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Control of vitamin B_{12} release from poly(ethylene glycol)/poly(butylene terephthalate) multiblock copolymers

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Abstract

The release of vitamin B_{12} (1355 Da) from matrices based on multiblock copolymers was studied. The copolymers were composed of hydrophilic poly(ethylene glycol)-terephthalate (PEGT) blocks and hydrophobic poly(butylene terephthalate) (PBT) blocks. Vitamin B_{12} loaded films were prepared by using a water-in-oil emulsion method. The copolymer properties, like permeability, could be varied by increasing the PEG-segment length from 300 up to 4000 g/mol and by changing the wt% of PEGT. From permeation and release experiments, the diffusion coefficient of vitamin B_{12} through PEGT/PBT films of different compositions was determined. The diffusion coefficient of vitamin B_{12} was strongly dependent on the composition of the copolymers. Although an increased wt% of PEGT (at a constant PEG-segment length) resulted in a higher diffusion coefficient, a major effect was observed at increasing PEG-segment length. By varying the copolymer composition, a complete release of vitamin B_{12} in 1 day up to a constant release for over 12 weeks was obtained. The release rate could be effectively tailored by blending copolymers with different PEG-segment lengths. The swelling and the crystallinity of the matrix could explain the effect of the matrix composition on the release behavior.

Keywords: Poly(ether ester); Controlled release; Peptides; Swelling; Crystallinity

1. Introduction

Extensive investigations have been carried out on polymers for controlled release systems for peptides and proteins [1,2]. Most of this work has focused on poly(lactide-co-glycolide) (PLGA) [3]. Recently, a series of poly(ethylene glycol) terephthalate/poly(butylene terephthalate) (PEGT/PBT) multiblock copolymers was studied as matrix for controlled drug delivery [4]. This polymer system is currently under (pre)clinical investigation for a wide range of biomedical applications, including bone replacement [5], and cartilage [6,7], muscle [7] and skin repair [8]. Many in vivo and in vitro studies have shown that PEGT/PBT multiblock copolymers are biocompatible and can be made biodegradable [8–15]. In 2000, a degradable cement restrictor composed of PEGT/PBT obtained market clearance from the FDA.

In earlier publications, PEGT/PBT copolymers were shown to be a successful matrix for (zero order) controlled release of large molecules, like proteins (lysozyme (14.5 kDa) and albumin (67 kDa)) [4]. A long time constant release of proteins was obtained by a combination of diffusion and degradation. The protein release rate could be tailored by varying the composition of the copolymer, with preservation of the protein activity. To our best knowledge, no experiments dealing with the release of small molecules from PEGT/PBT matrices have been reported. A lot of pharmaceuticals, however, are small molecules like peptides. Currently, leuprolide (1270 Da) loaded PLGA microspheres are on the market for the treatment of prostate cancer [16]. Also, research has been carried out on the development of salmon calcitonin (3500 Da) release systems based on, for example, poly(glycolic acid) [17]. In this study, the application of PEGT/PBT copolymer as matrix for controlled release of small water-soluble molecules, like

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peptides, was investigated. As a model, vitamin B_{12} (1355 Da) was used, because it has the same size as leuprolide and it is easily detectable by its red color. The vitamin B_{12} release was studied from solvent cast PEGT/PBT films. We investigated the effect of the matrix composition on the vitamin B_{12} release behavior by using a series of PEGT/PBT copolymers and blends of these copolymers.

2. Materials and methods

2.1. Materials

A series of poly(ethylene glycol) terephthalate/poly-(butylene terephthalate) (PEGT/PBT) copolymers were obtained from IsoTis (Bilthoven, The Netherlands). The poly (ether–ester) copolymers vary in PEGT/PBT weight ratio (80/20−30/70) and PEG segment length (300, 600, 1000 and 4000) and are indicated as **a**PEGT**b**PBT**c**, in which **a** is the PEG molecular weight, **b** the wt% PEG-terephthalate and **c** (=100−*b*) the wt% PBT. Phosphate buffered saline (PBS), (pH 7.4) was purchased from Life Technologies Ltd (Paisley, Scotland). Chloroform purchased from Fluka Chemie GmbH (Buchs, Switzerland) was of analytical grade. Vitamin B_{12} (cyanocobalamin, approximately 99%) and hexafluoroisopropanol (HFIP) were obtained from Sigma Chemical Co (St Louis, USA).

2.2. PEGT/PBT copolymer characterization

Proton NMR spectra were recorded on a Bruker ARX-400 operating at $\geqslant$200 MHz. $C_2D_2Cl_4$ was used as solvent without internal standard. The PEGT/PBT copolymer composition was calculated as described elsewhere [18]. The intrinsic viscosity [η] was determined of a solution of 0.5 g/dl of polymer in chloroform using a Schott Gerâte Ubbelohde viscosimeter (DIN) type 0c at 25°C. Thermal analysis of the polymers was carried out with a Perkin-Elmer Pyris 1 differential scanning calorimeter. Calibration was performed with pure indium and zinc. Samples (10–15 mg) were heated from 40°C to 250°C at a heating rate of 10°C/min. We calculated the % of crystallized polymer from the area under the curve (ΔH) as described in detail by Fakirov et al. [19,20].

