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A controlled release system for proteins based on poly(ether ester) block-copolymers: polymer network characterization

J.M. Bezemer[a], D.W. Grijpma[a], P.J. Dijkstra[a], C.A. van Blitterswijk[a,b,c], J. Feijen[a,*]

[a]*Institute for Biomedical Technology (BMTI), Polymer Chemistry and Biomaterials, Faculty of Chemical Engineering, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands,*

[b]*Biomaterials Research Group, University of Leiden, The Netherlands*

[c]*IsoTis BV, Prof. Bronkhorstlaan 10, 3723 MB Bilthoven, The Netherlands*

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Abstract

The properties of a series of multiblock copolymers, based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(butylene terephthalate) (PBT) blocks were investigated with respect to their application as a matrix for controlled release of proteins. The degree of swelling, *Q*, of the copolymers increased with increasing PEG content and with increasing molecular weight of the PEG segment. Within the composition range tested, *Q* varied from 1.26 for polymers with PEG segments of 600 g/mol and a PBT content of 60 weight.% up to 3.64 for polymers with PEG segments of 4000 g/mol and a PEG/PBT weight ratio of 80:20. Equilibrium stress (compression)–strain measurements were performed in order to estimate mesh sizes. The mesh size of the copolymers ranged from 38 to 93 Å, which was experimentally confirmed by diffusion of vitamin B_{12} (hydrodynamic diameter d_h=16.6 Å), lysozyme (d_h=41 Å) and bovine serum albumin (d_h=72 Å). The in vitro degradation of PEG/PBT copolymers with a PEG block length of 1000 g/mol and PEG/PBT weight ratios of 70:30, 60:40 and 40:60 was studied. Matrices with increasing PEG contents exhibited a faster weight loss in phosphate-buffered saline (pH 7.4) at 37°C. Over a degradation period of 54 days, M_n decreased by about 35–45%, while the composition of the matrices, determined by NMR, remained almost constant.

Keywords: Block-copolymer; Protein release; Mesh size; Permeability, Degradation

1. Introduction

Recent interest in the delivery of protein and peptide drugs has made the development of new controlled release systems necessary. Among them, hydrogels have been widely investigated because of good compatibility with tissue as well as with protein and peptide drugs due to their high water content [1]. Of particular interest are heterogeneous hydrogels, since properties of these networks can be precisely tailored by a proper combination of the different polymer segments, which may result in improved release kinetics, mechanical properties, processability, etc. [2]. Several types of heterogeneous hydrogels have been developed for drug release, including block and graft copolymers [3–5], interpenetrating networks [6,7], polymer blends [8,9] and polymer conjugates [10].

The heterogeneous hydrogels used in this study are poly(ether ester) multiblock copolymers, con-

*Corresponding author.

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taining repeating blocks based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(butylene terephthalate) (PBT). The poly(ether ester)s are typically prepared by a polycondensation in the melt from PEG, butanediol and dimethyl terephthalate, using a butyl titanate catalyst. They belong to a class of materials known as thermoplastic elastomers, which exhibit good physical properties like elasticity, toughness and strength in combination with easy processability. These properties result mainly from a phase-separated morphology [11,12]. At temperatures of usage, the poly(ether ester)s are cross-linked by the presence of 'hard' PBT domains, creating physical cross-links dispersed in the 'soft' amorphous phase consisting of both hydrophilic PEG segments and PBT [11–16]. In contrast to chemically cross-linked materials, the physical cross-links are reversible and can be disrupted at elevated temperatures or in solvents, which gives the material its good processability. By varying the PEG/PBT weight ratio and the length of the PEG segments, a series of copolymers can be obtained, with different morphologies and mechanical properties [13,14].

Several in vitro and in vivo studies show that PEG/PBT copolymers are well-tolerated and do not cause adverse tissue or systemic effects [17–23]. The copolymers are under clinical investigation for a wide range of biomedical applications, including bone replacement [24], anti-adhesive barrier [22] and artificial skin [20,21]. The objective of this study is to investigate the suitability of PEG/PBT copolymers as a matrix material for a controlled release system for proteins. For this reason, several parameters that determine the release characteristics of these poly(ether ester)s, such as equilibrium swelling, mechanical behavior, permeability and polymer degradation, have been analyzed as a function of the copolymer composition and are described in this paper.

2. Materials and methods

2.1. Materials

A series of poly(ethylene glycol)terephthalate/poly(butylene terephthalate) (PEG/PBT) copolymers was obtained from IsoTis BV (Bilthoven, The Netherlands). The poly(ether ester) copolymers vary in PEG/PBT weight ratio (80:20–30:70) and PEG segment length (600, 1000 and 4000 g/mol), and are indicated as **a**PEG**b**PBT**c**, in which **a** is the PEG molecular weight, **b** is the weight.% of PEG–terephthalate and **c** is the weight.% PBT. Typically, a polymer indicated as 1000PEG70PBT30 contains PEG segments of 1000 g/mol, a PEG–terephthalate content of 70 weight.% and a PBT content of 30 weight.%. The total soft segment length, including one terephthalate unit, is 1132 g/mol, and the PEG content of this particular polymer is (70×1000)/1132=61.8 weight.%. The chemical structure of the polymers is given in Fig. 10.

