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Synthesis and characterization of molecularly imprinted polymer for tramadol HCl using acryl amide and 2-hydroxyethyl meth acrylate as monomers

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ABSTRACT

Four electrodes were synthesized based on molecularly imprinted polymers (MIPs). Two MIPs were prepared by using tramadol hydrochloride (TRH) as the template, acryl amide (AA) and 2-hydroxy ethyl meth acrylate (2-HEMA) as monomers, divinyl benzene as a cross linker, and benzoyl peroxide as initiator, respectively. The same composition was used to prepare non-imprinted polymers (NIPs), but without the template (Tramadol hydrochloride). Different plasticizers were employed to prepare the membranes; tris (ethyl hexyl) phosphate (TEHP), tri Butyl phosphate (TBP), di-octyl phthalate (DOP) and nitrobenzene (NB) in PVC matrix. The electrode characteristics and properties were studied, including: slope, detection limit, life time and linearity range. The results of selectivity coefficient measurements using amino acids as interfering species showed no effect on tramadol electrode response. The prepared electrodes were intended for use in determining tramadol in pharmaceutical samples.

INTRODUCTION

Tramadol hydrochloride (1R,2R)-2-[(dimethylamino) methyl]-(3methoxyphenyl)cyclohexan-1-ol is a drug acting on opiate and non-opiate receptors, and is used mainly in treating moderate to severe pain [1], disquiet and depression. It is also indicated for treating liver metabolization and renal excretion disorders which may lead to side effects, especially in those suffering from kidney or liver failure. Tramadol is a white crystalline powder freely soluble in water and in chloroform which is given by mouth or parenteral [2].

Tramadol hydrochloride content can be determined via several ways. The most recently developed method employs a modified carbon paste electrode [3], spectrophotometry

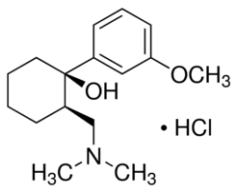

Figure 1. Chemical structure of tramadol hydrochloride. The selective electrodes approach is an effective technique for determining tramadol content because it has fast response time, and is rapid and easy to use, is of low cost and selective

[4-7], HPLC [7-9], GC [10], LC-MS/MS [11], capillary electrophoresis [12], voltammetry [13] and potentiometric [14-19].

The potentiometric sensor technique utilizes PVC membrane electrodes that are widely available and widely employed for the analysis of drugs and ionic species [20-28]. There are a variety drugs that are determined by the liquid selective electrode approach wherein MIPs being are used as recognition membranes. Among these are ibuprofen [29], warfarin [30], phenytoin [31] and metronidazole benzoate [32].

In this research, a polymerization process was used to prepare molecularly imprinted polymers (MIPs) for tramadol as the template and acrylamide and 2-hydroxy ethyl meth-acrylate as monomers, divinyl benzene as the cross linker, benzoyl peroxide as the initiator. In this study, different plasticizers were employed to construct electrode membranes which were then assessed in the task of determining tramadol in pharmaceutical samples.

EXPERIMENTAL

1. Preparation of MIP

For the preparation of the first tramadol hydrochloride molecularly imprinted polymer (TRH-MIP1), 0.569 mmol

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(0.15 g) of tramadol HCl was mixed with 1.803 mmol (0.475 g) acryl amide as the monomer. After this, 21.116 mmol (5.565 g) divinyl benzene was added to the solution as the cross linker, followed by (0.05 g) benzoyl peroxide as the initiator. All these materials were subsequently dissolved in 3 mL mixture of acetonitrile and chloroform. The second tramadol molecularly imprinted polymer (TRH-MIP2) was created by mixing together 0.301 mmol (0.0795 g) tramadol hydrochloride, 2.277 mmol (0.6 g) 2-hydroxyethyl meth acrylate as the monomer, 4.935 mmol (1.3) divinyl benzene as the cross linker and (0.05 g) benzoyl peroxide as the initiator. The product was then dissolved in 2 mL mixture of acetonitrile and chloroform, and the mixture was stirred for 5 minutes to obtain a homogenous solution. Afterwards, the gas N2 was passed through the solution for 30 minutes to remove oxygen from it, and the solution was placed in a water path at 65°C. When the reaction was complete, the molecularly imprinted polymer became hard, and, after the polymerization process, the polymer was dried and crushed to obtain it as particles. Finally, these particles was sonicated in CH_3OH / CH_3COOH (18:2 v/v) to remove the template from the MIP. The particles size of TRH- MIP1 and TRH-MIP2 were between (53 µm and 125 µm), respectively.

The preparation of non-molecularly imprinted polymers was done by way of the same procedure, using the same substances and under the same conditions as in the preparation of TRH-MIP1 and TRH-MIP2, but without the tramadol hydrochloride. To fabricate the electrode, a PVC tube (1-2 cm long) was flattened and polished by placing it on a glass plate and soaking it with THF. The membrane was then cut similar to the external diameter of the PVC tubing and pasted on the polished end. The other end of this was linked with an Ag-AgCl electrode.

