Synthesis and Characterization of Molecularly Imprinted Polymer for Prasugrel Drug Base on 2-Hydroxyethyl Methacrylate Monomer in Biological and Pharmaceutical Samples

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Abstract-In this research work, a molecularly imprinted polymer (MIP) was synthesized for Prasugrel. In this method, a polymer was first synthesized using 2-hydroxyethyl methacrylate (HEMA) as a monomer, N. crosslinker, N'-methylenebisacrylamide as and a azobisisobutyronitrile (AIBN) as an initiator. The successful synthesis of the MIP was confirmed by elemental analysis (CHN), Fourier-transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM). Then, the effects of various parameters such as pH, temperature, MIP capacity, selectivity, adsorption kinetics and adsorption isotherms were investigated by UV-Vis spectroscopy. The calibration curve was plotted for Prasugrel in these conditions and linear range, limit of detection (LOD) and limit of quantification (LOQ) were reported. Finally, the performance of the synthesized MIP as an adsorbent was studied under optimized conditions for the extraction and determination of Prasugrel in tablet matrix and biological fluids such as urine and plasma by high performance liquid chromatography (HPLC).

Index Terms—Prasugrel, molecular imprinting polymer, biological fluids, pharmaceutical sample.

I. INTRODUCTION

Prasugrel (chemical formula: C20H20FNO3S; molecular weight: 273.442 g.mol⁻¹) is a white powder that easily dissolves in methanol. It is a prodrug that must be converted to an active metabolite by the liver, and is rapidly transformed into an inactive thiolactone by hydrolases, and then into an active metabolite by CYP enzymes. Prasugrel is bound to proteins (by about 98 percent) and excreted in the urine (68%) and feces (27%). It is prescribed for patients with hypertension or heart failure in which blood is not pumped sufficiently to the body. Physical and chemical methods such as high performance liquid chromatography (HPLC) and UV spectrophotometry have been reported for detecting and measuring Prasugrel in biological fluids, and thin-layer chromatography (TLC) for drug, plasma, and urine samples. Most drugs are isolated and measured using HPLC because it enjoys high precision, polarity, sensitivity, etc [1]-[6]. In this method, sample preparation is very important because it reduces the matrix effect in biological fluids and yields reliable results [7]. Molecularly imprinted polymers (MIPs) are a new group of synthetic materials with specific recognition sites. MIPs are produced in the form of a 3-D polymeric network with strong crosslinkers in the copolymerization reaction between a functional monomer and a crosslinker in the presence of a template molecule [8], [9]. After the MIPs are produced, the template molecules are removed from the obtained structure as a result of which binding sites are created with the form, size, and functional groups matching those of the template. Various methods are used to produce MIPs. Liquid polymerization has been used in most reports on preparing these polymers because it is a simpler method. The best results in synthesizing MIPs are obtained by using small templates; therefore, Prasugrel was employed as the template in the present research. Monomer selection requires great care so that it can create active and selective sites for the template. We used 2-hydroxyethyl methacrylate as the monomer because the carboxyl group in its structure enables it to withdraw or donate hydrogen ions in response to pH changes in the environment and, therefore, can be considered pH sensitive.

We intended to produce a highly selective and pH sensitive polymer, and combine MIP-based solid phase extraction (SPE) and HPLC to detect and measure Prasugrel in biological fluids and drug samples.

II. EXPERIMENTS

A. Instruments

IR spectra were recorded on an IR Fourier transform spectrometer (FT-IR-4100, made by Jasco Inc., Easton, Maryland, USA), thermal gravimetric analysis (TGA) was performed employing a thermal analyzer (manufactured by the Shimadzu Corp. in Japan), a scanning electron microscope or SEM (Philips XL-30 FEG-SEM) was used for SEM analysis, elemental analysis by a CHN analyzer (made by the Lineis Messgerate GmbH Company in Hanau, Germany, Vario El), and UV/Vis spectroscopy by a UV/Vis spectrophotometer (V-530, produced by the Jasco Inc., Easton, Maryland, USA).

