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Design Molecular Imprinting Polymer Coupled with Solid-phase Extraction for Determination of Pregabalin in Pharmaceutical Formulations

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ABSTRACT

This research demonstrates an efficient method for the synthesis and storage of molecularly imprinted polymers (MIP) using bulk polymerization of Pregabalin (PGB), which is carried out at room temperature. This method has the advantages of high sensitivity, low cost, increased stability, and longer lifetime of the polymers. The research used specific ratios between template, monomer, and cross-linking agents to ensure suitable adsorption capacity. Benzoyl peroxide (BPO) was used as an initiator for the functional monomer styrene and ethylene glycol dimethacrylate (EGDMA) cross-linking to create the MIP of Pregabalin (PGB-MIP). The molecularly imprinted polymer was studied using ultraviolet-visible spectroscopy (UV-VIS) at 205 nm, a technique used for the detection of pharmaceutical drugs. Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were also used to study the structure of the polymer. The elution process applied to the template (PGB) of PGB-MIP showed cavities caused by porous mixture solvents such as methanol, chloroform, and acetic acid. The maximum adsorption capacity of the molecularly imprinted polymer was measured to be 117941.6 (µg/g) when 0.1 g of PGB-MIP was used, which is consistent with the Langmuir isotherm model. The optimal ratio of template to monomer was shown to be 1:1. For practical application, a solid phase extraction (SPE) syringe packed with molecularly imprinted polymers was used to selectively separate and concentrate Pregabalin in multiple source pharmaceutical drugs.

Keywords: Molecular imprinted polymers (MIP), Pregabalin, Isotherm process, Solid-phase extraction.

1. INTRODUCTION

For many reasons, including examining absorption, distribution, metabolism, and excretion, as well as figuring out half-lives, overdose, and forensic situations, it is crucial to ascertain how much of the drug dosage is still in the body. Furthermore, the scientific community faces this problem as one of its main obstacles **(Perrier et al., 1973; Shargel et al., 1999)**.

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Numerous drug overdoses, particularly those involving psychotropic chemicals, can have detrimental, irreversible effects **(Rinaldi et al., 2020)**. Lyrica and $C_8H_{17}NO_2$ are commercial names for pregabalin (PGB).

The chemical formula for this white to off-white crystalline chiral solid substance is (S)-3-(aminomethyl)-5-methylhexanoic acid, which is a 3-isobutyl derivative of gamma-aminobutyric acid (GABA) and a calcium channel blocker **Fig. 1 (Tůma et al., 2021)** . Similar in structure and pharmacology to gabapentin, it is utilized as an anticonvulsant, an analgesic in the treatment of painful neuropathy and fibromyalgia, epilepsy, a generalized anxiety condition in adults, and in critical care to relieve cough and pain associated with COVID-19 **(Desai et al., 2019; Pektaş et al., 2021; Chilkoti et al., 2022)**. Reproductive toxicity, vertigo, balance issues, seizures, agitation, somnolence, ataxia, hepatotoxicity, cholestatic, and diplopia are among the risks associated with an overdose of this medication **(Evoy et al., 2019)**. Numerous recent instances of fatalities indicate the likelihood of abuse, particularly in those with a history of opiate abuse. This medication has a 6-hour half-life, and its peak concentration in the Blood occurs one hour following ingestion **(Bonnet et al., 2021; Parisi et al., 2020)** . Since there is no counteragent for pregabalin overdose, this medication is eliminated from the body without any metabolism **(Yoon et al., 2023)**, making its measurement very crucial **(Rhee et al., 2018)**. Previously, a number of techniques for measuring PGB have been documented, such as spectroscopic approaches, ultraviolet (UV) and fluorescence spectroscopy **(Akhil et al., 2021)**, normal and reversed-phase high-performance square wave voltametric (SWV) electrochemical determination **(Akhil et al., 2021; Toudeshki et al., 2019)**, liquid chromatography (HPLC & RP-HPLC) **(Lin et al., 2019; Vyas et al., 2019)**, and bioanalytical techniques **(Bhaskar et al., 2020)**. The detection limit of the proposed method is compared with other previous research methodologies in **Table 1.**

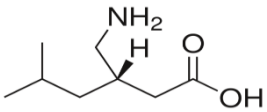


Figure 1. The chemical structure of pregabalin

Table 1. A comparison between the detection limit of the proposed method and that of various earlier research techniques.

