Self-Assembling Hydrogels Based on β-Cyclodextrin/Cholesterol Inclusion Complexes

Frank van de Manakker, Martin van der Pot, Tina Vermonden, Cornelus F. van Nostrum, and Wim E. Hennink*

Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Sorbonnelaan 16, P.O. Box 80082, 3508 TB Utrecht, The Netherlands

Received November 23, 2007; Revised Manuscript Received January 8, 2008

ABSTRACT: A novel self-assembling poly(ethylene glycol) hydrogel system based on inclusion complexes between β-cyclodextrin (β-CD) and cholesterol is described. Hydrogels are formed after hydration of a mixture of star-shaped 8-arm poly(ethylene glycol) (PEG) end-modified with β-CD groups and the same star-shaped PEG end-modified with cholesterol moieties. Rheological analysis as well as 2D-NMR spectroscopy demonstrated that the obtained gels are due to formation of β-CD/cholesterol inclusion complexes. As also observed by rheology, the hydrogels are fully thermoreversible upon repetitive heating and cooling steps. Hydrogel properties were dependent on polymer concentration, the β-CD/cholesterol stoichiometry, and the molecular weight of the star-shaped PEG. Because of their assumed biocompatibility and expected physiological clearance, hydrogels based on star-shaped PEG and β-CD/cholesterol inclusion complexes offer excellent opportunities as drug delivery matrices and for other pharmaceutical and biomedical applications.

1. Introduction

Hydrogels are hydrophilic polymer networks, which absorb substantial amounts of water and are under investigation for biomedical and pharmaceutical applications.[1−3] Hydrogels can be prepared by either chemical or physical cross-linking of hydrophilic polymers.[4] Chemical cross-linking methods introduce covalent cross-links between the polymer chains, e.g., by radical polymerization of methacrylate-derivatized polymers.[5−7] Because the cross-linking agents may potentially damage the loaded substances (e.g., pharmaceutical proteins, cells), physical cross-linking is preferred in which the network is retained by nonpermanent, reversible interactions between the polymer chains. Examples of physical interactions used for the design of hydrogels are ionic interactions,[8,9] hydrophobic interactions between amphiphilic polymers,[10−12] hydrogen bonding,[13,14] and stereocomplex formation between polymers with opposite chiralities.[15−17] Also, hydrogels have been designed making use of biomimetic interactions, like antigen−antibody interactions,[18] peptide−glycoprotein[19] interactions, and the integration of specific protein folding motifs.[20−22] Because of their self-assembling properties, hydrogels based on physical interactions are attractive systems for pharmaceutical and biomedical applications, like (injectable) in-situ gelling devices for drug delivery or tissue engineering.[23−25]

Physical interactions that potentially can be exploited for network formation are inclusion complexes formed by guest molecules and cyclodextrins (CDs). Cyclodextrins are cyclic oligosaccharides, joined by α-1,4-glucosidic linkages.[26,27] Subtypes are α-, β-, and γ-cyclodextrins, consisting of 6, 7, and 8 glucopyranose units, respectively. Because the hydroxyl groups are only located at the outer surface of the cyclodextrin molecule, the interior cavity is relatively hydrophobic. This feature gives CDs the ability to complex a wide range of lipophilic guest molecules (e.g., adamantane, cholesterol), which is mainly driven by hydrophobic effects (release of ordered water molecules from the CD cavity) and van der Waals interactions.[28−30] The capability of CD to form inclusion complexes with guest molecules has been exploited in pharmacy,[26,31−34] separation processes,[35] and material sciences[36,37] as well as in the food and cosmetic industry.[38]

Besides small guest molecules, linear polymers among which are poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), and poly(ϵ-caprolactone) (PCL) can also penetrate into the inner cavity of CDs to form so-called polypseudorotaxanes.[39] Based hereon, several hydrogel systems have been described, e.g., inclusion complexation of linear or multiarm PEG with α-cyclodextrins leads to α-CD microdomains formed by hydrogen bonds, which act as physical cross-links in hydrogels.[40−43] To obtain more stable hydrogels, amphiphilic triblock copolymers, PEG−poly(3-hydroxybutyrate)−PEG (PEG−PHB−PEG), PCL−PEG−PCL, or Pluronics, were complexed with α-CDs.[44−46] On the basis of this principle, β-cyclodextrin (β-CD) was used to complex with PPG.[47] For hydrogel formation, PPG, which is bulkier than PEG proved to be a suitable guest polymer for β-CD.

In the aforementioned studies of covalently cross-linked and self-assembling supramolecular physical hydrogels, these cyclodextrin-containing systems are based on covalently cross-linked CDs or polypseudorotaxane formation. The use of inclusion complexes between β-CD and low molecular weight guest molecules to design stimuli-sensitive hydrogels has recently been reported.[48−51] Hashidzume et al. described stimuli-responsive hydrogel systems by combining poly(acrylamide) derivatized with either β-CD or various guest molecules.[49] In another study, temperature sensitive hydrogels were formed after mixing aqueous solutions of adamantyl-containing *N*-isopropylacrylamide copolymers with an aqueous solution of β-CD dimer.[50] In this paper a novel self-assembling hydrogel system consisting of cholesterol- and β-CD-modified star-shaped 8-arm poly(ethylene glycol) (PEG_8) is described, in which the functional groups are coupled to the 8-arm PEG end groups via a succinyl linker to introduce hydrolyzable ester bonds. Cholesterol has the ability to form inclusion complexes with β-CDs.[32,52] In this system, physically cross-linked networks are therefore

* Corresponding author: Tel +31 30 253 6964; fax +31 30 251 7839; e-mail w.e.hennink@uu.nl.

