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Molecularly imprinted polymer-based potentiometric sensor for 2-aminopyridine as a potential impurity in piroxicam

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A molecularly imprinted polymer-based potentiometric sensor was fabricated for determination of 2-aminopyridine (2-AP). The electroactive component is the 2-APH$^+$. To form this specie the determination is carried out in a bufferic solution at pH 4.5 in which 2-AP is prevalently monoprotonated. Under these conditions, the membrane potential, increases with 2-AP concentration over a wide range of concentration (5 μM to 100 mM) with a near Nernstian response of 54.1 mV/decade and detection limit of 2.0 μM. The response time is less than 5 s and the sensor can be used for more than 3 months without any significant divergence in response. The selectivity coefficients of the proposed sensor were evaluated and exhibited good selectivity to 2-AP with respect to the electrode based on a non-imprinted polymer. The utility of the sensor was successfully tested by examining of 2-AP in piroxicam (PX) as a potential impurity.

Keywords: molecularly imprinted polymers; potentiometric sensor; 2-aminopyridine; piroxicam

Introduction

The concept underlying molecular imprinting is the assembly of a cross-linked polymer matrix around templating moieties. Upon removal of the templates, cavities or recognition sites are created which are specific or complementary in terms of both shape and functionality to the original template present in the sites.[1–3] Over the last two decades, different analytical applications have been described for molecularly imprinted polymers (MIPs). One of the many attractive features of MIPs are their application as adsorbents for solid-phase extraction and chromatographic purposes. [4–9] Development of analytical assays and sensors are other important applications of MIPs. Electrochemical sensing could offer good limits of detection (LODs), low cost, and the possibility of easy miniaturization and automation. Therefore many attempts have been made to develop electrochemical sensor-based MIP.[10–20] Ion-selective electrodes (ISEs) are one of the approaches to electrochemical transduction of MIP-based devices. Generally, this approach involves the incorporation of the imprinted polymer as the active ingredient in the membrane of an ISE,[21] Hutchins and Bachas,[22] and Murray *et al*.[23] have reported the first ion selective electrodes based on imprinted polymer using a potentiometric method. Recently, considerable attention has been paid to the development of the MIP-based potentiometric sensors. [24–32]

Aminopyridines are widely used in pharmacological and medical applications. Some of them show anesthetic properties and have been used as drugs for brain diseases.[33] 2-AP is one potential impurity in PX bulk drugs and pharmaceutical preparations. It is considered a synthesis precursor or a decomposition product through acid cleavage. The structures of the PX and 2-AP are illustrated in Figure 1.

The British Pharmacopoeia 2001 specifies the limit of 2-AP in PX to be 0.2% in bulk drugs and 0.25% in pharmaceutical preparations.[34] Derivative spectrophotometric determination of 2-AP in PX bulk material and pharmaceutical preparations has been reported.[35] Recently a sensitive spectrofluorometric method was developed to check for 2-AP, within the pharmacopoeia limit, in PX samples as an alternative to the British Pharmacopoeia chromatographic purity test.[36] Most of the reported methods are restricted by the attainable sensitivity and the requirement for bulky, expensive, and elaborate instrumentation. The design and development of simple devices such as sensors rather than laboratory-based instruments in monitoring 2-AP is very important. In view of the lack of selectivity of the conventional chemical sensors, molecularly imprinted polymer (MIP) recognition element-based sensors are gaining wide acceptance presently in scientific communities.[37] In the MIP-based ISEs, a signal is generated upon the reversible binding of analyte (2-APH$^+$ in this case) to the imprinted polymer recognition element. The binding of imprint ion results in change of potential across the membrane which is translated into an electronic signal. To our knowledge, an MIP-based potentiometric sensor has not been developed for 2-AP in particular and PX in general. In this paper, we describe a potentiometric sensor for 2-AP present in PX by dispersing MIP particles in orthonitropheny octhyl ether (*o*-NPOE) in presence of lipophilic salt additive and embedding in polyvinyl chloride (PVC) matrix to fabricate a membrane. This set-up allows detection of 2-AP present in pharmaceutical preparation.