2.3. Preparation of (vitamin B_{12}-loaded) PEGT/PBT films

PEGT/PBT copolymer (1 g) was dissolved in 7 ml chloroform and cast on a glass plate using a casting knife. Copolymers having a PBT content above 50 wt% were dissolved in a mixture of 6 ml chloroform and 1 ml hexafluoroisopropanol. The solvent was slowly evaporated at room temperature and, subsequently, the films were dried under vacuum overnight. The resulting films had a thickness of 50–100 μm.

For the preparation of vitamin B_{12} loaded films, a vitamin B_{12} solution in PBS was emulsified in a polymer solution using an ultra turrax (IKA Labortechnik T25) for 30 s at 19,000 rpm. The resulting water-in-oil emulsion was cast on a glass plate using a casting knife. After slow evaporation of the solvent, the films were removed from the glass plate and freeze dried for at least 16 h.

To study the influence of the matrix composition on the vitamin B_{12} release behavior, a series of copolymers was used, containing 20–70 wt% PBT and PEG-segments with molecular weights varying from 300 to 4000 g/mol. Besides changing the composition of the copolymer, blends of copolymers containing approximately 50 wt% PBT and PEG-segment lengths of 300 and 600 g/mol were also prepared to vary the polymer matrix properties. For these experiments, 0.6 ml of vitamin B_{12} in PBS (10 mg/ml) was incorporated per g copolymer.

2.4. Swelling in PBS

Dry films ($\pm$1.77 cm^2) were weighed and immersed in PBS at 37°C in a shaking bath. After at least 3 days, the weight of the samples was determined after surface water was removed by blotting the surface with a tissue. The water-uptake (in ml water per g polymer) was calculated from the weight increase. The equilibrium volume swelling ratio Q was determined according to the following equation (1.2 equals the density of all PEGT/PBT copolymers):

$$Q = 1 + \frac{1.2\,(M_{\text{swollen}} - M_{\text{dry}})}{M_{\text{dry}}} \tag{1}$$

From the water-uptake, also the number of water molecules per ethylene glycol (EG) unit in the copolymer could be calculated. The number of water molecules per gram of polymer was determined by using Eq. (2), in which 18 is the molar mass of water.

$$N_{H_2O} = \frac{(M_{\text{swollen}} - M_{\text{dry}})}{18\,M_{\text{dry}}} \tag{2}$$

The number of EG units per gram of polymer was calculated from the weight fraction of PEGT and the PEG segment length according to the following equation:

$$N_{\text{EG}} = \frac{\phi_{\text{PEGT}}\,\text{PEG}}{(\text{PEG} + 130)\,44} \tag{3}$$

in which ϕ_{PEGT} is the weight fraction of poly(ethylene glycol) terephthalate, PEG is the PEG segment length, 130 is the molecular weight of a terephthalate unit and 44 equals the molecular weight of one EG unit. The total

number of water molecules per ethylene glycol unit in a PEGT/PBT copolymer is the quotient of Eqs. (2) and (3).

2.5. Degradation in PBS

To determine the degradation of polymer matrices, dry films (approximately 0.25 g, 50–100 μm thickness) were immersed in 50 ml PBS at 37°C in a shaking bath. Each week, the buffer was refreshed. After seven weeks, the films were freeze dried and subsequently, the intrinsic viscosity [η] was determined as described above.

2.6. Permeability of equilibrium swollen membranes for vitamin B_{12}

The permeability of PEGT/PBT films for vitamin B_{12} (Mw = 1355 Da) 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 deionized 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 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). The permeability coefficients (P) were calculated from the UV absorbance data by the following Eq. (4):

$$\ln\left(1 - 2\frac{C_t}{C_0}\right) = -\frac{2A}{Vl}Pt \quad (4)$$

in which C_t is the vitamin B_{12} concentration in the receptor cell at time t, C_0 is the initial vitamin B_{12} concentration in the donor compartment, A is the membrane surface area, V is the volume of each cell, l is the membrane thickness, and P is the permeability coefficient [21]. The diffusion coefficient (D) of vitamin B_{12} in the various PEGT/PBT films was evaluated by

$$D = \frac{P}{K_d} \quad (5)$$

in which K_d is the partition coefficient. The partition coefficient defined as the equilibrium ratio of the solute concentration in the film to that in the surrounding solution was determined by solute uptake experiments. For each PEGT/PBT composition, three disks (2.85 cm^2), previously equilibrated in deionized water, were incubated in vitamin B_{12} solutions (2 mg/ml, 10 ml). After 14 days, the films were taken out of the solution, blotted with a tissue and immersed in 2 ml deionized water. The concentration of vitamin B_{12} released in the solutions was determined photospectroscopically as described above. The concentration of the solute in the films after immersion in the vitamin B_{12} solution was calculated from the total amount of released vitamin B_{12} and the volume of the equilibrium swollen films.

