DETERMINATION OF MORPHINE USING MOLECULARLY IMPRINTED POLYMERS BASED ON DIFFERENT MONOMERS

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ABSTRACT

This study was aimed to investigate for the particular adsorption of morphine, precipitation polymerization technology was used to create molecularly imprinted polymers. Using benzoyl peroxide as the initiator, ethylene glycol dimethacrylate as the crosslinker, and allyl chloride and styrene as the functional monomers. This MIP demonstrated a maximum morphine adsorption capacity of 38.24 mg/g at a bulk morphine concentration of (20,40,60,80,100) ppm in acetonitrile solution, one of the greatest talents recorded in the literature to date. Thermodynamic analysis, instrumental characterisation, adsorption studies, and suggested mechanisms all verified the molecularly imprinted polymers adsorption capacity, selectivity, reusability, and mechanical and chemical stabilities. FTIR, UV-vis, and scanning electron microscopy were used to monitor the analytes. The range of the relative standard deviations (RSD%) for three measurements made during two patient repeat tests at 20–100 ppm of morphine is (1.587–4.545) %. The range of (102-105) is the relative recoveries obtained for morphine in human urine samples that were spiked.

Keywords: solid phase extraction of toxic plants; toxicological analysis, botanical sources, drugs

تحديدالمورفين باستخدام بوليمرات الطبعة الجزيئية بالاعتماد على مونومرات مختلفة

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المستخلص

البحث يهدف الى تحديد المورفين عن طريق استخدام تقنية بلمرة الترسيب لإنشاء بوليمرات مطبوعة جزيئيًا .باستخدام بيروكسيد البنزويل كبادئ، وثنائي ميثاكريلات جلايكول الإيثيلين كرابط متشابك، وكلوريد الأليل والستايرين كمونومرات وظيفية. أظهر هذا الطبعات أقصى قدرة لامتصاص المورفين تبلغ 38.24 ملغم/غم ضمن تراكيز من المورفين يبلغ (20،40،60،80،100) جزء في المليون في محلول الأسيتونيتريل، وهي واحدة من أعظم المواهب المسجلة في الأدبيات حتى الآن. أثبت التحليل الديناميكي الحراري، والتوصيف الآلي، ودراسات الامتزاز، والآليات المقترحة قدرة امتزاز الطبعات المحضرة، والانتقائية، وقابلية إعادة الاستخدام، والثبات الميكانيكي والكيميائي. تم استخدام اطياف الاشعة تحت الحمراء، والأشعة فوق البنفسجية، والمجهر الإلكتروني الماسح لمراقبة التحاليل. مدى الانحراف القياسي النسبي لثلاثة قياسات تم إجراؤها خلال اختبارين متكررين للمريض عند 20–100 جزء في المليون من المورفين هو (1.587–4.545)٪. ويتراوح المدى (102–105) هو الاسترداد النسبي الذي تم الحصول عليه للمورفين في عينات الادرار البشرية التي تم فحصها.

الكلمات المفتاحية: استخلاص الطور الصلب للنباتات السامة؛ التحليل السمي، المصادر النباتية، ادوية

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INTRODUCTION

Morphine (MO) (5a, 6a-didehydro-4, 5-epoxy-17-methylmorphinan-3,6-diol), a strong opioid painkiller, is abused as an illegal substance and is derived from several botanical sources, including opium, poppy straw concentrate, and other poppy derivatives (1-3). On the other hand, indications of toxication and central nervous system disruption might be seen when excessive doses are consumed. MO at extremely high doses (120 mg) can be fatal. (4). As everyone is aware, MO is a medication that helps patients with extreme pain, but if taken excessively or repeatedly, it can have harmful side effects. Sensitive monitoring of MO in blood or urine is required to stop such MO misuse from happening. Thus far, gas chromatography/mass spectrometry (GC/MS) (5), high performance liquid chromatography (HPLC) , and immunoassays (6) have been the mainstays of MO detection methods (6). These analytical techniques are costly and time-consuming, though. Its drawbacks are the cost and stability of enzymes, particularly for enzymatic immunoassays . Therefore, the adoption of molecular imprinted polymers (MIP) is thought to be a more dependable and robust technique for specific binding to MO (10) , taking into account both stability and economic requirements. The sensitive polymer membrane we created as an SPE sensing transducer, which is then employed to create a potable instrument, can be utilized to determine MO on-site (7,8). The MIP-SPE sensor has the following benefits, according to the experimental results: it is highly sensitive and selective, more stable, doesn't require prior labeling, and is very easy to use. The aforementioned methods would also work for costly target compounds like poisons and just need modest amounts of polymer (11).

