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Research Article

Simultaneous Determination of Clopidogrel Bisulphate Based on Molecularly Imprinted Polymer and Its Pharmaceutical Application

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ABSTRACT
In the polymerization process, non-covalent molecularly imprinted polymers (MIP) were synthesized using clopidogrel bisulphate (Clop) as a template molecule, vinyl acetate (VA), 1-vinylimidizol (VIZ) as monomer, N, N-methylene bis-acrylamide (MBAA) as a cross-linker, and benzoyl peroxide (BPO) as an initiator. Four electrodes were synthesized using two different plasticizers, di-butyl subacute (DBS), di-octyl phthalate (DOP) in PVC matrix. Characterization of the obtained MIP was achieved by scanning electron microscopy (SEM), studying the electrodes properties, slope, detection limit, life time and linearity range the results showed that the membranes prepared gave linear range from (1×10^{-4}-1×10^{-1}, 1×10^{-3}-1×10^{-1}, 5×10^{-5}-$1\times10^{-2}$and 5×10^{-5}-1×10^{-2})M, respectively, with slops of (-17.94, -17.87, -17.68 and -18.2) as for the correlation coefficients are (0.9925, 0.9854, 0.9980 and 0.9886) and the detection limit it was (8.5×10^{-4}, 8×10^{-4}, $7\times10^{-4}$and 8×10^{-4})M respectively. Selectivity coefficient of MIP measurements using different interfering species, the prepared electrodes was intended excellent for use in determining Clopidogrel bisulphate drug (Clop) in pharmaceutical sample.

Keywords: Molecularly impressed electrodes, Clopidogrel bisulphate (Clop), vinyl acetate (VA) monomer, 1-vinylimidizol (VIZ) monomer.

INTRODUCTION

Molecular imprinting is a technique which creates recognition sites specific to a target molecule, called a template, within a synthetic polymer and has been widely used for analytical purposes for the selective adsorption of drugs and their metabolites[1, 2]. Comparable to immunosorbents, the different binding sites are allocated to the particular interactions within the polymer network between the template and the functional groups, working similarly to an antigen-antibody system[3-5]. The synthesis of an MIP involves first the complexation in solution of a template with a functional monomer (FM) by non-covalent or covalent interactions, followed by the polymerization of these monomers around the template in the presence of a cross-linker, a radical initiator and a suitable solvent. Following polymerization, the template is removed from the polymer network, leaving its imprint and the cavities complementary to the template in the polymer structure with size shape and chemical functionality[6-8]. The first and critical step in the preparation of MIPs is the formation of a complex between the template and FM by non-covalent interactions, i.e. hydrogen π-π bonding, van der Waals forces, and electrostatic interactions. The FM which can interact most strongly with the template provides the most stable complex[9-12]. The recognition properties of MIPs can also be tailored using a molecular templating method as shown in Scheme 1[13, 14].

Clopidogrel bisulfate, or methyl (+)-(S)-α-(o-chlorophenyl)-6, 7-dihydrothieno [3, 2-c] pyridine-5 (4H)-acetate sulphate, is a derivative of thienopyridine. It is an ADP-induced platelet aggregation inhibitor of acting by direct inhibition of adenosine diphosphate (ADP) binding to its receptor and the subsequent ADP-mediated activation of the GPIIb / IIIa glycoprotein complex[15, 16]. Clopidogrel is an analogue of ticlopidine which has similar actions and uses[17, 18]. The main circulating compound is the inactive derivative of carboxylic acid, formed by carboxylesterase hydrolysis of the ester function[19]. Clopidogrel is indicated to reduce atherosclerotic events in atherosclerosis patients documented by recent stroke, recent myocardial infraction or cardiovascular disease [20].

Varied procedures for quantitative clopidogrel determination in specific workers Clopidogrel was simultaneously determined in pharmaceutical dosage forms including HPLC effervescent tablets[21]. A GC – MS method for the study of carboxylic acid metabolite of Clopidogrel in plasma and serum was also reported[22]. Spectral photometry and CE methods were also used to measure aspirin in blood, urine and various

dosage forms[23, 24] and voltammetry[25]. Common disadvantages of these methods are the need for pure samples to prevent contact with other contaminants and to be time-consuming and repetitive while others use large volumes of biological fluids and require expensive instruments and toxic solvents, plus time-consuming procedures [26], Particularly in recent years, MIP used various functional group monomers to pay interest. MIPs have many advantages, including high selectivity and affinity to their target, high inertness of mechanical and chemical stability, and insolubility in water and most organic solvents. They can also be easily prepared have a high mechanical strength, heat and pressure and are cost-effective and accessible to harsh chemical media[27, 28].