10.1021/ma702607r CCC: $40.75
Published on Web 02/16/2008

established by the formation of inclusion complexes between the guest molecule cholesterol and β-CD units.

2. Experimental Part

Materials. Star shaped 8-arm poly(ethylene glycol)s (PEG_8-OH) were purchased from JenKem Technology USA (Allen, USA). Products with various MW's were used: $PEG_8$10K-OH (M_n = 9656 Da (MALDI), PDI = 1.10), $PEG_8$20K-OH (M_n = 20 185 Da (MALDI), PDI = 1.08), and $PEG_8$40K-OH (M_n = 42 680 Da (MALDI), PDI = 1.06). Linear monomethoxypoly(ethylene glycol) (mPEG5000-OH) and poly(ethylene glycol) (HO-PEG6000-OH) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands) and Fluka (Buchs, Switzerland), respectively. The M_n's of these linear PEG's were determined by ^{1}H NMR spectroscopy using trichloroacetyl isocyanate (TCAI, Sigma-Aldrich, Zwijndrecht, The Netherlands) as a shift reagent.[53] Using this method, M_n values of the mPEG5000-OH and HO-PEG6000-OH products were measured to be 5.1 and 6.8 kDa, respectively. Prior to use, all PEG's were dried on Sicapent (Merck, Darmstadt, Germany) for at least 24 h in vacuo. Cholesterol, succinic anhydride (SA), 4-(*N*,*N*-dimethylamino)pyridine (DMAP), 6-monodeoxy-6-monoamino-β-cyclodextrin (βCD-NH_2·HCl), *N*-ethyl-*N*′-(3-(dimethylamino)propyl)-carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), 1-adamantanecarboxylic acid (ACA), lithium chloride (LiCl), chloroform-d ($CDCl_3$, 99.8 atom % D), and deuterium oxide (D_2O, 99.9 atom % D) were provided by Sigma-Aldrich (Zwijndrecht, The Netherlands). Dicyclohexylcarbodiimide (DCC) was obtained from Acros Chimica (Geel, Belgium). Dichloromethane (DCM, peptide grade), tetrahydrofuran (THF, HPLC grade), *N*,*N*-dimethylformamide (DMF, peptide grade), and diethyl ether (Et_2O, AR stabilized) were supplied by Biosolve Ltd. (Valkenswaard, The Netherlands). DCM was dried and stored over 4 Å molecular sieves before use. Ammonium acetate (NH_4OAc), triethylamine (TEA), and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). For dialysis, Slide-A-Lyzer dialysis cassettes (MWCO = 10 000 Da, Perbio Science, Etten-Leur, The Netherlands) were used.

Characterization of Synthesized Products by ^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded of samples dissolved in $CDCl_3$ or D_2O on a Gemini 300 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA) using $CHCl_3$ at 7.26 ppm or HDO at 4.79 ppm as reference line, respectively. Typically 10−25 mg polymer was dissolved in 0.8 mL of deuterated solvent. To reduce baseline noise, 64−256 repetitive scans were taken. Autophasing and baseline correction in the region δ_H = 1.0−4.5 ppm were applied. DS values (degree of substitution; functionalized groups per 8-arm PEG molecule) of the synthesized products were determined with the use of the peak intensities of the signals corresponding to the protons of the coupled groups (e.g., cholesterol or succinyl) and the signal (3.4−3.8 ppm) corresponding to the known number of protons in the PEG chains and (in the case of 8-arm PEG) also the hexaglycerine backbone.

Spatial information was obtained from 2D-NMR (NOESY) spectra (τ_m = 300 ms) of a 1:1 (w/w) mixture of βCD- (DS: 7.4) and cholesterol-modified (DS: 6.1) $PEG_8$20K dissolved in D_2O (12 mg/mL). For comparison, a solution (1:1 (w/w)) of cholesterol-modified $PEG_8$20K and nonfunctionalized $PEG_8$20K was used (D_2O, 12 mg/mL). 2D-NMR spectra were recorded on a Gemini 500 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA). A spectral width of 5 kHz was used.

