RESEARCH ARTICLE

Synthesis of Molecularly Imprinted Polymers for estimation of Aspirin by Using Different Functional Monomers

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ABSTRACT

We have created a new technique with high sensitivity, low price, and high stability. This technique is based on a molecularly impressed polymer (MIP) using a functional monomer called methyl methacrylic acid (MAA) and styrene. Appropriate cross-linker and aspirin (AS) template to produce monolithic solid-phase micro-extraction (SPME) fiber. Also, a polymer was created without selective binding locations, which was called a non-imprinted polymer (NIP). (SPME) all these analytical techniques used for the extraction, preconcentration, and selective determination of aspirin (AS) and its derivatives with Uv-vis double beam spectrophotometer. The firmness, stability, and durability of the manufactured fiber offer it its essential position in SPME. Specimens were obtained from Aspirin devices Analytic testing was conducted using UV-vis and Electron Microscopy Scanning (SEM) and FTIR. The relative normal deviations (RSD percent) for four drug-repeated studies for three readings are a range of (at 30-60 ppm AS) percent (1.20-3.61). The comparative recoveries (93.63-99.71) acquired for AS are within the range.

Keywords: Aspirin, Molecularly imprinted polymer, Solid-phase extraction.

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INTRODUCTION

The molecular imprinting method (MIT) is an appealing synthetic method to mimic natural molecular recognition.[1-5] In this method, macromolecular entities are prepared through a polymerization method in which sites are introduced using a ligand as a template in a casting operation. The method is shown in a schematic way in Figure 1. First, a covalent or non-covalent bonding process arranges functional monomers around the template that Wulff proposes the covalent bonding process.[6] A complex of template monomers is created by a covalent, polymerized bond. Thus, selective breakage of the covalent bond leads to a polymer network that can recognize the imprinted model. The non-covalent bonding procedure is proposed by Mayes and Mosbach.[7] The molecular impression of cross-linked polymer interacts simultaneously with the template molecule through electrostatic interaction, hydrogen bonding, or analogous non-covalent bonds. The technique comprises polymerizing a functional monomer mixed with a template in the presence of a very big part of cross-linker. Following pre-arrangement between functional monomers and template, the template-functional monomers and cross-linkers are co-polymerized. The template will then be removed by extensive washing. Thus, the binding sites are left in addition to the template in size, shape, and functional groups. These binding sites have a molecular recognition impact that significantly enhances affinity and selectivity with their MIPs template as previously stated in work.[8] In addition to the drawback of the extraction of the template and the adsorbed quantity of MIPs, the MIPs are prepared using the third vinyl monomer, i.e., styrene, synthesized using an excess cross-linker. MIPs identification mechanism is the foundation for many biological procedures, such as binding ligand-receptor,

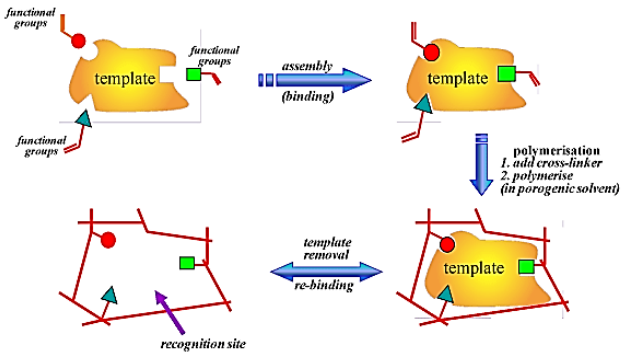

Figure 1: depiction of the molecular imprinting principle

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substratum-enzyme response, and translation and transcription of genetic code. MIPs are characterized by a combination of biological macromolecules, simple preparation, simple storage, and excellent stability.

