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Temperature-triggered liquefication of hydrogels for intentional implant removal

Abstract: The Diels-Alder (DA) reaction is one of the most studied thermoreversible reactions, suitable for the preparation of thermoreversible crosslinked materials. In this study, temperature-triggered liquefaction of DA hydrogels will be investigated. A DA model reaction is used to determine the *retro*-DA temperature of the two stereoisomers. A hydrogel based on furan-functionalized Poly-(*N*-(2-hydroxy-propyl) methacryl amide (PHPMA) and 4-arm-polyethylene glycol (PEG) with maleimide endgroups is rheologically investigated and liquefaction experiments are performed.

Keywords: thermoreversible hydrogel, Diels-Alder.

1 Introduction

In recent years, much research has been done in the field of implant coatings, mostly with a focus on improved tissue integration. However, due to persistent infection, inadequacies, or technical advancements, in some cases, the removal of the implant is unavoidable. Good tissue integration leads to problems when implants have to be removed. Tissue loss occurs and the insertion of new implants becomes much more difficult. The aim is therefore to develop coatings for implants that facilitate the removal of an implant by a trigger. Hydrogels are suitable for this application since their structure and mechanical properties are very similar to human tissue.[1] For the hydrogels to facilitate an intentional implant removal, the polymer chains must be reversibly crosslinked. One possible crosslinking method is the thermoreversible DA reaction between furan and maleimide. The necessary heating could be accomplished by superparamagnetic nanoparticles in the hydrogel, which can be heated locally by alternating magnetic fields. The heating decreases within a few nanometres, thus local temperatures up to a maximum of 110 °C can be reached without damaging surrounding tissue.[2] By removing the crosslinks, the hydrogel coating gets liquefied, and the implant can be easily removed without causing trauma.

2 Experimental

2.1 General

^{1}H NMR spectra were taken on a Bruker Biospin Avance III 400 (Rheinstetten, Germany). Rheometer measurements were carried out on a RheoStress® 100 system from Haake (Karlsruhe, Germany) at 20 °C in a plate-plate measuring arrangement. DSC measurements were performed on a Netzsch DSC204 Phoenix instrument at a heating rate of 2 K/min.

2.2 Materials

The solvents and reagents used for this investigation included Dimethylsulfoxide (DMSO, Merck), Dichloromethane (DCM, fisher scientific), diethyl ether (Et$_2$O, fisher scientific), 2,2′-Azobis(2-methylpropionitril) (AIBN, Sigma Aldrich), Bis-(1,11-maleimido)-triethylenglycol (BM(PEG)3, sigma Aldrich), *N,N′*-Dicyclohexylcarbodiimid (DCC, Sigma Aldrich), Dimethyaminopyrridin (DMAP, TCI), 3-(2-furyl) propionsäure (Alfar Aesar), *N*-(2-Hydroxypropyl) methacrylamid (HPMA, Polyscience).

2.3 Synthesis

Esterification of Isopropyl-3-(2-furyl)propionic acid. The ester was prepared by a STEGLICH reaction [3] The product is obtained as a brown liquid (1.24 g, 6.84 mmol, 68.4%). **¹H-NMR** (400 MHz, MeOD): δ [ppm]=7.34 (dd, *J*=1.8, 0.7 Hz, 1H, H-1), 6.27 (dd, *J*=3.2, 1.9 Hz, 1H, H-2), 6.06 – 6.01 (m, 1H, H-3), 4.96 (sept, *J* =6.3 Hz, 1H, H-6), 2.91 (t, *J*=7.3 Hz, 2H, H-4), 2.59 (t, *J*=7.4 Hz, 2H, H-5), 1.20 (d, *J*=6.3 Hz, 6H, H-7,8).

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DA reaction of Isopropyl-3-(2-furyl)propanoate with BM(PEG)3. The synthesis procedure was adapted from GAL-BIS et al[4] and the temperature was increased to 40°C. The crude product is purified via column chromatography (Et$_2$O to Et$_2$O/MeOH 10:1). The product (36 mg, 0.05 mmol, 34%) is obtained as a colorless viscous liquid. **^{1}H-NMR** (400 MHz, DCM): δ [ppm]=6.43 (dd, *J*=5.9, 1.5 Hz, 2H, H-2-*exo*), 6.30 (dd, *J*=5.8, 1.6 Hz, 2H, H-2-*endo*), 6.26 (d, *J*=5.7 Hz, 2H, H-3-*exo*), 6.15 (d, *J*=5.8 Hz, 2H, H-3-*endo*), 5.12 (dd, *J*=5.5, 1.6 Hz, 2H, H-1-*endo*), 5.07 (d, *J*=1.7 Hz, 2H, H-1-*exo*), 4.91 (hept, *J*=6.3 Hz, 2H, H-6), 3.62 – 3.35 (m, 16H, H-10,11,12, H-8-*endo*), 3.07 (d, *J*=7.6 Hz, 2H, H-9-*endo*), 2.89 (d, *J*=6.5 Hz, 2H, H-8-*exo*), 2.69 (d, *J*=6.5 Hz, 2H, H-9-*exo*), 2.56 – 2.11 (m, 10H, H-4,5), 1.14 (d, *J*=6.2 Hz, 12H, H-7).

