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Investigation of Poly(styrene-alt-maleic anhydride) Copolymer for Controlled Drug Delivery of Ceftriaxone Antibiotic

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Delivery of drugs by new technologies is a highly topical challenge of polymer chemistry. This report describes the preparation of antibacterial activated poly(styrene–alt–maleic anhydride) (PSMA) in which ceftriaxone antibiotic is covalently bonded onto the polymeric framework. The chemical grafting reaction of ceftriaxone antibiotic on PSMA was carried out in various ratios. In addition, PSMA was modified by isopropyle amine for preparation of PSMA-Isopropyle amide (PSMA-IPA) pH sensitive hydrogel. The physical loading of ceftriaxone antibiotic on PSMA-IPA in various ratios was carried out. The prepared copolymers were purified and their structures characterized by FT-IR and ^{1}H-NMR spectroscopy. *In vitro* drug releasing was performed under specific conditions to investigate the influence of pH on the releasing rate.

Keywords: Antibiotic, ceftriaxone, drug delivery system, poly(styrene-alt-maleic anhydride), graft copolymer

1 Introduction

Delivery of drugs by new technologies is a highly topical challenge of polymer chemistry. Thus far, numerous products, both on the market and in development, are being produced and studied to obtain precise and tunable control of molecular release. These controlled release drug delivery technologies have not only revitalized old pharmaceuticals, but have also been directed toward newer biopharmaceuticals produced by genetic research (1–4).

Polymers have played a major role in the development of controlled release systems; as matter of fact, the early polymeric drug delivery systems incorporated commercially available polymers (5–10). Successive extensive research efforts aimed to improve both the polymers and processes, as well as to apply them to the controlled release of a wide variety of pharmaceuticals or more general 'active' products. However, with the continued development of controlled release technology, the need has arisen for materials with more specific drug delivery properties (11, 12). These materials include new biodegradable polymers, polymers with both hydrophilic and hydrophobic characteristics, and hydrogels that respond to temperature or pH variations. In addition, methods to overcome some of the barriers associated with current drug delivery are necessary (13). The possibility of reducing or even eliminating, oral administration of possibly dangerous drugs (such as anti-inflammatory, antibiotics, etc.) using new biocompatible polymers in which drugs are directly incorporated is currently under investigation.

The continuing rise in microbial drug resistance has led to widespread problems in the treatment of bacterial infections (14). Of particular concern are those illnesses caused by methicillin-resistant Staphylococcus aureus (MRSA), which are responsible for a majority of hospital-acquired infections, clinical complications, and nearly 100,000 deaths each year in the United States alone (15, 16). The loss of effectiveness of commonly used antibacterial antibiotics such as penicillin and other ß-lactam drugs further adds to the dilemma, calling for the immediate need for improvements in drug design, discovery, and delivery. One of the major challenges in treating antibiotic-resistant bacterial infections is the need to develop agents that can stop the infection at the site of initiation, which frequently occurs in regions of the body where water-soluble drugs typically have poor access. However, the application

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of lipophilic agents to combat such infections has limited effectiveness due to uptake and delivery issues resulting from low water solubility and biodistribution (15). The ability to deliver antibacterial drugs to infections in fatty tissue or on the surface of implanted medical devices, for example, where microbial biofilms often develop, ultimately determines if the infection can be cleared without surgical intervention (17, 18).

Amphiphilic graft copolymers have been extensively studied due to their wide applications in pharmaceuticals. Styrene and maleic anhydride are known to produce alternating copolymers (19–21) that have been used in a variety of applications. Maeda and co-workers used low molecular weight PSMA copolymers clinically to deliver the antitumor protein neocarzinostatin (NCS) (22–24). The polymer protein conjugate, known as SMANCS, is formed using "partial half-esters" of SMA, in which 70% of the maleic anhydride groups were opened using butanol.

Stover and coworkers described the temperature- and pH responsive properties of poly(ethylene glycol) grafted amphiphilic copolymers based on alternating copolymers of maleic anhydride with styrene and 4-*tert*butylstyrene, respectively (25). The phase transitions of the resulting amphiphilic graft copolymers were attributed to the combination of hydrophobic interactions and intra/intermolecular hydrogen bonding. These copolymers had potential applications in developing hydrogel and surfactants with pH/temperature responsive properties (25).

Organic functional groups that decompose slowly in an aqueous environment have attracted wide interest as potential linkers for the covalent conjugation of drugs to polymers for a range of drug delivery applications. Herein, we describe the development of a type of pH sensitive molecules that hydrolyze with tunable rates under physiological conditions.

In this work, the chemical loading of the ceftriaxone antibiotic on poly(styrene-*alt*-maleic anhydride) (PSMA) was performed by direct grafting of the ceftriaxone on PSMA backbone. Also, the physical loading of ceftriaxone on amine modified PSMA hydrogel was carried out. *In vitro*, drug releasing from the antibiotic loaded polymers was investigated and compared with each other.

2 Experimental

2.1 Materials

Styrene was purchased from Merck and was purified by distillation in reduced pressure. Maleic anhydride, benzoyle peroxide (BPO), tetrahydrofuran (THF), N,N-dimethylformamid (DMF), isopropyl amine and triethylamine (TEA) were purchased from Merck and used without further purification. Pure ceftriaxone disodium antibiotic was purchased from Tabriz Dana Pharmacies. Sodium phosphate dibasic (Na_2HPO_4) and potassium phosphate monobasic (KH_2PO_4) were purchased from Aldrich.