Characterization of Synthesized Products by Gel Permeation Chromatography (GPC). Number-average (M_n) and weight-average (M_w) molecular weights of the end-modified PEG's were determined by gel permeation chromatography (GPC) on a Waters HPLC system (Alliance 2695 C, Waters, Milford MA) equipped with two linear PLgel 5 μm MIXED-D columns (300 mm length, 7.5 mm i.d.) in series. The column temperature was set to 40 °C. The eluent was DMF containing 10 mM LiCl, and the flow rate was 0.70 mL/min. Samples were dissolved overnight at a concentration of 5 mg/mL in the eluent and filtered through a 0.45 μm filter (Waters, Milford, MA) prior to analysis. Sample temperature was set to 25 °C, and the injection volume was 50 μL. Detection was done with a differential refractometer (sensitivity: 256; temperature: 40 ± 2 °C) (refractive index detector 2414, Waters, Milford, MA). Calibration was done using poly(ethylene glycol) standards of defined molecular weights (M_w from 194 to 439 600 Da; Fluka, Buchs, Switzerland). Sample molecular weights were determined with Empower Software Version 1154 (Waters, Milford, MA).

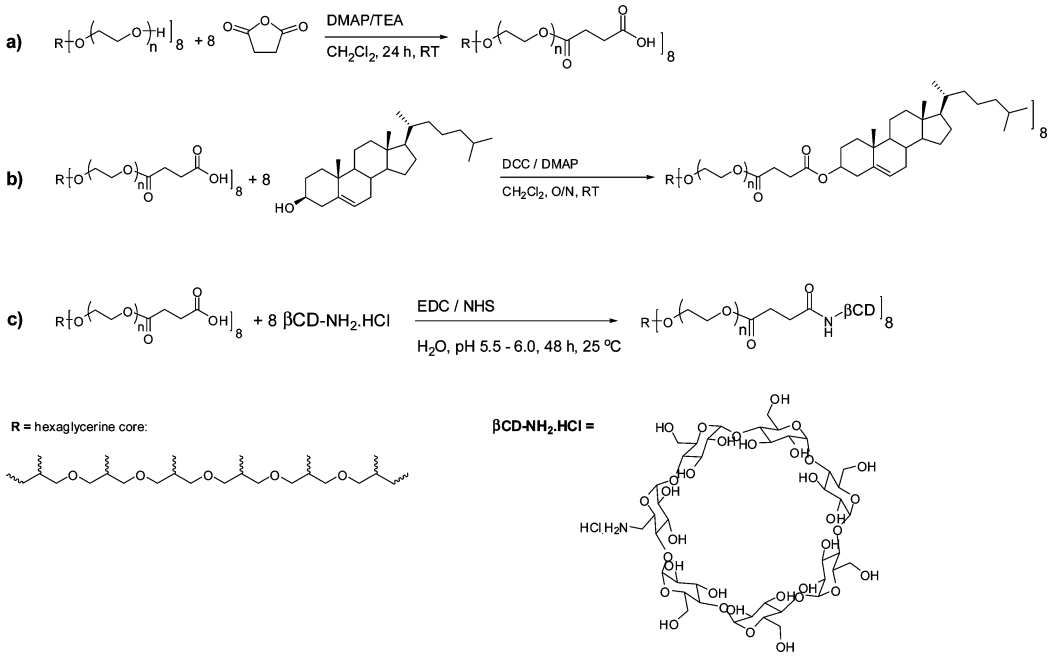
Characterization of β-CD-Modified Polymers by Polarimetry. Optical rotation of the different PEG_8-SA-βCD products was determined with a Jasco P-1010 polarimeter (Jasco Corp., Tokyo, Japan). Aqueous solutions of samples and standards were measured at λ = 589 nm in a thermostated (25 ± 2 °C) cell with a length of 10 cm. Standard solutions of βCD-NH_2·HCl in filtered (0.22 μm Millipore filter) demineralized water were measured, and the response was linear in a concentration up to 10 mM. The determined specific rotation of βCD-NH_2·HCl was $[\alpha]D^{25}$ = 141 deg cm^3 g^{-1} dm^{-1}. Since the 8-arm PEG polymers are not optically active, calculation of the β-CD content in the PEG_8-SA-βCD products was possible after measuring the optical rotation of aqueous solutions of these polymers.

Synthesis of 8-Arm Octasuccinylpoly(ethylene glycol) with Hexaglycerin Core (PEG_8-SA). The hydroxyl end groups of 8-arm PEG were reacted with succinic anhydride to yield 8-arm star shaped PEG's with succinic acid-functionalized end groups. As an example the synthesis of 8-arm SA-modified $PEG_8$20K ($PEG_8$20K-SA) is described. Other 8-arm PEG's (10 and 40 kDa) as well as linear PEG's (mPEG5000 and PEG6000) were modified accordingly. A 250 mL round-bottom flask with a Teflon stirring bar was dried at 150 °C for 12 h and flushed with N_2. After cooling to room temperature, 11 g of predried $PEG_8$20K-OH (0.55 mmol; 4.4 mmol of OH groups), 0.66 g SA (6.5 mmol, 1.5 equiv per OH group), and 0.53 g DMAP (4.4 mmol, 1 equiv per OH group) were dissolved in 110 mL of dry DCM under a N_2 atmosphere. After adding 355 μL TEA (4.4 mmol, 1 equiv per OH group), the solution was stirred under a N_2 atmosphere for 12 h at room temperature. Next, the solvent was evaporated under reduced pressure, and the residue was dissolved in 50 mL of THF, followed by repeated coevaporation (3×). The formed $PEG_8$20K-SA derivative was then precipitated by dropping its solution in THF (volume: 20 mL) into 400 mL of cold (−40 °C) diethyl ether under vigorous stirring. The resulting precipitate was filtered off using a glass filter and washed three times with 50 mL of diethyl ether. After drying in vacuo, 8.8 g of product (77%) was obtained as a white powder, which was analyzed with ^{1}H NMR spectroscopy and GPC.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.7 ppm (−OC(O)CH_2CH_2C(O)OH, 32H), 3.4−3.8 ppm (8-arm PEG H (−OCH_2CH_2O−, 1775H) + hexaglycerine backbone (−CH_2CH(O)CH_2O−, 30H)), 3.9 ppm (−OCH_2CH$_2$OC(O)C−, 16H), 4.3 ppm (−OCH$_2$CH_2OC(O)C−, 16H).

