

An optical sensor for zinc determination based on Zincon as sensing reagent

Saadat Rastegarzadeh*, Vida Rezaei

Department of Chemistry, College of Sciences, Shahid Chamran University, Ahvaz, Iran

Received 1 August 2006; received in revised form 4 August 2007; accepted 7 August 2007

Available online 11 August 2007

Abstract

An optical sensor has been designed for the determination of zinc by spectrophotometry. The sensing membrane is made by immobilizing Zincon as an ion pair with methyltriocetylammmonium ion on triacetylcellulose membrane. In the present of Zn^{2+} ions in ammonia/ammonium chloride buffer at pH 9 the color of the membrane changes from purple to blue. The calibration curve was linear in the range of 0.76–30.60 μM of Zn^{2+} ion with a limit of detection 0.16 μM (10.5 $ng\ ml^{-1}$). The response time of the optode was about 10–12 min, depending on the concentration of Zn^{2+} . The sensor can readily be regenerated with hydrochloric acid solution. This optode is stable and could be stored under water for three weeks without reagent leaching. The relative standard deviation for seven replicate measurements of 7.65 and 15.30 $\mu M\ Zn^{2+}$ was 3.09 and 2.82%, respectively. The sensor was successfully applied to the determination of zinc in powdered milk and hair samples.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Optical sensor; Zinc; Zincon; Triacetylcellulose; Methyltriocetylammmonium chloride

1. Introduction

Zinc is an essential element in nutrition of animals and human. It works with the enzymes that make genetic material, manufacture heme, digest food, metabolize carbohydrate, protein and fat, liberate vitamin A from storage in the liver and dispose of damaging free radicals. The zinc deficiency was marked by dwarfism or severe growth retardation, as well as, can result in metabolic changes such as impaired immune response, abnormal taste and abnormal dark adaptation. The foods with high in protein are abundant source of zinc, such as shellfish, meals and liver also milk which is a good source for infants.

Zinc is a relatively nontoxic element; however, it can be toxic if consumed in large enough quantities. A high zinc intake is known to produce copper-deficiency anemia by inducing the intestinal cells to synthesize large amounts of a protein that captures copper in a non-absorbable form [1]. Determination of zinc in excretive organ of the human body such as hair can reflect the cumulative status of this essential element in the body which evaluated nutrient status [2]. Therefore, there is an ongoing

need for a fast, simple, sensitive and economical method for the determination of zinc.

Over the past two decades the development and applications of optical chemical sensors (optodes) have grown rapidly which applied for determination of various analytes, including cations [3,4], anions [5,6], neutral [7,8] and gaseous [9,10] species.

Optical sensors required simple instrumentation and are suitable for multi-sensor array fabrication. In comparison with ion selective electrodes they do not require internal and external reference devices, long preconditioning time is not a prerequisite for use, and are not subjected to electrical noise [11]. A number of these optical sensors were prepared by immobilizing of lipophilic ligands as key component in a membrane [12,13]. The color complexing reactions between immobilized ligands and metal ions can determine its selectivity and, hence, its applicability in a given sample.

The immobilization of sensing reagents onto membranes is an important step in the development of optodes. The reagents are normally adsorbed onto, entrapped into or bounded to the supporting matrices by covalent bonds. Immobilization through adsorption can be done easily, but the reagents tend to leach from the supporting matrices. Different strategies have been pursued to slow down the process of leaching. Increasing the distribution coefficient of the components is so far the most successful

* Corresponding author. Fax: +98 611 3331042.

E-mail address: rastegarz@yahoo.com (S. Rastegarzadeh).

technique. For this reason, some highly lipophilic ligands were prepared and were indeed found to exhibit higher membrane lifetimes in optical membrane. Covalent immobilization is a method where the reagents are bound to insoluble carriers by covalent bonds. In this case, the reagents are bound tightly to the matrices, but have less freedom and the preparation procedure is complex [14].

Various polymeric membranes have been used as a supporting matrix for preparation of optodes. The rapid response results from the porous structure of the polymeric support, which minimizes barriers to mass transport between the analyte and immobilized indicator [15]. The hydrolyzed cellulose film is an example of these polymeric membranes which could be used for the preparation of optical sensors [15–18].

