



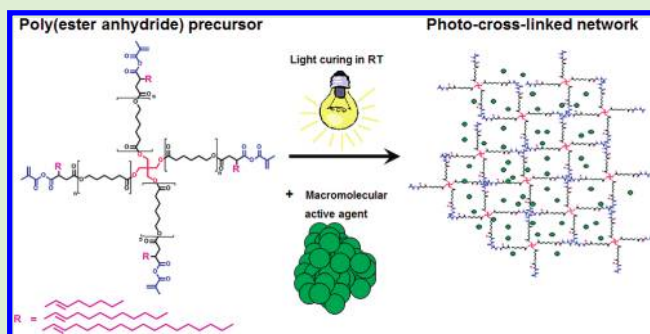
# Photo-Cross-Linked Biodegradable Poly(Ester Anhydride) Networks Prepared from Alkenylsuccinic Anhydride Functionalized Poly( $\epsilon$ -caprolactone) Precursors

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**ABSTRACT:** Biodegradable poly(ester anhydride) networks based on linear and star-shaped poly( $\epsilon$ -caprolactone)-based precursors were synthesized with the aim of obtaining matrixes suitable for release of macromolecular active agents. The ring-opening polymerization yielded hydroxyl telechelic oligomers, which were end-functionalized with succinic anhydride or with alkenylsuccinic anhydrides containing 8, 12, or 18 carbons in their alkenyl chains. Before cross-linking, the acid-terminated oligomers were reacted with methacrylic anhydride to obtain methacrylated precursors containing labile anhydride bonds. The degrees of substitution for the acid functionalization and methacrylation were >93%. Cross-linking of the precursors was carried out with visible light at room temperature. Gel contents and cross-linking densities were higher for networks cross-linked from the star-shaped precursors than for networks prepared from the linear precursors. In *in vitro* erosion tests, the presence of the alkenyl chain slowed down the erosion rate. The networks exhibited characteristic surface erosion: the mass loss was linear, whereas the dimensions of the specimens decreased steadily. A macromolecular release study showed the release of the model compound to be linear and in proportion to the mass loss.



## INTRODUCTION

Over the past decade, significant attention has been paid to biocompatible cross-linked resorbable polymeric networks for medical applications, such as controlled drug delivery and tissue engineering.<sup>1–3</sup> Cross-linking provides an effective tool to tailor the physical and mechanical properties as well as the degradation rates of biodegradable materials.<sup>4–7</sup> Among the cross-linking methods, photoinitiated cross-linking has many advantages over thermal or redox initiation for biomedical applications. It allows easy control of the reaction by the light source and fast cross-linking rates.<sup>3</sup> Furthermore, photo-cross-linking can be done without solvents under mild reaction conditions, thus reducing possible denaturation of biological active agents.<sup>8,9</sup>

Various syntheses routes have been examined for the preparation of cross-linked networks. According to literature, the most popular method of preparing biodegradable networks is via free-radical curing of prepolymers.<sup>3</sup> The networks have mostly been prepared from polyester precursors functionalized with acrylic,<sup>10</sup> methacrylic,<sup>11</sup> fumaric,<sup>12</sup> maleic,<sup>13</sup> or itaconic<sup>14</sup> double bonds. The properties of cross-linked polyesters can extensively be modified for various applications by varying the chemical composition and the architecture of the polymer.<sup>3</sup> Cross-linked lactide-based precursors have resulted in hard and rigid networks with high mechanical strength, which have potential for orthopedic

and bone tissue applications.<sup>15,16</sup> Biodegradable elastomers have been prepared from photo-cross-linked poly( $\epsilon$ -caprolactone-*co*-D, L-lactide) precursors for soft-tissue applications.<sup>17–19</sup> In general, polyester networks show bulk degradation, whereas surface erodible networks would be more preferable for controlled release applications. Surface erosion can be achieved by photo-cross-linking of polyanhydride networks from methacrylated sebacic acid (SA) or 1,3-bis(*p*-carboxy phenoxy) propane (CPP) or 1,6-bis(*p*-carboxy phenoxy) hexane (CPH) precursors. The degradation time of these cross-linked polyanhydrides can be adjusted between 2 days and 1 year through variation of the network composition.<sup>6,20–22</sup>

Poly(ester anhydrides) have been synthesized with the aim of combining the favorable properties of synthetic aliphatic polyesters and polyanhydrides.<sup>23–27</sup> In our previous work,<sup>28</sup> we found the anhydride bond to have a clear effect on the hydrolytic degradation of cross-linked poly( $\epsilon$ -caprolactone)-based poly(ester anhydrides): networks composed of short linear precursors containing hydrolytically labile anhydride bonds exhibited characteristics of surface erosion and complete mass loss in 2

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days. The advantage of cross-linked poly(ester anhydrides) is that polyester precursors can be readily modified by the monomer type, the molecular architecture (linear or star-shaped), and the molecular size during the synthesis of the oligomers.<sup>29</sup> As a consequence, the precursors can be modified to be liquid at room temperature, and thus drugs can easily be dispersed or dissolved in the precursor prior to the cross-linking process, even with high degree of drug loading. This was shown in our recent study, where photo-cross-linked poly(ester anhydride) networks containing 10–60 wt % of propranolol HCl were prepared. With all drug loadings, the networks exhibited surface-erosion-controlled drug release within 2 days.<sup>30</sup>

Although our previous study has indicated that cross-linked poly(ester anhydrides) can be used for controlled drug delivery, an erosion time of 2 days is too short for most drug release applications. The main objective of the present study was to slow down the erosion process of photo-cross-linked poly(ester anhydride) networks while maintaining the surface erosion properties. We have previously shown that the hydrophobicity of poly( $\epsilon$ -caprolactone) oligomers can be increased by lengthening the alkenyl chain of alkenylsuccinic anhydride (ASA)<sup>29</sup> and that the increased hydrophobicity has a pronounced effect on the degradation rate of thermoplastic poly(ester anhydrides).<sup>25</sup> In this study, it was investigated how the use of alkenylsuccinic anhydrides affects the hydrophobicity and the degradation rate of the cross-linked poly(ester anhydride) networks. The final aim of our research is to prepare drug delivery systems into which a wide spectrum of therapeutic agents, including proteins and peptides, can be entrapped under mild reaction conditions with the drug release being controlled by surface erosion of the polymer.

