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Research paper

Release of small water-soluble drugs from multiblock copolymer microspheres: a feasibility study

J. Sohier[a,*], R. van Dijkhuizen-Radersma[a], K. de Groot[b], J.M. Bezemer[a]

[a] *Chienna BV, Bilthoven, The Netherlands*
[b] *IsoTis NV, Bilthoven, The Netherlands*

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Abstract

Poly(ethylene glycol)-terephthalate/poly(butylene terephthalate) (PEGT/PBT) multiblock copolymer was investigated as a possible matrix for controlled delivery of small water-soluble drugs. Two molecules were selected as sustained release candidates from microspheres: leuprorelin acetate (peptide of Mw = 1270 D) and vitamin B_{12} (Mw = 1355 D). First, vitamin B_{12}-loaded microspheres were prepared using a double emulsion method and preparation parameters were varied (surfactant in the first emulsion and copolymer composition). The resulting microsphere structure, entrapment efficiency and release rate were evaluated. Vitamin B_{12}-loaded microsphere parameters could easily be tailored to achieve specific requirements. The addition of surfactant in the first preparation process led to a significant increase of the microsphere entrapment efficiency, whereas the decrease of the PEGT copolymer content allowed the release rates from microspheres to be precisely decreased. However, leuprorelin acetate-loaded microspheres did not show the same characteristics when prepared with the same parameters, possibly because of a high water solubility discrepancy between the vitamin B_{12} and the peptide. This study shows the suitability of PEGT/PBT microspheres as a controlled release system for vitamin B_{12}, but not for leuprorelin acetate. It also underlines the necessity of tailored development for each individual drug and emphasizes the risk of using model molecules.

Keywords: Microsphere; Peptide; Small water-soluble drug; Controlled release; Multiblock copolymer; Surfactant

1. Introduction

To facilitate the delivery of therapeutic peptides, many polymeric controlled release systems have been designed [1–3]. Polymers that have been studied as a matrix for protein release include poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers (PLGA) [4–6], poly(ε-caprolactone) (PCL) [7,8], poly(ether-ester) copolymers (poly(ethylene oxide)-poly(lactic-co-glycolic acid) (PEO/PLGA) or poly(ethylene glycol)-terephthalate/poly(-butylene terephthalate) (PEGT/PBT) for instance) [9–16], gelatin [17], collagen [18], alginates [19] and dextran [20]. Among these polymers, PEGT/PBT multiblock copolymers have the advantage that their properties, like permeability and biodegradability, can be easily tailored to meet specific requirements. Furthermore, their advantage compared to poly(D,L-lactide-co-glycolide) regarding protein biological activity has been reported when using lysozyme as a model [14,21]. Recently, it has been shown that a sustained release of small water-soluble molecules could be obtained from films based on these copolymers [22]. However, for therapeutical application, microspheres are often preferred since their administration does not require surgical intervention and can be considered as less painful than cylindrical implants.

In order to further investigate the possibilities offered by these copolymers, the aim of this study was to develop PEGT/PBT microspheres loaded with a small water-soluble drug. Two molecules were selected as sustained release candidates: leuprorelin acetate (leuprolide) and vitamin B_{12}. Leuprolide was selected because this highly water-soluble nona-peptide (Mw = 1270 D) is unstable in body fluid and excreted rapidly. In addition, PLGA-microspheres loaded with the peptide are currently available on the market, allowing us to compare release rates obtained from PEGT/PBT microspheres with a well-known system. The PLGA and PLA formulation provides a sustained release for 1 or 3 months that shows efficacy in the treatment of

* Corresponding author. Chienna BV, Prof. Bronkhorstlaan 10-D, 3723 MB, Bilthoven, The Netherlands. Tel.: +31-30-229-5149; fax: +31-30-228-0255.
E-mail address: jerome.sohier@chienna.com (J. Sohier).

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hormone dependent tumors, and more specifically prostate cancer and endometriosis [23,24]. Vitamin B_{12} was chosen because its size is comparable to that of leuprolide (Mw = 1355 D), and its quantification is easy.

We report first on the effects of preparation parameters and copolymer compositions on microsphere characteristics, using vitamin B_{12} as a small water-soluble drug model. Then leuprolide-loaded microspheres were prepared with the most promising processing parameters, and peptide release behavior was determined.