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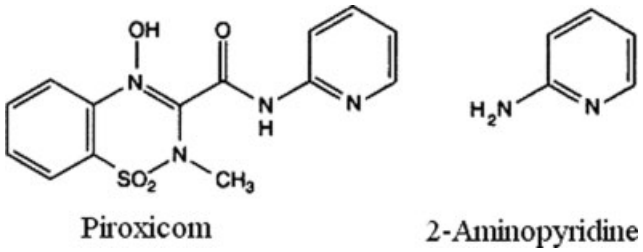

Figure 1. Chemical structures of pyroxicam and 2-aminopyridine.

Experimental

Reagents and materials

High molecular weight polyvinyl chloride (PVC) powder, sodium tetraphenyl borate (NaTPB) and 1-nitro-2-octyloxybenzene (*o*-NPOE) were obtained from Fluka, [bis(2-ethylhexyl) phthalate] (DOP) and sebacic acid dibutyl ester (DBS) were obtained from Merck and Aldrich, respectively. The imprinted polymer was synthesized using methacrylic acid (MAA) as monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker, azobisisobutyronitrile (AIBN) as initiator and chloroform as the porogen. 2-AP (template) and other chemicals used in the polymerizations were of analytical grade and were obtained from Merck. Standard solutions and buffers were prepared freshly with de-ionized water. Other reagents and solvents were the highest grade commercially available from Merck or Fluka.

Preparation of molecularly imprinted (MIP) and non-imprinted (NIP) polymer particles

Zhou and He have previously reported the preparation of 2-AP MIP by bulk polymerization method.[38] Here MIP was synthesized by precipitation polymerization. Briefly, the 2-AP target molecule (2 mmol) and functional monomer MAA (8 mmol) were dissolved in 150 ml chloroform in a glass container. The EGDMA cross-linker (60 mmol) and the AIBN initiator (130 mg) were added to this mixture and purged by nitrogen for 5 min. The glass container was sealed under vacuum and placed in a shaker bath at 60 °C for 24 h. After polymerization the 2-AP was removed from the fine MIP powder by extensive washing with 10% acetic acid in methanol solution (4 h) using a continuous extraction set-up. An NIP was synthesized in the same way but without the presence of the target molecule.

Rebinding experiments

Binding properties of the MIP and NIP were studied by batch-type rebinding assay in acetonitrile. The amount of analyte left after rebinding was determined with UV-Vis spectroscopy. All rebinding tests were performed using 20 mg of MIP or NIP in 2.0 ml acetonitrile at room temperature. In a typical rebinding experiment, known amounts of 2-AP was spiked into a suspension of MIP or NIP in acetonitrile. After each spiking, the suspension was sealed and was agitated for 15 h to ensure equilibration. The mixture was transferred into a centrifuge tube and centrifuged for 5 min. Then, concentration of free substrate in the solutions was determined using a spectrophotometer at λmax. The amount of substrate bound to the polymer, Q, was calculated by subtracting the concentration of free substrate from the initial substrate concentration. The average data of triplicate independent results were used for the following discussion.

Preparation of the membrane sensor

The PVC membrane sensors were fabricated as described below. 44 mg of PVC and 4 mg of NaTPB were dissolved in 3.0 ml of tetrahydrofuran (THF). 32 mg of MIP or NIP particles were dispersed in 120 mg of *o*-NPOE (DOP/DBS) and were added to the above solution, homogenized and then poured onto a glass plate of 25 mm of diameter. The THF was allowed to evaporate at room temperature. The PVC-based polymer membranes were obtained with thickness of ~0.6 mm. A desired piece of the membrane was attached to an end of a Pyrex tube (5.0 mm i.d. and 5.0 cm long) using a viscous solution of PVC in THF as an adhesive. The tube was then filled with internal filling solution of a mixture of 10^{-3}M of 2-AP and 0.1M HCl and conditioned for 24 h. The sensor was stored in the air when not in use. Finally, a step conditioning was carried out in 10^{-3} M 2-AP hydrochloride for stabilization of the sensor function before all measurements.