2.2 Characterization of Copolymers

^{1}H-NMR spectra were recorded on a Bruker DMX-300 spectrometer. FT-IR spectra were recorded on a Thermonicolet (Nexus 670) spectrometer and the transmission spectra were obtained by forming thin transparent KBr pellets. The molecular weight of the resulting polymer was obtained with a Maxima 820 GPC analysis instrument using polystyrene calibration standards with tetrahydrofuran (THF) as the mobile phase at a flow rate of 1.0 mL/min at room temperature.

2.3 Synthesis of Poly(styrene-alt-maleic anhydride)

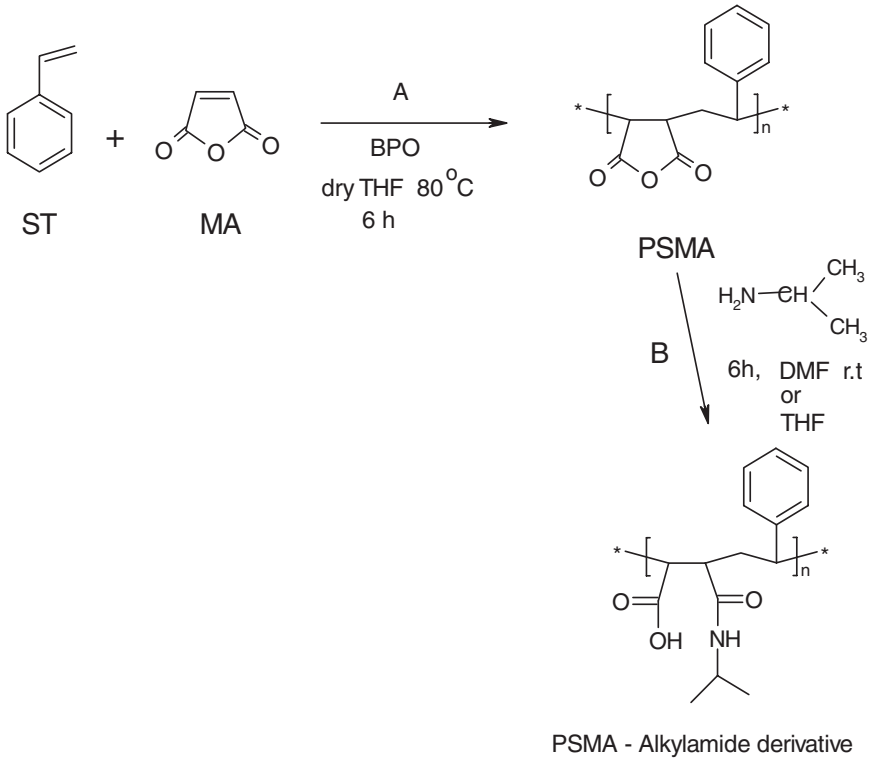
The poly(styrene-*alt*-maleic anhydride) was prepared through a thermally initiated free-radical polymerization of styrene and maleic anhydride according to the literature method (19). Briefly, equimolar amounts (0.043 mol) of styrene (5 *m*L) and maleic anhydride (0.043 mol) (4.24 g) were combined in a 100 mL round bottomed flask with benzoyl peroxide (BPO)(0.0624 g) in dried tetrahydrofouran (THF) (60 ml). The mixture was degassed by nitrogen to remove oxygen from the reaction vessel prior to polymerization. Polymerizations were carried out for 6 h in 80^{o}C by stirring under nitrogen atmosphere. The polymerization product was diluted in THF followed by dropwise addition into a 100-fold excess (v/v) of cold diethyl ether to precipitate pure PSMA polymer, which was then filtered and dried under vacuum at room temperature.

2.4 Preparation of Ceftriaxone Free Acid

Ceftriaxone disodium (10 g) was completely dissolved in a mixture of deionized water (50 ml) and methanol (30 ml); a yellowish solution was obtained. The ceftriaxone in free acid form was precipitated by adding dropwise concentrated formic acid (4 ml) to the above mentioned solution. The precipitate powder was filtered and dried under vacuum at room temperature.

2.5 Preparation of Ceftiaxone - g - PSMA (Chemical Loading)

The chemical graft reaction of the ceftriaxone antibiotic on the PSMA copolymer was carried out by following procedure: The PSMA (1 g) was dissolved in anhydrous DMF (40 ml) in a 100 ml round-bottomed flask. Then, the ceftriaxone free acid (0.0017 mol) (1 g) was added to above mentioned solution and TEA (0.0033 mol) (0.45 ml) was also added. The reaction was stirred overnight at room temperature under inert atmosphere. The workup of reaction was carried out by adding deionized water (200 ml) followed by the addition of hydrochloric acid (0.1 N)



Sch. 1. (A) Synthesis of PSMA alternating copolymer, (B) Modification by isopropyl amine to form PSMA–isopropyl amide.