MATERIALS AND METHODS

The medicolegal institution (Baghdad, Iraq) supplied morphine. Sigma-Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com) provided the allyl chloride, styrene, ethylene glycol methacrylate (EGDMA), and benzoyl peroxide, while Merck (Darmstadt, Germany, www.merck.com) provided the methanol, chloroform, and acetic acid. nitrogen gas (99.98) produced at the Arab Gulf facility in Baghdad.

Instrumentation

The control methods included UV (Shimadzu uv spectrophotometer 1800 pc) and scanning electron microscopy (SEM) (JSM.6390A). Heating and Shimadzu (FTIR) - 8000 series (Japan) FTIR. After washing to make sure all of the MO was gone, MIP-MO uptake—which had been pre-washed—was measured using ultraviolet radiation once more. Pure MO uptake, which has a wavelength of 278 nm, was first measured using this method. Using Sonerx (W.GERMANY), the prepolymer solution was agitated.

MIP procedure

A process for extracting morphine from opium is involve extracted the opium with a basic alcoholic solution (10 mL ethanol: 50 µLof NH4OH). morphine base was precipitated. Following the dissolution of 1 mmol of template MO in 2 ml of porogen solvent (1:9)Ethanol: acetonitrile, 0.528 mmol and 0.536 mmol of (styrene) and (allyl chloride), respectively, were added. The mixture was then stirred for 10 minutes with the help of ultrasonography. 6.31mmol of cross linker (EGDMA) for both was added, along with 0.4 mg of initiator (benzoyl peroxide). The tube was sealed by the stopper after 15 minutes of N_2 gas being passed through the prepolymerization stage solution. The tubs were then kept in the 60°C water bath for 72 hours. In that time, the polymerization steps were finished, and MO-MIPs were created. The mixture was cleaned with an excess of ethanol/acetic acid (9:1, v/v) solvent after the template was extracted and all non-reacted chemicals were removed from the MIP and NIP combination in the soxhlet. The results were vacuum-dried for two hours (12). The synthesized MIPs were made and placed in a drying oven for one hour at thirty. Next, using a mortar and pestle, smash and grind the mixture until the particles are 125µm in size. The column was filled with either a normal solution or pee, which was then suctioned down at a rate of 75 revolutions per minute (11).

Sampling

Make a stock solution containing (20,40,60,80,100) ppm of MO at pH 8 and run it through a column at 100 rpm. After removing the column from the MIP, it was

thrice cleaned with 2 mL of distilled water to get rid of any matrix interference. A variety of weights (0.4, 0.6, and 0.8) of MIP that had previously been sieved and ground (0.75 microns) were put into each 3 ml plastic syringe. Samples of probable morphine-containing urine were gathered, and the judge requested that they be transferred to forensic medicine in Baghdad, Iraq. For ten minutes, to get rid of any precipitation, at 5000 rpm the centrifuge sample was spun. After utilizing a column to extract the squid and non-pointed samples, morphine was promptly impregnated into the urine supernatant (13).

Extraction procedure

A MIP morphine SPE column was used to remove the morphine from the urine. 0.4 g of MIP were initially added to a 3 ml plastic syringe in order to create this column. The urine sample was centrifuged, and the supernatant was then poured at a flow rate of 75 rpm into the space above the SPE column packing. Eluent was collected in a tiny beaker and put to the column together with one milliliter of distilled water, one milliliter of acetonitrile, and four percent acetic acid. Following ten minutes of drying, after another drying cycle in a water bath heated to fifty degrees Celsius, the residue was removed. After the mixture cooled to room temperature and the solvent completely evaporated under a nitrogen stream, one milliliter of ethanol was added to the residue (14,15).