Analytical procedure

The sensor was preconditioned in a 0.1M hydrochloric acid solution for 0.5 h. The pH of the all test solutions (20 ml) were maintained at 4.5 $\pm$ 0.1 by use of 0.5 M acetic acid/acetate buffer. The potential of the test solution was measured with the following cell assembly for different concentrations of 2-AP in the range 1.0×10^{-7} to 1.0×10^{-1} M:

$$\text{Ag|AgCl|}1.0 \times 10^{-3}\text{M 2-AP, 0.1 M HCl|PVC membrane|test solution|}Hg_2Cl_2\text{|Hg}$$

The EMF was plotted as a function of the logarithm of 2-AP concentration. All measurements were made with a digital mV/pH meter Metrohm 691 at thermostated temperature (25.0 $\pm$ 0.2 °C).

Results and Discussion

Infrared spectroscopy

The interaction of 2-AP and MIP was investigated by IR spectroscopy. The results exhibited similar characteristic peaks due to similar backbone structure of the different polymers. As a result of the hydrogen binding with the COOH group of the methacrylic acid, the O-H stretching at 3417 cm^{-1} in leached MIP materials were shifted to 3411 cm^{-1} in corresponding unleached MIP. Moreover, there are also some differences between the IR spectra of the leached and unleached MIPs. In the leached polymer, there were two semi-sharp bands around 2987 cm^{-1} and 2958 cm^{-1} and a sharp band around 1163 cm^{-1}. These bands were slightly shifted in the unleached polymer to higher wave number (2989, 2960, and 1165 cm^{-1}, respectively).

Binding characteristics

Binding isotherms and Scatchard plot for the MIP and NIP are shown in Figures 2A and 2B, respectively. The polymers were incubated in a series of 2-AP solutions with different concentrations (0–5 mM). As seen in Figure 2A, due to the high affinity of the specific binding sites in the MIP, the amount of 2-AP bound to the MIP is higher than in the NIP. The number of imprinted sites in the

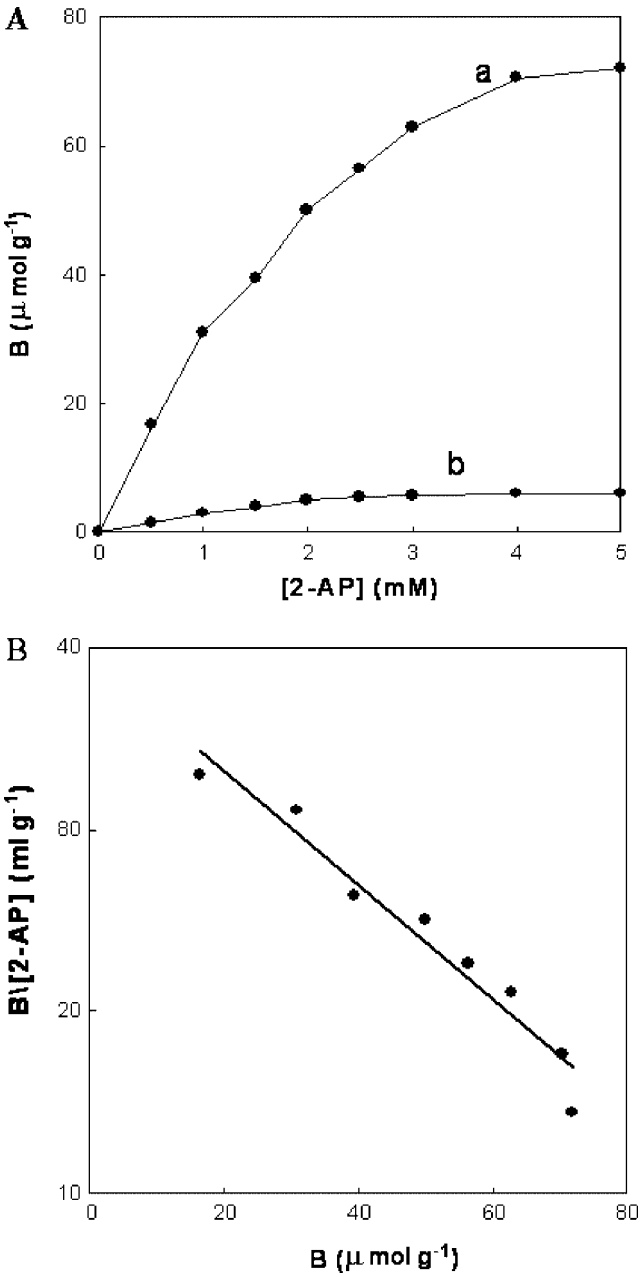

Figure 2. (A) Binding isotherm of the 2-AP. (a) MIP (b) NIP. (B) Scatchard plot for the binding between the 2-AP and the imprinted polymer.