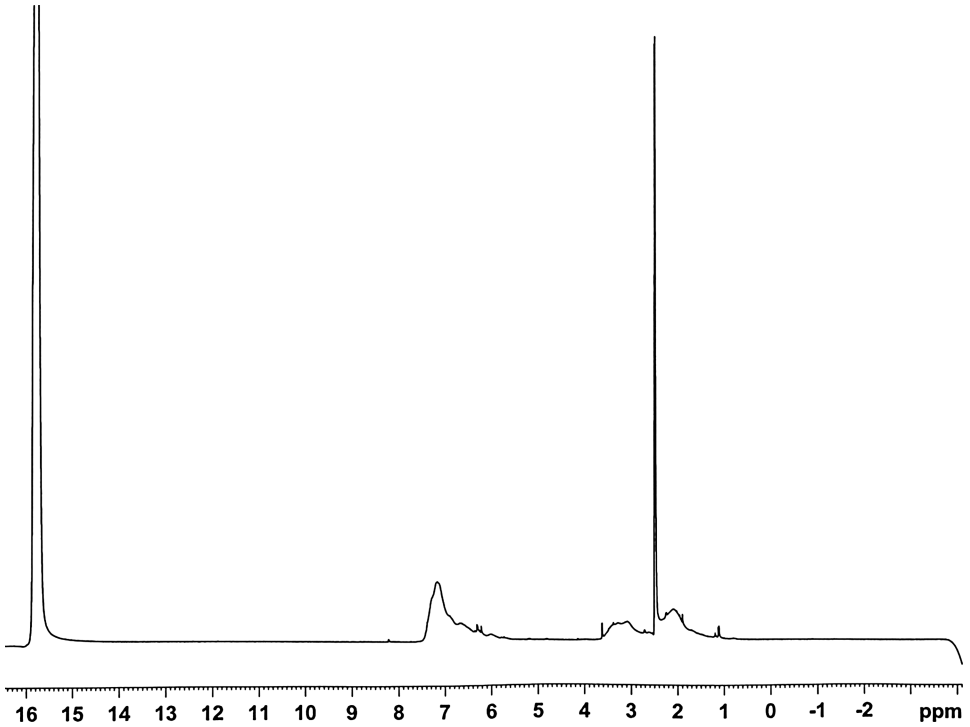


Fig. 1. ^{1}H-NMR spectrum of the Poly (styrene-alt-maleic anhydride) in DMSO-d_6.

until the pH decreased to 2.5–3. The obtained precipitate in gel form was recovered by centrifuge and washed with water several times. The product was dried under vacuum at room temperature for 3 days. The reaction was repeated for 1: 0.5 and 1: 2 w/w% ratios of copolymer: antibiotic, respectively.

2.6 Preparation of Isopropyl Amine Modified PSMA (PSMA-IPA)

Amine modified PSMA was synthesized by a reaction of primary alkyl amine as isopropyl amine with the repeated anhydride groups in backbone of PSMA. In a typical reaction, the PSMA (1 g) was dissolved in anhydrous DMF (40 mL) in a 100 mL round-bottomed flask. The copolymer solution was degassed with inert gas before adding a predetermined amount of isopropyl amine modifier. The reaction was stirred at room temperature for 5 h. The product was recovered by precipitation in cold diethyl ether followed by filtration and dried under vacuum at room temperature. The reaction was repeated for various molar ratios of amine to anhydride groups of the PSMA copolymer in DMF and THF as solvent and the results were listed in Table 1.

