

THE MICRO SCALE DETERMINATION OF NITRITE WITH THE WEISZ RING-OVEN TECHNIQUE

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In the present investigations, a method has been recommended for the determination of nitrite using 8-hydroxyquinoline as coupling agent with nitrite to yield a pink coloured end product. The Weisz ring-oven technique has been made use of for these studies. The effect of various cations and anions along with phenol on its determination has been investigated. Nitrite can be determined within the range from 2.0 to 200.00 ng with a maximum error of -8.5% in the case of the former. The tolerance limits of this determination in the presence of CO_3^{-2} , HCO_3^{-1} , Cl^- , NO_3^- , SO_4^{-2} , S^{-2} , SO_3^{-2} , NH^{+4} , CN , Mg^{+2} , Co^{+2} , Ca^{+2} , Cd^{+2} , Hg^{+2} , Pb^{+2} , Sn^{+2} , Sn^{+4} , and phenol have also been reported. The reaction is very fast and one determination can be made with 10-15 minutes. The method is recommended for routine use.

Key words: Weisz Ring-over technique, Determination of Nitrite Microscale delermination.

INTRODUCTION

Nitrites play a precusory role in the formation of N-nitrosamine among which majority of them have been reported to be potent carcinogens [1-3]. On the other hand with the increase in the interest of the quality of natural and sewage waters, the importance of nitrite has increased many fold. Keeping these factors in view, there is always a good scope for a rapid, accurate, simple and sensitive procedure for the determination of nitrite on microscale. The previously described methods [1-3] have one short-coming or the other therefore, this factor also enhances the need for evolving a method for its determination. Bashir and Flamerz have reported a spectrophoto-metric method for the determination of nitrite using 8-hydroxy quinoline as coupling agent and the absorbance has been measured at 499nm^3 . They have tried resorcinol, $\bar{\alpha}$ -naphthol, α -naphthol, tiron and 8-hydroxyquinoline as coupling reagents an have repeated the results were achieved with 8-hydroxyquinoline.

In the present work we have made use of the coupling reaction between nitrite and 8-hydroxyquinoline for the standardisation of a convenient, sensitive and accurate method for the determination of nitrite using the Weisz ring-oven technique [4]. The sensitivity of the reported reaction is greater with this method as compared to the previously reported one [3] and some of the results of this study have been presented here. The effects of the interferences on this determination have also been studied.

EXPERIMENTAL

Reagents

Sodium nitrite. 0.1499g of the A.R. Grade sodium nitrite (BDH) was dissolved in 100 ml distilled water, one pellet of sodium hydroxide and 1 ml of chloroform were added to prevent this liberation of nitrous acid and the inhibition of the bacterial growth respectively. The prepared solution was exactly diluted to contain 20 ng/ μl of nitrite. Further standard solutions were prepared to contain 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ng/ μl of the nitrite. These standard solutions were labelled as I, II, IV, VI, VIII and X respectively.

p-Aminobenzoic acid. The solution containing 0.2g of p-aminobenzoic acid (G.R. Merck) in 0.116 M-hydrochloric acid we diluted with the acid to 100 ml.

8-Hydroxyquinoline. The solution of 8-hydroxyquinoline was prepared by dissolving 0.5g of the substance (G.R. Merck) in 0.45% sodium hydroxide solution, which was further diluted to 100 ml with the later.

Sodium carbonate. Sodium carbonate 1.76g (G.R. Merck) was dissolved in distilled water and the volume was made to 100 ml. This solution was diluted as needed.

Sodium chloride. Sodium chloride 8.25g (G.R. Merck) was dissolved in 100.0ml distilled water, which was further diluted to contain 5 ng/ μl chloride.

Ammonium ferrous sulphate. A solution containing 100 ng/ μl iron was prepared by dissolving 0.8625g ammonium ferrous sulphate (Analar BDH) in double distilled water and diluted to 100 ml.

