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Estimation of Trimethoprim by using a New Selective Electrodes dependent on Molecularly Imprinted Polymers

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Abstract

Trimethoprim-MIPs were prepared by using (TMP) as the template as well as allylchloride (AYC) or allylbromide (AYB) as monomer, used (TMPTA) tri methylol propane tri acrylate or ethylene glycol di methyl acrylate (EGDMA) as cross-linker and initiator used (BP) benzyl peroxide. By using different plasticizers (di butyl Phthalate (DBPH), Nitrobenzene (NB), oleic acid (OA) and paraffin) for TMP-MIP1 and (Di butyl sebecate (DBS), Di methyl acrylate (DMA), Tributylphosphate(TBP) and Tris(ethylhexyl phosphate (TEHP)) for TMP-MIP2. Membranes of MIPs were prepared in PVC matrix. The characterizations of each electrode were determined The Slope range from (55.591 - 40.509) mV/decade, Limit of Detection (1 X 10 -5 - 5 X 10 -6) and Linearity range of electrodes MIPs from (1 X 10 -5 - 1 X 10 -1). Stable Signe of electrode pH from (3-9) and study the selectivity with additives of drugs synthesis (Glucose, Calcium stearate, sodium benzoate and benzoic acid) demonstrate strong selectivity.

Keywords: Trimethoprim; Sensor; Ion Selective electrodes (ISEs); MIPs Molecularly imprinted polymers.

1. Introduction

Trimethoprim (TMP) is a $C_{14}H_{18}N_4O_3$ compound with a molecular weight of 290.3 g/mole and the chemical name (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine. White or yellowish-white powder, partially soluble in water and ethanolDihydrofolate reductase inhibitors are a type of chemotherapeutic agent that belongs to the class of dihydrofolate reductase inhibitors [1,2]. Because of the inhibitor, trimethoprim has a synergistic impact. In bacteria, an occurrence that happens in more than one stage during the mandatory series of enzymatic reactions. [3]. Many common diseases, such as urinary, pulmonary, and gastrointestinal tract infections, are treated with this association [4]. As a result, it's critical to develop fast, convenient, and low-cost analytical methods for the simultaneous Quantitative analysis of these compounds for a good quality. control. According to the United States Pharmacopeia (USP) [5], high Performance Liquid Chromatography is the official procedure for the simultaneous study of trimethoprim in pharmaceutical formulations (HPLC). A large number of analytical papers have been published in the literature for determining interaction in industrial formulations and biological samples, these include mainly chromatography [6-9], spectrophotometric [10-13] and electrophoresis [14-16] methods.

Molecular Imprinting polymers (MIPs) is a technique for developing Receptors that are artificial for a given analyte with a predetermined specificity and selectivity that In various application areas, they can be used as ideal materials.. Polymeric matrix obtained using the imprinting technology, (MIPs), are robust molecular recognition components able to mimicking natural recognition entities such as biological receptors and antibodies that are used for distinguishing and the study of difficult samples such as environmental samples and biological fluids [17-19].The aim of MIT is the formation of a complex between an analyte (template) and a functional monomer. In the presence of a large excess of a cross-linking agent, a three-dimensional polymer network [20,21] is formed. After the polymerization process, the template is removed from the polymer, leaving specific recognition sites that are complementary in shape, scale, and chemical flexibility for the molecule prototype. Typically, molecular recognition phenomena are powered by intermolecular interactions such as the ionic

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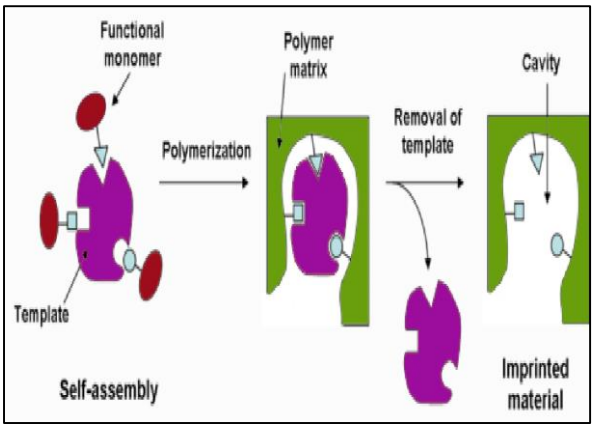
interactions, the hydrogen bonds H-bond and dipole-dipole between the functional groups of monomers and the template molecule in the matrix of polymer. The resulting polymer thus selectively recognizes and binds only the template molecules. [22-24]. In this work we determined the trimethoprim depended on molecularly imprinted polymers.

