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SPECTROPHOTOMETRIC DETERMINATION OF MICROQUANTITIES OF NITRITE

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A spectrophotometric method for the determination of nitrite in water and urine has been developed. It does not involve diazotization, therefore, it is reasonably convenient and simple to perform. It is sensitive, accurate and precise. The visual limit of identification is 2.5 μg/ml.

INTRODUCTION

Since long the determination of nitrities has been accomplished by colorimetery through diazotisation and coupling reactions. Nitrites are made to react with primary aromatic amines in acidic medium to give a diazonium salt which is coupled with certain compounds to produce intensely colored dyes. One of the most sensitive method is the ASTM method [1]. The reagent used in this method is (N–(1–naphthyl) ethylenediamine dihydrochloride which has been declared as carcinogenic [2]. Moreover, the diazotization and coupling is tedious and time consuming process. Another spectrophotometric method which is based on the development of color with Ce(IV) and promazine hydrochloride has been indirectly used for the determination of nitrites [3]. This method is also time consuming and cumbersome. N–nitrosodimethyl amine formed by the treatment of promethazine hydrochloride with $NaNO_2$ at a definite pH was separated and determined by pulse polarography. The method is sensitive, but stringent control of conditions is required [4].

There is another pulse polarographic method [5] which involves a reaction between NO_2 and diphenylamine at low pH with SCN as catalyst. A spectrophotometric method [6] which is based on diazotization of sulphanilic acid by NO_2 in presence of HCl and then finally coupling with 8–hydroxy quinoline–5 sulphonic acid has been described. The absorbance is measured at 530 nm. The method is good, but again involves diazotization which is troublesome. Another method, which is only a detection procedure is reaction of nitrite with mercaptoacetic acid [7]. The S–nitroso compound formed gives a salmon color. A method which is quite sensitive and is based on the measurement of chemiluminescence of NO released from nitrite [8] is described and it has been used for the determination of nitrite from food products. The method is good, but again requires an expensive instrument.

In the present investigation a color reaction [9] has been used for the determination of nitrite in microquantities. It does not involve diazotization and is reasonably convenient, sensitive, accurate, precise and rapid. It does not require expensive instrumentation. Visual limit of identification is 2.5 μg/ml.

EXPERIMENTAL

Apparatus

All the absorbance measurements were made with DB-Beckman spectrophotometer using 1–cm cells.

For pH measurements, Beckman pH meter with single combination electrode was used.

Reagents

All the reagents used were of analytical grade or of comparable purity.

Potassium nitrite solution was prepared by dissolving accurately weighed 100.0 mg of the reagent and diluted to 100 ml.

Barbituric acid solution (10.0 mg/ml) was prepared in warm distilled water.

Sodium carbonate solution (10.0 mg/ml) was prepared in distilled water.

Spot Test

To 1–2 drops of the test solution add one drop of 1 % solution of barbituric acid followed by a drop of 1 % sodium carbonate solution. Immediate appearance of pink color indicates the presence of nitrite. Instead of sodium carbonate, sodium hydroxide can also be employed.

Procedure

To 1 ml of a test solution containing 4 to 60 μg of nitrite add 1 ml of 1 % barbituric acid and 1 ml of 1 % sodium carbonate solution. Make up the volume to 50 ml with distilled water and shake. Measure the absorbance at 340 nm, against reagent blank using 1–cm glass cells. The calibration graph is shown in Fig. 2.

Table 1. Determination of nitrite.

Nitrite taken (μg)	Nitrite found (μg)	Deviation (%)
4.00	4.02	0.50
8.00	8.08	0.10
12.00	11.96	0.33
16.00	16.00	0.00
20.00	20.20	1.00
24.00	23.80	0.83
28.00	28.40	1.40
32.00	32.02	0.06

RESULTS AND DISCUSSION

Spectral Characteristics. The aquous solution of the complex exhibits absorption maxima at 340 nm and 520 nm. But the band at 340 nm is very intense as compared to one at 520 nm (Fig. 1), hence all the measurements were carried out at 340 nm. The reagent absorbs negligibly as compared to the colored product. The color reaction obeys Beer's Law in the range 4–60 μg/ml of nitrite. Calibration graph is shown in Fig. 2.

Effect of pH. The color reaction is stable at pH 9 to 11. The color intensity decreases with fall in pH (Fig. 3.).

