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Antibiotic residuals removal via novel fabricated hydrogel from 2-hydroxyethyl methacrylate and sodium methacrylate

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ABSTRACT

In this study, poly(2-hydroxyethyl-sodium methacrylate) (p(HEMA-SMA)) hydrogels were synthesized as a novel adsorbent to remove antibiotic residues from environmental samples. [p(HEMA-SMA)] co-polymers were synthesized by the free radical photopolymerization method. Synthesized hydrogels were characterized by different methods such as Fourier-transform infrared spectroscopy (FTIR), elemental and scanning electron microscope (SEM), and surface area calculations. The average size surface area of the synthesized hydrogels were 1.515 µm. Penicillin G (Pen. G) was used as the sample antibiotic for the adsorption process. The absorption of the drugs was studied under different environmental conditions. Medium pH, temperature, and hydrogel concentration were varied to achieve the highest absorption. The specific adsorption value (Qmax) of p(HEMA-SMA) copolymers was found 303.03mg/g for Penicillin G at the 0,35 mg/mL of initial Pen. G concentration. In conclusion, we suggest a novel microstructure, selective, low-cost adsorption polymeric material for the removal of Pen. G as the template antibiotic.

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1. Introduction

Antibiotics are secondary metabolites or semi-synthetic or synthetic derivatives that can inhibit or kill pathogenic microorganisms. According to the classification based on their chemical structure, β-lactam antibiotics, macrolides, aminoglycoside, and tetracycline are the four main antibiotic groups. β-lactam antibiotics are the most extensively used for antibacterial activity with low side effects. β-lactam antibiotics involve in carbapenem, Pen.G, monobactams, andcephalosporins. β-lactam antibiotics have a common ring structure [16].

Pen. G is one of the most commonly used β-lactam antibiotics, which is used effectively for the prevention and treatment of bacterial infection. The versatility of Pen. G has made it a choice for many applications, including agriculture and human health. However, the widespread use of antibiotics in animal husbandry may lead to residues in food which can stimulate allergic reactions in some hypersensitive individuals [17][25].

Antibiotics are rapidly losing their potency due to overuse or wrong use in human and animal health, known as antibiotic resistance. There are three major mechanisms in bacteria that make themselves resistant to β-lactam antibiotics. The first mechanism of β-lactam resistance is the production of β-lactamase which degrades β-lactam antibiotics before they reach the targets. The third mechanism is to prevent the β-lactam antibiotic from reaching the target by altering the permeability of the outer membrane or increasing the efflux pump activity [16]. Antibiotics were used as antimicrobial drug and as a result of unnecessary, wrong, and excessive antibiotic using, through the pharmaceutical companies, hospitals, and municipal waste, many antibiotics waste passes the environment and water [6]. Antibiotic residues in environment may also be responsible for the increase of the risk of development and spread of antibiotic resistance, posing a potential threat to public health, since they can be released into the environment after their application [14]. Therefore, it is important to minimize the inappropriate use of antibiotics and to detect the antibiotic residues in wastewater, and animal products such as milk, meat, and eggs [20].

In recent years, antibiotics have been detected in the effluent of pharmaceutical companies and hospitals, municipal wastewater, surface water, and groundwater. For the

determination of samples, liquid and mass chromatograhy techniques are frequently used [11][22]. In order to protect the public health, a rapid, accurate, and specific method is requested for the isolation and determination of penicillin G in food such as milk and environmental water [19][23][1].

In the current study, microstructure, selective, lowcost molecularly adsorption polymeric systems were produced using Pen.G as a template molecule for leading to improved away from the antibiotics. Firstly, Pen.G adsorption poly (2-hydroxyethyl methacrylate-sodium methacrylate) hydrogels were prepared by photopolymerization method.

Fourier-transform infrared spectroscopy (FTIR), elemental analysis, scanning electron microscopy (SEM), and surface area calculations were used for the characterization of hydrogels. Optimization studies of penicillin G adsorption on p(HEMA-SMA) hydrogels was investigated at different conditions and adsorption isotherms were calculated. Selectivity, specificity, and reusability studies were performed. According to all those interrelationships, we aimed to develop a nanomaterial that can be used for several purpose such as determination, purification and removal of Pen. G.

