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المجلة العراقية لبحوث السوق
وحماية المستهلك

SYNTHESIS AND DETERMINATION OF CALCIUM ION-IMPRINTED POLYMERIC AND ITS APPLICATION IN SERUM SAMPLE

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ABSTRACT

This study was aimed to synthesis new molecular imprinted polymers from different monomers which useful for determination of calcium ion in different serum samples. Calcium ion play an important role in blood clotting and bone mineralization. In plasma, 40 percent of circulating calcium is bound to proteins, 10 percent is in the form of inorganic complexes, and 50 percent is present as free (ionized) calcium. In this study, Blood samples were taken from patients with type 2 diabetes from Kadhimiya Hospital in order to determine the concentration of calcium ions in the blood, enter it into the column, and then calculate the final concentration in order to know the amount of dose taken by the patient. To acquire the highest adsorption capacity, molar ratios of the template, monomer, and cross-linking agent, as well as solvents and multiple monomers were investigated. Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) were used to analyze the calcium ion polymer. The elution of calcium has a small effect on the surfaces of the three-dimensional network structure. Calcium (II) ions were successfully eluted using a mixture of methanol and acetic acid. The calcium absorption capacities were7.989 µmol/g and 8.250 µmol/g (Q_{max}), respectively. Solid-phase extraction (SPE) syringes packed with ionic imprinted polymers (IIPs) were used to selectively separate and preconcentration the calcium (II) ion from serum to determine the calcium ion by flame atomic absorption spectroscopy (FAAS). Through the results obtained and compared to the atomic absorption device, which is considered the most accurate and sensitive device for the elements, there is no difference in the results, and because the atomic absorption device test is expensive and needs electricity all the time, we can use the molecular printing technique to separate, assign, and concentrate the elements.

Keywords: Molecularly imprinted polymer; calcium ion, styrene, monomers

تخليق وتحديد الطور الصلب البوليمري المطبوع بايون الكالسيوم في السيرم وتطبيقها في عينات سيرم

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الخلاصة

هدفت هذه الدراسة إلى تخليق بوليمرات جزيئية جديدة مطبوعة من مونومرات مختلفة والتي تفيد في تحديد أيون الكالسيوم في عينات مصل مختلفة. يلعب أيون الكالسيوم دورًا مهمًا في تخثر الدم وتمعدن العظام. في البلازما، 40 في المائة من الكالسيوم المنتشر مرتبط بالبروتينات، و 10 في المائة في شكل معقدات غير عضوية، و 50 في المائة موجود على شكل كالسيوم مجاني (مؤين) .في هذه الدراسة، تم أخذ عينات دم من مرضى السكري من النوع 2 من

مستشفى الكاظمية بهدف تحديد تركيز أيونات الكالسيوم في الدم، وثم أدخالها في العمود، ومن ثم حساب التركيز النهائي للكالسيوم لمعرفة مقدار الجرعة التي يأخذها المريض وقد تم تحديد أيونات الكالسيوم من خلال إضافة مونومر ستايرين. للحصول على أعلى سعة امتصاص، تم فحص النسب المولية للقالب، والمونمر، وعامل الربط المتبادل، وكذلك المذيبات والمونومرات المتعددة. تم استخدام المسح المجهري الإلكتروني (SEM) و Fourier Transform Infrared Spectroscopy (FTIR) لتحليل بوليمر أيون الكالسيوم. كان لشطف الكالسيوم تأثير ضئيل على أسطح بنية الشبكة ثلاثية الأبعاد. تمت تصفية أيونات الكالسيوم (II) بنجاح باستخدام خليط من الميثانول وحمض الخليك. كانت سعات امتصاص الكالسيوم 8.250 µmol/g و7.989 µmol/g على التوالي. تم استخدام حقنة للاستخلاص بالطور الصلب (SPE) المعبأة بالبوليمرات الأيونية المطبوعة (IIPs) للفصل الانتقائي والتركيزوالتقدير للايون الكالسيوم (II) في الدم بطريقة مطيافية الامتصاص الذري باللهب (FAAS). من خلال النتائج التي تم الحصول عليها ومقارنتها بجهاز الامتصاص الذري، والذي يعتبر الجهاز الأكثر دقة وحساسية للعناصر، لا فرق في النتائج. ولأن فحوصات جهاز الامتصاص الذري غالي الثمن ويحتاج للكهرباء طوال الوقت، يمكننا استخدم تقنية الطبعة الجزيئية لفصل العناصر وتعينها وتركيزها.

