RESEARCH ARTICLE

Selective Separation of Warfarin Sodium based on Molecularly Imprinted Polymer used Different Functional Monomers

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ABSTRACT

Liquid electrodes of warfarin-imprinted polymers synthesized based on precipitation polymerization mechanism. The synthesis of MIP is based on using Warfarin Sodium (WFS) as a template. Vinyl acetate (VA) and 1-vinylimidizol (VIZ) as a monomer, N, N-methylene bis-acrylamide (MBAA) as a cross-linker and benzoyl peroxide (BPO) as an initiator in the polymerization process. The di-butyl subacute (DBS) and di-octyl phthalate (DOP) using as plasticizers in PVC matrix. The characteristics properties of the electrodes membranes (WFS-VA+DBS), (WFS-VA+DOP), (WFS-VIZ+DBS), and (WFS-VIZ+DOP) were studied, including linearity, slope, correlation coefficients, and limit of detection; the results showed that the membranes prepared gave a linear range from (5×10^{-4}-1×10^{-1}, 1×10^{-3}-1×10^{-1}, 1×10^{-4}-1×10^{-1}, and 1×10^{-4}-1×10^{-1}) M, respectively, with slops of (-18.72, -18.2,-20.67, and -21.09) as for the correlation coefficients are (0.9699, 0.9958, 0.9997, and 0.998) and the detection limit it was (6.5×10^{-4}, 7×10^{-4}, 7.8×10^{-4}, and 7.5×10^{-4}) M respectively, the selectivity coefficient was measurements using analysis of methylparaben (MP), propylparaben (PP), and tri-sodium citrate (TSC), and the proposed electrodes were successfully applied to estimate warfarin in commercial drugs.

Keywords: Molecularly impressed electrodes, Potentiometric method, Warfarin sodium (WFS), Vinyl acetate (VA) monomer, 1-vinylimidizol (VIZ) monomer.

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INTRODUCTION

Warfarin sodium [4-hydroxy-3-(3-oxo-1-phenylbutyl)chromen-2-one] is antagonist vitamin K, and it's widely used in clinics as an oral anticoagulant for the prevention and treatment of venous and arterial thromboembolic disorders in patients and prevention of systemic embolism with cardiac fibrillation.[1] WFS was introduced as the first anticoagulant rodenticide.[2]

The warfarin sodium response range therapeutic is very important, specific, and varies from one patient to another.[3] Pharmacokinetics, pharmacodynamics, and technical parameters factors that affect the response range of warfarin sodium. The pharmacokinetics effect on warfarin sodium by reducing the intestine absorption,[4] therefore, knowledge the concentration of warfarin sodium is important for medical decisions and allows patients to be treated effectively.

Various analytical methods have been developed for determining WFS, including, High performance liquid chromatography (HPLC) with fluorescence detection,[4] LC–MS/MS,[5,6] CE,[7] and other methods. However, the instrument-strategies methods have a high energy and money consumption, a long time, tedious pretreatment, etc., this is particularly unsuitable for rapid monitoring of medicinal drugs. Electrochemical sensing is an alternative to the above techniques, which is widely used due to easy preparation, high sensitivity, low detection limit, etc.[8,9] However, specificity is a common problem with sensor-determination because the detection method usually involves no separation system. Physical, chemical, or biological modification is often necessary to provide a sensor with specific recognition ability for target molecules.[10,11]

Molecularly imprinted polymer (MIP) is the mechanism by which templates create different recognition sites in a polymer. Synthetic receptors prepared using molecular imprinting have robustness, high sensitivity, specificity, and relatively low cost,[12] these unique characteristics have made them desirable alternatives to natural receptors. The polymer science and Nano-technology improvements research have helped to enhanced performance sensors of MIP.[13] High-quality publications describe MIP sensors to determine biomolecules, drug abuse, and explosives have been encouraging in recent years, leading to this technology have applications in medical and diagnostics forensic. This review aims to provide a focused overview of the latest developments in MIP-based

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sensor technology, focusing on real-life applications research.[14] A very selective and sensitive electrochemical technique for warfarin-s detection and determination has present in this work. MIP is prepared using VA, VIZ as monomers, MBAA is a polymerizing cross-linker with DBS, DOP plasticizers in PVC matrix.