2. Materials and method

2.1. Materials

Pen.G, was obtained from biochemistry department at Ege University. In addition, 2-hydroxyethyl methacrylate (HEMA), sodium methacrylate (SMA) ethylene glycol di-methacrylate (EGDMA), 2,2-Dimethoxy-2-phenylacetone-phenone (DMPA) and other chemicals were obtained from Sigma Chemical Co (st.louis, USA).

2.2. Synthesis p(HEMA-SMA) copolymer.

Prior to the polymerization process, the template HEMA (2.5 mL), SMA (100 mg in 2 mL DW), and EGDMA (0.1 mL) were mixed in 50 mL bottom flat flaks for 5 min. Then, 5.4 mL of DMPA(25 mg) solution added the mixture as the initiator in the beaker to initiate the polymerization reaction. The flask was kept under the ultraviolet and polymer formation was observed in 3-5 min [15].

The prepared hydrogels were washed several times with deionized water. After washing, it was dryed at 30 °C in the oven for 24 hours and stored for further utilization. was used as an adsorption agent so that the template molecule of Pen.G, could be removed from the hydrogel structure at 6 h. This treatment was continued until the penicillin G could not be determined in the adsorption solution [5][4],**Ошибка! Источник ссылки не найден.**,[9] (Eq. (1)):

$$\text{Removal of penicillin G \%} = \frac{C_i - C_f}{C_i} * 100 \quad (1)$$

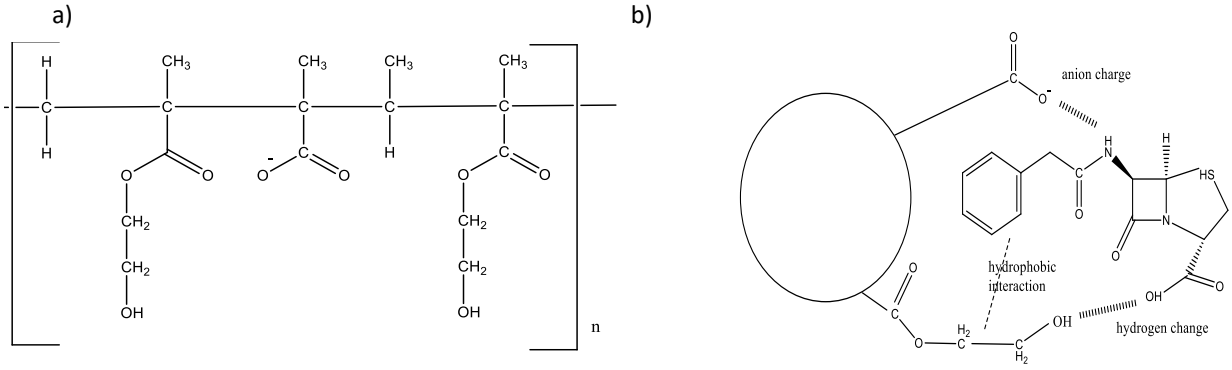

Figure 1. *a Molecular structure of p(HEMA-SMA).b. Penicillin G adsorption of p(HEMA-SMA) copolymer*.

C_{inital} initial concentration of penicillin G (ppm)

C_{final} the remaining amount of Penicillin G

Characteriation of penicillin G adsorption p(HEMA-MA) copolymers.For the chracterization of penicillin G adsorption p(HEMA-SMA) copolymers, SEM, FT-IR, elemental analysis, and surface area calculations were performed. Adsobtion studies of penicillin G p(HEMA-SMA) copolymers.

Pen.G adsorption on the p(HEMA-SMA) hydrogels were investigated with different parameters. The effects of time, pH, the initial Pen. G concentration, and temperature on adsorption were also investigated. For this reason, the initial concentration of Pen. G was changed between 100 and 350 ppm, and the pH of the solution was also changed between 2.0 and 12. For investigating of the effect of time, the experiments were performed in the range of 0 and 300 min and the experiments for the effect of temperature were studied in the range of 4 °C and 55 °C. At the end of the equilibrium time, the hydrogels were removed from the solutions with centrifugation. The adsorption amount of Pen. G was obtained by measuring the difference between the initial and the final Pen G concentration in the solution with UV spectrophotometry analysis (Fig. 2).