B. Reagents and Solutions

2-hydroxyethyl methacrylate (HEMA) and N, N[']methylenebisacrylamide, hexadecane, acetonitrile, methanol, dipotassium phosphate, and phosphoric acid were bought from the Merck Company (Darmstadt, Germany), azobisisobutyronitrile (AIBN) from the Across Company (New Jersey, USA), and sodium dodecyl sulfate (SDS) from the Fluka Chemical Corp. (Buchs, Switzerland).

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All reagents were HPLC grade. The stock solution (100 mg L^{-1}) was prepared by dissolving 5 mg of Prasugrel in 50 ml of methanol, and the pH of the solution was adjusted using 0.01M NaOH or 0.01M hydrochloric acid.

C. Polymer Synthesis

1) MIP preparation

We needed an organic and an aqueous phase to produce the MIP. To prepare the organic phase, 0.05g of N, Nmethylenebisacrylamide (the crosslinker), 5 ml of HEMA (the monomer), 0.2g of hexadecane, 0.01g of AIBN (the initiator), and 0.02g of Prasugrel were poured into an Erlenmeyer flask and put in an ultrasonic bath for 30 minutes [10]. Thirty ml of distilled water and 0.015g of SDS were used to prepare the aqueous phase. The aqueous phase was added to the organic phase, and placed in a nitrogen atmosphere at 85°C for 40 minutes. At the completion of the polymerization process, the resulting white polymer was immediately filtered, washed with 50 ml of methanol, and dried in an oven at 60°C. The non-imprinted polymer (NIP) was prepared in the same way but without using the template (Prasugrel). FT-IR spectra were obtained for the resulting polymers, and elemental analysis, TGA, and SEM analysis were performed on them.

2) Washing procedure

The polymer was ground using a mortar and pestle and passed through a 50 nm filter paper. It was then washed and ultrasonicated in 50 ml of methanol for 30 minutes. This process was repeated four times. Finally, the adsorbent was dried in an oven at 60° C and kept at ambient temperature.

3) Isotherm studies

One ml of the diluted Prasugrel solution (2.80 mg L⁻¹) and 1 ml of the optimum pH were added to each 1.5 ml microtube containing 0.01 g of the adsorbent. The microtubes were then vortexed at 25°C for 20 minutes, centrifuged, and the final concentration of Prasugrel in the supernatant was determined using the UV-Vis spectrophotometer. The quantity of Prasugrel in the q_e (mg g⁻¹) equilibrium adsorption capacity on the polymer was calculated from the following equation:

$$q_e = (C_0 - C_e) V/W \tag{1}$$

In the equation above, C_0 and C_c (mg L⁻¹) are the initial Prasugrel concentration and its concentration after reaching the equilibrium state, V (L) volume, and W (g) mass of the employed adsorbent.

4) HPLC system

An HPLC instrument was used to analyze Prasugrel. Chromatographic separation was performed on a C_{18} (4.6mm-250mm) column. The mobile phase was a mixture of acetonitrile and phosphate buffer (600-400 V/V) at pH3 with flow rate of 1 ml min ⁻¹ in isocratic elution mode. The injection volume of 20µl, the wavelength of 210 nm, and the ambient temperature were the same for all samples [11]-[15].

D. Real Sample

Blood plasma, urine samples (from a volunteer who was not taking Prasugrel), and real drug (pill) samples were used to evaluate performance of the synthesized polymer in adsorbing and recovering Prasugrel, and 100 µl of the Prasugrel stock solution in methanol of HPLC grade was poured in a 50 ml volumetric flask. Solutions containing 100 µl of the drug in the plasma, urine, and actual pill samples were then prepared. For this purpose, 100 µl of the stock solution was transferred to a 10 ml volumetric flask. After adding 1 ml of the optimum buffer, the volume was raised to the desired level by addition of plasma. 1.5 ml of these solutions was transferred to each of the 2 ml microtubes containing 0.05 g of the synthesized polymer. The microtubes were vortexed for 15 minutes and centrifuged for five minutes. The transparent supernatant was separated. 1.5 ml of the mobile phase was added to the remaining polymer, the mixture was vortexed for 15 minutes, put in an ultrasonic bath for 15 minutes, and centrifuged for five minutes. The supernatant was separated and measurements were made using the HPLC instrument. This was repeated three times, and the average and the relative standard deviation were calculated.