2. Instruments

In this work, we use an analyzer (WTW model, Germany), pH meter (WTW model pH 720, Germany) and a saturated calomel electrode (Gallenkamp, USA). The tramadol HCl-MIP electrodes were fabricated as previously described, in the laboratory. All potentiometric measurements was made at room temperature. For research purposes, the tramadol hydrochloride-MIP electrode was combined with an Ag-AgCl electrode, while 0.1 M of tramadol hydrochloride was used as internal solution, the electrode being soaked with this for at least 2 hours before use.

3. Materials and chemicals

1. Tramadol hydrochloride standard was obtained as a gift from the state company of drug and food industries and medical appliances (IRAQ-SDI-Samara). Coltra tablets (50 mg) (BRAWN, Haryana, India) were purchased from local pharmacies.
2. Plasticizers, tris (2-ethyl hexyl) phosphate (TEHP) (97.0% purity), tri-butyl phosphate (TBP) (99.0% purity), di-octyl phthalate (DOP) (99.5% purity), nitrobenzene (NB) (99.4% purity) were purchased from Sigma Aldrich. Other chemicals and reagents materials were obtained from Fluka, BDH and Sigma Aldrich.

4. Preparation of standard solutions

1. 50 mL of stock standard solution of 0.1 M tramadol hydrochloride was prepared by dissolving 1.317 g of standard tramadol hydrochloride in bi-distilled water. The other tramadol solutions ranged from 10^{-6}-10^{-2} M in 100 mL, and came from the stock solution of tramadol.
2. 100 mL of each amino acid solution was prepared from 10^{-6} to 10^{-2} M of a stock solution of 0.1 M amino acid.

5. Synthesis of membrane molecularly imprinted polymers

A tramadol-HCl membrane was immobilized into a PVC tube as previously describe in reference [33,34]. Herein, 0.04 g of TRH-MIP was mixed with 0.8 g plasticizer, either: TEHP (electrode A1), TBP (electrode A2), DOP (electrode B1), and NB (electrode B2). Following this, 0.34 g of PVC powder was scattered on 7 mL of tetrahydrofuran and stirred until a homogenous and clear viscous solution was acquired. The mixture was cast into a glass ring 30-35 mm diameter and unwound on a glass plate, with a ribbon of filter being positioned on top of the glass. The solvent was then allowed to evaporate at room temperature for more than 24 hours at least. The thickness of the obtained membrane was about

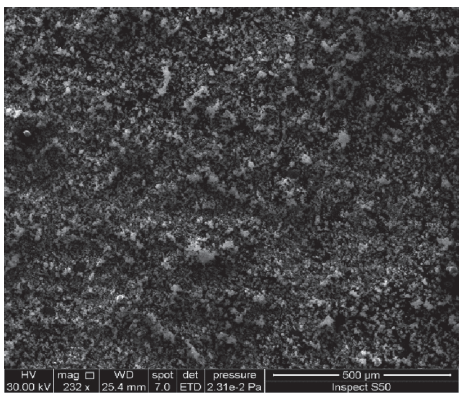

Figure 2a. SEM for MIP1 before washing

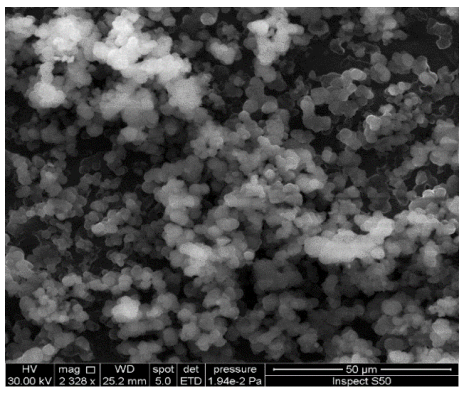

Figure 2b. SEM for MIP1 after washing

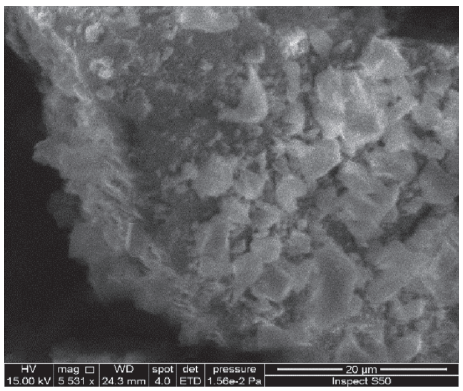

Figure 3a. SEM for MIP2 before washing

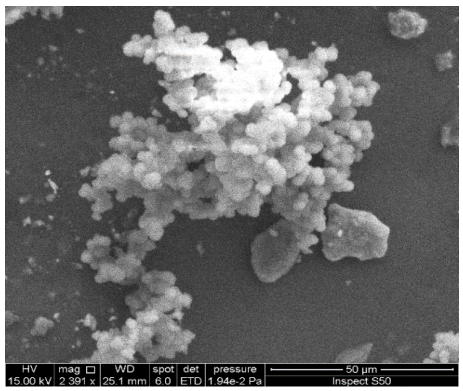

Figure 3b. SEM for MIP2 after washing

(0.4-0.7) mm. This size of membrane was considered adequate for electrode preparation.