Synthesis of PHPMA. HPMA is polymerized as 1 M solution in MeOH with addition of 1 mol% AIBN with a total volume of 5 mL at 60 °C for 16 hours in a shaking block. The reaction is stopped by immersing the vials in ice water. The polymer is precipitated in ice-cold diethyl ether. The white solid is centrifuged off, washed with cold diethyl ether, and dried overnight in vacuo. The polymer is reprecipitated from methanol in diethyl ether. **^{1}H-NMR** (400 MHz, DMSO): δ [ppm]=7.18 (s, 1H, H-5), 4.71 (s, 1H, H-3), 3.93 (m, 1H, H- 2), 3.10-3.21 (m, 2H, H-4); 1.78-1.87 (m, 2H, H-7), 0.99-1.2 (m, 6H, H-1,6).

Functionalization of PHPMA with furan. Under nitrogen atmosphere, a solution of DCC in dry DCM is added dropwise to a stirring solution of 3-(2-furyl)propionic acid in dry DCM. The solution is stirred overnight at room temperature. The precipitate is removed by filtration and the filtrate is evaporated on the rotary evaporator to approximately 1 mL. This concentrated filtrate is added dropwise to a solution of PHPMA in DMSO (all equivalents can be found in Table 1) and subsequently, a solution of DMAP (1.05 eq according to OH

groups) in DMSO is added. The reaction mixture is stirred at 30 °C for 48 h. The furyl-modified PHPMA is dialyzed against 0.1 M NaCl solution and water. Subsequently, the solution is freeze-dried and giving a slightly brown solid (71%). **^{1}H-NMR** (400 MHz, DMSO): δ [ppm]=7.49 (s, 1H, H-13), 7.10 (s, 1H, H-5), 6.33 (s, 1H, H- 12), 6.1 (s, 1H, H-11), 4.82 (s, 1H, H-3), 4.70 (s, 1H, H-8), 3.97-3.68 (m, 3H, H-2,4), 3.21-2.51 (m, 4H, H-9,10), 1.85-0.63 (m, 8H, H-1,6,7).

Preparation of Hydrogels. For the crosslinking reaction, stoichiometrically equivalent amounts of the furan-modified PHPMA and 4-arm-PEG with maleimide endgroups were each dissolved separately in water and then mixed. The mixture is shaken at 37 °C until gelation occurs. The gelation time is determined by the vial inverse method.

3 Results and Discussion

3.1 Determination of *retro*-Diels-Alder temperature for model compounds

In a DA reaction, two stereoisomers are formed: The kinetically favoured product (*endo*) and the thermodynamically more stable product (*exo*). The two products differ in their *retro*-DA temperature so that the model reaction as shown in Figure 1a was initially chosen to investigate the *retro*-DA temperatures of both products.

The differential scanning calorimetry (DSC) measurement of the DA product (Figure 1b) shows two endothermic signals, with the second signal appearing asymmetric, so two endothermic processes are suspected underneath. Accord-
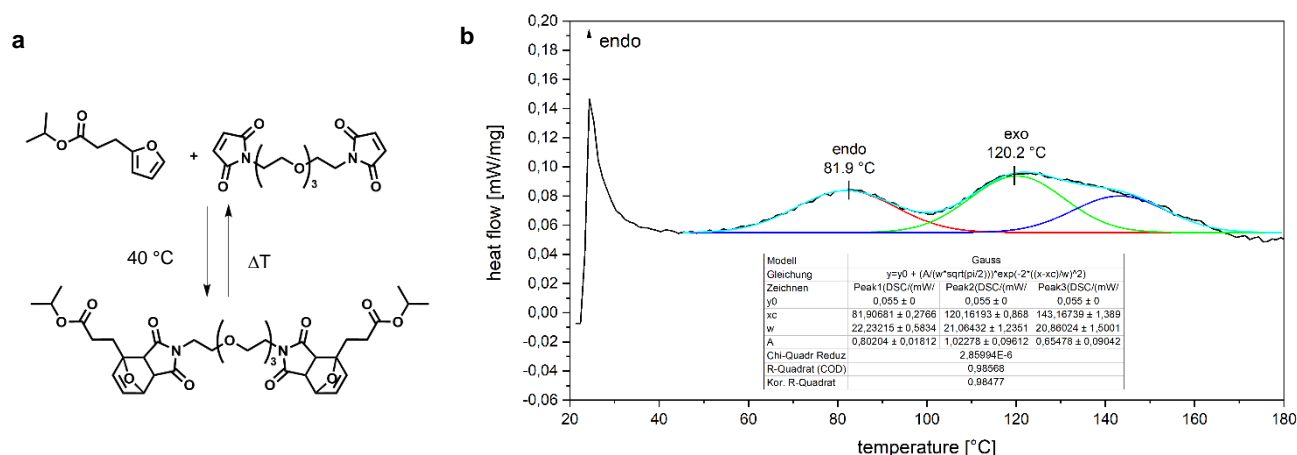

