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Synthesis of New Selective Electrodes for the Determination of Metronidazole Benzoate (MNZB) Based on a Molecularly Imprinted Polymer Combined With Poly Vinyl Chloride

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Abstract : Liquid electrodes of polymers imprinted with metronidazole benzoate (MNZB) were synthesized based on precipitation polymerization mechanism. The molecularly imprinted polymer (MIP) and non-imprinted polymers (NIP) were synthesized using MNZB as a template. In polymerization process, 2-Acrylamido-2-methyl-1-propane Sulphonic acid (AMPS) and 1-Vinylimidazole (VIZ) were used as monomers. Pentaerythritol triacrylate (PETRA) and Divinylbanzene (DVB) were used as cross-linkers and benzoyl peroxide (BPO) as an initiator. The molecularly imprinted membranes and the molecularly non-imprinted membranes were synthesized using Dibutyl Sebacate (DBS) and Tris (2-ethylhexyl) phosphate (TEHP) as plasticizers in PVC matrix. Slopes and detection limit of the liquid electrodes are ranged at (52.23– 58.94) mV/decade and (1.2×10^{-6} – 2.0×10^{-5}) M, respectively. Response time was 60 seconds. Liquid electrodes were filled with 10^{-1} M standard solution of drug and observed stable response for a pH ranged from 1.5 to 12 and with good selectivity for over several species. The new synthesis electrodes were successfully used for the analyte estimated in preparation pharmaceutical sample without any time consuming pretreatment steps.
Keywords : Molecularly imprinted electrodes; Metronidazole benzoate; potentiometric method; (AMPS); (VIZ) monomers.

Introduction

Metronidazole benzoate (MNZB), 1-(2-benzyloxy ethyl)-5-nitro methylimidazole, is one of the nitroimidazole derivative have antimicrobial properties. Effect against trichomonas, vincent's organisms, and anaerobic bacteria. MNZB is used before the Veterinarians to treat bacterial infections as well as giardia in dogs and cats[1]. Figure 1 appearance the chemical structure of MNZB. Different analytical methods have been applied for the determination of MNZB, including spectrophotometry[2], gas chromatography[3], and high performance liquid chromatography[4].

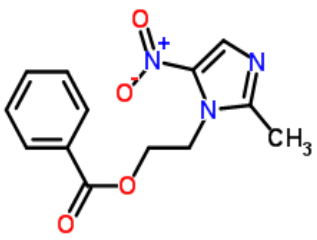


Fig.1. Structure of metronidazole benzoate (MNZB)

Metronidazole (MNZB) has been useful for the remediation of protozoa diseases including Giardia infections, bacterial vaginosis, amebic liver abscess[5, 6], etc. In general, MNZB is also harness for any potentially susceptible anaerobic infection by veterinary community or as a promoter to induce the growth of aquatic products[7]. However, MNZB has been prohibiting by many countries and areas from addition to food producing animals cause to carcinogenic properties according to the WHO International Agency for Research on Cancer (IARC)[8]. Different analytical methods were applied to determination of MNZB in diverse samples are crucial for food security, human health and investigations on the biological toxicity of MNZB. Different methods have been used for this purpose, most of which are on the basis of chromatography[9], spectrophotometry[10], and electrochemistry[11, 12].

Molecularly imprinted polymers (MIPs), generally behave as synthetic antibody snob, have been appear to be very promising candidates as highly selective adsorbents, because the advantages inherent such as reusability, physiochemical, molecular specificity, stability and applicability in harsh chemical media[13].MIPs are mainly based on the polymerization of functional monomers in the presence of a template molecule. The template is leached out leaving behind cavities which are integral in shape, size and functionality to the template. In recent years, MIP technology has develop to a valuable integral concept for biological activity with increased applicability in analytical chemistry, which show a different and rapid methods for synthesis a polymer matrix with molecule-specific activity properties with applications ranging from purification of racemic mixtures, to catalytic control and chemical sensing of complex chemical reactions[14].It was determination some drugs such as ibuprofen[15] and warfarin sodium[16] based on molecularly imprinted polymer method. In this study imprinted polymer electrodes were prepared based on metronidazole benzoate as a template in PVC matrix membrane and electrodes specification were studied.