6. Scanning Electron Microscope (SEM)

The SEM can be used to get an idea about the size, geometry and pore surface distribution of the membranes. SEM analysis indicates that molecular imprinted polymer in surface and in cross-section, had a highly ordered and regular pore structure which serves as the sites of interaction. Several papers have shown that a molecular imprinted membrane of this type recognizes the template molecule effectively and transports it with good efficiency due to the type and quality of the porous structures.

As shown by SEM, the morphology of MIP before and after washing is displayed in Figure 2a, 2b and Figure 3a, 3b. Herein, it can be seen that micro emulsion polymerization gives very small particles size around (4032-8064) nm for acryl amide (AA) polymer and (6923-9230) nm for 2-hydroxyethyl meth acrylate (2-HEMA) polymer.

7. Preparation of pharmaceutical samples

The drug tablets were ground to powder by using pestle and mortar. Subsequently, a required weight of the powder was used to prepare 100 mL solutions. Here, a certain amount of powder was dissolved in acetonitrile (CH_3CN) and stirred by magnetic stirrer for 30 minutes to completely dissolve the powder. The solution was completed to 100 mL by water to prepare 5×10^{-3} M and 5×10^{-4} M tramadol solutions.

RESULTS AND DISCUSSION

Several experiments were done to find out the optimal ratios of drug: monomer: cross linker for preparing molecularly imprinted polymers and non-imprinted polymers. The best ratios for forming MIPs and NIPs which give suitable performance characteristics are presented in Table 1.

Table 1. Different ratios of (D: M: C) and progeny used in the synthesis of MIPs and NIPs for (TRH)

No. of MIP	Ratio	Drug	Monomer	Cross linker	Initiator	Solvent	Result
		(TR)	(AA)	(DVB)	(BPO)		
MIP1	%	6.13	7.97	85.88	-	±5	Pile white gel
	mmol	0.3	0.39	4.2	0.24	mLC_2H_3N	
MIP1	%	2.56	46.15	51.28	-	±5	Pile white gel
	mmol	0.5	9	10	0.127	mLC_2H_3N	
MIP1	%	4	16	80	-	±5	Pile white gel
	mmol	0.5	2	10	0.2	mLC_2H_3N	
MIP1	%	1.14	13.366	85.49	-	±5	White rigid
	mmol	0.57	6.682	42.74	0.2	mLC_2H_3N	
NIP 1	%		13.52	86.47	-	±5	White rigid
	mmol	-	6.682	42.74	0.2	mLC_2H_3N	
		(TR)	(2HEMA)	(DVB)	(BPO)		
MIP2	%	1.14	13.366	85.49	-	±5	Pile yellow gel
	mmol	0.57	6.682	42.74	0.2	$mLCHCL_3$	
MIP2	%	12.73	27.53	59.72	2	±5	Pile yellow gel
	mmol	2.133	4.61	10	0.2	$mLCHCL_3$	
MIP2	%	2.01	30.96	67.02	-	±5	yellow rigid
	mmol	0.301	4.61	9.98	0.2	$mLCHCL_3$	
NIP 2	%	-	31.59	68.4	-	±5	yellow rigid
	mmol		4.61	9.98	0.2	$mLCHCL_3$	

All ratios of MIPs and NIPs were prepared in a water bath at 60-80ºC.

Table 2. The most identified peaks of FT-IR spectra for TRH-imprinted polymer using acryl amide (AA) as a monomer

No.	Functional Group	TRH	TRH-MIP (AA) before template removal	TRH-MIP (AA) After template removal
1	O-H str.(cm^{-1})	3305	3365	--------
2	C-H aliphatic.(cm^{-1})	2931,2860	2920,2860	2921,2856
3	C-H aromatic.(cm^{-1})	3068	3050	3043
4	C=C str. (cm^{-1})	1606	1604	1600
5	C=O str.amid.(cm^{-1})	-----	1674	1679
6	C=C str.olefin (cm^{-1})	------	1604	1629
7	C-O str.ether.(cm^{-1})	1047	1060	------
8	Out-of plane-para-sub	-----	815	835
9	Out-of plane-meta-sub	781,703	815,707	-----

The Fourier transmission infrared spectrometry (FTIR) spectra of leached and unleashed tramadol hydrochloride imprinted polymers MIP and NIP using acryl amide as monomer were recorded in the range of 400-4000 cm^{-1} by the KBr pellet method. These are listed in Table 2.

