higher than integral, the accuracy claimed being 0.01 per cent. In converting from the physical to the chemical scale, if the higher factor of Mecke and Childs, 1.00022, is used, the atomic weight of radium lead falls between 205.96 and 206.00. However, if the latter is always accompanied by actinium lead, Pb^{207}, in the proportions found in Katanga lead by Aston[4] (93.3:6.7) the average atomic weight of uranium lead should be 0.07 unit higher, a difference far outside the apparent accuracy of our experiments. This suggests the possible presence in uranium lead of lead isotopes of lower atomic mass than 206. An alternative is that the atomic weight of lead as found by us is too low, or that the packing fraction of lead determined with the mass spectrograph is incorrect.

The experimental result of this research is that the atomic weight of uranium lead from a specimen of cyrtolite is 205.92 ± 0.02.

[4] Aston, *Nature*, **129**, 649 (1932).

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Diphenylamine Sulfonic Acid as a Reagent for the Colorimetric Determination of Nitrates

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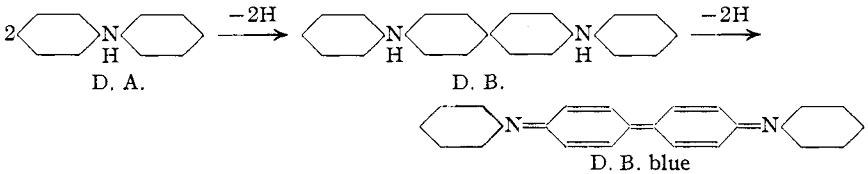
Since first suggested by E. Kopp,[1] the use of diphenylamine for the colorimetric determination of nitrates has been studied by numerous investigators.[2] Diphenylamine, mainly for the following reasons, is far from being an ideal reagent in the colorimetric determination of nitrates.

1. The blue color developed by nitrate in the presence of much sulfuric acid and some hydrochloric acid is to be attributed to the formation of an oxidation product of diphenylbenzidine, the latter being the first oxidation product of diphenylamine.



Diphenylamine therefore is not a specific reagent for nitrate, but the blue color is also developed by other oxidizing agents, such as ferric iron, nitrite, dichromate, etc.

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[1] E. Kopp, *Ber.*, **5**, 284 (1872).

[2] See especially the more extensive investigations of Tillmans and Sutthoff, *Z. anal. Chem.*, **50**, 473 (1911); L. Smith, *ibid.*, **56**, 28 (1917); Riehm, *ibid.*, **81**, 353 (1930); for bibliography compare J. H. Yoe, "Photometric Chemical Analysis," Vol. I, Colorimetry, John Wiley and Sons, New York, 1928, pp. 638–659.

2. The intensity of the blue color developed strongly depends upon the ratio of diphenylamine and nitrate in the reacting mixture. The concentration of the blue holoquinoid oxidation product decreases with increasing excess of diphenylamine, because part of the nitrate is consumed in the oxidation of the reagent to the colorless diphenylbenzidine. Still the use of the latter substance as a reagent does not seem to offer special advantages, as may be inferred from a study by H. Riehm.[3]

3. The blue oxidation product is not stable; in addition, it reacts on standing with diphenylbenzidine with formation of a green slightly soluble meriquinoid. Therefore the intensity as well as the shade of the color changes with time of standing.

4. The nitrate in the reacting mixture is reduced to nitrogen oxide (NO), the latter being partly oxidized by oxygen from the air to NO_2 which reacts again with the indicator with the formation of diphenylbenzidine blue and NO. The latter again is reoxidized, etc. Therefore the concentration of the blue dye is much larger than corresponds to the simple stoichiometric reaction between nitric acid and diphenylamine; hence the color intensity after a certain time of standing is much larger with nitrates than with equivalent amounts of other oxidizing agents. This explains why the reaction is much more sensitive for nitrates than for other oxidizing agents and also why the way of mixing the acid nitrate solution with the reagent has a pronounced influence upon the intensity of the color and its development with time of standing (see experimental part).

