Adsoption of Cationic Dye on Anionic Hydrogel and Its Second Use for Drug Delivery with Antibacterial Properties

Katyonik Boyanın Anyonik Hidrojel Üzerinde Adsorbsiyonu ve Antibakteriyel Özelliklerle İlaç Uygulamasında İkinci Kullanımı

Research Article

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ABSTRACT

In this work, a novel stimuli-responsive p(EPMA-co-AMPS) hydrogels containing 2,3-epoxypropylmethacrylate and 2-acrylamido-2-methyl-1-propanesulfonic acid were prepared through free radical copolymerization. In addition to the swelling properties of the obtained hydrogels, surface morphology, chemical and mechanical properties are fully characterized using SEM, FTIR and TPA. Firstly, resulted hydrogels were carried out for removal of cationic dyes from aqueous solution in environmental applications. The adsorption isotherms and kinetics of hydrogels were in good agreement with Langmuir equation and the pseudo-second-order equation, respectively. In addition, the adsorption capacity of p(EPMA-co-AMPS) hydrogels compete with other currently reported adsorbents. Secondly, the use of hydrogel for controlled drug release studies in biomedical applications has been investigated as a drug carrier. Finally, drug-loaded hydrogels were tested for antibacterial activities against Gram-positive bacteria (Bacillus cereus) and Gram-negative bacteria (Salmonella typhimurium) and demonstrated antibacterial activity. Results obtained from this study suggest that the resulted hydrogel could be promising materials for various applications.

Key Words
Hydrogels, dye, drug delivery systems, adsorption, antibacterial activity.

ÖZ

Bu çalışmada, serbest radikal kopolimerizasyonu ile 2,3-epoksipropilmetakrilat ve 2-akrilamido-2-metil-1-propansülfonik asit içeren yeni bir uyarana duyarlı p (EPMA-co-AMPS) hidrojeller hazırlandı. Elde edilen hidrojellerin şişme özelliklerinin yanında yüzey morfolojisi, kimyasal ve mekanik özellikleri SEM, FTIR ve TPA kullanılarak tamamen karakterize edilmiştir. İlk olarak, çevresel uygulamalarda sulu çözeltiden katyonik boyaların uzaklaştırılması için hidrojeller kullanılmıştır. Hidrojellerin adsorpsiyon izotermleri ve kinetikleri, sırasıyla Langmuir denklemi ve yalancı-ikinci mertebeden denklem ile iyi bir uyum içindedir. Ayrıca, p (EPMA-co-AMPS) hidrojellerin adsorpsiyon kapasitesi, halihazırda bildirilmiş olan diğer adsorbanlarla karşılaştırıldığında rekabet etmektedir. İkinci olarak, biyomedikal uygulamalarda kontrollü ilaç salımı çalışmaları için hidrojelin bir ilaç taşıyıcısı olarak kullanımı araştırılmıştır. Son olarak, ilaç-yüklü hidrojeller Gram-pozitif bakterilere (Bacillus cereus) ve Gram-negatif bakterilere (Salmonella typhimurium) karşı antibakteriyel aktiviteler için test edilmiş ve antibakteriyel aktivite göstermiştir. Bu çalışmadan elde edilen sonuçlar, hazırlanan hidrojelin çeşitli uygulamalar için umut verici materyaller olabileceğini göstermektedir.

Anahtar Kelimeler
Hidrojeller, boya, ilaç taşıyıcı sistemler, adsorpsiyon, antibakteriyel aktivite.

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INTRODUCTION

Hydrogels which are composed of hydrophilic homopolymer or copolymer network can absorb a large amount of water are also considered as smart materials due to their ability to sense pH , temperature, electrical and magnetic fields and etc. [1-4]. Stimuli-sensitive polymers have gained great interest recently in the design of intelligent materials for multi purposes such as controlled drug delivery systems, adsorbent for dye removal, heavy metal adsorption, solute separation, artificial organs and enzyme immobilization [5-10]. Typical examples of these polymers involve poly(acrylic acid), poly(N-isopropylacrylamide), poly(2-acrylamido-2-methyl-1-propanesulfonic acid)(PAMPS), poly(2-(dimethylamino) ethylmethacrylate) [11-13].

The pH responsive hydrogels are extensively studied in recent years [1, 14-16]. Within the group of pH hydrogels, PAMPS hydrogel is known for their reversible swelling/deswelling behavior in response to pH changes. PAMPS is a typical anionic component with sulfonate groups which is ionized in the higher pH area and negatively charged. In consequence, PAMPS, become expanded because of the increased osmotic pressure. The hydrogels derived from AMPS, exhibit swelling behavior depending on pH. Therefore, high swelling capacity and water-absorbing when AMPS is presented to a hydrogel are also well-suited for unique applications such as controlled drug delivery system and dye removal [17-19]. In addition, epoxypropyl methacrylate (EPMA) polymerizes practically as well as other vinyl monomers in aqueous and non-aqueous systems. Because of their functional groups of epoxy and methacrylate, EPMA containing copolymers have led to an interesting class of new materials. Epoxy groups can react with active hydrogen-containing groups such as amino, hydroxyl and carbonyl groups [20].

In this study, a novel pH sensitive hydrogel composed of EPMA and AMPS was prepared by free radical polymerization method using MBA as the cross-linking agent. The hydrogels were characterized by swelling behavior, FTIR, SEM, TPA and elemental analysis techniques. Hydrogels. have been shown to be very useful in various applications as an adsorbing agent and drug carrier. The experimental data were also examined by applying the well-known isotherm and kinetic models. Furthermore, these hydrogels were used as drug carrier in biomedical applications using GS as model drug. The loading and release amounts of GS were appraised by UV-Vis spectrometer (as a function of time at 324 nm) at pHs of 2.0 and 7.4 at 37 -C. Finally, the antibacterial activity of hydrogels was studied against E. coli by disk diffusion method.

MATERIALS and METHODS

Materials

The monomer 2,3-epoxypropylmethacrylate (EPMA) (Merck), comonomer 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) (99%, Sigma), initiator ammonium persulfate (APS) (99%, Sigma) and crosslinker N,N'methylenebisacrylamide (MBA) (99%, Sigma), accelerator N,N,N',N'tetramethylethylenediamine (TEMED) (99%, Merck) were used in the hydrogel preparation. Methylene Blue (MB) (82%, Sigma) was used as model dye for the batch adsorption experiments. The model drug Gentamicin Sulfate (GS) was purchased from a local medical store.

