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Determination of Nitrite and Nitrate by Forming Two 8-Hydroxyquinoline Based Azo Dyes

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Abstract

Two sensitive and selective spectrophotometric methods are presented for the determination of trace amounts of nitrite and nitrate. It relies on the reaction of nitrite with sulfadimidine (SD) and cisapride (CP) in acidic medium to form diazonium ions, which are coupled with 8-hydroxyquinoline (HQ) in basic solutions to form the related azo dyes. Absorbances were measured at 507 nm for SD-HQ product and at 532 nm for CP-HQ product. The calibration curves were linear in the ranges of 0.05-2.5 and 0.04-5.0 mg L^{-1} for SD-HQ and CP-HQ systems, respectively. The relative standard deviations for the nitrite determination methods were obtained as 0.3-0.9% and 0.1-0.8% for SD-HQ system and CP-HQ system, respectively. Nitrate was also determined after using a cadmium-copper reducing column. Interferences of various foreign ions were tested and the methods were successfully applied to the determination of nitrite and nitrate in various water samples.

Keywords: Nitrite, Nitrate, Sulfadimidine, Cisapride, Spectrophotometry.

1. Introduction

Nitrite and nitrate are two important metabolites in the biological nitrogen cycle [1]. Nitrate occurs naturally in soil by nitrogen-fixing bacteria, decaying plants, septic system effluent, and animal manure. Other sources of nitrate are nitrogenous fertilizers and airborne nitrogen compounds emitted by industries and automobiles. Nitrite is present in soil, surface water and food and can be generated by chemical transformation or biodegradation of nitrate. The most common food preservative for cured-meat products is nitrite. Nitrate is sometimes also added to food to serve as a reservoir for nitrite. Concentrations of nitrite and nitrate in water and food indicate their quality [2]. Nitrite and nitrate could be hazardous to health at high concentrations, especially for infants and pregnant women. Methaemoglobinaemia is the major acute toxic effect of nitrate and nitrite [3]. Nitrite converts hemoglobin to methaemoglobin which decreases the capacity of oxygen-carrying capacity of the blood. Also nitrosamines are formed in body due to high intake of nitrate and nitrite, and result the increasing of cancer risk [4]. Therefore, elucidation of the concentrations of nitrate, and of nitrite, is desirable from the standpoint of environmental chemistry, waste water treatment and biological chemistry.

Several analytical techniques have been presented in the literature, for the determination of nitrite and nitrate such as chromatography [5, 6], capillary electrophoresis [7], GC-MS [8] and electrochemical methods [9, 10]. However, most of them are not widely accepted, because of their complexities and intensive labor requirements. Spectrophotometric methods are simple, selective and valuable for nitrite determination when the Griess assay [11] is applied.

In the present study, two spectrophotometric methods have been established for determination of trace amounts of nitrite and nitrate in water samples. This study uses two simple diazotization reaction involving sulfadimidine (SD) and cisapride (CS) which are coupled with 8-hydroxyquinoline (HQ). Then, the produced azo dyes were monitored for the determination of nitrite. A copperized-cadmium reducing column was prepared to reduce nitrate to

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nitrite prior to nitrate determination. Various water samples were analyzed by the presented methods.

2. Experimental

2.1. Apparatus and chemicals

A Shimadzu Model UV-1601 PC double-beam spectrophotometer with one pair of 10.0 mm glass matched cells were used to obtain spectra and absorbances. A Jenway pH meter with a glass electrode was used for pH measurements.

All chemicals used were of analytical reagent grade, and deionized water was used in all experiments. Sodium nitrite, sodium nitrate, HQ, sodium dodecyl sulfate (SDS) and cadmium granules were purchased from Merck. CP and SD were purchased from Sigma and Acros companies, respectively.

All solutions were prepared with analytical grade reagents and deionized water. The stock nitrite and nitrate solutions (1000 mg L-1) were prepared by dissolving appropriate amounts of their sodium salts in water, diluting in 100.0 mL volumetric flask and then the solutions were standardized [3]. SD, CP and HQ solutions were prepared as 1.0, 5.0 and 40.0 mmol L-1, respectively, in ethanol.

