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Photo-crosslinked anhydride-modified polyester and –ethers for pHsensitive drug release

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Abstract

Photo-crosslinkable polymers have a great potential for the delivery of sensitive drugs. They allow preparation of drug releasing devices by photo-crosslinking, thus avoiding high processing temperatures. In this study, the hydrolysis behavior and drug release of three different photo-crosslinkable poly(ether anhydride)s and one poly(ester anhydride) were investigated. Three-arm poly(ethylene glycol) or polycaprolactone was reacted with succinic anhydride to obtain carboxylated macromers, and further functionalized with methacrylic anhydride to form methacrylated macromers with anhydride linkages. The synthetized macromers were used to prepare photocrosslinked matrices with different hydrolytic degradation times for active agent release purposes.

The hydrolysis was clearly pH-sensitive: polymer networks degraded slowly in acidic conditions, and degradation rate increased as the pH shifted towards basic conditions. Drug release was studied with two water-soluble model drugs lidocaine (234mol/g) and vitamin B₁₂ (1355g/mol). Vitamin B₁₂ was released mainly due to polymer network degradation, whereas smaller molecule lidocaine was released also through diffusion and swelling of polymer network. Only a small amount of vitamin B₁₂ was released in acidic conditions (pH 1.3 and pH 2.1). These polymers have potential in colon targeted drug delivery as the polymer could protect sensitive drugs from acidic conditions in the stomach, and the drug would be released as the conditions change closer to neutral pH in the intestine.

Keywords: photo-crosslinking, poly(ether anhydride), poly(ester anhydride), drug delivery, lidocaine, vitamin B₁₂

Introduction

Polymer-based active agent delivery offers tremendous possibilities for sustained, time-wise controlled and locally administered applications. Oral drug administration is the most common route for systemic delivery of drugs [1,2]. Some drugs, such as proteins, are labile in gastric acid, and therefore colon targeted drug delivery systems are needed [3–5]. In addition, some diseases require local drug delivery, such as Crohn's disease and colon cancer [6,7]. There are different ways to obtain targeted delivery to the colon, including pH-dependent, time-lag and enzyme-dependent mechanisms [3,8–10]. Traditional sustained release devices protect drugs from conditions in stomach and slow down the drug release, whereas controlled release systems locally release the drug at a predetermined rate [11]. Thus, controlled release devices maintain the drug release within the therapeutic window avoiding too low or too high drug concentrations [2].

Polymers are widely studied in medical engineering due to their versatility and possibility to tailor their properties for specific applications. Polymers are modified chemically to obtain materials with desired properties such as suitable mechanical strength, chemical compatibility and degradation [12–14]. Polymers can also be synthetized to have a low viscosity and photo-crosslinking ability, which enables the preparation of polymer structures under mild conditions. Moreover, a thermally sensitive drug can be mixed with liquid pre-polymer prior to photo-crosslinking, enabling the preparation of drug containing polymer matrices without heat or solvents. [12,13,15]

Polymers used in controlled drug release are both biostable and degradable [16,17]. Degradation is the process of polymer chain cleavage and it can be divided into surface and bulk erosion mechanisms [18]. In bulk erosion, the molecular weight is decreasing throughout the polymer and the size of the device remains constant until rapid fragmentation [18], whereas in surface erosion the material is lost from the exterior surface and the size of the device is constantly decreasing [14]. In surface eroding polymers, water penetration into the polymer matrix is slower than the hydrolytic degradation of the material [19]. Surface eroding polymers are usually favored in drug release devices, since the drug is released as the polymer is degrading, and thus larger drug molecules can be used [20]. Bulk degrading polymers on the other hand release the drug by diffusion or leaching, and finally via degradation of polymer matrix [21]. The degradation rate can be modified by copolymerization and functionalization [22]. By chemically modifying the polymer to be more hydrophobic, the hydrolytic degradation rate is lower, whereas enhancing hydrophilicity, the degradation rate is faster [14,19]. In addition, functionalizing the polymer with hydrolytically labile chemical bonds increases the degradation rate [12].