Experimental

Chemicals

Metronidazole benzoate was obtained from the State Company of Drug Industries and Medical Appliances (IRAQ-Medial East- Baghdad). The Commercial Metronidazole tablets obtained from local stores are; Framar Lyon-Flagyl 500 mg, Julphar- Negazole 500 mg and Microlabslimited -Metronidazole 500mg.

Tris (2-ethylhexyl) phosphate (TEPH) and Di-butyl sebacate (DBS) as well as metal salts were purchased from Sigma-Aldrich and were used as they were received. Pentaerythritoltriacrylate (PETRA) (99%), (2-Acrylamido-2-methyl-1-propane sulphonic acid (AMPS) (99%), or (1-Vinylimidazole)(99%), (pentaerythritol triacrylate (PETRA) or Divinylbanzene) and benzoyl peroxide (BPO) (78%) were purchased from Sigma-Aldrich. The chemicals used in the search were possesses high purity does not need to purify.

Apparatus

Potentiometric measurements were carried out with a digital voltmeter (HANA pH 211 instrument Microprocessor pH meter). pH measurements were made with a digital pH meter (wissenschaftlich-TechnischeWerkstätten GmbH WTW/pH meter in lab pH720-Germany), UV-Visible spectrophotometer double-beam model (UV-1650 PC) SHIMADZ (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10), using 1.00cm quartz cells, Infrared spectrophotometer SHIMADZU, FTIR-8000 (Japan), Scaning 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 Metronidazole benzoate solutions at room temperature with a concentrations range from10^{-1} to 10^{-6} M. For the accuracy the potential of solutions were measured after the arrival of the internal and external solution to the equilibrium, then the potential recorded.

Synthesis of the imprinted polymer (MIP)

Bulk polymerization method was use3d for preparation of MIP. The template (MNZB) of 0.5mmol was dissolved in a thick walled glass tube (50 mL capacity) filled with 10 mL chloroform. Two monomers were used for preparation of MIP, 3 mmole of (2-Acrylamido-2-methyl-1-propane Sulphonic acid (AMPS) with 30 mmole pentaerythritol triacrylate as a cross-linker, the second MIP based on 3mmol of 1-Vinylimidazole monomer 30 mmol Divinylbanzene as cross-linker. The initiator of 0.32 mmole BPO was used. The solution

was mixed in ultrasonic water bath for a period of 45 minutes, during this time the nitrogen gas was purged the mixture. 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. The templates were removed by repeated washing the MIPs successively with 100 mL portions of 30% (v/v) acetic acid /methanol solution by using soxhlet extraction. The polymer was dried at (35-45) ^{o}C for (24-48) hours, The polymers were then crushed and grounded using mortar and pestle and sieved to particles size 125 µm (using 100 mesh sieve); After the polymer was completely dried at ambient temperature, it was used as an active material in the selective sensor membrane. The non-printed polymer NIP was made at the same way but without the template drug.To prepare specific PVC membrane, high molecular weight PVC (0.17g) mixed together with the MIP (0.02g) and the plasticizer (0.4g) until the solution become homogenized, and then add THF (4-5 mL) and stirred. The solution was transferred to glass vessel based on glass board with 5cm dia. circular section to let this mixture evaporate for 24 hours. A glass tube contain a silver wire painted with silver chloride and filled with 0.1 M standard solution of Metronidazole benzoate was connected to one end of the Tygon tube tightly while the second end of the tube was attached to 10 mm dia. circular disk of the PVC membrane by using a concentrated PVC/THF solution as a glue in purpose of producing the electrode.

For the sake of clarity of the morphology and design of the particles and were used scanning electron microscope (SEM) .The morphology of MIP and NIP membranes for Metronidazole benzoate before and after washing is showed by electron microscope in figure 2. A porous on the surface (figure 2a) about 20 µm may indicate the binding sides to the polymer. Figure 2b shows clear holes about 50 µm in sizes have been obtained and which were removed by soxhlet extraction.