Fig. 1. Concept of Molecularly imprinted polymers[23]

2. Experiment

1-This work was conducted with an (Germany, WTW model), a pH meter (WTW model pH 720, Germany) expandable ion analyzer and a (Gallenkamp, USA) (SCE) saturated calomel electrode.



2- All of the highest purity chemical reagents used are: allyl chloride (99%), trimethylol propane triacrylate (99%) and benzoyl peroxide (78 percent). Sigma-Aldrich obtained plasticizers ((DBPH) dibutyl phthalate, nitrobenzene (NB), oleic acid (OA) and (PRF) Parrafin . Fluka was responsible for acquiring other chemicals and reagents.

3- Standard solution of 0.1 M trimethoprim (TMP) was prepared by dissolving 2.9032g of standard trimethoprim in methanol and diluting to 100 mL, ultrasonicator equipment was prepared used to aid in drug dissolution, and several 100 mL of standard solution range (10^{-6}-10^{-1} M) prepared freshly.

4-Interfering 0.1 M solution for (Glucose, Calcium stearate, sodium benzoate and benzoic acid) were prepared and then series (10^{-6}-10^{-1} M) were prepared. 0.1 M stock solution of each of interfering ;(1.8015)g of glucose , (1.2212)g of benzoic acid ,(1.4411)g sodium benzoate and (6.0702)g of calcium stearate were prepared by dissolving in Distilled water . 100mL standard solution ranged from (10^{-6} – 10^{-1}) M were freshly prepared

5-The preparation of the trimethoprim molecularly imprinted polymers (TMP-MIPs) 0.5 mmol of trimethoprim (TMP) was mixed with 3 mmol of (allyl chloride (AYC) or allyl bromide (AYB)) as monomer, after that was added 15 mmol of the (Ethyleneglycol dimethylacrylate (EGDMA) or Trimethylolpropanetriacrylate (TMPTA) as Cross-linker , and then added the initiator (BPO) benzoyl peroxide 0.3 mmol and 5mL of CHCl3 chloroform for obtaining homogeneous solution, the mixture was stirred for 5 minutes.

6- N2 gas passed for 30 min on the homogenous mixture to remove O2 from the solution. After that, the solution was put at 70 oC for 2 hours in a water bath. When the reaction completed and the TMP-MIPs formed as very hard material leave 24 hours to dried and then crushed and the trimethoprim extracted from polymers by using soxhlet using (1:9) (CH3COOH: CH3OH). After ensure the template removal completely. The polymer was dried at 45oC for 48 hours. The TMP-MIPs was then crushed and ground by a mortar, pestle and sieve to get 125-150 µm was collected and then used as an active substance in the selective sensors membrane.

7- 0.02g of TMP-MIPs polymers were mixed with different plasticizers used in this work such as DBPH, NB, O.A , PRF, DBS, DMA, TBP and TEHP. Then (0.2) g of PVC powder was added and dissolved in (7mL) of Tetra Hydro Furan with shaking until obtaining A pure, viscous solution. Then mix the solutions and shake until the mixture is homogeneous. Pour the mixture into a glass ring molds of diameter (30-35 mm) and spread it on a glass plate and places a filter paper over the mouth of the mug. The solvent was then kept to evaporate at room temperature for at least 24 hours. The thickness of the film obtained was different from one film to another, so the thickness was within (0.4-0.7 mm). This membrane size was suitable for preparing the electrodes. The construction and assembly of the electrodes by taking a PVC tube (1-2 cm long) that has been plunged into THF solution from one of it is sides and positioned vertically on the prepared membrane, cut to fit the outer diameter of the PVC tube. The other end of a glass container assembly was connected to plastic cover, Ag/AgCl through wire that was mounted. 3/4 glass tube filled with 0.1 M trimethoprim solution. The electrodes were placed in standard drug solution for three hours before using.

8- After good results were obtained when we used the prepared sensors depend on TMP-MIPs, in determination trimethoprim in pure form. Applied to estimate the trimethoprim drug in the pharmaceutical preparation whose found as tablets with 400 mg. These ISEs measurements have been tested by different potentiometric methods. Preparation solutions of trimethoprim at concentrations 1×10^{-3} and 1×10^{-4} M then calculation's the Erel.%, Rec.% and RSD% of trimethoprim in pharmaceuticals.