In this work we identify the preparation of four (MIP) with recognition materials, (VA), (VIZ) as monomer, (MBAA) as cross-linker and (BPO) as initiator in the polymerization method with the aid of analytical calculation studies. The performance of the MIPs was then evaluated. The MIPs performance was evaluated. The MIP with the highest binding capacity was chosen as PVC membrane recognition material using two different plasticizers; (DBS) and (DOP) for the determination of Clop. in pharmaceutical sample.

EXPERIMENTAL

MIP Preparation

Two electrode of (Clop) are prepare using MIP methods, the first electrode (Clop-MIP_1) prepared by mixed (0.5:3:15) mmol for (Clop), (VA) as monomer and (MBAA) cross linker; respectively, while the second electrode (Clop-MIP_2) was prepare by mixed (0.4:2.4:12) mmol for (Clop), (VIZ) as monomer and (MBAA) as cross linker, After this, (0.3 g) BPO as the initiator was added. All these materials were subsequently dissolved in 7mL mixture of methanol and 3ml chloroform for dissolve initiator, the mixture was stirred for 5 minutes to obtain a homogenous solutions. Afterwards, the gas N_2 was passed through the solution for 30 minutes to remove oxygen from it, and the solution was placed in a water path at 75°C. When the reaction was complete MIP became hard, after the polymerization process, the polymer was dried and crushed to obtain it as particles. Finally, these particles was sonicated in acetonitrile / CH_3COOH (18:2 v/v) to remove the template from the MIP. The particles size of (Clop-MIP_1) and (Clop-MIP_2) were between (53 µm and 125 µm), respectively. 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.

Instruments

In this work, we use potentiometric measurements were carried out with a digital voltmeter (HANA pH 211 instruments Microprocessor pH meter). An analy-zer (WTW model, Germany), pH meter (WTW model pH 720, Germany), SCE (Gallenkamp, USA). UV-VIS double-beam model (UV-1650 PC) SHIMADZ (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10), 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 Clop-MIP electrodes were fabricated as previously described, in the laboratory. All potentiometric measurements were made at room temperature. For research purposes, the Clop-MIP electrode was combined with an Ag-AgCl electrode, while 0.1 M of Clop. was used as internal solution, the electrode being soaked with this for at least 2 hours before use.

MATERIALS AND CHEMICALS

Clopidogrel standard was obtained from the state company of drug (IRAQ-SDI-Samara). Coltra tablets (75 mg) (England and India) were purchased from local pharmacies.

Plasticizers: (DBS) (97.0% purity) and (DOP) (99.5% purity), were purchased from Sigma Aldrich. Other chemicals and reagents materials were obtained from Fluka, BDH and Sigma Aldrich.

Preparation of standard solutions

About 100 mL of stock standard solution of 0.1 M Clopidogrel bisulphate was prepared by dissolving 4.1989 g of standard Clop. in distilled water. The other Clop. solutions ranged from 1×10^{-5}-1×10^{-1} M in 100 mL, and came from the stock solution of Clop.

A 100 mL of each interferences ion (K^+, Ca^{+2}, Al^{+3}) was prepared from 1×10^{-5}-1×10^{-1} M of a stock solution of 0.1 M interference ions.

Total of 100 mL of each (methyl paraben, propyl paraben, tri sodium citrate) interferences solution was prepared from 1×10^{-5}-1×10^{-1} M of a stock solution of 0.1 M interference.

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 methanol and stirred by magnetic stirrer for 30 minutes to completely dissolve the powder. The solution was completed

to 100 mL by methanol to prepare 1×10^{-3} M and 1×10^{-4} M Clopidogrel solutions.

Scanning Electron Microscope (SEM)

The SEM can be used to get an idea about the size, geometry and pores 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 after washing is displayed in Figure 1a, 1b. Herein, it can be seen that micro emulsion polymerization gives very small particles size around (369.7- 530.1) nm for Vinyl acetate (VA) polymer and (165.8-352.6) nm for vinylimidizol (VIZ) polymer.