Polyesters, especially polycaprolatone (PCL), are degrading hydrolytically through bulk erosion [13]. Polyanhydrides also degrade hydrolytically, however, they degrade significantly faster in water and are surface eroding [19]. Anhydride-linkages degrade by base-catalyzed hydrolysis [23] and degradation is pH dependent, being more stable in acidic conditions and more pronounced in basic conditions [19,24]. Since pH is changing along the gastro intestinal tract, pH-sensitive polymer degradation is a beneficial feature in intestine targeted oral drug delivery. In the stomach, the pH is acidic (pH 1.3-3.0) and in the intestine the pH is close to neutral conditions (pH 5.0-8.0) [8]. pH-sensitive polymers are able to protect sensitive drugs from acidic conditions in the stomach and release the drug in more neutral conditions in the intestine.

Poly(ethylene glycol) (PEG) is a widely researched and utilized polymer in medical applications. Unlike polyesters and polyanhydrides, PEG does not degrade in water. However, it does dissolve in water [25,26] and small PEG molecules can be removed from the body by metabolization or kidney filtration

[26–29]. Depending on the molecular weight, PEG is a viscous liquid (<1000g/mol) or solid higher at molecular weights. Polymer molecular weights of under 20 000g/mol are usually referred to as PEG, while higher molecular weight specimens are usually referred to as poly(ethylene oxide) (PEO).

Several photo-crosslinkable polymers have been developed for medical applications, since the required properties depend upon the application. Photo-crosslinkable polymers such as polyanhydrides [30], polyesters [31,32], PEGs [33], polyurethanes [34] and their different copolymers [12,27,30,35–38] have been utilized. Previously, we have researched photo-crosslinkable PCL-based poly(ester-anhydride) precursors for drug release [12,39]. The degradation of PCL-based (polyester anhydride) networks can be prolonged from days to several weeks by functionalizing with hydrophobic alkenylsuccinic anhydrides. Kim et al. have synthetized linear photo-crosslinkable dimethacrylated PEG-macromers with anhydride linkages [27]. The degradation time for these poly(ether anhydride) networks ranged between 20min to 2 days depending on the molecular weight of macromer.

In this article, we present a set of new, photo-crosslinkable tree-arm poly(ether anhydride)s and evaluate their potential as drug delivery matrices. The degradation of PEG-based poly(ether anhydride) and PCL-based poly(ester anhydride) networks in different pH conditions as well as drug release from poly(ether anhydride)s has not been previously studied.

The aim in this work was to synthetize polymer networks that have degradation rates of 5 hours to 24 hours in neutral conditions, since orally administered capsule is likely to arrive in the colon after 5 hours of dosing, and the average time for passing through the colon is 20-30 hours [4,9]. *In vitro* hydrolysis and swelling behavior tests were conducted in different pH conditions to simulate the conditions in gastrointestinal tract. pH-sensitive degradation would be useful in colon targeted drug release of sensitive drugs [8]. Stability in acidic conditions enable the polymer to protect drugs from enzymes and acidic conditions in stomach, and drug would be released as the polymer device is delivered to the intestine and pH is shifting towards neutral conditions.

Materials and methods

Materials

Prior to the synthesis, ϵ -caprolactone (CL, 97%, Sigma-Aldrich) was redistilled. Stannous octoate (SnOct₂, 95%), trimethylol propane (TMPE), trimethylolpropane ethoxylate (average M_n 170, 450 and 1014g/mol), succinic anhydride, methacrylic anhydride, hexane, d-chloroform and dichloromethane were purchased from Sigma-Aldrich and used as received. Photoinitiator TPO-L (ethyl phenyl(2,4,6-trimethylbenzoyl)phosphinate) was from Carbosynth Limited. Model drugs lidocaine and vitamin B₁₂ (Sigma-Aldrich) were used as received. The water solubility of lidocaine and vitamin B₁₂ are 4mg/ml[40] and 125mg/ml[41], and molecular weights 234mol/g and 1355g/mol, respectively. Phosphate buffer solutions (pH 2.1, 6.8, 7.4 and 12) were purchased from FF-Chemicals. Hydrochloric acid (HCl, 37%, Sigma-Aldrich) was used to prepare acidic solution of pH 1.3 by diluting HCl with distilled water.