## EXPERIMENTAL SECTION

**Materials.** Stannous octoate (SnOct<sub>2</sub>, 95%), ( $\pm$ )-2-octen-1-ylsuccinic anhydride (8-ASA, 97%), 2-dodecen-1-ylsuccinic anhydride (12-ASA, 95%), methacrylic anhydride (MAAH, 94%), and camphorquinone (CQ, 97%) were purchased from Sigma-Aldrich (Steinheim, Germany). Pentaerythritol (PERYT, 98%), 1,4-butanediol (BD, 99+%), and succinic anhydride (SAH, 99+%) were purchased from Acros Organics (Geel, Belgium). Dextran from *Leuconostoc* ssp. ( $M_r \approx 2\,000\,000$  g/mol) was purchased from FlukaBioChemica (Steinheim, Germany) and *n*-octadecylsuccinic anhydride (18-ASA, mixture of isomer) was purchased from Tokyo Kasei Organic Chemicals (Zwijndrecht, Belgium). All of the above chemicals were used as received. The  $\epsilon$ -caprolactone (CL) was purchased from Solvay Interlox (Warrington, England) and was redistilled and dried over molecular sieves.

**Synthesis of Poly(ester anhydride) Precursors.** Acid-functionalized linear and star-shaped oligomers were prepared as in our previous work<sup>29</sup> and are denoted in the form LIN-1000-8 or STAR-1000-8, where LIN and STAR indicate linear and star-shaped molecular architecture, 1000 indicates the molecular weight of the hydroxyl-terminated oligomer (molecular weight before acid-functionalization), and 8 indicates alkenyl chain lengths of eight carbons. Cross-linkable poly(ester anhydride) precursors with labile anhydride bonds were obtained by allowing the acid-terminated oligomers to react with methacrylic anhydride (MAAH). Methacrylated precursors are indicated by m in the end of precursor denotation (LIN-1000-8m or STAR-1000-8m).

**Cross-Linking of Poly(ester anhydride) Precursors.** Cross-linking was carried out by photoinitiation. To the liquid methacrylated precursors at room temperature was added 1 wt % of camphorquinone. The mixture was stirred until homogeneity was achieved and was then applied to a Teflon mold to obtain discoids (2 mm in thickness, 5 mm in

diameter, and 50 mg in weight). Photo-cross-linking was done with visible light (11 W, 16 mW/cm<sup>2</sup>) at room temperature with exposure times of 18 to 32 min depending on the precursor. Transparent Teflon film was applied to the top and bottom of the discoids to assist the molding. Cross-linked networks are indicated by nw in the end of network denotation (LIN-1000-8nw or STAR-1000-8nw).

For the macromolecular release study, the discoids were prepared as follows. The liquid methacrylated precursors, camphorquinone, and dextran powder (10 wt %) were stirred and applied to the Teflon mold as before. Cross-linked networks were produced by photo-cross-linking the discoids in a Triad 2000 light curing oven with visible light (350–550 nm, 220 mW/cm<sup>2</sup>, DeguDent). The exposure time in the curing chamber was 10 min. The light intensity at the surface of the discoids was measured with an HD2102.1 photoradiometer (Delta Ohm, Italy).

**Characterizations.** The number-average molecular weight ( $M_n$ ), weight-average molecular weight ( $M_w$ ), and molecular weight distribution (MWD) of methacrylated precursors were determined with respect to polystyrene standards by size exclusion chromatography (SEC). A Waters Associates system was equipped with a Waters 717plus Satellite auto-sampler, a Waters 510 HPLC solvent pump, four linear PL gel columns (10<sup>4</sup>, 10<sup>5</sup>, 10<sup>3</sup>, and 100 Å) connected in series, and a Waters 2414 differential refractometer. All samples were analyzed at room temperature. Chloroform (Riedel-de Haën Ag, stabilized with 1% ethanol) was used as eluent and was delivered at a flow rate of 1.0 mL·min<sup>-1</sup>. The samples were dissolved in chloroform to a concentration of 0.1% (w/v). The injection volume was 200  $\mu$ L. The dextran release study was performed with the same SEC system, except that it was equipped with four linear Ultrahydrogel water columns connected in series. All samples were analyzed at room temperature. Phosphate buffer solution (PBS, pH 7.4, FF-Chemicals) was used as eluent and delivered at a flow rate of 0.6 mL·min<sup>-1</sup>. The injection volume was 200  $\mu$ L.

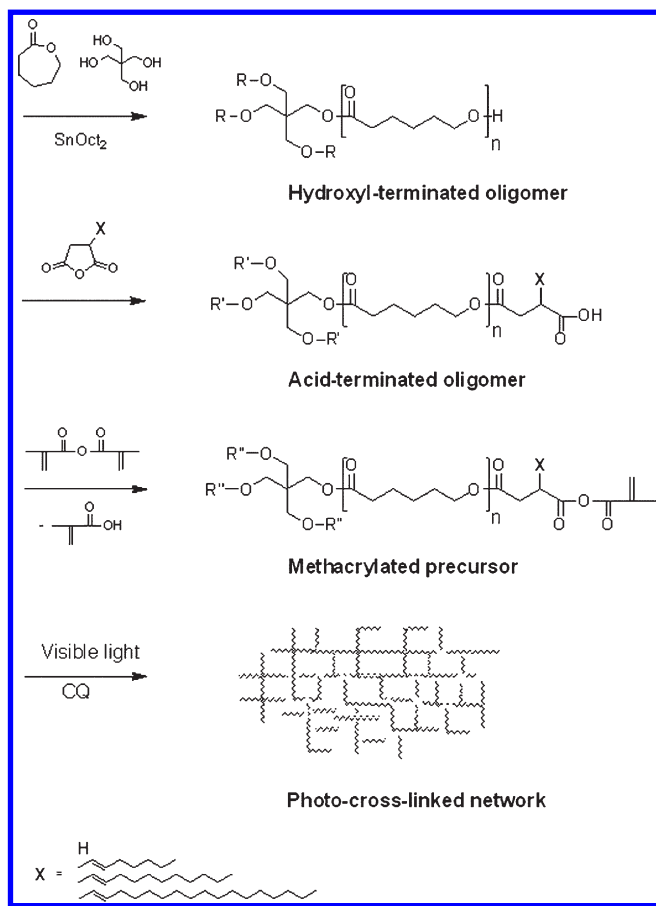
NMR spectra were recorded on a Varian Gemini 2000 300 MHz spectrometer. For <sup>1</sup>H NMR measurements, the samples were dissolved in chloroform-*d*<sub>1</sub> (Fluka Chemica) with deuteration not less than 99.8%, in 5 mm NMR tubes, at room temperature. The sample concentration was  $\sim$ 1.0% by weight. <sup>13</sup>C NMR was performed with 10% sample concentration in 10 mm tubes. Attenuated total reflectance infrared spectroscopy (ATR-FTIR, Nicolet Magna-FTIR spectrometer 750 equipped with PIKE Technologies GladiATR with diamond crystal plate) was used to monitor the amounts of the reacted double bonds. The amount of the reacted double bonds in the discoids was calculated from the decrease in absorbance near 1636 cm<sup>-1</sup>, a characteristic absorbance of the methacrylate double bond.<sup>28,31</sup>