Phosphate-buffered saline (PBS), pH 7.4, was purchased from NPBI (Emmercompascuum, The Netherlands). Chloroform ($CHCl_3$), $CDCl_3$ and methanol, obtained from Merck (Darmstadt, Germany), and hexafluoroisopropanol, obtained from Aldrich (Belgium), were of analytical grade. Lysozyme from chicken egg white (3× crystallized, dialyzed and lyophilized), vitamin B_{12} and bovine serum albumin (BSA, heat shock fractionate; fraction V powder minimum, 98%) were purchased from Sigma (St. Louis, MO, USA).

2.2. Preparation of PEG/PBT films

Poly(ether ester) copolymer (1 g) was dissolved in 7 ml of chloroform and cast on a glass plate using a 0.75-mm casting knife. Copolymers having a PBT content above 50 weight.% were dissolved in 7 ml of a mixture of chloroform and hexafluoroisopropanol (6:1, v/v). The solvent was slowly evaporated at room temperature and then the films were dried in vacuo for three days. The resulting films had a thickness of 50–100 μm. To prepare thick films (0.4–1 mm), more concentrated polymer solutions were used (10–20 weight.%, depending on the copolymer composition).

2.3. Swelling in PBS

Dry films (15 mm in diameter and 50–100 μm in thickness) were weighed and immersed in PBS at 37°C in a shaking bath. The equilibrium volume swelling ratio, *Q*, was determined from the equilibrium weight of the swollen samples using a density

of 1.2 g/ml for all polymers. Before measuring the weight, surface water was removed by blotting the surface with a tissue. Equilibrium swelling was reached within three days [26].

2.4. Polymer characterization and degradation

The molecular weight of the polymers was determined using gel permeation chromatography (GPC). Samples were eluted in chloroform containing hexafluoroisopropanol and acetonitrile through a Waters Styragel guard precolumn and two PCGel 5 μm Mixed-C columns of 30 cm. The flow-rate was 1 ml/min and a UV detector set at 245 nm was used. Column temperature was 35°C and sample concentration was 0.03%. The molecular weight was determined relative to polystyrene standards. The molecular weight of the polymers used in this study are presented in Table 1.

To determine the degradation of polymer matrices, dry films (15 mm in diameter and 50–100 μm in thickness) were weighed and immersed in PBS at 37°C in a shaking bath. After certain time intervals, samples were taken and, after drying in vacuo for three days, the weight loss was calculated by:

$$\text{weight loss}\,(\%) = 100 \times (W_0 - W_1)/W_0. \quad (1)$$

where W_0 and W_1 are the weights of the films before and after degradation, respectively. The change in molecular weight of the polymers was determined using GPC. The effect of degradation on the composition of 1000PEG70PBT30 was evaluated using ^{1}H NMR. Spectra were recorded on a Bruker AC 250 operating at 250.1 MHz. $CDCl_3$was used as solvent without internal standard.

2.5. Compression measurements on equilibrium-swollen samples

Poly(ether ester) discs (15 mm in diameter and 0.4–1 mm in thickness) were cut from dry films and allowed to swell to equilibrium in PBS at 37°C. Equilibrium stress (compression)–strain measurements were performed using an apparatus (Mitutoyo, Japan) as described by Cluff et al. [25]. The deformation of a swollen disc caused by different compression loads was measured at equilibrium. After each measurement, the films were placed in PBS at 37°C to recover to undeformed dimensions. The deformation was kept below 5% of the initial thickness.

2.6. Permeability of equilibrium swollen membranes

The permeability of PEG/PBT films was evaluated for three different solutes: vitamin B_{12} (M= 1355 g/mol), lysozyme (M=14,300 g/mol) and BSA (M=66,000 g/mol). The permeability for vitamin B_{12} was measured using a 2×7.5 ml two-chamber diffusion apparatus with an effective membrane area of 2.27 cm^2. Membranes were swollen to equilibrium in water at 37°C before they were placed between the two chambers. The donor compartment was filled with a 2-mg/ml vitamin B_{12} solution and deionized water was added to the receptor side. The compartments were stirred at 500 rpm in a thermostatic incubator at 37°C. Samples of the donor and

Table 1
Molecular weights and characterization of the network structure of equilibrium-swollen poly(ether ester) copolymer films in PBS at 37°C

Polymer	$\overline{M_n}$ (kg/mol)	$\overline{M_w}$ (kg/mol)	ν_e[a] (mol/m^3)	$\overline{M_c}$[b] (kg/mol)
600PEG77PBT23	40.8	77.6	177	5.07
600PEG55PBT45	53.2	114.3	215	4.60
1000PEG70PBT30	45.0	99.3	147	5.99
1000PEG60PBT40	45.0	94.2	150	5.88
4000PEG80PBT20	36.7	88.1	79	8.29
4000PEG55PBT45	45.5	97.0	98	7.97

[a] ν_e: elastically effective network chain concentration.
[b] $\overline{M_c}$: average molecular weight between cross-links.

receptor chambers were taken at various time points. The vitamin B_{12} concentration of the samples was determined using a SLT 340 ATTC microplate reader ($\lambda = 340$ nm).

Due to the low diffusion coefficients of lysozyme and BSA through PEG/PBT films, it was not possible to measure a significant quantity of permeated protein within a reasonable time interval using the diffusion apparatus. Therefore, the permeability of PEG/PBT membranes for lysozyme and BSA was evaluated from release experiments. Protein-containing films were prepared as described elsewhere [26]. In short, PEG/PBT copolymer (1 g) was dissolved in 7 ml of chloroform and emulsified with a protein solution (0.6 ml, 55 mg/ml) in PBS using ultra-turrax-mixing (30 s at 20.5 krpm, Ika Labortechnik T25). The resulting water-in-oil emulsion was cast onto a glass plate using a 0.75-mm casting knife. After slow evaporation of the solvent, films were removed from the glass plate and stored over $CaCl_2$ in a desiccator at 4°C. The final loading of protein in the films was 33 mg per g of polymer.