Instrumentation

The structural characterization of the hydrogels was performed by ATR technique using a Thermo Nicolet, IS 10 spectrometer with 4 cm^{-1} resolution between 4000 and 650 cm^{-1}. The SEM images of the particle were acquired from powder samples deposited on carbon tape attached on aluminum SEM stubs after Au coatings to a few nm under vacuum with SEM (Jeol JSM-5600 LV) operating with 20 kV. The uptake of dye amount and the amount of drug released were measured at UV 1800 spectrophotometer, Shimadzu. Elemental (C, N, H) analysis of the hydrogels was carried out by Thermo Flash 2000 C, H, N, O analyzer. A 5 kg load capacity texture analyzer, (TA texture analyzer plus XT Stable Micro System, Godalming, Surrey, UK) was used to measure the various mechanical strengths of the hydrogels.

Synthesis of p(EPMA-co-AMPS) Based Hydrogel

Cross-linked p(EPMA-co-AMPS) hydrogels were synthesized in a redox polymerization technique. In brief; in a typical one pot one-to-one (1:1) mol ratio of EPMA to AMPS hydrogel

synthesis: 0.0038 mol EPMA was dissolved in 0.5 mL isopropyl alcohol and 1 mol % MBA with respect to total monomer amounts was added. After vortex mixing an equal amount of AMPS in 1 mL water was added to solution and mixed to obtain isotropic clear solution. Polymerization was initiated by addition of 1 mL of 1 mol % APS solution in water with respect to total monomer amount and 50 μLTEMED. The solution was placed in plastic pipette with 5 mm diameter and then placed in temperature controlled oil bath at 45°C for 20 min. to complete the reaction. The hydrogels were taken out from the pipets and put in double distilled water for 36 hours in order to remove the unreacted species. The water was replaced every 6 hours. Then the hydrogels were dried at 25 -C till they attained constant weight. Finally, they were kept in sealed containers for swelling studies, characterization and application of experiments [21].

Swelling Studies of p(EPMA-co-AMPS) Hydrogels

The swelling properties of the hydrogels were performed by gravimetric analysis. Briefly, the dry hydrogel was immersed in the swelling medium at room temperature, (~30°C). After attaining the equilibrium, the gels were removed from the medium; the weight of the swollen hydrogel was determined after the removal of the surface water through blotting by filter paper. The percent equilibrium swelling ratio was calculated by the following equation 1:

$$\%S_{eq} = \frac{(W_{eq} - W_0)}{W_0} x100 \quad (1)$$

Where; W_{eq} is the weight of swollen gel at equilibrium, W_0 is the weight of dry gel and $\%S_{eq}$ is the swelling ratio.

Adsorption Studies of Hydrogels

Adsorption of dyes from aqueous solutions was investigated in batch system. Effects of the initial concentration of dye, effect of contact time on adsorption mechanism and also adsorption rate and capacity were studied. Aqueous solutions (50 mL) containing different concentrations of MB (in the range of 50-500 mg/L) were treated with 0.1 g p(EPMA-co-AMPS) hydrogel at room temperature until the adsorption reached to equilibrium. After equilibrium, the concentrations of MB remaining in the solution were analyzed by UV-visible spectrometer at wavelength of 664 nm for MB and maximum adsorption capacity of the hydrogel was measured. Adsorption capacity, Q_e, is the maximum dye amount adsorbed per gram of the dry hydrogel (mg MB/g dry hydrogel) at equilibrium. It was calculated from the following mass balance equation:

$$Q_e = \frac{(C_0 - C_e)V}{W} \quad (2)$$

Where; C_o and C_e are concentrations of the MB in the aqueous phase before-adsorption and after-adsorption at equilibrium, respectively (mg/L); V is the volume of the aqueous phase (L), and w is the weight of the dry hydrogel used (g).

Drug Loading and Release

0.1 g of the hydrogels were placed in drug (GS) solution (125 mg/L) at medium temperature and equilibrated for 3 days to fully absorb of drug. After loading, drug loaded hydrogels were washed with distilled water. To study the release of drugs, drug loaded hydrogels were immersed in 10 mL of buffer solutions at 37 - C (pH 2.0 and 7.4). After regular time-intervals, hydrogel was transferred into fresh release medium, and the amount of drug released was determined spectrometrically at 255 nm. The amount of drug was calculated using calibration plot obtained for drug solutions of known concentrations.

Antibacterial Study

Antibacterial activity of the hydrogels was tested against both Gram-positive bacteria (Bacillus cereus ATTC 14579) and Gram-negative bacteria (Salmonella typhimurium ATTC 14028) according to the disk diffusion test. For agar diffusion method, hydrogel sample was exposed to bacteria on nutrient agar, and the inhibition areas around each sample was measured and recorded as the antibacterial effect of the GS loaded and pure hydrogel. The agar plates were inoculated with about 100 μL suspensions of the bacteria. Swelled hydrogels were placed on the agar plate and incubated at 37°C for 24 h. Inhibition zone for bacterial growth was detected by disk diffusion model.

RESULTS AND DISCUSSION

Synthesis and Characterization of p(EPMA-co-AMPS) Hydrogel

In this work, new anionic hydrogels containing EPMA and AMPS were synthesized using APS and TEMED as a redox initiator system by free radical cross-linking copolymerization in aqueous solution. EPMA, a non-ionic monomer, contains dual functionality resulting from the presence of both methacrylate and epoxy groups in the same molecule. Otherwise, AMPS contains easily ionizable sulfonic acid group. A possible schematic representation for the p (EPMA-co-AMPS) hydrogel in synthesis reaction involving both functional groups is shown in Figure 1.

SEM images ensure great useful information about the formation of the hydrogel internal structure, which is cross-linked with MBA and the efficiency of loading the dye particles on the network. Figure 2 shows that the surface morphology of the hydrogels and the dye-loaded hydrogels. The hydrogel has a highly porous and crosslinked structure with more uneven density as seen in Figure 2a. The morphology of the hydrogel is not a homogeneous structure due to the high hydrophilicity of the gel. Comparing internal structure of the hydrogel network before and after absorption of dye, we found that the internal structure of the dye loaded hydrogels became rigid and displayed a relatively smooth surface in Figure 2b. The reason being that hydrogel pores are filled with dye molecules.