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Potassium nitrate. 0.3226g potassium nitrate (G.R. Merck) was dissolved in distilled water, diluted to 100 ml and was used as such.

Cobalt chloride. Cobalt chloride 0.4034g (G.R. Merck) was dissolved in distilled water and diluted to 100 ml. It was further diluted as desired by the working conditions.

Magnesium sulphate. Magnesium sulphate 0.5g (Riedel) was dissolved in distilled water and the volume was made to 100 ml, which was further diluted according to the requirement.

Sodium sulphate. A solution containing 1 mg/ml of sodium sulphate (G.R. Merck) was prepared by dissolving 0.1479g sodium sulphate in 100 ml double distilled water and further diluted accordingly.

Sodium bicarbonate. 0.1372g sodium bicarbonate (BDH) was dissolved in double distilled water diluted to 100 ml, which was further diluted according to the need.

Ammonia. 1 mg/ml ammonia solution was prepared by the dilution of ammonium hydroxide (BDH). It was standardised and diluted accordingly.

Solutions of all the other anions and cations used for interference studies were also made from the A.R. Grade reagents.

Similarly the other reagents used were also of A.R. Grade or equivalent purity.

Apparatus

The Weisz ring-oven [5] with 110^o working temperature, automatic filling micropipettes of 1 and 2 μ l capacity (Karl-Kolb Scientific and Technical Supplies, Buchschlage, Frankfurt, BRD) and Whatman filter paper No. 41 were used for the work. All glass-ware used was of A Grade officially calibrated.

Procedure

The ring-oven procedure, as reported earlier, was adopted [4]. Accordingly a definite aliquot of each of the unknown solutions and the two standard solutions were spotted at three different points marked around the centre of the filter paper so as to make an equilateral triangle and dried. Then aminobenzoic acid (1 μ l) was applied to each of these three points thereafter 1 μ l of 8-hydroxyquinoline solution was also applied to these points and the washing was done with distilled water from the centre to the ring zone in the usual way, which resulted in the appearance of three sharply outlined pink segments, each about 10-20 mm long. Only 5-6 washings were sufficient to

wash the reaction product to the ring zone. The unknown solution was evaluated for its exact strength following the usual procedure of comparing the colour intensity of the test segment with that of the standard segments [6]. In order to achieve higher accuracy, two more determinations with more volumes, i.e. higher concentrations of the test solution were carried out making use of the above procedure.

The effect of interferences on the determination of nitrite was studied following the usual method [4] as also described here. According to the procedure, only to the point, where the test solution was applied a definite aliquot of the interfering radical was also applied additionally and the above procedure of washing and evaluation of the test solution was adopted. In this way any deviation in the determination of nitrite was noted. The determination of nitrite in the presence of varied amounts of the interfering substances was carried out so as to find the tolerance limits.

RESULTS AND DISCUSSION

In the present investigations, we have made use of the coupling reaction between 8-hydroxyquinoline and nitrite which yield pink coloured end product. The reaction is spontaneous and pH dependent, the effect of which has also been noted while changing the concentration of sodium hydroxide solution as a solvent for the preparation of 8-hydroxyquinoline.

The utility of the standard scale is limited by the fact that the standard rings including IV, VI, VIII and X become so close to each other that the unknown ring cannot be evaluated in this range. In order to annual this diverse effect and consequently to make the standard scale practicable an attempt was made to reduce the initial concentration of the standard stock solution to various degrees but this could not be utilised because of the repeated undiscernibility of the standard rings. It was, therefore, imperative that one should adopt the Segment Technique for the evaluation of the unknown solutions of the test substance (nitrite). By using this latter technique, there was absolutely no inconvenience in the evaluation of the unknown rings. Hence this technique was conveniently employed during the present course of work.

The method does not involve any extraction step; rather the determination of nitrite in the present procedure is direct. Consequently, the method is simpler even when compared to the Griess Ilosvoy method [7]. According to Table 1 the determination of nitrite within the range

Table 1. Determination of nitrite by the segment technique.

