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Molecularly Imprinted Polymer Based PVC-Membrane-Coated Graphite Electrode for the Determination of Metoprolol

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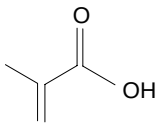
A PVC-based membrane containing metoprolol molecularly imprinted polymer (MIP) coated directly on graphite electrode for the determination of metoprolol in real samples is reported. This potentiometric sensor was designed by dispersing the MIP particles in dioctylphthalate plasticizer as solvent mediator and then embedded in polyvinyl chloride matrix. The electrode exhibited a near-Nernstian slope of 55.4 ± 1 mV decade^{-1} for metoprolol over a wide concentration range between 2.0×10^{-7}-8.0×10^{-3} M and a detection limit of 1.3×10^{-7} M. With a response time of about 14 s it could be used for at least 6 months without any divergence in potential. The proposed electrode can be used in the pH range of 3.5-10.5 and can reveal good selectivities for metoprolol over a wide variety of ions. Finally, the designed sensor was successfully applied as an indicator electrode to determine the concentration of metoprolol in tablets, human urine and plasma. The results were compared favorably with those obtained by HPLC method and showed satisfactory agreements with them.

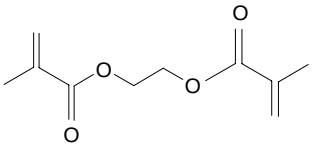
Keywords: Metoprolol, Ion-selective electrode, Molecularly imprinted polymer, Coated graphite, PVC-Membrane

INTRODUCTION

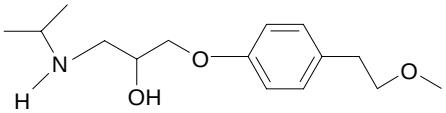
Metoprolol is a selective β_1 receptor blocker used in the treatment of several diseases of the cardiovascular system, especially hypertension. This drug is amongst the most widely prescribed drugs in the world, based on the aryloxy-propranololamine backbone (Fig. 1) [1-3]. Various methods have been developed for the determination of metoprolol including gas chromatography [4,5], and high-performance liquid chromatography (HPLC) with UV [6-8], fluorimetric [9,10] and mass spectrometric detection [11]. The main problems of employing chromatographic methods are the need for derivatisation and time-consuming extraction procedures. Since the use of techniques entails relatively expensive instrumentation and running costs, development of simpler,



Methacrylic acid (MAA)



Ethylene glycol dimethacrylate (EGDMA)



Metoprolol

Fig. 1. Chemical structure of functional monomer (MAA), cross-linking monomer (EGDMA) and imprint molecule (metoprolol).

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faster and less expensive, but still sensitive, electrochemical techniques could be an interesting alternative.

Among reported investigations in the field of molecularly imprinted polymers for β-blockers, there are only a few papers that are concerned with the metoprolol as template [12,13], and among the reported researches in the field of electrochemical methods for β-blockers [14-17], there are only a few sources that are concerned directly with metoprolol [17] and virtually no article is found to report on the detection of metoprolol using MIP based ion-selective electrode. On the whole, one could say that there are only a few researches dealing with potentiometric molecular sensors based on MIPs [18-25].

Molecular imprinting has been known as a polymerization technique for the preparation of synthetic polymers with recognition sites for given molecules [26]. Among the different methods available for the preparation of MIPs, the so-called non-covalent approach, which uses only non-covalent interactions between the template and the functional monomers, is probably the most flexible regarding the selection of the functional monomers and the possible template molecules. For these reasons, the non-covalent approach has been the most widely adopted [27]. The procedure for synthesizing an MIP is based on the chemical polymerization of a functional monomer and a cross-linking agent in the presence of a molecule used as a template. After the removal of the imprinted molecule, an imprinted polymer is obtained. This polymer contains sites with a high affinity for the template molecule, due to their shapes and the arrangement of the functional groups of the monomer units. The imprinted polymers are used as antibody-like materials for high selectivity and sensitivity, owing to their long-term stability, chemical inertness and insolubility in water and most organic solvents [28].

This study is concerned with the construction of a potentiometric sensor prepared from MIP-based membrane and its application for metoprolol determination in aqueous medium. The re-emergence of ion-selective electrodes as a strong research direction in the field of potentiometric sensors is largely due to their improved mechanistic understanding [29]. Compared with other methods of trace analysis, potentiometry is an extremely inexpensive technique. Potentiometric sensors are known to only minimally perturb the sample, *i.e.*, in comparison with other methods of trace analysis, these devices do not require any elaborate sample preparation including preconcentration or application of agents [30]. In the present work, we propose an MIP-based ion-selective electrode for the metoprolol present in tablets, human urine and plasma by casting a membrane after dispersing metoprolol imprinted polymer particles in dioctylphthalate (DOP) and embedding in polyvinyl chloride (PVC) matrix.

