Hydrolytic degradation and protein release studies of thermogelling polyurethane copolymers consisting of poly([R]-3-hydroxybutyrate), poly(ethylene glycol), and poly(propylene glycol)

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Abstract

This paper reports the hydrolytic degradation and protein release studies for a series of newly synthesized thermogelling tri-component multi-block poly(ether ester urethane)s consisting of poly([R]-3-hydroxybutyrate) (PHB), poly(propylene glycol) (PPG), and poly(ethylene glycol) (PEG). The poly(PEG/PPG/PHB urethane) copolymer hydrogels were hydrolytically degraded in phosphate buffer at pH 7.4 and 37 °C for a period of up to 6 months. The mass loss profiles of the copolymer hydrogels were obtained. The hydrogel residues at different time periods of hydrolysis were visualized by scanning electron microscopy, which exhibited increasing porosity with time of hydrolysis. The degradation products in the buffer were characterized by GPC, 1H NMR, MALDI-TOF, and TGA. The results showed that the ester backbone bonds of the PHB segments were broken by random chain scission, resulting in a decrease in the molecular weight. In addition, the constituents of degradation products were found to be 3-hydroxybutyric acid monomer and oligomers of various lengths (n = 1–5). The protein release profiles of the copolymer hydrogels were obtained using BSA as model protein. The results showed that the release rate was controllable by varying the composition of the poly(ether urethane)s or by adjusting the concentration of the copolymer in the hydrogels. Finally, we studied the correlation between the protein release characteristics of the hydrogels and their hydrolytic degradation. This is the first example that such a correlation has been attempted for a biodegradable thermogelling copolymer system.

Keywords: Poly(ether ester urethane); Poly([R]-3-hydroxybutyrate); Poly(ethylene glycol); Poly(propylene glycol); Hydrolytic degradation; Drug release

1. Introduction

In the rapidly developing field of biomaterials, thermogelling polymers have exhibited interesting properties that have made them potential candidates for drug delivery and tissue engineering applications [1–6]. Recently, our laboratory has synthesized a series of thermogelling multi-block poly(ether urethane)s consisting of poly([R]-3-hydroxybutyrate) (PHB), poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) using hexamethylene diisocyanate (HDI) as a coupling reagent [7].

PHB is a natural biodegradable and biocompatible polyester which degrades to d-3-hydroxybutyrate, a natural non-toxic human blood plasma component [8,9]. On the other hand, the PEG–PPG–PEG triblock copolymer is a biocompatible polymer that has been approved by the FDA for biomedical and food applications [10,11]. The biostability of polyurethane-based medical implants as well as the leaching out of potentially toxic degradation products are important considerations in the assessment of the suitability of the polymers for biomedical applications [12]. Chain scission of poly(ether urethane)s could either occur at the urethane linkage or the ester linkage, with the
ester linkage being the primary site of hydrolysis in poly(ester urethane)s [13,14].

The degradation of such thermogelling copolymers is different from that of chemically crosslinked hydrogels. In a crosslinked polymer hydrogel, the chemical bond of the crosslink or polymer backbones must be broken before erosion of the polymer fragments can take place [15–17]. The rate of degradation of the conventional crosslinked hydrogel can be controlled by the density and nature of the crosslink or degradability of the polymer backbone. In a physical hydrogel of a thermogelling copolymer, the formation of the hydrogel results from the physical packing of the polymeric segments in solution. Surface erosion can occur with the dissolution of the exposed polymer chains. However, it does not imply that the polymer chains have degraded into smaller fragments: these chains could have merely dissolved in the solution. Degradation of the polymer chains is expected to take place after further exposure to the aqueous environment, thus the degradation behavior is expected to be markedly different from that of chemical hydrogels.

Currently, there have been limited studies of the degradation process of a thermogelling copolymer. Jeong et al. [18] studied the degradation of PEG–PLGA–PEG triblock hydrogel, while Shim et al. [19] studied the biodegradability of a sulfonamide-modified poly(ε-caprolactone-co-lactide)–poly(ethylene glycol)–poly(ε-caprolactone-co-lactide) copolymer. The degradation of the thermogelling copolymers may involve three basic phases: an incubation period, a gel erosion period, and a chain scission period.