2. Materials and methods

2.1. Materials

PEGT/PBT multiblock copolymers were obtained from IsoTis NV, Bilthoven, The Netherlands, and were used as received. Vitamin B_{12} and bovine serum albumin (BSA, heat shock fractionate, fraction V powder minimum 98%, Mw = 66 kg/mol) were purchased from Sigma (St. Louis, MO). Leuprolide (leuprorelin acetate) was acquired from AKZO Nobel (Oss, The Netherlands). Poly(vinyl alcohol) (PVA, Mw = 22 kg/mol) was obtained from Aldrich Chemicals (Milwaukee, WI). Phosphate buffered saline (PBS) (pH 7.4) was obtained from Life Technologies Ltd. (Paisley, UK). Chloroform, purchased from Fluka chemica (Buchs, Switzerland), was of analytical grade.

2.2. Preparation of PEGT/PBT microspheres

Loaded microspheres were prepared using a water-in-oil-in-water (w/o/w) emulsion method [15]. An aqueous solution of the drug in PBS (0.6 ml, 10 mg/ml) was emulsified with a PEGT/PBT copolymer solution in chloroform (1 g in 7 ml), using an Ultra-Turrax (T25 Janke & Kunkel, IKA-Labortechnik) for 30 s at 19 krpm. This first emulsion (W/O) was poured in a 50 ml PBS buffer containing 4 weight (wt) % PVA, and stirred at 400 rpm for 5 min to prepare the second emulsion (W/O/W). Subsequently, 100 ml of PBS was added to facilitate chloroform removal (over 2 h). Microspheres were collected by centrifugation, washed with PBS three times and freeze-dried.

The effect of surfactants in the first emulsion on the internal/external structure of the microspheres, the drug entrapment efficiency and the release behavior was investigated. PVA and BSA were dissolved in the aqueous drug solution at different concentrations: 1 and 10 mg/ml for PVA; 1, 10 and 15 mg/ml for BSA. The copolymer used had a PEGT content of 55% and a PEG molecular weight of 300 g/mol.

To study the influence of copolymer composition on the above mentioned microsphere characteristics, different polymers were used. The PEGT content was varied from 55 to 80%, with a fixed PEG molecular weight of 300 g/mol. Microspheres were then prepared as described above.

2.3. Scanning electron microscopy

A Philips XL 30 ESEM-FEG was used to evaluate external and internal morphologies of microspheres. The internal structure was observed by breaking the microspheres in liquid nitrogen. All samples were gold sputter-coated using a Cressington 108 auto apparatus before analysis.

2.4. Particle size distribution

The size (number mean and volume mean diameter) and distribution of the microspheres were determined by a laser light diffraction method (master sizer S long bed ver. 2.18, Malvern instrument Ltd.).

2.5. Determination of drug content in the microspheres

To determine drug entrapment efficiency, about 15 mg of microspheres was dissolved in 5 ml of chloroform. Subsequently, the solutes were extracted by mixing with 5 ml of PBS. Concentrations of the aqueous phase were quantified using a spectrophotometer at 380 nm for vitamin B_{12} (El 312e, BioTek instruments) and a protein assay for leuprolide (Micro BCA assay, Pierce). The drug entrapment obtained with this method was always confirmed by the total amount released.

2.6. In vitro release

To evaluate the release, 50 mg of each type of loaded microsphere was incubated in 1 ml PBS (pH 7.4) at 37 °C. All samples were kept under constant agitation (25 rpm). The release medium was taken and refreshed at regular time intervals (at least seven times during the first 2 weeks and at least once every 2 weeks for the later time points) and concentrations were determined as described above.

2.7. Swelling in PBS

The swelling properties of the different copolymers were evaluated from experiments with films. Films of the same copolymer compositions, and the same loading as for the various microspheres were used. To prepare films, water-in-oil emulsions (obtained as described for microspheres above) were cast on a glass plate using a casting knife (set at 700 μm of thickness). After slow evaporation of the solvent, the films were removed from the glass plate and freeze-dried.

The swelling was obtained by immersing dry films of a known weight in PBS at 37 °C in a shaking bath. After at least 3 days, the weight of the samples was determined (after removal of the surface water with a tissue). The water

uptake (in ml of water per g of polymer) was calculated from the weight increase.

3. Results and discussion

3.1. Effect of surfactants in the first emulsion on vitamin B_{12}-loaded microspheres

In this study, loaded microspheres were prepared by a double emulsion method [15]. First, an emulsion was made from an aqueous drug solution and a polymer solution. This W/O emulsion was subsequently dispersed in an aqueous PVA solution, in which hardening of the microspheres was accomplished by evaporation of the organic solvent. In order to entrap the drug efficiently within the microspheres and to obtain a controlled long-term release, different parameters of the W/O emulsion were varied.

