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Synthesis and Characterization of Carbon Ceramic Composite Based on Molecularly Imprinted Polymer for Selective Absorption of Cloxacillin Antibiotic from Milk

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Abstract

With the emergence of the industrial age, the growing concern over environmental pollution has been to drug resistance in humans due to the use of antibiotics and their discharge in environment. The aim of this work was to synthesis of a carbon ceramic composite based on molecular imprinted polymers (MIP) particles via non-covalent procedure as selective adsorbent for removal of Cloxacillin (CLO) antibiotic from aqueous and biological media (MILK). The effect of operational parameters includes pH (3-10), contact time (1-120 min), MIP (0.1 – 5.0 %wt) and adsorbent dosage (0.1- 2.0 g/L) was studied for optimization of CLO removal condition. The synthesized MIP particles and fabricated carbon composites were characterized using scanning electron microscopy and nitrogen absorption/desorption (BET). The results showed that the removal efficiency was highest in neutral conditions and increased as amount of MIP particles increased. The optimum conditions for removal of CLO (94%) were determined at pH = 6.5, 87 minutes as contact time and the 1.6 g/L carbon composite contain 3.1 % wt MIP. The performance of the synthesized composites was evaluated for Cloxacillin removal from the milk sample. The performance of the MIP particles for the Cloxacillin removal of milk samples supplied from livestock showed that these adsorbents can help to effectively reduce the residues of drug contamination in dairy samples.

Keywords: Molecularly Imprinted Polymer, carbon ceramic composite, Cloxacillin, Milk.

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1. Introduction

Pharmaceutical compounds, including antibiotics has been penetrated in water sources and the environment through urban and industrial wastewaters, including pharmaceutical factories, hospitals, and livestock farms. Toxicity, non-biodegradability and creation of drug resistance in antibiotics have caused these compounds to be referred to as semi-persistent pollutants in the environment [1].

Cloxacillin (CLO) is a type of beta-lactamase-resistant penicillin. This antibiotic is used to treat infections caused by Staphylococcus species, microbial infections with golden staphylose (a bacterium producing penicillinase), skin infections, and respiratory system. CLO have oral absorption, but its absorption is reduced taking it with food. More than 50% of the CLO is absorbed by the oral route and its concentration reaches its peak after 1-2 hours. The half-life of the drug is between 1 up to 5 hours, which increases by up to 2.5 hours due to poor kidney function. About 30-60% of unchanged CLO is usually excreted by the kidney [2]. The presence trace antibiotics and hormones in treated water and raw sewage, may produce unwanted and unknown products during chlorination and ozonation processes, which may be toxic to humans and the environment [3]. Therefore, there is a need for an efficient system to remove these compounds from the aquatic environment. Conventional wastewater treatment processes are not capable of decomposing these compounds [4]. Various methods such as adsorption with activated carbon, reverse osmosis, air deposition, biological methods, as well as advanced oxidation processes include ozonation [5] photo Fenton [6], photosonooxidation [7], ultrasound radiation [8, 9], ultraviolet radiation [10] has been used to remove these drug compounds. Among these methods, absorption is one of the easiest and most cost-effective methods that have been widely used in recent years to remove industrial contaminants from wastewater [11].

The adsorption process seems attractive for a variety of new materials available for the recovery process. Most researchers are looking for synthesis and design a suitable adsorbent, not only having the ability to absorb compounds, but have high absorption capacity as well as it was enough stable in basic and acidic environments. Nowadays, a new generation of polymeric absorbers with unique properties is used by researchers for reduce compounds in aqueous [12]. It could be to mention the diverse characteristics of these adsorbents, including selective absorption, high absorption capacity, high specific surface, chemical, thermal and mechanical stability, and the reusing ability, in addition capability of using in aqueous and petroleum media. Molecularly imprinted polymers (MIPs) are polymeric particles contain selective identification sites relative to template molecule, which have attracted researchers in the last decade [13]. During imprinting process, template molecules are placed in the 3D polymer network based on shape, size and orientation functional groups [14].

Among these, carbon ceramic materials are one of the most commonly used sorbents used in water purification, due to several different properties, such as the ability to remove a wide range of pollutants and thermal stability. [15] Ceramic Matrix Composite (CMC) is a material consisting of a ceramic matrix combined with a ceramic (oxides, carbides) dispersed phase. CMC produced by conventional ceramic processes include polymer infiltration and pyrolysis, reactive melt infiltration, slurry infiltration, sol-gel infiltration and a combination of the above methods [16]. Sol gel based method involves preparation of a sol preceramic precursor and infiltrates into the preform, undergoes polymerization (gelation) and consequently it is converted into a ceramic at an elevated temperature. [17]

On the other hand, molecular imprinted polymers (MIP) are intelligent nano-systems that form in the presence of a molecule as a template and have a high specific chemical affinity to the template molecule, so that their mechanism is similar to antibodies or enzymes. [18] MIPs are used to make antibodies for protein, chromatography, sensors, separation and extraction. [19] The molecular imprinting is widely used as a suitable technique for creating specific recognition sites in polymeric matrices and has clear benefits such as easy preparation, cost-effectiveness and high chemical and mechanical strength. [20] Therefore, making MIPs as a widespread practical solution can provide the absorption and separation of specific chemical compounds in real environments such as water, wastewater, environments, biological systems, chemical and petrochemical industries. [21-23]

Composite materials are composed of two or more different components, which are referred to as so-called constituent materials, and its often displays many beneficial characteristics; in many cases, composites are stronger, of lower density, or less costly in comparison to established materials. [24]

In this study, selective MIP particles are synthesized by mass polymerization and then it used for fabrication of composite based adsorbent for remove the antibiotic CLO from milk. The effect of operational parameters includes pH, contact time, MIP dose and adsorbent dosage were considered for optimization of CLO removal condition. The synthesized MIP particles and fabricated carbon composites were characterized using scanning electron microscopy and nitrogen absorption/desorption (BET).

2. Materials and methods

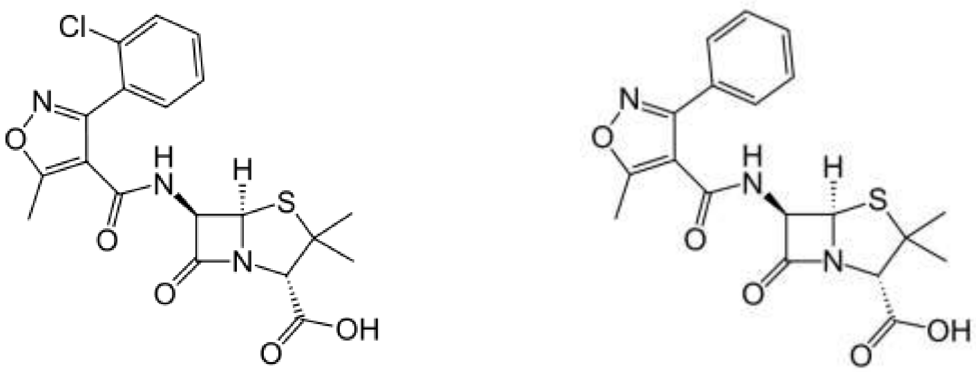
2.1. Chemicals and Devices

Cloxacillin antibiotic (CLO) (98%, See chemical structure in Fig 1) as template, and 2, 2′-Azobis (2-methylpropionitrile) (AIBN) as initiator (98%) was supplied from Floka Sigma- Aldrich (USA). Ethylene glycol di-methylacrylate (EGDMA) as cross linker, Methacrylic acid (MAA) as monomer was obtained from Merck Co. (Germany). The buffer phosphate solution as carrier electrolyte was prepared using 0.1 M H_3PO_4 or NaOH. Always use freshly prepared buffer solutions.

