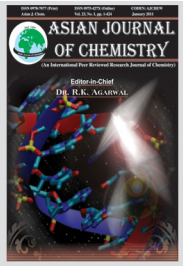
Asian Journal of Chemistry; Vol. 23, No. 9 (2011), 4122-4124

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Extraction and Determination of Nitrite using Ethanol-$(NH_4)_2SO_4$ Aqueous Two-Phase System

YING-HONG LIU*, QIAO CAI and WEI-XING MA

College of Chemical Engineering, Huaihai Institute of Technology, Lianyungang 222005, Jiangsu, P.R. China

*Corresponding author: Tel: +86 518 85857922; E-mail: liuyh9506@163.com; yinghong1978@yahoo.com.cn

(*Received*: 20 December 2010; *Accepted*: 20 May 2011) AJC-9981

The extraction and determination of nitrite using ethanol-$(NH_4)_2SO_4$ aqueous two-phase system was investigated. The diazotized product between nitrite and amino black 10B was found to be extracted to the ethanol phase with little interference. The absorbance variation, which was proportional to the concentration of nitrite, was determined at the wavelength of 520 and 620 nm. Beer's law was obeyed over the concentration range of 0-3.3 μg mL^{-1}. By superposing the absorbance at the two wavelength, the apparent molar absorption coefficient was 1.4×10^4 L mol^{-1} cm^{-1}. The proposed extraction method had been applied to the determination of nitrite in food samples. The results obtained by this method was in good agreement with the results obtained by naphthyl ethylenediamine spectrophotometry method.

Key Words: Aqueous two-phase extraction, Nitrite, Amino black 10B, Spectrophotometry.

INTRODUCTION

Nitrite, which exists widely in food and environment, is an intermediate product in the oxidation or reduction between ammonium and nitrate. Excessive intake of nitrite for human being will cause methemoglobin disease which leads to the disabilities of carrying oxygen for hemoglobin. Otherwise, nitrite can reacts with amine and amide compounds to generate nitrosamines, which is a kind of carcinogen[1]. So it is significant to study on the determination method of nitrite. Many analytical methods, such as spectrophotometry[2-5], ion chromatography[6,7], electrochemistry[8,9], chemiluminescence[10] and fluorimetry[11], have been applied for the determination of nitrite. Spectrophotometry method based on the diazotization coupling reaction is a commonly used method.

The aqueous two-phase system is formed by water-soluble polymers and certain inorganic salts or by mixing two dissimilar water-soluble polymers. Ordinary organic-inorganic salt system has been reported in recent years. The aqueous two-phase extraction system has been paid great attention for the reasons of less poisoning, quick separation, easy operation, clear boundary and no emulsification[12]. The extraction of metals, drugs and biological macromolecules used aqueous two-phase systems has been reported[13-17]. But the aqueous two-phase has not been reported for the extraction of nitrite.

In this work, a method for extraction and spectrophotometric determination of nitrite was established. Amino black 10B, as a primary aromatic amine, can react with nitrite to form diazo compound. The diazo compound is extracted into the upper phase of the aqueous two-phase system containing ethanol and $(NH_4)_2SO_4$. The change value of absorbance of the upper solution, due to the presence of nitrite, is correlated with its concentration. The proposed method has been applied for the extraction and determination of nitrite in food samples with satisfactory results.

EXPERIMENTAL

A 722-N spectrophotometer (Shanghai precision & scientific instrument Co. Ltd., China) with matched 1 cm glass cells was used for all the spectrophotometric determination.

The standard stock solution of nitrite (1 g L^{-1}) was prepared by dissolving 0.1500 g of $NaNO_2$ in 100 mL volumetric flask with water. The standard working solution of nitrite was obtained by diluting the above stock solution. Amino black 10B solution (0.5 g L^{-1}) was prepared by dissolving 0.5 g of amino black 10B in 1000 mL water. 1 mol L^{-1} HCl solution was used. All chemicals used were of analytical grade and double distilled water was used throughout the study.

