Amphiphilic poly(ether ester amide) multiblock copolymers as biodegradable matrices for the controlled release of proteins

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Abstract: Amphiphilic poly(ether ester amide) (PEEA) multiblock copolymers were synthesized by polycondensation in the melt from hydrophilic poly(ethylene glycol) (PEG), 1,4-dihydroxybutane and short bisester-bisamide blocks. These amide blocks were prepared by reaction of 1,4-diaminobutane with dimethyl adipate in the melt. A range of multiblock copolymers were prepared, with PEG contents varying from 23–66 wt %. The intrinsic viscosity of the PEEA polymers varied from 0.58–0.78. Differential scanning calorimetry showed melting transitions for the PEG blocks and for the amide-ester blocks, suggesting a phase separated structure. Both the melting temperature and the crystallinity of the hard amide-ester segments decreased with increasing PEG content of the polymers. The equilibrium swelling ratio in phosphate buffered saline (PBS) increased with increasing amount of PEG in the polymers and varied from 1.7 to 3.7, whereas the polymer that contained 66 wt % PEG was soluble in PBS. During incubation of PEEA films in PBS, weight loss and a continuous decrease in the resulting inherent polymer viscosity was observed. The rate of degradation increased with increasing PEG content. The composition of the remaining matrices did not change during degradation. A preliminary investigation of the protein release characteristics of these PEEA copolymers showed that release of the model protein lysozyme was proportional to the square root of time. The release rate was found to increase with increasing degree of swelling of the polymers. J Biomed Mater Res, 52, 8–17, 2000.

Key words: block-copolymer; protein release; degradation; poly(ether ester amide); swelling

INTRODUCTION

Application of biodegradable amphiphilic block copolymers as hydrogels for drug delivery systems offers attractive possibilities in the design of systems with tailor-made properties. Such delivery systems are needed to deal with the diverse characteristics of new drugs, such as the growing number of therapeutically active proteins and peptides.[1] Several protein delivery systems based on biodegradable amphiphilic block copolymers have been prepared from diblock,[2] triblock,[3–8] or multiblock copolymers.[9,10] As the hydrophilic segment, usually poly(ethylene glycol) (PEG) is selected, because of its nontoxicity, lack of immunogenicity, and its solubility in both organic solvents and water.[11] Various hydrophobic blocks have been applied to obtain physically crosslinked biodegradable hydrogels, e.g., polylactide,[12] polyglycolide,[13] poly(lactide-*co*- glycolide),[14] poly(ε-caprolactone),[15] poly(ethylene terephthalate) (PET),[16] and poly(butylene terephthalate) (PBT).[17]

The role of the hydrophobic block is to create physical crosslinks in the "soft" hydrophilic matrix, thereby providing the material its mechanical properties. In contrast to chemically crosslinked materials, the physical crosslinks are reversible and can be disrupted at elevated temperatures or in solvents, which gives the material a good processability. In particular, multiblock copolymers can be considered as suitable amphiphilic hydrogels for drug delivery applications. Tailoring of the properties of such polymers can be achieved by variation of the length and the weight fraction of the hydrophilic blocks, which results in polymer systems with a wide range of properties.

The mechanical properties, permeability, and degradation characteristics of amphiphilic multiblock copolymers are largely dependent on the chemical structure of the "hard" blocks. Aliphatic polyesters, based on lactic acid and glycolic acid are known to be com-

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pletely biodegradable.[18] Aliphatic polyamides (nylons) often show good mechanical and thermal properties, but their rate of degradation is generally so slow, that these polymers are considered as nonbiodegradable.[19] Therefore, the combination of the properties of both types of polymers in poly(ester amide)s has been proposed as a means of developing materials with improved mechanical properties and degradation characteristics.[20–26] Such polymers may be good candidates to be used as the biodegradable hard blocks in physically crosslinked hydrogels.

In this report, the synthesis and properties of poly(ether ester amide) (PEEA) multiblock copolymers, based on hydrophilic PEG oligomers, 1,4-dihydroxybutane, and bisester-bisamide blocks are described. The bisester-bisamide monomers were prepared from 1,4-diaminobutane and dimethyl adipate, as has been described previously by Stapert et al.[26,27] Small and symmetrical amide blocks of uniform length were selected, because it has been reported that poly(ester amide)s based on such blocks show rapid crystallization and have a high crystallinity, resulting in materials with good mechanical properties.[28,29] Furthermore, the short amide blocks contain only two amide bonds per unit and are water soluble, which facilitates the biodegradability.[26] By variation of the ratio between PEG diol and 1,4-dihydroxybutane, a series of polymers was obtained with a range of thermal, swelling, and degradation characteristics. A preliminary investigation of protein release from these hydrogels was performed, using lysozyme as a model protein.