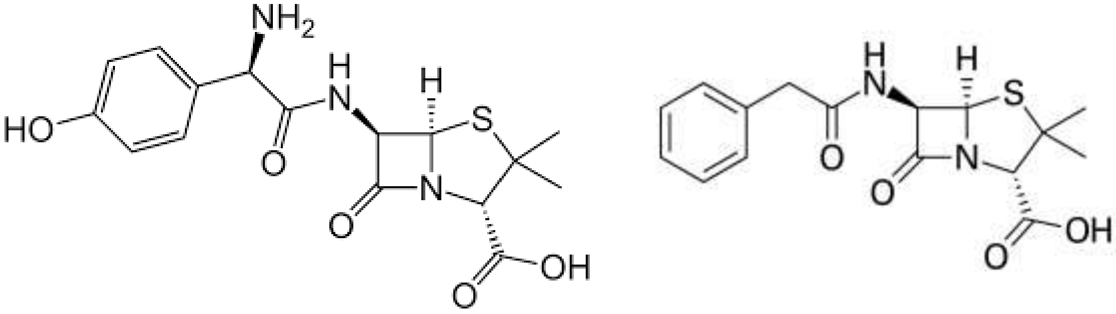
For assessing of MIP based composites performance in CLO removes from aqueous, residues CLO in solutions was determined by electrochemical method using galvanostate/potentiostate M101 Autolab device (Netherlands). The CLO was extracted from polymer matrix using soxhlet extractor (binder, Germany).

2.2. Preparation of MIP Particles

The CLO Molecularly imprinted polymer is preparing via following procedure: 9.0 mg CLO as analyte, 35.0 mL dry chloroform and 0.3 mL MAA adding to 100 mL balloon-flask and stirred for 15 min. Afterward, 20 mL EGDMA as cross linker was added to flask, then CLO (template, 9.0 mg) and MAA (functional monomer, 0.3 mL) were dissolved in chloroform (35 mL), and the resulting mixture was stirred for 15 min and then stood for another 4 h to prepare the pre-assembly solution. Then, EGDMA (cross linker, 20 mL) and AIBN (initiator, 0.22 g) were dissolved in the pre-assembly solution. The mixture was sealed and deoxygenated with a stream of nitrogen. The obtained pre-polymerization solution was shaken and stirred for at 65 C for 24 h. At the end of the reaction, the polymers were separated from the reaction medium. Following this, the polymers were extracted with methanol in soxhlex extractor to remove CLO until no CLO was detected by electrochemical determination in extraction solutions and washed with methanol for 24 h. Then, the wet polymers were dried at 60 °C in a vacuum oven for 24 h. The resulting CLO –MIP will use at sorption tests for separation of CLO from aqueous media. As a control experiment, non-imprinted polymer (NIP) particles were also prepared in similar procedure but without the addition of CLO.



Cloxacilin Oxacillin



AMOX Penicillin G

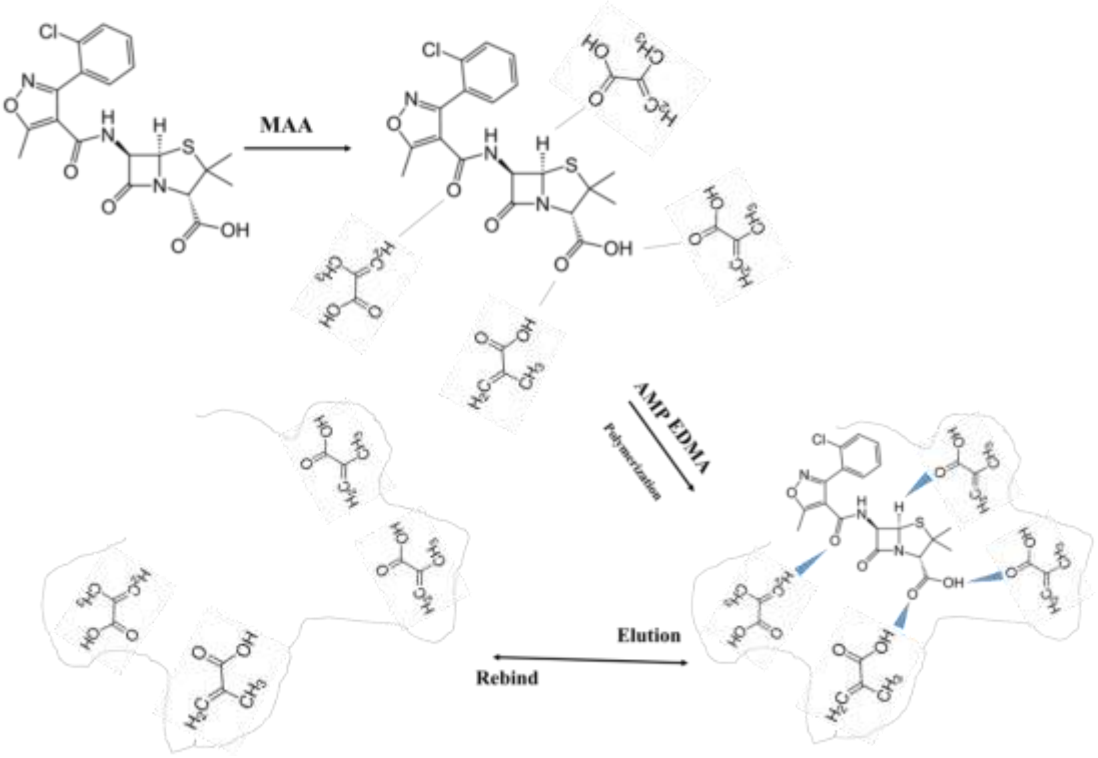
Figure 1. Chemical structures of four B-lactam antibiotics.

2.3. Preparation of carbon composite based on MIP

The adsorbent based on carbon composite modified with MIP was prepared by sol-gel method at ambient temperature based on scheme 1 follows. 20.0 µL potassium trimethoxy silan (PTMS, 90%, technical grade, Sigma Aldrich) was added to 4.0 mL water/ethanol mix (1:9) and pH was adjusted to 4.5 using 10 % v/v HCl. Then, the solution was agitated for 90 min to prehydrolyze the organosilane (scheme 2). After that, 0.34 g of the synthesized Fen-MIP and 1.0 g graphite powder was immersed in the prepared sol and homogenized for several minute. This was followed by evaporation of solvent to allow covalent bonding between the MIP/ silanol groups/graphite. The dried-sample, i.e. cured adsorbents were scoured with the 50/50 % v/v ethanol/water solution for several times until eliminate unreacted organosilane and then dried at 50 °C for 24 h. The dried sample was eventually well maintained in a desiccator for future use. A control sample was prepared under the same conditions without the addition of MIP particles, i.e. sol-gel solution and graphite powder (CC).