Differential scanning calorimetric (DSC) measurements were carried out on a Mettler Toledo Star<sup>e</sup> DSC 821<sup>e</sup>. For the methacrylated precursors, the first heating (to avoid premature cross-linking) was run from -100 to 60 °C and the second heating from -100 to 200 °C. In both cases, the heating and cooling rates were 10 °C min<sup>-1</sup>. Both ramps for the cross-linked samples were run from -100 to 200 °C, and heating and cooling rates were again 10 °C min<sup>-1</sup>. The glass-transition temperatures ( $T_g$ ), melting temperatures ( $T_m$ ), and melting endotherms ( $\Delta H$ ) of the samples were measured during the second heating period.

For the cross-linked samples, the isolated weight ( $m_{iso}$ ) was determined before the extraction and swelling tests. Swelling and extraction experiments were performed in dichloromethane at room temperature with three parallel specimens. A sample discoid was swollen for 24 h, the surface was carefully dried, and the discoid was weighed ( $m_{sw}$ ). After drying at room temperature under reduced pressure for 1 week, the sample was weighed again ( $m_{dry}$ ). The gel content ( $G$ ) was calculated using the following eq 1

$$G = \frac{m_{dry}}{m_{iso}} \quad (1)$$

**Scheme 1. Preparation of Photo-Cross-Linked Poly(Ester anhydride) Networks from Star-Shaped Hydroxyl-Terminated Poly( $\epsilon$ -caprolactone) Oligomers**

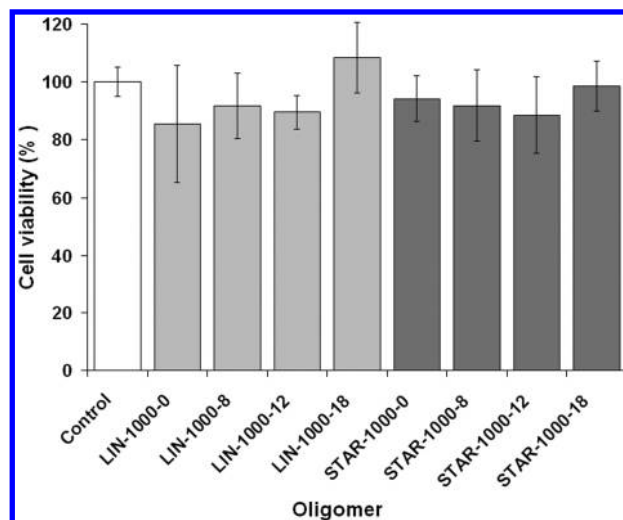


The degree of swelling ( $Q$ ) of the polymer networks in dichloromethane was calculated as follows<sup>32</sup>

$$Q = 1 + \rho_p \cdot \left( \frac{m_{sw}}{m_{dry} \cdot \rho_s} - \frac{1}{\rho_s} \right) \quad (2)$$

where  $\rho_s$  and  $\rho_p$  are the specific densities of the solvent (1.326 g cm<sup>-3</sup> for dichloromethane) and the polymer, respectively. The density of amorphous poly( $\epsilon$ -caprolactone) (1.094 g cm<sup>-3</sup>) was used as  $\rho_p$  for the cross-linked polymer samples.<sup>32</sup>

**Dissolution and Dextran Release Study.** A dissolution study of the networks was carried out with three parallel specimens for each time point. The discs (2 mm in thickness, 5 mm in diameter, and 50 mg in weight) were immersed in 30 mL of PBS in glass bottles at 37 °C. The airbath (Infors AG, Switzerland) was set to shake the bottles at a rate of 120 strokes/min. The pH was followed with Philips PW9420 pH meter equipped with Hamilton pH electrode. The discs were recovered from the test bottles at different time intervals and weighed. Specimens were subsequently vacuum-dried at room temperature for 1 week and weighed again. In the dextran release study ( $n = 3$ ), 5 mL of samples were collected and filtered (0.45  $\mu$ m) at predetermined time intervals, after which the amount of dextran in the solution was determined by SEC. The amounts of dextran released from the samples were determined by measuring peak heights, which were viewed against a linear calibration curve of dextran.



**Figure 1.** Cell viability after incubating 50  $\mu$ g/mL acid-terminated oligomers with human gingival fibroblasts for 24 h (mean  $\pm$  SD,  $n = 6$ ). There are no statistical differences ( $p < 0.05$ ) between groups. Acetone-treated cells were used as a control group.

**Cytotoxicity of Oligomers.** In vitro cytotoxicity of carboxyl acid-terminated oligomers was evaluated using the AlamarBlue test, which is based on the reduction of resazurin to resofurin in the cytosol of living cells. Human gingival fibroblasts were plated at a density of 25 000 cells/cm<sup>2</sup> in a 96-well plate and allowed to adhere for 24 h. A dilution series of acid-terminated oligomers (with or without alkenyl chain) were prepared in acetone and pipetted onto the top of the cells in six replicates. The final concentrations of acetone and oligomers were 2.5% and 50–400  $\mu$ g/mL, respectively. The cells were cultured for an additional 24 h and washed with PBS. Cell activities were determined using AlamarBlue assay (BioSource International) in colorimetric format. Measured absorbances (Multiskan MS ELISA plate reader, Labsystems, Finland; 560 and 595 nm) were used to calculate the reduction of AlamarBlue reagent in accordance with the manufacturer's instructions. The activity of acetone-treated cells was set to 100%.