Due to the low diffusion rate of vitamin B_{12} through PEGT/PBT films made from copolymers with PEG-segments of 300 g/mol, it was not possible to measure a significant quantity of permeated vitamin B_{12} within a reasonable time interval using the diffusion apparatus. Therefore, permeability of vitamin B_{12} through PEGT/PBT membranes with 300 g/mol-segments was evaluated from release experiments.

2.7. Vitamin B_{12} release from PEGT/PBT films

The vitamin B_{12} release from the vitamin B_{12} loaded PEGT/PBT films was investigated by incubating pieces of the films (± 1.77 cm^2) in 1.5 ml PBS. Vials were continuously shaken at 37°C and samples of the release medium were taken at various time points. The vitamin B_{12} concentration of the buffer was determined using an EL 312e microplate bio-kinetics reader ($\lambda = 380$ nm). The buffer was refreshed after sampling. The thickness of the swollen films was measured using a micrometer.

3. Results and discussion

3.1. Polymer matrix characteristics

To study the possibilities of applying PEGT/PBT copolymers as a matrix for the controlled release of vitamin B_{12}, a series of PEGT/PBT copolymers was investigated. The copolymers varied in PEG-segment length and in PEGT/PBT ratio. By varying the composition, the copolymer properties could be changed. The properties of PEGT/PBT multiblock copolymers are mainly determined by their phase separated morphology. The soft hydrophilic PEGT blocks are flexible and responsible for the water-uptake, whereas the hard hydrophobic PBT blocks are rigid and give the matrix its stiffness. The structure and morphology of segmented poly(ether–ester)s has been the subject of many studies [22 for a review].

Fakirov et al. found that domains of four different types may exist in PEGT/PBT copolymers: crystalline PBT, amorphous PBT, amorphous PEG and a mixed amorphous phase [23]. The ratio of the different phases is dependent on the polymerization conditions, copolymer composition and the thermal and mechanical history of the sample [22]. Concerning the effect of the 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-segments at a constant

PEGT/PBT ratio, or increasing the PBT content at a constant PEG segment length will increase the average block length of the PBT segments and thus facilitate crystallization. The copolymer is physically crosslinked by these crystalline domains. The more the copolymer is crosslinked, the smaller the mesh widths in the matrix and consequently, the less permeable the matrix will be [24].

The PEGT/PBT copolymers used in this study as a matrix for the controlled release of vitamin B_{12} varied in PEG-segment length from 300 to 4000 g/mol. The weight percentages of PBT were determined by NMR and are given in Table 1. The intrinsic viscosity of the copolymers was in the range of 0.64–1.24 dl/g, depending on the copolymer composition. For controlled release systems, the structure and properties of the water-swollen matrices are of primary interest. Therefore, the swelling behavior of solvent cast, dense PEGT/PBT films (50–100 μm thickness) was investigated as a function of the copolymer composition. The equilibrium swelling ratio Q was reached within three days (Table 1). For the copolymers used in this study, Q varied from 1.1 for copolymers with 300 g/mol PEG-segments up to 1.3–1.7 for 600 g/mol, 1.4–1.9 for 1000 g/mol and 1.7–3.7 for 4000 g/mol PEG-segments. Thus, as expected, the longer the PEG-segment length, the higher the swelling ratio Q. At increasing PEG-segment length, the effect of the PEG content on the swelling increased. This is in good agreement with earlier observation [18].

Generally, it is accepted that three molecules of water can be bound to each ethylene glycol unit [25]. This has been investigated using various techniques, including NMR spectroscopy, analysis of the water-PEG phase diagram and differential thermal analysis [26 and references herein]. However, in studies on segmented polyether polyurethanes with 2000 g/mol PEG-segments, although the number of bound water molecules increased with an increase in PEG content, it did not reach the generally accepted value of three [27]. This was caused by the interaction of the PEG segments with the other, more hydrophobic, segments [27,28]. This effect will be even larger for smaller PEG-segment lengths [26]. We calculated the number of water molecules for different of PEGT/PBT copolymers from the water uptake according to Eqs. (2) and (3). The number of water molecules increased with increasing PEG segment length from 0.5 for copolymers with 300 g/mol segment lengths up to almost 3 water molecules per ethylene glycol unit for 1000PEGT70PBT30. The copolymers with PEG-segment lengths of 4000 g/mol exceeded the value of 3 water molecules per ethylene glycol unit. This is probably caused by the presence of both bound and free water molecules [29], although it cannot be excluded that the other copolymer compositions also contained free water in the matrix. It has been described that the state of the water molecules influences the permeability characteristics of the matrix [30]. The presence of free water enhances the solute diffusion rate compared to matrices containing only bound water.