RESULTS AND DISCUSSION

Synthesis of MIPs for morphine

By using a non-covalent bulk polymerization technique called self-assembly, two MIPs of morphine were implanted. Functional monomers have proven to be invaluable in the research of interactions with templates. To synthesize MIPs and NIPs, two monomers were utilized: styrene and allyl chloride.

FTIR analysis

One crucial step in the chemical characterization process is the use of FTIR to identify the functional groups contained in a molecule. Table (1) displays the FTIR spectra of different MIPs and NIPs.

Table 1. Prominent peaks in the FT-IR spectra of the NIP employing styrene as a functional monomer and the morphine-imprinted polymer

	Functional Group	Morphine	Morphine-MIP Styrene before template removal	Morphine-MIP Styrene after template removal
1	OH (H_2O)	3411	3442	3470
2	CH-aliphatic.(cm^{-1})	2842, 2962	2873, 2952	2867, 2948
3	C=O ester.(cm^{-1})	1741	_____	_____
4	Ar-H.(cm^{-1})	3026	3073	3087
5	C=C aliphatic.(cm^{-1})	1591	1583	1556
6	C-O .(cm^{-1})	1270	1232	1260
7	$C=CH_2$.(cm^{-1})	_____	1632	1642
8	C=C alken .(cm^{-1})	_____	1542	1531
9	Out-of plane-mono-sub	750, 733	752,762	_____

According to Table 1, the morphine's FTIR spectra revealed the following bands: 3411, 2962 and 2842, 1741, 3026, 1591, 1270, 733, and 750. Stretching OH (H2O), C-H aliphatic, C=O ester, Ar-H, C=C vinyl, C-O, and C=CH2 are all characterized by a cm-1 value. For mono substituted rings, C=C alken stretching and out-of-plane bending are used. The following bands are visible in the morphine-MIP(Styrene) FTIR spectrum prior to template removal (16). The lengths of the OH (H2O) and C-H aliphatic and Ar-H stretching are 3446 cm-1, 2952 and 2873 cm-1, 3064 cm-1, and 1583 cm-1, respectively. The lengths of the out-of-plane bending for the mono substituted ring are 1228 cm-1, 1620 cm-1, 1535 cm-1, and 723, 678 cm-1 for the C=C and C-O aliphatic and C=O stretching. The absence of C=O ester stretching and out-of-plan bending for the mono substituted ring that excises in the template MO spectrum is seen in the FTIR spectrum of the MIP

(Styrene) after the template is removed, showing the drug's extraction from the template.Table (2) shows the FTIR spectra of the MIPs before and after NIP and template removal when Allyl Chloride is used as the monomer to synthesize more MIPs for MO consumption.

Table 2. The most prominent peaks in the FT-IR spectra of the NIP employing Allyl Chloride as a functional monomer and the morphine-imprinted polymer.

	Functional Group	Morphine	Morphine-MIP Allyl Chloride before template removal	Morphine-MIP Allyl Chloride after template removal
1	OH (H_2O)	3411	3443	3461
2	CH-aliphatic.(cm^{-1})	2842, 2962	2966,2842	2981,2961
3	C=O ester.(cm^{-1})	1741	1721	_____
4	Ar-H.(cm^{-1})	3026	3059	_____
5	C=C aliphatic.(cm^{-1})	1591	1545	_____
6	C-O .(cm^{-1})	1270	1261	1263
7	$C=CH_2$.(cm^{-1})	_____	1641	1744
8	C-Cl .(cm^{-1})	_____	1635	1631
9	Out-of plane-mono-sub	750, 733	751,708	_____