Preparation for real samples: A proper amount of samples were mixed and homogenized with a food grinder. 50 g of the homogenate was weighed into a 100 mL beaker and 12.5 mL of saturated borax solution (50 g L^{-1}) was added. After shaking up, the mixture with 300 mL of water was transferred into a 500 mL volumetric flask. The mixture was heated in boiling water bath for 15 min and then cooled to room temperature. After shaking the above solution, 5 mL of potassium ferrocyanide (106 g L^{-1}) and 5 mL of zinc acetate (220 g L^{-1})

were added to make the protein precipitation. The solution was diluted to the mark with water and kept aside for 0.5 h. After removing the upper fat, the supernatant was filtrate with filter paper. The filtrate, except 30 mL of early filtrate, was used for analysis by the proposed method and reference method.

Procedure: To obtain the aqueous two-phase extraction system, a suitable volume of NO_2^- working solution or sample solution, 1 mL of 1 mol L^{-1} HCl solution and 1 mL of 0.5 g L^{-1} amino black 10B solution were added to a 10 mL colorimetric tube. The mixed solution was diluted to 5 mL with water and then left to stand for 10 min. After adding 4 mL of ethanol and diluting the solution to the mark, 2.2 g of solid $(NH_4)_2SO_4$ was added. The mixture was shaken for 2 min and allowed to stand until the mixture was thoroughly separated into two phase. The upper ethanol phase was transferred completely into another tube and the solution was diluted to 10 mL with water. The absorbance of the solution was measured at 520 and 620 nm by using reagent blank solution as reference.

RESULTS AND DISCUSSION

Effect of salt species and amount on phase separation: In order to obtain the aqueous two-phase system, 2 g of NaCl, $NaNO_3$, Na_2CO_3, NH_4Cl and $(NH_4)_2SO_4$ were used, respectively. The results indicated that only Na_2CO_3 and $(NH_4)_2SO_4$ could cause the solution to separate into two phases. $(NH_4)_2SO_4$ had a greater ability of salting-out, better solubility, lower temperature coefficient and cheaper price[12], so $(NH_4)_2SO_4$ was chosen as the salting-out agent in this work. The effect of $(NH_4)_2SO_4$ amount on phase separation was then studied. The solution didn't separate to two phases when $(NH_4)_2SO_4$ amount was less than 1.0 g and the boundary of the two phases was not clear when the amount was at the range of 1.0-2.0 g. 2.0-2.5 g of $(NH_4)_2SO_4$ caused the solution to separate to two clear phases. Therefore, 2.2 g of $(NH_4)_2SO_4$ was selected for further study.

Absorption spectra: The absorbance spectra of amino black 10B in ethanol phase in absence and presence of nitrite was shown in Fig. 1. In the presence of nitrite, the absorbance of amino black 10B solution increased at 520 nm and decreased at 620 nm obviously, which indicated the occurring of diazo-reaction between amino black 10B and nitrite. In further study, 520 and 620 nm were selected as measuring wavelengths.

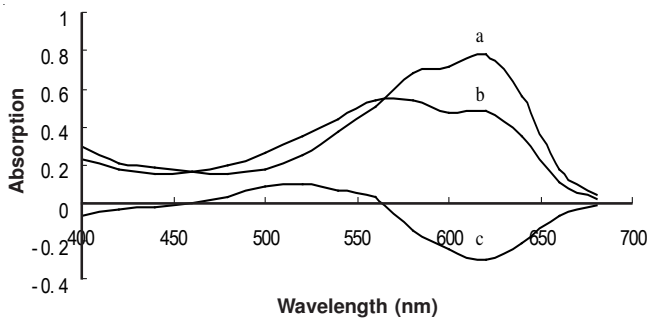


Fig. 1. Absorbance spectra of amino black 10B against ethanol (a), diazo compound against ethanol (b) and diazo compound against reagent black solution (c): 0.025 g L^{-1} amino black 10B, 1.7 μg mL^{-1} nitrite, 0.1 mol L^{-1} HCl

Effect of acidity: The experimental results showed that the absorbance of the system was larger in hydrochloric acid than in sulfuric acid and phosphate acid. So hydrochloric acid was selected as the medium of the diazo reaction. The effect of hydrochloric acid concentration was shown in Fig. 2. The optimum acidity in the experiments was controlled with 0.1 mol L^{-1} HCl from the figure.