Method	**Linear dynamic concentration range / pM**	**LOD/pM**	**Ref.**
Ion-selective electrode	1×10^3– 1×10^6	223000	**(Lotfy et al., 2012)**
HPLC	62×10^3– 62×10^7	62×10^2	**(Nirogi et al., 2009)**
Spectrophotometric	31.4×10^5– 47.6×10^5	119×10^4	**(Önal et al., 2009)**
Spectrofluorimetric	251×10^2 – 251×10^3	307	**(Önal et al., 2009)**
Impedimetric Detection	1×10^4 - 28×10^4	3000	**(Gholivand et al., 2014)**
GC-MS	226×10^4 – 628×10^5	226×10^4	**(Tafesse et al., 2018)**
RP-HPLC	628×10^5– 376×10^6	19×10^5	**(Patel et al., 2020)**
Capillary Electrophoresis	502×10^4 – 998×10^5	144×10^4	**(Hussein and Al-Bayati, 2021)**
Spectrophotometric	431×10^3– 219×10^5	154×10^3	**(Gujral et al., 2009)**
MIP	500-2500	225	**This Work**

The results and comparisons in the table suggest that the sensor's linear dynamic concentration range and detection limit differ significantly from those of alternative methods, with a significantly linear dynamic concentration range and a lower detection limit. In this work, benzoyl peroxide BPO served as the target molecule (pregabalin) initiator, and the MIP production was carried out in tandem with the recognition cite Butyl acrylate with crosslinking ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$.
The purpose of this study was to assess the suggested molecularly imprinted polymer (MIP) sensor's sensitivity and selectivity for pregabalin analysis in various matrices. Additionally, the effects of various solvent systems, cross-linking agents, and functional monomers on the adsorption and imprinting behavior of diazepam inside the polymer matrix were investigated. The MIP's surface morphology was examined using scanning electron microscopy, and the functional group interactions involved in the imprinting process were described using Fourier transform infrared spectroscopy.

2. EXPERIMENTAL WORK

2.1 Materials and Chemicals

The Iraqi State Corporation for Pharmaceutical and Medical Equipment Industries (Middle East Laboratories /Iraq) provided the reference standard for pregabalin. Additionally, local pharmacies sold commercial pregabalin-containing formulations, including 10 tablets of LYRICA (75 mg pregabalin each tablet) made by Germany-VIATRIS and 10 tablets of LYRICA/ (75 mg pregabalin per tablet) made by PFIZER-USA. Sigma-Aldrich provided the plasticizers; Sigma-Aldrich supplied 78% of the benzoyl peroxide (BPO) and ethylene glycol dimethacrylate (EGDMA), whereas styrene was used as a monomer. The chemicals that were employed were unpurified and had the purest reagent concentrations.

2.2 Preparation of MIP

A 0.0796 g of pregabalin was mixed with 0.2858 mL of functional monomer styrene in order to create the pregabalin molecularly imprinted polymer (PGB-MIP). 4.82 mL of cross-linker ethylene glycol dimethacrylate (EGDMA) was added to this solution after 0.075 g of initiator benzoyl peroxide. For five minutes, the mixture was shaken to create a uniform polymeric precursor solution. In order to eliminate any dissolved oxygen and facilitate polymerization at the start, nitrogen gas was bubbled through the solution for 20 minutes. An oxygen-free atmosphere that was appropriate for radical polymerization brought on by the initiator was guaranteed by the nitrogen purging. A pregabalin-imprinted polymeric matrix with recognition sites complementary to the target analyte was produced as a result of this procedure. Following that, the tube was placed inside a 65°C water bottle. Following the completion of the polymerization process, the molecularly imprinted polymer completely solidified and disintegrated into a minute polymer particle. Overnight, the polymer was allowed to dry at room temperature. Using porogenic solvent v/v (acetic acid, ethanol at a ratio of 1:9). Soxhlet solid liquid phase extraction was used to extract the template from MIP. This was accomplished successfully by repeatedly washing for a period of 24 to 72 hours. The polymer was allowed to dry and crushed in a mortar and sieved to 125 µm in size. Each plastic syringe (column) size(200MG/6ML/50PKG) was filled with 0.1 g of PGB-MIP and flow-capped at 70 mL/min of standard solution using a 3 mL solid phase extraction vacuum **(Al-Bayati, 2018)**.

In order to make a number of standard pregabalin solutions (2500, 2000, 1500, 1000, and 500 (µg/mL)), a stock solution was made by dissolving 0.5g of PGB in a 50 mL volumetric flask containing ethanol. A UV-VIS device measuring 205 nm was used to produce a calibration curve between an x-axis representing pregabalin concentration and a y-axis representing its absorption. The average weight of the powdered pregabalin Tablets, as shown in **Table 2**, was dissolved in 100 mm of methanol solution and filtered through 0.07µm cellulose filter paper to form pharmaceutical samples. This allowed for the calibration curve of 150 mg of pregabalin drugs (LYRICA /Germany-VIATRIS and LYRICA / PFIZER – USA) to have the lowest standard addition (SD) value.

Table 2. PGN-MIP polymer-prepared pharmaceutical medications for use in therapy.