The FTIR spectrum of TRH and TRH-MIP and after template removal showed a band at 3305 and 3365 cm^{-1} for hydroxyl group stretching, at 1047 and 1060 cm^{-1} for carbonyl ether group stretching and at 781,703,815 and 707 cm^{-1} for meta substitution, but disappearance at the TRH-MIP FTIR spectrum. FTIR spectrum of TRH-MIP after template removal showed a band at 1679 cm^{-1} for carbonyl amide group stretching, 1629 cm^{-1} for olefin group stretching and 835 cm^{-1} for para substitution.

All of the above indicate that the template was synthesized and the drug was removed from the polymer.

The Fourier transmission infrared spectrometry (FTIR) spectra of leached and unleashed tramadol hydrochloride imprinted polymers MIP and NIP using 2-Hydroxyethyl methacrylate as a monomer were recorded and listed in Table 3.

Table 3. The most identified FT-IR spectra peaks for the TRH-imprinted polymer, using 2-Hydroxyethyl methacrylate (2-HEMA) as a functional monomer

No.	Functional Group	TRH	TRH-MIP (2-HEMA) before template removal	TRH-MIP (2-HEMA) After template removal
1	O-H str.(cm^{-1})	3305	3477,3423	3442
2	C-H aliphatic.(cm^{-1})	2931,2860	2929	2925,2856
3	C-H aromatic.(cm^{-1})	3068	3083	3002
4	C=C str. (cm^{-1})	1606	1604	1600
5	C=O str.ester (cm^{-1})	-----	1714	1724
6	C-O str.ether.(cm^{-1})	-----	1081	1078
7	C=C str.olefin (cm^{-1})	------	1629	1629
8	Out-of plane-para-sub	-----	815	837

The FTIR spectrum of TR-MIP after template removal showed a band at 1078 cm^{-1} for carbonyl ether group stretching, at 1629 cm^{-1} for olefin group stretching and at 837 cm^{-1} for para substitution. It also showed disappearance at the TR FTIR spectrum.