The following bands were visible in the morphine's FTIR spectra, which was taken from Tables (3,6): cm-1 for stretching OH (H2O), C-H aliphatic, C=O ester, Ar-H, C=C aliphatic, C=Oester, Ar-H stretching aliphatic stretching in C=C, 3411, 2962 and 2842, 1741, 3026, 1591, 1270, 750, and 733. C is equal to CH2. Extending C-O Bending out of plane and stretching C-Cl to create a mono substituted ring. The following bands are visible in the MO-MIP (Allyl Chloride) FTIR spectrum prior to template removal (17). There are various types of stretching that can be performed on a mono substituted ring: 3460 cm-1 for OH (H2O) stretching, 2966 and 2842 cm-1 for C-H aliphatic stretching, 1721 cm-1 for C=O ester stretching, 3059 cm-1 for Ar-H stretching, 1545 cm-1 for C=C, 1261 cm-1 C-O stretching, 1641 cm-1 for C=CH2 stretching, 1635 cm-1 for C-Cl stretching, and 751, 708 cm-1 out of plan bending. Once the template is removed because there is no longer any C=O ester stretching, AR-H stretching=C aliphatic, or out of plan bending for the mono substituted ring that excises in the template MO spectrum, the FTIR spectrum of the MIP (Allyl Chloride) indicates that the medication has been extracted from the template. Many experiments with different ratios (D: M: C) were carried out in order to determine the optimal ratio for the synthesis of MIPs MO (18). A list of polymers with the necessary features has been produced by these trials using the molar ratios (D: M: C) of (4.5:6.244:89.252) and (4.504: 6.34: 89.16) for morphine -MIPs. This list is displayed in Table (3).

Table 3. The [D:M:C] variation ratios and progeny utilized in the manufacturing of MIPs and NIPs for morphine

		Drug Morphine	Monomer Styrene	Cross linker EGDMA	Initiator	Solvent	Result
MIP3	%	6.55	4.36	88.908	0.3	6ml	Pale
	mmole	0.45	0.30	0.12	0.32	CH_3OH	yellow
MIP3	%	6.82	8.93	84.3	0.3	6ml	Pale
	m mole	0.40	0.52	4.94	0.32	CH_3OH	yellow
MIP3	%	4.5	6.244	89.252	0.3	6ml	Pale
	mmole	0.30	0.416	5.946	0.32	CH_3OH	yellow
NIP3	%	------	6.244	89.252	0.3	6ml	Pale
	mmole		0.416	5.946	0.32	CH_3OH	yellow
		Drug Morphine	Monomer AIIyI Chlorid	Cross linker EGDMA	Initiator	Solvent	Result
MIP4	%	6.21	11.69	82.089	0.3	6ml	Pale
	mmole	0.25	0.47	3.30	0.32	CH_3OH	yellow
MIP4	%	5.99	6.33	87.76	0.3	6ml	Pale
	Mmole	0.35	0.37	5.12	0.32	CH_3OH	yellow
MIP4	%	4.504	6.34	89.18	0.3	6ml	Pale
	mmole	0.30	0.42	5.94	0.32	CH_3OH	yellow
NIP4	%	-------	6.34	89.18	0.3	6ml	Pale
	Mmole		0.42	5.94	0.32	CH_3OH	yellow

All ratio prepared in water bath at 60 ^{0}C

However, after the template is removed, the control NIPs and MIPs show similar spectra, suggesting that their backbone structures are similar. This suggests that using a Soxhlet extraction system to wash the template molecule can be effectively removed from the polymer framework by using MIP particles in a 70% methanol solution, which leaves behind certain recognition binding sites.

Adsorption isotherm

Isotherm adsorption can help in comprehending the adsorption mechanism of the adsorption template with a polymer surface. The results from the equilibrium of isotherm adsorption were assessed in order to identify the kind of isotherm Langmuir or Freundlich models. This was determined by plotting the drug's bind-ability (Q) against its free concentration; Q is calculated using the following equation:

$Q = [(Ci – Cf) Vs *1000] / MMIP$……1

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

Vs = volume (mL), MMIP = mass polymer (mg)

MIP/drug binding calculated by Scat chard analysis using the equation

$Q/ Cf = (Qmax - Q) / Kd$……2

Q max = maximum capacity, Kd = dissociation constant at binding side (19).

Table 4. Rebinding values of (morphine) using morphine -MIP particles based on (Styrene).

Morphine _MIP(Styrene)						
Mass of MIP g	Constriction ppm	Ci mM	C_{free} mM	Q µMole /g	Q/C_{free} L/g	T: P (templet: polymer)
0.2	20	0.2010	0.1974	0.180	0.9118	1:9
	40	0.4020	0.3952	0.340	0.8603	1:3
	60	0.6030	0.5830	1.000	1.7152	1:3
	80	0.8040	0.7810	1.150	1.4720	1:3
0.4	20	0.2010	0.1872	0.345	1.8429	1:9
	40	0.4020	0.3843	0.442	1.1514	1:3
	60	0.6030	0.5255	1.937	3.6869	1:3
	80	0.8040	0.7260	1.950	2.6859	1:3

Table 5. Rebinding values of (morphine) using morphine -MIP particles based on (Allyl Chloride).