Samples	Commercial name, Country Content 150mg	Average weight for 10 tablets (g)	The sample weight that corresponds to 0.03458 g (150mg) of the active component
1	LYRICA /Germany-VIATRIS	0.1361	0.1355
2	LYRICA / PFIZER - USA	0.1544	0.1546

3. RESULTS AND DISSUSSION

The residue with the least amount of absorbance was determined by UV-VIS spectroscopy after the pregabalin solution was forced through a syringe filled with PGB-MIP. This demonstrated that the benefits of using impressed polymers in SPE for the measurement of pregabalin had been effectively expressed through a decreased concentration during the final procedure, as shown in **Figs. 2 and 3**.

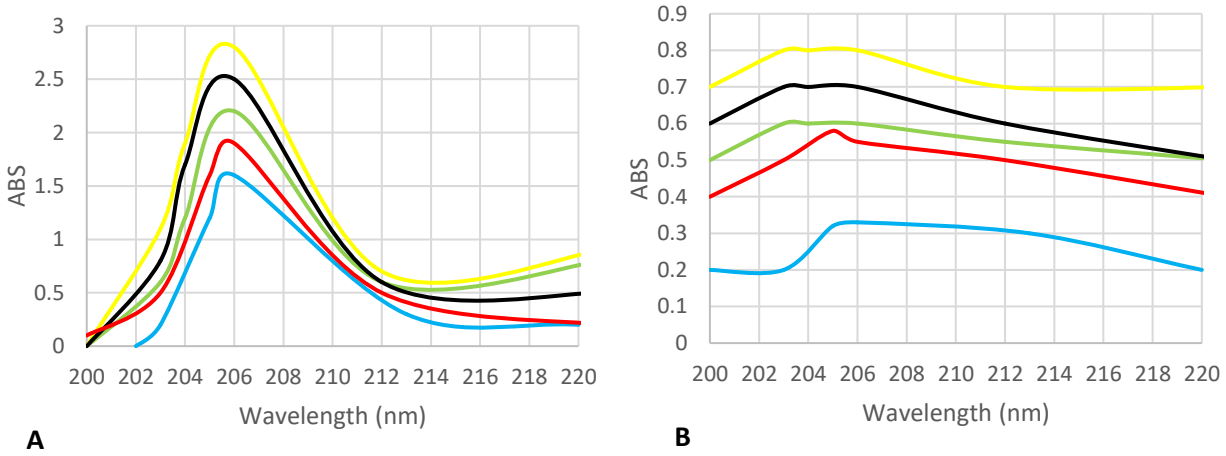


Figure 2. (**A**) The absorption of the pregabalin (Middle East Laboratories /Iraq) at a concentration of 25-150mg at 205 nm before passes through the MIP and (**B**) After pregabalin passes through the MIP column (■=150 µg/mL, ■=100 µg/mL, ■=75 µg/mL, ■=50 µg/mL, ■=25 µg/mL)

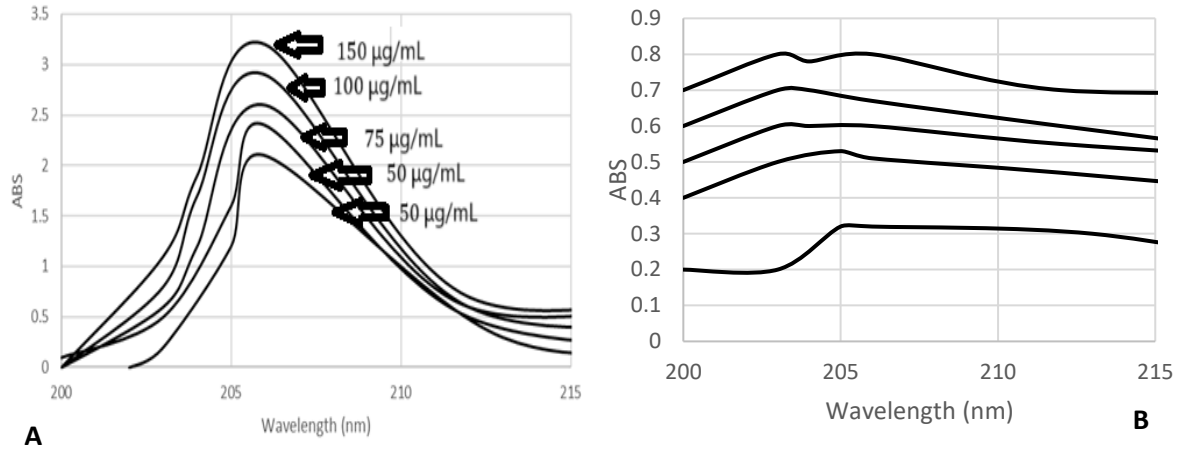


Figure 3. (A) The absorbance at 205 nm of the drug pregabalin concentration (LYRICA/VIATRIS) at (25-150 µg/mL) before the pregabalin passed through MIP column, and (**B**) After the pregabalin passed through MIP column.