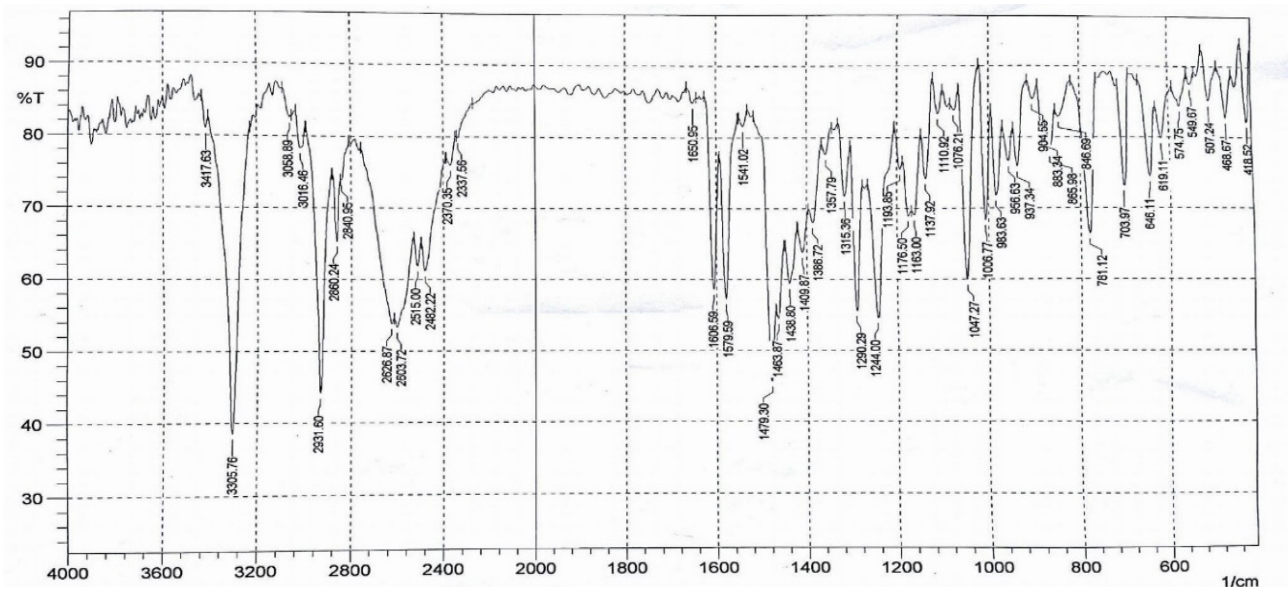

Figure 4. FTIR of (TRH) drug

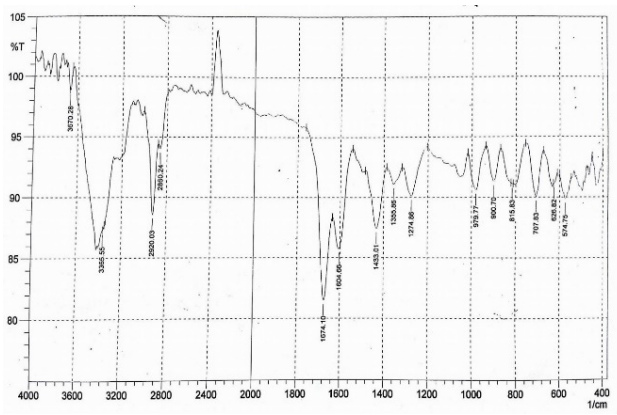

Figure 5. FTIR of TRH-MIP (AA) before the removal of TRH

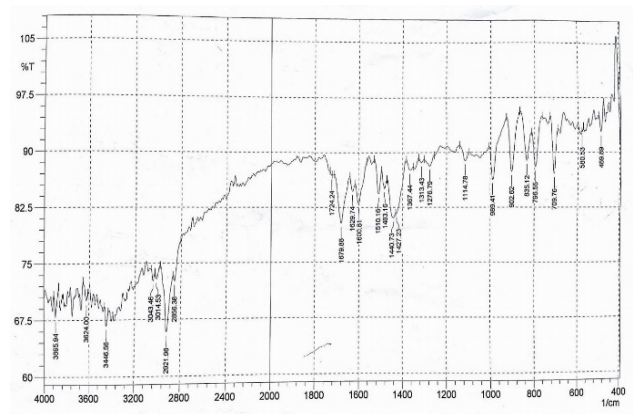

Figure 6. FTIR of TRH-MIP (AA) after the removal of TRH

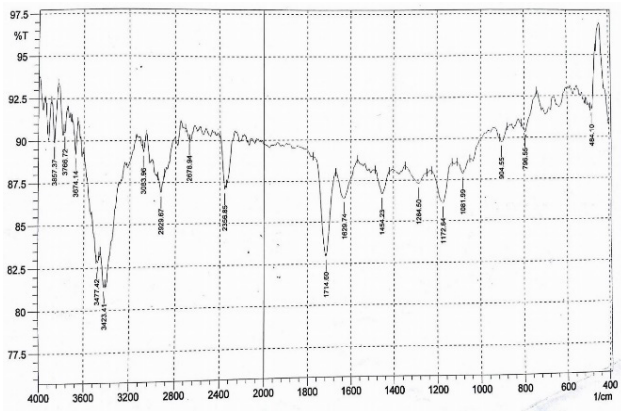

Figure 7. FTIR of TRH-MIP (2-HEMA) before the removal of TRH

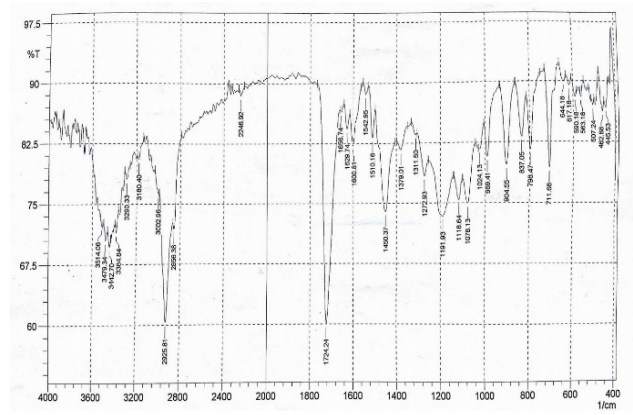

Figure 8. FTIR of TRH-MIP (2-HEMA) after the removal of TRH

All of the above indicate that the template was synthesized and the drug was removed from the polymer.

The plasticizer is an important component for membrane selective electrode and must have compatibility with the polymer and other membrane constituents to provide a homogeneous environment for the membrane. Four types of MIPs membranes were prepared with different types of plasticizers in order to study the viscosity, permeability and ability to avoid leaching of the plasticizer and MIP from the electrode (which otherwise would affect the electrode performance over time). The plasticizers are: tris (2-ethyl hexyl) phosphate (TEHP), tri- butyl phosphate (TBP), di-octyl phthalate (DOP) and nitrobenzene (NB). The characteristics and specification of each electrode parameter were studied based on TRH-MIP1 (A1, A2 membranes) and TRH-MIP2 (B1, B2 membranes). The examined electrode parameters are: linearity range, correlation coefficients, detection limit, and life time, respectively. The results obtained are shown in Table 4, while their calibration curves are shown in Figure 9.