Besides the biodegradability, thermogelling copolymers are interesting from the viewpoint of sustained drug release. PEG–PPG–PEG triblock copolymers have been thoroughly studied for drug delivery, wound covering and chemosensitizing for cancer therapy [20–22]. A high concentration of the copolymer is often needed in the thermogelling formulations (above 15 wt%). Such formulations exhibit poor resilience and burst effect of release of drugs. These shortcomings have made the system unsuitable for many biomedical applications [23,24]. Moreover, PEG–PPG–PEG triblock copolymers are non-biodegradable and have been reported to induce hyperlipidemia and increase the plasma level of cholesterol in rabbits and rats, suggesting that its use in the human body may not be an attractive option [25,26]. Our thermogelling poly(PEG/PPG/PHB urethane)s require extremely low polymer concentrations to form hydrogels from aqueous solutions upon temperature rising. The hydrogels are more robust than PEG–PPG–PEG triblock copolymer hydrogels, based on viscosity studies of the hydrogels [7].

In this paper, we present a detailed hydrolytic degradation and protein release study of the thermogelling multiblock poly(PEG/PPG/PHB urethane)s, and a discussion on the correlation between the protein release characteristics and the hydrolytic degradation. To the best of our knowledge, this paper represents the first attempt to correlate certain features of the protein release with hydrolytic degradation for a biodegradable thermogelling copolymer system.

2. Experimental methods

2.1. Materials

Natural source PHB, PEG, PPG, PEG–PPG–PEG triblock copolymer with a chain composition of EGEOEEOEGEO (also known as Pluronic F127), bis(2-methoxyethyl) ether (diglyme, 99%), ethylene glycol (99%), dibutyltin dilaurate (95%), 1,6-hexamethylene disiocyanate (HDI) (98%), methanol, diethyl ether and 1,2-dichloroethane (99.8%) were purchased from Aldrich. The Mn and Mw of the purified PHB were 8.7 × 104 and 2.3 × 104, respectively. The Mn and Mw of PEG were found to be 1890 and 2060, respectively. The Mn and Mw of PPG were found to be 2180 and 2290, respectively. Diglyme was dried with molecular sieves, and 1,2-dichloroethane was distilled over CaH2 before use. Bovine serum albumin (BSA) was purchased from Sigma (Mw = 66,000, according to manufacturer’s specifications). The Mn and Mw of the starting materials were determined by GPC, unless mentioned otherwise.

2.2. Synthesis of multi-block poly(PEG/PPG/PHB urethane)s copolymers

Telechelic hydroxylated PHB (PHB-diol) prepolymers (Mn = 1070) was prepared by transesterification between the natural source PHB and ethylene glycol using dibutyltin dilaurate in diglyme as reported previously [7,27,28]. The yields were about 80%. Poly(PEG/PPG/PHB urethane)s were synthesized from PHB-diol, PEG and PPG with molar ratios of PEG/PPG/PHB fixed at 2:1 and PHB content ranging from 5 to 15 mol% (calculated from the Mn of PHB-diol) using HDI as a coupling reagent. The amount of HDI added was equivalent to the reactive hydroxyl groups in the solution. Typically, 0.064 g of PHB-diol (Mn = 1070, 6.0 × 10−3 mol), 1.44 g of PEG (Mn = 1890, 7.6 × 10−4 mol) and 0.82 g of PPG (Mn = 2180, 3.8 × 10−2 mol) were dried in a 250-mL two-neck flask at 50 °C under high vacuum overnight. Then, 20 mL of anhydrous 1,2-dichloroethane was added to the flask and any trace of water in the system was removed through azotropic distillation with only 1 mL of 1,2-dichloroethane being left in the flask. When the flask was cooled down to 75 °C, 0.20 g of HDI (1.2 × 10−3 mol) and two drops of dibutyltin dilaurate (∼8 × 10−5 g) were added sequentially. The reaction mixture was stirred at 75 °C under a nitrogen atmosphere for 48 h. The resultant copolymer was precipitated from diethyl ether, and further purified by redissolving into 1,2-dichloroethane followed by precipitation in a mixture of methanol and diethyl ether to remove remaining dibutyltin dilaurate. A series of poly(PEG/PPG/PHB urethane)s with different compositions of PHB were prepared, and their number-average molecular weight and polydispersity values are given in Table 1 (see Scheme 1). The yield was 80% and above after isolation and purification.