Therefore, MIPs are pre-biomimetic materials that can substitute biological macro-molecules in certain areas where molecular recognition is needed. For instance, MIPs were used in distinct chromatography,[2,9,10] "ISSN" : "00032700", "abstract": "Molecular imprint polymers (MIPs strong phase extraction,[11-14] sensors,[15-18] and immunoassay aspirin (AS) is the first pre-steroidal anti-inflammatory drug (NSAID) group and has been used as of 1899 for its non-inflammatory, antipyretic and painkiller characteristics dating. More lately, aspirin is being assessed for the avoidance of two major causes of death, notably heart disease (including stroke, myocardial infarction, and thromboembolism) and cancer.[19,20] raising the possibility of clinical benefits by decreasing risks of occlusive vascular events. In secondary prevention among patients with a wide range of prior occlusive vascular events, including myocardial infarction (MI. In specific, AS is the most commonly used antiplatelet drug that reduces the aggregation of platelets. Generally, in the tiny intestine after intake, AS is hydrolyzed to salicylic acid (SA) and acetic acid figure 2. SA, obtained from AS hydrolysis, is taken into blood vessels and inhibits the generation of thromine buses in the blood. If the efficient concentrations of AS and SA in the blood could be evaluated, it would be feasible to predict a particular disease.

EXPERIMENTAL

Aspirin (AS), methyl methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM), styrene, benzoyl peroxide, were bought from Sigma Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com) ; ethanol, nitrogen gas (99.99) chloroform, acetic acid, were purchased from Merck (Darmstadt, Germany).

Instrumentation

UV-Vis (Shimadzu UV spectrophotometer 1800 pc (japan)) and Scanning Electron Microscopy (SEM) (JSM.6390A) (TOKYO JAPAN) and FTIR Shimadzu (FTIR) - 8000 (Japan), heating/ stirring(Germany) and centrifuge (Germany).

Preparation of MIPs

Dissolved 1 mmol of a template (AS) in 7 mL porogen (ethanol) and added 2.99 mmol of functional monomer (MAA). Ultrasonically stirred after the resulting blend for 5 min, 2 mmol of cross-linker (TRIM) and 0.32 mg of benzoyl peroxide were added to the solution, and 2 mmol of template (AS) was dissolved into 7 mL porogen (ethanol) and 2.99 mmol of functional monomer (styrene) was added to the solution. For 20 minutes, the two solutions were bubbled with nitrogen and used as a pre-polymer solution, sealing the rubber tube. Then at 60c overnight, the tub was leaved in the water bath. The polymerization process was completed and the formation of AS- MIPs was completed. The non-molecular polymer NIP was the same method of synthesis MIP but without the AS. MIP and NIP tubes were washed several times in the soxhlet extraction device for 24 hour with an excess quantity of a mixture containing ethanol / acetic acid 9:1(v/ v) until template and unreacted compounds were removed as much as possible and dried in vacuum for 1-hour. The MIP and NIP prepared were left in the oven for drying. Before extraction, of the sampling device and used as extraction needles. The plastic syringe (column) was packed with prepared MIP by using a plastic syringe.

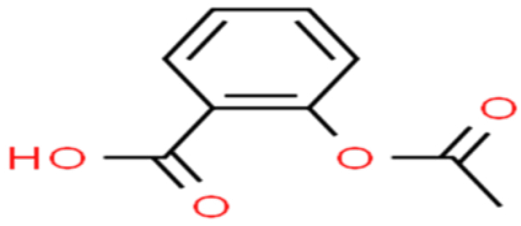

Figure 2: Aspirin chemical structures

Sampling

Stock solutions at the AS concentration (30, 60, 90,120,150 ppm) at pH 8 were prepared and passed through the column at a flow rate of 70 rpm. The extraction column was cleaned twice using 2 mL distilled water to remove matrix interferences and then separated from MIP.

The Sampling Device

A plastic syringe of 3 mL was used and each syringe was packed with distinct weights (0.3, 0.5 gm) from the earlier grinding and sifting MIP (0.75 microns).

