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TITLE: The Removal of the Organic Interference in the Analysis of Nitrate-Nitrogen in Waters by the Ultraviolet Spectrophotometric Screening Method.

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The determination of nitrate-nitrogen in water is difficult at best. The relatively complex procedures, the presence of interfering substances, and the limited concentration ranges of the various techniques all contribute to the difficulty. Screening methods can be used, but they only determine approximate concentrations. Once a sample has been screened, a method suitable for its concentration range must then be selected. If an easier method were available, or if an improvement on an existing method could be made, the nitrate-nitrogen analysis would be greatly facilitated.

The main purpose of this research is to refine and test the use of activated charcoal for the removal of the organic interference in various water samples. This removal would eliminate the unreliable organic corrections and would permit accurate analysis of nitrate-nitrogen by ultraviolet spectroscopy. A method of analysis using activated charcoal is proposed. The chromotropic acid method will be used as the reference/comparison method. The chromotropic acid method and the ultraviolet spectrophotometric screening method can both be found in the Standard Methods for the Examination of Water and Wastewater , 15th ed., 1980.

THE REMOVAL OF THE ORGANIC INTERFERENCE IN THE ANALYSIS OF NITRATE-NITROGEN IN WATERS BY THE ULTRAVIOLET SPECTROPHOTOMETRIC SCREENING METHOD

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In lakes and rivers, nitrogen as the nitrate ion usually occurs in trace amounts, 1 to 2 milligrams nitrate-nitrogen per liter, but in well water and other ground water it may reach much higher levels. At high concentrations, nitrate can cause the illness known as methemoglobinemia in infants. In Minnesota alone, from 1947-48, there were 139 cases with 14 deaths traced to a high nitrate content in well water [1,2]. As a result, a limit of 10 milligrams nitrate-nitrogen per liter has been imposed on domestic water supplies [3]. In domestic waste water, nitrate is found only in small amounts, but some effluents from treatment plants may contain nitrate in concentrations of up to 30 milligrams nitrate-nitrogen per liter. Nitrate reaches significantly high concentrations because it represents the final stage of the biological oxidation of organic nitrogen compounds. Nitrate is the highest oxidized form in the nitrogen cycle. Nitrate may also serve as an oxygen source, and is an essential nutrient for many photosynthetic autotrophs. As can be seen, the study of the nitrate content of water is of interest as an indication of bacterial activity and pollution.

There are a variety of methods available for the determination of nitrate-nitrogen in water [4]. These include the reduction of nitrate to ammonia, reduction to

nitrite, specific-ion electrode methods, direct spectrophotometry, and spectrophotometric methods utilizing color developing reagents. Unfortunately, the relatively complex procedures, the presence of interfering substances, and the limited concentration ranges of the various techniques make the determination of nitrate-nitrogen difficult.

Of the techniques available, a simple, rapid, and relatively reliable method is direct spectrophotometry in the ultraviolet (UV) region. Its advantage over chemical methods is seen in the work by Hoather and Rackham [5]. The nitrate concentrations determined from the ultraviolet absorption are much higher than the nitrate concentrations determined chemically. It appears that the total concentration of oxidized nitrogen is not completely measured by the usual chemical determinations of nitrite and nitrate. For such samples, ultraviolet absorption is beneficial because it indicates the presence of the oxidized nitrogen in a form that only appears gradually as nitrate when the sample is kept for a few weeks.

The direct UV method is especially suited for screening samples that have low dissolved organic matter contents, such as uncontaminated natural waters and potable water supplies. Reasonable results can be attained by applying a relatively small correction for dissolved organic matter in solution [1,4,5,6], on the empirical basis that the absorption at the nitrate wavelength due to

dissolved organic matter is anywhere from two to four times as great as that at the wavelength used to determine dissolved organic matter. It should be pointed out that dissolved organic matter refers to the absorbing materials measured at 275 nanometers (nm). The absorbance at 275 nm is used to determine the presence, or absence, of this dissolved organic matter that could also show absorbance at the wavelength selected for the nitrate-nitrogen determination. The most common wavelengths selected for nitrate-nitrogen determinations, in conjunction with the 275 nm wavelength, include either 210 nm [5,6] or 220 nm [1,4]. These techniques are not reliable when the dissolved organic matter concentrations are high and the nitrate-nitrogen concentration is low. The reason for the unreliability of the correction factor techniques comes from the different organic compounds present in different types of water.

In addition to the correction factor techniques briefly mentioned, other nitrate-nitrogen methods have been proposed to deal with the interference due to dissolved organic matter using wavelengths at 210 nm [7,8,9] and 230 nm [10]. Armstrong's [10] addition of an equal volume of concentrated sulfuric acid to a solution containing nitrate, when chloride is also present, causes a change in the nitrate absorption spectrum, the maximum being shifted to 230 nm. The measurement at 230 nm is easier because the adsorption of other substances is less than at 210 nm, and

may even be diminished in the presence of the sulfuric acid. The high acid concentration increases the reactivity of nitrate so that it may be destroyed by a suitable reducing agent, a hydrazine sulfate solution, allowing an accurate measurement of the non-nitrate absorbance of the sample. The difference in the two readings is proportional to the nitrate concentration. The sample should not contain more than 2.5 parts per million nitrate-nitrogen, but should contain more than 2 grams of chloride per liter. Samples of higher nitrate concentration may be diluted, and, if the chloride concentration is too low, hydrochloric acid may be added.

A rapid and accurate method for determining nitrate has been proposed by Bastian, et al. [7] for determining nitrate in alkaline earth carbonate mixtures. The method utilizes ultraviolet absorption of nitrate ion in dilute perchloric acid. The method consists of dissolving the sample in dilute perchloric acid and reading the absorbance at 210 nm. Interferences due to metal ions are removed by passing a weakly acid perchloric acid solution of the material through a cation exchange resin.

The proposed method for potable waters by Navone [8] is also based on the absorption of nitrate at 210 nm. The absorbance of a water sample containing nitrate ion is measured at 210 nm against a duplicate portion of the sample, a blank, in which the nitrate ion has been reduced to ammonia by the action of a zinc-copper couple in an

acidified medium. The use of the blank permits the cancelling out of all interfering substances except nitrite. It is assumed that nitrite ion concentrations in potable waters are low, and is therefore not a significant factor. Waters having a nitrate ion concentration above 8.8 mg/L must be diluted in order to use this proposed method.

The variability of the dissolved organic matter correction techniques and the possible lowering of precision by diluting a sample to bring the absorbance with the linear range of UV spectrophotometric analysis emphasizes the need for a non-arbitrary method with improved accuracy. Such a method has been proposed by Rennie, et al. [9], which removes not only the dissolved organic matter prior to analysis, but also some of the cationic interferences that affect the determination of nitrate.

Because of the difficulties encountered in dealing with dissolved organic matter plus the potential effectiveness of activated charcoal, Rennie and his associates investigate the organic matter content, nitrate content as received and the extent of nitrate retention on approximately 20 types of activated charcoals. Unfortunately, most of the charcoals tested contain nitrate and organic matter that is easily leached out. Also, the charcoals retained 19 to 84 per cent of the nitrate under acidic conditions. Never the less, methods using activated

charcoal have been investigated further.