In earlier publications, we reported a correlation between the equilibrium swelling ratio, the mesh size and the permeability of PEGT/PBT copolymers [18]. The mesh size is the space between neighboring chains in the polymer network, and can be considered as an indication for the available space in the matrix for solute diffusion [31]. An increase in the equilibrium-swelling ratio of the PEGT/PBT matrices resulted in an

Table 1
Equilibrium swelling of PEGT/PBT films and diffusion and partition coefficients of vitamin B_{12} as function of the copolymer composition[a]

Polymer composition (NMR)	Swelling ratio Q	Diffusion coefficient D (cm^2/s) from diffusion cells	Diffusion coefficient D (cm^2/s) from release	Partition coefficient K_d (—)
300PEGT32PBT68	1.11 ± 0.03	n.d.	$(1.7 \pm 0.2) \times 10^{-14}$	n.d.
300PEGT53PBT47	1.08 ± 0.01	n.d.	$(6.0 \pm 2.7) \times 10^{-13}$	n.d.
300PEGT61PBT39	1.09 ± 0.00	n.d.	$(1.2 \pm 0.4) \times 10^{-12}$	n.d.
600PEGT40PBT60	1.34 ± 0.02	$(2.4 \pm 0.4) \times 10^{-9}$	$(1.6 \pm 0.6) \times 10^{-9}$	0.25 ± 0.05
600PEGT51PBT49	1.29 ± 0.03	$(3.9 \pm 0.9) \times 10^{-9}$	$(1.7 \pm 0.0) \times 10^{-9}$	0.35 ± 0.08
600PEGT57PBT43	1.38 ± 0.02	n.d.	$(1.4 \pm 0.6) \times 10^{-9}$	n.d.
600PEGT77PBT23	1.66 ± 0.01	$(1.7 \pm 0.6) \times 10^{-8}$	n.d.	0.50 ± 0.20
1000PEGT41PBT59	1.41 ± 0.03	$(2.5 \pm 0.1) \times 10^{-8}$	n.d.	0.26 ± 0.01
1000PEGT60PBT40	1.73 ± 0.01	$(4.4 \pm 0.3) \times 10^{-8}$	n.d.	0.33 ± 0.01
1000PEGT71PBT29	1.92 ± 0.13	$(3.2 \pm 0.3) \times 10^{-8}$	n.d.	0.73 ± 0.07
4000PEGT32PBT68	1.66 ± 0.03	$(7.5 \pm 0.1) \times 10^{-8}$	n.d.	0.41 ± 0.05
4000PEGT55PBT45	2.62 ± 0.01	$(1.9 \pm 0.1) \times 10^{-7}$	n.d.	0.60 ± 0.02
4000PEGT82PBT18	3.66 ± 0.05	$(1.5 \pm 0.2) \times 10^{-7}$	n.d.	1.40 ± 0.20

[a] n.d.: not determined.

increasing mesh size. Therefore, at increasing PEG-segment length, an increase in diffusion coefficient of the vitamin B_{12} through the PEGT/PBT matrix is expected.

3.2. Permeability of PEGT/PBT films for vitamin B_{12}

This study investigated the diffusion rate of vitamin B_{12} through PEGT/PBT matrices for different copolymer compositions. By using diffusion cells, we could determine the permeability of copolymers with PEG-segments of 600, 1000 and 4000 g/mol for vitamin B_{12}. Figs. 1A and B present 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). Fig. 1A clearly shows

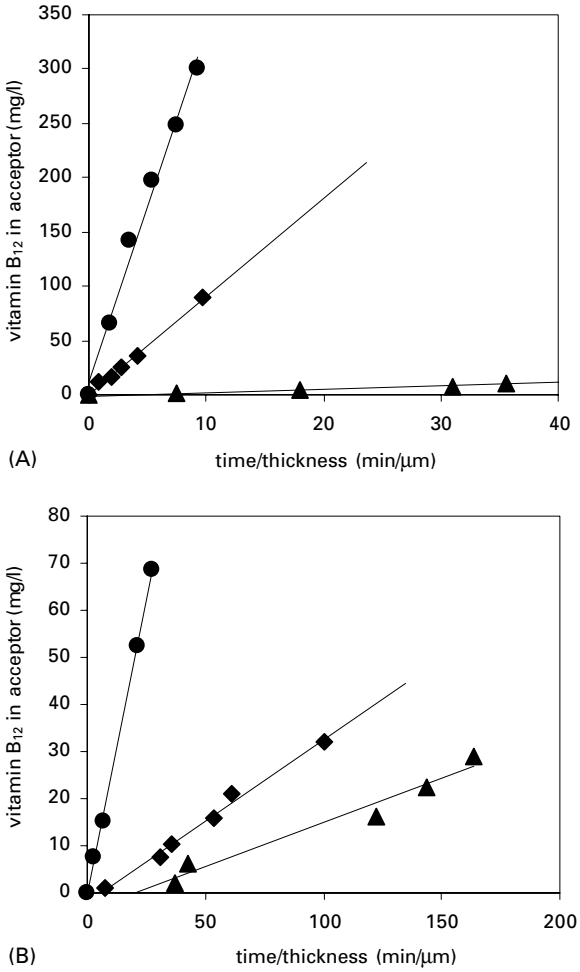

Fig. 1. Permeation of vitamin B_{12} through PEGT/PBT films with (A) various PEG segment lengths (4000PEGT55PBT45 (●), 1000PEGT60PBT40 (◆) and 600PEG51PBT49 (▲)) and (B) various wt% PEGT at constant PEG-segment length (600PEG77PBT23 (●), 600PEG51PBT49 (◆) and 600PEG40PBT60 (▲)). To correct for differences in thickness of the films, time is divided by the thickness.