Procedure of extraction method

To obtained the powder of tablets pharmaceutical samples using pestle and mortar to grinding the tablets, then taken a suitable weight for preparation in 100 mL of solutions. A used appropriate amount of ethanol (CH_3CH_2OH) for dissolved pharmaceutical samples as well as using the magnetic stirrer for more than 30 minutes. After that, filtered the solution by using 0.07µm cellulose filter paper for preparing and obtained the concentrations of 5×10^{-3} M and 8×10^{-4} M aspirin and was determination in pharmaceutical preparation by using a MIP aspirin solid-phase extraction (SPE) column. This column was previously prepared by packing a 3 ml plastic syringe with MIP (0.3, 0.5 g). The eluent was dried for 10 minutes, and 1 mL (1:100, v/v) acetic acid: acetonitrile was added, and the eluent was also collected in the beaker, and the residue was measured by Uv-Vis to ensure the aspirin was exited in the beaker that indicates aspirin was full in the column, the MIP was soxhlet and the filtred, the solution measured to know the concentration of aspirin.

RESULTS AND DISCUSSION

Synthesis of MIPs for Aspirin (AS)

Two AS MIPs were synthesized through the self-assembly (non-covalent) technique of bulk polymerization. The functional monomer played a key role in the research of the template interactions. For the synthesis of MIPs and NIPs, two monomers were used, MAA and styrene.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR is a significant technique of chemical characterization for the detection of functional groups in a compound. Table-1 shows the FTIR spectra of various MIPs and NIPs.

The KBr pellet technique (Table 1) registered the Fourier transmission infrared spectrometry spectrum of leached and unleached aspirin(AS) impressed polymers MIP and NIP in the range of 400–4000 cm-1. The following bands were shown from this table in the MAMP FTIR range: (3200,168 9,1759,3007,2872,1604,1188) cm^{-1} for O-H stretching ,C=O acid, C=O str.ester, C-H aromatic stretching, C-H, aliphatic stretching, C=C stretching, The FTIR spectrum of the AS –MIP(MAA) before template removal showed the following bands (3539,1633,1732,2958,1151) cm^{-1} for O-H stretching of carboxylic acid, C-H aromatic stretching, C-H aliphatic stretching, carbonyl acid stretching, C=C stretching, C-O-C stretching out of plan bending for monosubstituted ring. The FTIR spectrum of the MIP(MAA) after template removal shows the absence of C-H aromatic stretching, C = C stretching, and out of plan bending for a monosubstituted ring, which excises in template (AS) spectrum which indicates the extracted of a drug from a template. When using the methyl methacrylic acid (MAA) as a monomer for synthesis of another MIPs for aspirin. The KBr pellet technique (Table 2) registered the Fourier transmission infrared spectrometry spectrum of leached and unleached aspirin(AS) impressed polymers MIP and NIP in the range of 400–4000 cm^{-1}. The following bands were shown from this table in the MAMP FTIR range: (32 00,1689,1759,3007,2872,1604,1188) cm^{-1} for O-H stretching, C = O acid, C = O str.ester, C-H aromatic stretching, C-H. Aliphatic stretching, C = C stretching, The FTIR spectrum of the AS –MIP(MAA) before template removal showed the following bands (3539,1633,1732,2958,1151) cm^{-1} for O-H stretching of carboxylic acid, C-H aromatic stretching, C-H aliphatic stretching, carbonyl acid stretching, C = C stretching, C-O-C stretching out of plan bending for monosubstituted ring. The FTIR spectrum of the MIP (MAA)after template removal shows the absence of C-H aromatic stretching, C = C stretching, and out of plan bending for a monosubstituted ring, which excises in template (AS) spectrum which indicates the extracted of the drug from a template, when using the styrene as the monomer. Several experiments were carried out using different ratios (D: M: C) to reach the optimum ratio for the preparation of MIPs (AS). Among these experiments of the molar ratios (D: M: C) of (2.95:8.93:88.11), (2.14: 8.75: 89.1) for AS -MIPs have produced polymers suitable characteristics list in Table 3.

