Full Paper

PVC Membrane and Coated Graphite Sensors for the Determination of Vitamin B1

Farnoush Faridbod,* and Tahereh Jamshidpour

Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran, Tehran, Iran

*Corresponding Author, Tel.: +98-21-61113813

E-Mail: faridbodf@khayam.ut.ac.ir

Abstract- Vitamin B1, also known as thiamine or thiamin or "thio-vitamine" is used by all living organisms. The compound is synthesized by bacteria, fungi, and plants, and hence animals have to obtain it from external sources. Vitamin B1 is an essential nutrient for human beings too. The present work focuses on the development of symmetric and asymmetric PVC membrane sensors for the selective determination of Thiamine. The membranes were prepared through the incorporation of thiamine-tetraphenyl borate ion-pairs, as the ionophore, in PVC membranes, based on different plasticizers (i.e. dibutyl phthalate (DBP), benzyl acetate (BA) and nitrobenzene (NB)). Based on the results of the optimization experiments, the best sensor performance was found in the case of DBP-based membranes. The optimal symmetric electrodes were found to produce fast and stable Nernstian responses from in thiamine solution in the concentration window of 5.0×10^{-6}-1.0×10^{-1} mol L^{-1}, while the asymmetric sensors (the graphite coated electrodes) led to linear responses in the range of 5.0×10^{-7} – 1.0×10^{-2} mol L^{-1}. The electrodes revealed pH independent responses in the pH range of 2.0-5.0. The validation of the methods, based on the application of the developed sensors, revealed the devices to be suitable for application in the analysis of the target species in pharmaceutical formulations.

1. INTRODUCTION

3-((4-Amino-2-methyl-5-pyrimidinyl)methyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride (Fig. 1), also known as thiamine or vitamin B1, is a co-enzyme which functions in its pyrophosphate (TPP) form also referred to as co-carboxylase, which takes part in a number of critical conversions in the metabolism of carbohydrates. Consequently, the level of thiamine required is a function of the amount of carbohydrates in the diet. Thiamine deficiency leads to impaired carbohydrate deficiency, which in turn causes 'biochemical lesions'. This deficiency can also lead to clinical conditions such as anorexia and neurological disorders, which can be followed by weakness, heart failure and death [1].
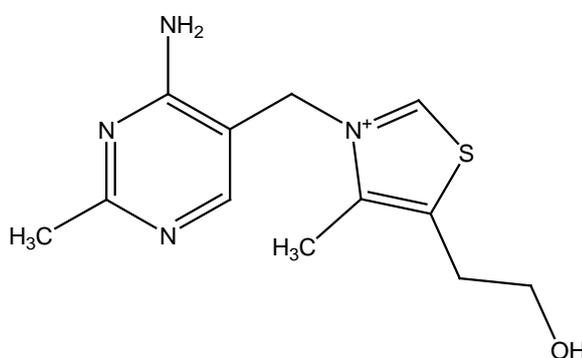

Fig. 1. Chemical structure of Thiamine

Among the analytical methods used for the determination of pharmaceuticals, electrochemical techniques play a key role due to their simplicity, high speed and accuracy as compared to other commonly used analytical techniques such as spectrophotometry and HPLC and other methods, which are sophisticated and time consuming, and require the application of high-tech expensive equipment, which might be unaffordable for many laboratories.

The concentration of thiamine has reportedly been determined through different chromatographic [2-5], spectroscopic [6] and electroanalytical [7,8] techniques.

To achieve better ionophore-based sensors the design of a robust membrane matrix is very important. In this light, and given the unique properties of polyvinyl chloride (PVC), specially the fact that the polymer can be plasticized using polar and non-polar water-immiscible solvents, PVC based membranes are very good candidates to this end [9-20].

The present work was started with the intention of developing a thiamine potentiometric membrane electrode using an ion-pair of thiamine and tetraphenyl borate as the selectophore. Then, the resulting sensors were used in the determination of thiamine in the pharmaceutical formulations.

2. EXPERIMENTAL SECTION

2.1. Apparatus

R684 Analion Ag/AgCl double junction electrodes were used as the internal and external references, and were connected to a Corning ion-analyzer with a 250 pH/mV meter with ±0.1 mV precision in the external part of the circuit.