For identification of the hydrogel, infrared spectroscopy was used. The FTIR spectra of the dried crosslinked p(EPMA-co-AMPS) hydrogels is shown in Figure 3. In these situation of p(EPMA-co-AMPS) copolymer, the peak at 1648 cm^{-1} was attributed to the stretching vibration of C=O double bond, and that at 1457 cm^{-1} was the vibration of C-N

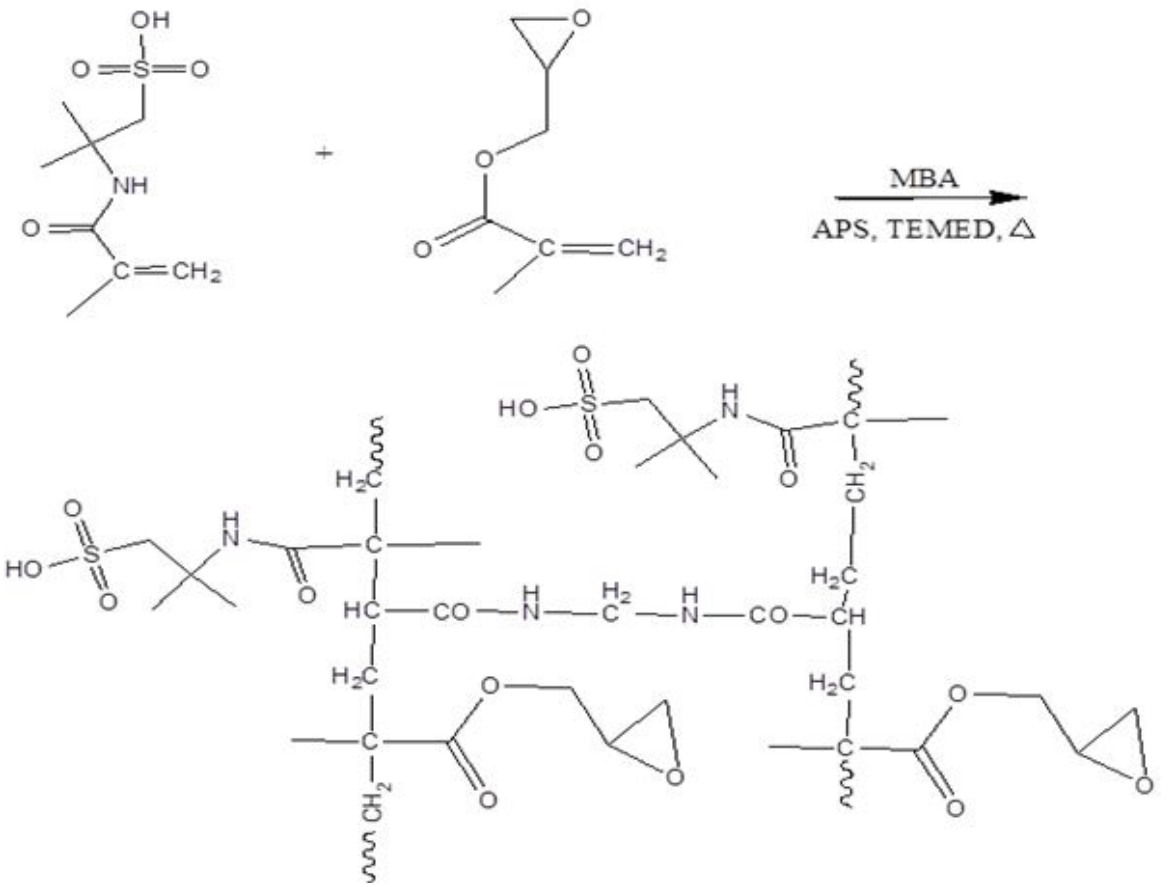

Figure 1. The schematic representation of the synthesis of p(EPMA-co-AMPS) hydrogel.

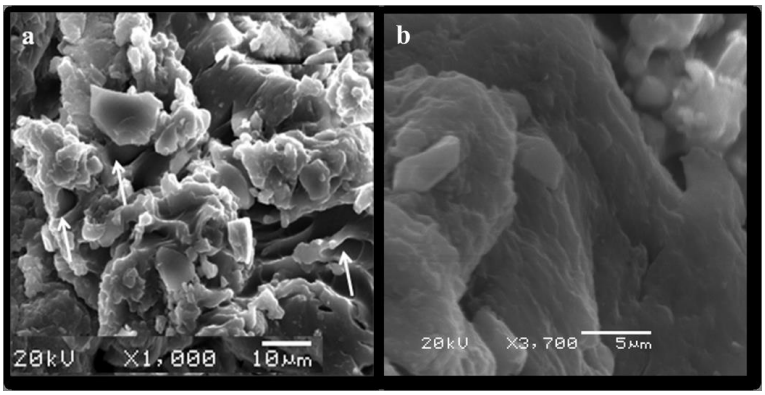

Figure 2. (a) SEM image of p(EPMA-co-AMPS) hydrogel and (b) p(EPMA-co-AMPS) hydrogel adsorbed MB.

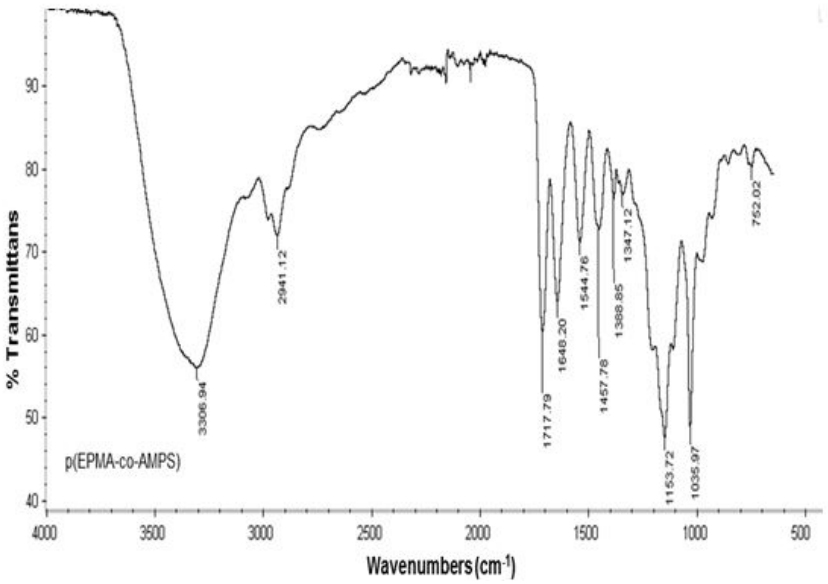

Figure 3. FTIR spectrum of p(EPMA-co-AMPS) hydrogel.