## RESULTS AND DISCUSSION

The reaction scheme of the polymerizations is presented in Scheme 1. The synthesis and properties of the polyester oligomers functionalized with different succinic anhydrides have been previously described.<sup>25,29</sup> Cross-linkable poly(ester anhydride) precursors with labile anhydride bonds were obtained by allowing acid-terminated oligomers to react with methacrylic anhydride. Methacrylation of acid-terminated oligomers has been previously applied by Anseth et al.<sup>6</sup> to the preparation of cross-linked polyanhydrides, by Kim et al.<sup>33</sup> to the preparation of cross-linked poly(ether anhydrides), and by us<sup>28</sup> to the preparation of cross-linked poly(ester anhydrides). After purification, the methacrylated precursors were photo-cross-linked to obtain poly(ester anhydride) networks.

**In Vitro Cytotoxicity of the ASA-Functionalized Oligomers.** We have recently studied the in vivo and in vitro cytotoxicity of hydroxyl- and acid-terminated poly( $\epsilon$ -caprolactone) oligomers, methacrylated poly(ester anhydride) precursors, and cross-linked poly(ester anhydride) networks; the oligomers and precursors did not decrease cell viability in vitro, and the implanted discs (s.c., rats) did not evoke any cytokine activity in vivo.<sup>30</sup> The chemical components of the present study were the same as those previously used, except that ASA functionalization was

**Table 1. Properties of Methacrylated Poly(ester anhydride) Precursors**

precursor					<sup>13</sup> C NMR	theoretical		SEC	
	type of coinitt. <sup>a</sup>	amount of coinitt. (mol %) <sup>b</sup>	theoret. segment length (units) <sup>c</sup>	alkenyl chain length (carbons) <sup>d</sup>	DS (%)	M <sub>n</sub> (g/mol)	M <sub>n</sub> (g/mol)	M <sub>w</sub> (g/mol)	MWD
LIN-1000-0m	BD	10	4.5	0	93	1450	3300	3900	1.19
LIN-1000-8m	BD	10	4.5	8	97	1670	3400	4000	1.15
LIN-1000-12m	BD	10	4.5	12	94	1790	3900	4300	1.13
LIN-1000-18m	BD	10	4.5	18	95	1950	4800	5500	1.16
STAR-1000-0m	PERYT	10	2.25	0	94	1830	3800	4800	1.27
STAR-1000-8m	PERYT	10	2.25	8	98	2280	4300	5300	1.22
STAR-1000-12m	PERYT	10	2.25	12	95	2500	4800	5600	1.17
STAR-1000-18m	PERYT	10	2.25	18	96	2840	6400	7600	1.18

<sup>a</sup> Type of coinittiator, BD = 1,4-butanediol, PERYT = pentaerythritol. <sup>b</sup> Amount of the coinittiator (mol %). <sup>c</sup> Theoretical  $\epsilon$ -caprolactone segment length of the precursor ( $\epsilon$ -caprolactone units). <sup>d</sup> Amount of the carbons in the alkenyl chain.

used instead of SAH functionalization. Burkoth et al.<sup>21</sup> reported that during the hydrolytic degradation of the photo-cross-linked polyanhydride networks, the anhydride bonds were cleaved, leading to the formation of constituent diacids and water-soluble poly(methacrylic acid). It is presumed that similar to cross-linked polyanhydrides, degradation of the poly(ester anhydrides) cross-linked with methacrylate double bonds yields constituent acid-terminated oligomers and poly(methacrylic acid). For the preliminary safety studies, we evaluated the cytotoxicity of the most likely degradation products, that is, acid-terminated oligomers containing 8, 12, or 18 carbon alkenyl chains.

As seen in Figure 1, in *in vitro* cytotoxicity tests, the low-molecular-weight linear and star-shaped ASA-functionalized oligomers were noncytotoxic at the level of their solubility. Cell activities with oligomer concentrations below 50  $\mu\text{g}/\text{mL}$  were in the range of 85–108%, and no statistically significant differences were found between the acetone-treated control cells and oligomer-treated cells. At concentrations above 100  $\mu\text{g}/\text{mL}$ , the oligomers precipitated onto the cells in aqueous solution. These precipitates dose-dependently decreased cell activities (data not shown) because they hindered the cellular transport of gases and nutrients essential for viability. The experiments indicated that adding the alkenyl chain did not influence the cytotoxicity and that ASA-functionalized oligomers are therefore suitable for the subsequent steps toward biodegradable active agent release applications.

**Methacrylated Precursors.** An overview of the polymerizations is shown in Table 1. The chemical structures of the resulting methacrylated precursors were confirmed from ATR-FTIR and NMR spectra. The ATR-FTIR spectra were dominated by the ester carbonyl absorbance of lactone units at  $\sim 1728\text{ cm}^{-1}$ . The methacrylation created absorbance due to the anhydride bond and the methacryl double bond at 1788, 1803, and  $1636\text{ cm}^{-1}$ , and, as expected, the broad acid absorbance from  $3000\text{ to }3500\text{ cm}^{-1}$  disappeared. The characteristic peaks of a methacrylate double bond can be seen in the <sup>1</sup>H NMR spectra at 6.20 and 5.79 ppm (Figure 2a) and in the <sup>13</sup>C NMR spectra at 129.41 ppm (Figure 2b). For the ASA-functionalized methacrylated precursors, the extent of methacrylation was calculated from the <sup>13</sup>C NMR spectra (Figure 2b) as the ratio of the sum of the peak integrals of carbons c and b' before and after the methacrylation.<sup>29</sup> The degree of substitution for the methacrylations was found to be >93% for all linear and star-shaped network

precursors, in agreement with previous methacrylations of acid-terminated oligomers.<sup>28,33</sup>

The changes in molecular weight during the methacrylation were followed by SEC (Table 1). Helminen et al.<sup>28</sup> and Kim et al.<sup>33</sup> found the molecular weight of dimethacrylated anhydride precursors to increase during drying under high vacuum and suggested that this was due to polycondensation of the anhydride linkages. In the present study, the vacuum drying was done as rapidly as possible, and the disappearance of the solvent was followed by <sup>1</sup>H NMR. As in the ASA functionalizations,<sup>29</sup> methacrylation did not change the molecular weights significantly, and the molecular weight distributions remained narrow.