Various authors have reported the beneficial effect of the presence of PVA and BSA in the first water phase on the W/O emulsion stability and the drug entrapment efficiency [25,26]. In these studies, the microspheres were made from PLGA, PCL, and PLA, which are hydrophobic polymers. Here, we applied amphiphilic PEGT/PBT multiblock copolymers, which may interact in a different way with surfactants in the first water phase. The composition of the copolymer (PEG molecular weight of 300 g/mol) was selected based on previous release experiments that showed a long-term release of vitamin B_{12} from films [22].

The type of surfactant in the W/O emulsion and the concentration used did not influence the size of the microspheres. In all cases, the average particle size was between 50 and 70 μm (volume mean), with a unimodal narrow size distribution (data not shown).

Externally, the particles were spherical and had a wrinkled and non-porous surface, as shown in Fig. 1. No influence of the first water phase composition on the external morphologies of the microspheres was observed.

The internal structures of the different microspheres are presented in Fig. 2. Irrespective of the type and concentration of the surfactant, the microspheres were porous (pore sizes between 1 and 3 μm). When BSA was used in the first water phase, the resulting microspheres were additionally often hollow (microcapsules-like). This might be explained by the better stabilization of water/air interfaces due to the presence of BSA. During the course of the first emulsion, small air bubbles might be stabilized by BSA and therefore induce hollowness of the microspheres during the second emulsion. The porosity of the microspheres was surprising, since in previous studies on PEGT/PBT microspheres of different copolymer compositions, the formation of non-porous particles was predominantly observed [14]. This lack of porosity was attributed to the amphiphilic nature of the polymer, which acts as an intrinsic emulsifier. Here, it has to be noted that the short length of the PEG segments (300 g/mol) and the high PBT weight percentage (45 wt%) of the

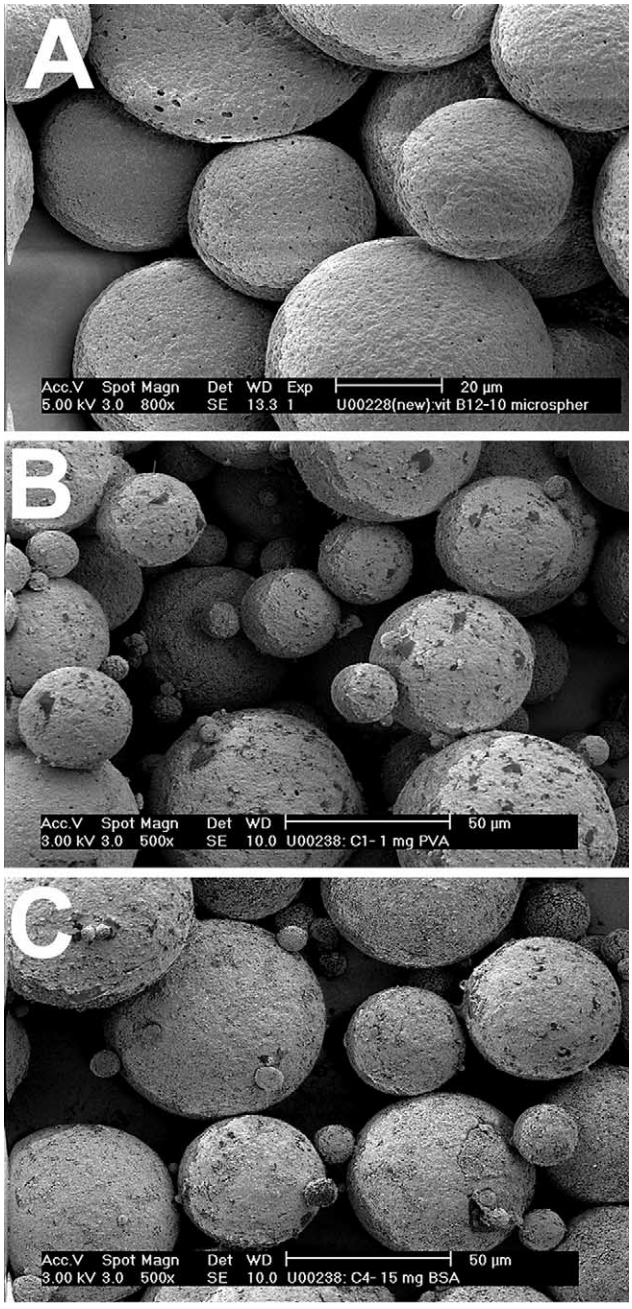

Fig. 1. Effect of surfactant in the first water phase on the external morphology of vitamin B_{12} microspheres (A, no surfactant; B, PVA, 1 mg/ml; C, BSA, 15 mg/ml).