3. RESULT AND DISCUSION

The molecular imprinted polymer of trimethoprim using after extraction the template (trimethoprim) to constructed four membranes for

FTIR Spectral studies on trimethoprim (TMP) polymers.

The results in table (1) found the difference between the infrared spectra of the standard TMP. Drug from the imprinted polymer MIP1 spectrum. This indicates occurrence of interference between allyl chloride (AYC) monomer and TMP. Drug, the bands were shown from this table in Trimethoprim. FT-IR spectrum is: (3469, 3319, 3010, 2974, 2833, 1236, 1595 and 1633) cm-1 for stretching N-H, str. C-H aromatic, aliphatic C-H. O-C str. C=C aromatic str. and C=N str.) Respectively, while, the TMP- MIP3) FT-IR bands spectrum before template removal is: (3460, 3373, 3042, 2968, 2891, 1294, 1564, 1724 and 1637) cm-1 for stretching stretching N-H, str. C-H aromatic, aliphatic C-H. , O-C str. , C=C aromatic str., C=O ester and C=C allyl str..). Then the FTIR spectrum of the TMPZ-MIP3 after removal of the template shows the different their locations of bands in addition to the absence of N-H, str. C-H aromatic ,C=C aromatic and C=N stretching. Which indicate to remove the trimethoprim drug and formation the molecularly imprint polymer. The results in table (2) found the difference between the infrared spectra of the standard TMP. Drug from the imprinted polymer MIP2 spectrum. This indicates occurrence of interference between allyl bromide (AYB) monomer and TMP. Drug, the bands were shown from this table in Trimethoprim. FT-IR spectrum is: (3469, 3319, 3010, 2974, 2833, 1236, 1595 and 1633) cm-1 for stretching N-H, str. C-H aromatic, aliphatic C-H. O-C str. C=C aromatic str. and C=N str.) Respectively, while, the TMP- MIP4) FT-IR bands spectrum before template removal is: (3467, 3423, 2983, 2958, 2891, 1244, 1595, 1637, 1724 and 1637) cm-1 for stretching stretching N-H, str. C-H aromatic, aliphatic C-H. , O-C str. , C=C aromatic str., C=N str., C=O ester and C=C allyl str.). Then the FTIR spectrum of the TMP-MIP4 after removal of the template shows the different their locations of bands in addition to the absence of N-H, str. C-H aromatic ,O=C str. C=C aromatic and C=N stretching. Which indicate to remove the trimethoprim drug and formation the molecularly imprint polymer.

Table 1. . FT-IR spectrum for (TMP-MIP1) imprinted polymer

	Functional group (cm-1)	*Trimethoprim.*	*(MIP1) Before template removal*	*(MIP1) after template removal*
1	*N-H str.*	*3469* *3319*	*3460* *3373*	-------
2	*C-H aromatic*	*3010*	*3042*	------
3	*C-H aliphatic.*	*2974* *2833*	*2968* *2891*	*2970*
4	*O-C str.*	*1236*	*1294*	*1149*
5	*C= C aromatic str.*	*1595*	*1564*	------
6	*C=N str.* *1633*	-------	----	
7	*C=O ester* ------	*1724*	*1728*	
8	*C=C allyl str.* ------	*1637*	*1635*	

