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The Determination of Nitrate.

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ABSTRACT

The increased interest in the problem of surface and ground water pollution by nitrate has emphasized the need for a simple, sensitive and reliable method for the determination of nitrate in waters. According to methods of analysis commonly employed for the determination of nitrate in water, the presence of appreciable amounts of chloride would result in a low value for nitrate. Even the most widely used methods for nitrate determination are not specific and involve lengthy procedures such as separation of interferences or concentration of sample solution.

A simple, sensitive and specific method for the determination of nitrate in microgram ranges applicable for water and air samples is described. The method is directly applicable to water samples without recourse to evaporation or separation procedures. The proposed method was evolved as a result of the exhaustive study of the nitrate-chromotropic acid reaction in concentrated sulfuric medium.

Nitrate reacts with chromotropic acid in about 75% v/v sulfuric acid medium giving a water-soluble yellow product. Spectrophotometric measurement of the absorbance of the color at 410 millimicrons provides a means for the determination of nitrate in the concentration range of 0.2 to 20 milligrams per liter. Many commonly occurring materials such as oxidizing

agents, nitrite, chloride and iron (III) were found to interfere with the nitrate-chromotropic acid reaction. As a result of systematic studies of the nitrate-chromotropic acid system with respect to these interferences, a simple, direct procedure has been developed by which all the above mentioned interferences were either eliminated or masked. Thus the interference due to oxidizing agents was overcome by using sodium sulfite and that of nitrite was eliminated through the use of urea. The chloride interference was overcome by masking chloride as an antimony(III) chloro complex using antimony (III) sulfate solution. The use of antimony (III) also suppressed the interference of iron (III) by demasking the yellow iron (III) chloro complexes. The method has been applied to the analysis of water and air samples. The proposed method is simpler, more rapid and more specific than any other existing method.

CHAPTER I

INTRODUCTION

The increased use of nitrogen fertilizers added to soil in the production of food and fiber during the past quarter century has stimulated interest in the effect of nitrates on the surface and ground water. In the United States the use of ammonia alone - as the immediate precursor of nitrates - added to the soil as a fertilizer, has increased from 1,800,000 tons in 1951 to 56,000,000 tons in 1963. The increased use of fertilizers results not only in increase of crop production but also adds the possibility of the loss of this nitrate and other forms of nitrogen to streams, lakes and ground water. This is especially true if the fertilizers are not properly used and if those used are not completely utilized by plants.

Nitrate is the highest oxidized state of nitrogen and, as the nitrogen of most waste nitrogeneous organic matter becomes oxidized to nitrates, these substances - principally as the potassium, sodium, and calcium salts - are widely distributed in all parts of the world. In many places these nitrates occur as deposits and are known as niter beds. According to Mansfield and Boardman (36) the conditions under which nitrate deposits may occur are so widely distributed that almost any part of the United States might be expected to yield at least minor amounts

of these salts. They also point out that one field in the Armagosa Valley of California contained 168 acres that could produce 1,980 tons of refined nitrate.

The sodium nitrate in Northern Chile, commonly called Chile saltpeter, is the only known extensive deposit of nitrogen-bearing mineral in the world. It is an important source of nitrogen for the manufacture of explosives, fertilizers and many other substances. Saltpeter or nitrates occur elsewhere, but seldom in strata, being for the most part a product continually formed by the action of ammonifying organisms, followed by nitrification by another group of bacteria. Niter deposit formation constitutes, therefore, a process identical to that taking place in the soil, continuously, in the mineralization of nitrogeneous organic matter. In the soil layer, which may extend from a few inches to several feet in depth, there are continuous transformations of compounds of nitrogen brought about by a variety of microorganisms. Under the influence and action of these living organisms, ammonia may be produced from protein, urea, amino acids, and other nitrogeneous compounds. The ammonia may then become oxidized to nitrites by a group of organisms consisting of the species, Nitrosomonas, Nitrosococcus, Nitrosopira and Nitrosogloea. Probably concomitantly, there are other groups such as Nitrobacter and Nitrocystis, that carry the oxidation of the nitrite to nitrate which is the highest state of oxidation of nitrogen. To carry on the transformation of ammonia to nitrate, conditions of oxidation must be aerobic and

the pH must be slightly on the alkaline side. This means that a buffered soil with a calcium carbonate-carbon dioxide system is needed to maintain the pH. The carbon dioxide is produced by bacterial action on the carbohydrate material. The calcium carbonate may be a high lime soil or one treated with agricultural lime. Nitrification by bacteria proceeds best at 30-35°C and continues at a slowing rate down to 5°C. When proper conditions are met, the production of nitrates on the surface of the earth from waste organic nitrogeneous material proceeds at an enormous rate. It is this conversion that makes possible the continuous reuse of waste nitrogen, usually in preferred form of nitrates, to feed all plant and animal life on the earth and to prevent the accumulation of unwanted waste material. Nitrates as they become available for plant growth must be taken up by the crop. Otherwise, leaching may take place and they thus become a constituent of ground water.

Production water supplies which pose no threat to the consumer's health depend upon continuous protection. Well waters obtained from aquifers beneath impervious strata, and not connected with fragmented or cavernous rock, are usually considered sufficiently protected to preclude need for purification. However, ground waters are becoming polluted with increasing frequency, and the resulting hazard requires special surveillance. Chemical pollutants originating from either sewage or industrial effluents are illustrations of such pollution. Surveillance of these water supplies should include chemical, physical, radiological and biological examination (46).

The concentration of pollutants within a political and geographical urban area can arise from combustion or industrial process sources within a given area, from similar sources outside the boundaries of that area, or from natural processes taking place over large areas of the earth's surface or upper atmosphere. The pollution arising from man-made sources outside a given area may come from nearby cities or industrial areas.

Even if all man-made sources of pollution could be completely eliminated, concentrations of many natural pollutants would remain. These concentrations therefore represent the lowest possible concentrations of pollutants achievable with perfect pollution control. These natural concentrations of pollutants also can be of some importance in the calibration of instruments which measure pollutants. Many gaseous pollutants react with each other and also undergo photolysis in the presence of sunlight. There exists several natural processes for the production of oxides of nitrogen which can contribute to atmospheric pollution and which have been brought forth in the following discussion.

Nitrous Oxide

Infrared (4), mass spectrographic (54) and combustion analysis(32) have shown that nitrous oxide is evolved in appreciable quantities from soils under anearobic conditions. Poorly aerated soils which are approaching saturation with moisture rapidly release large amounts of their available

nitrogen as nitrous oxide. It has also been proposed that nitrous oxide is formed from nitrogen in the ozonosphere (altitudes of about 10 to 45 kilometers) and at ground level by the following reactions (22, 23).

$$N_2 + O + M \rightarrow N_2O + M$$

where M is a third body.

$$N_2 + O_3^* \rightarrow N_2O + O_2$$

where O_3^* is an activated ozone molecule. Nitrous oxide would be destroyed by photolysis owing to radiation below 2100 Å. Radiation of this wavelength would penetrate to the top of the ozonosphere. The amount of nitrous oxide at ground level as determined by mass spectrographic and infrared analysis is 0.5 (± 0.1) parts per million by volume (49, 9).

Nitric Oxide and Nitrogen Dioxide

It has been established that nitric oxide (and nitrogen dioxide by air oxidation) is formed under anaerobic conditions in silos containing alfalfa and cabbage (34, 45). Several hundred parts per million of nitrogen dioxide have been detected in silos immediately after storage of silage (34). The process apparently involves the reduction of nitrates to nitrites by forage bacteria (45). The nitrites are converted to nitrous acid which breaks down to nitric oxide. Just how widespread such bacteriological processes are on the earth's surface is difficult to estimate. Some nitric oxide also can be formed in

the upper atmosphere through photolysis of nitrous oxide. Laboratory experiments indicate that about 5 to 10 percent of nitric oxide is formed upon photolysis of nitrous oxide at 1850 Å (and up to 2000 Å) probably by means of the following reactions (65)

$$N_2O \rightarrow N_2 + O$$

$$O + N_2O \rightarrow 2NO.$$

From the second reaction it is evident that nitric oxide could be formed by direct attack of atomic oxygen on nitrous oxide at altitudes about 80 kilometers. Nitrogen molecule can be ionized and dissociated by ultraviolet radiation below 800 Å and by x-ray bombardment in the E-layer above 100 kilometers. Nitric oxide can then be formed by the reaction

$$N + O + M \rightarrow NO + M.$$

Small amounts of nitric oxide are also formed by electrical storms. The twilight and night air glow also may be due to the reaction (40)

$$NO + O \rightarrow NO_2$$

or the reaction (6)

$$N + O_3 \rightarrow NO + O_2.$$

A mass 46 peak probably due to nitrogen dioxide was detected at altitudes between 88 and 80 kilometers with a neutral gas spectrometer.

Chemical measurement of nitrogen dioxide at remote sites in Florida and Hawaii gives values between 0.1 to 1 parts per hundred million (29, 30). All of the evidence appears to

indicate that nitric oxide and nitrogen dioxide are present in the atmosphere from natural sources but at concentrations of a few parts per hundred million or less. The presence of oxygen and moisture in the atmosphere would lead to the formation of nitrite and nitrate and can be brought down to earth by rain.

$$2NO + O_2 \rightarrow NO_2$$

$$2NO_2 + H_2O \rightarrow HNO_3 + HNO_2$$

$$2HNO_2 + O_2 \rightarrow 2HNO_3$$

Nitrite

Nitrites occur in water as an intermediate in the oxidation of ammonia or through the reduction of nitrates. The natural sources of ammonia are urea, fertilizers of both organic and inorganic form and by aerobic and anaerobic deamination of amino acids. In raw surface supplies, trace amounts of nitrites indicate the presence of pollution.

Nitrate

Nitrate in water usually indicates the final stages of biological oxidation. In waters with excessive amounts of nitrate, an illness known as infant methemoglobinemia may be indicated. Most aquatic plants utilize nitrogen in the form of ammonia or nitrate. During the warm seasons streams and lakes get depleted in both forms of nitrogen and during winter seasons they reach maximum concentrations. A level of 0.30 milligrams per liter of ammonia or nitrate is considered to be the level which supports growth of algae forms. Nitrate appears in water as a result of

the runoff from fertilized soils and from irrigation return water, or as a result of the leaching of nitrate rock formations. Also, nitrates are formed by the oxidation of nitrites by bacteria

$$NO_2^- + {}^1/_2\, O_2 \rightarrow NO_3^- .$$

Significance of Pollution by Nitrogen Oxides, Nitrites and Nitrates

Nitric oxide is formed by the high temperature combustion processes occurring in internal combustion engines, incinerators, power plants, etc. The presence of nitrogen oxides in urban atmosphere is important because they initiate photochemical reactions with organic substances (1). The major products observed in the photooxidation of hydrocarbon in the presence of nitrogen oxide system include ozone, aldehydes, ketones, and carbon monoxide. Alkyl nitrates and the class of substances that are claimed to correspond to peroxyacyl nitrates are consistently identified as important products of photooxidation reactions. In many qualitative aspects irradiated auto exhaust resembles that of photooxidized hydrocarbon-nitrogen oxide mixtures. Significant formation of aerosols or "smog" from hydrocarbon-nitrogen dioxide systems, and from auto exhaust systems in the presence and absence of sulfur trioxide have been reported. Among the number of experimental systems used to produce eye irritation, irradiated hydrocarbon-nitrogen oxide and irradiated gasoline-nitrogen oxide mixtures are of particular significance. Of the products thus far

identified only formaldehyde, acrolein and peroxylacyl nitrates have been shown to be eye irritants. Widespread damage to leaves of crop plants have been observed as a result of photochemical air pollution. Plant damage effects have been produced in the laboratory by the use of irradiated organic substance-nitrogen oxide system and by irradiated auto exhaust. Members of the peroxyacyl nitrates series can cause glazing, bronzing or silvering of lower leaf surface of susceptible plant species at concentrations even below 0.01 parts per million.

Well waters shown to contain nitrates have caused serious and occasional fatal poisoning in infants. This has occurred with sufficient frequency and widespread geographic distribution to compel recognition of the hazard by assigning a limit to the concentration of nitrate in drinking water. In Minnesota alone from 1947 to 1950, 139 cases of methemoglobinemia, including 14 deaths due to nitrate in farm well water supplies have been reported (11). The sources of pollution may be wastes from chemical fertilizer plants and field fertilization. The causative factor producing serious blood change in infants was first reported in 1945 in polluted water containing 140 milligrams per liter of nitrate nitrogen and 0.4 milligrams per liter of nitrite ion in one case; in the second case, 90 milligrams per liter of nitrate nitrogen and 1.3 milligrams per liter nitrite ion (15). Similar occurrences have been recorded in other countries.

The 1958 International Drinking Water Standards have indicated that ingestion of water containing nitrates in excess of 50 milligrams per liter (as nitrate) may give rise to

infantile methemoglobinemia. Taylor (53) in England has suggested the limit of 20 milligrams per liter nitrate nitrogen. Bosch, et al., (11) considers nitrate nitrogen concentrations in excess of 10 to 20 milligrams per liter capable of producing cyanosis in infants. Various South American countries have recommended that maximum permissible levels range from 0.5 to 228 milligrams per liter nitrate (0.1 to 51 milligrams per liter nitrate nitrogen) (12). According to Campbell's reports (13), cases of infantile nitrate poisoning are variable and have occurred from concentrations ranging from 15 to 250 milligrams per liter, or more, nitrate nitrogen.

Nitrate poisoning appears to be confined to infants during their first few months of life; adults drinking the same water are not affected, but breast fed infants of mothers drinking such water may be poisoned (16). Cows drinking water containing nitrate may produce milk sufficiently high in nitrate to result in infant poisoning (13). If nitrate concentration is too great, both man and animal can be poisoned.

Among the more acceptable hypotheses for the specificity of nitrate poisoning of infants is the following: the gastric, free acidity of infants is low (a pH 4), permitting the growth of nitrate-reducing flora in a portion of the gastrointestinal tract from which nitrite absorption can occur. Foetal hemoglobin forms methemoglobin more readily than the adult form. According to a recent study from Germany (26), the primary causes of toxicity are an elevated nitrate concentration and the presence of an unphysiologic amount of nitrate forming bacteria, especially in the upper

portion of the digestive tract. Members of the coliform group and the genus clostridium are capable of reducing nitrate to nitrite. In infants whose diet is mainly carbohydrate, it is believed that the coliform organisms are the group responsible; organisms capable of reducing nitrite to nitrogen are not normally present in the infant.

There are no reports of methemoglobinemia in infants fed water from public water supplies in the United States. Sodium nitrate has been fed (33) at levels below 1% (10,000 ppm) to rats for a lifetime without adverse effects. Two dogs tolerated, for 105 and 125 days, respectively, 2% nitrate in their diet without adverse effects. Nitrite is equally dangerous in water supplies. Although concentrations that naturally occur are generally of no health significance, nevertheless, they may enter water supplies inadvertently as a result of intentional addition to private supplies as anticorrosion agents.

A limit of 200 parts per million of nitrite in "corned" products has been set by Federal Regulation on the basis that 100 grams of corned beef could convert maximally from 10 to 40 grams hemoglobin to methemoglobin, (1.4 to 5.7% of total hemoglobin). Adult human blood normally contains on the average of 0.7 percent methemoglobin; the blood of "heavy" smokers may contain 7 to 10 percent carboxyhemoglobin, another blood pigment conversion product incapable of transporting oxygen.

Because of the great difference in molecular weight between sodium nitrite, 69, and hemoglobin, 64,000, small increments of nitrite produce large quantities of methemoglobin (1 gram nitrite converts 460 to 1850 grams hemoglobin). The margin of safety is still further narrowed in infants whose blood volume is small; their total blood hemoglobin is decreased after birth (from 17 to 20 grams to 10.5 to 12 grams), and their foetal hemoglobin is more readily converted to methemoglobin. An instance of nitrate poisoning of children has been reported (43) in which the children ate frankfurters and bologna containing nitrite considerably in excess of the 200 parts per million permitted.

Evidence in support of the recommended limit for nitrate is given in detail by Walton (56) in a survey of the reported cases of nitrate poisoning of infants in this country to 1951. No cases of poisoning were reported when the water contained less than 10 milligrams per liter nitrate nitrogen. In many instances the samples for analysis were not obtained until several months after the occurrence of the poisoning.

According to methods of analysis commonly employed for nitrate in water, the presence of appreciable amounts of chlorides would result in a low value for nitrate, and the presence of considerable amounts of organic matter would give a high value for the nitrate. In the light of the above information and uncertainty introduced by tardy analysis, the frequent lack of attention to

possible interfering factors, and the uncertainties associated with bacterial pollution, the Public Health Service Drinking Water Standards recommend 10 milligrams of nitrate nitrogen (45 milligrams nitrate) per liter as a limit which should not be exceeded.

At present there is no method of economically removing excessive amounts of nitrate from water. A timely analysis is therefore important in areas in which nitrate content of water is known to be in excess of the recommended limit, to warn the population of the potential dangers of using the water for infant feeding and to inform them of alternative sources of water that may be used with safety.

CHAPTER II

METHODS AVAILABLE FOR THE DETERMINATION OF NITRATE

The determination of a substance free from interfering materials seldom presents difficulties. As a rule such a determination can be made with almost any desired degree of accuracy. The state of affairs is likely to be much more different when the substance is to be determined in an actual sample. Natural materials are almost always complex and many man-made materials are also mixtures. Thus selective or specific methods should be developed so as to allow the determination substances with requisite accuracy even in the presence of diverse substances. Furthermore, it is highly desirable to develop a simple method which will give rapid and reliable results even at the hands of nonspecialized personnel. New or improved methods of analysis developed during the past several years have produced many methods complying to most of the above mentioned requirements, but such is not the case with the determination of nitrate.

Nitrates serve as an index of pollution of water and oxides of nitrogen have now been recognized as one of the more important pollutants in air. The determination of nitrate up to the present has depended upon methods which are either nonspecific or

nonsensitive. The following review of the available analytical methods for the determination of nitrate reveals the present status of the determination of nitrate in the microgram range.

In aqueous media under ordinary conditions no reagent has been reported which can give a direct color reaction with nitrate ions. Anionic complexes of nitrates are weak and do not have properties useful enough for analytical studies. All inorganic nitrates are quite soluble, hence there exists to precipitation reactions for nitrates using metal ions. Precipitation reactions of nitrate by certain organic nitrogen bases have been reviewed by Feigl (18). Williams (61) has descirbed analytical methods for the gravimetric determination of nitrates using nitron, di(1-naphthylamine)acetate, cinchonamine, diethylamino-1-phenyl ethyl-p-[nitro] benzoate, 6-ethoxy-5-nitro-quinoline, p-tolylisothiourea, N-benzyhydryldiethylamine and dicyclohexylthallium. From a systematic study, Salam and Stephen (47) have proposed new and improved organic precipitating agents for nitrate having a sensitivity of 0.5 milligrams per milliliter of nitrate. The gravimetric method for the determination of nitrate using these reagents have been limited to pure nitrite solutions since the reagents are also capable of precipitating other anions.