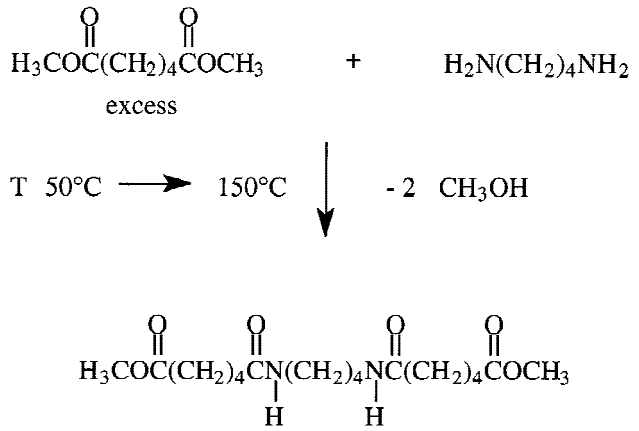
MATERIALS AND METHODS

Materials

Dimethyl adipate, 1,4-diaminobutane, 1,4-dihydroxybutane, tetrabutylorthotitanoate [$Ti(OBu)_4$], PEG, MW = 1000 g/mol, α-tocopherol, $CDCl_3$, and DMSO-d_6 were obtained from Merck (Darmstadt, Germany). Phosphate buffered saline (PBS), pH 7.4 was purchased from NPBI (Emmercompascuum, The Netherlands). Lysozyme from chicken egg white (3× crystallized, dialyzed, and lyophilized) was obtained from Sigma Chem Corp. (St. Louis, MO). All solvents used were of analytical grade.

Synthesis of diamide-dimethyl ester monomer and PEEAs

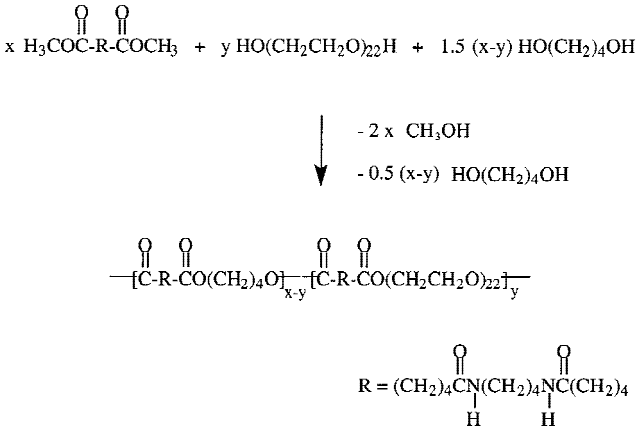
The synthesis of the diamide-dimethyl ester monomer (Scheme 1) was performed as described by Stapert et al.,[26] with some slight modifications. Dimethyl adipate (116.9 g, 0.67 mol) was heated to 50°C under a constant nitrogen flow.



Scheme 1. Synthesis of the bisester-bisamide monomer from 1,4-diaminobutane and dimethyl adipate.

Subsequently, 0.25 g $Ti(OBu)_4$ was added, followed by slowly adding warm 1,4-diaminobutane (5.91 g, 0.067 mol) during a period of 1 h. After methanol was distilled off for 4 h, every 1/2 h the temperature was increased with 25°C up to a final temperature of 150°C. The melt was cooled to room temperature and filtered to remove the excess dimethyl adipate. The product was then dissolved in chloroform at 50°C and filtered again. The chloroform was evaporated under reduced pressure and the product was washed three times with cold tetrahydrofuran (THF) to remove remaining dimethyl adipate. Finally, the obtained product was dried at 40°C *in vacuo.*

PEEA multiblock copolymers were synthesized by melt polycondensation (Scheme 2). Polymerization was performed in a glass reactor with nitrogen inlet and a mechanical stirrer. As a typical example, the synthesis of 5 g of a PEEA containing 20 wt % PEG is described. In a vessel, diamide-dimethyl ester monomer (3.80 g, 1.02×10^{-2} mol), PEG1000 (1.02 g, 1.02×10^{-3} mol), 1,4-dihydroxybutane (1.24 g, 1.38×10^{-2} mol), and antioxidant (α-tocopherol) (0.06 g, 1.0 wt %), were heated to 175°C under a nitrogen flow. A solution of the catalyst $Ti(OBu)_4$ in toluene (0.12 mL, 0.05 g/mL) was added to the melt and methanol was distilled off during 4 h. Subsequently, the temperature was increased to 220°C and the pressure was slowly reduced to 0.3 mbar.



Scheme 2. Synthesis of PEEAs from PEG1000, 1,4-dihydroxybutane, and bisester-bisamide blocks.

During 8 h, the condensation product 1,4-dihydroxybutane was distilled off. After the reaction was stopped, the melt was slowly cooled to room temperature under nitrogen flow. Several copolymers were synthesized, with PEG contents varying from 20–65 wt %. The molecular weight of the PEG segment was 1000 g/mol.

Polymer characterization

Proton NMR spectra were recorded on a Bruker AC 250 operating at 250.1 MHz. $CDCl_3$ or DMSO-d_6 were used as solvents without internal standard.

Thermal analysis of polymers was performed with a Perkin-Elmer DSC7 differential scanning calorimeter equipped with a PE-7700 computer and TAS-7 software. Calibration was performed with pure indium. Samples (5–10 mg) were heated from −60° to 200°C at a heating rate of 20°C/min, annealed for 5 min and cooled to −60°C (20 °C/min). Subsequently, a second heating curve was recorded. Melting (T_m) and crystallization (T_c) temperatures were taken from peak maxima of the second heating and the cooling curves, respectively, and the area under the curves as ΔH.

The inherent viscosity η_{inh} of the polymers was determined using a Cannon 55 L117 viscometer at 20°C and polymer solutions of 5 mg/mL in a mixture of chloroform and methanol (1:1, v/v). The intrinsic viscosity [η] was determined using concentrations of 20, 10, 6.7, and 5 mg/mL of polymer in a mixture of chloroform and methanol (1:1, v/v).