Synthesis of oligomers

In this study, five photo-crosslinkable macromers were synthetized. No solvent was used in any of the synthesis and functionalization steps. Three of the macromers were anhydride-modified PEGs with different molecular weights and one of them was anhydride-modified PCL, used as a reference for the novel macromers. In addition, one methacrylated PEG was synthetized.

Similar type of synthesis of PCL-based poly(ester-anhydride) macromer is described earlier [12,22,42,43]. Briefly, ε -caprolactone was weighed with co-initiator trimethylol propane (10mol-%) and catalyst SnOct₂ (0.02mol-%) in a three-neck flask. The ring opening polymerization of ε -caprolactone was carried out at 160°C for 5h under a nitrogen atmosphere until all monomer was reacted, resulting in three-arm ε -caprolactone oligomer with OH-termination (PCL-OH).

Functionalization of oligomers

Trimethylolpropane ethoxylate is a hydroxyl-terminated 3-arm poly(ethylene glycol). The 3-arm PEGs and PCL oligomer were functionalized with succinic anhydride (1.03mol-% per hydroxyl groups) to obtain macromers with carboxylic acid end groups. The functionalization was carried out for 2h at 140°C.

Acid-terminated macromers were further reacted at 60° C for 3 days using 1.5-fold excess methacrylic anhydride relative to the carboxylic acid-groups. This step results in anhydride bond and methacrylate end group. In addition, hydroxyl-terminated trimethylolpropane ethoxylate with M_n average of 450g/mol was functionalized with methacrylic anhydride in the same conditions.

Precipitation of macromers

Macromers were precipitated in hexane (10 times in 100ml of hexane) to remove unreacted methacrylic anhydride and methacrylic anhydride residuals (methacrylic acid) formed in the functionalization step. Subsequently, macromers were dried in vacuum to remove all hexane.

Photo-crosslinking of macromers and sample preparation

Functionalized macromers were mixed with photo-initiator TPO-L (5wt-% relative to macromer) and poured into a Teflon mold to obtain cylinders (approximately 10mg) with 2mm height and 2mm diameter. The samples were crosslinked in a Triad 2000 light curing unit with visible light (350–550 nm, DeguDent) for 18 minutes.

To prepare samples with lidocaine and vitamin B_{12} , 10 and 1wt-% of drugs relative to macromer were added and mixed on a heated plate (T=75°C) until drugs were dissolved. Lidocaine dissolved fast, since its melting temperature is 68°C. The dissolution of vitamin B_{12} was significantly slower compared to that of lidocaine and it was monitored with an optical microscope. No solvents were used in the preparation of the polymer-drug samples. Due to the slow dissolution of vitamin B_{12} into the polymer, the amount was kept lower compared to that of lidocaine.

When using anhydrides, it should be noted that they degrade easily. Due to the hydrolytic lability of anhydride bond, polymers containing anhydride-linkages should be stored under moisture-free conditions, such as under nitrogen or in refrigerator [44]. Therefore, both macromers and crosslinked samples were stored in freezer before further use.

Characterization

The synthesis of the macromers was monitored using ¹H and ¹³C NMR spectroscopy (Brüker NMR Spectrometer AV III 400). The samples were dissolved in chloroform-d with average concentration of 20mg/ml (¹H) and 120mg/ml (¹³C). ATR-IR spectra was obtained with FT-IR spectrometer (Spectrum Two, Perking Elmer). The gel content G (1) and the degree of swelling in dichloromethane Q (2) of cross-linked samples was evaluated by weighing the initial mass of the sample (m_i) immersing the samples in dichloromethane for 2 hours, weighing the samples immediately (m_{sw}) drying the samples in vacuum for 24h and weighing the remaining mass (m_r). Density of dichloromethane (ρ_s) is 1.326g/cm³ and used densities of polymers (ρ_p) are 1.094g/cm³ for polycaprolactone and 1.125 g/cm³ for poly(ethylene glycol).

$$G(\%) = \frac{m_r}{m_i} * 100 \tag{1}$$

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

To analyze the glass transition (Tg) and melting (Tm) temperatures of macromers and crosslinked polymers as well as evaluate the drug dissolution in the polymer matrix, differential scanning calorimetry (DSC Q2000, TA Instruments) was used. The amount of the sample was 5-7mg. The samples were first heated at rate of 10°C/min from 40°C to 80°C, cooled to -90°C, heated to 85°C and cooled to 40°C. The T_g was analyzed from the second heating scan. The results were analyzed with TA Universal Analysis software.