5. Other factors, such as temperature during the mixing and at the time of standing, the concentration of sulfuric acid and chloride in the reacting mixture, shaking after mixing, and even the size of the tubes or vessels used affect the intensity of the color. Although various factors can be controlled by working under standardized conditions, it is evident that it will be hard to get reproducible results. From all this it is not surprising that no linear relation is found between the concentration of nitrate and the intensity of the color developed (Beer's law).

The advantages of diphenylamine sulfonic acid as an oxidation–reduction indicator[4] suggested the possibility of using it in the colorimetric determination of nitrates. The oxidation mechanism of the sulfonic acid is similar to that of diphenylamine; therefore some of the disadvantages involved in the use of diphenylamine (or benzidine) will also occur with diphenylamine sulfonic acid as a reagent. Preliminary experiments showed that the color developed in the reaction between nitrate and the sulfonic acid is fairly stable, whereas that formed in the reaction with diphenylamine or benzidine under identical experimental conditions fades relatively rapidly.

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(3) H. Riehm, *Z. anal. Chem.*, **81**, 439 (1930); however, see also K. Pfeilsticker, *ibid.*, **56**, 397 (1931); G. W. Monier Williams, *Analyst*, **56**, 397 (1931); H. Stromberg, *Proc. Staff Meetings Mayo Clinic*, **7**, 254 (1932).

(4) L. A. Sarver and I. M. Kolthoff, THIS JOURNAL, **53**, 2902 (1931).

These experiments indicated that diphenylamine sulfonic acid might be superior to diphenylamine or benzidine in the quantitative determination of nitrates. The oxidation product formed in the strongly acid medium has a bluish-violet color, whereas diphenylamine yields an indigo-blue solution.

Experimental

Materials Used.—C. P. products of potassium nitrate and potassium chloride, both recrystallized twice, and nitrogen-free sulfuric acid were used.

Sodium Diphenylamine Sulfonate.—Standard solution 0.006 molar. This solution was prepared by dissolving 0.512 g. of the barium salt[4] in about 125 cc. of water. To this solution was added 0.117 g. of sodium sulfate (anhydrous) dissolved in a little water, and the barium sulfate formed filtered off. The filtrate was diluted with water to 250 cc. Various dilutions of this standard solution have been used in the experimental work.

Diphenylamine, Eastman Kodak Company product.

The conditions which affect the reproducibility and the intensity of the colored product formed in the reaction between nitrate and the reagent were systematically studied.

Effect of Method of Mixing.—The object of this set of experiments was to find a way of eliminating the uneven heating caused by heat of dilution of sulfuric acid in mixtures containing the indicator. Two series of experiments were run, one in small Erlenmeyer flasks, the other in test-tubes. Each sample contained 1 cc. of 0.0003 molar diphenylamine sulfonate (standard solution 1:20), 1 cc. of a nitrate solution containing 1 mg. of NO_3 and 18 g. of potassium chloride, respectively, per liter. To each mixture were added 2, 3, 4, 5, 6 and 7 cc. of sulfuric acid, respectively. The formation of colors with respect to sulfuric acid concentration was quite irregular, but the two series as a whole showed that the mixtures in Erlenmeyer flasks were lighter in color than those in the corresponding test-tubes. A variation of the chloride and indicator concentration did not change the order of the effect. Similar results were obtained with diphenylamine instead of diphenylamine sulfonate as an indicator. In order to find a procedure yielding reproducible results, the methods of mixing the nitrate, indicator and acid solutions were varied systematically. The best results were obtained according to the following procedure: to an aqueous solution containing the nitrate and a suitable amount of potassium chloride a measured volume of sulfuric acid was added, the mixture after shaking cooled to room temperature, two drops of indicator (standard solution) dropped in and the whole mixed by careful stirring. By this method, which was applied in the later work, the uneven heating of the reaction mixture was eliminated and reproducible results were obtained.