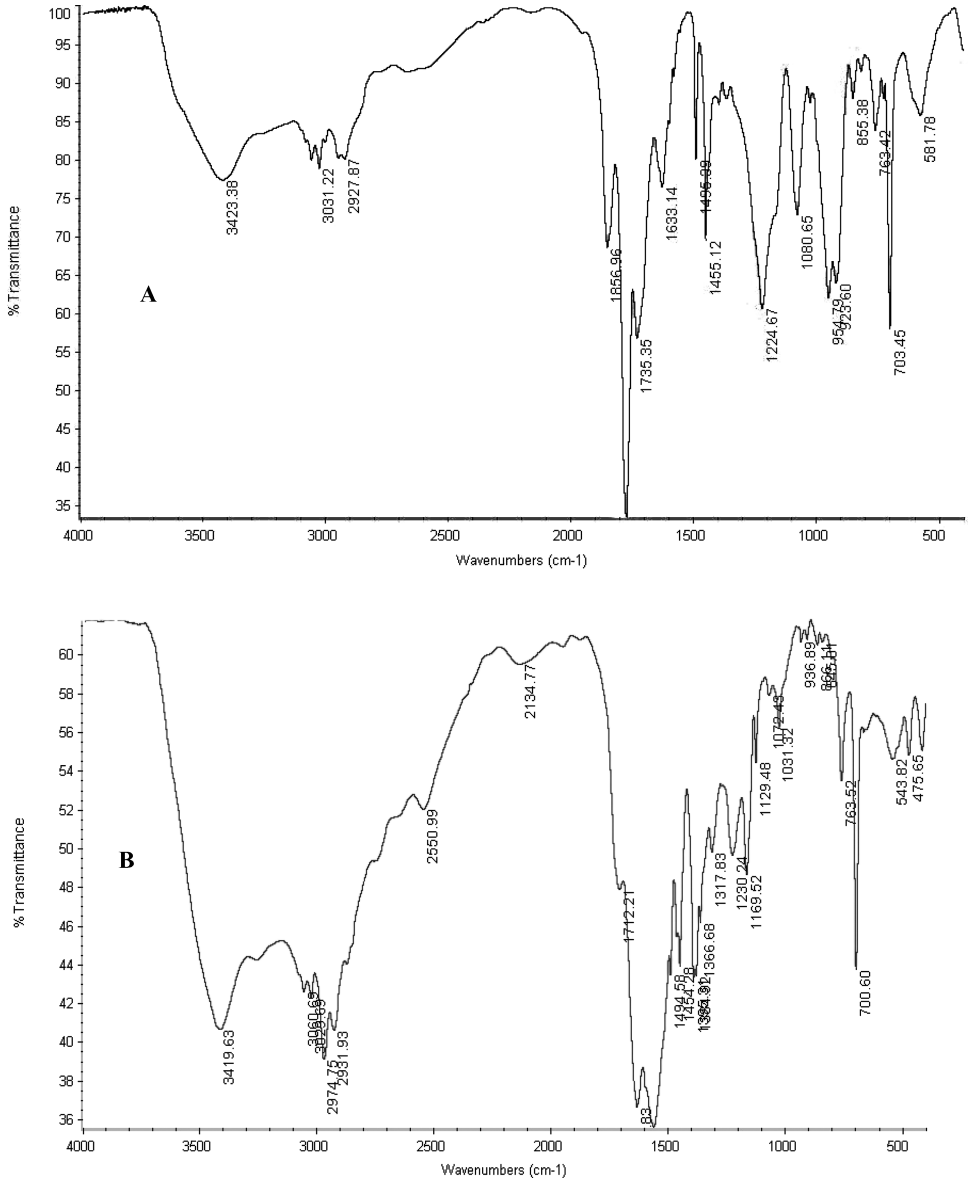


Fig. 2. (A) FT-IR spectrum of the poly(styrene-alt-maleic anhydride) and (B) FT-IR spectrum of the poly(styrene-alt-maleic anhydride) grafted by isopropyle amine.

Table 1. Isopropylamine derivative of the PSMA copolymers and pH of sol-gel transition

Solvent of reaction	*Molar ratio of amine to PSMA in feed of reaction*	*pH of sol-gel transition*
THF	0.5	3.9
THF	1	4.84
THF	2	5.23
DMF	0.5	4.77
DMF	1	5.45
DMF	2	6.07

2.7 Measurement of Cloudy Point

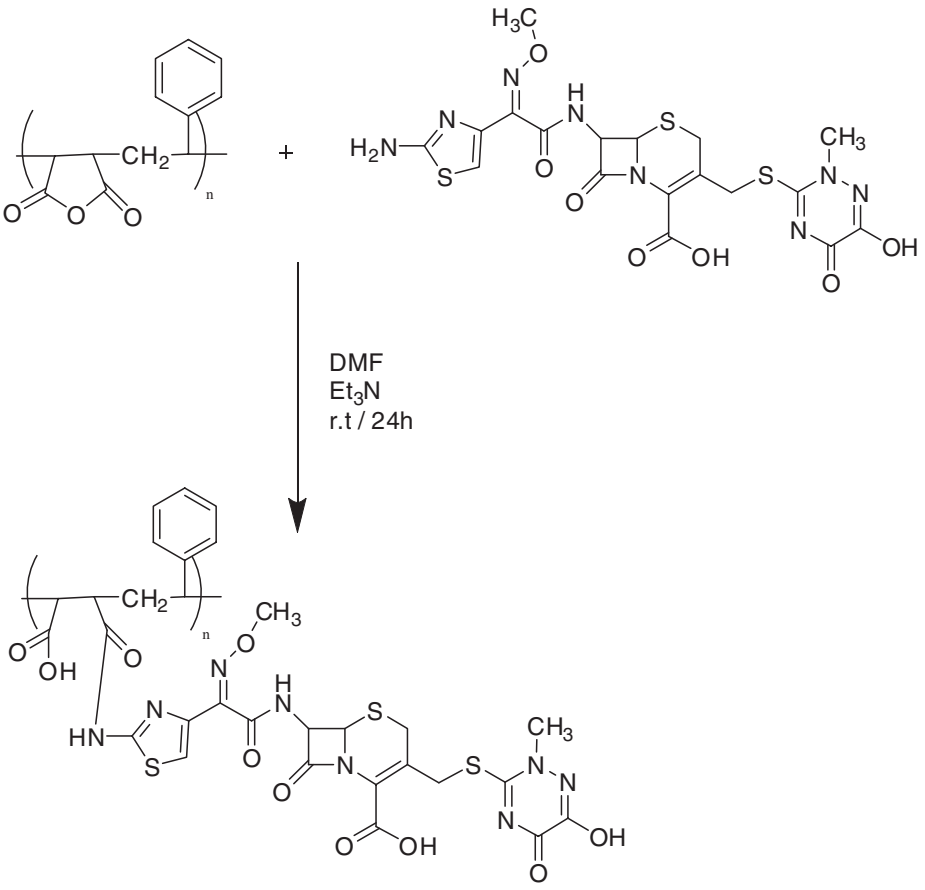
A Metller MA235 pH/ion meter was used for titration of selected isopropyl amine modified PSMA until cloudy point. The polymer solutions (33 mg/mL) were prepared in NaOH (3 mL of 0.33 M) and stirred for 1.5 h to hydrolyze any remaining anhydrides. A total of deionized water (12 mL) was added, and the solution was titrated with 0.1N HCl from pH 11.00 to cloudy point pH with constant stirring.

