Synthesis and Characterization of New Functional Poly(ester-anhydride)s Based on Succinic and Sebacic Acids

Katarzyna Jaszcz

Summary: New functional poly(ester-anhydride)s with allyl pendant groups in the side chains were obtained by polycondensation of sebacic acid (SBA) and poly(3-allyloxy-1,2-propylene succinate) (OSAGE) terminated with carboxyl groups. The carboxyl groups in OSAGE and in SBA were converted to mixed anhydride groups by acetylation with acetic anhydride. After that, prepolymers obtained were condensed in vacuum to yield higher molecular weight poly(ester-anhydride)s. The influence of SBA and OSAGE content in poly(ester-anhydride)s on their selected properties e.g. molecular weight, thermal and solubility characteristics as well as degradation rate and mode, were examined. Poly(ester-anhydride)s were degraded in aqueous buffer of pH 7.4 at 37 °C. The hydrolytic degradation was monitored by determination of weight loss of samples and by determination of ester to anhydride groups ratio.

Keywords: allyl groups; hydrolytic degradation; poly(ester-anhydride)

Introduction

Polyanhydrides represent one of the most extensively studied classes of biodegradable polymers due to their biocompatibility and excellent controlled release characteristics[1] and due to that have been proposed for medical and pharmaceutical applications for more than two decades.[2] They have been especially extensively studied as controlled drug delivery matrices for short-term release of drugs because of their high degradation rates.[3–5] Recently, poly(ester-anhydride) copolymers have been synthesized that combine the individual properties (such as biodegradation rate and mode) of these two classes of polymers.[6–9] We had already reported the synthesis of functional poly(ester-anhydride) based on succinic acid.[10]

In this work new family of functional poly(ester-anhydride)s with allyl pendant groups in the side chains, based on succinic and sebacic acids was obtained. Pendant functionality in biodegradable polymers creates interesting perspectives for chemical coupling of drugs to such a carrier. That may be also utilized to form crosslinked matrix with enhanced mechanical and physical properties.

Experimental Part

Materials

Succinic acid 99% (Aldrich), allyl glycidyl ether 99% (Aldrich) and acetic anhydride (POCH S.A.) were used as supplied. Sebacic acid (Aldrich) was crystalized from methanol. Solvents were purified according to known procedures.

Methods

^{1}H NMR spectra were recorded at Varian UNITY/INOVA spectrometer (300 MHz) in $CDCl_3$, DMSO–d_6 or D_2O with TMS or TSP as an internal standard. Viscosity

---

Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, 44-100 Gliwice, ul. M.Strzody 9, Poland
Fax: +48 32/2371509
E-mail: Katarzyna.Jaszcz@polsl.pl

measurements were performed in $CHCl_3$ or THF, at 23 °C using Ubbelohde viscometer.

VPO analysis of oligoester was performed in chloroform using Knauer vapor pressure osmometer. The molecular weight of poly(ester-anhydride)s was determined in chloroform by gel permeation chromatography (GPC) using a Spectra-Physics 8800 chromatograph equipped with refractive index detector (Shodex SE 61) and calibrated with polystyrene standards. Thermal analyses were performed using 822^e DSC Mettler Toledo differential scanning calorimeter. Samples were tested in temperature range from −70 °C to 250 °C at a heating rate of 10 °C/min. Microspheres were investigated using light microscope (DELTA Optical) with magnification of 40–1000.

Preparation of OSAGE

OSAGE was synthesised as described earlier[10] by melt condensation of succinic acid (SAc) and allyl glycidyl ether (AGE) using twofold excess of SAc. The reaction was carried out at 150 °C as long as the acid value (AV) decreased to a constant value. The crude product was dissolved in chloroform and filtered to remove unreacted SAc. The oligoester was precipitated in diethyl ether/petroleum ether (1:1 v/v) mixture, separated by sedimentation and dried under vacuum. The structure of oligoester was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The oligoester obtained did not contain detectable amount of hydroxyl groups (HV = 0), while its AV = 236 mg KOH/1 g. Molecular weight of OSAGE determined by VPO was Mn(VPO) = 896 g/mol. Thermal properties of the oligoester were evaluated with DSC. Tg = −39 °C and no melting transitions were observed for the oligomer obtained. Exothermal transition was observed however above 115 °C due to polymerization of allyl groups.