Pen.G measurements were monitored with UV-vis spectrophotometer analysis and 290 nm wavelength in UV-vis spectrophotometer detector was selected. The calibration graph was prepared in standard solutions (in water) at a range of 100 to 350 ppm.

The Q values were calculated with the following equation (Eq. (2)). It also should be noticed that all adsorption curves are averages of at least triplicated experiments.

$$Q=[\frac{(C_i-C_f)}{m}]*V \quad [\frac{Pen.G\ (mg)}{dry\ hydrogel\ (g)}] \tag{2}$$

Q is the hydrogel adsorption capacity of Pen. G on p(HEMA-SMA) hydrogels (mg/g), C_i is the initial concentrations of Pen. G in the solution (ppm), C_f is the final concentration of Pen. G in the solution (ppm), and V is the volume of the aqueous phase (mL). The calibration curve was prepared with the Pen. G solution concentration between 100 and 350 ppm.

2.4. Adsorption isotherms

Adsorption isotherm is used to characterize the interaction of each molecule with the adsorbent. The adsorption isotherm provides an association between the concentration of molecules in the solution and the amount of adsorbed molecules in the solid phase when the two phases are in equilibrium. In this context, Langmuir and Freundlich adsorption isotherms were calculated.

The Langmuir adsorption model assumes that there is a certain number of defined adsorption sites, each of which is capable of binding only one molecule. It is assumed that the energy levels of these regions are equal and far away from each other by the adsorbed molecules. The Langmuir adsorption model is defined as follows (Eq. (3)):

$$Q=\frac{Q_{max}bC_d}{(1+bC_d)} \tag{3}$$

Here, Q is the amount of Pen. G removed (mg/g), C_d is the equilibrium concentration (mg/L), b is the Langmuir constant (mL/mg), and Q_{max} is the maximum adsorption capacity (mg/g). By linearizing of the equations,

$$Q = [\frac{1}{Q_{max}}] = [\frac{1}{Q_{max}b}](\frac{1}{C_d}) \tag{4}$$

equality is achieved. When $[1/C_d]$ is plotted against to $[1/Q]$, the y axis of the line gives the cut point $[1/Q_{max}]$ and the slope of the line gives $1/Q_{max}.b$.

The Freundlich adsorption model, which is another commenly used adsorption model, accepts the exponential adsorption system in contrast to the Langmuir adsorption model. After the initial surface adsorption, it shows the strong soluble-soluble interaction with the condensation effect.

The Freundlich adsorption model is defined as (Eq. (5)):

$$Q = K_F C_{eq}(\frac{1}{n}) \tag{5}$$

Here, K_F and n are the Freundlich constants. $1/n$ indicates the surface heterogeneity and takes values ranging from 0 to 1. K_F, Freundlich constant. The adsorption constant, which indicates the size of the adsorption capacity, depends on the temperature, the adsorbent, and the adsorbed compound. n, Freundlich constant, is degree of adsorption which shows the severity of adsorption. As the value approaches, surface heterogeneity increases [21], [7] [2]. By linearizing the logarithm of the equations:

$$\ln Q=\ln K_F+(\frac{1}{n})\ln C_{eq} \tag{6}$$

equality is achieved. When $\ln C_{eq}$ is plotted against $\ln Q$, $\ln K_F$ is the value ofcut off point of the y-axis and (1/n) is the value of slope of the line.

The Temkin isotherm assumes linear decrease of heat adsorption while ignoring extremely low and very high concentration. It also assumes uniform distribution of bounding energy up to some maximum bonding energy. It is expressed by Eq. (7) below.

$$q_e=(\frac{RT}{b})\ln AT+(\frac{RT}{b})\ln C_e \tag{7}$$

where q_e is the amount of adsorbate adsorbed at equilibrium (mg/g), C_e is concentration of adsorbate in solution at equilibrium (mg/L).