The methods investigated include batchwise addition of powdered or granular charcoals followed by filtration to remove the charcoal, the use of a charcoal column, and the construction of a filter stack using materials impregnated with charcoal. Because of the drawbacks of the batchwise addition, and the insufficient removal of the organic matter in the column, the development of a method using a particular analytical-grade filter paper impregnated with activated charcoal under alkaline conditions was pursued. Tests with 4, 6, and 16 layers of the charcoal paper have shown that at least 16 layers are required in order to allow a sufficient contact time to remove the organic matter from the samples. Tests with standard nitrate solutions also show that nitrate ion is not absorbed under alkaline conditions by the increased number of layers.

The main purpose of this investigation has been to refine and test the batchwise addition of activated charcoal for the removal of dissolved organic matter in natural water samples. This removal process would eliminate the need for a correction wavelength, making the analysis of nitrate-nitrogen by UV spectroscopy a simpler and more accurate method. The drawbacks encountered by Rennie and his associates have also been investigated.

EXPERIMENTAL

I. Chromotropic Acid Method

A. Apparatus

Absorbance was measured at 410 nm with a HACH DR/3000 Spectrophotometer using a 1-inch path-length glass cell. Analysis of the yellow reaction product followed the procedure outlined in Standard Methods [4] using five times (5X) the volumes listed (final volume of 50 ml).

B. Reagents

1. Distilled, deionized water, stored in a plastic container, was used for all solutions and dilutions.

2. A stock nitrate solution was prepared by taking 0.7218 g of potassium nitrate, dried in an oven at 105 C for 24 hours, dissolving it in water and diluting to 1000 ml. This was then preserved with 2 ml of chloroform per liter giving a final concentration of 100 mg of nitrate-nitrogen per liter.

3. A standard nitrate solution was prepared by diluting 50.0 ml of stock nitrate solution to 500 ml with water, giving a final concentration of 10.0 mg of nitrate-nitrogen per liter.

4. Nitrate standards of 0.1 to 5.0 mg of nitrate-nitrogen per liter were prepared prior to analyses.

5. The sulfite-urea reagent was prepared by dissolving 5 g of urea and 4 g of sodium sulfite in water and diluting to 100 ml.

6. The antimony reagent was prepared by heating 500 mg of antimony metal powder in 80 ml of concentrated sulfuric acid until all the metal had dissolved. This was done in a hood to prevent the sulfuric acid fumes from entering the room. The solution was allowed to cool and then it was cautiously added to 20 ml iced water. Since crystals formed overnight, and redissolving by heating proved difficult, this reagent was prepared just prior to analyses.

7. The purification of the chromotropic acid sodium salt (4,5-dihydroxy-2,7-naphthalene disulfonic acid disodium salt) was performed according to the procedure outlined in Standard Methods [4]. Recrystallization proved to be more of a problem then expected. When the crystals did finally form, they were not white, needle crystals, but a blue granular mass that didn't dry in a desiccator, and melted when placed in an oven. Therefore, the certified A.C.S. chromotropic acid sodium salt received from the Fischer Scientific Company, Chemical Manufacturing Division was used as received. Visual and absorbance comparisons were made between the yellow reaction products formed when using the purified chromotropic acid and the chromotropic acid sodium salt as reagents on various nitrate standards. The results showed little, if any, difference in absorbance

readings and color development. So, the chromotropic acid reagent was prepared by dissolving 0.1 g of the chromotropic acid sodium salt in 100 ml concentrated sulfuric acid. This was stored in a brown glass bottle, and prepared fresh every two weeks.

C. Procedure

Standard curves were prepared by using a linear least squares curve fitting program [11]. Standard nitrate-nitrogen concentration levels of 0, 0.1, 0.5, 1.0, 2.5, 4.0, and 5.0 mg/L were used to prepare the standard curves. After it was confirmed that the standard curve was linear, two standards, 1 and 5 mg/L, were analyzed with each group of samples. Samples were filtered through 4.25 cm GF/C Whatman Glass Microfibre filters to remove any suspended matter that might be present. Color development followed the outlined procedure. The absorbance at 410 nm was set at zero absorbance with a distilled, deionized water blank. Readings were made directly for all samples, alternating with the water blank. Concentrations were calculated from the least squares, best-fit slope of the standard curves.

II. Ultraviolet Spectrophotometric Screening Method

A. Apparatus

Absorbance was measured with an EU-700 Series

GCA/McPherson Spectrophotometric Instrument using matched 1-cm path-length silica cells. The determination of nitrate-nitrogen followed the procedure outlined in Standard Methods [4].

B. Reagents

1. Distilled, deionized water, stored in a plastic container, was used to prepare the stock nitrate solution. Distilled water, stored in a glass container, was used to prepare the standard nitrate solutions.

2. Stock nitrate solution: Prepared as described in the Chromotropic Acid Method reagent section (reagent #2).

3. Standard nitrate solution: Prepared as described in the Chromotropic Acid Method reagent section (reagent #3).

4. Nitrate standards of 0.5 to 10.0 mg nitrate-nitrogen per liter were prepared prior to analyses by diluting the standard nitrate solution to the appropriate nitrate concentrations using the distilled water that was stored in the glass container.

C. Procedure

Standard curves were prepared using a least-squares curve fitting routine[11]. Standard nitrate-nitrogen concentration levels were 0, 0.5, 1.0, 4.0, 5.0, and 10.0 mg/L. After it was confirmed that the

standard curve was linear, two standards, 1 and 5 mg/L, were analyzed with each group of samples. To obtain a clear sample, 4.25 cm GF/C Whatman Glass Microfibre filters were used. To 50 ml of clear sample, 1 ml of 1 N hydrochloric acid was added and mixed in thoroughly. Absorbance was read against distilled water, stored in a glass container, set at zero absorbance. A wavelength of 220 nm was used to obtain a nitrate-nitrogen reading, and a wavelength of 275 nm was used to determine the interference due to dissolved organic matter. Readings were made directly for all samples at both wavelengths. As described in Standard Methods [4], two times the absorbance at 275 nm was subtracted from the absorbance reading at 220 nm. This corrected absorbance value was converted to a nitrate-nitrogen concentration from the standard curves.

III. Ultraviolet Spectrophotometric Screening Method With Charcoal Treatment

A. Apparatus

1. Absorbance was read using the EU-700 Series GCA/McPherson Spectrophotometric Instrument with matched 1-cm path-length silica cells.