that the permeability of PEG/PBT films for vitamin B_{12} is strongly dependent on the PEG-segment length. Copolymers with relatively long PEG segments, such as 4000PEGT55PBT45 display a high permeability compared to copolymers with less shorter PEG segments, such as 600PEGT51PBT49. At a constant PEG-segment length, the permeability increased with increasing wt% PEGT (Fig. 1B). For copolymers with PEG-segments of 300 g/mol, the permeability was too low to measure within a reasonable time interval.

From the permeation experiments, the permeability coefficients P of the PEGT/PBT membranes for vitamin B_{12} were determined using Eq. (4). P ranged from $1.2\times10^{-9}\,cm^2/s$ for the 600PEGT40PBT60 composition to $2.1\times10^{-7}\,cm^2/s$ for 4000PEGT80PBT20. To obtain the vitamin B_{12} diffusion coefficient for the various PEGT/PBT copolymers, K_d had to be determined. In Table 1, the partition coefficients are presented as a function of the copolymer composition. The K_d values observed are close to one, indicating that no interaction between solute and matrix occurred. As can be expected for a hydrophilic drug in the absence of interaction with the matrix, the partitioning of vitamin B_{12} in PEGT/PBT films increased with increasing degree of swelling of the copolymers. The vitamin B_{12} diffusion coefficients, calculated from the results of the permeability and partition experiments, are given in Table 1.

Due to the low diffusion rate of vitamin B_{12} through copolymers with 300 g/mol PEG-segment lengths, it was not possible to detect the vitamin B_{12} concentrations in the diffusion cell. Therefore, the diffusion coefficients of vitamin B_{12} through the copolymers with 300 g/mol PEG-segments were calculated from the release experiments. To compare the two methods, the diffusion coefficients of vitamin B_{12} through some matrices of PEGT/PBT copolymers with 600 g/mol PEG-segments lengths were determined using both methods. For this monolithic system, in which the vitamin B_{12} solution is expected to be homogeneously mixed with the matrix, the total release can be described according to the following equations [32]:

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{Dt}{\pi l^2}} \tag{6}$$

for $M_t/M_\infty < 0.6$;

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 Dt}{l^2}\right) \tag{7}$$

for $M_t/M_\infty > 0.4$; in which, M_∞ is the total amount of vitamin B_{12} in the matrix, M_t is the amount of vitamin B_{12} released at time t and l is the thickness of the film. The diffusion coefficients of vitamin B_{12} through the PEGT/PBT series with 300 g/mol PEG-segment lengths were obtained by plotting the fractional vitamin B_{12} released M_t/M_∞ versus the square root of time. Representative plots are given in Fig. 2. From the first

part of the curves ($M_t/M_\infty < 0.6$), the diffusion coefficient D was calculated by using Eq. (6). The vitamin B_{12} release from copolymers with 600 g/mol PEG-segment lengths was too fast to be able to use Eq. (6). Therefore, the diffusion coefficient D of vitamin B_{12} through these copolymers was calculated from the vitamin B_{12} release by using Eq. (7).

The diffusion coefficients calculated from the diffusion cell experiments and from the initial release are both given in Table 1. The diffusion coefficient of vitamin B_{12} was approximately 10^{-7} and 10^{-8} cm^2/s for PEGT/PBT matrices with 4000 g/mol PEG-segment lengths and 1000 g/mol PEG-segments, respectively. The diffusion coefficients for vitamin B_{12} through copolymers with 600 g/mol PEG-segments calculated by both methods were in the same range (10^{-9} cm^2/s), indicating agreement between the methods. In matrices with PEG-segment lengths of 300 g/mol, the diffusion coefficient was in the range of 10^{-12}–10^{-14} cm^2/s. In comparison, the diffusion coefficient of vitamin B_{12} in water is 3.79×10^{-6} cm^2/s [21]. From these results, it can be concluded that the PEG-segment length is the important determining factor of the diffusion coefficient. At a constant PEG-segment length, the diffusion coefficient can be modulated to a certain extent by varying the PEGT content. A similar trend was observed in the equilibrium volume swelling ratio Q of the polymers with different PEG-segments lengths (Table 1).