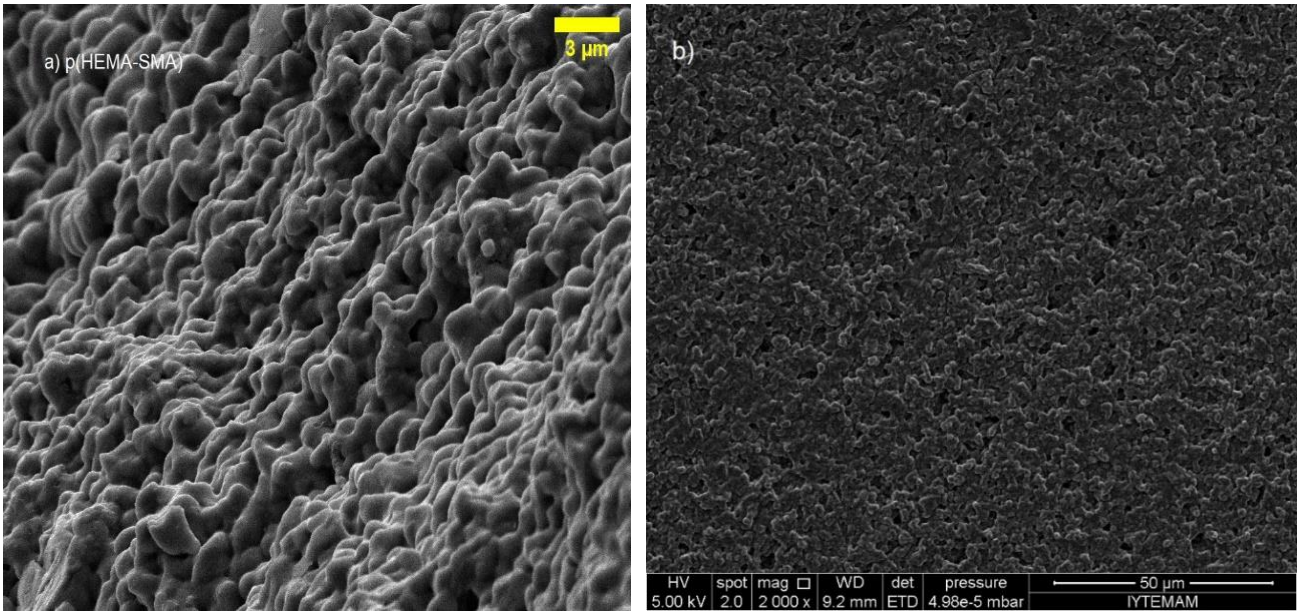

Figure 2. *The SEM images of the p(HEMA-SMA) copolymers with (in the scale of 3 µm), b (in the scale of 50 µm)*

B is a constant related to the heat of adsorption and it is defined by the expression B = RT/*b*, *b* is the Temkin constant (J/mol), *T* is the absolute temperature (K), *R* is the gas constant (8.314 J/ mol K), and *A* is the Temkin isotherm constant (L/g). From the plot of q_e vs. lnCe, *B* and *A* can be calculated from the slopes (*B*) and intercepts (BlnA) respectively [10].

3. Results

Adsorption yield of the Pen. G p (HEMA-SMA) copolymer.

After adsorption at 6 hr, temperature 40 °C removal of Pen. G 69%, utilizing the equation (Eq. (1)).

Characterization of p(HEMA-SMA) copolymers.

In this study p(HEMA-SMA) copolymers were characterized using FTIR, elemental and SEM, and surface area calculations.

The morphological structure of p(HEMA-SMA) copolimers was determined by SEM. As shown in Fig. 2, penicillin p(HEMA-SMA) hydrogels include spherical particles.

