Artigo

DEVELOPMENT OF AN OPTICAL REDOX CHEMICAL SENSOR FOR NITRITE DETERMINATION

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An optical chemical sensor for the determination of nitrite based on incorporating methyltrioctylammonium chloride as an anionic exchanger on the triacetylcellulose polymer has been reported. The response of the sensor is based on the redox reaction between nitrite in aqueous solution and iodide adsorbed on sensing membrane using anion exchange phenomena. The sensing membrane reversibly responses to nitrite ion over the range of 6.52×10^{-6} – 8.70×10^{-5} mol L^{-1} with a detection limit of 6.05×10^{-7} mol L^{-1} (0.03 μg mL^{-1}) and response time of 6 min. The relative standard deviation for eight replicate measurements of 8.70×10^{-6} and 4.34×10^{-5} mol L^{-1} of nitrite was 4.4 and 2.5 %, respectively. The sensor was successfully applied for determination of nitrite in food, saliva and water samples.

Keywords: optical sensor; nitrite; methyltrioctylammonium chloride.

INTRODUCTION

Nitrite has long been recognised as a versatile preservative within meat products through its ability to inhibit microbial growth, which it provide color and taste of meat.[1,2] Consumed nitrite through foods or present nitrite in salivary can react readily with secondary and tertiary amines and amides within the food in the acidic environment of human stomach or other parts of the body to producing toxic and carcinogenic nitrosamines compounds.[3] Also, combination of nitrite with blood pigments reduce the oxygen carrying capacity of hemoglobin.[4,5] Moreover, nitrites can be used in fertilizers, detergent, wood pulp, dye and synthetic fiber industries which caused serious pollution problems.[6] Due to toxic effects of nitrite, it is important to develop specific, simple and low-cost methods for nitrite determination.

A number of methods have been described in the literature for nitrite determination, which include spectrofluorometry,[7,8] chromatography,[9,10] electrochemical detection,[11,12] capillary electrophoresis,[13,14] flow injection analysis.[15,16] Most of these methods are not suitable for routine determination of nitrite because some of them require expensive instruments or reagents, and others involve difficult and time-consuming separation procedures.

On the other hand, the most of the analytical methods which are being used for environmental problems generate chemical wastes. In some circumstances, the used chemical materials are even more hazardous than the species being monitored. However, it has drawn much attention recently focusing on the development of chemical processes that minimized or eliminate the use of toxic substances in the prevention of environmental pollution and human hazards.[17]

During the last decade, the development and construction of optical chemical sensors or optodes has increased considerably. They are more interested by analytical chemists because they are extensively employed in routine and research laboratories by the capability to decrease the reagent consumption. Other advantages include simple and low cost preparation, ease of miniaturization, possibility of remote, in situ monitoring and no need for separate reference devices.[18,19] These features make optochemical sensors as a powerful tool for environmental and industrial process monitoring.

The present paper reports on the development of an optical chemical sensor based on immobilization methyltrioctylammonium chloride onto a triacetylcellulose membrane and its application for the determination of nitrite using redox reaction with iodide.

EXPERIMENTAL

Apparatus

A GBC UV-Vis spectrophotometer model Cintra 101 was used for recording the spectra, and the absorbance measurements were made using a Jasco UV-Vis spectrophotometer model 7850. The sensing membrane was placed in a glass cell and all measurements were performed in a batch mode.

Measurement of pH was performed using a Metrohm 632 pH-meter with a combined glass electrode.

Reagents and solutions

All solutions were prepared using reagent grade chemicals and doubly distilled water.

Stock solution of nitrite, 2.17×10^{-2} mol L^{-1}, was prepared by dissolving 0.150 g of sodium nitrite (Merck, dried at 105 °C for 2 h before use) in 100 mL water. This nitrite solution was prepared every week and stored in dark. The working solution was prepared freshly by appropriate dilution. A 0.07 mol L^{-1} of iodide ion solution was prepared by dissolving 1.162 g of potassium iodide (Merck) in water and diluting to 100 mL in a volumetric flask. This solution was prepared freshly. Sulphuric acid solution was prepared by appropriate dilution of concentrated sulphuric acid (Merck).

