Spectrophotometric Determination of Nitrous Acid with Disodium 1-(4'-Aminobenzeneazo)-amino-8-hydroxy-naphthalene-2', 6-disulfonate

By Yukiteru KATSUBE and John H. YOE

(Received July 27, 1959)

Colorimetric methods in general use for a small quantity of nitrous acid are based on the formation of intensely colored azo dyes. Nitrous acid reacts with primary aromatic amines in acid solution to form diazonium salts, which react with a second molecule of the amine to produce colored cations of an aminoazo compound. Although the intense color of the azo dyestuffs formed in this way is available for the determination of nitrous acid, the coupling reagent used has an important influence on the determination.

The best amine that has been used for the determination of nitrous acid is sulfanilic acid (*p*-anilinesulfonic acid), with subsequent coupling of the resulting diazonium compound with α-naphthylamine to give the red dyestuff *p*-benzene sulfonic acid-azo-α-naphthylamine[1-6].

---

[1] P. Griess, *Ber.*, **12**, 427 (1879).

[2] F. L. Hahn and G. Jaeger, ibid., **58**, 2335 (1925).

[3] J. Blom, ibid., **59**, 121 (1926).

[4] F. P. Treadwell, "Analytical Chemistry", English Ed., Vol. II, John Wiley & Sons, Inc., New York (1951), p. 306.

[5] F. Feigl, "Spot Tests", English Ed., Vol. I, Elsevier Publishing Co., New York (1954), p. 302.

[6] N. A. Tananaeff and A. M. Schapowalenko, *Z. anal. Chem.*, **100**, 350 (1935).

This paper describes a new method for the determination of trace quantities of nitrous acid based on the formation of a colored compound with disodium 1-(4′-aminobenzeneazo)-2-amino-8-hydroxy-naphthalene-2′, 6-disulfonate.

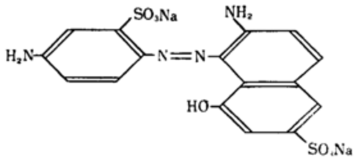


Fig. 1. Disodium 1-(4′-aminobenzeneazo)-2-amino-8-hydroxy-naphthalene-2′, 6-disulfonate.

Experimental

Apparatus and Reagents. — *Spectrophotometer.* — A Shimazdu Spectrophotometer with matched 1 cm. glass cells, was used.

Standard solution of nitrous acid. — The standard solution of nitrous acid was prepared according to Treadwell[4]. His instructions are as follows. Add silver nitrate to a concentrated solution of commercial potassium nitrite, filter off the precipitated silver nitrite and wash it several times with cold distilled water. To obtain pure silver nitrite, dissolve the precipitate in as little distilled water as possible and cool quickly. Drain off the mother liquor through a funnel provided with a platinum cone using gentle suction. Wash the silver nitrite on the funnel with a small volume of cold distilled water. Place the washed silver nitrite in a calcium chloride desiccator and allow to dry in the dark. Dissolve 1.5388 g. of the dried silver nitrite in a liter volumetric flask with hot distilled water and add 0.8 g. of pure sodium chloride to convert the silver nitrite into silver chloride and sodium nitrite. After cooling, make the solution up to volume with distilled water and allow the precipitate to settle. Pipet 10 ml. of the clear solution into a second liter-volumetric flask, make up to the mark with distilled water and thoroughly mix. One milliliter of this solution contains 4.7 mg. of nitrous acid.

Reagent solution. — Prepare the reagent solution by dissolving disodium 1-(4′-aminobenzeneazo)-2-amino-8-hydroxy-naphthalene-2′, 6-disulfonate in distilled water to give a 0.05% solution. (The reagent may be obtained from the LaMotte Chemical Products Co., Chestertown, Maryland, U. S. A.)

0.4% Ethylenediaminetetraacetic acid (disodium salt) solution.

6 N Hydrochloric acid.

Procedure. — Transfer 20 ml. of EDTA solution to a 25 ml. volumetric flask and add 1 ml. of the sample solution, which has been adjusted so as to contain 5 to 60 p. p. m. of nitrous acid. Add 2.5 ml. of the reagent solution, and 0.3 ml. of 6 N hydrochloric acid and shake well. Make up to the mark with distilled water, thoroughly mix and, after ten minutes, measure the absorbance at 625 mμ, using a reagent blank solution as a reference. Determine the weight or the concentration of nitrous acid in the sample from a calibration curve.