The usual reactions of nitrate are redox or nitration reactions. Under the influence of a strong acid such as concentrated sulfuric acid, nitrates yield nitric acid and nitronium ion. The oxidation reaction of nitric acid as well as the nitration of various substances have been used in methods for the

detection and estimation of nitrate. The reduction of nitrate in alkaline, neutral or slightly acidic media can give nitrite or ammonia. A number of characteristic reactions are available for nitrite or ammonia. Hence, in the methods based on reduction, nitrates are estimated indirectly by the determination of nitrites or ammonia. Methods based on the redox principle are not selective since a number of other oxidizing agents or reducing agents occurring in the sample can interfere at various stages. There is no convenient method for the separation of these interferences without making the method unduly lengthy. According to Muller [9] methods based on redox reactions are inferior to nitration methods.

There are many methods based on the oxidation power of nitrate. These methods involve the use of such reagents as diphenylamine, diphenylbenzidine, o-tolidine, barbatic, brucine, narcotine, strychnine, indigocarmine, hydroquinone, ferrous sulfate, and others, have been summarized by Welcher [7]. In a strongly acidic medium nitrate reacts with the above mentioned reagents producing the corresponding colored oxidation products. None of these reagents have been shown to be specific for nitrate, since similar reactions occur with other oxidizing agents. None of the methods based on the use of the above reagents appears to have been widely adopted since reproducible and reliable results are difficult to obtain due to the lack of color stability. Use of brucine as a reagent for nitrate was suggested by Snell [10], and Noll [11] has applied it for the

determination of nitrate in boiler water. A critical study of the standard brucine method was made by Fardus and Maly (17). Stability of the reagent, the effect of the temperature rise when sulfuric acid is added, and oxidative decomposition during the period of color development are extremely critical factors. Jenkins and Medsker (27) have modified the standard brucine method by the addition of a large excess of chloride to mask the variations caused by minor amounts of chloride in the sample.

In the reductive method for the determination of nitrate, the nitrite formed is determined by the diazocoupling reaction and subsequent spectrophotometric measurement of the intensity of the resultant azo-dye. The most commonly used coupling reactions involve diazotized sulfanilic acid or sulfanilamide with 1-napthylamine or N-1-napthylethylene diamine. The variations in methods depend upon the nature and conditions of the reduction of nitrate to nitrite. Thus Garner (19) used the microbiological reduction of nitrate to nitrite, Nelson (39) used zinc and citric acid, and Chow (12) recommended a manganese (IV) catalyzed ammonical zinc reduction. Critical conditions have to be maintained for reproducible nitrate to nitrite reduction, and appear to be more difficult in practice than the reduction of nitrate to ammonia. Macdonald (35) has reviewed methods of determining nitrate by reduction to ammonia. The ammonia formed has to be separated by steam distillation and is usually determined with Nessler's reagent. These methods are

lengthy, high blanks are usually encountered, and they cannot differentiate nitrate from nitrite. There is no successful way of eliminating nitrite interference in this method. Pappenhagen (44) has used pyrindine-pyrazolone as a reagent for determining ammonia. Devarda's alloy is the most widely used reducing agent for nitrate, but chromium (II) chloride (21), and copper catalyzed ferrous hydroxide (51) have also been employed.

In a strong sulfuric acid medium a large number of organic compounds (58) undergo nitration to give colored reaction products. The most investigated of these reagents for nitrate determinations are phenol-2,4-disulfonic acid and the xylenols. The phenoldisulfonic acid method as proposed by Grandval and Lajoux (20) is essentially the same as used today (50), although it has subsequently undergone extensive investigation by several workers as cited in Welcher (59). The method essentially consists of treating a dry sample with a solution of phenoldisulfonic acid and measuring the yellow color of nitrophenolate disulfonate anion in an alkaline medium. Taras (53) has reported a photometric modification of the above method; and Komary, et al., (31), and Johnson (28) have used the method for the determination of nitrate in plant materials and dry solids, respectively. The method is time-consuming in that a relatively large volume of sample has to be evaporated carefully and even then it is not sensitive enough to be applicable for low levels of nitrates. This method is also subjected to a large number of interferences, the most serious being chloride. Nitration of 2,4-xylenol has been used for nitrate

determinations by Werr (60). In the methods using this reagent, the 6-nitro-xylenolate formed is separated by steam distillation (60, 64), or by solvent extraction (5) and subsequently measured as the yellow 6-nitroxylenolate in aqueous alkali. Bartely and Asai (24) developed the method involving the use of 2,6-xylenol and found that (25) nitrate determinations could be done satisfactorily only after the complete removal of chloride and nitrite. Montgomery (37) used a large excess of chloride in the 2,6-xylenol method to make the chloride concentration a parameter. Actually, in an acidic medium excess chloride reduces nitrate to nitrite and the resulting product is a mixture of nitroso-and nitroxylenols and oxidation products. The removal of nitrite interference in the original samples becomes a problem since any excess of sulfamic acid used will cause low or even negative values for nitrate. Iron (III) and oxidizing agents cause serious interference. Andrews (2) has improved the 2,6-xylenol method to tolerate up to 700 parts per million of chloride by using mercury (II) sulfate as a masking agent for chloride, yet it involves a careful solvent extraction for the removal of other interferences.

Vagi (54) has reported that chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) gives a color reaction with nitrate in a concentrated sulfuric acid medium. The reagent seems to have received little attention until West and Sarma (62), using this reagent, developed a specific and sensitive spot test for nitrate. West and Lyles (63) have shown that the nitrate-chromotropic acid system is essentially free from interferences.

Of special interest was the observation that chloride enhances the nitrate-chromotropic acid color formation. Using a large excess of chloride, they found the reaction complied to Beer's law in the range of 0.1 to 1 part per million of nitrate and applied it to the determination of nitrate in water and air samples. Above 1 part per million the working curve showed a discontinuity and a positive deviation from Beer's law. Bastain (8) has used the reagent for the determination of nitrate in samples containing traces of formaldehyde but no chloride, the interference due to formaldehyde being removed by oxidation to formic acid.

Other methods proposed for the determination of nitrate are as follows: Bloomfield (10) has reported that nitrate could be determined indirectly by the suppressing action of nitrate on the color reaction of rhenium (VII) - furfuraldoxime and tin (II) chloride.

Direct ultraviolet absorption methods for determining nitrate in water have been described by Bastain et al. (7). A number of anions and cations interfere. Armstrong (3) has reported that in samples containing chloride, the addition of an equal volume of sulfuric acid caused the maximum absorbance wavelength of nitrate to shift from 210 millimicrons to 230 millimicrons. Novone (42) has described a direct ultraviolet procedure in which the absorbance of nitrate is measured at 210 millimicrons against a blank in which the nitrate ions have been

destroyed by the action of the zinc-copper couple in an acidic medium. This eliminates most of the interfering substances except for nitrite.

Thus, the literature records numerous methods and modified methods which require lengthy separation procedures to overcome even the most commonly occurring interferences. It is clear, therefore, that there is no simple method of high sensitivity and specificity for nitrate determination. There is a need for such a method, and as a result of the work described in this dissertation, it is hoped that the requirements have been met.

CHAPTER III

CONVENTIONAL METHODS FOR THE DETERMINATION OF NITRATES

The phenoldisulfonic acid method and the brucine method have been suggested as standard methods for the determination of nitrate in water samples (50). The limitations of these methods are well known, but decades of work have been unsuccessful in significantly overcoming the difficulties. Of the several recently developed methods, the ultraviolet spectrophotometric method has been given a tentative status until enough data have been accumulated to determine its reliability.

The details of these methods and their use and limitations are presented below.

A. PHENOLDISULFONIC ACID METHOD

Principle

The yellow color produced by the reaction between nitrate and phenoldisulfonic acid obeys Beer's law up to at least 12 milligrams per liter of nitrate nitrogen at a wavelength of 480 millimicrons when a light path of 1 centimeter is used. At a wavelength of 410 millimicrons, the point of maximum absorption, the range for determination extends up to 2 milligrams per liter with the same cell path.

Procedure

1. Color removal. Decolorize 150 milliliters of water sample by stirring with aluminum hydroxide suspension. Allow the sample to stand for a minute and filter.

2. Nitrite conversion. To 100 milliliters of sample add 1 milliliter of sulfuric acid and stir. Add dropwise with stirring potassium permanganate solution until a faint pink color persists. Allow the sample to stand for 15 minutes to complete the conversion of nitrite to nitrate. Nitrite must be determined by an alternative method and properly deducted from the total nitrate.

3. Chloride removal. Determine the chloride content of the sample and treat 100 milliliters of sample with an equivalent amount of silver sulfate. The precipitate is removed by filtration or by centrifugation, after coagulation of the silver chloride by heat. Excellent removal of silver chloride can be achieved by allowing the treated sample to stand overnight.

4. Neutralize the clarified sample to approximately pH 7, transfer to a casserole, and evaporate to dryness over a hot water bath. Using a glass rod, rub the residue with 2.0 milliliters of phenoldisulfonic acid reagent to insure dissolution of all solids. (The reagent is prepared by heating on a water bath for 2 hours, a mixture containing 25 grams phenol, 150 milliliters concentrated sulfuric acid, and 75 milliliters of fuming sulfuric acid.) Dilute with 20 milliliters distilled water and add 6 to 7 milliliters of

strong ammonium hydroxide, or 5 to 6 millliliters of 12 normal potassium hydroxide until the maximum color develops. Remove any hydroxide precipitates or turbidity by filtration. Transfer the filtrate to a 50 or 100 millliliter volumetric flask or Nessler tube, dilute to mark and mix.

5. Photometric readings can be made at 410 millimicrons. Readings should be made against a blank prepared from the same folumes of phenoldisulfonic acid reagent and ammonium hydroxide or potassium hydroxide as used for the samples.

6. Preparation of standard. Evaporate 50 millliliters of stock nitrate solution which contains 100 milligrams per liter of nitrate nitrogen. Dissolve the residue by rubbing with 2 millliliters of phenoldisulfonic acid reagent and dilute to 500 millliliters with distilled water (1.00 millliliters of this solution = 10 micrograms nitrogen = 44.3 micrograms nitrate ions.) Transfer 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 3.5, 6, 10, 15, 20, and 30 millliliters of the above prepared solution to 50 or 100 millliliter volumetric flasks. To each, add 2.0 millliliters of phenoldisulfonic acid reagent. Dilute and neutralize with 6 to 7 millliliters of ammonium hydroxide or 5 to 6 millliliters of 12 normal potassium hydroxide. Make up the volume and measure absorbances at 410 millimicrons using a 1 centimeter cell against the blank.

Interferences

As even small concentrations of chloride introduce nitrate losses in this method, it is important that the chloride content

reduced to a minimum, preferably below 10 milligrams per liter. However, the silver sulfate used for this purpose presents problems with some water samples due to the incomplete precipitation of the silver ion, which leads to an off color or turbidity when the final color is developed. Ammonium hydroxide was preferred over potassium hydroxide due to complexation of the latter with silver ions. Colored ions and materials physically modifying the color system should be absent.

Minimum detectable concentrations. In the absence of interferences, the phenoldisulfonic acid method is sensitive to 1 microgram of nitrate nitrogen (4.43 micrograms nitrate) which represents 0.01 milligrams per liter nitrogen in a 100 milliliter sample.

Precision and Accuracy

Accuracy of the order of ± 0.1 milligram per liter nitrate nitrogen can be obtained only by the proper treatment of the chloride and nitrite interferences. Many laboratories appear to have difficulty in coping with these interferences.

A synthetic sample containing 1.1 milligrams per liter nitrate nitrogen, 0.25 milligrams per liter nitrite nitrogen, and 241 milligrams per liter chloride was analyzed in 40 laboratories with a standard deviation of ± 1.1 milligrams per liter. One half of the reported results were within ± 0.4 milligrams per liter of the known nitrate nitrogen concentration. The individual laboratories reproduced their own results within ± 0.1 milligrams per liter.

B. BRUCINE METHOD

Principle

The reaction between nitrate and brucine yields a yellow color employed for colorimetric estimation. The color system does not obey Beer's law. Standards and samples must be treated simultaneously for color development and the intensity is measured at 410 millimicrons. The intensity of the maximum color developed varies inversely with the temperature, while the rate of color development varies directly with temperature.

Procedure

Nitrate standards in the range of 0.0 to 10.1 milligrams per liter nitrate were prepared. If the sample contains chlorine, it must be removed by adding 1 drop of 0.5% w/v sodium arsenite. Avoid excess arsenite.

Pipet 2 milliliters of sample or standard containing not more than 10 milligrams per liter nitrate nitrogen into a 50 milliliter beaker. Add 1 milliliter of brucine-sulfanilic acid reagent (dissolve 1 gram brucine and 0.1 gram sulfanilic acid in 70 milliliters hot distilled water. Add 3 milliliters concentrated hydrochloric acid and make up to 100 milliliters.) Into a second 50-milliliter beaker, measure 10 milliliters of 20 : 3 sulfuric acid-water mixture. The intensity of color may be affected slightly by the heat capacity of the beakers. It is important, however, that only 50-milliliter beakers be used. Mix the contents of the two beakers by carefully adding the sample

with brucine-sulfanilic acid reagent to the beaker containing acid. Pour from one beaker to the other four to six times to insure mixing. Allow the treated sample to remain in the dark for 10 ± 1 minutes. While the sample is standing for color development, measure 10 milliliters distilled water into the empty beaker. After 10 minutes, add the water to the sample and mix as before. Allow to cool in the dark for 20 to 30 minutes. Set the blank at 100 percent transmittance at a wavelength of 410 millimicrons. Although Beer's law is not obeyed, plotting transmittance against nitrate concentration can produce a smooth curve. Standards must be run simultaneously with the sample.

Interferences

All strong oxidizing or reducing agents interfere. The interference by residual chlorine may be eliminated by the addition of sodium arsenite, provided that the residual chlorine does not exceed 5 milligrams per liter. Only a slight excess of sodium arsenite may be present as an excess of this will affect the determination. Iron (II and III) and quadrivalent manganese give slight positive interferences, but in concentration less than 1 milligram per liter, these are negligible. The interference due to nitrite is eliminated by the use of sulfanilic acid. Chloride is not reported to interfere; however, experiences (27) have shown this is not the case.

Precision and Accuracy

A synthetic unknown containing 1.1 milligram per liter nitrate nitrogen, 0.25 milligrams per liter nitrite nitrogen, and

241 milligrams per liter chloride was analyzed in seven laboratories with a standard deviation of ± 0.49 milligrams per liter nitrogen. One half of the reported results were within ± 0.1 milligram per liter of the known nitrate nitrogen concentration. The individual laboratories reproduced their own results within ± 0.1 milligrams per liter.

C. ULTRAVIOLET SPECTROPHOTOMETRIC METHOD (Tentative)

Principle

Measurement of the ultraviolet absorption at 210 millimicrons enables a rapid means of determining nitrate. Beer's law is obeyed up to 11 milligrams per liter nitrate nitrogen. Because dissolved organic substances may also absorb at 220 millimicrons, and nitrate does not at 275 millimicrons, a second measurement is made at 275 millimicrons for the purpose of correcting the nitrate value. The extent of this empirical correction is related to the nature and concentration of the organic matter, and consequently, may vary from one water sample to another. Filtration of the sample is intended to remove possible interference due to suspended particles. Acidification with 1 normal hydrochloric acid is designed to prevent interferences from hydroxide or carbonate at concentrations up to 1,000 milligrams per liter as calcium carbonate. Chloride is without effect on the determination.

Procedure

1. Preparation of standard curve. Using a 10 milligram per liter nitrate nitrogen stock solution, calibration standards

are prepared in the range of 0 to 0.35 milligrams nitrate nitrogen. Add to each standard, 1 milliliter of hydrochloric acid, mix and measure the absorbance at 220 millimicrons against redistilled water. Check the standard curve periodically in the area of interest.

2. Treatment of the sample. Pass sufficient sample through an appropriate filter and collect 50 milliliters filtrate in a Nessler tube. Reject the first few portions of filtrate. Add 1 milliliter of 1 normal hydrochloric acid, and invert the tube a dozen times to insure complete reaction of the carbonate.

3. Spectrophotometric measurement. Read the absorbance of the sample against redistilled water and set a zero absorbance. Use a wavelength of 220 millimicrons to obtain the nitrate reading and a wavelength of 275 millimicrons to obtain the interference due to dissolved organic matter.

4. Preparation of correction curves for nitrite, hexavalent chromium, and surfactants. When nitrite, chromium and anionic surfactants are known to be present in the sample, prepare correction curves for each of these substances at 2 milligrams per liter intervals up to 10 milligrams per liter. Use potassium nitrite, potassium dichromate and alkyl benzene sulfonate or linear alkylated sulfonate, and redistilled water for this purpose. Measure the absorbance given by varying concentrations of each substance at a wavelength of 220 millimicrons against redistilled water and plot a separate curve for each of these interfering materials.

5. Correction for dissolved organic matter. Convert the absorbance or transmittance measurement at 275 millimicrons into equivalent nitrate by reading the nitrate value from the standard calibration curve obtained at 220 millimicrons. Multiply the value by a suitable correction factor which has been determined on a sufficient number of samples of the particular water. In some cases, a factor of 2 may apply, while in other instances, the empirical factor of 2 may be too high. A good general rule is to rely on the ultraviolet method only when the correction for organic matter amounts to less then 20 percent of the total apparent nitrate reading. Deduct the organic correction from the gross nitrate result.

6. Correction for nitrite, chromium (VI), or surfactants. Deduct the equivalent nitrate values for each of these interfering substances from the gross nitrate result.

7. Interferences. Dissolved organic matter, nitrite, hexavalent chromium and surfactants interfere. The latter three substances may be compensated for by the preparation of individual correction curves.

Organic matter can cause a positive but variable interference, the degree depending on the nature and concentration of organic material. For this reason, sufficient data must be accumulated in order to obtain a factor which can be used for a given water. This factor may not apply to another water containing organic matter of a different chemical structure.

Occasionally high quality well waters and river water give nitrate values by the ultraviolet method higher than those obtained by the usual determination of nitrite and nitrate. When such samples are kept for several weeks, the nitrate values obtained by the customary colorimetric methods eventually rise to those found by the ultraviolet method. Thorough cleaning and rinsing of all glassware must be practiced in order to reduce the error which might result from streaks or particles, surfactants and dichromate wash solutions.

Sulfate, ammonium bicarbonate, phosphate and fluoride in concentrations normally present in drinking water offer negligible interference.

CHAPTER IV

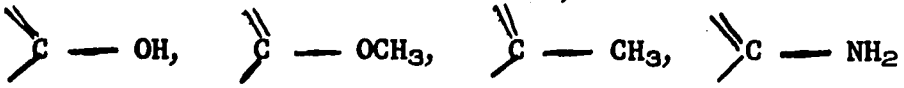
SEARCH FOR A NEW ORGANIC REAGENT FOR NITRATE

Among those properties which make appeal directly to the senses, that of color stands out prominently. The color of a substance is also useful for its determination provided a certain number of conditions are satisfied. The high intensity of the colors of numerous reaction products of different ions with organic reagents make the latter indispensible for the determination of trace quantities of substances by photometric methods.

A search for a new and better reagent for nitrate was made in which a number of organic compounds were investigated for the first time. In selecting those organic compounds to be tested as a reagent for nitrate, the following approach was used.

The organic compound should readily react with nitrate in an aqueous sulfuric acid medium. The reaction should be fast and reproducible. The concentration of sulfuric acid medium for conducting the reaction should be limited to a maximum value of 75 to 80% v/v so as to provide enough room for the sample or standard nitrate solution. The reaction product should have a high intensity of color so as to provide sensitivity and the color must be stable with respect to time. It was desirable that the

reagent used be stable and have a low background color. Aromatic compounds containing the following groups,



offered the best possibilities of color reactions with nitrates and were therefore investigated.