2. Polycarbonate centrifuge tubes, 50 ml capacity.

3. IEC International Centrifuge, Model HT.

4. Burrell Wrist-action Shaker.

5. Whatman Glass Microfibre filters, 4.25 cm, GF/C.

B. Reagents

1. Ground, charcoal, Norit-A, alkaline, decolorizing carbon.

2. All other reagents are the same as described in the Ultraviolet Spectrophotometric Screening Method reagent section.

C. Procedure

The charcoal was dried in an oven at 105 C for 24 hours, and then kept in the oven between analyses. A sample was prepared for analysis by placing 0.1 g of charcoal into the centrifuge tubes. For each sample, a pair of centrifuge tubes were used. To each of the tubes, 30 ml of the water sample was added. The centrifuge tubes were placed in the shaker for 10 minutes at the highest rotation setting. They were then placed in the centrifuge for 20 minutes at approximately 7500 rpm. Being careful to avoid shaking, the samples were filtered, using a suction filtration apparatus with glass fiber filter paper, to remove the charcoal from the solution. To 50 ml of the clear sample, 1 ml of 1 N HCl was added and mixed thoroughly. Absorbance was measured against distilled water, stored in a glass container, set at zero absorbance. A wavelength of 220 nm was used to obtain a

nitrate-nitrogen absorbance value, and a wavelength of 275 nm was used to determine the effectiveness of the charcoal in the removal of the interference due to the dissolved organic matter. Readings were made directly for all samples at both wavelengths.

IV. A Proposed Method For Determining Nitrate

A. Apparatus

1. Absorbance was read from the EU-700 Series GCA/McPherson Spectrophotometric Instrument using matched 1-cm path-length silica cells.

2. 50 ml capacity Polycarbonate Centrifuge tubes.

3. IEC International Centrifuge, Model HT.

4. Burrell Wrist-action Shaker.

5. Whatman GF/C Glass Microfibre filters, 4.25 cm.

B. Reagents

1. Fresh distilled water, stored in glass, was used for all solutions and dilutions.

2. Stock Nitrate Solution: 0.7218 grams of dried potassium nitrate was dissolved in water and diluted to volume in a 1000-ml volumetric flask. The solution was preserved by adding 2 ml of chloroform per liter of solution for a final concentration of 100 milligrams

nitrate-nitrogen absorbance value, and a wavelength of 275 nm was used to determine the effectiveness of the charcoal in the removal of the interference due to the dissolved organic matter. Readings were made directly for all samples at both wavelengths.

IV. A Proposed Method For Determining Nitrate

A. Apparatus

1. Absorbance was read from the EU-700 Series GCA/McPherson Spectrophotometric Instrument using matched 1-cm path-length silica cells.

2. 50 ml capacity Polycarbonate Centrifuge tubes.

3. IEC International Centrifuge, Model HT.

4. Burrell Wrist-action Shaker.

5. Whatman GF/C Glass Microfibre filters, 4.25 cm.

B. Reagents

1. Fresh distilled water, stored in glass, was used for all solutions and dilutions.

2. Stock Nitrate Solution: 0.7218 grams of dried potassium nitrate was dissolved in water and diluted to volume in a 1000-ml volumetric flask. The solution was preserved by adding 2 ml of chloroform per liter of solution for a final concentration of 100 milligrams

nitrate-nitrogen per liter.

3. Standard Nitrate Solution: 50.0 ml of stock nitrate solution was diluted to volume in a 500-ml volumetric flask with water for a final concentration of 10.0 milligrams nitrate-nitrogen per liter.

4. Sodium Hydroxide solution, 3.5 % m/v, stored in a plastic (polyethylene) bottle.

5. Mixed Acid Reagent: 5.0 g of sulphamic acid was dissolved in 500 ml of 5 % v/v sulphuric acid solution. The solution was stored in a glass bottle.

6. Ground charcoal, Norit-A, alkaline, decolorizing carbon.

C. Procedure

To 100 ml of sample, add 5 ml of 3.5 % m/v sodium hydroxide solution. Using two centrifuge tubes for each sample, place 0.1 g of dried powdered charcoal in each tube, and then place 35 ml of the sample solution into each centrifuge tube. Save the remaining 30 ml of sample. Place the centrifuge tubes in the shaker for 10 minutes at full rotation. Balance the pair of centrifuge tubes on a balance and place in the centrifuge for 20 minutes at 7500 rpm. Using a suction filtration apparatus with GF/C filter paper, pass the remaining 30 ml of the sample solution through the filter and discard the filtrate. Being careful to avoid shaking, filter the samples to remove the charcoal. Place 5 ml of the mixed acid reagent and 5 ml of

distilled water into a 50-ml volumetric flask and fill to volume with filtrate. Measure the absorbance at 210 nm against distilled water in the reference cell. A blank determination is carried out by taking 100 ml of distilled water through the full procedure in place of the sample. Correct absorbances for the blank and convert to milligrams nitrate-nitrogen per liter from a calibration curve.

To prepare the calibration curve, prepare nitrate calibration standards in the range of 0 to 5 milligrams nitrate-nitrogen per liter by diluting to 100 ml the following volumes of standard nitrate solution: 0, 1.00, 5.00, 10.0, 25.0, and 50.0 milliliters. Treat the nitrate standards in the same manner as the samples.

PRELIMINARY STUDY OF THE METHODS

A study of the methods involved in this work is necessary in order to see what effect dissolved organic matter has on nitrate-nitrogen determinations. In conjunction with a research project conducted by the American Chemical Society Student Affiliates Chapter at Emporia State University, the nitrate-nitrogen concentrations of samples taken from six different sampling sites were determined by the chromotropic acid method and the ultraviolet spectrophotometric screening method. This section deals with the problems encountered due to interferences from dissolved organic matter, and also with the problems in the methods themselves.

In Table 1, the resulting nitrate-nitrogen concentrations for each of the six sampling sites are listed. The table also lists the day the samples were taken, and the method used for analysis. The chromotropic acid method, C.A.M., is taken as the reference method. The ultraviolet spectrophotometric screening method is represented in two different ways. The first, listed as the U.V. method, represents the nitrate-nitrogen concentrations determined from the absorbance measurements taken at a wavelength of 220 nm. This method shows the direct effect of the dissolved organic matter interference. The second, listed as the UV(org. corr) method, represents the nitrate-nitrogen concentrations determined from the

Table 1

A COMPARISON OF THE NITRATE-NITROGEN CONCENTRATIONS FROM THE PRELIMINARY STUDY OF THE METHODS

Sampling Date	Method Used	Sampling Site # 1	# 2	# 3
9-14-85	C.A.M.	4.10	1.99	3.00
9-27-85	C.A.M.	1.57	0.35	2.09
	U.V.	2.51	1.66	2.85
10-16-85	C.A.M.	0.92	0.41	0.49
	U.V.	1.56	1.54	1.02
10-25-85	C.A.M.	1.27	0.29	1.23
	U.V.	1.77	1.79	1.73
	UV (org corr)	1.21	0.33	1.19
11-13-85	C.A.M.	1.46	0.82	1.45
	U.V.	1.72	2.25	1.71
	UV (org corr)	1.36	0.76	1.34
11-27-85	C.A.M.	1.53	2.37	1.53
	U.V.	1.88	4.71	1.90
	UV (org corr)	1.45	2.74	1.45
1-20-86	C.A.M.	1.44	7.16	1.53
	U.V.	1.70	8.93	1.83
	UV (org corr)	1.47	7.47	1.57
1-25-86	C.A.M.	1.37	7.69	1.60
	U.V.	1.91	9.50	1.98
	UV (org corr)	1.34	7.81	1.56
2-01-86	C.A.M.	0.82	8.21	0.87
	U.V.	1.34	9.85	1.37
2-08-86	C.A.M.	1.21	7.30	1.30
*4-14-86	C.A.M.	0.35	17.6	0.31
	U.V.	0.73	19.9	0.68
	UV (org corr)	0.31	18.0	0.35