EXPERIMENTAL

Chemicals and Materials

Warfarin sodium (WFS) has been obtained from the Pharmaceutical Industries and Medical Appliances State Company (IRAQ- SID- Samara). The commercial tablets of WFS obtained from local stores are; warfarin 14 tablets 1 mg PL Holder: Bristol laboratories Ltb and WFS 14 tablets 1 mg MA Holder: TEVA UK Ltd. 01056-B, Sigma-Aldrich purchased DBS and DOP as well as metal salt and used as received. VA and VIZ (99%) were purchased from Sigma-Aldrich as a monomer, MBAA (99%), and BPO (78%). The chemical used was the highest purity reagent grade and was used without further purification as received.

Apparatus

A voltmeter digital (HANA pH 211 instruments Microprocessor) was used to perform potentiometric measurements. pH meter digital (wissenschaftlich-Technische Werkstätten GmbH WTW / pH meter pH720:Germany) pH measurements were performed; Double-beam UV-VIS spectrophotometer (UV-1650 PC, SHIMADZ-japan), infrared spectrophotometer (FTIR-8000 SHIMADZU:Japan), Scanning Electron Microscopy (SEM)- JSM-6390A,Tokyo:Japan and Sensitive electronic balance (ACS120-4 Kern & Sohn GmbH:Germany).

The quality of electrodes was monitored by measuring at room temperature the potential of warfarin solutions ranging from $5x10^{-5}$ to 10^{-1} M. After the internal and external solution arrived at the equilibrium the potential was measured for accuracy, and then the potential registered**.**

Molecular Imprinted Polymer (MIP) Synthesis

MIPs prepare using bulk polymerization method, two different functional group monomer need for MIP preparation, The first MIP is prepared by dissolving 0.5 mmol of temple (WFS) in methanol then mixed with 3 mmol of (VA) as monomer and 15 mmol of a cross-linker (MBAA), while the second MIP is prepared by dissolving 4 mmol of the temple (WFS) in methanol, 2.4 mmol of (VIZ) monomer, and 12 mmol of cross-linker (MBAA) all these constituent also dissolved in methanol solvent, The 0.32 mmole (BPO) initiator has been used, for 45 minutes, ultrasonic water bath using for mixed the solution, during this time the mixture was purged from the nitrogen gas after 45 minutes sealed the tube and put it in a water bath at 70°C for 3 hours to allow complete the reaction. By using soxhlet extraction, remove the templates from MIP using 100mL acetic acid/acetonitrile solution (2:8 v/v) portions. The polymer was dried for (42 to 75) hours at (35 to 45)˚C, crushed and ground the polymers by mortar and pestle and sieve to get 125 µm particle size (using 125 µm mesh sieve); after dried completely at room temperature, It was used as active material in the selective sensor membrane. The specific PVC membrane is prepared by mixed (0.2 g) PVC with (0.036 g) MIP after removing template drug, (0.45 g) plasticizer, and (4–5 mL) THF with stirring until homogenization of the solution, evaporate the solution mixture for 24 hours by transferred to glass vessel based on glass board with 5 cm dia. circular section. A glass tube contained Ag-wire painted with AgCl and filled with standard 0.1 M WFS solution, one end connected with Tygon tube while the other attached to a diameter of 10 mm. the PVC membrane circular disk uses a combined PVC/THF solution as glue for electrode processing.

The scanning electron microscope (SEM) has been used to study morphology and particle structure of MIP membranes in WFS, and Figure 1 show the morphology of MIP_1 and MIP_2 after washing by electron microscope, which shows very small particles and spherically shaped polymeric particles with small sizes around (296.8–75.7) nm for VA polymer, (179.9–214.2) nm for VIZ polymer.