band which are the primary amide carbonyl group peaks of AMPS. Also, the characteristic adsorption peak of AMPS at 1035 and 1153cm^{-1} corresponded to attached the symmetric and asymmetric vibration of S=O bond of SO_3^- groups, respectively. The peak at 1720, 900 and 752 cm^{-1} and indicates C=O stretching vibration of EPMA ester group and epoxy group present in the backbone, respectively. for each individual peak compared with all the other peaks is in compatible with the stretching frequencies of the functional groups in FTIR atlas which provide evidence that the reaction of EPMA and AMPS. The elemental analysis results for mass percentage values of C, H, N and S amounts in p(EPMA-co-AMPS) were 43.65%, 7.11%, 4.21% and 5.96%, respectively. The results obtained from elemental analysis support the FTIR results for the polymers which were shown in Figure 3.

One of the most important point in various applications such as environmental and medical is swelling behavior of the adsorbent. The swelling behavior of sensitive hydrogel materials can be affected by salt-sensitivity and pH of media. Swelling capacity of hydrogel is investigated for distilled water (DW) and dye (MB) solution in graphic which is plotted swelling ratio versus of time in Figure 4. As can be clearly seen, firstly, the rate of water uptake of dry hydrogels in distilled water remained sharply within 150 minutes and later took place the stable mode. The hydrogels were reached their maximum swelling capacity in 270 min. and they absorbed water about 500% by mass. Furthermore, it was observed that the equilibrium swelling capacity of hydrogel was higher in DW than in dye solution. The reason being that the decreasing in the available spaces for water retention due to the filling up of

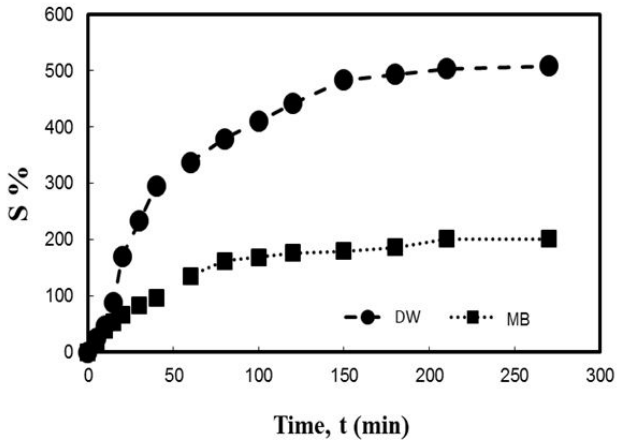

Figure 4. Swelling rate of p(EPMA-co-AMPS) hydrogel in DW and MB solution.

the dye molecules was responsible for reducing the water absorption rate.

Figure 5 indicates the swelling behaviors of hydrogel samples in various potential medium at room temperature. Also these hydrogels were treated with distilled water, tap water, lake water (from Lake Van in Turkey) and MB dye solution for 24 h. The results showed swelling of hydrogels vary between 200-500% by mass. It can be seen from the Figure 5 the hydrogel has the lowest water uptake capacity in lake water due to the salinity media compared to the tap water while has the highest water uptake capacity in distilled water. The less water absorbing capacity of the hydrogel can be explain with the presence of dissolved anions and cations in tap water, lake water and dye solution.

Figure 6 indicates pH sensibility of the equilibrium swelling ratio for hydrogels at room temperature in distilled water from pH 2.0 to 12.0. P(EPMA-co-AMPS) hydrogels are ionic in nature so their swelling behavior depends on pH of the medium. As appeared in this Figure, the equilibrium mass swelling percentage for pure EPMA is not influenced by changing the pH of the swelling medium due to the fact that EPMA is non-ionic hydrogel and does not have any group that could be ionized in an aqueous solution [21]. With the overview of the AMPS groups which contains ionic ($-SO_3^-$) units into the main chain, the swelling ratios are increased as the pH values of solutions are enhanced. It can be concluded that the hydrogels revealed higher swelling capability in a basic media while lower swelling capability in an acidic media. It depends on the truth that the sulfonate groups could accept or release protons in response to aqueous media of various pH. Thus, in this case, the charges on polymeric side chains are lower than that in the distilled water, whence the swelling ratios in this low pH solution are smallest [22]. In basic medium, the degree of substitution of protons decreases, and the

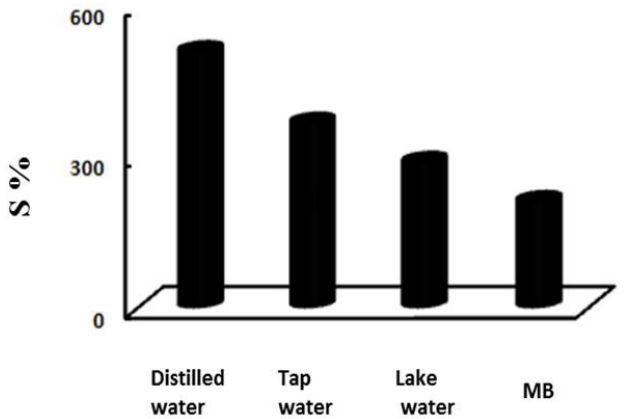

Figure 5. Water absorbency of hydrogels in different medium.

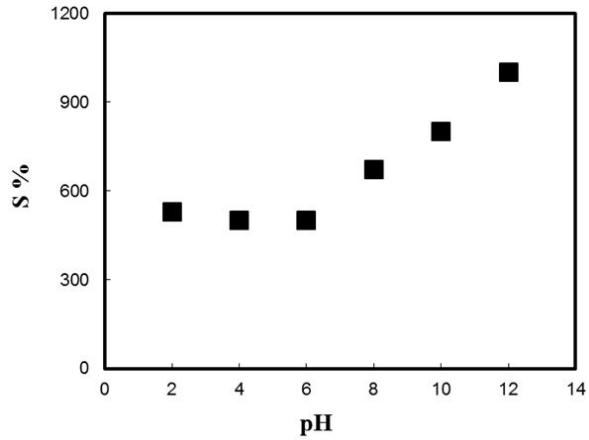

Figure 6. Effect of pH on water absorbency of p(EPMA-co-AMPS) hydrogel.

charges on the polymeric chains increase. Because of this situation the hydrogel expands and swelling capacity is gradually increases.