Effect of Time. After mixing the reactants at room temperature (about 20°) the color reaches its maximum intensity within few seconds and remains stable for 3 to 4 hr.

Effect of Temperature. The color intensity of the complex remains constant in the range 18°–80°.

Effect of Concentration. The concentration of barbituric acid should be approximately ten times the concentration of nitrite for maximum development of color.

Order of Mixing of Reactants. The order of mixing of reagents is very important for the development of color. The addition of alkali (Na_2CO_3) should be made after

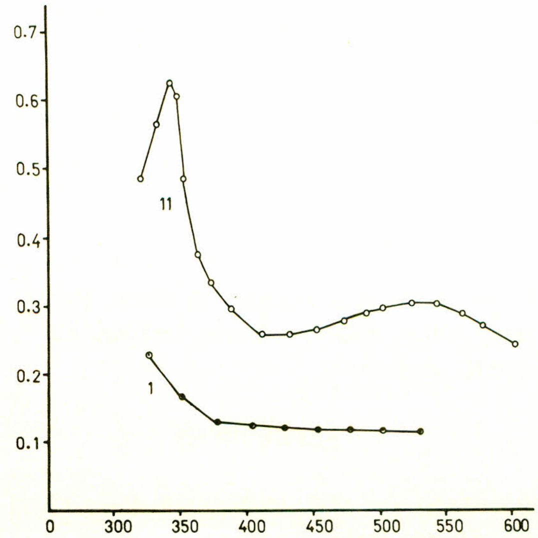

Fig. 1. (i) Maximum absorption of barbituric acid in alkaline medium. (ii) Maximum absorption of coloured complex.

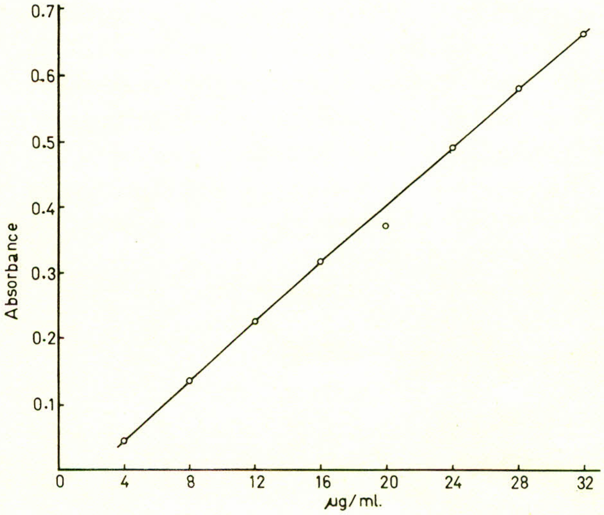

Fig. 2. Beer's law plot.

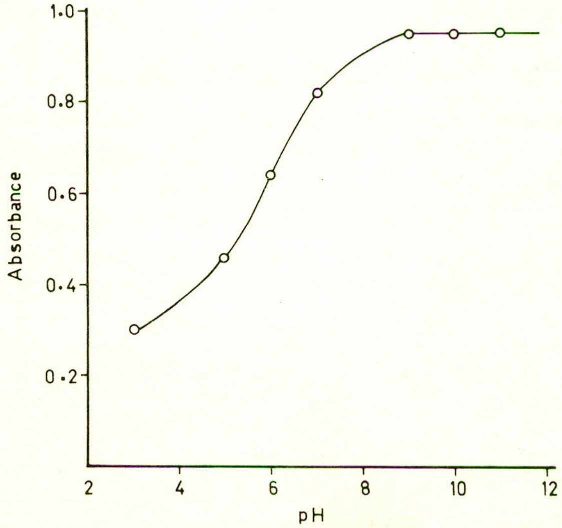

Fig. 3. Effect of pH on colour intensity.

barbituric acid has been added to nitrite solution.

Sandell's Sensitivity. The (SS) of this color complex is 2.3 $\mu g/cm^2$.

Precision and Accuracy. The precision and accuracy of the method were studied by analysing solutions containing known amounts of nitrite. The results are summarised in Table 1.

Effect of Diverse Ions. Nitrite can be determined accurately even when large amounts of nitrate, glucose, ascorbic acid, sulfite, sulfate, chloride, bromide, Ca^{++}, Mg^{++}, Na^+ and K^+ are present.

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