To investigate protein release, the films (dry dimensions: 1.76 $cm^2 \times 50$ μm) were incubated in 1.5 ml of PBS (pH 7.4). Vials were shaken at 37°C and samples were taken at various time points. Protein content was determined using a standard Coomassie Blue assay (Pierce). Buffer was refreshed after sampling. The thickness of the swollen membranes was measured using a micrometer.

3. Results and discussion

3.1. PEG/PBT network properties

To evaluate multiblock copolymers based on poly(ethylene glycol) (PEG) and poly(butylene terephthalate) (PBT) for use as drug delivery systems, knowledge of the structural characteristics of these materials is important. The structure and morphology of segmented poly(ether ester)s has been the subject of many studies (see [27] for a review). Of particular interest for this study is the work of Fakirov et al. [13–16], dealing with the relationship between morphology and the composition of PEG/PBT copolymers. Using differential scanning calorimetry (DSC), small angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS), it was found that domains of four different types may exist in PEG/PBT copolymers: crystalline PBT, amorphous PBT, amorphous PEG and a mixed amorphous phase [15]. The ratio of the different phases is dependent on the polymerization conditions, copolymer composition and the thermal and mechanical history of the sample [27]. Concerning the effect of copolymer composition, for polymers with a low PBT content and, consequently, short PBT sequences, no crystalline PBT phase was found. Increasing the molecular weight of the PEG diols at a constant PEG/PBT weight ratio, or increasing the PBT content at a constant PEG block length will increase the average block length of the PBT segments and thus facilitate crystallization. The average distance between the crystalline domains is strongly dependent on the molecular weight of the PEG diol used whereas the PEG/PBT weight ratio does not alter the long spacing significantly [13,14].

For biomedical applications such as controlled release devices, the structure and properties of water-swollen matrices is of primary interest. Therefore, the swelling behavior of solvent cast, dense PEG/PBT films (50–100 μm in thickness) was investigated as a function of the copolymer composition. Equilibrium swelling (Q) was reached within three days. Within the range of copolymers used in this study, Q varied from 1.26 for 600PEG40PBT60 up to 3.64 for 4000PEG80PBT20. The equilibrium swelling ratio of PEG/PBT copolymers was less than that of other PEG-containing multiblock copolymers with equivalent PEG content and PEG molecular weight, such as copolymers of PEG and poly(ethylene terephthalate) (PET) [28], PEG-based poly(urethanes) [29] and copolymers of PEG and poly(lactic–glycolic acid) (PLGA) [30]. Since swelling of a polymer network in a solvent is dependent on the degree of cross-linking, the lower equilibrium swelling ratio found for the PBT-based hydrogels indicates that the PBT blocks are more efficient in forming physical cross-links than hard segments based on PET and PLGA, for example.

As expected, the equilibrium swelling ratio increased with increasing amount of the hydrophilic component, PEG, in the hydrogel (Fig. 1). Within the composition range tested, a linear relation between PEG content and swelling was found (correlation

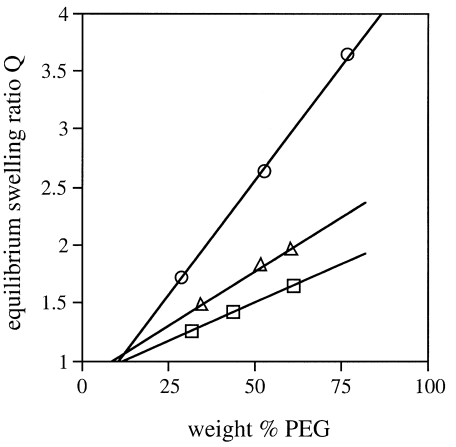

Fig. 1. Equilibrium swelling ratio of PEG/PBT copolymers as a function of PEG weight.% of the copolymers. Polymers have PEG block lengths of 600 (□), 1000 (△) and 4000 (○) g/mol ($n=3$; ±s.d.).

coefficient $r^2>0.99$). Furthermore, at a constant PEG weight percentage, the swelling increased as the molecular weight of the PEG segment was increased (Fig. 1). In order to find a relationship between swelling and the molecular weight of PEG, the slopes of the three curves in Fig. 1 were plotted as a function of the molecular weight of PEG. As shown in Fig. 2, the variation of the swelling with the PEG weight percentage of the copolymers is proportional to the square root of the molecular weight of the PEG blocks used in the copolymer (correlation coefficient $r^2>0.999$) and, thus, to its radius of gyration, which is related to the distance between junction zones [16].

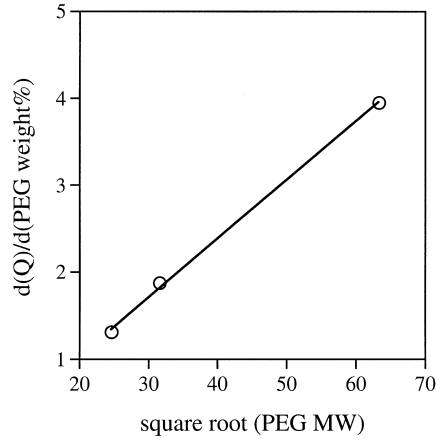

Fig. 2. Relationship between the variation of the swelling with PEG weight percentage and the PEG molecular weight.