2.2. Preparation of Cu-Cd reducing column

To prepare Cu-Cd reducing column, 8.0 g of cadmium granules were washed with acetone (30 mL), water (2×30 mL) and then with hydrochloric acid as 2.0 mol L^{-1} (20 mL). Then, the granules were immediately rinsed with 3×50 mL water and then immediately 10.0 mL of $CuSO_4$ solution as 2.0% (wt/v) was added. The mixture was stirred until decolorizing of the Cu(II) solution. Then, the prepared Cu-Cd granules were washed with 50 mL of dionized water and 50 mL of ammonium chloride 0.10 mol L-1, respectively. The prepared Cu-Cd granules were preserved in the ammonium chloride solution. The prepared Cu-Cd reducing granules were transferred to a column with an i.d. equal to 5 mm. The nitrate to nitrite conversion efficiency of the column was also evaluated [12, 13]. To reduce nitrate of the samples, the samples were passed through the column by a flow rate of 5.0 mL min^{-1}, twice.

2.3. Procedure using SD-HQ system

First, 5.0 mL of the sample solution containing nitrite, 1.0 mL of hydrochloric acid 0.005 mol L^{-1} and 1.0 mL of 3.0 mmol L^{-1} SD were transferred to a 10.0 mL volumetric flask and allowed to stand for 7 min for complete diazotization. Then 1.0 mL of sodium hydroxide 0.10 mol L^{-1} and 1.0 mL of 8.0 mmol L^{-1} HQ were sequentially added and the contents were diluted to 10.0 mL using dionized water. After 2 min, the absorbance of the produced azo dye was measured at 507 nm against the reagent blank.

2.4. Procedure using CP-HQ system

First, 5.0 mL of the sample solution containing nitrite, 2.0 mL of 7.0 % SDS, 0.5 mL of hydrochloric acid 0.016 mol L^{-1} and 1.0 mL of 7.0 mmol. L^{-1} CP were transferred to a 10.0 mL volumetric flask and allowed to stand for 5 min for complete diazotization. Then, 0.2 mL of sodium hydroxide 0.50 mol L^{-1} and 1.0 mL of 5.0 mmol. L^{-1} HQ were sequentially added and the contents were diluted to 10.0 mL using dionized water. After 5 min, the absorbance of the produced azo dye was measured at 532 nm against the reagent blank.

3. Results and discussion

The method involves the coupling between SD and HQ also CP and HQ in the presence of nitrite. The preliminary experiments showed that CP is not soluble in aqueous solutions, therefore, SDS was added to solubilize CP in the solutions. The absorption spectra of the azo dyes formed by SD and CP are given in Figure 1 and 2.

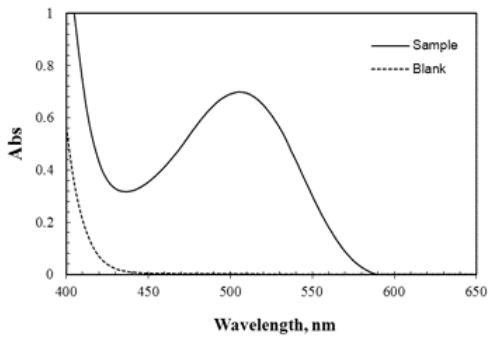


Figure 1. Absorbance spectra of blank and 10 µg nitrite containing sample solution.Condition: 10.0 mL solutions containing hydrochloric acid 0.01 mol L^{-1}, SD 1.0 mmol L^{-1}, HQ 4.0 mmol L^{-1} and sodium hydroxide 0.10 mol L^{-1}.

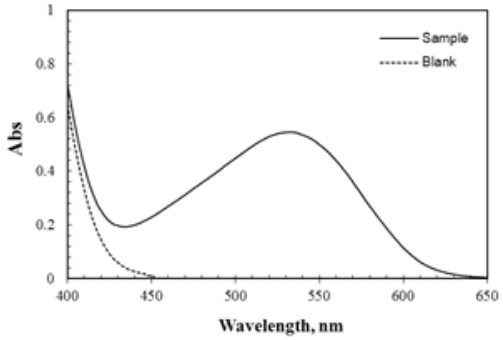


Figure 2. Absorbance spectra blank and 10 µg nitrite containing sample solution. Condition: 10.0 mL solutions containing hydrochloric acid 0.005 mol L^{-1}, CP 0.5 mmol L^{-1}, HQ 1.0 mmol L^{-1}, sodium hydroxide 0.10 mol L^{-1} and SDS 1.0% (wt/v).

The absorption maxima for the SD-HQ and CP-HQ systems are at 507 and 532 nm. The wavelengths were used throughout all of the experiments.