Figure 1: a) DA model reaction, b) corresponding DSC measurements for the determination of *retro*-DA temperature.

ingly, a curve fitting with three Gaussian curves was performed. The first two processes can be assigned to the *retro*-DA reactions of the two stereoisomers. The *retro*-DA temperature of the *endo*-product (81.8 °C) is about 40 °C lower than that of the *exo*-product (120.2 °C). Such a difference has already been found for other furan-maleimide-DA products.[5] The third endothermic process is assigned to the evaporation of the furyl ester formed in the *retro*-DA reaction. These results indicate that during the synthesis of the hydrogels, the formation of the *exo*-product should be largely prevented in order to keep the temperature for liquefication as low as possible.

3.2 Preparation of PHPMA-Furan

For hydrogels with biomedical application, polymers must be selected that exhibit both long-term stability and high biocompatibility. Poly-(*N*-(2-hydroxy-propyl) methacryl amide (PHPMA) has shown promise as a polymer, as it is known to have high biocompatibility and can form hydrogels through crosslinking.[6] Steglich esterification is used here to functionalize the PHPMA with furan groups and to adjust various degrees of substitution (DS) (Table 1). The DS was determined by UV-Vis spectroscopy employing a calibration with isopropyl-3-(2-furyl)propanoate.

Table 1: Results of Steglich-esterification of PHPMA.

OH [eq]	Furan [eq]	DCC [eq]	DS [%]
1	0.5	0.32	10.4±3.1
1	0.25	0.16	7.2±2.1
1	0.125	0.08	2.0±0.7

The experimentally determined values of the DS are significantly below the theoretical values, thus coupling efficiency is only in the range of 30-40%. However, only a small DS of 1-15% is required for the synthesis of a hydrogel, which can be easily achieved with this reaction (Table 1).

3.3 Hydrogel synthesis and characterization

A 4-arm-PEG with maleimide endgroups is used to crosslink PHPMA (Figure 2a). For the hydrogel preparation, both polymers are dissolved in water and mixed in equimolar amounts. Gelation was proven by vial-inverse tests (Figure 2c). All solutions have shown gelation at 37 °C within three days.

Dynamic rheological analyses were performed to characterize the viscoelastic properties of the hydrogels obtained from PHMPA with different DS. Rheometer measurements were performed at 20 °C using a plate-plate geometry. For this purpose, amplitude sweeps were performed in advance with all hydrogels to identify the linear viscoelastic region where both the storage modulus G' and the loss modulus G" are independent of the applied load and the material remains undamaged. Frequency sweeps at this amplitude then can be used to characterize the dynamic properties of the hydrogel and determine the linear equilibrium plateau. For the following frequency sweeps, a constant shear stress of 10 Pa was chosen, and the frequency was increased from 0.1 Hz to 100 Hz.

All three hydrogels demonstrate a typical behavior in frequency-dependent measurements. The hydrogels first show a plateau of the storage modulus G' at low frequencies and then a strong increase of the storage modulus.[7] The lower the DS the lower the crosslink density will be. Higher crosslinking density results in stronger gels.[8] The hydrogel with a DS of 2% is very soft and less stable and accordingly shows a lower storage modulus G'. A DS of 6% already leads to an increase in the storage modulus by a factor of 10. A DS of 14% is still another order of magnitude higher, which is also noticeable in the haptics of the hydrogels. The plateau in the storage modulus G', the gel shear modulus (Figure 2b), can be used to calculate the mesh size. To do this, a linear fit with a slope of zero is first placed through the plateau to obtain a constant value. The mesh sizes $\xi_{s,i}^{rheo}$ were calculated as described by PRES-COSOLIDO et al[9] with equation (1) and are compiled in Table 2.

$$\frac{3}{4}\pi\left(\xi_{s,i}^{rheo}\right)^3 = \frac{RT}{G'N_A} \qquad (1)$$

As expected, the mesh size for a hydrogel of the same concentration consisting of a polymer backbone with a DS of 2% is significantly larger than with a DS of 6% or 14%. For a DS of 2%, the mesh size is 15.4 nm, while for a DS of 6%, a value of 6.2 nm is already reached. For a DS of 14%, the mesh size is again significantly smaller at 3.6 nm. Therefore, hydrogels of different stiffness can be prepared using prepolymers of different DS. The hydrogel with a DS of 2% is thus in a similar range to brain and nervous tissue, whereas hydrogels with higher DS are more similar to relaxed muscle tissue.[10]

Table 2: Storage modulus and calculated mesh size of hydrogels with different compositions.

