RESEARCH ARTICLE

Synthesis New Liquid Electrodes for Determination Pyrazinamide Based on a Molecularly Imprinted Polymer

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ABSTRACT

Liquid electrodes of Pyrazinamide (PZD) imprinted polymer were synthesis based on precipitation polymerization mechanism. The molecularly imprinted (MIP) and non-imprinted (NIP) polymers were synthesized using PZD as a template. By 1-Vinylimidazole (VIZ) and methyl methacrylate (MMA) as monomers, trimethylolpropane trimethacrylate(TPT) cross-linkers and benzoyl peroxide (BPO) as an initiator. The molecularly imprinted membranes were synthesis using Dibutyl Sebacate (DBS), Dibutyl phthalate (DBPH), Dioctyl phthalate(DOPH), and Nitrobenzene (NO) as plasticizers in PVC matrix. The slopes and limit of detection of liquid electrodes obtained from the calibration curves ranged from (-27.86– 29.96) mV/decade and 1.8 x 10^{-5} – 6.0 x 10^{-6} M, respectively, and the response time was about 60 seconds. The Liquid electrodes were filled with 10^{-1} M standard solution of the drug and observed stable response for a pH ranged from 2.0 to 11.0 and with good selectivity for over several species. The fresh electrodes of synthesis were effectively used in the pharmaceutical sample to determine PZD without any time-consuming pretreatment measures.

Keywords: (MMA), Molecularly imprinted electrodes, Potentiometric method, Pyrazinamide, (VIZ) monomers.

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INTRODUCTION

Molecularly impressed polymers (MIPs) are a promising solution to tailor-made binding receptor locations by rearranging templates and rearranging functional monomers.[1-4] Functional monomers and crosslinkers involving the formation of cavities in which the model is placed in the presence of template molecules. By bonding with hydrogen In the first step, the template interacts with a functional monomer, reversible covalent bonds, electrostatic interactions, and van der Waals. In a second phase, In the presence of a large excess cross-linking agent, the monomer-template complex is polymerized. The chemical bonds between the monomer and the cross-linker make room for the functional monomer model. Finally, the template can be separated from the polymer framework after polymerization, which shows binding sites with additional shape, size, and chemical features.[5-6] Pyrazinamide (pyrazine-2-carboxamide, $C_5H_5N_3O$) Figure 1 occurs as a white crystalline powder with a molecular weight of 123.11. It melts within (188-193 ^oC). It's odorless and has a bitter taste. It is sparsely water-soluble, mildly ethanol-soluble (95%), and very mildly diethyl ether-soluble.[7] Pyrazinamide plays a Distinctive role in shortening therapy from 9–12 months to 6 months as it kills a semi-dormant tubercle bacilli population in acidic pH environments that are not killed by other TB medications.[8]

The model is Leached and left cavities in form, size, and shape function that are essential to the model. In latest years,[9] MIP technology has created a precious extensive idea for biological activity with enhanced applicability in analytical chemistry,[10] demonstrating a distinct and quick technique of synthesizing a polymer matrix with molecular activity characteristics for applications ranging from the purification of racemic mixtures to catalytic control and chemical sensing of complicated chemical reactions. Some drugs such as ibuprofen[11] and warfarin sodium[12] were determined based on a polymer technology that was molecularly impressed. Pyrazinamide-based Polymer electrodes have been prepared as a PVC matrix membrane template, and electrode specifications have been studied in this research (Figure 1).

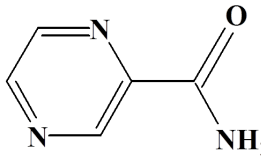

Figure 1: Structure of pyrazinamide.

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EXPERIMENTAL

Chemicals

Pyrazinamide was obtained from the State Company of Drug Industries and Medical Appliances (IRAQ-Medial East-Baghdad). The Commercial Pyrazinamide tablets B.P (400 mg), India. All drugs were purchased from local pharmacies.

Dibutyl Sebacate (DBS), Dibutyl phthalate (DBPH), Dioctyl phthalate(DOPH), and Nitrobenzene (NO) In addition to metal salts, they were bought from Sigma-Aldrich and used as obtained. Vinylimidazole (VIZ) (99%), methyl methacrylate (MMA) (99%), trimethylol propane trimethacrylate (TPT) (99Benzoyl peroxide (BPO) (78%) and benzoyl peroxide (BPO) were bought from Sigma-Aldrich. The chemicals that have been used in the quest have elevated purity that need not be purified.