3.1. Optimization of parameters

For receiving the best sensitivity, optimization is necessary. The net absorbances of the formed azo dyes against their appropriate blanks were considered as the analytical signals. A one-at-a-time optimization procedure was evaluated for optimizing the affecting parameters.

All the experiments were performed at ambient temperature. The influence of hydrochloric acid concentration on the sensitivity of the proposed method was studied. The experimental results reveal that the absorbance increases by increasing hydrochloric acid up to 0.005 and 0.008 mol L^{-1} for SD-HQ and CP-HQ systems, respectively. The absorbances then decreases at higher concentrations of the acid.

The results are given in Figure 3. Increasing the hydrochloric acid concentration accelerate the production of diazonium cation and, consequently, increases the yield of the azo dye formation. The higher concentrations of hydrochloric acid will neutralize some amounts of sodium hydroxide; therefore lowers the efficiency of the coupling reactions with HQ. For the next studies, hydrochloric acid as 0.005 and 0.008 mol L^{-1} were selected for SD-HQ and CP-HQ systems, respectively. The diazonium cations are coupled fast and efficient in alkaline solutions with phenolic and naphtholic reagents [14]. To evaluate the influence of sodium hydroxide concentration on the sensitivity of the methods, sodium hydroxide concentration in the range of 0.00-0.16 mol L^{-1} was investigated and the procedures were followed. According to Figure . 4, it appeared that the best sensitivities were observed in 0.10 mol L^{-1} sodium hydroxide. Therefore, a 0.10 mol L^{-1} sodium hydroxide was accepted for both systems for the subsequent experiments.

In order to evaluate the effect of SD and CP concentrations on the sensitivity of the presented methods, their concentrations in the range of 0.0-1.3 mmol L^{-1} were studied and the procedures were followed. The obtained results showed that the sensitivities of the methods were maximum and constant for concentrations higher than 0.1 and 0.5 mmol L^{-1} for SD-HQ and CP-HQ systems, respectively. Therefore, 0.3 mmol L^{-1} SD and 0.7 mmol L^{-1} CP were used for the further experiments.

The influence SDS concentration on the absorbance of CP-HQ system was investigated in the range of 0.2-2.0%. The results showed that the sensitivity of the system increased by increasing the SDS concentration up to 1.4% and then remained constant. Therefore, SDS as 1.4% was used for further studies.

The effect of the diazotization reaction time was also studied in the range of 1-15 min according to the procedures. The results showed that the rate of the diazotization reactions were relatively fast, and the reactions were completed after 7 and 5 min for SD-HQ and CP-HQ systems, respectively. The time durations were selected for the diazotization reactions.

Figure 3. Effect of hydrochloric acid on the sensitivity of the procedures. Condition for SD-HQ system: 10.0 mL solutions containing 10 µg nitrite, SD 1.0 mmol L^{-1}, HQ 4.0 mmol L^{-1}, sodium hydroxide 0.04 mol L^{-1}.Condition for CP-HQ system: 10.0 mL solutions containing 10 µg nitrite, CP 0.5 mmol L^{-1}, HQ 1.0 mmol L^{-1}, sodium hydroxide 0.01 mol L^{-1} and SDS 1.0% (wt/v).

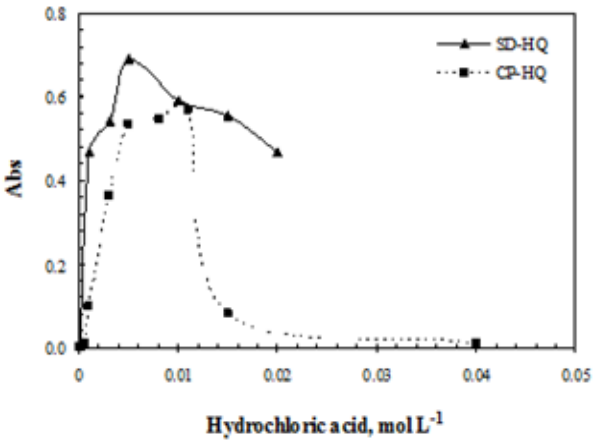
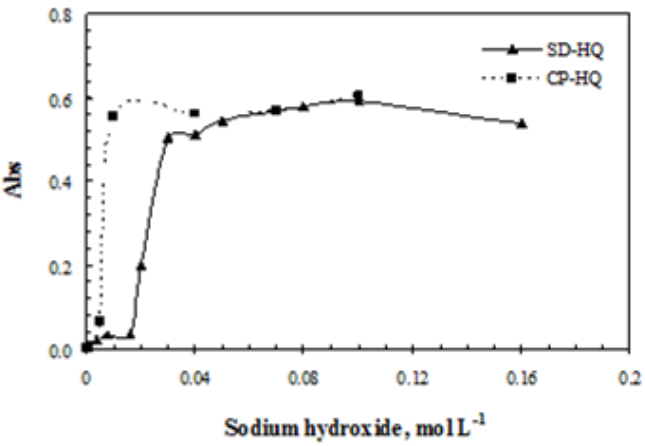


