2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Synthesis of Molecularly Imprinted Polymers for Selective Extraction Followed by Solid Phase Determination of Metformin in Pharmaceutical Preparation and in Human Serum

Rana A. Kamal Aldeen**, Yehya K. Al-Bayati

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received 13/12/2022, Revised 30/04/2023, Accepted 02/05/2023, Published Online First 20/10/2023, Published 01/05/2024

Abstract

This paper demonstrates that the synthesising and storage of molecular-imprinted polymers (MIP) at room temperature using bulk polymerisation of Metformin (Met) is characterised by high sensitivity, reduced costs, increased stability, and extended life. The research used 0.8:4:20 mmol ratios of template, monomer and cross-linking agents for the polymerisation in order to ensure an appropriate adsorption capacity. Benzoyl peroxide BPO was employed as the initiator for the functional monomer styrene C_8H_8 , cross-linked with Ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$, thereby creating MIP for Metformin(Met-MIP) that could be characterised with UV-Visible Spectrophotometry at 236nm, for pharmaceutical drugs and human serum. Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) was used for MIP drug. The elution process that was applied to the template (Met.) from the Met-MIP created cavities that were caused by the porogenic mixture solvents that were created from methanol, chloroform and acetic acid (70:20:10) mL respectively. The maximum adsorption capacity was 1.2320, 2.4448 µmol/g for two studies using 0.1 and 0.2 g weights of Met-MIP respectively. A solid-phase extraction (SPE) syringe packed with molecular imprinted polymers (MIPs) was employed to selectively separate and pre-concentrate the Metformin in multiple pharmaceutical drugs from several sources. The human serum was based on the use of deionized water to dilute the serum, followed by the heating of the serum with methanol. Subsequently, a few drops hydrochloric acid 1M were applied to gate transparence solution and detect Metformin at UV region 236 nm by applying the standard addition method.

Keywords: Adsorption process, Metformin, (Molecular Imprinted Polymers) MIP, Serum, (Solid-Phase Extraction) SPE.

Introduction

Metformin (Met) or Glucofage is an oral diabetes medication that helps control blood sugar levels and is used together with diet and exercise to improve blood sugar control in adults with type 2 diabetes mellitus. It is a component of drugs like metformin-alogliptin (Kazano) and metformin-canagliflozin (Invokamet). In the last stages of type 2 diabetes, metformin can also be used with insulin. Metformin

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

decreases hepatic glucose production, and increases insulin-mediated peripheral glucose uptake, and decreases intestinal glucose absorption, it is also used off-label to treat polycystic ovary syndrome (PCOS)[1,2].

Common adverse effects include diarrhea, nausea, and abdominal pain. It has a low risk of causing low blood sugar. High blood lactic acid levels are a concern if the medication is used in overly large doses or prescribed to patients with severe kidney problems. It is not recommended for those with significant liver disease [3]. Metformin is a biguanide antihyperglycemic agent that works by decreasing glucose production by the liver, increasing the insulin sensitivity of body tissues, and increasing GDF15 (growth differentiation factor, a protein coding gene) secretion, which reduces appetite and caloric intake [1]. Fig. 1 shows the structure of metformin.

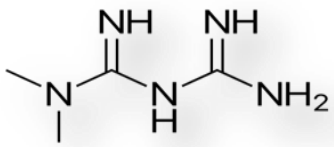


Figure 1. Structure of Metformin[1].

In the beginning, the imprint molecule with the present monomers forms a complex in molecular imprinted polymers (MIP). The functional groups are maintained in situ following the polymerization cycle[4], as depicted in Fig. 2, by a strongly cross-linking polymer structure.

In addition, the steric configuration of all these connections around a given substratum and the template is really an important characteristic for the formation of binding sites[5,6], providing additional shape, size, and flexibility to promote the selective identification followed by a high target affinity. As a result, the process of recognition in MIPs can be characterized in resemblance to enzyme-proven mechanisms – substratum- Complex is formed in the (lock and key) model [7,8].

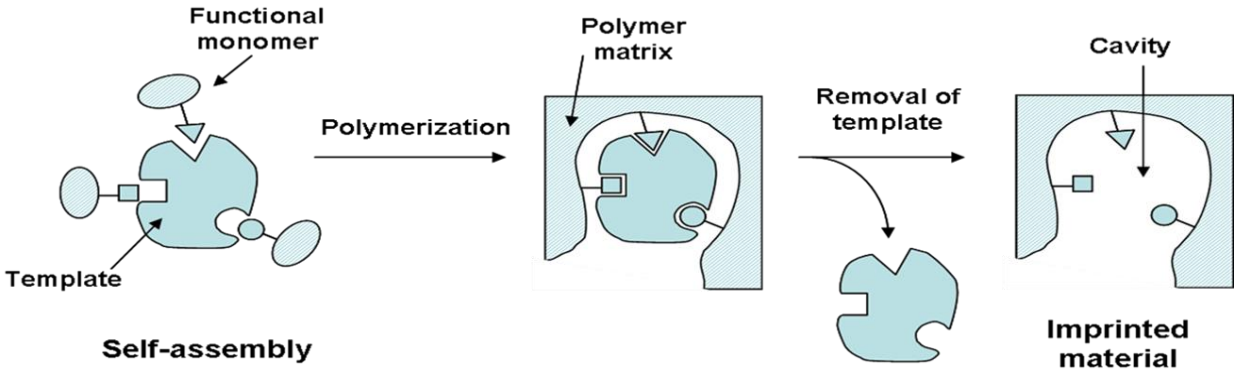


Figure 2. Molecular imprinted polymer cycle [9].

Certain MIP applications were prepared in SPE [10,11]. The concentration of the solute in the fluid phase at constant temperature provides the adsorption isotherm. An isotherm is the relation between the concentrations of a solid and fluid, used to describe states of sorption process[12].

- Solid phase extraction (SPE) is a technique designed for rapid, selective sample preparation and purification prior to the chromatographic analysis (e.g. HPLC(high performance liquid chromatography) , GC(gas chromatography), TLC(thin layer chromatography)) [13,14]. In SPE, one or more analytes from a liquid sample are isolated by extracting, partitioning, and adsorbing onto a solid stationary phase, Fig. 3 [15].

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

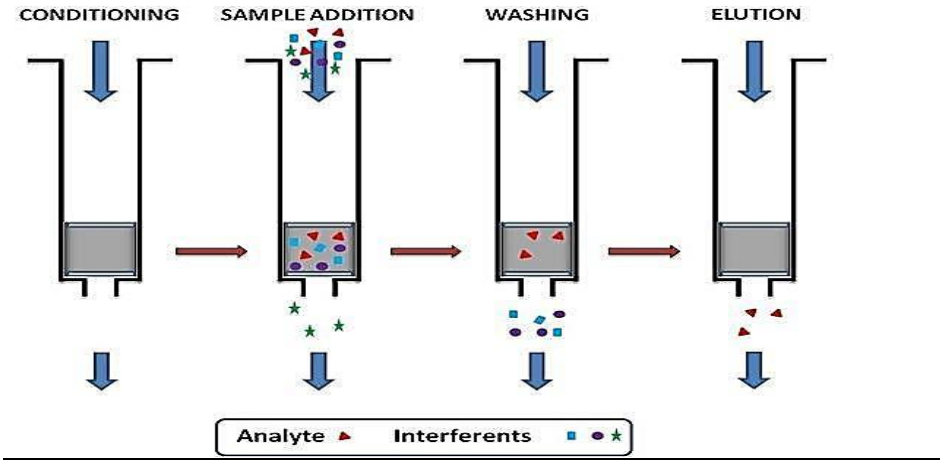


Figure3. Illustrate the process of SPE.