2.2. Reagents

All chemicals were of analytical reagent grade and were used without any further purifications. High-molecular weight poly(vinylchloride) (PVC) (Fluka Co., USA), sodium tetraphenyl borate (NaTPB), dibutyl phthalate (DBP), nitrobenzene (NB), benzyl acetate (BA) and tetrahydrofuran (THF) (Merck Co., Germany), were used in the construction of the PVC membranes. Thiamine hydrochloride and its samples were from the courtesy of Jalinus Co. Tehran, Iran (a domestic pharmaceutical company).

2.3. Preparation of the sensing element

The thiamine-tetraphenyl borate ion-pair was prepared by mixing about 10 mL acidic solution of 0.01 mol L^{-1} thiamine and appropriate amount of sodium tetraphenyl borate solutions, let it precipitates, filtering off the result precipitate, washing and drying it. To be used in the membrane preparation.

2.4. Preparation of the PVC membrane and coated graphite electrodes

To prepare the general PVC membranes electrodes various quantities of the ion-pair were mixed with proper amounts of PVC, the plasticizer and an ionic additive. These components were dissolved in a small volume of tetrahydrofuran (THF) in a 2 cm diameter glass dish. Next, the mixture was left to lose some of its THF content to form a viscose oily mixture. Into this solution was dipped a plastic tube of about 4 mm o.d. for about 10 s. This way a translucent membrane of about 0.3 mm in thickness was formed at the tip of the tube, which was left to completely dry for about half a day. Then, the inside of the tube was filled with a 1.0×10^{-3} mol L^{-1} thiamine solution, as the internal filling solution. Eventually the so-prepared electrode was conditioned by being soaked in another volume of the 1.0×10^{-3} mol L^{-1} thiamine solution for 18 hours.

In case of coated graphite electrodes, spectroscopic grade graphite rods (2 and 10 mm in diameter and length) were used. First, the surface of the graphite rods were polished with a slurry of fine alumina spread on a polishing cloth. Then, the electrodes were sonicated in distilled water and dried in air, before the polished surface of the electrodes was dipped into a membrane prepared as described above, and left for the solvent to evaporate overnight. The electrodes were eventually conditioned by soaking in a 1.0×10^{-3} mol L^{-1} thiamine solution for one day.

2.5. Standard Thiamine solutions

Initially, a 0.1 mol L^{-1} solution of thiamine was prepared as the stock solution. This was done by dissolving 2.653 g of the pure compound in 100 mL of distilled water. This solution was used to prepare the other working standard solutions in the range of 1.0×10^{-7} to 1.0×10^{-2} mol L^{-1} through dilution .

2.6. Potential measurements

The cell assemblies as below were used for the potential measurements :

I: Ag-AgCl ‖ internal solution, 1.0×10^{-3} mol L^{-1} thiamine | PVC membrane | sample solution ‖ Ag-AgCl, KC1 (satd.)

II: Coated graphite-PVC membrane | sample solution ‖ Ag-AgCl, KC1 (satd.)

The sample concentrations were measured using the calibration curve method and with the standard solutions.

3. RESULTS AND DISCUSSION

Given that the sensitivity and selectivity behavior of the membrane electrodes based on ion-pairs, is highly dependent on the nature and amount of the membrane ingredients which should be carefully examined [21-25]. It is noteworthy that the characteristics of ion selective electrodes (ISEs) can be greatly modified through adjusting the relative amounts and proportions of the electrode membrane components, like the PVC matrix, the plasticizer and the ion-carrier.

3.1. Membrane composition

The results of membrane ingredient optimization is listed in Table 1. As can be seen, the 5% of the ion-pair reagent shows the better potential response. Also, addition of 3% an ionic additive to the membrane composition improve the sensor response. The role of the membrane solvent (plasticizer) is mainly to act as a fluidizer, which allows for the dissolution, mobility and diffusion of the ion-pair throughout the membrane. Like any other ingredient the nature and the amount of this component has a critical role in controlling the electrical asymmetry of the membrane, as well as the limit fouling of the sensor. It is a well-established fact that the nature of the plasticizer has a clear influence on the slope, linearity concentration window and the selectivity of membrane sensors [26-30]. To evaluate the effect of the nature of the solvents on the response of the sensor, dibutyl phthalate (DBP), nitrobenzene (NB) and benzyl acetate (BA), were tested and the results are given in Table 1.