In principle, the equilibrium swelling ratio provides quantitative information about the network structure of PEG/PBT hydrogels [31]. However, to calculate structural parameters such as the molecular weight between cross-links and the mesh size, the polymer–solvent interaction parameter, χ, must be known accurately. Network parameters can also be determined by measuring equilibrium elastic moduli. It is common practice to express the deformation of a swollen network, cross-linked in the dry state, as follows [31]:

$$\tau = RT\phi_p^{1/3}\nu_e\left(\alpha - \frac{1}{\alpha^2}\right) \quad (2)$$

where τ is the reduced stress, expressed as applied force per unit area of the unswollen sample, α is the ratio of deformed to undeformed length of the sample, ν_e is the elastically effective network chain concentration, ϕ_p is the polymer volume fraction in the equilibrium swollen state (taken as $1/Q$), R is the gas constant and T is the absolute temperature. Eq. (2) is based on the assumptions that network junctions are fixed at their mean positions and that, under strain, the distance between junctions changes in the same ratio as the macroscopic dimensions (affine deformation) [32]. More advanced theories take into account the fluctuations of the junctions [33]. In the present case of a hydrophilic network physically cross-linked by PBT microdomains, it can be expected that the functionality of the cross-links is rather high, which restricts fluctuations of the junctions. Moreover, at the small deformations used in this study, the deformation is close to the affine limit [33]. Based on these considerations, Eq. (2) was used to describe the stress–strain characteristics of the swollen poly(ether ester) copolymers.

Uniaxial compression–extension measurements were performed on the six polymers presented in Table 1. Polymers having a PBT content of more than 45 weight.% could not be used to prepare flat solvent cast films with adequate thickness and were not included in this study. In Fig. 3, plots of the applied stress, τ, against $\alpha - 1/\alpha^2$ are shown. For all polymers tested, a linear relationship was found over the deformation range covered. The slopes of these

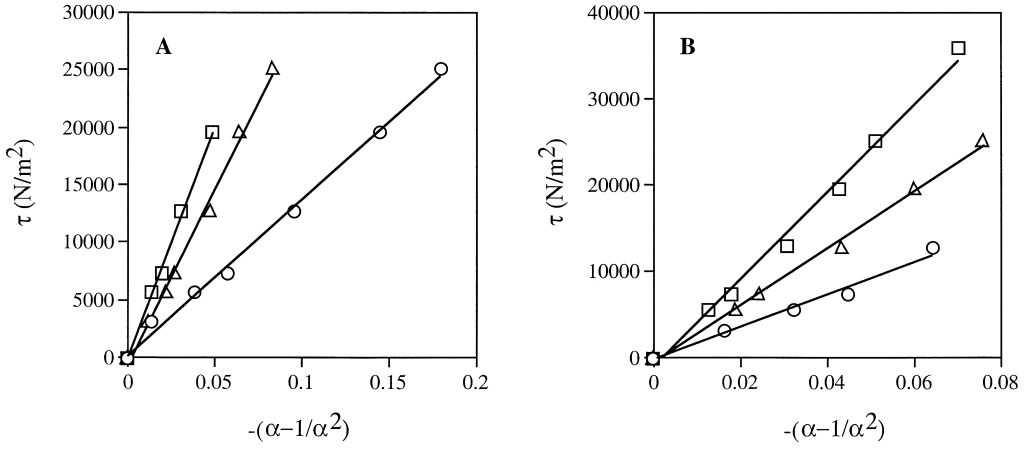

Fig. 3. Plots of stress (τ) vs. ($\alpha - 1/\alpha^2$) for poly(ether ester) copolymers swollen in PBS at 37°C. (A) 4000PEG80PBT20 (○), 1000PEG70PBT30 (△) and 600PEG77PBT23 (□); (B) 4000PEG55PBT45 (○), 1000PEG60PBT40 (△) and 600PEG55PBT45 (□) ($n=3$; s.d. always <10%).

curves, obtained by linear regression, were used to calculate ν_e. As was found for the equilibrium swelling, ν_e was dependent on the copolymer composition: with increasing PEG content and PEG block length in the copolymers, ν_e decreased (Table 1).

The effective network chain concentration can be used to calculate the average molecular weight between cross-links ($\overline{M}_c$) [31,34]:

$$\nu_e = \frac{1}{\bar{v}\overline{M}_c}\left(1 - 2\frac{\overline{M}_c}{\overline{M}_n}\right) \quad (3)$$

where $\bar{v}$ is the specific volume of the dry polymer (0.83 cm^3/g) and $\overline{M}_n$ is the number average molecular weight. The term $(1-2\overline{M}_c/\overline{M}_n)$ is a correction for network imperfections resulting from chain ends, which equals unity for perfect networks. The results of this calculation are given in Table 1. It is important to note here that values of $\overline{M}_c$ calculated by Eq. (3) can only be used as an indication of the network structure. The physical cross-links in the swollen poly(ether ester) networks consist of ordered PBT segments of a certain volume, rather than of well-defined point cross-links, which is a deviation from the rubber elasticity assumptions. However, Patterson et al. [35] showed that the rubber elasticity theories could still be applied with some approximation to analyze the structure of semi-crystalline plasticized poly(vinyl chloride), when the degree of crystallinity is low. Another factor that will affect the validity of Eq. (3) is that the network consists of polydisperse and heterogeneous block copolymers, with a certain distribution of block lengths.