Morphine _MIP(Allyl Chloride)						
Mass of MIP g	Constriction ppm	Ci mM	C_{free} mM	Q µMole /g	Q/C_{free} L/g	T: P (templet:polymer)
0.2	20	0.2010	0.2005	0.025	0.1246	0.5:10
	40	0.4020	0.3978	0.210	0.5279	1:15
	60	0.6030	0.5962	0.340	0.5702	1.15
	80	0.8040	0.7969	0.355	0.4454	1:15
0.4	20	0.2010	0.1707	0.757	4.4376	0.5:10
	40	0.4020	0.3643	0.942	2.5871	1:15
	60	0.6030	0.5377	1.632	3.0360	1:15
	80	0.8040	0.7381	1.647	2.2320	1:15

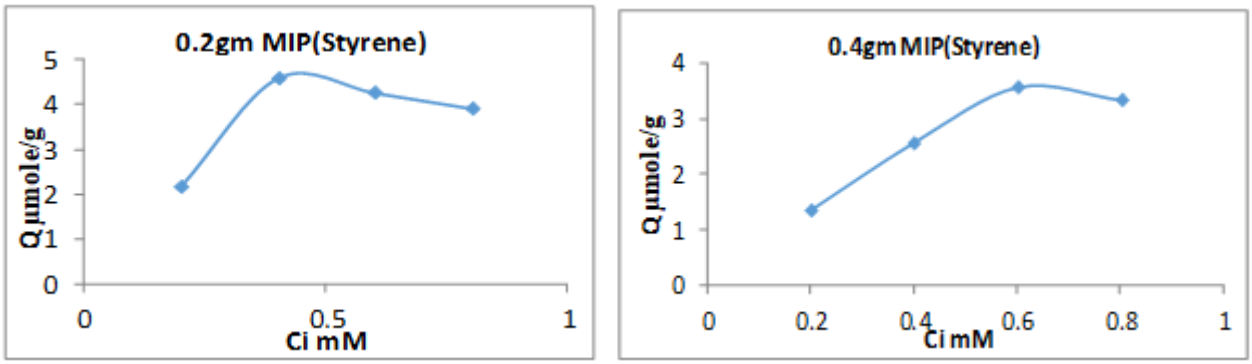


Figure 1. Binding isotherm of Styrene monomer by plotting Q against Ci

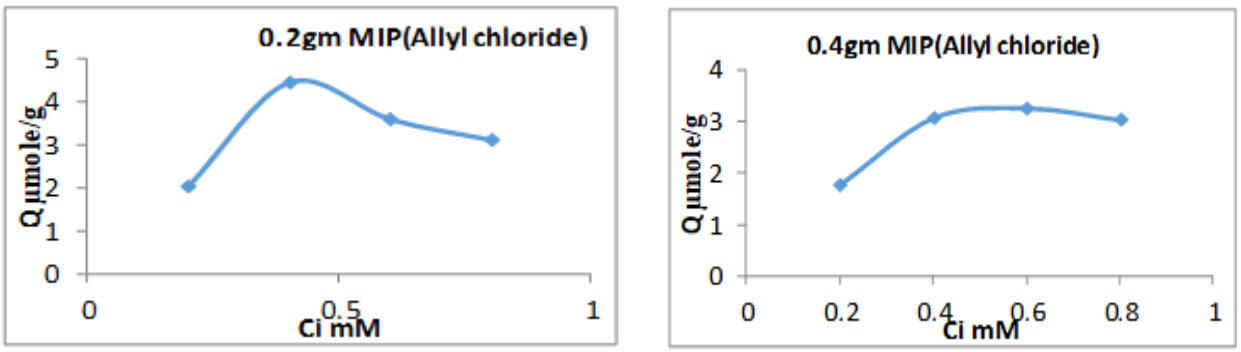
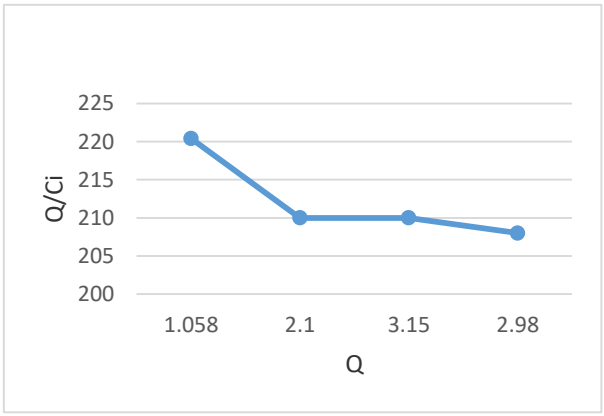
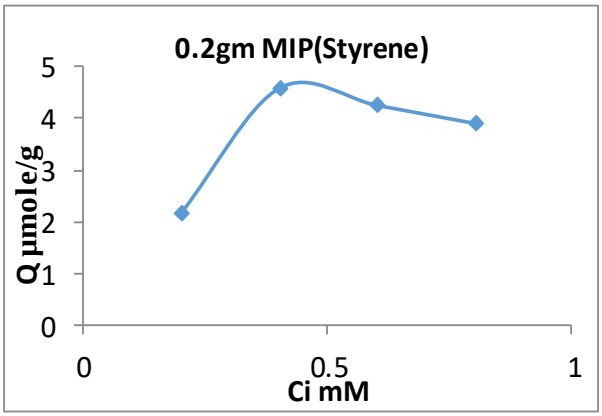


Figure 2. Binding isotherm of Allyl chloride monomer by plotting Q against Ci



A



B

Figure 3. Styrenr MIP =0.2g

A) relationship between Q/Ci vs Q **B) relationship between Ci vs Q**