Apparatus

A UV-visible double-beam spectrophotometer (UV-1650 PC) SHIMADZ (Japan), computer interfaced via the SHIMADZU UV probe information scheme (version 1.10), using 1.00 cm quartz cells, SHIMADZU infrared spectrophotometer; digital voltmeter (HANA pH 211 instrument Microprocessor pH meter) was used to perform potential measurements. Digital pH meter pH measurements (Wissenschaftlich-TechnischeWerkstätten GmbH WTW/pH meter in laboratory pH720-Germany) were performed, FTIR-8000 (Japan), Scanning Electron Microscopy (SEM) [JSM-6390A] (Tokyo, Japan) and sensitive balance (Electronic balance ACS120-4 Kern & Sohn GmbH, Germany. The performance of the electrode was investigated by measuring the potential of Pyrazinamide solutions at room temperature with concentrations range from10^{-1} to 10^{-6} M. For the accuracy, the potential of solutions was measured after the arrival of the internal and external solution to the equilibrium, then the potential recorded.

Synthesis of the imprinted polymer (MIP)

The method of bulk polymerization was used to prepare MIP. The 0.5 mmol model (PZD) was placed in a dense walled glass tube filled with 10 mL chloroform (50 mL capacity). Two monomers have been used to prepare MIP, 5 mmole of 1-VIZ with 12.5 mmoles TPT as a cross-linker, the second MIP based on 3mmol of MMA monomer 12.5 mmol TPT as cross-linker. The initiator of 0.32 mmole BPO was used. The solution was mixed in ultrasonic water bath for 45 minutes. The nitrogen gas was purified during this time. After 45 minutes seal the tube and put the tube in 55°C water bath to permit starting the reaction which continued for 1 hr through the use of soxhlet extraction, the templates were removed by repeated cleaning the MIPs with 100 mL parts of 30 percent (v/v) acetic acid / methanol solution. The paper was washed for (24–48) hours at (35-45) ºC, the polymers were then crushed and ground using mortar and pestle, and 125 µm of particle size was sieved (using 100 mesh sieves).); it was used as active material in the selective sensor membrane after the polymer had been completely dried at ambient temperature. The unprinted polymer NIP was produced in the same manner, but without the drug for the template. For the preparation of specific PVC membranes, high molecular weight PVC (0.17 g) is mixed with MIP (0.02 g) and plasticizer (0.4 g) until the solution is homogenized, then add THF (4-5 mL) and stirred. The solution was transferred to a 5 cm dia glass board based glass vessel. Circular section for 24 hours to allow this combination to evaporate. A glass tube contained a silver wire painted with silver chloride and filled with 0.1 M normal metronidazole benzoate solution was tightly connected to one end of the Tygon tube while the second end of the tube was tightly connected to 10 mm dia. PVC membrane circular disk using a focused PVC/THF solution as a glue for electrode production. For the sake of clarity of the particle morphology and layout, a scanning electron microscope (SEM) has been used. Figure 2 shows the morphology of MIP and NIP membranes for Metronidazole benzoate before and after washing. The binding sides to the polymer may be indicated by a porous surface (Figure 2a) about 1 mm. Figure 2b indicates clear holes that were collected in dimensions of around 50 µm and removed through soxhlet extraction.

Potential measurements

Measurements were carried out in a 50 mL double-walled glass cell, and magnetic stirring was used to obtain a homogeneous solution and under laboratory. The effectiveness of the electrodes was scrutinized by measuring the ability of conventional medication alternatives prepared with a