2.4. Removal experiments of CLO

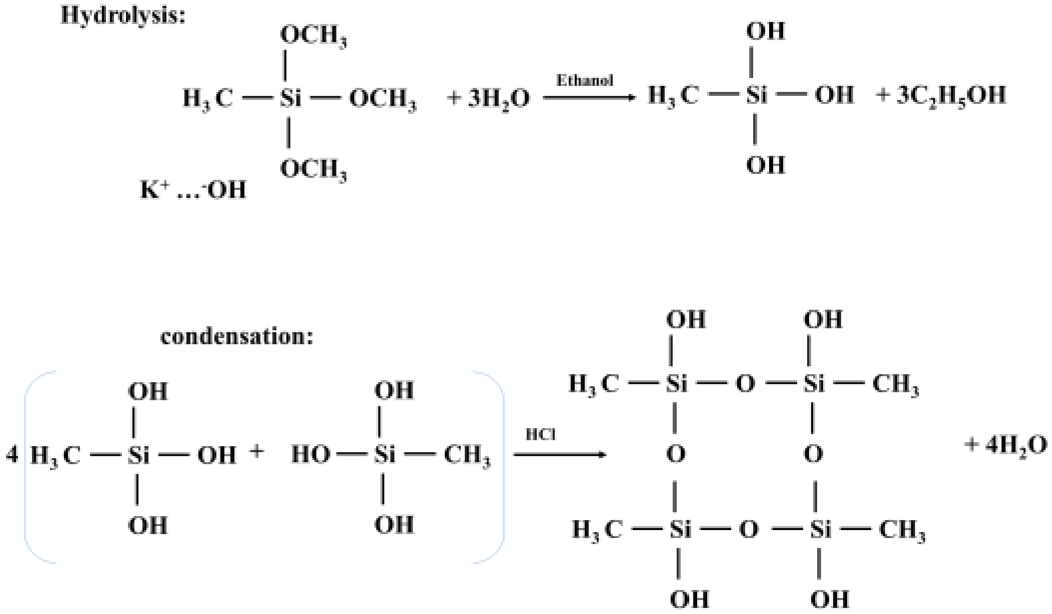
The operational parameters include contact time of MIP as adsorbent, pH, MIP amount and adsorbent dose was affected on removal efficiency of CLO. In this research, removal conditions of CLO were optimized using respond surface method (RSM) by changing of contact time, pH, MIP dose and MIP based adsorbent dosage. The RSM consists of a group of statistical and mathematical methods that allow the optimal conditions to be achieved in complex systems. For the study, the composite statistical design (CCD) was used; this model is a combination of second-order (quadratic) functions and interaction coefficients.



Scheme 1. Steps for preparation of CLO MIP

Table 1 lists the variables and their intervals. By introduction of intervals the mentioned parameters to the Design-Expert software, twenty tests were designed based on the CCD (table 2). The CLO removal tests were performed out by following way: identified amount composite prepared with proper MIP particles was added to a solution contain 0.05 g/L CLO (its pH was adjusted by PBS according to each test listed in table 2). The mixture was gently stirred for given time. Finally, residues CLO concentration in solution is determines by differential pulse voltammetry.

In order to measure the concentration of CLO in solution, a calibration curve is first required to measure the concentration of the unknown samples. The calibration curve was plotted based on electrochemical sensor response of AuNPs/GO SPCE towards different concentrations of CLO, as shown in Fig. 2. As shown in this figure, the oxidation peak current is increased with the gradual increase of CLO concentration, and there is a direct linear relationship between the concentration and the oxidation peak current. This linear relationship is the CLO calibration curve, as shown in inset of fig 2. The residues CLO concentration at any time of the removal process was calculated based on the peak-oxidation current and using the equation shown in fig 2.



Scheme 2. Schematic of sol-gel process on potassium trimethoxysilane as precursor.

Table 1. Variables and studied intervals

Variable	Sign	-α	-1	0	1	α
MIP (% wt)	A	0.1	1.325	2.55	3.755	5
Time (min)	B	5	28	62.5	96	120
pH	C	3	4.2	6	8.5	10
Adsorbent dose (g/L)	D	0.01	0.21	0.51	0.8	1

Table 2. Designed tests for optimization of CLO removal by MIP based on CCD.

Run	A:MIP (%wt)	B:pH	C: time min	D:AD g/L	Removal %
1	2.55	6.5	70	1.05	87
2	2.55	6.5	70	2	94
3	1.325	4.25	45	1.525	45
4	2.55	6.5	20	1.05	82
5	3.775	8.75	95	0.575	27
6	2.55	6.5	70	1.05	80
7	2.55	6.5	120	1.05	50
8	1.325	8.75	95	0.575	21
9	1.325	4.25	45	0.575	43
10	1.325	8.75	95	1.525	29
11	2.55	6.5	70	0.1	58
12	2.55	11	70	1.05	13
13	2.55	6.5	70	1.05	84
14	1.325	4.25	95	1.525	18
15	3.775	8.75	45	1.525	43
16	3.775	4.25	95	1.525	45
17	3.775	8.75	95	1.525	90
18	2.55	6.5	70	1.05	88
19	3.775	8.75	45	0.575	20
20	2.55	6.5	70	1.05	96
21	0.1	6.5	70	1.05	15.2
22	3.775	4.25	45	1.525	64
23	1.325	8.75	45	1.525	42
24	1.325	4.25	95	0.575	20
25	1.325	8.75	45	0.575	35
26	5	6.5	70	1.05	24
27	3.775	4.25	95	0.575	45
28	2.55	6.5	70	1.05	86
29	3.775	4.25	45	0.58	18
30	2.55	2	70	1.05	9