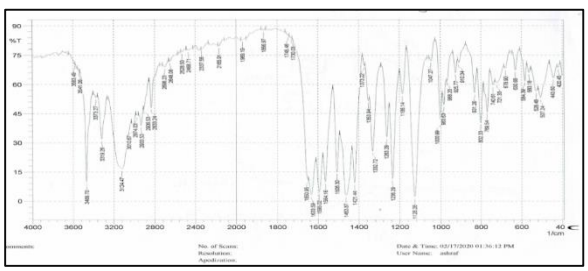


Fig. 2. FTIR of (TMP) drug

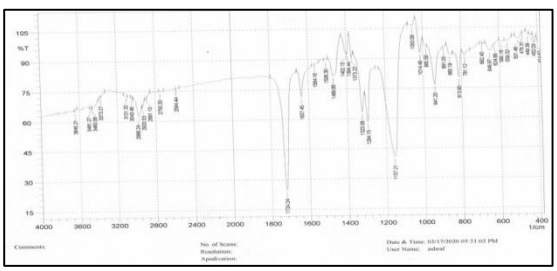


Fig. 3. Spectrum of TMP-MIP1 before the extraction of TMP

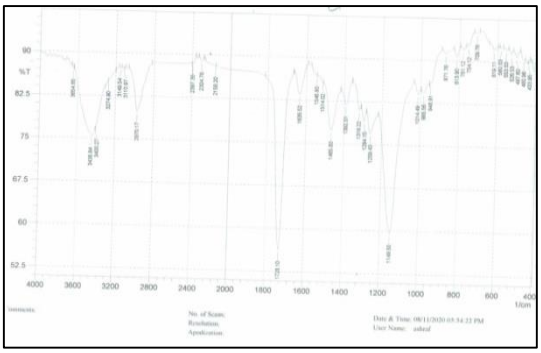


Fig. 4. Spectrum of TMP-MIP1 after the extraction of TMP.

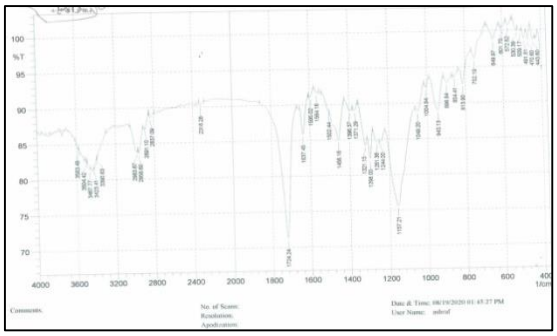


Fig. 5. Spectrum of TMP-MIP2 before the extraction of TMP.

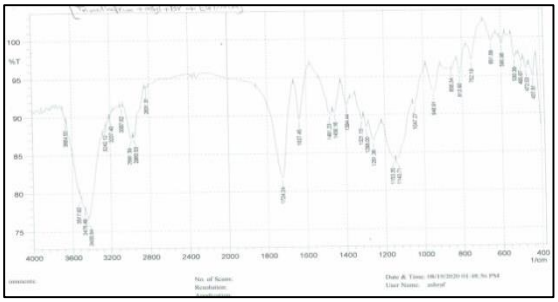


Fig. 6. Spectrum of TMP-MIP2 after the extraction of TMP

Table. 2 FT-IR spectrum for (TMP-MIP2) imprinted polymer.

	Functional group (cm^{-1})	Trimethoprim.	(MIP_2) before template removal	(MIP_2) after template removal
1	N-H str.	3469, 3319	3467, 3423	-------
2	C-H aromatic	3010	2983	------
3	C-H aliphatic.	2974 2832	2958 2891	2960 2831
4	O-C str.	1236	1244	-------
5	C= C aromatic str.	1595	1595	------
6	C=N str.	1633	1637	----
7	C=O ester	------	1724	1724
8	C=C allyl str.	------	1637	1635

Morphological analysis

SEM of TMP-MIPs.

scanning Electron Microscopy (SEM) used to Examination and analysis surface and topography of TMP-MIP1 and TMP-MIP2 before and after Template removal that show in figures (7) for TMP-MIP1 and figures (8) for TMP-MIP2 .

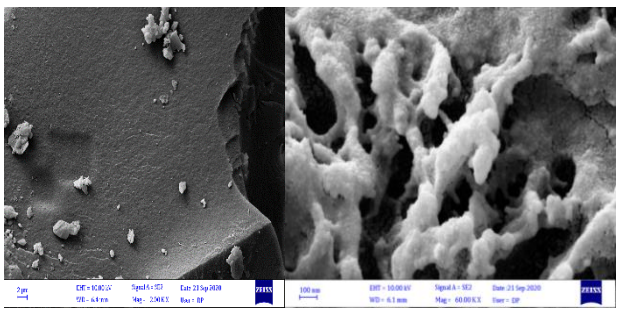


Fig. 7. Scanning Electron Microscopy for TMP-MIP1, (a) before (b) after extraction the template

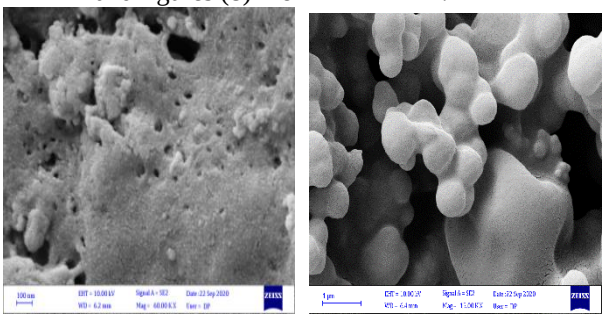


Fig.8. Scanning Electron Microscopy for TMP-MIP1, (a) before (b) after extraction the template.