Table 4. Characteristics of the tramadol HCl-MIP electrode based on different functional monomers and plasticizers

Membrane composition	TRH-MIP1+ TEHP (A1)	TRH-MIP1+ TBP (A2)	TRH-MIP2+ DOP (B1)	TRH-MIP2+ NB (B2)
Slope (mV/decade)	33.15	32.09	16.17	30.21
Linearity range (M)	10^{-2}-10^{-5}	10^{-3}-10^{-6}	10^{-2}-10^{-5}	10^{-3}-10^{-6}
Correlation coefficient	0.9813	0.9896	0.9869	0.9940
Detection limit (M)	3×10^{-6}	1×10^{-6}	1×10^{-6}	2×10^{-6}
Life time (day)	16	12	7	4

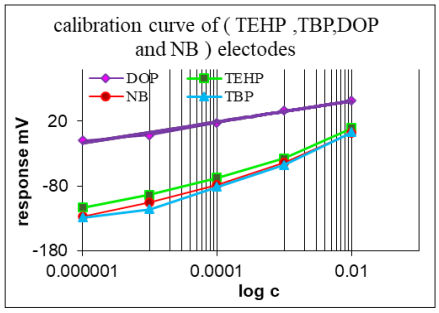

Figure 9. Calibration curve for TRH-MIP1 and TRH-MIP2 membrane electrodes

The three electrodes A1, A2, and B2 gave Nernstian slopes of 33.15, 32.09, and 30.21 mV/decade, respectively. These slopes indicate that the drug interacts with the polymer via two covalent bonds. Electrode B1, however, shows a non-Nernstian slope of 16.17 mV/decade. The low slope of 16.17 mV/decade revealed by electrode TRH-MIP2+DOP is due to the incompatibility of the DOP plasticizer with the species of the membrane (the monomer). This brought about a leaching of the plasticizer from the membrane into the external solution. As the life time of electrodes A1 and A2 as given in Table 4 are longer than that of electrodes B1 and B2, therefore, electrodes A1 and A2 are better for use in the determination of tramadol content in pharmaceutical samples.

All experiments for the calibration of NIP electrodes gave a constant potential for the tramadol solutions ranging from 10^{-1} to 10^{-6} M.

1. Effect of pH on electrode response

Three concentrations of tramadol solution (5×10^{-3}, 5×10^{-4} and 5×10^{-5}) were used to study the effect of pH on electrode response. The electrode potential as measured for the tested solutions has pH ranging from 1 to 10. The low and high pH were fixed by using hydrochloric acid (0.1 M,1 M) and/or ammonium hydroxide (0.1 M,1 M), respectively. The results obtained by adding the appropriate volume of HCl/NH_4OH are shown in Table 5, while the typical plot for electrode A1 is shown in Figure 10. At high acidity of less than 4, the tramadol begins to hydrolyzed and the electrode responds to hydrogen ions. The best pH for calibrating the electrodes is, thus, between 4 to 8.

Table 5. Working pH range for tramadol hydrochloride selective electrodes

Number and composition of MIPs	Membranes	Membrane composition	pH range		
			5×10^{-3}	5×10^{-4}	5×10^{-5}
MIP1 TR+AA+DVB	A1	TR-MIP1 +TEHP	4-8.5	4.5-9	4-8
	A2	TR-MIP1 +TBP	4-7.5	2.5-7.5	4-8
MIP2 TR+2-HEMA + DVB	B1	TR-MIP2+DOP	3-8	4-7.5	3-8
	B2	TR-MIP2 +NB	4.5-8.5	4.5-8	5.5-9.5

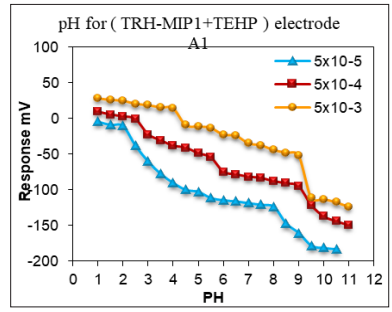

Figure 10. Effect of pH on the tramadol hydrochloride ((TRH-MIP2+DOP (A1)) electrodes at concentrations 5×10^{-3}, 5×10^{-4} and 5×10^{-5}

2. Interference study

For calculating the selectivity coefficient measurement, we used the separate solution method. The procedure is to plot the calibration curve for tramadol solutions ranging from 10^{-1} to 10^{-6} M, then to plot calibration curves for the same concentrations (10^{-1} to 10^{-6} M) of amino acid, and subsequently apply the following equation to calculate the selectivity coefficient.

$$\text{Log Kpot} = [(EB - EA)/(2.303RT/z\,F)] + (1 - zA/zB)\log aA$$

EA, EB; zA, zB; and aA, represents the potentials, charge numbers and activities for the primary A and interfering B ions, respectively, at aA = aB. Interfering species of amino acids: asparagine, arginine, glycine, and tryptophan were employed in order to simulate the effect of the amino acid when a patient co-self-administers tablets containing the aforementioned amino acids with a tablet of tramadol. The interference depends upon the ability of the amino acid to fit in the cavity of the polymer. The selectivity coefficients of the amino acids using electrodes based on TRH-MIP1-TEHP are listed in Table 6. Because of the obtained low selectivity coefficients values, the results indicate that there

Table 6. Selectivity coefficients for (TRH–MIP1+TEHP) electrode at different concentrations of tramadol hydrochloride