Dye Adsorption Studies

To further study the adsorption of MB, the hydrogel was placed in aqueous concentrated dye solutions and allowed to equilibrate for six days. At the end of this time, the aqueous solution of dye containing hydrogel showed almost colorless compared to the original dark colorations of dye solutions and the shape of hydrogel is bulgy due to the swelling. This situation can be explained by the stronger interactions between the active sites of adsorbent and adsorbate molecules. Electrostatic interaction is very dominant which can form between sulfonate group on the backbone of p(EPMA-co-AMPS) and quaternary nitrogen group of cationic dye and hence dye adsorption is better [21]. Digital image of color changes of dye solutions and the hydrogel during the adsorption process and a possible interaction between the hydrogel and the cationic MB molecule is presented in Figure 7 and Figure 8, respectively.

Adsorption of the MB on the hydrogel was investigated depending on the initial dye concentration, contact time and properties of the samples. The initial dye concentration is one of the important parameters in adsorption process due to determining the amount of dye adsorption by an adsorbent. The effect of the initial dye concentration factor depends on the immediate relation between the dye concentration and the available binding sites on an adsorbent surface [23]. The effect of initial concentration of MB was investigated by varying concentrations over the range of 50-500 mg/L and shown with Figure 9.

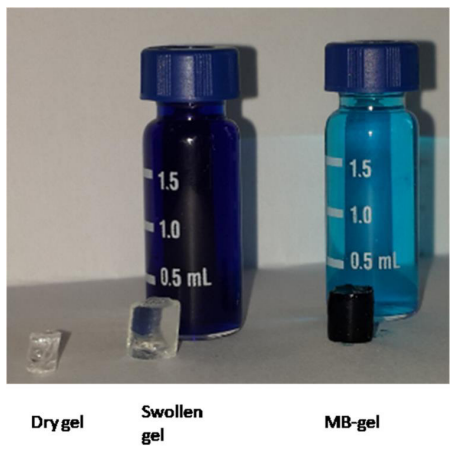

Figure 7. Digital image of color changes of dye solutions and p(EPMA-co-AMPS) hydrogel during the adsorption process.

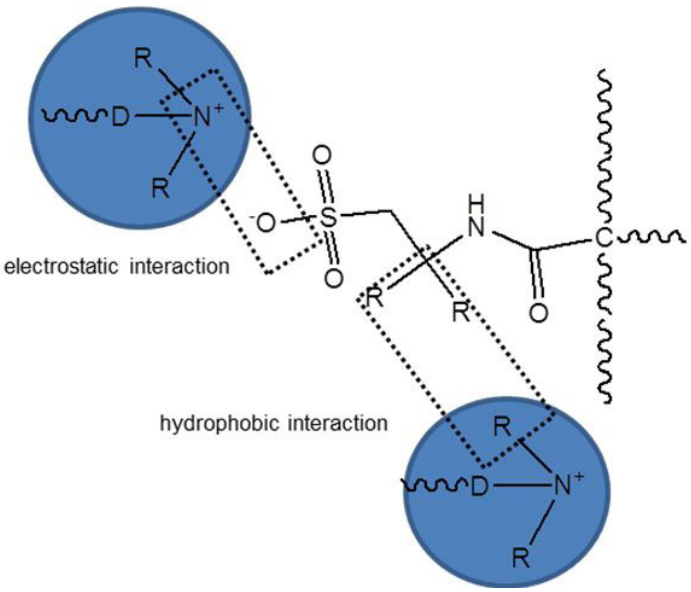

Figure 8. A possible interaction between dye molecules and p(EPMA-co-AMPS) hydrogel.

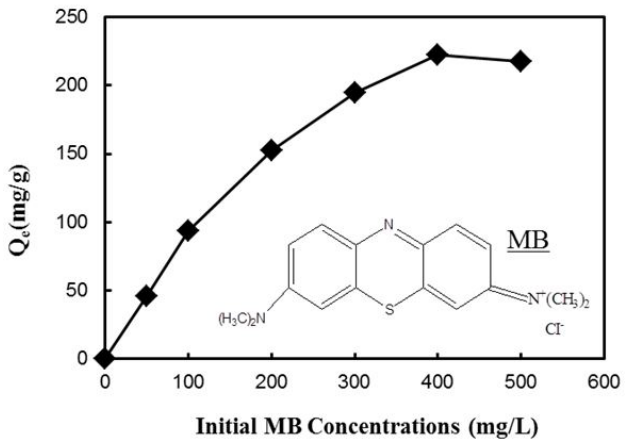

Figure 9. The effect of the initial MB concentrations on adsorption.

As clearly seen the Figure that the adsorption capacity (Q_e) of the hydrogel rapidly increased from 46.2 to 217.6 mg/g when C_o increased from 50.00 to 400 mg/L. The reason for why the amount of adsorbed dye is low in low concentrations can be explain by the weakness of the adsorbent-adsorbate forces and this case can cause the dye uptake at low concentrations to be less. In contrast, with increasing dye molecules concentration, there is an increase in the amounts of dye molecules adsorbed due to increasing driving force of the dye molecules toward the active sites on the hydrogel. Dye uptake reached maximum at concentration of 400 mg/L. At this stage the dye molecules uptake reached equilibrium and all sites were saturated with dye molecules. It is clear that the adsorption capacity tended to decrease when Co was between 400 and 500 mg/L. This can be observed due to cationic nature of MB dye, increased dye concentration can produce positive charge density on solution which results an electrostatic repulsion between adsorbed cations on hydrogel surface and non-adsorbed cations in solution.