* data collected by Dr. David Schroeder

Table 1

A COMPARISON OF THE NITRATE-NITROGEN CONCENTRATIONS FROM THE PRELIMINARY STUDY OF THE METHODS

Sampling Date	Method Used	Sampling Site # 4	# 5	# 6
9-14-85	C.A.M.	2.62	4.92	2.86
9-27-85	C.A.M.	1.97	4.05	1.63
	U.V.	2.46	5.90	2.36
10-16-85	C.A.M.	0.85	2.80	0.97
	U.V.	1.12	8.60	1.38
10-25-85	C.A.M.	1.15	3.40	1.34
	U.V.	1.61	5.28	1.87
	UV (org corr)	1.10	3.71	1.30
11-13-85	C.A.M.	1.41	4.20	1.46
	U.V.	1.69	6.15	1.74
	UV (org corr)	1.29	4.52	1.33
11-27-85	C.A.M.	1.55	3.58	1.56
	U.V.	1.87	5.83	1.90
	UV (org corr)	1.46	3.90	1.49
1-20-86	C.A.M.	1.56	3.90	1.58
	U.V.	1.85	6.27	1.92
	UV (org corr)	1.60	4.56	1.64
1-25-86	C.A.M.	1.57	3.46	1.47
	U.V.	1.91	6.68	1.81
	UV (org corr)	1.48	4.57	1.45
2-01-86	C.A.M.	0.89	3.54	0.93
	U.V.	1.32	5.74	1.42
2-08-86	C.A.M.	1.28	3.68	1.29
*4-14-86	C.A.M.	0.31	3.32	0.43
	U.V.	0.73	5.17	0.82
	UV (org corr)	0.37	3.97	0.43

* data collected by Dr. David Schroeder

first absorbance measurement at 220 nm followed by a second measurement at a wavelength of 275 nm. This second wavelength is used to empirically correct for the dissolved organic matter interference.

The first comparison is between the U.V. results and the C.A.M. results. From the table, it can be seen that every U.V. nitrate-nitrogen concentration is higher than the C.A.M. concentrations. On the average, the U.V. results are 30 to 40 per cent higher. There is an exception at Site #5, the sewage treatment plant. The average U.V. nitrate-nitrogen concentration is 70 per cent higher. This is beleived to be due mainly to the excess detergents and surfactants present. These higher U.V. nitrate-nitrogen concentrations prove that there is dissolved organic matter present, and that it is causing an increase in the nitrate-nitrogen concentrations. The U.V. method, using the 220 nm wavelength alone, is not an effective, accurate method for nitrate-nitrogen determinations.

The use of the organic correction is definitely more effective in determining nitrate-nitrogen concentrations. The UV(org. corr) concentrations listed in the table are provide better agreement with the C.A.M. concentrations. The concentrations range from an average concentration per site of 4 per cent below to 3 per cent above the C.A.M. concentrations. Once again, the exception is Site #5 where the average UV(org. corr) concentration is 15 per cent

higher. Even though the UV(org. corr) method has a definite advantage over the U.V. method, there is still room for improvement, especially when high levels of dissolved organic matter are present.

The selection of the chromotropic acid method as the reference method over the other methods described in Standard Methods [4] is based on its detection range being better suited for our samples. It also seems to be the least complicated. Unfortunately, this method is more of a problem than first anticipated. First of all, concentrated sulfuric acid is used extensively throughout the procedure. Extreme caution must be used when preparing a sample. An example of the difficulties involved with the use of concentrated sulfuric acid is seen in the preparation of the antimony reagent. The antimony metal is first dissolved in the sulfuric acid. To achieve this, the solution needs to be heated, causing acid fumes to be evolved. Even in a good fume hood, this is not a desirable occurrence. This reagent also tends to recrystallize upon standing. When trying to redissolve the crystals, the reagent bottle is heated in a water bath. The result in one case was the explosion of the bottle. Needless to say, the acid in the reagent caused some damage. Additional caution needs to be taken when analyzing a sample on the spectrophotometer. The sample cell should be filled carefully to avoid trapping air bubbles.

Another problem with the chromotropic acid method is

the inconsistency in the blank absorbance readings. Table 2 shows the variance of the blank absorbance readings. The readings range from 0.015 absorbance units to 0.062 absorbance units. The mean value of 0.031 absorbance units has a relative standard deviation that is 50 per cent of the mean value. This uncertainty in the blank readings is carried into the blank corrected absorbance readings, and then into the calculated nitrate-nitrogen concentrations of the samples. With this much uncertainty, there is a lack of confidence in the final results.

The observed uncertainties suggest that the chromotropic acid method is in error. To try to determine where the error is coming from, a closer look at the method is needed. The chromotropic acid reagent is the color developing reagent. It reacts with nitrate to form a yellow reaction product. The hypothesis is that there is a background interference in the chromotropic acid reagent. If this is true, then the absorbance of a sample prepared without the chromotropic acid reagent will be greater than the absorbance of a blank that is prepared without the reagent.

A sample of rain water was used to test this hypothesis. Samples are made ready for analysis by preparing duplicate rain water samples that follow the normal chromotropic acid method preparation. Duplicate samples are also prepared without the addition of the chromotropic acid reagent. Along with these samples, a

Table 2

VARIANCE OF THE BLANK ABSORBANCE READINGS
IN THE CHROMOTROPIC ACID METHOD

Analysis Date	Blank Absorbance Reading	Range
9-25-85	0.032	0.015
10-05-85	0.039	to
10-26-85	0.062	0.062
11-12-85	0.030	
11-24-85	0.019	
12-08-85	0.028	
1-24-86	0.018	Average
2-05-86	0.021	0.031
2-18-86	0.058	±
2-18-86	0.061	0.016
2-27-86	0.015	
6-10-86	0.021	
7-05-86	0.023	
9-20-86	0.034	
10-12-86	0.022	
10-30-86	0.020	

blank solution is prepared using distilled-deionized water. The blank solution is used for comparison with a blank solution prepared without the chromotropic acid reagent. The results from this test can be seen in Tables 3 and 4.

Obviously, without the chromotropic acid reagent in the solution, the yellow reaction product will not form. Therefore, the absorbance readings for the rain water samples without the reagent should be low, and indeed they are. Their absorbance readings are almost zero. This same result is obtained from the blank solution without the chromotropic acid reagent. When comparing these readings to the normally prepared blank absorbance readings, the chromotropic acid reagent seems to have some absorbing substance(s) present. As a result, the blank correction is needed. There may also be substances in the rain water that are not present in the distilled-deionized water which cause high results. Is the blank correction effective in correcting for the absorbing substances? Does it also correct for the inconsistent blank readings, and minimize the uncertainty? Answering these and other questions about the chromotropic acid method would definitely benefit this method. This is also more of a reason to either improve an existing method, or propose a new and better method for the analysis of nitrate-nitrogen.