copolymer render it relatively hydrophobic. Indeed, for the copolymer composition used, a low water uptake of vitamin B_{12}-loaded films was observed (approximately 0.28 ml of water per g of polymer). This very low swelling could explain the porosity observed. As the polymer does not swell during the course of the microsphere preparation, the pores may represent the water droplets in the initial W/O emulsion.

Entrapment efficiencies within the microspheres were strongly influenced by the concentration and type of surfactant in the first emulsion (Fig. 3). Entrapment

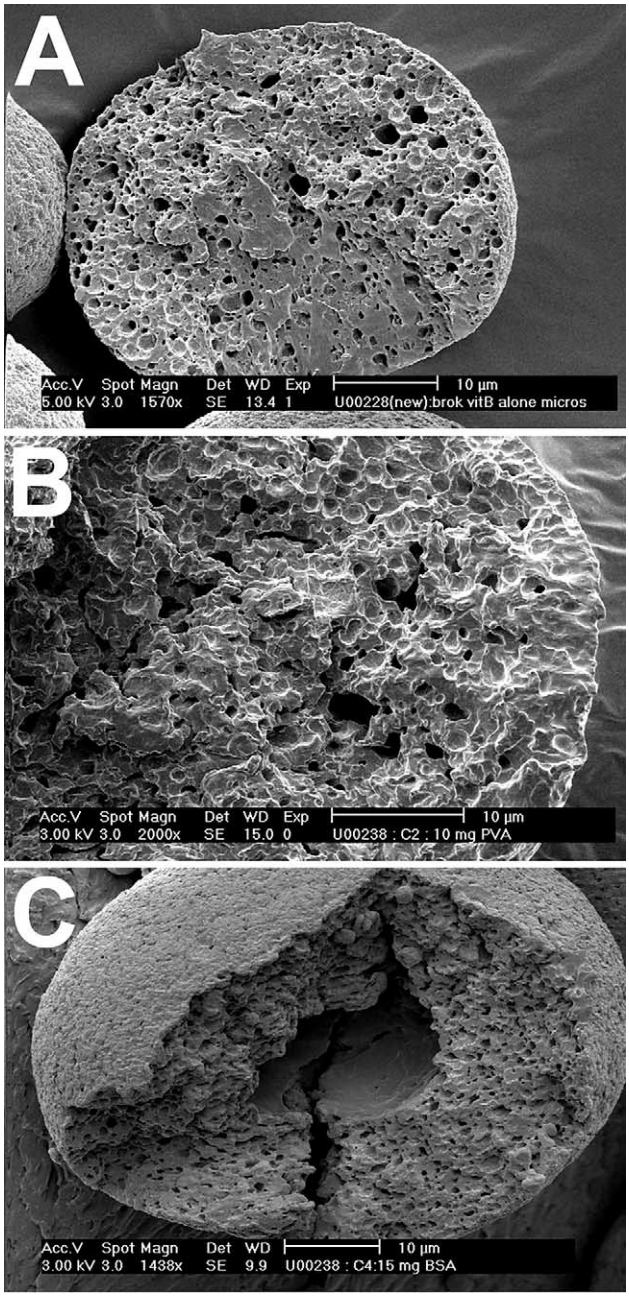

Fig. 2. Effect of surfactant in the first water phase on the internal morphology of vitamin B_{12} microspheres (A, no surfactant; B, PVA, 10 mg/ml; C, BSA, 10 mg/ml).

efficiency was relatively high in the absence of surfactants in the W/O emulsion (56%). The presence of PVA increased it up to approximately 80%, regardless of the concentration used. Concerning BSA, a beneficial effect was only noticed for the highest concentration (15 mg/ml), which enhanced the entrapment efficiency to 77%.