EXPERIMENTAL

Reagents and Apparatus

All chemicals were of analytical grades and all solutions were prepared in double-distilled water. Metoprolol tartrate was obtained from SUN Pharmaceutical Industries LTD (Maharashtra, India). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), chloroform, acetic acid, acetonitrile, methanol, hydrochloric acid, trifluoroacetic acid (TFA) triethylamine, sodium chloride and the nitrate salts of all cations used (all from Merck), dibutyl phthalate (DBP), dioctyl phthalate (DOP), *ortho*-nitro phenyl octyl ether (*o*-NPOE), poly(vinyl chloride) (PVC) (with high relative molecular weight), oleic acid (OA) (all from Fluka), potassium tetrakis (*p*-chlorophenyl) borate (KTpClPB), sodium tetraphenyl borate (NaTPB) and 2,2′-azobisisobutyronitrile (AIBN) (from Aldrich) were used as received.

All potentials were measured on a digital Hioki 3200 multimeter *vs*. a Philips saturated calomel reference electrode (SCE) at 25.0 ± 0.1 °C. The Fourier transform infrared (FTIR) spectra of non-imprinted and molecularly imprinted polymers were obtained using a Nexus870 Thermo Nicolet FTIR spectrometer. A Shimadzu-UV-1700 PC controlled double-beam spectrophotometer equipped with quartz cell with a 1-cm path length was used for recording absorbances in the comparative study with pharmacopoeia method [31]. HPLC-comparative analysis was performed using a Perkin-Elmer 200 Series chromatographic workstation. These HPLC measurements were carried out on a C_{18} column. The mobile phase consisted of acetonitrile:methanol:0.5% acetic acid:triethylamine (56:18:26:0.1, v/v) at a flow rate of 1.0 ml min^{-1} [32]. The HPLC system was equilibrated for approximately 30 min at the same flow rate before the analysis commenced. 20 µl of sample was injected manually with a

100 µl syringe. The UV detection wavelength was 225 nm. Chromatography was performed at ambient temperature and each analysis was repeated in triplicate. A model MP225 Mettler-Toledo pH-meter was used for the pH adjustments.

Stock solution of metoprolol (5.0×10^{-2} M) was prepared by dissolving an appropriate amount of metoprolol tartrate in bidistilled water. Other dilute solutions (1.0×10^{-2} M-1.0×10^{-8} M) were prepared by serial dilution at constant ionic strength (0.05 M NaCl) and all pHs were adjusted with HNO_3 or NaOH.

Preparation of Metoprolol MIP or Non-Imprinted Polymer (NIP) Particles

The procedure for the synthesis of MIP and NIP polymers was as follows:

To be briefe, to a three-necked round-bottom flask were added template (metoprolol tartrate; 0.25 mmol, or 0.171 g or metoprolol; 0.5 mmol), functional monomer (MAA; 2 mmol, or 0.17 ml), cross-linker (EGDMA; 10 mmol, or 1.89 ml) and initiator (AIBN; 0.25 mmol, or 0.042 g) in chloroform (20 ml). The mixture was purged with nitrogen for 10 min to remove any dissolved oxygen, which would inhibit free radical polymerization. The polymerization was allowed to continue in a water bath at 60 °C for 18 h. After polymerization, a hard polymer monolith was obtained, which was crushed and ground into a fine powder with a mortar and pestle. Soxhlet extraction was performed to remove the template with 70:30 (V/V) methanol:acetic acid overnight. Then, the polymer was washed several times with pure methanol to remove the acetic acid and facilitate drying. The dried polymer was now ready for use. The method for the preparation of NIP was exactly similar to the procedure for the synthesis of MIP with the exception of metoprolol (imprint molecule) omitted in the preparation of NIP.

Electrode Preparation

The general procedure used to prepare the PVC membrane was similar to works reported by Sales and coworkers [20], Rao and coworkers [21,22], and Shamsipur and coworkers [33]. Exactly 60 mg PVC was dissolved in 2.5 ml of THF. A 40 mg of MIP or NIP particles were dispersed in 0.2 ml of DOP which were added to the above solution and homogenized. Graphite electrodes (3 mm diameter and 10 mm long) were prepared from spectroscopic grade graphite. The electrode was polished with fine alumina slurries on a polishing cloth, sonicated in distilled water and dried in air. A shielded copper wire was glued to one end of the above graphite rod with epoxy resin, and the electrode was sealed into the end of a PVC tube. The polished electrode was dipped into the membrane solution, and the solvent was evaporated. A layer was formed on the graphite surface, which was allowed to set for 3 h. The electrode was finally conditioned for 18 h by being soaked in a 1.0×10^{-4} M solution of metoprolol.