Membrane preparation

The general procedure to prepare of sensing membrane was performed according to previously reported work.[20] For this purpose, the transparent triacetylcellulose (TAC) membranes were produced from waste photographic films that had been previously treated with commercial sodium hypochlorite in order to remove colored gelatinous layers. These membranes were placed in the solution containing 0.15 g methyltrioctylammonium chloride (Merck) and 10 mL ethylenediamine (Merck) for 12 min at ambient temperature. Then, they were washed with water for removing the additional reagent. After

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washing for removing the additional reagent, the optode membrane was stored under water.

Analytical procedure

The prepared membrane was placed in a solution of potassium iodide (0.07 mol L^{-1}) for 70 s, then it was washed and mounted into a spectrophotometer cell. A few mL of a solution containing nitrite ion and sulfuric acid was transferred to cell, and the absorbance was measured at 363 nm against a blank membrane after 6 min.

Preparation of real samples

Saliva and fresh water samples were collected and filtered through Whatman filter paper, stored at 5 °C and analyzed within 24 h.

Sausage sample was treated according to the method recommended by AOAC.[21] 2.00 g of sample was mixed and homogenized in a mortar. The thoroughly mixed sample was then taken in a 250 mL beaker and 40 mL of hot water was added and shaken for 5 min. The suspension was incubated for 15 min in a warm water bath at 50 °C. To precipitate the proteins, 5 mL of 20% zinc acetate was introduced. After cooling, the volume was diluted to 50 mL with water and then filtered through a Whatman filter paper. An aliquot of this solution was used for analytical procedure.

RESULTS AND DISCUSSION

It has been reported that the methyltrioctylammonium chloride (MTA^+Cl^-) immobilized on triacetylcellulose membrane shows an excellent tendency toward adsorption of anions.[22] Accordingly, an optical redox sensor was prepared by immobilization of methyltrioctylammonium chloride on the triacetylcellulose (TAC) membrane for determination of nitrite. The redox characteristics of membrane are achieved by exchanging the counter ion (Cl^-) of immobilized methyltrioctylammonium chloride with iodide ion. When this membrane was placed in acidic solution of nitrite ions, the colorless membrane changes to yellow. This change is due to oxidation of iodide to I_3^- by nitrite ions solution acidified with sulphuric acid.[23] Then the formed I_3^- adsorbed on membrane by an anionic exchange mechanism.

The following scheme presents the stages of preparation and response mechanism of proposed optical sensor.

$$TAC_{(mem.)} + MTA^+Cl^- \xrightarrow{\text{Ethylenediamine}} TAC - MTA^+Cl^-_{(mem.)} \quad (1)$$

$$TAC - MTA^+Cl^-_{(mem.)} + I^-_{(aq.)} \longrightarrow TAC - MTA^+I^-_{(mem.)} + Cl^-_{(aq.)} \quad (2)$$

$$\underset{\text{(colorless)}}{TAC\text{-}MTA^+I^-_{(mem.)}} + NO_2^-{}_{(aq.)} \xrightarrow{H^+} \underset{\text{(yellow)}}{TAC\text{-}MTA^+I_3^-{}_{(mem.)}} + NO \quad (3)$$

Figure 1 shows the characteristic spectra of the membrane which were obtained in the absence and presence of nitrite ion at different concentrations. An increase in the absorbance at maximum wavelength, 363 nm, appeared as the nitrite concentration increased. Therefore this wavelength was selected for measuring the absorbance of optode.