Preparation of Prepolymers

OSAGE and SBA prepolymers were prepared according to the literature.[11] Typically, oligoester or SBA were refluxed in acetic anhydride (1:10 w/v) under nitrogen for 30 min. Excess of acetic anhydride and acetic acid formed as a by-product were removed under vacuum. The prepolymers obtained were dissolved in methylene chloride, precipitated in diethyl ether/petroleum ether (1:1 v/v), separated and dried under vacuum. The prepolymers were stored at −18 °C. The complete conversion of the acid groups was confirmed by ^{1}H NMR. There was no signal of carboxyl end groups proton at low-field side of ^{1}H NMR spectra ($\delta > 10$ ppm) while the signal of methyl protons of the acetyl end groups appeared at $\delta = 2.22$ ppm.

Polycondensation of Prepolymers

OSAGE and SBA prepolymers were mixed in defined ratios (Table 1), stirred with magnetic stirrer and heated at 150 °C for 2 h under high vacuum conditions (0.10–0.01 mm Hg) to yield poly(ester-anhydride)s. In a typical procedure ca. 10 g of prepolymers were used. The poly(ester-anhydride)s obtained were disolved in methylene chloride, precipitated in diethyl ether/petroleum ether (1:1 v/v), washed with petroleum ether and dried under vacuum. The polymers obtained were stored in freezer.

Hydrolytic Degradation

Hydrolytic degradation was performed in phosphate buffer solution (pH 7.4) at 37 °C. Disc shaped samples (6 mm diameter, 1 mm thickness and weight 0.2 g) were placed in vials containing 20 ml of buffer solution. The vials were incubated at 37 °C for various time (0.5 h to 30 days). After incubation, the samples were separated and washed with water, dried in vacuum and weighted to estimate weight loss. The ^{1}H NMR spectra of non-degraded parts of samples (solid residues after degradation) were recorded.

Formation of Microspheres

Microspheres were prepared using a solvent removal technique. The polymer solution in methylene chloride (with concentration of 20–50 mg/ml) was dispersed in 0.5–2.0% aqueous solution of poly(vinyl alcohol) (PVA 88%, Mn = 100000). The

Table 1.
Characteristics of poly(ester-anhydride)s

Symbol	Feed ratio [%]		[η] [dl/g]	Mn (^{1}H NMR)	Molecular weight (GPC)		Thermal properties (DSC)		
	OSAGE	SBA			Mn	Mw	Tg [°C]	Tm [°C]	ΔH [J/g]
PSB100	0	100	0.195[a)]	10200	3800	12300	–	82,1	−94,6
PSAGESB80	20	80	0.074[a)]	18100	1100	4800	−43,3	78,7	−101,4
PSAGESB60	40	60	0.063[a)]	19300	1600	5100	−39,1	71,9	−79,1
PSAGESB40	60	40	0.052[a)]	19900	4500	10400	−38,9	60,7	−30,9
PSAGESB20	80	20	0.020[b)]	37500	960	3800	−37,2	43,7	−2,6
PSAGESB0	100	0	0.029[b)]	14600	2700	21000	−21,3	–	–

[a)] Determined in chloroform solution; [b)]Determined in THF solution.

dispersion was stirred at room temperature for 3 h to evaporate the organic solvent. The microspheres were than filtered, washed 3 times with distilled water, dried under vacuum and stored in freezer.

Results and Discussion

A series of functional poly(ester-anhydride)s were obtained by polycondensation of sebacic acid (SBA) and poly(3-allyloxy-1,2-propylene succinate) (OSAGE) prepolymers. The polymers obtained were solid materials amorphous and elastic when containing more OSAGE and crystalline when containing more SBA fragments.