III. RESULTS AND DISCUSSION

A. Characterization

FT-IR spectra were taken and elemental analysis, TGA, and SEM analysis were performed to study the polymer that was molecularly imprinted using the polymerization method with Prasugrel as the template. Data obtained from FT-IR spectra and from CHN analysis is presented in Table I. This table shows the main FT-IR peaks. The band at 299cm⁻¹ is that of the aliphatic CH bond in the main structure of the polymer. The data obtained from CHN analysis shows that there was one crosslinker for every six monomers.

Polymer morphology was studied using the SEM. The SEM images in Fig. 1 show that the synthesized polymeric adsorbent was in the form of agglomerated particles the surfaces of which were rough highly porous. The particle sizes were in the range of about 20-60 nm.

In TGA, the polymer was stable at up to about 230°C, and had the weight loss of about 3.2 percent at up to 100°C (Fig. 2) due to the adsorbed water. At about 240°C, the polymer skeleton burned and weight loss began.

TABLE I: FOURIER TRANSFORMATION-INFRARED AND ELEMENTAL ANALYSIS INFORMATION

FT-IR		-		-	
spectra					
	3675 cm ⁻¹	2992 cm ⁻¹	1731 cm ⁻¹	1492 cm ⁻¹	1068 cm ⁻¹
	O-H	Aliphatic	C=O	CH_2	C-O
	stretchi	CH	stretch	stretc	stretchi
	ng	stretching	ing	hing	ng
Elemental					
analysis					
	Carbon (%)	Nitrogen (%)	Oxyge n (%)		
	51	2.89	50.32		

B. Optimization of Conditions

To determine the optimum amount of the N, N-methylenebisacrylamide crosslinker, various amounts of it

(0.03-0.18) were used in synthesizing the polymer. For this purpose, 0.01g of each produced adsorbent together with 1 ml of the stock solution and 1 ml of the optimum pH were poured into each microtube, the microtubes were shaken on the vortex for 20 minutes, and the UV-Vis spectrophotometer was employed for quantification. As shown in Fig. 4, the adsorbent synthesized had the highest capacity when 0.15 g of the crosslinker was used; i.e., it adsorbed the largest amount of the drug in Fig. 3

The quantities of adsorbed Prasugrel were measured at different pH values. The synthesized polymer is pH sensitive. Fig. 5 shows that the largest amount of the drug was adsorbed at pH 6. This was because at lower pH values the polymer chain was wrinkled and had low adsorption capacity, whereas the ring system of the polymer chain was opened at higher pH values and the polymer had higher adsorption capacities [16]-[18]. Therefore, pH 6 was considered the optimum pH. The point of zero charge (PZC) was also studied. PZC is the pH value at which the net charge of the adsorbent is zero. As shown in Fig. 4, pH 6 was the PZC for the synthesized adsorbent. The adsorbent had a positive charge at lower pH values and a negative charge at higher pH values.



Fig. 1. SEM images of imprinted polymer.







Prasugrel adsorption as a function of time at pH 6 is shown in Fig. 6. It was found that 15 minutes was enough for more than 96 percent of Prasugrel to be adsorbed. This indicates that the adsorption sites on the polymer were activated and enjoyed good accessibility.



Fig. 4. Effects of pH value on Prasugrel adsorption by the synthesized polymer. Each 10 ml contained 20 mg L^{-1} of Prasugrel. Buffer solutions (Magic Buffer) were used to adjust the pH. The synthesized adsorbent (0.01 g) was added to each solution and the solution was shaken with a vortex mixer for 20 minutes.