PEEA films were prepared from solutions of 1 g copolymer in 7 mL chloroform/methanol (1:1 v/v). The polymer solutions were cast onto a glass plate using a 0.75-mm casting knife. The solvent was slowly evaporated at room temperature and then the films were dried *in vacuo* for 3 days. To measure the degree of swelling of the polymers, 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 kinetics of swelling was evaluated by periodically measuring the weight of films after blotting the surface with a tissue, until equilibrium was reached. The equilibrium swelling ratio q was determined from the ratio of the equilibrium weight of the swollen samples and the dry samples.

Polymer degradation in PBS at 37°C

To determine the degradation of the copolymers, 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 weighed after drying *in vacuo* for 3 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 inherent viscosity of the polymers during degradation was monitored, as well as the effect of degradation on the composition of the polymers (^{1}H NMR).

Release of lysozyme from PEEA films

A protein solution (0.6 mL, 55 mg/mL) in PBS was emulsified in a polymer solution (1 g polymer in 9 mL chloroform/methanol, 8:1 v/v) 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 solvents, films were removed from the glass plate and stored over $CaCl_2$ in a desiccator at 4°C. Lysozyme (14.5 kD) was used as a model protein.

The lysozyme containing films (15-mm diameter) were incubated in 1.5 mL PBS (pH 7.4). Vials were shaken at 37°C and samples were taken at various time points. The protein concentration in the buffer 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.

RESULTS AND DISCUSSION

Synthesis of bisester-bisamide monomer and PEEAs

A series of PEEA multiblock copolymers was synthesized from PEG 1000, 1,4-dihydroxybutane, and aliphatic diamide-dimethyl ester monomers. The bisester-bisamide monomers were prepared in the melt from 1,4-diaminobutane and dimethyl adipate according to a procedure as has been described by Stapert et al.[26] (Scheme 1). After purification, the obtained product yield was 71%. ^{1}H-NMR analysis confirmed the chemical structure and the purity of the diamide-dimethyl ester monomers (Fig. 1). The melting temperature of the product was 141°C.

Polymerization was performed using a two-step process. The first step involves transesterification of

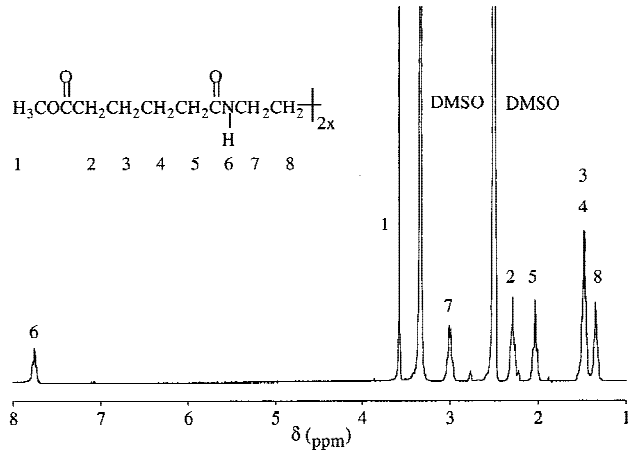


Figure 1. ^{1}H-NMR spectrum of the bisester-bisamide monomer in DMSO-d_6.

the diamide-dimethyl ester monomers with PEG and an excess of 1,4-dihydroxybutane at 175°C for 4 h, using $Ti(OBu)_4$ as a catalyst and α-tocopherol as an antioxidant to prevent thermal degradation of PEG. Then, polycondensation was performed at 220°C, using vacuum to facilitate removal of the condensation product 1,4-dihydroxybutane. The overall reaction is given in Scheme 2. PEEA multiblock copolymers with a mol fraction of hydrophilic blocks varying from 0.1 (20 wt % PEG) to 0.7 (65 wt % PEG) were prepared. The results of the polymerizations are given in Table I. The composition of the copolymers was calculated from ^{1}H-NMR spectra; a typical spectrum is shown in Figure 2. The ratio between integral intensities originating from protons 6 (amide segment), 8 (butanediol), 10, 11, and 12 (PEG), was used to calculate the PEG content of the PEEA copolymers. As shown in Table I, the obtained composition was in good agreement with the polymer composition expected from the feed composition. It was not possible to determine the number-average molecular weight of the polymers from ^{1}H-NMR spectra, because integral intensities of the endgroups were too small. This indicates that the molecular weight of the PEEA copolymers is in the range of 20 kg/mol or higher. The intrinsic viscosity of the polymers varied from 0.58–0.78.