In this work identify the MIP preparation was performed in conjunction with the recognition cite styrene C_8H_8 with crosslinking ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$, whereby benzoyl peroxide BPO functioned as the target molecule (Metformin) initiator. Subsequently, the impact of the monomer dosages on the adsorption performance was observed. The study also examined adsorption behaviors with diverse functional monomers, cross-linking agents, and solvents. SEM, FTIR was employed to characterise the primed MIPs. Furthermore, this research investigated the impact of solid phase extraction and initial Metformin concentration on adsorption capacity.

Materials and Methods

Metformin. HCl from Samarra/Iraq was provided, Styrene, EGDMA, Benzoyl peroxide was purchased from Sigma Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com), Methanol, Nitrogen gas (99.99) were supplied from Al-Watan factory (Al-Nahda Street/ Baghdad/Iraq), Chloroform and Acetic acid were purchased from Merck (Darmstadt, Germany).

Preparation and Processing:

The Met-MIP preparation process used high-purity chemicals:

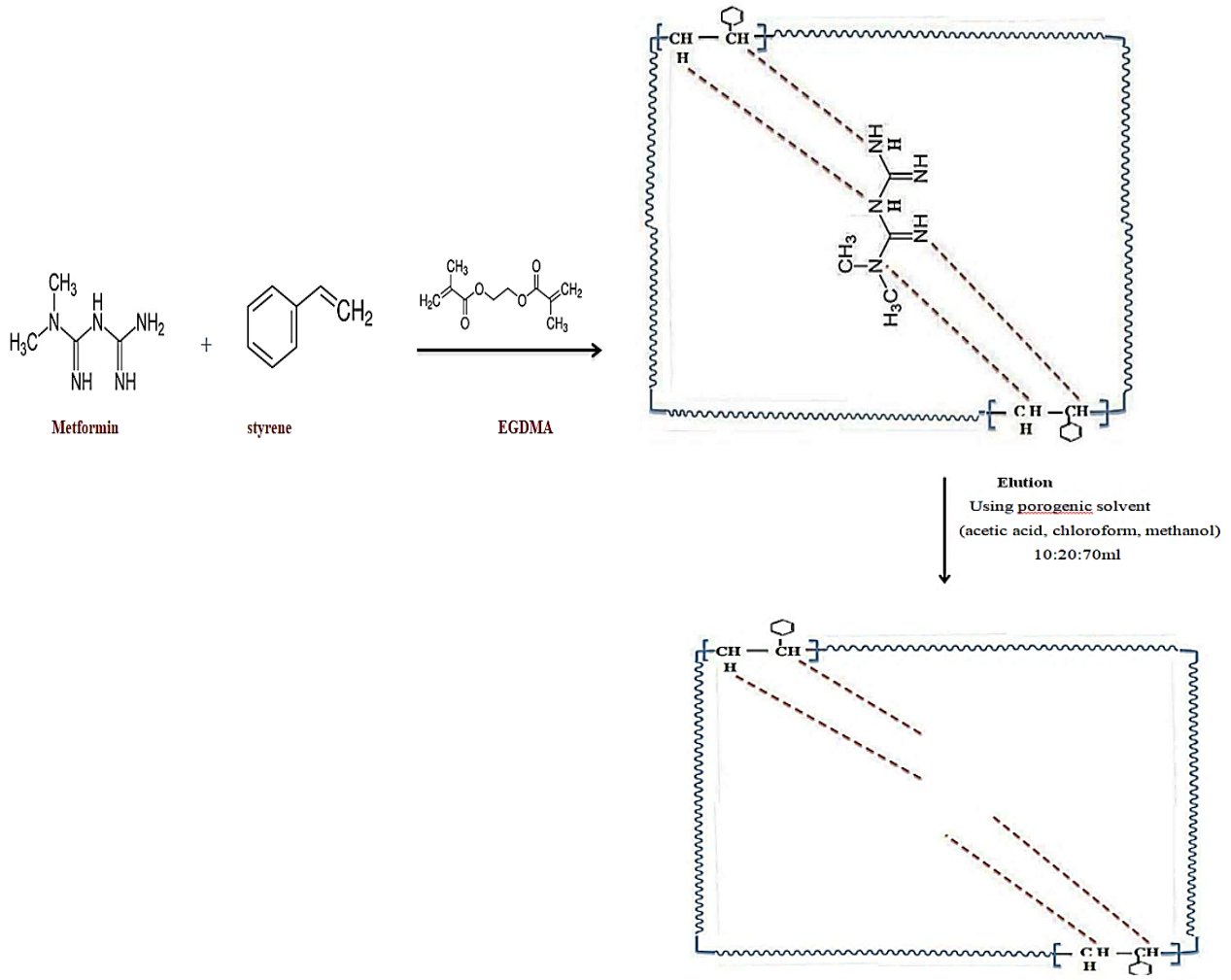
Specifically, 0.8 mmol of Metformin (Metformin. HCl) 0.1325g was dissolved in 4 mL of methanol with stirring, 4 mmol of Styrene 0.4180g with 2 mL methanol was added, waiting for few seconds at room temperature. Subsequently, 20 mmol of cross-linker Ethylene glycol dimethacrylate EGDMA 3.9644g, 2mL of methanol, and 0.3 g of benzoyl per-oxide were dissolved in chloroform to create an initiator. These were added to the mixture, which was shaken well in order to produce a clear solution. The solution was bubbled with Nitrogen gas for 20 minutes in order to eliminate the dissolved oxygen from the monomer solution. It was then sealed in the tube.

The solution was placed in a water bath, where it remained overnight at a temperature of 60 ºC. Thus, the Met-MIP polymerization process 0.8:4:20 was completed, leading to the formation of a white polymer with a fluff structure has been formed. This was left to dry overnight at room temperature. The Met- MIP was synthesized using the self-assembly (non-covalent) bulk polymerization method.

Soxhlet solid liquid extraction was performed to remove template (Metformin) from MIP. This relied on the use of a porogenic solvent consisting of 10 mL of acetic acid, 20 mL of chloroform, and 70 mL of methanol. The removal process was successful following repeated for 16-18 hours, following which the particles were rinsed in methanol and water in order to eliminate residual acetic acid. Subsequently, the polymer was dried at room temperature, before being crushed with a mortar and sieved to produce 125µm particles. A proposed successive mechanism for Met-MIP can be represented by the following scheme 1 and illustrate the structure of synthesized

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

polymer after elution process by using a suitable porogenic solvent.



Scheme 1. Suggested mechanism of the synthesized of Met-MIP, using styrene as a functional monomer, EGDMA as cross-linker and the Structure of synthesized polymer after elution process.

Sample Preparation of (Metformin. HCl) (Glucophage)

The samples of pharmaceutical were prepared by taking the average weight of ten tablets of Metformin, they were crushed and grinded. Tablets containing 500 mg of Metformin were weighed 0.5264, 0.5598, 0.5567 g of Metformin drugs (Metforal/Germany, Glucophage/ Italy and Piophage / Iraq) dissolved in 100 mL of methanol solution, then filtered through cellulose filter paper 0.07µm in order to obtain the concentrations 1.0×10^{-4} , 1.4×10^{-4} and 1.6×10^{-4} mmol/mL (0.1, 0.14, 0.16µmol/mL) (equivalent to 0.00166, 0.00232, 0.00265)g of active ingredients (Table 1), which have lowest standard deviation (SD) value and these concentrations were used with MIP in a solid phase extraction (SPE) column MIP-SPE which was prepared.