Emf Measurements

The following cell assembly was used for the measurement of all emfs:

SCE||Sample solution|MIP-based membrane|Graphite electrode

The coated electrode containing MIP was used as the measuring electrode in conjunction with an SCE and the activity coefficients were calculated according to the Debye-Huckel equation:

$$\log \gamma = \frac{-0.51 z^2 \sqrt{\mu}}{1 + \sqrt{\mu}}$$

where γ is the activity coefficient, μ and z are the ionic strength of the solution and charge of the ions, respectively.

RESULTS AND DISCUSSION

Preparation and Characterization of MIP

It is noteworthy that in the preparation of many non-covalent MIPs a template:functional monomer:cross-linker molar ratio of about 1:4:20 has resulted in very suitable performance characteristics [19,21,28,34-41]. Therefore, this ratio was used for the synthesis of metoprolol molecularly imprinted polymer in the present work. The most commonly used functional monomer is methacrylic acid (MAA). In addition to the strong ionic interactions that MAA can form with basic functional groups on the template, the carboxyl group on this monomer is an excellent hydrogen bond donor and acceptor [28]. The chemical environment and the morphology of the MIP are greatly affected by the choice of cross-linking monomer, so that careful consideration must be

given to its choice. The most commonly used cross-linker EGDMA has two acrylate groups that enable it to form more rigid polymers. This enhanced rigidity has been shown to lead to MIPs with higher capacities and selectivities [28]. For efficient template-monomer interactions, generally, aprotic organic solvents with low polarities are required. A solvent with such characteristics as chloroform leads to an MIP which will provide binding sites with higher fidelity and increased capacity [28]. Accordingly, we used MAA, EGDMA and chloroform as functional monomer, cross-linker and porogenic solvent, respectively, for the synthesis of MIP.

The control polymer (NIP) and molecularly imprinted particles obtained using non-covalent imprinting protocol were subjected to characterization by FT-IR and binding batch static methods.

FT-IR Spectra. The FT-IR spectra of control non-imprinted and molecularly imprinted polymer materials prepared using radical bulk polymerization are shown in Fig. 2. In the IR spectra, the absorptions due to carboxyl OH stretching (~3500 cm^{-1}), carbonyl group stretching (~1730 cm^{-1}), C-O stretching (~1260 cm^{-1}) and C-H vibrations (~756, ~1390, ~1460 and ~2956 cm^{-1}) were observed. By evaluation and comparison of the spectra (A, B and C), the important results obtained are as follows:

(1) The absorbances pertaining to metoprolol (spectrum C), stretching of aromatic C=C (~1512 cm^{-1}), stretching of aromatic C-H (~1250 cm^{-1}), C-N stretching (~1111 cm^{-1}), bending of aromatic C-H (~821 cm^{-1}) and combination bands for para substitution (~1891 cm^{-1}, ~2067 cm^{-1}) aren't observed in the MIP spectrum (B). This difference proves that imprint molecule (metoprolol) has been sufficiently leached from MIP in the soxhlet extraction step.

(2) The peak attributed to the C-H stretching of methylene group (~2956 cm^{-1}), carbonyl group stretching (~1730 cm^{-1}), C-O stretching (~1260 cm^{-1}) and C-H bending of $-CH_2$ (~1460 cm^{-1}) for the MIP were relatively stronger than those of NIP. Therefore, it can be concluded that the presence of imprint molecule (metoprolol) causes increased incorporation of ethylene glycol dimethacrylate into the polymer during the preparation step.

Binding experiment by batch static method. Six portions of standard or sample solutions (100 ml) containing metoprolol (0.3 mg) were individually transferred into 250 ml beakers, and the pH value was adjusted to 6.00 with 0.1 M, HNO_3 or NaOH. Then 50 mg NIP or MIP adsorbent was added to each sample, and the mixtures were shaken vigorously for 30, 60, 90, 120, 150 and 180 min, respectively, to facilitate adsorption of the metoprolol onto the imprinted polymer particles. After the solutions were centrifuged, free concentration of metoprolol in the filtrate solutions was directly determined by spectrophotometry. It was found from the resulting data that the time period of 150 min incubation was enough to achieve equilibrium. The amount of metoprolol bound to the polymer was calculated by subtracting the amount of free substrate from the initial amount of the template. The same procedure was followed for NIP particles. The phase distribution ratio (K_d) and adsorption capacity (Q) were calculated using the following equations:

$$K_d = \frac{C_i - C_f}{C_f} \times \frac{V}{W} \quad (1)$$

$$Q = \frac{(C_i - C_f)V}{W} \quad (2)$$

where C_i and C_f represent the initial and equilibrium concentration of metoprolol in the aqueous phase (mg ml^{-1}), W is the weight of the polymer (g) and V is the volume of the aqueous phase (ml). Data from adsorption capacity studies were substituted in Eqs. (1) and (2) and thus K_d and Q were calculated to be 307.8 ± 17.8 ml g^{-1} and 0.8 ± 0.04 mg g^{-1} for MIP and 36.6 ± 2.9 ml g^{-1} and 0.114 ± 0.01 mg g^{-1} for NIP, respectively. By the evaluation of these data, it can be concluded that the adsorption of metoprolol onto NIP is based on non-specific interactions; consequently, K_d and Q quantities of NIP are negligible in comparison to MIP. This can be related to the introduction of shape-selective binding sites into three-dimensional polymeric network of MIP.

Optimal Membrane Composition

In chemical sensors and biosensors, a chemical or physical signal is generated upon binding of the analyte to the recognition elements: a transducer then translates this signal into a quantifiable output signal. The same general principle applies if a molecularly imprinted polymer is used as the recognition element instead of a biomolecule [18]. The possibility of developing ion-selective electrodes for a few

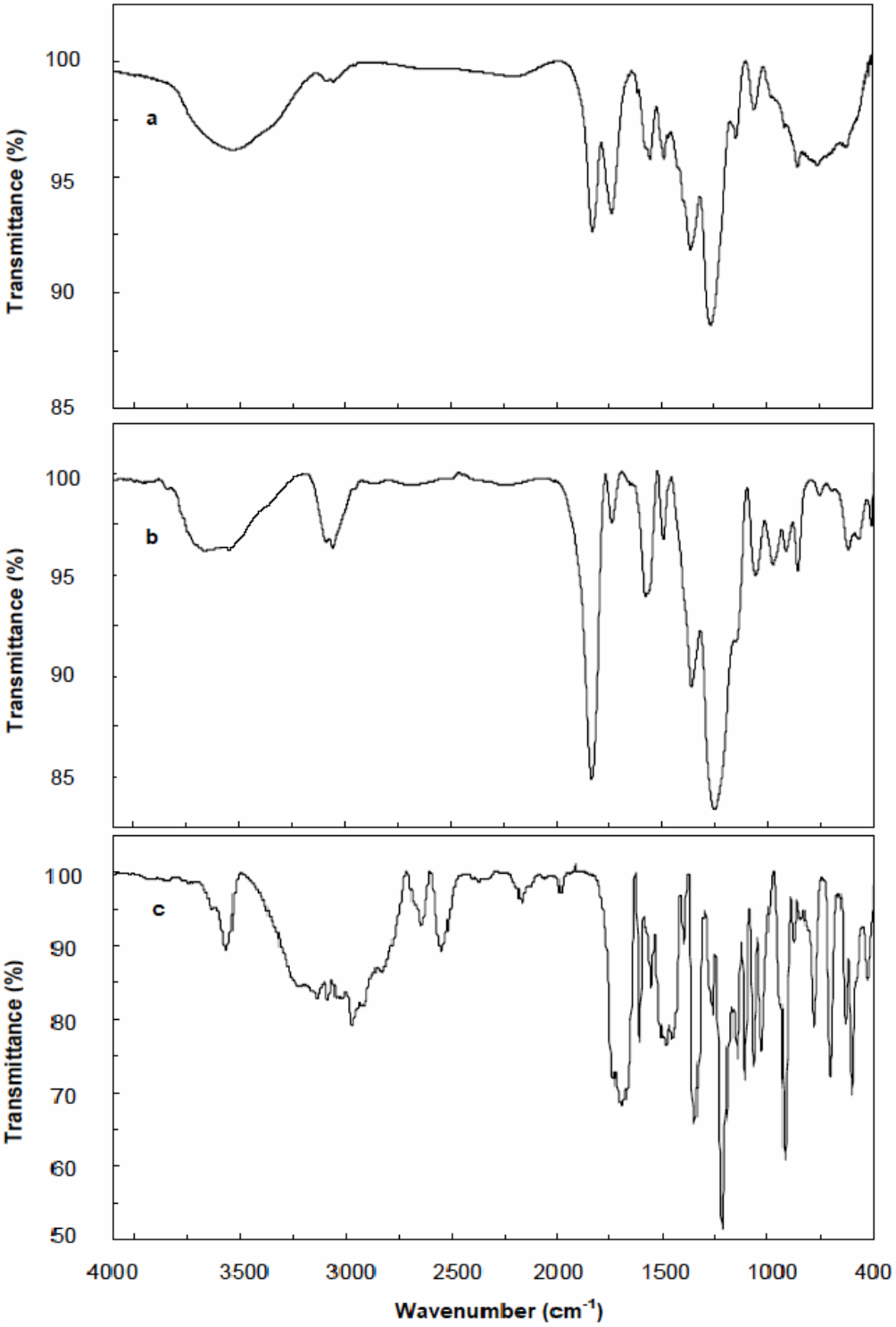

Fig. 2. FT-IR spectra of NIP (a), MIP (b) and metoprolol tartrate (c) by KBr pellet method.