Moreover, the synthesized polymer was studied at different temperatures. As shown in Fig. 7, adsorption decreased at higher temperatures. Mobility declined at low temperatures resulting in reduced adsorption. We can say that the synthesized adsorbent had suitable efficiency at various temperatures and temperature did not influence its adsorption capacity.

C. Isotherm Studies

Langmuir, Freundlich, and Temkin isotherms were employed to study Prasugrel adsorption by the synthesized polymer at ambient temperature. Equations 1-3 were used to obtain adsorption parameters. Results are listed in Table II [19]-[21].

Langmuir equation: $C_e/q_e = (1/q_{Max}K_L) + (C_e/q_{Max})$ (2)

Freundlich equation: $\ln q_e = \ln K_F + 1/nC_e$. (3)

Temkin equation:
$$q_e = BlnA + BlnC_e$$
 (4)

In Table II, q_{Max} represents the highest adsorption capacity of a full layer on the adsorbent surface (mg g⁻¹), K_L Langmuir constant (L mg⁻¹), R_L the Langmuir dimensionless constant separation factor, K_F the Freundlich constant (mg g⁻¹) (L mg⁻¹), 1/n the heterogeneity factor, B the constant related to the heat of adsorption and defined by the expression B=RT/b (where b is the Temkin constant (J mol⁻¹), A the Temkin constant (L g⁻¹), R the gas constant (8.314 J mol⁻¹K⁻¹), and T the absolute temperature (K).

Langmuir adsorption isotherm, one of the most widely used adsorption isotherms, was used to determine adsorption capacity (the amount of Prasugrel adsorbed by 1g of the adsorbent). The values of adsorption capacity at equilibrium (q_e) were plotted against C_e , and the C_e/q_e curve indicated a linear relationship. The Langmuir constant (q_{Max}) and K_L can be calculated from the slope and y-intercept of the curve. The value of R_L at pH was 0.02 (Table II), which suggests that Prasugrel uptake was at a desirable level.



Fig. 5. Presents the kinetics of Prasugrel adsorption on the MIP. Each 10ml contained 20mg L^{-1} of Prasugrel. The MIP (0.01 g) was added to each solution and the solution was shaken with a vortex mixer for different durations at pH 6.

Freundlich adsorption isotherm indicates the relationship between the amounts of the drug adsorbed for every mass unit of the adsorbent q_e and shows Prasugrel concentration in the solution in the C_e equilibrium state. The diagram of $\ln q_e$ (mg g⁻¹) against C_e (mg L⁻¹) was drawn. The Freundlich adsorption isotherm suggested that Prasugrel concentration on the adsorbent improved as long as its concentration in the solution increased.

The Temkin equation demonstrates that sorption energy declines linearly on completion of the sorption centers of an adsorbent. Plotting q_e against $\ln C_e$ helps in determining the A and B constants. Results are presented in Table II.



Fig. 6. Shows the effects of the adsorbent on temperature. Each 10 ml contained 20 mg L^{-1} of Prasugrel. The MIP (0.01 g) was added to each solution, and the mixture was shaken with a vortex mixer at various temperatures at pH 6.

D. Selectivity of the Sorbent

The quantities of Prasugrel adsorbed on the NIP and MIP were studied and compared. The equation for the selectivity coefficient β , which is used to investigate bonds in MIPs, is as follows [22] :

$$\beta = Q_{(imprinted)} / Q_{(non-imprinted)}$$
 (5)

TABLE III: SIGNIFICANT PARAMETER FOR SELECTIVITY OF PRASUGREL

	MIP	NIP
β	1.32	
DC Prasugrel	3.19	
DC _{Clonazepam}	0.67	
$K'_{Prasugrel/Clonazepam}$	4.76	0.18
$K^{\prime\prime}_{\rm \ Prasugrel/Clonazepam}$	26.44	

In the equation above, $Q_{(imprinted)}$ represents the capacity of Prasugrel for bond formation in the MIP (mg g⁻¹) and $Q_{(non-imprinted)}$ the capacity of Prasugrel for bond formation in the NIP (mg g⁻¹). Table III illustrates that the selectivity coefficient for the synthesized polymer was 1.32. In fact, we can say that the MIP cavities create specific sites in the MIP networks that selectively adsorb Prasugrel, whereas the NIP lacks these MIP sites and has a lower capacity for Prasugrel-binding. The drug Clonazepam was also employed to study and compare adsorption by MIP and NIP.

