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A new potentiometric sensor based on molecularly imprinted polymer for analysis of a veterinary drug imidocarb dipropionate

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ABSTRACT

A new potentiometric sensor based on molecularly imprinted polymer (MIP) was fabricated for the recognition and determination of imidocarb dipropionate. The MIP was synthesized using imidocarb as the template material, methacrylic acid as a functional monomer, and ethylene glycol dimethacrylate as a cross linking agent. The sensor showed a high selectivity and a sensitive response to the template molecule in aqueous solution. The MIP-electrode exhibited a near-Nernstian response in a wide concentration range 10^{-5} - 10^{-2} M with a lower detection limit of 2×10^{-6} M. The potentiometric conditions were carefully studied and all measuring parameters were optimized including pH, buffer type, plasticizer type, response time and stability. The applicability of the sensor was tested through potentiometric determination of imidocarb dipropionate in pure drug as well as in pharmaceutical formulation. The proposed method was statistically compared with a reported one showing no significant difference regarding accuracy and precision, which assured a good reliable novel sensor for imidocarb estimation.

1. Introduction

Molecularly imprinted polymers are synthetic matrices that mimic biological receptor systems, which have the capability to bind target molecule with similar affinity and specificity to antibodies and enzymes [1,2]. This unique feature of MIPs was introduced during synthesis stage, where functional and cross-linking monomers are co-polymerized in the presence of a target analyte (the imprint molecule). The polymer forms binding pockets around the template molecule. Complexation process (with covalent or non-covalent bond) will occur among these species, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Upon removal of the imprint molecule, binding sites that are complementary in size and shape to the analyte will be revealed. Thus, a molecular memory is introduced into the polymer, which is now capable of selectively rebinding the analyte [3]. In recent years, molecularly imprinted polymers (MIPs) have attracted much attention due to their outstanding advantages, such as predetermined recognition ability, stability, relative ease and low cost of preparation, and potential application to a wide range of target molecules [4,5]. Detection applications are employing transduction mechanisms including conductometry [6], amperometry [7-9], voltammetry [10,11], Quartz microbalance [12,13] and surface plasmon resonance [14]. Potentiometric technique is also well-known versatile, simple, rapid and inexpensive method for determination of target ion (molecule). Potentiometric technique is another approach to electrochemical transduction of ion selective sensors based on MIP [15,16]. Generally this approach utilizes MIP as a selective molecular recognition membrane or layer in chemical sensing systems.

Imidocarb is 1,3-*bis*-[3-(4,5-dihydro-1*H*-imidazol-2-yl) phenyl] urea. Among a variety of drugs that has been advocated over the years as therapeutic or prophylactic agents against infection with hemoprotozoa in domestic animals, imidocarb is considered the most efficacious and safest of all available medications [17]. It is a chemotherapeutic agent with antiprotozoal activity. It is usually administered as dipropionate and has been used over 20 years in the treatment and prophylaxis of some protozoal diseases such as babesiosis (red water fever) and anaplasmosis in food-producing species. In cattle imidocarb dipropionate is administered at a dose of 3 mg/kg body weight for the treatment of red water disease [17-19]. The concentration of imidocarb decreased from 5.40±0.61

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to 0.12±0.01 µg/g in liver and from 1.05±0.31 to 0.06±0.02 µg/g in muscle, this decrease occurred between days 14 and 224 after treatment. The depletion kinetics of imidocarb fitted a two-compartment model where the half-lives of alpha and beta phase in liver were 31.7 and 48.5 days, while in muscle they were 34.9 and 120.7 days, respectively. 7-Ethoxycoumarin metabolism was found in all *in vitro* systems where umbelliferone glucuronide was the predominant metabolite produced by hepatocyte and liver slice cultures [17]. The codex alimentarius reported that maximum residual limits (MRL's) in cattle different tissues; 300, 1500 and 2000 µg/Kg in muscle, liver and kidney, respectively.