In vitro hydrolysis of polymer networks

 m_d

Hydrolysis and drug release studies were conducted in the same conditions. The samples (10mg on average) were immersed in 10ml phosphate buffer solution (pH 2.1, 6.8, 7.4 and 12) and mildly agitated at 100rpm at 37°C. The mass loss (3) and swelling (4) was monitored by weighing the samples initial mass (m_i), swollen mass (m_s) after removing the sample from buffer and drying the sample surface and mass after drying in vacuum until constant mass (m_d).

Remaining mass
$$(\%) = \frac{m_d}{m_i} \cdot 100$$
 (3)
Swelling $(\%) = \frac{m_s - m_d}{m_s} \cdot 100$ (4)

Drug release and stability

The lidocaine and vitamin B₁₂ release were monitored using UV/Vis-spectrophotometry (3100PC UV-VIS, VWR). The calibration curves analyzed from absorption peak at 271nm for lidocaine and 361nm for vitamin B_{12} were linear in the range of 10-200ppm (y=0.0015x+0.0461, R^2 =0.9996) and 1-20ppm (y=0.019x+0.0011, R²=0.9999), respectively. The drug release study was conducted in similar conditions as the hydrolysis study. In addition, to simulate the GI tract, samples were immersed in pH 1.3 HCl-solution for 2h, and subsequently transferred to pH 6.8 phosphate buffer solution for 24h. The HCl-solution simulates the gastric fluid, whereas phosphate buffer of pH 6.8 mimics the intestinal fluid [10].

The stability of lidocaine and vitamin B₁₂ in different pH conditions was monitored and no change in the absorbance was observed during 48h. Except with vitamin B₁₂ in pH 12, the concentration decreased slightly and absorption maximum shifted to 350nm indicating that pH 12 changes the molecule. The thermal stability of the drugs was evaluated by thermogravimetric analysis (TGA Q500, TA Instruments). Specimens (sample size \sim 10–20 mg) were heated from 30°C to 600°C at 20 °C/min under nitrogen with a purge rate of 60 ml/min and decomposition temperatures were analyzed with TA Universal Analysis software. As vitamin B12 has chemical and physical bounded water [45], the temperature was first heated from 30°C to 70°C and kept isothermal for 15 minutes to remove the water.

Results and discussion

The synthesis of PEG-anhydrides is expected to progress similarly as previously reported with PCLanhydrides [13]. A reaction scheme of synthesis of PEG-anhydrides is presented in Figure 1.



Figure 1. Reaction scheme of PEG-anhydride synthesis. a) Functionalization of TMPE with succinic anhydride results to acid terminated macromer. b) Methacrylization with methacrylic anhydride results to methacrylated macromer and formation of methacrylic acid. Anhydride bond in the methacrylated macromer is presented in green and reactive double bond is presented in red.

The chemical structures of macromers were confirmed with ¹H NMR, ¹³C NMR and ATR-IR. ¹³C NMR was used to monitor the PEG-anhydride synthesis (Figure 2). As hydroxyl groups react, the carbon in end groups at δ 61ppm (peak h) react and shifts to δ 64ppm (peak h') and a peak around δ 177pm (COOH-group) appears. Degree of functionalization is calculated from peaks h and h'. The carboxylic acid group reacts further during the methacrylation and the peak at δ 177pm disappears. The degree of functionalization was high (96-99%) and degree of methacrylization was 100% for all of PEG-macromers (Table 1). ¹H spectra of PEG-anhydride synthesis can be found in the supporting information (Figure S1).



Figure 2. ¹³C NMR spectra of PEG-anhydride 450 synthesis. a) three-arm PEG (450g/mol), b) acid-terminated PEG-macromer and c) methacrylated PEG-macromer (PEG-anhydride).