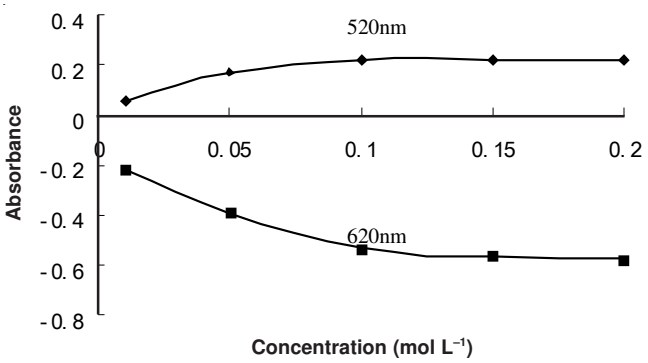


Fig. 2. Effect of HCl concentration: 0.05 g L^{-1} amino black 10B, 3.0 μg L^{-1} nitrite

Effect of amino black 10B concentration: The effect of amino black 10B concentration on the absorbance was investigated. The results of Fig. 3 showed that the absorbance of the solution was maximum and constant in the range of 0.04-0.1 g L^{-1}. In this work, 0.05 g L^{-1} amino black 10B was chosen as optimum concentration.

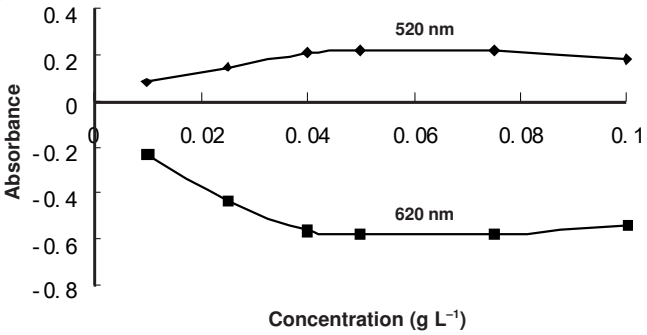


Fig. 3. Effect of amino black 10B concentration: 3.0 μg L^{-1} nitrite, 0.1 mol L^{-1} HCl

Stability of the system: The results showed that the diazo reaction could be complete within 10 min. Therefore, the extraction was carried out after keeping the mixture of amino black 10B and nitrite for 10 min in HCl medium. Under the conditions of 3.5 μg L^{-1} nitrite, 0.05 g L^{-1} amino black 10B and 0.1 mol L^{-1} HCl, the absorbance of the upper phase solution kept steady within at least 90 min, which indicated that the diazo product was stable within 90 min.

Calibration curves and detection limits: A series of nitrite working solutions with different concentrations were prepared. Under the chosen experimental conditions, the absorbance of these solutions was measured at 520 and 620 nm. Regression analysis for the results was carried out using least-square method. The regression equations and related parameters were shown in Table-1. In all cases, Beer's law was obeyed with good correlation coefficients in the concentration

TABLE-1
REGRESSION EQUATIONS OF CALIBRATION CURVES AND RELATED PARAMETERS

Wavelength (nm)	Regression equation	Correlation coefficient	Molar absorption coefficient (L mol^{-1} cm^{-1})	LOD (μg mL^{-1})
520	$A_{520\ nm} = 0.0972C - 0.0686$	0.9990	0.5×10^4	0.05
620	$A_{620\ nm} = -0.2057C + 0.0732$	0.9992	1.0×10^4	0.02
520 + 620	$A_t = 0.3029C + 0.1409$	0.9994	1.4×10^4	0.01

TABLE-3
ANALYTICAL RESULTS OF REAL SAMPLES

Samples	Proposed method*		Reference method*	
	Found (mg Kg^{-1})	RSD (%)	Found (mg Kg^{-1})	RSD (%)
Ham sausage 1	8.2	3.5	7.9	2.3
Ham sausage 2	9.6	2.5	9.8	1.8
Lunch meat 1	12.5	1.6	12.3	2.8
Lunch meat 2	13.7	1.3	13.7	1.0

*Mean for seven replicate determinations.

ranges of 0-3.3 μg mL^{-1}. By superposing the absorbance of the two measurement wavelengths ($A_t = A_{520\ nm} - A_{620\ nm}$), the apparent molar absorption coefficient increased 1.5-2.0 times which meant increasing of sensitivity. The limits of detection (LOD) were determined using the formula: LOD = 3σ/b, where σ is the standard deviation of the reagent blank for 11 times determination and b is the slope of the calibration curves and the results were also shown in Table-1.