Through **Figs. 2 and 3**, after passing 150 µg/mL of the commercial pregabalin solutions through the MIP column, it was seen that the highest wavelength of pregabalin was at 205 nm. The wavelength of the (LYRICA /VIATRIS) disappearance indicates that the pregabalin has been trapped in the column.

The active groups of pregabalin are clearly visible in **Figs. 3 and 4**, as indicated in **Table 2**, which will be utilized to identify and produce the molecularly imprinted polymer for pregabalin. The 400–4000 cm^{-1} range was used to record the leached and unleached pregabalin (PGB) imprinted polymer MIP Fourier transmission infrared spectrometry spectra. **Table 3** shows the infrared spectrum of pregabalin (PGB) drug beams of high to medium intensity frequencies. The distinctive peak at 3500 cm^{-1} is attributable to the stretching vibration of the aromatic OH str.(cm^{-1}). the stretching vibrations of the aliphatic (C-H) bond (2970,2965) cm^{-1}, the vibration version the carbonyl of the amide group - NH_2 is allocated at 3088 cm^{-1}, the absorption band of -O-C=O at 1733 cm^{-1} is attributed to the stretching vibrations of the aromatic ring. **Fig. 4 (Song et al., 2019)**.

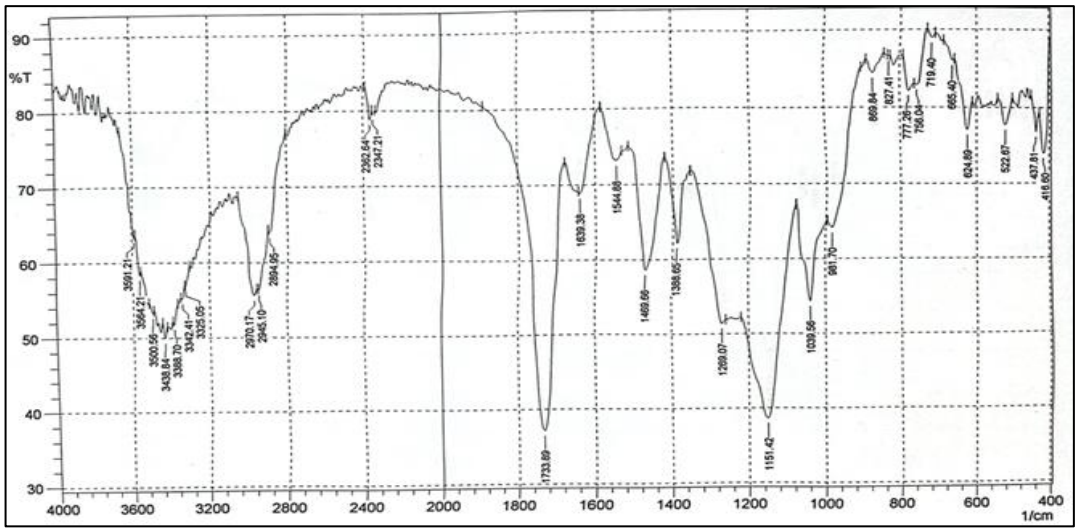


Figure 4. FTIR spectra of pregabalin standard.

Table 3. FT-IR spectra (cm^{-1}) peaks for pregabalin drug-MIP imprinting polymer

F.G.	Drug (pregabalin)	Before	After
-CH aromatic	-------	3084,307	3066
-OH str.(cm-1)	3500	3442	-------
NH2- str.(cm-1)	3088	3047	------
H-C=C str.(cm-1)	----	1600	1625
N-C=O str.amid(cm^{-1})	-----	1718	1660
-CH-aliphatic (cm^{-1})	2970,2965	2923,3010	2927,2850
O-C=O str.ester (cm^{-1})	1733	1631	1733

4. EXPERIMENTAL WORK

For the purpose of determining effective aggregates, FTIR characterization analysis using KBr was performed. it can be seen that the characteristic absorption peaks at (3442) cm^{-1} are attributed to the vibrational frequency of the band (O-H), the band at (1718) cm^{-1} attributed to the stretching vibrations of the band (O=C-N), the band at (3074) cm^{-1} attributed to the stretching vibrations of the band (N-H) before the washing stage, after drug removal , the band of (O-H) and (N-H) were disappearance that FT-IR spectra before and after template removal proves that template has been fully extracted from MIP in the extraction stage by Soxhlet. **Figs. 5 and 6.**