Some methods have been reported for the determination of imidocarb in different tissues, from which an HPLC method was developed for the determination of imidocarb in cattle kidney with cation-exchange clean-up followed by ultraviolet detection at 260 nm [20]. Also HPLC with mass spectrometric detection was performed for the analysis of imidocarb in livestock and seafood products [21], in equine tissues [22] and in bovine tissues and milk samples [23].

In this paper a versatile, reliable and rapid potentiometric technique based on molecularly imprinted polymer for the determination of imidocarb in bulk powder and in dosage form will be attempted.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical grade. High molecular weight polyvinyl chloride (PVC) powder, nitophenyloctyl ether (*o*-NPOE), nitrophenylphenyl ether (NPPE), dioctylphthalate (DOP), dioctylsebacetate (DOS), were obtained from Fluka-Biochemica (St. Louis, USA). Methacrylic acid (MAA), ethylene glycol dimethaacrylate and benzoyl peroxide (BP) were all purchased from Merck (Darmstadt, Germany). Acetonitrile, methanol, acetic acid and tetrahydofuran (THF) were obtained from Sigma-Aldrich (Steinheim-Germany). Sodium chloride, potassium dihydrogen phosphate and sodium hydroxide were purchased from BDH (Poole, UK).

Imidocarb dipropionate (Batch number 110309) of purity 100.48% (as determined by a reported method [20]); was kindly obtained from National Organization of Drug Control and Research (NODCAR), Giza, Egypt, and was used as provided. A Stock solution of 0.1 M imidocarb dipropionate was prepared in deionized water.

Phosphate buffer (pH = 7.0) was prepared by adding 29.1 mL of 0.2 M NaOH to 50 mL 0.2 M potassium dihydrogen phosphate and the volume was completed to 200 mL with water.

Imidox injection (Batch No. 10-027A) labeled to contain 120 mg/mL imidocarb dipropionate which is equivalent to 85 mg/mL imidocarb base (Afrivet Business Management (Pty) Ltd, Faerie Glen, South Africa). It was obtained from commercial sources in the local market.

2.2. Instrumentations

Spectrophotometric measurements at λ_{max} = 260 nm were performed on Milton Roy spectronic 21D. Scanning electron microscope JEOL JXA-840A electron probe microanalyzer. FT-IR Shimadzu 8900 controlled by IR solution FTIR control software.

All potential measurements were made at 25±1 °C with a Jenway (UK) Model 3305 pH/mV meter using imidocarb membrane sensor in conjunction with an Orion Ag/AgCl single-junction reference electrode (Model 90-20). A combination Orion Ross glass electrode (81-02) was used for pH adjustments; the reference method for imidocarb determination [20] was carried out on Agilent 1200 HPLC connected to multi-variable UV detector.

2.3. Synthesis of molecular imprinted polymer

The polymerization was accomplished by adding 0.1 mM imidocarb dipropionate (template molecule), 1.0 mM MAA (monomer), 10.0 mM ethyleneglycoldimethacrylate (cross linker) and 0.06 g benzoyl peroxide (initiator) to 3 mL aceto-nitrile solution as a porogen solvent. The mixture was sonicated and degassed with nitrogen for 10 min before heated to 60 °C until it solidified. After removing the solvent, the polymer was isolated and dried. The resulting polymer was crushed, grounded and sieved. The particle size < 75 µm was used as a sensing material. Leaching studies of template were carried out using methanol, acetic acid and alkaline solutions several times to remove the unreacted ingredients and templated molecule. After the polymer was completely dried at ambient temperature, it was used as an active medium in the selective sensor. The blank polymer, non-imprinted polymer (NIP), was similarly prepared by omitting the imprint molecule in the same manner.

2.4. Preparation of the membrane sensor

The sensing membrane was prepared by mixing 24.624 mg of PVC powder and 1.296 mg of prepared imidocarb dipropionate templated polymer (MIP) particles with 45.36 mg of a plasticizer. The mixture was stirred until the PVC is well moistened, and then the mixture was dispersed in 3.0 mL THF. The resulting mixture was adequately mixed and then was poured onto a glass cup of 18 mm i.d. The solvent was evaporated slowly at room temperature until a solid membrane with about 0.3 mm thickness was formed. A desired piece of the membrane was cut and then was attached to an end of a Tygon tube (3.00 mm i.d. and 3 cm long) using a viscous solution of PVC in THF as an adhesive. The resulting sensor was then filled with an internal solution of 10^{-2} M imidocarb dipropionate and conditioned for 24 h. Finally a step conditioning was carried out in 10^{-2} M imidocarb dipropionate for stabilization of the sensor function before each series of measurements.