The following procedure was adopted in testing the selected reagents. A 0.1% w/v solution of the organic compound was made in concentrated sulfuric acid. Two solutions of nitrates of 1 milligram of nitrate per liter, and 10 milligrams of nitrate per liter were prepared from analytical grade sodium nitrate. One milliliter of each of the nitrate solutions was added respectively to two test tubes, and was cooled in a cold water bath of about 15°C. Two milliliters of concentrated sulfuric acid were added to each. The test tubes were cooled in a bath for about 5 minutes and 1 milliliter of the reagent was added to each tube. The solutions were mixed and observations were made on the colors for several hours. The same experiment was repeated using 1 milliliter of water.

The results of the above investigations are given in TABLE I. Reagents which gave a high and proportional intensity of color with nitrate were selected for further studies. Those reagents which gave a weak or unstable color reaction were tested in higher and lower sulfuric acid concentrations. No encouraging results other than the one presented for 75% v/v sulfuric acid was observed.

The result of the investigation showed that the reagent 2,6-dimethoxyphenol gave a very sensitive and apparently a stable color reaction with nitrate. West and Sarma (62) have shown that chromotropic acid (1,8 dihydroxy-3,6-napthalene disulfonic acid) is a sensitive reagent for nitrate, and therefore other naphthol-sulfonic acids were tested. Those naphthalene derivatives which gave stable and reproducible color reactions were found to have less sensitivity compared to chromotropic acid-nitrate reaction.

Detailed investigations were carried out on the 2,6-dimethoxyphenol-nitrate system. The results showed that although this is the most sensitive color reaction known so far, a method based on this reagent for the moment would be highly conditional for several reasons. The nature of the absorption spectrum and maximum were critical to sulfuric acid concentration. In a medium of 80% v/v sulfuric acid concentration, the system obeyed Beer's law from 1 to 10 milligrams per liter nitrate only after a 2 hour period of color development. The absorbance of the system changed slowly with respect to time due to the oxidation by air and a large excess of reagent was needed for satisfactory color development. Study of interferences by other ions with the system showed that Cu^{+2}, Hg^{+2}, Fe^{+2}, Fe^{+3}, Ce^{+4}, VO^{+2}, I^-, MoO_4^{-2}, WO_4^{-2}, IO_3^{-2}, SeO_4^{-2} and TeO_4^{-2} interfere seriously. Even trace amounts of iron (III) interfere by forming the highly colored phenolate complex. No simple technique has been found for overcoming the iron (III) and copper (II) interferences, and these ions are to be expected in water samples.

On the other hand, chromotropic acid was found to have several advantages compared to any other reagent for nitrate. Chromotropic acid was very stable in sulfuric acid medium. The nitrate-chromotropic acid system was extremely reproducible and the conditions governing this were not at all critical. The reagent had the least number of interferences of those studied and can be made specific for nitrate in the simplest manner. It was therefore concluded that the development of a method based on the use of chromotropic acid alone would offer a satisfactory and reliable solution to the difficult problem of nitrate determination.

TABLE I

COLOR REACTION OF NITRATE WITH ORGANIC REAGENTS
IN 75% v/v SULFURIC ACID MEDIUM

Reagents Used	Blank Color	1 Microgram Nitrate Used	10 Microgram Nitrate Used	Remarks
1. alizarin	dark pink	dark pink	dark pink	
2. p-aminoacetanilide	no color	no color	no color	
3. 2-amino-1-phenol-4-sulfonic acid	no color	no color	no color	
4. anthraquinone	no color	no color	no color	
5. anthraquinone-4-sulfonic acid	no color	no color	no color	
6. benzoin oxime	no color	no color	no color	
7. benzophenone	slight yellow	slight yellow	slight yellow	
8. catecholdisulfonic acid	no color	no color	slight yellow	insensitive
9. 2,4-dihydroxybenzoic acid	no color	no color	slight yellow	insensitive

TABLE I (continued)

10.	2,4-dihydroxybenzaldehyde	pink	pink	pink	
11.	2,6-dimethoxyphenol	yellow	pale red	dark violet	sensitive and color proportional
12.	2,6-dimethylphenol	no color	no color	violet	color not proportional
13.	p-hydroxybenzaldehyde	red	red	red	
14.	ethyl-p-aminobenzoate	no color	slight yellow	slight yellow	insensitive
15.	gallic acid	no color	no color	no color	
16.	4,5-dihydroxy-1,3-benzenedisulfonic acid	pink	pink	pink	
17.	p-hydroxybenzenearsonic acid	no color	no color	no color	
18.	β-naphthol	brown	red	red	darkens by air oxidation
19.	β-naphthylamine	pink	pink	pink	
20.	Di-β-naphthol	violet	violet	violet	
21.	1,3-naphthalene diol	violet	violet	violet	
22.	1,7-naphthalene diol	violet	violet	violet	

TABLE I (continued)

23.	1-naphthol-4-sulfonic acid	slight yellow	slight yellow	slight yellow	insensitive
24.	1-naphthol-5-sulfonic acid	slight yellow	slight yellow	slight yellow	insensitive
25.	6,7-dihydroxy naphthalene 2-sulfonic acid	slight pink	slight pink	slight pink	
26.	2-naphthol-6-sulfonic acid	no color	no color	slight yellow	insensitive
27.	2-naphthol-3,6-disulfonic acid	no color	no color	slight yellow	insensitive
28.	1-naphthol-8 amino-3,6 disulfonic acid	no color	no color	slight yellow	insensitive
29.	oricinol (3,5-dihydroxytoluene)	pink	pink	pink	
30.	orthophthalic acid	no color	no color	no color	insensitive
31.	phenolphthalein	no color	no color	slight yellow	insensitive
32.	phenolphthalein disulfonic acid	no color	no color	slight yellow	insensitive
33.	pholorglucinol	slight yellow	yellow	yellow	darkens by air oxidation

TABLE I (continued)

34.	pyrogallol	no color	pink	pink	Darkens by air oxidation
35.	resorcinol	slight yellow	slight yellow	slight yellow	insensitive
36.	resorsolic acid	slight yellow	slight yellow	slight yellow	insensitive
37.	saccharin	pink	pink	pink	
38.	tyrosine	no color	slight yellow	slight yellow	insensitive

CHAPTER V

EXPERIMENTAL

A. PREPARATION OF REAGENTS

Double distilled water was used for the preparation of all aqueous solutions. All inorganic chemicals used in this work were of analytical reagent grade.

1. STANDARD NITRATE SOLUTION

A stock nitrate solution having a concentration of 1 gram per liter nitrate was prepared by dissolving 1.371 grams of dried sodium nitrate in water and diluting to one liter. By diluting the stock solution, nitrate solutions of the suitable concentrations were prepared.

2. SULFURIC ACID

Concentrated sulfuric acid used was (from Mallinckrodt Chemical Works or du Pont Company) found to be free from nitrate by testing with chromotropic acid.

3. PREPARATION OF PURIFIED CHROMOTROPIC ACID (1,8-dihydroxy-3,6-naphthalene disulfonic acid, $C_{10}H_8O_8S_2 \cdot 2H_2O$)

About 125 milliliters water were boiled in a beaker and disodium salt of chromotropic acid (1,8-dihydroxy-3,6-naphthalene disulfonic acid, disodium salt, Eastman Kodak Company) was added in small lots until the solution was just saturated. About 5 grams of

decolorizing charcoal was added and the mixture boiled for about 10 minutes. Water was added to replenish loss due to evaporation. The solution was filtered hot through cotton wool plugged in a funnel. The filtrate was again decolorized and filtered hot through a funnel fitted with double filter paper. Chromotropic acid was precipitated from the filtrate by cooling and adding 10 milliliters of concentrated sulfuric acid. The product was filtered under suction, washed several times with alcohol, and dried at about 80°C. The product obtained was almost white and was stored in low actinic bottles.

4. CHROMOTROPIC ACID REAGENT

The reagent was prepared by dissolving 0.1 gram of purified chromotropic acid in 100 milliliters of concentrated sulfuric acid and stored in an amber colored bottle. The reagent solution was colorless (a check on the absence of nitrate in sulfuric acid) and was found to be adequately stable for at least a month.

5. UREA SOLUTION

Five grams of urea were dissolved in 100 milliliters of water. A drop of this solution contains about 2.5 milligrams of urea.

6. SODIUM SULFITE SOLUTION

Twelve grams of anhydrous sodium sulfite was dissolved in 100 milliliters of water. One drop of this solution contained about 3.6 milligrams of sulfite.

7. SODIUM SULFITE-UREA MIXTURE

This solution was prepared by dissolving 5 grams of urea and 12 grams of anhydrous sodium sulfite and diluting to 100 milliliters. This solution was used for removal of interferences such as nitrite or oxidizing agents.

8. ANTIMONY (III) SOLUTION

A 0.5% w/v solution was prepared by heating 0.5 grams of fine antimony metal powder (General Chemical Company) in 85 milliliters of concentrated sulfuric acid until all of the metal was dissolved. The solution was cooled and added to 20 milliliters of ice cold water. The solution remained clear for hours at room temperature; however, overnight standing caused crystallization of the antimony (III) sulfate. The crystallized salt was easily redissolved by heating, and was cooled to room temperature before use. Two milliliters of this solution contained about 10 milligrams of antimony (III) and can mask up to 5 milligrams of chloride. A 1% w/v solution of antimony was also prepared whenever needed by using 1 gram antimony metal and following the above procedure.

B. PREPARATION OF SOLUTIONS OF MISCELLANEOUS IONS

Stock solutions of the more common type of chemical interferences encountered in nitrate determination were prepared as follows.

1. IRON (III) SULFATE

Ferric ammonium sulfate (0.860 grams) was dissolved and made up to 100 milliliters to give 1 milligram per milliliter of iron (III) solution.

2. CHLORIDE SOLUTION

A solution containing 10 milligrams per milliliter of chloride was prepared by dissolving 1.65 grams of sodium chloride in water and diluting to 100 milliliters.

3. NITRITE SOLUTION

Sodium nitrite (0.15 gram) was dissolved and made up to 100 milliliters with water. The solution contained 1 milligram nitrite per milliliter.

4. HYDROGEN PEROXIDE

Fresh 3% hydrogen peroxide (0.33 milliliters) was diluted to 100 milliliters with water. The solution contained approximately 1 milligram of hydrogen peroxide per milliliter.

5. POTASSIUM PERMANGANATE

Potassium permanganate (0.145 gram) was dissolved and made up to 100 milliliters with water to give 100 micrograms of permanganate per milliliter.

6. GENERAL INTERFERENCES

For general interference studies, solutions of diverse ions were prepared: each solution had a concentration of 10 milligrams of the respective ions per milliliter.

C. APPARATUS

The apparatus used was as follows:

Volumetric flasks of 10 milliliter capacity;

Graduated pipets of 10, 2 and 0.1 milliliter capacities;

Two 50 milliliter beakers; and

A cold water bath for cooling purposes.

Interfering Ions (10 mg/ml)	Compound Used for Solution Preparation	Weight In Grams per 100 ml
Li^{+}	Lithium Chloride, $LiCl$	6.16
Na^{+}	Sodium Sulfate, $Na_2SO_4 \cdot 10H_2O$	7.00
K^{+}	Potassium Hydrogen Sulfate, $KHSO_4$	3.50
Cu^{+2}	Cupric Sulfate, $CuSO_4 \cdot 5H_2O$	3.94
Be^{+2}	Beryllium Sulfate, $BeSO_4$	11.17
Mg^{+2}	Magnesium Sulfate, $MgSO_4 \cdot 7H_2O$	10.15
Ca^{+2}	Calcium Chloride, $CaCl_2$	2.78
Zn^{+2}	Zinc Sulfate, $ZnSO_4 \cdot 7H_2O$	4.40
Sr^{+2}	Strontium Chloride, $SrCl_2 \cdot 2H_2O$	3.06
Cd^{+2}	Cadmium Chloride, $CdCl_2\ 2^1/_2H_2O$	2.04
Ba^{+2}	Barium Chloride, $BaCl_2 \cdot 2H_2O$	1.78
Hg^{+2}	Mercuric Chloride, $HgCl_2$	1.33
BO_2^{-}	Boric Acid, H_3BO_3	1.42
$B_4O_7^{-2}$	Sodium Tetraborate, $Na_2B_4O_7 \cdot 10H_2O$	2.48
Al^{+3}	Aluminum Sulfate, $Al_2(SO_4)_3 \cdot 8H_2O$	12.30
CO_3^{-2}	Sodium Carbonate, Na_2CO_3	1.77
Ti^{+4}	Titanium Oxide, TiO_2	1.67
Zr^{+4}	Zirconium Sulfate, $Zr(SO_4)_2$	3.12
Sn^{+2}	Stannous Chloride, $SnCl_2 \cdot 2H_2O$	1.90
Sn^{+4}	Stannic Chloride, $SnCl_4$	2.20
Pb^{+2}	Lead Acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$	1.83
NH_4^{+}	Ammonium Sulfate, $(NH_4)_2SO_4$	7.35
HPO_4^{-2}	Disodium Hydrogen Phosphate, $Na_2HPO_4 \cdot 12H_2O$	3.73

(continued)

$P_4O_7^{-4}$	Tetrasodium metaphosphate, $Na_4P_4O_7$	1.43
VO^{+2}	Vanadyl Chloride, $VOCl_2$	2.06
As^{+3}	Sodium Arsenite, Na_3AsO_3	2.56
Bi^{+3}	Bismuth Chloride, $BiCl_3$	1.51
S^{-2}	Sodium Sulfate, $Na_2S \cdot 9H_2O$	7.50
Cr^{+3}	Chromium Sulfate, $Cr_2(SO_4)_3 \cdot 5H_2O$	4.65
CrO_4^{-2}	Potassium Dichromate, $K_2Cr_2O_7$	2.82
SeO_3^{-2}	Sodium Selenite, Na_2SeO_3	1.36
SeO_4^{-2}	Sodium Selenate, $Na_2SeO_4 \cdot 10H_2O$	1.87
MoO_4^{-2}	Sodium Molybdate, $Na_2MoO_4 \cdot 2H_2O$	1.51
WO_4^{-2}	Sodium Tungstate, $Na_2WO_4 \cdot 2H_2O$	1.33
VO_3^-	Ammonium Metavanadate, NH_4VO_3	1.18
F^-	Sodium Fluoride, NaF	2.21
OCl^-	Sodium Hypochlorite, NaCl solution	29 ml per 5% sol.
ClO_3^-	Sodium Chlorate, $NaClO_3$	1.28
ClO_4^-	Sodium Perchlorate, $NaClO_4$	1.23
Mn^{+2}	Manganous Sulfate, $MnSO_4 \cdot H_2O$	3.20
MnO_4^-	Potassium Permanganate, $KMnO_4$	1.45
Br^-	Potassium Bromide, KBr	1.49
BrO_3^-	Potassium Bromate, $KBrO_3$	1.32
I^-	Potassium Iodide, KI	1.31
IO_3^-	Potassium Iodate, KIO_3	1.22
Fe^{+2}	Ferrous Ammonium Sulfate, $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	7.00
Co^{+2}	Cobaltous Sulfate, $CoSO_4 \cdot 7H_2O$	4.76
Ni^{+2}	Nickel Sulfate, $NiSO_4 \cdot 7H_2O$	4.78

A Beckmann D K Spectrophotometer equipped with 1 centimeter quartz cells was used in recording absorption spectra. Absorbance measurements were made in a Beckmann Model D U Spectrophotometer equipped with matched 1 centimeter quartz cells.

D. PRELIMINARY EXPERIMENTS

Nitrate reacts with chromotropic acid in strong sulfuric acid mediums to form an intense yellow solution. Preliminary experiments were deemed essential to establish the significant parameters of the nitrate-chromotropic acid system.

1. ABSORPTION SPECTRA OF NITRATE-CHROMOTROPIC ACID SYSTEM

Absorption spectra of the nitrate-chromotropic acid system, and the chromotropic acid reagent were recorded in three different sulfuric acid concentrations. This was necessary to obtain the wavelength of maximum absorption of nitrate-chromotropic acid system corresponding to a minimum absorption of chromotropic acid, and to ascertain whether there was any alteration in the nature of the spectrum with change in sulfuric acid concentration. A set of experiments for recording absorption spectra were carried out as follows:

First, 0.5 milliliter of a nitrate standard (100 milligrams per liter) equivalent to 50 micrograms nitrate was transferred into three 10 milliliter volumetric flasks containing 0.5, 2.5, and 3 milliliters of water respectively. Thus, 50 micrograms of nitrate were contained in 1, 3, and 5 milliliters of water in the three flasks respectively. The flasks were immersed in a tray of

cold water of about 10°C and 2 milliliters of concentrated sulfuric acid were added to each. The flasks were swirled and allowed to cool for about 4 minutes. One milliliter of 0.1% chromotropic acid reagent was added to each and after swirling and cooling them for about 5 minutes, the volume of solution in each flask was made up to 10 milliliters by the addition of concentrated sulfuric acid. The flasks were stoppered and the contents were mixed thoroughly. The flasks were allowed to stand at room temperature (about 25°C) for approximately an hour. In each flask the volume was again made up to the 10 milliliter mark by adding concentrated sulfuric acid and the solution was mixed. A set of experiments for a reagent blank was run using 1, 3, and 5 milliliters of water and following the above procedure. The concentration of sulfuric acid in the three cases was approximately 75% v v, 77% v, and 80% v v. In exact terms the above media should be expressed as the ratio of aqueous volume to sulfuric acid volume, namely as 1:9.5 ; 1:12.5 ; and 1:15.0 respectively.