S. No.	Amount of nitrite		Error (%)
	Given (ng)	Found (ng)	
1.	1.00	1.15	+ 15.00
2.	2.00	1.80	- 8.50
3.	6.00	6.30	+ 5.00
4.	8.00	8.00	± 0.00
5.	10.00	9.70	- 3.00
6.	40.00	39.00	- 2.50
7.	60.00	60.00	± 0.00
8.	80.00	80.00	± 0.00
9.	100.00	101.00	- 1.80
10.	200.00	200.00	± 0.00

from 2.0 to 200.00 ng can be done with a maximum error of -8.5% in the case of the former amount. According to our observation as presented in Table 2. CO_3 , HCO_3^- , Cl^- , NO_3^- , SO_4^{2-} , S^{2-} , SO_3^{2-} , NH_4^+ , CN^- , Mg^{+2} , Co^{+2} , Cu^{+2} , Ca^{+2} , Cd^{+2} , Hg^{+2} , Pb^{+2} , Sn^{+4} , and phenol show a great tolerance for the determination of nitrite.

The sensitivity of the method here reported is far more than the study made by Bashir and Flamerz [3].

One determination of the unknown nitrite solution took only about 10-15 min with good accuracy for the method.

It is beyond any doubt to say that the method is quite tolerant towards anions and cations with the limits as exhibited by the Table 2. Keeping in view the simplicity, accuracy, sensitivity, specificity and high tolerance of the diverse ions, studied on this determination, the method proposed here is recommended for routine analysis.

Table 2. Study of interferences on the determination of nitrite

S. No.	Amount of nitrite taken (ng)	Interfering substance added (ng)	Amount of nitrite found (ng)	Error (%)
1.	4.00	200.00 ng	4.00	± 0.00
2.	4.00	250.00 "	4.00	± 0.00
3.	4.00	300.00 "	4.00	± 0.00
4.	4.00	325.00 "	3.75	- 6.25
5.	4.00	350.00 "	3.50	- 12.50
Carbonate				
1.	4.00	50.00 "	4.00	± 0.00
2.	4.00	100.00 "	4.00	± 0.00
3.	4.00	150.00 "	4.00	± 0.00
4.	4.00	200.00 "	3.75	- 6.25
5.	4.00	250.00 "	3.50	- 12.50
Bicarbonate				
1.	4.00	4.00 µg	4.00	± 0.00
2.	4.00	10.00 "	4.00	± 0.00
3.	4.00	15.00 "	4.00	± 0.00
4.	4.00	20.00 "	3.50	- 12.50
5.	4.00	25.50 "	3.25	- 18.75
Choloride				
1.	4.00	20.00 ng	4.00	± 0.00
2.	4.00	150.00 "	4.00	± 0.00
3.	4.00	450.00 "	4.00	± 0.00
4.	4.00	500.00 "	3.75	- 6.25
5.	4.00	600.00 "	3.50	- 12.50
Nitrate				

(Continued.....)

(Table 2, Continue)