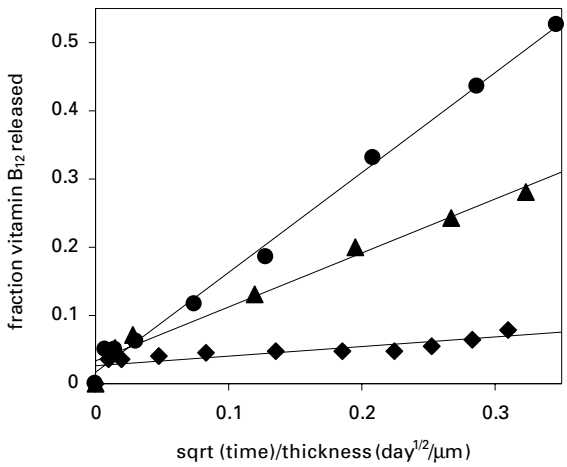

Fig. 2. Initial release of vitamin B_{12} as function of the square root of time from 300PEGT32PBT68 (◆), 300PEGT53PBT47 (▲) and 300PEGT61PBT39 (●) films for determination of the diffusion coefficients. To correct for differences in thickness of the films, the square root of time is divided by the thickness. ($n = 3$).

However, the swelling can only partly explain the trend in diffusion coefficient. For example, within the 300 g/mol PEG-segment length series a difference in diffusion coefficient is observed, while no significant difference in swelling can be noticed. Probably, the diffusion rate is also affected by the matrix morphology. At a constant PEG-segment length of 300 g/mol, with increasing wt% of PBT, a higher percentage of crystallinity in the film was expected as described above. DSC measurements confirmed this hypothesis (Table 2). The crystallinity in the film increased from 1.3% at 40 wt% of PBT up to 11% of crystallinity at 70 wt% of PBT. Because the crystallites can be considered as impermeable [33], most of the vitamin B_{12} transport takes place through the amorphous domains. Thus, the crystallites reduce the available space for solute diffusion and increase the characteristic diffusion length [33]. Moreover, due to the existence of a crystalline polymer fraction, the solute diffusion coefficient through the amorphous phase is modified [34,35]. This is caused by a decrease in swelling of the amorphous phase at increasing crystallinity. Expansion of the network is hampered, because the crystallites act as crosslinks and impede the swelling process. Thus, the decreasing diffusion rate of vitamin B_{12} through the 300 g/mol matrices could be the result of a higher crystallinity at higher wt% of PBT.

3.3. Sustained release of vitamin B_{12}

For biomedical applications as controlled release devices, the long-term release of drugs is of particular interest. Therefore, only copolymers with 300 g/mol PEG-segment lengths and copolymers with 600 and 1000 g/mol segments and low wt% PBT were selected for the release study. As could be expected from the diffusion coefficients, the release of vitamin B_{12} from films was strongly dependent on the composition of the

Table 2
Crystallinity and intrinsic viscosity prior to and after 12 weeks of in vitro degradation in PBS as function of polymer composition[a]

Polymer composition (NMR)	Crystallinity (%) (DSC)	Intrinsic viscosity ($t = 0$) (dl/g)	Intrinsic viscosity ($t = 7$ weeks) (dl/g)
300PEGT32PBT68	11.0	n.d.	n.d.
300PEGT53PBT47	2.3	0.64	0.66
300PEGT61PBT39	1.3	0.68	0.65
600PEGT57PBT43	n.d.	0.92	0.69
1000PEGT71PBT29	n.d.	0.90	0.54

[a] n.d.: not determined.

copolymers. Although an increased wt% of PEGT (at a constant PEG-segment length) resulted in an increasing vitamin B_{12} release rate, a major effect was observed at increasing PEG-segment length. The release of vitamin B_{12} was complete in 1 day for copolymers with PEG-segments of 600 and 1000 g/mol, while the release from copolymers with 300 g/mol PEG-segments continued for over 12 weeks (Fig. 3).

The release profiles of both copolymers with 300 g/mol PEG-segment lengths, shown in Fig. 3, indicate an almost constant release of vitamin B_{12} in time. If the release were only determined by diffusion, a first order release would be expected. Zero order release was also obtained for proteins, like lysozyme, from PEGT/PBT matrices with 600 and 1000 g/mol PEG-segments [4]. This was explained by an increase in diffusion coefficient in time due to polymer degradation. However, considering the time period of the release, degradation of copolymers with 300 g/mol PEG-segment lengths will not play an important part. Viscosity measurements proved this hypothesis. Unloaded PEGT/PBT films were degraded in PBS at 37°C for 7 weeks. The intrinsic viscosity of the polymer was measured prior to and after degradation of the films in PBS. From Table 2, it can be seen that the intrinsic viscosity decreased for the PEGT/PBT copolymers with 600 and 1000 g/mol PEG-segments lengths. However, the intrinsic viscosity remained unchanged for films of copolymers with 300 g/mol PEG-segments after 7 weeks in PBS, indicating that no degradation has taken place. Instead, the zero order release of vitamin B_{12} from 300 g/mol PEGT/PBT matrices might be caused by the low water-uptake of these copolymers and the solubility of vitamin B_{12} in water. The relatively short PEG-segments resulted in a low water-uptake (Table 1). The solubility of vitamin B_{12} in water is 12 mg/ml [36]. Due to the low amount of water present in the PEGT/PBT matrix, only a fraction of the vitamin B_{12} will be dissolved. The vitamin B_{12} loaded matrices prepared from copolymers with 300 g/mol PEG-segments should be considered as a dispersed system [32]. As long as solid vitamin B_{12} is present, the concentration gradient within and outside the matrix is constant, resulting in a zero order release. In this mechanism, the dissolving of the vitamin B_{12} is assumed to be the rate-limiting step and the concentration gradient inside the matrix is neglected. Although this mechanism can describe the zero order release, it does not explain the difference in release rate within the 300 g/mol series. As discussed before, also the morphology of the matrix plays a role. It has to be noted that the Eqs. (6) and (7) describe a dissolved system. Since our system may contain undissolved drug, the obtained values can only be considered as a first approximation.