2.3. Hydrolytic degradation of poly(PEG/PPG/PHB urethane) hydrogels

Aqueous solutions of 5 wt% copolymer were mixed and left to equilibrate overnight at 4 °C. In a typical preparation, 1 mL of polymer solution was injected into a porous cellulose cassette (pore size: ∼100 μm) and left to equilibrate at 37 °C to form a polymer gel. The polymer gel had dimensions of 10 mm × 25 mm × 4 mm. Each hydrogel sample was placed into 25 mL of phosphate buffer solution in a test tube, which was incubated and shaken at 50 rpm in a water bath at 37 °C. The buffer solution had a pH of 7.4, and contained 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4, and 0.24 g of K2HPO4 in 1 L of solution.

The buffer solutions were replaced with fresh ones at predetermined time intervals. The process was allowed to proceed for up to 6 months, and
The experiments were done in triplicate. The collected buffer solutions were lyophilized and weighed. In order to determine the weight attributed to the salt contents of the buffer solution, a blank containing only 25 mL of phosphate buffer solution was lyophilized and the residue was weighed to give the weight of the dry salts per 25 mL of the buffer solution. The dry weights of the copolymers dissolved and/or degraded into the buffer solutions were obtained by the difference between the samples and blank weights.

The dissolved and/or degraded copolymers in the buffer solutions were extracted using chloroform, followed by evaporation of chloroform and drying in vacuum at 50°C for 1 week. The aqueous phase (buffer solutions) was lyophilized and dried in vacuum at 50°C for 1 week to give the salt residues, which was kept for further analysis.

2.4. Mass loss of poly(PEG/PPG/PHB urethane) hydrogels

The mass loss of the copolymer gels after dissolution and degradation was defined as

\[ \text{Mass loss (\%) } = \frac{1 - (W_t/W_0)}{100\%} \]  

where \( W_0 \) and \( W_t \) were the initial weight and the weight of the copolymer dissolved or degraded in the buffer solution at time \( t \), respectively. \( W_t \) was obtained after drying the collected buffer solution samples at 50°C under vacuum for one week.

2.5. Protein release study of poly(PEG/PPG/PHB urethane) hydrogels

Aqueous solutions of 5 wt% copolymer were mixed and left to equilibrate overnight at 4°C. Appropriate amounts of BSA were loaded to make the concentration of BSA in the polymer solution 5 mg mL\(^{-1}\). For comparison, 30 wt% of EG\(_{100}\)PG\(_{65}\)EG\(_{100}\) triblock copolymer in aqueous solutions was prepared. The concentration of BSA in these solutions was also 5 mg mL\(^{-1}\). In a typical example, 1 mL of polymer solution was injected into a porous cellulose cassette (pore size: \( \approx 100 \mu\text{m} \)) and left to equilibrate at 37°C. The polymer hydrogel obtained had dimensions of 10 mm \( \times 25 \text{ mm} \times 4 \text{ mm} \) and was placed in 25 mL of phosphate buffer release solutions in a test tube, which was incubated and shaken at 50 rpm in a water bath at 37°C. The buffer solutions were replaced with fresh ones at predetermined time intervals, and the experiments were done in triplicate. The collected buffer solutions were lyophilized and kept at 80°C for further analysis. The BSA content was determined using the Pierce BCA Protein Assay kit. Quantitation of BSA was based on a calibration curve, obtained using the BSA standards provided, in the range of 20–2000 \( \mu\text{g mL}^{-1}\).

2.6. Gel permeation chromatography

Molecular weight of the degradation products in the chloroform-soluble fraction were determined by gel permeation chromatography (GPC) using a Shimadzu SCL-10A and LC-8A system equipped with two Phenogel...
5 \mu m 50 \text{ and } 1000 \text{ \AA} \text{ columns (size: } 300 \times 4.6 \text{ mm) in series and a Shimadzu RID-10A refractive index detector. THF was used as eluent at a flow rate of 0.20 mL/min at 45 {'C}. Monodispersed PEG standards were used to obtain a calibration curve.

2.7. \textit{H NMR spectroscopy}

The \textit{H NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at 400 MHz at room temperature. The \textit{H NMR measurements were carried out with an acquisition time of 3.2 s, a pulse repetition time of 2.0 s, a 30° pulse width, 5208 Hz spectral width, and 32 K data points. Chemical shift was referred to the solvent peaks (δ = 7.3 for CHCl\(_3\)).

