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Photo-crosslinked networks prepared from fumaric acid monoethyl ester-functionalized poly(D,L-lactic acid) oligomers and N-vinyl-2-pyrrolidone for the controlled and sustained release of proteins

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ABSTRACT

Photo-crosslinked networks were prepared from fumaric acid monoethyl ester-functionalized poly (D,L-lactic acid) oligomers and N-vinyl-2-pyrrolidone. Two model proteins, lysozyme and albumin, were incorporated into the network films as solid particles and their release behavior was studied. By varying the NVP content and macromer molecular weight the degradation behavior and protein release profiles of the prepared networks could be tuned. The more hydrophilic and less densely crosslinked networks released albumin and lysozyme at a faster rate. Although active lysozyme was released from the networks over the complete release period, lysozyme release was often incomplete. This was most likely caused by electrostatic and/or hydrophobic interactions between the protein and the degrading polymer network.

1. Introduction

In the past decade several researchers have investigated the use of photo-crosslinked biodegradable polymer networks for controlled drug delivery applications [1,2]. A major advantage of photo-crosslinking is that a drug can easily be entrapped in a network by dispersing or dissolving it in a macromer solution prior to crosslinking. In this way large amounts of drug can be loaded at high efficiencies. Furthermore, photo-polymerization is rapid and can be accomplished with minimal heat generation, allowing the incorporation of heat-sensitive compounds such as proteins.

Poly(D,L-lactic acid) (PDLLA) is a well-known polymer that has been studied extensively for application in biodegradable drug delivery systems [3]. Networks based on PDLLA can be prepared by photo-initiated crosslinking of functionalized PDLLA oligomers. Besides the frequently used methacrylate derivatives, fumaric acid derivatives are also attractive compounds for endfunctionalization reactions. It can be expected that residual unreacted fumarate endgroups will not lead to toxicity upon implantation since fumaric acid is a compound naturally found in the body [4]. The relatively low reactivity of fumarate endgroups can be overcome by choosing an appropriate co-monomer for the photo-initiated crosslinking reaction, such as N-vinyl-2-pyrrolidone (NVP) [5].

Many photo-crosslinked polymer networks have been studied as drug delivery systems. These include photo-crosslinked highly swollen hydrogels [6–8] and more hydrophobic networks based on PDLLA, poly(ε-caprolactone), poly(trimethylene carbonate) (PTMC) or co-polymers of these three [9–12]. Drug release profiles can be tuned by varying the crosslink density or by adjusting the hydrophilicity of crosslinked polymer networks. Several authors have used the incorporation of poly(ethylene glycol) (PEG) to increase network hydrophilicity and adjust drug release profiles [13–15]. Increasing the NVP content of networks prepared from fumaric acid derivatives was shown to also increase network hydrophilicity and drug release rates [16–18].

Controlled and sustained release of protein drugs is challenging due to the large size and the relative instability of these molecules. During preparation, storage and release a range of conditions may affect the stability of a protein [19,20]. Important in this respect is the possible presence of residual organic solvents. When incorporating protein drugs in photo-crosslinked polymer networks care

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should be taken to prevent side reactions of free radicals with the protein [21]. However, when solid protein particles are dispersed in an organic phase containing the macromer and photo-initiator contact with free radicals is limited since the protein remains in the solid form during the photo-polymerization reaction [22].

In previous research we prepared biocompatible tissue engineering scaffolds by stereolithography from fumaric acid monoethyl ester (FAME) functionalized PDLLA oligomers and NVP [18]. Furthermore, we investigated PTMC oligomers functionalized with FAME endgroups for the controlled release of model drugs and proteins [5,23]. Due to slow degradation of the PTMC-based networks only low molecular weight model drugs and proteins could be released over a time period of several months. To allow for the release of high molecular weight proteins in a controlled and sustained way in this paper we describe the preparation of photo-crosslinked polymer networks from PDLLA-FAME macromers and NVP. The crosslink density and hydrophilicity of the networks were varied by using macromers of different molecular weights and different NVP concentrations. The degradation behavior of the networks and the release of two model proteins, lysozyme and bovine serum albumin, from the prepared networks were studied.

2. Materials and methods

2.1. Materials

D,L-Lactide (DLLA) was purchased from Purac Biochem (The Netherlands). Tin 2-ethylhexanoate ($Sn(Oct)_2$), trimethylol propane (TMP), fumaric acid monoethyl ester (FAME), deuterated chloroform, bovine serum albumin, lysozyme, *Micrococcus lysodeikticus* and acetate buffer solution (pH 4.7) were obtained from Sigma Aldrich (St. Louis, MO). N-Vinyl-2-pyrrolidone (NVP) and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Fluka (Switzerland). 4-Dimethylaminopyridine (DMAP) was purchased from Merck (Germany). Irgacure 2959 (2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) was obtained from Ciba Specialty Chemicals (Switzerland). The BCA (bicinchoninic acid) protein assay kit was obtained from Thermo Fisher Scientific (Waltham, MA). Phosphate-buffered saline (PBS), pH 7.4 was obtained from B. Braun (Germany). Analytical grade dichloromethane (DCM) was obtained from Biosolve (The Netherlands). DCM was dried over CaH_2 and distilled. The other solvents were of technical grade and were used as received (Biosolve, The Netherlands).