Figure 1 presents the record of the absorption spectra of chromotropic acid (I) and nitrate-chromotropic acid (II, III, and IV) systems. These were taken with reference to water in 1 centimeter quartz cells in a Beckmann Model DK spectrophotometer between 300 to 525 millimicrons. The wavelength of absorption maximum was at about 415 to 420 millimicrons and 410 millimicrons was chosen for further absorption measurements. The general nature of the spectrum and the wavelength of absorption maximum of the nitrate-chromotropic acid system did not change with sulfuric acid

TABLE II

[illegible] SYSTEM
IN SULFURIC ACID MEDIUM USED FOR RECORDING SPECTRUM

Figure Number	Spectrum Number	Amount of Nitrate in Micro-grams	Volume of Aqueous Phase in Milliliters	Volume of Sulfuric Acid Used in Making Up to 10 Milliliters	Approximate [illegible] of Sulfuric Acid
1	I	1	3	7.5	75
	II	50	3	3.5	35
	III	50	1	9.5	95
	IV	50	3	7.5	75
2	I	1	3	7.5	75
	II	2.5	3	7.5	75
	III	25	3	7.5	75
	IV	50	3	7.5	75

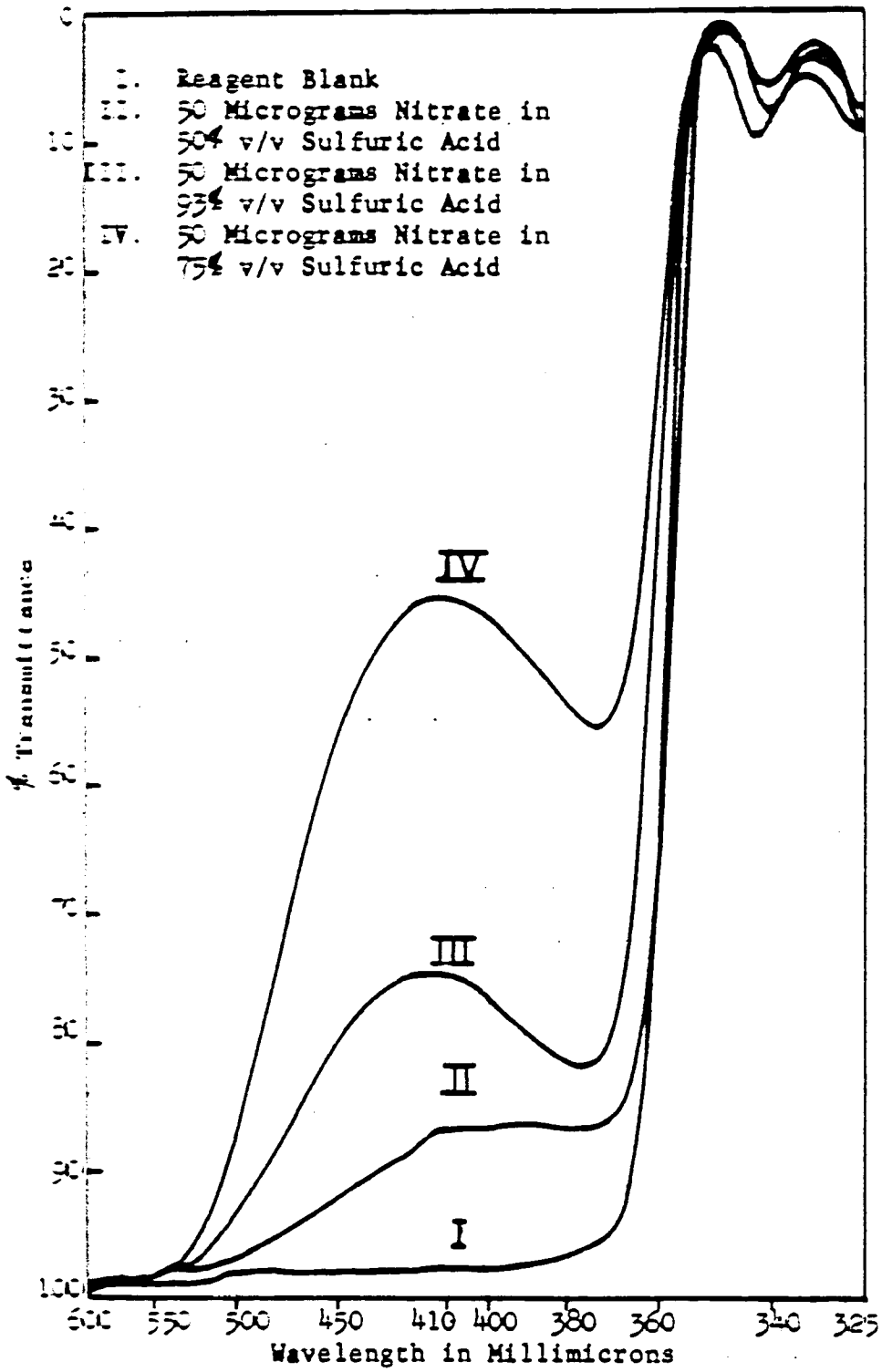


Figure 1

Absorption Spectra of Nitrate-Chromotropic Acid Systems in Various Sulfuric Acid Concentrations

concentration. However, the intensity of the color developed depended on the sulfuric acid concentration and was maximum for a 75% sulfuric acid medium. The blanks gave almost the same spectra irrespective of the difference in acid concentration, and at the wavelength of 410 millimicrons the absorption due to chromotropic acid was minimum.

It was also necessary to check the consistency of the region of absorption maximum with varying amounts of nitrate. Absorption spectra of nitrate-chromotropic acid system in 75% sulfuric acid were recorded for three different amounts of nitrate; namely, 2.5, 25, and 50 micrograms. The solutions were studied following the preliminary procedure described previously. Figure 2 presents the spectra and shows that the wavelength of absorption maximum was at about 410 millimicrons for the range of nitrate concentration tested.

2. OPTIMUM SULFURIC ACID CONCENTRATION FOR THE REACTION

The intensity of the nitrate-chromotropic acid color reaction depends upon the sulfuric acid concentration of the medium. The optimum sulfuric acid concentration of the medium which gave the maximum intensity and stability for the color reaction was established.

Fifty micrograms of nitrate in various aqueous volumes ranging from 0.5 to 4.5 milliliters were prepared in 10 milliliter volumetric flasks. The flasks were cooled to 10°C and 2 milliliters of concentrated sulfuric acid were added. After

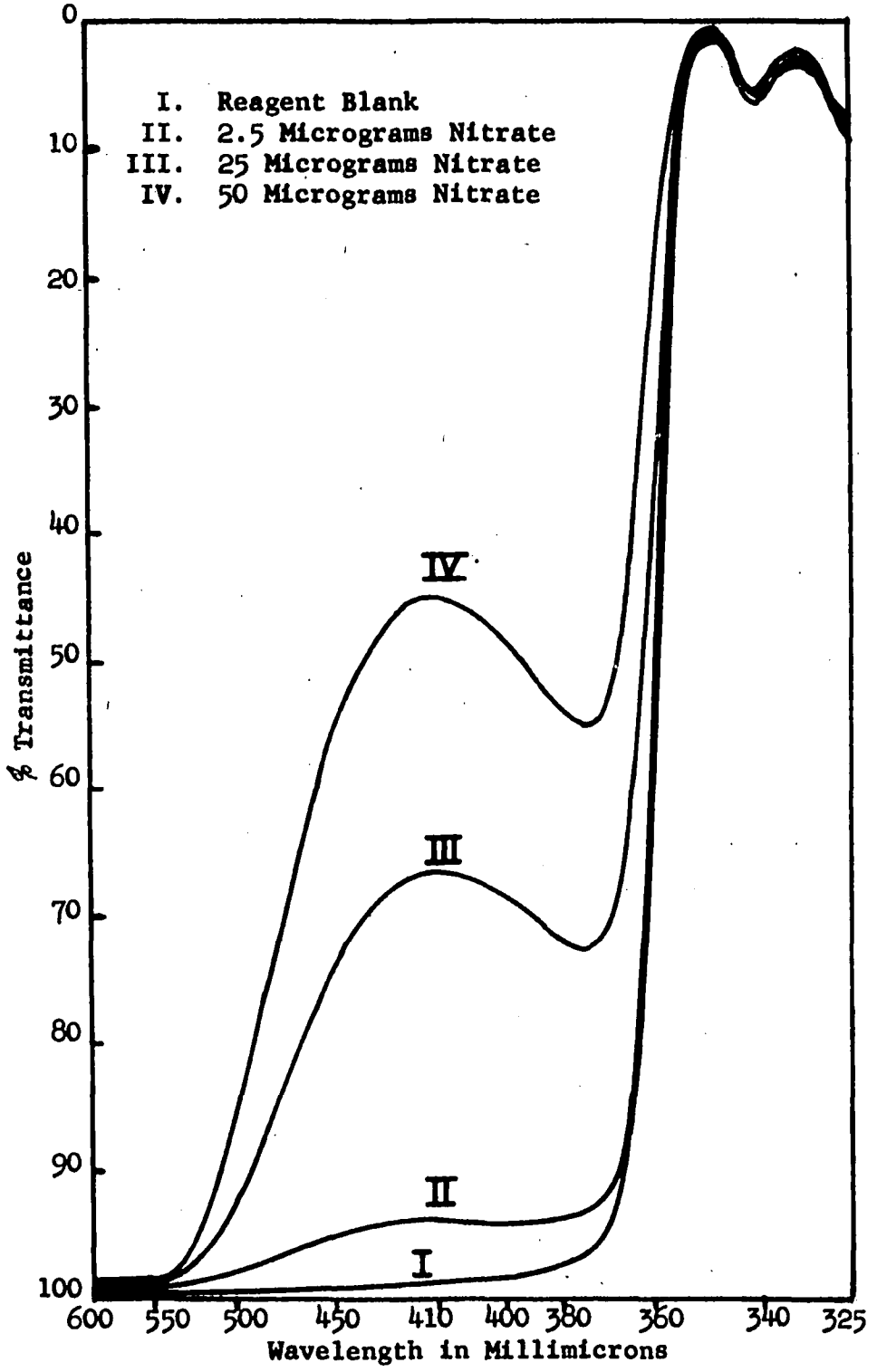


Figure 2

Absorption Spectrum of Nitrate-Chromotropic Acid System in 75% v/v Sulfuric Acid

about 4 minutes, 1 millliliter of 0.1% chromotropic acid reagent was added to each. The flasks were swirled after each addition and were allowed to cool in the bath for about 3 minutes. The final volume was made up to 10 millliliters with concentrated sulfuric acid and the flasks were allowed to cool at room temperature. After one hour the volume was again made up to 10 millliliters with concentrated sulfuric acid, and was mixed gently by turning each flask upside down. The absorbance of the solutions were measured against water at 410 millimicrons in a Beckmann Model D U Spectrophotometer using 1 centimeter quartz cells. The reagent blanks were also prepared at the respective concentrations of sulfuric acid and their absorbances measured at 410 millimicrons. The absorbance measurement of standards and blanks were repeated after 24 hours. The blank corrected absorbances due to the nitrate-chromotropic acid system were plotted against the respective volumes of aqueous phases used in each case. The results are shown in TABLE III and Figure 3. The absorbance due to the nitrate chromotropic acid system was maximum and stable when 2.75 to 3.1 millliliters of aqueous phase containing the nitrate was made up to 10 millliliters with concentrated sulfuric acid. The acid concentration range corresponded approximately to 75 - 79% of sulfuric acid. Although color was developed above 80% sulfuric acid, it was unstable and decreased with respect to time. At lower concentrations of sulfuric acid the color developed was incomplete and increased slowly with respect to time. This can be readily seen from Figure 3 where the

TABLE III

VARIATION OF ABSORBANCE WITH RESPECT
TO AQUEOUS VOLUME OF THE REACTION MEDIUM

Volume of Aqueous Phase Containing 50 Micrograms Nitrate	Corrected Absorbance of Nitrate-Chromotropic Acid System	
	1 Hour Color Development	24 Hour Color Development
0.50	0.285	0.080
1.00	0.310	0.090
1.50	0.340	0.110
2.00	0.350	0.200
2.50	0.354	0.330
2.75	0.354	0.350
3.00	0.354	0.354
3.25	0.350	0.350
3.75	0.310	0.320
4.00	0.290	0.300
4.50	0.130	0.235

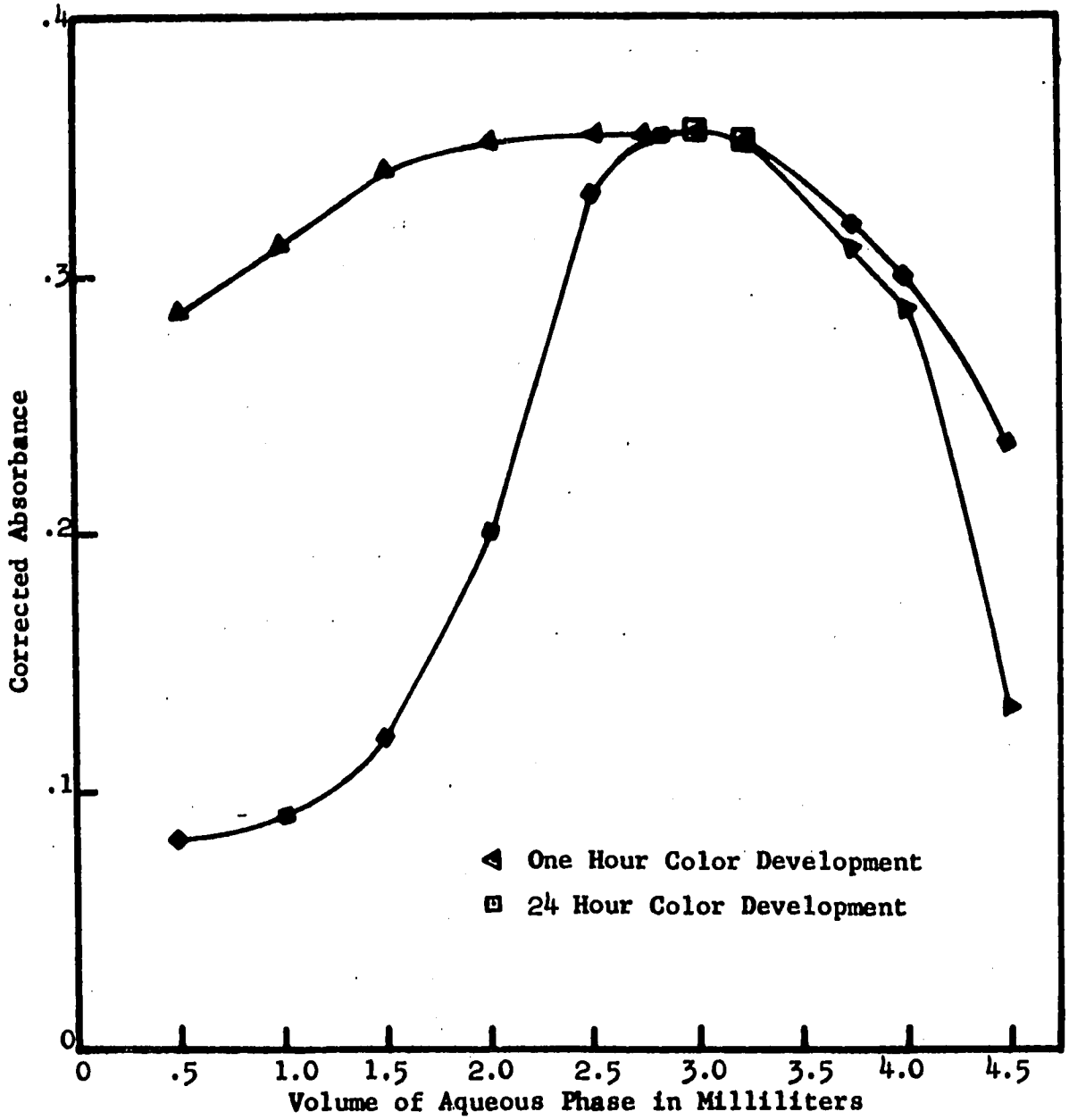


Figure 3

Absorbance Variation with Aqueous Phase Volume of the Reaction Medium

corrected absorbances after 1 hour and 24 hours were plotted against the respective dilutions.

3. RATE OF COLOR DEVELOPMENT

In a 75% sulfuric acid medium, the intensity of the color developed by the nitrate-chromotropic acid system was maximum and stable. It was necessary to find the minimum time needed for the maximum color development of the nitrate chromotropic acid system. The absorbance readings of a nitrate-chromotropic acid system against a reagent blank was taken with respect to time immediately after the preparation. The readings were taken up to 24 hours. The experiment was carried as follows:

Three portions of nitrate solution containing 2.5, 25 and 50 micrograms of nitrate respectively, were transferred into three 10 millliliter volumetric flasks and the volume was made up to 3 millliliters by the addition of distilled water. Three millliliters of double distilled water were also transferred into another 10 millliliter flask. The flasks were cooled to 10°C and 2 millliliters of sulfuric acid was added to each. After 4 minutes, 1 millliliter of 0.1% reagent solution was added. The flasks were swirled, after 3 minutes the volume was made up, and the solutions were mixed. A stop watch was started when the mixing was done. The solutions were transferred to 1 centimeter cells and the absorbance of the nitrate-chromotropic acid system was measured against blank at 410 millimicrons at various intervals of time as shown in TABLE IV. Figure 4 presents the variation of absorbance with time and shows that the maximum absorbance was reached

TABLE IV

VARIATION OF ABSORBANCE OF THE
NITRATE-CHROMOTROPIC ACID SYSTEM WITH TIME

Time	Absorbance Measured Against Blank At 410 Millimicrons		
	2.5 Micrograms Nitrate	25 Micrograms Nitrate	50 Micrograms Nitrate
Minutes			
5	0.020	0.178	0.355
10	0.020	0.178	0.354
15	0.020	0.177	0.353
30	0.019	0.178	0.353
45	0.019	0.178	0.353
Hours			
1	0.019	0.178	0.353
2	0.019	0.178	0.353
5	0.019	0.178	0.353
10	0.019	0.177	0.350
20	0.019	0.177	0.352
25	0.019	0.177	0.352

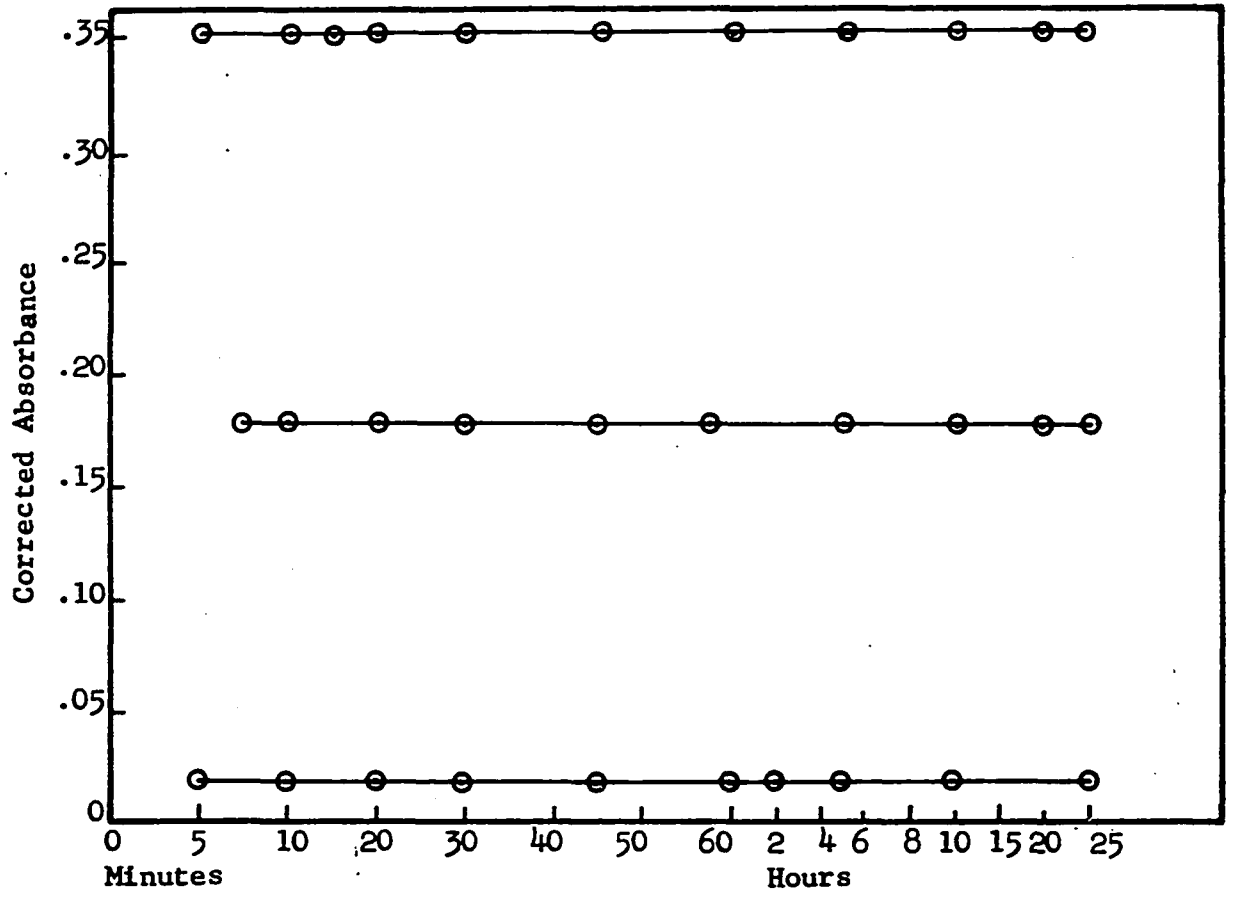


Figure 4

Variation of Absorbance of the
Nitrate-Chromotropic Acid System with Time

in 5 minutes and was stable for 24 hours. Although the maximum absorbance was reached within 5 minutes, the minimum time for color development could not be reduced less than one hour due to practical reasons. First, the solution was warm due to mixing of acid and water, and sufficient time must be allowed for cooling before the final adjustment of volume. Second, small gas bubbles were released when solution became warm and these may interfere in the absorbance measurement. By allowing the solution to stand, the air bubbles can be removed from the system.