Interestingly, the results in Table 1 suggest that the average molecular weight between cross-links is mainly dependent on the length of the PEG blocks. This is in good agreement with the results of SAXS measurements performed by Fakirov et al. [13,14]. They proposed that, at a fixed PEG block length, the long spacing, which is the average distance between crystals (including the thickness of the crystal plus the amorphous region between the crystals), is independent of the PEG/PBT ratio. However, the long spacing was strongly dependent on the molecular weight of the PEG diol. For PEG/PBT copolymers with PEG segments of 600, 1000 and 2000 g/mol, the average long spacings were around 102, 110 and 144 Å, respectively.

From the average molecular weight between cross-links, the mesh size, ξ, of the swollen PEG/PBT networks can be estimated. For this, it has to be assumed that all PBT is located within small physical cross-link zones and that PEG is located in between the cross-link points only. Then, the mesh size can be approximated using [34,36]:

$$\xi = \phi_p^{-1/3} l (nC_\infty)^{1/2} \quad (4)$$

where l is the bond length, C_∞ is the characteristic ratio (3.8 for PEG) [37] and n is the average number of bonds between cross-links. Since it is assumed that only PEG is located between cross-links, n can be expressed as:

$$n = 3\frac{x_{PEG}\overline{M_c}}{M_r} \quad (5)$$

where x_{PEG} is the weight fraction of PEG in the copolymer, M_r is the molecular weight of the repeating unit of PEG (44 g/mol). The bond length was taken as 1.50 Å, which is the average of one carbon–carbon bond and two carbon–oxygen bonds. In Fig. 4, the values of the mesh size of the different poly(ether ester) networks are plotted as a function of the equilibrium swelling ratio. It was found that, except for the polymer with the lowest degree of swelling, the mesh size was proportional to the equilibrium swelling ratio of a particular polymer, and could be described by:

$$\xi = 21.8Q + 15.9 \quad (6)$$

with the correlation coefficient $r^2 = 0.99$. A linear relationship between ξ and Q for gels that had an equilibrium swelling ratio smaller than ten was also found by Canal and Peppas [36] for cross-linked poly(vinyl alcohol) and poly(2-hydroxy ethyl methacrylate) gels and by Am Ende and Peppas [38] for cross-linked poly(acrylic acid–*co*-2-hydroxy ethyl methacrylate) gels.

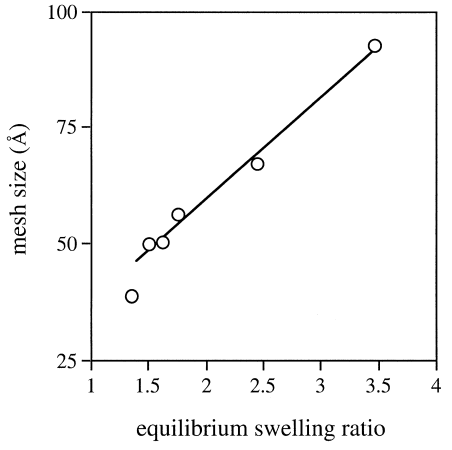

Fig. 4. Mesh size of poly(ether ester) copolymers as a function of the equilibrium swelling ratio.

3.2. Permeability of equilibrium swollen PEG/PBT membranes

To validate the obtained mesh sizes, diffusion experiments were performed. Three solutes of different molecular weights were used: vitamin B_{12} (M = 1355 g/mol), lysozyme (M = 14,300 g/mol) and BSA (M = 66,000 g/mol). The hydrodynamic diameters of the solutes are 16.6 [39], 41 and 72 Å [40], respectively. The effect of interactions between polymer matrix and solutes has been neglected in this study. As the size of vitamin B_{12} is much smaller than the mesh size of the copolymers, it was expected that all copolymers were permeable for this solute. This was confirmed by the results of diffusion experiments: vitamin B_{12} transport from donor to receptor compartment was found for all PEG/PBT films, irrespective of the copolymer composition. Fig. 5 presents typical results of the amount of vitamin B_{12} permeated as a function of time. In order to compare the different membranes with each other, a correction was made for the membrane thickness by dividing time by the thickness of the swollen films (usually between 50 and 100 μm). From Fig. 5, it is obvious that the permeability of PEG/PBT films

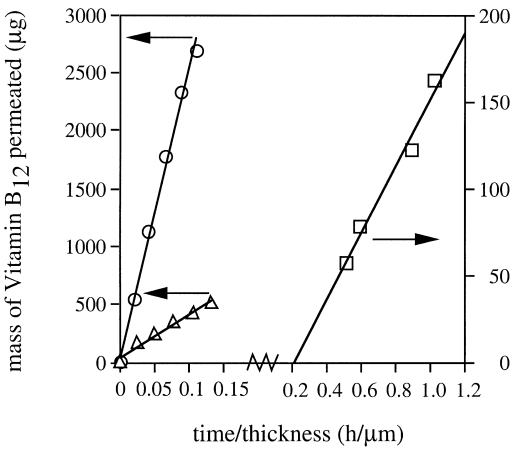

Fig. 5. Amount of vitamin B_{12} permeated through 4000PEG80PBT20 (○), 1000PEG70PBT30 (△) and 600PEG55PBT45 (□) films. To correct for differences in the thickness of the films, time is divided by the thickness (n = 3; ± s.d.).

for vitamin B_{12} is strongly dependent on the copolymer composition. Compared to polymers with a high degree of swelling, such as 4000PEG80PBT20, the permeability of relatively hydrophobic polymers such as 600PEG55PBT45 was small, resulting in a lag time of around one day. From the permeation experiments, the permeability coefficients, P_m, of the PEG/PBT membranes for vitamin B_{12} were determined as described in detail elsewhere [39]. P_m ranged from 1.2×10^{-9} cm^2/s for the polymer with the lowest degree of swelling (600PEG40PBT60) to 2.1×10^{-7} cm^2/s for the polymer with the highest degree of swelling (4000PEG80PBT20).