2.2. Synthesis and characterization of three-armed FAME-functionalized PDLLA oligomers

Three-armed poly(D,L-lactide) oligomers were synthesized by ring opening polymerization of D,L-lactide in the presence of trimethylol propane (TMP) as a trifunctional initiator (Fig. 1). The oligomer syntheses were carried out on the 40 g scale. DLLA, initiator and $Sn(Oct)_2$ (approximately 0.2 mmol mol^{-1} monomer) as a catalyst were reacted in the melt at 130 °C for 48 h under argon. The targeted molecular weights were 6.1, 9.1 and 12.1 kg mol^{-1}, corresponding to approximately 14, 21 and 28 D,L-lactide units per arm, respectively. To achieve this 42, 63 and 84 mol of monomer were used per mol of initiator.

The oligomers were functionalized by coupling fumaric acid monoethyl ester to the hydroxyl termini of the oligomers (Fig. 1) [24,25]. An amount of oligomer was charged into a three-necked flask and dried for 2 h at 110 °C in vacuo and cooled to room temperature under argon. The oligomers were dissolved in dried DCM, and after addition and dissolution of FAME the system was further cooled to 0 °C. Then a DCM solution of DCC and DMAP was added

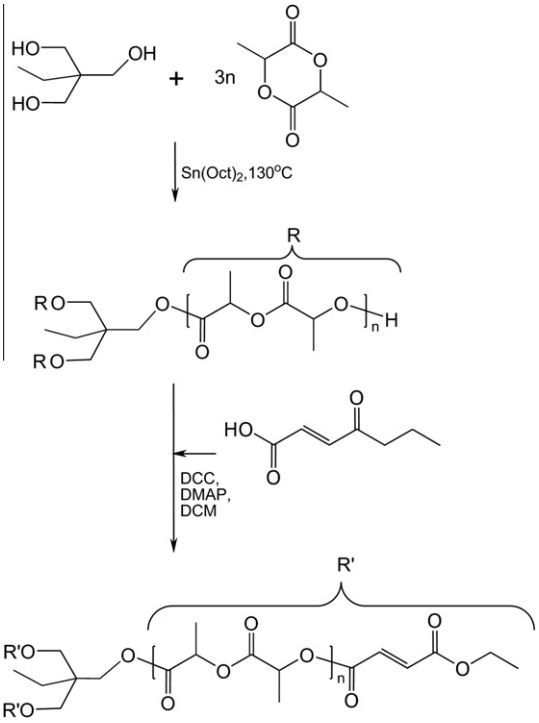

Fig. 1. Synthesis of FAME-functionalized PDLLA oligomers.

drop-wise to the vigorously stirred oligomer solution. In the coupling reaction 1.2 mol of FAME and DCC and 0.03 mol of DMAP were used per mole of hydroxyl endgroups. The coupling reaction was continued overnight, letting the contents slowly warm up to room temperature. After completion of the reaction the formed dicyclohexylurea was removed by filtration. The macromers were purified by precipitation in cold isopropanol, washing with water and freeze-drying.

The macromers are labeled PDLLA 3XMW-FAME, in which 3 is the number of arms (the same for all macromers) and MW is the molecular weight per arm. For example, a PDLLA 3X3K-FAME macromer has a molecular weight of 3000 g mol^{-1} per arm and an overall molecular weight of approximately 9000 g mol^{-1}.

Proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded in a Varian Inova 300 MHz NMR spectrometer. Deuterated chloroform was used as a solvent. The oligomer and macromer number average molecular weights (M_n) and the degrees of functionalization of the macromers were determined from the spectra.

2.3. Preparation and characterization of PDLLA-FAME/NVP network films

Disk-shaped network specimens were prepared from FAME-functionalized PDLLA oligomers. Macromers were dissolved in NVP at different concentrations (30, 40 and 50 wt.% NVP of the total mass). To each formulation 1 wt.% (with respect to the macromer) of Irgacure 2959 photo-initiator was added. The solutions were poured into a Teflon mold, covered with a thin fluorinated ethylene propylene(FEP) foil, and photo-crosslinked in an Ultralum crosslinking cabinet (365 nm, 3–5 mW cm^{-2}, 15 min). Disk-shaped samples with a diameter of approximately 10 mm and thickness of approximately 0.5 mm were obtained.

To determine the gel content after crosslinking a network specimen ($n = 3$) was weighed (m_0), extracted with acetone overnight and dried at 90 °C until a constant weight was achieved. The mass

of the dry network (m_1) was then determined. The gel content is defined as:

$$\text{gel content} = \frac{m_1}{m_0} \times 100\%$$

To determine the water uptake a network specimen (non-extracted, $n = 3$) was dried at 90 °C overnight, weighed (m_d), and incubated in distilled water for 1 day. Then the samples were removed from the water, blotted dry, and weighed again (m_s). The water uptake was calculated using:

$$\text{water uptake} = \frac{m_s - m_d}{m_d} \times 100\%$$

The thermal properties of macromers and non-extracted networks were evaluated using a Perkin Elmer Pyris 1 differential scanning calorimeter. Network specimens were dried at 90 °C overnight prior to the measurements. Samples were heated from −50 °C to 150 °C at a heating rate of 10 °C min^{-1} and quenched rapidly at 300 °C min^{-1} to 70 °C. After 5 min a second heating scan was recorded. The glass transition temperature (T_g) was taken as the midpoint of the heat capacity change in the second heating run.