Zincon is a sensitive reagent for spectrophotometric determination of metallic ions [19]. Several optical sensors have been reported using this indicator by different immobilization methods. Oehme et al. presented a Cu^{2+} optode based on Zincon-tetraoctylammonium ion pair immobilized on a membrane consisted of a polyester support, an active layer composed of hydrogel, and a hydroxylic plasticizer. Because the layer irreversibly extracted copper from the solution, a kinetic approach has been applied for quantitation [20]. In another research Oehme et al. used Zincon as colorimetric reagent for a comparative study on the effect of different immobilization methods and matrix materials on the performance of an optical Cu^{2+} -sensitive membrane [21]. Kovacs and Nagy constructed an opto-electrochemical sensor utilizing a planar conductive indium-tin-oxide glass support, which was coated by plasticized polyurethane matrix containing Zincon as ion pair with tetraoctylammonium ion [22]. Cano-Raya et al. used Zincon in composition a disposable sensor for the measurement of Cu^{2+} based on a change in the fluorescence of porphyrine 2,7,12,17-tetra-*tert*-butyl-5,10,15,20-tetraaza-21H,23H-porphine (TP). This sensor was prepared by spin-coating a polyester support with a PVC solution containing TP, a plasticizer, Zincon and the ion-pairing benzetonium chloride [23]. The immobilization of Zincon on ion exchange resin was also reported. Liu et al. described a flow injection analysis system with an anion-exchange resin which presorbed by Zincon. This chelating agent-loaded resin was added into a flow through cell and has been applied to the direct determination of zinc in the hair of children [24].

In this paper, we described an optode for sensitive and selective determination of zinc. This optical chemical sensor is prepared by immobilizing Zincon-methyltriethylammonium ion pair as lipophilic sensing reagent on triacetylcellulose membrane according to a simple method.

2. Experimental

2.1. Apparatus

A Jasco UV–vis spectrophotometer model 7850 was used for recording the spectra, and the absorbance measurements were made using a Perkin-Elmer UV–vis spectrophotometer model

550S. The sensing membrane was placed in a disposable plastic 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.

2.2. Reagents and solutions

All reagents used were of analytical grade and were purchased from Merck and Sigma companies. Also double distilled water was used throughout.

A 1.2×10^{-2} M zinc stock solution was prepared by dissolving 0.785 g of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in exactly 200 ml of water and standardizing with EDTA [25]. Standard solutions were prepared by adequate dilution of the stock solution.

Buffer solution at pH 6 was prepared from acetic acid/sodium acetate, pH 7 and 11 from borax and pH 8–10 from ammonia/ammonium chloride [26].

2.3. Preparation of optical sensor

The following procedure for the preparation of sensor was performed in order to immobilize Zincon on triacetylcellulose membrane [17,27]. For this purpose, the transparent triacetylcellulose membrane was produced from waste photographic film that had been previously treated with commercial sodium hypochlorite in order to remove colored gelatinous layers. This clean and dry membrane was placed in the solution containing 0.01 g Zincon, 0.01 g methyltriethylammonium chloride and 5 ml ethylenediamine for 20–22 min at ambient temperature. Then it was washed with water for removing the additional reagents. The obtained membrane was stored under water when not in use.

2.4. Analytical procedure

An aliquot of Zn^{2+} solution and 2 ml of ammonia/ammonium chloride buffer at pH 9 were added to 25 ml volumetric flask and diluted to mark with water. A few ml of this solution was transferred to spectrophotometer cell which optical membrane was mounted into it. The cell was shaken and after 12 min the absorbance was measured at 645 nm.

2.5. Preparation of hair sample

Hair sample was washed with acetone and water to remove the surface contamination. Then 1 g of clean hair sample was accurately weighed and dry-ashed in furnace at 450°C . The residue was dissolved in nitric acid. The pH of the solution was adjusted to about 5 with sodium hydroxide and the solution diluted to 50 ml with water [2].

2.6. Preparation of powdered milk sample

Two grams of the sample was treated with 1 g of sodium hydroxide dissolved in a few ml of water and evaporated to dryness in a platinum dish. The residue was placed in a furnace at 500°C for 20 min. Then it was removed, cooled and 15 ml of

water and 2 ml of concentrated nitric acid were added to dissolve the residue [28]. The pH of the solution was adjusted to about 5 with sodium hydroxide and the solution diluted to 50 ml with water.

