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A novel fiber optic spectrophotometric determination of nitrite using Safranin O and cloud point extraction

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ABSTRACT

A novel fiber optic spectrophotometric method for nitrite determination in different samples is suggested, based on the reaction of nitrite with Safranin O in acidic medium to form a diazo-safranin, which is subsequently coupled with pyrogallol in alkaline medium to form a highly stable, red azo dye, followed by cloud point extraction (CPE) using a mixed micelle of a nonionic surfactant, Triton X-114, with an anionic surfactant, sodium dodecyl sulphate (SDS). The reaction and extraction conditions (e.g., acidity for diazotization and alkalinity for pyrogallol coupling, and other reagent concentrations, time, and tolerance to other ions) were optimized. Linearity was obeyed in a concentration range up to 230 $\mu g\,L^{-1}$, and the detection limit of the method is 0.5 $\mu g\,L^{-1}$ of nitrite ion. The molar absorptivity for nitrite of the Safranin–diazonium salt ($\varepsilon_{610\,nm} = 4 \times 10^3\,L\,mol^{-1}\,cm^{-1}$) existing in literature was greatly enhanced by pyrogallol coupling and CPE enrichment ($\varepsilon_{592\,nm} = 1.39 \times 10^5\,L\,mol^{-1}\,cm^{-1}$). The method was applied to the determination of nitrite in tap water, lake water and milk samples with an optimal preconcentration factor of 20.

1. Introduction

The determination of nitrite in environmental samples plays an important role in the monitoring of environmental pollution. Nitrite ion is one of the pollutants found in the atmosphere and natural water. Nitrites are sometimes used as preservative in the food industry and a corrosion inhibitor in industrial process water. Nitrite is an active intermediate in the nitrogen cycle, resulting from incomplete oxidation of ammonia or from reduction of nitrates. This ion is an important precursor of nitrosamines, which are potential carcinogens. It is an environmental concern due to its harmful effects to aquatic plants, animals and human health. Thus, its determination at $\mu g\,L^{-1}$ levels is important [1–4].

A number of techniques have been recently proposed in the literature for the determination of nitrite in water and food samples, such as high-performance liquid chromatography (HPLC) with post column photochemical reaction and chemiluminescence detection [5], ion-pair HPLC–ultraviolet (UV) [6], ion chromatography with UV–visible (vis) diode array detection [7], capillary electrophoresis with capacitatively coupled contactless conductivity detection [8] gas chromatography–mass spectrometry [9], electrochemical methods [10,11] and kinetic methods [12,13] spectrofluorimetric [14,15] and spectrophotometric methods [16,12,17–23]. Growing interest in the use of flow injection analysis (FIA) in conjunction with UV/vis spectrometric detection has been demonstrated in several papers. Generally, FI-spectrophotometric techniques are based on the diazotization of a suitable aromatic amine by acidified nitrite with subsequent coupling reaction providing a highly coloured azo chromophore from which the concentration of nitrite can be assessed [5]. The classical Griess method 1879 is based on this principle [4,24]. The control of such reactions and/or manifolds in FIA is still complicated, and the cost of instrumentation may be restrictive to many laboratories. Direct UV/vis spectrometry, though less sensitive, is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity, flexibility, and easy operation, often demanding low cost equipment. Most spectrophotometric methods for the determination of nitrite and nitrate in different kinds of samples are based on the classical Griess reaction [4,24].

Since direct UV–vis spectrophotometry has a limited sensitivity for nitrite, a preconcentration step is often required to improve the detection limit. Recently, preconcentration/matrix separation procedures such as liquid–liquid extraction [25], solid phase extraction [26,27], column preconcentration [28–31], microphase sorbent extraction [32] and cloud point extraction (CPE) [33,34] were applied. These not only provide an improvement in the detection limits, but also reduce interference from the matrix. CPE offers a convenient alternative to more conventional extraction systems. It has been successfully used in the preconcentration of divergent species such as metal ions, proteins and other

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biomaterials, or organic compounds of intensely differing polarity. Relevant literature provides a deeper discussion on the physical chemistry of CPE [35–39]. To date, only two CPE approaches have been reported in literature for the determination of nitrite ions. Afkhami et al. [33] described a CPE-spectrophotometric determination of nitrite, based on the color reaction of nitrite with *p*-nitroaniline in acidic medium in the presence of diphenylamine, followed by the micelle-mediated extraction of the azo product. Afkhami et al. [34] presented a CPE-spectrophotometric methodology for the determination of hydroxylamine and nitrite in mixture in water and biological samples after the formation of the azo dye. The method was based on the combination of two well-known reactions: oxidation of hydroxylamine to nitrite, and nitrite determination with *N*,*N*-dimethylaniline and *p*-nitroaniline followed by micelle-mediated extraction of the produced azo dye.