Table 3

CHROMOTROPIC ACID METHOD DATA FOR THE TEST OF THE HYPOTHESIS THAT THERE IS A BACKGROUND INTERFERENCE FROM THE CHROMOTROPIC ACID REAGENT

Solution	Absorbance (410 nm)	Blank Corrected Absorbance	Concentration (mg/L)
Blank	0.034	0.000	0.0
std	0.893	0.859	5.0
# 1	0.154	0.120	0.698
# 2	0.155	0.121	0.704
# 3	0.002	---	---
# 4	0.003	---	---
# 5	0.119	0.085	0.495
# 6	0.119	0.085	0.495

Blank : Distilled-deionized water.

std : 5.0 milligrams nitrate-nitrogen per liter.

1 : Rain water sample, normal chromotropic acid method.

2 : duplicate of # 1.

3 : Rain water sample, chromotropic acid method omitting the chromotropic acid reagent.

4 : duplicate of # 3.

5 : Rain water sample, chromotropic acid method after charcoal treatment.

6 : duplicate of # 5.

Table 4

CHROMOTROPIC ACID METHOD DATA FOR THE TEST OF THE HYPOTHESIS THAT THE CHARCOAL TREATMENT IS REMOVING FROM THE SOLUTION

Solution	Absorbance (410 nm)	Blank Corrected Absorbance	Concentration (mg/L)
Blank A	0.022	0.0	0.0
Blank B	0.005	---	---
# 1	0.106	0.084	0.491
# 2	0.109	0.087	0.509
# 3	0.088	0.066	0.386
# 4	0.079	0.057	0.333

Blank A : Distilled-deionized water, normal chromotropic acid method.

Blank B : Distilled-deionized water, chromotropic acid method omitting the chromotropic acid method.

1 : 0.5 milligrams nitrate-nitrogen per liter, normal chromotropic acid method.

2 : duplicate of # 1.

3 : 0.5 milligrams nitrate-nitrogen per liter, chromotropic acid method after charcoal treatment.

4 : duplicate of # 3.

RESULTS AND DISCUSSION

I. Chromotropic Acid Method

West and Ramachandran [2] show that Beer's law is obeyed up to 20 milligrams nitrate-nitrogen per liter (mg/L) when using 2.5 milliliters (ml) of standard and a final volume of 10 ml. They state that color development will also occur from 5 ml of standard or sample by adding a proportional amount of reagents and adjusting the final volume to 25 ml. Using 10 ml of standard or sample and the proportional amounts of reagents, and adjusting the final volume to 50 ml also develops the color. Standards prepared in this manner, ranging from 0 to 10 mg/L, obey Beer's law up to 5 mg/L (see Figure 1). Above 5 mg/L the curve shows a positive deviation from Beer's law. Table 5 Part A gives the mean absorbances and the standard deviations of the nitrate-nitrogen / chromotropic acid system at standard concentrations up to 5 mg/L. It can also be seen that this method is accurate to within a 6 per cent standard deviation at the 1 mg/l level.

Using a sample of the effluent from the city sewage treatment plant, which has a high level of dissolved organic matter, and a Cottonwood River sample, which has a low dissolved organic matter level, the precision of the chromotropic acid method can be seen. The results are shown in Table 6. For the five analyses of the sewage

Table 5

STANDARD CURVE DATA

Mean Values and Relative Standard Deviations

A. Chromotropic Acid Method

Absorbance (410 nm)	Concentration (mg/L)
0.0	0.0
0.085 ± 0.006	0.5 ± 0.02
0.169 ± 0.015	1.0 ± 0.06
0.837 ± 0.032	5.0 ± 0.03

B. Ultraviolet Spectrophotometric Screening Method

Absorbance (220 nm)	Concentration (mg/L)
0.0	0.0
0.116 ± 0.005	0.5 ± 0.02
0.242 ± 0.016	1.0 ± 0.05
1.187 ± 0.035	5.0 ± 0.03

C. Proposed Method for Determining Nitrate

Absorbance (210 nm)	Concentration (mg/L)
0.0	0.0
0.208 ± 0.003	0.5 ± 0.01
0.440 ± 0.003	1.0 ± 0.01
1.062 ± 0.004	2.5 ± 0.01
2.046 ± 0.004	5.0 ± 0.02

Figure 1 : **Calibration curve for the chromotropic acid method.**

ABSORBANCE
.9
.6
.3
0
0
1
2
3
4
5
6
ABSORBANCE
CONCENTRATION, Mol-1

Table 6

PRECISION OF THE CHROMOTROPIC ACID METHOD

Sampling Site #5 : Sewage Treatment Plant

Solution	Absorbance (410 nm)	Concentration (mg/L)
# 1	0.676	3.78
# 2	0.639	3.57
# 3	0.644	3.60
# 4	0.667	3.73
# 5	0.681	3.81
Mean Value	0.661	3.70
	±	±
	0.019	0.11

Sampling Site #6 : Cottonwood River

Solution	Absorbance (410 nm)	Concentration (mg/L)
# 1	0.152	0.934
# 2	0.151	0.928
# 3	0.153	0.940
# 4	0.151	0.928
# 5	0.153	0.940
Mean Value	0.152	0.934
	±	±
	0.001	0.006

treatment plant sample, the precision is within 3 per cent relative standard deviation of the mean value for both the absorbance and concentration. The five analyses of the river sample show the chromotropic acid method to be precise to within 1 per cent relative standard deviation of the mean absorbance and concentration values.

II. Ultraviolet Spectrophotometric Screening Method

Figure 2 shows a plot of the mean absorbances at 220 nm against the standard nitrate-nitrogen concentrations, agreeing well with Beer's law. According to Standard Methods [4], the nitrate calibration curve follows Beer's law up to 11 mg N/L. Our labatory results show that Beer's law is obeyed up to at least 10 milligrams nitrate-nitrogen per liter (mg/L). Goldman and Jacobs [1] have shown that Beer's law is obeyed up to 30 mg/L. Figure 2 and Table 5 Part B give results from 0 to 5 mg/L. This is because most of the nitrate-nitrogen concentrations for our samples fall between 0 and 5 mg/L. If a sample has a concentration above 5 mg/L, the concentration is assumed to be correct when read from the standard curve. In Table 5 Part B we see that for a concentration of 1 mg/L there is a 5 per cent standard deviation, and a corresponding absorbance value with a 7 per cent standard deviation.