Effect of Concentration of Sulfuric Acid.—It was found that the sensitivity of the reaction increases with the sulfuric acid concentration in the mixture. If the acid concentration in the reaction mixture was lower than 50%, the sensitivity of the reaction became too small and the color formation very slow. A small variation of the acid concentration in the neighborhood of 50% did not affect the reproducibility of the colors.

Effect of Concentration of Potassium Chloride.—The presence of chlorides is essential for obtaining the highest sensitivity. A concentration of 10 g. per liter of potassium chloride in the nitrate solution to be investigated was found to be a satisfactory amount. A change in this chloride content within 20% was without influence upon the intensity of the color.

Effect of Concentration of Diphenylamine Sulfonate.—If the concentration of nitrate was kept constant in a series of solutions and that of diphenylamine sulfonate varied, the intensity of the color produced increased to the point where nitrate and indicator were present in equivalent amounts. With an excess of reagent the intensity of color produced became less as the excess was increased. The stability of the color was the greatest in the solutions in which the nitrate and diphenylamine sulfonate were present in approximately equivalent quantities. In the final procedure the concentration of the reagent is kept constant in a series of standards and unknowns.

Effect of Temperature.—Experiments showed that regular and reproducible results were obtained when the indicator was added to the acid nitrate–chloride mixture at room temperature.

If the mixtures were heated above room temperature irregular results were obtained and the colors were less intense than if developed at ordinary temperature.

Relation between Color and Nitrate Concentration.—From the above it is evident that there will not be exact proportionality between the intensity of the color produced and the nitrate content of the mixture. In the final procedure the use of a series of standards (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg. nitrate per liter) is recommended. In this range of concentrations there is sufficient proportionality between any unknown and the closest two standards to make a fairly accurate determination. The proportionality is most linear when the colors are first formed, and decreases as the solutions are allowed to assume deeper colors; this is especially true at the higher concentrations of the range. The changes taking place on standing are caused mainly by the effect of oxygen from the air (see introduction in this paper), irregular results being obtained with slight changes of conditions.

For demonstration of the time effect some experimental figures are offered in Table I. The solutions were prepared according to the standard procedure given in this paper, and comparisons made in a colorimeter.

TABLE I

EFFECT OF TIME OF STANDING ON COLOR INTENSITY

Solutions compared, NO_3 per liter	Theoretical ratio	Found, ratio after minutes							
5 and 4 mg.	1.25	3	5	10	15	20	30	90	
Ratio		1.19	1.19	1.19	1.18	1.14	1.11	1.08	
4 and 3 mg.	1.33	3	5	10	15				
Ratio		1.35	1.31	1.20	1.10				
3 and 2 mg.	1.5	2	5	10	15	30	60		
Ratio		1.7	1.53	1.48	1.43	1.30	1.18		
2 and 1 mg.	2.0	5	15	20	30	35	40	50	65
Ratio		2.65	2.4	2.25	2.05	1.98	1.90	1.82	1.7
1 and 0.5 mg.	2.0	5	20	40	55	70			
Ratio			2.0	1.86	1.7	1.68			

These data show that the best time to make comparisons varies with the concentrations of the solutions under comparison. Generally it was found that comparisons should be made as soon as colors were intense enough to allow their matching in a colorimeter. Incidentally it may be mentioned that the violet colors are much more stable than the blue ones obtained with diphenylamine or diphenylbenzidine as an indicator.

Procedure.—A series of standards is prepared each containing 10 g. of potassium chloride and 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg. of nitrate ion, respectively, per liter. To the aqueous solution of the nitrate to be analyzed is added potassium chloride to a concentration of 8–12 g.

per liter. If one does not know the approximate concentration of the unknown, a preliminary experiment with standards containing 0.5, 1.0 and 3.0 mg. of nitrate per liter is run.