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Table 1. Pharmaceutical drugs prepared for treating with Met-MIP polymer

No. of Samples	Commercial name, Country Content 500mg	Average weight for 10 of tablets (g)	Weight of sample equivalent to 0.00166g (1.0×10^{-4}) mmol/mL of the active ingredient	Weight of sample equivalent to 0.00232g (1.4×10^{-4}) mmol/mL of the active ingredient	Weight of sample equivalent to 0.00265g (1.6×10^{-4}) mmol/mL of the active ingredient
1	Glucophage/Germany	0.5264	0.0017	0.0024	0.0028
2	Metforal / Italy	0.5598	0.0019	0.0026	0.0030
3	Piophage / Iraqi	0.5567	0.0018	0.0026	0.0029

Accuracy of the work for extraction and determination of Metformin

1. **FT-IR Spectrum of Molecularly Imprinted Polymers for (MET):**

The functional groups present in a compound can be detected using FT-IR Fourier transmission infrared spectrometer, which comprises a significant chemical characterization process. The Metformin FT-IR spectra presents multiple functional groups, in addition to Met-MIP both prior to and following the Metformin template removal.

Figs. 4, 5 A, B for Met-MIP and Table 2 show the details of bands.

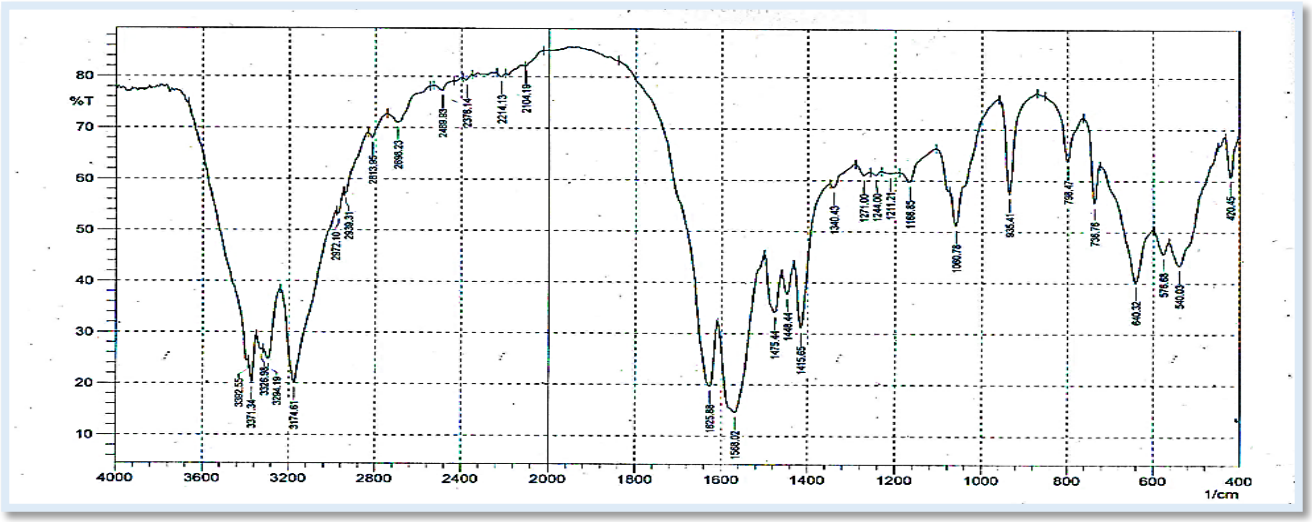


Figure 4. FT-IR spectra of Metformin standard.

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

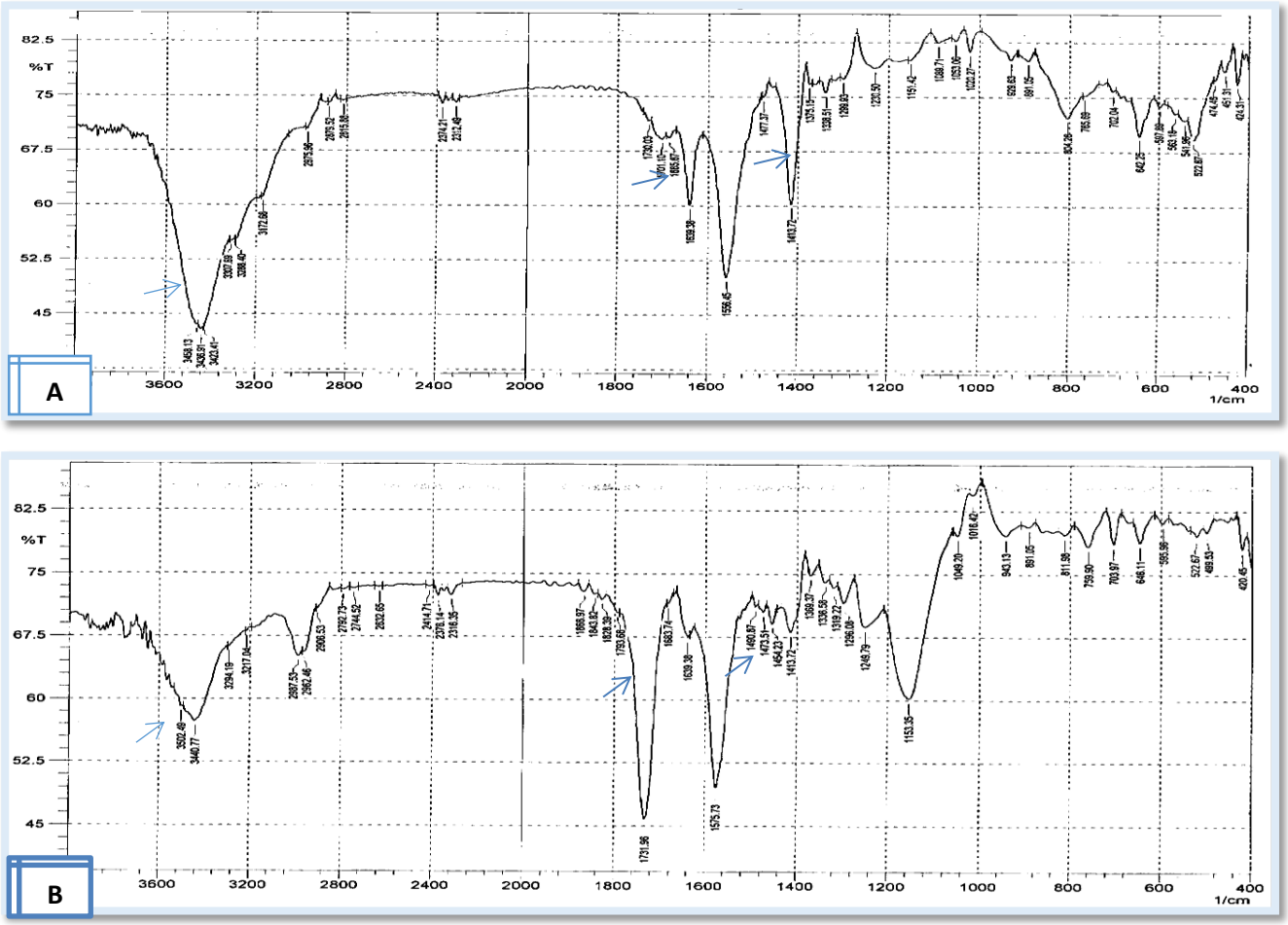
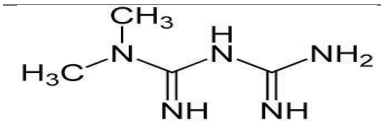


Figure 5. A, B FT-IR spectrum of Met-MIP before and after extraction.