In Tables 7A and 7B we can also see the precision and accuracy of the ultraviolet spectrophotometric

Figure 2 : **Calibration curve for the ultraviolet spectrophotometric screening method.**

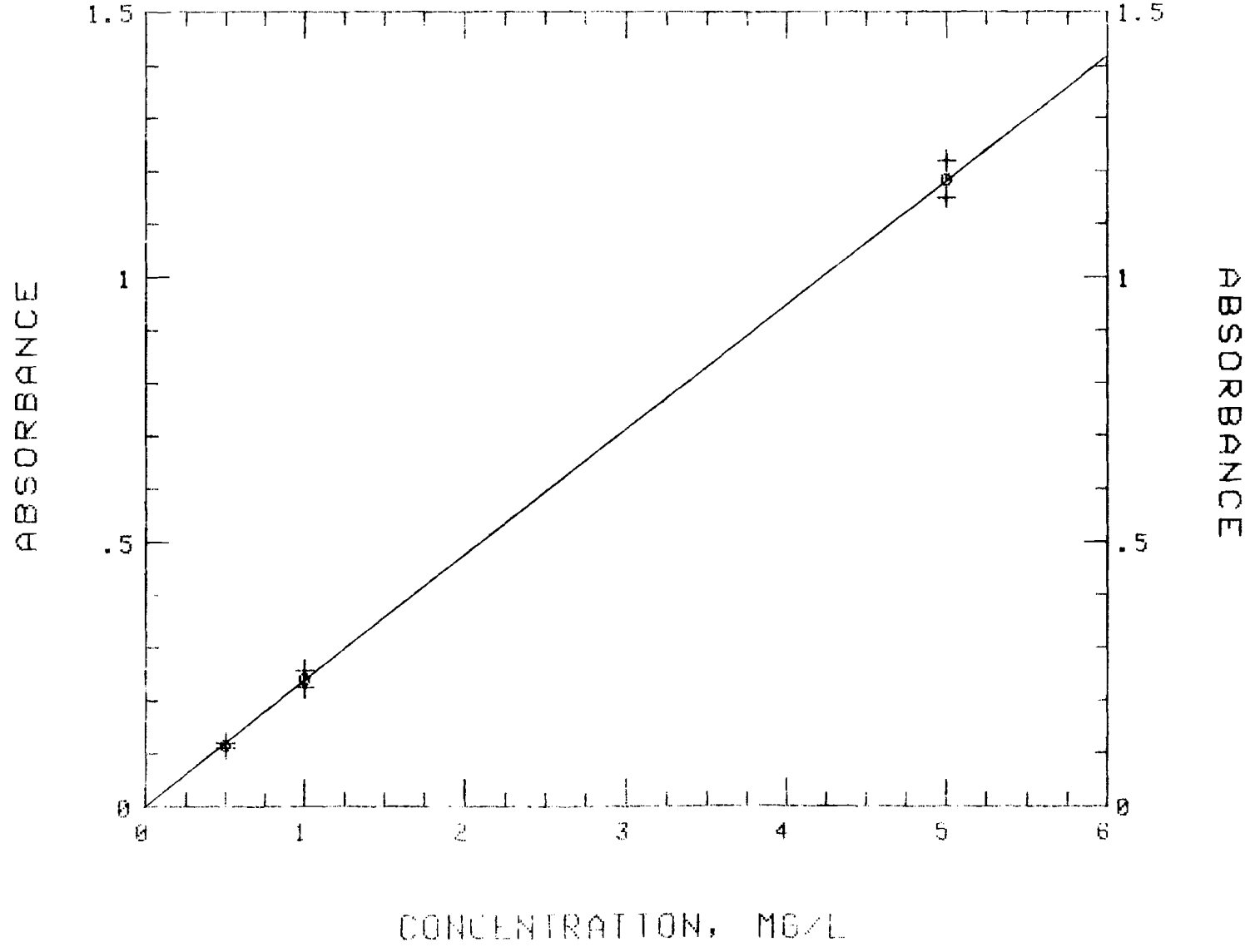
1.5
1
.5
0
ABSORBANCE
0
1
2
3
4
5
6
CONCENTRATION, MG/L

Table 7A

ULTRAVIOLET SPECTROPHOTOMETRIC SCREENING METHOD DATA FOR THE TEST OF THE HYPOTHESIS THAT THE CHARCOAL TREATMENT IS REMOVING NITRATE FROM THE SOLUTION

Solution	Absorbance (220 nm)	Average Absorbance	Concentration (mg/L)
Blank	0.000	0.000	0.000
# 1	0.118	0.118	0.501
	0.118		
	0.119		
# 2	0.118	0.118	0.498
	0.117		
	0.118		
# 3	0.117	0.118	0.499
	0.118		
	0.119		
# 4	0.118	0.118	0.499
	0.117		
	0.119		
# 5	0.088	0.090	0.379
	0.092		
	0.089		
# 6	0.088	0.089	0.375
	0.089		
	0.089		
# 7	0.087	0.088	0.372
	0.088		
	0.089		

Table 7B

ULTRAVIOLET SPECTROPHOTOMETRIC SCREECING METHOD DATA FOR THE TEST OF THE HYPOTHESIS THAT THE CHARCOAL TREATMENT IS REMOVING NITRATE FROM THE SOLUTION

Solution	Average Absorbance (220 nm)	Concentration (mg/L)
Blank	0.000	0.000
# 1	0.118	0.501
# 2	0.118	0.498
# 3	0.118	0.499
# 4	0.118	0.499
# 5	0.090	0.379
# 6	0.089	0.375
# 7	0.088	0.372

Blank : Distilled water.

1 : 0.5 milligrams nitrate-nitrogen per liter, normal U.V. analysis.

2 : duplicate of # 1.

3 : 0.5 mg/L filtered through Whatman GF/C glass fiber filter, U.V. analysis.

4 : duplicate of # 3.

5 : 0.5 mg/L charcoal treated, U.V. analysis.

6 : same as # 5.

7 : same as # 5.

screening method. Using a standard solution of 0.5 mg/L, duplicate solutions are analyzed. For solutions #1 and #2, there is less than 1 per cent standard deviation in the absorbance values, and less than 1 per cent standard deviation in the concentrations. For solutions #3 and #4, similar results are obtained. Solutions #1 and #2 differ from solutions #3 and #4 in that #3 and #4 have been filtered through Whatman GF/C glass fiber filter paper. The reason for doing this was to see if the filter paper caused an error in the final results by either adding or removing nitrate from the solution. As can be seen, the filter paper does neither. Therefore, one can be relatively sure that no error is introduced into the analysis as a result of filtering a sample.

III. Ultraviolet Spectrophotometric Screening Method with Charcoal Treatment

The first step in investigating the batchwise addition of activated charcoal is to see if it actually does remove dissolved organic matter from the samples. Tables 8A and 8B show the results of the charcoal treated samples compared to the chromotropic acid method and the ultraviolet spectrophotometric screening method including the organic correction. It can be seen from a comparison of the absorbance readings at 275 nm, before and after charcoal treatment, that the charcoal is removing the

dissolved organic matter. This is evident from the near zero values obtained. It is relevant here to discuss how the results in Tables 8A and 8B may be used to support the assumption that the absorbance readings for nitrate at 220 nm and for dissolved organic matter at 275 nm are inter-related. A range of sample types is covered with the expectation that the samples contain different organic species. The results show that for the samples with a significant absorbance at 275 nm before charcoal treatment, the absorbance falls to zero after charcoal treatment. This indicates that the dissolved organic matter has been removed by the charcoal. If any species other than nitrate were present, the absorbance at 220 nm would give a higher nitrate concentration than the chromotropic acid method. The results in the table show that, in every instance but one, the nitrate concentrations after charcoal treatment are lower than the concentrations obtained from the chromotropic acid method. This suggests that the charcoal is removing more than just the dissolved organic matter from the samples.