The relation between the presence of a surfactant in the first emulsion and the internal porosity and entrapment efficiency of the microspheres has been the subject of many studies. Nihant et al. [25,27] reported that BSA could interact with polymers (PLA and PLGA) at the water/oil

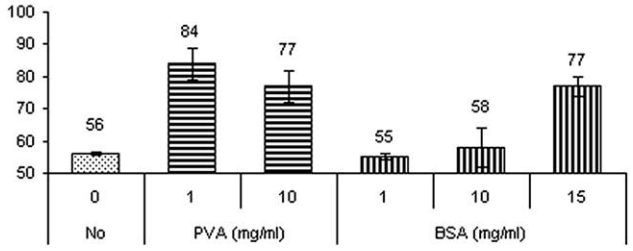

Fig. 3. Effect of surfactants on vitamin B_{12} entrapment efficiency ($n = 3$, $\pm$SD).

interface to form a film. This film increased the first emulsion stability, preventing the original fine water droplets of the W/O emulsion from coalescence and disruption in the external water phase during solvent evaporation. As a result, microspheres presented smaller internal porosities and higher entrapment efficiencies. When a water-in-oil surfactant was added to the oil phase (Pluronic F68), it displaced the polymer chains at the interface and prevented the formation of this stabilizing interfacial film. The resulting microspheres presented bigger internal pores and lower entrapment efficiency of a hydrophilic model drug (indigocarmine). In the present study, the relations between internal structures and entrapment efficiencies of the microspheres appeared to be different. Whereas the improvement of entrapment efficiencies indicates a more stable emulsion in the presence of surfactants, no effect of the surfactants on the internal pore size of the microspheres was observed. The internal pores of the different batches were all in the same size range (1–3 μm). To further investigate potential interactions between the vitamin B_{12}, the copolymer and the surfactants, vitamin B_{12}-free microspheres were prepared with W/O emulsions containing BSA or PVA. The microspheres showed similar internal porosity to vitamin B_{12}-loaded ones (Fig. 4). This indicates that the internal structure of the hardened microspheres is mainly determined by the copolymer itself. As mentioned earlier, PEGT/PBT copolymers are amphiphilic and possess intrinsic emulsifying properties [14]. Indeed, the small pore size range observed in the different microspheres suggests a very stable first emulsion, which explains the relatively high entrapment efficiency in the absence of surfactant. These tensio-active properties might also explain the lack of a decrease of internal porosity with increasing entrapment efficiency of the microspheres. If the W/O emulsion is already stabilized efficiently by the copolymer, surfactants will not induce a decrease of the pore size of the microspheres, but they may still prevent more efficiently the disruption of the water droplets in the external aqueous phase, leading to higher entrapment efficiencies.

In addition to the previous theory, another phenomenon could play a role in the beneficial effect of surfactants on the entrapment efficiency of vitamin B_{12}-loaded microspheres. Maa and Hsu [28] considered the effect of inner aqueous droplet size and viscosity on entrapment efficiency and showed an increase of entrapment efficiency of BSA when

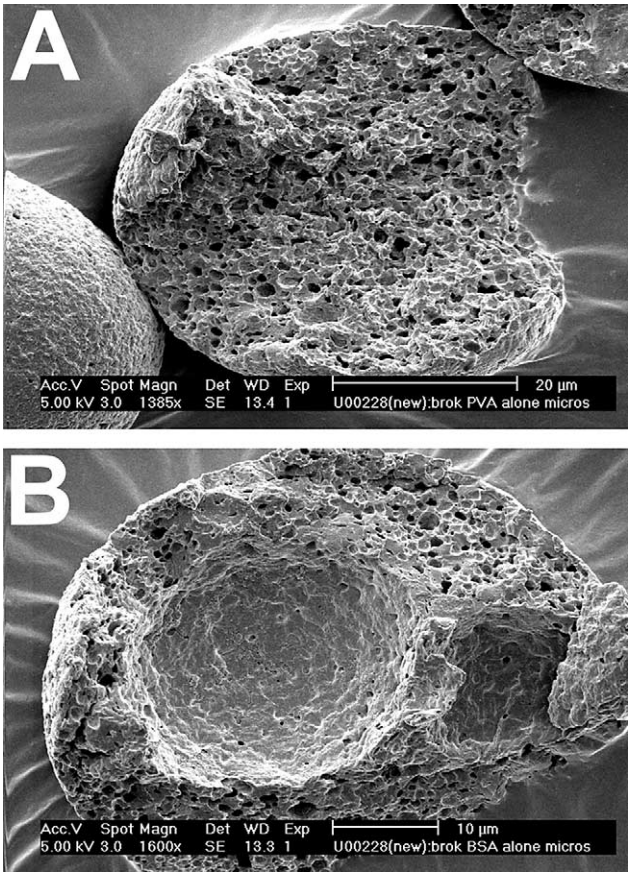

Fig. 4. Internal structures of microspheres prepared using BSA and PVA in the first water phase, without vitamin B_{12} (A, PVA, 10 mg/ml; B, BSA, 10 mg/ml).