4. REAGENT CONCENTRATION AND REACTION STOICHIOMETRY

In order to assess the reliability of the nitrate-chromotropic acid system, it was found essential to study the influence of varying amounts of chromotropic acid on the absorbance of the system. Three milliliters of aqueous phase containing 50 micrograms of nitrate ions were placed in each of ten volumetric flasks. The flasks were cooled to 10°C and 2 milliliters of concentrated sulfuric acid were added to each. A series of chromotropic acid solutions varying in concentration from 50 to 1000 milligrams per liter were prepared in concentrated sulfuric acid. One milliliter of each chromotropic acid solution was added to a corresponding nitrate solution and allowed to stand for 3 minutes. The volume was made up to 10 milliliters with concentrated sulfuric acid and mixed. After 45 minutes the volume was adjusted to the 10 milliliter mark and after 15 minutes the absorbance was read at 410 millimicrons against water. The readings were also taken after 24 hours.

TABLE V presents the data and Figure 5 shows the plot of absorbance against varying amounts of chromotropic acid used. The absorbance readings reached maximum and constant values beyond 300 micrograms of chromotropic acid. The molar ratio of nitrate to chromotropic acid for the reaction is 1:2 with respect to the equivalence point obtained by the intersection of the linear portions of the curve. (At the equivalence point, 160 micrograms of chromotropic acid react with 50 micrograms nitrate.) The curvature at the intermediate region is due to the slower reaction kinetics between nitrate and chromotropic acid compared to the two extreme regions. The absorption spectra of the solution in this region also showed presence of traces of unreacted chromotropic acid and an increase in absorbance with respect to time. Thus, in order to obtain consistent absorbance readings and a representative reagent blank, large excesses of chromotropic acid; namely 1 milliliter of 0.1% chromotropic acid in sulfuric acid should be used for each color development.

The same molar ratio of two for nitrate to chromotropic acid was obtained by using a fixed amount of chromotropic acid and varying the amount of nitrate. The slope ratio method also gave the same figure for nitrate-chromotropic acid reaction.

The exact chemical nature of the substance responsible for the color reaction could not be identified because the product was so highly soluble in the medium that no satisfactory method for isolating it from the solution could be achieved.

TABLE V

VARIATION OF ABSORBANCE WITH CHROMOTROPIC ACID CONCENTRATION

Amount of Nitrate (Micrograms*)	Amount of Chromotropic Acid		Blank Corrected Absorbance	
	(Micrograms)	(Micromoles)	1 Hour	24 Hours
50	0	0	0.000	0.000
50	50	0.1405	0.103	0.102
50	100	0.2810	0.215	0.215
50	150	0.4214	0.295	0.317
50	200	0.5620	0.323	0.340
50	250	0.7025	0.345	0.348
50	300	0.8430	0.350	0.350
50	350	0.9835	0.351	0.350
50	400	1.1240	0.352	0.352
50	500	1.4050	0.352	0.352
50	750	2.1075	0.350	0.350
50	1000	2.8100	0.350	0.350

(* 50 micrograms nitrate = 0.8075 micromoles)

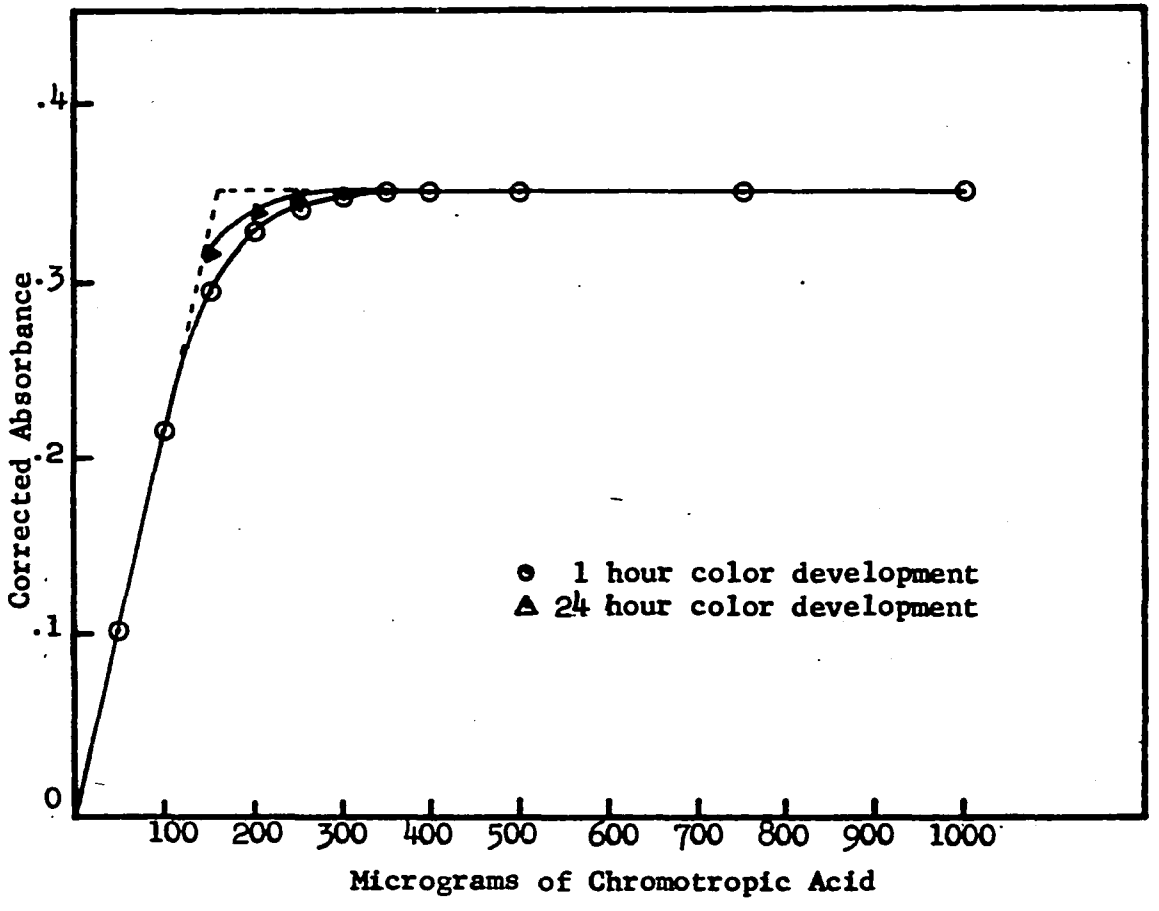


Figure 5

Variation of Absorbance with
Chromotropic Acid Concentration

5. BEER'S LAW OBEDIENCE OF THE NITRATE-CHROMOTROPIC ACID SYSTEM

The significant parameters of the nitrate-chromotropic color reaction was established by which a consistant absorbance reading stable for 24 hours could be obtained. The system was next studied to find the range of nitrate concentration through which absorbances are proportional to concentration. The studies were carried out as follows.

Two and a half milliliters of nitrate standard in the concentration range of 0.2 to 30 milligrams per liter were transferred to 10 milliliter volumetric flasks. The volume was made up to 3 milliliters by adding 0.5 milliliters water to each. The flasks were cooled to 10°C in a cold water bath and 2 milliliters of concentrated sulfuric acid were added to each. After 4 minutes, 1 milliliter of 0.1% solution of the reagent was added to each flask. The flasks were swirled and allowed to cool for about three minutes. The volume was made up to the 10 milliliter mark by adding sulfuric acid. The flasks were stoppered and mixed gently by inverting them four to five times. The flasks were allowed to cool for 45 minutes and the volume again made up to the mark with concentrated sulfuric acid. At the end of 15 minutes after the last make up, the absorbances were measured at 410 millimicrons in a 1 centimeter cell against water. A reagent blank was also run simultaneously using 3 milliliters of water. The absorbance readings were corrected for the blank and are shown in the third column of TABLE VI. A plot of absorbance vs. concentration of nitrate as shown in Figure 6 is a straight

TABLE VI

BEER'S LAW OBEDIENCE OF THE
NITRATE-CHROMOTROPIC ACID SYSTEM

Nitrate Milligrams per Liter	Total Amount of Nitrate in Micrograms	Corrected Absorbance	Corrected Absorbance After 24 Hours
0.0	Blank	0.015	0.017
0.20	0.50	0.0035	0.004
0.30	0.75	0.005	0.005
0.40	1.00	0.007	0.007
0.80	2.00	0.015	0.015
1.60	4.00	0.028	0.028
4.00	10.00	0.071	0.070
6.00	15.00	0.107	0.108
8.00	20.00	0.142	0.141
12.00	30.00	0.211	0.215
16.00	40.00	0.277	0.275
20.00	50.00	0.347	0.350
25.00	62.50	0.418	0.435
30.00	75.00	0.498	0.515

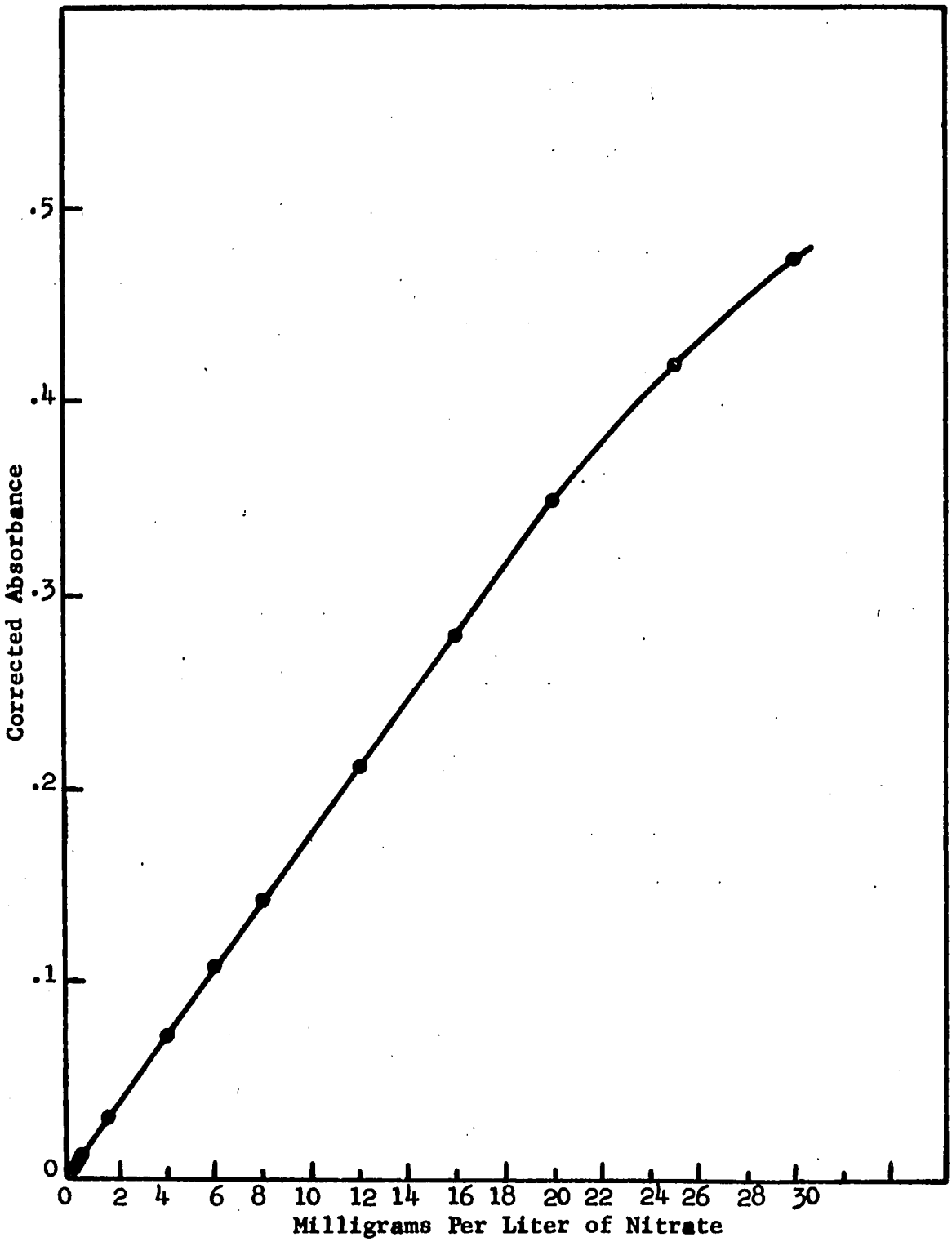


Figure 6

Beer's Law Obedience of the
Nitrate-Chromotropic Acid System

line up to 20 milligrams per liter and shows a negative deviation from linearity above 20 milligrams per liter. Hence the system obeys Beer's law from 0.2 to 20 milligrams per liter nitrate. The readings were repeated after 24 hours and are presented in the last column of TABLE VI. The system was found to be extremely stable and reproducible. It was found in the course of the work that the following practice obviated certain difficulties for accurate absorbance measurement. The final mixing of solutions for color development was done very gently so as not to introduce gas bubbles. For filling the cell it was found expedient to keep the cell in a slanting position (about 30° to the horizontal) with a ground side of the cell facing up and pouring the solution very slowly down the side of the cell. Even if some trapped air bubbles were introduced while filling the cell, they were surfaced easily along the ground side of the cell.

The time allowed for cooling after the addition of 2 milliliters of concentrated sulfuric acid, and 1 milliliter of chromotropic acid reagent was the minimum time necessary. These time factors were not critical. The final absorbances of the system did not show any difference even if greater time was allowed for cooling after the addition of sulfuric acid, and reagents respectively.

6. THE TEMPERATURE OF THE COOLING BATH

The influence of the initial temperature of the cooling bath was studied. Four sets of standard solutions in the range of 0 to 20 milligrams per liter of nitrate analyzed using cooling

baths of initial temperatures of 10, 15, 20 and 25 degrees centigrade. The procedure followed was the same as that described in the previous experiment. The temperature of the cooling bath did not remain fixed throughout the experiment. Especially after addition of 2 milliliters concentrated sulfuric acid, and 1 milliliter chromotropic acid reagent, the final equilibriated temperature rose to approximately 25, 30, 35 and 40°C respectively. Thus, in the last stage of mixing of the system after making up with sulfuric acid, the final temperatures produced were different for each set, but never rose above 70°C.

The results of the experiment as presented in TABLE VII show that initial temperature of the bath need not be critical and may vary anywhere from 10 to 20°C. The cooling bath essentially served to dissipate sufficient initial heat to avoid the final temperature going above 70°C.

The use of a cooling bath with an initial temperature equal to 25°C was difficult in practice. The sulfuric acid must be added very slowly and carefully to avoid localized ebbulition. More time had to be spent for cooling, and even then during the final mixing the flask may become somewhat uncomfortable to handle. The result of the experiments run at 25°C shows good agreement.

If cooling baths were not used to dissipate the initial heat, the temperature will rise to about 95°C in the final make up and mixing stage. This will cause an inconsistant color development, due at least in part to the charring of the organic materials present.

TABLE VII

ABSORBANCE OF THE NITRATE-CHROMOTROPIC ACID SYSTEM
USING COOLING BATHS OF DIFFERENT INITIAL TEMPERATURES

(Measurements at 410 millimicrons using a 1 centimeter cell)

Nitrate in Milligrams per Liter	Amount of Nitrate in Micrograms	BLANK CORRECTED ABSORBANCE			
		Using 10°C Water Bath	Using 15°C Water Bath	Using 20°C Water Bath	Using 25°C Water Bath
0.0	Blank	0.015	0.015	0.015	0.015
0.20	0.50	0.0035	0.004	0.0035	0.004
0.30	0.75	0.005	0.005	0.005	0.005
0.40	1.00	0.007	0.007	0.007	0.007
0.80	2.00	0.015	0.014	0.014	0.013
1.60	4.00	0.028	0.028	0.029	0.025
4.00	10.00	0.071	0.073	0.073	0.070
6.00	15.00	0.107	0.107	0.107	0.105
8.00	20.00	0.143	0.142	0.141	0.138
12.00	30.00	0.210	0.210	0.210	0.205
16.00	40.00	0.275	0.277	0.278	0.270
20.00	50.00	0.348	0.350	0.350	0.345

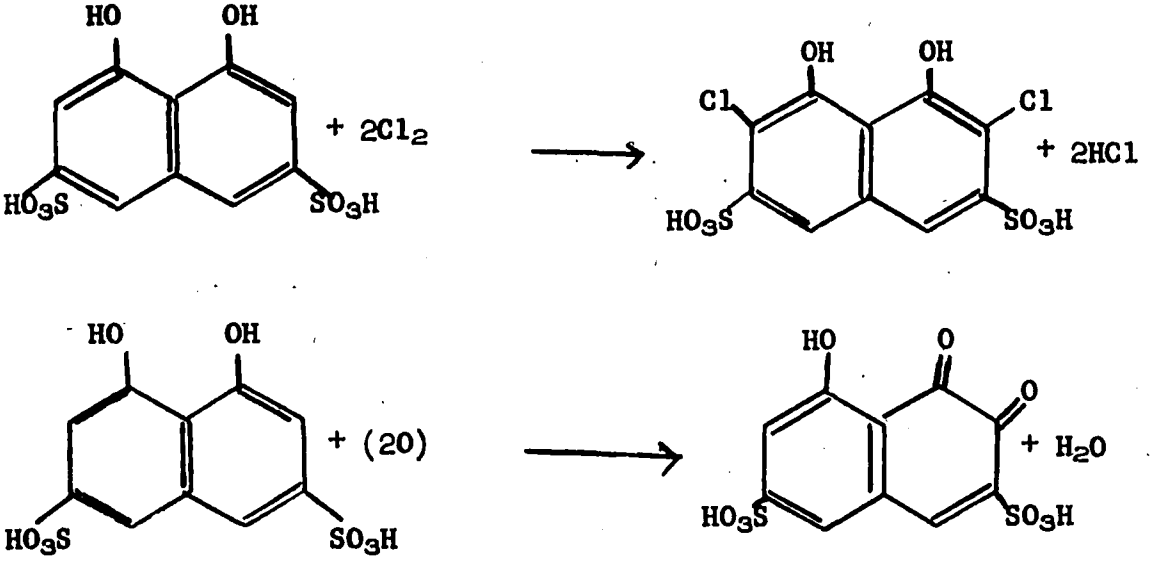
CHAPTER VI

MAJOR INTERFERENCE STUDIES

Substances which interfere seriously in many methods for nitrate determination are nitrite, chloride, oxidizing agents, and iron (III). Because of the common occurrence of these substances in water samples, a special study was made of their interference in the nitrate-chromotropic acid system.