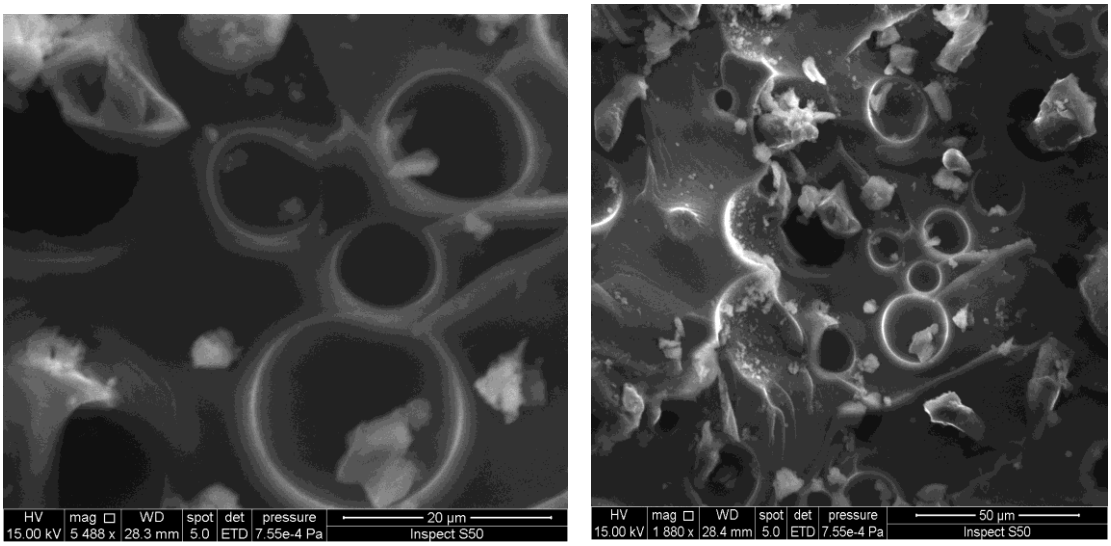


Fig.2. SEM photograph of the surface of MIP, a) after washing b) before washing

Potential Measurements

Measurements were carried out in a 50 mL double walled glass cell, magnetic stirring was used for obtain a homogeneous solution and under laboratory. The efficacy of the electrodes was scrutinized by measuring the potential of standard solutions for drugs prepared with a concentration range of 10^{-1} to 10^{-6} M by serial dilution. The slope, detection limit, and response time operative life were calculated from the calibration curve.

Preparation of Pharmaceutical Samples

Three types of tablets were used to determine the concentration of Metronidazole benzoate,France-(Framar Lyon): B.P(500) mg (Flagyl) tablets, U.A.E-(Julphar) B.p (500) mg (Negazole tablets), India-(MICRO LABS LIMITED) B.P(500mg) (Metronidazole) capsules were grinded(0.0275g) and dissolved in 1M (HCl) and completed in volumetric flask to (100ml).

Results and Discussion

Liquid Membranes Electrode

MIP based liquid electrodes, their concentrations range and slopes response to Nernstian equation has been investigated. The membranes of MIP made of the monomers AMPS and VIZ with a PVC matrix using two plasticizers DBS and TEHP. The internal solution was used 0.1M aqueous standard solution of drug for all liquid electrodes. Experimental results of synthesis of molecularly imprinted (MIP) and non-imprinted polymers (NIP) based on two monomers AMPS and VIZ indicate that both monomers can be used for the preparation of effective MIP for MNZB. 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 membrane. Both of the plasticizers that are used, DBS and TEHP, are suitable for the fabrication of MIP-based MNZB electrodes. Table 1 show the parameters of the fabricated and tested electrodes, Four membranes of the different compositions were prepared using two different plasticizers with different viscosities, dibutylsebacate (DBS) (ν=11.0042cSt) and tris (-2-Ethyl hexyl) phosphate (TEPH)(ν =8.015 cSt). The results of electrode specification were obtained from the calibration curves that listed in Table 1. The slopes of the electrodes ranged between 19.62-57.36 mV/decade and linear dynamic ranges between $1.2x10^{-6}$- $2.0\ x10^{-5}$ M. In generally the preparation electrodes have a short response time (about 60 second) mostly at high concentrations. The values listed in table 1 also indicate the electrodes IT and IVT give the good results therefore, the liquid electrode were used to determine both drugs in pharmaceutical samples.