Figure 4. Effect of sodium hydroxide concentration on the sensitivity of the procedures. Condition for SD-HQ system: 10.0 mL solutions containing 10 µg nitrite, SD 1.0 mmol L^{-1}, HQ 4.0 mmol L^{-1}, hydrochloric acid 0.005 mol L^{-1}.Condition for CP-HQ system: 10.0 mL solutions containing 10 µg nitrite, CP 0.5 mmol L^{-1}, HQ 1.0 mmol L^{-1}, hydrochloric acid 0.008 mol L^{-1} and SDS 1.0% (wt/v).



In this step, HQ concentration was optimized. The same procedures were examined by adding various concentrations of HQ in the range of 0.00-0.03 mol L^{-1}. HQ concentration as 0.008 mol L^{-1}

showed maximum sensitivity for SD-HQ system, and the sensitivity of CP-HQ system was maximum and constant in the HQ concentration range of 0.001-0.030 mmol L^{-1}. For the subsequent studies, HQ concentrations as 0.008 and 0.005 mol L^{-1} were selected for SD-HQ and CP-HQ systems, respectively. The effect of the coupling reaction times were also studied in the range of 1-15 min according to the procedures. The results reveal that the rate of the coupling reactions were fast, and the reactions were completed after 2 min. Therefore, a 2 min coupling time was selected for both SD-HQ and CP-HQ systems. The effect of ionic strength on the determination of nitrite was investigated. Various concentrations of sodium chloride in the range of 0.0-1.6 mol L^{-1} were tolerated, but no considerable differences in sensitivity of the systems were observed.

3.2. Analytical characteristics

The optimal conditions were used and dependency of the absorbances to nitrite concentrations was evaluated. The calibration graphs were constructed at 507 and 532 nm for SD-HQ and CP-HQ systems, respectively. The calibrations equations were Abs = 0.607×Cnitrite-0.016 (R2=0.9993) for SD-HQ system and Abs = 0.620×Cnitrite-0.012 (R2=0.9997) for CP-HQ system, respectively in the ranges of 0.05-2.50 and 0.04-5.00 mg L^{-1} nitrite. Accuracy and precision of the procedures were also evaluated. For SD-HQ system, nitrite concentrations as 0.6 and 2.0 mg L^{-1} were tested. The recovery and RSD percents of the SD-HQ system were 103.3 and 0.3 and 100.0 and 0.9 (n=6), respectively for nitrite concentrations as 0.6 and 2.0 mg L^{-1}. The CP-HQ also showed recovery and RSD percents as 105.0 and 0.8 and 100.8 and 0.1, respectively for nitrite concentrations as 0.2 and 4.0 (n=6). The obtained limit of detections (LODs) were 4 (for SD-HQ system) and 7 µg L^{-1} (for CP- HQ system) for ten times blank analysis.

3.3. Effects of the potentially interfering ions

. To assess the possible analytical applications of the proposed procedures, the influences of concomitant ions on the determination of nitrite were studied by analyzing some synthetic samples. The individual samples were made with nitrite (0.5 mg L^{-1}) and one ion. The tolerance limit was defined as the concentration of every foreign ion where the ion caused an error in the range of ±5%. The tolerance limits for the foreign ions studied are shown in Table 1. The results showed that the most of the ions tested did not interfere with their concentrations up to at least 500-fold excess.

3.4. Applications

The effectiveness of the presented methods on the determination of nitrite and nitrate were evaluated for some water samples. Water samples were collected from various sources, filtered through filter paper (No.1 Whatman) and analyzed within 1 h of sampling. The standard addition of nitrite to the samples was also performed to show the accuracy of the developed procedures. A standard method [15] was also used to evaluate the results of the presented procedures. The results are given in Table 2 and 3. The nitrite and nitrate determination procedures were successful, according to the results obtained.

