple, rapid, precise, spectrophotometric method has been developed and proposed for the determination of nitrite in natural waters in the presence of chloride and nitrate. Nitrite reacts with zinc 1-naphthol-4-sulfonate at pH 2.4 at room temperature in about 30 minutes to form 2-nitroso-1-naphthol-4-sulfonic acid, which reacts with cobalt(II) at pH 7 to form an intensely red-colored complex. The complex obeys Beer's law as a function of nitrite over a wide range, 0.02 - 9 ppm nitrite.

The precision and upper limit of the method are delimited by the spectrophotometer used.

Nitrate does not interfere at 1000 ppm nitrate, and only causes 10 % relative error at 100,000 ppm nitrate.

The nitrosolation reaction is regularly consummated in the presence of 175 ppm chloride, but much larger concentrations of chloride have no effect. Even when the nitrosolation reaction is consummated in 1.1 $\underline{M}$ hydrochloric acid at 0 °C, no interference is noted.

VI. LITERATURE CITED

(1) O. N. Witt and H. Kaufmann, *Ber.*, 24, 3157 (1891).

(2) W. M. Wise and W. W. Brandt, *Anal. Chem.*, 26, 693 (1954).

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Chapter 3

HIGHLIGHTS OF THE RESEARCH -- A SUMMARY

Albert L. Caskey

Many research results remain hidden in the dusty, bound volumes of sophisticated journals on out-of-the-way shelves in scientific libraries. Many other research results are lost in the scientific jargon of authors who write only to their professional colleagues. Personnel in other disciplines and the practicing engineer and scientist do not often come in contact with such research results, and if they do, the words that are used leave the readers with little enlightenment. In this Chapter is recorded an attempt to report in non-scientist's terms the significant accomplishments of the research detailed in Chapters 1 and 2. Perhaps this summary will aid those non-scientists who read it.

In manufacturing and chemical industries, in hospitals, in environmental-protection laboratories, in pollution control, in the evaluation of natural resources, in water resources, in the food industry, and in many other areas of modern life, the chemist in

the control laboratory serves to evaluate natural resources, starting materials, intermediate products, and final products. Thus, the control chemist is essential to modern society. Without the quality-control laboratory, high quality goods and services, that we take for granted, would not exist.

Quality-control personnel desire some ideal kind of "probe" which can be inserted into the sample in such a way that the concentration of the constituent of interest can then be read on a dial or recorded by a recorder. In an environmental situation, the probe possibly would measure the concentration of a pollutant. In a natural-resource situation, perhaps the probe would measure the relative quantity of a valuable ore present.

Probes can take many shapes and forms. Perhaps one of the simplest probes is a chemical reagent which form a color when added to a solution of a sample. The intensity of the color formed depends upon the amount of the constituent to be determined. A high constituent concentration results in an intense color. By measuring the intensity of the color, or the amount of the color, the amount of the constituent to be determined is measured "spectrophotometrically."

In water-resource situations such as those which instigated this research, the ideal probe is a water-soluble, specific, cheap, readily available reagent which can be added to a water sample with little or no prior treatment and with simple techniques, using simple instruments, and the amount of pollutant measured. The long-term objective of this research is to design, prepare, study, and evaluate such ideal reagents.

The herein reported search for new reagents involved two interrelated and yet independent projects. In research, as in any endeavor, a real measure of the practical value is direct application to current, difficult problems. Effort was directed toward solving specific problems associated with pollution in natural-water systems such as rivers, lakes, and other streams. These efforts focused on methods for determining nitrate, a common chemical found in fertilizer and sewage, and nitrite, a common species found in water polluted with farm-lot and urban sewage.

The work reported in Chapter 1 describes a new method for the determination of nitrate in natural waters. The proposed reagent is zinc 1-naphthol-4-sulfonate, which reacts with nitrate to form a colored compound, 2-nitro-1-naphthol-4-sulfonic acid. A water sample is taken and simply treated to remove any solid particles which are present in it and also any interfering substances, and then evaporated to dryness. The residue then is treated with the reagent. An acid is added and the reaction mixture heated at 50 °C for about thirty minutes. The reaction is stopped, the solution diluted to volume, and the intensity of the color measured on a spectrophotometer.

The method can be accomplished using very complex instrumentation available only in very sophisticated chemical laboratories or very simple instrumentation. Hence, it is readily adaptable to the simple techniques and instruments used in water-treatment plants. This method is similar in procedure to the standard Phenoldisulfonic Acid Method in common use today, but uses a purer reagent and gives better precision.

Nitrate is often released from farm-lot sewage and urban sewage into ponds and streams. When organic matter is also present in the streams, nitrate is reduced to nitrite, which is toxic to man and animals. Nitrite reacts within the human body to block the ability of hemoglobin to carry oxygen from the lungs. Nitrite may also form nitroso-amines, which are carcinogens, or cancer-former substances.

A method is reported herein for the determination of nitrite in water. The sample is treated with the reagent, zinc 1-naphthol-4-sulfonate, in a weakly acid solution to form a color-forming substance. The latter substance then reacts with cobalt to form a highly colored, red cobalt complex. The amount of red color is related to the amount of nitrite in the sample.

The method can see nitrite in the presence of 1000-times larger concentrations of nitrate, and of 1,000,000-times larger concentrations of chloride, and can be used in any laboratory.

The communication of results of research efforts to the public is of prime importance, but so also is the rapid presentation of laboratory findings to professional colleagues. The findings of the research herein reported have been presented at various scientific meetings. The titles and other pertinent information for the papers so presented at scientific meetings have been summarized in Table 1.

The public records show the support for this project from funds provided by the Office of Water Resources Research of the United States Department of Interior through the Water Resources Center, Urbana, Illinois as authorized under the Water Resources Research Act of 1964, P. L. 88-379. However, these public records do not show the support by Southern Illinois University at Carbondale. The full commitment

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Frances Baumann, Paula Palmer, and Dainee Serna, who typed the
manuscript, including the many preliminary drafts.

Table 1. Papers Presented

1. R. J. Antepenko, F. N. Abercrombie and A. L. Caskey. "Essential
   Conditions for the Use of 1-Naphthol-4-sulfonic Acid as a Spectro-
   photometric Reagent for Nitrate in Water." *Abstracts 7th Midwest
   Regional Meeting American Chemical Society*, St. Louis, 104 (1971).

2. J. C. Lewis and A. L. Caskey. "Zinc 1-Naphthol-4-sulfonate --A
   Spectrophotometric Reagent for the Determination of Nitrite in
   Natural Waters. *Abstracts 10th Midwest Regional Meeting American
   Chemical Society, Iowa City, Iowa*, 20 (1974).