KINETIC-SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF NITRITE BY THE REACTION OF METHYL ORANGE FORMATION

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Abstract

The composite diazotization coupling reaction of sulfanilic acid and N, N-dimethylaniline was used for the precise, accurate, and rapid determination of trace amounts of nitrite ion (0.02-3 μg/ml). The reaction was monitored spectrophotometrically by measuring the absorbance of the produced methyl orange at 510 nm by a fixed time method of 5.0 minutes. The relative standard deviation for the determination of 0.50 μg/ml of nitrite was 1.8% (n=11). The method was used for the determination of nitrite in sausage and water samples.

Introduction

The determination of micro amounts of nitrite is important owing to its biological effects [1-3]. Many different methods are available for the determination of micro amounts of nitrite in solution [4-6]. Among these the spectrophotometric methods, which are based on the formation of an azo dye [7-9], are important. Numerous kinetic methods for the determination of nitrite have also been reported [10-15].

In this article we wish to describe a kinetic spectrophotometric method for the determination of low concentrations of nitrite ion using its reaction with sulfanilic acid and N,N-dimethylaniline. The method is rapid, accurate, precise, selective and has a relatively large linear dynamic range.

Experimental Section
Reagents and Chemicals

Triply distilled water was used throughout. All chemicals were of analytical-reagent grade.

Nitrite standard solution was prepared by dissolving 1.5000 g of dried $NaNO_2$ (Merck) in water and diluting to the mark in a one-liter volumetric flask. This solution was standardized [16]. Working solutions were prepared by appropriate dilution of the stock solution with water. A

0.040 M sulfanilic acid solution was prepared by dissolving 1.7319 g of sulfanilic acid (Merck) in water and diluting to the mark in a 250-ml volumetric flask. Acetic acid solution (6 M) was prepared by appropriate dilution of glacial acetic acid (Merck) with water.

A 0.5 M N,N-dimethylaniline solution was prepared by dissolving 6.3 ml N,N-dimethylaniline (Merck) in water containing 20 ml of glacial acetic acid and diluting to 100 ml with water. This solution was stored in a dark bottle in a refrigerator.

Apparatus

A Shimadzu model UV-120-01 spectrophotometer with a 1-cm glass cell was used for absorbance measurements.

Recommended Procedure

All the measurements were performed at 30±0.1°C. Into a 10-ml standard flask introduce an aliquot of sample containing 0.20-30.0 μg of nitrite and 1.0 ml of 0.04 M sulfanilic acid and 1.3 ml of 6 M acetic acid. Dilute to about 9 ml with water. Add 0.60 ml of 0.5 M N,N-dimethylaniline and dilute to the mark with water. Transfer a portion of the solution into a 1-cm glass cell and measure the absorbance at 510 nm during the first 5.0 minutes.

Results and Discussion

Composite diazotization coupling reaction of sulfanilic acid, as a diazotizable aromatic amine, and N,N-dimethylaniline, as a coupling agent, proceeds in acidic media to produce methyl orange. The rate of the reaction is proportional to the concentration of nitrite ion. This reaction was used for the determination of trace amounts of nitrite ion. The reaction was monitored spectrophotometrically by measuring the absorbance of the reaction mixture at 510 nm (Figure 1) by a fixed time method of 5.0 minutes.

Effect of Variables

Since the reaction proceeds in acidic media, the effect of type and concentration of acid on reaction rate was studied. Hydrochloric acid, sulfuric acid, phosphoric acid and acetic acid were tested and acetic acid was found to be the best.

The study of the effect of acetic acid concentration on reaction rate showed that its optimum value is dependent on the concentration of N,N-dimethylaniline. As Figure 2 shows, a concentration of 0.03 M N,N-dimethylaniline and 1 M acetic acid was found to be suitable.

The influence of the concentration of sulfanilic acid on reaction rate was also studied (Figure 3) and the concentration of 4.0×10^{-3} M was selected for the study. A change in the ionic strength of the solution had no considerable effect on reaction rate up to about 1.0 M.

As Figure 4 shows, the reaction rate is increased by increasing temperature up to 30°C then remains nearly constant and decreases at temperatures higher than 40°C. This decrease may be due to instability of nitrous acid at high temperatures. The temperature of 30°C was selected for routine works.

Analytical Parameters

Under the optimum conditions, a linear calibration graph was obtained in the concentration range of 0.02-3.0 μg/ml with a correlation coefficient of 0.9999 and a regression equation of:

$$A = 1.021 \times 10^{-3} + 0.6436\, C$$

where C is the concentration of nitrite in μg/ml. The relative standard deviation of eleven replicate determinations was 1.8% for a 0.50 μg/ml nitrite solution.