**Photo-Cross-Linked Networks.** The final aim of our work was to prepare matrixes for controlled delivery of sensitive protein- and peptide-based drugs. Our strategy in this work was, through photo-cross-linking of low-molecular-weight liquid precursors, to use poly(ester anhydride) networks that could be prepared at room temperature and without solvents. The cross-linked samples were analyzed by ATR-FTIR and DSC as well as in extraction and swelling tests. These methods are suitable for cross-linked samples because they do not require the sample to be soluble. The gel contents, degree of swellings, and amounts of reacted double bonds of the photo-cross-linked poly(ester anhydride) networks are presented in Table 2. Unwanted degradation of poly(ester anhydrides)<sup>34</sup> and polyanhydrides<sup>35</sup> during storage has been reported. Because anhydride bonds are moisture-sensitive, polymers containing anhydride bonds are best stored at low temperatures under an inert atmosphere.<sup>34</sup> The effect of the alkenyl chain to the stability of the photo-cross-linked samples was also in our interest; therefore, gel contents of the samples kept at  $-20\text{ }^{\circ}\text{C}$  for 50 days were also studied.

The ASA-functionalized linear precursors appeared as viscous liquids and were photo-cross-linkable at room temperature, whereas the SAH-functionalized linear precursor (LIN-1000-0m) had to be heated to  $37\text{ }^{\circ}\text{C}$  to obtain less viscous precursor suitable for the photo-cross-linking. Networks prepared from the ASA-functionalized linear precursors had gel contents from 78 to 84% and swelling degrees from 380 to 490%, whereas the SAH-functionalized network (LIN-1000-0nw) had clearly lower gel content (48%) and higher swelling degree (800%) (Table 2). The low gel content and looser network affected the storage stability of the SAH-functionalized network. When the samples were kept in a freezer ( $-20\text{ }^{\circ}\text{C}$ ) for 50 days, the gel content of SAH-functionalized discs decreased 90%, whereas the ASA-

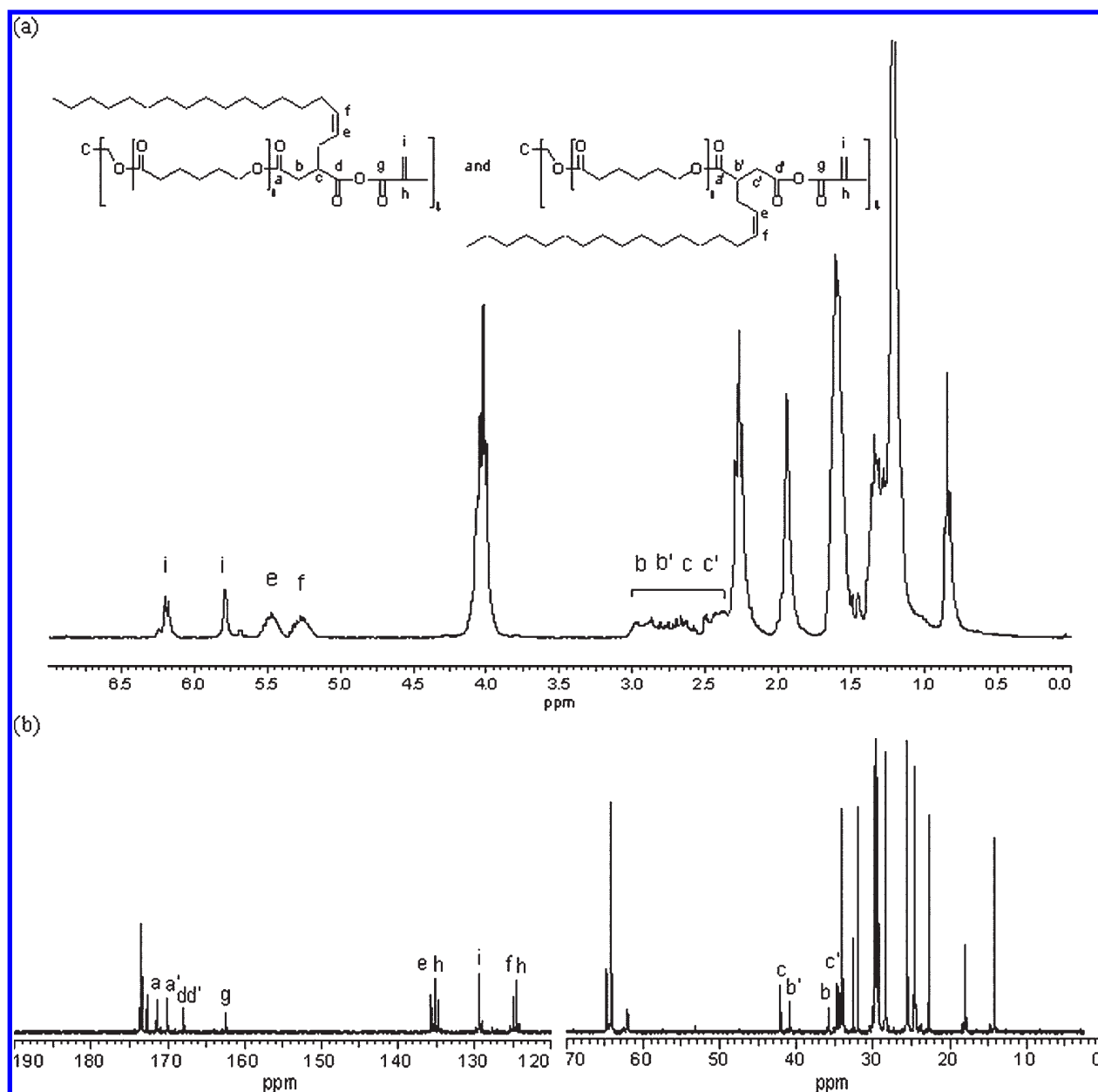
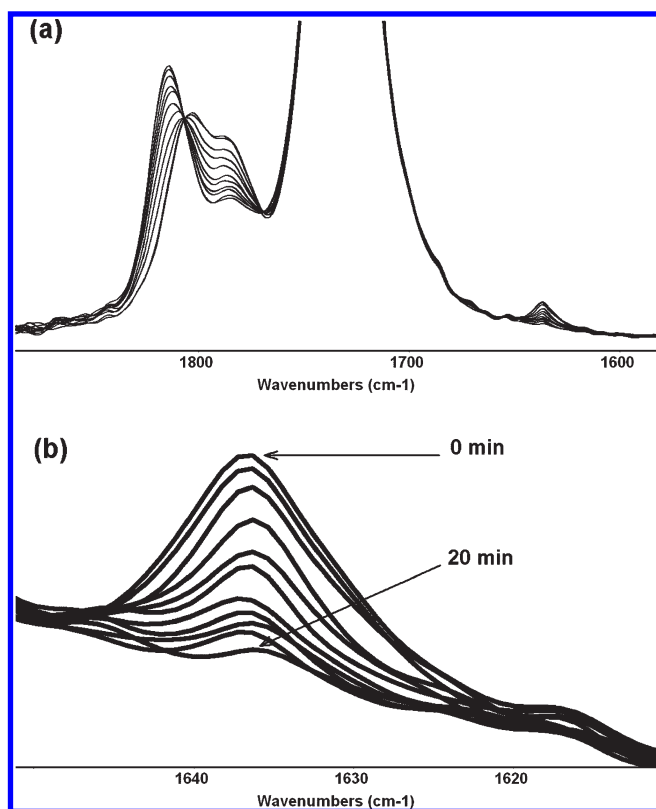


Figure 2.  $^1\text{H}$  NMR (a) and  $^{13}\text{C}$  NMR (b) spectra of methacrylated STAR-1000-18m precursor.