In contrast to vitamin B_{12}, no significant transport of lysozyme and BSA from donor to acceptor cell could be measured within a period of one week. For this reason, the permeability of PEG/PBT polymers for proteins was evaluated from release experiments [41]. Protein-containing films, prepared as described elsewhere [26], were extracted in PBS at 37°C. A representative plot of the amount of released lysozyme as a function of the square root of time is given in Fig. 6. Within a period of one week, no significant amount of lysozyme released from 600PEG55PBT45 membranes could be determined, whereas lysozyme was released from polymers with a higher degree of swelling than that of 600PEG55PBT45. This suggests that, for this particular polymer, the network mesh size is too small for diffusion of lysozyme (with a hydrodynamic diameter of 41 Å), which confirmed that the calculated mesh size for 600PEG55PBT45 (38 Å) is of the correct order of magnitude.

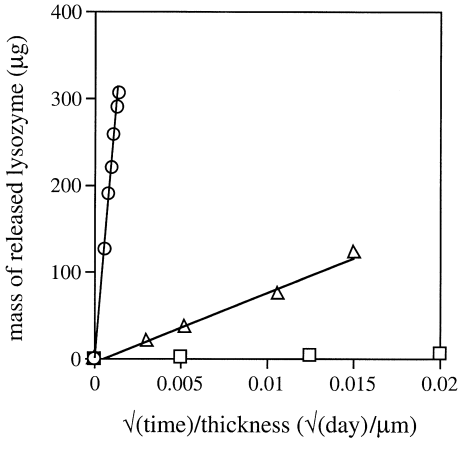

Fig. 6. Amount of lysozyme released from 4000PEG80PBT20 (○), 1000PEG70PBT30 (△) and 600PEG55PBT45 (□) films. The initial lysozyme content of the films was 33 μg/mg. To correct for differences in the thickness of the films, the square root of time is divided by the thickness ($n=3$; ± s.d.).

In a previous paper, the dependence of lysozyme release from PEG/PBT films on the copolymer composition was studied in more detail [26]. It was found that the diffusion coefficients are very small compared with the diffusion of lysozyme in water (10^{-6} cm^2/s). Furthermore, the diffusion coefficients were strongly dependent on the swelling ratio and, thus, on the composition of the copolymers. An increase in the equilibrium swelling ratio of the films from 1.47 up to 3.66 caused an almost 50,000-fold increase in the effective lysozyme diffusion coefficient, from 8×10^{-14} to 3.9×10^{-9} cm^2/s. These small values of the lysozyme diffusion coefficients can explain why no significant protein transport could be measured in the diffusion apparatus within a reasonable time period. For example, for the polymer 1000PEG70PBT30, the effective lysozyme diffusion coefficient is 5×10^{-12} cm^2/s [26]. In the case of a donor compartment filled with a lysozyme solution of 10 mg/ml, it can be easily calculated that even after 150 h, the concentration of lysozyme in the acceptor chamber of the diffusion apparatus is only 2 μg/ml, which is too low for accurate determination.

For the most bulky protein, BSA (72 Å), release could only be determined from 4000PEG80PBT20 membranes (Fig. 7). For all other polymers, no significant BSA release was found within a period of two weeks. Once more, these results confirm the data presented in Fig. 4, as the calculated hydrogel mesh size of the poly(ether ester) copolymers was less than 72 Å, except for that of 4000PEG80PBT20 (93 Å).

The amount of released lysozyme from the highly swollen polymers 4000PEG80PBT20 and 4000PEG55PBT45 ($Q>2.4$), with a high permeability for lysozyme, was proportional to the square root of time up to 60% protein release, which indicates that the release profile follows Fickian diffusion. However, from the films prepared from PEG/PBT copolymers with PEG segments of 1000 and 600 g/mol, which were swollen to a lesser extent ($Q<2$) but were still permeable for lysozyme

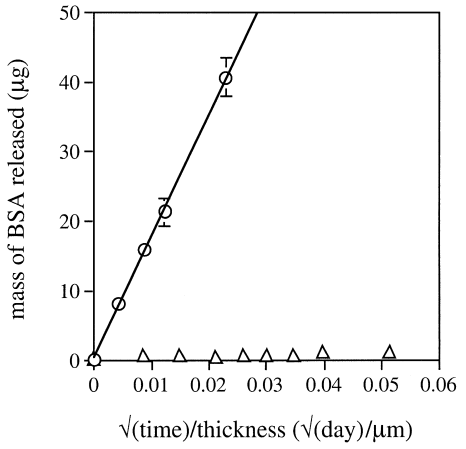

Fig. 7. Amount of BSA released from 4000PEG80PBT20 (○) and 1000PEG70PBT30 (△) films. The initial BSA content of the films was 33 μg/mg. To correct for differences in the thickness of the films, the square root of time is divided by the thickness ($n=3$; ±s.d.).