3.4. Effect of copolymer blending on vitamin B_{12} permeability

To overcome the gap between the release rates of vitamin B_{12} of the copolymers with different PEG-segments lengths, blends of 300PEGT53PBT47 and 600PEGT51PBT49 were prepared. Because of the large difference in the diffusion coefficients of these two copolymers, adding of only 5 wt% 600PEGT51PBT49 is sufficient to increase the release rate of vitamin B_{12} to a large extent (Fig. 4). By blending of copolymers with different PEG-segment lengths, the release rate can be effectively tailored from 1 day up to 12 weeks.

The effect of blending on the release rate was further evaluated by calculating the diffusion coefficients from the release curves using Eqs. (6) and (7). The experimental diffusion coefficients are given in Fig. 5. In this

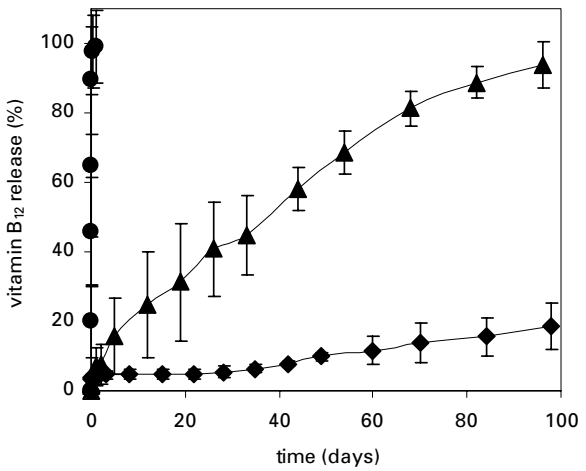

Fig. 3. Cumulative release of vitamin B_{12} from PEGT/PBT films of different compositions (◆: 300PEGT32PBT68, ▲: 300PEGT61PBT39, ●: 600PEGT57PBT43) ($n = 3$; ±S.D.).

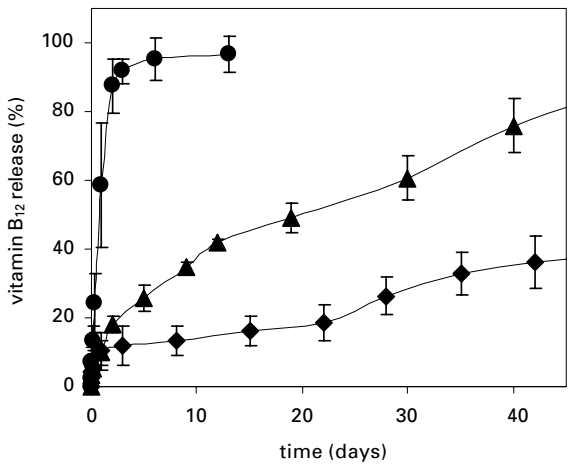

Fig. 4. Cumulative release of vitamin B_{12} from 300PEGT53PBT47/600PEGT51PBT49 blends (◆: 100% 300PEGT53PBT47, ▲: 95% 300PEGT53PBT47/5% 600PEGT51PBT49, ●: 75% 300PEGT53PBT47/25% 600PEGT51PBT49) ($n = 3$; ±S.D.).

figure, also the average diffusion coefficients are shown as function of the blend composition, calculated from Eq. (8):

$$D_{av} = \phi_{300} D_{300} + \phi_{600} D_{600} \quad (8)$$

in which D_{300} is the diffusion coefficient of vitamin B_{12} through 300PEGT53PBT47, D_{600} is the diffusion coefficient through 600PEGT51PBT49 and ϕ_{300} and ϕ_{600} are the weight fractions of the copolymers. Eq. (8) is based on the assumption that the blends can be considered as molecularly mixed matrices, as the two closely related copolymers were blended in solution.

By comparison of the experimental diffusion coefficient calculated from the release curves and the average diffusion coefficient calculated by Eq. (8), the following can be observed. The experimental and the average diffusion coefficients of the blends containing more than 25 wt% of 600PEGT51PBT49 were in the same range. However, a large difference could be seen for low percentages (2–15 wt%) of 600PEGT53PBT47 in the matrix. The experimental diffusion coefficients of these blends were significantly lower than the calculated average diffusion coefficient. So, the effect of blending on the release rate cannot be simply explained by calculating the average diffusion coefficient from the diffusion coefficients of the copolymers (300PEGT53PBT47 and 600PEGT51PBT49). To determine the mechanism behind the effect of blending on the diffusion coefficient, another parameter that could be affected by blending, the swelling, was determined.