Figure 10 shows the adsorbed amount of MB onto hydrogel as a function of time (until 150 h). All the experiments for effect of contact time studies were conducted with 50 mL of volume of 500 mg/L MB solutions and treating with 0.1 g hydrogel at room temperature It is clear from the Figure 10 that in first 72 h, adsorption of MB on the hydrogel increased quickly. This might be due to the reason that during this time, a large number of vacant surface

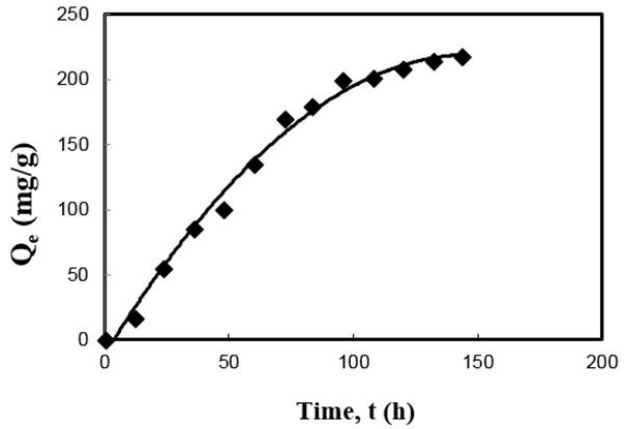

Figure 10. The adsorption of dye onto p(EPMA-co-AMPS) hydrogel as functions of time.

sites were available for adsorption of dye molecules quickly filled the active surface [24]. The amount of MB adsorbed from 72 to 144 h increased gradually but slowly. Such a situation may be attained due to the fact that the adsorption gradually reaches an equilibrium point. After 144 h, adsorption was not increased anymore which can be due to the fact that the anionic surface sites of the hydrogel were filled with dye molecules. Another possibility of this stationary phase of the adsorption may be explained with the saturation of the possible monolayer coverage of MB on the surface of the hydrogel. Similar observations are reported in literature [1,21,25].

Adsorption Isotherms and Kinetics

Adsorption isotherms are the basic requirements for investigating any sorption system. In this study, the most common adsorption isotherm equations, including Langmuir and Freundlich, were tested to understand the nature of the adsorption mechanism and the equilibrium conditions [26]. The constants of isotherms were determined using slopes and intercepts of linear plots. The Langmuir isotherm model assumes a monolayer adsorption on a surface with a finite number of identical sites, where all sites are energetically equivalent and there is no interaction between adsorbed molecules. The Freundlich model is an empirical equation based on the distribution of solute between the solid phase and the aqueous phase at equilibrium and can be applied to nonideal adsorption on heterogeneous surfaces as well as multilayer [26]. Figure 11 and 12 show the Langmuir and Freundlich isotherms models for adsorption of MB on the hydrogel, respectively. In all isotherm studies, temperature and pH were kept constant at 25°C and 6.0, respectively.

One of the most essential characteristics to elucidate the application of an adsorptive material is kinetics. The research of adsorption kinetics describes the dye adsorption capacity rate evidently

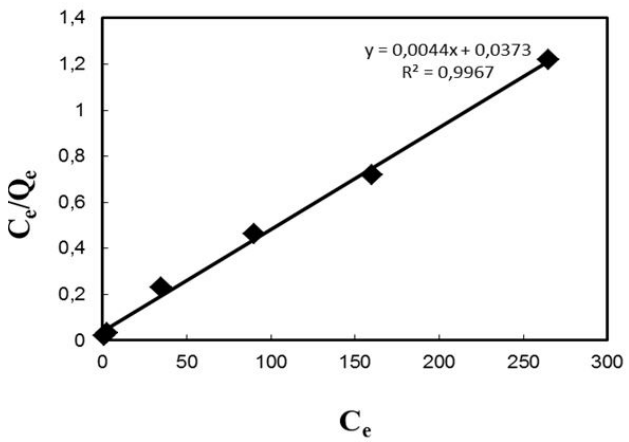

Figure 11. Langmuir isotherm for adsorption of MB on p(EPMA-co-AMPS) hydrogel.

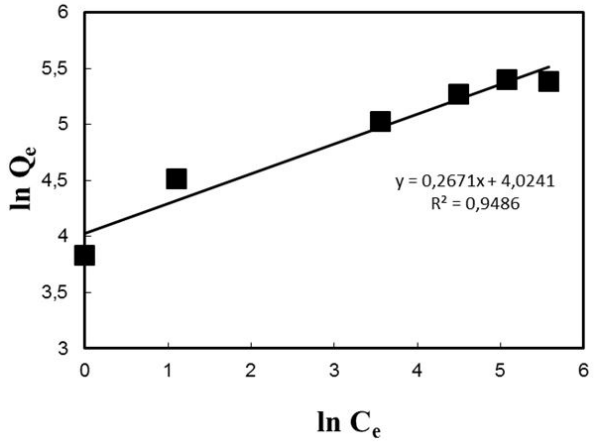

Figure 12. Freundlich isotherm for adsorption of MB on p(EPMA-co-AMPS) hydrogel.

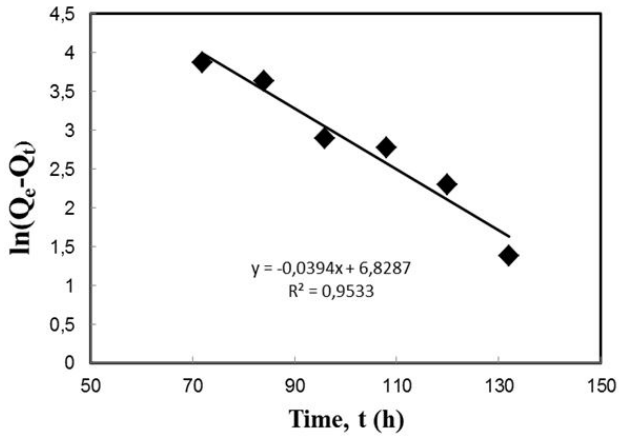

Figure 13. Pseudo-first- order kinetic plot for adsorption of MB on p(EPMA-co-AMPS).