Results and Discussion

Absorption Spectra. — Disodium 1-(4′-aminobenzeneazo) - 2 - amino - 8 - hydroxy - naphthalene-2′,6-disulfonate is a red-brown solid, soluble in water. It is stable indefinitely in the solid state, and for, at least, three months when dissolved in water. The aqueous solution of the reagent is red; the nitrite ion in hydrochloric acid reacts with it to give a blue solution. Absorption spectra of the reagent solution and of the blue solution produced by the color reaction between the reagent and nitrite in diluted hydrochloric acid solution are given in Fig. 2.

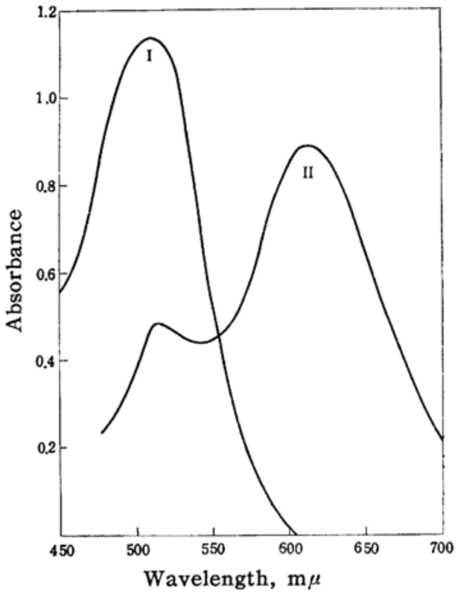
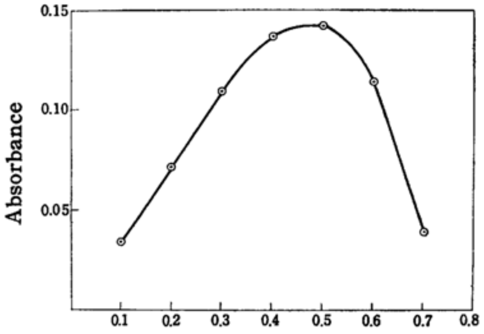


Fig. 2. Absorption spectra.
I. Reagent, 50 p. p. m.
II. Reagent (50 p. p. m.) + HNO_2 (2.3 p. p. m.)

As seen in Fig. 2, the compound produced by the color reaction has a maximum absorption at 625 mμ; the reagent solution does not absorb light at this wavelength. Hence, for analytical use absorbance measurements are made at 625 mμ. A large exess of the reagent must be avoided because its solution has a slight absorption at 625 mμ when the reagent concentration is greater than 50 p. p. m. It is not necessary to use exactly the same concentration of the reagent for standards and unknowns so far as the concentration of unreacted reagent is below 50 p. p. m. and water is used as reference solution. However, color intensities measured with the reagent solution as a blank obey Beer's law better than those with a water blank.

Application of the method of continuous variations[7,8] to this color reaction indicates that the mole ratio of reagent to NO_2^- is 1 to 1. The result is shown in Fig. 3.



x ml. of reagent solution added to $(1-x)$ ml. of NO_2^- solution

Fig. 3. Application of method of continuous variations: The nitrite and the reagent solutions were mixed in proportion to give the x to $(1-x)$ ratio of component in 1.0 ml. total volume in a 100 ml. volumetric flask, where x is equivalent to ml. of 10^{-3} M reagent and $(1-x)$ is equivalent to ml. of 10^{-3} M nitrite ion (the concentration of hydrochloric acid in the mixed solution was adjusted to 0.1 N).

Effect of Concentration of Hydrochloric Acid. — In order to determine the effect of the concentration of hydrochloric acid on the color reaction, the blue colored solution containing 2.8 p. p. m. of nitrous acid and 50 p.p.m. of the reagent were prepared with hydrochloric acid of various strengths, and absorbances of these solutions were measured. The results are shown in Fig. 4.

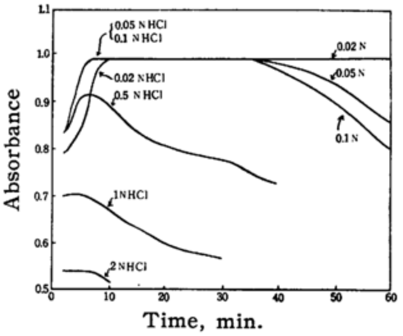


Fig. 4. Effect of hydrochloric acid.