Selectivity coefficient Calculation.

For the selectivity coefficient calculation, a different solution approach was used and was determined by following the equation (1)[24,25].

$$\text{Log Kpot} = [(E_B - E_A)/(2.303RT/ZF)] + (1 - \mathbf{z}_A/\mathbf{z}_B)\ \text{logaA} \quad \ldots\ldots\ldots(1)$$

Table 5 Selectivity Coefficients for (TMP-MIP2+DBS) electrode at different concentrations of TMP.

Conc. (M)	Interfering ions							
	Glucose		Calcium stearate		Sodium benzoate		Benzoic acid	
	E_B mV	$K_{A,B}$	E_B mV	$K_{A,B}$	E_B mV	$K_{A,B}$	E_B mV	$K_{A,B}$
$1x\ 10^{-1}$	213.1	1.160×10^{-3}	186.9	3.893×10^{-4}	113.5	1.827×10^{-5}	96.2	8.887×10^{-6}
$1x\ 10^{-2}$	163.4	2.002×10^{-3}	165.4	2.176×10^{-3}	93.5	1.087×10^{-4}	81.7	6.651×10^{-5}
$1x\ 10^{-3}$	98.7	1.032×10^{-3}	103.5	1.260×10^{-3}	86.9	6.312×10^{-4}	73.5	3.611×10^{-4}
$1x\ 10^{-4}$	83.2	4.550×10^{-3}	96.5	7.921×10^{-3}	71.5	2.794×10^{-3}	61.7	1.857×10^{-3}
$1x\ 10^{-5}$	46.1	7.078×10^{-3}	71.7	2.057×10^{-2}	52.3	9.165×10^{-3}	52.5	9.241×10^{-3}
$1x\ 10^{-6}$	32.6	1.023×10^{-1}	52.2	2.316×10^{-1}	31.7	9.853×10^{-2}	44.2	1.659×10^{-1}

Table 6 Selectivity Coefficients for (TMP-MIP1+DBPH) electrode at different concentrations of TMP.

Conc. (M)	Interfering ions							
	Glucose		Calcium stearate		Sodium benzoate		Benzoic acid	
	E_B mV	$K_{A,B}$	E_B mV	$K_{A,B}$	E_B mV	$K_{A,B}$	E_B Mv	$K_{A,B}$
$1x\ 10^{-1}$	-211.7	1.704×10^{-10}	-86.1	4.990×10^{-2}	-11.2	1.298×10^{-5}	-39.1	2.716×10^{-6}
$1x\ 10^{-2}$	-209.3	2.088×10^{-9}	83.6-	2.401×10^{-6}	-16.3	1.044×10^{-4}	-61.3	8.383×10^{-6}
$1x\ 10^{-3}$	-203.6	2.769×10^{-8}	-80.3	2.782×10^{-5}	-17.2	9.568×10^{-4}	-76.8	3.386×10^{-5}
$1x\ 10^{-4}$	-196.2	3.277×10^{-7}	-71.9	3.429×10^{-4}	-23.7	5.115×10^{-3}	-89.5	1.278×10^{-4}
$1x\ 10^{-5}$	-182.1	6.298×10^{-6}	70.7-	3.248×10^{-3}	-39.1	1.909×10^{-2}	-106.2	4.438×10^{-4}
$1x\ 10^{-6}$	-171.6	2.190×10^{-4}	69.2-	6.819×10^{-2}	-46.5	2.434×10^{-1}	-121.4	3.653×10^{-3}

PH Effects

The effect of changing the pH function with which the Sulfamethoxazole electrodes for SMT-MIP5 depend on (DBPH, NB, O.A and PRF) as plasticizers operate was studied by determining the electrode response by measuring the potential of the Sulfamethoxazole electrode for three different concentrations 10-4,10-3,10-2 M for ranges of pH from 10 -1.0, the pH values of the solutions were adjusted using a solution.

Dilute hydrochloric acid or dilute sodium hydroxide solution. Different responses appear with the difference in the pH range of the solution, but there is a specific range of pH in which the response to a particular electrode stabilizes and represents the appropriate range within which potentiometric measurements can be made, followed by a decrease in response values with increasing pH values Solution the results shown in table(7).