Adsorption Isotherm

Isotherm adsorption is helpful in knowing the adsorption mechanism of the polymer surface adsorption model. The information acquired from the isotherm adsorption equilibrium was evaluated to demonstrate the sort of isotherm Langmuir or Freundlich models[21] 4-vinylpyridine (4-VPy. This was determined by plotting the capacity to bind (Q) against the drug's free concentration; the Q is calculated as follows:

$$Q = [(Ci - Cf)\ Vs \times 1000] / MMIP \quad ...1$$

Ci = initial drug concentration (µmol / mL), Cf = final drug concentration (µmol/ mL)

Vs = volume of solution tested (mL), MMIP = mass of dried polymer (mg), Than measuring binding parameter, MIP/drug binding calculated by Scatchard analysis using the equation

$$Q/Cf = (Q_{max} - Q)/Kd \quad ...2$$

Q_{max} = maximum capacity, Kd = dissociation constant at binding side.

Isotherm adsorption obtained after shaking different AS concentrations with a two-hour synthesis particle in a 25 ° C heat water bath, as shown in Figures (2, 3). Table 4 included experimental information for regrouping experiments.

Morphological Characterization

Morphological analysis is a significant feature for understanding the size and layout of locations that removed polymer AS.

Table 1: The most identified peaks of the FTIR spectra (cm^{-1}) used as a functional monomer for MAA-imprinted polymer and NIP.

	Functional group	*AS*	*AS–MIP (MAA) before template removal*	*AS–MIP (MAA) after template removal*
1	O-H str. (cm^{-1})	3200-2500	3539-3421	3559-3419
2	C=O. (cm^{-1}) acid	1689	1633	1618
3	C=O str.ester.(cm^{-1})	1759	1732	1734
4	C-H aromatic.(cm^{-1})	3007	----	----
5	C-H aliphatic.	2872-2831	2958	2958
6	C=C	1604	-----	1558
7	C-O-C	1188	1151	1149

Table 2: The most identified peaks of the FTIR spectra (cm^{-1}) used as a functional monomer for Styrene-imprinted polymer and NIP.

	Functional group	*AS*	*AS–MIP (MAA) before template removal*	*AS–MIP (MAA) after template removal*
1	O-H str. (cm^{-1})	3200-2500	3539-3421	3559-3419
2	C = O. (cm^{-1}) acid	1689	1633	1618
3	C = O str.ester.(cm^{-1})	1759	1732	1734
4	C-H aromatic.(cm^{-1})	3007	----	----
5	C-H aliphatic.	2872-2831	2958	2958
6	C = C	1604	-----	----
7	C-O-C	1188	1151	1149

Table 3: The variation ratios of [D: M: C] and progeny used in the preparation of MIPs and NIPs for AS

		Drug AS	*Monomer (MAA)*	*Crosslinker TRIM*	*Initiator*	*Solvent*	*Result*
MIP1	%	4.22	9.85	85.91	0.5	7mLCH_3CH_2OH	white rigid
	mmole	1.5	3.5	30.5	0.5		
MIP1	%	1.6	8.2	90.1	0.5	7mLCH_3CH_2OH	white rigid
	mmole	0.51	2.52	27.5	0.5		
MIP1	%	2.95	8.93	88.11	0.5	7mLCH_3CH_2OH	white rigid
	mmole	0.99	2.99	29.5	0.5		
NIP1	%	----	8.93	88.11	0.5	7mLCH_3CH_2OH	white rigid
	mmole		2.99	29.5	0.5		
		Drug AS	Monomer Styrene	Crosslinker TRIM	Initiator	Solvent	Result
MIP2	%	1.98	6.4	91.61	0.5	7mLCH_3CH_2OH	white rigid
	mmole	0.9	2.9	41.5	0.5		
MIP2	%	2.1	8.15	89.74	0.5	7mLCH_3CH_2OH	white rigid
	mmole	0.9	3.5	38.5	0.5		
MIP2	%	2.14	8.75	89.1	0.5	7mLCH_3CH_2OH	white rigid
	mmole	1.1	4.5	45.8	0.5		
NIP2	%	----	8.75	89.1	0.5	7mLCH_3CH_2OH	white rigid
	mmole		4.5	45.8	0.5		

All ratios prepared in a water bath at 60 C0.

Table 4: Rebinding values of (AS) using AS -MIP particles based on (MAA) and (styrene).

