Spectrophotometric Determination of Nitrite Based on Its Reaction with *p*-Nitroaniline in the Presence of Diphenylamine in Micellar Media

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In the present work a very simple, sensitive and selective spectrophotometric method for the determination of nitrite in micellar media is described. The method is based on the color reaction of nitrite with p-nitroaniline in the presence of diphenylamine in acid media. In order to remove the extraction step, Triton X-100, a non-ionic surfactant was used as micellar media. The optimum reaction conditions such as acid concentration, reagents concentration and effect of time have been studied and the analytical characteristics of the method such as limit of detection, linear range and molar absorptivity have been obtained. The interference of some anions and cations was also tested. The method was applied to the determination of nitrite in real samples.

Key Words : Spectrophotometric determination, Nitrite, *p*-Nitroaniline, Diphenylamine, Micellar media

Introduction

The nitrite ion is an important intermediate in biological nitrogen cycle and is present in soils and surface waters.[1] Nitrite is also a versatile chemical agent, which has found numerous applications ranging from dye manufacture to food preservation.[2] Moreover the determination of nitrite in natural waters is important, being harmful to human health.[3] Several reports have been published on the determination of nitrite including chromatography,[4,5] electrochemical detection,[6-8] capillary electrophoresis[9,10] and spectrophotometric methods.[3,11-17] In some of these methods, selectivity is poor; some demand expensive and complicated instruments or reagents and the others need to do difficult and time-consuming separation procedures.

Spectroscopic methods are by far the most widely used for nitrite determination due to the excellent limit of detection obtained and facile assay-type protocols.[18] The most common approach to the spectrophotometric detection of nitrite is the Griess Assay.[18] The assay typically relies on the diazotisation of a suitable aromatic amine by acidified nitrite with the subsequent coupling reaction providing a highly colored azo chromophore from which the concentration of nitrite can be assessed.

In the present work a very simple, sensitive, selective and low cost procedure for the spectrophotometric determination of nitrite is described. The method is based on the reaction of acidified nitrite with p-nitroaniline and diphenylamine in micellar media. The produced azo chromophore is insoluble in water, therefore Triton X-100, a neutral surfactant, was used to dissolve it in water. By using surfactant for dissolving the insoluble product in water, solvent extraction, solid phase extraction or mixed solvent systems have been avoided.

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Experimental Section

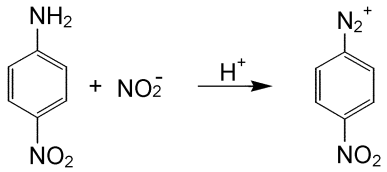
Apparatus. UV-Visible absorbance spectra were recorded on a Shimadzu UV-265 scanning spectrophotometer using 1-cm quartz cells. Metrohm model 713 pH-meter with a combined glass electrode was used for pH measurements.

Reagents. All chemical reagents used were of analytical reagent grade and triply distilled water was used throughout the experiments. Stock nitrite solution (1000 μg mL^{-1}) was prepared by dissolving appropriate amount of its sodium salt (Merck) in water. p-nitroaniline solution (0.2% m/v) and diphenylamine (0.5% m/v) were prepared in ethanol. A 5.0 mol L^{-1} sulfuric acid solution was prepared by appropriate dilution of concentrated sulfuric acid (Merck). Triton X-100 stock solution (14% v/v) was prepared by dissolving 14 mL of concentrated solution (Merck) in hot distilled water.

Procedure. An aliquot of the solution containing 0.5-8.0 μg of nitrite ion, 2 mL of sulfuric acid solution, 1 mL of triton X-100 solution and 1 mL of p-nitroaniline solution were transferred into a 10-mL volumetric flask. The solution was diluted to *ca* 9 mL with water. The solution was then allowed to stand for 1 min. After that 1 mL of diphenylamine solution was added and made up to the mark with the water. The solution was then allowed to stand for 10 min at room temperature. Then a portion of the solution was transferred into a quartz cell to measure its absorbance at 500 nm.

Results and Discussion

Composite diazotization coupling reaction of p-nitroaniline, as a diazotizable aromatic amine, and diphenylamine, as a coupling agent, proceeds in acidic media to produce an azo product.