Table1. Parameter of MNZB-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
IT	MNZB-MIP1 (AMPS PETRA + DBS)	58.23	0.9984	$1\times(10^{-6}\text{-}10^{-1})$	$2x10^{-5}$	50
IIT	MNZB-MIP1 (AMPS PETRA +TEHP)	57.36	0.9997	$1\times(10^{-6}\text{-}10^{-1})$	$1.2x10^{-6}$	40
IIIT	MNZB-MIP2 (VIZ+DVB + DBS)	56.62	0.9999	$1\times(10^{-5}\text{-}10^{-2})$	$4x10^{-6}$	30
IIVT	MNZB-MIP2 (VIZ+DVB + TEHP)	58.94	0.9999	$1\times(10^{-6}\text{-}10^{-1})$	$3.2x10^{-5}$	50

Influence of pH

The effect of pH on the potential values of the four electrodes was studied over pH range 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 1×10^{-4} to 1×10^{-2} M of standard solutions of drugs. The obtained results are shown in Table 2 and the typical plot of electrode potential versus pH for electrode IT and IVT are shown in Figure 3.

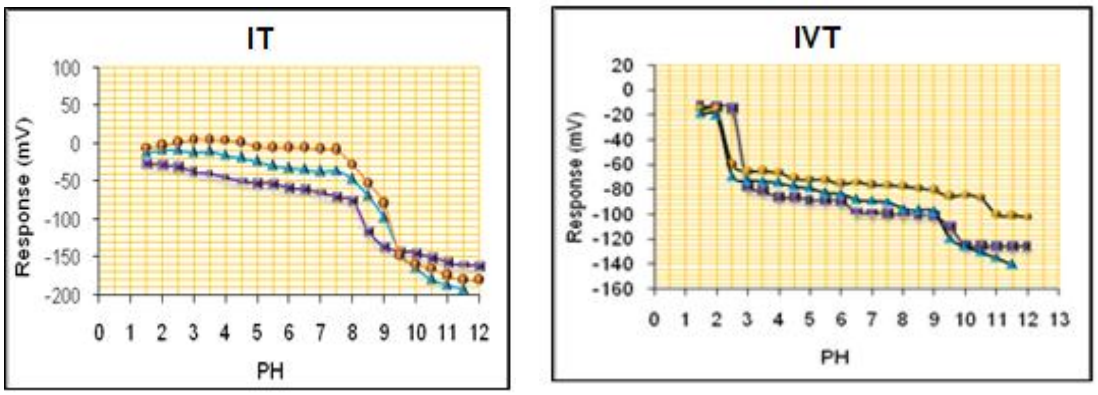


Fig.3. Typical plot of electrode response versus pH of MNZB-MIP electrodes at different concentrations

Table2.Working pH ranges for MNZB-MIP electrodes

Electrode No.	Membrane composition	pH range		
		$1x10^{-2}$	**$1x10^{-3}$**	**$1x10^{-4}$**
IT	MNZB-MIP1 + DBS	1.5-12	1.5-12	1.5-12
IIT	MNZB-MIP1 +TEHP	1.5-12	1.5-12	1.5-12
IIIT	MNZB-MIP2 +DBS	1.5-10	2.0-9	2.5-8.5
IVT	MNZB-MIP2 +TEHP	2.5-9	2.3-8.7	3-9.2

Response time and life time

The response time for all MZNB.MIP electrodes was obtained from the dynamic potential response at 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 metronidazole benzoate 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 MZNB.MIP during 30-50 days as shown in Table 3.

Table 3. Response time of Metronidazole benzoate electrode

Membrane	Conce. (M)	(mV) at t/100	Time (s) at 95%	Time (s) at 100%
MNZB-MIP1 + DBS (IT)	10^{-1}	-4.6	5.2	5.5
	10^{-2}	-8	21	22.1
	10^{-3}	-8.1	44	45.8
	10^{-4}	-16.2	55.8	58.7
	10^{-5}	-9.1	57	60.0
	10^{-6}	-13.8	59	62.1
MNZB-MIP1 + TEHP (IIT)	10^{-1}	-0.4	15.2	16.0
	10^{-2}	-4.2	15.3	16.1
	10^{-3}	-10	29	30.5
	10^{-4}	-22.7	34	35.8

	10^{-5}	26.1	38	40.0
	10^{-6}	-5.4	46	48.4
MNZB-MIP2 + DBS (IIIT)	10^{-1}	-10.4	25.2	26.0
	10^{-2}	-14.2	25.3	26.1
	10^{-3}	-10.6	29.7	30.5
	10^{-4}	-22.7	36.3	38.8
	10^{-5}	-24.1	38	40.0
	10^{-6}	-25.4	45	48.9
MNZB-MIP2 + TEHP (IVT)	10^{-1}	-10.4	19.5	20.2
	10^{-2}	-24.4	19.9	20.4
	10^{-3}	-29.2	22	23.6
	10^{-4}	-30.1	28	30.4
	10^{-5}	-32.6	34	35.7
	10^{-6}	-34.7	40	41.3