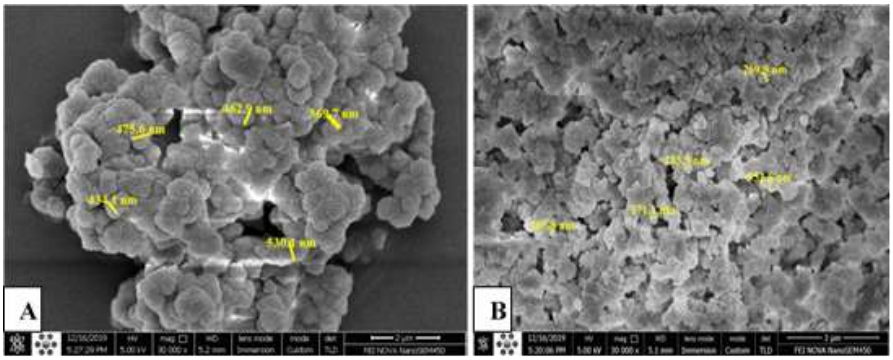

Fig.1: A. SEM of [Clop-MIP(VA)], B. [Clop-MIP(VIZ)] obtained by bulk polymerization.

RESULTS AND DISCUSSION

Four MIP membranes made using two different monomer VA and VIZ with PVC matrix and two different plasticizers DBS and DOP, the characteristic and properties of these membranes were studied, including: slope, detection limit, linearity, life time and the response to the Nernstian equation were investigated, the results in table 1 indicated that both monomer and both plasticizers can be used for preparing effective MIP for Clopidogrel bisulphat.

FTIR- Analysis

The Fourier transmission infrared spectrometry (FTIR) spectra of MIP before and after extraction the Clop. template were recorded in the range of 400-4000 cm^{-1}.The listed in Table 2 showed a band that before/after removal clop-template.

The infrared spectrum of Clop-drug shows the presence of bands at 3080, 2956,2848, 1753,1591 and 588 cm^{-1} for stretching C-H arom, C-H aliph, C=O, C=C, and C-Cl respectively, these bands differ when preparing Clop-MIP_1 in presence and absence the Clop-template. The results showed a change in bands location and appearance of new bands for O-H and C=O at 3396, 1762 cm^{-1} respectively, but the spectrum of Clop-MIP_1 after template removal showed absence of C=O at 1762 cm^{-1} and C-Cl at 601 cm^{-1}. All of the above indicate that the template was synthesized and the drug was removed from the polymer. While the infrared spectrum of Clop-MIP_2 given different bands location as recorded and listed in table 3

The results shown in table 3 a difference in bands location for clop- drug and MIP before/after removing the template, the FTIR spectrum of Clop-MIP_2 before removal Clop-template showed a bands at (3265, 3062, 2939, 1656 shoulder, 1656, 1527 and 621) cm^{-1} for O-H enol, C-H arom, C-H aliph, O=C-C=N, N-C=CH_2, C=C and C-Cl respectively, and notes absence C=O at 1753 cm^{-1}, while showed absence C-H aromatic at 3062 cm^{-1}, C=C str. at 1527 cm^{-1} and 621 cm^{-1} for para substitution disappearance at the Clop-MIP_2 after removal Clop-template.

Analysis of commercial Clopidogrel bisulphate tablets:

The commercial Clop. drug was measured by two ways: direct and standard addition methods, the concentration of synthetic commercial drug used at (1×10^{-3},1×10^{-4})M. the results listed in table 4, 5 showed that the electrodes prepared were suitable for estimating the commercial Clop. drug as the recovery percent range between (102-104.5) with the relative error value decreased.

Table 1: The characteristics of Clop-MIP electrode using two different a monomer and two different plasticizer

Electrode no.	Membrane composition	Parameter			
		Slope mv/decade	Correlation coefficient (r)	Linearity range (M)	Detection limit/ M
I	Clop-MIP$_1$ (VA+MBAA+DBS)	-17.94	0.9925	1×10^{-4}-1×10^{-1}	8.5×10^{-4}
II	Clop-MIP$_1$ (VA+MBAA+DOP)	-17.87	0.9854	1×10^{-3}-1×10^{-1}	8×10^{-4}
III	Clop-MIP$_2$ (VIZ+MBAA+DBS)	-17.68	0.9980	5×10^{-5}-1×10^{-2}	7×10^{-4}
IV	Clop-MIP$_2$ (VIZ+MBAA+DOP)	-18.2	0.9886	5×10^{-5}-1×10^{-2}	8×10^{-4}

