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Synthesis of a Novel pH-Sensitive Polyurethane–Alginate Blend with Poly(ethylene terephthalate) Waste for the Oral Delivery of Protein

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ABSTRACT: With the aim of using poly(ethylene terephthalate) (PET) waste for the synthesis of a value added product, we prepared polyurethane (PU) from bishydrohxyethylene terephthalate (BHET), a byproduct obtained from the glycolysis of PET. Biodegradable, water-swelling PU was synthesized by the reaction of BHET, hexamethylene diisocyanate, and poly(ethylene glycol) (PEG). Both BHET and PU were characterized by Fourier transform infrared spectroscopy, and the formation of PU was further confirmed by NMR analysis. The swelling behavior of PU in water was examined in terms of the various molecular weights of PEG. Semi-interpenetrating network beads of PU and sodium alginate were prepared with calcium chloride ($CaCl_2$) as a crosslinker to attain a pH sensitivity for successful oral protein/drug delivery. Bovine serum albumin (BSA) was used as a model protein. The pH-responsive swelling behavior and protein (BSA) release kinetics in different pH media corresponding to the gastrointestinal tract (pH 1.2 and 7.4) were investigated. The degree of swelling in the case of the PU–alginate beads at pH 1.2 was found to be at a minimum, whereas the degree of swelling was significantly elevated (1080%) at pH 7.4. This substantiated the pH sensitivity of the polymeric beads with a minimum loss of encapsulated protein in the stomach and the almost complete release of encapsulated protein in the intestine. This revealed good opportunities for oral protein/drug delivery with a polymer derived from waste PET. Moreover, the fungal biodegradation study confirmed its compatibility with the ecological system. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40650.

KEYWORDS: biodegradable; biomedical applications; drug-delivery systems; polyurethanes

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INTRODUCTION

Poly(ethylene terephthalate) (PET) is one of the most resourceful engineering plastics; it has outstanding thermal and mechanical properties and is generally used to manufacture textiles and bottles for packaging.[1,2] In recent times, a potential concern has been taken in the utilization of PET wastes for the production of specialized goods such as unsaturated polyesters,[3,4] polyurethane (PU) foams,[5] and polymer concrete.[6] Chemical techniques for recycling PET waste are suitable only for condensation polymers and include the breakdown of waste plastics of a single polymer type into oligomers, dimers, and monomers that can be purified and repolymerized for _valued end uses.[5,7,8] Three distinct processes–glycolysis, methanolysis, and hydrolysis—can accomplish it.[9–11] Although a variety of degradable and nondegradable polymers have been used as matrices to incorporate the drugs, biologically degradable polymers are preferred for drug-delivery applications.[12–14] Therefore, our aim was to synthesize PU for the purpose of drug delivery with the glycolized product of PET waste. PUs are one of the most captivating biodegradable polymers and show their novelty in biomedical applications.[15–19] Several researchers have worked with PU systems for the sustained and controlled delivery of anticancer drugs,[14,20] colon-specific drugs,[16] antifungal drugs,[18] and anti-inflammatory drugs.[21] The field of the controlled delivery of proteins has grown enormously in recent times.[22] Proteins are poorly absorbed and easily degraded by proteolytic enzymes in the gastrointestinal (GI) tract; hence, their oral bioavailability is generally poor. Thus, a potential system for delivering proteins is currently in demand. Patient acquiescence of the administration of drugs in the form of oral delivery is the main cause for delivering proteins and peptides by mouth.[23] In the development of oral protein-release devices, another important parameter is the controlled release of the encapsulated protein at different pHs in the GI tract. To fulfill that criterion, sodium alginate (SA) was blended with the synthesized PU, and partially crosslinked beads were prepared with a calcium chloride solution. The stability of the encapsulated protein during the processing, release, storage, safety, and biocompatibility of the degradable polymer is also very much necessary for controlled release applications.[20] In the systemic delivery of proteins, biodegradable polymeric microspheres occupy an important place because of several aspects, such as

Figure 1. Washed and dried BHET synthesized from waste PET. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the protection of sensitive proteins from degradation, the maintenance of the protein structure, sustained release patterns, and improved bioavailability.

In this study, PU was synthesized with bishydrohxyethylene terephthalate (BHET), and the polymeric blend was prepared at three different ratios of PU to SA. A swelling study of alginate beads and beads of the PU–alginate blend at different pHs was done. The encapsulation of bovine serum albumin (BSA) as a model protein and its release pattern from the PU–alginate beads was investigated at different pHs as BSA is universally used for protein delivery studies and standardization. The encapsulation and release profile of BSA were improved with increasing amounts of alginate. The significance of this study is the utilization of waste products for the production of cost-effective protein delivery devices. Thus, it is a potential approach for encapsulating protein drugs within the biodegradable PU–alginate blend matrix, protecting the BSA from systemic degradation, providing rapid clearance, and finally providing a sustained release.

EXPERIMENTAL

Materials

PET flakes were prepared from used clear PET bottles. Ethylene glycol and zinc acetate (as the transesterification catalyst) were purchased from Merck, India. Hexamethylene diisocyanate (HMDI) was purchased from Sigma Aldrich, and nitrogen was purged through it before use. Poly(ethylene glycol)s (PEGs), with molecular weights of 400 and 600 (Merck, India), were vacuum-dried before use. Chloroform was also supplied by Merck, India. SA was supplied by LOBA Chemie, and calcium chloride ($CaCl_2$), BSA, copper sulfate, sodium carbonate, and sodium hydroxide were purchased from Merck, India. Sodium potassium tartrate and folin and Ciocalteu's phenol reagents were supplied by Sisco Research Laboratory.

Methods

Depolymerization of PET. Hydroxyl terminal groups were exposed by the reaction of glycolysis as these hydroxyl groups were prerequisites for the synthesis of PU. The glycolysis of PET was carried out in a 500-mL, round-bottom flask on a magnetic stirrer equipped with a heating oil bath and a reflux condenser. The reaction was carried out with postconsumer PET at a temperature of 190°C in the presence of zinc acetate catalyst (1 wt %) with an ethylene glycol/PET ratio of 9:1 under an N_2 blanket. After the glycolysis reaction, boiling water was added to the product under vigorous agitation for 30 min at a temperature of 100°C. Then, the mixture was filtered to remove unwanted solid materials. The recovered liquid was kept at 4°C overnight to obtain the crystallized BHET.[24] Crystals of BHET (Figure 1) were separated by filtration and kept in a vacuum drier until they were dried completely.