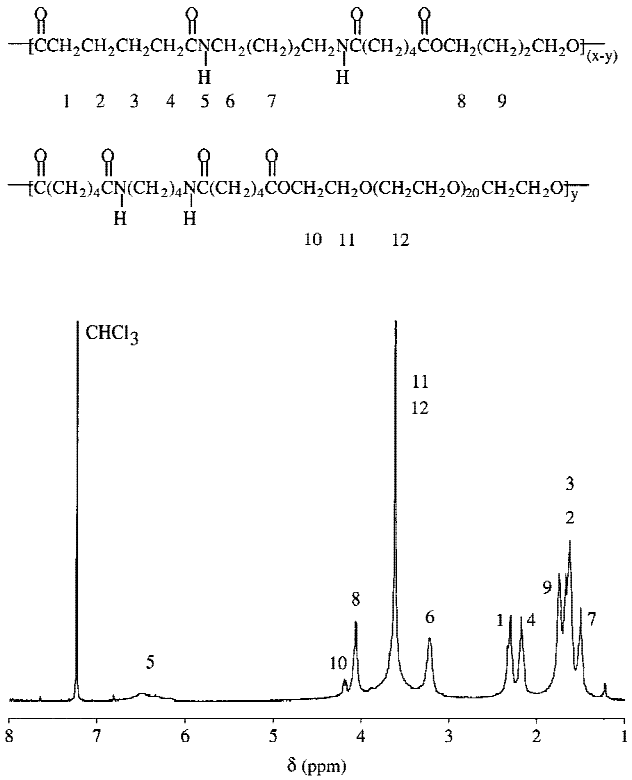


Figure 2. ^{1}H-NMR spectrum of poly(ether ester amide) PEEA1 in $CDCl_3$.

Thermal properties of PEEAs

The thermal properties of the synthesized PEEA copolymers were determined by differential scanning calorimetry (DSC). In Figure 3, the second heating and cooling traces for PEEA1 and PEEA5 are shown to illustrate the data listed in Table II. For comparison, the characteristics of the homopoly(ester amide) PEEA0 are included (obtained from reference[27]). Over the temperature range of –50° to 200°C two transitions were observed, which confirmed the phase separated structure of the copolymers. The high temperature transitions can be attributed to the melting and crystallization of the amide block, and the transition at low temperature to melting or crystallization of PEG. Crystallization of the PEG segments was not observed for PEEA copolymers with less than 40 wt % PEG. Probably, the PEG content of these copolymers was too low to be able to form semicrystalline domains.[30] In the other cases, PEG crystallization increased with increasing PEG content.

TABLE I
Results of the Polymerization of Poly(ether ester amide)s

Polymer	PEG Content Based on Feed (wt %)	PEG Content in Polymer (wt %)[a]	Intrinsic Viscosity[b] (dL/g)
PEEA1	20	23	0.63
PEEA2	31	32	0.68
PEEA3	41	40	0.58
PEEA5	55	54	0.78
PEEA7	65	66	0.68

[a]PEG content in the copolymer after purification as determined by ^{1}NMR.
[b]Determined using a mixture of chloroform and methanol (1:1 v/v) at 20°C.

Over the whole composition range studied, none of the polymers was completely amorphous (Table II). The melting enthalpy corrected for the ester-amide block weight content, which can be taken as a measure for the crystallinity of the ester-amide segments, is almost constant up to a PEG content of 40 wt %, although ΔH_m of PEEA1 was lower than expected. A further increase in the PEG content results in a rapid decrease of the crystallinity of the polymers. Possibly, the sharp decrease in ΔH_m of the ester-amide segments of polymers with relatively high PEG contents is related to the length of the hard blocks. With increasing PEG wt %, the average sequence length of the blocks will decrease. The number average degree of polymerization (P_n) of the hard blocks can be calculated as $1/(1 - X_a)$, in which X_a is the mol fraction hard blocks. For PEEA1, the average length of the ester-amide sequences is 8.6, whereas for PEEA7 P_n is 1.5. Crystallization might require a certain minimum length of the blocks. The concentration of sufficiently long blocks

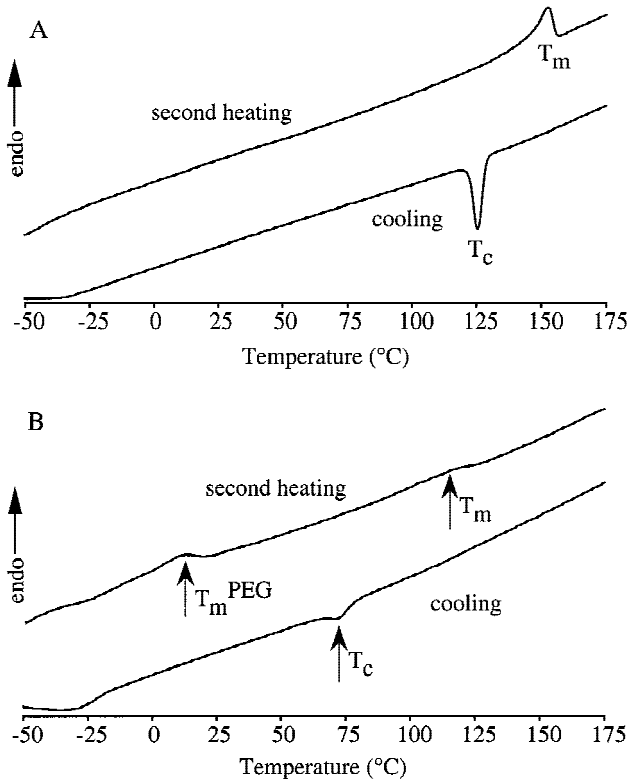


Figure 3. DSC (second) heating and cooling scans (20°C/min) of poly(ether ester amide)s PEEA1 (A) and PEEA5 (B).