3. Results and discussion

3.1. Sensing reagent and spectral characteristics

Zincon is well known as sensitive spectrophotometric reagent for zinc [19]. Preliminary experiments denoted that Zincon was not immobilized effectively on triacetylcellulose membrane. We used methyltrioctylammonium chloride for the preparation of a lipophilic ion pair with Zincon in order to help immobilization of Zincon on membrane (Fig. 1). So addition of a little amount of methyltrioctylammonium chloride to solution of Zincon in ethylenediamine caused a change of membrane color to purple which indicated the adsorption of Zincon-methyltrioctylammonium ion pair on triacetylcellulose. The color of this membrane was changed to blue in the presence of Zn^{2+} solution containing ammonia/ammonium chloride buffer at pH 9.

The absorption spectra of Zincon in solution and immobilized on membrane in the presence of Zn^{2+} at pH 9 was investigated. The obtained results indicated that the maximum absorption wavelength of Zn-Zincon in membrane was 645 nm whereas for this complex in solution was 623 nm. So, 645 nm was selected for measuring the absorbance of optode in further studies.

3.2. Study of variables on the optode response

In order to achieve the highest sensitivity in the determination of zinc by purposed optode, the effect of various parameters on response of the optical sensor was investigated.

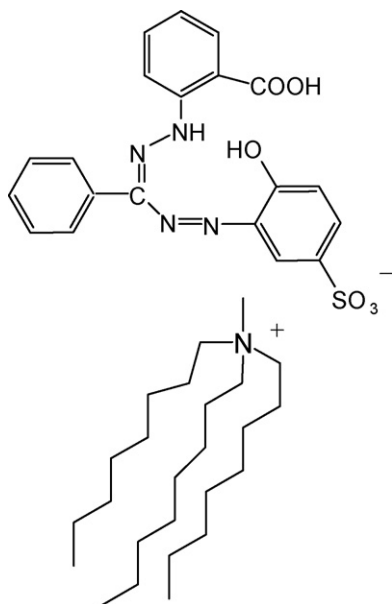


Fig. 1. Structure of Zincon-methyltrioctylammonium ion pair.

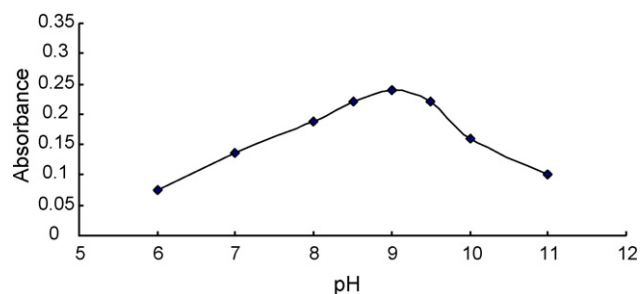


Fig. 2. Effect of pH on the optode response.

In preliminary experiments, the effect of variables on preparation of optode to provide a stable, sensitive and homogeneous membrane was studied. It was found that the reactivity of immobilized reagent could differ according to the solution that was formed. For this purpose, the influence of methyltrioctylammonium chloride/Zincon ratio (w/w) in sensor characteristics was investigated. The results denoted that the maximum response was obtained at ratio 1. In low and high amounts of methyltrioctylammonium chloride the immobilization of Zincon did not performed well. Therefore, the best optode was prepared by treating transparent triacetylcellulose membrane with a mixture of 0.01 g of Zincon and methyltrioctylammonium chloride in 5 ml ethylenediamine for 20–22 min.

One of the effective variables on the sensor response is pH of Zn^{2+} solution. For this means, the influence of pH over the range 6–11 on the response of optode was studied. The pH was adjusted by addition of appropriate buffer solutions. As it is obvious from Fig. 2 the maximum response was obtained in the presence of ammonia/ammonium chloride buffer at pH 9. The presented results were similar to reported studies on formation of Zn-Zincon complex in solution [2,29].

In another experiment the effect of electrolyte concentration on the sensor response in the range 0.05–1 M of sodium nitrate solution was investigated. The obtained results indicated that the change in ionic strength of Zn^{2+} solution had no effect on the absorbance of the purposed optode.

3.3. Regeneration of the optode

A good sensor should fully regenerate at short time. For this purpose, the effect of acetic acid/sodium acetate and formic acid/sodium formate buffers in pH range of 3–4, hydrochloric acid and EDTA were studied as regenerating reagent. The best result was obtained by hydrochloric acid. A solution of 0.01 M hydrochloric acid provided a short membrane regeneration time of lower than 1 min without any leaching the reagent. The membrane could be regenerated about 20 times and used without any loss of sensitivity.