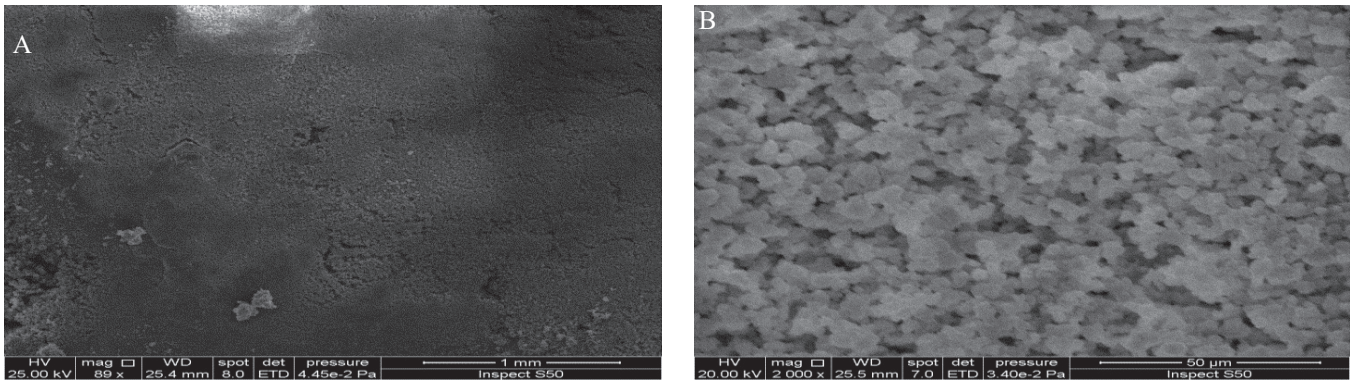

Figure 2: SEM photograph of the surface of MIP, a) before washing b) after washing

concentration range of 10^{-1} to 10^{-6} M through serial dilution. From the calibration curve, the operating life of the slope, detection limit, and response time were calculated.

Preparation of Pharmaceutical Samples

To determine the concentration of two kinds of tablets Pyrazinamide Two kinds of tablets have been used to determine Pyrazinamide concentration, India: B.P (400)mg Pyrazinamide tablets, India (Pyrazinamide in mixture) B.P(400)mg (Rifampicin 150mg, Isoniazid 75mg, pyrazinamide 400mg and Ethambutol Hydrochloride 275mg Tablets) manufactured in India by MACLEODS PHARMACEUTICAL and Dissolved in 1 M (HCl) and finished in (100ml) volumetric flask.

RESULTS AND DISCUSSION

Characterization

The fourier-transform infrared spectroscopy (FTIR) spectrum of drugs, MIP basecl on (1-vinylimidazole) as the fundamental functional monomers (before and after removing the template) and their NIPs .The primary peaks appearing in these statistics were summarized in Table 1 .

From the Table 1 show two sharp bands for asymmetrical and symmetrical N-H stretching 3413-3290 cm-1 for PZD and a band for Ar-H stretching at 3066 cm-1, which disappeared after soxhlet extraction system also appearance bands for C=O (ester) 1726 cm-1. And C-H (aliphatic)2972-2887 cm-1 stretching all this data indicated to removal PZD from the template. The FTIR spectrum of drugs, MIP based on (methyl methacrylate) as the functional monomers (before and after moving the template) and their NIPS. The primary peaks appearing in these statistics were summarized in Table 2.

Table 2 show two sharp bands for asymmetrical and symmetrical N-H stretching 4313-3290 cm^{-1} for PZD, a band for Ar-H stretching at 3066 $cm^{-1}$and C=O (amide)stretching at 1714 cm-1 which disappeared after soxhlet extraction system also appearance bands for C=O (ester) 1731cm^{-1} and C-H (aliphatic)2981-2891 cm^{-1} and C=C 1614 cm^{-1}stretching all this data indicated to removal PZD from the template.

Liquid Membranes Electrode

MIP based liquid electrodes, their concentrations range and slopes response to the Nernstian equation have been investigated. The membranes of MIP made of the monomers MMA and VIZ with a PVC matrix using two plasticizers DBS and NO. The internal solution was used 0.1M standard aqueous solution of a drug for all liquid electrodes. Experimental results of the synthesis of MIP and non-imprinted polymers (NIP) based on two monomers MMA and VIZ indicate that both monomers can be used for the preparation of effective MIP for PZD. The plasticizer is an essential part of the sensing membrane, which have important role as a solvent for the different components and determines the mobility of the analyte in the membrane. Both of the plasticizers that are used, DBS and NO, are suitable for the fabrication of MIP-based PZD electrodes. Table 3 shows parameters of the fabricated and tested electrodes. Four membranes of the different compositions were prepared using two different plasticizers with different viscosities, dibutyl sebacate (DBS) (ν = 11.0042cSt) and nitrobenzene (NO) (ν = 2.030cSt). Electrode specification findings were acquired from the calibration curves mentioned in Table 3. The slopes of the electrodes ranged between -27.866-29.962 mV/decade and linear dynamic ranges between $3.0x10^{-5}$- 6.0 $x10^{-6}$ M. In general, the preparation electrodes have a short response time (about 60 seconds) mostly at high concentrations. The values listed in Table 3 also indicate the electrodes IQ and IVQ give good results; therefore, the liquid electrode were used to determine both drugs in pharmaceutical samples.