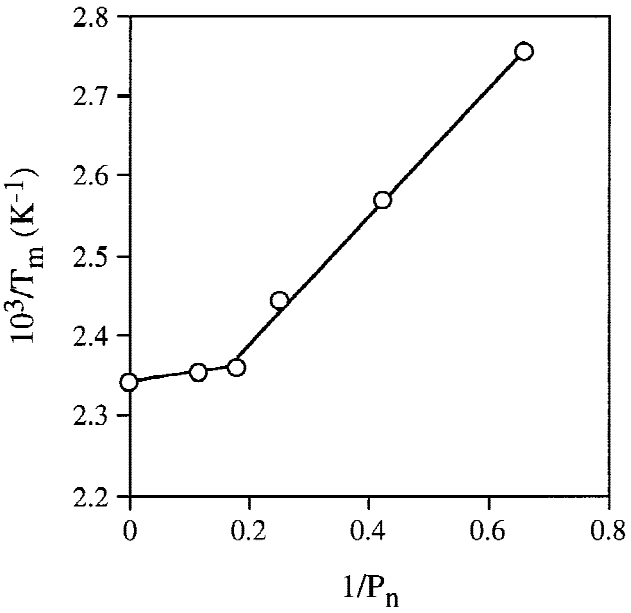


Figure 4. Reciprocal melting temperature of PEEA multiblock copolymers as a function of the reciprocal number average degree of polymerization of the amide-ester hard segments.

will be low in copolymers with a high PEG content, resulting in a decrease in crystallinity.

A similar trend as for the melting enthalpy was observed for the melting temperature. Only a slight decrease in the melting temperature of the amide blocks with increasing soft segment content was found for block copolymers containing less than 40 wt % PEG (Table II). However, a further increase of the PEG content up to 66 wt % resulted in a large decrease of T_m from about 150° to 90°C. In Figure 4, the melting temperature is related to the average length of the hard segments. In agreement with the melting point depression theory of Flory,[31,32] a linear relation was found between the reciprocal melting temperature and the reciprocal number average degree of polymerization of the hard blocks. However, at a block length of approximately 6 a sharp transition in the slope of the curve was observed. Such behavior has also been reported for block poly(ether ester)s, based on PBT and poly(tetramethylene oxide).[33]

Information about the rate of crystallization can be obtained from the undercooling, defined as the difference between T_m and T_c at a certain heating and cooling rate. Table II shows that incorporation of 23 wt % PEG in the poly(ester amide) homopolymer caused a decrease in the undercooling from 37°C (for PEEA0) to 26°C (for PEEA1). Probably, a faster growth of the crystals is achieved in the presence of a small amount of the flexible PEG segments. A further increase of the PEG content resulted in an increase of the undercooling up to 43°C (for PEEA5), which is indicative for an increased hindrance of the formation of crystals. The values of the undercooling are comparable to those of other fast crystallizing polymers, such as PBT.[34]

TABLE II
Thermal Properties of the Poly(ether ester amide)s

Polymer	T_m (°C)	$T_m - T_c$ (°C)	ΔH_m (J/g)	ΔH_m (J/g h.b.)[a]	T_m^{PEG} (°C)	ΔH_m^{PEG} (J/g)
PEEA1	152	26	19	28	—	—
PEEA2	151	30	19	33	—	—
PEEA3	136	41	16	33	5	10
PEEA5	116	43	6	20	12	15
PEEA7	90	—	2	15	23	25
PEEA0[b]	154	37	34	34		

[a]Melting enthalpy per gram hard block.
[b]The characteristics of the homopoly(ester amide) PEEA0 are obtained from reference[27].

Swelling of PEEA films in PBS buffer at 37°C

For controlled release devices, the properties of the water-swollen matrices are of primary interest. For example, the extent of swelling largely determines the rate at which water-soluble drugs, such as proteins, are released from controlled release devices. Therefore, the swelling behavior of solvent cast, PEEA films was investigated as a function of the copolymer composition. Equilibrium swelling (q) in PBS at 37°C was

reached within 5 h [Fig. 5(A)]. As expected, the equilibrium swelling ratio increased with increasing amount of the hydrophilic component, PEG, in the hydrogel [Fig. 5(B)]. An increase in PEG content from 23 to 40 wt % resulted in an increase in q from 1.7 to 2.1. However, above a PEG content of 40 wt %, an abrupt increase in the degree of swelling was observed. The polymer with the highest PEG content (PEEA7, 66 wt % PEG) dissolved in the buffer after 15 min.

The degree of swelling q was in the same range as the swelling of PEG-nylon 66 multiblock copolymers.[35] Interestingly, these poly(ether amide) copolymers show a similar abrupt increase in swelling at higher PEG wt %. The transition in swelling behavior of the PEEA copolymers corresponds well with the DSC data, because it was found that the crystallinity of the ester-amide blocks was almost constant up to a PEG content of 40 wt %, whereas a further increase in the PEG content resulted in a rapid decrease of the crystallinity. Consequently, the amount of physical crosslinks will decrease rapidly at PEG contents above 40 wt %. Because swelling of a polymer network in a solvent is dependent on the degree of (physical) crosslinking, this may explain the transition in swelling behavior of the PEEA multiblock copolymers at a PEG content higher than 40 wt %.