Interferences

The potential interference of a large number of cations and anions on the determination of 0.5μg/ml of nitrite was examined. The results are given in Table 1. The tolerance limit of each foreign ion was taken as the amount of added ion causing less than a ±3% relative error. As can be seen from Table 1, the selectivity achieved by the proposed method was very good, since most of the ions tested did not interfere even when present in 200-1000 fold of nitrite.
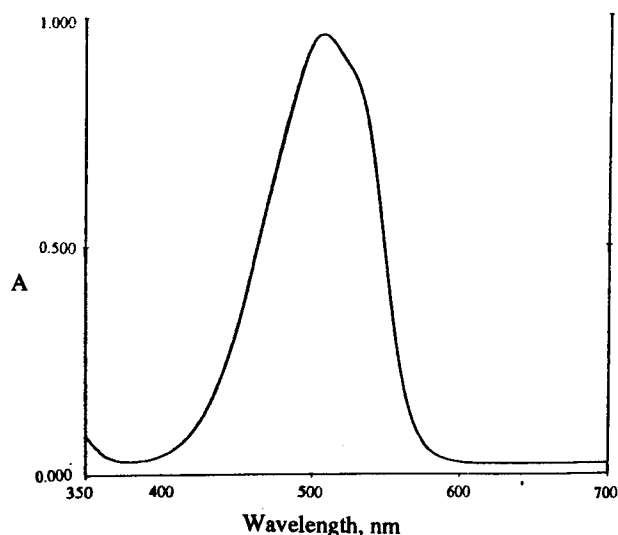

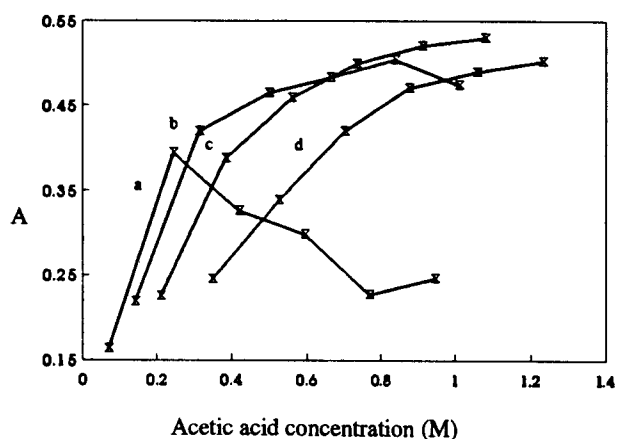**Figure 1.** Absorption spectrum of methyl orange in acidic media

Acetic acid concentration (M)

Figure 2. Effect of acetic acid concentration on the reaction rate in a)0.01, b) 0.02, c)0.03, d)0.04 M N,N-dimethylaniline
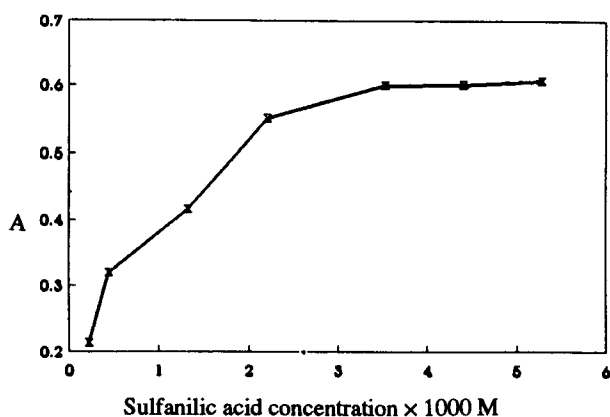

Sulfanilic acid concentration × 1000 M

Figure 3. Effect of sulfanilic acid concentration on the reaction rate

Table 1. Effect of foreign ions on the determination of 0.5 μg/ml of nitrite

Ion	Tolerance limit (μg/ml)
Ce(III), Ba(II), Pb(II), Cd(II), Zn(II), Mg(II), Sr(II), Mn(II), Ca(II), Ag(I), Na(I), Li(I), K(I), NH_4^+, PO_4^{3-}, $C_2O_4^{-}$, CO_3^{-}, SO_4^{-}, NO_3^-, Cl^-, F^-, IO_3^-,	500[a]
CN^-, SCN^- ClO_4^-	400
Ni(II)	200
Co(II)	
SO_3^{-}[b]	100
Cu(II)	80
I^-	20
Fe(III)	15
Fe(II)	4