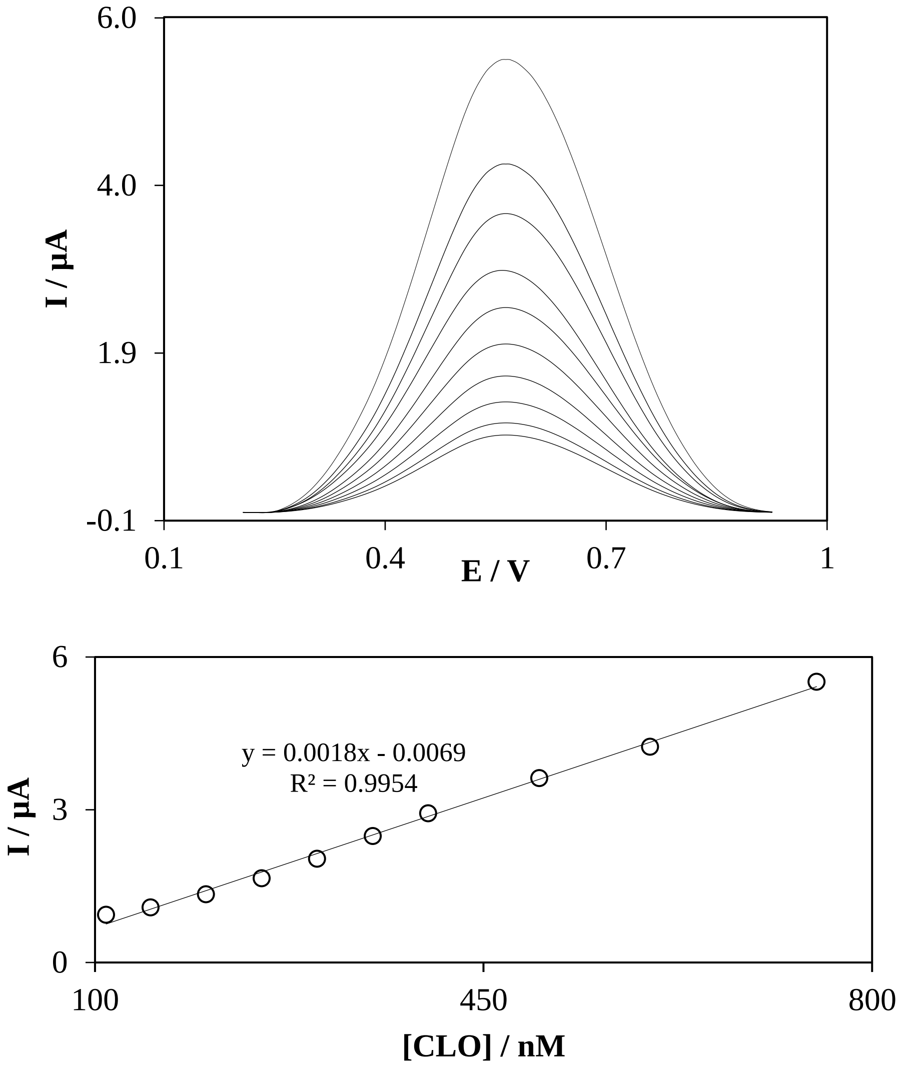


Figure 2. Differential pulse voltammetry of modified screen printed carbon electrode with Au nanoparticles and graphene oxide in buffer phosphate solution (pH 7.0) containing different CLO concentration. Numbers 1-10 represents CLO concentration range in 110 – 750 nM. Inset 1: calibration curve of CLO.

2.5. Determination of adsorption capacity of MIP based adsorbent and residues CLO content

Absorption capacity of the MIP based adsorbent for CLO is determines from the following equation:

$$\boldsymbol{q = \frac{(C_i - C_f)\times V}{m}} \qquad \textbf{(1)}$$

Where, q is absorption capacity (mg/g), V is volume solution (L), Ci and Cf are initial and final CLO concentration in sample (mg/L) and m is used mass of MIP based adsorbent (g). The CLO removal percentage from aqueous was determined by equation 2:

$$\text{Removal (\%)} =(1- C_f/C_i)100 \qquad (2)$$

3. Results and Discussion

A Molecularly Imprinted Polymer (MIP) is a polymer that has been processed using the molecular imprinting technique, as a result is form the regions with special molecular arrangement in polymer matrix. This has highly affinity to interact with the molecule chemically equivalent or at least resembling the chosen template molecule [25].

3.1. Characterization of used nanostructures and proposed sensor

The TEM image, Fig. 3A, is presenting the appropriate conditions of single layer GO and its compatibility for nanosensor application. Also, the TEM image of the purchased AuNPs (Fig. 3B) is representing the correct shape and size and also lack of agglomeration. Furthermore, the FE-SEM imaging of the nanomaterials-modified working area of the SPCEs was shown the correct assemble and decoration of the nanomaterials. In FE-SME image 3C, a single layer of the GO on the bare SPCE is shown while in the image of Fig. 3D, the decoration of the AuNPs on the GO-modified SPCE are characterized. This observation suggests that AuNPs /GO hybrid is a promising material that can improve the electrochemical sensitivity of electrodes for CLO detection.

The CV and EIS are the most frequently used electrochemical methods for evaluating the process of sensor fabrication step by step. In both of the methods, the $[Fe(CN)_6]^{3-/4-}$ solution is the preferred electrolyte which can also indicate the effects of each surface modification on the electrochemical behavior of electrode.

The cyclic voltammograms of the modified electrodes were recorded in a 0.1 M phosphate buffer containing 5.0 mM K_3 [Fe (CN) $_6$] at pH 7 and shown in Figure 4A. It can be seen CLO has a weak oxidation peak at 0.17 V at the bare SPCE. Meanwhile, the oxidation peak intensity decreased after the modification of SPCE using GO (Fig 4A, curve b). This can be interpreted in that GO has a partially negative charge, and K_3 [Fe (CN) $_6$] in an aqueous environment has a negative charge too. The presence of K_3 [Fe (CN) $_6$] reduced at the surface of the GO/SPCE due to the repulsion among the same charges; therefore, the oxidation current was lower than that at the bare electrode. In addition, GO is known as a semiconductor in many reports, which reduces the anodic or cathodic peak current. Curve c in Fig. 4A shows the CV of the SPCE modified with AuNPs in the presence of K_3 [Fe (CN) $_6$]. It can be seen that the oxidation current of K_3 [Fe (CN) $_6$] was increased due to the presence of AuNPs at the SPCE surface. Due to its high conductivity, partially positive charge and very small dimensions, AuNPs increased the effective surface area of the electrode and the sensitivity of the sensor to K_3 [Fe (CN) $_6$].

A comparison between curves (b) and (d) shows that the oxidation peak of K_3 [Fe (CN) $_6$] was highly increased due the addition of AuNPs to the GO/ SPCE. This observation suggests that AuNPs/GO hybrid is a promising material that can improve the electrochemical sensitivity of electrodes for K_3 [Fe (CN) $_6$] detection.

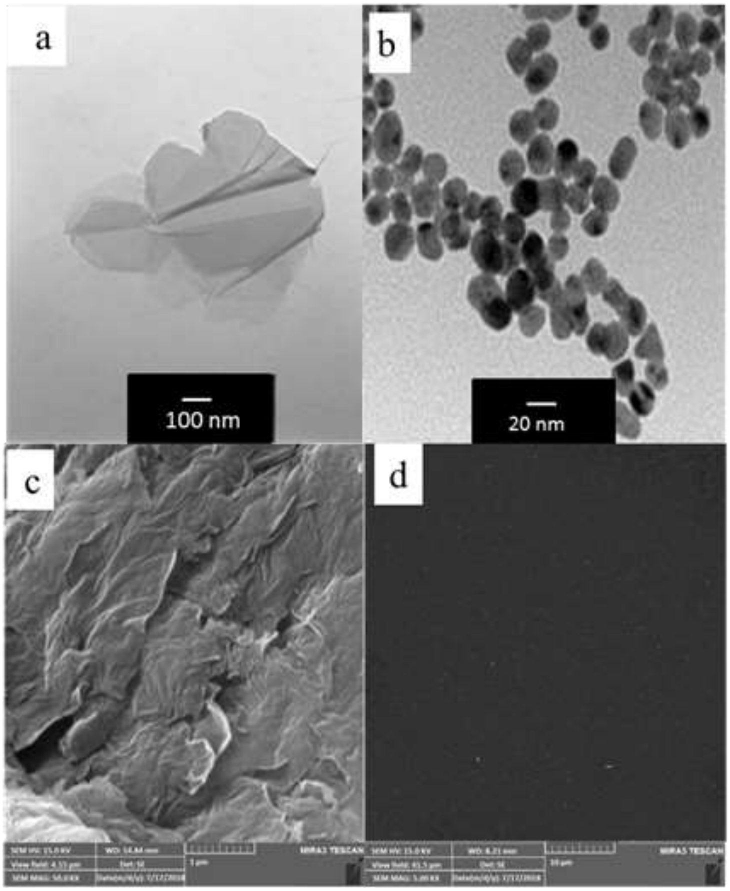


Figure 3. Characterization of the modified electrodes in different fabrication steps. The TEM images of the Exfoliated Graphene Oxide (EGO) (a) and the Gold Nanoparticles (AuNPs) (b). The FE-SEM images of the EGO-modified SPCE electrode (c) and EGO/AuNPs-modified SPCE electrode (d).