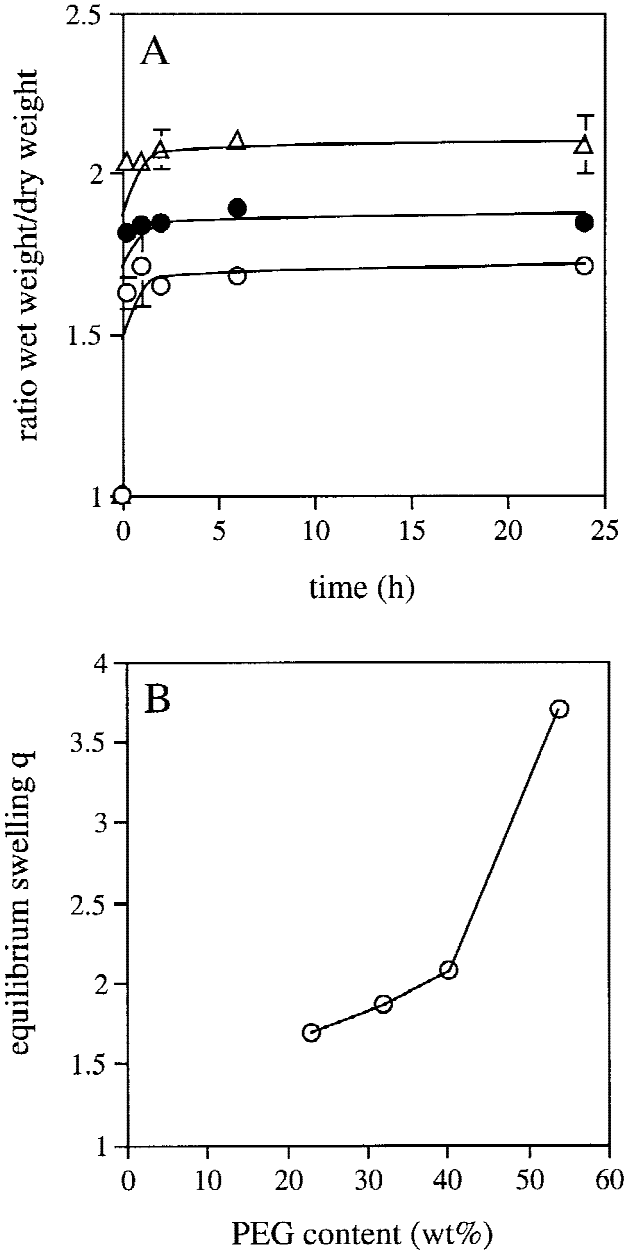


Figure 5. Swelling behavior of PEEA films in PBS at 37°C. (A) Swelling kinetics of PEEA1 (○), PEEA2 (●), and PEEA3 (△). (B) Equilibrium swelling as a function of the PEG content of the copolymers (n = 3; ±SD).

Compared with PEG containing multiblock copolymers based on aromatic polyesters, such as PET[16] and PBT,[10] the degree of swelling of the PEEA copolymers is rather high. This suggests that the amide blocks are less efficient in forming physical crosslinks in the water-swollen hydrogel than hard segments based on aromatic polyesters. The more hydrophilic nature of the amide segments in comparison with the aromatic polyesters, may also contribute to the efficiency of crosslinking. Furthermore, it has to be noted that the PEEA films were prepared from mixtures of chloroform and methanol. A difference in the rate of evaporation of the two solvents may result in a shift in composition of the solvent mixture during the drying process of the films. This may affect the ultimate structure of the films. For example, a demixing process may cause the formation of pores in the matrices. Although a dense internal structure of the films was observed by SEM, an effect of a possible shift in composition of the solvent mixture during formation of the films on the swelling behavior of the PEEA films cannot be excluded.

In vitro degradation of PEEA matrices

The *in vitro* degradation of PEEA copolymer films in PBS was examined at 37°C. PEEA7 was not selected because this copolymer was rapidly dissolved in the buffer. Figure 6 shows that immediately after immersion in the buffer, a decrease in inherent viscosity was observed for the polymers, except for PEEA1. For this copolymer, the decrease in viscosity started after 6 days of incubation. During the first days of incubation, PEEA1 showed a small increase of the inherent viscosity. Probably, this is the effect of leaching out of low molecular weight oligomers, which were formed during the polycondensation process. Indeed, an initial release of material was observed for PEEA1, as shown in Figure 7. The decrease in viscosity was more pronounced with increasing PEG content of the co-

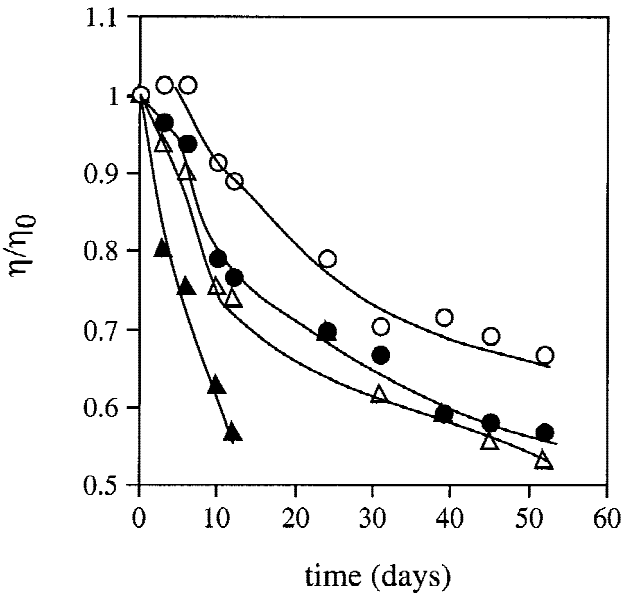


Figure 6. Change of the inherent viscosity as a function of degradation time in PBS at 37°C of PEEA1 (○), PEEA2 (●), PEEA3 (△), and PEEA5 (▲).

polymers. A similar relation was found for the weight loss of the films (Fig. 7). Matrices having increasing PEG content exhibited a faster weight loss, ranging from 12% for PEEA1 up to 50% weight loss for PEEA3 after a degradation time of 40 days. Extensive fragmentation of PEEA5 films was observed during the incubation period, which made it impossible to determine accurately the weight loss of these films.