Influence of pH

The impact of pH on the possible values of the four electrodes over the pH range was researched from 1.5 to 12 and adjusting the pH by adding drops of 0.1 M HCl, and 0.1 M NaOH to the aqueous solutions of the drugs and the obtained potentials at each value were recorded. The effect of pH on the electrode potential was recorded for concentrations range from 5×10^{-4} to

Table 1: FTIR Spectra peaks for PZD - printed polymer using (VIZ) as a functional monomer

No	*Functional group*	*Drug (PZD)*	*PZD-MIP (VIZ) before template removal*	*PZD-MIP (VIZ) after template removal*
1	N-H. (cm^{-1})	3413-3290	3923-3380	-----
2	C = N. (cm^{-1})	1610	1643	1637
3	C = O (amide). (cm^{-1})	1714	1726	1726
4	Ar-H.(cm^{-1})	3066	3103	-----
5	C = C.(cm^{-1})	1579	1643	1637
6	C = O (ester).(cm^{-1})	-----	1726	1726
7	C-H (aliphatic). (cm^{-1})	-----	2972-2887	2970-2831

Table 2: FTIR Spectra peaks for PZD – printed polymer using (VIZ) as a functional monomer

No	*Functional group*	*Drug (PZD)*	*PZD-MIP2 (MMA) before template removal*	*PZD-MIP2 (MAA) after template removal*
1	N-H(cm^{-1})	4313-3290	3476-3392	-----
2	C = N(cm^{-1})	1610	1556	-----
3	C = O (amide)(cm^{-1})	1714	1639	-----
4	Ar-H(cm^{-1})	3066	3100	-----
5	C = C(cm^{-1})	1579	1514	1541
6	C = O (ester)(cm^{-1})	-----	1731	1731
7	C-H (alphatic)(cm^{-1})	-----	2956-2860	2981-2891
8	C = C(cm^{-1})	___	___	1614

Table 3: Parameter of PZD-MIP electrodes based on different plasticizers

Electrode No.	*Membrane composition*	*Parameter*				
		Slope mV/decade	*Correlation Coefficient(r)*	*Linearity range/M*	*Detection limit/M*	*Life time/day*
IQ	PZD-MIP1(VIZ TPT + DOPH)	-19.867	0.9992	(1×10^{-4}-1×10^{-1})	7×10^{-5}	17
IIQ	PZD-MIP1(VIZ TPT +NO)	-27.866	0.9990	(1×10^{-5}-5×10^{-1})	3×10^{-5}	21
IIIQ	PZD-MIP2(MMA+TPT + DBPH)	29.962	0.9982	(1×10^{-5}-1×10^{-1})	6×10^{-6}	28
IIVQ	PZD-MIP2(MMA+TPT + DBS)	20.26	0.9895	(1×10^{-4}-1×10^{-1})	4×10^{-5}	32

5×10^{-3} M of standard solutions of drugs. The obtained results are shown in Table 4, and the typical plot of electrode potential versus pH for electrode IQ and IVQ are shown in Figure 3.

Response time and lifetime

The response time for all PZD. MIP electrodes were obtained from the dynamic potential response at a concentration range between $1 \times 10^{-6} - 1 \times 10^{-1}$ M by measuring the time required to reach 95 % equilibrium potential. The results indicate that the response time of the electrodes were approximately 25.2 seconds for the solution of Pyrazinamide at high concentration 10^{-1} M and about 59 seconds at low concentration10^{-6} M. The electrode lifetime was obtained by measuring the slope periodically from calibration curves for PZD. MIP during 17–32 days, as shown in Table 5.