Table 1. Various membrane ingredients used in making PVC membrane sensor

No.	%Ion pair	%PVC	% Plasticizer	% NaTPB	Linearity range (mol L^{-1})	Slope* (mV/decade)	R^2
1	4	30	66 DBP	0	$1.0\times10^{-5} - 1.0\times10^{-1}$	18±0.5	0.982
2	5	30	65 DPB	0	$1.0\times10^{-5} - 1.0\times10^{-1}$	26.9±0.6	0.991
3	6	30	64 DBP	0	$1.0\times10^{-5} - 1.0\times10^{-1}$	26.3±0.3	0.981
4	7	30	63 DBP	0	$1.0\times10^{-5} - 1.0\times10^{-1}$	17.5±0.4	0.990
5	5	30	64 DBP	1	$5.0\times10^{-6} - 1.0\times10^{-1}$	26.5±0.4	0.996
6	5	30	63 DBP	2	$5.0\times10^{-6} - 1.0\times10^{-1}$	28.3±0.6	0.991
7	5	30	62 DBP	3	$5.0\times10^{-6} - 1.0\times10^{-1}$	29.4±0.3	0.993
8	5	30	62 NB	3	$5.0\times10^{-5} - 1.0\times10^{-1}$	11.2±0.5	0.988
9	5	30	62 BA	3	$5.0\times10^{-5} - 1.0\times10^{-1}$	6.3±0.5	0.981
10	0	30	62 DBP	3	$5.0\times10^{-4} - 1.0\times10^{-2}$	4.4±0.6	0.983

* The results are based on five replicate measurements

The results showed that DBP, having a relatively lower dielectric constant than other two solvents tested was found to lead to better results in terms of the linearity range of the response and the detection limit and slope, and was hence as the optimal solvent due chosen to be employed in the construction of the sensor. These modified effects were attributed to the better extraction of thiamine into the organic layer of the membrane, in the presence of DBP.

In sum, based on the results membrane no. 7 (with a composition of 30% PVC, 5% ion-pair, 3% NaTPB and 62% DBP) was found to lead to the best results and was hence chosen as the optimum membrane composition.

Table 2, summarizes the characteristics of the membranes with various amounts of the ingredients for coated graphite electrode. The data in this table reveals that membranes with a composition according to no.6 (30% PVC, 5% ion pair, 63% DBP, and 2% NaTPB) led to the optimal Nernstian slope of 29.7 mV per decade concentration of thiamine over a very wide concentration window. The results in the table make it clear that the presence of 2% of NaTPB can lead to a Nernstian potential response. It has been demonstrated that the presence of such

negatively charged lipophilic additives, as NaTPB, can improve the potentiometric response of selective electrodes through reducing their Ohmic resistance and improving their selectivity and response behavior. Also in the case of ionophores with poor extraction capabilities, the sensitivity of the membrane electrodes, is enhanced as the result of the presence of the additives.

Table 1. Optimization of the membrane ingredients for the coated graphite electrode

No.	%Ion pair	%PVC	% Plasticizer	% NaTPB	Linearity range (mol L^{-1})	Slope* (mV/decade)	R^2
1	4	30	66 DBP	0	$1.0\times10^{-5} - 1.0\times10^{-2}$	21.5±0.5	0.945
2	5	30	65 DPB	0	$1.0\times10^{-6} - 1.0\times10^{-2}$	26.9±0.4	0.990
3	6	30	64 DBP	0	$1.0\times10^{-6} - 1.0\times10^{-2}$	21.7±0.7	0.981
4	7	30	63 DBP	0	$1.0\times10^{-6} - 1.0\times10^{-2}$	18.3±0.5	0.975
5	5	30	64 DBP	1	$5.0\times10^{-7} - 1.0\times10^{-2}$	28.8±0.8	0.987
6	5	30	63 DBP	2	$5.0\times10^{-7} - 1.0\times10^{-2}$	29.7±0.4	0.991
7	5	30	62 DBP	3	$5.0\times10^{-7} - 1.0\times10^{-2}$	29.3±0.4	0.987