2.4. Hydrolytic degradation of PDLLA-FAME/NVP network films

To study the hydrolytic degradation of the photo-crosslinked networks disk-shaped specimens (non-extracted, diameter 10 mm, thickness 0.5 mm) were immersed in 2 ml of PBS (37 °C) containing 0.02 wt.% NaN_3 as a bactericide. At different time points specimens ($n = 2$) were blotted dry and weighed to determine the wet mass, dried at 90 °C under a nitrogen flow until constant weight, and then weighed to determine the dry mass.

2.5. Protein release from PDLLA-FAME/NVP network films

Lysozyme (14 kDa) and bovine serum albumin (66 kDa) were used as model protein drugs, and were ground and sieved into particles smaller than 106 μm. The proteins (10 wt.%) were dispersed in different solutions of FAME-functionalized PDLLA oligomers (30, 40 or 50 wt.% NVP and 1 wt.% Irgacure 2959). The mixtures were stirred and poured into Teflon molds (diameter 10 mm, height 0.5 mm). The dispersions were crosslinked in a nitrogen atmosphere for 15 min at 3–5 mW cm^{-2} (Ultralum crosslinking cabinet, 365 nm). Crosslinking was done immediately after preparing the dispersions, therefore sedimentation of the particles did not occur.

The protein-loaded specimens ($n = 3$) were removed from the molds and immersed in 3 ml of PBS (pH 7.4, 37 °C) containing 0.02 wt.% NaN_3 as a bactericide. In the release experiments aliquots of 0.5 ml were taken at different time points and replaced by 0.5 ml of fresh PBS. In the determination of the amounts released corrections were made for these dilutions. Lysozyme and albumin concentrations were determined using the BCA (bicinchoninic acid) protein assay [26]. To determine the activity of the released lysozyme additional lysozyme-loaded specimens were incubated in 2 ml of PBS and all PBS was replaced each week. The lysozyme concentration in these solutions was determined using the BCA assay and the lysozyme activity was determined using the *M. lysodeikticus* lysis assay [27]. Sink conditions were maintained at all times.

Scanning electron microscopy (SEM) (Philips XL30 operated at 2 keV) was used to analyze the protein particles and protein-loaded specimens. Samples were sputter-coated with gold prior to analysis.

Table 1
Characteristics of the prepared macromers.

Macromer	M_n (kg mol^{-1} per arm)[a]	p (%)[a]	T_g (°C)[b]
PDLLA 3X2K-FAME	2.3	100	44.7
PDLLA 3X3K-FAME	3.0	97	47.7
PDLLA 3X4K-FAME	3.8	100	49.6

[a] Determined by ^{1}HNMR.
[b] Determined by DSC.

3. Results and discussion

3.1. Synthesis and characterization of three-armed FAME-functionalized PDLLA oligomers

With trimethylol propane as a trifunctional initiator D,L-lactide was polymerized by ring opening polymerization to yield three-armed oligomers with targeted molecular weights of 2000, 3000 and 4000 g mol^{-1} per arm. The molecular weights obtained, as determined from the TMP to D,L-lactide ratios in ^{1}HNMR spectra, were close to the targeted molecular weights (Table 1).

Fumaric acid monoethyl ester was coupled to the oligomers to obtain crosslinkable macromers. The successful coupling of FAME to the PDLLA oligomers was confirmed by the appearance of the –CH=CH–, –CH_2– and –CH_3 peaks of FAME at δ 6.91, δ 4.28, and δ 1.22 in the ^{1}HNMR spectra of the precipitated reaction products. The degrees of functionalization (p) of the macromers were determined using the integral values of the –CH_3 peak of FAME at δ 1.22 and the –CH_3 of TMP at δ 0.88. For all macromers degrees of functionalization close to 100% were obtained (Table 1).

The glass transition temperatures of the macromers were determined by DSC and are presented in Table 1. As expected, the glass transition temperatures of the macromers increase with increasing molecular weight.

3.2. Characterization of PDLLA-FAME/NVP network films

Networks were formed from FAME-functionalized PDLLA oligomers by UV irradiation using NVP as a reactive diluent and Irgacure 2959 as a photo-initiator. Macromers of different molecular weights where used and the NVP content was varied from 30 to 50 wt.%. The influence of NVP content and macromer molecular weight on the network properties were evaluated.

In all cases the network gel content was close to 90% (Fig. 2), indicating efficient network formation. As was previously found [18], the water uptake of the networks increased with increasing

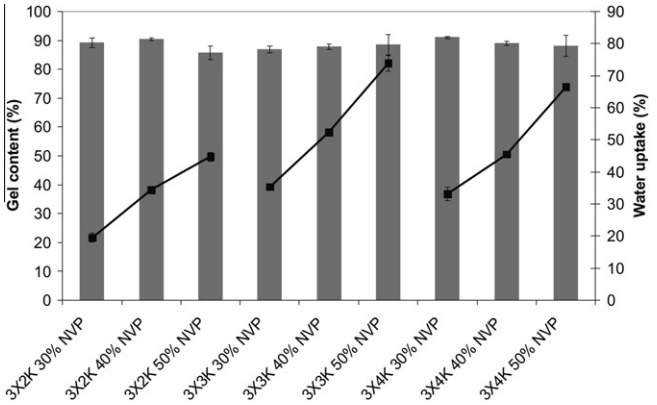

Fig. 2. Gel content (bars) and water uptake (lines) of networks prepared from PDLLA-FAME macromers of different molecular weights and with different amounts of NVP.