Synthesis of PEG_8-SA-Cholesterol. The synthesized $PEG_8$20K-SA was derivatized with cholesterol moieties using DCC as a condensing agent. In a typical procedure, $PEG_8$20K-SA was dried at room temperature under reduced pressure in the presence of Sicapent. Then, in a predried 100 mL round-bottom flask under N_2 flow, 3 g of $PEG_8$20K-SA (1.1 mmol of COOH end groups), 0.55 g of cholesterol (1.4 mmol, 1.3 equiv per succinyl moiety), and 44 mg of DMAP (0.36 mmol, 0.3 equiv per succinyl moiety) were dissolved in 50 mL of dry DCM. After cooling the solution to 0 °C, 0.19 g of DCC (0.93 mmol, 0.8 equiv per succinyl moiety) was added. After 1 h, the solution was brought to ambient temperature and stirred overnight under a N_2 atmosphere. Next, the mixture was cooled to −20 °C to precipitate dicyclohexylurea (DCU), which was filtered off. The filtrate was evaporated to dryness under reduced pressure, and the obtained residue was dissolved in 70 mL of THF followed by repeated coevaporation (3×) with THF. The polymer was dissolved in ∼20 mL of THF and precipitated into 400 mL cold (−40 °C) diethyl ether under vigorous stirring. The precipitate was collected by filtration and washed three times with 50 mL of diethyl ether. After drying in

Scheme 1. Synthetic Route toward (a) PEG_8-SA, (b) PEG_8-SA-chol, and (c) PEG_8-SA-βCD



vacuo, 2.62 g of product (79%) was obtained as a white powder, which was analyzed with ^{1}H NMR spectroscopy and GPC.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.7 ppm (3H cholesterol group × DS), 0.9 ppm (6H cholesterol group × DS), 0.9−2.2 ppm (32H cholesterol group × DS), 2.3 ppm (2H cholesterol group × DS), 2.6 ppm (−OC(O)CH_2CH_2C(O)O−Chol, 32H), 3.4−3.8 ppm (8-arm PEG H (−OCH_2CH_2O−, 1775H) + hexaglycerine backbone (−CH_2CH(O)CH_2O−, 30H)), 3.9 ppm (−OCH_2CH$_2$OC(O)C−, 16H), 4.4 ppm (−OCH$_2$CH_2OC(O)C−, 16H), 4.6 ppm (1H cholesterol group × DS), 5.4 ppm (1H cholesterol group × DS).

Coupling of cholesterol groups to the 10 and 40 kDa star-shaped PEG_8-SA and linear mono- and bifunctional mPEG5000-SA and SA-PEG6000-SA polymers was established in a similar manner with slight modifications. To obtain $PEG_8$40K-SA-cholesterol with DS 6, 1.6 equiv instead of 0.8 equiv of DCC was added and 1.8 equiv instead of 1.3 equiv of cholesterol was added to the solution of 40 kDa PEG_8-SA. Synthesis details of linear PEG-SA-cholesterol can be found in the Supporting Information.

Synthesis of PEG_8-SA-βCD. 6-Monodeoxy-6-monoamino-β-cyclodextrin (βCD-NH_2·HCl) was coupled to $PEG_8$20K-SA to obtain 8-arm β-CD-functionalized PEG. Coupling of β-CD to the 10 and 40 kDa PEG_8-SA polymers was done accordingly. Below a typical procedure to synthesize $PEG_8$20K-SA-βCD is given.

1.63 g of $PEG_8$20K-SA (0.62 mmol of succinyl moieties), 1.08 g of βCD-NH_2·HCl (0.93 mmol, 1.5 equiv relative to succinyl moieties), and 0.14 g of NHS (1.24 mmol, 2 equiv relative to succinyl moieties) were introduced into a 100 mL round-bottom flask with a Teflon stirring bar and dissolved after addition of 32 mL of demineralized water. Next, the solution was cooled to 0 °C, and 220 μL of an EDC solution (1.24 mmol, 2 equiv relative to succinyl moieties) in demineralized water was added. The pH was adjusted to 5.5−6.0 with a 4 N NaOH solution. After 1 h, the solution was brought to 25 °C and stirred for 48 h, while the pH was kept at 5.5−6.0. The reaction mixture was dialyzed (Slide-A-Lyzer dialysis cassettes (MWCO = 10 000 Da)) for 3 days against 5 L of 10 mM ammonium acetate buffer, pH 4.7 at 4 °C. Buffer was refreshed twice a day. The polymer was collected by lyophilization and further dried in vacuo in the presence of Sicapent at room temperature for 48 h. A white fluffy product was obtained in high yield (94%). The polymer was analyzed by ^{1}H NMR spectroscopy, GPC, and polarimetry.