drugs and herbicides with MIPs has recently opened up new horizons for sensor technology [19-25]. It is well-known that MIPs have binding sites that are complementary in size and shape to the imprint molecule. In other words, a molecular memory is introduced into the polymer, which is capable of selectively rebinding the analyte. In this paper, the optimal conditions for the best performance of metoprolol-selective electrode based on a membrane containing MIP have been investigated systematically.

It is well-known that the important features of the PVC membrane such as the nature and amount of ion recognizing material, solvent mediator (plasticizer) and especially, the nature of the additives significantly influence the sensitivity and selectivity of ion-selective electrodes [42,43]. Thus, different aspects of the membrane based on synthesized imprinted polymer were optimized whose results are summarized in Table 1.

Since the nature of the *plasticizer* influences the dielectric constant of the membrane phase and the mobility of the attendant species [42], it is expected to play a fundamental role in determining the electrode characteristics. It was observed that among the three different solvent mediators used, the use of 69% DOP in the presence of 10% MIP (entry 3, Table 1) provided better sensitivity, with a slope of 49.2 ± 1 mV decade^{-1}. The results indicated that the membrane with *o*-NPOE offered lower sensitivity in the range 1.0×10^{-7} M to 1.0×10^{-2} M. These findings signify that the plasticizers with low dielectric constants, *i.e.*, DOP (ε = 5.0) and DBP (ε = 8.5) give better response characteristics compared to *o*-NPOE with high dielectric constant of 24.0. Observation of better response in the case of the membranes containing DOP is most likely due to highly energetic ionic interactions that can occur between carboxylic groups of the binding sites and positively charged metoprolol in this plasticizer with its lowest dielectric constant.

The influence of MIP quantity and MIP/PVC ratio in the

Table 1. Effect of Membrane Composition on Response of the MIP-Based Metoprolol Electrode

Membrane no.	Composition (%)								Slope (mV decade^{-1}) [a]
	MIP	PVC	o-NPOE	DBP	DOP	KTpClPB	NaTPB	OA	
1	10	21	69	-	-	-	-	-	19.8
2	10	21	-	69	-	-	-	-	34.7
3	10	21	-	-	69	-	-	-	49.2
4	4	22	-	-	74	-	-	-	18.1
5	7	22	-	-	71	-	-	-	35.9
6[b]	13	20	-	-	67	-	-	-	54.3
7	16	19	-	-	65	-	-	-	41.5
8	19	19	-	-	62	-	-	-	Unstable
9	12	20	-	-	68	-	-	-	52.4
10	15	20	-	-	65	-	-	-	55.1
11	13	20	-	-	65	2	-	-	54.8
12	13	19	-	-	64	4	-	-	55.9
13	13	20	-	-	65	-	2	-	45.2
14	13	19	-	-	64	-	4	-	45.7
15	13	20	-	-	65	-	-	2	40.3
16	13	19	-	-	64	-	-	4	40.6

[a]Concentration range = 1.0×10^{-7}-1.0×10^{-2} M; pH of solutions = 6. [b]Optimum composition.

membrane was investigated, whose results are shown in Table 1 (entries 3-10). The sensitivity of the electrode increased with increasing MIP content until a value of 15% and MIP/PVC ratio of 0.75 was reached. Further addition of MIP, however, resulted in some decrease of the electrode response, most probably due to some inhomogeneities and possible saturation of the membrane [44].

It should be noted that, even though the addition of the KTpClPB resulted in responses that were closer to the additive free membrane, the selectivity coefficients of the membranes 11 and 12 (with incorporated KTpClPB) were somehow larger. Therefore, the additive free electrode based on membrane 6 was selected as the optimum membrane.