Table 2
Glass transition temperatures of dry, non-extracted network specimens.

Macromer	NVP content (wt.%)	T_g (°C)
PDLLA 3X2K-FAME	30	56.9
PDLLA 3X2K-FAME	40	66.0
PDLLA 3X2K-FAME	50	66.3
PDLLA 3X3K-FAME	30	59.1
PDLLA 3X3K-FAME	40	60.5
PDLLA 3X3K-FAME	50	60.9
PDLLA 3X4K-FAME	30	62.8
PDLLA 3X4K-FAME	40	65.6
PDLLA 3X4K-FAME	50	69.1
High MW PDLLA[a]	0	55.1
High MW PVP[a,b]	100	179

[a] T_g values of high MW PDLLA and high MW PVP are given as a reference.
[b] Data from Brandrup et al. [29] $M_w = 9 \times 10^5$ g mol^{-1}.

NVP content. This is due to the hydrophilic character of NVP. When networks with the same NVP content prepared from PDLLA 3X2K-FAME macromers and PDLLA 3X3K-FAME macromers are compared it can be seen that the networks prepared from the higher molecular weight macromers absorb more water. This is in agreement with previous findings [5,23] and is most likely a result of the larger meshsize of these networks. However, if the molecular weight of the macromers is increased from 3000 to 4000 g mol^{-1} per arm no further increase in water uptake is observed. Phase separation possibly plays a role here. With increasing macromer molecular weight the network meshsize increases and the number of FAME groups per FAME/NVP chain decreases. This may enable some phase separation of the hydrophobic PDLLA chains and the hydrophilic FAME/NVP chains and lead to a different network structure in the swollen state. Another possible explanation could be that in networks prepared from higher molecular weight macromers more entanglements are present that can restrict network swelling.

The glass transition temperatures of dry non-extracted networks were determined by differential scanning calorimetry (DSC) and are presented in Table 2. It can been seen that the networks show higher T_g values than the macromers (Table 1). Furthermore, the T_g increases with increasing NVP content and the T_g values found are higher than that of high molecular weight PDLLA. It is expected that in the dry state the T_g of the networks will increase with increasing NVP content, because the T_g of high molecular weight poly(N-vinyl-2-pyrrolidone) (PVP) is relatively high (T_g is 170 °C). For all networks a single T_g was observed, which indicates that the NVP-containing chains formed a single phase with the macromers. All PDLLA-FAME/NVP networks showed T_g values between 56 °C and 70 °C. No clear trend as a function of macromer molecular weight was found. Although it could be expected that networks prepared from lower molecular weight macromers would have higher crosslink densities and would therefore display a higher T_g [28], this was not observed. It should be noted that non-extracted samples were analyzed and any unreacted NVP may have acted as a plasticizer and have decreased the T_g. Furthermore, the glass transitions of these networks are rather broad and therefore small differences between samples are difficult to detect.

3.3. Hydrolytic degradation of PDLLA-FAME/NVP network films

In the prepared networks both the NVP content and the macromer molecular weight were varied. To understand the effect of these factors on network degradation a clear view of the network structure is required. In Fig. 3 the proposed network structure and the effect of macromer molecular weight and NVP content are depicted schematically.

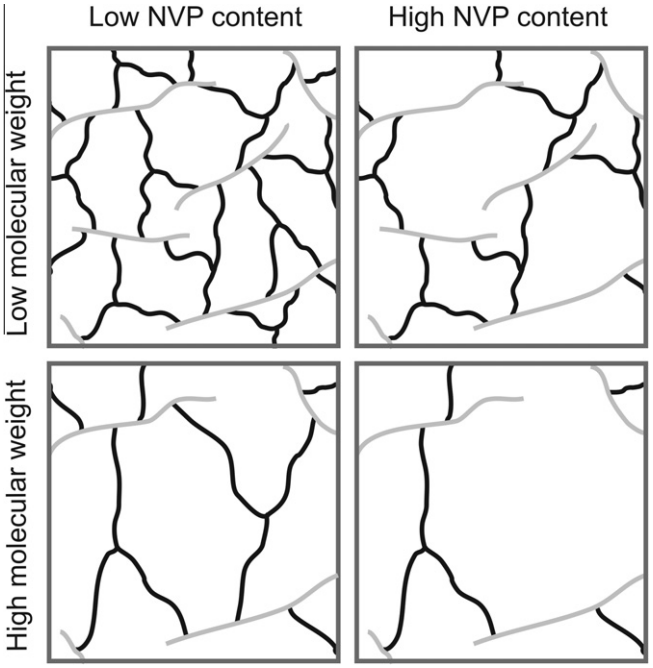

Fig. 3. Proposed structure of the synthesized networks. PDLLA chains are shown in black, FAME/NVP chains are shown in gray.

The networks consist of hydrophobic, degradable PDLLA chains and hydrophilic, non-degradable FAME/NVP chains. These FAME/NVP chains are formed when FAME and NVP co-polymerize through light-induced radical polymerization to form the network. Based on the co-polymerization constants of fumaric acid and NVP the formation of alternating co-polymers would be expected [29]. However, the number of NVP molecules present is much higher than the number of FAME end groups and the mobility of the FAME endgroups is restricted. Furthermore, in a previous study we found that both FAME and NVP are consumed throughout the polymerization reaction [5]. We therefore expect that the network structure is as depicted in Fig. 3: PDLLA chains connected by FAME/NVP co-polymer chains in which the FAME end groups are separated by a number of NVP moieties.