2.8. MALDI-TOF mass spectrometry

MALDI-TOF was performed on a Bruker (Karlsruhe, Germany) AutoFlex MALDI Tandem TOF/TOF mass spectrometer. Dithranol or trans-2-[3-tert-butylphenyl]-2-methyl-2-propenylidene malononitrile was used as the matrix and silver trifluoromethanesulfonate as the ion source.

2.9. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of the polymer films were recorded on a Bio-Rad 165 FT-IR spectrophotometer; 64 scans were signal-averaged with a resolution of 2 cm\(^{-1}\) at room temperature.

2.10. Thermal analysis

Thermogravimetric analyses (TGA) were made using a TA Instruments SDT 2960. Samples were heated at 20 °C min\(^{-1}\) from room temperature to 800 °C in a dynamic nitrogen atmosphere (flow rate = 70 mL min\(^{-1}\)).

2.2. Field emission scanning electron microscopy (SEM)

SEM images were obtained at acceleration voltage of 5 kV on a JSM-6700F microscope (JEOL, Japan). The samples were sputter-coated with a thin layer of gold for 15 s to make the sample conductive before testing.

3. Results and discussion

3.1. Experimental setup

The hydrogel samples were enclosed in a porous cellulose cassette and immersed in a large excess of phosphate buffer solution. The buffer solutions were replaced with fresh ones at regular time intervals to simulate the dynamic flow of fluids in the body. The hydrolytic degradation experiments were carried out at pH 7.4 and 37 °C to simulate physiological conditions. The water bath was set in motion at 50 rpm to account for bodily movements upon injection of the gel depot. At various time points, the gel residues and buffer solutions were collected and lyophilized. The contents in the buffer solutions were divided into two parts: the part that could be extracted by chloroform (mainly copolymer with long chains) and the part that remained in the aqueous buffer solution phase (mainly salts and low-molecular weight final degradation products). A similar setup was used for the protein release study with protein-loaded hydrogels.

3.2. Hydrolytic degradation of the poly(PEG/PPG/PHB urethane) hydrogels

3.2.1. Mass loss of the hydrogels and the chain scission of the copolymers

The hydrolytic degradation process was accompanied by the mass loss of the hydrogels, as shown in Fig. 1. For all the gels, an incubation period (period during which there was little mass loss) was observed. The incubation period of the gels decreased with increasing PHB content, following the sequence of EPH(2%) > EPH(5%) > EPH(8%). The incubation period was followed by a period of steady mass loss. At the end of the erosion process, the rate of mass loss was observed to have decreased and a plateau feature was observed at the terminating end of the erosion profile. The copolymer gel erosion could be controlled by the composition of the copolymer. With decreasing PHB content, the time required for complete erosion increased. EPH(8%) erodes completely after 30 days, EPH(5%) after 40 days and EPH(2%) after 70 days.

Visual examinations of the remained hydrogel samples were carried out using SEM in order to observe changes in the surface structure of the gel. The micrographs are shown in Fig. 2 for EPH(2%), EPH(5%) and EPH(8%) at degradation time of 0, 14, and 30 days. The surface of the gel residue before erosion was devoid of pores and packing of the lyophilized gel appeared to be compact. After 14 days of erosion, structural deterioration of the gel was observed and pores (ca. 5–10 \mu m) developed on the surface of the films. After 30 days of erosion, the pores became more numerous and an enlargement of the pores was observed.
FTIR was used to probe the molecular changes occurring in the polymer segments after various periods of degradation (Fig. 3). In the original un-degraded sample, the PHB ester peak corresponding to $1721\text{ cm}^{-1}$ can be observed, along with a small peak at $1660\text{ cm}^{-1}$ which corresponds to the $-\text{C}=\text{O}$ stretch of the urethane peak. The gel residue obtained after 1 month of hydrolysis showed a broadening as well as a shift of the peak to $1632\text{ cm}^{-1}$ (attributed to the $-\text{C}=\text{O}$ carboxylic stretching) and a concomitant decrease in the height of the $1721\text{ cm}^{-1}$ ester peak was observed. The water-soluble fraction of the hydrolysis products after 1 month show that the ratio of the peak height at $1721\text{ cm}^{-1}$ to the peak height at $1632\text{ cm}^{-1}$ greatly decreased. This confirms that the ester bonds were hydrolysed to the carboxylic acid groups. After 6 months of hydrolysis, the ratio of the peak height at $1721\text{ cm}^{-1}$ to the peak height at $1632\text{ cm}^{-1}$ decreased even further implying further scission of the PHB segments in the water-soluble fraction after 6 months of hydrolysis.