Selectivity coefficient

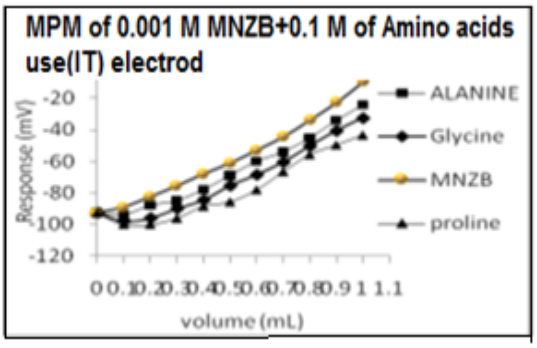
MPM is used for electrodes to determine the potentiometric selectivity coefficients (KpotA,B) associated with two ions whatever their charge, as MPM theory is basis upon layers of electrical diffuse on both sides (the aqueous and the membrane of the interface), so it is not depend on equation of Nicolsky-Eisenman. With respect to MPM, the coefficients of selectivity for equal charge ions (i.e. ZA=ZB) are stated as the ratio of the primary and interfering ions concentrations within aqueous solutions at which as much as the permeability of the primary and interfering ions which passing through the membrane surface selectively . The selectivity coefficients of unequal charge ions (i.e. ZA≠ZB), that are not only represented the primary and interfering ions amounts which permeated through the surface of membrane (as a function), but they are also identify the concentration of primary ion within the initial reference solution and the value of delta EMF. In this method the selectivity coefficient is given by using equation below:-

$K^{pot}_{A,B} = (a'A - aA) / aB$

The results have shown in Figure 4 and in Tables 4 regarding the coefficient of selectivity have been computed through the interfering ion concentration which gave a potential difference as much as that the amount induced due to the increasing in the concentration of primary ion.

Table 4 and Fig.4. Result of selectivity coefficients using separate solution method for some interfering species (cations and amino acids)

Membrane composition	Interfering-Ion(10-3 M)	KMPM ΔE=10	KMPM ΔE=5
MNZB-MIP1 + DBS	Na^{1+}	0.615	0.731
	Mg^{2+}	0.810	0.804
	Al^{3+}	0.457	0.580
	Alanine	0.938	0.733
	Glycine	0.806	0.653
	proline	0.502	0.642



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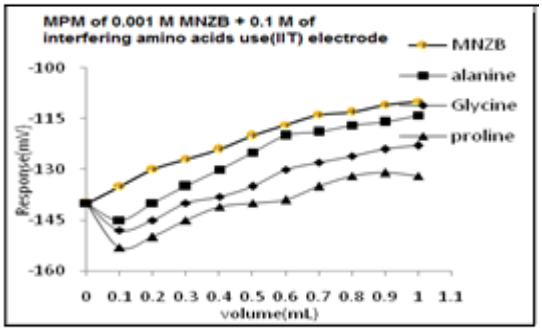
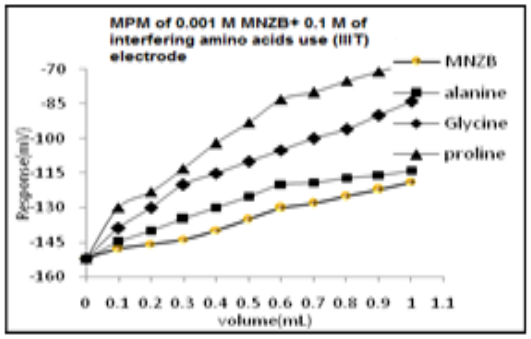
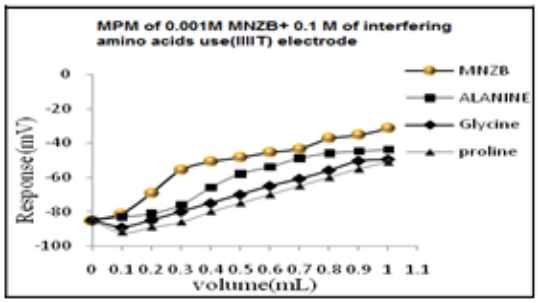
MNZB-MIP1+ TEHP	Na^{1+}	0.615	0.731
	Mg^{2+}	0.810	0.804
	Al^{3+}	0.457	0.580
	Alanine	0.938	0.733
	Glycine	0.806	0.653
	proline	0.502	0.642