The mixtures are prepared in the following way: to 10 cc. of the nitrate–chloride solution is added 10 cc. of sulfuric acid from a pipet. Immediately after addition of the acid the flask is put into cold water and stirred sufficiently to mix its content. After cooling to room temperature 0.1 cc. of a 0.006 molar sodium diphenylamine sulfonate solution is added and the whole carefully mixed. The colors are compared in a colorimeter as soon as the more dilute of the two standards between which the unknown appears to belong has become sufficiently colored. As already mentioned, there is no exact proportionality between intensity of color and nitrate concentration. Therefore the method of calculating the nitrate concentration of the unknown is an empirical one. Suppose the unknown was found between 2 and 3 mg.; then with the 3 mg. standard set at a reading of 20, the 2 mg. standard read 35 and the unknown 30. The concentration of the unknown then is equal to

$$2 + \frac{35 - 30}{35 - 20} = 2.33 \text{ mg. per liter}$$

After the preliminary series has given an approximate value the procedure just given is repeated with the unknown and its two closest standards. This determination gives an accuracy of the order of 5% as shown by the data in Table II.

TABLE II

COLORIMETRIC NITRATE DETERMINATION

NO_3 present, mg. per liter	NO_3 found, mg. per liter	Relative error in %	NO_3 present, mg. per liter	NO_3 found, mg. per liter	Relative error in %
0.32	0.34	+5	1.50	1.60	+7
.48	.47	−2	2.80	2.72	−3
.47	.50	+6	3.50	3.54	+1
.70	.70	0.0	3.68	3.65	−1
1.40	1.50	+7	4.50	4.47	−1

Interfering Substances.—Since diphenylamine and its derivatives act as oxidation–reduction indicators in the nitrate test, all oxidizing agents with high enough oxidation potential will react with the indicator. The sensitivity of diphenylamine sulfonic acid for ferric iron amounts to 2 mg. of Fe^{III} per liter, if the standard procedure is applied. Hydrofluoric acid does not eliminate the interference by ferric iron. Most oxidizing agents, however, can be easily separated from nitrate or made harmless by proper reduction. Of great practical importance is the interference by nitrite; the sensitivity toward this ion according to the standard procedure is 0.2 mg. of NO_2 per liter. Tillmans and Sutthoff[2] have recommended making nitrous acid harmless by the addition of urea. This procedure has been applied by various authors; however, it was found by us that the excess of urea interferes very strongly with the color development of small amounts of nitrate and gives rise to quite erroneous results. This is true with diphenylamine as well as diphenylbenzidine and diphenylamine sulfonic acid as reagents. The method therefore should be eliminated from the literature. It was also found that hydrazoic acid interferes strongly. The reason probably is that urea as well as hydrazoic acid reacts with the nitrous acid formed in the reoxidation of nitric oxide by oxygen from the air. Good results were obtained by another method, also recommended by Tillmans and Sutthoff, in which the nitrite is removed by boiling with ammonium chloride:

$$NH_4NO_2 \longrightarrow N_2 + 2H_2O$$

To 100 cc. of solution containing nitrate and no more than 10 mg. of nitrite per liter is added 0.5 g. of ammonium chloride. The mixture is boiled down to a volume of 25 cc. and made up with water and 0.5 g. of potassium chloride to 100 cc. Sodium nitrite C. P. was recrystallized twice; solutions of this salt containing 10 mg. of NO_2^- per liter were treated according to the above procedure and did not develop any color with diphenylamine sulfonate when treated according to the standard procedure after boiling. Similar experiments were made with solutions containing 10 mg. of nitrite per liter and quantities between 0.5 to 5 mg. of nitrate per liter. The colors developed were exactly the same as those developed in nitrate solutions of corresponding strengths in the absence of nitrite.

Summary

Sodium diphenylamine sulfonate can be used as a reagent in the colorimetric determination of nitrates and has advantages over diphenylamine or diphenylbenzidine as reagents. A procedure has been developed which allows the determination of 0.001 to 0.05 mg. of nitrate in 10-cc. samples with an accuracy of 5%. The factors affecting the reproducibility of the color formation have been studied systematically. Nitrite interferes with the determination and has to be removed by boiling with ammonium chloride. Urea cannot be used since its excess interferes with the color formation. The same is true with diphenylamine and diphenylbenzidine as reagents.

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