Safranin O (3,7-diamino-2,8-dimethyl-5 phenylphenazinium chloride), is a phenazine dye that has been used as a photosensitizer in electron- and energy-transfer reactions. Safranin (also Safranin O or basic red 2) is widely used as a redox indicator in analytical chemistry. Safranin O has been used as an analytical reagent for the determination of nitrite [40–43]; in acidic medium, Safranin O reacted with nitrite to form a diazonium cation, which caused the change of the reddish-orange color of the dye solution to blue.

There has been considerable progress in recent years in the development of microfabricated systems for use in chemical and biological sciences. The microfabricated chemical measurement devices have several advantages over macroscopic systems, including design flexibility, size, cost, and sensitivity. Much development has been driven by a need to perform rapid measurements on small sample volumes [44–47]. The aim of this study is to propose a novel method for the fiber optic spectrophotometric determination of nitrite after a simple CPE preconcentration step to increase sensitivity and selectivity. The method is based on the color reaction of nitrite with Safranin O in the presence of pyrogallol in alkaline medium and mixed micelle-mediated extraction of the red azo product. A combination of a nonionic surfactant, Triton X-114, with an anionic surfactant, SDS, was used as extraction aid. In principle, trace levels of nitrate can also be determined by the same reaction following quantitative reduction to nitrite with a cadmium reductor column.

2. Experimental

2.1. Apparatus

Experiments were carried out using a commercially available miniature fiber-optic based spectrometer (Ocean Optics Inc., HR4000CG-UV-NIR), which utilises a small tungsten halogen lamp (Ocean Optics Inc.) as the light source and a charge-coupled device (CCD)-based detector for absorbance measurements (Fig. 1). The spectral resolution declared by the manufacturer was 0.02 nm. A thermostated bath maintained at the desired temperature was used for reaction (Hettich, Universal). The pH values of the solutions were measured by a Hanna HI 221 pH-meter using the full range of 0–14. Conical bottom disposable plastic centrifuge tubes (10 mL) made of clear and autoclavable polypropylene were used for phase separations. All glassware was rinsed carefully with 1:3 diluted HCl, followed by distilled water to prevent nitrate or nitrite contamination. Milk samples were defatted by centrifugation (Selecta Medifriger-BL, 10,000 rpm, 30 min, 4 °C)

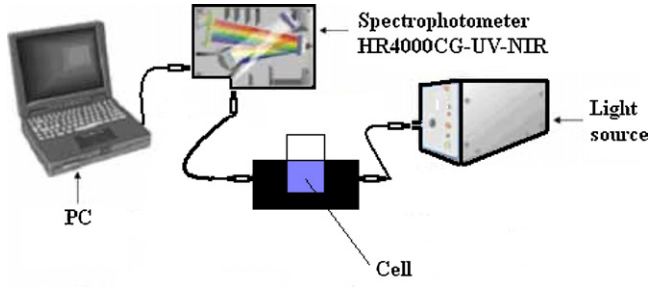

Fig. 1. Instrumental setup of the fiber optic spectrophotometric assay for nitrite.

2.2. Reagents

All chemicals used were analytical reagent grade and distilled water was used throughout the experiments. Stock nitrite solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving appropriate amount of its sodium salt (Merck) in water, and preserved with 2 mL chloroform. Safranin O (1.0×10^{-3} mol L^{-1}) solution was prepared in distilled water. A 1×10^{-3} mol L^{-1} coupling reagent solution (pyrogallol) was prepared in distilled water. A 5% Na_2CO_3 solution was prepared by dissolving 5.0 g of Na_2CO_3 in 100 mL-distilled water. A 3.0 mol L^{-1} HCl solution was prepared by appropriate dilution of concentrated HCl (Merck). Triton X-114 stock solution (5.0% w/v) was prepared from the concentrated solution (Merck) in distilled water. Working standards were prepared by appropriate dilution of the corresponding stock solutions. Aqueous metal ion solutions were prepared by dissolving appropriate quantities of reagent grade metal nitrate or chloride salts.

2.3. Sample preparation and storage

A sample taken for the determination of nitrite should be collected in glass or polyethylene bottles. Acids should never be used for sample preservation due to the decomposition tendency of nitrous acid into nitrogen oxides, i.e., disproportionation of HNO_2 into NO and NO_3^- [43]. The samples collected were then filtered through a 0.45 μm membrane filter and determined at once. The determination was conducted on fresh samples to prevent bacterial degradation.