membrane was estimated from Figure 2B based on the Scatchard equation:[39]

$$\frac{B}{[A]} = \frac{B_{max} - B}{K_d} \qquad (1)$$

where B is the amount of 2-Ap bound to the MIP and [A] is the corresponding concentration of 2-AP, B_{max} is the apparent maximum number of the binding sites, K_d is the dissociation constant. The obtained Scatchard regression equation is:

$$\frac{B}{[A]} = 39.5 - 0.314B \qquad (r = 0.975) \qquad (2)$$

Therefore, the K_d is 3.18 mM and the B_{max} is 125.8 $\mu molg^{-1}$.

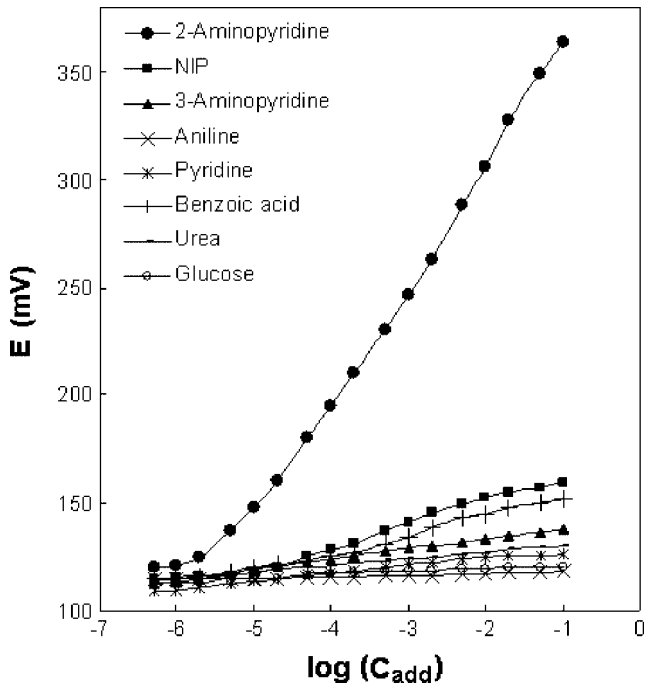

Figure 3. Potentiometric response curves of the 2-AP MIP-based membranes to 2-AP, other similar structures and some small organic molecules.

Potentiometric response of the MIP and NIP membranes

Increasing concentrations of the 2-AP (C_{add} in M) were added to the buffered test solutions at the pH value of 4.5. The potential increases immediately after the 2-AP addition at concentrations higher than around 10^{-6} M, for the membrane conditioned as described in the experimental part. Figure 3 shows typical potential response curves of the sensors based on MIP and NIP to 2-AP and its analogs (or some small organic molecules) in the concentration range of 5.0×10^{-6} to 1.0×10^{-1} M. As seen, a specific response to 2-AP could be observed with MIP but not with NIP, suggesting that the molecular imprinting is more effective in 2-AP sensing than to the compounds with similar structures or that of the untemplated polymer.

Effect of membrane composition

The effect of various components including of MIP, PVC, plasticizers on the performance of MIP-based sensors were studied. Thus, different aspects of the membrane preparation using 2-AP MIP particles were optimized. Addition of appropriate plasticizer leads to optimum physical properties and ensures high mobility of the 2-AP ions in the membrane. These solvent mediators strongly influence the working concentration range of potentiometric sensors. As it is well known that the plasticizers improve the electrochemical properties of conventional potentiometric sensors. The effect of different plasticizers on the performance of MIP-based sensors was first investigated. Figure 4 shows the potential response obtained for MIP-based sensors with three different plasticizers; *o*-NPOE, DOP and DBS. As seen, the membrane with *o*-NPOE offered a linear response of 54.1 ± 0.1 mV over the range 5.0×10^{-6} to 1×10^{-1} M. On the other hand, there is a limited linear response range for DOP and DBS. In spite of plasticizer type, its quantities in membrane (membrane composition) are essential. As seen from Table 1, the PVC membrane sensors