¹H and ¹³C spectra of PCL-anhydride synthesis can be found in the supporting information (Figure S2). In PCL-anhydride synthesis the first step is ring-opening polymerization. During the oligomer synthesis, CL-monomer (at δ 4.2ppm, peak h in the Figure S2) reacts with a yield of 98.5%, as calculated from ¹H NMR peaks h and h'. The degree of functionalization (99.8%) is calculated from peaks j and k,l in ¹H NMR spectra. Methacrylization of acid terminated macromer can be seen in the ¹H spectra as split in the peaks m and I (δ 2.7ppm) to m' and I' (δ 2.8ppm and 2.6ppm). The degree of methacrylization (95%) was calculated from ¹³C NMR spectra from the peaks k and k'.

The M_n values of oligomers and methacrylated macromers were calculated from ¹H NMR spectra. The molecular weight of oligomers and macromers was slightly higher compared to the theoretical values and values for trimethylol propane provided by the manufacturer. The molecular weights are reported in the Table 1.

Since the macromers are small in molecular weight, the relative amount of functional end-groups in the macromers is high. For example, PEG-anhydride 170 has relatively the highest amount of functional groups in the macromer and only 27% on the macromer is PEG. Because of this, the network consists mainly of functional groups. PEG-anhydride 1014 consists of 72% of PEG. Thus, it has a very different amount of PEG in the network. The difference in the relative amount of PEG is expected to affect the hydrolysis behavior. The relative amounts of PEG and PCL in the methacrylated macromers are reported in the Table 1.

Table 1. Average molecular weights of oligomers (TMPE 170, TMPE 450, TMPE 1014, and PCL) and methacrylated macromers. Relative amount of PEG and PCL in the macromer. Degrees of functionalization (DF) and methacrylization (DM) of macromers calculated from NMR spectra. ^a M_n values from the manufacturer, ^b theoretical M_n value and ^c calculated from NMR.

| | Oligomer average M _n | | Functionalized macromer M _n | | Relative amount of PEG/PCL in | | DF (%) | DM (%) |
|--------------------|------------------------------------|-------------------|--|-------------------|----------------------------------|------------------|-----------|-----------|
| | (g/ | mol) | (g/mol) | | the macromer | | | |
| PEG-anhydride 170 | 170ª | 170 ^c | 677 ^b | 620 ^c | 25% ^b | 27% ^c | 99% | 100% |
| PEG-anhydride 450 | 450 ^a | 460 ^c | 957 ^b | 920 ^c | 47% ^b | 51% ^c | 99% | 100% |
| PEG-anhydride 1014 | 1014ª | 1300 ^c | 1521 ^b | 1800 ^c | 67% ^b | 72% ^c | 96% | 100% |
| PCL-anhydride | 1276 ^b | 1400 ^c | 1783 ^b | 1900 ^c | 72% ^b | 73% ^c | 99.8% | 95% |

The gel content (G) and degree of swelling (Q) are reported in the Table 2. High gel content values indicate that the macromers have crosslinked and formed networks. Figure 3 presents the IR spectra of PEG-anhydride 450 macromer and crosslinked network. The methacrylate double bond (1640cm⁻¹) disappears as it reacts during photo-crosslinking.

A low degree of swelling indicates high crosslinking density and it increases with increasing macromer size [46]. In this study, the degree of swelling is similar for each of the polymer networks. Previously, with relatively small macromers (around 2000g/mol) the degrees of swelling have been similar to macromers with different sizes, whereas there are larger differences in swelling degrees when the molecular weight range is larger [47–49]. The low swelling degrees obtained in this study indicate that the networks have high crosslinking densities.

Table 2. Gel content G (n=3) and swelling degree Q (n=3) of networks in dichloromethane.

| | G (%) | Q (%) |
|--------------------|----------|-----------|
| PEG-anhydride 170 | 88.6±1.0 | 204.5±1.8 |
| PEG-anhydride 450 | 88.8±0.7 | 202.1±0.1 |
| PEG-anhydride 1014 | 85.1±2.4 | 197.0±3.4 |
| PCL-anhydride | 91.1±0.1 | 195.2±1.3 |



Figure 3. IR spectra of PEG-anhydride 450 macromer and photo-crosslinked network.