الكلمات المفتاحية :الطبعة الجزيئية البوليمرية ,كالسيوم ايون, ستايرين ,مونمر

INTRODUCTION

Calcium is a metallic element that comprises more than 3% of the earth's crust, ranking fifth in abundance. It is found in leaves, bones, teeth, shells, etc. It has never been found in nature alone uncombined calcium seems to be silver. Calcium is an essential component of human and animal bodies because it protects the bone system and serves as a regulatory ion both within and outside the cell **(Aljabari & Al-Bayati, 2021)**. Bulk polymerization is the most straightforward way to create pure polymer forms **(Al-Bayati &Hadi, 2022; Andac & Denizli, 2004; Irshad Ahmad *etal*., 2018)**. The ion-imprinting process consists of three steps: (i) template (metal ions) complexation with a polymerizable ligand; (ii) polymerization of this complex; and (iii) template removal after polymerization. The specificity of the ligand, the coordination geometry, and the coordination number of the ions, as well as their charges and sizes, all have an influence on the selectivity of a polymeric adsorbent in the ion imprinting process **(Al-Bayati & Aljabari, 2016).** The template can be delivered into the system in a variety of ways, including standing alone or being bonded to a surface, resulting in 3D or 2D imprinting environments that respect polymerization. This could be covalent, non-covalent, or semi-covalent in nature **(Zaheer *eta*., 2021*)*.** Non-covalent imprinting is by far the most prevalent approach due to its ease of production and the vast range of monomers available **(Al Fatease *et al*., 2021; Pedro Melendez *etal*., 2017).** By virtue of their existence, they are required for molecular interactions. Acceptance procedures are widely employed since they are considered the most efficient and successful method for molecularly imprinted polymer (MIP) synthesis. The researchers are currently working on creating a method for selective preconcentration of sorbents utilized in solid-phase extraction (SPE) **(Al-Batatyi & Abd, 2017; Beeregowda, 2014).** Waste water or river water is examples of complicated matrices. SPE is a more straightforward, quick, and cost-effective method of extraction that is also environmentally friendly. The most significant issue is the use of standard stationery stacked in SPE columns **(Mohsen & Al-Bayati, 2021; Nose *et al*., 1988)**. The retention phase's low selectivity mechanism it's possible to achieve a desired level of selectivity **(Agarwal & Kasana, 2019)**. Here, we investigate the selective separation and preconcentration of the calcium (II) ions from aqueous solutions by the addition of an allyl chloride monomer, resulting in bulk polymerization formation, to determine the calcium ion by flame atomic absorption spectroscopy (FAAS). This study was aimed to synthesis new molecular imprinted polymers from different monomers which useful for determination of calcium ion in different serum samples.

Chemicals and materials

Calcium chloride dihydrate (99.9%), styrene (99.9%), ethylene glycol methacrylate (EGDMA) (99.9%), benzoyl peroxide were purchased from Sigma Aldrich, methanol, nitrogen gas and acetic acid.

Preparation and Processing:

Preparation of ionic imprinted polymer: For preparation of the number one calcium ionic imprinted polymer (calcium-IIP), calcium chloride dihydrate (0.147g, 1 mmol) was dissolved in methanol (2 mL), then mixed with styrene (4 mmol) as a monomer in methanol (2 mL) and left for few seconds at room temperature. Then, ethylene glycol methacrylate (EGDMA) (3.9 g, 20 mmol) was dissolved in methanol (2 mL) (as a cross-linker), and benzoyl peroxide (300 mg) as an initiator) was dissolved in chloroform (2 mL) and then added to the solution to obtain a homogeneous solution, and the mixture was shaken for 5 minutes. After wards, nitrogen gas was passed for 30 minutes through the mixture to extract oxygen from it. The solution was then placed in a water bath at 60 °C for 6 hours. When the reaction was completed and the Ca-IIP were formed. they were left for 24 hours to dry and then they were crushed and ground by a mortar and pestle, the sieve was used to obtain the particles with a diameter of 125-150 μm and then collected. The Ca ion was extracted from polymers Ca-IIP by using soxhlet of (60:10:20) (methanol: acetic acid: acetonitrile) for weak. After that, the polymer was dried for 24 hours at room temperature and collected to be used as a substance in a solid-phase extraction syringe**.** Each plastic syringe (column) was packed with Ca-IIP (200 mg) and used 3 mL solution for solid-phase extraction by a peristaltic pump.