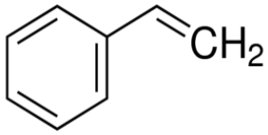
The spectrum of Met shows strong bands at 3392 and 3326 $cm^{-1}$for N-H_2 stretching, 3307, 3288 cm^{-1} for Met –MIP before elution while in 3294, 3217 cm^{-1} after elution become smallest. As it explain in Table 2, C=N stretching bands appear at 1625, 1639 cm^{-1} in both Met and MIP before extraction respectively but after extraction it be very smallest, C-H aliphatic bands in Metformin have been seen at 2972, 2939 cm^{-1}, in Met -MIP before elution at 2975 cm^{-1} and after elution at 2987and 2962 cm^{-1} . To improve that Metformin had been removed successfully in addition to N-H_2, NH str. and C=N, there is C=C group of monomer in 1413 cm^{-1} which disappears after eluting that means there is an interaction between C=C of monomer and N-H of template(Met.), C=O takes two form ketone1730,1731 cm^{-1} and enole form in (3423,3436,3458), (3502, 3440) cm^{-1} respectively. (The bands values [16]).

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

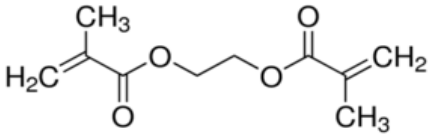
Table 2. The structures of the main three compositions of Met-MIP and the bands indicate MIP before and after removal template.



Template
(Metformin)



Monomer
(Styrene)



Cross linker
(EGDMA)

Band	Drug(Template)	MIP before extraction	MIP after extraction
N-H$_2$ N-H str.	3392,3326	3307, 3288	/ 3294, 3217
C=N str.	1625	1639	/
C-H aliph.	2972 2939	2975	2987 2962
C=O est.	/	/1730	1731
C=O enol.		3423,3436,3458	3502, 3440
C=C aro.	/	1413	/1413

2. Scanning Electron Microscopy SEM for MIP-Met:

The morphological evaluation is critical to the appreciation of the morphological traits, cavity sizes, and surface configurations of MIPs both prior to and following the Metformin template removal. SEM images were used to analyze the morphology of the Met-MIP . Fig. 6 (A,B) and the results of calculated the dimentions of six cavities are cleared in Table 3 by using image j program.

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

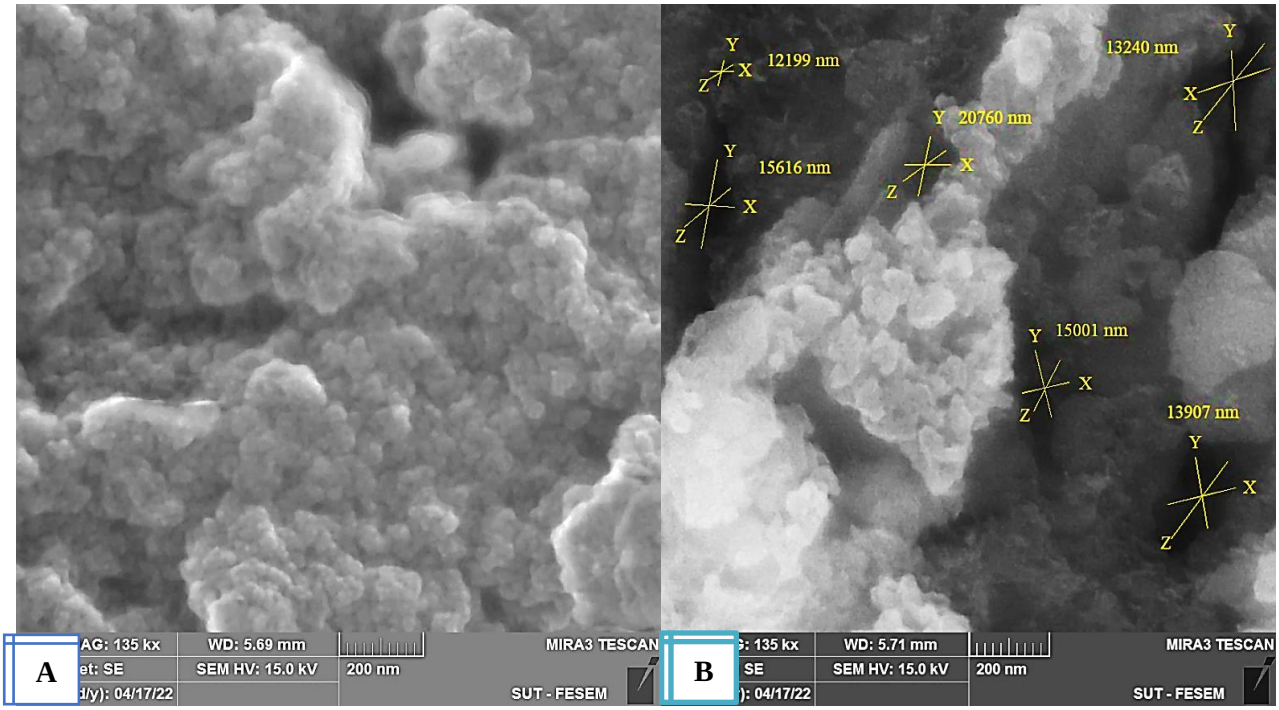


Figure 6. A, B surface morphologies of the particles before and after elution for Met- MIP respectively, and three dimensions of cavities with their areas.

Table 3. Calculated mean, angle, lengths of some cavities (selected six of them) and their areas using image j program.

Cavities	Area	Mean	Min	Max	Angle	Length
1	119.898	20760.62	19717.79	22748	180	62.961
2	143.212	15001.08	12886.43	17185	178.636	76.67
3	316.398	13907.2	11822	16873	-168.93	171.08
4	226.474	13240.39	10985.6	15765.67	-177.732	122.977
5	193.169	12199.21	9740.667	15734.51	177.99	104.087
6	73.271	15616.87	13623.65	18701.75	174.644	39.103
Total Mean	178.737	15120.9	13129.35	17834.65	60.768	96.147
SD	86.354	3020.344	3507.103	2643.141	181.362	47.186
Total Min-Max	73.271-316.398	12199.21-20760.62	9740.667-19717.79	15734.51-22748	-177.732-180	39.103-171.08

3D of Cavities between min = 12199.21nm (12.19921µm) to max = 20760.62nm (20.76062µm) that mean the number of molecules of Metformin fill cavities ranging around this range.

We notice that the holes vary in diameter range between (12.19921-20.76062) µm and most of the holes are large, which leads to the retention of large quantities of the drug and this is consistent with the high value of the capacity in isotherm.

3. UV-VIS Spectrophotometry

A column (10 mL solid phase extraction of plastic syringe (height=7cm, diameter=1.5cm) (was used and each syringe was packed with different weights 0.1 and 0.2g from Met- MIP. The resulting solutions (standard solution, pharmaceutical drugs of Metformin and serum) was poured from the top of the column and the movement of the solution was 70 rpm by electric vacuum.