Table 2. Properties of Photo-Cross-Linked Poly(ester anhydride) Networks

cross-linked network	cross-linking time (min)	gel content		swelling degree (%)	amount of reacted double bonds (%)
		cross-linked (%)	cross-linked and stored (%) <sup>a</sup>		
LIN-1000-0nw	28	47.5 ± 3.7	4.8 ± 1.3	800 ± 150	90
LIN-1000-8nw	30	83.0 ± 0.2	74.6 ± 0.5	380 ± 30	81
LIN-1000-12nw	32	84.1 ± 0.9	79.7 ± 1.2	380 ± 30	80
LIN-1000-18nw	32	78.2 ± 0.5	74.5 ± 0.2	490 ± 60	75
STAR-1000-0nw	18	94.6 ± 0.3	93.6 ± 0.9	250 ± 10	93
STAR-1000-8nw	20	95.6 ± 0.7	95.1 ± 0.3	190 ± 10	93
STAR-1000-12nw	22	96.6 ± 0.2	95.9 ± 0.5	190 ± 10	91
STAR-1000-18nw	22	96.0 ± 1.2	95.6 ± 0.4	220 ± 10	91

<sup>a</sup> Stored in freezer (−20 °C) 50 days.

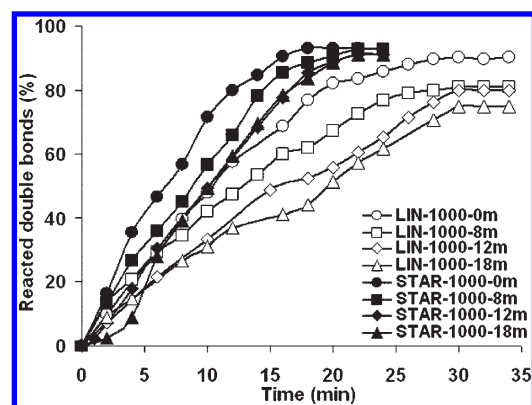


**Figure 3.** ATR-FTIR spectra of anhydride bond at  $1814\text{ cm}^{-1}$  (a) and double bond at  $1636\text{ cm}^{-1}$  (b) of STAR-1000-18m precursor recorded during photo-cross-linking.

functionalized discoids lost only 4–10% of their gel content. The better storage stability of the ASA-functionalized discoids was evidently due to the increased hydrophobicity of the discoids, which was achieved by adding hydrophobic alkenyl chains to the oligomers.

Changing the molecular architecture of the precursor from linear to star-shaped had a clear increasing effect on the gel content and decreasing effect on the swelling degree of the cross-linked networks. As seen in Table 2, the gel contents of the networks prepared from star-shaped precursors were all >94%, whereas those of the corresponding networks prepared from linear precursors were 12–47% lower. The difference was evidently due to the larger amount of double bonds in the star-shaped precursors. The swelling results showed the networks formed from star-shaped precursors to be denser (swelling degrees 190–250%) than those formed from the corresponding linear precursors (swelling degrees 380–800%). The denser networks were due to the chain length of the precursor arm, which was half as long in the star-shaped precursor as in the linear counterpart. Finally, adding an alkenyl chain or increasing the alkenyl chain length in the star-shaped precursors did not affect the gel content, swelling degree, or stability in storage (50 days at  $-20\text{ }^{\circ}\text{C}$ ).

The photo-cross-linking was also followed by ATR-FTIR. The experimental set up, that is, light source, light intensity, light distance, sample size, and use of Teflon film, was the same as in the preparation of molded samples. During photo-cross-linking of 20 min, the anhydride bond moved from 1788 and  $1803\text{ cm}^{-1}$  to  $1814\text{ cm}^{-1}$ , as seen in Figure 3a, and the methacryl double bond at  $1636\text{ cm}^{-1}$  decreased in intensity, as shown more closely in Figure 3b.



**Figure 4.** Amounts of the reacted double bonds of linear and star-shaped precursors as a function of photo-cross-linking time.

Figure 4 shows the amounts of reacted double bonds as a function of time for the photo-cross-linked poly(ester anhydride) networks. In general, when the molecular architecture of the precursor was changed from linear to star-shaped, or when the alkenyl chain length of ASA was decreased from 18 to 8 carbons, the crosslinking time decreased, and the amounts of reacted double bonds increased. In addition, the amounts of the reacted double bonds were at the same level as the gel contents, except for the network prepared from SAH-functionalized linear precursor (Table 2). For the LIN-1000-0nw network, the amount of the reacted double bonds was high (90%), although the gel content was low (48%), indicating that most of the double bonds react, but the labile anhydride bonds degrade during preparation of the sample.