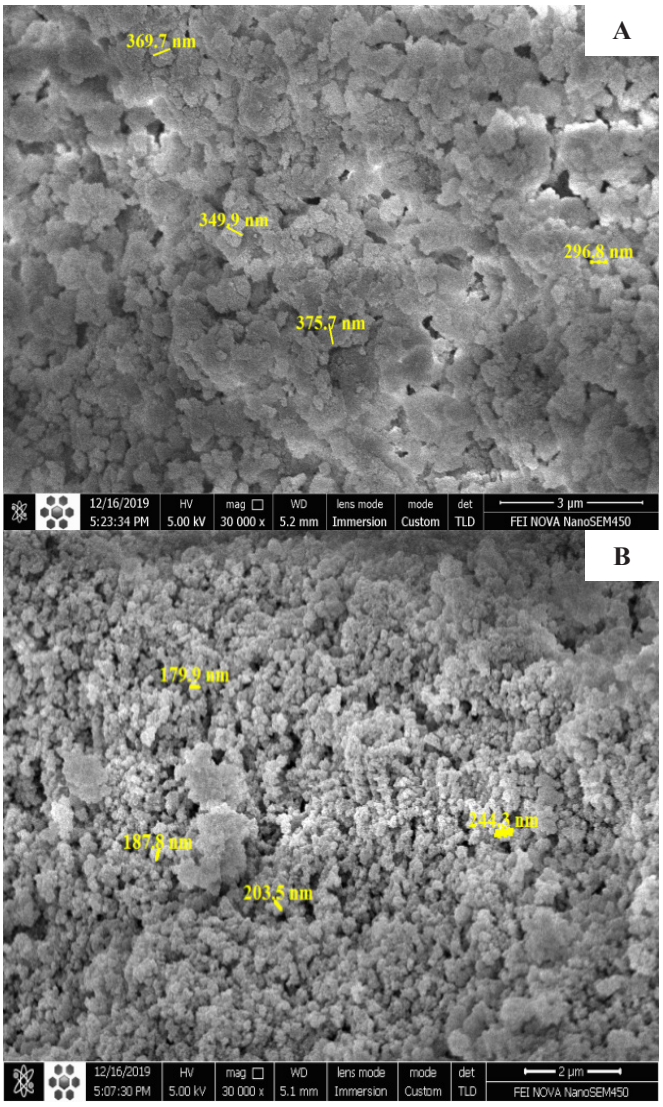

Figure 1: SEM of [WFS-MIP_1(VA)] and [WFS-MIP_2(VIZ)] obtained by bulk polymerization.

Potential Measurements

Measurements were performed in a 50 mL double-walled glass container; magnetic stirring was used to produce a homogeneous solution under laboratory conditions. The serial standard solutions drug prepared with a concentration range of 5×10^{-5} to 1×10^{-1} M and tested the efficiency by measuring the electrode's potential; slope, detection limit, and response time were calculated from the calibration curve.

Pharmaceutical Samples Preparation

For determined the concentrations of WFS two types of tablets were used WFS-14 tablets 1 mg PL Holder: Bristol laboratories Ltb and WFS-14 tablets 1 mg MA Holder: TEVA UK Ltd. 01056-B were grinded (0.0275g) and dissolved in methanol and finished to (100mL) in volumetric flasks.

RESULTS AND DISCUSSION

Liquid Membranes Electrode

MIP-based liquid electrodes, their range of concentrations, and the response of slopes to the Nernstian equation were investigated. MIP membranes made of VA, VIZ monomers with a PVC matrix using DBS, DOP as a different plasticizer. The internal solution was used a 0.1M standard solution of WFS for all liquid-electrodes. Experimental results of MIP_S synthesis based on two monomers VA and VIZ indicate that both monomers can be used to prepare effective MIP for WFS. The plasticizer is an essential part of the sensing membrane that plays an important role as a solvent for the various components and determines the mobility of the analyte in the membrane. The results inducted both plasticizers are suitable, table 1 shows the parameters of the electrodes produced and tested, Four membranes of the various compositions were prepared using two different viscosity plasticizers, (DBS) (π=11.0042cSt), (DOP) (π=16.6cSt). The characteristics of WFS-MIP based electrodes were studied as shown in Table 1, the electrodes I and IV also indicate the value given good results, and the liquid electrode was used in pharmaceutical samples to determine warfarin drugs.