The observed mass loss of PEEA3 and PEEA5 was much faster as compared with PEEA1 and PEEA2. As

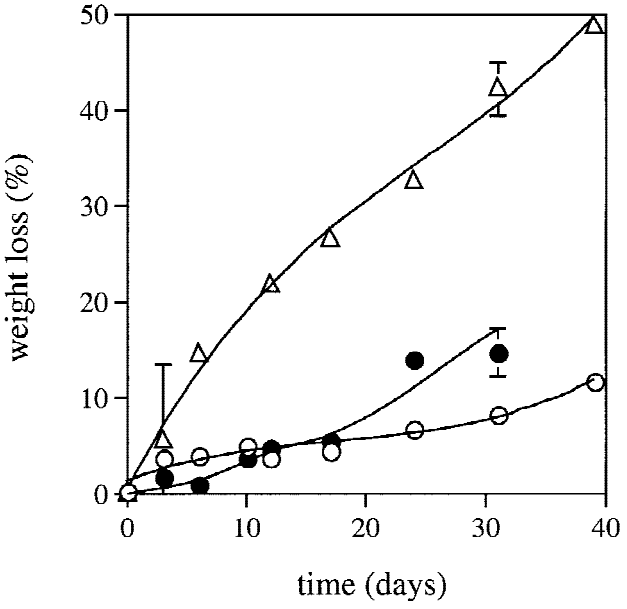


Figure 7. Weight loss as a function of degradation time in PBS at 37°C of PEEA1 (○), PEEA2 (●), and PEEA3 (△).

was found for the thermal and swelling properties, these data suggest that the PEEA copolymers can be divided in two groups. One group of polymers, in which the hard ester-amide phase forms the continuous phase, displays a rather slow rate of degradation (PEEA1 and PEEA2). In the other group, the continuous phase may be mainly composed of PEG. These polymers degrade relatively fast (PEEA3 and PEEA5).

Most likely, degradation of the copolymers takes place by hydrolysis of the ester bonds.[23,25] Because it can be expected that the ester bond between PEG and the amide block is the most labile bond, an increase in PEG content probably results in an increase in degradation rate. In addition, incorporation of hydrophilic PEG segments in poly(ester amide)s facilitates the solubilization of larger polymer fragments, leading to an increase in the rate of weight loss with increasing PEG content as shown in Figure 7. The presence of PEG increases the accessibility of the polymers for water and consequently ensures degradation in the bulk of the matrices. This is in agreement with the data of the polymer inherent viscosity analysis (Fig. 6). For all polymers tested, a decrease of the inherent viscosity in time was observed for the residual matrix. In the case of surface erosion the molecular weight of the remaining matrix is expected to be constant. Also, SEM analysis showed that degradation did not result in significant changes in the surface morphology of the films (data not shown).

The mass loss profiles cannot be predicted by inherent viscosity changes only. Indeed, the change of inherent viscosity for PEEA2 is almost similar to that of PEEA3, whereas the mass loss profiles are different. This can be explained as follows. First, it has to be noted that in Figure 6 the inherent viscosity of the remaining matrix is presented. Because mass loss was more pronounced for PEEA3, it can be expected that low molecular weight products, which lower the inherent viscosity, were released from the matrix to a larger extent than for PEEA1 and PEEA2, keeping the inherent viscosity of the remaining of PEEA3 relatively high. Furthermore, mass loss is not only determined by the rate of decrease in molecular weight, but is also related to the solubility of the polymer fragments in the degradation medium. As described above, incorporation of hydrophilic PEG segments facilitates solubilization of polymer fragments, resulting in a more pronounced mass loss.

As the ester bond between PEG and the amide block is expected to be the most susceptible one to hydrolysis, the composition of the remaining matrix might change during degradation because of release of PEG-rich products. To investigate this, ^{1}H-NMR spectra were recorded for the PEEA copolymers, recovered after a certain degradation period. The copolymer composition was calculated from the integral intensities as described above. Over a period of 52 days, no

significant changes were observed in copolymer composition (data not shown). This is in agreement with reports on the degradation of other PEG-containing multiblock copolymers, such as PEG/PET,[36] PEG/PBT,[37] and PEG/PCL[38] copolymers.

Degradation *in vivo* of the PEEA copolymers might be faster than was observed *in vitro.* Several reports describe that the degradation of poly(ester amide)s is enhanced in the presence of enzymes.[22,27,35] In addition, oxidative cleavage of the PEG segments may contribute to the degradation pathway, as has been demonstrated for poly(ether urethane)s, for which *in vivo* degradation takes place essentially at the ether linkage of the soft segment.[39] Further studies should address the biocompatibility of the PEEA multiblock copolymers and their degradation products.