Quantitative analysis

The accuracy of electrodes IQ and IVQ were measured by determining Pyrazinamide in synthetic solutions of 5×10^{-3} and 5×10^{-4} M using the standard addition method. Excellent results

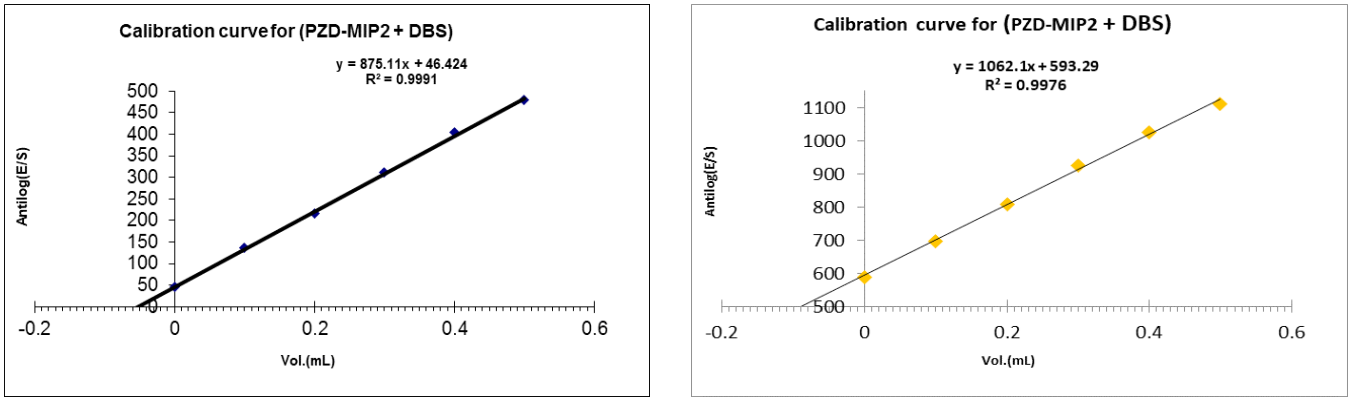

Figure 11: Variation of antilog(E/S) of a synthetic solution of $5x10^{-3}$, $5x10^{-4}$ M versus of standard PZD added using electrode (IVQ)

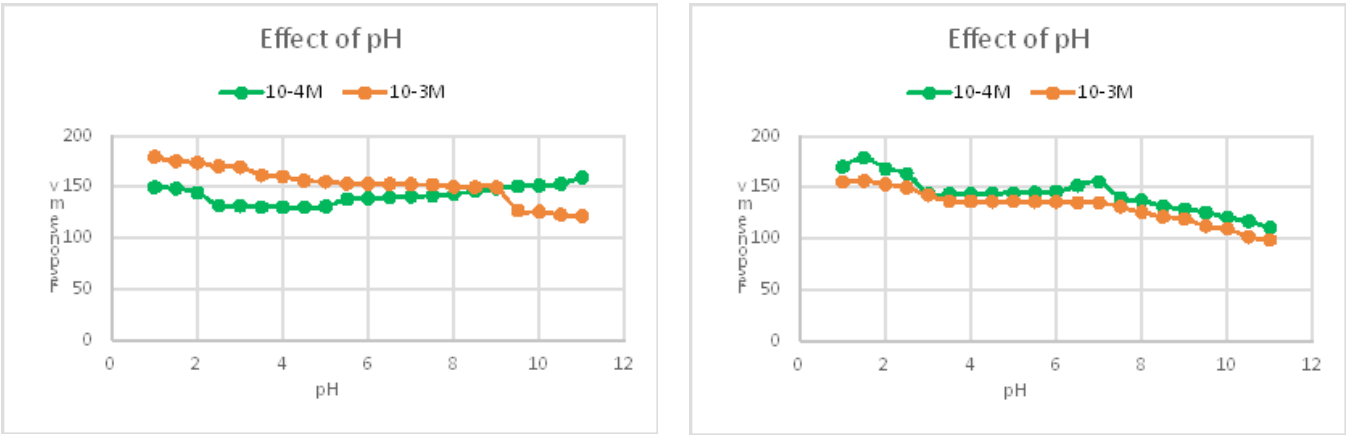

Figure 8: Typical plot of electrode response versus pH of PZD-MIP electrodes at different concentrations