The isotherm adsorption of MO- MIP based on styrene, allyl chloride, and monomers displays a two-site non-covalent bond link between morphine and the polymer. With styrene and allyl chloride, the scat chard plot for morphine MIP was nonlinear, showing that the binding sites are different in relation to the MO. The plot is divided into two separate portions, each of which is represented by a straight line with an associated equation. This indicates that there are two types of morphine binding sites with the polymer; we propose that MO binds to styrene and allyl chloride via the polymer's two oxygen atoms (20). The binding sites can be categorized based on their unique binding characteristics, apparent maximum amount of capacity (Qmax), and equilibrium dissociation constant (Kd).

The equation of the first part is: Y = -10.42 x + 230.84

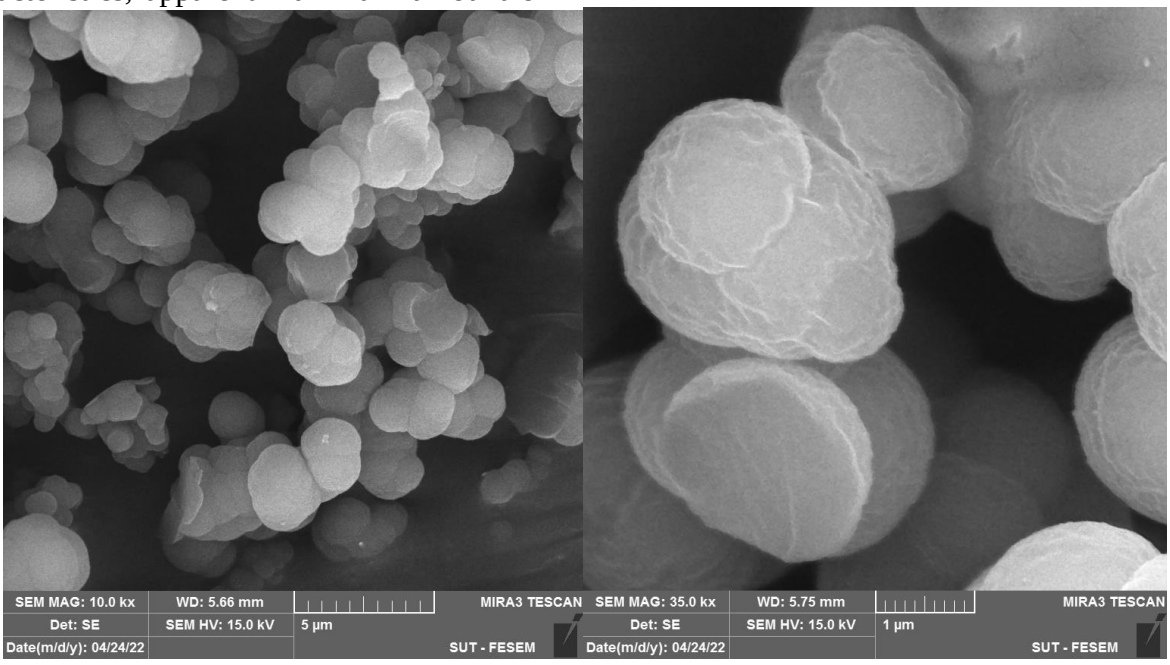
The equation of second part is: Y = -0.95 x + 211.27