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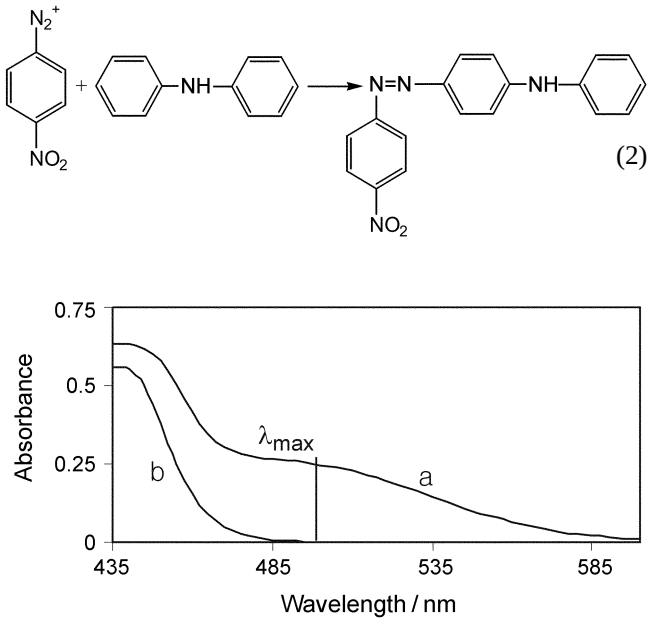

Figure 1. (a) The spectra of produced azo compound and (b) the blank solution against water. Conditions: nitrite ion, 0.8 μg mL^{-1}; sulfuric acid, 1 mol L^{-1}; Triton X-100, 1.4% (v/v); p-nitroaniline, 0.02% (m/v); and diphenylamine, 0.05% (m/v). The blank solution was prepared in the same way except that distilled water was used instead of nitrite solution.

The produced azo compound is insoluble in water. But it was observed that it is soluble in water by the addition of the neutral surfactant of Triton X-100.

The absorption spectrum of produced azo compound is shown in Figure 1. The amount of the absorbance of the solution at 500 nm, the λ_{max} of the product, is proportional to the nitrite concentration. Therefore, the system is suitable for spectrophotometric determination of nitrite.

Optimization of the system. The nitrozation reaction takes place in acid media. Various acids were tested and sulfuric acid was found as the best one. The effect of the concentration of sulfuric acid was studied. As it is observed from Figure 2 the absorbance of the solution increased by increasing sulfuric acid up to 1 mol L^{-1} and remained nearly constant at higher concentrations. Therefore 1 mol L^{-1} acid sulfuric was used as optimum concentration.

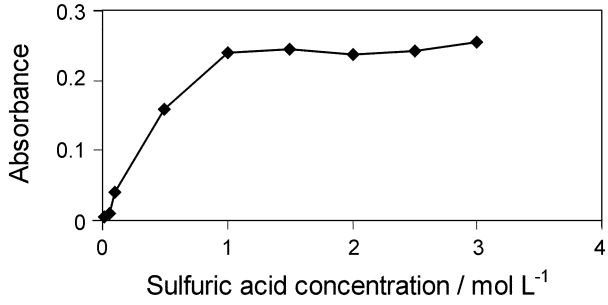

Figure 2. Effect of sulfuric acid concentration on the absorbance of the system. Conditions: nitrite ion, 0.6 μg mL^{-1}; Triton X-100, 1.4% (v/v); p-nitroaniline, 0.02% (m/v); and 0.04% m/v diphenylamine.

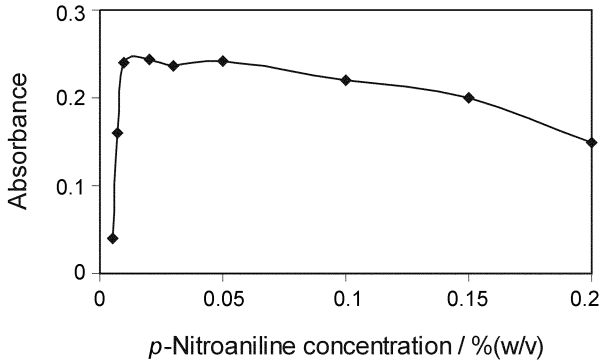

Figure 3. Effect of p-nitroaniline concentration on the absorbance of the system, Conditions: nitrite ion, 0.6 μg mL^{-1}; sulfuric acid, 1 mol L^{-1}; Triton X-100, 1.4% (v/v); and diphenylamine, 0.04% (m/v).

As mentioned above the produced azo chromophore is insoluble in water, therefore, Triton X-100, a neutral surfactant, was used to dissolve it in water. In order to obtain optimum concentration of Triton X-100, the effect of surfactant concentration on the absorbance of the system was investigated. The results showed that the absorbance of the solutions increased by increasing Triton X-100 concentration in the range 0.8-1.2% v/v, remaining constant between 1.2 and 1.6% v/v and slightly decreased at higher concentrations. Therefore, 1.4% v/v Triton X-100 was applied to provide micellar media in order to prevent extraction procedure or using mixed solvent systems.

As Figure 3 shows, changing the concentration of p-nitroaniline in the range 0.01-0.05% (m/v) had no effect on the absorbance of the system. Therefore, a 0.02% (m/v) concentration of the p-nitroaniline was used for further works.