Table 7 Effect pH ranges used for TMP-MIP1-selective electrodes.

Number	Membrane composition	pH range		
		$1 X 10^{-2}$	$1 X 10^{-3}$	$1 X 10^{-4}$
I	TMP-MIP3+DBPH	4 - 8	3.5 - 8.5	4 – 9
II	TMP-MIP3+NB	4 - 9	3.5 – 9	3.5 – 9
III	TMP-MIP3+O.A	4 - 8	4 – 9	4 – 9
IV	TMP-MIP3+PRF	3.5 - 9	4 – 9	3.5 – 9

Table 8 Effect pH ranges used for TMP-MIP2-selective electrodes**.**

Number	Membrane composition	pH range		
		$1 X 10^{-2}$	$1 X 10^{-3}$	$1 X 10^{-4}$
I	TMP-MIP4+DBS	3.5 – 8.5	3 – 8.5	3.5 – 8
II	TMP-MIP4+DMA	3.5 – 8	3 – 8.5	3 – 8.5
III	TMP-MIP4+TBP	3.5 – 9	3 – 9	3.5 – 8.5
IV	TMP-MIP4+TEHP	3 – 8	3.5 - 8.5	3 – 9

Sample analysis:

Three techniques were used to determination Trimethoprim via direct, standard addition method (SAM) and multi-standard addition (MSA) method in pure form and pharmaceutical preparation. Equation (2)[25] used to SAM.

$$C_U = C_S / 10^{\Delta E/S} [1+ (V_U / V_S)] - (V_U / V_S) \ldots\ldots\ldots (2)$$

Where the volume and concentration of an unknown and standard solution respectively are VU, VS, CU and CS

Table 9 Determination of Trimethoprim pure samples by ion selective electrodes techniques based on PVC membranes for TMP-MIPs.

	Measurement by using ISEs methods				
	(Standard sample)	1×10^{-4} (M)			
	Methods	)Con. Found(M	E rel.%	Rec. %	RSD%
	Direct	0.983 X 10^{-4}	-1.7	98.3	1.512
	SAM	1.006X 10^{-4}	0.6	100.6	0.976
TMP-MIP3+DBPH	MSA	1.010X 10^{-4}	1	101	0.613
	(Standard sample)	1×10^{-3} (M)			
	Direct	1.035 X 10^{-3}	3.5	103.5	2.341
	SAM	0.967 X 10^{-3}	-3.3	96.7	1.805
	MSA	1.02 X 10^{-3}	2	102	1.213
	(Standard sample)	1×10^{-4} (M)			
	Direct	0.970 X 10^{-4}	-3	97	3.001
	SAM	0.975 X 10^{-4}	-2.5	97.5	2.737
	MSA	1.029 X 10^{-4}	2.9	102.9	0.839
TMP-MIP3+NB	(Standard sample)	1×10^{-3} (M)			
	Direct	0.978 X 10^{-3}	-2.2	97.8	3.501
	SAM	0.984 X 10^{-3}	-1.6	98.4	2.611
	MSA	1.02 X 10^{-3}	2	102	1.943
	Measurement by using ISEs methods				
	(Standard sample)	1×10^{-4} (M)			
	Methods	Con. Found(M)	E rel.%	Rec. %	RSD%
	Direct	1.029 X 10^{-4}	2.9	102.9	2.6
	SAM	0.983 X 10^{-4}	-1.7	98.3	0.36
TMP-MIP4+DBS	MSA	1.040 X 10^{-4}	4	104	3.2
	(Standard sample)	1×10^{-3} (M)			
	Direct	0.962 X 10^{-3}	-3.8	96.2	2.73
	SAM	0.967 X 10^{-3}	-3.3	96.7	2.42
	MSA	1.009 X 10^{-3}	0.9	100.9	0.13
	(Standard sample)	1×10^{-4} (M)			
	Direct	0.975 X 10^{-4}	-2.5	97.5	1.04
	SAM	0.996 X 10^{-4}	-0.4	99.6	0.23
	MSA	0.977 X 10^{-4}	-2.3	97.7	0.77
	(Standard sample)	1×10^{-3} (M)			
TMP-MIP4+TEHP	Direct	0.988 X 10^{-3}	-1.2	98.8	2.6
	SAM	0.994 X 10^{-3}	-0.6	99.4	0.44
	MSA	0.992 X 10^{-3}	-0.8	99.2	0.36

- **Trimethoprim estimation in pharmaceutical preparation.**

After good results were obtained when we used the prepared sensors depend on TMP-MIP1 and TMP-MIP2, in determination Trimethoprim in pure form. Applied to estimate the Trimethoprim drug in the pharmaceutical preparation whose found as tablets with 80 mg. These ISEs measurements have been tested by different potentiometric methods. Preparation solutions of Trimethoprim at concentrations 1×10^{-3} and 1×10^{-4} M then calculation's the Erel.%, Rec.% and RSD% of Trimethoprim in pharmaceuticals. The results obtained represented in the table (10) and (11) .