Synthesis of PU. The overall BHET/PEG (chain extender) molar ratio was varied in the synthesis of PU. BHET was placed into a three-necked round-bottom flask with a magnetic stirrer with a hot oil bath, reflux condenser, and under an N_2 atmosphere. The temperature of the oil bath was increased to 110°C. The ratios of BHET to the chain extender (PEG) for the synthesis of PU are tabulated in Table I. When the BHET was completely melted, HMDI (1.682 g, 0.02 mol) was added to it, and the temperature was increased to 130°C. It took almost 1 h to obtain the isocyanate group (NCO) terminated prepolymer. The conversion of the prepolymer into the final PU was carried out by continuous stirring of the prepolymer throughout the reaction. The temperature was increased again to 180°C after the chain extender was added. When homogeneity was obtained in the reaction mixture, the dispersion of the chain extender was considered to be completed. Then, the mixture was cooled, and the polymer was dissolved in chloroform and cast onto a Petri plate to form a sheet. The synthesized polymer was placed in a circulating hot-air oven and dried for 24 h.

Preparation of the PU–SA Blends. The required ratios of SA and PU were kept overnight in distilled water under continuous stirring to obtain the solutions. The PU–SA blends were prepared to introduce pH sensitivity into the polymer matrix for the oral delivery of the protein (BSA).

Preparation of the BSA-Loaded PU–Alginate Blend. To prepare the BSA incorporated PU–alginate semi-interpenetrating network (semi-IPN) beads, BSA with final concentration of 0.1% w/v was added to the dissolved PU–alginate solution with continuous stirring to form homogeneous PU–alginate–BSA blend solution.

Table I. PU Compositions

PU name	HMDI/BHET/PEG molar ratio	Molecular weight of PEG
PU-1208P4	2:1.2:0.8	400
PU-1307P4	2:1.3:0.7	400
PU-1406P4	2:1.4:0.6	400
PU-1505P4	2:1.5:0.5	400
PU-1604P4	2:1.6:0.4	400
PU-1307P6	2:1.3:0.7	600

Preparation of Blank and BSA-Loaded PU–Alginate Beads. The PU–alginate blend(5 mL) was added dropwise to 20 mL of a 2% w/v calcium chloride solution through a 26-gauge stainless steel needle to prepare the semi-IPN beads. The dropping rate of the blend solution into the $CaCl_2$ solution was 1 mL/min. In the case of the PU–alginate beads, only SA was crosslinked with Ca^{2+}. So, the beads were considered to be semi-IPN beads. The distance between the edge of the needle and the surface of the calcium solution was kept apart at 10 cm. The beads were left in the gelling medium for 30 min. Then, the beads were separated from the solution through a Buchner's filter and left to dry for 24 h at room temperature in the vacuum oven before further use.

For the preparation of drug-loaded beads, a calculated amount of BSA was added to the solution of the PU–alginate blend and kept under magnetic stirring. The subsequent processes were the same as in the preparation of the PU–alginate blank beads.

Characterization of the Synthesized Polymers

Acid and Hydroxyl Values of BHET. The acid value of BHET was determined by the titration of 1 g of sample with a 0.700 mol/L KOH solution with phenolphthalein as an indicator. The hydroxyl value of BHET was determined by a standard procedure (Lubrizol test procedure TP-TM-007C). The molecular weight was determined from the values of titration.

Fourier Transform Infrared (FTIR) Spectroscopy Studies. FTIR analysis was carried out with attenuated total reflection–FTIR (spectrometer model Alpha E, Bruker, Germany), with scanning from 4000 to 500 cm^{-1} for 42 consecutive scans at room temperature. Dried BHET was mixed with potassium bromide (KBr) at a 1:10 weight ratio, and KBr pellets were prepared with 10 tons of hydraulic pressure for 10 min at room temperature. The PU film, HMDI, and PEG were used directly for analysis in the attenuated total reflection mode.

NMR Analysis. ^{1}H-NMR spectra were recorded on a Bruker Av 3000 Supercon NMR system (Germany) operating at 300 MHz. The polymeric material was dissolved in deuterated chloroform ($CDCl_3$) in NMR tubes having polytetrafluoroethylene/silicone septum caps. A total of 30 scans of the solution were averaged to obtain the final data.

Water Uptake of PU. The swelling study of five PU films with different compositions based on PEG400 along with a PU film based on PEG600 (as tabulated in Table I) was performed in distilled water at room temperature. The experiment was continued for 2 h, and readings were taken with a regular interval of 15 min. The degree of swelling was calculated and plotted with time (shown later in Figure 4).

Biodegradation Study of PU Films. *Phamerochaete crysosporium* (MTCC no. 787, ATCC no. 24725) was grown in media in a conical flask. PU films were cut into medium-size pieces and kept in the conical flask at 37°C to perform a 30-day-long biodegradation study. The PU films were collected on the 15th and 30th day. The films were washed with ethanol and dried. Scanning electron microscopy (SEM) was performed to investigate fungal biodegradation.

Drug Encapsulation of PU. A polymer solution was prepared with a final concentration of 1 mg/mL. The pH was adjusted to 5.5, and the BSA solution was prepared with a final concentration of 1 mg/mL with the pH maintained at 8.2. Both solutions were mixed vigorously with a vortex and were kept at 37°C for another 30 min to form the drug–polymer complexes. Then, the mixture was centrifuged to separate the supernatant. The drug entrapment efficiency was calculated with a Lowry's protein assay with standard curve of BSA.

Morphological and Bead Size Analysis of the PU–Alginate Blends. The shape of the PU–alginate beads was observed through the images taken with the help of a scanning electron microscope (EVO-18, Carl Zeiss, Germany). The mean diameter of the beads was measured by a digital screw gauge (Mitutoyo, Japan). The average values were taken from at least 25 beads.

Swelling Study of the PU–Alginate Beads in Different pH Media. Swelling studies were carried out with PU–alginate beads of three different ratios in two buffer solutions with different pHs: simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). Accurately weighed amounts of beads were immersed in 10 mL of each media at 37 ±0.5°C for 6 h. The swollen beads were taken out of the medium and weighed immediately in a microbalance; this was followed by blotting with a paper towel to remove excess water on the surface at fixed time intervals. The degree of swelling was calculated with the following formula:[25]

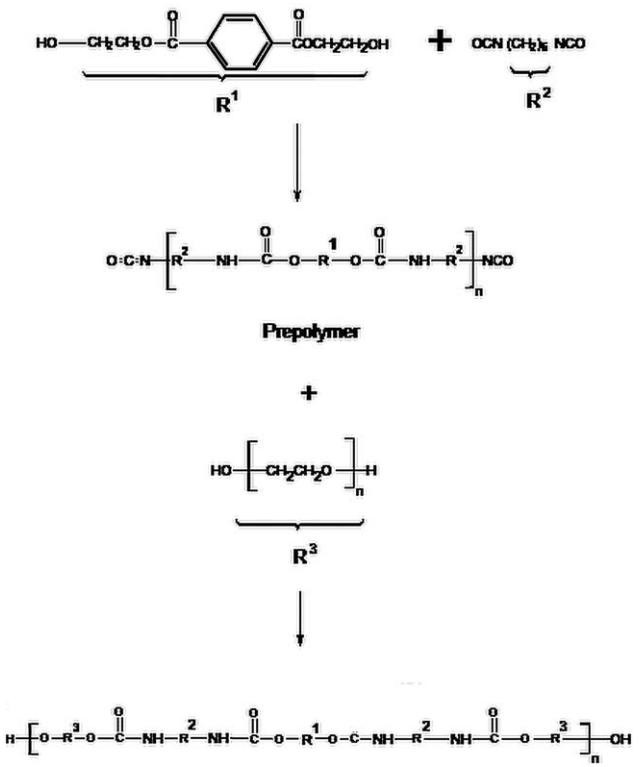
$$\text{Swelling degree } (\%) = \frac{(W - W0)}{W0} \times 100\% \quad (1)$$

where W is the weight of the beads after swelling and W_0 is the initial weight of the beads.