AS-MIP(MAA)					*AS-MIP(styrene)*			
Mass of MIP g	*Ci mM*	C_{free} *mM*	*Q µMole/g*	*Q/Cfree L/g*	*Ci mM*	C_{free} *mM*	*Q µMole/g*	Q/C_{free} *L/g*
0.3	0.2010	0.1960	0.1512	0.7714	0.2010	0.1995	0.0450	0.2255
	0.4020	0.3865	0.4696	1.2150	0.4020	0.3845	0.5303	1.3791
	0.6030	0.5736	0.8909	1.5531	0.6030	0.5728	0.9151	1.5975
	0.8040	0.7845	0.2878	0.3668	0.8040	0.7846	0.5878	0.7491
0.5	0.2010	0.1892	0. 14	1.1173	0.2010	0.1923	0.1241	0.6453
	0.4020	0.3689	0.3771	1.0222	0.4020	0.3886	0.1914	0.4925
	0.6030	0.5827	0.3781	0.6488	0.6030	0.5826	0.2911	0.4996
	0.8040	0.7803	1.3120	1.6814	0.8040	0.7726	0.4451	0.5761

Figures (11, 12) noted from the SEM images that AS-MIP powders were successfully hybridized into polymer membranes, while the printed membranes show a smooth surface area after removal of the AS. Morphological assessment also stated that the structure of AS – MIP (MAA) is more porous than that of AS-MIP(Styrene) Micro-analysis indicates very tiny particles and spherically formed polymer particles with tiny dimensions around (2.941-11.64) µm for MAA polymer and (2.50-4.16) µm for STY polymer, these can be differentiated in Figures 11, 12 associated picture.

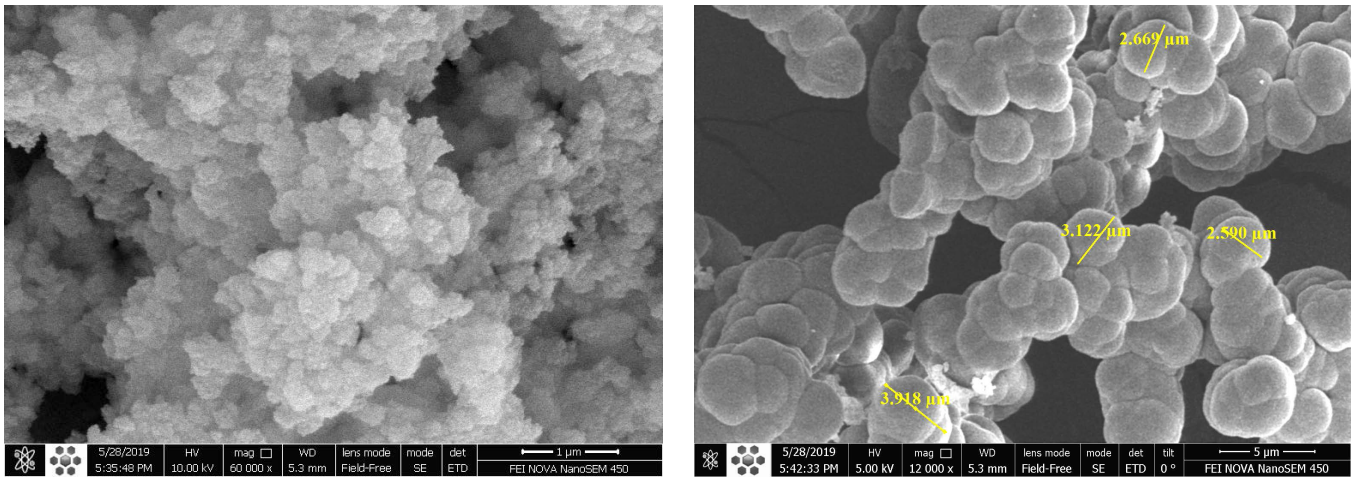

Figure 11: SEM photograph of the surface of AS-MIP (MAA), a) after AS removal b) before AS removal