the inner aqueous droplets were more viscous. During the formation of the microspheres, a dynamic process of disruption of the W/O emulsion droplets takes place due to the turbulent flow of the second emulsion. These disruptions occur along 'weakening' routes created by the aqueous droplets of the first emulsion, since their viscosity is lower than that of the polymeric phase. The exposure of the aqueous drug-containing domains, inside the W/O droplet, to the external aqueous phase causes protein leakage [25]. More viscous water droplets and thus W/O emulsion droplets will be disrupted less easily, resulting in less drug loss. The use of PVA in the first water phase has been reported to increase the viscosity of the water-in-oil emulsion [26], which may explain the increase of entrapment efficiency of the vitamin B_{12}.

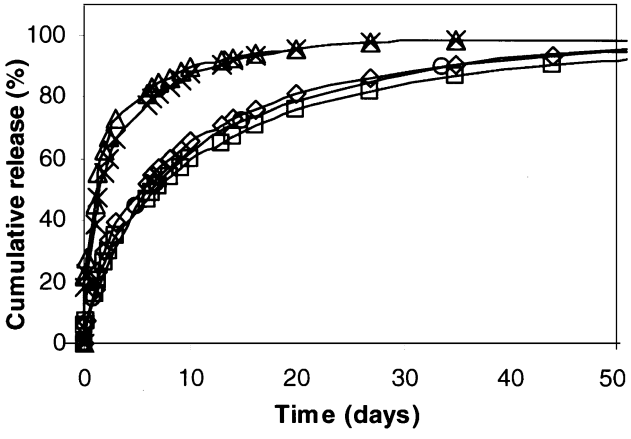

Fig. 5. Vitamin B_{12} release from microspheres made using different surfactant concentrations (◇, PVA, 1 mg/ml; □, PVA, 10 mg/ml; △, BSA, 10 mg/ml; ×, BSA, 15 mg/ml; ○, no surfactant in the first water phase) ($n = 3$, ± s.d.).

The vitamin B_{12} release from the microspheres was also dependent on the presence of surfactants, as shown in Fig. 5. The microspheres prepared from W/O emulsions containing BSA were characterized by a fast release of 70% of the entrapped vitamin B_{12} within the first 5 days, followed by a slow release for 2 weeks. The PVA-containing microspheres and those prepared without surfactants showed a slow release for 60 days. The surfactant concentrations did not have any influence on the release rates. Since the size of all microspheres was comparable, the explanation for these differences has to be found in their internal structure. BSA-containing microspheres were more hollow, which can account for the faster release due to shorter length of diffusion paths.

3.2. Effect of copolymer compositions on vitamin B_{12}-loaded microspheres

To study the effect of copolymer composition on the characteristics of vitamin B_{12}-loaded microspheres, the PEGT content of the copolymers was varied while the PEG molecular weight was kept constant (300 g/mol). The variation of the PEGT content at fixed PEG molecular weight is a known factor in adjusting the release rate of proteins and small water-soluble drug from this type of copolymeric matrix [14,16,22]. The microspheres were prepared using 1 mg/ml of PVA in the water-in-oil emulsion, to achieve the best entrapment efficiency.

No influence of copolymer compositions on microsphere size and morphology was observed. The average particle size was around 60 μm. All internal structures were porous, although the pore size decreased with increasing PEGT content (data not shown). This is probably related to the increase of elastomeric properties and the hydrophilic nature of the polymers when the PEGT content of the copolymer increases.

Vitamin B_{12} entrapment efficiency and release rates were dependent on the PEGT content of the copolymers. With increasing PEG content, a decreasing encapsulation efficiency was observed, from 84% recovery for microspheres prepared from polymers with a PEGT wt% of 55, to only 34% for the copolymer containing 80 wt% PEGT (Fig. 6). The dependency of the entrapment efficiency on the copolymer composition can be ascribed to premature release of vitamin B_{12} during the 2 h of solvent evaporation in the aqueous PVA solution. With increasing release rate,