Table 1. Effect of foreign ions on the determination of nitrite.

Foreign ions	(wtion / wtNitrite)	
	SD-HQ	CP-HQ
Cl^-, SO_4^{2-}, NO^{3-}, $CH3COO^-$, Br-, SCN^-, CO_3^{2-}, I^-, IO^{3-}, H_2PO^{4-}, $C_2O_4^{2-}$, NH_4^+, Cr(VI), Al(III), Mo(VI), K(I), Ca(II), Pb(II),	500[a]	500[a]
Hg(II), V(III), Ag(I), Ni(II), Zn(II), Ba(II), Co(II)		500
Cd(II), Mg(II), Cu(II)		500[b]
Cr(III), Fe(III), Mo(VI)		200[b]
Cd(II), Ni(II), Cr(III), Hg(II), V(III), Mg(II)	200	
Ag(I)	100	
Ba(II), Zn(II), Co(II)	70	
Cu(II), Fe(III)	50	

[a]Maximum value tested.

[b]In the presence of 0.005 mol L^{-1} EDTA , 0.005 mol L^{-1} tartarat and 0.002 mol L^{-1} citrate.

Table 2. Determination of nitrite in some water matrices.

Samples	Nitrite added (mg L^{-1})	Nitrite found (mg L^{-1})	
		SD-HQ	CP-HQ
Tap water	0.50	0.50±0.01	0.50±0.01
Mineral water	0.50	0.50±0.01	0.50±0.03
Lake water	0.50	0.50±0.01	0.49±0.01
River water	0.50	0.50±0.01	0.50±0.04

Table 3. Determination of nitrate in some water samples.

Samples	Standard method (mg L^{-1})	Nitrite found (mg L^{-1})	
		SD-HQ	CP-HQ
Tap water	3.21±0.02		3.32±0.08
Mineral water 1	1.40±0.05		1.50±0.17
Mineral water 2	2.51±0.12		2.54±0.11
Mineral water 3	2.29±0.02	2.29±0.01	
Mineral water 4	4.85±0.25	4.86±0.08	
River water	1.43±0.04	1.43±0.02	

4. Conclusion

Two new simple spectrophotometric determination procedures for nitrite and nitrate were developed based on the diazotization of sulfadimidine and cisapride. The procedures use 8-hydroxyquinoline as the coupling agent. Nitrate was reduced through a laboratory made Cd-Cu reducing column. The methods were sensitive and low-cost for the determination of nitrite and nitrate ions. As the procedure was free from the interference of diverse ions, it has been applied to analyze lower concentrations of nitrite and nitrate in water samples.

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اندازه گیری نیتریت و نیترات بوسیله تشکیل ۸-هیدروکسی کوئینولین بر پایه رنگهای آزو

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چکیده:

دو روش اسپکتروفتومتری حساس و گزینش پذیر برای اندازه گیری مقادیر ناچیز نیتریت و نیترات ارائه می گردد. دراین دو روش واکنش نیتریت با سولفادیمیدین (SD) و سیزاپراید (CP) در محیط اسیدی انجام شده، یون های دیازونیوم تشکیل شده و سپس این یون ها در محیط بازی با ۸-هیدروکسی کینولین (HQ) واکنش می دهند تا رنگ های آزو مربوطه تشکیل شوند. جذب محلول ها در طول موج های ۵۰۷ نانومتر برای محصول SD-HQ و در طول موج ۵۳۲ نانومتر برای محصول CP-HQ اندازه گیری شدند. منحنی های درجه بندی در محدوده های غلظتی ۲/۵-۰/۰۵ و ۵/۰-۰/۰۴ میلی گرم در لیتر برترتیب برای سیستم های SD-HQ و CP-HQ خطی بودند. انحراف استاندارد نسبی برای این روش های اندازه گیری نیتریت برترتیب برای سیستم های SD-HQ و CP-HQ، ۰/۹-۰/۳ و ۰/۸-۰/۱ درصد بدست آمدند. نیترات نیز پس از استفاده از ستون کاهنده کادمیوم-مس به روش های ارائه شده، اندازه گیری شد. مزاحمت های انواع یون های خارجی مورد بررسی قرار گرفتند و روش های اندازه گیری ارائه شده، با موفقیت برای اندازه گیری نیتریت و نیترات در نمونه های آبی مختلف بکار برده شدند.

کلمات کلیدی: نیتریت، نیترات، Sulfadimidine، Cisapride، اسپکتروفتومتری

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