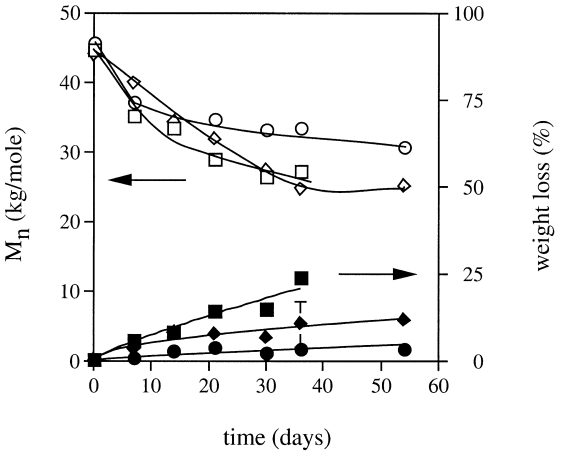

Fig. 8. Degradation of 1000PEG70PBT30 (□), 1000PEG60PBT40 (◊) and 1000PEG40PBT60 (○) films in PBS at 37°C. Filled symbols indicate weight loss ($n=2$±s.d.), open symbols represent M_n.

($\xi>42$ Å), the square root of time dependency was only found during the first days of lysozyme release. After this initial period, lysozyme was released with an almost constant rate (data not shown). In a previous publication, this rather unexpected release behavior was attributed to the effect of polymer degradation on diffusion [26].

3.3. Polymer degradation

To study the in vitro degradation of PEG/PBT copolymers, three polymers, with a PEG block length of 1000 g/mol and PEG/PBT weight ratios of 70:30, 60:40 and 40:60 were selected. Profiles of the weight loss of solvent cast films in PBS at 37°C are shown in Fig. 8. The data show that matrices having increasing PEG content exhibited a faster weight loss. Fragmentation of 1000PEG70PBT30 films was observed during the incubation period, while films with PEG/PBT ratios of 60:40 and 40:60 remained intact.

Fig. 8 also shows the change in the number average molecular weight (M_n) of the remaining polymer as a function of time. Over the time period of 54 days, M_n decreased by about 35–45%. A plot of the reciprocal number average molecular weight, M_n, as a function of degradation time in PBS is a straight line (Fig. 9), which can be described by the following relationship:

$$\frac{1}{M_n} = \frac{1}{M_{n,0}} + k_1 t \quad (7)$$

where M_n is the number average molecular weight at time t, $M_{n,0}$ is the initial number average molecular weight and k_1 is the degradation rate constant. This

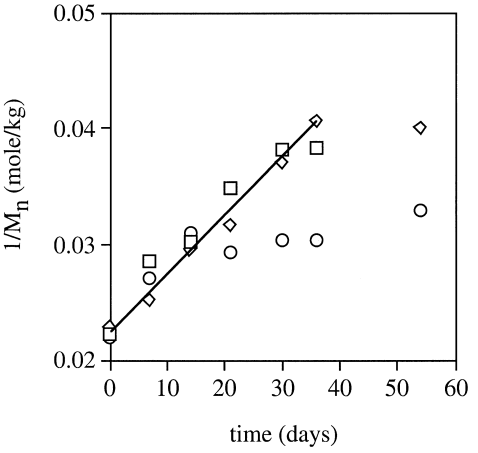

Fig. 9. Change of the reciprocal M_n as a function of incubation time in PBS at 37°C of 1000PEG70PBT30 (□), 1000PEG60PBT40 (◊) and 1000PEG40PBT60 (○) films.

indicates that in vitro degradation is caused by a non-catalyzed degradation reaction. Furthermore, Fig. 9 shows that, within experimental error, degradation of polymers with a similar PEG block length but with a different PEG/PBT weight ratio could be described by the same relationship and degradation rate constant (6.3×10^{-12} mol/g/s) until a plateau value was reached.

For the most hydrophobic polymer (1000PEG40PBT60), the plateau value had already been reached by 15 days, while the decrease in the molecular weight of the residues of the 70:30 and 60:40 matrices proceeded for at least 35 days. Assuming that degradation is caused by hydrolysis, the plateau value may be explained by the fact that the number of ester bonds susceptible to hydrolysis is limited for PEG/PBT matrices. As suggested by Reed and Gilding [42] for PEG/PET block copolymers, primary cleavage will be at the ester linkages between PEG and terephthalate, since they are located in the most hydrophilic environment. The number of these bonds is less for 1000PEG40PBT60 than for the polymers with only 30 and 40 weight.% PBT, which may explain the fact that the M_n plateau value was reached faster. Additionally, solubilization of the low-molecular-weight fraction of the molecular weight distribution tends to increase the average molecular weight of the remaining matrix, which may counteract the simultaneous decrease of the molecular weight caused by random chain scission. This hypothesis was supported by the fact that, during degradation, the polydispersity M_w/M_n decreased from 2.2 to 1.8 for all copolymer compositions.

As the ester bond between PEG and terephthalate is expected to be the most susceptible one to hydrolysis, the composition of the remaining matrix might change during degradation due to release of PEG-rich products. This was investigated by ^{1}H-NMR for the polymer that displayed the most pronounced weight loss (1000PEG70PBT30). A typical NMR spectrum of this polymer (before degradation) is shown in Fig. 10. The ratio between integral intensities originating from protons a and c was used to calculate the PEG/PBT ratio during degradation. Initially, a small increase in PBT content was found, from 28 to 31 weight.%. After that, the composition remained constant. The small increase may be caused by the extraction of low-molecular-weight PEG oxidation products, which had been formed during synthesis of the poly(ether ester)s due to insufficient thermostabilization [19].