Table 1: WFS-MIPs electrode characteristics based on different functional monomers

Electrode no.	Membrane composition	Parameter			
		Slope Mv/decade	Correlation coefficient (r)	Linearity range (M)	Detection limit/ M
I	WFS-MIP_1 (VA+MBAA+DBS)	-18.72	0.996	5×10^{-4}-1×10^{-1}	6.5×10^{-4}
II	WFS-MIP_1 (VA+MBAA+DOP)	-18.2	0.9958	1×10^{-3}-1×10^{-1}	7×10^{-4}
III	WFS-MIP_2 (VIZ+MBAA+DBS)	-20.67	0.9997	1×10^{-4}-1×10^{-1}	7.8×10^{-4}
IV	WRS-MIP_2 (VIZ+MBAA+DOP)	-21.09	0.998	1×10^{-4}-1×10^{-1}	7.5×10^{-4}

FTIR- Analysis

The FTIR spectrum has been studied over a range of (400-4000 cm^{-1}). It's an important method for detecting the functional groups present in a compound. FTIR spectra of different MIPs before and after extract using KBr pallet methods are shown in Table 2a and 2b.

The results in table 2a found the difference between the infrared spectra of the standard warfarin drug from the MIP_1 spectrum. This indicates the occurrence of interference between MIP_1 and WFS drug, and the bands were shown from this table in WFS is (3423, 3058, 2968, 2889, 1718, 1660 and 1600) cm^{-1} for stretching (O-H, aromatic C-H, aliphatic C-H. O-C=O, O=C, and C=C) respectively, while, the WFS-MIP_1 bands before template removal is: (3390, 3064, 2998, 1654 and 1535) cm^{-1} for stretching O-H, C-H aromatic, C-H aliphatic, O-C=O, and C=C. Then FTIR spectrum of MIP_1 after removing the template shows the different locations of bands, and the absence of C=O stretching is 1660 cm^{-1}. Also, when using (VIZ) as a monomer to synthesize another warfarin MIP_2 the FTIR spectrum is shown in tablet 2b before and after removing the template.

Table 2a: The FT-IR spectra (cm^{-1}) peaks for WFS-MIP_1 imprinting polymer:

	Functional group (cm^{-1})	*WFS.*	*WFS–VA (MIP_1) before template removal*	*WFS–VA (MIP_1) after template removal*
1	O-H str.	3423	3390	3402
2	C-H aromatic	3058	3064	3064
3	C-H aliphatic.	2968,2889	2998	2952,2867
4	O-C=O str.	1718	1654	1656
5	O= C str.	1660	---------	--------
6	C=C str.	1600	1535	1527

Table 2b: The FT-IR spectra (cm^{-1}) peaks for WFS-MIP_2 imprinting polymer:

	Functional group (cm^{-1})	*WFS.*	*WFS–VIZ (MIP_2) before template removal*	*WFS–VIZ (MIP_2) after template removal*
1	O-H str.	3423	3326	3446
2	C-H aromatic	3058	3070	3066
3	C-H aliphatic.	2968,2889	2956, 2885	2952, 2929
4	O-C=O str.	1718	-----	-----
5	C=O str.	1660	1658	-----
6	C=C str.	1600	1529	1652

Table 3: Recovery results and standard deviation of commercial drugs obtained using membrane I (MIP_1+DBS).

Drug	*Concentration Prepared/ M*	*Potentiometric methods*	*Concentration Found/ M*	*%Rec.*	*%RE*	*%RSD*
Warfarin pure material	$1X10^{-3}$	Direct method	$1.038x10^{-3}$	103.81	3.81	1.05
		SAM	$1.036x10^{-3}$	104.09	4.09	3.21
	$1X10^{-4}$	Direct method	$1.030x10^{-4}$	103.31	3.31	0.99
		SAM	$1.040x10^{-4}$	102.75	2.75	3.35
Bristol laboratories Ltb	$1X10^{-3}$	Direct method	$1.045x10^{-3}$	104.55	4.55	1.60
		SAM	$1.044x10^{-3}$	105.10	5.01	3.25
	$1X10^{-4}$	Direct method	$1.030x10^{-4}$	103.32	3.22	1.30
		SAM	$1.020x10^{-4}$	102.73	2.73	2.11
TEVA UK Ltd.	$1X10^{-3}$	Direct method	$1.042x10^{-3}$	104.18	4.18	1.61
		SAM	$1.045x10^{-3}$	104.34	4.34	2.65
	$1X10^{-4}$	Direct method	$1.040x10^{-4}$	103.61	3.61	0.86
		SAM	$1.030x10^{-4}$	102.75	2.75	3.45

Table 4: Recovery results and standard deviation of commercial drugs obtained using membrane III (MIP_2+DBS).