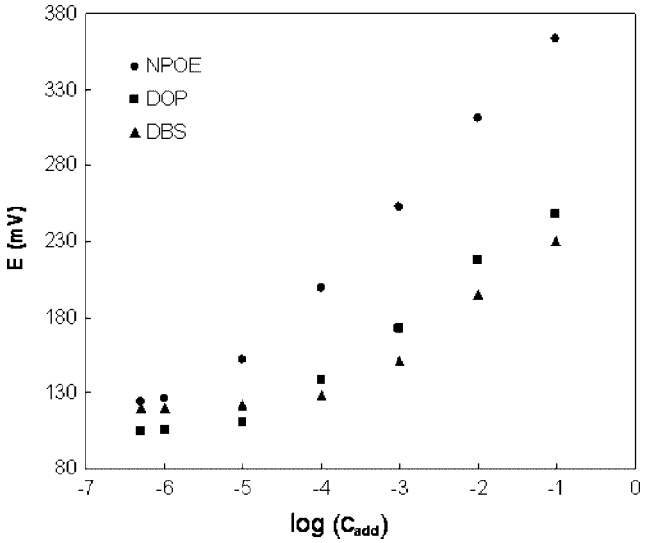

Figure 4. Potential response of MIP-based 2-AP potentiometric sensor fabricated with different plasticizers.

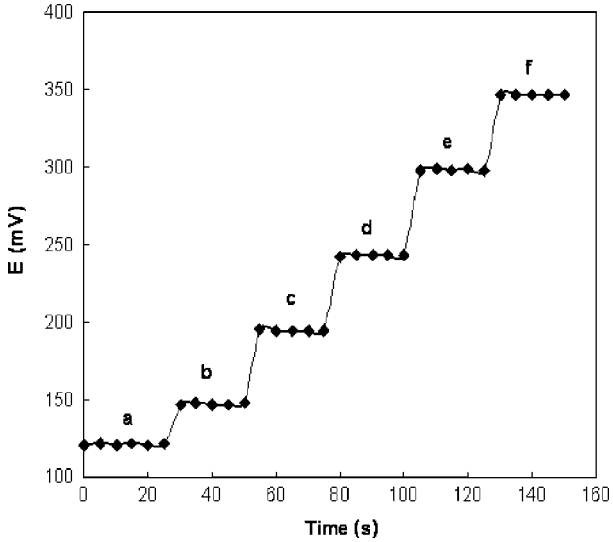

Figure 5. Dynamic response of MIP-based 2-AP potentiometric sensor for stepwise concentration change of 2-AP (a) 1.0×10^{-6} M, (b) 1.0×10^{-5} M, (c) 1.0×10^{-4} M, (d) 1.0×10^{-3} M, (e) 1.0×10^{-2} M and (f) 1.0×10^{-1} M.

with the PVC/MIP/NaTPB/*o*-NPOE percentage ratio of 22%, 16%, 2%, 60% were selected as the one with the optimal membrane ingredient composition.

Dynamic response time

Dynamic response is another factor that measures the sensing ability of the sensor. The response time was recorded by changing the 2-AP concentration in test solution over a concentration range of 1.0×10^{-6} to 1.0×10^{-1}M. The actual potential versus time traces is shown in Figure 5. The time for the sensor to reach the equilibrium potential for the electrode was very short (<5 s) in the whole concentration range.

Effect of pH on the sensor response

The effect of pH of the test solution on the performance of an MIP-based 2-AP sensor was studied by varying the pH in the range 1.0–12.0 that was adjusted with NaOH and HNO_3 solutions. The results show that the best pH for constant and maximum response characteristic is 3.0–5.0 (Figure 6). Hence, the pH of the test solution was adjusted to 4.5 ± 0.1 by means of 0.5 M acetic acid/acetate buffer. The small potential change at lower pH values(pH $<$ 3) could be due to interference of H^+ and the observed diminishing of 2-AP signal at higher pH values (pH $>$ 6) may be attributed to 2-APH$^+$ de-protonation ($pK_a = 6.9$) in the sample solutions.