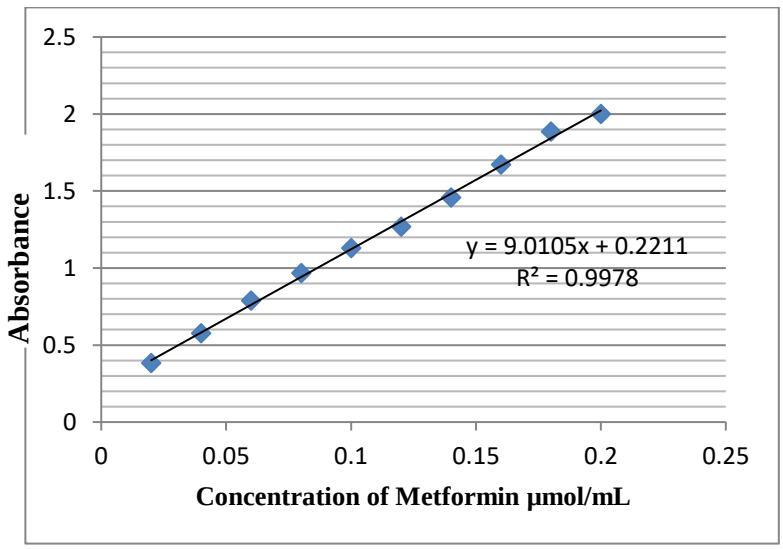
A series of standard solutions of Metformin. HCl 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, 0.2

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

µmol/mL was prepared by dissolving 0.0116g in methanol volumetric flask 100 mL as a stock solution, after passing the solution of Metformin in syringe packed with Met-MIP, the residue which has less absorption was measured by UV-VIS instrument at 236 nm [17], that indicates to lower concentration at final process, for good expressive example of the advantages of the use of impressed polymers in SPE in the quantification of the Metformin.

A calibration curve between standard solutions of different concentrations of Metformin. HCl (0.02-0.2)

µmol /mL and their absorbance are plotted in Fig. 7.



Concentration of Metformin µmol /mL	Absorbance
0.02	0.3836
0.04	0.5749
0.06	0.7889
0.08	0.9656
0.10	1.1281
0.12	1.2688
0.14	1.4575
0.16	1.6705
0.18	1.8855
0.20	1.9987

Figure 7. Calibration curve between concentration of Metformin standard µmol/mL and its absorbance.

- **Adsorption Capacity and Pre-concentration:**

A series of adsorption achievement for different initial concentrations of Met-MIP

ranging from 0.02 to 0.2 µmol/mL on adsorption capacity µmol/g was studied for 0.1and 0.2 g weight of MIP using the following equation Eq. 1 [18]:

$$\mathbf{Q} = (\boldsymbol{Ci} - \boldsymbol{Cf})(\boldsymbol{\mu mol/mL}) * \frac{\boldsymbol{vol\ (ml)}}{\boldsymbol{Wof\ Mip(g)}} \quad \textbf{.....1}$$

Ci- initial concentration, Cf - final concentration (after passing through column packed with Met-MIP)

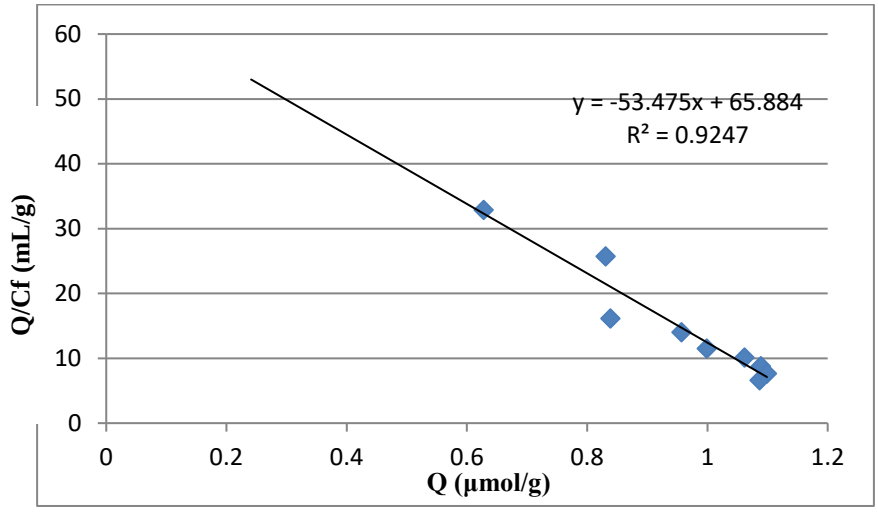
Table 4. The optimal synthesis conditions for the molecularly imprinted polymer for Metformin developed in this study in 0.1 g of MIP

W mip(g)	Ci(µMol/mL)	Cf(µMol/mL)	Vol (mL)
0.1	0.02	0.0119	3
	0.04	0.0191	3
	0.06	0.0323	3
	0.08	0.0520	3
	0.10	0.0681	3
	0.12	0.0867	3
	0.14	0.1046	3
	0.16	0.1240	3
	0.18	0.1434	3
	0.20	0.1638	3

The relation between initial concentration Ci (µmol/ml) and capacity Q (µmol/g):

The relation between capacity Q (µmol/g) and Q/Cf (mL/g):

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal



Q µmol/g	Q/Cf (mL/g)
0.241	20.252
0.628	32.880
0.831	25.728
0.839	16.135
0.957	14.053
0.999	11.522
1.062	10.153
1.089	8.782
1.099	7.664
1.087	6.636

Figure 8. Illustrate the relation between capacity Q (µmol/g) and Q/Cf (mL/g)

From slope equation:

Slope = -1/kd ……2

-53.475 = -1/ kd

Kd = 0.0187

Intersept = 65.884

Intersept = Qmax/ kd ……3

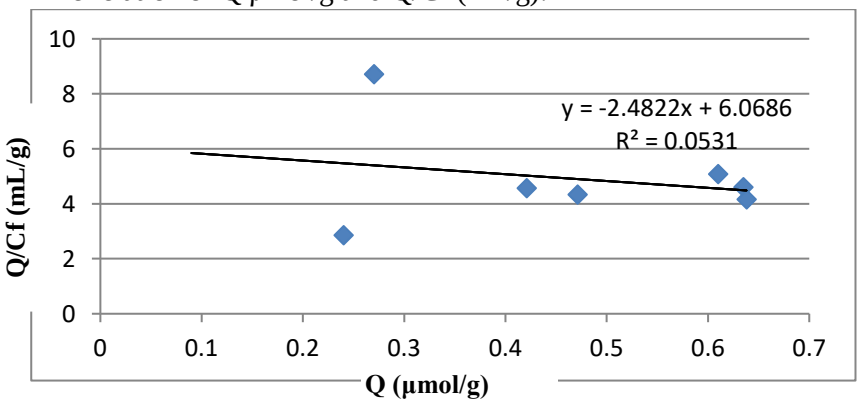
Qmax = 65.884 * 0.0187

= 1.2320 µmol/g for Met-MIP using 0.1g weight of MIP

Table 5. The optimal synthesis conditions for the molecularly imprinted polymer for Metformin.HCl developed in this study in 0.2 g of MIP