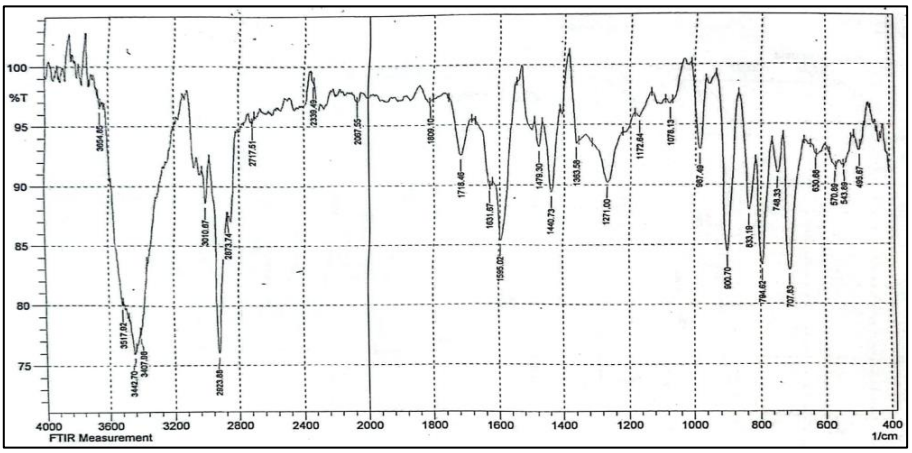


Figure 5. Pregabalin drug-MIP spectrum before drug removal.

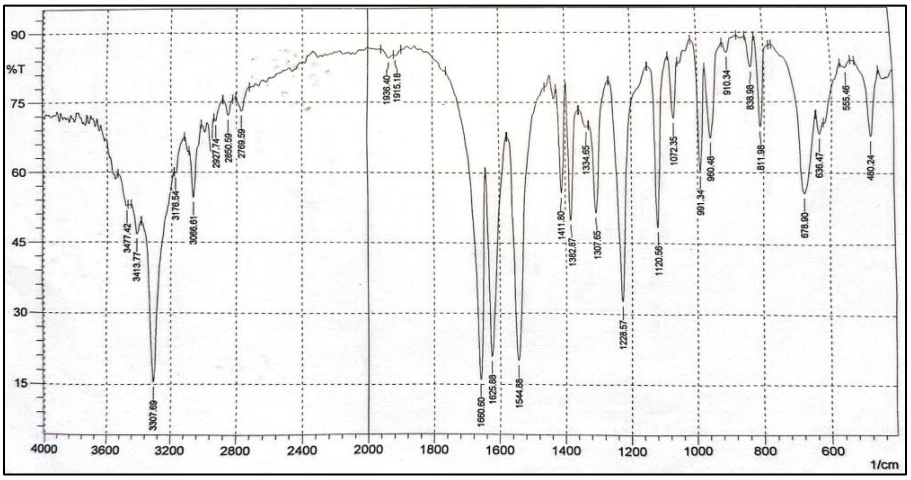
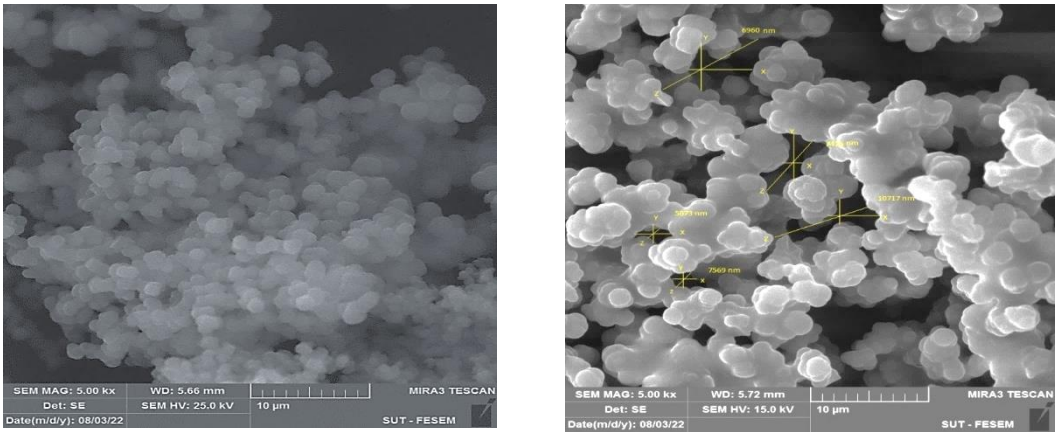


Figure 6. Pregabalin drug-MIP spectrum after drug removal.

Electron scanning microscopy diazepam is a SEM that shows very small particles and irregularly shaped polymeric particles with small sizes around 5673- 10771 nm. These irregular holes played a role in trapping the PGB and separating it from other mixtures, and this helped in the rapid estimation of the PGB. The understanding of certain morphological properties depends on the morphological evaluation **(Rydz et al., 2019)**. SEM images were used to examine the morphology of the PGB-MIPs, as shown in **Fig. 7A and B**, and **Table 4**.