^{1}H NMR (300 MHz, D_2O, δ): 2.6 ppm (−OC(O)CH_2CH_2C-(O)NH-βCD, 32H), 3.2−3.9 ppm (8-arm PEG H (−OCH_2CH_2O−, 1775H) + hexaglycerine backbone (−CH_2CH(O)CH_2O−, 30H) + βCD glucosidic H (42H × DS)), 4.4 ppm (−OCH$_2$CH_2OC(O)C−, 16H), 5.0 ppm (βCD anomeric H (7H × DS).

Preparation and Rheological Characterization of PEG_8-SA-Cholesterol/PEG_8-SA-βCD Hydrogels. PEG_8-SA-cholesterol and PEG_8-SA-βCD mixtures (molar ratios cholesterol/β-CD varying from 0 to 10) were dissolved in 5 mM NH_4OAc buffer (pH 4.7) to obtain 2% (w/w) solutions. These solutions were then lyophilized, and hydrogels were obtained by hydration of the lyophilized mixtures (10−40 mg) for 16 h at 4 °C with appropriate amounts of 100 mM NH_4OAc pH 4.7 buffer (160−190 μL).

Adamantanecarboxylic acid (ACA) was used to competitively displace cholesterol from the β-CD cavities. Therefore, increasing amounts of ACA (0−2 equiv relative to the amount of present cholesterol moieties) were added to a 2% (w/w) solution of PEG_8-SA-cholesterol and PEG_8-SA-βCD (molar ratio cholesterol/ β-CD = 1) in 5 mM NH_4OAc buffer (pH 4.7). After lyophilization of the resulting mixtures, hydrogels were prepared as mentioned above.

Rheological characterization of the hydrogels was done with a AR1000-N rheometer (TA Instruments, Etten-Leur, The Netherlands) equipped with a 1° steel cone geometry of 20 mm diameter and solvent trap. Using a spatula or pipet (for weak gels), an ∼55 μL sample was placed between the preheated (40 °C) plates of the rheometer. Rheological gel characteristics were monitored by oscillatory time sweep, strain sweep, and temperature sweep experiments. During time sweep experiments the G' (shear storage modulus) and G'' (loss modulus) were measured at 4, 20, and 37 °C for a period of 5 min. Temperature sweep experiments were done to investigate the temperature dependency of the hydrogels' viscoelastic properties, after increasing the temperature from 4 °C (heating rate: 1 °C/min) to 50 °C. After each temperature increment (1 °C) and 30 s equilibration, G' and G'' were measured. The point at which G''/G' (= tan δ) = 1 is considered as the gel temperature (T_{gel}).[54] In both time and temperature sweep experiments, a constant strain of 1% and frequency of 1 Hz were used.

3. Results and Discussion

Synthesis and Characterization of PEG_8-SA-Cholesterol and PEG_8-SA-βCD. The synthesis of β-CD- and cholesterol-functionalized 8-arm PEG polymers was performed according to Scheme 1. The degree of substitution (DS) is defined as the number of either cholesterol or β-CD per PEG molecule.

Star-shaped PEG_8-OH was first converted into PEG_8-SA by a base-catalyzed reaction with succinic anhydride (SA). SA-

Table 1. M_n, M_w, and Polydispersity (PD) of the Star-Shaped 8-Arm PEG's and Their Derivatives, Determined by GPC Using Linear PEG as Calibration Standards

	DS[a]	M_n (kDa)	M_w (kDa)	PD
$PEG_8$40K-OH		24.1	26.0	1.1
$PEG_8$20K-OH		12.2	13.2	1.1
$PEG_8$10K-OH		6.5	6.9	1.1
$PEG_8$40K-SA	8	18.6	20.5	1.1
$PEG_8$20K-SA	8	6.9	7.5	1.1
$PEG_8$10K-SA	8	1.0[b]	1.4	1.4
$PEG_8$40K-SA-chol	6.4	19.7	22.8	1.2
$PEG_8$20K-SA-chol	6.1	9.0	9.7	1.1
$PEG_8$10K-SA-chol	6.3	3.3	3.8	1.1
$PEG_8$40K-SA-β**CD**	6.9	31.1	42.9	1.4
$PEG_8$20K-SA-β**CD**	7.1	16.7	21.5	1.3
$PEG_8$10K-SA-β**CD**	7.2	11.5	14.4	1.3