Membrane composition	Interfering-Ion(10-3 M)	KMPM ΔE=10	KMPM ΔE=5
MNZB-MIP2 + DBS	Na^{1+}	0.815	0.931
	Mg^{2+}	0.852	0.690
	Al^{3+}	0.889	0.758
	Alanine	0.689	0.733
	Glycine	0.806	0.653
	proline	0.870	0.642



Membrane composition	Interfering-Ion(10-3 M)	KMPM ΔE=10	KMPM ΔE=5
MNZB-MIP2 + TEHP	Na^{1+}	0.937	0.648
	Mg^{2+}	0.959	0.584
	Al^{3+}	0.784	0.829
	Alanine	0.562	0.673
	Glycine	0.725	0.584
	proline	0.687	0.496



Quantitative analysis

The accuracy of electrodes IT and IVT were measured by determining Metronidazole benzoate in synthetic solutions of 10^{-3} and 10^{-4} M using standard addition method. Excellent results of % recovery were obtained in the range 94.95 to 105.6. A typical plot for membrane IT and IVT at concentration of synthetic solution ($10^{-3,}$ 10^{-4}) M is shown in Fig. (5, 6) and the standard solution added was 0.1 M.

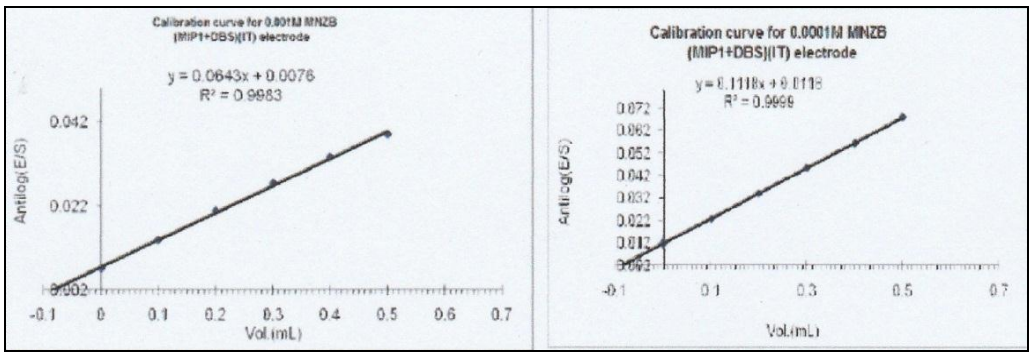


Fig.5. Variation of antilog (E/S) of synthetic solution of 10^{-3}, 10^{-4} M versus of standard MNZB added using electrode (IT)

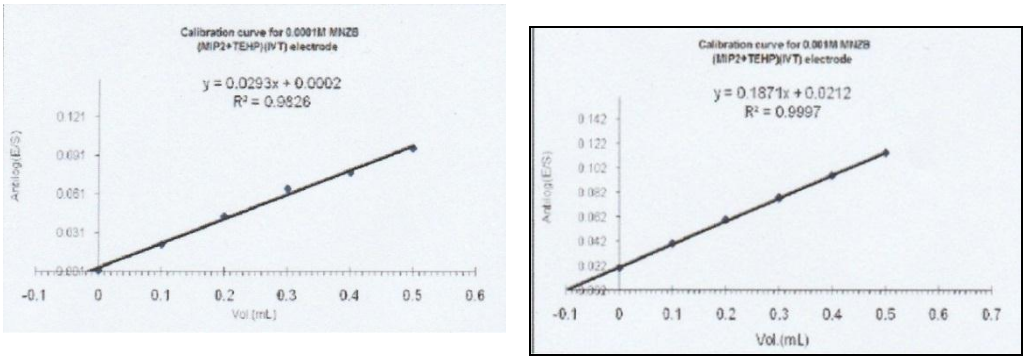


Fig.6. Variation of antilog(E/S) of synthetic solution of 10^{-3},10^{-4} M versus of standard MNZB added using electrode (IVT)

Direct method and standard addition method were applied for the determination Metronidazole benzoate in commercial pharmaceutical tablets (Framar Lyon-Flagyl 500 mg, Julphar- Negazole 500 mg and Micro labs limited -Metronidazole 500mg) obtained from local stories using membrane IT based on DBS and IVT based on TEHP as plasticizer. The values of the % recovery (table 5and 6) agree with the value given in British Phamacopoeia[17]. There is no interference of all species on electrode response, therefore, the values of recovery obtained by standard addition method agree with the results of direct method.