2.8 The Physical Loading of Cefriaxone Antibiotic on PSMA-IPA Hydrogel

PSMA–IPA (0.5 g) was dissolved in deionized water (40 ml). To the above obtained solution, the cefriaxone disodium salt (0.5 g) which was dissolved in deionized water (10 mL) was added. The mixture was stirred at room temperature for 6 h. The product was recovered by acid precipitation using hydrochloridric acid (0.1 N). The precipitate was filtered and washed completely with water and dried under vacuum for 48 h.

2.9 *In vitro* Drug Release

The release of cefriaxone was determined with a UV spectrophotometer at $\lambda_{max} = 296$ nm as a function of time. The procedure was as follow: The sample of copolymer–drug adduct (20 mg) was placed in a dialysis tube in a 100 ml container, containing 50 ml of phosphate–buffered solutions at pH 2, 7.4 and 9 and kept at 25°C. Then, in the specific times, the release of drug was determined with measuring the absorption by spectrophotometer. Each experiment was carried out in triplicate.



Sch. 2. The chemical loading of ceftriaxone antibiotic on PSMA.

3 Results and Discussion

Polymer-based drug delivery systems are used to optimize the therapeutic properties of drugs and render them safer and more effective and reliable. Moreover, polymeric drugs and macromolecules, when used as drug carriers, can be easily synthesized, freely water-soluble, and nontoxic (26). One important goal in drug delivery is that the attached drugs can be targeted to specific areas of the body, tissues, or cells. This method decreases the toxic side effects of the drugs as well as achieves controlled drug delivery.

3.1 PSMA Copolymer Synthesis

The free-radical polymerization of the PSMA is facile, and the copolymer is readily recovered. The overall reaction scheme is shown in Scheme 1. To clarify the nature of the obtained PSMA copolymer, ^{1}H-NMR studies were undertaken in dimethyl-d_6 sulfoxide (Fig. 1). A few drops of trifluoroacetic acid were added so that the peak due to H_2O can be shifted outside the region shown in Figure 1.

The broad signals from 1.45 to 3.70 ppm of the ^{1}H-NMR spectrum show signals of CH and CH_2 of the main chain. The signal related to the methyl group, near 1.16 ppm is from the terminal cumene residue in the PSMA chain. The signal near 7.1 ppm indicates a proton signal of the styrene residue. Calculation of the ratio of styrene and maleyl residues in the proton peak area indicated a character of 1:1 copolymer as expected from the tendency of these monomers to polymerize together as one unit.

The molecular weight of the PSMA copolymers could be controlled by varying the molar ratio of free-radical initiator and volume of added solvent (19). The molecular weight and polydispersity (PD) of the obtained PSMA was determined by Gel permeation chromatography (GPC) and $M_w = 13845$ with PD = 3.02 was obtained.

3.2 Isopropyl Amine Modified PSMA

Modified PSMA copolymers are suitable candidates for biomaterial applications due to their facile synthesis and functionalization by the introduction of hydrophobic/ hydrophilic moieties. The utilization of PSMA in the synthesis of functionalized polymers has been reported (19).

Modification of PSMA was carried out by isopropyl amine amidation of anhydride moieties in various ratio of amine to repeated anhydride groups of PSMA in DMF or THF as solvent. The resulting copolymers have pH-sensitive property and show a reversible sol-gel transition, induced by a narrow pH change in the range of pH 3.8–3.0. Alkylamine modification of the PSMA backbone is used to control its pH-solubility response and resultant membrane-disruption activity too. Each isopropyl amine reacts with one maleic anhydride group in the PSMA backbone to form an alkylamide linkage and one carboxylic acid group, which confers pH-sensitivity to the modified copolymer. Amide bonds are significantly more resistant to hydrolysis than esters which lead to the greater stability of the resulting PSMA copolymer derivatives *in vitro* and *in vivo* condition. In the higher ratio of isopropyl amine to anhydride moieties of PSMA, the ^{1}H-NMR analyses of ISO-PSMA copolymer indicates that the reaction is nearing completion, allowing stochiometric control of the degree of alkyl chain modification. Table 1 shows the sol-gel transition pH of modified isopropyl amine copolymer in THF and DMF solvents.

As the hydrophobicity of the PSMA backbone is increased by modification with alkylamine groups, the p*K*a of the copolymer is shifted upward. An increase in p*K*a causes the sol-gel transition pH to increase. Also, the results of reactions in THF and DMF solvents in Table 1 show that, the modification of PSMA with isopropyl amine in DMF solvent leads to hydrogels with the greater sol-gel transition pH in comparison to the same reactions in THF. This can be interpreted by the fact that, the ISO-PSMA copolymers have weaker solubility in THF than DMF so the reactions in DMF proceed in higher yields.