Sampling procedure

Serial concentrations (20, 40, 60, 80, 100ppm) were prepared from $CaCl_2.2H_2O$ (0.147g, 1 mmol) by dissolving in methanol in a 100 mL volumetric flask. Calibration curve between concentration of calcium. and its absorption A, this was achieved using at (274nm by UV-VIS instrument).as shown in figure.1.

Concentration of Ca^{2+}ion ppm	Absorption
20	.03940
40	0.0543
60	0.0748
80	0.1002
100	0.125

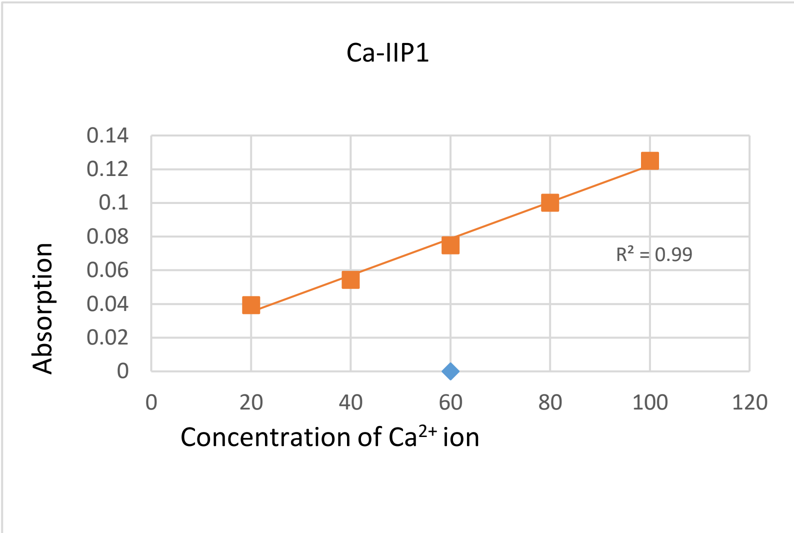


Figure. (1): Calibration curve between concentration of Calcium ion standard ppm and its absorptions in UV-VIS spectrophotometer technique.

RESULTS AND DISCUSSION

Transform Infrared Spectroscopy (FTIR) analysis:

To detect the functional groups, present in a compound, FTIR an important chemical characterization process. The FTIR method was successfully applied to the study of molecularly printed materials and has been beneficial in identifying the functional groups of polymers. FTIR spectroscopy was especially sensitive to the structural characteristics of polymer formation. The spectrum of Ca-IIP before elution where appears peaks at 1639 cm^{-1} for Ca-Cl stretching,3433cm^{-1} for O-H stretching , 1730 cm^{-1} for C=O stretching,3172 cm^{-1} for C-H stretching, 1232 cm^{-1} for C-O-C stretching, 3016 cm^{-1} for C-H aromatic stretching, 1556 cm^{-1} for C=C aromatic stretching (Agarwal & Kasana, 2019).When compare with FTIR after removal the calcium ion show disappearance the peak of Ca-Cl which indicate that calcium ion was removed and form the ionic imprint polymer are shown in Figure (4) .