When the PHB segment hydrolyses to form 3-hydroxybutyric acid, the number of hydroxyl and carboxylic acid groups increases. In Figs. 4b–d, two peaks are observed in the $-\text{OH}$ stretching region. The $3500\text{ cm}^{-1}$ peak corresponds to the $-\text{OH}$ stretch of the hydroxyl moiety while the peak observed at between $3250$ and $3300\text{ cm}^{-1}$ corresponds to the $-\text{OH}$ stretch of the carboxylic acid moiety. It can be observed that the peak corresponding to the $-\text{OH}$ stretch of the carboxylic acid moiety is absent in the FTIR spectrum of the original un-degraded polymer sample.

3.2.2. Characterization of the degradation products extracted by chloroform

At various time points during the hydrolytic degradation experiments, the buffer solutions containing degradation products were extracted by chloroform. We hypothesize that the chloroform extracts mainly contained the copolymer degradation products which still have large molecular weight. The very short fragments such as 3-hydroxybutyric acid or its oligomers tend to remain in the buffer solution.
The molecular weight of the copolymers in the chloroform extracts were measured by GPC. The GPC profiles of the copolymers were unimodal, as shown in Fig. 4a. The molecular weight of the copolymer samples decreased quite sharply until the first 2 months of hydrolysis, as shown in Fig. 4b. Throughout the period of 6 months of hydrolytic degradation, the molecular weight of the polymer chains continuously decreased, reducing to a final value of just slightly more than half their original molecular weight. The results are consistent with those of the FTIR spectra presented in the previous section which show that hydrolytic degradation was continuous from 1 to 6 months. In addition to the molecular weight information, the PHB content of the copolymer degradation products were determined by 1H NMR and TGA, and the results are listed in Table 1. The PHB content of the polymer chains decreased as the hydrolysis proceeded, indicating that a significant amount of water-soluble products containing PHB leached into the buffer solution during hydrolysis.

The mode of chain scission was determined by the method of Shih [29,30]. The number of ester bonds in the polymer chains were calculated based on Eq. (2):

\[
\text{Number of ester bonds} = \frac{\text{Weight fraction of PHB segments} \times M_d(\text{polymer chain})}{M_{\text{m}}(\text{HB repeating unit})}.
\]  

The fractional ester bonds (%) were calculated based on Eq. (3):

\[
Es = \frac{\text{Number of ester bonds at time } t}{\text{Initial number of ester bonds}} \times 100\%,
\]  

where \(Es\) refers to the fractional ester bonds (%) remaining at time \(t\). The rate of decrease of the ester bonds will follow a pseudo-first order kinetics [29,30] as described by

\[
-d[Es]/dt = kEs,
\]  

where \(k\) is the pseudo-first-order rate constant.

The extent of cleavage of ester bonds was found to be dependant on the total number of ester bonds in the polymer chain and the rate of decrease of the ester bonds followed pseudo-first order kinetics, giving linear natural logarithm plots (Fig. 4c). Chain scission of the ester bonds occurred at a faster rate with decreasing PHB content. The values of the rate constant \(k\) obtained were as follows: EPH(2%) (–0.40 month\(^{-1}\)), EPH(5%) (–0.35 month\(^{-1}\)), and EPH(8%) (–0.31 month\(^{-1}\)). In a study of the hydrolysis process of \(d,l\)-lactic acid oligomers, Schliecker et al. reported that the rate of decrease of the number of ester bonds follows Eq. (4). A random chain scission of the \(d,l\)-lactic acid oligomers was proposed [31]. Similarly, degradation of the copolymer chains in our study can be expected to occur via the random hydrolytic ester cleavage.