As seen in the FTIR spectrum (Fig. 3), the p(HEMA) (A) and p(HEMA-SMA) (B) structures have characteristic hydrogen-bonded alcohol, -OH,

tensile vibration band around 3451 cm−1. By way of the incorporation of the SMA monomer into the polymer structure, the intensity of the tensile vibration band of the hydrogen-bonded alcohol, OH, increased according to the p(HEMA). This may be due to the fact that -C-O^- stretching vibrations in the structure of the p (HEMA-SMA) copolymer also have vibrations around 3300– 3400 cm−1. FTIR spectrum of the p(HEMA-SMA) has -C-O^- stretching vibrations around (1277-1160) cm^{-1}. However, this vibration is not seen in the FTIR spectrum of p(HEMA). This is also an indication that the SMA co-monomer incorporate into the polymer structure. In addition, the extra -C═O groups from the SMA monomer in the p(HEMA-SMA) structure caused the -C═O stretching vibration observed around 1728 cm^{-1} in the p(HEMA-SMA) structures severely.

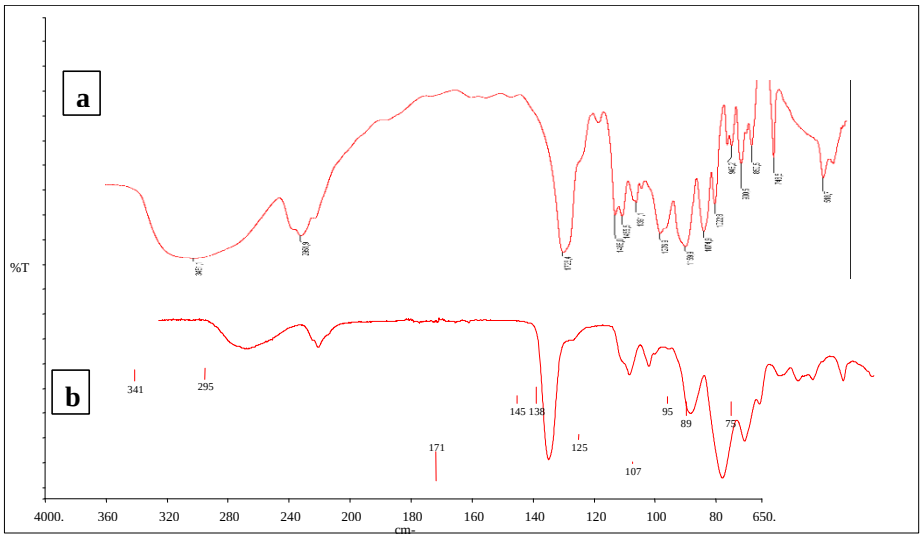

Figure 3. *FTIR spectra of p(HEMA-SMA) copolymer, b p(HEMA) co-polymers.*

The time-dependent Pen.G adsorption on p(HEMA-SMA) copolymers was shown in Fig. 1b. As seen from Fig. 6, the adsorption amount of Pen.G was increased by the time of progress, and the saturation levels were reached within 300 min. Also, the maximum adsorption of Pen.G was found as 29.93 mg/g. The effect of the initial concentration of penicillin G on the adsorption of Pen.G on the copolymer is given in Fig. 7. As seen from the graph, the amount of Pen.G adsorbed per unit mass by the micropolymers increased with increasing concentration of Pen.G in the solution. Also, at a concentration of about 350 ppm Pen.G, it reached a high adsorption capacity. In addition, 29.93 mg/g Pen.G adsorption at the initial concentration of 0.350 mg/mL penicillin G per unit mass of the p(HEMA-SMA) copolymer is associated surface area of the microstructure.

Also, the homogeneous distribution of molecular specific wells to Pen.G and the anionic character of the SMA functional monomer significantly increased the Pen.G adsorption of the copolymer by increasing the affinity to Pen.G.

Increasing the initial concentration of Pen.G raises the concentration difference (ΔC), which is the driving force for adsorption. Furthermore, Pen.G adsorption capacity is also increased by the increase of the driving force.

When the concentration-dependent adsorption curve is examined, it appears that the adsorption is compatible with the Freundlich model. The Freundlich isotherm is derived by assuming exponentially increasing adsorption, with adsorption where the hydrophobic interaction predominates, and multiple adsorption behavior is expected [21].

The change in adsorption capacity of the Pen.G copolymer is given in Fig. 6. The effect of Pen.G adsorption on the temperature was studied at (4–55) °C. It was observed that from the obtained graph, the Pen.G adsorption capacity of the co-polymer increased until 40 °C with increasing temperature values.