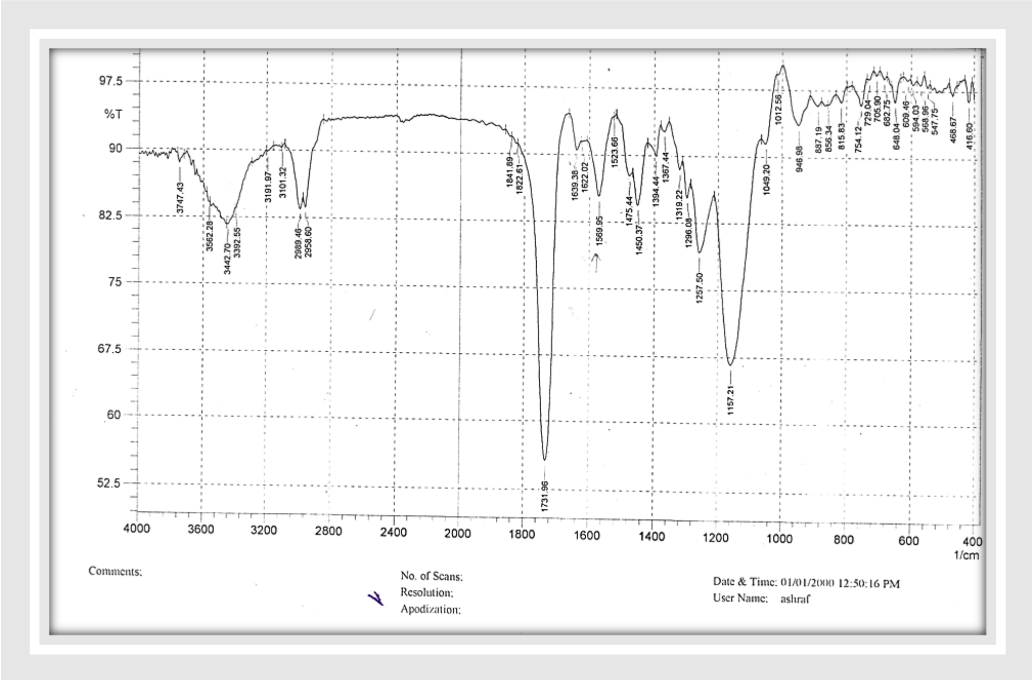


Figure (2): FTIR spectra of salt $CaCl_2 .2H_2O$.