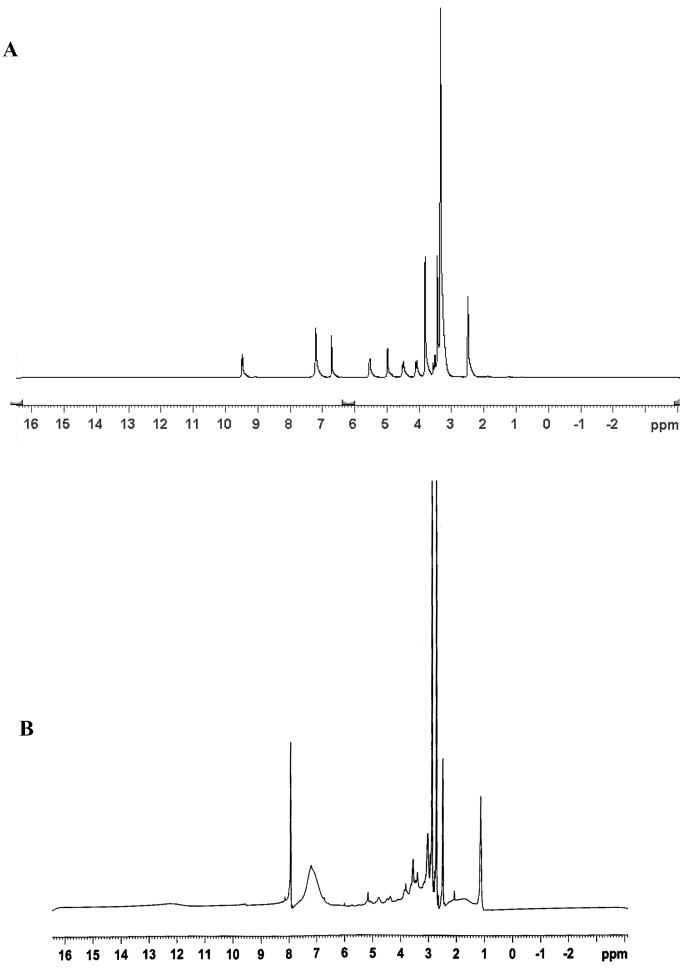


Fig. 3. (A) ^{1}H-NMR spectrum of the cefriaxone antibiotic; (B) ^{1}H-NMR spectrum of the PSMA-g-ceftriaxone antibiotic.

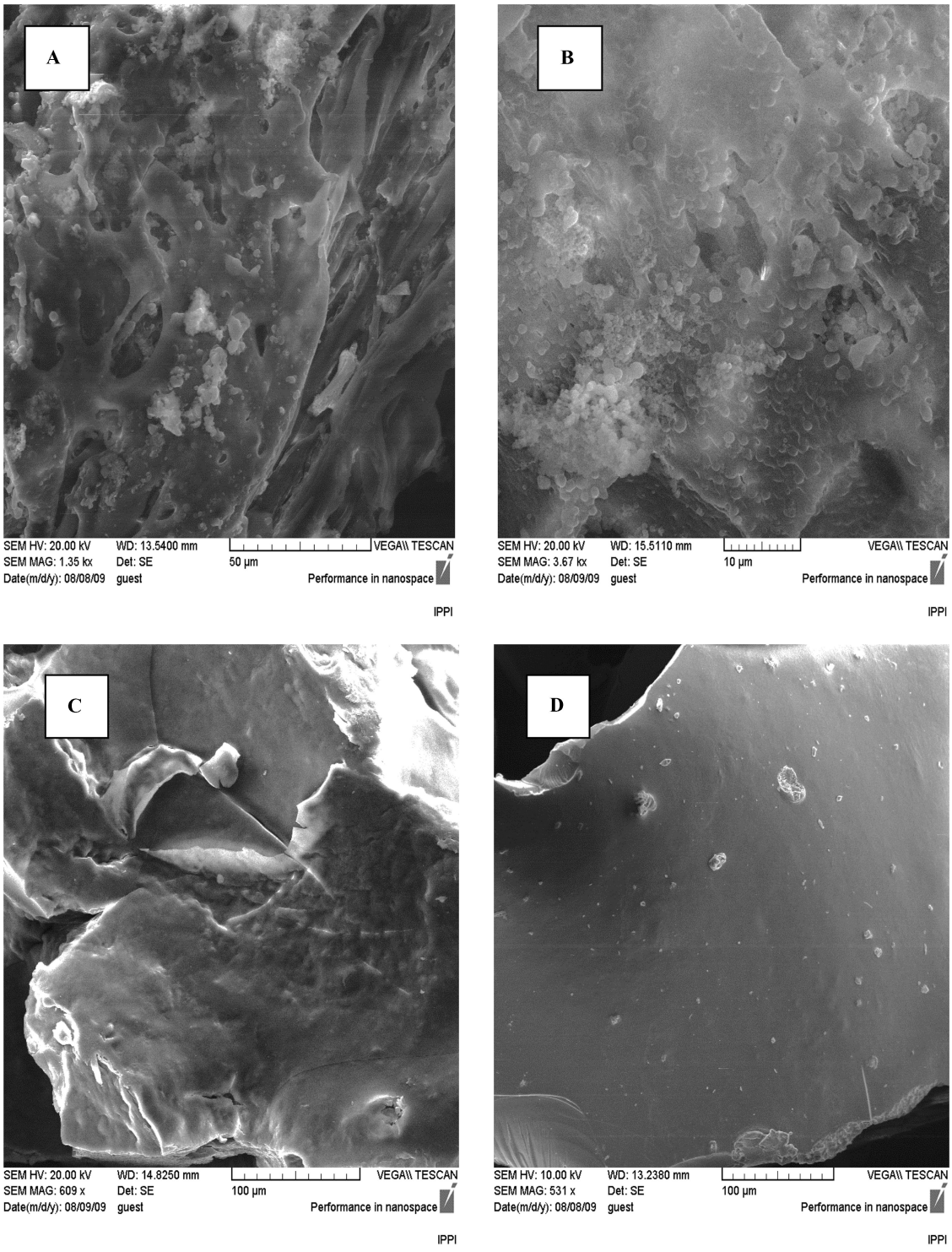


Fig. 4. Scanning electron microscopic images of the PSMA (A and B) and the PSMA-g-ceftriaxone (C and D).