1. OXIDIZING AGENTS

Chlorine and other oxidizing agents can react with chromotropic acid producing yellow color. The yellow color might be due to the following reactions



The absorption spectrum of the yellow oxidation product is shown by Spectrum I in Figure 7. Although the spectrum appears similar to that obtained with nitrate and chromotropic acid (Spectrum III-Figure 7), the maximum absorption of the oxidation product lies at about 460 millimicrons. This clearly shows that there is a difference in the nitrate-chromotropic acid reaction compared to the reaction of chromotropic acid and an oxidizing agent. Several oxidizing agents such as hydrogen peroxide, potassium permanganate, and chromate gave yellow colored products having almost the same absorption maximum. Since the oxidation product has considerable absorbance at 410 millimicrons, it would seriously interfere in the determination of nitrate by chromotropic acid. The only course open to overcome the interference was to eliminate all oxidizing agents using a suitable reducing agent. It was found that the addition of an excess of sodium sulfite before the addition of reagnet completely eliminated interferences due to the oxidizing agents studied. The results are shown in TABLE VIII. The influence of excess sodium sulfite on the nitrate-chromotropic acid system was studied at various concentrations of nitrate. The procedure followed for this study was the same as that used in the case of testing the Beer's law obedience of the nitrate-chromotropic acid system. Five milligrams of sulfite as sodium sulfite were introduced into the nitrate solution before adding sulfuric acid and chromotropic acid reagent. The results in colume 3 of TABLE IX show the excess sulfite did not interfere with the nitrate-chromotropic acid system. Negligibility slow kinetics of

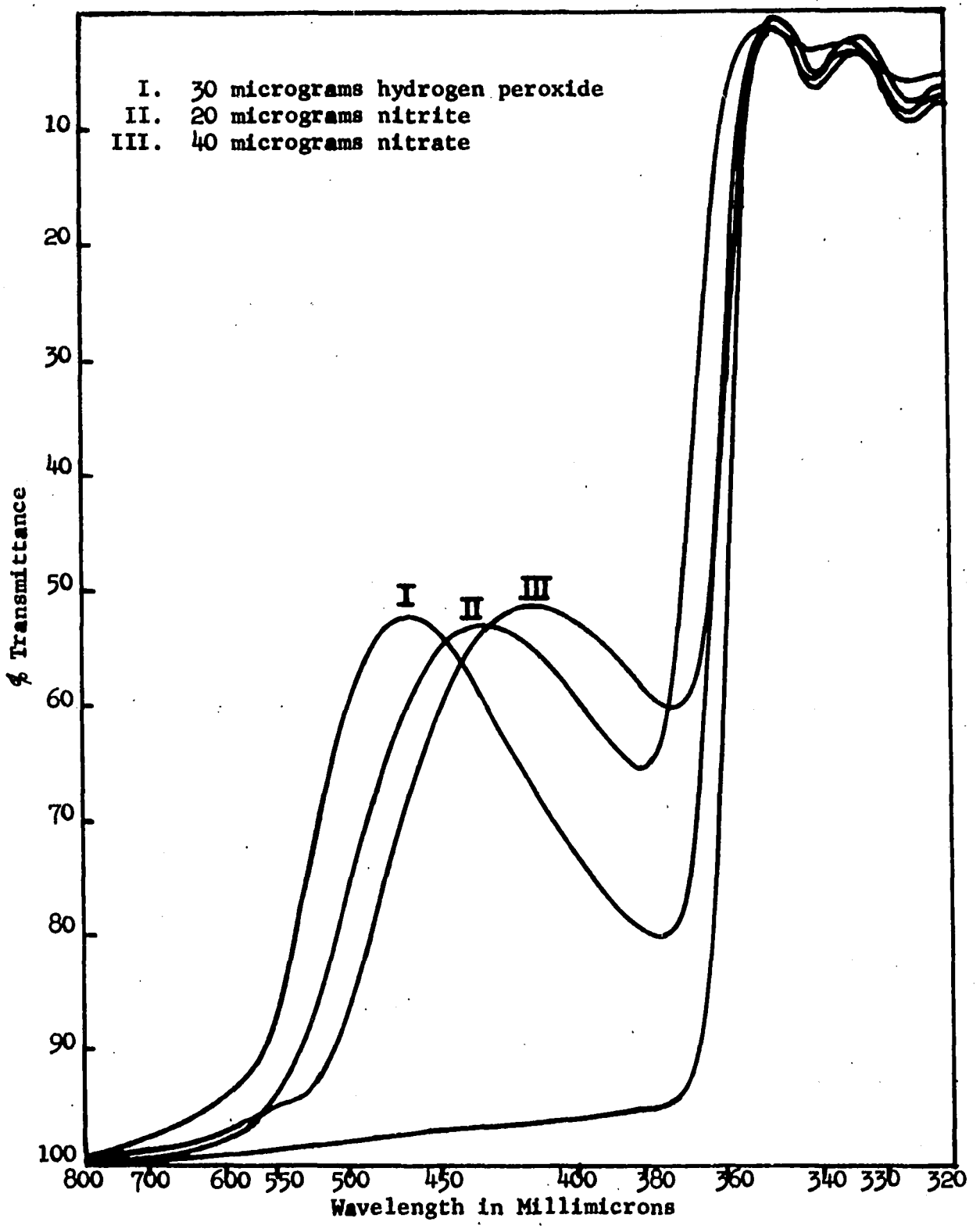


Figure 7

Absorption Spectra of Chromotropic Acid in 75% v/v
Sulfuric Acid with Hydrogen Peroxide, Nitrite, and Nitrate

TABLE VIII

ELIMINATION OF THE INTERFERENCE
DUE TO OXIDIZING AGENTS

Interference Milligrams per Liter	Nitrate Milligrams per Liter	Amount of Sulfite Added in Milligrams	Absorbance	Corrected Absorbance
0.0	0.0	0.0	0.015	Blank
0.0	0.0	3.6	0.015	0.000
0.0	1.6	3.6	0.043	0.028
0.0	4.0	3.6	0.088	0.073
Permanganate (MnO_4^-)				
40	0.0	0.0	0.620	0.615
40	0.0	3.6	0.015	0.000
40	1.6	3.6	0.043	0.028
40	4.0	3.6	0.087	0.072
Peroxide (H_2O_2)				
200	0.0	0.0	2.00	1.985
200	0.0	3.6	0.015	0.000
200	1.6	3.6	0.042	0.027
200	4.0	3.6	0.088	0.073
Chlorine (OCl^-)				
200	0.0	0.0	2.00	2.00
200	0.0	3.6	0.015	0.000
200	1.6	3.6	0.43	0.028
200	4.0	3.6	0.087	0.072

TABLE IX

ABSORBANCE OF THE NITRATE-CHROMOTROPIC ACID SYSTEM IN THE PRESENCE OF SULFITE AND UREA

(At 410 millimicrons using a 1 centimeter cell)

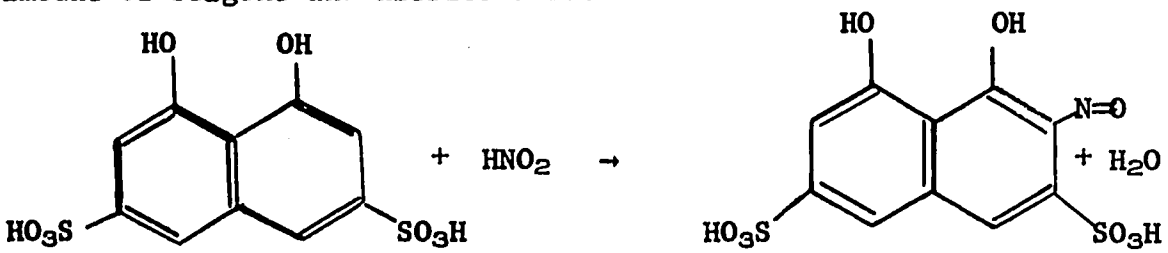
Series No. 1 With Nitrate only
Series No. 2 Nitrate in presence of 5 milligrams of sulfite
Series No. 3 Nitrate in presence of 5 milligrams of urea

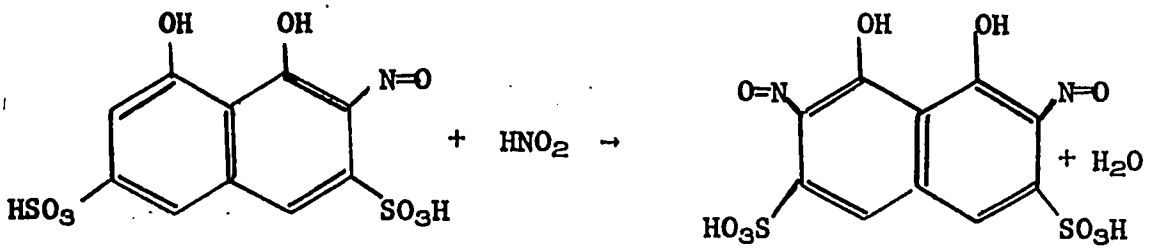
Nitrate in Milligrams per Liter	Corrected Absorbance Series No. 1	Corrected Absorbance Series No. 2	Corrected Absorbance Series No. 3
0.2	0.0035	0.0035	0.004
0.3	0.005	0.005	0.005
0.4	0.007	0.007	0.008
0.8	0.015	0.014	0.015
1.6	0.028	0.028	0.029
4.0	0.071	0.073	0.072
6.0	0.107	0.107	0.107
8.0	0.142	0.141	0.144
12.0	0.211	0.212	0.211
16.0	0.277	0.280	0.278
20.0	0.347	0.348	0.350

the sulfite-nitrate reaction under the experimental condition and the fast reaction between nitrate and chromotropic acid might account for the lack of influence of sulfite on the system.

2. NITRITE

Nitrite produced a yellow reaction product with chromotropic acid in 75% sulfuric acid medium. The absorption maximum of the color lies at about 340 millimicrons as shown by spectrum II in Figure 7. This color reaction was found to be almost two times more intensive than the nitrate-chromotropic acid color reaction. The product may be a mono or dinitroso derivative depending on the amount of reagent and nitrite used.





The interference due to nitrite can be avoided by destroying nitrite as nitrogen gas using compounds such as urea, sulfamic acid, acid amides or amines. Among these compounds urea was found

to be simple and effective in destroying nitrite. Excess urea was hydrolyzed in the system to carbon dioxide and ammonium ions.

$$2HONO + H_2N-\underset{\underset{O}{\|}}{C}-NH_2 \rightarrow 2N_2\uparrow + CO_2\uparrow + 3H_2O$$

$$H_2N-\underset{\underset{O}{\|}}{C}-NH_2 + H_2O + 2H^+ \rightarrow 2NH_4^+ + CO_2\uparrow$$

Thus, addition of urea prior to the addition of the chromotropic acid eliminated all nitrite from the system as nitrogen gas, yet did not affect the nitrate-chromotropic acid system. The results of these studies are presented in TABLE X and in the fourth column of TABLE IX.

3. CHLORIDE

Chloride can react with nitrate in highly acidic solutions giving the following products:

$$HNO_3 + 3HCl \xrightarrow{H_2SO_4} NOCl + 2H_2O + Cl_2$$

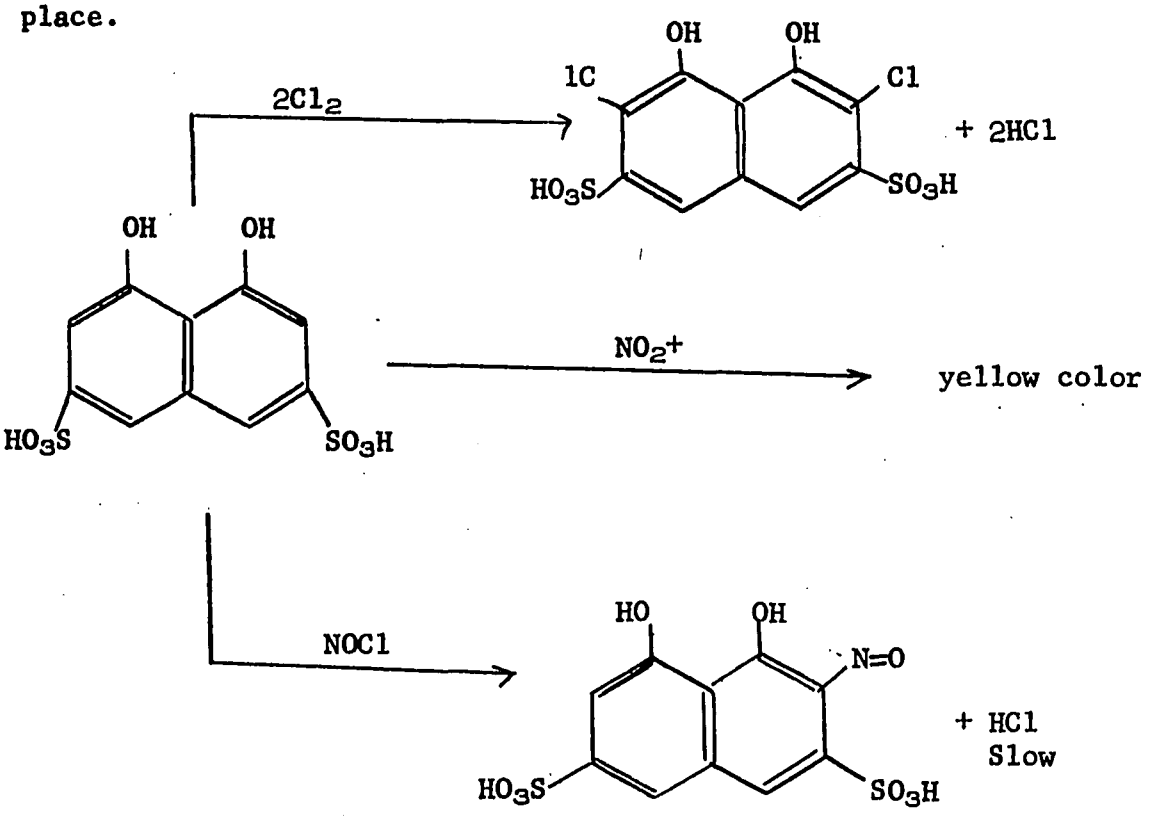
Chloride is thus a serious and potential interference in the methods of nitrate determination where a relatively high acid concentration is used in the reaction medium. The nitrate result will be lower if the products of the above reaction do not produce a color reaction with the reagent. On the other hand, a higher result for nitrate will be obtained if the products react with the reagent to form a more sensitive color reaction. Chromotropic acid and chlorine react to produce yellow color which is far more sensitive than the nitrate-chromotropic acid reaction. Thus, the increased sensitivity of the nitrate-chromotropic acid

TABLE X

ELIMINATION OF NITRITE INTERFERENCE

Interference Milligrams per Liter	Nitrate Milligrams per Liter	Urea Added in Milligrams	Absorbance	Corrected Absorbance
Nitrite				
0.0	0.0	0.0	0.015	Blank
0.0	0.0	2.5	0.015	0.000
30.0	0.0	0.0	1.20	1.18
30.0	0.0	2.5	0.017	0.002
30.0	1.6	2.5	0.043	0.028
30.0	4.0	2.5	0.087	0.072
100.0	4.0	2.5	0.088	0.073

color reaction in the presence of excess chloride in 75% v/v sulfuric acid medium as observed by West and Lyles (64) was due to the reaction of chromotropic acid with the products of the nitrate-chloride reaction. In their procedure, 3 milliliters of nitrate solution were made up to 10 milliliters with a concentrated sulfuric acid solution of 0.01% chromotropic acid and 1% concentrated hydrochloric acid. Thus the nitrate as well as the product of nitrate-chloride reaction has an immediate access to chromotropic acid and the following reactions could take place.



The yellow color developed in the presence of chloride and nitrate resembled more closely the chromotropic acid-chlorine color reaction than the nitrate-chromotropic acid color reaction. This

is shown in Figure 8 where a comparison is made of the spectra of chromotropic acid with nitrate, chlorine, and nitrate and chloride respectively in a 75% sulfuric acid medium. The color developed using the West-Lyles method was not stable and the color intensity was found to be increasing even after 24 hours as the reagent was being converted to mixtures of several yellow products. These products might involve nitro, nitroso, and chloro derivatives of chromotropic acid and probably included the formation of some quinones due to oxidation reactions.

An induced air oxidation reaction of hydrochloric acid to chlorine in the presence of nitrosyl chloride might account for the indefinite increase of the yellow color.

$$2NOCl + {}^{1}/_{2}(O)_2 + H_2O \rightarrow 2NO_2 + 2HCl \quad \text{Slow}$$

$$2NO_2 + 4HCl \rightarrow 2NOCl + 2H_2O + Cl_2 \quad \text{Slow}$$

$$Cl_2 + \text{Chromotropic acid} \rightarrow \text{yellow color} \quad \text{Fast}$$

Since chloride will almost always occur along with nitrate in the sample solution, the chloride interference was studied as follows.