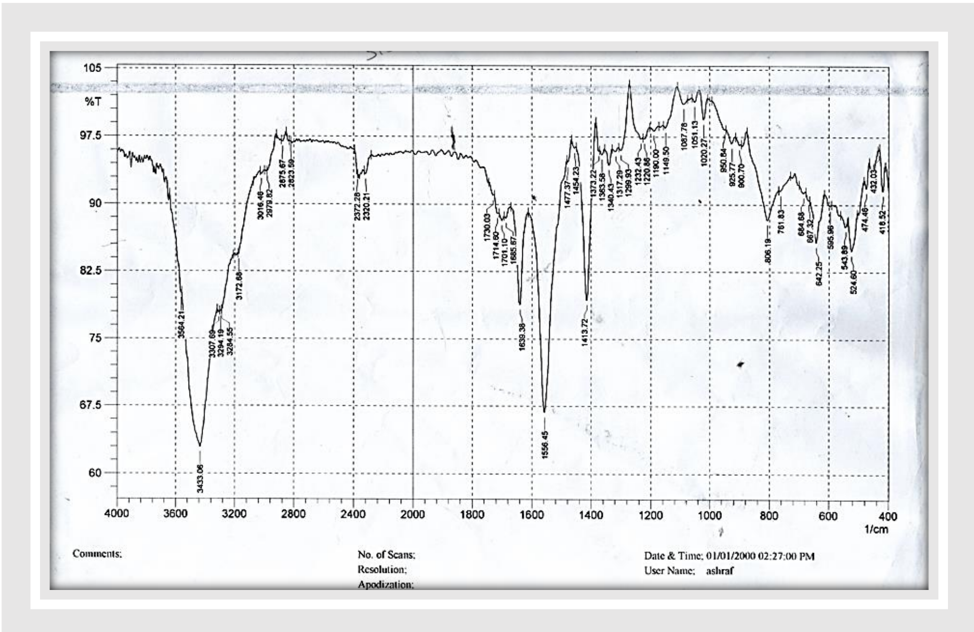


Figure (3): FTIR spectra of Ca-IIP1 (styrene) before remove the Ca^{2+}ion

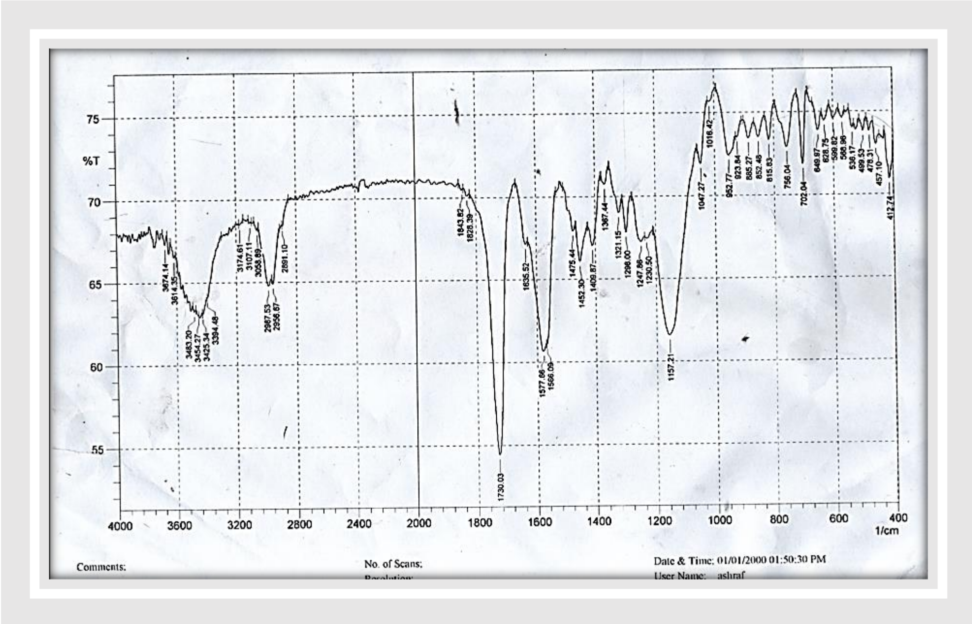


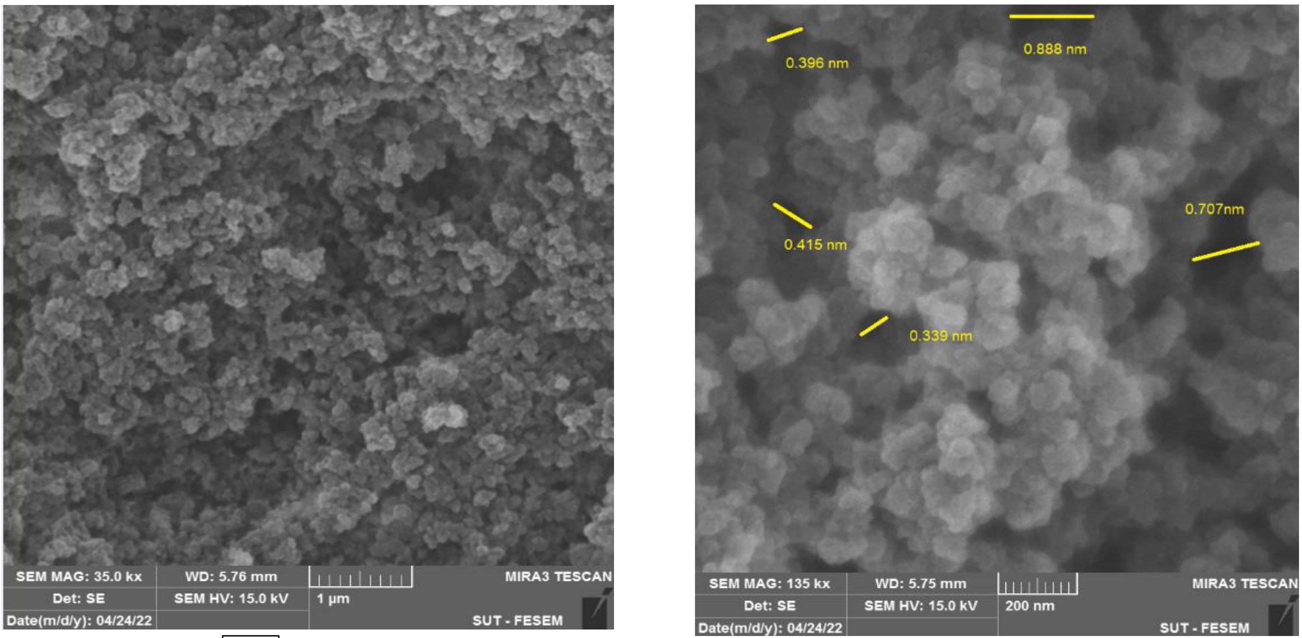
Figure (4): FTIR spectra of Ca-IIP (styrene) after remove the Ca^{2+}ion

Table (1): The most identified peaks of FTIR spectra for Ca-IIP using (styrene) as a functional monomer.