Study of parameters affecting the response

In order to facilitate the establishment of interaction between the membrane and the sample, a mass transfer of methyltrioctylammonium chloride into the triacetylcellulose membrane is required. The results indicated that the effective immobilization of

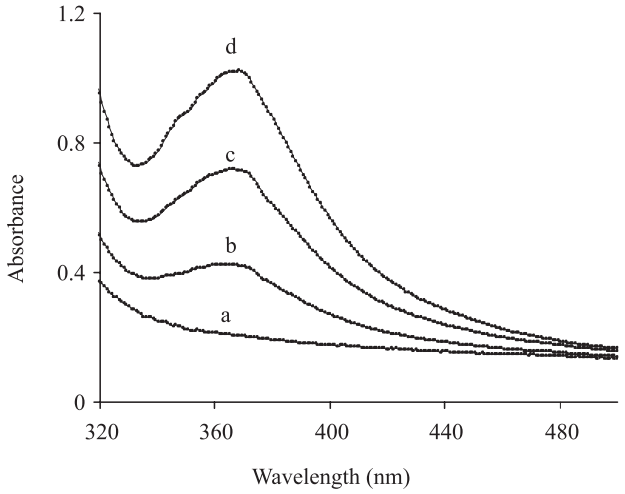

Figure 1. *Absorption spectra of optode (a) in the absence and (b-d) in the presence of 2.17 x 10^{-5}, 4.34 x 10^{-5} and 8.70 x 10^{-5} mol L^{-1} of nitrite, respectively*

methyltrioctylammonium chloride into membrane was obtained when using ethylenediamine as solvent. This is due to the hydrolyzed cellulose film in ethylendiamin shaped the porous structure in the polymer, which minimizes barriers of mass transport.[24,25]

To study the influence of membrane composition, several optodes with different amount of methyltrioctylammonium chloride in the range of 0.05-0.25 g were prepared.

The results denoted that the highest response was obtained when using 0.15 g of methyltrioctylammonium chloride. Also the resulting data showed that the response increased with preparation time of membrane, but above 15 min the membrane began to become opaque. Finally, a suitable optode with maximum response and higher reproducibility was prepared by treating transparent triacetylcellulose membrane with a solution of 0.15 g methyltrioctylammonium chloride in 10 mL ethylenediamine for 12 min.

The redox reaction of nitrite with iodide ion is affected by the acidity of solution. Thus the influence of sulfuric acid concentration of test solution on the response of the proposed nitrite optical sensor was studied. As can be seen from Figure 2, the response of the sensor increased by increasing acid concentration up to 0.07 mol L^{-1} while beyond this concentration, the optical response decreased. The diminished response could be due to instability of nitrite ion at high concentration of acid.[23] Thus a sulfuric acid concentration of 0.07 mol L^{-1} was selected in further studies.

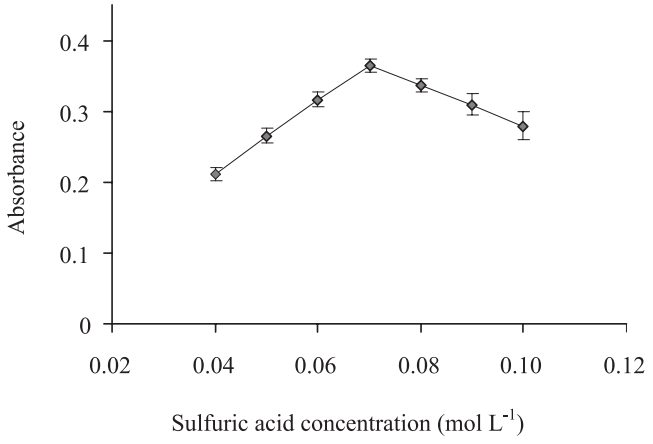

Figure 2. *Effect of sulfuric acid concentration on the optode response for solution containing 4.34 x 10^{-5} mol L^{-1} of nitrite, each point refers to the mean of triplicate measurements*

The effect of iodide concentration over the range of 0.04-0.10 mol L^{-1} on the absorbance of optical sensor was studied. The results shown in Figure 3 indicated that the maximum response was obtained at 0.06-0.08 mol L^{-1} of iodide ions and above this value the optical response decreased. This is due to saturation of the membrane at higher concentration of iodide resulting in a decrease adsorption of I_3^- on membrane. Therefore 0.07 mol L^{-1} of iodide ion was used throughout the study.