Thermal analysis

DSC was used to evaluate if the drugs were dissolved or dispersed in the polymer matrix. The results are presented in Table 3. Lidocaine has a melting peak at 68°C and vitamin B12 does not melt. No melting peak was observed, which indicates that the lidocaine was dissolved in the polymer matrix. It can be noticed from Table 3 that the networks have higher T_g compared to those of macromers. PEG-anhydride 170 had the highest difference in T_g (45°C) between macromer and network. This might be due to the shortest arms of the PEG-anhydride 170. Shorter arms result to higher crosslink density and thus tighter network, which restricts the movement of polymer chains and leads to higher T_g . Networks with lidocaine have slightly higher T_g compared to polymer networks (except PEG-anhydride 170). This might be due to interactions between the polymer networks and lidocaine. Vitamin B₁₂ did not affect the T_g of polymer network, most probably due to low amount the active agent (1wt-%).

| Sample | Macromer (°C) | Network (°C) | Difference (°C) | Network +Lidocaine (°C) | Network +Vitamin B ₁₂ (°C) |
|--------------------|------------------|-----------------|--------------------|----------------------------|--|
| PEG-anhydride 170 | -37±0.2 | 8±2.1 | 45 | 4±0.3 | 8±1.3 |
| PEG-anhydride 450 | -41±0.0 | -23±1.4 | 19 | -21±0.2 | -23±1.7 |
| PEG-anhydride 1014 | -47±0.0 | -40±0.9 | 7 | -36±0.6 | -39±0.6 |
| PCL-anhydride | -56±0.3 | -44±1.6 | 12 | -40±0.2 | -44±0.7 |

Table 3. Tg values of macromers, photo-crosslinked networks and photo-crosslinked networks with model drugs.n=2.

TGA of drugs confirmed that they degrade in higher temperatures than used in this study (Supplementary Figure S3). Decomposition of lidocaine is initiated at 104°C and B12 at 187°C. TGA graph of vitamin B12 shows 9.5% mass loss between 30-70°C, which corresponds to resorption of physical and chemical bounded water [45].

Hydrolysis and swelling

The mass loss of the polymers in different pH conditions is shown in Figure 4. Hydrolytic degradation is slowest in acidic conditions (pH 2.1) and faster approaching neutral (pH 6.8 and 7.4) and basic (pH 12) conditions, as is expected since degradation of anhydride-linkage is pH-dependent [19,50]. By comparing the degradation rates of networks in pH 12, it can be seen that the networks degrade with quite similar rate during the first 8 hours. It is assumed, that the anhydride-linkage has the dominant role in the degradation, and thus the degradation rates are similar.

PCL-anhydride networks have the greatest difference in degradation rate in different pH conditions. Furthermore, it has the slowest degradation rate in acidic conditions. Amongst PEG-anhydrides, PEG-anhydride 170 networks degrade slowest and PEG-anhydride 1014 networks fastest. Kim et al. reported similar effects with crosslinked PEG-anhydride networks made from linear macromers; networks prepared from low molecular weight macromers degraded more slowly due to the fact that they are more tightly crosslinked [27]. Tight crosslinking hinders the swelling and water penetration into the network, thus there is less water available for the hydrolytic degradation.



Figure 4. Mass loss of polymers in different pH conditions. a)PEG-anhydride 170, b) PEG-anhydride 450, c)PEG-anhydride 1014 and d) PCL-anhydride.

PEG-anhydride networks swell slightly in acidic conditions (pH 2.1) and more considerably in other pH conditions (pH 6.8, 7.4 and 12) (Figure 5). Again, PEG-anhydride 170 networks swell the least and PEG-anhydride 1014 networks the most. The molecular weight of the macromer has shown to affect swelling previously [27,37]. PCL-anhydride networks swell as well, however the swelling is significantly lower compared to PEG-anhydride networks. Hydrophilicity in polymers enhances the water uptake, resulting in faster polymer degradation [14,51]. Therefore hydrophilic PEG-anhydride networks swell more and anhydride-linkages degrade faster compared to hydrophobic PCL-anhydride network. PEG-anhydride 1014 network swells the most, since it has the highest relative content of PEG (Table 1) and thus assumable highest hydrophilicity. Furthermore, it has the longest arms in the macromer, resulting in the loosest network. As anhydride-linkages degrade hydrolytically, two carboxylic acid groups are formed [23]. These carboxylic acids increase the hydrophilic nature of polymer network further enhancing the swelling [8].