A B

Figure 7. A. show the particle surface morphologies for PGB-MIP before elution and B. show the particle surface morphologies for PGB-MIP after elution, respectively, as well as the cavities' three dimensions and areas.

Table 4. Calculated mean, angle, lengths of some cavities (selected six of them) and their areas using the ImageJ program.

No.	Parameters	Area	Mean(nm)	Min(nm)	Max(nm)	Angle(nm)	Length(nm)
1		693.982	5444.295	3058.557	11354	-179.349	348.52
2		2583588	26388.92	2787	65535	0	0
3		192.119	15809.25	14467.47	17941.47	178.21	95.091
4		90.178	17179.67	16420.74	18487.5	-176.583	44.301
5		176.436	19270.16	17353.27	22026	-172.235	87.931
6		113.703	18535.67	15622	29285	-177.955	55.478
7	Total Mean	430809	17104.66	11618.17	27438.16	-87.985	105.22
8	SD	1054642	6790.912	6802.379	19560.54	148.318	123.978
9	Total Min-Max	90.178-2583588	5444.295-26388.92	2787-17353.27	11354-65535	-179.349-178.21	0-348.52

*** Total mean of Cavities between min = 5444.295nm (5.44429) µm to max = 26388.92nm (26.38892) µm.**

The holes' diameter ranges from 5444.295-26388.92 nm, as shown in **Table 4,** 3D of Cavities from min = 5444.295 nm (5.44429µm) to max = 26388.92 nm (26.38892 µm). Some of the holes are extremely large, which results in the retention of significant amounts of the medication and is in line with the high isothermal capacity value. **(Omar et al., 2017; Al-Safi et al., 2018)**. The amount of adsorbed (mg/g) was calculated using the formulae reported by Vanderborght and Van Griekenm. Adsorption capacity and pre-concentration:
The following equation was used to analyze a series of absorption successes for various initial concentrations of PGB-MIP, ranging from 0.7 to 0.2 mol/mL Eq. 1 was used to calculate the **(Emad and Al-Bayati, 2025)** concentration of MIPs during the determination of the adsorption time

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

MMIP = mass polymer (mg), Ci, Cf = initial and final drug concentration (µmol/mL), V_s = volume (mL), and MIP/drug binding determined by Scatchard analysis using Eq. 1 in **Fig. 8.** which were described by binding parameters that were established using Eq. 2 and the Scatchard equation **(Omar et al., 2017; Ndunda, 2020):**

$$Q / Cf = (Qmax - Q) / Kd \quad (2)$$

Q max = maximum capacity, Kd = dissociation constant at the binding side **(Quinto et al., 2020; Rahman et al., 2021).** The concentrations from 2500 to 500 µg/mL consume an 8 mL volume when using 0.1000g of weight of PGB-MIP, **Table 5**. The results obtained are indicated in **Tables 5 and 6**. Note that the concentration Ci equals 2500(µg/mL) is the best to obtain the highest capacity. This means that at this concentration, the largest amount of the pregabalin is retained in the prepared (PGB-MIP).

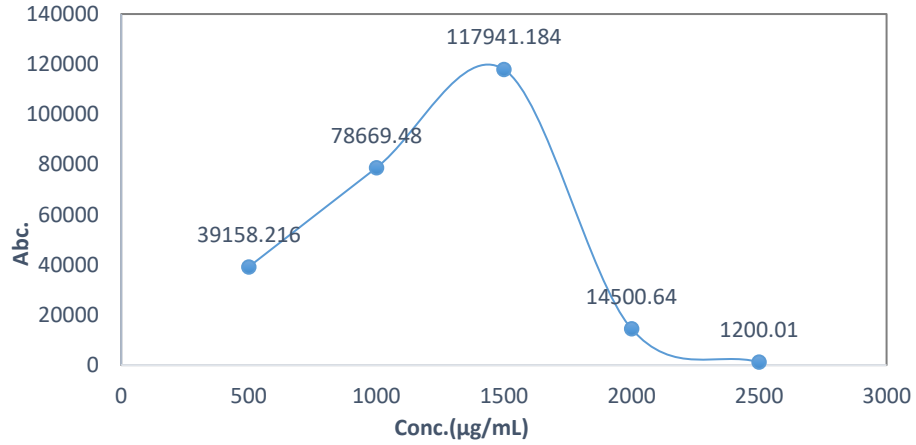


Figure 8. Pregabalin standard concentrations (µg/mL) and their absorptions are calibrated using a calibration curve.

According to the information obtained between capacity Q (µg/g) and initial concentration Ci (µg/mL). shows what type of isotherm it is Langmuir. In this model, a monolayer is created when adsorbate particles stick to particular locations on the surface, as **Fig. 9** illustrates. The highest capacity may be achieved by using a weight of 0.1 g and a concentration of 2500 µg/mL.