From the equation 1 Kd = 0.045µmole/ L and Qmax = 9.95µmole/g

For equation 2 Kd = 0.11 µmole/ L and Qmax = 4.26 µmole/g

Characterization of morphological

Understanding the size and layout of the sites that extracted the morphine from the polymer is crucial, and this can be done by morphological analysis.



A **B**

Figure 4. SEM photograph of the surface of morphine -MIP (A-styrene and B-allyl chloride)

The SEM images in Figure 5 demonstrate the successful hybridization of MO-MIP powders into polymer membranes; the printed membranes have a smooth surface.

Additionally, morphological analysis showed that, in comparison to morphine-MIP (allyl chloride), MO-MIP (styrene) has a higher spherical structure. The corresponding image in Figure 5 illustrates the small and spherically shaped polymeric particles found in micro analysis. The diameters of these particles are around (50-150 nm) for styrene polymer and (120-200 nm) for allyl chloride polymer.

Urine samples analysis: To detect MO in urine samples, MIP-styrene was uniformly sprayed and MIP-Allyl chloride was applied using a Freundlich isotherm under optimal circumstances. The urine sample matrix was a part of both the initial procedure and the post-extraction wash stage. The cleaning procedure will be finished by using a peristatic pump to let the carrier and solution pass through the plastic syringe. During the washing stage, the components that suck weakly into a homogenous column should be removed. It was shown that in order to suppress the matrix peaks, the washing duration has to be increased from 70 seconds to 3 minutes. To illustrate this, take a sample of empty pee and wash it for three minutes. Using the same washing technique, the urine sample reached sufficient MO levels; no drop in In a perfect world, 0.2–0.4 g of MIP (styrene) and MIP (allyl chloride) were successfully injected into urine samples to produce a range of 20–100 ppm of morphine. The outcomes are displayed in Table 7.

Table 7. Using solid phase extraction and the imprinted polymer approach, the standard addition method for drug determination

Wt. of MIP(g)	Type of MIP	NO.of patient	Conc. Taken (ppm)	Conc. Found (ppm)	% Recovery	RSD%	RE%
0.2	MIP1 Styrene	1	60	62	103	2.437	3.33
		2	20	21	105	4.545	5
0.2	MIP2 Allyl chloride	1	80	82	102	1.86	2.5
		2	60	62	103	1.587	3.33

The urine samples utilized in this study were used to extract MO using MIP- MO solid phase extraction (SPE) Colum. Furthermore, it is known that each imprinted polymer has the capacity to bind MO and other similar drugs (9). The first step involved producing the molecularly imprinted polymers of morphine, which enable the measurement and concentration of minute amounts of the medication at different times in order to facilitate drug metabolism. Using solid phase extraction to obtain a concentration was the second stage, which improved selectivity, sensitivity, and precision. sample volume and flow rate. The time reduced as the flow rate grew after we set a 75 rpm flow rate, and it was 5 minutes. For MO, quantities smaller than 10 mL should be chosen since they showed good repeatability and were thought to be appropriate for tracing levels. To determine the kind of isotherm Langmuir or Freundlich models .Extremely low detection limits of 0.8–1.2 ng mL−1 were attained. ultimately, urine samples containing MO were successfully extracted using MIP fibers, with relative recoveries between 102-105.

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