The GPC, NMR and mass loss data presented above are in good agreement with the work of Reed et al. [42] on the degradation of PET/PEG multiblock copolymers, who proposed that the mechanism of in vitro degradation for these kind of copolymers is by hydrolysis. Prior work on the in vivo degradation of PEG/PBT copolymers has indicated that biological response to the implant material (cellular and/or enzymatic) will also contribute to degradation [19]. It has been demonstrated that in vivo degradation of poly(ether urethane)s takes place essentially at the ether linkage of the soft segment [43]. To endorse the prior long-term in vivo degradation studies [19,44], detailed studies on the contribution of hydrolysis and oxidation to the degradation of PEG/PBT copolymers are being carried out in our laboratory.

With respect to the application as a matrix for the controlled release of proteins, the results of the in vitro degradation experiments indicate that the release of drugs from PEG/PBT copolymers will not be governed by mass loss. In particular, for polymers with a relatively high PBT content, the rate of mass loss is too small to determine the release rate of incorporated proteins. Thus, diffusion will be the primary mechanism of drug release. For these systems, the release rate can be tailored precisely by controlling the copolymer composition. Release rates will increase with increasing PEG/PBT weight ratio and increasing molecular weight of the PEG component, because of an increase in swelling and mesh size. However, characterization of the network structure showed that the application of the PEG/PBT block copolymers used in this study as a protein release system is limited to protein drugs with a hydrodynamic radius of less than approximately 90 Å. In order to release larger proteins from PEG/PBT systems, matrices with a controlled microporosity have to be prepared.

Although mass loss may not contribute considerably to release of proteins, the role of polymer degradation cannot be neglected. During the release period, permeability of the matrix for the incorporated drug may increase due to chain scission. This

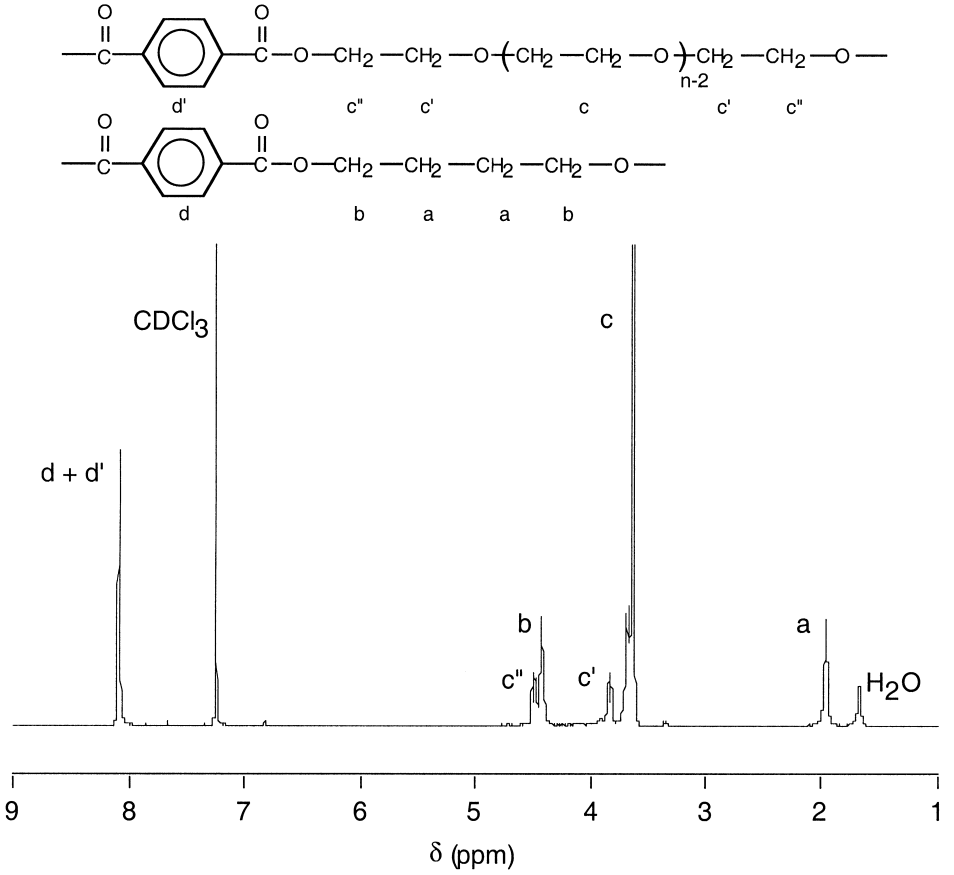

Fig. 10. ^{1}H NMR spectrum of 1000PEG70PBT30 in $CDCl_3$.

offers the possibility of obtaining a constant release rate [26].

4. Conclusions

The results of this study show that poly(ether ester) multiblock copolymers, based on hydrophilic PEG and hydrophobic PBT, have a good potential as a matrix material for a controlled release system for therapeutically active proteins and peptides. By controlling the copolymer composition, hydrogel properties, such as swelling, cross-link density and mesh size, can be tailored. The calculated mesh size of the evaluated copolymers ranged from 38 to 93 Å, which was experimentally confirmed by diffusion measurements. This range in mesh sizes allows modulation of peptide and protein release from the poly(ether ester)s. Finally, it was shown that, at 37°C in PBS buffer (pH 7.4), degradation of the PEG/PBT copolymers occurs, which may also affect the ultimate release profile.

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