To investigate the degradation behavior of PDLLA-FAME/NVP network films specimens were incubated in PBS at 37 °C. At regular time intervals samples were weighed to determine their wet mass and dried and weighed to determine their dry mass. The results are shown in Fig. 4.

The networks prepared with more NVP lose more dry mass over time (Fig. 4d). Since these networks are most hydrophilic and therefore display the highest water uptake (Fig. 2) they show relatively fast degradation. Furthermore, the networks prepared with high amounts of NVP contain a large number of hydrophilic FAME/NVP chains that can readily enter into solution as hydrolytic degradation of the PDLLA chains occurs. Additionally, in the networks prepared with high amounts of NVP fewer FAME groups are present in the FAME/NVP chains, which connect the PDLA chains in the network (Fig. 3). This means that the extent of degradation required before these FAME/NVP chains can enter into solution is lower compared with networks prepared with a smaller amount of NVP. No clear effect of the macromer molecular weight on the dry mass loss was found. The NVP content appears to be the most important factor in determining degradation behavior.

Not only the NVP content but also the macromer molecular weight seems to play an important role in the wet mass profiles

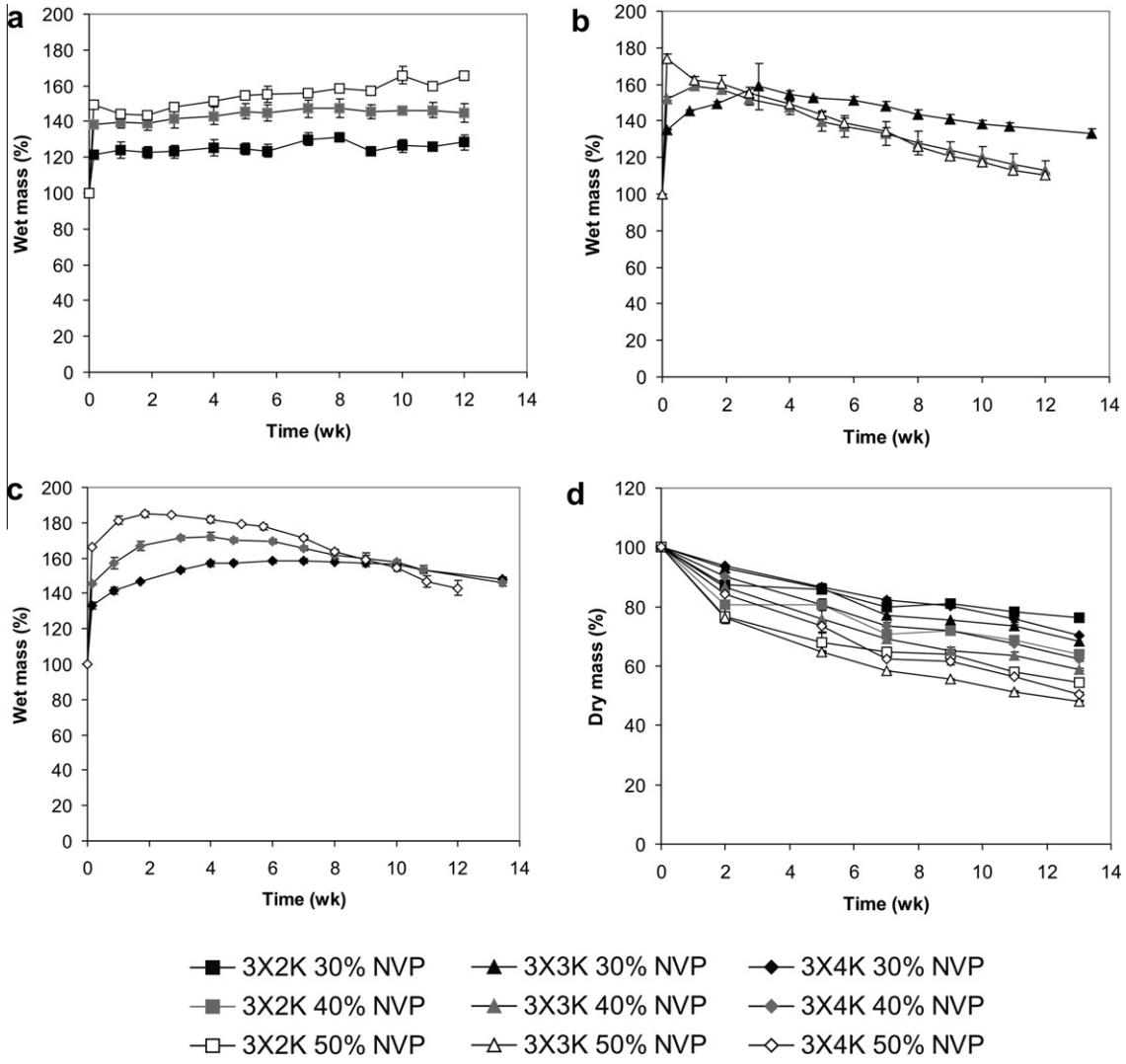

Fig. 4. Degradation of PDLLA-FAME/NVP networks. Swelling ratio in PBS (a–c) and dry mass (d) as a function of time.

overtime (Fig. 4a–c). For the networks prepared from PDLLA 3X3K-FAME and PDLLA 3X4K-FAME macromersa maximum in the wet mass is first observed, followed by a steady decrease (Fig. 4b and c). The initial increase in the wet mass could be caused by hydrolytic chain scission of the PDLLA chains that results in an increased meshsize and network swelling. As this process continues the hydrophilic FAME/NVP chains and other degradation products start to enter into solution and the wet mass decreases. Again it is found that degradation is fastest for the networks with the highest NVP contents.