If the charcoal is removing nitrate from the samples, then the absorbance after the charcoal treatment will be less than the absorbance of the untreated sample. Using a standard nitrate solution of 0.5 mg/L, the chromotropic acid method and the ultraviolet spectrophotometric screening method are both used to test this hypothesis. Table 4 has the results of the

Table 8A

COMPARISON OF THE RESULTS OBTAINED BY THE METHODS INVOLVED FOR FILTERED AND CHARCOAL TREATED SAMPLES

Sampling Site	Chromotropic Acid Method	UV Spectrophotometric Screening Method			
	Abs(410 nm)	Abs(220 nm)		Abs(275 nm)	
		# 1	# 2	# 1	# 2
Iowa Beef Processing					
- filtered	1.115	1.883	1.998	0.308	0.297
- charcoal treated	---	1.350	1.971	0.016	0.002
Sewage Treatment Plant					
- filtered	0.272	0.815	0.789	0.349	0.313
- charcoal treated	---	0.236	0.282	0.014	-0.002
Lake Wooster					
- filtered	0.083	0.245	0.258	0.124	0.120
- charcoal treated	---	0.058	0.070	0.001	-0.010
Melvern Lake					
- filtered	0.084	0.301		0.205	
- charcoal treated	0.048	0.072		0.010	
John Redmond Reservoir					
- filtered	0.013	0.178		0.160	
- charcoal treated	-0.002	0.0		0.0	
Cottonwood River					
- filtered	0.270	0.432		0.063	
- charcoal treated	0.188	0.285		0.002	
Neosho River					
- filtered	0.263	0.397		0.055	
- charcoal treated	0.186	0.278		0.002	
Rain Water					
- filtered	0.121	0.277		0.087	
- charcoal treated # 1	0.084	0.113		0.003	
# 2	0.083				
# 3	0.083				

Table 8B

COMPARISON OF THE RESULTS OBTAINED BY THE METHODS INVOLVED FOR FILTERED AND CHARCOAL TREATED SAMPLES

Sampling Site	Chromotropic Acid Method	UV Spectrophotometric Screening Method			
	Conc (mg/L)	Abs (Corr)		Conc (mg/L)	
		# 1	# 2	# 1	# 2
Iowa Beef Processing					
- filtered	6.46	1.575	1.701	7.02	7.35
- charcoal treated	---	1.334	1.968	5.94	8.50
Sewage Treatment Plant					
- filtered	1.58	0.466	0.476	2.07	2.06
- charcoal treated	---	0.222	0.284	0.987	1.23
Lake Wooster					
- filtered	0.48	0.121	0.138	0.539	0.597
- charcoal treated	---	0.057	0.080	0.254	0.344
Melvern Lake					
- filtered	0.486	0.096		0.395	
- charcoal treated	0.278	0.062		0.255	
John Redmond Reservoir					
- filtered	0.075	0.018		0.075	
- charcoal treated	0.0	0.0		0.0	
Cottonwood River					
- filtered	1.56	0.369		1.60	
- charcoal treated	1.09	0.283		1.23	
Neosho River					
- filtered	1.52	0.342		1.48	
- charcoal treated	1.07	0.276		1.19	
Rain Water					
- filtered	0.700	0.190		0.782	
- charcoal # 1 treated	0.486	0.110		0.451	
# 2	0.480				
# 3	0.480				

chromotropic acid method, and Tables 7A and 7B have the results for the ultraviolet method. For both methods, the charcoal is removing approximately 30 per cent of the nitrate from the solution. This is also consistent with the various samples listed in Table 8B.

The removal of nitrate ions from aqueous solutions by activated charcoal may be pH dependent. The effect of altering the pH is investigated by Rennie, et al. [7]. In a pH range from 1.5 to 10.4, they find that while organic removal is from 90 to 97 per cent, nitrate-ion retention is anywhere from 24 to 63 per cent. Increasing the pH to 12.6 gives 100 per cent organic removal and 0 per cent nitrate-ion retention. Their results confirm the pH dependence of nitrate retention and dissolved organic matter adsorption.

While the removal of nitrate ions from aqueous solutions by activated charcoal is pH dependent, the adsorption for nitrate is independent of pH over a wide range according to Hoather and Rackham [4]. In the pH range 1.5 to 1.9, Rennie and his associates find that the variation of adsorption of nitrate solutions with pH to be less than 7 per cent of the absorbance reading.

IV. Proposed Method for Determining Nitrate

Rennie, et al. [7] describes a batchwise addition method using a particular brand of activated charcoal.

This method consists of a membrane filtration of the sample and an addition of 0.5 grams of the powdered charcoal to 100 ml of the filtrate. The pH is adjusted above 12 by the addition of a sodium hydroxide solution. This mixture is stirred for 5 minutes before removing the charcoal by filtration. The pH of the filtrate is reduced to below 2 by adding a mixed acid reagent, and the absorbance is measured at 210 nm against distilled water. There is no absorbance due to dissolved organic matter, i.e., at 275 nm. A calibration curve is constructed using standards treated in the same manner. The drawbacks to this procedure, according to Rennie and his associates, are the need to weigh out the charcoal, contamination of the glassware, and the need for two filtrations.

The drawbacks in the procedure by Rennie and his associates, particularly the need for two filtrations, can be improved upon by using a centrifuge. To 100 ml of sample, add the sodium hydroxide solution to adjust the pH to above 12. This addition eliminates the interference of ferric and ferrous ions by forming insoluble hydroxides at the elevated pH. 35 ml portions of the sample are added to two individual centrifuge tubes, each containing 0.1 grams of charcoal. The remaining 30 ml of sample should be saved in order to rinse the filter paper. This mixture is shaken for 10 minutes to ensure a sufficient contact time for total removal of the dissolved organic matter. After 20 minutes of centrifuging, the sample is filtered through

Whatman GF/C filter paper to remove the charcoal. The pH of the filtrate is reduced to below 2 by the addition of a mixed acid reagent. The mixed acid reagent contains sulfamic acid which eliminates interference from nitrite. The pH adjustment to below 2 eliminates interferences from hydroxyl and carbonate ions. The absorbance is measured at 210 nm, and a calibration curve is constructed from standards treated in the same manner. Table 5 Part C and Figure 3 show the standard curve data and calibration curve for the proposed method, respectively. Beer's law is obeyed up to 5 mg/L. The mean absorbances are all within a 2 percent standard deviation. This same result can be seen with the concentrations, they are within a 2 per cent standard deviation. There is no noticeable contamination of the glassware, and there is only the need for one filtration of the sample.