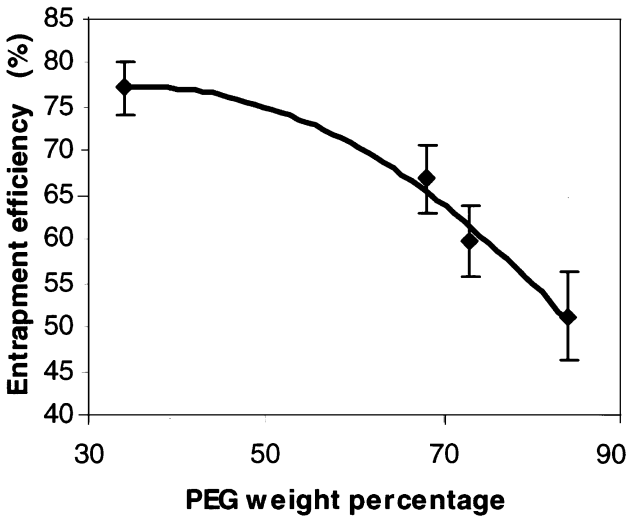

Fig. 6. Vitamin B_{12} entrapment efficiency as a function of PEGT content ($n = 3$, ± SD).

the entrapment efficiency will decrease. Indeed, increasing release rates were observed with increasing PEGT content. Whereas release from 55 PEGT-wt% microspheres was completed after 2 months, release from 80 PEG-wt% microspheres was already complete after 5 days (Fig. 7). These release profiles are in agreement with the release of vitamin B_{12} obtained from films of the same composition [22].

As stated before, the PEGT/PBT copolymers possess hydrogel properties and the swelling levels increase with increasing PEGT ratio or PEG segment length [12]. This can usually explain release rate differences between PEGT/PBT compositions: the permeation of solutes increases with an increasing swelling because of increasing polymer network mesh sizes. However, no differences in swelling were detected between the four copolymer compositions used in this study. For all compositions, the water uptake of vitamin B_{12}-loaded films was close to 0.28 ml of water per g of polymer. Therefore, vitamin B_{12} release behavior has to be determined by the phase separated morphology of PEGT/PBT copolymers. The copolymers can be described as biphasic, composed of hard, partly crystalline, PBT domains, and soft, amorphous PEG domains. As reported before, with increasing PBT content at constant PEG segment, the crystallinity of PEGT/PBT multiblock copolymers increased [12,22]. As the crystallites are considered as impermeable, there is an increase of the length of diffusion paths with increasing crystallinity [29]. As a result, vitamin B_{12} release is slower when the proportion of soft domains is lower, thus at lower PEGT content.

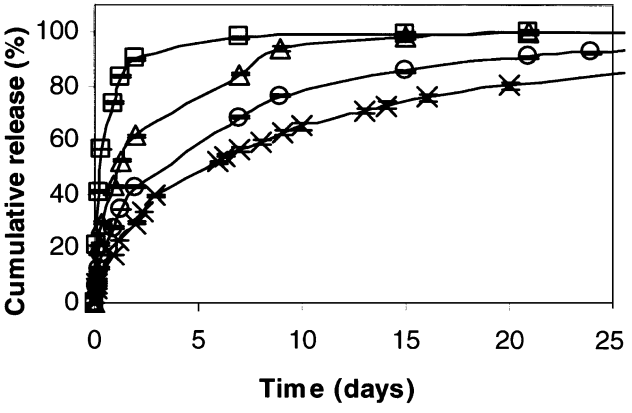

Fig. 7. Vitamin B_{12} release from microspheres of different PEGT wt% compositions (□, 80%; △, 70%; ○, 60%; ×, 55%) ($n = 3$, ± SD).

Besides the polymer morphology, the characteristics of the drug will determine the ultimate release profile. In the case of vitamin B_{12}, a relatively low solubility in water has been reported (12 mg/ml) [22]. In combination with the low water uptake of the copolymers selected for this study, only a fraction of the vitamin B_{12} will be in the dissolved state in the PEGT/PBT microspheres during the release. Thus, the release rate is determined by a combination of dissolution of the vitamin B_{12} and diffusion out of the matrix.

3.3. Leuprolide release from PEGT/PBT microspheres

Based on the vitamin B_{12} experiments, the following parameters were selected to prepare leuprolide-loaded microspheres: (1) 1 mg/ml of PVA in the water-in-oil emulsion, in order to select the best entrapment efficiency; (2) a copolymer with a PEGT content of 55% and a PEG molecular weight of 300 g/mol, to prevent a fast release of the peptide. For comparison, microspheres without surfactant in the first water phase and of the same copolymer composition were also prepared.

As expected, the external characteristics of the microspheres corresponded to those described for vitamin B_{12}-loaded microspheres. The average diameter (volume mean) was approximately 60 μm. The internal structure of the microspheres was porous, regardless of the presence of PVA in the first water phase.