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Fig. 4. (a) GPC profiles of the copolymer degradation products in chloroform extracts from the PBS buffer at various hydrolysis periods; (b) changes in molecular weight of the copolymer degradation products with time of hydrolysis up to 6 months (▲: EPH(2%), ■: EPH(5%), ×: EPH(8%)); (c) plot of the natural logarithm of the fractional ester bonds remaining versus degradation time of the polymers after various periods of degradation (▲: EPH(2%), ■: EPH(5%), ×: EPH(8%)).
along the PHB segments, which accounted for the decrease in the molecular weight of the polymers in the degradation process at pH 7.4. The decreasing $k$ values show that with increasing hydrophobicity of the polymers (due to the increasing PHB content), the rate of degradation of the polymers decrease. The rate of degradation of this series of thermogelling poly(ester urethane)s can be tuned by adjusting the copolymer composition.

### 3.2.3. Characterization the degradation products remained in PBS buffer

After chloroform extraction, short fragments of the degradation products such as 3-hydroxybutyric acid or its oligomers and some PEG–PPG fragments may still remain in the buffer solution together with the salts. This part of the degradation products was characterized using NMR and MALDI-TOF spectroscopy. The $^1$H NMR spectrum of the degradation products of EPH(5%), obtained after 6 months of hydrolysis, is shown in Fig. 5. The products were identified to be 3-hydroxybutyric acid, PEG and PPG segment blocks. The PEG and PPG peaks did not display any shifts in the peak position and indicates that the chain scission did not occur at the polyether segments. The MALDI-TOF spectrum of the degradation products is shown in Fig. 6. The $m/z$ values of the peaks correspond to the monomers up to pentamers of 3-hydroxybutyric acid. In general, for each fragment, three different peaks can be identified, corresponding to the molecules with no sodium ions, 1 sodium ion, and 2 sodium ions, respectively.

### 3.2.4. Hydrolytic degradation mechanism of poly(PEG/PPG/PHB urethane)s

Thermogelling copolymers have high water content and behave differently from conventional crosslinked hydrogels. Chemically crosslinked hydrogels require degradation

**Fig. 5.** $^1$H NMR spectrum of the degradation products of EPH(5%) after 6 months of hydrolysis remained in the PBS buffer.

**Fig. 6.** Characteristic matrix-assisted laser desorption ionization mass spectra of the degradation products of EPH(5%) after 6 months of hydrolysis remained in the PBS buffer.
of the crosslinks before polymer erosion takes place. Thermogelling copolymers behave differently. The physical copolymer gels could either undergo gel erosion with or without degradation of the polymer chains. The initial stage of the hydrolytic degradation of the gel is characterized by an erosion of the gel material into the buffer solution. The erosion process can be broken down into 3 sub-stages. The first sub-stage is the incubation period. Anseth et al. [15] calculated that the length of incubation time increases with an increase in the extent of crosslinking in a hydrogel. In our system, a lower PHB content increases the efficiency of the gel packing and increases the effective physical crosslinks in the gel, thereby prolonging the incubation period. Similar observations have been made in a study of chemically crosslinked hydrogels based on poly(vinyl alcohol) macromers [16]. This has been attributed to a "high degree of interconnectivity" present in the chemically crosslinked hydrogels. In our case, the enhanced interactions between the hydrophobic segments hold the polymer chains together in the polymer gel for a certain period of time. The tight compact packing can be seen in the SEM micrographs of the polymer gels before the hydrolysis. Upon equilibration with the buffer system, the gel surface slowly dissolved. The onset of the dissolution depends on the composition of the copolymer. With increasing PHB content, the dissolution process of the polymer gel begins earlier. We can begin to understand this phenomenon by relating the gelation process to a micelle packing process. Previously, we have postulated that the polymer micelles of this work are of an associated micelle nature [7]. At higher temperatures, an aggregation of the micelles results in the formation of a gel state. An increase in the PHB content in the polymer gels could lead to a disruption on the micelle packing which would further disturb the packing of the gel. Adept use of this knowledge allows control of the onset of dissolution by the variation of the PHB content in the copolymer. Sub-stage 2 is characterized by a period of constant mass loss. This happens when the physical crosslinks have been broken, allowing a constant dissolution of the polymer gel. From the GPC profiles, a rapid decrease in the molecular weight of the polymer was observed in the first month of degradation. This leads to the formation of polymer chains which are much more soluble in the buffer solution than the original polymer sample, consequently, the erosion of the gel takes place at a faster rate. Sub-stage 3 shows that there is little mass loss towards the end of the erosion process. This feature of the erosion profile was not predicted by the theoretical studies of the erosion of hydrogels [15–17]. This strongly suggests that the erosion of the polymer gel is dependent on the volume of the gel depot. When the volume of the gel is small, there appears to be a lower driving force for erosion to occur. From the combined GPC and FTIR results, it appears that erosion of the polymer gel occurs in parallel with the chain scission of the polymer chains. The molecular weight of the polymer chains decreased greatly in the initial stages of the hydrolytic degradation process via a random chain scission occurring at the ester linkages of the PHB segments.