3.4. Response characteristics

The absorbance response of the optode versus time in the selected experimental conditions for 7.65 and 15.30 μM of Zn^{2+} solutions is shown in Fig. 3. As can be seen, the proposed sensor was reached about 95% of the steady state

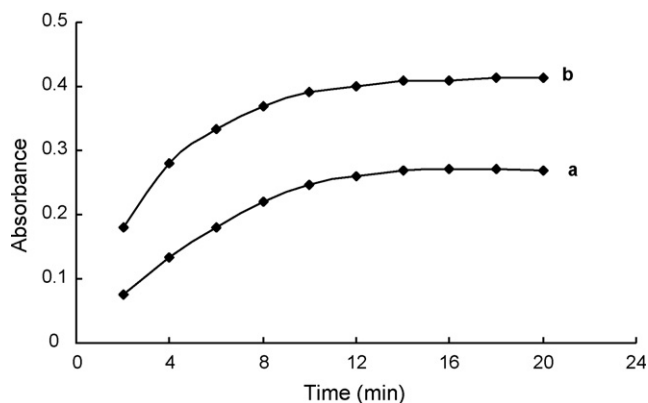


Fig. 3. Absorbance as a function of time when the concentration of Zn^{2+} was (a) 7.65 and (b) 15.30 μM .

response at 10–12 min, depending on the concentration of Zn^{2+} ions.

The calibration graph based on absorbance of the optode versus Zn^{2+} ion concentration was linear in the range of 0.76–30.60 μM with equation $A = 0.0182C + 0.1297$ and correlation coefficient (r) of 0.9996, where C is concentration of Zn^{2+} in μM and A is absorbance of optode at 645 nm.

The limit of detection based on three times the standard deviation of blank was 0.16 μM (10.5 $ng\ ml^{-1}$).

The precision using a single membrane was tested by performing seven replicate measurements for 7.65 and 15.30 μM of Zn^{2+} solutions. The relative standard deviation (R.S.D.) for these determinations was 3.09 and 2.82%, respectively. Between membranes reproducibility was evaluated by carrying out the same procedure on four membranes, the R.S.D. for the measurement of 15.30 μM Zn^{2+} was 3.50%.

In addition repeat measurements showed that the changes in the absorbance, after keeping the membrane in water for 3 weeks, were less than 4%. This result denoted that the optode is very stable, so it could be stored for more than a week without losing its characteristics.

3.5. Selectivity

The selectivity of the optode was studied for 7.65 μM of Zn^{2+} solution in the presence of different amount of foreign ions using the proposed method. The tolerance limit was set as the amount of foreign ion causing $\pm 5\%$ error in determination zinc. The results are summarized in Table 1. The interference of Cu^{2+} , Ni^{2+} and Mn^{2+} on the determination of Zn^{2+} was eliminated up

Table 1
Effect of interfering ions on determination of 7.65 μM Zn^{2+}

Foreign ions	Tolerance limit (M)
Al^{3+} , Ca^{2+} , CO_3^{2-} , SO_4^{2-} , Na^+ , PO_4^{3-} , NO_2^- , CN^- , SCN^- , Cr^{3+} , K^+ , Mg^{2+} , I^- , Cl^- , Br^- , BrO_3^- , IO_3^-	1000
Cd^{2+}	300
Pb^{2+} , Fe^{3+}	200
Cu^{2+} , Ni^{2+} , Mn^{2+}	20 ^a

^a Masked as discussed in Section 3.5.

Table 2
Determination of zinc in real sample

Sample	Zinc found ($\mu g\ g^{-1}$)		Relative error (%)
	Proposed method	AAS method	
Hair	163.9	162.4	0.9
Powdered milk	32.2	32.0	0.6

to 20 times using 0.1 M glycine for Cu^{2+} , 8×10^{-4} M cyanide ion for Ni^{2+} and 6×10^{-3} M oxalate ion for Mn^{2+} . The Fe^{3+} and Pb^{2+} ions precipitated under optimum conditions and were separated by centrifuge.

3.6. Analytical application

The presented optical sensor has been applied to the determination of zinc in hair and powdered milk samples under optimum conditions. For evaluating the accuracy of the method, a comparison between results obtained by proposed method and AAS was performed. As can be seen in Table 2, the results obtained for both methods have good agreements.