this rate controls the residence time of adsorbent capacity at the solid-solution interface [27]. The constants of kinetic models were determined using slopes and intercepts of linear plots. Pseudo-first-order and Pseudo-second-order kinetic models were applied to find out adsorption mechanism [28] and showed by Figure 13 and 14, respectively. It can be clearly seen from the Figure 13 and Figure 14 that the adsorption of MB on the hydrogel follows the pseudo second-order kinetic model due to the high correlation coefficient (R^2). The isotherm and kinetic model's constants for MB adsorption by p(EPMA-co-AMPS) are shown with Table 1. As can be seen from the Table 1 that all the constants and regression coefficients were computed. RL constant, a dimensionless separation factor, is used to predict whether an adsorption system is ''favorable'' or ''unfavorable'' [29]. R_L is defined as; where C_o(mg/L) is the highest C_o and b (L/mg) is the Langmuir constant. The RL value in range of 0-1 computed shows that the adsorption of MB onto the hydrogel is favorable and relatively high MB adsorption by the adsorbent is achievable at high concentration. K_F (L/g) is a constant relating the adsorption capacity and n is an empirical parameter relating the adsorption intensity. It is clear from Table 1 Langmuir adsorption equation fitted well (R^2:0.99) with the adsorption data under the concentration range studied for experiments of batch adsorption for the hydrogel. It is evident that MB adsorbed on the hydrogel has a monolayer adsorption. The maximum adsorbed MB amount was found 217.6 mg/g. A comparison between p(EPMA-co-AMPS) hydrogel and other hydrogels reported in the literature for MB adsorption is given in Table 2.

Drug Release Studies

While analysing the release of an antibacterial drug from hydrogels that are targeted to be used as drug carrier for biomedical applications, the nature of release media is very important. The drug release behavior of hydrogels depends on

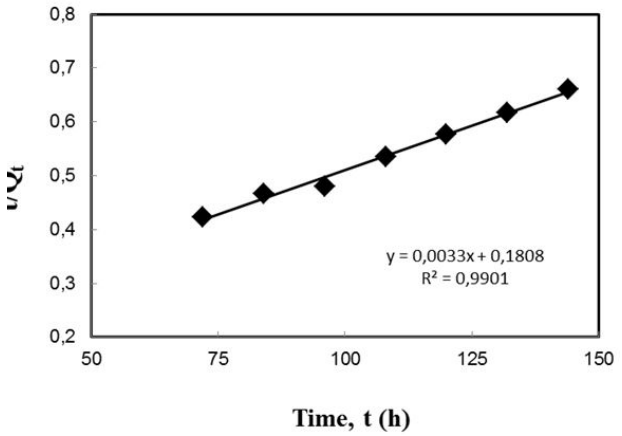

Figure 14. Pseudo-second-order kinetic plot for adsorption of MB on p(EPMA-co-AMPS).

Table 1. Isotherm models and kinetic constants for MB adsorption by p(EPMA-co-AMPS).

Isotherm/ Kinetic* models	Linear equations	Plot	Constants	R^2
Langmuir	$Q_e=(Q^o.bC_e)/(1+bC_e)$ $R_L=1/(1+bC_0)$	C_e/Q_e vs C_e	b = 0.108 = 250 R_L = 0.018-0.156	0.996
Freundlich	$lnQ_e=lnK_f+[(1/n)lnC_e]$	ln Q_e vs ln C_e	K_F = 55.9 n = 3.75	0.94
Pseudo-first-order*	$ln(Q_e-Q_t)=ln(Q_e)-k_1t$ $t_{1/2}=ln2/k_1$	$ln(Q_e-Q_t)$ vs t	k_1 = 0.039 Q_e = 923.3 $t_{1/2}$ = 17.8	0.953
Pseudo-second-order*	$t/Q_t=(1/k_2\ Q^2_e)+(1/Q_et)$ $t_{1/2}=1/(k_2Q_e)$	t/Q_t vs t	$k_2 = 5*10^{-5}$ Q_e = 333.3 $t_{1/2}$ = 60	0.990

Table 2. Comparison between p(EPMA-co-AMPS) hydrogel and other hydrogels discussed in the literature for MB adsorption capacity.

Hydrogel adsorbent	MB adsorption capacity (mg/g)	References
Polyacrylamide/polyacrylate/gum Arabic	48	[30]
Acrylamide/N-vinylpyrrolidone/ 3-(2-hydroxyethyl carbamoyl) acrylic acid	5.50	[31]
Magnetic polyvinyl alcohol/laponite RD	251.0	[32]
Polyacrylamide/chitosan IPN cryogel	750	[33]
Chitosan hydrogel beads	226.24	[34]
P25–Graphene hydrogels	87.63	[35]
p(EPMA-co-AMPS) hydrogel	217.6	This work

the pH of the solution owing to the presence of pH sensitive groups on the hydrogel structural network. The dynamic release of model antibacterial drug (GS) from the drug-loaded hydrogel was carried out at pH of 2.0 and 7.4. The drug release kinetics analyzed by plotting the amount of drug release versus time and shown in Figure 15. The release study shows that the drug release from hydrogels is comparable. It is clear that GS release increased with increasing pH of the medium and almost 80 % drug is released in 5 h. After 77 h observation, a high amount of drug release equal to 8.0 mg/g was observed in pH 7.4 as compared to in acidic solutions at pH 2.0 which was 3.7 mg/g. This was once more dedicated to the ousting of the captured drug from the collapsed hydrogels in acidic pH solution. It should be noticed that these results are in good agreement with the water swelling behavior of the hydrogels in the event of the collapsed structure in acidic solution and the swollen state in basic solutions [36]. Sohail reported that cross-linked chitosan-co-poly(AMPS) hydrogels wirh containing a higher concentration of AMPS produced 26% at pH 1.2 and 91% at pH 7.4 drug release in 24 h [38]. Similar observations have been reported in literature [36-38].

Mechanical Properties of Hydrogel

Texture profile analysis (TPA) is a useful technique that could provide a reliable overview of mechanical properties of hydrogel in environmental conditions. It can be presented in Figure 16 that, the hardness of the hydrogels also rised with rising some topological interactions of hydrogels incorporated with the solution such as electrostatic interactions, hydrogen bonds covalent bonds, physical entanglement,

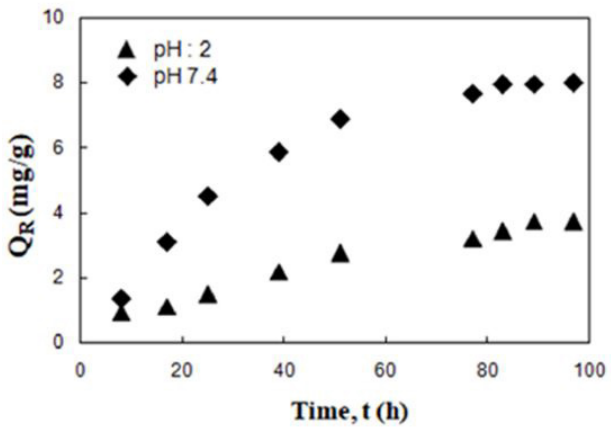

Figure 15. The release of drug by p(EPMA-co-AMPS) hydrogel as functions of time.