2.3.1. Preparation of milk samples for analysis

For the preparation of milk samples for analysis, the elimination of fatty compounds is necessary, because lipids can affect the test results. Milk samples were defatted by cooling and centrifugation, and deproteinized with the addition of Carrez reagents, according to the procedures described by Bando et al. [48]. Briefly, commercial milk samples (10 mL) were defatted by centrifugation at 10,000 rpm and at 4 °C for 30 min. Then the sample was treated with Carrez reagents (zinc sulphate and potassium hexacyanoferrate(II)). A volume of 0.5 mL Carrez I (15%, w/v, $K_4Fe(CN)_6.3\ H_2O$) and 0.5 mL Carrez II (30%, w/v, $ZnSO_4.7H_2O$) were added [49], the mixture was shaken manually, and precipitated material was removed by centrifugation at 4000 rpm for 5 min. After the necessary operations, the pretreated sample was diluted to 10 mL with distilled water, and 2.5 mL was taken for analysis. The filtrate was stored at room temperature until being analysed for nitrite on the same day.

2.4. Recommended procedure

An aliquot of the solution containing 2–230 μg L^{-1} of nitrite, 1.0 mL of 2 mol L^{-1} HCl solution and 0.5 mL of Safranin O solution was transferred into a 10-mL tube. The solution was diluted to approximately 5 mL with water and allowed to stand for 5 min at room temperature for completion of the diazotization reaction. Then, 1.0 mL of 1.0×10^{-3} pyrogallol and 1.0 mL of 5% Na_2CO_3 solution were added to form an azo dye. Thus, the diazonium salt of the

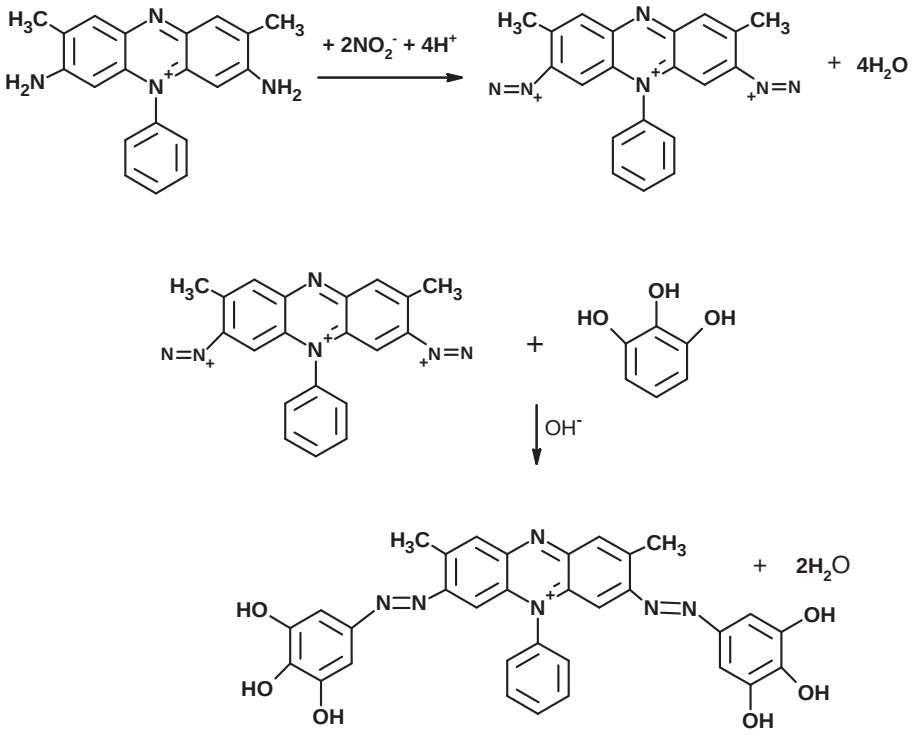

Fig. 2. Schematic representation of the coupling reaction.

dye was formed in acidic medium while the pyrogallol-coupled product in alkaline medium. The sample was shaken and left to stand for 10 min to allow the coupling reaction to go to completion. Then, 0.5 mL of 5% (w/v) Triton X-114, 0.5 mL of 2.0% (w/v) SDS and 0.5 mL of 10% (w/v) NaCl were added, made up to the mark with water, and the mixture was shaken. Separation of the two phases was achieved by centrifugation for 5 min at 4000 rpm. The mixture was cooled in an ice bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily decanted or pipetted. The micellar extract was collected, diluted with 0.3 mL of acetonitrile, and transferred into a 0.5-mL quartz cell to measure its absorbance at 576 nm against a reagent blank.