Encapsulation Efficiency of the PU–Alginate Beads. The BSA content of the PU–alginate beads was determined with a UV spectrophotometer (Optizen, India). Dried PU–alginate beads containing a known amount of BSA was added to 10 mL of a phosphate-buffered saline (PBS) solution with a pH of 7.4 and sonicated for 30 min. When the beads were completely mixed in the buffer solution, the mixture was centrifuged, and the clear supernatant was collected for measurement. The content of BSA was calculated from a standard curve of BSA with Lowry's protein assay, and eq. (2)[26] was used to determine the encapsulation efficiency. The encapsulation efficiency of each blend formulation was studied and the data are represented as the mean value. The statistical analysis was done using one way analyses of variance (ANOVA). On the encapsulation efficiency data, and the p value was used as a limit to indicate statistical significance:

$$\text{Encapsulation efficiency} = \frac{\text{Drug present in the beads}}{\text{Amount of drug used}} \times 100 \quad (2)$$

Study of the *In Vitro* BSA Release from the PU–Alginate Beads. The BSA release study was carried out in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). An accurately weighed amount of dried PU–alginate beads with three different compositions were separately put into three glass beakers containing 10 mL of buffer solution and kept at 37 ± 0.5°C with a rotation speed of 60 rpm. Each composition contained 1 mg of BSA. After specific time intervals (the initial first hour with



Scheme 1. Structure of PU obtained by a two-step reaction of BHET, HMDI, and PEG. Where, $R^1 = CH_2CH_2O(OC)Ph(CO)OCH_2CH_2—$, Ph stands for Benzene ring®, $R^2 = —(CH_2)_6—$, $R^3 = —[CH_2CH_2O]n—$.

15-min intervals, the second hour with 30-min intervals, the third and fourth hours with 60-min intervals, and finally, the fifth hour onward at time intervals of 120 min), 1 mL of dissolution media was collected from the release medium, and this was replaced with the same amount of fresh PBS solution each time after withdrawal of the sample. The collected samples were centrifuged, and the supernatants were used to measure the unknown concentrations by Lowry's assay with a UV spectrophotometer at the absorbance of 660 nm. All of the dissolution runs were performed in triplicate. The cumulative amount of drug released at different time intervals were calculated from the following formula[27] and plotted against time to study the BSA release pattern:

$$\text{Cumulative amount released} = \frac{CD}{\text{Amount of drug used}} \times 100\% \quad (3)$$

where C and D refer to the concentration of drug (BSA) in the release medium and the volume of the release medium, respectively.

RESULTS AND DISCUSSION

Synthesis of PU Based on BHET

The preparation of PU was two-step process, as shown in Scheme 1. The first step was the reaction between BHET and the aliphatic diisocyanate HMDI. The hydroxyl value of BHET was found to be between 180 to 250 for different batches. According to the calculation deduced from the values of titration, 1 mol of —OH was present in approximately 126 g of BHET. PEG as the chain extender was used in a little excess intentionally to compose the polymer product (PU) with hydroxyl terminals. We managed the distribution of BHET and PEG by the side of the polymer chain reaction by carrying out the reaction in two steps. The formation of PU was confirmed by FTIR and NMR studies.

FTIR Analysis

FTIR spectra of BHET, HMDI, PEG600, and PU are shown in Figure 2(A–D), respectively. The FTIR spectrum of BHET [Figure 2(A)] showed a peak at a wave number of 3449 cm^{-1}; this corresponded to OH stretching, whereas the peak at 1717 cm^{-1} corresponded to ester carbonyl groups (C═O). This was similar to that previously reported by Xi et al.,[8] and peaks at 2879–2964 cm^{-1} for the C—H stretching, 1285 and 1133 cm^{-1} for asymmetrical and symmetrical C—O—C stretching, and 727 cm^{-1} for the stretching of the C—H para position in the aromatic ring were also detected, as reported by Badri et al.[5] and Syariffuddeen et al.[7] The FTIR spectrum of PEG600 [Figure 2(C)] showed peaks at 3467 cm^{-1} for OH terminal stretching, 2867 cm^{-1} for C—H stretching, 1300–1460 cm^{-1}

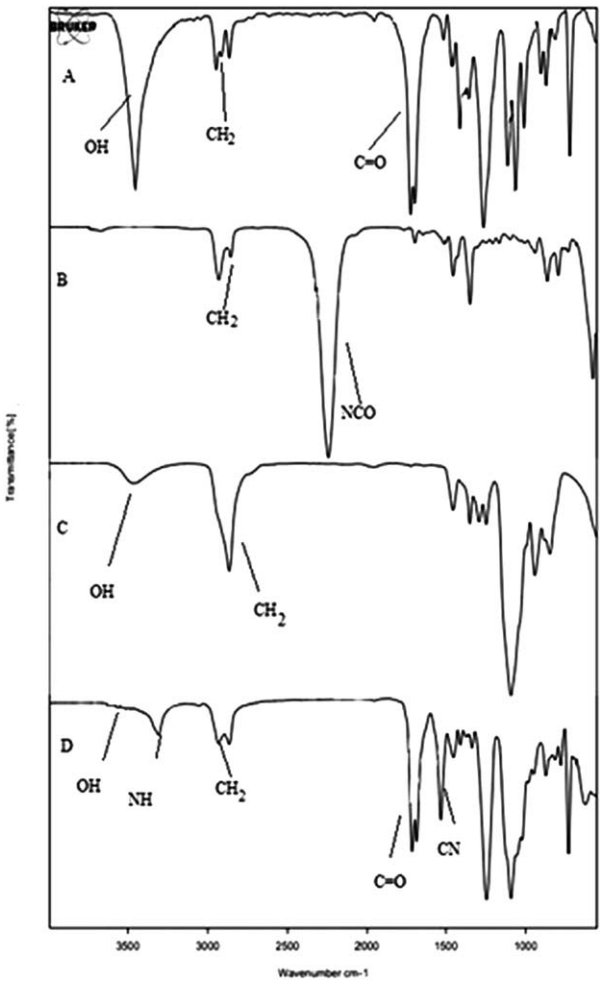


Figure 2. FTIR spectra of (A) BHET, (B) HMDI, (C) PEG600, and (D) PU.