1.	400	2.00	µg		4.00	± 0.00
2.	400	6.00	"		4.00	± 0.00
3.	4.00	9.00	"		4.00	± 0.00
4.	4.00	10.00	"	Sulphate	4.00	± 0.00
5.	4.00	15.00	"		3.50	- 12.00
1.	4.00	0.20	ng		4.00	± 0.00
2.	4.00	0.40	"		4.00	± 0.00
3.	4.00	0.60	"		4.00	± 0.00
4.	4.00	0.80	"	Sulphide	3.75	- 6.25
5.	4.00	1.00	"		3.50	- 12.50
1.	4.00	0.50	"		3.95	- 1.25
2.	4.00	0.80	"		3.85	- 3.75
3.	4.00	1.00	"		3.70	- 7.50
4.	4.00	1.50	"	Sulphite	3.50	- 12.50
5.	4.00	2.00	"		3.20	- 20.00
1.	4.00	0.50	"		4.00	± 0.00
2.	4.00	1.10	"		4.00	± 0.00
3.	4.00	1.5	"		4.00	± 0.00
4.	4.00	2.0	"	Ammonia	3.50	- 12.50
5.	4.00	3.0	"		3.00	- 25.00
1.	4.00	0.5	"		4.00	± 0.00
2.	4.00	1.0	"		4.00	± 0.00
3.	4.00	2.0	"		3.75	- 6.25
4.	4.00	4.0	"	Cyanide	3.50	- 12.00
5.	4.00	6.0	"		3.25	- 18.75
1.	4.00	10.00	ug		4.00	± 0.00
2.	4.00	20.00	"		4.00	± 0.00
3.	4.00	50.00	"		4.00	± 0.00
4.	4.00	80.00	"	Magnesium	3.75	- 6.25
5.	4.00	90.00	"		3.50	- 12.50
1.	4.0	0.1	"		4.00	± 0.00
2.	4.0	0.3	"		4.00	± 0.00
3.	4.0	0.4	"		4.00	± 0.00
4.	4.0	0.5	"	Copper (II)	3.50	- 12.50
5.	4.0	0.6	"		3.00	- 25.00
1.	4.0	0.1	"		4.00	± 0.00
2.	4.0	0.3	"		4.00	± 0.00
3.	4.0	0.5	"		4.00	± 0.00
4.	4.0	1.0	"	Calcium (II)	3.75	± 6.25
5.	4.0	1.5	"		3.50	- 12.50

(Continued.....)

(Table 2, Continued)

1.	4.00	0.20	''		4.00	± 0.00
2.	4.00	0.30	''		4.00	± 0.00
3.	4.00	0.50	''		4.00	± 0.00
4.	4.00	0.80	''	Cobalt	4.00	± 0.00
5.	4.00	1.00	''		3.25	- 18.75
1.	4.00	0.29	''		4.00	± 0.00
2.	4.00	0.40	''		4.00	± 0.00
3.	4.00	0.60	''		4.00	± 0.00
4.	4.00	0.80	''	Iron	3.75	- 6.25
5.	4.00	1.00	''		3.00	- 25.00
1.	4.0	1.0	ng	Cadmium	4.0	± 0.00
2.	4.0	10.0	''		4.0	± 0.00
3.	4.0	20.0	''		4.0	± 0.00
4.	4.0	30.0	''		3.75	- 6.25
5.	4.0	45.0	''		3.0	- 25.00
1.	4.0	0.2	''		4.00	± 0.00
2.	4.0	0.4	''		4.00	± 0.00
3.	4.0	0.5	''		4.00	± 0.00
4.	4.0	1.0	''	Mercury (II)	3.60	- 10.00
5.	4.0	1.5	''		3.75	- 18.25
1.	4.0	0.5	''		4.00	± 0.00
2.	4.0	0.8	''		4.00	± 0.00
3.	4.0	1.5	''		4.00	± 0.00
4.	4.00	2.5	''	Lead (II)	3.75	- 6.25
5.	4.0	3.0	''		3.59	- 12.50
1.	4.0	2.0	''		4.00	± 0.00
2.	4.0	5.0	''		4.00	± 0.00
3.	4.0	20.0	''		4.00	± 0.00
4.	4.0	30.00	''	Tin (II)	3.50	- 12.50
5.	4.0	45.0	''		3.00	- 25.00
1.	4.0	1.0	''		4.00	± 0.00
2.	4.0	5.0	''		4.00	± 0.00
3.	4.0	15.0	''		4.00	± 0.00
4.	4.0	25.0	''	Tin (IV)	3.50	- 12.50
5.	4.0	40.0	''		3.75	- 18.25
1.	4.0	25.0	''		4.00	± 0.00
2.	4.0	100.0	''		4.00	± 0.00
3.	4.0	400.0	''		3.75	- 6.25
4.	4.0	450.0	''	Phenol	3.50	- 12.50
5.	4.0	500.0	''		3.00	- 25.00

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