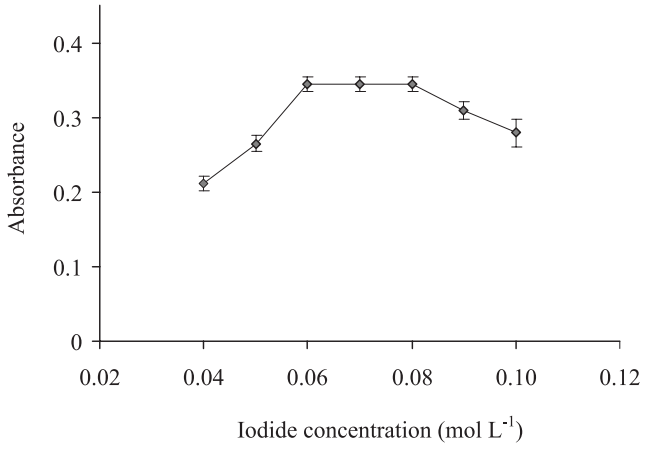

Figure 3. *Effect of iodide ion concentration on the optode response for the solution containing 4.34 x 10^{-5} mol L^{-1} of nitrite and 0.07 mol L^{-1} sulfuric acid, each point refers to the mean of triplicate measurements*

In another experiment, the presence time of membrane in iodide solution was also investigated. The results revealed that the response of the sensor is independent of the presence time of membrane in iodide solution in a range of 60-90 s, and higher than this time range the optical response reduced.

Response time of optode

The dynamic response time, which is an important characteristic for any membrane sensor, was studied by plotting absorbance versus time for different concentrations of nitrite in selected experimental conditions. As it is observed from Figure 4 the sensor response increased with time and not reached to steady-state response up to 16 min. Therefore in order to select an appropriate time for measuring the absorbance, the calibration curve for different times was studied. The results indicate that the calibration curve in the range of 6-10 min has the best sensitivity and correlation coefficient. Thus 6 min was selected as response time of optode.

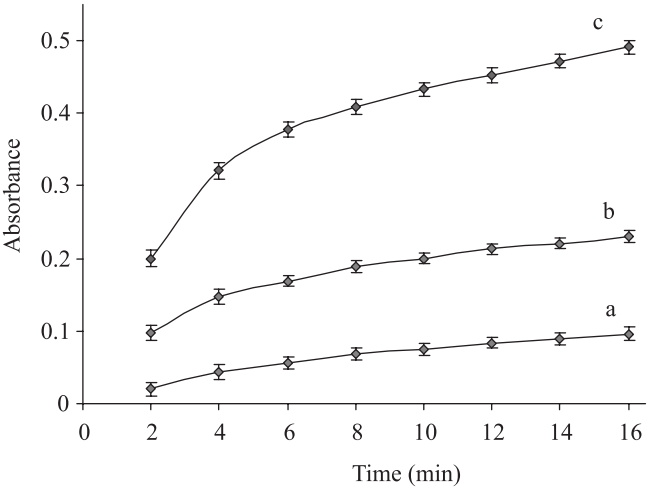

Figure 4. *Absorbance as a function of time when the concentration of nitrite as (a) 1.09 x 10^{-5}, (b) 2.17 x 10^{-5} and (c) 4.34 x 10^{-5} mol L^{-1}, each point refers to the mean of triplicate measurements*

Analytical figures of merit

Under the optimum experimental conditions, a calibration graph for nitrite was constructed by plotting absorbance values of sensing membrane as a function of the nitrite concentration. The calibration graph was linear in the range of 6.52 x 10^{-6}–8.70 x 10^{-5} mol L^{-1} (0.30-4.00 µg mL^{-1}) of nitrite and the equation using least square linear regression fit was A = 9.467 x 10^3 C (mol L^{-1}) - 0.045 with correlation coefficient (r) of 0.9994. The detection limit which was estimated as the concentration of nitrite producing an analytical signal equal to three times the standard deviation of the blank signal (n = 10) was found to be 6.05 x 10^{-7} mol L^{-1} (0.03 µg mL^{-1}) .