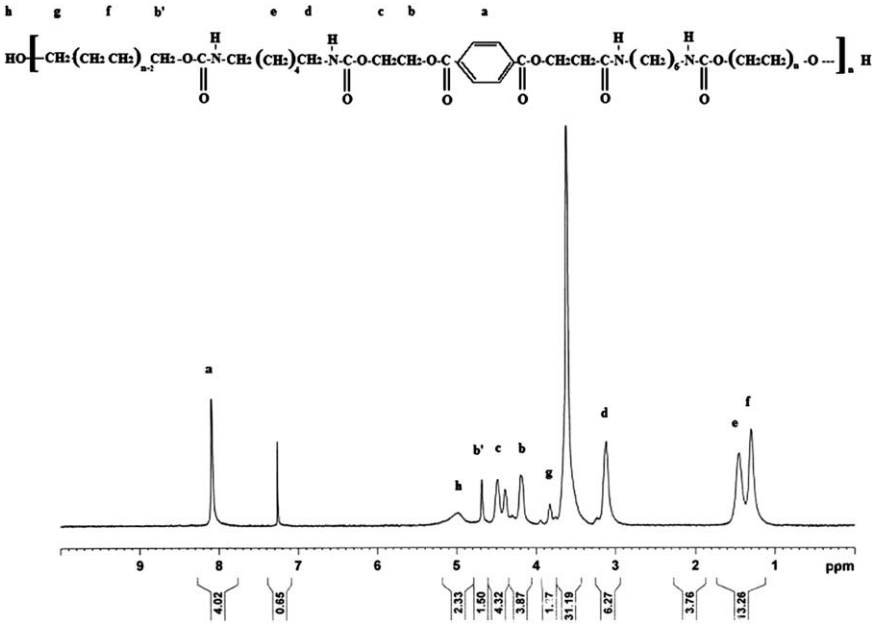


Figure 3. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of PU.

due to C—H scissoring and bending, 1000–1260 cm^{-1} due to alcohol stretching, and 1050–1150 cm^{-1} due to C—O—C stretching.

A characteristic band of isocyanate was present at 2248 cm^{-1} in the FTIR spectrum of HMDI [Figure 2(B)]; this indicated the terminal N—C—O group. The reaction of BHET, HMDI, and the chain extender PEG resulted in the formation of PU, as demonstrated by a strong band at 1716 cm^{-1} in the IR spectrum. This peak was attributed to the formation of urethane carbonyl bond (—NHCOO—) groups with the concomitant disappearance of the isocyanate groups (—NCO), as shown in Figure 2(D). FTIR analysis by Maafi et al.[13] also revealed a similar attribute with regard to the presence of isocyanate groups in the PU prepolymer and the disappearance of that characteristic peak after complete chain extension. The existence of a band at 3314 cm^{-1} was characteristic of the valence vibrations of bound N—H linkages; the band at 1532 cm^{-1} for C—NH stretching agreed with a previous report by Sadeghi and Sayaf.[11] Peaks for CH_2 appearing between 2860–2935 and 727 cm^{-1} represented the characteristic of aromatic C—H linkage deformation. The band at 1250 cm^{-1} was attributed to C—O—C. The broad peak around 3400–3500 cm^{-1} adjacent to the peak for the N—H group indicated the presence of the hydroxyl group in the polymer.

NMR Analysis

The chemical structure of PU was analyzed by NMR spectroscopy. The ^{1}H-NMR spectrum of PU is shown in Figure 3. The proton spectrum of PU indicated the presence of characteristic peaks of PU. The peak labeled *a* showed the resonance of protons of the aromatic benzene group ($\delta_H = 8.09$ ppm), as reported by Tawfik and Eskander.[6] The b, b′, and c peaks show the methylene groups (—CH_2—) near the —COO— groups ($\delta_H = 4.21$,4.69, and 4.48 ppm, respectively). Peak d indicates the methylene (—CH_2—) groups next to the amine (—NH—) groups ($\delta_H = 3.12$ ppm). Peaks e and f represent the peaks for the long methylene (—CH_2—) groups ($\delta_H = 1.45$ and 1.30 ppm, respectively). Peak g shows the peak of the methylene groups (—CH_2—) near the outer hydroxyl groups($\delta_H = 3.82$ ppm), and peak h indicated the outer hydroxyl groups ($\delta_H = 4.98$ ppm), as reported by Sadeghi and Sayaf.[11] The peaks for chloroform and water accounted for solvent and water contamination in the PU sample.

FTIR analysis,[20,21,28,29] along with NMR spectroscopy,[13,15] indicated that the PU was successfully synthesized.

Water Uptake of PU

Different samples composed of different ratios of BHET to PEG are given in Table I. The sample notation indicated the feed compositions in the molar ratios of BHET to PEG and

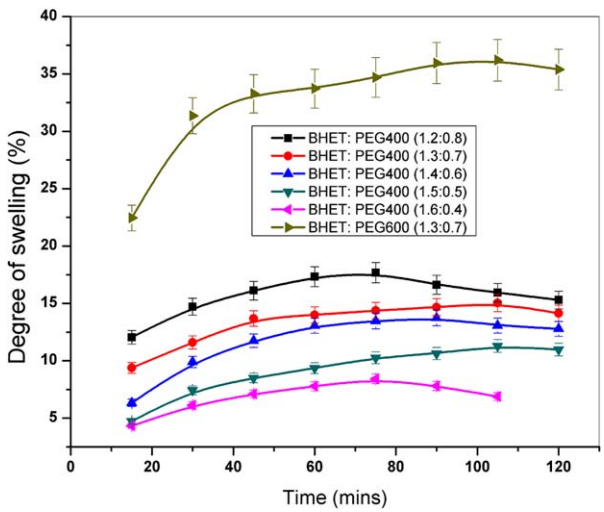


Figure 4. Comparative swelling study of the PU films synthesized with various molar ratios of BHET to PEG. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the molecular weight of PEG. The higher the amount of PEG was, the higher the observed swelling percentage was. BHET and PEG600 were used at a ratio of 1.3:0.7 in PU-1307P6. A similar sample, PU-1307P4, consisting of PEG400, was used to identify the difference between their water uptake characteristics. It is clearly visible in Figure 4 that the PU composed of PEG600 swelled far more than the PUs containing PEG400. The PU macromolecules had PEG chains as end fragments because excess PEG was used for its synthesis. These results demonstrate that the polymer–water interaction varied greatly with the amount and molecular weight of PEG. The swelling properties of PU in water were reasonable, as PEG is itself hydrophilic in nature. The physicochemical properties and the swelling degree of polymers synthesized from PEG depended on the molecular weight of PEG.[27] This could be explained from the fact that the hydrophilic content of the PU helped in the greater binding of water molecules to the polymer network, and the degree of swelling increased accordingly. The swelling study of the PU films were also executed in different pH media, but these results did not establish a valid difference to support pH sensitivity.