Selectivity of the system: To study the selectivity of the proposed method, the effect of foreign species on the determination of 3.0 μg mL^{-1} nitrite was studied. When the relative error was within ± 5 %, the allowable concentrations of foreign ions were shown in Table-2.

TABLE-2
ALLOWABLE AMOUNT OF FOREIGN IONS

Foreign ions	Allowable amount (mg mL^{-1})
K^+, Na^+, Ca^{2+}, SO_4^{2-}	10.0
Al^{3+}, F^-, Cl^-	5.0
NO_3^-, Pb^{2+}, Fe^{3+}	3.0
Ni^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+}, Hg^{2+}, Zn^{2+}	1.5

Application: In order to investigate the application of the proposed method, it was applied to the extraction and determination of nitrite in food samples. The results obtained for the analysis of real samples using the proposed method and naphthyl ethylenediamine spectrophotometry method as reference method were summarized in Table-3. As shown in Table-3, the results of the both methods were in very good agreement. The standard addition experiment were made and the recoveries were in the range of 95.8-102.4 %.

Conclusion

The aqueous two-phase of ethanol and $(NH_4)_2SO_4$ mixture could be successfully applid to the extraction of nitrite without interference from several existing metals and inorganic anions. Spectrophotometric determination of nitrite was carried out subsequently. The proposed method could be applied to the determiantion of nitrite in food samples and environmental samples.

ACKNOWLEDGEMENTS

This work is supported by Nature Science Research Project of Huaihai Institute of Technology, China (No. 2010150014).

REFERENCES

1. A.A. Ensafi and M. Amini, *Sens. Actuators B*, **147**, 61 (2010).
2. V.V. Kuznetsov and S.V. Zemyatova, *J. Anal. Chem*., **62**, 637 (2010).
3. B.P. Dayananda and H.D. Revanasiddappa, *Chem. Pap*., **61**, 446 (2007).
4. H.D. Revanasiddappa, K. Kumar and M. Bilwa, *Microchim. Acta*., **137**, 249 (2001).
5. T.A. Doane and W.R. Horwáth, *Anal. Lett*., **36**, 2713 (2003).
6. X.L. Jiang, L.W. Lim and T. Takeuchi, *Anal. Bioanal. Chem*., **393**, 387 (2009).
7. P. Niedzielski, I. Kurzyc and J. Siepak, *Anal. Chim. Acta*, **577**, 220 (2006).
8. H.S. Yin, Y.L. Zhou and T. Liu, *Microchim. Acta*, **171**, 385 (2010).
9. K. Zhao, H.Y. Song, S.Q. Zhuang, L.M. Dai, P.G. He and Y.Z. Fang, *Electrochem. Commun*., **9**, 65 (2007).
10. D.Y. He, Z.J. Zhang, Y. Huang and and Y.F. Hu, *Food Chem*., **101**, 667 (2007).
11. X.Q. Zhan, D.H. Li, H. Zheng and J.G. Xu, *Anal. Lett*., **34**, 2761 (2001).
12. Y.T. Gao and W. Wang, *Precious Met.*, **27**, 45 (2006).
13. N. Yoshikuni, T. Baba, N. Tsunoda and K. Oguma, *Talanta*, **66**, 40 (2005).
14. Y. Akama and A. Sali, *Talanta*, **57**, 681 (2002).
15. Q. Gao, S. Li and C. He, *Anal. Chim. Acta*, **590**, 187 (2007).
16. L. Zapala and J. Kalembkiewicz, *Talanta*, **69**, 601 (2006).
17. F. Qu, H. Qin and M. Dong, *Chin. Chem. Lett*., **20**, 1100 (2009).