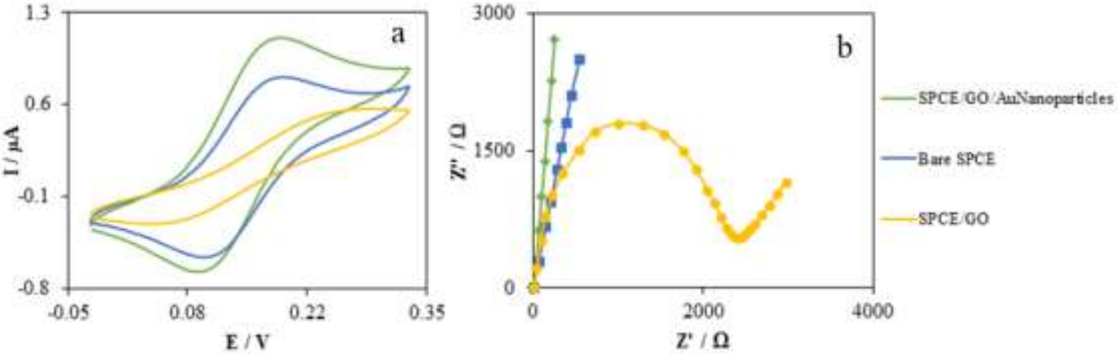


Figure 4- The cyclic voltammetry (CV) of the different modified electrode in the 1.0 mM $K_3[Fe(CN)_6]$ solution in phosphate buffer solution (a) and the Nyquist plot of the different electrodes in the solution of 5.0 mM $K_3[Fe(CN)_6]$ containing 1.0 M KCl (b).

Curve d in Fig. 4B presents the Nyquist curve of the SPCE modified with GO and AuNPs. As it can be seen, the diameter of the semicircle in this curve is smaller than that of the GO/SPCE. In fact, the R_{ct} has decreased to 14.16 Ω. This reduction could be due to the good electrical conducting properties of AuNPs which enhanced the electron transfer rate.

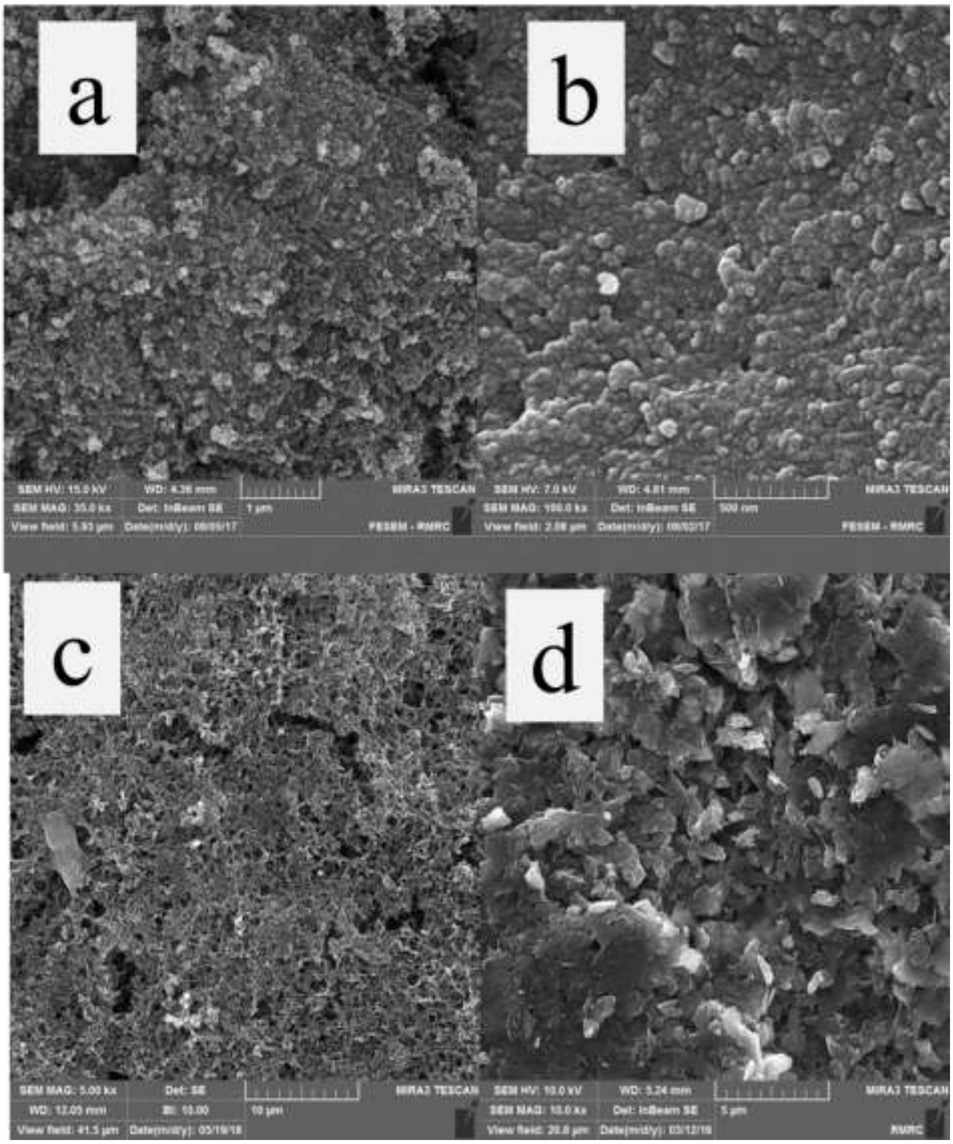


Figure 5- The morphologies of (a) NIP and (b) MIP, c) MIP based carbon composite and d) carbon composite without MIP. Magnification 10Kx.

3.2. Characterization of CLO – MIP based Composite

The morphology of prepared polymer particles in presences (MIP) and absence (NIP) of CLO molecules (as template) was observed by field emission scanning electron microscopy. Figure 5b shows the particle morphology of the MIP prepared from MAA/EGDMA. As shown, we can see a smoother structure than the MIP due to no CLO adding to mixture of MAA/EGDMA during bulk polymerization. While, due to the trapping of the CLO molecules in MAA molecular chains during bulk polymerization and their extraction by solvent in soxhelt extractor, numerous cavities are observed at the polymer surface (fig 5a).

In this study, during the synthesis of NIP (polymer without molecular imprint), due to the absence of the (CLO) molecule at polymerization process, it forms a continuous structure with less porosity than MIP. However, in the SEM image of the MIP, there is an enormous number of pores. This was due to the formation of a polymer network around the template molecule, which these pores create during the extraction process of CLO and its remove from the polymer matrix. The presence of these pores can help to increase the effective surface area for contacting the polymer surface with the CLO molecule and thus separating it from the solution.