Biodegradation Study (SEM)

It is clearly visible from the SEM images (Figure 5) that the fungus degraded the films. No fungal growth was visible on the films, as several investigators have also reported that microbial attack on PUs could be achieved through the enzymatic action of extracellular hydrolytic enzymes.[30] These organisms do not grow solely on PUs, and the enzymes secreted in the growth media break down the polyester of PU. Microorganisms use PUs as their sole carbon source and are dependent on the PU molecular structures and the types of their chemical links. Previous studies have shown that many fungi inhabit PU under different conditions. Some remains only on the surface, whereas others penetrate into the bulk. Several of these fungi produce esterases that are capable of degrading PU.[31] Some fungus degrades PU if provided with additional nutrients. The presence of nutrients promote the growth of fungi in the media. Polyesters are polymers in which component monomers are bound via ester linkages. Ester linkages are generally easy to hydrolyze, and hence, a number of synthetic polymers are biodegradable. Figure 5(A) indicates the control, which had a rough surface, whereas Figure 5(B) shows a smooth surface along with some fissures. It is clearly visible from Figure 5(C) that the surface layer of the film was degraded, and the inner layer came out from the extensive fissures. The presence of the crack on the surface of the PU films demonstrated the occurrence of fungal degradation. A long-term study will be performed to investigate complete degradation of the PU films by the fungus. The results suggest that the PU synthesized with PET waste should not cause any environmental setbacks after their disposal because fungus can degrade it.

Drug Encapsulation of PU

PU was suspended in distilled water. Simultaneously, an aqueous solution of BSA was prepared, and the pH values of both were maintained as previously mentioned before their mixing. The BSA encapsulation efficiency was calculated three times to obtain an average value, and it showed a nearly 76% encapsulation efficiency. The result indicates that the self-assembling of BSA with PU was successful, and the polymer could be used as a means of carrying protein drugs. The difficulty in executing this job was the swelling properties of PU. As discussed earlier in the swelling study of the PUs, the synthesized PUs did not show any pH sensitivity during swelling. Therefore, a blend of SA and PU was prepared to include this quality in the PU based drug-delivery vehicle.

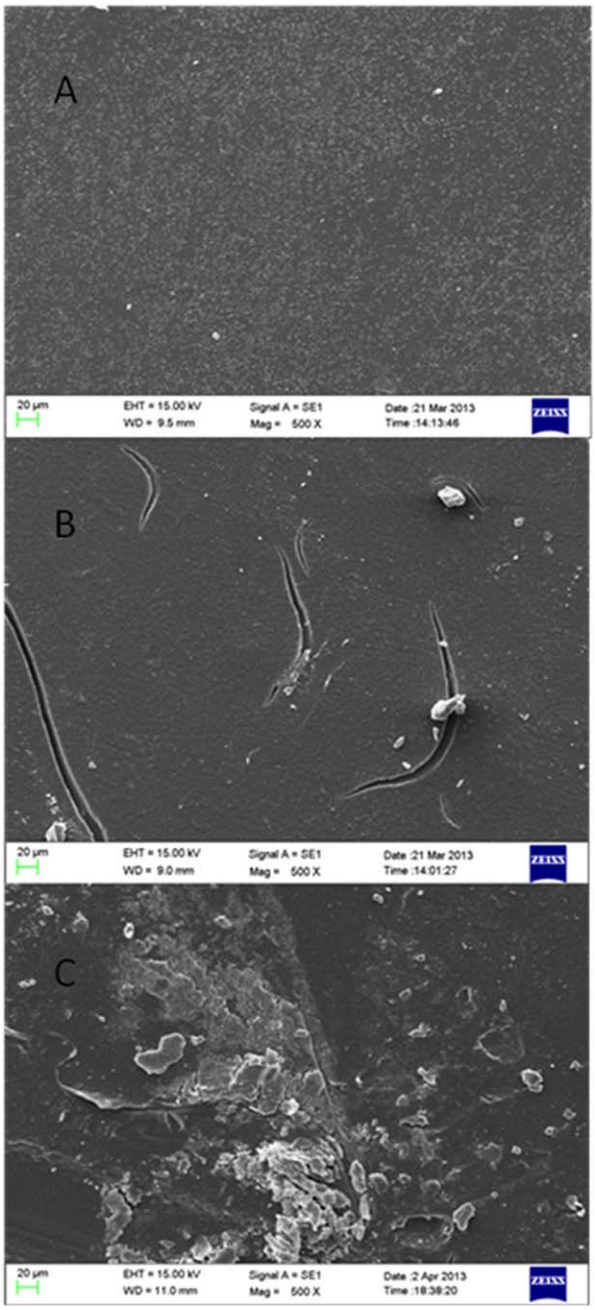


Figure 5. SEM images of (A) the control PU film, (B) the film after 15 days of fungal biodegradation, and (C) the film after 30 days of fungal biodegradation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Compositions of the PU–Alginate Beads

Blend name	PU/alginate molar ratio	Amount of PU (g)	Amount of alginate (g)	$CaCl_2$ (%)	Water (mL)	BSA (mg)
PU-Alg1	9:1	0.9	0.1	2	10	10
PU-Alg2	8:2	0.8	0.2	2	10	10
PU-Alg3	7:3	0.7	0.3	2	10	10

Blends of PU and SA for the Preparation of Semi-IPN Beads and Characterization of the Beads

The different PU–alginate blend compositions are listed in Table II. The overnight agitation of PU and SA in an aqueous medium was performed to prepare various homogeneous mixtures of different sets of blends. PU–alginate polymeric beads were prepared by the crosslinking of alginate chains by calcium chloride[32,33] by the addition of polymeric blends into the crosslinking solution of calcium chloride. Alginate was crosslinked immediately in a Ca^{2+} solution, and thus, microencapsulated beads were formed.[25] On the contrary, PU did not get crosslinked in the calcium chloride solution. PU–alginate blends were partially crosslinked because of the presence of SA, and semi-IPN beads were prepared. pH-responsive, alginate-containing PU blends were designed to allow sustained BSA release through the prevention of premature protein release in the acidic environment. We expected that this combination would allow us to acquire the strength of both PU and alginate hydrogel.

Morphological and Bead Size Analyses

The pictures of air-dried PU–alginate beads with different ratios (9:1, 8:2, and 7:3) along with the enlarged pictures of their surface and alginate beads with different concentrations (1, 2, and 3%) taken under the scanning electron microscope are shown in Figure 6. Alginate beads of low concentration were of uneven surface and not spherical in shape, whereas with increasing concentration, the beads had a circular shape and smoother surface [Figures 6(B–D)]. In the case of the photographs of the beads of the PU–alginate blend, beads with a maximum amount of PU had a rough, uneven surface and were not quite circular in shape; the had severe cracks and wrinkles all over the beads [Figure 6(E,F)]. Beads with a ratio of 8:2 were almost circular in appearance, were relatively even, and had minimal cracks on the surface [Figure 6(G,H)]. Beads with the highest amount of alginate were very smooth and were perfectly circular in shape [Figure 6(I,J)]. The pores and fissures were reduced in the beads as the amount of alginate increased gradually in the blend and retarded the rapid release of BSA at an early stage.