Concentration of Conditioning Solution

The proposed membrane electrode was further examined with different concentrations of conditioning solution from 1.0×10^{-3} M to 1.0×10^{-5} M. Functioning of the membrane sensor with the solution of various metoprolol tartrate concentrations was found to cause no significant difference in the intercept of the resulting potential-log $C_{metoprolol}$ plots. However, in the case of 1.0×10^{-4} M more stable responses were obtained compared to 1.0×10^{-3} M and 1.0×10^{-5} M. Thus, a 1.0×10^{-4} M concentration of the conditioning solution was quite appropriate for a smooth functioning of the membrane electrode system. It should be noted that the optimum equilibrium time for the membrane sensor was found to be 18 h, after which stable potentials were generated in contact with the metoprolol solutions.

Response Time and Reversibility

In this study, the practical response time or the average time required for the metoprolol sensor to reach 90% of the maximum potential was recorded after immersion into metoprolol solutions with a 10-fold difference in concentration (Fig. 3; two isolated jumps in concentration, 5.0×10^{-5} M to 5.0×10^{-4} M and 5.0×10^{-4} M to 5.0×10^{-3} M) [42]. As can be seen, it was found that the electrode reached to its equilibrium response in 14 s. The other points after the time to reach equilibrium potential indicated that reproducibility of the electrode response was acceptable even during longer periods (up to 300 s). To evaluate the reversibility of the electrode, the measurements were performed in the sequence of high-to-low

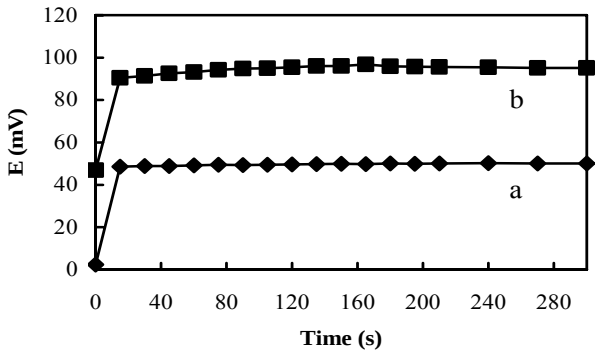

Fig. 3. Response time of the metoprolol selective electrode with two isolated jumps in concentration; (a) 5.0×10^{-5} M to 5.0×10^{-4} M, (b) 5.0×10^{-4} M to 5.0×10^{-3} M.

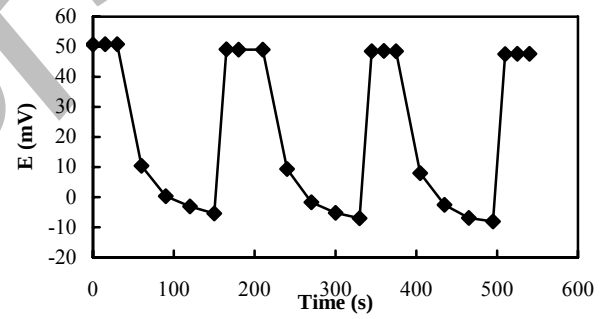

Fig. 4. Reversibility of the metoprolol selective electrode in several high-to-low sample cycles (5.0×10^{-4} M to 5.0×10^{-5} M).

sample concentrations the results of which are shown in Fig. 4. It could be seen that the potentiometric response of the sensor was reversible, although the time needed to reach the equilibrium values was longer than that for the low-to-high sample concentration procedure. It is well-documented that, in the case of high-to-low concentrations, the time needed to attain a stable potential was some 100 times longer than that required for the case of low-to-high concentrations (for a 10 times change in the concentration) [42].

Effect of pH

The pH dependence of the membrane sensor was tested over the pH range 2.0-12.0 at 1.0×10^{-4} M and 1.0×10^{-5} M metoprolol concentrations (Fig. 5). Adjustments of pH were

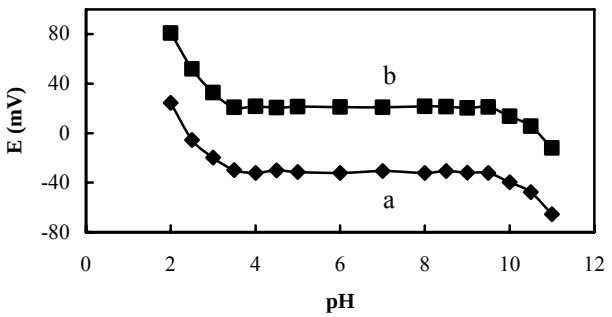

Fig. 5. Effect of pH of test solution on the potential response of the metoprolol selective sensor at (a) 1.0×10^{-5} M and (b) 1.0×10^{-4} M metoprolol concentrations.