It is seen that the optimum concentration of hydrochloric acid is between 0.05 and 0.1 N and hence, the solution containing nitrite must be adjusted to within this concentration range. Additional acid decreases the color intensity, causing rapid decolorization. With less acid, the color does not attain a maximum intensity within a reasonable time. In the optimum acid concentration, the color increases during the first ten minutes after the preparation of the blue solution, and then remains constant for about thirty minutes. It is necessary, therefore, to allow the solution to stand ten minutes and then to measure the absorbance within thirty minutes.

When sulfuric, phosphoric and acetic acid are used in place of hydrochloric acid, a less intense color is obtained.

Effect of Temperature. — There is no variation in the absorbance of the color system over a temperature range of 15 to 30°C. Hence, normal temperature changes in the laboratory introduce no error.

Interfering Ions. — The reagent was tested with 67 ions on a spot plate to determine the effect of other ions. The tested ions are given in Table I as the periodic table style.

Sixteen ions shown by italics in Table I were found to cause interference (As^{+3},

TABLE I. EFFECT OF OTHER IONS

$\mathrm{Li^+}$	$\mathrm{Be^{+2}}$										$\mathrm{B_4O_7^{-2}}$	$\mathrm{CO_3^{-2}}$	$\mathrm{NO_3^-}$		F^-
$\mathrm{Na^+}$	$\mathrm{Mg^{+2}}$										$\mathrm{Al^{+3}}$	$\mathrm{SiO_3^{-2}}$	$\mathrm{HPO_4^{-2}}$	$\mathrm{S^{-2}}$	$\mathrm{Cl^-}$
$\mathrm{K^+}$	$\mathrm{Ca^{+2}}$	$\mathrm{Sc^{+3}}$	Ti^{+4}	$\mathrm{Cr^{+3}}$	Mn^{+2} MnO_4^-	Fe^{+3} $\mathrm{Fe^{+2}}$	$\mathrm{Co^{+2}}$	$\mathrm{Ni^{+2}}$	$\mathrm{Cu^{+2}}$	$\mathrm{Zn^{+2}}$	$\mathrm{Ga^{+3}}$	$\mathrm{Ge^{+4}}$	As^{+3}	CeO_3^{-2}	Br^-
$\mathrm{Rb^+}$	$\mathrm{Sr^{+2}}$	$\mathrm{Y^{+3}}$	Zr^{+4}	MoO_4^{-2}		$\mathrm{Ru^{+3}}$	$\mathrm{Rh^{+3}}$			$\mathrm{Cd^{+2}}$	$\mathrm{In^{+3}}$	$Sn^{+2\ +4}$	$\mathrm{Sb^{+3}}$		I^-
$\mathrm{Cs^+}$	$\mathrm{Ba^{+2}}$		$\mathrm{Hf^{+4}}$		$\mathrm{ReO_4^-}$	$\mathrm{OsO_5^{-2}}$	$\mathrm{Ir^{+4}}$			Hg^{+} $^{+2}$	$\mathrm{Tl^{+3}}$	$\mathrm{Pb^{+2}}$	$\mathrm{Bi^{+3}}$		
				$\mathrm{UO_2^{+2}}$											
*$\mathrm{La^{+3}}$	Ce^{+3}	$\mathrm{Pr^{+3}}$	$\mathrm{Nd^{+3}}$	$\mathrm{Sm^{+3}}$	$\mathrm{Eu^{+3}}$			$\mathrm{Dy^{+3}}$		$\mathrm{Er^{+3}}$	$\mathrm{Tm^{+3}}$	$\mathrm{Yb^{+3}}$			

---

7) P. Job, *Ann. chim.*, (10), **9**, 113 (1928).

8) W. C. Vosburgh and G. R. Cooper, *J. Am. Chem. Soc.*, **63**, 437 (1941).

Br^-, Fe^{+3}, Ce^{+3}, CeO_3^{-2}, F^-, $Hg^{+,2+}$, I^-, Mn^{+2}, MnO_4^-, MoO_4^{-2}, $Sn^{+2,+4}$, Ti^{+4}, Zr^{+4}). Ferric ions cause a serious error and must be removed before the addition of the reagent. Chemical methods of separation of ferric ions did not yield good results. However, when EDTA solution is added to the sample solution before the addition of the reagent, interference of ferric ions is effectively eliminated. EDTA has no influence on the absorption spectrum of the blue colored solution. The effect of some common ions was tested spectrophotometrically; Table II summerizes the tolerance.

In Table II, the limiting concentration of an ion is taken to be the concentration that causes a deviation of more than 0.01 unit in the absorbance of a solution containing 0.94 p. p. m. of nitrous acid.