Table 5. The ideal circumstances for the development of the molecularly imprinted polymer of PG

Wt. MIP (g)	Ci (µg/mL)	Cf (µg/mL)	Vol (mL)
0.1000	500	10.5223	8
	1000	16.6315	8
	1500	25.7352	8
	2000	32.8131	8
	2500	32.7871	8

Table 6. The connection between capacity Q(µg/g) and initial concentration Ci (µg/mL).

Ci (µg/mL)	Q (µg/g)
500	39158.2160
1000	78669.4800
1500	117941.1840
2000	14500.6400
2500	1200.0100

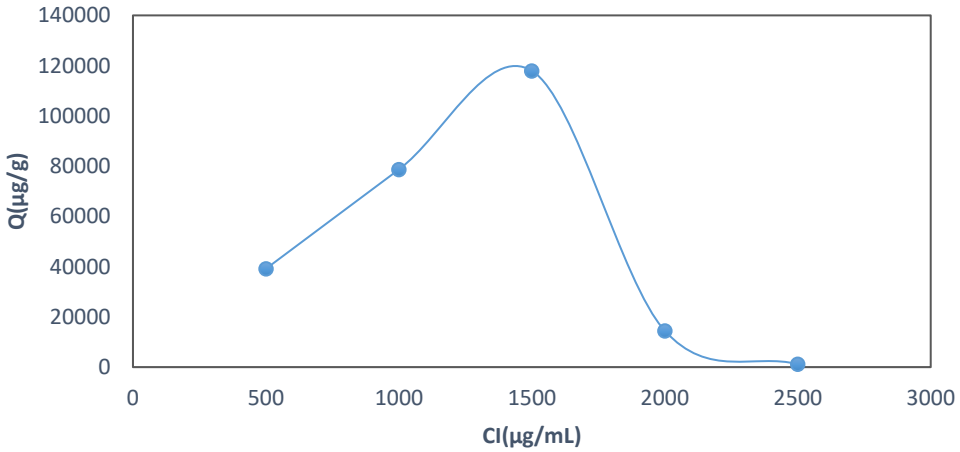


Figure 9. The Langmuir isotherm model

The value of the dissociation constant in the binding site based on the values of capacity Q (µg/g) versus Q/Cf (mL/g) to know the amount of pregabalin that reaches saturation in the column, as shown in **Fig. 10**. According to the obtained calculations, the K_d value is 0.0356926 and Q_{max} is 3295.1408 µmol/g. This indicates that PGN-Mip had a single capacity of 3295.1408 µmol/g, which is consistent with the Langmuir isotherm model, which has a single slope and scattered values.

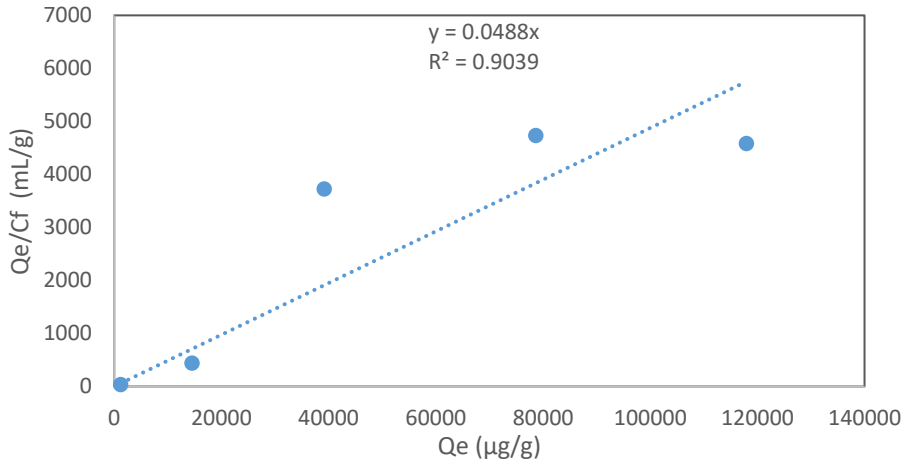


Figure 10. The relationship between capacity Q (µg/g) and Q/Cf (mL/g)

The statistical results derived from the successful capture, separation, and estimation of pregabalin are displayed in **Table 7**. The real value is shown by the pregabalin calibration curve's absorption at 2500 (µg/mL). The acceptable statistical data show that the ideal circumstances for capturing, separating, and calculating the pregabalin in pharmaceutical

preparations were chosen. When compared with other works, pores with a high ability to retain the PGN and the ability to separate with high efficiency were obtained due to the formation of many bonds between the drug and the monomer, including hydrogen and ionic bonds, due to the presence and difference in the functional groups that characterize both the drug, the monomer, and the cross linker.

Table 7. Pre- and post-isotherm process pharmaceutical drug analysis precision and accuracy in UV-VIS spectrophotometry.