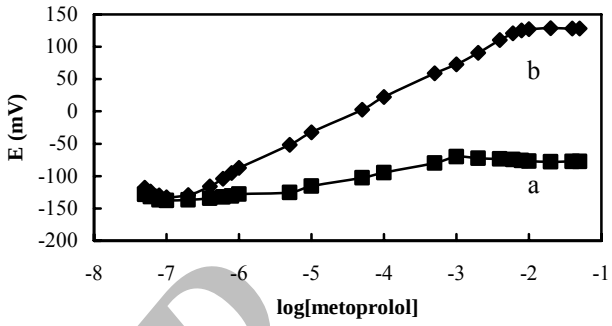

Fig. 6. Calibration plot for the proposed metoprolol selective electrode with optimized membrane composition based on NIP (a) and MIP (b) particles.

made with nitric acid or sodium hydroxide solutions. As is seen from Fig. 5, potentials stay constant from pH 3.5 to 10.5, beyond which potential drifts are observed. Thus, this range was taken as the working pH range of the proposed sensor and pH 6.0 was selected for the measurements. This behavior of the prepared membrane could be interpreted as follows:
(1) The pK_a of metoprolol is about 9.5 [45-47], *i.e.*, the observed potential drift at high pH values (pH > 10.5) could be due to the formation of metoprolol in non-ionic form.
(2) Below pH 3.5, the membrane may respond to H^+ as a consequence of which an increase in the potentials was observed.

Calibration Curve

After the optimization of the membrane composition, we continued to prepare a membrane with NIP using the same amount of the active material (13%) and compared the electrode performance of both polymers. The typical potential response curves of the sensors based on NIP and MIP to metoprolol in the concentration range of 5.0×10^{-8} M to 5.0×10^{-2} M are shown in Fig. 6. The figure illustrates that a specific response to metoprolol could be observed with MIP but not with NIP, suggesting that the molecular imprinting was more effective for metoprolol sensing than for the NIP. A slope of ~23 mV decade^{-1} for the membrane based on NIP in the concentration range of 5.0×10^{-6} M to 1.0×10^{-3} M metoprolol is most probably due to the carboxyl groups on the polymer surface which could have a non-specific interaction with the metoprolol.

The emf response of the MIP-based membrane at varying concentrations of the metoprolol (Fig. 6) indicates a rectilinear range from 2.0×10^{-7} M to 8.0×10^{-3} M. The slopes of the calibration curves were 55.4 ± 1 mV per decade of the metoprolol concentration. The limit of detection, as determined from the intersection of the two extrapolated segments of the calibration graph, was 1.3×10^{-7} M. The electrodes exhibit a stable potential response and almost unchanged metoprolol selectivity for at least 6 months.

Potentiometric Selectivity

The influence of interfering ions on the response behavior of ion-selective membrane electrodes is usually described in terms of selectivity coefficients. The potentiometric selectivity coefficients, $K_{Met.,J}^{Pot.}$ of the NIP and MIP-membrane sensors were evaluated by the separate solutions method [48,49]. As could be seen from Table 2, the selectivity coefficients obtained for the examined species indicated that most of these compounds did not disturb the functioning of the metoprolol-selective electrode significantly. As previously mentioned, non-specific interactions could occur in both MIP and NIP materials in comparison to specific shape-selective interactions. The carboxyl groups on the surface of MIP and NIP particles are the origin of non-specific interactions. Based on these findings, it is clear that the selectivities of the electrodes based on MIP and NIP are comparable for most of the ionic species.

Table 2. Selectivity Coefficient Values ($K^{Pot.}_{Met.,J}$) of the Proposed Electrodes Based on the MIP (I) and NIP (II) Particles

Interferent (J)	$K^{Pot.}_{Met.,J}$		Interferent (J)	$K^{Pot.}_{Met.,J}$	
	I	II		I	II
Urea	3.6×10^{-5}	7.7×10^{-4}	Mg^{2+}	8.3×10^{-5}	1.3×10^{-4}
Benzoic acid	2.7×10^{-2}	8.3×10^{-2}	Ca^{2+}	1.8×10^{-4}	2.9×10^{-4}
Oxalic acid	2.1×10^{-1}	9.9×10^{-1}	Ba^{2+}	3.2×10^{-5}	5.1×10^{-5}
Glucose	6.9×10^{-6}	2.3×10^{-4}	Pb^{2+}	3.4×10^{-4}	3.6×10^{-3}
Na^+	9.5×10^{-4}	1.1×10^{-3}	Cu^{2+}	9.9×10^{-5}	4.7×10^{-4}
K^+	5.1×10^{-4}	4.1×10^{-4}	Ni^{2+}	2.2×10^{-3}	7.1×10^{-3}
NH_4^+	7.5×10^{-4}	8.5×10^{-4}	Zn^{2+}	1.1×10^{-3}	5.2×10^{-3}

PRELIMINARY APPLICATIONS

Potentiometric Titration

It should be noted that the metoprolol-selective membrane electrode introduced here could be used for the indirect (titration) and direct potentiometric determination of the metoprolol ions. We successfully applied the MIP-based electrode as an indicator in the potentiometric titration of 25 ml of 0.005 M metoprolol against 0.1 M sodium tetraphenyl borate solution. It is well-known that metoprolol as a secondary amine reacts with tetraphenylborate to form a precipitate ion-pair product [50]. The resulting titration curve is shown in Fig. 7, indicating that the amount of metoprolol in solution could be determined with the proposed electrode.