Regeneration and reproducibility of optical sensor

It would be desirable for a sensor to be reproducible. For this purpose the regeneration of optode was investigated using sodium sulfite, sodium thiosulfate and sodium hydroxide. The fully regeneration of sensor at a short time was observed by 0.1 mol L^{-1} sodium hydroxide less than 30 s.

The reproducibility is important characteristic in determining the suitability of an optical sensor for determination of the species of interest in different test solutions. For evaluating the reproducibility of optical sensor eight replicate measurements for 8.70 x 10^{-6} and 4.34 x 10^{-5} mol L^{-1} of nitrite solutions were performed using a single membrane. The relative standard deviation (RSD) for these determinations was 4.4 and 2.5%, respectively. The results show that the reproducibility is satisfactory and the membrane could be regenerated easily by using sodium hydroxide. The reproducibility between days (n = 4) and between different membranes (n = 4) was assayed by measuring the absorbance under optimum conditions for 4.34 x 10^{-5} mol L^{-1} of nitrite solution. The results showed that RSD value for these measurements was less than 3.5%.

Short-term stability and life time

In order to study of stability of the sensor the response of the membrane at 363 nm in contact with 4.34 x 10^{-5} mol L^{-1} of nitrite solution was recorded over a period of 12 h with an interval of 1 h. The mean absorbance value and standard deviation was found to be 0.378 ± 0.005 (n = 12). This indicated that the optode membrane has good short-term stability. Furthermore, the absorbance values of the optode membrane dropped about 5% during the measurement of sample solution over 1 month. However, prepared membranes were kept under water when not in use to prevent them from drying out.

Study of interferences

The effect of interferences on the determination of nitrite was investigated using the optimized optical membrane sensor system. For this purpose, various known amounts of ions were added to a sample containing 2.17 x 10^{-5} mol L^{-1} of nitrite and the general procedure was applied. The tolerance limit was defined as the maximum concentration of potentially interfering ions causing ± 5% error in the determination of nitrite. The results are shown in Table 1. The results indicate that except $S_2O_3^{2-}$ and SO_3^{2-} most of the cations and anions do not interfere in the analysis of nitrite under the experimental conditions.

Table 1. Effect of potentially interfering ions on determination of 2.17 x 10^{-5} mol L^{-1} of nitrite

Ions	Tolerance ratio ([M]/[NO_2^-])
NO_3^-, Na^+	1000
K^+ , PO_4^{3-} ,Cl^-	500
Mg^{2+} ,CO_3^{2-}	200
Mn^{2+}, Zn^{2+},Co^{2+}, Ni^{2+}, Ca^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, tartrate	100
Cu^{2+}, Cd^{2+}, Br^-, Citrate, Oxalate	50
$S_2O_3^{2-}$, SO_3^{2-}	0.5

Therefore, the present method had good selectivity and could be applied to the direct analysis of samples. The interfering of $S_2O_3^{2-}$ and SO_3^{2-} could be due to these reducing properties which prevent the formation of I_3^- and diminished sensor response to nitrite ion.

Application of the method

In order to evaluate the practical utility of the optode, nitrite was determined in several samples including sausage, saliva and water samples. Parallel determination was carried out to validate the recommended method with the standard method involving sulphanilamide and N-(1-naphthyl) ethylendiamine dihydrochloride.[21] The results obtained can be seen in Tables 2 and 3. The possibility of applying the present method was tested by determining the recovery of known amounts of nitrite ion added to the water samples. The good recoveries obtained showed that nitrite can be analysed in water with different composition.

The accuracy of the recommended method was statistically assessed by performing a t-test. As obvious from Tables 2 and 3 calculated | t | values were less than critical value at 95% confidence level, so there are no significant differences between the accuracy of the suggested method and the standard method.