[a] Degree of substitution (DS) defined as the number of coupled groups per PEG molecule determined by ^{1}H NMR spectroscopy or polarimetry. [b] Underestimated M_n likely due to column interactions.

modified 8-arm PEG's (10, 20, and 40 kDa) were obtained in high yields (>85%), and the peak integrals in the ^{1}H NMR spectra showed that the hydroxyl end groups were quantitatively derivatized. Next, PEG_8-SA-cholesterol was synthesized by reaction of cholesterol with PEG_8-SA using dicyclohexylcarbodiimide (DCC) as coupling agent. In this study we focused on the synthesis of PEG_8-SA-cholesterol with 5−6 cholesterol groups per 8-arm PEG molecule because quantitative conjugation of the polymer end groups yielded a PEG_8-SA-cholesterol with very limited solubility in water. ^{1}H NMR spectra showed that cholesterol moieties were coupled (DS 5.1−6.7) to PEG_8-SA end groups. For the 10, 40, and 20 kDa PEG_8-SA-cholesterol, mass yields were in the range 60−90%.

PEG_8-SA-βCD was also synthesized using carbodiimide chemistry. Because 6-monodeoxy-6-monoamino-β-cyclodextrin (βCD-NH_2·HCl) is not soluble in DCM, the reaction was done in water (pH 5.5−6.0) using EDC and NHS as coupling agent and catalyst, respectively. After dialysis and freeze-drying, PEG_8-SA-βCD polymers with DS ranging from 6.9 to 8.0 were obtained with high mass yields of more than 90%. The DS of the PEG_8-SA-βCD was determined with polarimetry because the partial overlap and/or disturbance of the CD proton peaks in the ^{1}H NMR spectra did not allow an accurate analysis.

We further refer to a specific polymer as PEG_8*xx*K-SA-chol$_y$ or PEG_8*xx*K-SA-βCD_y, where *xx* stands for the used 8-arm PEG (10, 20, or 40 kDa) and *y* indicates the number of coupled groups per 8-arm PEG molecule (DS).

The molecular weights of the different functionalized PEG's as well as the starting polymers were determined using GPC with DMF/10 mM LiCl as eluent (Table 1). Because calibration was done with linear PEG's, the observed M_n's of the star-shaped PEG's were lower than expected. Modification of these polymers with SA led to a decrease in M_n. Probably the succinic acid moieties deteriorate solvation of the polymer chains by the eluent resulting in a decrease of its hydrodynamic volume. After modification of the different PEG_8-SA polymers with cholesterol or β-CD, an increase in M_n compared to the succinyl-modified polymers was observed.

Linear monofunctional (mPEG-OH (5 kDa)) and bifunctional (HO-PEG-OH (6 kDa)) PEG's were also quantitatively derivatized with succinic anhydride, followed by coupling of cholesterol. In these polymers 90% of the terminal COOH groups was derivatized with cholesterol groups (^{1}H NMR); these polymers are further denoted as mPEG5000-SA-chol$_{0.9}$ and chol$_{0.9}$-SA-PEG6000-SA-chol$_{0.9}$.

More detailed ^{1}H NMR, GPC, and polarimetry data of the different polymers can be found in the Supporting Information.

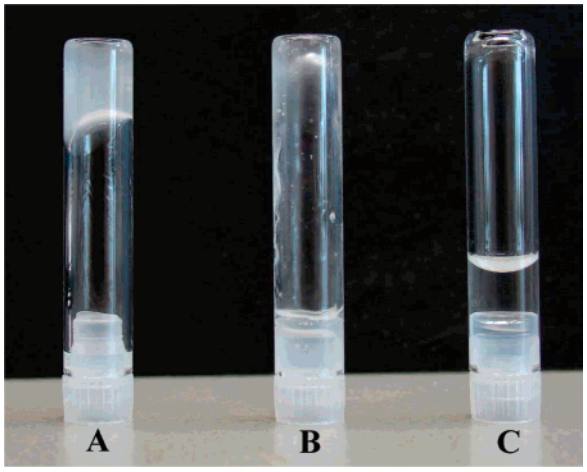


Figure 1. Photographs of a 17.5% (w/w) $PEG_8$20K-SA-chol$_{5.7}$/PEG_8-20K-SA-$\beta CD_{8.0}$ (molar ratio β-CD/chol 1:1) hydrogel at room temperature (A) and two controls, namely mixtures of $PEG_8$20K-SA-chol$_{5.7}$/$PEG_8$20K-OH (B) and $PEG_8$20K-SA-$\beta CD_{8.0}$/$PEG_8$20K-OH (C).

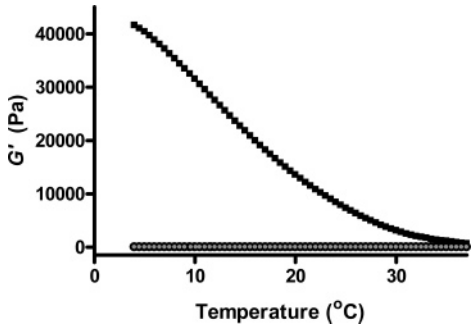


Figure 2. Storage modulus (G') of a 17.5% (w/w) $PEG_8$20K-SA-chol$_{5.7}$/$PEG_8$20K-SA-$\beta CD_{8.0}$ hydrogel (■), a $PEG_8$20K-SA-chol$_{5.7}$/PEG_8-20K-OH mixture (●), and a $PEG_8$20K-SA-$\beta CD_{8.0}$/$PEG_8$20K-OH (gray ○) mixture as a function of temperature.