* The results are based on five replicate measurements

3.2. Calibration curves

The range in which the calibration curve of the potentiometric sensors has been linear can be observed as the calibration graphs [31-37]. Measurements could be performed in this lower range, but noted that more closely spaced calibration points are required for more precise determinations. The slope of the calibration graph obtained for the PVC membrane electrode was 29.4 mV per decade of the thiamine concentration, with a standard deviation of ±0.3 mV for three replicate measurements (Fig. 2). In the case of the optimal coated graphite electrodes (CGEs), the potential responses towards the concentration of thiamine were found to be linear from 5.0×10^{-7}-1.0×10^{-2} mol L^{-1}, and the slope of the calibration graph equaled 29.7 mV per decade of thiamine concentration and a standard deviation of ±0.4 mV based on five replicate measurements (Fig. 3). Based on the calibration curves, the detection limits of the PVC electrodes and CGEs were estimated to be 7.8×10^{-6} mol L^{-1}and 4.1×10^{-7} mol L^{-1} respectively. This was done by extrapolating the two linear segments at the lower part of the calibration curves.
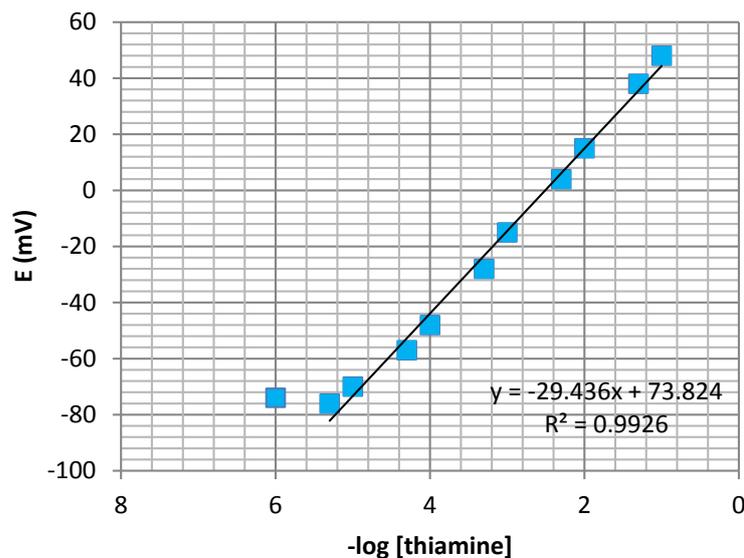
Fig. 2. Calibration curve of Thiamine-PVC membrane sensor; the results are based on 5 replicate measurements
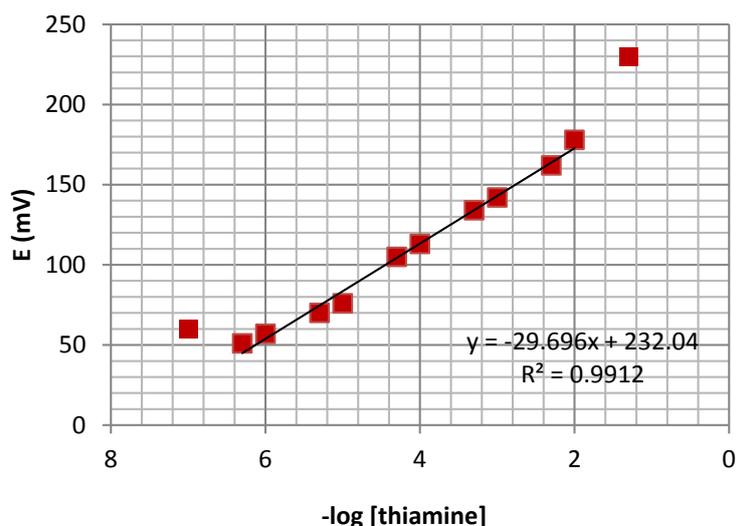

Fig. 3. Calibration curve of Thiamine-PVC membrane sensor; the results are based on 5 replicate measurements

In terms of the structure of the indicator electrode, potentiometric membrane sensors are classified in to two wide categories of symmetrical and asymmetrical ion-selective electrodes. Based on the definitions, symmetrical ISEs are the devices in which the selective membrane is between two solutions, while with asymmetrical ISEs the inner side of the membrane is actually in contact with a solid contact, while its other side is exposed to the test solution [11, 24]. Absence of internal filling solution in asymmetric electrodes improve the linear range and lower detection limit of the sensor. However, the upper detection limit (UDL) due to the saturation of the site in the small surface of the electrode causes the decrease in UDL.