4. Conclusion

The basis of the work reported here is the development of an optical sensor for selective and sensitive determination of zinc based on immobilization of Zincon-methyltriocetylammmonium ion pair on triacetylcellulose. The purposed optode can be used for the determination of zinc in the range of 0.76–30.60 μM with a detection limit of 0.16 μM (10.5 $ng\ ml^{-1}$). In comparison with other methods [20–23] the sensing membrane preparation is simple, fast, uses low cost materials and the sensing layer is laid on both sides of triacetylcellulose membrane. In addition the presented optode is more selective and sensitive than other reported zinc optical sensors [30,31]. The proposed method could be widely applied to the determination of zinc in real sample with different compositions.

Acknowledgement

The authors are grateful to Shahid Chamran University Research Councils for financial support.

References

- [1] C.B. Cataldo, L.K. De Bruyne, E.N. Whitney (Eds.), Nutrition and Diet Therapy, fourth ed., West Publishing Company, Minneapolis, 1995, pp. 199–201.
- [2] R. Liu, D. Liu, A. Sun, G. Liu, Simultaneous determination of copper and zinc in the hair of children by pH gradient construction in a flow-injection system, Analyst 120 (1995) 569–572.
- [3] N. Pourreza, S. Rastegarzadeh, Optical test strip for spectrophotometric determination of palladium based on 5(*p*-dimethylaminobenzylidene)rhodanine reagent, Can. J. Anal. Sci. Spectrosc. 49 (2004) 314–319.
- [4] L.F. Capitan-Vallvey, C. Cano Raya, E. Lopez Lopez, M.D. Fernandez Ramos, Irreversible optical test strip for mercury determination based on neutral ionophore, Anal. Chim. Acta 524 (2004) 365–372.