Table 9 shows a comparison of the proposed method with the chromotropic acid method, and also with a high pressure liquid chromotography (HPLC) method [12]. Three different samples, containing different species and amounts of dissolved organic matter, are used. The Cottonwood River results show the proposed method nitrate concentration to be higher than the other methods; 4 per cent higher than the chromotropic acid method, and 13 per cent higher than the HPLC method. For the sewage treatment plant, the proposed method concentration is 10 per cent higher than the chromotropic acid method, and 3 per cent

Figure 3 : Calibration curve for the proposed method.

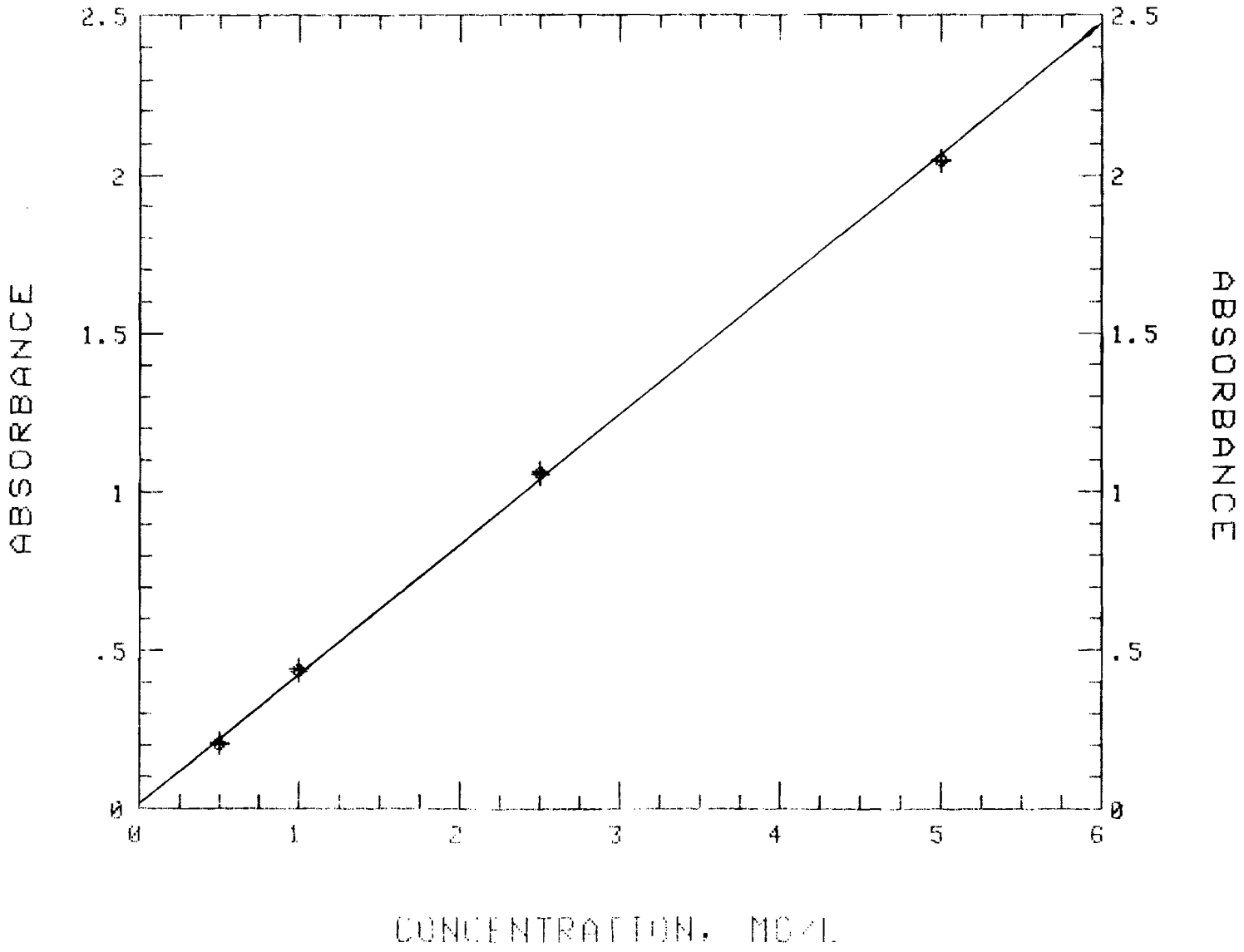
2.5
2
1.5
1
.5
0
0
1
2
3
4
5
6
ABSORBANCE
ABSORBANCE
CONCENTRATION, MG/L

Table 9

A COMPARISON OF THE PROPOSED METHOD NITRATE RESULTS WITH THE CHROMOTROPIC ACID METHOD RESULTS, AND ALSO A COMPARISON WITH A HIGH PRESSURE LIQUID CHROMOTOGRAPHY METHOD

Solution	Proposed Method		Chromotropic Acid Method		HPLC Method
	Avg Abs(210 nm)	Concentration	Abs(410 nm)	Concentration	Concentration
Cottonwood River	0.435	1.06 mg/L	0.200	1.02 mg/L	0.922 mg/L
Sewage Treatment Plant	1.202	2.93	0.486	2.65	3.02
Lake Wooster	0.094	0.23	0.067	0.27	----

lower than the HPLC method. Finally, the Lake Wooster sample has a proposed method nitrate concentration that is 15 per cent lower than the chromotropic acid method. The HPLC method was not used to determine nitrate for this sample. The results for the proposed method are not consistently higher or lower than the other methods, but the results are comparable. The proposed method gives promising results, especially with standard nitrate solutions, but, more work needs to be done with natural water samples.

CONCLUSION

When determining nitrate by ultraviolet spectroscopy, the presence of dissolved organic matter in a water sample causes an increase in the nitrate-nitrogen concentration. Through the use of a second measurement, the dissolved organic matter interference may be empirically corrected. Unfortunately, this technique is unreliable when the dissolved organic matter content is high.

The chromotropic acid method is not bothered by dissolved organic matter, but the method itself has problems. Concentrated sulfuric acid is used extensively throughout the procedure. In addition to the obvious precautions associated with the handling of sulfuric acid, care must also be taken when preparing and heating reagents containing the acid. Sample cells need to be filled carefully to avoid trapping air bubbles. The chromotropic acid method also has a problem with inconsistent blank absorbance readings from one analysis to another.

As a result of the difficulties encountered in dealing with dissolved organic matter and the problems with the chromotropic acid method, a method investigating the potential effectiveness of activated charcoal is proposed. The resulting nitrate-nitrogen concentrations of the proposed method are comparable to those obtained by the chromotropic acid method and an HPLC method. The precision and accuracy of the method using standard nitrate solutions

gives satisfactory results. The method is potentially suitable for a variety of samples.

From its use as a decolorizing agent in the organic laboratory to its regular use in some treatment plants, the adsorption of organic matter by activated charcoal is well known. Its use in the determination of nitrate-nitrogen by direct ultraviolet spectroscopy seems to be an effective means of removing the interference encountered by dissolved organic matter.

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