^{1}H and ^{13}C NMR spectra of the poly(ester-anhydride)s confirmed their expected structure. The ^{1}H NMR spectra of poly(ester-anhydride)s obtained from OSAGE, OSAGE and SBA as well as that of poly(sebacic anhydride) are shown in Figure 2 with detailed assignments of the peaks. ^{1}H NMR technique was used to determine the OSAGE and SBA ratio in

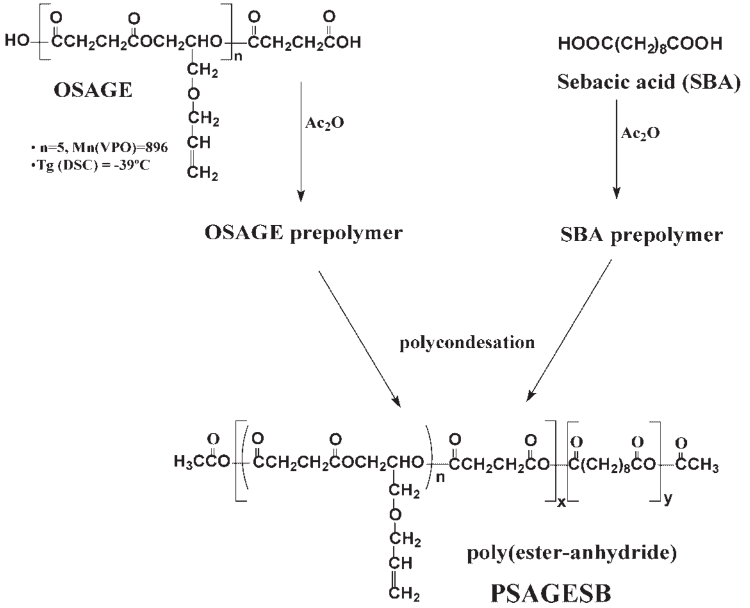

Figure 1.
Reaction scheme of synthesis of poly(ester-anhydride)s based on succinic and sebacic acids.

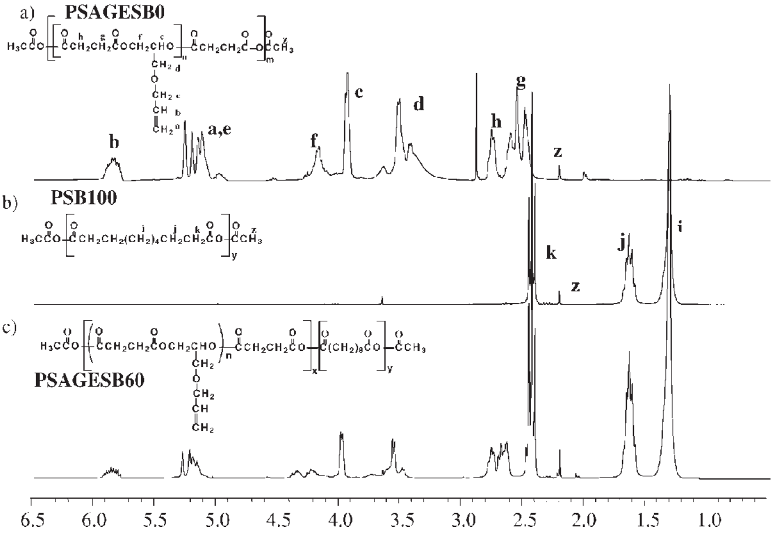

Figure 2.
^{1}H NMR spectra of (a) poly(ester-anhydride) obtained by polycondensation of acetylated OSAGE (DMSO-d_6), (b) poly(sebacic anhydride) ($CDCl_3$), (c) poly(ester-anhydride) obtained by copolycondensation of acetylated OSAGE and SBA ($CDCl_3$).

the copolymers as well as their molecular weight. The molecular weight of poly(ester-anhydride)s were calculated by comparison of intensities of the **h, k** and **z** signals (Figure 2). The content of OSAGE and SBA incorporated into the polymers, calculated by comparison of intensities of the **b, h** and **k** signals (Figure 2) corresponded to the prepolymers feed ratio.

Molecular weight values of poly(ester-anhydride)s calculated from ^{1}H NMR and determined by GPC are summarized in Table 1. No correlation was found between the molecular weights determined by GPC and calculated from ^{1}H NMR spectra. The molecular weight determined by GPC showed relatively broad dispersity (Mw/Mn were in the range of 3.2 to 7.8) and were much lower than Mn calculated from ^{1}H NMR spectra.

The GPC chromatograms of all poly(ester-anhydride)s exhibited bimodal character and indicated the presence of low molecular fraction (Mn below 1000 g/mol), probably due to unreacted prepolymers or degradation products. Intrinsic viscosity $[\eta]$ values (Table 1) of poly(ester-anhydride)s determined in $CHCl_3$ or THF solution were rather low suggesting low molecular weight of poly(ester-anhydride)s corresponding better with GPC results. The highest $[\eta]$ value was determined for poly(sebacic anhydride) (PSB100) and the lowest one for PSAGESB20 with OSAGE content equal to 80%.