3. Results and discussion

3.1. Spectral features

Gonçalves et al. [43] reported a spectrophotometric method for the determination of nitrite using safranin as color reagent. The reaction between nitrite and safranin produces a safranin–HNO_2 species, which exhibits absorption peaks at 280, 349, 420 (shoulder) and 610 nm. Lambert–Beer's law was obeyed in the concentration range 7.0×10^{-6}–5.0×10^{-5} M. For the diazotization process, it would be expected that the two open NH_2 groups in Safranin O would be readily diazotized in a HCl medium, and that each diazonium group would then react with a molecule of pyrogallol by electrophilic substitution at *p*-position to produce an intense red azo dye. The dye showed an absorption maximum at 576 nm. According to Venkataraman [50] the second amino group of Safranin O requires special techniques for its diazotization, such as diazotization in concentrated sulfuric acid solution. Hence, diazonium salt, with a remaining open amino group, is capable of acid coupling with other molecules. A schematic representation of this reaction is shown in Fig. 2. An investigation using Job's method of continuous variations for the reactants showed that diazotized Safranin O reacts with pyrogallol at the stoichiometric ratio of 1:2. Similar results have been observed with the mole-ratio method. Fig. 3 shows the absorption spectra of Safranin O diazonium cation formed with nitrite (c), and coupled to pyrogallol in alkaline medium (a) and in acidic medium (b), showing

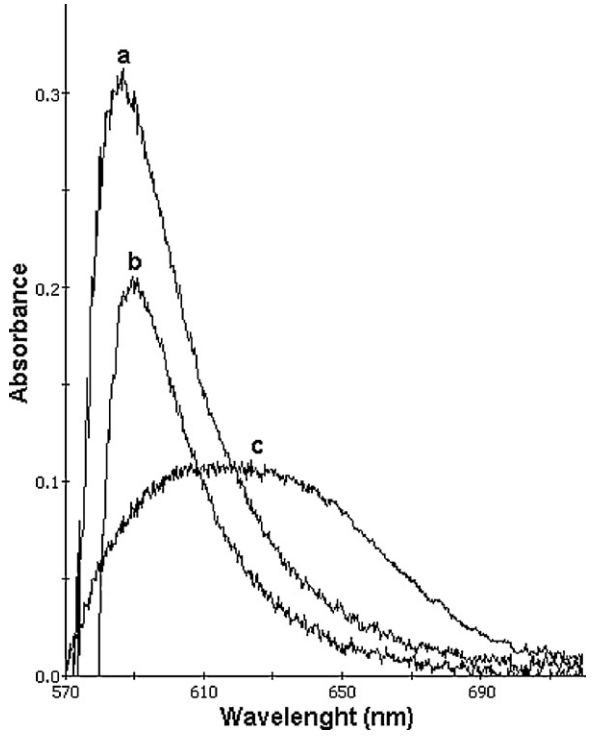

Fig. 3. Absorption spectrum of red azo-dye produced from Safranin O diazonium cation (nitrite: 138 $\mu g\,L^{-1}$) and pyrogallol (a) in alkaline medium and (b) in acidic medium. (c) Absorption spectrum of Safranin O diazonium cation (without coupling).

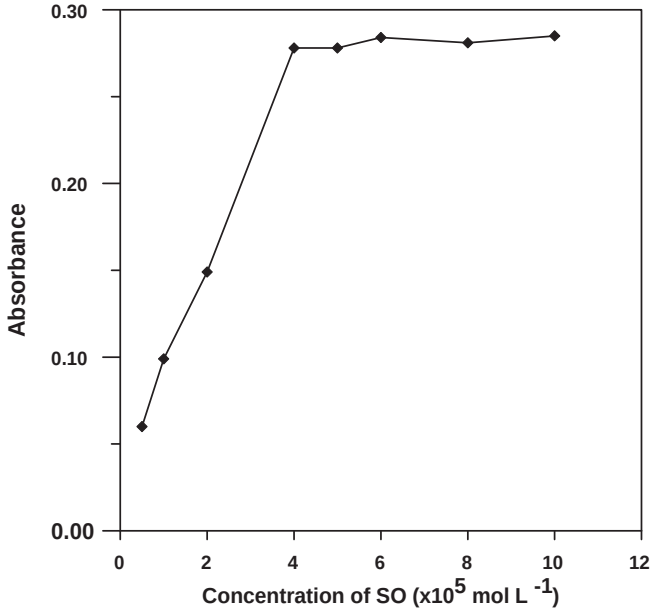

Fig. 4. Effect of Safranin O concentration on the CPE of nitrite (92 μg L^{-1}). Extraction conditions: 1.0 mL of 2.0 mol L^{-1} HCl, 0.5 mL of 1.0×10^{-3} mol L^{-1} Safranin O, 0.5 mL of 1.0×10^{-3} mol L^{-1} pyrogallol, 0.5 mL of 5% (w/v) Triton X-114, 0.5 mL of 2.0% (w/v) SDS and 0.5 mL of 10% (w/v) NaCl.