	Functional Group	CaCl2.2H2O	Ca -IIP(Styrene) before template removal	Ca –IIP (Styrene) After template removal
1-	v Ca-Cl cm-1	1639	1639	-----------
2-	v O-H cm-1	3442	3433	3454
3-	v C=O cm-1	-----------	1730	1730
4-	v C-H cm-1 aliphatic	-----------	2979 2875	2987 2956
5-	v C-H cm-1 olf	-----------	3172	3174
6-	v C-O-C cm-1 ester	-----------	1232	1230
7-	v C-H cm-1 aromatic	-----------	3016	3058
8-	v C=C cm-1 aromatic	-----------	1556	1577

Scanning electron microscope (SEM)

SEM creates a high-resolution image by scanning the surface of a comparison surface; this figure (5) depicts the morphology of IIP for calcium before and after washing. The figure(5) reveals obvious calcium holes in the sizes eliminated by soxhlet extraction(**Zaheer *et al.*, 2021**).



A B

Figure (5): SEM photograph of the surface of Ca-IIP (styrene), (A) before calcium removal, (B) after calcium removal.

Table (2): Results obtained using different ratios of [D:M:C] and progeny for the synthesis of IPs and NIPs for Ca-IIP

NO. of IIP	Ratio%	Salt $CaCl_2.2H2O$	Monomer Styrene	Cross linker EGDMA	Initiator Benzoyl peroxide	Solvent	**Result**
IIP1	**%**	**9.302**	**18.604**	**72.093**	**0.3**	**6ml CH_3OH**	**White**
	mmole	**2**	**4**	**15.5**	**0.32**		
IIP1	**%**	**6.976**	**18.604**	**74.418**	**0.3**	**6ml CH_3OH**	**White**
	mmole	**1.5**	**4**	**16**	**0.32**		
IIP1	**%**	**4.048**	**16.177**	**79.772**	**0.3**	**6ml CH_3OH**	**White**
	mmole	**0.999**	**4**	**19.675**	**0.32**		
NIP1	**%**	**------**	**16.177**	**79.772**	**0.3**	**6ml CH_3OH**	**White**

The optimum ratios employed in the synthesis of Ca-ion-imprinted polymers (IIPs) and non-imprinted polymers (NIPs) are summarized in Table 2. After the calcium ion is removed, the control NIPs and IIPs, however, exhibit the same spectra and structural similarities. This demonstrates that removing the template molecule and leaving particular recognition binding sites in the polymer structure may be accomplished by washing the IIP particles in a (methanol:acetic acid, 60:10) solution using the soxhlet extraction method.

Table (3): The optimal synthesis conditions for the ionic imprinted polymer for Ca-IIP1 (styrene) developed in this study used UV-VIS technique.

Ca-IIP1 (styrene)					
Mass of IIP mg	Ci ppm	C_i µM	C_{free} µM	Q µMole/g	Q_{free} mL/g
0.2	ppm20	0.136	0.097	1.935	19.886
	ppm40	0.272	0.195	3.465	17.769
	ppm60	0.408	0.285	4.305	15.105
	ppm80	0.544	0.392	4.560	11.632
	ppm100	0.680	0.526	4.620	8.783