3.3. Protein release from poly(PEG/PPG/PHB urethane) hydrogels

The in-vitro release kinetics of the model protein BSA released from the copolymer hydrogels was studied and

![Fig. 7. (a) Protein release profiles for poly(PEG/PPG/PHB urethane) hydrogels of different copolymer compositions in comparison with PEG–PPG–PEG triblock copolymer [▲: EPH(2%), ■: EPH(5%), ×: EPH(8%), ●: EG100–PG65–EG100 triblock copolymer (30 wt%)]. (b) Expanded protein release profiles of up to 1 day of (a). (c) Protein release profiles for EPH(5%) hydrogels of different copolymer concentrations (*: 3 wt%, ●: 4 wt%, ■: 5 wt%). Experiments were carried out in triplicate and the standard deviation for all data points were within ±5%.](image-url)
Table 2
Release characteristics of BSA from different hydrogel formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>n</th>
<th>k (day⁻¹/₂)</th>
<th>Dᵥ × 10⁷ (cm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH(2%) (5%)</td>
<td>0.491</td>
<td>0.122</td>
<td>0.054</td>
</tr>
<tr>
<td>EPH(5%) (5%)</td>
<td>0.300</td>
<td>0.183</td>
<td>0.121</td>
</tr>
<tr>
<td>EPH(8%) (5%)</td>
<td>0.302</td>
<td>0.246</td>
<td>0.220</td>
</tr>
<tr>
<td>EPH(5%) (3%)</td>
<td>0.475</td>
<td>0.283</td>
<td>0.291</td>
</tr>
<tr>
<td>EPH(5%) (4%)</td>
<td>0.493</td>
<td>0.227</td>
<td>0.187</td>
</tr>
<tr>
<td>EPH(5%) (5%)</td>
<td>0.300</td>
<td>0.183</td>
<td>0.121</td>
</tr>
</tbody>
</table>

*For all protein release kinetics calculations, data set in the range of Mᵣ/Mₓ ≤ 0.6 was used. Value of n was calculated by the following equation: \( \log (Mᵣ/Mₓ) = n \log t + \log k \). Value of k was calculated by the following equation: \( Mᵣ/Mₓ = kt^{1/2} \). Value of Dᵥ was calculated by the following equation: \( Mᵣ/Mₓ = 4/l × (Dᵥ × t/π)^{1/2} \).

compared with EG₁₀₀–PG₆₅–EG₁₀₀ triblock copolymer hydrogel (Fig. 7). The EG₁₀₀–PG₆₅–EG₁₀₀ triblock copolymer hydrogel released its entire content of BSA within 4 h but the gels formed by the new thermogelling copolymers were able to achieve a sustained release of up to 80 days.

The release profile of all the polymers can be fitted to the following equation for drug release from a gel slab in the range of \( Mᵣ/Mₓ ≤ 0.6 \) [32,33]:

\[
Mᵣ/Mₓ = kt^n,
\]

where \( Mᵣ \) and \( Mₓ \) are the mass of protein released at time \( t \) and infinite time, respectively; \( k \), a characteristic exponent of the mode of transport of the protein. The values of \( n \) and \( k \) were calculated from the slopes and intercepts, respectively, of the plot of \( \log (Mᵣ/Mₓ) \) versus \( \log (t) \) and tabulated in Table 2. For Fickian diffusional release, in which the rate of diffusion of the protein is rate limiting, \( n = 0.5 \), while values of \( n \) between 0.5 and 1 indicate the anomalous (non-Fickian) transport [32].