Drug	*Concentration Prepared/ M*	*Potentiometric methods*	*Concentration Found/ M*	*%Rec.*	*%RE*	*%RSD*
Warfarin pure material	$1X10^{-3}$	Direct method	$1.004x10^{-3}$	100.43	0.43	1.46
		SAM	$1.034x10^{-3}$	103.82	3.82	0.84
	$1X10^{-4}$	Direct method	$1.030x10^{-4}$	102.19	2.19	0.77
		SAM	$1.030x10^{-4}$	102.13	2.31	2.08
Bristol laboratories Ltb	$1X10^{-3}$	Direct method	$1.020x10^{-3}$	102.04	2.04	1.90
		SAM	$1.041x1^{-3}$	104.98	4.98	1.2
	$1X10^{-4}$	Direct method	$1.040x10^{-4}$	104.25	4.25	0.45
		SAM	$1.050x10^{-4}$	104.79	4.79	1.45
TEVA UK Ltd.	$1X10^{-3}$	Direct method	$1.050x10^{-3}$	104.99	4.99	0.96
		SAM	$1.035x10^{-3}$	103.61	3.61	2.01
	$1X10^{-4}$	Direct method	$1.050x10^{-4}$	104.77	4.77	3.75
		SAM	$1.050x10^{-4}$	104.58	4.58	3.13

From the value in table 2b, showing the difference in the spectrum of WFS, WFS-MIP_2 (VIZ) before and after template removal, in WFS spectra found the following bands: (3423, 3058, 2968, 1718, 1660, and 1600) cm^{-1} for stretching O-H, C-H aromatic, C-H aliphatic, O-C=O, C=O, and C=C, had shifted some and removal, other bands, when WFS-MIP_2 (VIZ) prepared, while when the template removal shows the absence of O-C=O stretching and C=O at 1718 cm^{-1} and 1658 cm^{-1}, this results that excise in template spectrum (WFS) indicating the drug extracted from the template.

Quantitative analysis

The accuracy of electrodes membrane (I-IV) was measured using the standard addition method to determine WFS in 1×10^{-3} and 1×10^{-4} M synthetic solutions. Excellent percentage recovery results were obtained between 94.93 and 105.1 Table 3 and 4) shows a typical plot for membrane I and IV at synthetic solution concentration (1×10^{-3}, 1×10^{-4}) M, and 0.1 M standard solution was added

For determination of WFS in commercial pharmaceutical tablets, England-(actavis company), 1 mg warfarin tablets, England-(almus) 1 mg (warfarin tablets), (WFS) obtained from local stories using a membrane (I-IV) based on DBS, DOP as a plasticizer, direct method, and standard addition method, were applied, The percentage recovery values (Table 3 and 4) are consistent with the British Pharmacopoeia value. There is no interference of all species with the electrode response, so the recovery values obtained by the standard addition method are in agreement with the direct method results.

CONCLUSION

The construction of molecularly imprinted electrode sensors (MIP) using Vinyl acetate (VA),1-vinylimidizol (VIZ) as a monomer, N, N-methylene bis-acrylamide (MBAA) as a cross-linker and benzoyl peroxide (BPO) as an initiator in the polymerization process. Using di-butyl subacute (DBS), di-octyl phthalate (DOP) as plasticizers. MIP results that show high sensitivity, reasonable selectivity, fast static response, long-term stability, and applicability over a wide pH range were obtained using electrodes based on DBS, DOP plasticizers. Good results of recoveries were obtained for warfarin-s

determination in the commercial tablets compared to the British Pharmacopoeia.

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