W mip(g)	Ci(µMol/mL)	Cf(µMol/mL)	Vol (mL)
0.2	0.02	0.012	14
	0.04	0.031	6
	0.06	0.054	3
	0.08	0.0720	3
	0.10	0.0840	3
	0.12	0.0920	3
	0.14	0.1086	3
	0.16	0.1200	3
	0.18	0.1436	3
	0.20	0.1575	3

The relation of Q µmol/g and Q/Cf (mL/g):



Q µmol/g	Q/Cf (mL/g)
0.560	46.667
0.270	8.709
0.090	1.667
0.120	1.660
0.240	2.857
0.421	4.570
0.471	4.337
0.610	5.083
0.635	4.608
0.638	4.162

Figure 9. Illustrate the relation between capacity Q (µmol/g) and Q/Cf (µmol/g)

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Slope = -1/kd

-2.4822= -1/ kd

Kd= 0.4029

Intersept = 6.0686

Intersept = Qmax/ kd

Qmax =0.4029 *6.0686

= 2.4448 µmol/g for Met-MIP using 0.2g weight of MIP

- **Effect of Weight of Solid Phase Met-MIP:**

Weight of MIP has an effect on the separation process because this process occurs by using solid phase extraction technique, and depending on the sites in the MIP. Two tubes contain 0.1, 0.2 gm from Met-MIP and series of different concentrations from 0.02 to 0.2 µmol/mL of standard solutions of metformin were studied for 0.1 and 0.2 g weight of MIP and comparison the adsorption capacity µmol/g.

The volumes solution of the analytes was 3mL for all concentrations 0.02-0.2 µmol/mL for 0.1g weight of Met- MIP, (Table 4), while the volumes for concentrations 0.02- 0.2 µmol/mL for 0.2g weight of Met- MIP were 14-3 mL (Table 5), therefore, a good capacity 2.4448 µmol/g has been achieved when using 0.2g weight of Met- MIP than 1.2320 µmol/g for Met-MIP using 0.1g, that mean a larger amount of interaction position available in 0.2 g so a promote linking sites has been done and the interaction taking place in more than site.

Table 6. Precision and accuracy of the analysis of pharmaceutical drugs.

Drug name 500mg	MIP g	Concentration Ci µMol/mL	Absorption before isotherm process	Absorption after isotherm process	Concentration Cf µMol/mL	Vol mL	Q µ Mol/g	RSD% = (δn-1 /Mean) *100 Precision	Rec. % =(practical value/True value)*100 Accuracy	Re %
Metfo ral/ Germ any	**0.1 0.2**	0.08	1.0016	0.9221 0.8988	0.0737 0.0718	8 15	0.504 0.615	0.124 0.118	103.73	3.73
Gluco phage / Italy	**0.1 0.2**	0.10	1.1377	1.0081 1.0819	0.0827 0.0551	7 12	1.211 2.694	0.153 0.162	100.85	0.85
Pioph age/ Iraq	**0.1 0.2**	0.08	0.9056	0.7221 0.5431	0.0638 0.0479	7 15	1.134 2.407	0.114 0.121	97.47	-2.53

* For n=5 absorptions of drugs before isotherm process.

Thetrue value is the absorption at 0.08 ,0.1µmol/ml in calibration curve for MIP.

* For n=5 absorptions of drugs before isotherm process (passing through MIP column), * The true value is the absorption at 0.1,0.12 µmol/mL in calibration curve of Metformin.

In Table 6 the volumes passing through MIPs column for pharmaceutical drugs consuming mils more than standard due to interferences and additions using in manufacture drugs.

- In Human Serum

1- Sample Collection

In total, 5 ml of blood was gathered and placed in serum separator tubes (SST). The clot activator SST contained a gel in the form of an inert

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

thixotropic polymer [19, 20], which was located at the bottom, its purpose being to separate blood cells from serum through centrifugation. This was performed for each patient and healthy individual. Blood samples were allowed to stand for 5 minutes following centrifugation at ~ 2000 rpm. The serum was frozen at 20ºC , so that it could later be employed for the estimation of Metformin.

2- Procedure

This method uses one ml of each human serum. In other words, it requires serum from the control group (healthy individuals who do not take Metformin) and the patients' group (who took Metformin drug), both of which were diluted in 10 mL of deionized water. Subsequently, 1 mL of diluted serum was placed in a 10 ml volumetric flask, to which was added 2-3 drops of 1 N HCl solution, the purpose being to eliminate the viscosity of the serum[21,22]. Methanol was used to make the volume up to 10 ml. The solutions were then warmed in a water bath for 10-15 minutes at a temperature not exceeding 60 ºc in order to create a transparent solution.

Several series of solutions were created for each control and patient group. This was realized through the transferal of 1 ml to each eleven volumetric flask (10 mL) (we doubled the amount of serum to get the quantity needed for 11 volumetric flask) followed by the addition of constant volumes of standard Metformin (0.1 mL) from different concentrations 0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, 0.2 µmol/mL to obtain 0, 0.0002, 0.0004, 0.0006, 0.0008, 0.001, 0.0012, 0.0014, 0.0016, 0.0018, 0.002 µmol/mL. Flask No.1 is the sample (serum). The findings were subjected to mathematical evaluation ($M_1V_1=M_2V_2$ for the standard addition method) (see Table 7). Furthermore, the absorption recorded for each volumetric flask was gauged with the assistance of UV-Visible spectrophotometry, which focused on the control serum and then measured the patient serum at the maximum 236 nm absorption, the objective being to eradicate the majority of interferences. Subsequently, the resultant solution was scanned in the 190-300 nm range. Fig. 15 presents the calibration curve that was plotted between the concentrations and absorptions.

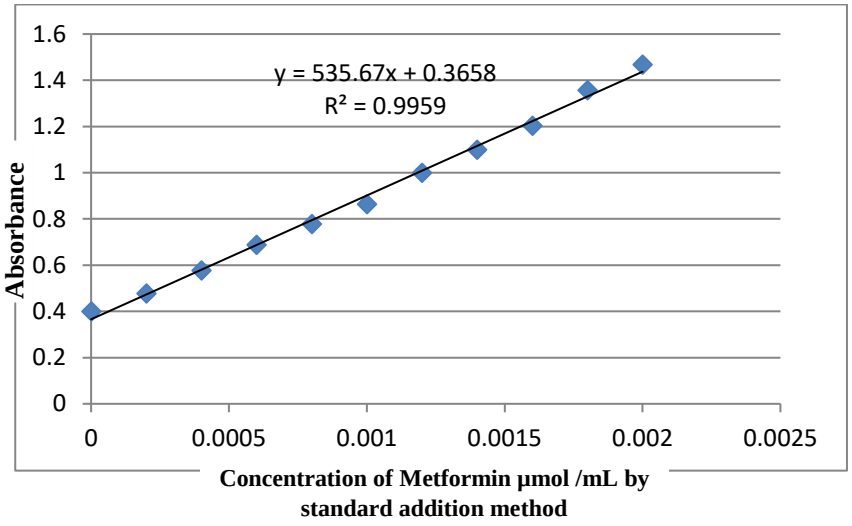
Table 7. Results of standard addition for the determination of Metformin in human serum.

Human	Diluted Serum	1N HCl	Metformin µMol/mL										
Control	1 mL	2-3 drops	0	0	0	0	0	0	0	0	0	0	0
Patient	1 mL	2-3 drops	0	0.0002	0.0004	0.0006	0.0008	0.001	0.0012	0.0014	0.0016	0.0018	0.002

Metformin in serum was statistically evaluated by considering the length of time the drug was in the body of the patient, the rate at which it was metabolized, and the medication dose. These variables differ between patients. In addition, it has an onset of action of about 1.5 hours, half-life in the circulation of about 1.5–4.9 hours, and duration of action of 16–20 hours [23]

Calibration curve between concentrations and absorptions.