2.5. EMF measurements

The performance of the sensor was investigated by measuring the EMF values of various imidocarb dipropionate solutions. Potentiometric evaluation of the electrodes was carried out using the following cell (Ag/AgCl|MIP membrane| internal solution imidocarb dipropionate|Ag/AgCl). All measurements were made at room temperature.

2.6. Procedures

2.6.1. Calibration of the prepared sensor

The above mentioned imidocarb biomimetic sensor in conjunction with a single-junction Ag/AgCl reference electrode were immersed in 25 mL beaker. Aliquot volumes from standard imidocarb dipropionate solution (0.1 M) were diluted with phosphate buffer (pH = 7.0) to obtain a final concentrations of 10^{-5} - 10^{-2} M. 20 mL of these solutions were transferred to the beaker and the potential readings after each addition were recorded after stabilization to ±0.2 mV and the calibration curve was plotted. The calibration plot was used for measuring unknown concentrations under the same conditions.

2.6.2. Application of the proposed method for imidocarb in pharmaceutical preparation

Imidox injection was assayed without sample pretreatment. Aliquot volume of the injection was diluted with deionized water to obtain a stock solution of 0.1 M imidocarb

dipropionate, then, the procedure was completed as under calibration of the prepared sensor.

3. Results and discussion

3.1. Physical characterization of imidocarb MIP

The micrographs of imidocarb MIP and NIP were investigated by the scanning electron micrography (SEM). Figure 1 shows appreciable differences in the morphology of the polymers. The non-imprinted polymer had a more uniform, smooth shape than the imprinted polymer which had an irregular, rough morphology (rather like micro particles with small cavities). The regular structure of the non-imprinted polymer was due to the fact that no specific binding sites had been created for the analyte. The cavities in the MIP were probably caused by the structure of the target molecule (Imidocarb).

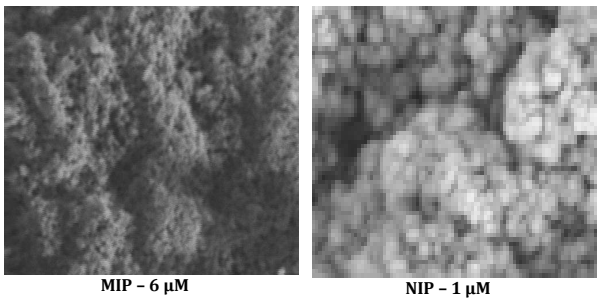

Figure 1. Surface morphology of both MIP and NIP.

3.2. Binding of imidocarb to molecularly imprinted polymer

Binding experiments of imidocarb to the methacrylic imprinted polymer were performed in 10 mL of imidocarb aqueous solution having 5-20 mM concentrations at 25 °C. The change of imidocarb concentrations in the aqueous solution was determined by monitoring UV absorbance at 260 nm with UV detector. Amounts of imidocarb bound to the imprinted polymer were calculated by subtracting the amount of free substrate from the initial amount of the template. The adsorption capacity (Q), was calculated by the equation (1).

$$Q = (C_0 - C_t)\, V / W \quad (1)$$

where C_0 and C_t represent the initial and equilibrium concentration of imidocarb, V is the volume of imidocarb solution (10 mL) and W is the weight of dry polymer (0.015 g) used for the binding experiment. It was found that the values of (Q) increased with time and became constant at longer time than 12 h. By increasing the concentration of imidocarb from 1000 to 10000 µM, the value of (Q) was 1.9 and 3.4 µmol/g polymer, respectively, which indicates that the binding sites of the imprinted polymer were filled with imidocarb.