A series of 2.5 milliliters of nitrate standards was transferred into 10 milliliter volumetric flasks. To each series, 250, 500, 1000 and 2500 micrograms of chloride ion in the form of 0.5 milliliter aqueous sodium chloride solutions were added. The flasks were allowed to stand in a cold water bath (10°C), 2 milliliters of concentrated sulfuric acid were added, and the mixtures were allowed to cool for four minutes. One milliliter

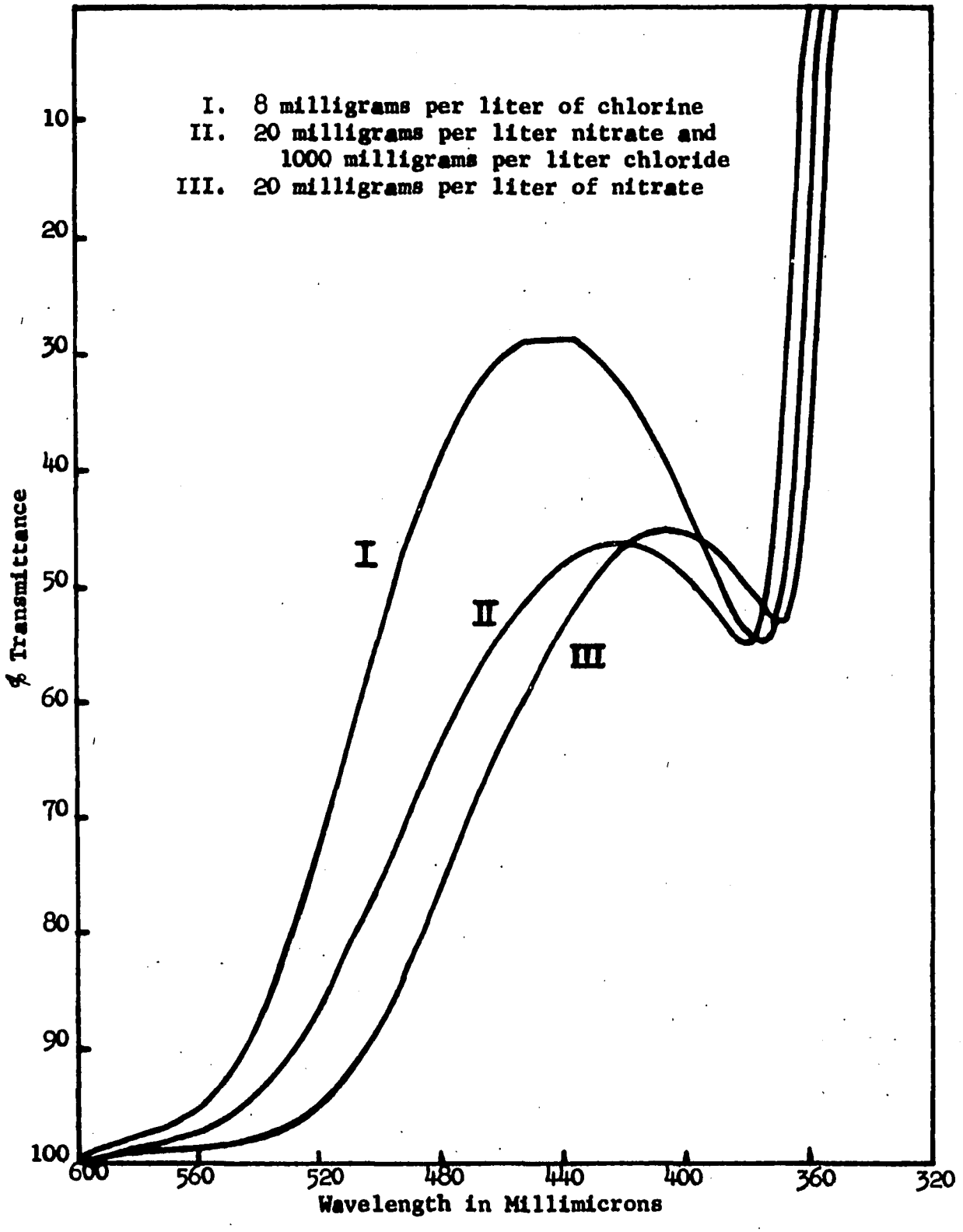


Figure 8

Absorption Spectra of Chromotropic Acid
with Chlorine, Nitrate, and Nitrate and Chloride

of the chromotropic acid reagent was added and the solutions cooled for 3 minutes. The volume was made up to the 10 milliliter mark and the solution mixed to uniformity. The absorbance readings were taken exactly after one hour at 410 millimicrons employing a 1 centimeter cell. The data presented in TABLE XI show that if chloride is added separately before adding chromotropic acid, contrary to the West-Lyles procedure, measured absorbances were lower compared to that obtained by using pure nitrate solution. This suppression of sensitivity not only depended on the amount of chloride added, but also on the time allowed for cooling especially after the addition of the sulfuric acid in the procedure. It thus appears that in the absence of immediate access to the chromotropic acid reagent, nitrate and chlorine might be undergoing the following reactions reducing the fast reacting species, namely nitrate and chlorine.

$$HNO_3 + NOCl \rightarrow 2NO_2 + HCl$$

$$2NO_2 + Cl_2 \rightarrow 2NO_2Cl$$

The rate of color development of the nitrate-chromotropic acid system at 15 milligrams per liter nitrate level was studied in the presence of various amounts of chlorides as shown in Figure 9, plots II, III, and IV. Plot I is that of the nitrate-chromotropic acid system without chloride. Although the color developed was suppressed initially in the presence of chloride, the intensity was gradually increasing with time and eventually the absorbance even exceeded that of the system without chloride.

TABLE XI

INFLUENCE OF CHLORIDE IN THE
NITRATE-CHROMOTROPIC ACID SYSTEM

Nitrate Used Milligrams per Liter	Blank Corrected Absorbance	Corrected Absorbances in Presence of Chloride			
		I 250 µgm Chloride	II 500 µgm Chloride	III 1000 µgm Chloride	IV 2500 µgm Chloride
0.2	0.004	0.003	0.000	0.000	0.000
0.3	0.005	0.004	0.003	0.000	0.000
0.4	0.007	0.005	0.005	0.003	0.000
0.8	0.015	0.014	0.014	0.012	0.005
1.6	0.028	0.027	0.025	0.023	0.010
4.0	0.073	0.069	0.065	0.060	0.038
6.0	0.107	0.101	0.098	0.093	0.068
8.0	0.142	0.138	0.135	0.130	0.105
12.0	0.211	0.200	0.197	0.195	0.160
16.0	0.280	0.270	0.265	0.255	0.180
20.0	0.350	0.345	0.340	0.330	0.240
Blanks	0.015	0.015	0.015	0.015	0.015

Figure 9

VARIATION OF ABSORBANCE WITH RESPECT TO TIME AND THE INFLUENCE OF CHLORIDE ON COLOR DEVELOPMENT

I 15 milligrams per liter of nitrate and no chloride

II 15 milligrams per liter of nitrate with 200 milligrams per liter chloride ions

III 15 milligrams per liter of nitrate with 400 milligrams per liter chloride ions

IV 15 milligrams per liter of nitrate with 1000 milligrams per liter chloride ions

V 20 milligrams per liter of nitrate with 2000 milligrams per liter chloride and 10 milligrams antimony III for masking

VI 9.15 milligrams per liter of nitrate with 4000 milligrams per liter chloride and 20 milligrams antimony (III)

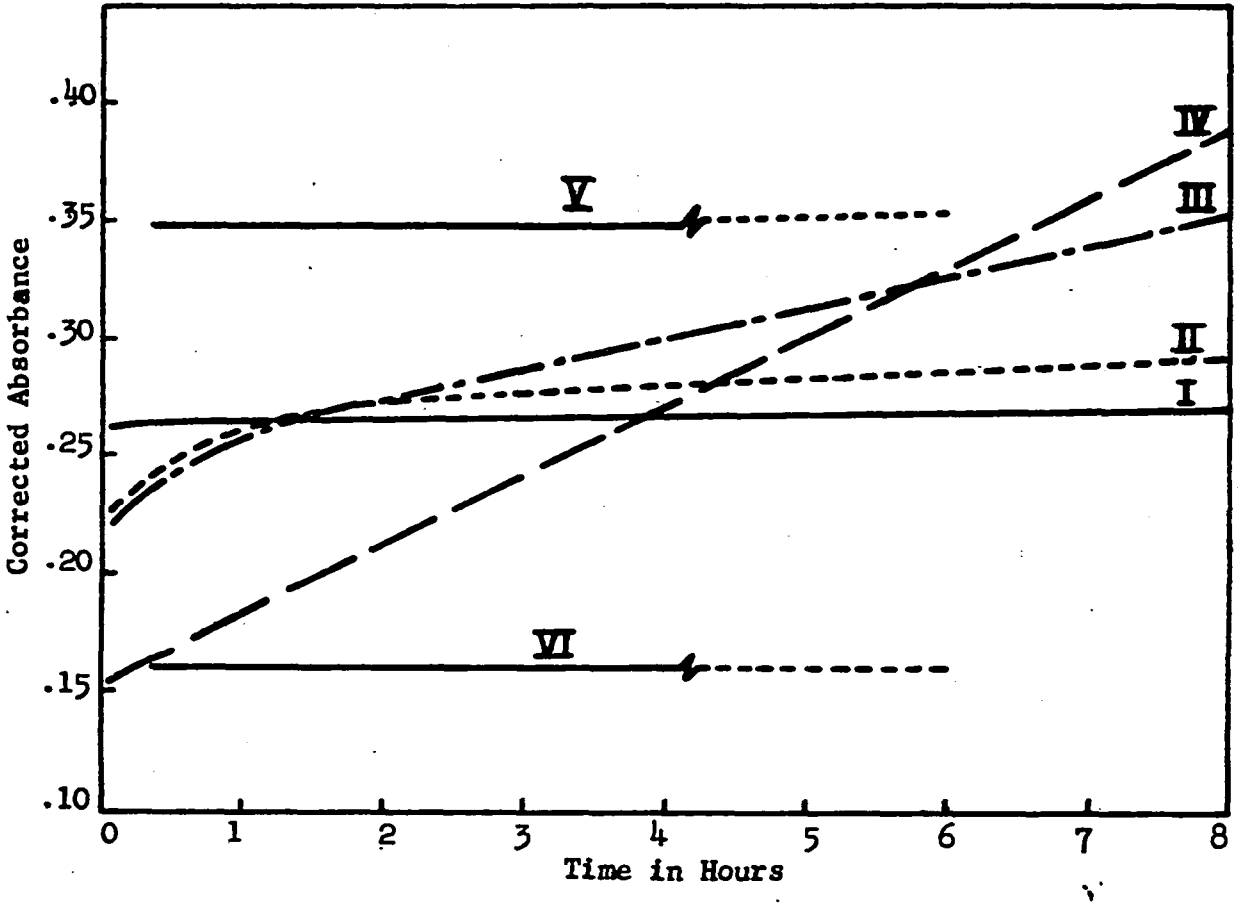


Figure 9

Variation of Absorbance with Respect to Time and the Influence of Chloride on Color Development

Because of the unreliability of the reactions involving nitrate and chloride with chromotropic acid, a procedure which would eliminate chloride or prevent the nitric acid-hydrochloric acid reaction was sought. Precipitation of chloride with silver may be effective, but is very tedious and costly. The only simple course open was to find a suitable method to mask the effect of chloride on nitrate. Therefore, a suitable cation which can form a strong chlorocomplex under the experimental condition was sought.

Among possible masking agents for chloride that were tested, only mercury (II) and antimony (III) showed promise to form strong chlorocomplexes in a strongly acidic medium. However, mercury (II) could not be used in large excesses in the system because it was easily reduced to insoluble mercury (I) chloride. Antimony (III) was found to mask the effect of chloride in the system so completely that true test colors developed and remained stable even after 24 hours. This is shown in plots V and VI in Figure 9, and the experimental solutions for these plots were prepared as described below.

Two and a half milliliters of a nitrate solution containing 20 milligrams per liter nitrate and 2000 milligrams per liter chloride were pipetted into a 10 milliliter volumetric flask. Two milliliters of the 0.5% antimony (III) sulfate solution was added. The antimony (III) solution also contributed about 0.4 milliliters of water for appropriate dilution to keep the final acid concentration in the optimum range. After allowing the

mixture to cool for about 4 minutes, 1 milliliter of reagent solution was added. After about 3 minutes of cooling the volume was made up to 10 milliliters with concentrated sulfuric acid and the solution was mixed. The absorbance of the solution at 410 millimicrons was measured with respect to time. The experiment was repeated using a 2.5 milliliter nitrate solution containing 9.15 milligrams per liter nitrate and 4000 milligrams per liter chloride. Two milliliters of 1% antimony (III) solution was used to mask chloride.

The efficiency of chloride masking by antimony (III) was determined by studying the influence of varying concentrations of chloride on a nitrate-chromotropic acid system containing a fixed amount of antimony (III). The results of such a study are presented in TABLE XII and Figure 10. It can be seen that in the presence of 5 milligrams of antimony (III), chloride concentrations ranging up to 3 milligrams did not affect the absorbance of the nitrate-chromotropic color reaction. Therefore, 5 milligrams of antimony (III) can effectively mask up to 3 milligrams of chloride, or for 2.5 milliliter samples, up to 1200 parts per million of chloride can be tolerated. Color developed was not stable and consistent if the amount of chloride ion exceeded 3 milligrams because the excess unmasked chloride interfered with the nitrate-chromotropic acid system.

4. IRON

Iron (III) is a commonly occurring ion in water samples, although it is expected to be present only in amounts of a few milligrams per liter. Even small amounts, however, were causing

TABLE XII

VARIATION OF ABSORBANCE OF THE NITRATE-CHROMOTROPIC ACID SYSTEM CONTAINING ANTIMONY WITH RESPECT TO CHLORIDE VARIATION

Nitrate Milligrams per Liter	Total Antimony Used in Milligrams	Total Chloride Concentration Used in Milligrams	Corrected Absorbance After 1 Hour	Corrected Absorbance After 22 Hours
20	5	0	0.350	0.350
20	5	1	0.350	0.350
20	5	2	0.350	0.350
20	5	3	0.350	0.350
20	5	4	0.312	0.375
20	5	5	0.275	0.355
20	5	6	0.240	0.325
20	5	8	0.170	0.250

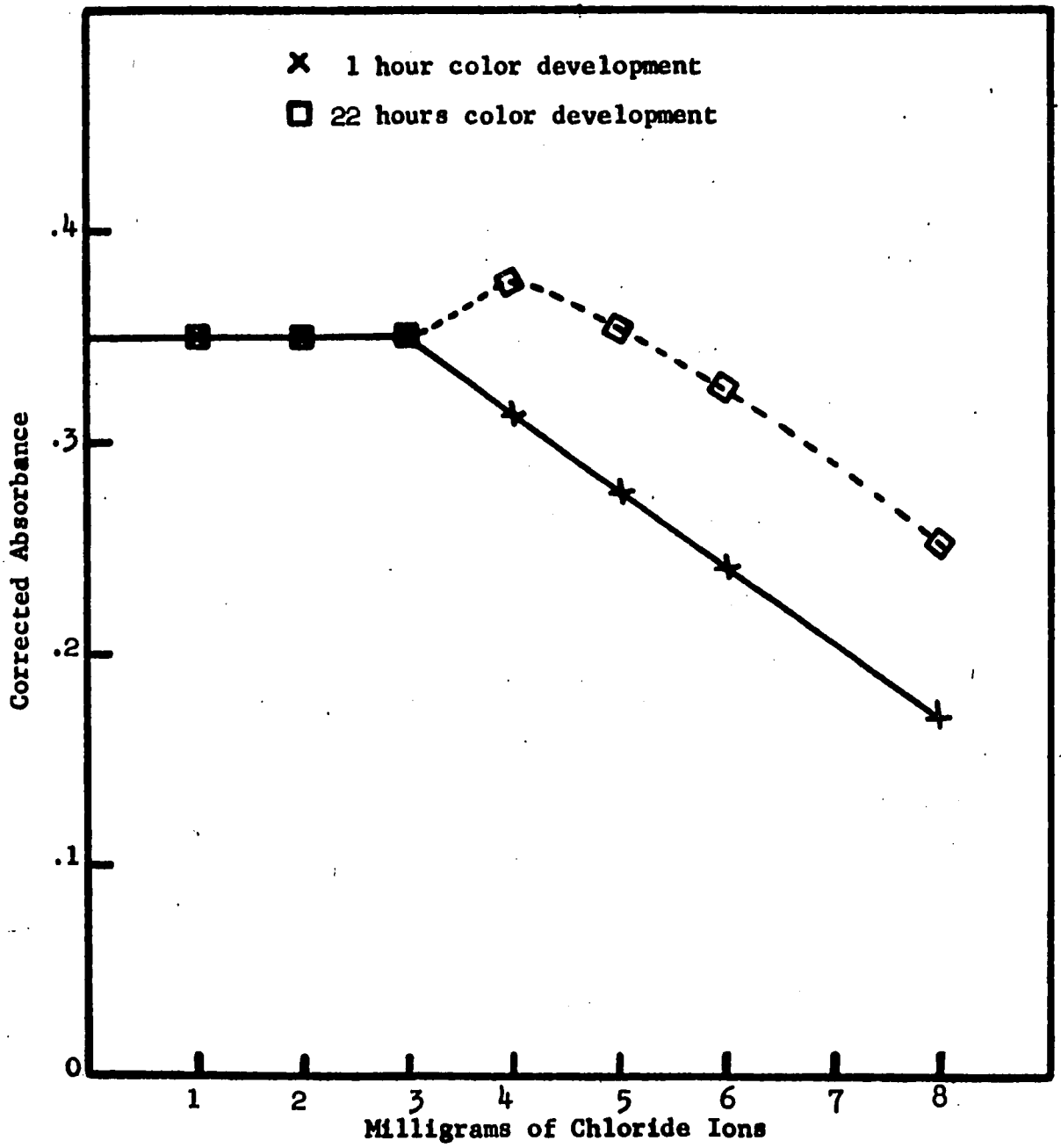


Figure 10

Influence of Varying Chloride on the
Nitrate-Chromotropic Acid System Containing
20 Milligrams per liter Nitrate and
5 Milligrams of Antimony (III)

serious interference in the nitrate-chromotropic acid system, because of the formation of yellow chloroferrate (III) complexes in the presence of chloride. This interference was also easily overcome by the addition of antimony (III), which completely discharged the interferring color by demasking the chloroferrate (III) complexes. In the presence of antimony, up to 40 milligrams per liter of iron and 1000 milligrams per liter of chloride showed no significant interference. The results of these studies are presented in TABLE XIII.

TABLE XIII

ELIMINATION OF IRON (III) INTERFERENCE

Iron (III) (+Chloride*) Concentration Milligrams per Liter	Nitrate Concentration Milligrams per Liter	Amount of Antimony Used Milligrams	Absorbance Readings	Corrected Absorbance
0.0	0.0	0.0	0.015	0.000
0.0	0.0	10.0	0.015	0.000
40.0	0.0	0.0	0.115	0.100
40.0	0.0	10.0	0.015	0.000
40.0	0.8	10.0	0.030	0.015
40.0	4.0	10.0	0.087	0.072
0.0	0.8	10.0	0.030	0.015
0.0	4.0	10.0	0.088	0.073

* Chloride was added with iron (III) in each case in amounts equivalent to 1000 milligrams per liter.

CHAPTER VII

NITRATE DETERMINATION USING CHROMOTROPIC ACID

A. THE STANDARD RECOMMENDED PROCEDURE

The significant parameters of the nitrate-chromotropic acid system have been obtained by preliminary investigation, and the procedures for eliminating major interferences have been described in the previous chapters. This information was utilized to evolve a simple procedure for the determination of nitrate. The interference elimination steps were incorporated in the procedure in the most appropriate manner to make the method as direct as possible. Thus, the addition of sulfite, urea, and antimony in sulfuric acid should eliminate interferences due to oxidizing agents and chlorine, nitrite, chloride and iron (III), respectively. The following procedure was proposed for the nitrate determination using chromotropic acid and was investigated further for its reliability.

Two and a half milliliters of nitrate solution (standard or sample) having a nitrate content in the range of 0.2 to 20 milligrams per liter were transferred to dry 10 milliliter volumetric flasks. The flasks were placed in a tray of cold water of 10-20°C. To each flask a drop of the sulfite-urea solution and 2 milliliters of antimony solution were added. When the chloride concentration was 0 to 2000 milligrams per liter, a 0.5% antimony solution was

used, and a 1% solution was employed when the chloride was in the range of 2000-4000 milligrams per liter. The flasks were swirled after each addition. After cooling the flasks in the bath for at least 4 minutes, 1 milliliter of chromotropic acid reagent was added. The flasks were again swirled and then allowed to cool for about 3 minutes. Concentrated sulfuric acid was added to adjust the volume to the 10 milliliter mark, the flasks were stoppered, and the contents mixed by inverting four times. The flasks were allowed to stand for 45 minutes at room temperature and the volume was again adjusted to the 10 milliliter mark with concentrated sulfuric acid. Final mixing should be done very gently to avoid trapping of gas bubbles. Distilled water was substituted for nitrate solution in running blank for each set of experiments.

The absorbance reading was taken 15 minutes or more after the last adjustment of volume. The absorbance was measured in a Beckmann Model D U Spectrophotometer using 1 centimeter quartz cells at 410 millimicrons against water in the reference cell. The cell must be rinsed with the solution and then filled carefully so as to avoid trapping bubbles. For this operation, it was found expedient to keep the cell in a slanting position (30° to horizontal) with a ground side facing up and pouring the solution very slowly down the side of the cell.