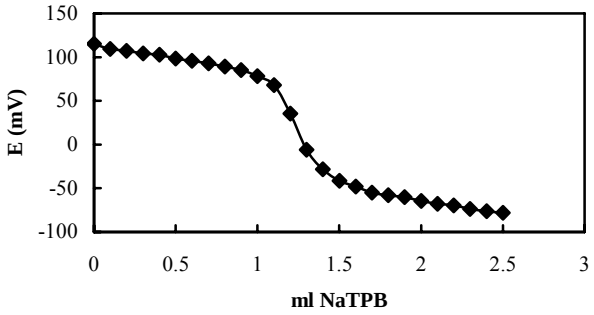

Fig. 7. Potentiometric titration curve of 25 ml of 0.005 M metoprolol solution with 0.1 M NaTPB, using the proposed sensor as an indicator electrode.

Determination of Metoprolol Tartrate in Metoral® Tablet

We successfully applied the introduced metoprolol-selective membrane electrode for the direct determination of metoprolol tartrate in the metoral® tablets obtained from a local pharmaceutical shop. These tablets are composed of metoprolol tartrate (50 mg per tablet) and some common excipients. Ten tablets were finely ground, homogenized and a portion of the powder (0.1812 g, average tablet mass) was weighed accurately, transferred into a 500 ml volumetric flask and diluted with water. The mixture was sonicated for at least 15 min to aid dissolution and then filtered. A 10 ml of the filtrate was diluted further to 50 ml with water so that the concentration of metoprolol in the final solution was within the working range, and then analyzed by the proposed MIP electrode. The metoprolol tartrate content of the metoral® tablet was found to be 49.1 ± 1.8 mg per tablet by the proposed potentiometric method. This accorded satisfactorily with those obtained by pharmacopoeia method [31] (48.1 ± 1.4 mg) and HPLC analysis [32] (48.7 ± 0.9 mg).

Recoveries of Spiked Metoprolol in Human Urine and Plasma Samples

The high degree of metoprolol selectivity exhibited by the membrane electrode makes it potentially useful for monitoring concentration level of metoprolol in real samples. In this regard, experiments were performed to determine the

Table 3. Recoveries of Spiked Metoprolol in Human Urine and Plasma Samples

Sample[b]	Metoprolol added (μg)	Metoprolol found (μg)	Recovery (%)[a]	
			Proposed method	HPLC
Urine	150.0	152.4	101.6 (3.8)	102.8 (2.3)
Plasma	150.0	153.3	102.2 (4.1)	103.9 (2.1)

[a]Average of three measurements with RSD% in parenthesis. [b]Sample volumes for the proposed method were 50 ml.

feasibility of using the electrode to measure metoprolol in human urine and plasma samples.

Blood samples (5 ml) were collected in heparin-containing tubes, protected from light and kept at 4 °C. Plasma was obtained after blood centrifugation at 1800 *g* for 5 min and was diluted ten times with water before potentiometric measurements. Urine sample (10 ml) was deproteinated with TFA (100 μl), vortexed for 3 min, and centrifuged for 5 min at 1500 g. The treated sample was diluted 100 times with water prior to potentiometric measurement by the proposed method. The results of the recovery tests are summarized in Table 3. The results are in satisfactory agreement with those determined by the HPLC method. Excellent recoveries were observed indicating that the constituents of the two analyzed samples did not interfere in any way with the detection of the metoprolol.

CONCLUSIONS

In conclusion, this report proposes a new strategy to construct a potentiometric biomimetic sensor for direct, rapid and highly selective detection of metoprolol. The strategy involves the dispersion of metoprolol imprinted polymer particles in dioctylphthalate, embedding the resulting mixture in PVC matrix and then casting the final composite after dissolving the template in tetrahydrofuran. The proposed graphite-coated membrane electrode revealed a near-Nernstian response over a wide metoprolol concentration range, fast response time and selectivity over a large number of metal ions. The stability, reusability and dynamic response time are analogous to the conventional chemical sensors. The analytical applicability of the proposed sensor was examined by the determination of metoprolol in tablets and recovery tests of metoprolol added to human urine and plasma samples.

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