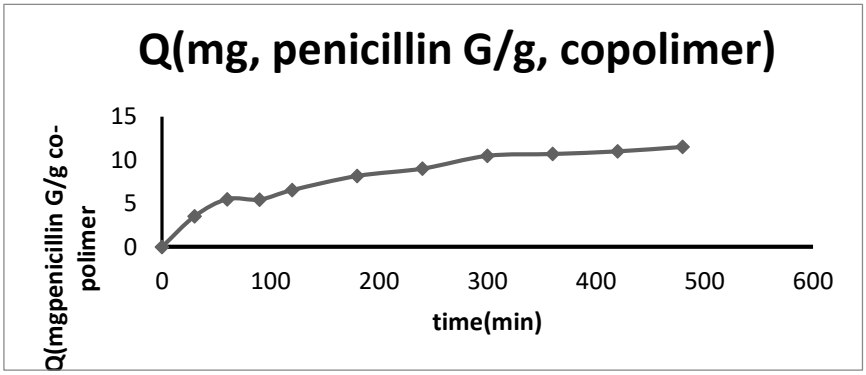

Figure 4. *The effect of time on Pen.G adsorption ($C_{initial}$: 350ppm Pen G; pH= 2.0 acid buffer; 20 °C)*

According to the [ΔG = (ΔH-TΔS)] theory, the interaction increases with temperature. Where ΔH can be positive or negative, control of ΔG is achieved by a positive entropy change. For this, increasing entropy is achieved with heat. However, there are also weak interactions with hydroxyl groups on the HEMA surface, such as hydrogen bonds and van der Waals interactions. The van der Waals interaction forces seen in hydrophobic interactions have also been observed in other studies that have been associated with increased temperature [8]. With this theory, the raising Pen. G adsorption of the co-polymer with increasing temperature up to a certain temperature can be explained. However, penicillin G adsorption is decreased at high temperatures after 40 °C. It is known that the lactam ring of beta-lactam antibiotics is chemically very unstable and is not resistant to acid, temperature, and beta-lactamase enzyme [26].

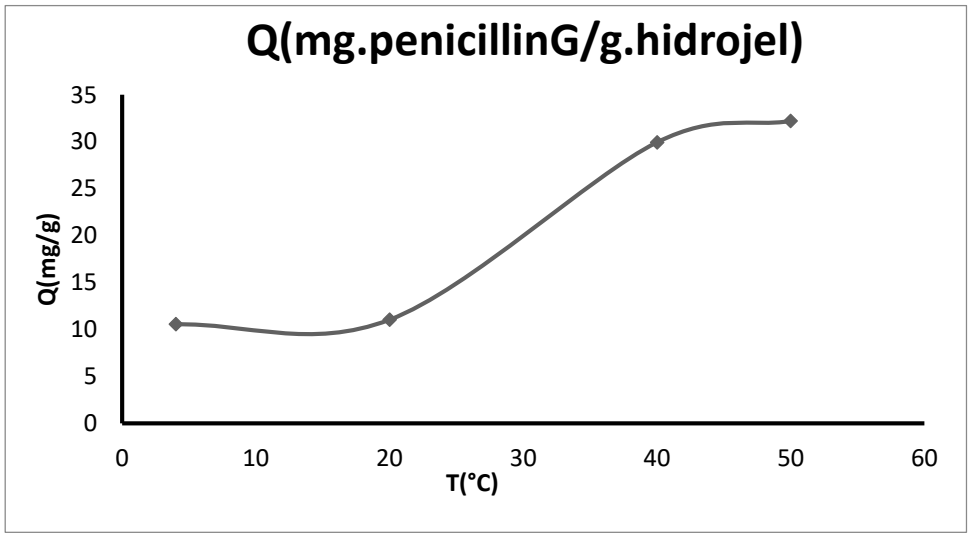

Figure 5. *The effect of initial concentration on Pen G adsorption (pH = 2.0 acid buffer; 20 °C; 300 min)*