Table II. INTERFERENCES

Ion	Added as	Limiting concn., p. p. m.
Co^{+2}	$CoCl_2$	300
Cr^{+3}	$Cr_2(SO_4)_3$	200
Cu^{+2}	$CuSO_4$	300
Fe^{+3}	$Fe_2(SO_4)_3$	100
Ni^{+2}	$NiSO_4$	300

TABLE III. ACCURACY AND PRECISION OF THE METHOD

Nitrous acid Taken p. p. m.	Nitrous acid Found* p. p. m.	Absorbance** at 625 mμ	Standard deviation*** in absorbance unit	Error %
0.19	0.18	0.075	0.002	−5.3
0.38	0.37	0.151	0.001	−2.6
0.56	0.57	0.231	0.001	+1.8
0.75	0.76	0.307	0.002	+1.3
0.94	0.95	0.386	0.001	+1.1
1.13	1.14	0.463	0.002	+1.0
1.31	1.31	0.534	0.003	0.0
1.50	1.51	0.611	0.002	+0.7
1.69	1.65	0.669	0.003	−2.4
1.88	1.85	0.749	0.004	−1.6
2.07		0.807	0.002	
2.26		0.864	0.002	
2.63		0.95	0.010	
2.82		0.99	0.013	
3.38		0.15	0.018	

* These values were calculated by use of 0.405 of average value, from 0.19 to 1.88 p. p. m. HNO_2, of absorbances calculated per one p. p. m. HNO_2.

** These values are the averages of six replicate determinations.

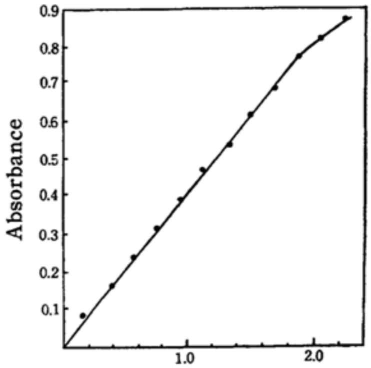
*** The standard deviations were calculated by the equation, $\sigma = \sqrt{\sum d^2/(n-1)}$, d being the deviation of each absorbance measured from the average and n the number of measurements.

Calibration Curve and Precision. — In order to construct a calibration curve and to determine the precision of the method, fifteen series of six replicate determinations were made. The precision, expressed as the standard deviation from the mean for each of the fifteen series, is shown in Table III.

The spectrophotometric sensitivity of the color reaction is 0.004 γ of nitrous acid per sq. cm. A more practical sensitivity may be taken as one part of nitrous acid in 10,000,000 parts of solution. A solution containing this concentration has an absorbance of 0.040 unit.

Beer's Law. — Beer's law is obeyed up to about 2 p. p. m. of nitrous acid and the absorbances are sufficiently reproducible to allow the range of the determination to be extended to about 3 p. p. m. (see Fig. 5).

Determination of Nitrous Acid in Synthetic Solutions Containing Various Ions. — The determination of nitrous acid in the presence of certain other ions has made. The results are given in Table IV.



Concentration of nitrous acid, p. p. m.

Fig. 5. Beer's law plot.

TABLE IV. DETERMINATION OF NITROUS ACID

	Solutions added	Analyzed p. p. m.	Found HNO_2 p. p. m.	Error %
1)	Fe^{+3}	200		
	HNO_2	0.20	0.21	+5.0
2)	Fe^{+2}	400		
	Fe^{+3}	400		
	Cu^{+2}	200		
	HNO_2	0.60	0.62	+3.3
3)	Cu^{+2}	200		
	Ni^{+2}	200		
	Fe^{+3}	500		
	HNO_2	1.80	1.78	−1.1
4)	Fe^{+2}	200		
	Fe^{+3}	200		
	Ca^{+2}	600		
	Mg^{+2}	600		
	HNO_2	0.20	0.20	0.0

Summary

A procedure has been developed for the spectrophotometric determination of trace amounts of nitrous acid with disodium 1-(4′-aminobenzeneazo)-2-amino-8-hydroxy-naphthalene-2′, 6-disulfonate. The method is accurate and precise and has a sensitivity of 0.004 γ of nitrous acid per sq. cm. Beer's law is obeyed up to about 2 p. p. m. of nitrous acid. A study of the effect of many ions on the color reaction has been made. The interference of ferric ions is eliminated by the use of EDTA.

Himeji Technical Institute, Himeji (Y. K.)

John Lee Pratt Trace Analysis Laboratory
University of Virginia
Charlottesville, Va., U. S. A. (J. H. Y.)