Table 2. Determination results of nitrite in sausage and saliva samples and comparison with the results obtained by standard method

Sample	Concentration of nitrite found [a] (mol L^{-1})		Relative error %	t-test [b]
	Proposed method	Standard method		
Sausage	$8.83(\pm0.38)\times10^{-6}$	$8.91(\pm0.27)\times10^{-6}$	-0.9	0.34
Saliva	$5.31(\pm0.22)\times10^{-5}$	$5.58(\pm0.18)\times10^{-5}$	-4.8	1.91

[a] Mean ± standard deviation (n=4). [b] Critical value of $|t|$ for P value of 0.05 and six degrees of freedom is 2.45

CONCLUSION

This study has shown that the optode membrane containing methyltrioctylammonium chloride as an anionic exchanger can be used for the determination of nitrite ions contained in different aqueous matrices. The sensing mechanism is based on formation of I_3^- by redox reaction between iodide and nitrite in membrane interface. The optode described in this work shows a high selectivity for nitrite over other common ions except for $S_2O_3^{2-}$ and SO_3^{2-}. The main advantage of purposed sensor in comparison with previously reported optodes[26-28] (Table 4) is its simple and fast preparation of sensing membrane using low-cost materials. In addition it is fully reversible and has a long lifetime. The recommended method is non-toxic and safer than other methods where organic solvents are used. The obtained results revealed that, the purposed optical sensor is on par with the standard methods currently being used for the determination of nitrite in real samples.

Table 3. Determination results of nitrite in water samples and comparison with the results obtained by standard method

Sample	Nitrite added (mol L^{-1})	Proposed method		Standard method		t-test [b]
		Nitrite found [a] (mol L^{-1})	Recovery %	Nitrite found [a] (mol L^{-1})	Recovery %	
Tap	-	N.D[c]	-	N.D	-	-
	1.96×10^{-5}	$2.04(\pm0.12)\times10^{-5}$	104	$2.03(\pm0.12)\times10^{-5}$	103	0.12
Spring	-	N.D	-	N.D	-	-
	1.52×10^{-5}	$1.59(\pm0.12)\times10^{-5}$	105	$1.56(\pm0.07)\times10^{-5}$	103	0.43
Karun River	-	N.D	-	N.D	-	-
	1.09×10^{-5}	$1.13(\pm0.11)\times10^{-5}$	104	$1.12(\pm0.07)\times10^{-5}$	103	0.15

[a] Mean ± standard deviation (n=4). [b] Critical value of $|t|$ for P value of 0.05 and six degrees of freedom is 2.45. [c] Not Detected

Table 4. Comparison of proposed optode with previously reported optodes

Reagent	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Technique of sensing film preparation	Estimate of sensor preparation time	Ref.
RBOE [a]	5-5000	0.5	Entrapment of RBOE into PVC	30 min	26
BCB [b]	1.7×10^{-4} – 2.99	8×10^{-5}	Adsorption reagent on membrane	4 h	27
SFA & NEDA[c]	0.05 - 10	0.02	Inserting an optical fiber into a capillary tube	not reported	28
Gallocyanine	0.008–1.50	0.001	Binding reagent to a polymer	86 h	29
Safranine O	0.005–2.00	0.001	Binding reagent to a polymer	86 h	30
MTA^+Cl^- [d] , I^-	0.3-4.0	0.03	Adsorption reagent on membrane	12 min	This work

[a] Rhodamin B octadecyl ester perchlorate (RBOE). [b] Brilliant cresyl blue (BCB). [c] Sulfanilamide (SFA) & N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDA). [d] Methyltrioctylammonium chloride (MTA^+Cl^-)