Table 4: Working pH ranges for PZD-MIP electrodes

Electrode No.	*Membrane composition*	*pH range*	
		$5x10^{-3}$	*$5x10^{-4}$*
IQ	PZD-MIP1 + DOPH	4.5-7.5	2.5-5.0
IIQ	PZD-MIP1 + NO	3.0-7.0	3.5-6.0
IIIQ	PZD-MIP2 +DBPH	4.0-7.5	2.5-5.0
IVQ	MNZB-MIP2 +DBS	3.5-7.0	3.0-6.0

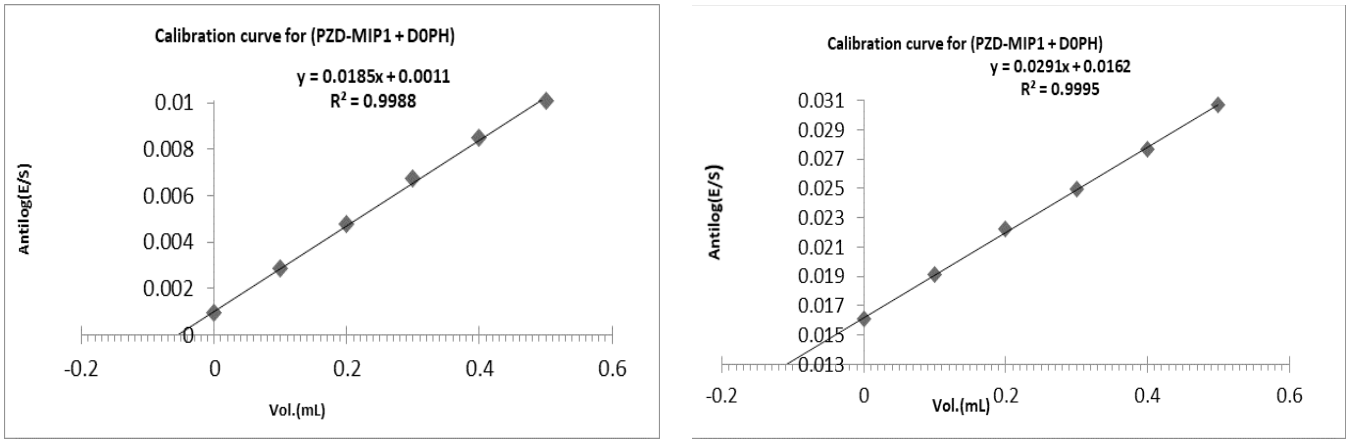

Figure 10: Variation of antilog (E/S) of a synthetic solution of 5×10^{-3}, 5×10^{-4} M versus of standard PZD added using electrode (IQ)

Table 5: Response time of Pyrazinamide electrode

Membrane	*Conce. (M)*	*(mV) at t/100*	*Time (s) at 95%*	*Time (s) at 100%*
PZD-MIP1 + DOPH (IQ)	10^{-1}	16.15	55	57
	10^{-2}	39.23	52	54
	10^{-3}	28.59	51	53
	10^{-4}	21.94	51	53
	10^{-5}	35.90	55	57
	10^{-6}	25.65	50	52
PZD-MIP1 + NO (IIQ)	10^{-1}	23.37	46	48
	10^{-2}	22.80	48	50
	10^{-3}	35.34	55	54
	10^{-4}	33.34	51	53
	10^{-5}	23.94	56	58
	10^{-6}	21.94	50	57
PZD-MIP2 + DBPH (IIIQ)	10^{-1}	28.78	46	48
	10^{-2}	24.03	53	55
	10^{-3}	33.63	53	55
	10^{-4}	25.36	43	45
	10^{-5}	25.27	57	60
	10^{-6}	28.69	46	48
PZD-MIP2 + DBPH (IVQ)	10^{-1}	21.09	36	37
	10^{-2}	26.70	46	48
	10^{-3}	23.84	52	54
	10^{-4}	34.67	52	54
	10^{-5}	27.64	48	50
	10^{-6}	31.54	49	51

Table 7: Results of recovery and standard deviation of commercial drugs obtained by using membrane IQ.