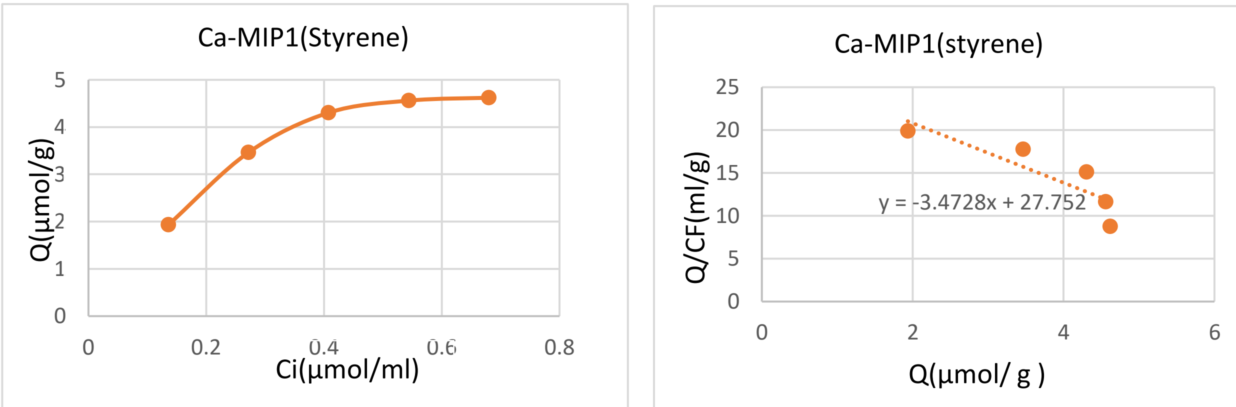


Figure (6): Illustrate Langmuir isotherm model.

Slop=-1/kd
-3.4728=-1/ kd =0.2879
Intercept=Q max/kd
27.752=Q max/0.2879
`Q max=7.989 µmol/g

Atomic absorption spectroscopy (AAS)

Standard solutions with concentrations of 20, 40, 60, 80and 100ppm were prepared and measured by atomic absorption at wavelength 422.7 nm, as shown in Figure 7

Concentration of Ca^{2+} ion ppm	Absorption
20	0.053
40	0.078
60	0.115
80	0.147
100	0.199

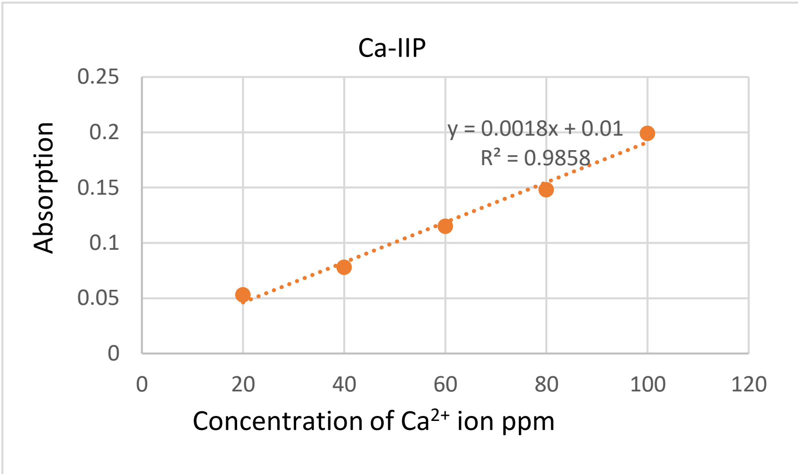
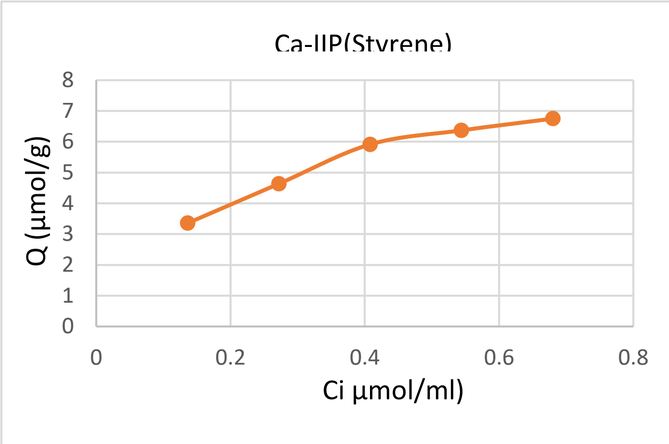


Figure (7): Calibration curve between concentration of calcium ion standard ppm and its absorptions in A.A.S technique

Table (4): The optimal synthesis conditions for the molecularly imprinted polymer for IIP1 - Ca (styrene) developed in this study used A.A.S technique.