Conc. (M)	Concentrations of tramadol hydrochloride (M): Concentrations of interference ions (M)							
	Interfering ions							
	Asparagine		Arginine		Glycine		Tryptophan	
	EB (mV)	KA,B	EB (mV)	KA,B	EB (mV)	KA,B	EB (mV)	KA,B
10-2	-74.2	4.7336×10^{-3}	-98.1	9.0446×10^{-4}	-101.9	6.9519×10^{-4}	-114.2	2.9661×10^{-4}
10-3	-97.6	2.3765×10^{-2}	-109.4	1.0497×10^{-2}	-128.1	2.8751×10^{-3}	-119.9	5.0730×10^{-3}
10-4	-107.4	2.0196×10^{-1}	-129	4.5252×10^{-2}	-136.4	2.7107×10^{-2}	-129.3	4.4322×10^{-2}
10-5	-134.9	1.0317×10^{-1}	-124.6	2.1053×10^{-1}	-149.1	3.8589×10^{-2}	-132.6	1.2098×10^{-1}
10-6	-141.1	6.0738×10^{-1}	-139.8	6.6459×10^{-1}	-155.7	2.2098×10^{-1}	-146.9	4.0646×10^{-1}

is no interference of these amino acids. A typical selectivity coefficient plot of these amino acids based on use of electrode TRH-MIP1-TBP is shown in Figure 11.

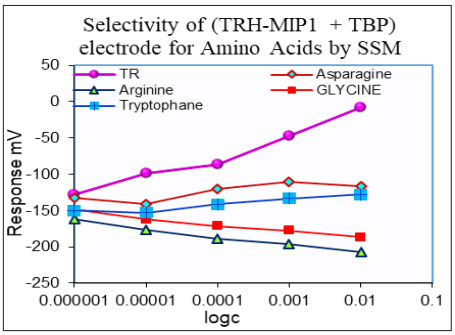

Figure 11. Selectivity coefficient of TRH-MIP 1 + TBP electrodes with amino acids

3. Analysis of tramadol in commercial pharmaceutical tablets

Three methods were used for measuring the tramadol in pharmaceutical tablets: direct, the single method and the standard addition method. The method performances were checked by applying them to synthetic tramadol solutions. The concentrations of synthetic tramadol used were 5×10^{-3} and 5×10^{-4} M, and the results are listed in Table 7. Herein, excellent results were obtained, the percent recovery ranged from 88-102 with low values of errors. A typical plot for the standard addition method is shown in Figure 12. This used electrode TRH-MIP2-DOP and 5×10^{-3} M and 5×10^{-3} M concentrations of tramadol. The results indicate the prepared electrodes were extremely suitable for determining tramadol content in commercial tablets.

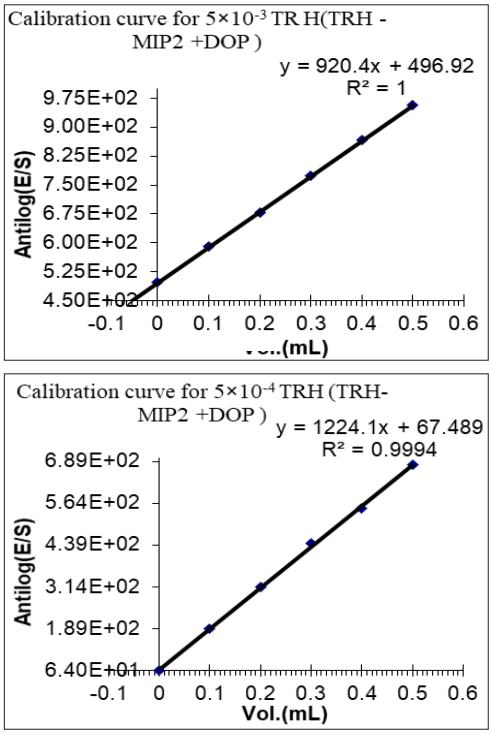

Figure 12. Standard addition method for determining tramadol hydrochloride solution content (5×10^{-3} and 5×10^{-4}) by MSA, using the TRH–MIP2+DOP electrode

Table 7. Determination of tramadol hydrochloride in synthetic solutions of tramadol