SEM images of carbon composite and carbon composite containing MIP particles prepared by sol-gel method are shown in Figure 5c and 5d. In the presented images, a porous and uneven structure can be seen in both carbon composites prepared by the sol-gel method. Although numerous pores and pores are visible on the surface of the carbon composite containing MIP particles compared to the carbon composite, which helps to increase the surface area and pore volume of the composite, the histological results obtained from the BET test is confirming of this.

Table 3 summarizes data of the N_2 adsorption and desorption analysis on MIP based composite and composite without MIP (CC). As shown in the table, the specific surface area, total pore volume and pore size for MIP based composite are 28.11 cm^2 / g, 0.44 cm^3 / g and 54.91 nm, respectively. By comparing the values of the specific surface, it is confirmed that the imprinting in the polymers is well done.

Table 3. Data analysis from N_2 adsorption – desorption Isotherm.

sorbent	Special surface area (cm^2/g)	Total pores volumes (cm^3/g)	Average of pores sizes (nm)
MIP based carbon composite	28.11	0.44	54.91
Carbon composite	7.43	0.12	20.45

The FT-IR spectra of the synthetized MIPs and NIPs were obtained in the range of 500–4000 cm^{-1} by Bruker Tensor 27 FTIR Spectrometer. Figure 6 shows FT-IR spectra of CLO- MIP after and before removal the template (washed and unwashed, respectively) and NIP. It can be seen that spectrum of MIPs and NIP are similar, indicating similarity in the structure of the network. There is a broad band between 1100 -1200 cm^{-1}, corresponding to the stretching vibrations of -C-O-C- that could be attributed to the link of the MAA monomer and cross-linker EGDMA.

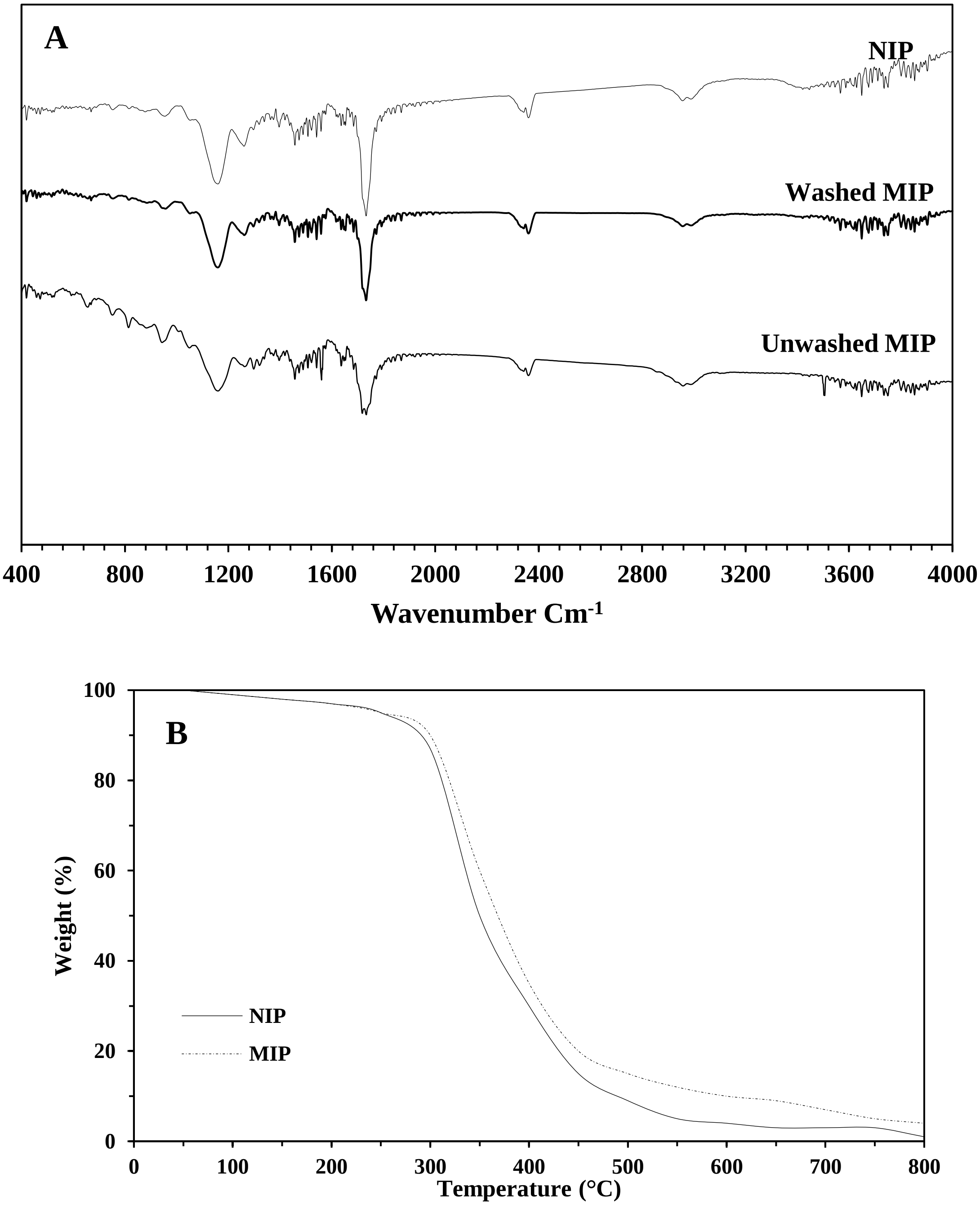


Figure 6. A) FT-IR spectra of NIP and MIPs before and after washing CLO. B) Thermogravimetric analysis of MIPs and NIPs.

In addition, there are absorptions due to stretch of the carbonyl group (1733 cm^{-1}), C-O stretch (1263cm^{-1}), and vibration C-H (756, 1396, 1458 and 2962 cm^{-1}). At 2995 and 2962 cm^{-1}, are the stretching vibrations of C-H, band at 1458 cm^{-1} appears to be due to -CH_2-, and the band in 1398 cm^{-1} may be due to CH_3. The sharp peak at 1733 cm^{-1} is the characteristic peak of C=O stretch, due to C=O vibration of EGDMA. Likewise, it was also identified similarities between the spectrum of template and MIP unwashed mainly in the C-N and C-C bonds (500-1600 cm^{-1}). After washing, the spectra of the MIP are comparable to the NIP (blank polymer), which indicates that the entire template was removed from the MIP unwashed. At FT-IR spectrum of MIP unwashed, there are two clear absorptions at 3500 and 1560 cm^{-1} related to O-H stretch and N-H stretching, respectively. The presence of these peaks can be attributed to the interaction between hydrogen of the carbonyl groups as well as nitrogen groups of the CLO with the functional groups of MAA. Similar results were obtained by Soledad-Rodríguez et al. [26], they were synthesized MIPs based in MAA as monomer and EGDMA as cross linker, found in general, that the FT-IR spectra for all the MIPs are very similar each to other, consistent with the fact that all the products were synthesized based on same monomer and cross linker, and also there were no significant difference between MIPs and NIPs.