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal



Concentration of Metformin in serum µmol /mL by standard addition method	Absorbance of Metformin in serum by standard addition method
0	0.4001
0.0002	0.4775
0.0004	0.5778
0.0006	0.6889
0.0008	0.7779
0.0010	0.8644
0.0012	0.9997
0.0014	1.1005
0.0016	1.204
0.0018	1.3568
0.0020	1.4689

Figure 10. Calibration curve between concentrations of Metformin in serum using standard addition method µmol/ml and its absorbance.

When y= 0.4001 that mean the absorbance of Metformin in this sample of serum is 0.4001

It found that the absorption 0.4001 are nearest to the absorption 0.3836 which has concentration 0.0200 µmol /mL in calibration curve (Fig. 10) and substituting for y= 0.4001 the concentration is 0.0209µmol /mL. This means the concentration of Metformin in this sample of serum is 0.0209 µmol /mL by ratio and proportion. So a comparison for absorption of this concentration after passing through Met-MIP column was studied in pharmaceutical drugs solution and human serum.

*To know the concentration of drug in human serum we must multiply this concentration 0.0209µmol /mL x 10 (Dilution coefficient).

Discussion

This paper presents a comparison between two approaches to the drug Metformin. The T-Test statistical evaluation [24, 25] was designed to facilitate a comparison between the identification of Metformin once it had passed through the Met-MIP syringe solid phase extraction process and the human serum at 236 nm:

$$/t/= \bar{X}i1 - \bar{X}i2/(S(\sqrt{1/n1 + 1/n2}))$$

If $\bar{X}i1 = \bar{X}i2$ ⟶ Null hypothesis when $t_{calculated} < t_{tab}$

That mean $\bar{X}i1 - \bar{X}i2$ = zero

$\bar{X}i1 \neq \bar{X}i2$ ⟶ Alternative hypothesis when $t_{calculated} > t_{tab}$

That mean $\bar{X}i1 - \bar{X}i2 > <$ zero

* $\bar{X}i1 =$ 0.0967 Mean for n1=3 absorption value after passing through Met-MIP column in pharmaceutical drugs solution with S1 variance= 0.065

$\bar{X}i2$ = 0.1651 Mean for n2=3 absorption value after passing through Met-MIP column in human serum with S2 variance=0.044

S^2 = (n1 -1)*S^2_1+n2-1)*S^2_2 / n1+n2- 2

t calculated=1.537, t tab =$t_{0.05/2, (n1+n2)-2}$= 2.776

It found $t_{calculated} <$ t_{tab} at confidence level 95% therefor there is no significant difference between two approaches, So Null hypothesis will be accepted.

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Conclusion

New and bulk polymers were created by using styrene and crosslinking ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$ as Met-MIP, different studies and experiments were used to reach for selective molecular imprinted polymer by prepare and optimize required monomers, cross-linker using suitable solvents , porogen solvent for template removal and the optimal molar ratios of Template (Metformin)to monomer to cross-linker. Irregular shapes three-dimension network structure of polymers can be seen by SEM before and after removal template, FTIR, all improve the healthy work.

One slope gain when studying the capacity of adsorption of Met-MIP with uniform values (homogeneous structure) in this study proves that the capacity increases with increasing the weight of the MIP. The maximum adsorption capacity was 1.2320, 2.4448 µmol/g for two studies of 0.1,0.2 g of Met-MIP respectively. A standard addition method using to eliminate the interferences when detect the concentration of Metformin in human serum. T-Test statistical evaluation was designed to facilitate a comparison between the identification of Metformin once it passed through the Met-MIP syringe solid phase extraction process and the human serum at 236 nm and when it found that t calculated < t tab at confidence level 95% by UV for Metformin drug therefor there is no significant difference between two methods. So Null hypothesis will be accepted.

Acknowledgment

The authors would like to thank the Editor-in Chief and all referees for their suggestions and helpful comments that have improved the paper's quality and clarity.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' Contribution Statement

Both authors conceived the idea and supervised the findings of this work. R.A. K. developed the theory, investigated the topic of the article, and performed the computations. Both authors verified the analytical methods, discussed the result and contributed to the final manuscript.

References

1. Mohammed SK, Al-Taweil HI, Salih NM, Muhi SA, Taha EM. Effects of Duration of Diabetes and Diabetes Therapy (Metformin) on Fbxw7 Levels in Iraqi Type II Diabetic Patients. Med. Legal Update. 2021 Jan 1;21(1):1308-. https://doi.org/10.37506/mlu.v21i1.2501
2. Lomteva SV, Shkurat TP, Bugrimova ES, Zolotykh OS, Alexandrova AA, Karantysh GV. Violation of the Hormonal Spectrum in Polycystic Ovaries in Combination with Insulin Resistance. What is the Trigger: Insulin Resistance or Polycystic Ovary. Baghdad Sci J. 2022 Oct 1; 19(5): 990-998. http://dx.doi.org/10.21123/bsj.2022.6317
3. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. Metabolism. 2016 Feb 1; 65(2): 20-9. https://doi.org/10.1016/j.metabol.2015.10.014
4. Hadi EA, Al-Bayati YK. Preparation and characterized study of new molecularly imprinted polymers for determination Cocaine by GC-Mass based on different Functional Monomers. Egypt J Chem. 2022 Jan 1; 65(1): 107-16.https://doi.org/10.21608/EJCHEM.2021.78126.3817