The pH sol-gel transition can be adjusted by degree of modification of PSMA by amine. Table 1 shows that when we reduce the ratio of amine to PSMA, the pH sol-gel transition is reduced.

Figure 2 shows the typical IR spectra of both the starting and the grafted copolymers; it confirms that the grafting reaction is very efficient. The spectrum of the PSMA copolymer displays characteristic anhydride peaks at 1778

and 1857 cm^{-1}. In the spectrum of the PSMA-ISA, the anhydride peaks have disappeared, and instead, the spectrum shows the characteristic of absorption peaks of the amide carbonyl and the carboxylic acid at a lower frequency.

The amount of isopropyl amine in the grafted copolymer is easily estimated from ^{1}H-NMR spectrum. ^{1}H-NMR spectrum (not shown) of the grafted copolymer reveal a resonance for proton of amide at $\delta = 7.94$ ppm and broad aromatic resonance at $\delta = 7.01$ ppm and peaks at $\delta = 1.05$ corresponded to the methyl groups of the isopropyl amine. The extent of isopropyl amine reaction with anhydride units in the PSMA can be estimated from the ratio of peak areas for the amide proton (1H) vs. the phenyl protons (5 H for PSMA).

3.3 Chemical Loading of Ceftriaxone Antibiotic on PSMA

The chemical loading of ceftriaxone antibiotic on PSMA copolymer was performed by direct amidation reaction between antibiotic and repeated anhydride groups of PSMA. The overall reactions are shown in Scheme 2.

Figure 3 shows the typical ^{1}H-NMR spectrum of the PSMA-ceftriaxone adduct. For comparison, the ^{1}H-NMR of ceftiaxone in free acid form in DMSO d_6 was also included in Figure 8. The peaks related to ceftriaxone spectrum were clearly shown in the spectrum of PSMA-g-ceftriaxone. Some of the distinguished peaks in this spectrum are as follows; the broad peak at $\delta = 7.88$ ppm corresponds to aromatic protons of styrene units and obtained carboxylic acid groups have been revealed at $\delta =$ 12.32 ppm in copolymer structure. The peak of ceftriaxone NH_2 group ($\delta = 5.55$ ppm) that reacted with repeated maleic anhydride groups in PSMA backbone disappeared in the spectrum of PSMA-g-ceftriaxone and instead, the peak at $\delta = 8.13$ ppm related to amides N-H was revealed. The aliphatic groups of copolymer backbone and drug were revealed at $\delta = 1.45$–3.75 ppm.

The scanning electron microscopy images were taken for PSMA and PSMA-g-ceftriaxone and depicted in Figure 4. As one can observe in Figure 4, the change of morphology in the PSMA and the PSMA-g-ceftriaxone images was clearly seen.

3.4 *In vitro* Drug Release

The rate of release usually depends on the polymer microstructure, pH of medium, and temperature. In this work, the rate of ceftriaxone release from the prepared copolymer-drug adducts was studied at various pH values.

The effect of the copolymer microstructure on the release rate of the ceftriaxone was also studied. Two series of copolymer-drug adducts were investigated: The PSMA-g-ceftriaxone and the PSMA–IPA copolymer which had ceftriaxone physically loaded on it. The release rate profile of both copolymers in various pH values (pH = 2, 7.4 and 9) are depicted in Figures 5 and 6. It was found that in the case of both copolymer-drug adduct, the release rate of antibiotic was increased as the pH of medium increased. In addition, the copolymer–ceftriaxone adducts showed high hydrolytic stability in an acidic medium (i.e., within the stomach pH), while the release rate of cefriaxone was greatest in an alkaline medium (i.e., within colon pH). In both series of copolymer-drug systems, pH sensitive properties were shown, but dependence of copolymer-drug systems which were drug chemically loaded are more than the physically loaded cases. In fact, with chemically loaded drug on the copolymer, the rate of drug release depends on hydrolysis rate of drug from backbone of the copolymer. Also, in different ratio of drug to copolymers with increased drug ratio, the release rate was increased. In comparison of the two series of copolymer-drug adducts (chemically and physically), it can be seen that the release rate profiles in the case of PSMA-g-ceftriaxone systems was performed in longer duration than the PSMA–IPA with the physically drug loaded on it.