Table 2: The FT-IR spectra for Clop-VA polymer before/after removal clop-template

	Functional group (cm^{-1})	Clop.	Clop–MIP$_1$ (VA) before template removal	Clop–MIP$_1$ (VA) after template removal
1	O-H enol.	------	3396	3371
2	C-H aromatic	3080	3062	3060
3	C-H aliphatic.	2956,2848	2943	2941
4	C=O str.Keton.	1753	1654	1654
5	C=C str.	1591	1525	1525
6	O= C-ester.	------	1762	--------
7	C-Cl	588	601	--------

Table 3: The FT-IR spectra for Clop-VIZ polymer before/after removal Clop-template

	Functional group (cm^{-1})	Clop.	Clop–MIP$_2$ (VIZ) before template removal	Clop–MIP$_2$ (VIZ) after template removal
1	O-H enol.	------	3265	3415
2	C-H aromatic	3080	3062	-----
3	C-H aliphatic.	2956,2881	2939, 2871	2952,2941
4	C=O str.keton	1753	-----	-----
5	O= C-C=N.	------	1656 (shoulder)	1654 (shoulder)
6	N-C=CH_2	-----	1656	1525
7	C=C str.	1591	1527	-----
8	C-Cl	588	621	--------

Table 4: Recovery results and standard deviation of Clop. drugs obtained through the use of (MIP1+DBS).

Drug	Conc. Prepared/ M	Potentiometric methods	Conc. Found/ M	%Rec.	%RE	%RSD
Clopidogrel bisulphate pure material	1×10^{-3}	Direct method	1.024×10^{-3}	102.41	2.41	1.60
		SAM	1.020×10^{-3}	102.35	2.35	1.49
	1×10^{-4}	Direct method	1.037×10^{-4}	103.74	3.74	1.99
		SAM	1.027×10^{-4}	102.49	2.49	1.50
Rich plavex/ clopidogrel tablet U.S.P.75 mg/3204 AUS	1×10^{-3}	Direct method	1.032×10^{-3}	103.16	3.16	1.11
		SAM	1.032×10^{-3}	102.75	2.75	2.47
	1×10^{-4}	Direct method	1.043×10^{-4}	104.35	4.35	0.19
		SAM	1.027×10^{-4}	102.48	2.48	0.76
Clopidogrel tablets USP 75 mg/ plagerine/ INDIA	1×10^{-3}	Direct method	1.035×10^{-3}	103.47	3.47	1.27
		SAM	1.028×10^{-3}	103.85	3.85	1.62
	1×10^{-4}	Direct method	1.049×10^{-4}	104.89	4.89	0.52
		SAM	1.035×10^{-4}	104.14	4.14	2.07

Table 5: Recovery results and standard deviation of commercial drugs obtained through the use of (MIP2+DBS).

Drug	Conc. Prepared/ M	Potentiometric methods	Conc. Found/ M	%Rec.	%RE	%RSD
Clopidogrel bisulphate pure material	1×10^{-3}	Direct method	1.031×10^{-3}	103.07	3.07	1.72
		SAM	1.036×10^{-3}	102.69	2.69	2.69
	1×10^{-4}	Direct method	1.028×10^{-4}	102.82	2.82	1.80
		SAM	1.031×10^{-4}	102.89	2.89	1.34
Rich plavex/ clopidogrel tablet U.S.P. 75 mg/3204 AUS	1×10^{-3}	Direct method	1.047×10^{-3}	104.68	4.68	1.12
		SAM	1.034×10^{-3}	103.18	3.18	2.51
	1×10^{-4}	Direct method	1.028×10^{-4}	102.81	2.81	0.90
		SAM	1.023×10^{-4}	104.28	4.28	1.33
Clopidogrel tablets USP 75 mg/ plagerine/ INDIA	1×10^{-3}	Direct method	1.035×10^{-3}	103.52	3.52	1.01
		SAM	1.043×10^{-3}	104.40	4.40	3.05
	1×10^{-4}	Direct method	1.034×10^{-4}	103.44	3.44	1.38
		SAM	1.037×110^{-4}	103.37	3.37	2.04

CONCLUSION

In this research, four electrodes were prepared based on MIP method using two monomer (VA, VIZ) and two different plasticizer (DBS, DOP), as it was observed that the intraction between template and the monomer was non-covently, therefor the Clop. drug was extracted easly to form selective cavity for estimation commercial Clop and excellent results obtained at lowest costs and with high accuracy.

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