Selectivity of the MIP membrane

Potentiometric selectivity coefficients ($K^{pot}_{I,J}$) of the sensor towards different species were determined by the matched potential method (MPM), recommended by IUPAC.[40] According to this method, the concentration of the primary ion ($C_I = 2.0 \times 10^{-5}$ M) is added to a reference solution (1.0×10^{-5} M of 2-AP) and the potential is measured. In separate experiments, interfering organic materials are successively added to an identical reference solution, until the measured potential matched with that obtained on the addition of the primary ions. The selectivity coefficient is given by the resulting primary ion to interfering ion activity (a_J) (concentration) ratio. The results of selectivity experiments obtained with an MIP-based sensor are shown in Table 2. As seen from Table 2, the interfering compounds could not affect the selectivity of the proposed sensor to 2-AP, showing that they would not disturb the functioning of the electrode. However, the potential response of the sensor based on NIP or MIP to benzoic acid may be caused by non-specific binding to the carboxyl group on the surface of the NIP or adsorption. Hydrogen bonding of benzoic acid with the carbonyl groups in the templated polymer might be a reason for their interferences.

The sensor's characteristics

The potential responses of MIP- and NIP-based sensors under best conditions, as obtained from the above studies, were checked

Table 1. Optimization of membrane ingredients for fabrication of MIP-based 2-AP potentiometric sensor

Membrane	PVC	MIP	NaTPB	*o*-NPOE	DOP	DBS	Working concentration range (M)
1	17.5	20	2.5	60	–	–	2.0×10^{-5} to 1.0×10^{-2}
2	25	20	2.5	52.5	–	–	5.0×10^{-5} to 5.0×10^{-2}
3	25	18	2	55	–	–	1.0×10^{-5} to 5.0×10^{-2}
4	22	16	2	60	–	–	5.0×10^{-6} to 1.0×10^{-1}
5	22	16	2	–	60	–	1.0×10^{-4} to 1.0×10^{-2}
6	22	16	2	–	–	60	2.0×10^{-4} to 5.0×10^{-2}

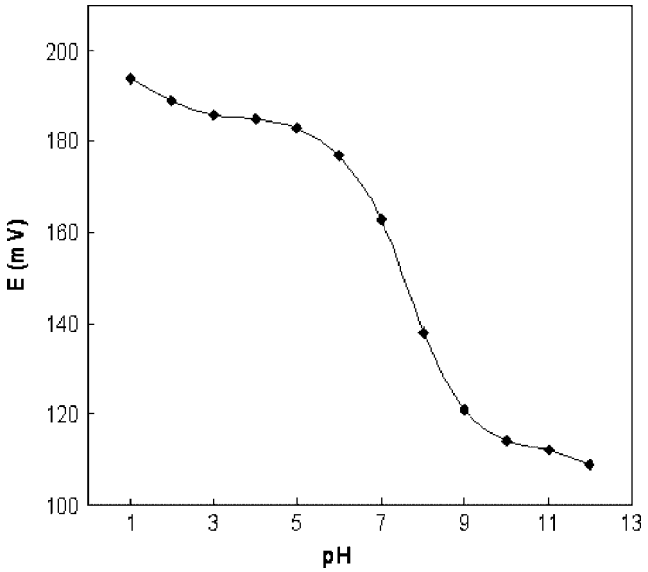

Figure 6. Effect of pH solution on the potential response of the MIP-based sensor in presence of 1.0×10^{-3} M 2-AP solution.

Table 2. Comparison of selectivity coefficients of 2-AP with respect to some compounds as interference

Interfering, *J*	$K^{pot}_{2AP,J}$	Interfering, *J*	$K^{pot}_{2AP,J}$
Piroxicam	3.45×10^{-4} (0.65)[a]	Ca^{+2}	3.26×10^{-5}
3-Aminopyridine	6.59×10^{-3} (0.78)	Na^{+}	7.14×10^{-5}
Aniline	4.32×10^{-3} (0.36)	Zn^{+2}	9.86×10^{-4}
Pyridine	8.41×10^{-3} (0.53)	K^{+}	2.48×10^{-5}
Urea	2.86×10^{-4}	Mg^{+2}	4.61×10^{-5}
Benzoic acid	8.16×10^{-2} (7.49×10^{-2})	Ba^{+2}	5.24×10^{-5}
Glucose	2.10×10^{-5}		

[a] The values in the parentheses are the selectivity coefficients for the sensor based on NIP.