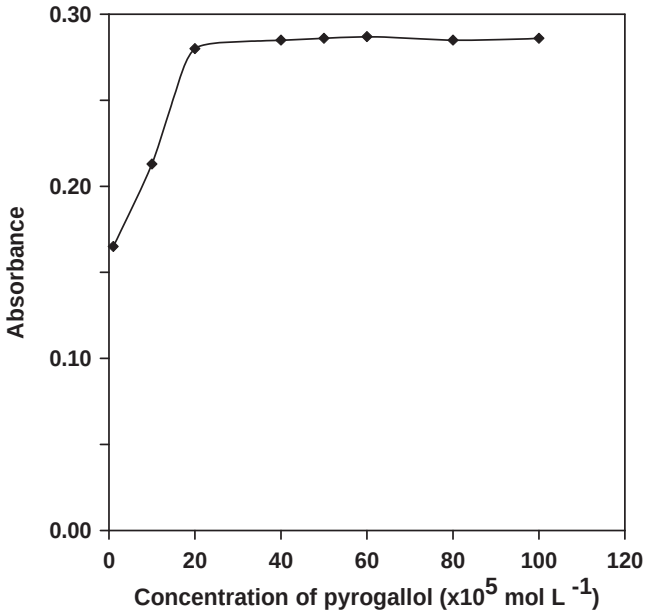

Fig. 5. Effect of pyrogallol concentration on the extraction of nitrite ion (92 μg L^{-1}). Extraction conditions: 1.0 mL of 2.0 mol L^{-1} HCl, 0.5 mL of 1.0×10^{-3} mol L^{-1} Safranin O, 0.5 mL of 5% (w/v) Triton X-114, 0.5 mL of 2.0% (w/v) SDS and 0.5 mL of 10% (w/v) NaCl.

significant sensitivity enhancement with pyrogallol coupling in alkaline medium. It can be seen that sensitivity is enhanced with pyrogallol coupling, producing a stronger intensity band at a much lower concentration of nitrite.

3.2. Effect of Safranin concentration

In the study of nitrite CPE behaviour, the variation of absorbance signal with reagent (Safranin O) concentration in the range 5.0×10^{-6}–1.0×10^{-4} is shown in Fig. 4, where nitrite extraction reached a maximum plateau at a reagent concentration of 4.0×10^{-5} mol L^{-1}. A Safranin O concentration of 5.0×10^{-5} mol L^{-1} was chosen to account for other extractable species that might potentially interfere with nitrite determination.

3.3. Effect of acidity on diazotization

As shown in Fig. 2, pyrogallol was bonded to Safranin O by using a two-step reaction, namely diazotization and coupling. The diazotization reaction takes place in acidic medium, and diazonium salt is produced by reaction of nitrous acid ($NaNO_2$ + HCl) with the amino group of Safranin dye. This diazonium salt can then be coupled to pyrogallol. The effect of acidity on the diazotization reaction was studied in the range 0.1–1.0 M HCl, and constant absorbance is observed in this range. Therefore, diazotization is carried out at room temperature, and optimum acidity for the formation of diazonium chloride is fixed at 0.2 M.

3.4. Effect of sodium carbonate on coupling

The coupling reactions are carried out sometimes in acidic and sometimes in alkaline media. However, when a phenolic compound (α- and β-naphthol, phenol, resorcinol, *o*,*m*-cresol and pyrogallol) is used for coupling, the medium should be adjusted to alkaline range after diazotization [50]. Pyrogallol was selected as a coupling agent in this study. The effect of Na_2CO_3 concentration on the absorbance was studied; volumes from 0.5 to 2.0 mL of 5% (w/v) Na_2CO_3 solutions were examined. The investigations showed that 1–1.5 mL of Na_2CO_3 gave maximum absorbance and 1.0 mL was chosen for the procedure. Other alkaline (NaOH and aqueous NH_3) solutions were tested, but best results were obtained by using Na_2CO_3.

3.5. Effect of reaction time

This method involves the diazotization of Safranin O in acidic medium followed by the coupling with pyrogallol in alkaline medium to give a coloured dye. Effect of time on both reaction steps (i.e., diazotization and coupling), as well as on the CPE procedure, was investigated. The dependence of absorbance upon diazotization and coupling reaction time was studied within range of 1–15 min. Diazotization was very fast and completed within 5 min [37–40], and the coupling reaction was completed within 10 min at room temperature. It was also observed that a temperature of 25 °C is sufficient for nearly complete recovery of the nitrite.