Conclusion

Trimethoprim molecularly imprinted electrodes based on allyl chloride (AYC) and allyl bromide (AYB) as a monomer , the cross-linker trimethylolpropanetri acrylate (TMPTA) and Etheleyene glycol dimethyl acrylate (EGDMA) was used and (BP) benzoyl peroxide as (initiator)was constructed based on PVC matrix membrane. Excellent electrode parameters were obtained including Nernstain slopes, detection limit and pH. The prepared electrodes were used for Trimethoprim determination in commercial drugs which gives recovery ranged from 94.8 to 103.9.

Table 10 Sample analysis of pharmaceuticals Trimethoprim by using MIPs membrane electrode (TMP-MIP1+DBPH).

PHARMACEUTICAL	Cotrimstada (STADA, GER)		
	Direct	SAM	MSA
Concentration (prepared) M		$1 X 10^{-3}$	
Value founded	$0.948X 10^{-3}$	$0.955X 10^{-3}$	$0.973X 10^{-3}$
Recovery%	94.8	95.5	97.3
Erel %	-5.2	-4.5	-2.7
% RSD	4.810	4.244	3.006
Concentration (prepared) M		$1 X 10^{-4}$	
Value founded	$0.978X 10^{-4}$	$0.981X 10^{-3}$	$1.015X 10^{-3}$
Recovery%	97.8	98.1	101.5
Erel %	-2.2	-1.9	1.5
% RSD	1.286	1.041	1.903
PHARMACEUTICAL	Trimethoprim (actaivs, UK)		
Concentration (prepared) M		$1 X 10^{-3}$	
Value founded	$0.963X 10^{-3}$	$1.025X 10^{-3}$	$1.011X 10^{-3}$
Recovery%	96.3	102.5	101.1
Erel %	-3.7	2.5	1.1
% RSD	2.888	1.304	1.003
Concentration (prepared) M		$1 X 10^{-4}$	
Value founded	$1.039X 10^{-3}$	$0.979X 10^{-3}$	$0.993X 10^{-3}$
Recovery%	103.9	97.9	99.3
Erel %	3.9	-2.1	-0.7
% RSD	2.080	1.887	0.521

Table 11 Sample analysis of pharmaceuticals Trimethoprim by using MIPs membrane electrode (TMP-MIP2+DBS).

PHARMACEUTICAL	Cotrimstada (STADA, GER.)		
	Direct	SAM	MSA
Concentration (prepared) M		$1 X 10^{-3}$	
Value founded	$0.978 X 10^{-3}$	$0.985 X 10^{-3}$	$0.989 X 10^{-3}$
Recovery%	97.8	98.5	98.9
Erel %	-2.2	-1.5	-1.1
% RSD	1.824	1.047	0.851
Concentration (prepared) M		$1 X 10^{-4}$	
Value founded	$0.972 X 10^{-4}$	$0.984 X 10^{-4}$	$0.990 X 10^{-4}$
Recovery%	97.2	98.4	99.0
Erel %	-2.8	-1.6	-1
% RSD	2.067	1.830	0.947
PHARMACEUTICAL	Trimethoprim (actaivs, UK)		
Concentration (prepared) M		$1 X 10^{-3}$	
Value founded	$1.032 X 10^{-3}$	$0.985 X 10^{-3}$	$0.996 X 10^{-3}$
Recovery%	103.2	98.5	99.6
Erel %	3.2	-1.5	-0.4
% RSD	2.954	1.037	0.629
Concentration (prepared) M		$1 X 10^{-4}$	
Value founded	$0.964 X 10^{-4}$	$0.970 X 10^{-4}$	$0.983 X 10^{-4}$
Recovery%	96.4	97.0	98.3
Erel %	-3.6	-3	-2.7
% RSD	2.719	3.051	1.058

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