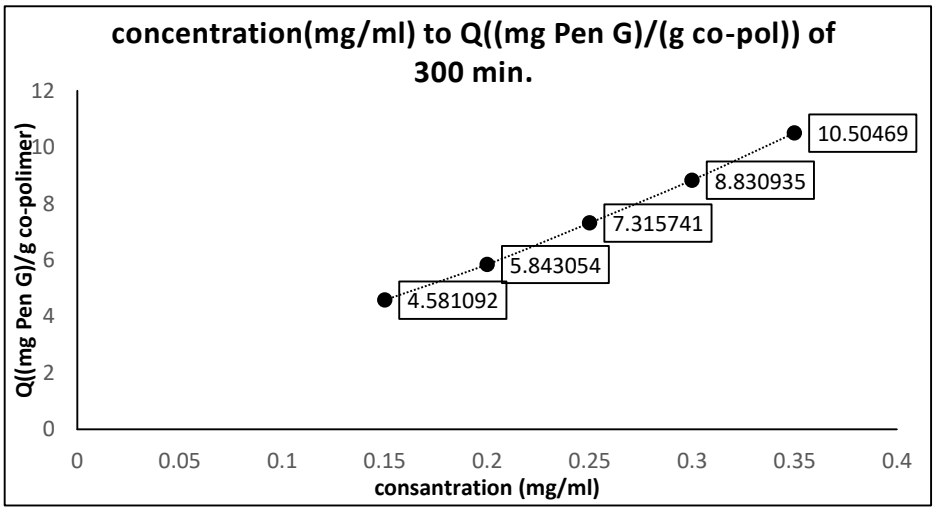

Figure 6. *Effect of temperature on Pen G adsorption ($C_{initial}$: 0.35 mg/mL Pen G; pH=2.0 ortho-phosphoric acid buffer; 300 min)*

In connection with this, the decrease in adsorption of penicillin G in the copolymer is significant due to the β-lactam ring which may be unstable and degradable at high temperatures.

As a result, shown in the graph, the effect of temperature change on penicillin G adsorption of the copolymer can be supported thermodynamically.

The apparent increase in penicillin G adsorption capacity on synthesized copolymers with increasing temperature and followed by a decrease in adsorption by degradation of penicillin G structure at high temperatures indicates and supports binding between the hydrophobic ligand alanine and penicillin G. The apparent increase in penicillin G adsorption capacity on synthesized co-polymers with increasing

temperature and followed by an increase in adsorption of penicillin G at high temperatures indicates and supports binding between the hydrophobic ligand alanine and penicillin G.

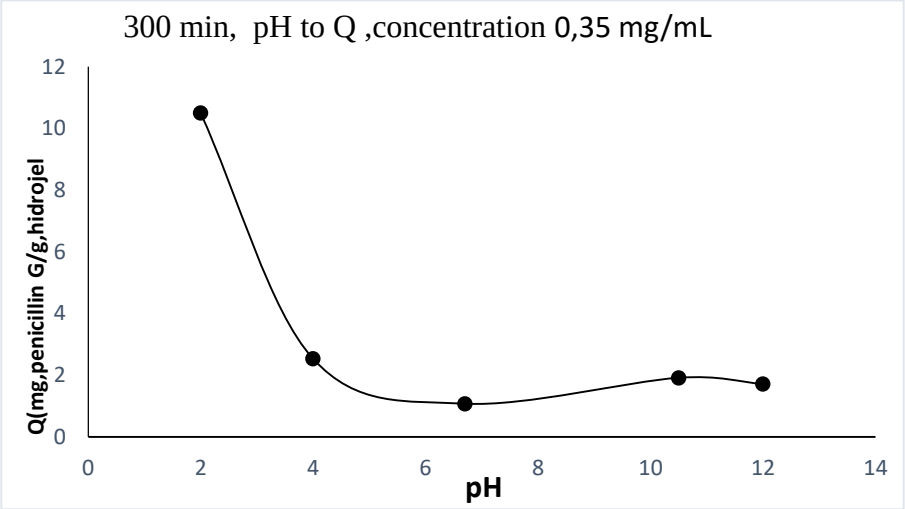

Figure 7. *Effect of pH on Pen G adsorption ($C_{initial}$: 0,35 mg/mL; pH 2.0, 4.0, 6.7,10.5,12.0 ortho-phosphoric acid buffer, 20 °C; 300 min*)

3.1. Adsorption isotherms

The adsorption isotherm of the penicillin G p(HEMA-SMA) copolymer was determined from the initial adsorption effect of penicillin G concentration. The results of the calculations show that the correlation value R^2 = 0.9948 indicates that the adsorption corresponds to the Freundlich adsorption model.