3.3. FT-IR spectroscopy

Infrared spectra were used as a tool in order to consider the origin of the selectivity of imidocarb by the imprinted polymer. The FT-IR spectra provided information on the interaction between the imprinted polymer and templates via hydrogen bonding [24,25]. The IR band of free amide group near 3000 cm^{-1} was monitored. In the IR spectra, the characteristic IR bands of amide-I and amide-II of methacrylate appeared near 1642 and 1546 cm^{-1}, which were assigned to C=O stretching vibration (amide-I) and N–H deformation (amide-II), respectively. There are another absorption peaks near 1265, 695 and 578 cm^{-1} for amide-III, amide-IV, and amide-V, respectively.

We noted the spectra difference of the amide-I and II region. A variation in the absorption peak height of amide-II stretching was observed before and after the L-glutamine extraction. This is assigned to inter- and intramolecular hydrogen bonding. The IR absorption band of amide-I at 1642 cm^{-1} became slightly broader than that obtained without the template extraction. In addition, the absorption peak of amide-II at 1546 cm^{-1} was slightly shifted to lower wave number side of 1539 cm^{-1} after the template extraction.

The peak heights corresponding to amide-II was apparently reduced and the peak widths became slightly broad after the extraction. Thus, the spectral data obtained here explained that the hydrogen networks between the methacrylate chains was the origin to the recognition of the imidocarb molecules.

3.4. Optimization of the parameters for imidocarb determination

3.4.1. Effect of pH

The pH effect of the tested solution on the electrochemical behavior of the sensor was studied under a constant concentration of imidocarb dipropionate and varying the content of the hydrogen ions in the pH range of 3.0-11.0 which was adjusted with H_2SO_4 or NaOH solutions. As shown in Figure 2, the potentials were kept constant in the range of 6.0-7.0. The observed potential drift at lower pH values might be attributed to the membrane response to H^+ and at higher pH values (pH > 7) could be due to formation of hydroxo species. Consequently pH of 7 is selected as the best condition for further investigation and the phosphate buffer is then employed.

As the selected appropriate pH is 7, the employed buffers might be phosphate, saline sodium citrate and HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid). The phosphate is of high buffer capacity, cheap and available in most of laboratories so it is selected for further investigations.

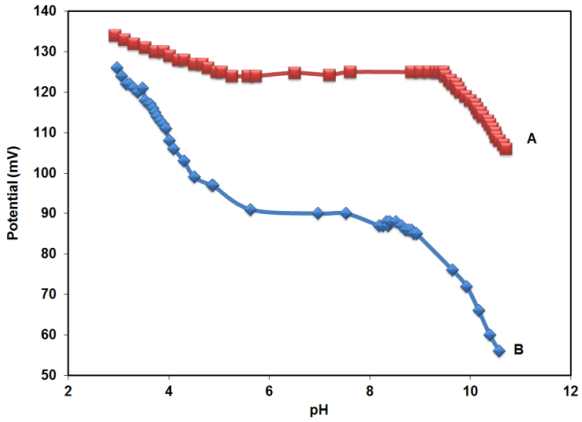

Figure 2. Effect of pH, (A): 1×10^{-3} M; (B): 1×10^{-4} M.

3.4.2. Effect of type of plasticizer

Upon embedding different plasticizers; dibutylsebacate (DBS), dioctylphthalate (DOP), nitrophenyloctylether (NPOE) and nitrophenylphenylether (NPPE) into the plastic liquid polymeric membrane mixture, the best response of the sensors was shown by NPPE, NPOE followed by DOP, respectively (Figure 3). While the sensor membrane embedded with DBS showed sluggish response so both NPOE and NPPE could be used.

Table 1. Potentiometric selectivity coefficients $K_{M,X}^{pot}$ for the imidocarb MIP based sensor.

Interfering compound	$K_{M,X}^{pot}$
Sodium sulphate	-1.969
Magnesium sulphate	-2.125
Sodium chloride	-2.125
Potassium nitrate	-2.125
Calcium chloride	-2.090
Ferric chloride	-1.031
Diminazene aceturate	-2.120
Glycine	-1.031

Table 2. Determination of imidocarb in its pure form using the proposed membrane sensor method.