The wet beads of the PU–alginate blend were white in color [Figure 6(A)], and on drying, their color changed to dark yellow. The diameter of the alginate beads were found to increase with increasing concentration of alginate solution. The diameters of the beads prepared from 1, 2, and 3% alginate solutions were 0.50 ± 0.5, 0.67 ± 0.10, and 0.79 ± 0.10 mm, respectively. The diameter of the beads increased to 1.04 ± 0.05, 1.15 ± 0.05, and 1.27 ± 0.05 mm, respectively, with increasing amount of PU in the blends. No difference was observed in the pictures of the BSA-loaded and unloaded beads (these pictures are not included in the figure).

Swelling Study

Swelling experiments at pH 1.2 and 7.4 buffers were performed for alginate beads and PU–alginate beads to check its sensitivity toward changing the pH in the GI tract. A sharp change in the pH range from 1–2 (gastric fluid) to 7–7.4 (intestinal fluid) occurred when the drug-loaded polymer has to travel from the stomach to the small intestine. The swelling measurements of the alginate beads and PU–alginate beads prepared at different compositions are shown in Figures 7 and 8, respectively. The swelling characteristics of the beads at pH 1.2 are shown in Figures 7(a) and 8(a). At pH 1.2, with increasing amount of alginate, the degree of swelling of the beads decreased. The ultimate swelling of the beads at pH 7.4 increased with increasing content of alginate. Previous studies reported in the literature also suggested similar features. The increase in the degree of swelling of the beads with increasing amount of alginate continued over a long period.[34–36] The nature of the swelling characteristics gave an obvious viewpoint toward the practical applicability of the polymeric blend in the form of beads. The beads disallowed the release of an extensive amount of drug while traversing through the acidic medium of the gastric fluid and released the mass of entrapped drug in the small intestine.[37] The swelling rate decreased because of the increasing content of SA. At pH 1.2, the degree of swelling was found to be very low for the blend beads because of hydrogen-bond formation between the —OH groups of PU and the —OH groups of alginate. The degree of swelling for alginate beads (1, 2, and 3%) at pH 1.2 were 48, 36, and 29%, respectively. Yuan et al.[33] reported that at pH 1.2, alginate beads were hydrolyzed because of the low-molecular-weight fraction of alginic acid. Un-ionized carboxylic groups were generated because of the conversion of COO^- group, and the disappearance of electrostatic attraction between the Ca^{2+} ions and COO^- ions in the egg-box junction took place. Ion exchange between H^+ ions and free Ca^{2+} ions inside the beads assumed to be protecting the beads. In the case of the composition with a PU/alginate ratio of 9:1, the maximum degree of swelling degree was 29.60%, but with an increase in the content of alginate in the blends (8:2 and 7:3), the degree of swelling decreased to 26.36 and 25.55%, respectively. This result also signified that the further addition of SA in the blends did not help in significantly decreasing the degree of swelling.

PU–alginate blend beads showed remarkable swelling properties at pH7.4. It has been reported that alginate easily swells at alkaline pH because it can react with the alkali, and this leads to its complete dissolution.[38] The polymer blend (at a 9:1 ratio) swelled up to 5 h and showed about a 940% degree of swelling,

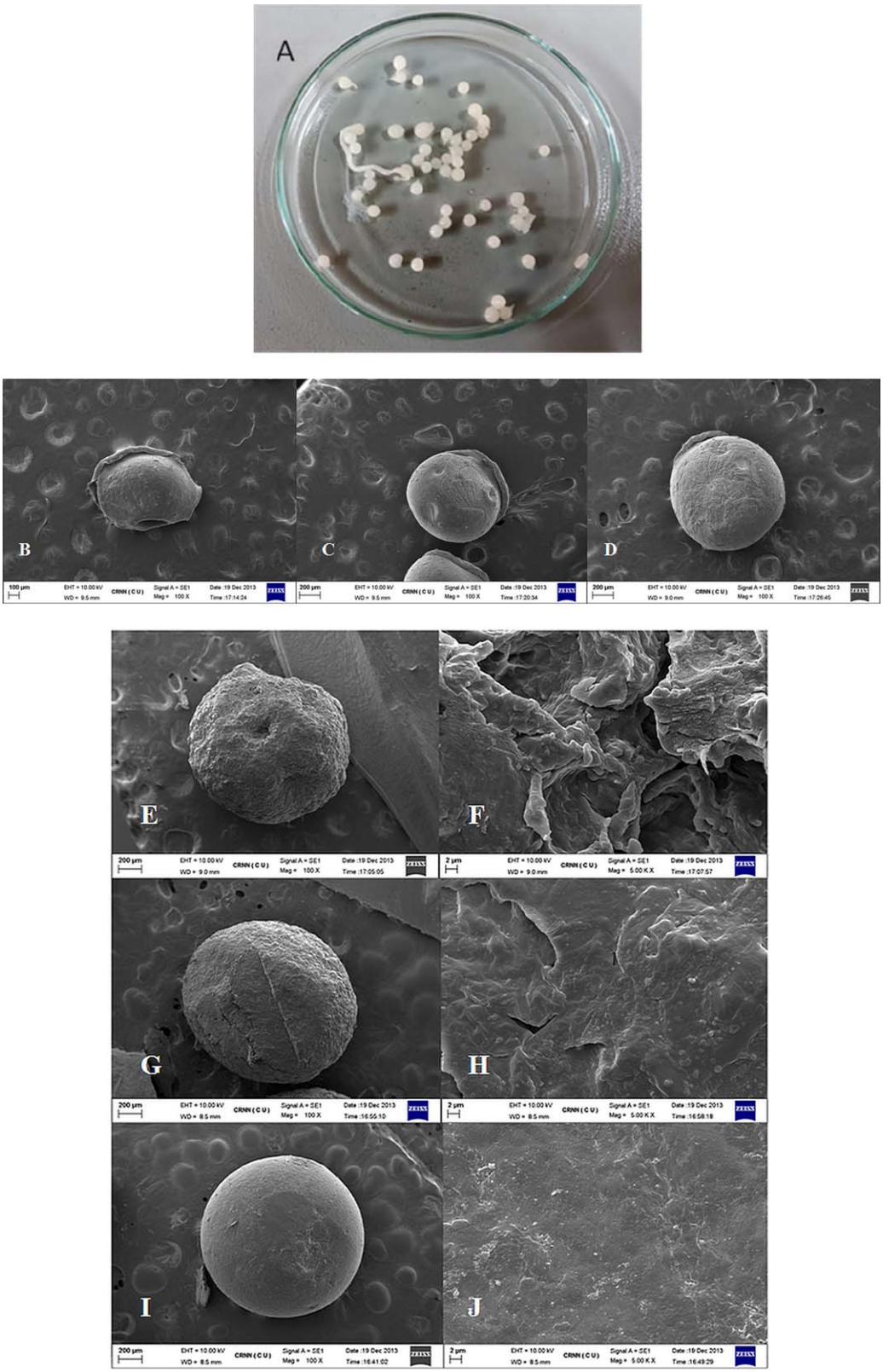


Figure 6. (A) Image of the wet PU–alginate beads and SEM images of (B) a 1% alginate bead, (C) a 2% alginate bead, (D) a 3% alginate bead, (E,F) a PU–alginate (9:1) bead, (G,H) a PU–alginate (8:2) bead, and (I,J) a PU–alginate (7:3) bead. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