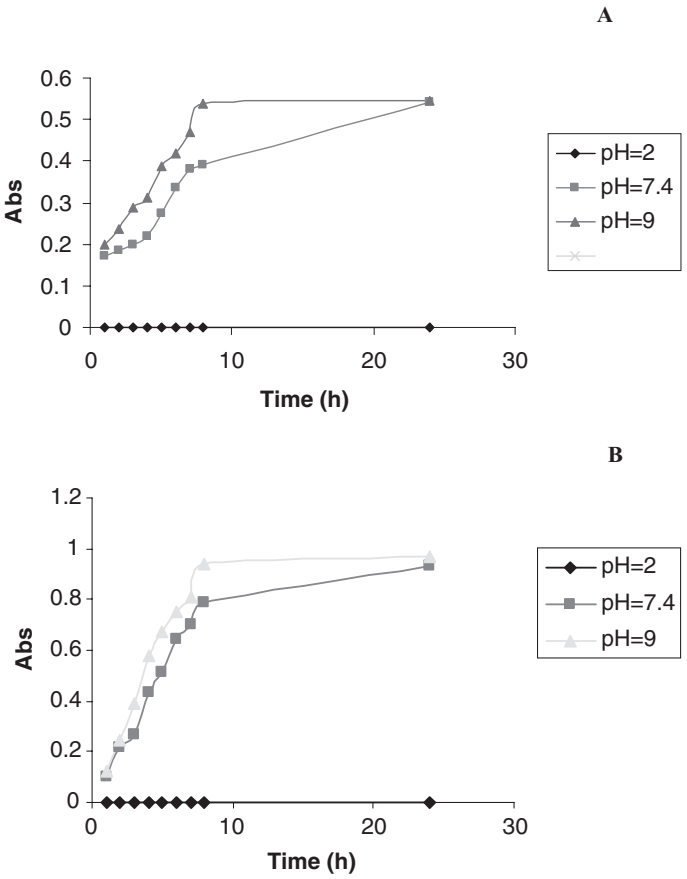


Fig. 5. *In vitro* drug release profiles of the ceftriaxone antibiotic from the PSMA-g-ceftriaxone ((A) 0.5:1 w/w% and (B) 1:1 w/w% in feed of chemical reaction) in phosphate buffer in various pH values.

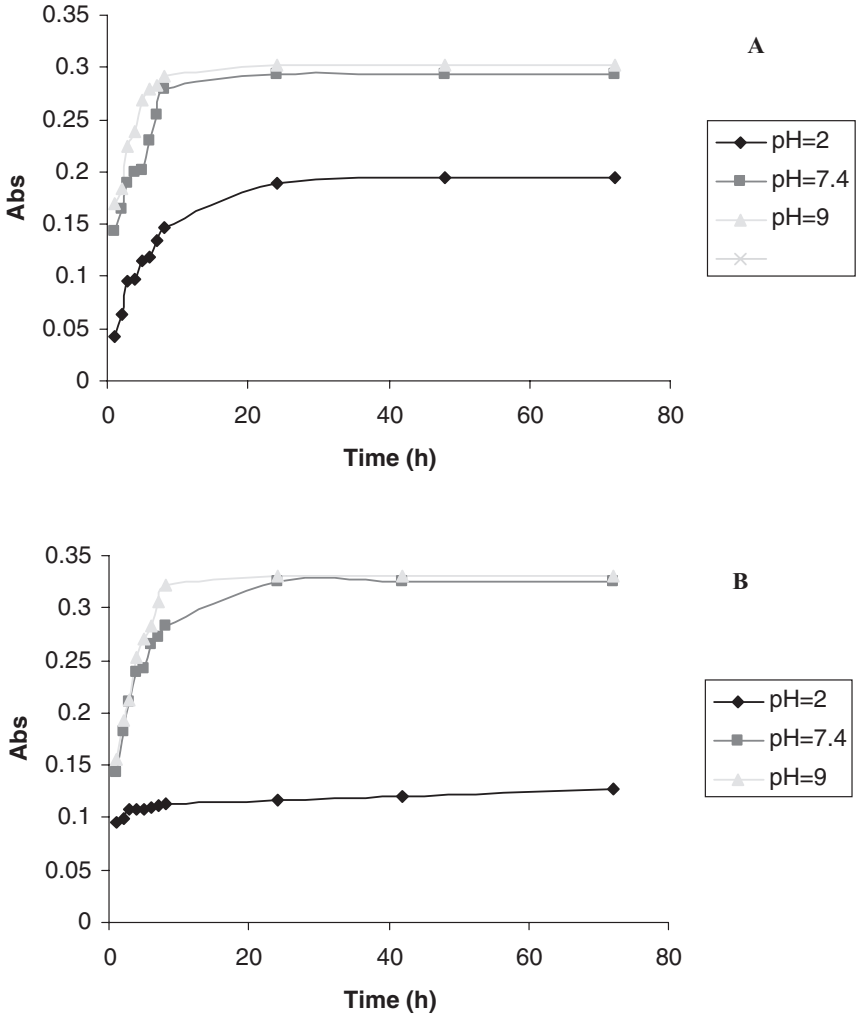


Fig. 6. *In vitro* drug release profile of the physically loaded ceftriaxone antibiotic from the PSMA-isopropyl amide ((A) 0.5:1 w/w% and (B) 1:1 w/w% in feed of physical loading reaction) in phosphate buffer with various pH values at room temperature.

4 Conclusions

Two systems for controlled delivery of ceftriaxone antibiotic were developed. These systems were based on PSMA copolymer. In the case of PSMA-g-ceftriaxone, the polymeric system was represented, in which the degradable amide linkage can control the release rate of conjugated drug in various medium. The physical loading of ceftriaxone antibiotic on modified PSMA was investigated. *In vitro* release of ceftriaxone was determined with a UV spectrophotometer at $\lambda_{max} = 296$ nm as a function of time. The release was monitored in phosphate–buffered solutions of pH 2, 7.4, 9 at room temperature (25°C). *In vitro* release profile showed that the amount of drug release in chemical loading was higher than the physical loading. Also the release rate showed a high dependence on the pH of the release medium. In addition, at pH 2, the release in chemical loaded form was not shown, whereas in the physical loaded form, there was a slight release at pH 2. The release dependence of the cefriaxone antibiotic on the pH from the obtained loaded copolymeric system has made it a favorite system for applying as targeting drugs to specific locations such as the small intestine, since the polymeric formulation is not highly affected by the low pH such as stomach pH.

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