3.6. Effect of coupling agent

With diazo-safranin coupling α- and β-naphthols gave blue colors, phenol, resorcinol and pyrogallol fairly red purples [51]. The effect of varying the concentration of coupling agent was studied using the proposed procedure. The absorbance of the solutions increased by increasing the pyrogallol concentration up to 2.0×10^{-5} mol L^{-1} and then remained constant at higher concentrations (Fig. 5). Therefore, an optimal concentration of 1.0×10^{-4} mol L^{-1} pyrogallol was applied in the proposed method. An excess of pyrogallol has no effect on color.

3.7. Effect of SDS and Triton X-114 concentration

The concentration of surfactant that is used in CPE is a critical factor. Both surfactants in the mixed micelle positively influenced nitrite extraction. Effect of SDS concentration (individually) on the extractive determination of nitrite was investigated in the range 0.02–0.2% (w/v) for a fixed level of Triton X-114. The absorbance of the surfactant-rich phase increased by increasing SDS concentration between 0.01% and 0.08% (w/v) and remained nearly constant

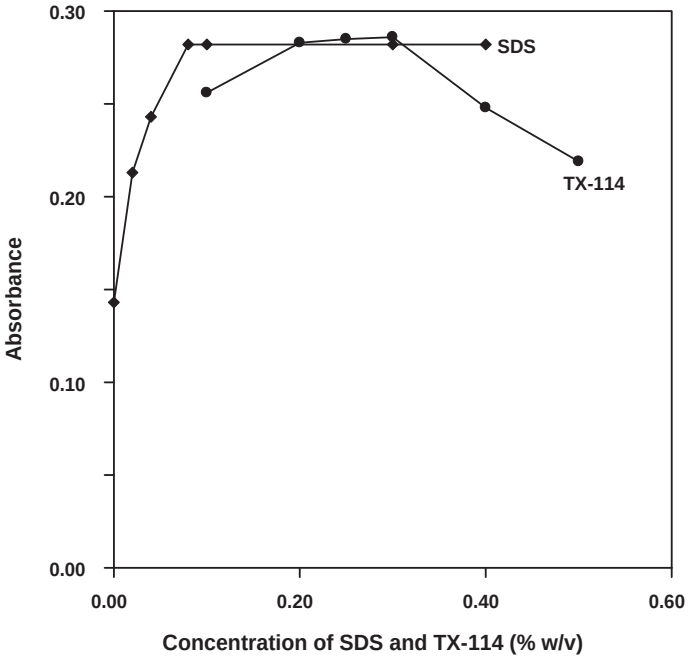

Fig. 6. Combined effect of SDS and TX-114 on the extraction of nitrite ion (92 μg L^{-1}). (The response to one surfactant of variable concentration was studied at a fixed concentration of the other, i.e., SDS: 0.1% and TX-114: 0.25%). Mutual extraction conditions: 1.0 mL of 2.0 mol L^{-1} HCl, 0.5 mL of 1.0×10^{-3} mol L^{-1} Safranin O, and 0.5 mL of 10% (w/v) NaCl.

at higher concentrations. Therefore, a 0.1% (w/v) SDS was used as optimum concentration. Without SDS, a low absorption signal was observed.

The variations in the analytical signal as a function of Triton X-114 concentration in the range of 0.1–0.5% (w/v) were investigated for a fixed level of SDS. Quantitative extraction was observed when Triton X-114 concentration was above 0.25%, whereas at lower concentrations, the absorbance was low probably due to the inadequacy of the micellar assemblies to entrap the hydrophobic complex quantitatively. The highest synergistic response was obtained when 0.1% SDS and 0.25% (w/v) Triton X-114 were used in combination (Fig. 6). With an increase of surfactant concentration above these values, the preconcentration efficiency decreased due to the increments in the volume of surfactant-rich phase.

3.8. Effect of NaCl concentration

The study was performed at the cloud point formation, which was induced by NaCl. The electrolyte effect on the cloud point from mixed nonionic–ionic surfactant system plays an important role. It was reported that the cloud point of Triton X-114 increased on adding small amounts of either anionic surfactant (SDS) or cationic surfactant (CTAB) [52]. However, when inorganic electrolytes were added to the Triton X-114 solution in the presence of SDS or CTAB, the cloud point decreased drastically [52]. The salt effect was studied by the addition of NaCl to the solution in the range of 0.1–2.0%(w/v). NaCl was found to quantitatively extract the safranin-pyrogallol dye from aqueous sample at NaCl concentrations above 0.5% (w/v), using single step extraction. It should be added that, in the conventional Safranin–diazonium salt assay for nitrite [43], chloride is an interferent, whereas in the proposed assay, it is an enhancer of nitrite extraction. A concentration of 0.5% NaCl was chosen for subsequent experiments.