3.3. pH study

To evaluate the influence of pH on the response of both electrodes, the potentials of the two devices were measured at a fixed thiamine concentration (1.0×10^{-3} mol L^{-1}), while changing the solution pH in the range of 1.0 to 7.0 through adding concentrated NaOH or HCl solutions. The observations revealed that the potential response of the both PVC and CGE sensors remained constant in the pH range of 2.0 to 5.0 (Fig 4). This indicates that the electrode is applicable to the analysis of the analytes in this pH window without expecting disturbances caused by the pH changes. The fluctuations in the response above pH=5.0 were attributed to the neutralization of the positive charge on the drug molecule above this pH, while below pH=2.0, the variations in the response were attributed to the removal of the ion-pair present in the membrane or the analyte in the solution.
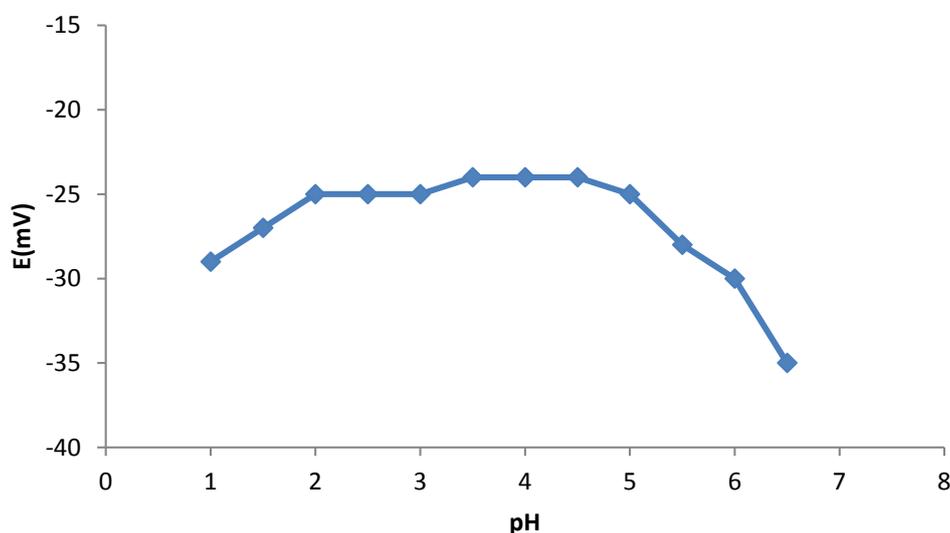

Fig. 4. pH effect on the potential response of the PME in a solution of Thiamine (1.0×10^{-3} mol L^{-1})

3.4. Dynamic Response Time

The dynamic response time of the sensors, which can be defined as the time required for and electrode to reach response values within ±1 mV of its final equilibrium potential, after successive immersions into solutions with 10 fold concentration differences, was also measured in this case. To this end, the time required for the sensors to reach the equilibrium potential values upon changes in the concentration of the test solutions, was measured. Both sensors were found to quickly reach their equilibrium responses in the whole concentration range, and the average time for the CGE and PVE sensors, in the whole concentration range, were found to be about 18 s and 20 s, respectively.

3.5. Selectivity

Selectivity, or in other terms an ion-selective electrode's specific tendency towards the target ion, as compared to other present interfering species can be considered as the most prominent characteristic of these devices. The selectivity coefficients of both sensors were evaluated by the matched potential method (MPM) and the resulting MPM [36,37] selectivity coefficients are shown in Table 3.