Hydrogel Formation. To prepare homogeneous hydrogels, $PEG_8$20K-SA-chol$_{5.7}$ and $PEG_8$20K-SA-$\beta CD_{8.0}$ were first dissolved at a low concentration (2% (w/w)) in a 5 mM ammonium acetate buffer (pH 4.7). After lyophilization, the resulting mixture was hydrated in 100 mM ammonium acetate buffer (pH 4.7) at 4 °C to minimize hydrolysis of ester linkages.

Figure 1 shows photographs of hydrated mixtures of one of the functionalized polymer components ($PEG_8$20K-SA-chol$_{5.7}$ or $PEG_8$20K-SA-$\beta CD_{8.0}$) and nonfunctionalized $PEG_8$20K-OH (B and C) as well as both functionalized components together (A). Both controls (B and C) yielded viscous solutions, whereas a gel was formed when a mixture of βCD- and cholesterol-modified 8-arm PEG's was hydrated (Figure 1A). In line herewith, Figure 2 shows that hydration of the βCD- and cholesterol-modified 8-arm PEG's resulted in the formation of a viscoelastic hydrogel, whereas if one of the functionalized polymers was replaced by nonfunctionalized PEG_8, solutions with only fluidlike properties were obtained.

The results presented in Figures 1 and 2 indicate that both cholesterol- and β-CD-modified star-shaped PEG polymers are required to form hydrogels and that this gelation is likely caused by the formation of inclusion complexes between β-CD and cholesterol (Figure 3).

Figure 4A shows the temperature-dependent rheological characteristics of a 10% (w/w) $PEG_8$20K-SA-chol$_{6.1}$/$PEG_8$20K-SA-$\beta CD_{7.4}$ (chol/β-CD = 1) mixture. At 4 °C, a G' of 13.3 ± 0.9 kPa and tan δ of 0.32 ± 0.01 (n = 3) were observed, demonstrating that under these conditions a viscoelastic hydrogel

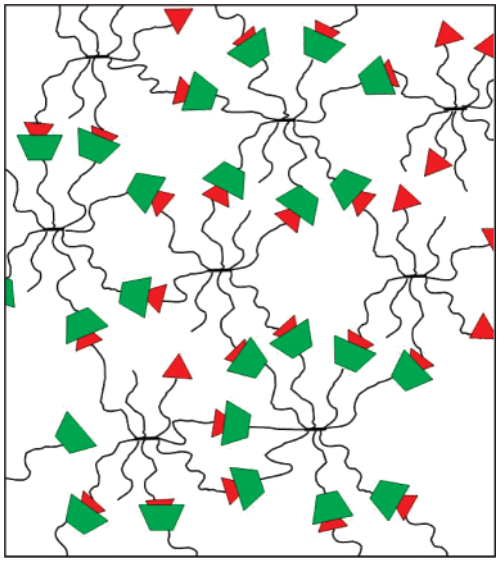


Figure 3. Hydrogel concept based on inclusion complexes between cholesterol (triangles) and β-CD (cone-shaped structures) moieties coupled to star-shaped 8-arm PEG.

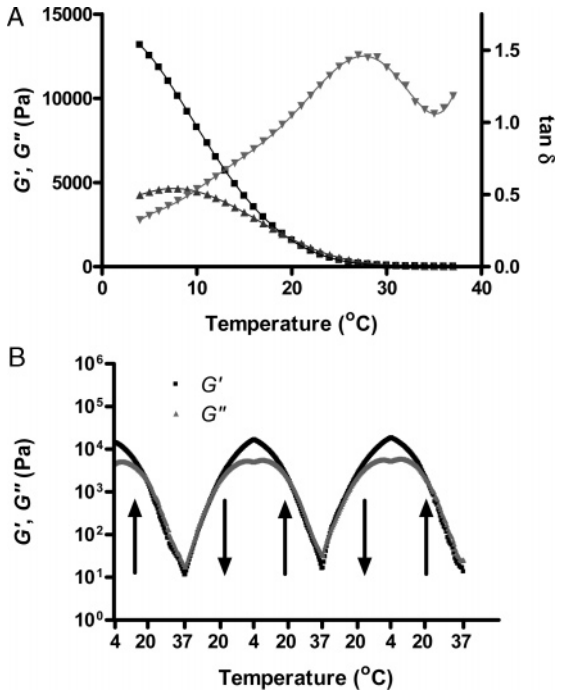


Figure 4. (A) Rheological properties of a 10% (w/w) $PEG_8$20K-SA-chol$_{6.1}$/$PEG_8$20K-SA-$\beta CD_{7.4}$ hydrogel as a function of temperature. G' (■, left axis), G'' (▲, left axis), and tan δ (▼, right axis). (B) Thermoreversibility of a 10% (w/w) $PEG_8$20K-SA-chol$_{6.1}$/$PEG_8$20K-SA-$\beta CD_{7.4}$ system. G' (■) and G'' (▲) upon repeated heating (↑) and cooling (↓) in the temperature range 4−37 °C.

was formed. However, with increasing temperature, G' gradually decreased, which was associated with a concomitant increase of tan δ, indicating that a gel-to-sol transition occurred. T_{gel}, the temperature where G' equals G'' (tan δ = 1), was 20 ± 1 °C for this hydrogel.