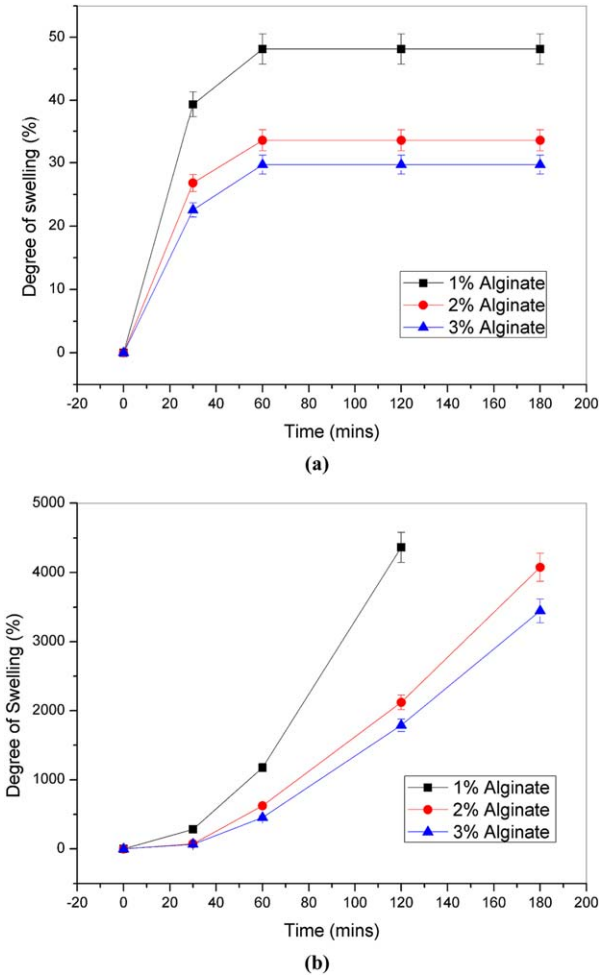


Figure 7. Comparative swelling study of three different types of alginate beads with different percentages of SA at pHs of (a) 1.2 and (b) 7.4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

whereas beads prepared from the 1% alginate solution swelled up to 2 h with a very high degree of swelling (4364%), and then, dissolution occurred in both cases. The blend beads of PU and alginate at a ratio of 8:2 showed a degree of swelling similar to that of the previous blend at 5 h. However, the beads survived in the swelling medium for a few more hours but lost their shape and were destroyed at 6 h. The alginate beads (from the 2 and 3% solutions) were dissolved in the swelling medium at pH 7.4 at 3 h after they showed swelling degrees of 4229 and 3445, respectively. The degree of swelling was about 1080% at 6 h for the 7:3 PU/alginate, but further measurement was not possible because of bead disintegration in the swelling medium and the loss of material in blotting cloth and because of the highly hydrated structure and the almost complete removal of calcium ions. The results of the swelling studies at pH 1.2 and 7.4 indicated that beads prepared with alginate easily dissolved at pH 7.4, and their fast swelling behavior limited their application at this pH. In comparison to the beads prepared, the incorporation of PU with alginate at different ratios resulted in sustained swelling behavior over long period. The swelling properties varied with the changing ratio of PU to alginate. In a comparison of Figures 7(b) and 8(b), the disintegration rate of the PU–alginate beads in PBS (pH 7.4) was significantly lower than that for the alginate beads. At this pH, the swelling and disintegration rates were found to decrease with increasing amount of alginate. This may have been related to the increased elastic force resulting from hydrogen bonding between the amine and hydroxyl groups of PU and SA or the physical cross-linking because of polymer chain entanglement.

Encapsulation Efficiency

The encapsulation efficiencies were checked for BSA-encapsulated beads at different ratios of the PU–alginate blends. The result indicates that an increase in the concentration of SA in the blend improved the encapsulation efficiency. Three batches of BSA-loaded beads prepared from the PU–alginate blends were taken to estimate the encapsulation efficiency. Figure 9 illustrates that encapsulation efficiency of the PU–alginate beads of all three formulations were much better and were statistically significant ($p \leq 0.05$) compared to the alginate bead control. When the amount of PU was 9% w/v and that of

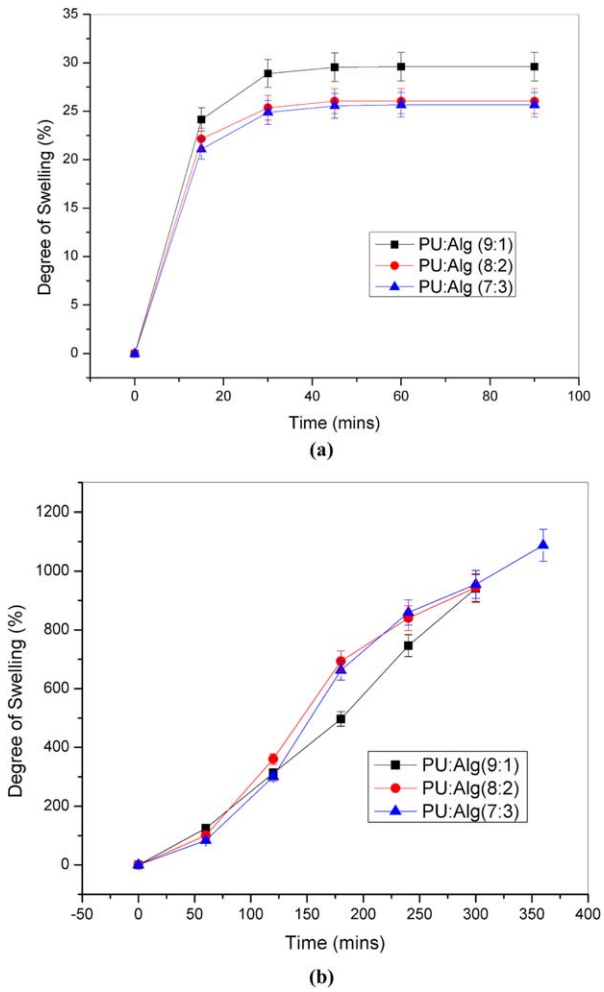


Figure 8. Comparative swelling study of three different compositions of PU–alginate (Alg) beads at pHs of (a) 1.2 and (b) 7.4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