- [5] J.A. Ortuno, M.I. Albero, M.S. Garcia, C. Sanchez-Pedreno, M.I. Garcia, R. Exposito, Flow-through bulk optode for the fluorimetric determination of perchlorate, *Talanta* 60 (2003) 563–569.
- [6] W.H. Chan, A.W.M. Lee, J.Z. Lu, Optode for the specific determination of anionic surfactants, *Anal. Chim. Acta* 361 (1998) 55–61.
- [7] H.H. Zeng, K.M. Wang, C.L. Liu, R.Q. Yu, A reversible optode membrane for picric acid based on the fluorescence quenching of pyrene, *Talanta* 40 (1993) 1569–1573.
- [8] R.H. Yang, K.M. Wang, D. Xiao, X.H. Yang, H.M. Li, A selective optical chemical sensor for the determination of Tween-60 based on fluorescence enhancement of tetraphenylporphyrin, *Anal. Chim. Acta* 404 (2000) 205–211.
- [9] A. Mills, A. Lepre, L. Wild, Effect of plasticizer-polymer compatibility on the response characteristics of optical thin CO₂ and O₂ sensing films, *Anal. Chim. Acta* 362 (1998) 193–202.
- [10] F.L. Alves, I.M. Raimundo Jr., I.F. Gimenez, O.L. Alves, An organopalladium-PVC membrane for sulphur dioxide optical sensing, *Sens. Actuators B* 107 (2005) 47–52.
- [11] I.H.A. Badr, A. Plata, P. Molina, M. Alajarin, A. Vidal, L.G. Bachas, Hydrogen sulfite optical sensor based on a lipophilic guanidinium ionophore, *Anal. Chim. Acta* 388 (1999) 63–69.
- [12] E.K. Paleologos, M.I. Prodromidis, D.L. Giokas, A.Ch. Pappas, M.I. Karayannis, Highly selective spectrophotometric determination of trace cobalt and development of a reagentless fiber-optic sensor, *Anal. Chim. Acta* 467 (2002) 205–215.
- [13] S.H. Kim, S.K. Han, S.H. Park, Sang M. Lee, Su M. Lee, K.N. Koh, S.W. Kang, Use of squarylium dyes as a sensing molecule in optical sensors for detection of metal ions, *Dyes Pigments* 41 (1999) 221–226.
- [14] X. Liu, W. Xing, G. Ou, J. Liang, A new reagent immobilization method for a portable fiber optic probe for determination of ferrous ions, *Anal. Sci.* 16 (2000) 473–476.
- [15] T.P. Jones, M.D. Porter, Optical pH sensor based on the chemical modification of a porous polymer film, *Anal. Chem.* 60 (1988) 404–406.
- [16] Y. Kostov, S. Tzonkov, L. Yotova, M. Krysteva, Membranes for optical pH sensors, *Anal. Chim. Acta* 280 (1993) 15–19.
- [17] A. Safavi, H. Abdollahi, Optical sensor for high pH values, *Anal. Chim. Acta* 367 (1998) 167–173.
- [18] M. Krysteva, M. Al Hallak, Optical enzyme sensor for urea determination via immobilized pH indicator and urease onto transparent membranes, *Scientific World J.* 3 (2003) 585–592.
- [19] Z. Holzbecher, L. Divis, M. Kral, L. Sucha, F. Vlácil, *Handbook of Organic Reagent in Inorganic Analysis*, Ellis Horwood Limited, Chichester, 1976, p. 421 and 711.
- [20] I. Oehme, B. Prokes, I. Murkovic, T. Werner, I. Klimant, O.S. Wolfbeis, LED-compatible copper(II)-selective optrode membrane based on lipophilized Zincon, *Fresenius' J. Anal. Chem.* 350 (1994) 563–567.
- [21] I. Oehme, S. Prattes, O.S. Wolfbeis, G.J. Mohr, The effect of polymeric supports and methods of immobilization on the performance of an optical copper(II)-sensitive membrane based on the colourimetric reagent Zincon, *Talanta* 47 (1998) 595–604.
- [22] B. Kovacs, G. Nagy, Opto-electrochemical planar wave-guide sensor for copper(II) ion, *J. Biochem. Biophys. Methods* 53 (2002) 177–187.
- [23] C. Cano-Raya, M.D. Fernandez-Ramos, L.F. Capitan-Vallvey, Fluorescence resonance energy transfer disposable sensor for copper(II), *Anal. Chim. Acta* 555 (2006) 299–307.
- [24] R.M. Liu, D.J. Liu, A.L. Sun, Application of ion-exchange-resin phase spectrophotometry to flow injection analysis system-I. Determination of trace zinc in hair of children, *Talanta* 40 (1993) 381–384.
- [25] A.I. Vogel, *A Text-Book of Quantitative Inorganic Analysis*, Longman, London, 1961, p. 433.
- [26] J.A. Dean, *Analytical Chemistry Handbook*, McGraw-Hill, New York, 1995, pp. 14.30–14.34.
- [27] A. Abbaspour, L. Baramakeh, Novel zirconium optical sensor based on immobilization of Alizarin Red S on a triacetylcellulose membrane by using principle component analysis artificial neural network, *Sens. Actuators B* 114 (2006) 950–956.
- [28] N. Pourreza, S. Kamran-Hekani, S. Rastegarzadeh, Kinetic-catalytic determination of trace amounts of Mn(II) based on a reduced phenolphthalein-periodate reaction, *Can. J. Anal. Sci. Spectrosc.* 43 (1998) 148–151.
- [29] P. Richter, M.I. Toral, A.E. Tapia, E. Fuenzalida, Flow injection photometric determination of zinc and copper with Zincon based on the variation of the stability of the complexes with pH, *Analyst* 122 (1997) 1045–1048.
- [30] B. Kuswandi, A.A. Vaughan, R. Narayanaswamy, Simple regression model using an optode for the simultaneous determination of zinc and cadmium mixtures in aqueous samples, *Anal. Sci.* 17 (2001) 181–186.
- [31] M.I. Albero, J.A. Ortuno, M.S. Garcia, C. Sanchez-Pedreno, R. Exposito, Determination of zinc(II) in pharmaceuticals based on a flow-through bulk optode, *J. Pharm. Biomed. Anal.* 29 (2002) 779–786.

Biographies

Saadat Rastegarzadeh received her MSc (1994) from Shiraz University and PhD (2002) from Shahid Chamran University in analytical chemistry. She is currently an assistant professor at the Department of Chemistry of the College of Sciences in Shahid Chamran University. Her main research interest is to study the optical sensors and their application to determination of essential elements in human life and pharmaceutical analysis.

Vida Rezaei received her MSc degree in analytical chemistry from Shahid Chamran University in 2006. Her interest field is optical sensors.