and the results are shown in Figure 3. As seen from Figure 3, the plot obtained for MIP-based sensors offers linear response in the ranges 5×10^{-6} to 1×10^{-1} M. The detection limit of the sensor was 2×10^{-6} M. The repeatability of the potential response was evaluated with six repeated potentiometric measurements of the 5×10^{-6} M 2-AP solution. The precision of the described procedure in terms of relative standard deviation was 4.5%. The long-term stability of the MIP-based sensor was also investigated. The results show that the sensor was stable (deviation less than 1 mV for 1×10^{-5} 2-AP M) during 10 weeks and can be reused for more than 20 times without any loss in sensing ability. The accuracy of the measurements by the MIP-based sensor was checked by calculating the recovery of spiked PX sample with known 2-AP concentration The mean percentage recoveries, obtained by applying the calibration curve method, were indicated in Table 3.

Analytical application

The sensor was successfully used for detection and determination of 2-AP as impurity in PX capsules. The PX capsules with a nominal content of 10 mg were obtained from Razak Pharmaceutical Company, Iran. Ten capsules were weighed accurately and spiked

Table 3. Results of determination of 2-AP in piroxicam capsule by using MIP particle-based potentiometric sensor

Sample	Nominal Value (mg/capsule)	Concenteration of 2-AP(μg/ml) Amount added (2-AP%)	Found[a]	Recovery (%)
Capsule	10 mg	–	–	–
	10 mg	5 (0.125)	4.8 ± 0.16	96
	10 mg	10 (0.25)	10.3 ± 1.51	103
	10 mg	40 (1)	40.6 ± 1.12	101.5
	10 mg	200 (5)	198.4 ± 1.04	99.2

[a] Average of three successive determinations

with adequate amount of 2-AP for preparation of different experimental samples (Table 3). Adequate amounts of the samples were dissolved in water and then filtered. Further dilution was made into a 25 mL volumetric flask to obtain a final solution containing different 2-AP concentration. The results obtained are clearly shown in Table 3, indicating that the MIP-based 2-AP sensor can reliably be used for determination of 2-AP in PX as impurity with different percentages.

Conclusions

In conclusion, we have successfully demonstrated and designed a new 2-AP potentiometric sensor based on MIP particles. Scatchard analysis shows that the maximum binding sites of the 2-AP imprinted polymer (B_{max}) toward 2-AP is 125.8 μmol g^{-1}. It was shown a high selectivity to 2-AP. The membrane was rigid enough to bear the filling solution in contact with the internal reference electrode. To obtain a good potentiometric response, the membrane must be previously conditioned. An interesting characteristic of the potentiometric sensor was that a short time, only a few seconds, was required to reach the equilibrium potential. The prepared membranes exhibited the same recognition characteristics over a period of three months. The potentiometric performance of the proposed sensor satisfies the requirement for the assay of 2-AP as potential impurity in PX.

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References

[1] C. Alexander, L. Davidson, W. Hayes, *Tetrahedron* **2003**, *59*, 2025.
[2] K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495.
[3] K. Haupt, *Analyst* **2000**, *126*, 747.
[4] J. Olsen, P. Martin, I. D. Wilson, G. R. Jones, *Analyst* **1999**, *124*, 467.
[5] K. Ensing, C. Berggren, R. E. Majors, *LC-GC Eur.* **2002**, *14*, 162.
[6] D. Stevenson, *Trends Anal. Chem.* **1999**, *18*, 154.
[7] H. Sanbe, J. Haginaka, *Analyst* **2003**, *128*, 593.
[8] B. Sellergren, *J. Chromatog. A* **2001**, *906*, 227.
[9] D. Wistuba, V. Schurig, *J. Chromatog. A* **2000**, *875*, 255.
[10] S. A. Piletsky, A. P. F. Turner, *Electroanal.* **2002**, *14*, 317.
[11] M. C. Blanco-Lopez, M. J. Lobo-Castanon, A. J. Miranda-Ordieres, P. Tunon-Blanco, *Trends Anal. Chem.* **2004**, *23*, 36.
[12] S. A. Piletsky, E. V. Piletskaya, A. V. El'skaya, K. Yano, I. Karube, Y. P. Parhometz, A. V. Elskaya, *Biosens. Bioelectron.* **1995**, *10*, 959.
[13] D. Kriz, M. Kempe, K. Mosbach, *Sensor. Actuat. B* **1996**, *3*, 178.