It was found that thermal and solubility characteristics of poly(ester-anhydride)s were dependent on SBA and OSAGE content in polymers.

The thermal properties of copolymers such as melting temperature (Tm), heat of fusion (ΔHm) and glass transition temperature (Tg) were determined by DSC. The results (Table 1) indicated that the PSAGESB0 obtained without SBA was completely amorphous (no crystallinity was observed within the temperature ranging from -70 to 250 °C) and had Tg equal to -21 °C. In the DSC thermogram of PSB100, the sharp melting peak at 82 °C and no Tg was observed. Relative crystallinity estimated by dividing the determined heat of fusion of PSB100 ($\Delta Hm = -94{,}6$ J/g) by theoretical value (-115 J/g) was equal

Table 2.
Solubility characteristics of poly(ester-anhydride)s.

	$CHCl_3$	THF	Toluene	Acetone	DMSO	DMF	MMA
PSB100	+	−	+/−	−	−	−	−
PSAGESB80	+	−	+/−	−	−	−	−
PSAGESB60	+	+	+/−	−	−	−	−
PSAGESB40	+	+	−	+	−	+/−	−
PSAGESB20	+/−	+	−	+	+	+	+
PSAGESB0	+/−	+	−	+	+	+	+

+ soluble, **+/−** partialy soluble, **−** non-soluble.

to 80%. The melting temperature and heat of fusion of poly(ester-anhydride)s decreased with increasing the OSAGE content. It indicated on lower crystallinity of copolymers. Due to the lower Tm of copolymers compared to poly(sebacic anhydride), they could be fabricated into devices under mild conditions, especially suitable for drug delivery systems. The Tg of the copolymers increased gradually with decreasing SBA content. The Tg for PSB100 was not detected.

Solubility characteristics of poly(ester-anhydride)s was also dependent on SBA and OSAGE content (Table 2). Poly(ester-anhydride)s containing more than 60% SBA exhibited solubility characteristics similar to that of poly(sebacic anhydride). They were soluble only in chlorinated solvent. Increase of OSAGE content in copolymers caused worse solubility in chlorinated solvents but much better in polar and aprotic solvents eg. acetone, THF, DMF, DMSO.

Due to solubility of PSAGESB0 and PSAGESB20 in methyl methacrylate (MMA), the application of poly(ester-anhydride)s in biodegradable bone cements could be taken into consideration.

Poly(ester-anhydride)s obtained were subjected to hydrolytic degradation in aqueous buffer of pH 7.4 at 37 °C. The degradation was monitored mainly by determination of weight loss. The ester (E) to anhydride groups (formed from succinic acid A(SAc) or from sebacic acid A(SBA)) ratios were also controlled during degradation process, using ^{1}H NMR technique.

The weight of all samples decreases as a function of time. Figure 3 displays the weight loss of poly(ester-anhydride)s of different SBA to OSAGE ratios. Two different degradation periods can be distinguished: a rapid weight loss in the first stage (ca. one week, linear for poly(ester-anhydride)s containing more than 60% SBA) (Figure 3a), was followed by much

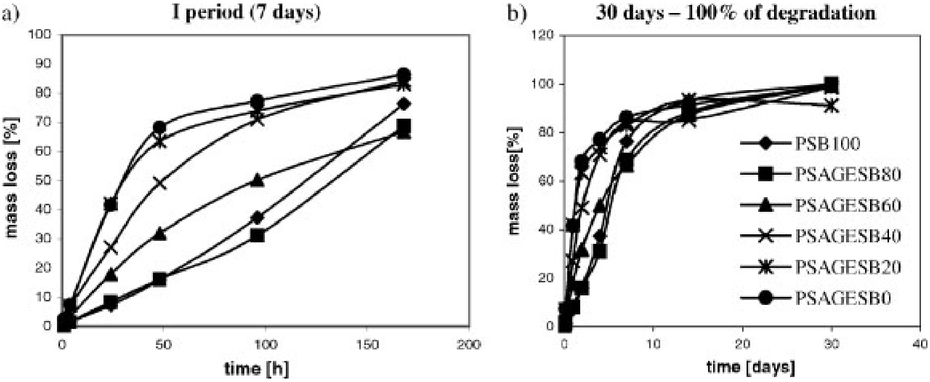

Figure 3.
The weight loss of samples of poly(ester-anhydride)s during hydrolytic degradation as a function of immersion time in buffer solution.