Parameter	Amount taken in µg/mL	Amount found in µg/mL	% Recovery [b]	Comparative method [20]
Imidocarb	5.00	5.01	100.20	100.73
	500.00	498.75	99.75	99.96
	1000.00	1007.80	100.78	100.89
	2000.00	1991.20	99.56	100.16
	3000.00	3009.00	100.30	100.66
	4000.00	3950.00	98.75	
	5000.00	5032.00	100.64	
Mean ± S.D			99.99±0.70	100.48±0.40
Variance			0.49	0.16
t test (2.23) [a]			1.37	
F test (6.16) [a]			3.11	

[a] The values in parenthesis are the corresponding theoritical values of *t* and *F* at $p = 0.05$, where n = 7 for the proposed method and n = 5 for the reported method.
[b] Average of three determinations.

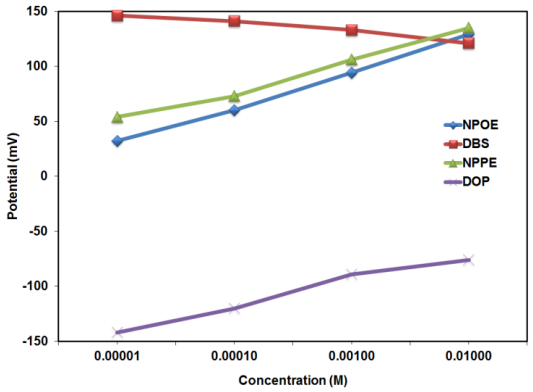

Figure 3. Effect of plasticizer type on imidocarb sensor response.

3.4.3. Response time and stability

The response time is an important factor for the operation of each potentiometric sensor. The response time of the sensors, which was evaluated by measuring the time required to achieve a steady–state potential (within ±0.5), was less than 10 sec for all imidocarb solutions in the linear range of calibration curves. The stability and reproducibility of the response of the electrode were also tested. The potentials remained constant for ~10 min.

3.4.4. Interference study

Since tissue samples contain rather high concentrations of interfering ions, it is necessary to consider the interference of co-existing ions in liver and kidney samples. The selectivity of the MIP-based sensor was characterized [26] to evaluate the influence of the discriminated ions (Figure 4). The selectivity coefficients $K_{M,X}^{pot}$ for imidocarb ions over other cations were estimated. Potentiometric selectivity coefficient values for the MIP based sensor are summarized in Table 1.

Sodium sulphate, magnesium sulphate, sodium chloride, potassium nitrate, calcium chloride, ferric chloride, glycine and tetracycline hydrochloride were not tolerated up to ten- fold excess than 10^{-4} M imidocarb. It can be seen that the proposed electrode shows high selectivity for imidocarb ions over most inorganic and organic cations normally found in tissue (liver or kidney), which is promising for us to try the use of the synthesized electrode in the determination of imidocarb in different animal tissues as a future work.

3.5. Validation of the proposed method

3.5.1. Linearity and range

Calibration curve was plotted and the linear range was from 10^{-5} - 10^{-2} M with a lower detection limit of 2×10^{-6} M and the regression equation was y = 218.96-24.71 x (r^2 = 0.9992). However, for the NIP-based membranes that exhibit a near-Nernstian response in a rather narrow concentration range, only the nonspecific interaction of the imidocarb ions with the ion-exchanger occurs. Evidently, it can be demonstrated that the MIP is effective for specific recognition of the target ions.

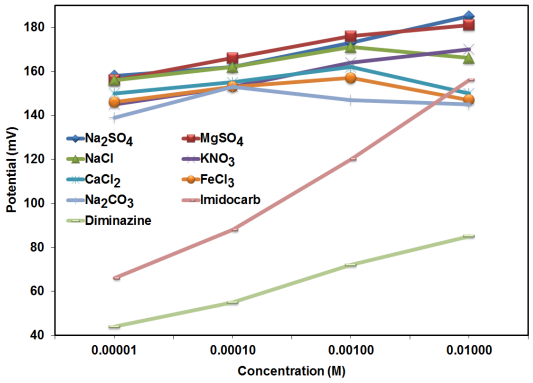

Figure 4. Effect of interferents on imidocarb sensor response.