[14] S. A. Piletsky, E. V. Piletskaya, T. A. Sergeyeva, T. L. Panasyuk, A. V. El'skaya, R. Levi, I. Karube, G. Wulff, *Macromolecules* **1998**, *31*, 2137.
[15] R. Shoji, T. Takeuchi, H. Suzuki, I. Kubo, *Chem. Senses B* **2002**, *18*, 7.
[16] D. Kriz, K. Mosbach, *Anal. Chim. Acta.* **1995**, *300*, 71.
[17] M. C. Blanco-L'opez, M. J. Lobo-Castanon, A. J. Miranda-Ordieres, P. Tunon-Blanco, *Biosens. Bioelectron.* **2003**, *18*, 353.
[18] E. Hedborg, F. Winquist, I. Lundström, L. I. Andersson, K. Mosbach, *Sensor. Actuat. A* **1993**, *37*, 796.
[19] A. Kugimiya, T. Takeuchi, *Biosens. Bioelectron.* **2001**, *16*, 1059.
[20] S. P. Pogorelova, T. Bourenko, A. B. Kharitonov, I. Willner, *Analyst* **2002**, *127*, 1484.
[21] C. Malitesta, I. Losito, P. G. Zambonin, *Anal. Chem.* **1999**, *71*, 1366.
[22] R. S. Hutchins, L. O. Bachas, *Anal. Chem.* **1995**, *67*, 1654.
[23] G. M. Murray, A. L. Jenkins, A. Bzhelyonsky, O. M. Uy, *J. Hopkins Apl. Tech. D.* **1997**, *18*, 464.
[24] Y. Zhou, B. Yu, E. Shiu, *Anal. Chem.* **2004**, *76*, 2689.
[25] G. D'Agostino, G. Alberti, R. Biesuz, M. Pesavento, *Biosens. Bioelectron.* **2006**, *22*, 145.
[26] P. Metilda, K. Prasad, R. Kala, J. M. Gladis, T. Prasada Rao, G. R. K. Naidu, *Anal. Chim. Acta.* **2007**, *582*, 147.
[27] K. P. Prathish, K. Prasad, T. Prasada Rao, M. V. S. Suryanarayana, *Talanta* **2007**, *71*, 1976.
[28] S. Sadeghi, F. Fathi, J. Abbasifar, *Sensor. Actuat. B* **2007**, *122*, 158.
[29] M. Javanbakht, S. Eynollahi Fard, A. Mohammadi, M. Abdouss, M. R. Ganjali, P. Norouzi, L. Safaralie, *Anal. Chim. Acta.* **2008**, *612*, 65.
[30] A. H. Kamel, F. T. C. Moreira, S. A. A. Almeida, M. G. F. Sales, *Electroanal.* **2008**, *20*, 194.
[31] R. Liang, R. Zhang, W. Qin, *Sensor. Actuat. B* **2009**, *141*, 544.
[32] X. Chen, Z. Yang, S. Si, *J. Electroanal. Chem.* **2009**, *635*, 1.
[33] M. Okamato, K. Takahashi, T. Doi, Y. Takimoto, *Anal. Chem.* **1997**, *69*, 2919.
[34] British Pharmacopoeia Commission, *British Pharmacopoeia*, The Stationery Office: London, **2001**.
[35] G. Carlucci, A. Colanzi, P. Mazzeo, M. G. Quaglia, *Int. J. Pharm.* **1989**, *53*, 257.
[36] M. H. Barary, M. H. Abdel-Hay, S. M. Sabry, T. S. Belal, *J. Pharm. Biomed. Anal.* **2004**, *34*, 221.
[37] C. Alexander, H. S. Andersson, L. L. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony, M. J. Whitcombe, *J. Mol. Recognit.* **2006**, *19*, 106.
[38] J. Zhou, X. He, *Anal. Chim. Acta.* **1999**, *381*, 85.
[39] H. I. Yamamura, S. J. Enna, M. J. Kuhar, *Neurotransmitter Receptor Binding*, Raven Press: New York, **1985**.
[40] Y. Umezawa, K. Umezawa, H. Sato, *Pure Appl. Chem.* **1995**, *67*, 507.