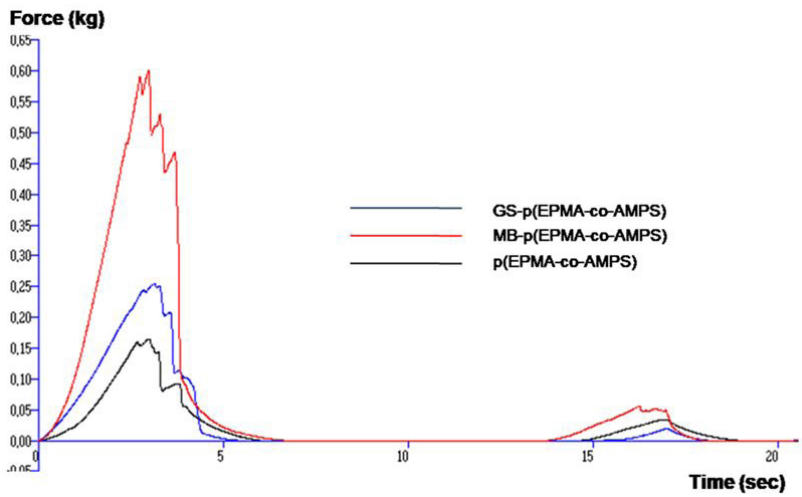

Figure 16. The hardness curves of drug loaded hydrogel (GS-p(EPMA-co-AMPS)), dye loaded hydrogel (MB-p(EPMA-co-AMPS)) and pure hydrogel p(EPMA-co-AMPS).

ionic bond, van der Walls forces, or hydrophilic interactions. These samples behave as strong gel materials. The p(EPMA-co-AMPS) gels incorporated with 250 ppm dye solution (MB-p(EPMA-co-AMPS)) showed the highest hardness values compared with other samples (GS-p(EPMA-co-AMPS) and pure p(EPMA-co-AMPS)). The hardness was increased from 166 to 596 g due to the electrostatic interactions between negatively charged regions of the hydrogel network with anionic dye molecules.

Antibacterial Studies

The antibacterial activity is the most valuable property in the field of biomedical applications. The ability of antibacterial activity of the pure and GS loaded p(EPMA-co-AMPS) hydrogel was tested by disk diffusion method both Gram-positive bacteria (B. cereus) and Gram-negative bacteria (S. typhimurium) [39]. Inhibition diameter around the hydrogel was shown in Figure 17. Although the pure hydrogels did not show antibacterial activity, the drug loaded hydrogels showed good resistance to bacterials. It was viewed that the hydrogel was effective in decreasing the viable cell count of S. typhimurium (inhibition zone diameter 22 mm/sample) and B. cereus (inhibition zone diameter 13 mm/sample. Based on this result, it can be concluded that the drug loaded hydrogel exhibits good fair antibacterial action and it can be used as an antibacterial agent in the application of many fields [40].

CONCLUSION

In this study, crosslinked p(EPMA-co-AMPS) hydrogels were prepared by free radical polymerization in solution. Swelling capacity of the hydrogels were analyzed under different experimental conditions, according to the results hydrogels swelled in the range of 200-500% by mass and the best swelling was observed in distilled water in comparison with lake water, tap water and MB solutions. The ability of hydrogel as an adsorbent to remove MB from aqueous solution was examined in batch system. Obtained results were applied two well-known isotherm models: Langmuir and Freundlich. Equilibrium isotherms were well defined by the Langmuir equation, giving maximum adsorption capacity of 217.6 mg MB/g dry hydrogel. Also, evaluation of the adsorption results obtained on the basis of Pseudo-first and Pseudo–second-order kinetic models analyzed that hydrogel-dye system was best defined by the pseudo-second-order model. It can be concluded that, taking into consideration the results, the hydrogel has a good adsorption capacity for the MB. Also the dynamic release of model antibacterial drug (GS) from the drug-loaded hydrogel was studied for gastrointestinal system. A high amount of drug release (8.0 mg/g) was observed at pH (7.4) of intestinal system as compared to that (3.7 mg/g) in acidic solutions at pH (2.0) of gastric system. We believe that hydrogel reported here can be a better alternate of other similar adsorbents for high-capacity

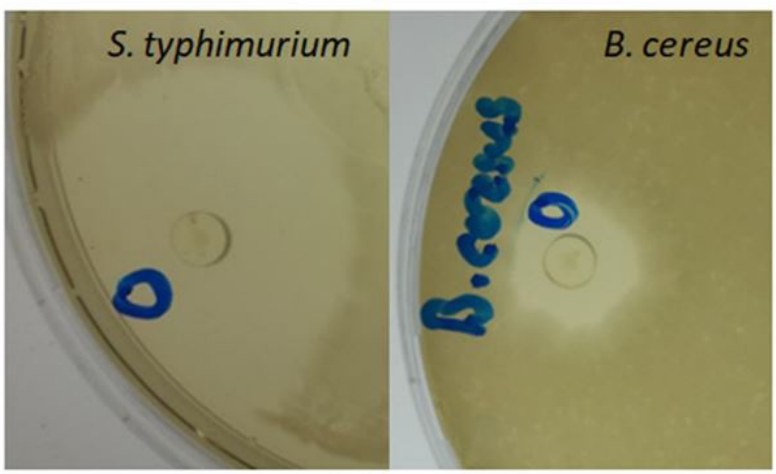

Figure 17. In Vitro antibacterial activities of drug loaded hydrogel.

adsorbents for cationic dye removal and also will shed light onto future investigations. Also, due to the combination of variety of properties of the hydrogel such as water binding capacity, antimicrobial effect and good adsorbent activity, these hydrogel systems can be used for agricultural applications, in preparing antimicrobial material, controlled drug release systems and wastewater treatment processes.

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