3.5.2. Accuracy

The accuracy of the proposed method was evaluated by analyzing standard solutions of the studied drug. The results obtained by the proposed method were favorably compared with those obtained by a comparison HPLC method [20]. The percentages found were calculated, the results obtained in Table 2, showed excellent accuracy.

Table 3. Precision data for imidocarb by the proposed membrane sensor method.

Parameter		Concentration of imidocarb in µg/mL		
		35.00	**100.00**	**1000.00**
Intra-day	% Recovery [a]	102.40	100.72	98.90
		102.12	100.50	99.79
		101.11	99.34	100.10
	Mean	101.88	100.19	99.60
	± S.D	0.68	0.74	0.62
	% R.S.D	0.67	0.74	0.63
Inter-day	% Recovery [a]	99.85	100.09	100.40
		100.50	99.45	101.15
		101.31	99.65	99.97
	Mean	100.55	99.73	100.51
	± S.D	0.73	0.33	0.60
	% R.S.D	0.73	0.33	0.59

[a] Average of three determinations.

Table 4. Determination of imidocarb in its dosage form using the proposed membrane sensor method.

Parameter	Amount taken in µg/mL	Amount found in µg/mL	% Recovery [b]	Comparative method [20]
Imidocarb	5.00	4.96	99.20	100.15
	500.00	504.50	100.90	99.15
	1000.00	996.70	99.67	100.36
	2000.00	2010.60	100.53	99.81
	3000.00	3024.00	100.80	100.62
	4000.00	3940.00	98.50	
	5000.00	4967.50	99.35	
Mean ± S.D			99.85 ±0.91	100.02±0.57
Variance			0.83	0.32
t test (2.23) [a]			0.36	
F test (6.16) [a]			2.57	

[a] The values in parenthesis are the corresponding theoritical values of *t* and *F* at *p* = 0.05, where n = 7 for the proposed method and n = 5 for the reported method.
[b] Average of three determinations.

Statistical analysis [27] of the results obtained by the proposed and comparison methods using student´s t-test and variance ratio F-test revealed no significant difference between the performances of the two methods.

3.5.3. Precision

The repeatability was evaluated through replicate analysis of three different concentrations of imidocarb. The mean percentages found are based on the average of three separate determinations as shown in Table 3.

The Intermediate precision was performed through replicate analysis of three different concentrations of imidocarb on three successive days. The mean percentages found are based on the average of three separate determinations as shown in Table 3. The data indicate that the proposed method is highly precise during one run and between different runs.

3.5.4. Ruggedness and robustness

The ruggedness of the proposed method was ascertained using two different potentiometers (Orion710A and Jenway 3305). The robustness of the methods was evaluated by observing the influence of small variations of experimental variables, i.e., the volume of the reagent (±0.1 mL), mV drift of detection ±1 mV and the reaction temperature (25±5 °C). These minor changes that may take place during the experimental operation did not greatly affect the measured potential.

3.6. Application to pharmaceutical preparation

The proposed MIP-based sensor was used to analyze imidocarb in imidox injection samples and the results are given in Table 4. It can be seen that good recoveries of imidocarb in imidox injection upon direct determination vary from 98.50 to 100.90 %.

4. Conclusion

This study demonstrated the potential of imidocarb imprinted polymer which was synthesized as smart material for recognition of imidocarb. In addition, a novel potentiometric PVC polymeric membrane sensor based on a molecularly imprinted polymer was fabricated for selective recognition and determination of imidocarb dipropionate. Validation of the assay method according to the quality assurance standards (range, within-day repeatability, between-day variability, standard deviation, accuracy, and precision) was justified ensuring a reliable novel sensor for imidocarb estimation. Application of the proposed assay method for routine determination of imidocarb in pure drug and pharmaceutical formulation was verified. Samples of liver and kidney from bovine animals will be analyzed in a future work to ensure their suitability for human consumption.

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