Figure 5. Swelling (see Equation 4) of polymers in different pH conditions. a)PEG-anhydride 170, b) PEG-anhydride 450, c) PEG-anhydride 1014 and d) PCL-anhydride.

The networks retain their shape during hydrolysis and swelling. Figure 6 shows the picture of vacuum dried B₁₂ containing polymer samples at different time points and pH conditions. Polymer networks containing lidocaine degrade faster compared to polymer and vitamin B₁₂ containing samples. Table 4 shows time for total degradation, i.e. the time point where each network is totally degraded. Previously, it has been shown that the drug chemistry affects the mass loss and swelling of polymer. Solubility of drug affects the hydrolytic degradation: water-soluble drugs may increase the water absorption into the polymer and thus increase the hydrolytic degradation rate. Lidocaine is used in both basic form (as in this study) and in salt form. The basic lidocaine has been shown to accelerate hydrolytic degradation rate of polyesters (PLA and PLGA) through base catalysis effect. [21,52,53] Hydrolytic degradation of anhydride-linkage is also base-catalyzed and therefore the lidocaine accelerates the degradation of the networks and the mass loss of lidocaine containing samples is faster than that of neat polymers and networks containing vitamin B₁₂.



Figure 6. Pictures of partly degraded samples with vitamin B₁₂. a)PEG-anhydride 170, b) PEG-anhydride 450, c)PEG-anhydride 1014 and d) PCL-anhydride.

| Polymer | Time for t | Time for total degradation (h) | | | | |
|--------------------|-------------------------|--------------------------------|--------|-------|--|--|
| _ | | pH 6.8 | pH 7.4 | pH 12 | | |
| PEG-anhydride 170 | polymer | >24 | >24 | 24 | | |
| | lidocaine | 24 | 20 | 16 | | |
| | vitamin B_{12} | >24 | >24 | 20 | | |
| PEG-anhydride 450 | polymer | >24 | 20 | 18 | | |
| | lidocaine | 22 | 16 | 12 | | |
| | vitamin B ₁₂ | >24 | 20 | 18 | | |
| PEG-anhydride 1014 | polymer | 12 | 10 | 8 | | |
| | lidocaine | 8 | 6 | 4 | | |
| | vitamin B ₁₂ | 20 | 18 | 6 | | |
| PCL-anhydride | polymer | >24 | 20 | 14 | | |
| | lidocaine | 12 | 10 | 6 | | |
| | vitamin B ₁₂ | 24 | 20 | 14 | | |

Table 4. Time for total degradation for each of the sample groups.

Lidocaine and vitamin B₁₂ release

According to the calibration curves, the maximum absorbance of 100% active agent release should be 0.2 for lidocaine and 0.25 for vitamin B₁₂ samples. However, the absorbance of degraded samples was higher. Therefore, it was assumed that degrading polymer network absorbs light, which was confirmed by measuring the absorbance of degraded networks. The absorption was especially high in the UV-region. To eliminate the effect of degraded polymer network on absorption, maximum absorption values were determined by using reference samples. Used reference samples were similar active agent containing polymers as used in the release studies. Reference samples were analyzed by immersing the samples (n=3) in phosphate buffer solution and measuring absorption at 271nm for lidocaine and 361nm for vitamin B₁₂ in pH conditions of 6.8, 7.4 and 12 after the polymer network was degraded. Polymer network degradation rate in acidic pH conditions slow and therefore the effect of degraded polymer network in absorbance is not determined in acidic conditions.