Table5. Results of recovery and standard deviation of commercial drugs obtained by using membrane IT.

Pharmaceutical Drug	Potentiometric methods	Concentration Prepared/ M	Concentration Found/ M	%Rec.	%RE	%RSD	F-experimental	F theoretical
Framar Lyon-Flagyl 500 mg	Direct method	$1.0x10^{-3}$	$0.10448\ x10^{-3}$	104.48	4.48	0.45	14.2	19.2
	SAM		$9.9851\ x10^{-3}$	99.85	-0.15	-0.10	15.4	
	Direct method	$1.0x10^{-4}$	0.9495×10^{-4}	94.95	-5.05	1.21	5.3	19.2
	SAM		1.0472×10^{-4}	104.72	4.72	-0.22	7.4	
Julphar-Negazole 500 mg	Direct method	$1.0x10^{-3}$	0.1056×10^{-3}	105.60	5.60	1.20	13.2	19.2
	SAM		1.0515×10^{-3}	105.15	5.15	-0.07	12.5	
	Direct method	$1.0x10^{-4}$	0.10580×10^{-4}	105.79	5.79	0.79	10.6	19.2
	SAM		0.9356×10^{-4}	99.36	-0.64	-0.16	15.7	
Micro labs limited - Metronidazole 500mg	Direct method	$1.0x10^{-3}$	0.9938×10^{-3}	99.38	-0.62	0.79	15.4	19.2
	SAM		1.0496×10^{-3}	104.58	4.58	-0.13	10.9	
	Direct method	$1.0x10^{-4}$	$0.1052\ x10^{-4}$	105.24	5.24	0.45	13.5	19.2
	SAM		$1.0186\ x10^{-4}$	101.86	1.86	0.96	18.2	

Table6. Results of recovery and standard deviation of commercial drugs obtained by usingmembrane IVT.

Pharmaceutical Drug	Potentiometric methods	Concentration Prepared/ M	Concentration Found/ M	%Rec.	%RE	%RSD	F-experimental	F theoretical
Framar Lyon-Flagyl 500 mg	Direct method	$1.0x10^{-3}$	1.0302×10^{-3}	103.02	3.02	4.70	9.5	19.2
	SAM		1.0491×10^{-3}	104.91	4.91	-0.52	11.3	
	Direct method	$1.0x10^{-4}$	1.0337×10^{-4}	103.37	3.37	3.58	11.1	19.2
	SAM		0.97870×10^{-4}	97.87	-2.13	-0.80	15.4	
Julphar-Negazole 500 mg	Direct method	$1.0x10^{-3}$	0.10392×10^{-3}	103.92	3.92	10.13	12.6	19.2
	SAM		1.0495×10^{-3}	104.95	4.95	-0.99	14.2	
	Direct method	$1.0x10^{-4}$	1.0200×10^{-4}	102.00	2.00	3.22	8.8	19.2
	SAM		0.98115×10^{-4}	98.12	-1.88	-1.10	6.7	
Micro labs limited - Metronidazole 500mg	Direct method	$1.0x10^{-3}$	0.97724×10^{-4}	97.72	-2.28	5.11	9.5	19.2
	SAM		0.97110×10^{-3}	97.11	-2.89	-4.81	7.5	
	Direct method	$1.0x10^{-4}$	1.0487×10^{-4}	104.87	4.87	1.77	2.17	19.2
	SAM		1.0622×10^{-4}	106.22	6.22	-0.46	4.42	

Conclusion

The construct ion of molecularly imprinted electrodes sensors (MIP) using Metronidazole benzoate as a template and(pentaerythritol triacrylate (PETRA) ,(Divinylbanzene) (DVB) as cross-linkers and (2-Acrylamido-2-methyl-1-propane Sulphonic acid (AMPS), (1-Vinylimidazole)(VIZ) 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 TEHP plasticizers. Good results of recoveries were obtained for the determination of Metronidazole benzoate in the commercial tablets in comparison with the British Pharmacopoeia.

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