Drug name 100mg	MIP	Concentration Ci µg/mL	Absorption before isotherm process	Absorption after isotherm process	Concentration Cf µg/mL	Vol. ml	Q (µg/g)	RSD% =(δn-1/Mean)*100 Precision	Rec. % = (practical value/True value)* 100 Accuracy	Re%= Rec-100
LYRICA /Germany-VIATRIS	MIP 0.1g	150	0.1671	0.0761	0.0713	8	118641.6	3.72	97.41	2.59
LYRICA/ PFIZER- USA		150	0.1796	0.0702	0.0651	8	116341.5	2.95	95.92	4.08

* For n=5 drugs were absorbed before the isothermal process (via the column of MIP).

5. CONCLUSIONS

According to the information obtained between capacity Q (µg/g) and initial concentration Ci(µg/mL). shows what type of isotherm it is Langmuir. In this model, a monolayer is created when adsorbate particles stick to particular locations on the surface show as **Fig. 9** illustrates that the highest capacity may be achieved by using a weight of 0.1 gm and a concentration of 2500µg/mL. Using various functional groups as monomers, a novel bulk polymer was produced. PGN-MIP was made from styrene and cross-linked ethylene glycol dimethyl acrylate. Numerous analytical techniques to produce polymers with particular molecular fingerprints, experiments were carried out. This was achieved by preparing and optimising the required monomers, cross-linking them with the right solvents, and removing the template with porogenic solvents, and adhered to the ideal molar ratios of template (pregabalin) to monomer for cross-linking. Both before and after the template is removed, the irregularly formed three-dimensional network structure of the polymer can be observed via SEM; the precision of this work is further enhanced by FT-IR and isotherm processing. Studying the PGN-MIP's adsorption capacity, which has a 1:1 template to monomer ratio and fits the Langmuir isotherm model with scatter values (heterogeneous structure), yields significant results. PGN-MIP has a maximum adsorption capacity of 117941.6 µmol/g.

Credit Authorship Contribution Statement

Firas Sattar Abdullah: Writing – original draft, Validation, experimental work, and Methodology. Yehya Kamal Al-Bayati: The corresponding author and owner of the project idea, Writing – review & editing, Methodology, and proofreading.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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تصميم بوليمر البصمة الجزيئية مقترناً باستخلاص الطور الصلب لتحديد مادة البريجابالين في المستحضرات الصيدلانية

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الخلاصة

يوضـــح هذا البحث طريقة فعّالة لتخليق وتخزين البوليمرات المطبوعة جزيئيًا (MIP) باســـتخدام بلمرة بريجابالين (PGB) بكميات كبيرة، والتي تُجرى في درجة حرارة الغرفة. تتميز هذه الطريقة بحساسية عالية، وتكلفة منخفضة، واستقرار متزايد، وعمر أطول للبوليمرات. استخدم البحث نسبًا محددة بين القالب، والمونومر، وعوامل الربط المتقاطع لضمان قدرة امتزاز مناسبة. استُخدم بنزويل بيروكسيد (BPO) كبادئ للربط المتقاطع بين المونومر الوظيفي أكريلات البيوتيل وإيثيلين جليكول ثنائي ميثاكريلات (EGDMA) لإنشـاء MIP للديازيبام (PGB–MIP). دُرست البوليمرات المطبوعة جزيئيًا باستخدام مطيافية الأشعة فوق البنفسجية المرئية (UV–VIS) عند طول موجة 228 نانومتر، وهي تقنية تُستخدم للكشف عن الأدوية الصيدلانية. كما استُخدمت مطيافية الأشعة تحت الحمراء بتقنية تحويل فوريه (FTIR) والمجهر الإلكتروني الماســح (SEM) لدراســة بنية البوليمر. أظهرت عملية الإيلوشــن المُطبقة على قالب PGB–MIP وجود تجاويف ناتجة عن مذيبات خليط مســامية مثل الميثانول والكلوروفورم وحمض الأســيتيك. قُيســت أقصــى ســعة امتزاز للبوليمر المطبوع جزيئيًا ووجدت أنها 3.978 (ميكروغرام/غرام) عند استخدام 0.1 غرام من PGB–MIP، وهو ما يتوافق مع نموذج لانغمير متساوي الحرارة. وتبين أن النسبة المثلى للقالب إلى المونومر هي 1:1. للتطبيق العملي، اســتُخدمت حقنة اســتخلاص الطور الصــلب (SPE) مُعبأة ببوليمرات مطبوعة جزيئيًا لفصل وتركيز الديازيبام بشكل انتقائي في أدوية صيدلانية متعددة المصادر.

الكلمات المفتاحية: البوليمرات المطبوعه جزيئيًا(MIP)، بريجابالين، العمليات الحراريه، استخلاص الطور الصلب.