IIP1 - Ca (styrene)					
Mass of MIP mg	Ci ppm	C_i μM	C_{free} μM	Q μMole/g	Q_{free} mL/g
0.2	ppm20	0.136	0.068	3.355	48.693
	ppm40	0.272	0.169	4.635	27.290
	ppm60	0.408	0.239	5.915	24.740
	ppm80	0.544	0.331	6.369	16.500
	ppm100	0.680	0.455	6.750	14.835



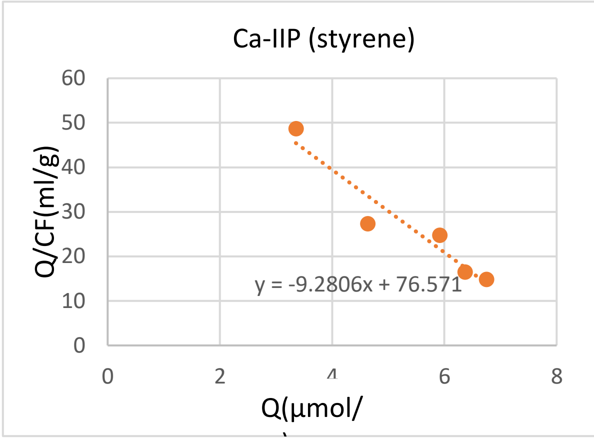


Figure (8): Illustrate Langmuir isotherm model.

Slop=-1/kd
-9.2806=-1/ kd =0.10775
Intercept=Q max/kd
76.571=Q max/0.10775
Q max= 8.250µml/g

In human serum

1- Sample collection

10 ml of blood was collected in plain tubes from each patient . 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 the calcium in the serum of patients with type II diabetes.

2- Procedure

1 ml of serum transferred to volumetric flask (10) ml was diluted in 10 ml of deionized water., and it was examined in the atomic absorption instrument for determination the calcium ion in the serum. A concentration of a serum sample containing calcium, were taken and applied to the ion-imprinted polymers. The rang value for calcium in the serum (0.0083-0.0106) ppm.

$S_1=2.541\times10^{4-}$

$S2=2.645\times10^{4-}$

F-test = $\frac{S1^2}{S2^2}$

F-test= 1.084

Table (5): The statistical values for F test between tabular values and observed values.

	S1	S2	F-test	F- table
1	2.541×10^{-4}	2.645×10^{-4}	1.084	19.2

It found F-test calculated < F-tab at confidence level 95% therefor there is no significant difference between two methods, So Null hypothesis will be accepted.

CONCLUSION

Bulk polymerization was used to create a novel calcium-IIP. EGDMA was chosen as the cross-linker and styrene as the functional monomer. In addition, benzyl peroxide was utilized as an initiator when chloroform was the solvent. The ideal calcium (II) ion to monomer and crosslinker dosage molar ratios were investigated. Three-dimensional network structure of polymers and their unpredictable shapes were studied using SEM. The results of FT-IR demonstrated that the Ca (II) ion was successfully eluted by a solution of methanol: acetic acid: acetonitrile (60:10:20v/v). The exceptional stability and regeneration capabilities of calcium-IIP are illustrated by the fact that the elution process has little to no impact on the chemical characteristics of the polymer or the shape of the cavity. According to the previous results, between two methods analytical technique by atomic absorption and our method IIP by UV for Ca^{2+}ion, there was no significant difference between two methods evidence of the method's efficiency and reliability in the analysis and estimation of the elements. Therefore, we can dispense with the atomic absorption device, which is costly and requires continuous electricity, and use the molecular print to estimate the elements.

Future works:

- Study the selectivity of prepared ionic imprinted polymer towards the other metals.
- Study the possibility of using the prepared polymers on extraction several times and the effect of this on the structure and properties of the polymer and its adsorption capacity.
- Prepare polymer by using a new molar ratio and study the effect of that on the adsorption capacity.
- Several salts of the same metals are used as a template in preparing imprinting polymers and studying the characterization to compare them with each other.
- Prepare new polymer by using a different type of monomers and crosslinkers and also by using two monomers in the same polymer.

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