The effect of the diphenylamine concentration on the absorbance of the system was also investigated. The results showed that the absorbance of the solutions increased by increasing diphenylamine concentration up to 0.01% (m/v), remained nearly constant between 0.02 and 0.1% m/v and decreased at higher concentrations (Fig. 4). Therefore, 0.05% (m/v) diphenylamine was applied in the proposed method.

Effect of time on both the reaction steps (diazotisation and

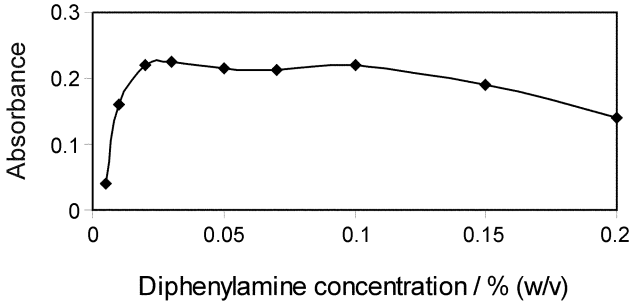

Figure 4. Effect of diphenylamine concentration on the absorbance of the system, Conditions: nitrite ion, 0.6 μg mL^{-1}; sulfuric acid, 1 mol L^{-1}; Triton X-100, 1.4% (v/v); and p-nitroaniline, 0.02% (m/v).

Table 1. Analytical features of the proposed method

Regression equation (n = 7)[a]	A = 0.3098 × C 0.008, r = 0.9998 [b]
molar absorptivity	$1.425 \times 10^{+4}$ L mol^{-1} cm^{-1}
Linear range (μg mL^{-1})	0.05-0.8
Limit of Detection ($3\sigma_{blank}$) (μg mL^{-1}, n = 5)	0.01
Repeatability (R.S.D., %) (n=5)	2.97 (for 0.3 μg mL^{-1} nitrite)

[a]The concentration of nitrite is in mg mL^{-1}. [b]Regression coefficient.

Table 2. Tolerance ratio of diverse ions on the determination of 0.2 μg mL^{-1} nitrite ion

Ion	Tolerance ratio[a] ($w_{ion}/w_{nitrite}$)
I^-, Br^-, PO_4^{3-}, F^-, CO_3^{2-}, Cl^- , tartarat, Nitrate, CH_3COO^-	500
Cd^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Ba^{2+}, Ca^{2+}, Al^{3+}, Mg^{2+}, Ag^+, Cr^{3+}	500 (EDTA)
Fe^{3+}	250 (tartarat)

[a]Masking agents are given in parenthesis.

coupling steps) was investigated. The results showed that these reactions were completed in 1 and 10 min, respectively.

Analytical characteristics. Table 1 summarizes the characteristics of the optimized method, including regression equation, linear range, limit of detection (LOD) and reproducibility.

Interference study. The effect of different cations and anions on the determination of 0.2 μg mL^{-1} nitrite ion by the proposed method was studied. An ion was considered as interferent, when it caused a variation greater than ± 5% in the absorbance of the sample. The results (Table 2) indicate that the investigated anions did not interfere in the determination of nitrite by the proposed method. The investigated cations interfered on the determination of nitrite, probably due to their complexation with the organic reagents or the product. In the presence of 1.0×10^{-3} mol L^{-1} EDTA as masking agent the interfering effect of all the investigated cations except that of Fe^{3+} was completely removed. The interfering effect of Fe^{3+} was completely removed in the presence of 1.0×10^{-3} mol L^{-1} tartrate.

Table 3. Determination of nitrite added to water samples by the proposed method[a]

Sample	Nitrite added (μg mL^{-1})	Nitrite found (μg mL^{-1})	Recovery (%)
Well water (Hamadan, Maryanaj)	0.10	0.10 ± 0.01	100
	0.25	0.24 ± 0.02	96
	0.40	0.42 ± 0.03	105
River water (Hamadan, Ekbatan)	0.10	0.11 ± 0.01	110
	0.30	0.31 ± 0.02	103
	0.50	0.47 ± 0.02	94

[a]Average of three determinations ± SD.

Application. The proposed method was successfully applied to the determination of nitrite ion in several water samples. The tested waters was found to be free from nitrite and so synthetic samples were prepared by adding known amounts of nitrite to the water samples. The results are shown in Table 3. The recoveries are close to 100% and indicate there is no serious interference in such water samples.

Conclusion

The new proposed procedure gives a rapid, sensitive and very low cost spectrophotometric procedure for determination of nitrite ion that can be applied to real samples. Using surfactant caused dissolving the insoluble product in water, and therefore solvent extraction, mixed solvent systems or solid phase extraction has been avoided. The sensitivity, linear range and detection limit are nearly the same as the reported methods used solvent extraction or solid phase extraction.[3,19-21] Addition of surfactant also increased the rate of reaction.

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