Pharmaceutical PZD	*Potentiometric methods*	*Concentration Prepared/ M*	*Concentration Found/ M*	*%Rec.*	*%RE*	*%RSD*	*F-experimental*	*F theoretical*
Pure	Direct method	5.0×10^{-3}	$10^{-3}\times5.1801$	103.60	3.60	0.70	7.8	19.2
	SAM		$10^{-3}\times4.8792$	97.58	-2.42	0.19	4.2	
	Direct method	5.0×10^{-4}	4.8680×10^{-4}	97.37	-2.63	4.72	5.8	19.2
	SAM		4.8884×10^{-4}	97.77	-2.23	4.32	7.2	
Pyrazinamide MACLEODS India	Direct method	5.0×10^{-3}	5.2475×10^{-3}	104.96	4.96	4.19	12.7	19.2
	SAM		5.0804×10^{-3}	101.61	1.61	4.10	4.6	
	Direct method	5.0×10^{-4}	4.8270×10^{-4}	96.54	-3.46	4.89	6.8	19.2
	SAM		5.0892×10^{-4}	101.78	1.78	2.84	3.6	
Pyrazinamide Mixture MACLEODS India	Direct method	5.0×10^{-3}	4.9599×10^{-3}	99.22	-0.78	0.74	7.1	19.2
	SAM		5.0127×10^{-3}	100.25	0.25	1.58	5.7	
	Direct method	5.0×10^{-4}	4.7740×10^{-4}	95.50	-4.50	2.33	9.2	19.2
	SAM		5.0410×10^{-4}	100.82	0.82	4.40	3.8	

Table 8: Results of recovery and standard deviation of commercial drugs obtained by using membrane IVQ.

Pharmaceutical PZD	*Potentiometric methods*	*Concentration Prepared/ M*	*Concentration Found/ M*	*%Rec.*	*%RE*	*%RSD*	*F-experimental*	*F theoretical*
Pure	Direct method	5.0×10^{-3}	5.144×10^{-3}	102.88	2.88	1.36	3.6	19.2
	SAM		4.919×10^{-3}	98.38	-1.62	4.47	7.9	
	Direct method	5.0×10^{-4}	4.949×10^{-4}	98.98	-1.02	4.04	6.4	19.2
	SAM		4.969×10^{-4}	99.39	-0.61	3.56	8.2	
Pyrazinamide MACLEODS India	Direct method	5.0×10^{-3}	4.919×10^{-3}	98.38	-1.62	1.22	2.5	19.2
	SAM		5.114×10^{-3}	102.28	2.28	4.69	6.3	
	Direct method	5.0×10^{-4}	4.773×10^{-4}	95.46	-4.54	2.35	8.9	19.2
	SAM		5.080×10^{-4}	101.60	1.60	3.15	3.4	
Pyrazinamide Mixture MACLEODS India	Direct method	5.0×10^{-3}	5.063×10^{-3}	101.26	1.26	3.95	6.2	19.2
	SAM		4.868×10^{-3}	97.36	-2.64	3.29	8.3	
	Direct method	5.0×10^{-4}	4.883×10^{-4}	97.66	-2.34	4.09	2.5	19.2
	SAM		4.868×10^{-4}	97.35	-2.65	2.67	5.8	

of % recovery were obtained in the range 94.95 to 105.6. A typical plot for membrane IQ and IVQ at a concentration of synthetic solution($5\times10^{-3,}$ 5×10^{-4})M is shown in Figures. (5, 6) and the standard solution added was 0.1 M.

The direct method and standard addition method were applied for the determination of Pyrazinamide in commercial pharmaceutical tablets (Asia-duspataline 135 mg, Epico-colospasmia135 mg, and Abbott-duspataline 135 mg) obtained from local stories using membrane IQ based on DBS and IVQ based on NO as a plasticizer. The values of the % recovery (Table 7 and 8) agree with the value given in British pharmacopoeia.[13] There is no interference of all species on the electrode response. Therefore, the values of recovery obtained by the standard addition method agree with the results of the direct method.

CONCLUSION

The construction of molecularly imprinted electrodes sensors (MIP) using Pyrazinamide as a template and TPT as cross-linkers and 1-VIZ, MMA as monomers in different plasticizers. Results of MIP that show high sensitivity, reasonable selectivity, fast static response, long-term stability, and applicability over a wide pH range were obtained by using electrode based on DBS and NO plasticizers. Good results of recoveries were obtained for the determination of Pyrazinamide in the commercial tablets in comparison with the British Pharmacopoeia.

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