Networks prepared from the PDLLA 3X2K-FAME macromers show different wet mass profiles overtime than the networks prepared from higher molecular weight macromers. Although the dry mass of these networks decreases over time (Fig. 4d), the wet mass does not, but stays more or less constant, or even increases slightly (Fig. 4a). This may be related to the high crosslink density of these networks. As chain scission occurs the meshsize increases and the network can take up more water. On the other hand, soluble degradation products exit the network. Most likely these effects cancel each other out, leading to relatively stable wet masses in time.

These results indicate that the degradation behavior of these network films is affected by both the macromer molecular weight and the NVP content.

3.4. Protein release from PDLLA-FAME/NVP network films

To investigate the drug release behavior from different networks prepared from PDLLA-FAME macromers and NVP the networks were loaded with protein particles. By dispersing solid protein particles in the macromer solutions loaded networks could easily be obtained after photo-crosslinking (Fig. 5). Disks measuring 10 mm in diameter and approximately 0.5 mm in thickness that contained 10 wt.% protein were prepared. Lysozyme (14 kDa) and bovine serum albumin (66 kDa) were used as model proteins. The effect of the NVP content and macromer molecular weight on the protein release profiles was investigated. The results of the release experiments are shown in Figs. 6 and 7.

Fig. 6 shows that the NVP content of PDLLA-FAME/NVP networks has a significant influence on the protein release profiles. Proteins are released fastest from the networks with the highest NVP content. This is a result of the high water uptake of these networks (Fig. 2). Both lysozyme and albumin are released from the networks containing 50% NVP within a few weeks. This was also the case for networks containing 50% NVP prepared from PDLLA 3X3K-FAME and PDLLA 3X4K-FAME macromers (data not shown). Release from the networks containing 30% or 40% NVP is more sustained. Lysozyme is not released much faster than albumin, although it is a smaller protein. While often the major part of the

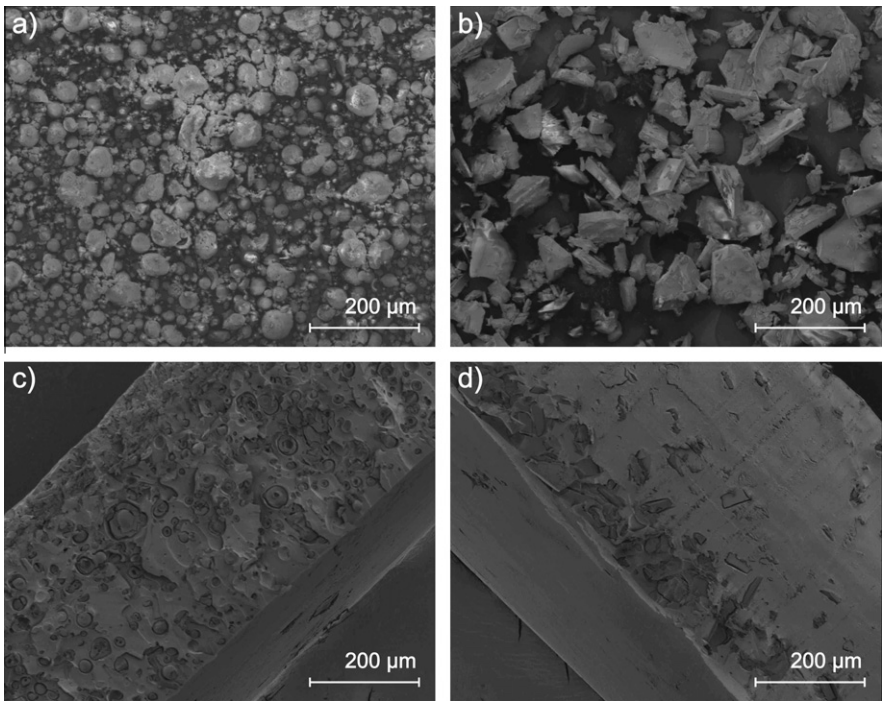

Fig. 5. SEM images of (a) lysozyme particles, (b) albumin particles, (c) a PDLLA 3X2K-FAME/40% NVP network specimen loaded with lysozyme particles and (d) a PDLLA 3X2K-FAME/40% NVP network specimen loaded with albumin particles.

loaded albumin was released, in many cases lysozyme release was found to be incomplete.

Both degradation and diffusion can play a role in release from these PDLLA-FAME/NVP networks. These networks degrade during the release period, resulting in an increasing meshsize over time. The release of albumin from the PDLLA 3X2K-FAME networks containing 30% NVP is initially slow, while after approximately 8 weeks the release rate starts to increase, due to the increased meshsize. Diffusion of the protein becomes less restricted and the release rate increases.

In Fig. 7 the release of lysozyme and albumin from networks prepared from PDLLA-FAME macromers of different molecular weights is displayed. All these networks contain 40% NVP. Protein release is slowest from the PDLLA 3X2K-FAME/NVP networks, which is due to the high crosslink density and low water uptake of these networks (Fig. 2). Differences between the PDLLA 3X3K-FAME/NVP and PDLLA 3X4K-FAME/NVP networks are small, which is in agreement with the water uptake measurements (Fig. 2) and degradation characteristics (Fig. 4).