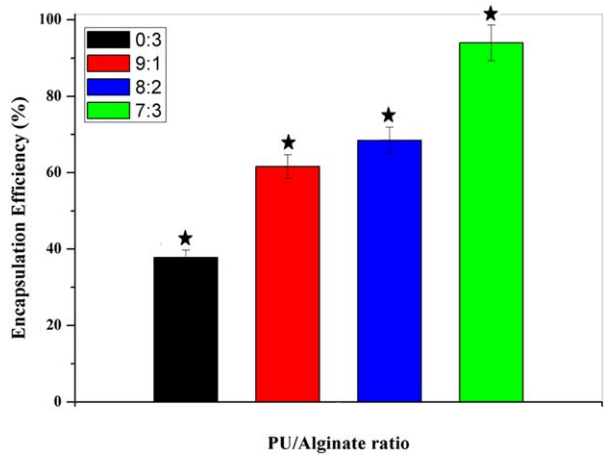


Figure 9. Encapsulation efficiencies of the PU–alginate beads. The values are shown as means and standard errors ($n = 3$). $^{*}p \leq 0.05$ (significant). *denotes the significant change in comparison to control (alginate bead). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

alginate was only 1% w/v, the encapsulation was 61.6%, but with the intensifying amount of SA, for a PU-to-alginate ratios of 8:2 and 7:3, the encapsulation efficiency increased up to 68.5 and 94%, respectively. The cation diffused between the alginate chains and bonded to unoccupied binding sites on the polymer. Thus, with increasing number of biopolymer molecules per unit solution volume, the number of binding sites for Ca^{2+} ions also increased. As a result, a more densely crosslinked gel structure probably formed. This explained the result in which the encapsulation efficiency increased and the release of BSA decreased with increasing alginate concentration.

BSA Release from the Calcium–PU–Alginate Beads

The *in vitro* release of BSA from the PU–alginate beads prepared by the previously described method was studied in both acid (pH 1.2) and alkaline (pH 7.4) dissolution media under sink conditions. The BSA released at specific time intervals is shown in Figure 10. The *in vitro* release profile of BSA indicated a prolonged release time with increasing alginate content in the blends. The BSA release profile signified that the BSA release from the beads at pH 1.2 was very low and ceased after 45 min as the beads did not swell further after 45 min. This was due to the limited degree of swelling of the polymer network and the low solubility of BSA at this pH. Subsequently, the rate of BSA release decreased with increasing content of SA in the blends. This was very similar in characteristics to the swelling behavior. The results of this study prove that the maximum amount of BSA was protected from acid and enzymes in gastric juice. The PU–alginate beads prepared at ratios of 9:1, 8:2, and 7:3 released approximately 99, 86, and 68% of BSA in the pH 7.4 PBS medium after 5 h. Beads from the blend ratio of 8:2 released 93.4% of BSA in 7 h, whereas the blend with the maximum amount of alginate (7:3) released 84% of BSA in 9 h. The samples were collected from the dissolution media until the beads were disintegrated. It was clear from this study that the beads with more alginate survived in the PBS (pH 7.4) medium for the maximum time. Various polymeric systems, such as chitosan, gelatin, and hydroxyapatite, have been studied to investigate the release patterns of protein or drug from them.[20] It was reported that a burst release of BSA was found in the case of a chitosan–alginate blend as a delivery system.[26] Jin et al.[27] reported that 50% of the encapsulated BSA was released within 30 min, and it was more than 60% by 1 h at pH 1.2. The cumulative release of BSA in PBS (pH 7.4) was more than 80% at 2 h. As reported by Mladenovska et al.,[39] an initial rapid release followed by a continuous slower release of BSA was observed in case of the gelatin microspheres. Initial burst release was observed in case of hydroxyapatite microspheres. As investigated by Boonsongrit et al.,[40] 70% of the BSA was rapidly desorbed after 30 min, and 80% was desorbed after 24 h. As per our results, the protein (BSA) entrapped in the PU–alginate blend matrices was released by diffusion through the pores of the polymer network and further by disintegration of the beads. The disruption of the calcium alginate matrix occurred more quickly in phosphate buffer at pH 7.4. The affinity of calcium toward phosphate was greater than that to alginate, and consequently, BSA was released from the PU–alginate blend beads through the continuous erosion

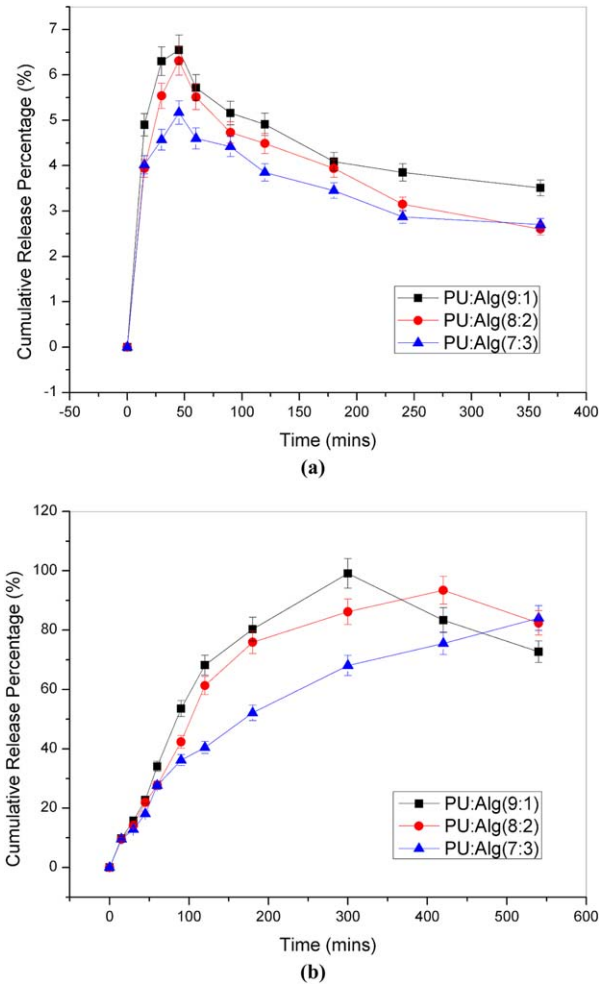


Figure 10. Cumulative percentage release of BSA from the PU–alginate (Alg) beads at pH (a) 1.2 and (b) 7.4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mechanism. Therefore, the prepared beads of the PU–alginate blends showed better entrapment, swelling, and sustained release of BSA as compared to the other polymeric formulations reported in the literature.

CONCLUSIONS

Partially water-soluble biodegradable PU was synthesized with the depolymerized product of PET, and it was confirmed by FTIR and NMR studies. Synthesized PU was blended with SA to induce pH-responsive swelling properties in the semi-IPN beads. PU–alginate bead samples showed excellent pH-responsive swelling. At pH 1.2, the degree of swelling was much lower, whereas at pH 7.4, it was observed to be the highest (ca. 1080%) at 6 h, as found in the *in vitro* swelling studies. BSA as a model protein was entrapped within the bead samples at different ratios (9:1, 8:2, and 7:3) of PU to SA. Consistent with the swelling properties, the PU–alginate beads showed persuasive results of the release studies in the pH 7.4 PBS medium. So, we concluded that pH-responsive PU–alginate beads could serve as potent devices for the oral delivery of protein and other drugs. Thus, the PU–alginate blend could be used as a vehicle for the delivery of BSA and other proteins.

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