slower degradation in next period. Such degradation profile is characteristic for other polyanhydrides.[7]

In the first period, degradation process of poly(ester-anhydride)s containing more OSAGE parts was faster than degradation of poly(sebacic anhydride) or poly(ester-anhydride)s with higher SBA content. This is caused by the differences in crystallinity of copolymers. In general, a crystallinity of polymers is one of main factors to control the polymer degradation and erosion.[12] The higher crystallinity of poly(ester-anhydride)s containing more SBA, resulted in their slower degradation during the first period due to surface character of degradation process. No crumbs or fragments were found to be generated during 10 days of degradation process for PSB and PSAGESB80. It was also observed that the size of those samples in buffer solution remained nearly unvaried in his time. The samples of copolymers obtained with use of 40% or more OSAGE underwent changes (cracked, crumbled or transferred into viscous resin) after two to six days of immersion in buffer. It is associated with bulk degradation process of the poly(ester-anhydride)s, different from that of PSB100 where mainly surface degradation was observed. Bulk degradation process caused fast hydrolysis of anhydride bonds in whole volume of a sample. Next stage of degradation process was due mainly to hydrolysis of more stable ester bonds.

Analysis of ^{1}H NMR spectra of non-degraded samples (residues after degradation) confirmed that the anhydride bonds (formed by sebacic or succinic acids) were cleaved faster than ester ones. The anhydride bonds were lost the faster the bigger OSAGE content in poly(ester-anhydride) was. It was also stated that the anhydride bonds formed by succinic acid were lost faster than the anhydride bonds formed by sebacic acid (Figure 4 and 5).

Poly(ester-anhydride)s were used for the preparation of microspheres using emulsion/solvent evaporation technique. The particles obtained from poly(ester-anhydride)s containing more than 60% of OSAGE had tendency to aggregation. The tendency was increased with increasing content of OSAGE in poly(ester-anhydride)s. Stable microspheres were not obtained when content of SBA in poly (ester-anhydride)s was lower than 60%. Low crystallinity of such copolymers and Tg below 0 °C resulted in gluing of microspheres already in aqueous dispersion or after their separation. Probably it is also due to their partial hydrolytic degradation. For copolymers containing a high amount of ester bonds, fast surface and bulk

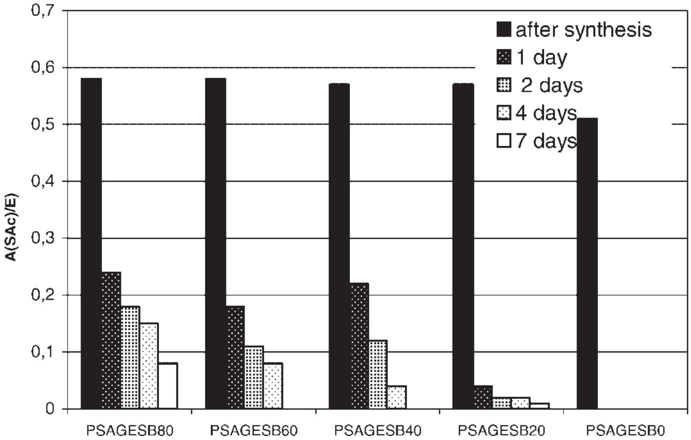

Figure 4.
The anhydride (formed by succinic acid) to ester groups ratio in poly(ester-anhydride)s as a function of imersion time in buffer solution.

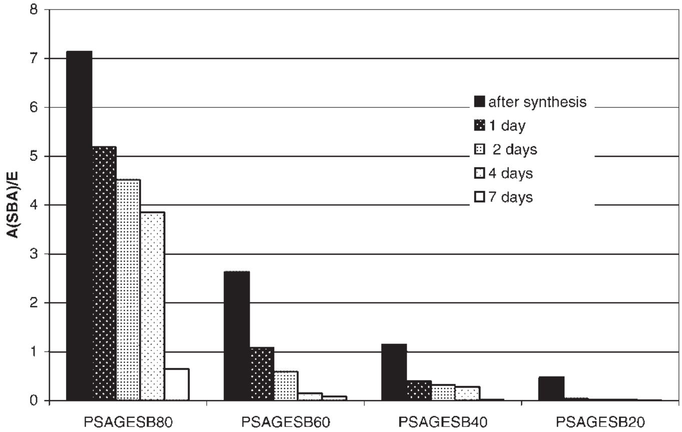

Figure 5.
The anhydride (formed by sebacic acid) to ester groups ratio in poly(ester-anhydride)s as a function of imersion time in buffer solution.