From these results it can be concluded that the model proteins used can be released from PDLLA-FAME/NVP networks over prolonged periods of time ranging from several weeks to a few months. Both the NVP content and the macromer molecular weight can be varied to obtain optimal release profiles.

Since it is of great importance that a released protein has retained is activity the activity of the released lysozyme was determined (Fig. 8). It was found that active lysozyme was released over the complete release period. This indicates that the protein remains active within the polymer matrix. The activity of the released lysozyme varied from 60% to 100%. No clear effects of the composition of the network films on the activity of the released protein were found.

While in the case of albumin often most of the protein was released, lysozyme release was frequently incomplete (Figs. 6 and 7). Incomplete release of lysozyme from release systems based on poly(lactic-co-glycolic acid) has been repeatedly observed and investigated [30–33]. Many factors during device preparation, storage and release can play a role [19,20]. Since photo-initiated free radical polymerization was used to prepare the protein-loaded network films, side reactions may have occurred between the radicals and the proteins which may lead to incomplete protein release [21]. However, in this case the protein was incorporated in the form of solid particles, minimizing the contact between protein and radicals [22]. It is therefore expected that interaction of the protein with the polymer matrix, through hydrophobic or electrostatic interactions, is a more important explanation for the incomplete release. In some cases the lysozyme concentration in the medium even decreased over time (Fig. 6a), also indicating lysozyme adsorption to the polymer matrix. To investigate the effect of lysozyme adsorption on the release profile the release study was repeated in different release media (Fig. 9). It was found that a release medium with a lower pH and the addition of extra salt both resulted in a significant increase in overall lysozyme release.

Lysozyme is a basic protein that is positively charged at neutral pH (isoelectric point pH 11). In contrast, albumin has an isoelectric point of 5. When chain scission of PDLLA occurs negatively charged carboxyl end groups are formed that may interact with the positively charged amino acids of lysozyme [33,34]. To confirm that ionic interactions play a role in the incomplete release of lysozyme sodium chloride was added to the release medium. Salt ions present in the release medium reduce the ionic interactions by shielding the charged groups. An increase in salt concentration was indeed found to increase the amount of lysozyme released (Fig. 9), indicating that electrostatic interactions play an important role in the incomplete protein release.

A release medium of lower pH (acetate buffer, pH 4.7) also resulted in the release of more lysozyme compared with release in PBS (Fig. 9). At lower pH the carboxylic acid end groups present in the networks are protonated, leading to a lower extent of ionic interaction with lysozyme [33]. This may explain why a release medium of lower pH results in more complete release of lysozyme.

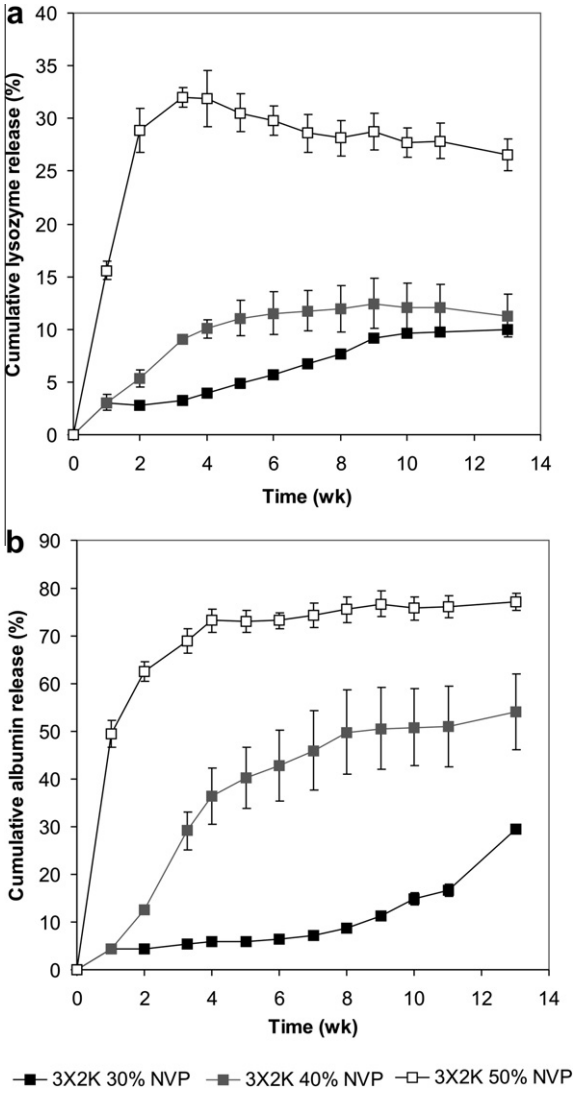

Fig. 6. Influence of the NVP content of PDLLA 3X2K-FAME/NVP networks on the release of (a) lysozyme and (b) albumin.