Table 2. Selectivity coefficients of the thiamine sensor for various interfering species

interference	-log K_{MPM}
Ca^{2+}	-3.91
Mg^{2+}	-3.38
K^+	-3.98
NH_4^+	-3.98
NO_3^-	-4.54
Vitamin B_{12}	-3.35

3.6. Real sample analysis

To show the applicability of the sensor in analysis of active ingredient of Thiamine in pharmaceutical formulations, both sensors were evaluated by being used in measuring the concentration of the Thiamine as analyte in some pharmaceutical Tablets. The results of real analysis has been presented in Table 4.

Table 4. Potentiometric determination of Thiamine in pharmaceutical formulations

Sample	Stated content (mg/tablet)	Found by PME*	Recovery for PME	Found by CGE*	Recovery for CGE
Sample 1	300	307.5±0.5	2.5 %	308.5±0.6	2.8 %
Sample 2	300	291.3±0.6	2.9 %	290.7±0.4	3.1 %
Sample 3	300	310.1±0.5	3.3 %	308.2±0.5	2.7 %

*The results are based on 5 replicate measurements.

As can be seen, both sensors can be successfully used for the determination of Thiamine in real samples.

Repeatability and reproducibility of the proposed sensors were also studied to assess precision of the technique. For the repeatability monitoring, 8 replicate standard samples with various concentrations of Thiamine were measured. The RSD values of the determination was not exceed 3.2%. Regarding the reproducibility of the sensors, the same three sensors from each types were made and three concentrations of Thiamine were measured by them. The associated RSD values less than 5.4% were obtained.

4. CONCLUSION

In this work, a thiamine potentiometric sensors based on PVC membrane and coated wire was made. Both sensor responded according to the well-known ion-exchange mechanism and used ion-pair complex of Thiamine-Tetraphenyl borate as their sensing materials. The electrodes shows a fast, stable and Nernstian response over a wide thiamine concentration range of 1.0×10^{-1}-5.0×10^{-6} mol L^{-1} in case of PVC membrane electrode and 1.0×10^{-2}-5.0×10^{-7} mol L^{-1} in case of coated graphite electrode. Both sensors were successfully used in analysis of Thiamine active ingredient of some pharmaceutical tablets. The RSD less than 4% was found in real sample analysis.

REFERENCES

[1] A. M. Committee, Analyst 125 (2000) 353.

[2] M. Kimura, T. Fujita, and Y. Itokawa, Clin. Chem. 28 (1982) 29.

[3] M. Kimura and Y. Itokawa, Clin. Chem. 29 (1983) 2073.

[4] N. Yantih, D. Widowati, and T. Aryani, Can. J. Sci. Ind. Reas. 2 (2011) 269.

[5] J. Tan, R. Lia, and Z. Jiang, Anal. Method 3 (2011) 1568.

[6] M. Márquez, M. Silva, and D. Pérez-Bendito, Anal. Lett. 22 (1989) 2485.

[7] J. Oni, P. Westbroek, and T. Nyokong, Electroanalysis 14 (2002) 1165.

[8] G. B. Slepchenko, L. S. Anisimova, V. F. Slipchenko, E. V Mikheeva, and N. P. Pikula, Pharm. Chem. J. 39 (2005) 166.

[9] F. Faridbod, M. R. Ganjali, B. Larijani, M. Hosseini, K. Alizadeh, and P. Norouzi, Int. J. Electrochem. Sci. 4 (2009) 1528.

[10] M. R. Ganjali, M. Rezapour, M. Pirali-Hamedani and H. Rashedi, Int. J. Electrochem. Sci. 10 (2015) 6924.

[11]   M. R. Ganjali, Z. Memari, F. Faridbod, and P. Norouzi, Int. J. Electrochem. Sci. 3 (2008) 1169.

[12]   M. R. Ganjali, I. Alahdadi, M. Aghazadeh and E. Pourbasheer, Int. J. Electrochem. Sci. 10 (2015) 6913.

[13]   M. R. Ganjali, F. Faridbod, N. Davarkhah, S. J. Shahtaheri and P. Norouzi, Int. J. Environ. Res. 9 (2015) 333.

[14]   H. A. Zamani, M. T. Hamed-Mosavian, E. Aminzadeh, M. R. Ganjali, M. Ghaemy, H. Behmadi and F. Faridbod, Desalination 250 (2010) 56.