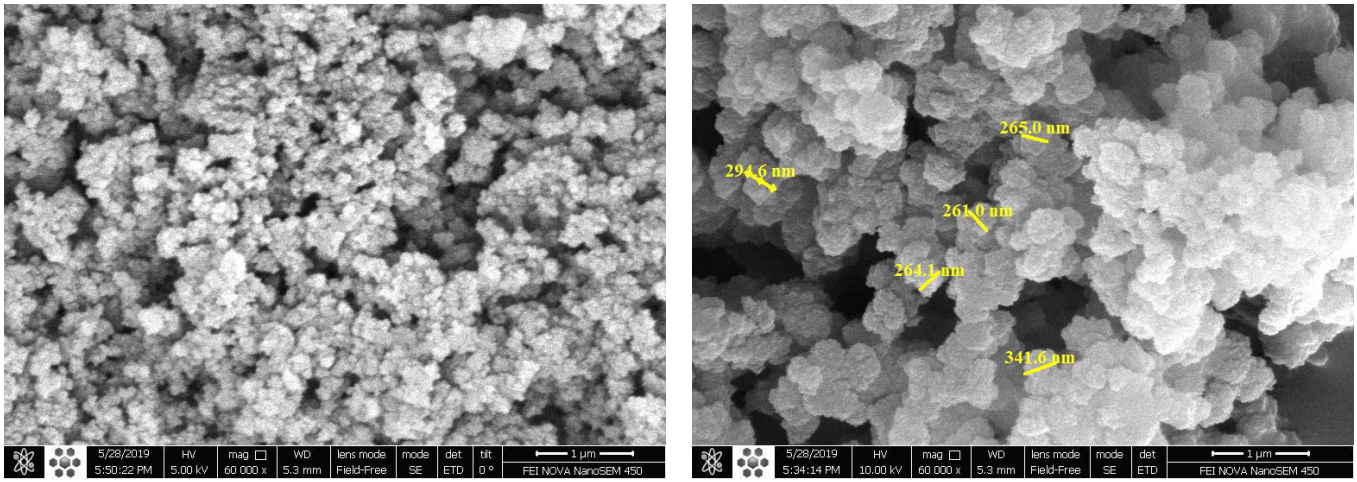

Figure 12: SEM photograph of the surface of AS –MIP (Styrene), after AS removal b) before AS removal.

Table 6: Standard addition method for drug determination using the imprinted polymer method solid-phase extraction used MIP-MAA.

Wt. of MIP (g)	*Conc. of solution (ppm)*	*Bristol tablets*			
		Conc. Taken (ppm)	*Conc. Found (ppm)*	*Recovery %*	*RSD%*
0.3	30	30	29.45	98.50	1.86
0.3	60	60	59.65	97.22	1.62
0.5	30	30	29.55	98.38	2.07
0.5	60	60	59.72	98.88	1.78

Average of three measurements

Table 7: Standard addition method for drug determination using imprinted polymer method solid-phase extraction used MIP- Styrene

Wt. of MIP (g)	*Conc. of solution (ppm)*	*Bayer tablets*			
		Conc. Taken (ppm)	*Conc. Found (ppm)*	*Recovery %*	*RSD%*
0.3	30	30	29.70	98.36	1.42
0.3	60	60	59.30	97.22	1.12
0.5	30	30	29.54	98.21	1.24
0.5	60	60	59.72	97.63	1.13

Average of three measurements

Investigation of chemical and thermal stability

Different experiments have shown that manufactured needles are chemically stable and that the ability to extract manufactured columns has not changed until 300°C when the temperature limiting application of these columns is extracted (discussed in detail in ESM).

CONCLUSION

The study involves the application of chemical devices using separate monomers with cross-linker to give the suitable geometric shape to achieve the molecularly impressed polymers (MIP), as well as an understanding of the ability of each print ready for the drug. It is thus possible to estimate the drug on the grounds of tiny levels and various mixtures. Planning of molecularly impressed aspirin materials included: the first step was to prepare the molecular print, and the second step was to acquire a concentration process low-dose medicine using solid-state extraction, thus achieving a method per sample and assessment in one step.

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