**Thermal Properties.** Thermal properties of the methacrylated poly(ester anhydride) precursors and the photo-cross-linked networks are summarized in Table 3. In general, methacrylation of the acid-terminated oligomers resulted in lower  $T_g$  and  $T_m$  values and changed the physical state at room temperature to less viscous. As for the previous reported acid-terminated low-molecular-weight oligomers,<sup>29</sup> the star-shaped 18-ASA-functionalized methacrylated precursor (STAR-1000-18m) was the only star-shaped precursor to show melting transitions. This was apparently due to the 18-carbon alkenyl chain, which was long enough to crystallize. Similar crystallization of the alkenyl chain has been reported for stearyl side chains.<sup>36,37</sup>

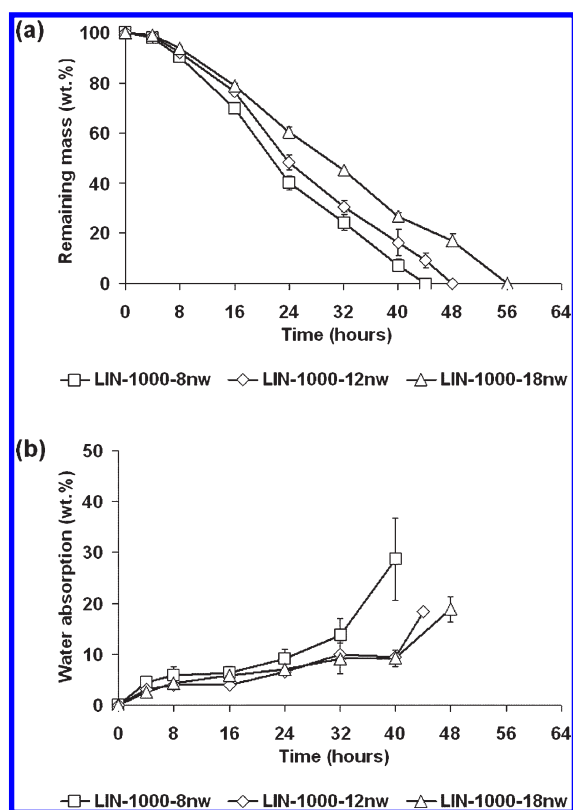
As expected, photo-cross-linking increased  $T_g$  and changed the state of all samples from viscous liquid to solid elastic network (Table 3). The greater increase in  $T_g$  for the star-shaped networks ( $\sim 10\text{ }^{\circ}\text{C}$ ) than for the linear networks ( $\sim 3\text{ }^{\circ}\text{C}$ ) may be related to the shorter arms of the star-shaped precursors and the higher cross-linking density because the distance between the cross-links decreases and the precursor chains become more tightly connected to each other. The presence of the alkenyl chains did not markedly affect  $T_g$  and  $T_g$  could not even be detected in the STAR-1000-18nw network, just as it could not be detected in the corresponding methacrylated precursor. Melting transitions of cross-linked networks were observed only for networks prepared from linear precursors and the STAR-1000-18nw network. Evidently, in these networks, the polymer main chain or the 18 carbon alkenyl chain was long enough to crystallize.

**Mass Loss and Water Absorption.** During the dissolution studies of the cross-linked discoids, no change in pH was observed. Mass loss and water absorption of the cross-linked

**Table 3. Thermal Properties of Methacrylated Poly(ester anhydride) Precursors and the Photo-Cross-Linked Poly(ester anhydride) Networks**

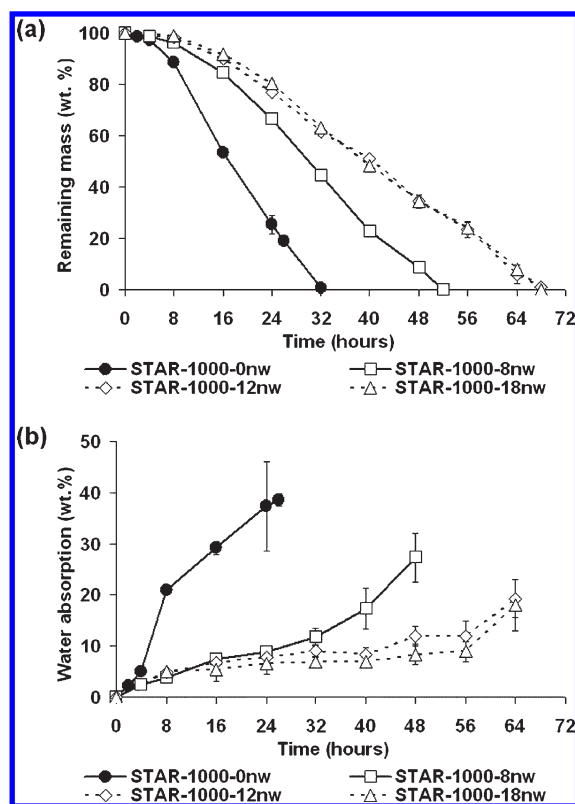
oligomer	methacrylated precursor (m)			cross-linked network (nw)		
	$T_g$ (°C)	$T_m$ (°C)	$\Delta H$ (J/g)	$T_g$ (°C)	$T_m$ (°C)	$\Delta H$ (J/g)
LIN-1000-0	-60	25	66	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>
LIN-1000-8	-63	12	42	-61	14	34
LIN-1000-12	-64	12	46	-61	14	38
LIN-1000-18	-60	8	36	-57	22	27
STAR-1000-0	-54	<sup>c</sup>	<sup>c</sup>	-44	<sup>c</sup>	<sup>c</sup>
STAR-1000-8	-58	<sup>c</sup>	<sup>c</sup>	-48	<sup>c</sup>	<sup>c</sup>
STAR-1000-12	-58	<sup>c</sup>	<sup>c</sup>	-48	<sup>c</sup>	<sup>c</sup>
STAR-1000-18	<sup>a</sup>	9	39	<sup>a</sup>	16	35

<sup>a</sup> Not detected. <sup>b</sup> Not measured because of low gel content. <sup>c</sup> Amorphous.



**Figure 5.** Mass loss (a) and water absorption (b) of poly(ester anhydride) networks prepared from linear precursors.

samples prepared from the linear precursors are shown in Figure 5. In view of the clearly lower degree of cross-linking, the SAH-functionalized network (i.e., network prepared from the linear precursor without alkenyl chain) was not studied in the dissolution test. As shown in Figure 5a, lengthening of the alkenyl chain from 8 to 12 or 18 carbons, that is, increasing the hydrophobicity, did not significantly affect the erosion time. The water absorption of all samples was low (<15%) until ~80 to 90% of the sample mass was lost (Figure 5b). Evidently, the increase in hydrophobicity was not sufficient to decrease the

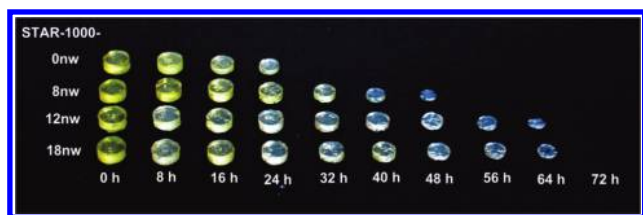


**Figure 6.** Mass loss (a) and water absorption (b) of poly(ester anhydride) networks prepared from star-shaped precursors.