When the concentration-dependent adsorption curve is examined, it is seen that the adsorption is compatible with the Freundlich model. The classical Freundlich adsorption model accepts the exponential adsorption system as opposed to the Langmuir adsorption model. After the initial surface adsorption, it exhibits a strong solute-solute interaction with the condensation effect. The Freundlich isotherm is derived assuming an exponential increasing adsorption, in other words, multiphase adsorption behavior is expected for

As seen in Fig. 7, penicillin G adsorption of p(HEMA-SMA) co-polymer is pH dependent. Penicillin G adsorption capacity of the co-polymer increases as the acidic region moves to the basic region. The maximum penicillin G adsorption was found to be 10.50 mg/g in pH 2.0 ortho-phosphoric acid buffer. heterogeneous amorphous surfaces [21] [2]. The 1/n coefficient in the equation shows this feature. There is heterogeneous distribution of discrete interaction regions.

Freundlich equation is an exponential function of the amount of adsorbent adsorbed on the adsorbent surface. The Freundlich isotherm is generally used for adsorption from liquid solutions. Freundlich isotherm is based on approaches and can be thought of as the sum of the distributions of Langmuir adsorption isotherms. Freundlich isoterm does not predict saturation of the bonding surface by solvated [3], [7].

Adsorption of antibiotics, steroids, and hormones onto commonly used adsorbents follows this isotherm. Reversed phase and hydrophobic interaction type adsorption are usually followed by Freundlich type isotherm. Like the Langmuir equation, the Freundlich isotherm equation does not apply Henry's law at low concentrations and a stable constant adsorbent value cannot be obtained after equilibration [18]. In the initial

Table 1. *Langmuir, Freundlich and Temkin adsorption isotherm constants*

experimental	**Langmuir constants**			**Freundlich costants**			**Temkin costants**			**Shape of izotherm**
Q_{ex}(mg/g)	Q_{max}(mg/g	b(ml/mg)	R^2	K_f	n	R^2	A(L/g)	B(j/mol)	R^2	0<R<1
29.93	303.03	0.0033	0.9933	0.04709	1.0098	0.9948	1.00	347.435	0.9761	

4. Conclusions

In this study, a HEMA-based micro-size, the highly selective, environmentally friendly, and cost-effective polymeric system has been developed for the first time in the literature. These copolymers have the potential to use direct determination and purification of penicillin G which is widely used for antimicrobial agents or growth factors in animal breeding from environmental wastes and food products. Antibiotic residue analysis is a problem in countries in terms of public health. In this study, the synthesized copolymer, which has a molecular memory, can be used to separate, purify, and determine the target molecule penicillin G from environmental wastes and food products. Moreover, the copolymer provides reasonable specificity, ease, reliability, and quick response to the specific molecule. This polymeric system is a convenient method with high adsorption capacity, allowing easy and rapid analysis without the need for a ligand system. In this context, it is necessary to use only the advantageous aspects of the use of antibiotics which is an integral part of public health to detect the essential removal of adverse effects and to minimize antibiotic residues in environmental pollution. The synthesized copolymers can provide a promising method for control of the different pharmaceutical residues caused by the use of high doses of antibiotics.

(Table 1). Temkin equation is excellent for predicting the gasphase equilibrium, conversely complex adsorption systems including the liquid-phase adsorption isotherms are usually not penicillin G concentration experiment, *Q*max was obtained as 303.03 mg/g; in the Freundlich adsorption model equation, the *n* value was calculated as 1.0098 and the *K*f value as 0.04709 appropriate to be represented [7]. In the Temkin adsorption model equation, the *A* value was calculated as 1.00 and the *B* value as 347.435 and R^2 as 0.9761 (Table 1).

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