Table 1
Effect of interfering ions on the determination of 40 μg L^{-1} nitrite ion.

Species	Tolerance ratio (ion/nitrite; w/w)
Al(III), Cd(II), Zn(II), Ni(II),	2000
Pb(II), Mn(II), Ca(II), Mg(II),	2000
Fe(III)[a], Co(II), Cu(II), Cr(III)	500

[a] In the presence of 0.5 mL of 1% (w/v) EDTA disodium salt.

3.9. Effect of centrifugation time

The coupling reaction was completed within 10 min at room temperature. The effect of centrifugation time (following coupling) upon extraction efficiency was examined in the range of 1–10 min at 4000 rpm for 5 min. Complete phase separation was achieved for time periods ≥5 min (optimal), and no appreciable improvements were observed for longer periods.

3.10. Effect of dilution agent

The volume of the surfactant-rich phase formed was too small (0.2 mL) to measure the absorbance directly in a conventional optical cell. Organic solvents could aid the transfer of the highly viscous surfactant-rich phase to a spectrophotometric cell. Among organic solvents comprising acetonitrile, ethanol, methanol and acetone, acetonitrile was found to yield the highest absorbance. At the cost of slightly reduced sensitivity, the lowest possible volume (0.3 mL) of acetonitrile was used for dilution of the surfactant-rich phase.

3.11. Analytical figures of merit

Because nitrite is extracted with almost 100% efficiency from 10 mL of sample solution, and measured after preconcentration by CPE to a final volume of 0.5 mL (0.2 mL surfactant rich phase + 0.3 mL acetonitrile), the solution was concentrated by a factor of 20. The regression equation of the analytical calibration curve obtained was $A = 0.0029C + 0.0031$, where A is the final absorbance (following CPE enrichment) at 592 nm and C is the initial NO_2^- concentration in μg L^{-1}, with a linear range of 2–230 μg L^{-1} NO_2^-. The calibration graph was linear with a correlation coefficient of 0.9984, and the relative standard deviation was 4.0% for nitrite (C = 40 μg L^{-1}). The limit of detection (LOD), defined as $C_L = 3S_B/m$ (where C_L, S_B, and m are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively),

Table 2
Determination of nitrite ion in real samples with CPE-spectrophotometric detection.

Sample	Proposed method		
	Added (μg L^{-1})	Found (μg L^{-1})	Recovery (%)
Lake water[a]	–	375 ± 1	–
	40	415 ± 1	100
	60	435 ± 2	100
Tap water	–	63 ± 1	–
	40	103 ± 2	100
	60	123 ± 2	100
Drinking water	–	3.2 ± 0.04	–
	4	7.2 ± 0.07	100
	6	9.2 ± 0.08	100
Milk sample A	–	< LOD	–
	5	5.0 ± 0.02	100
	10	10.1 ± 0.02	101
Milk sample B	–	< LOD	–
	5	5.1 ± 0.02	102
	10	10.1 ± 0.01	101

Table 3
Comparison of the performance of the proposed CPE-spectrophotometric method with those of other reported preconcentration methods.

Preconcentration method	Technique	Linear range($\mu g\,L^{-1}$)	Detection limit($\mu g\,L^{-1}$)	Reference
Liquid–liquid extraction	FAAS	100–2200	40	[25]
Solid-phase extraction	UV–vis	1–10	0.46	[26]
Solid-phase extraction	UV–vis	0–140	5	[27]
Column preconcentration	UV–vis	0.4–24	0.173	[28]
Column preconcentration	UV–vis	6.6–130	4.6	[29]
Column preconcentration	UV–vis	6.6–130	4.6	[30]
Column preconcentration	UV–vis	21.2–432	4.6	[31]
Micro-phase sorbent extraction	UV–vis	1.5–30	NR	[32]
Cloud-point extraction	UV–vis	2–40	0.87	[33]
Cloud point extraction	UV–vis	5.06–150	1.196	[34]
Proposed cloud point extraction	UV–vis	2–230	0.5	This method

was 0.5 $\mu g\,L^{-1}$, with a concomitant limit of quantification (LOQ) of 1.6 $\mu g\,L^{-1}$.