a) Above of which was not tested.
b) After removed as described in text.
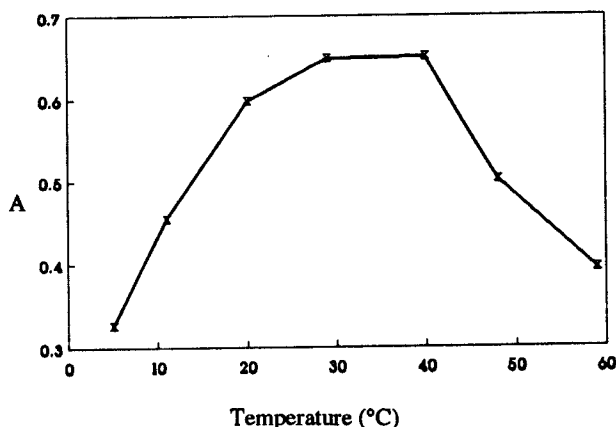
Figure 4. Effect of temperature on the reaction rate

The results showed that more than 40-fold iodide, 30-fold Fe(III), 10-fold sulfite and 8-fold Fe(II) interfere. Iodide, Fe(II), and sulfite interfere by reducing nitrite ion. The interfering effect of sulfite ion was effectively removed using formaldehyde as a masking agent before acidification of the solution.

Applications

In order to evaluate the analytical applicablity of the method, the procedure established was used for the determination of nitrite in a sample of sausage and four samples of water.

For the determination of nitrite in sausage sample, dissolution was carried out and its nitrite content was determined by both the proposed and standard methods [17]. The nitrite concentration in the sample was found to be 34.1±0.2 ppm (n=5) by the proposed method which was in satisfactory agreement with that obtained by the standard

Table 2. Determination of nitrite in waste water

Sample number	Concentration of nitrite (μg/ml)	
	Proposed method (n=5)	Standard method (n=5)
1	0.098	0.098
2	0.203	0.197
3	0.258	0.252
4	0.642	0.651

method (34.4±0.2 ppm).

Four samples of waste water were also analyzed. As Table 2 shows, these results agreed well with the standard method [17].

Conclusion

The proposed method could be used for rapid and accurate determination of nitrite in water and meat samples. Although the sensitivity and selectivity of the proposed method is not much of an improvement on some previously reported methods, it could be considered superior with regard to the rapidity, simplicity and large linear dynamic range.

References

1. Lijinsky, W. and Epstein, S. S. *Nature*, **223**, 21, (1970).
2. Wolff, I. A. and Wasserman, A.E. *Science*, **177**, 15, (1972).
3. Gabbay, J., Almog, Y., Davidson, M. and Donagi, A. E. *Analyst*, **102**, 371, (1977).
4. Williams, W. J. *Handbook of anion determination*, p. 143, Butterworth, London, (1979).
5. Fiddler, W., Doerr, R.C., Gates, R. A. and Fox, J.B. *J. Assoc. Off. Anal. Chem.*, **67**, 525, (1984).
6. Fox, J. B. *CRC Crit. Rev. Anal. Chem.*, **15**, 283, (1985).

7. Boltz, D.F. and Howell, J. A. *Colorimetric determination of nonmetals*, p. 216. Wiley, New York, (1978).
8. Norwitz, G. and Keliher, P. N. *Analyst*, **109**, 1281, (1984).
9. Tarafder, P. K. and Rathor, D. P. S. *Ibid.*, **113**, 1073, (1988).
    10. Afkhami, A. and Mogharnesband, A. *Anal. Lett.*, **27**, 991, (1994).
11. Koupparis, M. A., Walczak, K.M. and Malmstadt, H. V. *Analyst*, **107**, 1309, (1982).
12. Doerr, R. C., Fox, J. B., Lakritz, L. and Fiddler, W. *Anal. Chem.*, **53**, 381, (1981).
13. Karagannis, M. I., Piperaki, E. A. and Maniadaki, M. M. *Anal Lett.*, **19**, 13, (1986).

14. Jiang, M., Jiang, F., Dvan, J., Targ, X. and Zha, Z. *Anal. Chim. Acta*, **234**, 403, (1990).
15. Tomas, P. R., Garmen, M. L. and Virginia, T. *Ibid*, **265**, 103, (1992).
16. Welcher, F. J. Standard methods of chemical analysis, (6th edn), Vol. 2, part B, p. 2448. Krieger Publishing Company, New York, (1975).
17. Horwitz, W. (ed.). *Official methods of analysis of the association of Official Analytical Chemists*, (13th edn), p. 381. Association of Official Analytical Chemisst, Washington, (1980).