The thermogravimetric analysis (TGA) curves of the MIPs and NIPs are given in Fig. 6b. It can be seen with the temperature increase at range of 250–600 ° C, weight of both polymers was significantly decreased due to thermal decomposition the residual organic substrate. There existed about 6.34% difference in the weight retention between NIP and MIPs. This small difference in the weight retention could be owing to the residual template molecules in MIPs.

3.2 Optimization of CLO removal by MIP

The response surface graphs are designed to show and estimate the efficiency of the effective factors in the CLO absorption on MIP by applying non-dependent variables, as shown in Fig. 7. These graphs show the effect of interactions between the two factors from the three factors investigated on the efficiency of CLO remove from the solution, while other variable is constant. Figure 7 (a) shows the effect of simultaneous changes in the contact time (1-120 min) and the amount of MIP (0.01 -1.0 g) on the removal percentage of CLO, when pH equal to 7.92. It can be seen, by increasing the contact time of MIP in a CLO solution up to 90 min, the CLO removal efficiency increases. The prolonging of contact time of MIP in the solution has no significant effect on the removal efficiency of CLO.

Adsorbent mass is one of important factors in removing contaminants by adsorption. If this amount is less than the required amount, the absorption step may not be complete and all of the analyte will not be adsorbed by the sorbent. In this study, with the increase in the amount of MIP particles, which is used as an adsorbent to remove CLO, the removal efficiency has increased. Due to the fact that the number of CLO molecules is constant in the solution, and by increasing the number of active sites for the adsorption of CLO molecules, it is possible to improve the CLO removal process by increasing the amount of MIPs. Similar results have been reported about increasing the amount of ampicillin removal by MIPs [26].

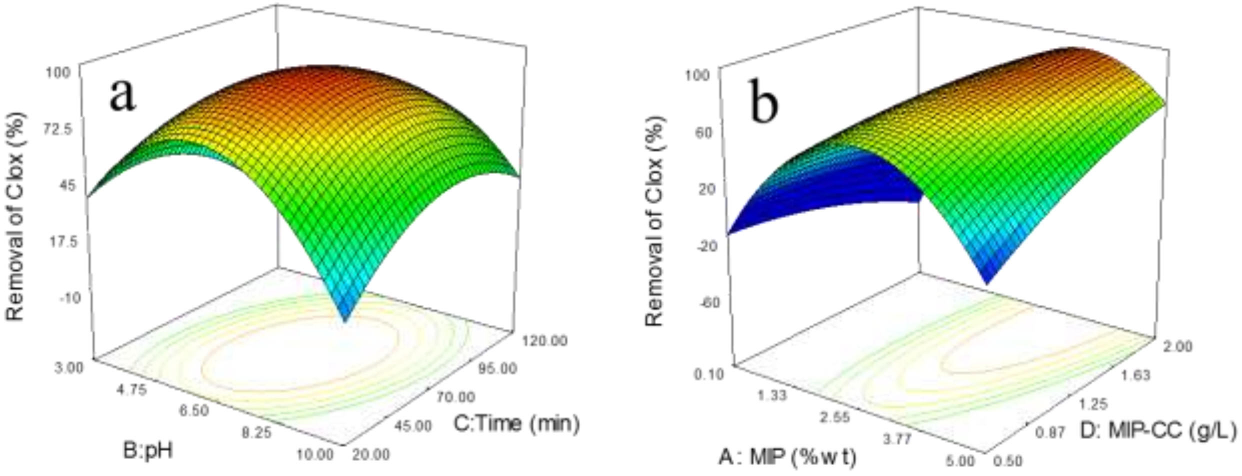


Figure 7. The 3D surface plot of simultaneous effect a) pH and contact time (constant pH= 7.92) and b) MIP and MIP CC dose on removal percentage of CLO from aqueous.

Figure 7 (a) shows the effect of simultaneous changes in pH (3-10) and contact time (20- 120 min) on the removal percentage of CLO while a MIP value is 0.68 g. It is observed that with increasing pH from 3 to 7, the CLO removal rate with a rather steep slope increased and then decreased in the range of 7 to 10.

The pH of the solution is one of the affecting factors on chemical surface properties of the adsorbent, in other words, the charge surface of absorbent may vary in acid or alkaline conditions. By reviewing literature, it can be abundantly found that pKa of MAA, which is the main body of the polymer, is reported to be about 4.65. This means that the functional groups of COOH are converted to $COOH_2^+$ in acidic environments (> 4.65) and have a positive partial charge [27]. While CLO is also positive in acidic environments, repulsive positive charges interfere with the interaction between the CLO functional groups and the carbonyl groups of MAA, as a result, a less amount of CLO will be adsorbing by the MIP. On the other hand, in the mildly acidic environment (4.65<) to neutral, the COOH converts to COO^-, that is, a negative species in the environment, therefore, conditions are appropriate for interactions between CLO and MIP particles, consequently, the highest remove is observed in the neutral region. With increasing pH, electrostatic repulsion between CLO and functional groups at the MIP surface also increased, resulting in less amounts of CLO will adsorb on MIP. What can be learned from the results of the effect of contact time is that the rate of CLO absorption on the MIP particles is slowly, so that the balance time is observed in 90 min. It seems that CLO molecules have saturated MIP surface over the time, and it can be said that the adsorption process has reached a balance. On the other hand, with the increase in the MIP amount in solution, the removal efficiency of CLO is gradually increased.

In the adsorption process, there are ion exchange site and specific proprietary positions on the adsorbent surface, where the antibiotic will interact to them. The prolonging times, there will be enough time for interaction of antibiotic molecules with specific absorption sites. On the other hand, the prolonging the contact time after reaching the equilibrium caused to some of the adsorbed molecules on MIP with weak interaction exits from its place and replaced by another molecule. Thus, we have seen no significant change in efficiency. Similar studies have been carried out using different adsorbents on antibiotic remove, where these conducted to investigation the contact time and the amount of adsorbent.

Du et al. have been used modified silica gel particles for removal of Cloxacillin. It has been reported that the equilibrium time for antibiotic absorption on these particles is about 2 hours [28]. In another study, kaolin was used to elimination of tetracycline, where a balance time of about 5 hours has been reported [29]. By comparing the equilibrium times, we can conclude that the highest removal efficiency shorter time using proposed adsorbent is obtained than the similar ones.

Optimum conditions for the removal of CLO were obtained in pH 8.5, the contact time of 89 minutes, and 0.79 g MIP. The absorption capacity of both synthesized polymers of MIP and NIP for CLO removal in optimal conditions at various concentrations of CLO was investigated, the results are presented in Fig. 8. Regarding the graph, it can be seen that the polymer capacity of MIP increased with increasing CLO concentration.