3.12. Effect of interfering species

The effects of possible interfering species, which commonly accompany nitrite in natural waters, were studied in the determination of 40 $\mu g\,L^{-1}$ of nitrite following the recommended procedure (Table 1). The present method is based on the oxidation of Safranin O with nitrite followed by coupling with phenols. Therefore strong oxidizing or reducing species are expected to interfere. The interferent cations were completely eliminated by chelation in the presence of 1.0×10^{-3} mol L^{-1} ethylenediaminetetraacetic acid (EDTA) disodium salt. The tolerance limit is defined as the concentration of added ion causing less than ±5% relative error for nitrite ion determination (Table 1).

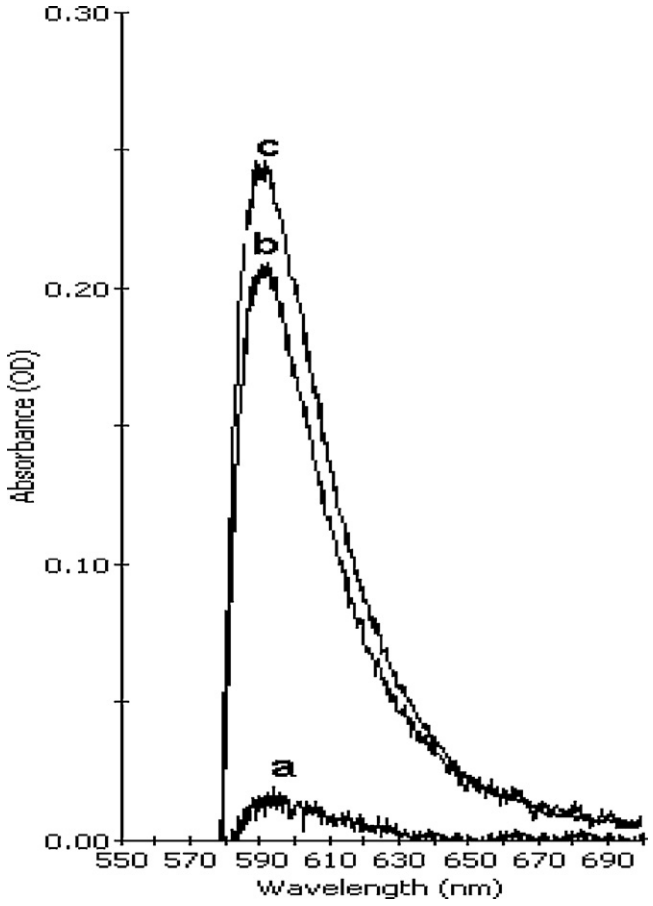

Fig. 7. Spectra of (a) milk sample, diluted to 10 mL after the necessary operations, and a 2.5-mL aliquot taken for analysis; (b) 2.09 μM nitrite standard (92 $\mu g\,L^{-1}$), (c) milk sample + 92 $\mu g\,L^{-1}$ nitrite standard, i.e., ($a+b$). Common extraction conditions for (a)–(c): 1.0 mL of 2.0 mol L^{-1} HCl, 0.5 mL of 1.0×10^{-3} mol L^{-1} Safranin O, 0.5 mL of 1.0×10^{-3} mol L^{-1} pyrogallol, 1 mL of 5% (w/v) Na_2CO_3, 0.5 mL of 5% (w/v) Triton X-114, 0.5 mL of 2.0% (w/v) SDS, and 0.5 mL of 10% (w/v) NaCl.

3.13. Applications

The method was applied to determination of nitrite in tap, lake water and milk samples (Table 2). The water samples are collected from different sources and are filtered before analysis. The recovery of added nitrite at different levels was quantitative (about 100%) for water and milk samples (Table 2). Typical spectra of a milk sample, nitrite standard, and standard-spiked milk sample are shown in Fig. 7.

4. Conclusion

The introduction of safranin and pyrogallol as new diazotization and coupling agents, respectively, provides good sensitivity and wide applicability to spectrophotometric nitrite assay. The reaction product, Safranin O azo dye, is very stable under the working conditions. The high tolerance limits for a relatively large number of foreign ions are the apparent advantages of the proposed method. In principle, nitrate-containing samples can be determined in the same way following quantitative reduction to nitrite. A comparison of the proposed method with the previously reported methods for preconcentration and spectrophotometric determination of nitrite (Table 3) indicates that it is competent in terms of sensitivity and selectivity [25–34]. The molar absorptivity for nitrite of the Safranin–diazonium salt ($\varepsilon_{610\,nm} = 4 \times 10^3$ L mol^{-1} cm^{-1}) existing in literature [43] was greatly enhanced by pyrogallol coupling and CPE enrichment ($\varepsilon_{592\,nm} = 1.39 \times 10^5$ L mol^{-1} cm^{-1}).

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