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REFERENCES

1. Hassan, S. S. M.; Marzouk, S. A. M.; Sayour, H. E. M.; *Talanta* **2003**, *59*, 1237**.**
2. Yilmaz, Ü. T.; Somer, G.; *J. Electroanal. Chem.* **2008**, *624*, 59.
3. Helaleh, M. I. H.; Korenaga, T.; *J. Chromatogr., B: Anal Technol. Biomed Life Sci.* **2000**, *744*, 433.
4. Santos , W. J. R.; Lima , P. R.; Tanaka, A. A.; Tanaka, S. M. C. N.; Kubota, L. T.; *Food Chem.* **2009**, *113*, 1206.
5. Nagaraja, P.; Al-Tayar, N. G. S.; Shivakumar, A.; Shrestha, A. K.; Gowda, A. K.; *Spectrochim. Acta, Part A* **2010**, *75*, 1411.
6. Burakham, R.; Oshima, M.; Grudpan, K.; Motomizu, S.; *Talanta* **2004**, *64*, 1259.
7. Huang, K.; Wang, H.; Guo, Y.; Fan, R.; Zhang, H.; *Talanta* **2006**, *69*, 73.
8. Chen, T.; Tong, A.; Zhou, Y.; *Spectrochim. Acta, Part A* **2007**, *66*, 586.
9. Akyüz, M.; Ata, S.; *Talanta* **2009**, *79*, 900.
10. Kage, S.; Kudo, K.; Ikeda, N.; *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2000**, *742*, 363.
11. Kalimuthu, P.; John, S. A. ; *Electrochem. Commun.* **2009**, *11*, 1065.
12. Mori, V.; Bertotti , M.; *Talanta* **1998**, *47*, 651.
13. Barciela-Alonso, M. C.; Prego, R.; *Anal. Chim. Acta* **2000**, *416*, 21.
14. Gaspar, A.; Juhasz, P.; Bagyi, K.; *J. Chromatogr., A* **2005**, *1065*, 327.
15. Baeza, M.; Bartrol´I, J.; Alonso, J.; *Talanta* **2005**, *68*, 245.
16. Monser, L.; Sadok , S.; Greenway, G. M. ; Shah, I. ; Uglow, R. F. ; *Talanta* **2005**, *57*, 511.
17. Leelasattarathkul, T.; Liawruangrath, S.; Rayanakorn, M.; Liawruangrath, B.; Oungpipat, W.; Youngvises, N.; *Talanta* **2007**, *72*, 126.
18. Kalyan, Y.; Pandey, A. K.; Bhagat, P. R.; Acharya, R.; Natarajan, V.; Naidu, G. R. K.; Reddy, A. V. R.; *J. Hazard. Mater.* **2009**, *166*, 377.
19. Safavi, A.; Bagheri, M.; *Anal. Chim. Acta* **2005**, *530* , 55.
20. Rastegarzadeh, S.; Moradpour, Z.; *Instrum. Sci. Technol.* **2007**, *35*, 637.
21. AOAC; *Official Methods of Analysis,* Method 36.1.21, 16th ed., 1995**.**
22. Rastegarzadeh, S.; Pourreza, N.; Saeedi, I.; *Talanta* **2009**, *77*, 1032.
23. Vogel, A. I.; *Vogel's Qualitative Inorganic Analysis*, 7th ed., Revised by Svehla, G.; Pearson Education Ltd., 1996.
24. Jones, T. P.; Porter, M. D.; *Anal. Chem.* **1988**, *60*, 404.
25. Kostov, Y.; Tzonkov, S.; Yotova, L.; Krysteva, M.; *Anal. Chim. Acta* **1993**, *280*, 15.
26. Mohr, G. J.; Wolfbeis, O. S.; *Analyst* **1996**, *121*, 1489.
27. Ensafi, A. A.; Amini, M. ; *Sens. Actuators B* **2010**, *147*, 61.
28. Xiong, Y.; Zhu, D.; Duan, C.; Wang, J.; Guan, Y.; *Anal. Bioanal. Chem*. **2010**, *396*, 943.
29. Kazemzadeha, A.; Daghighi, S.; *Spectrochim. Acta, Part A* **2005**, *61*, 1871.
30. Ensafi, A. A.; Kazemzadeh, A.; *Microchem. J.* **2002**, *72*, 193.