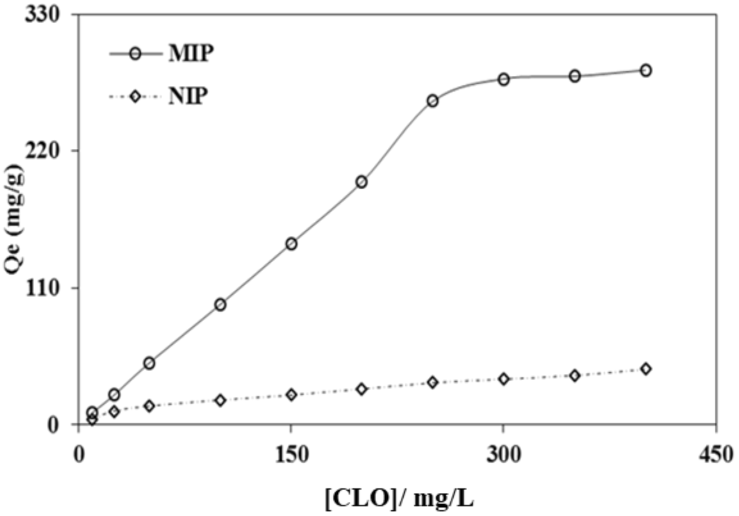


Figure 8. The comparison of adsorption capacity the NIP and MIP toward CLO. (Conditions: pH=8.5, 0.79 g MIP and 89 min as contact time

Table 4. Results of CLO Measurement in MILK.

sample	Added (nM)	Founded (nM)	Measured after adding MIP (nM)
Milk	-	164	>100
	50	215	115
	100	264	118
	200	366	125
	300	467	140

3.3 Evaluation of the adsorption Capacity and selectivity

The maximum absorption capacity (q) of MIP particles was determined to 280 mg/g. However, the maximum NIP capacity was 40 mg/g. These results confirm that MIP has a high ability to absorb CLO. It is likely that low-absorbed amounts on the NIP are also due to poor physical interactions between CLO and polymer particles. In the removal of CLO by modified silica particles, researchers have reported that the maximum absorption capacity by particles is 6.5 mg/g per gram of absorbent [28]. In another study, the authors reported that one gram of synthesized MIP particles for ampicillin can absorb 13.5 mg of antibiotics [30]. Compared to these cases, the polymer particles prepared in this study are much more efficient.

High selectivity is one of the most important characteristics of MIPs to the template molecule. This is probably due to the fact that, during molecular imprinting, the functional groups of the template molecules (here CLO) form a unique arrangement toward the functional groups of monomer units, which is specific to itself. In order to verify the selectivity of MIPs toward CLO, other β-lactam antibiotics with similar chemical structures as competitive molecules: amoxicillin (AMOX), oxacillin (OXA), and penicillin G (PEN-G) were examined (see Fig .1). For interference studies, recoveries of these antibiotics at concentration levels of 1 μM were tested by procedure developed for the MIPs. The values found were 11.2 % for AMOX, 19.3 % for OXA and 8.4 % for PEN-G and for CLO 101 %. This demonstrated that the synthesized MIP particles for CLO exhibited specific selectivity for CLO in the presence of other structurally related compounds.

The comparison between specifications of some adsorbents for removal of antibiotics are listed in Table 5. As can be seen, proposed adsorbent are comparable to other adsorbents. Although, surface area of the proposed composite is less than other adsorbents, but manufacturing method and its separating from aqueous solution is easy. The sol gel procedure is easy and cheap, low temperature technique and low-risk procedures. It is able also to achieve uniform and small sized powders with sol gel method.

Table 5. Comparison of the proposed method and some other methods for pesticide removal.

Antibiotic	adsorbent	Qmax (mg/g)	Surface Area (m^2/g)	Adsorption Efficiency (%)	Ref
Clox	MIP-CC	280	121.3	87	This work
Tetracycline	BSA/Fe_3O_4 magnetic composite	104.25	-	94	[31]
Erythromycin	BSA/Fe_3O_4 magnetic composite	144.25	-	97	[31]
cefazolin	activated carbon/Fe_3O_4 nanocomposite	59.2	184	96	[32]
Doxycycline	activated Carbon and Magnetic Oxide/Activated Bioinorganic Nanocomposite	80.3	120.1	89	[33]

3.4 Application of MIP particles in CLO removal from MILK

Finally, in order to evaluate the performance of synthesized MIP particles for absorbing residues CLO values in real samples such as milk, 0.79 g of MIP particles were added to 20 mL of milk, which prepared from a livestock farm in Yazd, after stirring of solution for 89 min, polymer particles were removed from the milk using a paper filter. The concentration of CLO in the milk was calculated before and after the addition of MIP particles by electrochemical measurements, and the average value of 10 replicates was reported in Table 4. To confirm the measurement method and high adsorption capability, different volumes of CLO with given concentration were added to the milk. The results of this test indicate the proper performance of MIP adsorbent particles in the effective reduction of the antibiotic residues of CLO from aqueous media.

To estimate the reliability and accuracy of presented method, residues obtained were also analyzed by HPLC-UV. At chromatograms shown in fig. 9, it is observed that the absorption peak located at 3.0 min, which correspond to the CLO, disappears after contact with MIP particles.

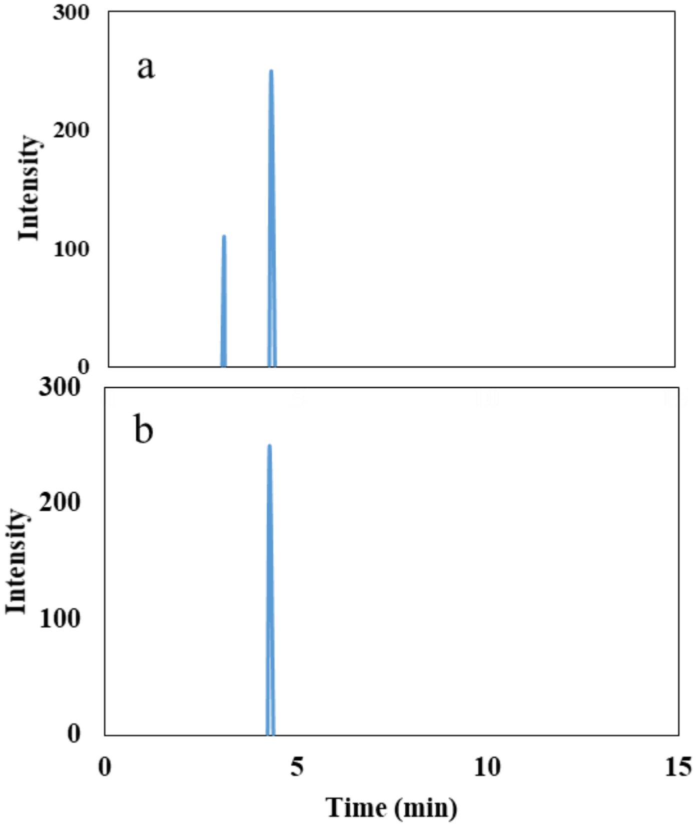


Figure 9. A chromatogram of the analyte before (a) and after (b) contact with MIP particles

4 Conclusions

This research focused on evaluation of MIPs as a sorbent for the selective recognition and adsorption for CLO. The CLO was firstly determined in aqueous by DPV using glassy carbon electrode modified with gold nanoparticles and graphene oxide. The interaction between the template and the monomer was demonstrated by FTIR, TGA and DPV. The maximum CLO adsorption capacity on MIP adsorbent in the optimum conditions, including pH, 8.5 and contact time of 89 minutes, is 280 mg/g. The adsorption selectivity experiments suggested that the MIPs exhibited high affinity toward CLO when compared with NIPs, even in the presence of other compounds. This high capacity indicates that synthesized MIP particles can be used to effectively reduce the residue of drug contamination in dairy samples.

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