Figure 7 and 8 show the release of lidocaine and vitamin B₁₂, respectively. Lidocaine is releasing faster than vitamin B₁₂ from all polymer networks in all pH conditions. Several factors affect the drug release, such as polymer degradation, swelling, diffusion and properties of drug. Polymer networks containing

lidocaine degraded faster compared to neat and vitamin B_{12} containing polymers, which explains part of the faster release of lidocaine. However, the release of lidocaine is faster than the degradation of polymer networks. The release mechanisms of lidocaine was further studied by using methacrylated 3-arm PEG and preparing samples containing 10wt-% of lidocaine. No lidocaine release or mass loss was observed at pH 7.4 in 24h, however, the samples swell 4-6% which is less compared to PEGanhydrides. Therefore, it is assumed that lidocaine does not diffuse through the polymer matrix if the polymer does not swell first. Thus, swelling and polymer network degradation are effecting the release of lidocaine. Vitamin B_{12} release follows more clearly the polymer network degradation. Therefore, it is hypothesized that vitamin B_{12} is released mainly due to polymer network degradation.

Properties of a drug, such as water solubility and molar size are also affecting the release. Water soluble drugs and small molecules are releases faster compared to less water soluble drugs and larger molecules. [21,51,54–57] Water solubility and molar mass of lidocaine are 4 mg/ml and 234 g/mol, respectively and vitamin B_{12} 125 mg/ml and 1355g/mol, respectively. Lidocaine is less water soluble compared to vitamin B_{12} and it releases faster. Therefore, the molar mass has the greater effect on the release in this study than water solubility.

Vitamin B_{12} release is pH dependent; the release is the slowest in acidic conditions, the release rate increases in higher pH conditions and is fastest in pH 12. The release of vitamin B_{12} follows the degradation of polymer network. Lidocaine release on the other hand is not clearly pH sensitive, since it is released also due to swelling of the polymer network.



Figure 7. Lidocaine release in different pH conditions. a)PEG-anhydride 170, b) PEG-anhydride 450, c) PEG-anhydride 1014 and d) PCL-anhydride.



Figure 8. Vitamin B_{12} release in different pH conditions. a)PEG-anhydride 170, b) PEG-anhydride 450, c)PEG-anhydride 1014 and d) PCL-anhydride.

To further study the pH sensitive active agent release and simulate the GI tract, samples were immersed in pH 1.3 HCl-solution for 2h, and subsequently transferred to pH 6.8 phosphate buffer solution for 24h. Figure 9 presents the drug release in changing pH conditions. Release of B₁₂ is low in pH 1.3, whereas lidocaine is released in acidic conditions. After pH change to 6.8, vitamin B₁₂ release begins as the polymer network starts to degrade and swell. Lidocaine has a plateau in the release probably due to the release mechanism change. In acidic conditions polymer networks swell slightly and as the pH changes, the swelling is enhanced due to the degradation, which may cause the plateau. These *in vitro* studies show that the polymers have potential as pH sensitive drug delivery materials especially for larger molecules.



Figure 9. Drug release of a) lidocaine and b) vitamin B₁₂. Samples were first in HCl-solution (pH 1.3) for 2 hours, subsequently they were moved to pH 6.8 phosphate buffer solution for 24 hours.

Conclusion

The synthesis of novel, photo-crosslinkable three-arm poly(ether anhydride)s was reported. *In vitro* hydrolytic degradation of networks and drug release in different pH conditions was studied to evaluate the potential of such polymers for colon targeted drug delivery. The degradation rate of photo-crosslinked poly(ether anhydride)s and poly(ester anhydride) can be controlled by changing the molecular weight and hydrophilicity of macromers. More hydrophilic macromers result in networks, which degrade faster and swell more.

Drug chemistry and size significantly affect drug release rate and polymer network degradation. The larger molecule B₁₂ released more slowly and the release was mainly degradation controlled, whereas the small molecule lidocaine released faster in all conditions and the release was more diffusion and swelling controlled. Furthermore, lidocaine containing networks also degraded faster due to the basic nature of lidocaine, which accelerated the hydrolytic degradation of anhydride-linkages.

The release of vitamin B_{12} was pH controlled; the release was minimal in acidic conditions (pH 1.3 and 2.1) and it was enhanced in neutral and basic conditions. Thus, these polymers have potential especially in colon targeted controlled delivery of larger molecules, such as proteins.

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