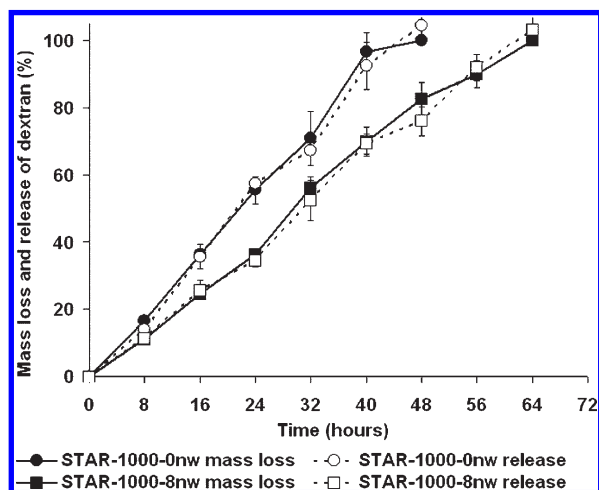
erosion rate or water absorption of the discs prepared from linear precursors. However, the samples showed clear signs of surface erosion: a linear mass loss but an almost intact core.

Changing the molecular architecture of the precursor from linear to star-shaped doubled the amount of alkenyl chains and thus increased the hydrophobicity of the cross-linked discs. The sample prepared from the star-shaped SAH-functionalized precursor (STAR-1000-0m), that is, network without alkenyl chains, eroded linearly from the surface, after 4 h of lag time, in 32 h (Figure 6a). Adding the eight-carbon alkenyl chain to the star-shaped precursor had a clear effect on the erosion time of the network: lag time was doubled to 8 h and erosion time to 52 h (Figure 6a). The eight-carbon alkenyl chain also exhibited decreased water absorption relative to the network without alkenyl chain (Figure 6b). As shown in Figure 7, 8-ASA-functionalized cross-linked discs eroded from the surface, similarly to the SAH-functionalized cross-linked discoid. Degradation products of the 8-ASA-functionalized networks caused the buffer solution to become slightly opaque, whereas even after complete degradation of SAH-functionalized networks the buffer solution was transparent.

Increase in the alkenyl chain length in the star-shaped precursors from 8 to 12 or 18 carbons prolonged the erosion of the photo-cross-linked discoid (Figure 6a). Moreover, the erosion was clearly different from that of the discs prepared from the star-shaped precursor without alkenyl chain or with eight-carbon alkenyl chain. Whereas the degradation products of the other discs dissolved in the buffer solution, the degradation products of the networks prepared from star-shaped 12- and 18-ASA-functionalized precursors (STAR-1000-12nw and STAR-1000-18nw) precipitated on the surface of the discs.



**Figure 7.** Photograph of poly(ester anhydride) discoids prepared from star-shaped precursors.



**Figure 8.** Mass loss of the dextran containing discoids and dextran release of photo-cross-linked poly(ester anhydride) networks (STAR-1000-0nw and STAR-1000-8nw).

Beneath the degradation products was intact discoid, the erosion having occurred only on the surface (Figure 7). As Göpferich et al.<sup>38</sup> have reported for polyanhydride copolymers consisting of a fatty acid dimer and sebacic acid, precipitation is due to poor solubility of the degradation products, which accumulate on the surface of the cross-linked discoids.

**Macromolecular Release.** In our previous drug release study on photo-cross-linked poly(ester anhydrides) without alkenyl chains (STAR-1000-0nw), we found samples with water-soluble small-molecule drug (propranolol HCl,  $M_w$  296 g/mol) to display high degrees of drug loading (10–60 wt %) and to be capable of surface erosion-controlled active agent release in vitro and in vivo.<sup>30</sup> In the present study, we used high-molecular-weight dextran ( $M_r \sim 2\,000\,000$  g/mol) as a model compound to simulate the release of macromolecular active agents that cannot diffuse out from the cross-linked matrix. The macromolecular release studies were carried out on two photo-cross-linked poly(ester anhydride) networks: one prepared from the star-shaped precursor without alkenyl chain and the other from the more hydrophobic precursor containing eight-carbon alkenyl chain. These precursors were selected for study because they were viscous liquids and photocurable, and the cross-linked networks prepared from them had high degrees of cross-linking and exhibited clear surface erosion without precipitation of degradation products. We presumed that surface erosion of the cross-linked network would enable linear release of the macromolecular model compound just as for the smaller model compound (propranolol HCl) and that increasing the hydrophobicity would delay the release. Photo-cross-linking was done in the same way, in the light-curing oven, as in our previous study of propranolol

HCl release. After photo-cross-linking, the dextran-containing samples were elastic and had gel contents of >95%.

The mass loss and the quantity of dextran released during the erosion as a function of time are shown for the photo-cross-linked STAR-1000-0nw and STAR-1000-8nw samples in Figure 8. The quantity of dextran released was nearly linear and proportional to the decrease in the mass. In addition, the dimensions of the specimen decreased steadily. The degradation behavior and the release of the model compound from these two samples are typical for surface erodable polymers. It is also evident from Figure 8 that the network prepared from the 8-ASA-functionalized precursor, that is, the more hydrophobic precursor, degraded more slowly. Tailoring the precursor structure, in this case by increasing the hydrophobicity of the cross-linkable precursor, clearly affects the degradation and release rates of the photo-cross-linked samples. The results show the potential of these materials for applications requiring controlled short-term release of high-molecular-weight compounds.

## CONCLUSIONS

Biodegradable poly(ester anhydride) networks based on alkenylsuccinic anhydride functionalized linear and star-shaped poly( $\epsilon$ -caprolactone) precursors were synthesized. Change in the molecular structure of the precursor from linear to star-shaped increased cross-linking density and raised the gel content from  $\sim 80$  to 95%. The networks showed clear signs of surface erosion: a linear mass loss but practically intact core. Tailoring the star-shaped precursor with alkenyl chains prolonged the degradation of the network. Whereas the degradation products of the less hydrophobic networks were readily dissolvable in the buffer solution, the degradation products of the networks prepared from star-shaped 12- and 18-ASA-functionalized precursors precipitated on the surface of the discoids. Study of high-molecular-weight dextran release showed linear release of the model compound, which was proportional to the mass loss. These photo-cross-linked poly(ester anhydride) networks are expected to be suitable for applications requiring controlled release of macromolecular pharmaceutical agents.

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