Surprisingly, entrapment efficiencies and release behavior differed from vitamin B_{12}-loaded microspheres. A maximum of 17% of the peptide was entrapped when using 1 mg/ml of PVA in the first water phase and only 13% could be recovered without surfactant. The release was in both cases completed after 20 days, with a very high burst within the first 2 days (Fig. 8). This fast release and low encapsulation of leuprolide have to be explained by the peptide characteristics, since all processing parameters were similar to those of the vitamin B_{12}-loaded microspheres. Although molecular weights of model and peptide are comparable, other properties like hydrodynamic radius and water solubility may also be of importance. The fact that leuprolide is more soluble in water than vitamin B_{12} (at least 900 mg/ml for leuprolide [24], and 12 mg/ml for vitamin B_{12}) could explain the differences observed. Leuprolide may have a higher diffusion coefficient resulting in a fast release, already during the hardening of the microspheres, and consequently in a low peptide encapsulation efficiency.

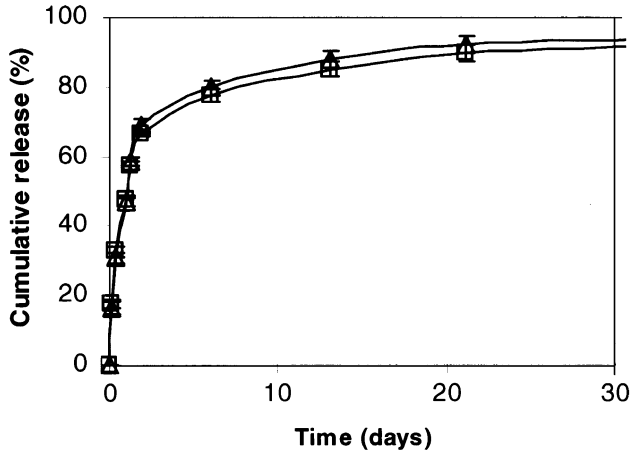

Fig. 8. Leuprolide release from PEGT/PBT microspheres (□: no surfactant, △: PVA-1 mg/ml) (n = 3, ± s.d.).

Furthermore, vitamin B_{12} may be released more slowly due to an incomplete dissolution as mentioned above. On the contrary, the leuprolide solubility is high enough to suppose that all of it is dissolved within the swollen copolymer matrix, inducing a faster release.

In comparison with PLA and PLGA microspheres loaded with leuprolide, the release rates and entrapment efficiencies of the PEGT/PBT microspheres were significantly faster and lower [23,24]. This can be ascribed to the different release mechanism of the copolymers: whereas release from the PEGT/PBT matrix of this composition is mainly achieved by diffusion, the one from PLGA and PLA is mostly done by degradation of the matrix. In addition, high entrapment efficiency of leuprolide in the PLGA and PLA formulations was obtained by increasing the viscosity of the W/O emulsion (lowering of the W/O temperature and addition of gelatin, increase of drug and polymer concentrations). Such modifications of the microsphere preparation parameters could be useful for PEGT/PBT copolymers to enhance leuprolide entrapment efficiency. But they would probably not induce a slower release of the small drug, which can apparently diffuse too easily through the polymer matrix.

4. Conclusions

This study shows different behavior of the PEGT/PBT copolymer towards the controlled delivery of small water-soluble drugs of similar size. Using vitamin B_{12} as a model, the external morphology of the microspheres was regular and their size was suitable for injection. Adjustments of the processing parameters, such as addition of PVA to the water-in-oil emulsion, resulted in a high vitamin B_{12} entrapment efficiency within the microspheres. Furthermore, variation of the copolymer composition allowed adjustment of release rate and entrapment efficiencies to meet specific requirements. However, when leuprolide was used instead of vitamin B_{12}, low entrapment efficiency and fast release were observed. The release differences may be explained by the high water solubility of leuprolide compared to vitamin B_{12}. In addition, the release characteristics of the leuprolide-loaded microspheres were not comparable to the ones reported for PLGA and PLA formulations. This was probably due to the dissimilar release mechanism of these different copolymers.

These results illustrate the necessity of tailored development for each individual drug and emphasize the risk in using model molecules in the context of drug delivery.

Nevertheless, the study demonstrates the suitability of PEGT/PBT copolymer microspheres as a controlled release system for vitamin B_{12}. More experiments should be done with other compounds to confirm the potential interest of PEGT/PBT copolymers as release systems for small water-soluble drugs.

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