The collective diffusional coefficients of the gels, \( Dᵥ \), were determined from the gradient of the plot of the initial release rate versus the square root of time, using the following equation, within the same range of \( Mᵣ/Mₓ ≤ 0.6 \):

\[
Mᵣ/Mₓ = 4/l × (Dᵥ × t/π)^{1/2},
\]

where \( l = 0.4 \) cm, the thickness of the gel. The protein release profile can be divided into two stages, the diffusion-controlled stage and the combined erosion/diffusion-controlled stage. The initial release profile is defined by the Fickian diffusion for the first 60% of the release process, followed by a linear release of the protein with respect to time.

Sustained release profiles of the gels with the same polymer concentration were compared. The polymer with the highest PHB content (EPH(8%)%) formed a gel with the fastest sustained release characteristics. The period of sustained release (15–40 days) can be controlled by adjustment of the concentration of the polymer EPH(5%)% in the thermogelling solution from 3 to 5 wt%.

3.4. Correlation between protein release and copolymer hydrogel degradation

The protein release profiles can be correlated with the polymer gel erosion profiles. Erosion sub-stage 1 (period of no mass loss) coincides with the diffusional controlled release of the protein. At the initial stages of the release experiment, the gel exists as a tightly packed structure. Protein appears to be only released via the diffusion through water-rich regions of the gel structure. Diffusivity of a solute through physically crosslinked hydrogels decreases with an increase in the crosslinking density and with a decrease of the volume fraction of solvent within the hydrogel [38]. Increasing the concentration of the poly(ester urethane)s in our thermogelling formulation reduced the proportion of the solvent-rich regions, leading to a decrease in the diffusional coefficient of BSA as observed. The effect of the composition of the poly(ester urethane)s was studied in relation to the sustained protein release. We observed that the diffusivity of the protein increased with increased PHB content. Cohn et al. [39] reported that for a multiblock PEG, PPG and PCL copolymer, the inclusion of PCL induces possible spatial hindrances in the gel structure. The presence of PHB segments could have reduced the packing efficiency of the polymer chains and reduced the amount of effective physical crosslinking in the gel structure. As such, gels made from the poly(ester urethane)s with the highest PHB content gave the shortest release period in our experiments. The diffusion coefficients reported in this study can be compared with the calculated diffusion coefficient of BSA of \( 9.35 \times 10^{-7} \) cm² s⁻¹ in an infinite solution at 37 °C [38]. It can be seen that the all the gels of different compositions and different concentrations reduced the diffusion coefficient markedly. The values
show that the presence of the polymer chains in the gel structure could have caused an obstruction effect to the free diffusion of the BSA protein molecule.

After some time, erosion of the polymer gel structure follows. During the linear polymer erosion phase (sub-stage 2), a linear release of the protein with respect to time was observed (compare Figs. 1 and 7a). The release of the protein is dominated by the erosion of the polymer gel leading to a linear profile in the release of the protein. The rate of release of the protein is affected by the concentration of the poly(ester urethane)s in the formulation and the PHB composition of the poly(ester urethane)s. This example further illustrates that the packing of the gel structure is an important factor in the determination of the release rate of the protein. Hennink et al. [40] performed Monte Carlo simulations which showed that higher crosslink density in a gel led to a slower rate of release. Our experimental results showed that better packing tends to result in greater resistance against erosion and manifests itself as a longer lasting sustained release profile. The rates of release of the protein decrease as follows: EPH(8%) > EPH(5%) > EPH(2%). For the formulations with different concentrations, the rates of release of the protein decrease as follows: 3 wt% > 4 wt% > 5 wt%.

4. Conclusions

The hydrolytic degradation and protein release characteristics of a series of new thermogelling poly(ether ester urethane)s consisting of PEG, PPG, and PHB were investigated. The correlation between the protein release of the hydrogels and the hydrolytic degradation was studied for the first time for such a thermogelling copolymer system. The hydrolytic degradation process was characterized by an initial incubation period, followed by the erosion of the polymer gel and the random scission via the ester bonds of the PHB segments in buffer solution. The rate of chain scission could be controlled by adjusting the copolymer composition. In the protein release studies, we observed that the gels released the entire loaded model protein. The initial stage of the drug release was diffusion controlling, the later stage was erosion controlling. The release rate of the protein could be tuned by the formulation and copolymer composition.

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