B. RESULTS

TABLE XIV gives the corrected absorbances of the nitrate-chromotropic acid system at various concentrations of nitrate, both in the absence and presence of concentrated chloride. When

TABLE XIV

ABSORBANCE OF THE NITRATE-CHROMOTROPIC ACID SYSTEM

At 410 millimicrons using a 1 centimeter cell

Nitrate Milligrams per Liter	Corrected Absorbance Series 1	Corrected Absorbance Series 2	Corrected Absorbance Series 3	Corrected Absorbance Series 4
Blank	0.015	0.015	0.015	0.015
0.2	0.0035	0.0035	0.004	0.0035
0.3	0.005	0.005	0.005	0.005
0.4	0.007	0.007	0.008	0.007
0.8	0.015	0.014	0.015	0.016
1.6	0.028	0.028	0.029	0.028
4.0	0.071	0.073	0.072	0.071
6.0	0.107	0.107	0.107	0.107
8.0	0.142	0.141	0.144	0.142
12.0	0.211	0.212	0.211	0.211
16.0	0.277	0.280	0.278	0.278
20.0	0.347	0.347	0.350	0.351

Series 1 With nitrate only
Series 2 Nitrate in presence of urea, sulfite and antimony (III)
Series 3 and 4 Nitrate in presence of urea, sulfite and antimony (III) together with 1000 milligrams per liter and 2000 milligrams per liter chloride respectively.

chloride concentrations were below 2000 milligrams per liter, a 0.5% antimony solution was used and a 1% solution was used at higher concentrations. Beer's law was obeyed from 0.2 to 20 milligrams per liter. The standard graph is shown by Figure 11. TABLE XV presents the results of a statistical evaluation of nitrate determinations at the respective levels, and with the chloride concentration varying from 0 to 2000 milligrams per liter. The standard deviations and coefficients of variation at the 95% confidence limit are given at the respective level. For low absorbance measurements, 5 centimeter cells could be used. The required volume of the solution can be prepared by developing the color using 5 millliliters of standard or sample, adding proportional amounts of the reagnets, and adjusting the final volume to 25 millliliters.

C. EXAMINATION OF OTHER INTERFERENCES

The influence of diverse ions on the nitrate-chromotropic acid system was studied following the recommended procedure to determine the possibility of interference by other ions. A solution containing 200 milligrams per liter of each ion, both in the presence and in the absence of 4 milligrams per liter nitrate, was treated according to the recommended procedure. An ion was considered as non-interfering if the absorbances of the glank as well as that of the standard in the presence of the ion agreed correspondingly to those without the ion.

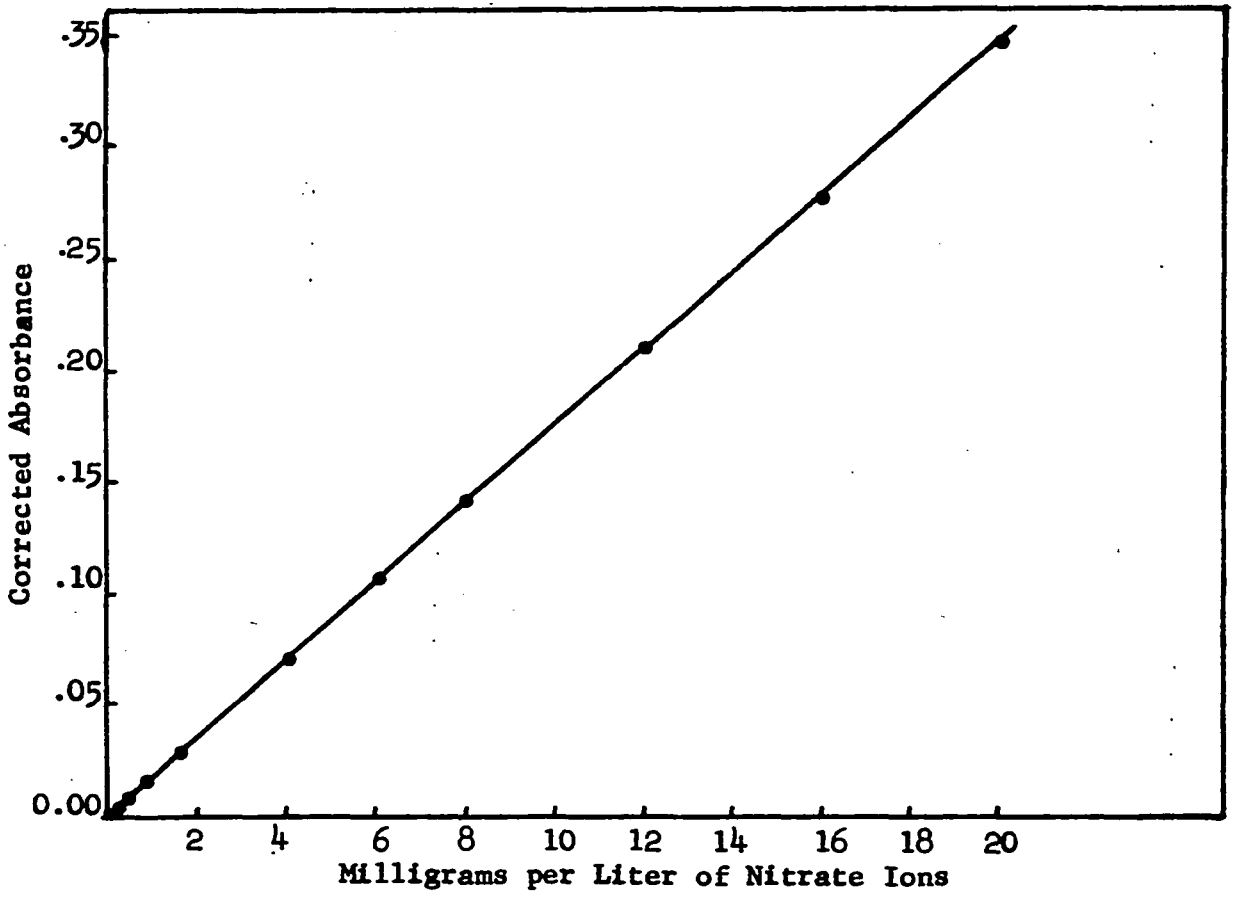


Figure 11

The Standard Graph of the
Chromotropic Acid Method

TABLE XV

STATISTICAL ANALYSIS OF ABSORBANCE
AT VARIOUS LEVELS OF NITRATE

Number of Determinations	Nitrate Concentration Milligrams per Liter	Average Corrected Absorbance	Standard Deviation in Milligrams per Liter	Coefficient of Variance for 95% Confidence Limit
20	0.3	0.0049	± 0.030	± 20%
10	0.6	0.0115	± 0.030	± 10%
10	1.2	0.0227	± 0.024	± 4%
10	3.1	0.0560	± 0.0042	± 3%
10	6.5	0.1150	± 0.093	± 3%
10	10.0	0.1780	± 0.100	± 2%
10	16.6	0.2900	± 0.160	± 2%
10	19.5	0.3400	± 0.180	± 2%

When applied to the following ions, no significant interference was disclosed.

Li^+, Na^+, K^+, Cu^{+2}

Be^{+2}, Mg^{+2}, Ca^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2}, Sr^{+2}

BO_2^-, $B_4O_7^{-2}$, Al^{+3}

CO_3^{-2}, Ti^{+4}, Zr^{+4}, Sn^{+2}, Sn^{+4}

NH_4^+, HPO_4^{-2}, $P_4O_7^{-4}$, VO^{+2}, As^{+3}, Bi^{+3}

S^{-2}, SO_3^{-2}, Cr^{+3}, CrO_4^{-2}, MoO_4^{-2}, WO_4^{-2}

F^-, Cl^-, ClO_3^-, ClO_4^-, Mn^{+2}, MnO_4^-, Br^-, BrO_3^-, OCl^-

Fe^{+2}, Co^{+2}, Ni^{+2}

CH_3OO^-, $HCOO^-$.

Barium and lead form turbidities at concentrations of 200 milligrams per liter but can be tolerated in the system in concentrations up to 50 milligrams per liter. The following ions interfere in the system due to formation of precipitates of antimony (III) iodide or selenium metal, respectively: I^-, IO_3^-, SeO_3^{-2}, and SeO_4^{-2}. Centrifugation did not succeed in removing the precipitates. These ions would not be expected to be present in significant amounts in ordinary water samples, and so do not offer any complications. If present in concentrations greater than 20 milligrams per liter, chromium (III) interferes due to the absorbance of its sulfato complexes. Formaldehyde interferes and its presence is indicated by the formation of violet color with chromotropic acid under the experimental conditions. When encountered in samples, the interference due to formaldehyde can

be overcome by oxidizing it to formic acid with a slight excess of potassium permanganate. The excess permanganate will be reduced by the sulfite added in the recommended procedure.

D. APPLICATION TO ANALYSIS OF WATER SAMPLES

To prevent any change in the nitrogen balance through biological activity, the nitrate determination should be started promptly after sampling. If such a step is impractical, storage near freezing temperature or addition of 0.8 millliliters of concentrated sulfuric acid to 1 liter of sample may serve to maintain the nitrogen balance.

The most reliable results are obtained with fresh samples and a prompt analysis. The chromotropic acid method can be directly applied to clear and colorless water samples without recourse to evaporation or precipitation procedures. The results of such an analysis on drinking water, rain water and river waters are presented in TABLE XVI. If samples contain appreciable amounts of suspended impurities, they should be removed by centrifugation or filtration. TABLE XVI also presents data from analysis of turbid water samples (stagnant and river water). If the sample has a color, it may be removed by stirring thoroughly with aluminum hydroxide suspension. After allowing to stand for a few minutes, the sample is filtered and subjected to analysis.

The results obtained by this method are more reliable than by conventional methods. Reliability of the method on any new type of water sample must be tested as follows. A distilled water blank and a sample blank in the absence of chromotropic acid must

TABLE XVI

ANALYSIS OF WATER SAMPLES

Nature of Sample	Nitrate Added in Milligrams per Liter	Chloride Present in Milligrams per Liter	Mean Value of Nitrate Found in Milligrams per Liter	Number of Determinations
Baton Rouge tap water	0.00	50	nil	5
Baton Rouge tap water	2.50	50	2.5±0.02	10
Synthetic Sample*	2.0	4000	2.0±0.02	5
Synthetic Sample*	4.0	4000	4.0±0.05	5
Muddy Stagnant Rain Water	0.0	nil	6.2±0.10	3
Muddy Stagnant Rain Water	8.0	nil	14.3±0.30	3
Direct Rain Water	0.0	nil	2.0±0.04	3
Direct Rain Water	4.0	nil	6.1±0.05	3
WATER SAMPLES**				
No. 37	0.00	1120	4.30	2
	3.00	1120	7.30	2
No. 38	0.00	1176	4.35	2
	3.00	1176	7.35	2
No. 39	0.00	1006	4.70	2
	3.00	1006	7.70	2
No. 40	0.00	1016	8.10	2
	3.00	1016	11.10	2
No. 41	0.00	950	4.60	2
	3.00	950	7.60	2
No. 42	0.00	1016	4.60	2
	3.00	1016	7.60	2

* The antimony (III) solution used was 1%

** Water samples containing suspended impurities, received from Kem-Tech Laboratories, Inc., Baton Rouge, Louisiana. Suspended matters removed by centrifugation.

agree closely in the absorbance at 410 millimicrons. This would indicate the absence of any substance in the sample which would cause a positive interference. Again a standard addition of nitrate to the sample must agree closely to the amount of added nitrate. This would obviate any negative interference or interference which consumes nitrate. For analysis of every series of samples, it is advisable to apply the reliability test.

E. APPLICATION TO THE ANALYSIS OF AIR SAMPLES

1. DETERMINATION OF NITRATE-NITROGEN IN AIR

To prepare the sample for analysis, air was scrubbed at the rate of 200 milliliters per minute for more than an hour through 10 milliliters of 0.2 normal sulfuric acid containing 10 milligrams of urea. The apparatus used is shown in Figure 12. After the scrubbing, the solution was treated with a drop of 0.1 normal permanganate to oxidize any interference. The sample was analyzed according to the recommended procedure. TABLE XVII presents results obtained on laboratory air samples.

2. DETERMINATION OF NITROGEN DUE TO NITROGEN OXIDES, NITRITE AND NITRATE

For sampling total nitrogen present in the form of nitric oxide, nitrogen dioxide, nitrite and nitrate, the scrubbing solution was replaced by 10 milliliters of 0.2 normal sulfuric acid containing a drop of permanganate. In this case, nitric oxide, nitrogen dioxide, and nitrite were oxidized to nitrate; and nitrogen present in these forms was easily obtained by multiplying the nitrate estimated by chemical factor of nitrogen to nitrate.

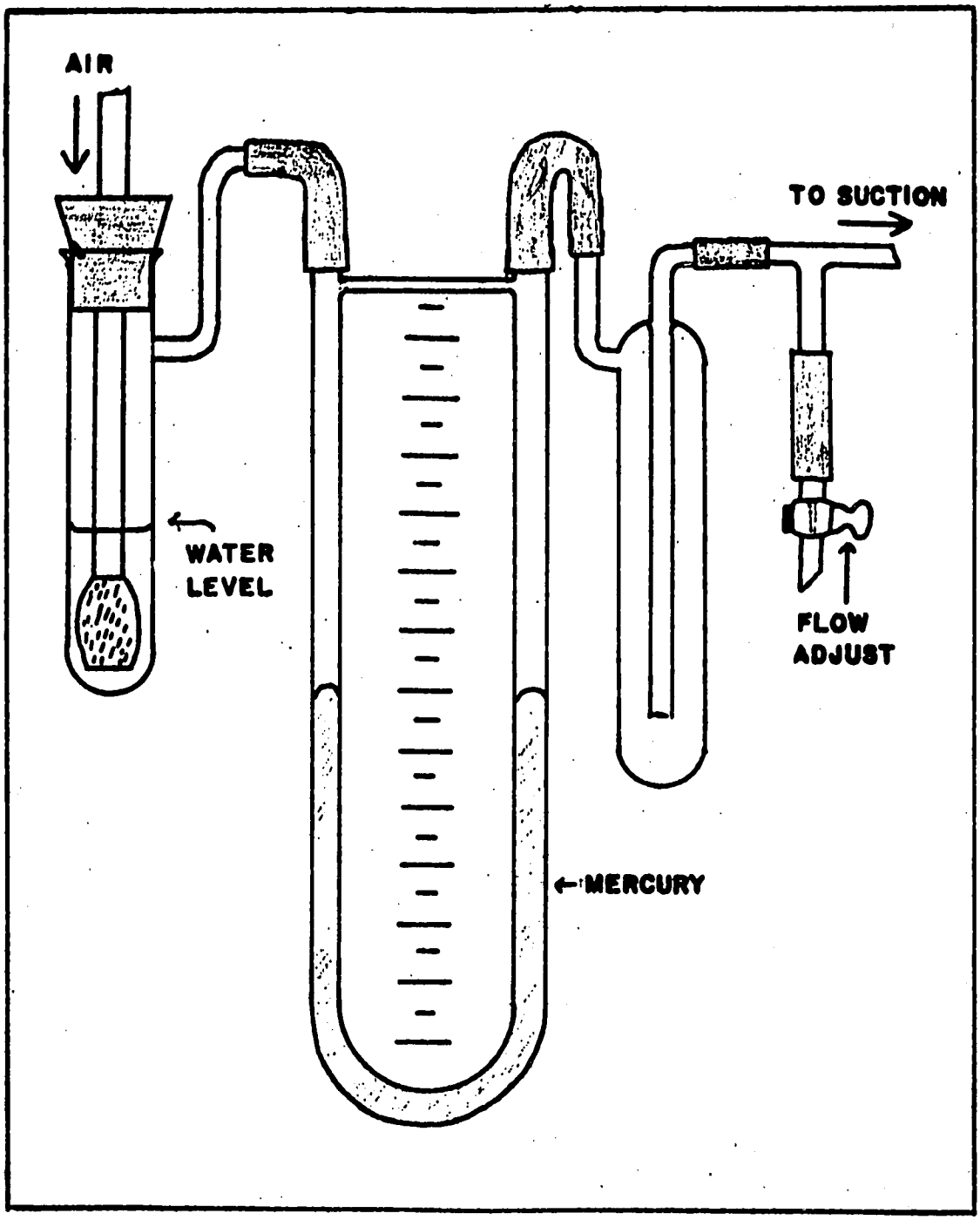


Figure 12

Apparatus for Scrubbing Air Samples

TABLE XVII

DETERMINATION OF NITROGEN DUE TO NO, NO_2, NO_2^- and NO_3^- in AIR

Scrubbing Solution	Liters of Air Scrubbed	Corrected Absorbance	Nitrate Nitrogen in Parts per Hundred Million	Remarks
10 milliliters of 0.2 normal H_2SO_4 Containing 10 milligrams Urea	15	0.015	0.0130	Nitrogen due to Nitrate in Air
	15	0.016	0.0130	
	27	0.060	0.0285	
	15	0.016	0.0130	
10 milliliters of 0.2 normal H_2SO_4 Containing Permanganate	15	0.029	0.0124	Nitrogen due to NO, NO_2, NO_2^- and NO_3^-
	15	0.027	0.0220	
	27	0.060	0.0285	
	15	0.020	0.0166	

F. DETERMINATION OF TRACES OF NITRATE IN PURE SODIUM NITRITE

Interest in this determination was primarily to find out the extent of air oxidation sodium nitrite might be undergoing in an aqueous solution. It is reported that nitrites are easily oxidized to nitrate. A 400 milligram per liter aqueous solution of nitrite was prepared using sodium nitrite. The nitrate content of this solution was determined at the time of preparation and after some definite periods of time. The nitrite in the solution was completely destroyed by adding excess urea and the recommended procedure was applied. The results presented in TABLE XVIII show that the aqueous solution of sodium nitrite is stable.

G. CONCLUSION

The color reaction of nitrate and chromotropic acid in strong sulfuric acid medium has been investigated spectrophotometrically. A simple, sensitive and specific method for the determination of nitrate has been developed using chromotropic acid as a reagent. Major interferences due to oxidizing agents, nitrite, chloride and iron (III) have been eliminated by a simple addition of sulfite, urea and antimony (III).

Considering the complexities involved in the determination of nitrate, it can be concluded that the chromotropic acid method offers a simple and reliable solution to the problem of nitrate determination. The range of determination extends from 0.2 to 20 milligrams of nitrate per liter. The method is simple,

TABLE XVIII

DETERMINATION OF NITRATE IN NITRITE
AND STABILITY OF AQUEOUS SODIUM NITRITE SOLUTION

Time of Standing of Nitrite Solution (months)	Nitrite Analyzed (Micrograms)	Corrected Absorbance	Nitrate Found (Micrograms)	Percent Nitrate
0	1000	0.035	5.00	0.50
1	1000	0.038	5.30	0.53
2	1000	0.040	5.5	0.55
4	1000	0.042	5.8	0.58

sensitive and has no significant interference. Because of this fact the method is directly applicable to the analysis of water samples and requires no preliminary separation or concentration procedure. The method has been applied to water samples and reliable results consistent with the standard addition technique have been obtained. Statistical evaluation has shown that the method can give better and more reproducible results than the phenoldisulfonic acid or the brucine methods.

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Title of Thesis: The Determination of Nitrate

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Date of Examination:

May 3, 1966