Electrode NO. and composition	Measurement by using ISE methods				
TRH-MIP1+TEHP (A1)	Standard sample 5×10^{-3}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	0.69	98.62	-1.38	4.9309×10^{-3}
	SAM	0.72	98.92	-1.08	4.9459×10^{-3}
	MSA	-	99.05	-0.95	4.9527×10^{-3}
	Standard sample 5×10^{-4}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	1.38	98.28	-1.72	4.9140×10^{-4}
	SAM	1.9	102.85	2.85	5.1423×10^{-4}
	MSA	-	98.85	-1.15	5.0268×10^{-4}
TRH-MIP1+TBP (A2)	Standard sample 5×10^{-3}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	0.72	98.86	-1.14	4.9430×10^{-3}
	SAM	0.83	100.74	0.74	5.0368×10^{-3}
	MSA	-	100.65	0.65	5.0327×10^{-3}
	Standard sample 5×10^{-4}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	1.09	98.56	-1.44	4.9278×10^{-4}
	SAM	0.93	98.40	-1.60	4.9201×10^{-4}
	MSA	-	98.94	-1.06	4.9472×10^{-4}
TR-HCl-MIP2+ DOP (B1)	Standard sample 5×10^{-3}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	2.18	100.89	0.89	5.0443×10^{-3}
	SAM	0.56	99.74	-0.26	4.9868×10^{-3}
	MSA	-	99.65	-0.35	4.9823×10^{-3}
	Standard sample 5×10^{-4}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	1.42	101.4	1.4	5.0701×10^{-4}
	SAM	0.6	99.30	-0.70	4.9650×10^{-4}
	MSA	-	101.21	1.21	5.0606×10^{-4}
TRH-MIP2 + NB (B2)	Standard sample 5×10^{-3}				
	Parameter	RSD%	RC%	RE%	Con. found
	Direct	2.03	99.06	-0.94	4.9529×10^{-3}
	SAM	0.57	99.57	-0.43	4.9784×10^{-3}
	MSA	-	100.35	0.35	5.0174×10^{-3}
	Standard sample 5×10^{-4}				
	Parameter	RSD%	RC%	RE%	Con. founded
	Direct	1.92	98.63	-1.37	4.9314×10^{-4}
	SAM	0.84	98.61	-1.39	4.9306×10^{-4}
	MSA	-	101.44	1.44	5.0718×10^{-4}

The results of commercial tramadol tablet content research using the three methods of analysis are listed in Table 8, with their statistical results listed in Table 9.

Table 8. Results of analysis of different types of commercial tramadol tablets

Membrane composition	TRH-MIP1+TEHP		
Pharmaceutical	COLTRA 50 (Haryana, India)		
	DM	SAM	MSAM
Concentration (taken) M	5×10^{-3}		
Conc. founded	4.9171×10^{-3}	4.9217×10^{-3}	5.0765×10^{-3}
Recovery %	98.34	98.43	101.53
RE%	-1.66	-1.57	1.53
RSD%	1.24	1.9	-
Concentration (taken) M	5×10^{-4}		
Conc. founded	4.8920×10^{-4}	5.1012×10^{-4}	5.0860×10^{-4}
Recovery %	97.84	102.02	101.72
RE%	-2.16	2.02	1.72
RSD%	1.62	1.46	-
Membrane composition	TRH-MIP1+TBP		
Pharmaceutical	Haryana, India		
Concentration (taken) M	5×10^{-3}		
Conc. founded	4.9157×10^{-3}	5.0850×10^{-3}	5.0837×10^{-3}
Recovery %	98.31	101.70	101.67
RE%	-1.69	1.70	1.67
RSD%	0.77	1.29	-
Concentration (taken) M	5×10^{-4}		
Conc. founded	5.1099×10^{-4}	5.0998×10^{-4}	5.0955×10^{-4}
Recovery %	102.2	102	101.91
RE%	2.2	2	1.91
RSD%	0.77	1.44	-

*each measurement was repeated three times

Table 9. Statistic results for commercial tramadol tablets using the three methods of analysis

Membrane composition	TRH-MIP2+DOP		
Pharmaceutical	COLTRA 50 (Haryana, India)		
	DM	SAM	MSAM
Concentration (taken) M	5×10^{-3}		
Conc. founded	4.9161×10^{-3}	5.1086×10^{-3}	5.0935×10^{-3}
Recovery %	98.32	102.17	101.87
RE%	-1.68	2.17	1.87
RSD%	1.01	1.54	-
Concentration (taken) M	5×10^{-4}		
Conc. founded	4.8975×10^{-4}	4.9248×10^{-4}	5.0925×10^{-4}
Recovery %	97.95	98.50	101.85
RE%	-2.05	-1.50	1.85
RSD%	0.66	0.89	-
Membrane composition	TRH-MIP2+NB		
Pharmaceutical	COLTRA 50 (Haryana, India)		
Concentration (taken) M	5×10^{-3}		
Conc. founded	5.0787×10^{-3}	5.0834×10^{-3}	5.0740×10^{-3}
Recovery %	101.57	101.67	101.48
RE%	1.57	1.67	1.48
RSD%	1.4	1.27	-
Concentration (taken) M	5×10^{-4}		
Conc. founded	4.9034×10^{-4}	5.0761×10^{-4}	5.1056×10^{-4}
Recovery %	98.07	101.52	102.11
RE%	-1.93	1.52	2.11
RSD%	1.4	1.2	-

*each measurement was repeated three times.

CONCLUSION

Electrodes based on a molecular imprinted polymer for tramadol HCl were prepared using two different monomers and plasticizers. The values of Nernstian slope research indicate that the tramadol drug is bonded to the polymer by two covalent bonds. No interference of amino acids response was seen in the tramadol determination, and excellent results for the determination of tramadol in pharmaceutical samples were indicated. The recovery of synthetic tramadol solutions ranged from 88 to 102.

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