Lysozyme release from PEEA films

A preliminary investigation was performed on the suitability of the PEEA copolymers as a matrix for the controlled release of proteins. Lysozyme, a 14.5 kD cationic enzyme, was used as a model protein. Protein containing films were prepared by a water-in-oil emulsion method as described previously.[10] The swelling of loaded films in PBS was slightly greater than the swelling of unloaded films (Table III). Such a small effect of loading on the swelling has been described before for a similar system.[10] Release profiles of lysozyme from PEEA films varying in copolymer composition are presented in Figure 8. The cumulative release is plotted as a function of the square root of time, which gave a linear relationship for the first part of the release curve. This indicates that the release is governed by Fickian diffusion.

From Figure 8, lysozyme diffusion coefficients were obtained using Eq. 2:

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{D_{\text{initial}}t}{\pi l^2}} \qquad (2)$$

in which M_t/M_∞ is the fraction released lysozyme, l is the thickness of the film, and t is the time. The results, presented in Table III, show that the diffusion coefficients are very small compared with the diffusion of lysozyme in water (10^{-6} cm^2/s). Although a small increase in the diffusion coefficient was observed with increasing swelling ratio, the influence of the swelling on the magnitude of the diffusion coefficient was only limited. An increase in the swelling from 1.8 to 4.0 resulted in an increase of the diffusion coefficient of only 225%. In contrast, it has been reported for PEG/PBT copolymers that an increase in the equilibrium swelling ratio from 1.47 up to 3.66 caused an almost 50,000-fold increase in the lysozyme diffusion coefficient. This might be attributed to a more pronounced phase separation of the PEG/PBT copolymers in comparison with the PEEA copolymers.

TABLE III
Equilibrium Swelling Ratio and Diffusion Coefficient for Lysozyme in PBS at 37°C of PEEA Films

Polymer	q (Unloaded Films)	q (Loaded Films)	$D_{\text{lysozyme}} \times 10^{10}$ (cm^2/s)
PEEA1	1.70 ± 0.04	1.79 ± 0.02	0.8 ± 0.2
PEEA2	1.88 ± 0.04	1.98 ± 0.02	1.3 ± 0.2
PEEA3	2.09 ± 0.09	2.04 ± 0.01	1.3 ± 0.1
PEEA5	3.70 ± 0.12	3.97 ± 0.03	1.8 ± 0.1

Protein content is 33 mg lysozyme per gram copolymer.

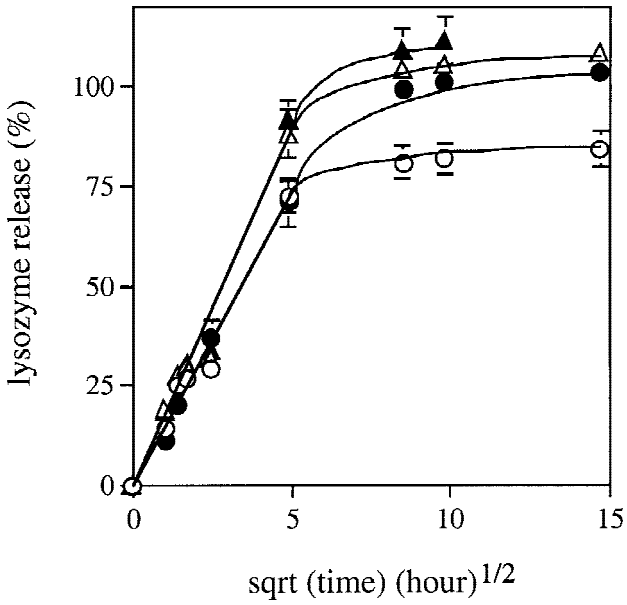


Figure 8. Release of lysozyme in PBS at 37°C from PEEA1 (○), PEEA2 (●), PEEA3 (△), and PEEA5 (▲) as a function of the square root of time (n = 3; ±SD).

Another factor that might influence the release rate is the polymer molecular weight. It has been found that the diffusion coefficient of proteins through amphiphilic multiblock copolymer films is strongly dependent on the molecular weight of the polymers.[10] The diffusion rate increases with decreasing polymer molecular weight. Thus, the higher intrinsic viscosity of PEEA5 compared with the other PEEA polymers (Table I) may contribute to the fact that the lysozyme diffusion coefficient through PEEA5 was lower than expected from the equilibrium swelling data (Table III).

The model protein used in this study is rather small (14.5 kD). It can be expected that release of larger proteins is much slower and is more dependent on the copolymer composition. Furthermore, in the present study the length of the PEG segment was fixed at 1000 g/mol. Most likely, the use of PEG segments of various lengths offers possibilities to manipulate the struc-

ture of the PEEAs to a larger extent, allowing a more precise control of protein release rates.

CONCLUSIONS

The results of this study show that biodegradable amphiphilic multiblock copolymers can be prepared successfully by copolymerization of hydrophilic PEG oligomers, 1,4-dihydroxybutane, and bisesterbisamide monomers. The properties of the polymers, such as crystallinity, water-uptake ,and rate of degradation, can be modulated by variation of the PEG content in the copolymers. A preliminary investigation of the protein release characteristics of the PEEA copolymers showed that the diffusion of the model protein lysozyme through the films was slow in comparison with the diffusion in water. The protein release rate could be controlled by the degree of swelling of the polymers. These properties make the PEEA multiblock copolymers promising candidates for application as matrix material for controlled release systems for proteins.

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