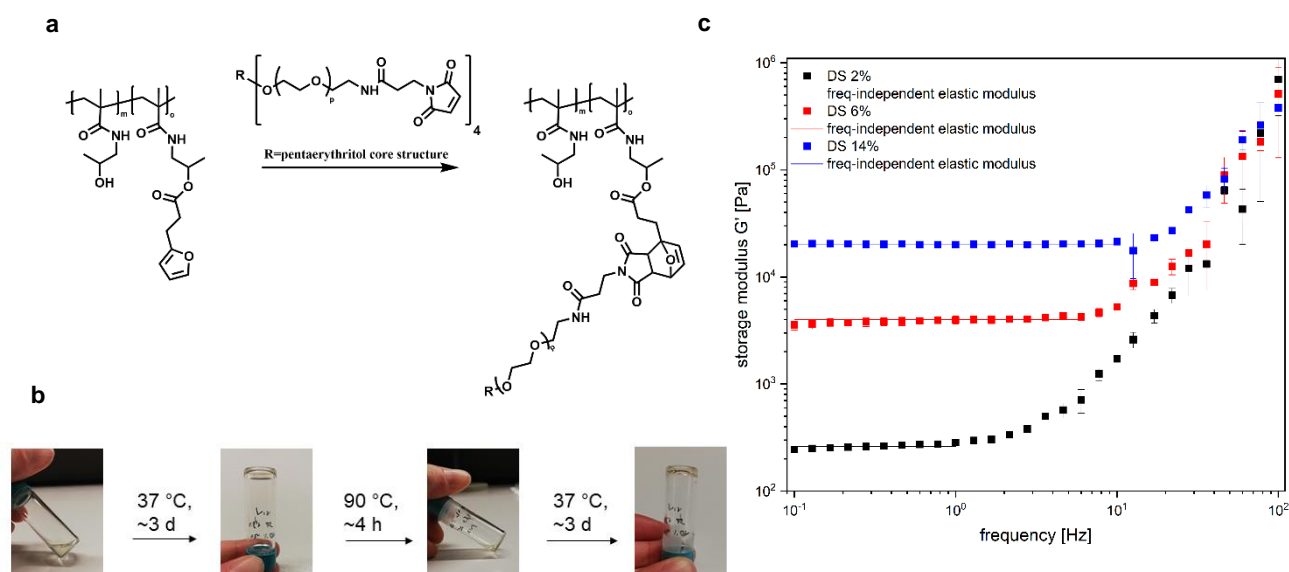| DS [%]	Concentration [% w/v]	G' [Pa]	$\xi_{s,i}^{rheo}$ [nm]
2	10	260.7	15.4
6	10	3991.8	6.2
14	10	20243.6	3.6

Figure 2: a) reaction scheme for synthesis of Diels-Alder hydrogel on the base of furan-functionalized PHPMA and 4-arm-PEG-maleimide, b) gelation-liquefication cycle for a hydrogel with DS of 2%, c) rheologic measurements.

3.4 Hydrogel liquefaction

The gelation should be thermoreversible due to the crosslinking via DA reaction. To test this, the hydrogels were heated to 90 °C. For most hydrogels, this process caused the water to separate from the structure and the gel to dry out. However, for the hydrogel with a DS of 2%, liquefaction was observed at 90 °C via the vial inverse method (Figure 2b). Regelation could be achieved again at 37 °C.

This test shows that DA crosslinking can be reversed at temperatures around 90 °C. One explanation for the fact that liquefication of the hydrogels only occurs at a low DS is that a high concentration of diene/dienophile leads to an increase in the *retro*-DA temperature.[4] Also, the *retro*-DA temperature for the *exo*-product is around 120°C (compare Figure 1b) so that the proportion of the *exo*-product may be too high. However, an attempt to liquefy the hydrogels at higher temperatures also resulted only in desiccation. The evaporation of the water seems to be much faster than the *retro*-DA reaction.

4 Conclusion

In summary, reversible gelation of furan-functionalized PHPMA crosslinked via DA reaction with 4-arm-PEG-maleimide is possible. However, for higher DS, the *retro*-DA temperature increases such that only the water evaporates, and no liquefaction could be achieved. At this point, further investigations are needed to make liquefaction possible.

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