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

5. Andersson LI. Application of molecular imprinting to the development of aqueous buffer and organic solvent based radioligand binding assays for (S)-propranolol. Analytical chemistry. 1996 Jan 1;68(1):111-7.. https://doi.org/10.1021/ac950668+
6. Alvarez-Lorenzo C, González-Chomón C, Concheiro A. Molecularly imprinted hydrogels for affinity-controlled and stimuli-responsive drug delivery. Smart materials for drug delivery. 2013 Apr 17; 2: 228-260. https://doi.org/10.1039/9781849734318-00228
7. Al-Nisani MM, Al-Bayati YK, Osman ME. A Synthesis of New Liquid Electrodes for the Determination of Ibuprofen, based on a Molecularly Imprinted Polymer. Int J Drug Deliv Technol. 2021, 11(2): 308–312. https://doi.org/10.25258/ijddt.11.2.12
8. Ansell RJ. Characterization of the Binding Properties of Molecularly Imprinted Polymers. Molecularly Imprinted Polymers in Biotechnology. 2015; 150: 51-93. https://doi.org/10.1007/10_2015_316
9. Vasapollo G, Sole RD, Mergola L, Lazzoi MR, Scardino A, Scorrano S, Mele G. Molecularly imprinted polymers: present and future prospective. Int J Mol Sci. 2011 Sep 14; 12(9): 5908-45. https://doi.org/10.3390/ijms12095908
10. Lim HH, Oh YS, Shin HS. Determination of N-nitrosodimethylamine and N-nitrosomethylethylamine in drug substances and products of sartans, metformin and ranitidine by precipitation and solid phase extraction and gas chromatography–tandem mass spectrometry J Pharm Biomed Anal. 2020 Sep 10; 189: 113460. https://doi.org/10.1016/j.jpba.2020.113460
11. Knoll S, Jacob S, Mieck S, Triebskorn R, Braunbeck T, Huhn C. Development of a capillary electrophoresis–mass spectrometry method for the analysis of metformin and its transformation product guanylurea in biota. Anal Bioanal Chem. 2020; 412(5): 4985–96 https://doi.org/10.1007/s00216-020-02759-6.
12. Yu H, Chen Z, Fu Y, Kang L, Wang M, Du X. Synthesis and optimization of molecularly imprinted polymers for quercetin. . Polym Int.. 2012 Jun; 61(6): 1002-1009. https://doi.org/10.1002/pi.4172
13. Apffel A, Zhao L, Sartain MJ. A novel solid phase extraction sample preparation method for lipidomic analysis of human plasma using liquid chromatography/mass spectrometry. Metabolites. 2021 May 4;11(5):294. https://doi.org/10.3390/metabo11050294
14. Qasim B, Razzak AA, Kamil GM, Motelica-Heino M, Domenico M. Quantitative Determination of Fluoroquinolones in Contaminated Soils by HPLC with Solid-Phase Extraction. Baghdad Sci .J. 2020 Mar 1; 17(1): 48-56. https://doi.org/10.21123/bsj.2020.17.1.0048
15. Al-Janabi KW. Determination of Some Polychlorinated Biphenyls in River Tigris within Baghdad City Ibn al-Haitham J Pure Appl Sci. 2017 Feb 20; 29(3): 118-31. https://jih.uobaghdad.edu.iq/index.php/j/article/view/16.
16. Morrison Boyd, Bhattacharjee, 2010, Organic chemistry, 7th Ed., Pearson Education India, p: 1508. https://www.amazon.ae/Organic-Chemistry-Seventh-Morrison-Bhattacharjee/dp/8131704815
17. Mali AD. Seeta Mali, AshpakTamboli and Ritesh Bathe, Simultaneous UV spectrophotometric methods for estimation of metformin HCl and glimepiride in bulk and tablet dosage form. Int J Adv Pharm. 4 (6). 2015: 117-24. https://jih.uobaghdad.edu.iq/index.php/j/article/view/16
18. Huang YJ, Chang R, Zhu QJ. Synthesis and characterization of a molecularly imprinted polymer of spermidine and the exploration of its molecular recognition properties. Polymers. 2018 Dec 14; 10(12): 1389. https://doi.org/10.3390/polym10121389
19. Yasar NE, Konukoglu D. Atypic separator gel flotation in blood collection tube in a patient with hyperproteinemia. Int J Med Biochem. 2020; 3(3): 186-188. https://doi.org/10.14744/ijmb.2020.53824
20. Schrapp A, Mory C, Duflot T, Pereira T, Imbert L, Lamoureux F. The right blood collection tube for therapeutic drug monitoring and toxicology screening procedures: Standard tubes, gel or mechanical separator?. Clinica Chimica Acta. 2019 Jan 1; 488: 196-201. https://doi.org/10.1016/j.cca.2018.10.043
21. Constable P, Trefz FM, Stämpfli H. Effects of pH and the plasma or serum concentrations of total calcium, chloride, magnesium, l-lactate, and albumin on the plasma ionized calcium concentration in calves. . J Vet Intern Med.. 2019 Jul; 33(4): 1822-32. https://doi.org/10.1111/jvim.15509
22. Cuthbertson L, Turner SE, Jackson A, Ranson C, Loosemore M, Kelleher P, Moffatt MF, Cookson WO, Hull JH, Shah A. Evidence of immunometabolic dysregulation and airway dysbiosis in athletes susceptible to respiratory illness. eBioMedicine. 2022 May 1;79:104024. https://doi.org/10.1016/j.ebiom.2022.104024
23. Akhtar J, Ahmad U, Khan MI, editors. Metformin: Pharmacology and Drug Interactions. BoD–Books on Demand; 2021 Dec 15. https://books.google.iq/books?hl=en&lr=&id=B69aEAAAQBAJ&oi=fnd&pg=PP12&ots=A0JCj_c2W2&sig=vxC7cNBqTKxmfcEJbC_WtvLEb4M&redir_esc=y#v=onepage&q&f=false

2024, 21(5): 1561-1576
https://dx.doi.org/10.21123/bsj.2023.8225
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

24. Meier PC, Zünd RE. Statistical methods in analytical chemistry. John Wiley & Sons. 2nd Ed. 2005;153: 48. https://books.google.iq/books?id=Pfnb8fZE6QgC&source=gbs_navlinks_s

25. Deckard ND. Book review: elementary statistics: a step by step approach. Teach Sociol. 9th Ed. 2016; 44(4). P. 296–298. https://doi.org/10.1177/0092055X16666513

تصنيع بوليمرات مطبوعة جزيئيًا متبوعا بالاستخلاص الانتقائي بالطور الصلب لتقدير الميتفورمين في المستحضرات الصيدلانية وفي مصل الانسان

رنا عدنان كمال الدين، يحيى كمال البياتي

قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة

يوضح هذا البحث تحضير وتخزين البوليمرات الجزيئية المطبوعة (MIP) في درجة حرارة الغرفة عن طريق البلمرة الصلدة لـ Metformin (Met) والتي تتميز بالحساسية العالية والتكلفة المنخفضة والاستقرار العالي. اذ تم أخذ نسب 0.8: 4: 20 ملي مول للقالب ، و للمونومر ولعوامل الربط المتصالب للبلمرة من أجل ضمان قدرة امتزاز مناسبة. المونومر الوظيفي الستايرين C_8H_8 تم ربطه مع إيثيلين جليكول ثنائي ميثاكريلات $C_{10}H_{14}O_4$ EGDMA رابط التشابك وبالتالي إنشاء MIP لـ Metformin كـ Met-MIP تم تمييزه باستخدام مقياس الطيف الضوئي UV-VIS عند 236 نانومتر في الادوية الصيدلانية ومصل الانسان ، والتحليل الطيفي بالأشعة تحت الحمراء والمسح المجهري الإلكتروني للطبعة الدوائية. أنشأت عملية الشطف التي تم تطبيقها على القالب اي انتزاع القالب ال Met من Met-MIP تجاويف ناتجة عن استخدام المذيب المكون للفجوات المكون من الميثانول والكلوروفورم وحمض الخليك (10:20:70) مللترعلى التوالي. كانت السعة القصوى للامتزاز Met-MIP هي 1.2320 ، 2.4448 ميكرو مول / غم عند استخدام وزن 0.1 و0.2 غم من Met-MIP على التوالي. استخدمت سرنجة الطور الصلب (SPE) المعبأة ببوليمرات مطبوعة جزيئيًا (MIPs) للفصل الانتقائي والتركيز للميتفورمين في العديد من الأدوية الصيدلانية من عدة مصادر. اعتمد المصل البشري على استخدام الماء منزوع الأيونات لتخفيف المصل ، يليه تسخين المصل مع الميثانول ، ثم اضافة بضع قطرات من حمض الهيدروكلوريك M1 للحصول على محلول رائق ليتم الكشف عن الميتفورمين في منطقة الأشعة فوق البنفسجية عند طول موجي 236 نانومتر من خلال تطبيق طريقة الإضافة القياسية.

الكلمات المفتاحية: عملية الامتزاز ، ميتفورمين ، بوليمرات الطبعة الجزيئية ، المصل ، استخلاص الطور الصلب.