[15]   M. R. Ganjali, T. Poursaberi, M. Hosseini, M. Salavati-Niasari, M.Yousefi and M. Shamsipur, Anal. Sci. 18 (2002) 289.

[16]   M. R. Ganjali, R. Kiani-Anbouhi, M. Shamsipur, T. Poursaberi, M. Salavati-Niasari, Z. Talebpour, M. Emami, Electroanalysis 16 (2004) 1002.

[17]   H. A. Zamani, M. Mohammadhosseini, S. Haji-Mohammadrezazadeh, F. Faridbod, M. R. Ganjali, S. Meghdadi, and A. Davoodnia, Mater. Sci. Eng. C 32 (2012) 712.

[18]   V. K. Gupta, A. K. Singh, and B. Gupta, Anal. Chim. Acta 575 (2006) 198.

[19]   M. R. Ganjali, M. Tahami, M. Shamsipur, T. Poursaberi, S. Haghgoo and M. Hosseini, Electroanalysis 15 (2003) 1038.

[20]   M. R. Ganjali, A. Karimi, S. J. Shahtaheri, and P. Norouzi, Int. J. Electrochem. Sci. 8 (2013) 1999.

[21]   H. A. Zamani, G. Rajabzadeh, M. R. Ganjali, and S. M. Khatami, Electroanalysis 17 (2005) 2260.

[22]   M. R. Ganjali, H. A. Zamani, P. Norouzi, M. Adib, and M. Accedy, Acta Chim. Slov. 52 (2005) 309.

[23]   F. Faridbod, M. R. Ganjali, B. Larijani, P. Norouzi, S. Riahi, and F. S. Mirnaghi, Sensors 7 (2007) 3119.

[24]   M. R. Ganjali, P. Norouzi, F. S. Mirnaghi, S. Riahi, and F. Faridbod, IEEE Sensors J. 7 (2007) 1138.

[25]   F. Faridbod, F. Mizani, M. R. Ganjali, and P. Norouzi, Int. J. Electrochem. Sci. 8 (2013) 10461.

[26]   F. Faridbod, M. R. Ganjali and P. Norouzi, Int. J. Electrochem. Sci. 8 (2013) 6107.

[27]   M. Shamsipur, S. Rouhani, H. Shaghi, M. R. Ganjali, and H. Eshghi, Anal. Chem. 71 (1999) 4938.

[28]   H. A. Zamani, J. Abedini-Torghabeh, and M. R. Ganjali, Electroanalysis 18 (2006) 888.

[29]   H. A. Zamani, M. R. Ganjali, P. Norouzi, and S. Meghdadi, Anal. Lett. 41 (2008) 902.

[30]   H. A. Zamani, A. Imani, A. Arvinfar, F. Rahimi, M. R. Ganjali, F. Faridbod, and S. Meghdadi, Mater. Sci. Eng. C 31 (2011) 588.

[31]   M. R. Ganjali, P. Norouzi, A. Atrian, F. Faridbod, S. Meghdadi, and M. Giahi, Mater. Sci. Eng. C 29 (2009) 205.

408

[32]   M. R. Ganjali, J. Ravanshad, M. Hosseini, M. Salavati-Niasari, M. R. Pourjavid, and M. R. Baezzat, Electroanalysis 16 (2004) 1771.

[33]   M. Shamsipur, M. Javanbakht, M. F. Mousavi, M. R. Ganjali, V. Lippolis, A. Garau and L. Tei, Talanta, 55 (2001) 1047.

[34]   M. R. Ganjali, A. Roubollahi, A. R. Mardan, M. Hamzeloo, A. Mogimi and M. Shamsipur, Microchem. J., 60 (1998) 122.

[35]   M. Shamsipur, S. Rouhani, M. R. Ganjali, H. Eshghi and H. Sharghi, Microchem. J. 63 (1999) 202.

[36]   M. R. Ganjali, S. Rasoolipour, M. Rezapour, P. Norouzi, A. Tajarodi, and Y. Hanifehpour, Electroanalysis 17 (2005) 1534.

[37]   H. A. Zamani, M. T. Hamed-Mosavian, E. Hamidfar, M. R. Ganjali, and P. Norouzi, Mater. Sci. Eng. C 28 (2008) 1551.