Distribution coefficients (DCs) of the selected substrates between the solution and the polymer were calculated using the following equation:

$$DC = C_p / C_s \tag{6}$$

In the equation above, C_p and C_s are the concentrations of the substrates in the polymer and in the solution, respectively. Higher calculated value of DC for Prasugrel compared to Clonazepam suggests that the MIP was able to create special sites that were able to recognize the best molecule for the MIP cavity. Results are presented in Table III. Furthermore, the selectivity coefficient of the adsorbent K' (which represents the two materials adsorbed by one adsorbent) and the relative selectivity coefficient K'' (which stands for the difference between the two adsorbents) were studied and calculated using the following equations [23]:

$$K' = DC_A / DC_B$$
(7)

In the equation above, DCA and DCB represent template distribution coefficients and distribution coefficients of other materials adsorbed by the adsorbent, respectively [24]-[25].

$$\underline{K''} = K'_{imprinted} / K'_{non-imprinted}$$
(8)

The higher value of K' for the MIP compared to the NIPs indicates that the MIP had higher selectivity for Prasugrel.

E. Application of the Method

Drug extraction from urine, plasma, and drug samples Fig. 8 was carried out to see whether the method proposed for Prasugrel determination was suitable for real samples. Extraction was performed under optimal conditions using the method presented in this article. Results are listed in Table IV. Extraction was performed under optimal conditions for human plasma and urine. The separately controlled extracted samples were negative for HBS antigen, hepatitis C antibodies, and HIV I and II. Results in Table IV indicate that this method is suitable for measuring amounts of Prasugrel in urine samples. The reason for the low recovery of the drug in plasma samples is that the proteins in these samples occupy the existing sites on the adsorbent. We can say that the method introduced in this article enjoys good precision.



F. Analytical Performance of the Proposed Method

Table V presents the linear relationship and selectivity of this method for the drug Prasugrel. The calibration curve was drawn using the standards at various concentrations under optimal conditions. The following equations were employed to calculate the limit of detection (LOD) and the limit of quantification (LOC) for the drug of interest:

$$LOD = 3\sigma/m \tag{9}$$

$$LOQ = 10\sigma/m \tag{10}$$

In the equations above, σ is the standard deviation (SD, n=6) and m the slope of the calibration curve. As results in Table V indicate, the R² value (0.9774) and the values for LOD and LOQ are relatively good. Therefore we can conclude that the analytical performance of the proposed method was good.

Samples	Concentra tion of Prasugrel	Adde d	Found (µg.m L ⁻¹)	Recove ry	RSD (%)
	$(\mu g.mL^{-1})$	$(\mu g. mL^{-1})$	L)	(70)	
Drug	1.00		1.02	102	5.85
Urine		1.00	0.89	89	1.86
Plasma		1.00	0.28	28	13.58

TABLE V: ANALYTICAL F	PERFORMANCE OF PRASUGREL
Analytical calibration curve	Y=0.0128X+0.0034

\mathbb{R}^2	0.9774
Liner range	0.1-50
$(\mu g.mL^{-1})$	
RSD	0.86
Repeatability	
	0.97
Reproducibility	
LOD	0.09
(µg. L ⁻¹)	
LOQ	0.3
(µg. L ⁻¹)	

IV. CONCLUSIONS

The synthesized MIP is pH sensitive and releases the

adsorbed drug at pH 6. Moreover, we can say the synthesized MIP has high selectivity for Prasugrel and can be reused for up to four times. Results obtained for Prasugrel extraction by the MIP reported 89% recovery for the urine samples and 28% for the plasma samples. This suggests that the synthesized MIP can be used in analytical studies.

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