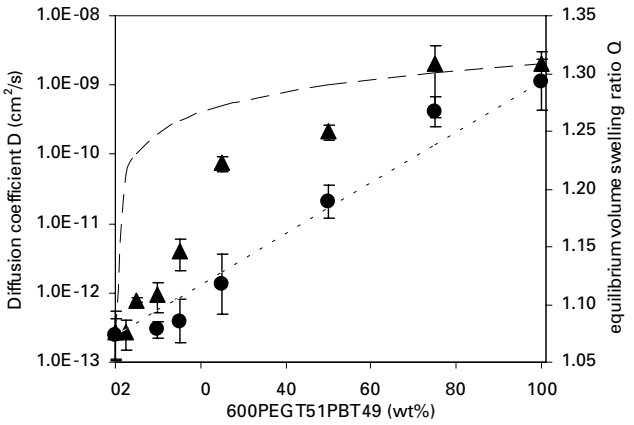

Fig. 5. Experimental diffusion coefficients calculated from the release experiments of vitamin B_{12} through films of 300PEGT53PBT47/600PEGT51PBT49 blends (▲) and the volume swelling ratio Q of these films (●) as a function of the wt% 600PEGT51PBT49. The calculated average diffusion coefficients (- - -) and swelling ratios (....) are indicated by dotted lines.

The equilibrium volume swelling ratio Q of films cast from solutions of 300PEGT53PBT47 and 600PEGT51PBT49 mixtures in chloroform is also given in Fig. 5. Identical to the diffusion coefficient, the average volume swelling ratios of the blends are calculated and indicated in the figure. It can be seen, that the swelling ratio Q is not directly proportional with the amount of 600PEGT51PBT49. Although the swelling increased with increasing amount of 600PEGT51PBT49, the swelling was only affected significantly after adding of 25 wt%. At low wt% of 600PEGT51PBT49 the experimentally determined swelling is lower than expected from the calculated average swelling. Apparently, the hydrophilic 600 g/mol segments have difficulties expanding in the stiff hydrophobic matrix based on the 300PEGT53PBT47.

However, the release rate was already affected after adding of 5 wt% of 600PEGT51PBT49 (Fig. 4). Therefore, as seen for the different copolymer compositions the increase in release rate cannot fully be explained by an increase in swelling. To investigate whether the increase in release rate is caused by a difference in matrix structure, DSC analyses were carried out. The crystallinity of the matrix, calculated from the DSC measurements, is given in Table 3 as a function of the blend compositions. The crystallinity depends on the PBT block length and the average distance between the PBT blocks. The latter is strongly dependent on the PEG-segment length, whereas the PEGT/PBT ratio does not alter the spacing significantly [19,20]. Although the PBT blocks in 600PEGT51PBT49 are larger than in 300PEGT53PBT47, which could increase crystallinity, the distance between these blocks is larger in 600PEGT51PBT49, which could decrease crystallinity. This might explain why no difference in crystallinity was

Table 3
Swelling ratio Q and crystallinity for blends of 300PEGT53PBT47 and 600PEGT51PBT49[a]

wt% 300PEGT53PBT47	wt% 600PEGT51PBT49	Swelling ratio Q	Crystallinity (%) (DSC)
100	0	1.07±0.02	2.3
90	10	1.08±0.01	5.3
85	15	1.09±0.02	4.2
75	25	1.12±0.01	1.8
50	50	1.19±0.01	2.3
25	75	1.27±0.01	n.d.
0	100	1.29±0.03	2.0

[a] n.d.: not determined.

observed between these two polymers (Table 2). However, the crystallinity increased significantly by adding 10 and 15 wt% of 600PEGT51PBT49. Possibly, the longer PBT blocks of the 600PEGT51PBT49 copolymer facilitate crystallization with the smaller, but more available, PBT blocks of the 300PEGT53PBT47. Thus, the fact that the diffusion coefficient of vitamin B_{12} through the blends (2–15 wt% of 600PEGT53PBT47) is lower than expected, may be caused by a combination of unexpected swelling and crystallinity behavior.

4. Conclusion

The results of this study show that PEGT/PBT multiblock copolymers can be successfully used as matrix for controlled release of small water-soluble molecules. By varying the copolymer composition, a complete release of vitamin B_{12} in 1 day up to a constant release for over 12 weeks can be obtained. The release rate can be effectively tailored by blending copolymers with different PEG-segment lengths. The swelling and the crystallinity of the matrix determine the diffusion coefficient of vitamin B_{12} through PEGT/PBT copolymers. In general, the swelling can mainly be varied by changing the PEG-segment length. At a constant PEG-segment length, the crystallinity increases at increasing wt% PBT. For a number of PEGT/PBT copolymers used in this study, no in vitro degradation was observed after 7 weeks. Further studies will focus on in vivo degradation of a series of PEGT/PBT copolymers. Furthermore, the results of this study will be used to develop controlled release systems for pharmaceutically relevant peptides.

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