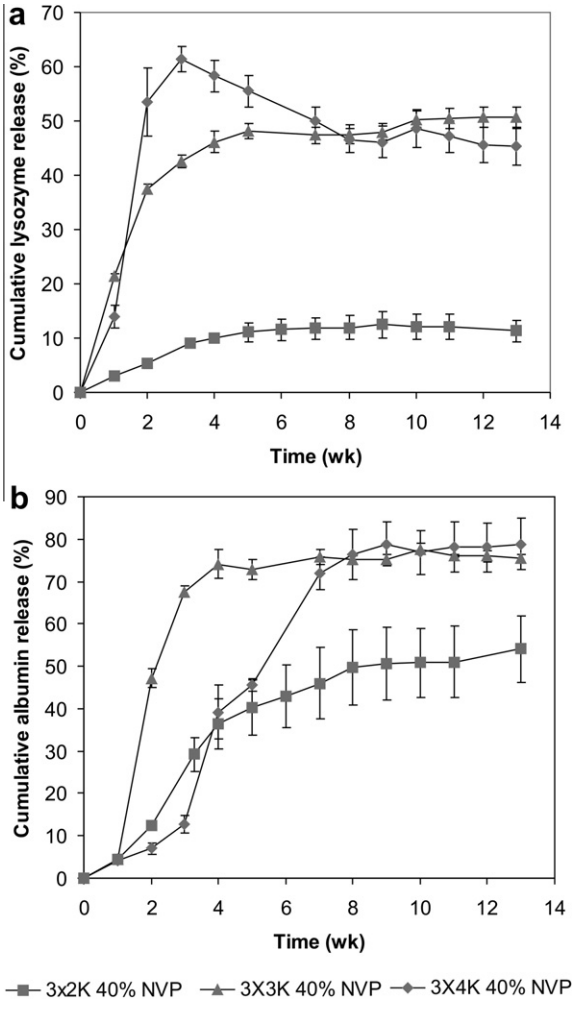

Fig. 7. Influence of the macromer molecular weight of PDLLA-FAME/NVP networks containing 40% NVP on the release of (a) lysozyme and (b) albumin.

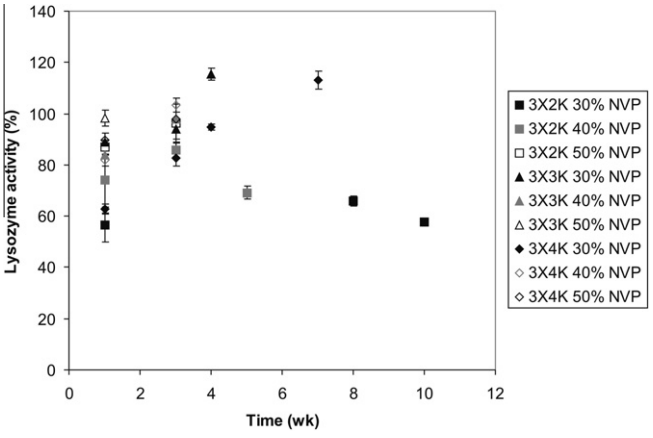

Fig. 8. Lysozyme activity after release from different networks prepared from FAME-functionalized PDLLA oligomers and NVP. The activity of the released lysozyme was compared with that of a freshly prepared lysozyme solution (taken as 100% activity). The presented activity values should be considered as approximations of the enzyme activity.

However, it has also been suggested that lysozyme is not optimally folded at pH 7.4 and that its hydrophobic domains are partially exposed at this pH [31]. This may result in enhanced adsorption of the protein to the hydrophobic polymer matrix through hydrophobic interactions. This suggests that hydrophobic interactions may also play a role in the incomplete release of lysozyme from PDLLA-FAME/NVP networks. It should be noted that a change in pH of the release medium may have an effect on network degradation and in this way may also affect protein release rates.

These results indicate that the controlled and sustained release of proteins from degradable polymer matrices remains challenging. Release kinetics do not only depend on the polymer characteristics and protein size, but also on other properties of the protein, such as charge and stability under the release conditions. Therefore, every protein release system based on degradable polymers needs careful optimization to obtain the desired release profile and complete release of active protein.

4. Conclusions

Photo-crosslinked networks were prepared from PDLLA-FAME macromers and NVP. By changing the NVP content and macromer molecular weight the degradation behavior and protein release profiles of the prepared networks could be controlled. Two model proteins, lysozyme and albumin, could be released from the

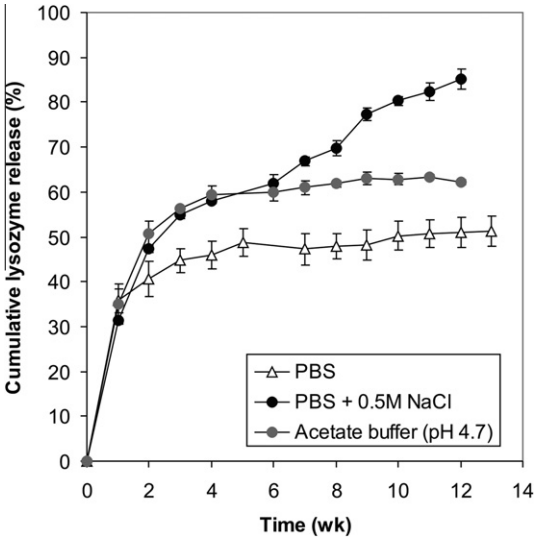

Fig. 9. Effect of the release medium on lysozyme release from PDLLA 3X3K-FAME/50% NVP networks.

networks in a controlled and sustained way. The more hydrophilic and less densely crosslinked networks released the proteins at a faster rate. Although active lysozyme was released from the networks over the complete release period, lysozyme release was often incomplete. This was most likely caused by electrostatic and/or hydrophobic interactions between the protein and the degrading polymer network.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2012.06.011.

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