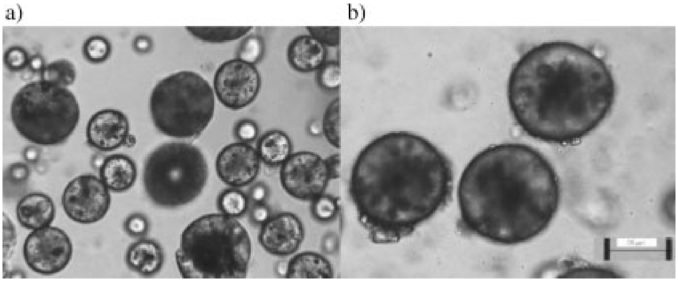

Figure 6.
Photomicrographs of microspheres (PSAGESB80) obtained from polymer solutions a) 30 mg/ml and b) 50 mg/ml, dispersed in 1% PVA solution in water (magnification 400).

degradation results in transformation of the solid particles into viscous resin.

The particles obtained from PSAGESB80 or PSAGESB60 were stable, spherical in shape, however those prepared with polymers containing 80% SBA possessed smoother surfaces compared to those prepared from PSAGESB60. Microspheres were obtained from polymer solutions of different concentration (20–50 mg/ml) and using aqueous PVA of various concentration (0.5–2.0%). Increase of the concentration of a polymer resulted in receiving microspheres with bigger dimensions (Figure 6), while increased concentration of PVA solution resulted in smaller microspheres.

Conclusion

In this work new family of poly(ester-anhydride) copolymers, based on succinic and sebacic acids was obtained and characterized. The poly(ester-anhydride)s differs with their solubility and thermal properties as well as their degradation rate and mode. Due to solubility of some copolymers in methyl methacrylate, the application of poly(ester-anhydride)s in biodegradable bone cements could be taken into consideration. The lower Tm of copolymers compared to that of poly(sebacic anhydride), could allow to be fabricated into devices in mild conditions, especially suitable for drug delivery systems. Susceptibility to formulate poly(ester-anhydride)s into microspheres would enable their eventual use in drug delivery systems.

[1] N. Kumar, R. S. Langer, A. J. Domb, *Adv. Drug Delivery Rev.* **2002**, *54*, 889.
[2] H. B. Rosen, J. Chang, G. E. Wnek, R. J. Linhardt, R. Langer, *Biomaterials* **1983**, *4*, 131.
[3] E. Shen, R. Piszczek, B. Dziadul, B. Narasimhan, *Biomaterials* **2001**, *22*, 201.
[4] E. Fournier, C. Passirani, C. N. Montero-Menei, I. P. Benoit, *Biomaterials* **2003**, *24*, 3311.
[5] J. P. Jain, S. Modi, A. J. Domb, N. Kumar, *J. Controlled Release* **2005**, *103*, 541.
[6] L. Erdmann, K. E. Uhrich, *Biomaterials* **2000**, *21*, 1941.
[7] H. L. Jiang, K. J. Zhu, *Biomaterials* **2001**, *22*, 211.
[8] U. Edlung, A. C. Albertsson, *Adv. Drug Delivery Rev.* **2003**, *55*, 585.
[9] B. A. Pfeifer, J. A. Burdick, R. Langer, *Biomaterials* **2005**, *26*, 117.
[10] K. Jaszcz, J. Łukaszczyk, *React. Funct. Polymers* submitted for publication.
[11] A. J. Domb, E. Mathiowitz, E. Ron, S. Giannos, R. Langer, *J. Polym. Sci. : Part A: Polym. Chem.* **1991**, *29*, 571.
[12] A. Gopferich, J. Tessmar, *Adv. Drug Deliv. Rev.* **2002**, *54*, 911.