Figure 4B shows the G' and G'' of the 10% (w/w) PEG_8-20K-SA-chol$_{6.1}$/$PEG_8$20K-SA-$\beta CD_{7.4}$ hydrogel system upon repetitive heating and cooling. After heating from 4 to 37 °C and subsequent cooling from 37 to 4 °C, the G' and G'' reached their original values, demonstrating the thermoreversibility of the PEG_8-SA-chol/PEG_8-SA-βCD hydrogel system. When cholesterol/β-CD complexes are formed, the PEG chains will lose mobility, which translates in a decrease in entropy ($\Delta S < 0$). Below T_{gel}, the system is in a gel state because the binding enthalpy of the cholesterol/β-CD complexes compensates for the entropy loss. As the change in Gibbs free energy (ΔG), given by $\Delta H - T\Delta S$, increases with temperature at $T > T_{gel}$ due to the larger positive $T\Delta S$, the binding enthalpy of the cholesterol/β-CD complexes (ΔH) cannot compensate for this larger positive $T\Delta S$, which results in disruption of the gel.

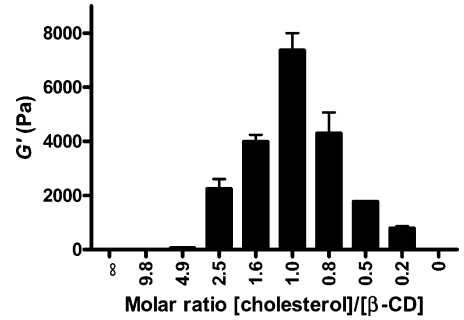


Figure 5. Storage modulus (G') of 15% (w/w) $PEG_8$20K-SA-$\beta CD_{8.0}$/$PEG_8$20K-SA-chol$_{6.1}$ hydrogel mixtures at 20 °C in relationship to the cholesterol/β-CD stoichiometry (expressed as molar ratio of cholesterol/β-CD groups). To control the molar ratio of cholesterol/β-CD groups, different amounts of one of the hydrogel components were replaced by nonfunctionalized $PEG_8$20K-OH. The data are shown as average and SEM, n = 3.

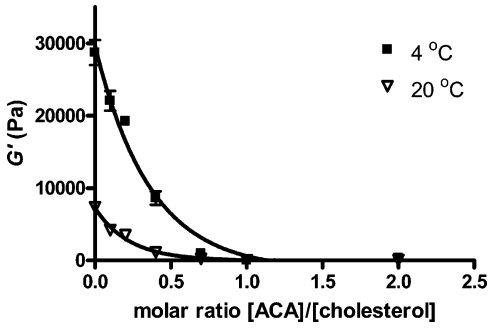


Figure 6. Storage modulus (G') of 15% (w/w) $PEG_8$20K-SA-$\beta CD_{8.0}$/$PEG_8$20K-SA-chol$_{5.7}$ hydrogel system (molar ratio cholesterol/β-CD = 1/1) at 4 and 20 °C with increasing amounts of the competitive β-CD-interacting compound 1-adamantanecarboxylic acid. The data are shown as average and SEM, n = 3.

Figure 5 shows the storage moduli of $PEG_8$20K-SA-$\beta CD_{8.0}$/$PEG_8$20K-SA-chol$_{6.1}$ gels as a function of the molar ratio between present β-CD and cholesterol moieties in the hydrogels. The highest G' was observed at an equimolar ratio of cholesterol and β-CD. When either β-CD or cholesterol was present in excess, a decrease in G' was found. For mixtures comprising ~10× more cholesterol than β-CD groups and vice versa, G' was negligible and viscous solutions remained. This suggests that gel formation is indeed caused by formation of cholesterol/β-CD inclusion complexes.

To further demonstrate the occurrence of β-CD/cholesterol interactions as a driving force for network formation, increasing amounts of the competitive agent 1-adamantanecarboxylic acid (ACA has a 2-fold higher binding affinity toward β-CD compared to cholesterol[55−57]) were added to $PEG_8$20K-SA-$\beta CD_{8.0}$/$PEG_8$20K-SA-chol$_{5.7}$ hydrogels. Figure 6 shows that G' of the gels decreased with increasing concentrations of ACA at both 4 and 20 °C. At an equimolar ACA/cholesterol group ratio the gel structure was completely broken, and a viscous solution remained. These results give further evidence that network formation in this hydrogel system is due to reversible β-CD/cholesterol inclusion complexes.

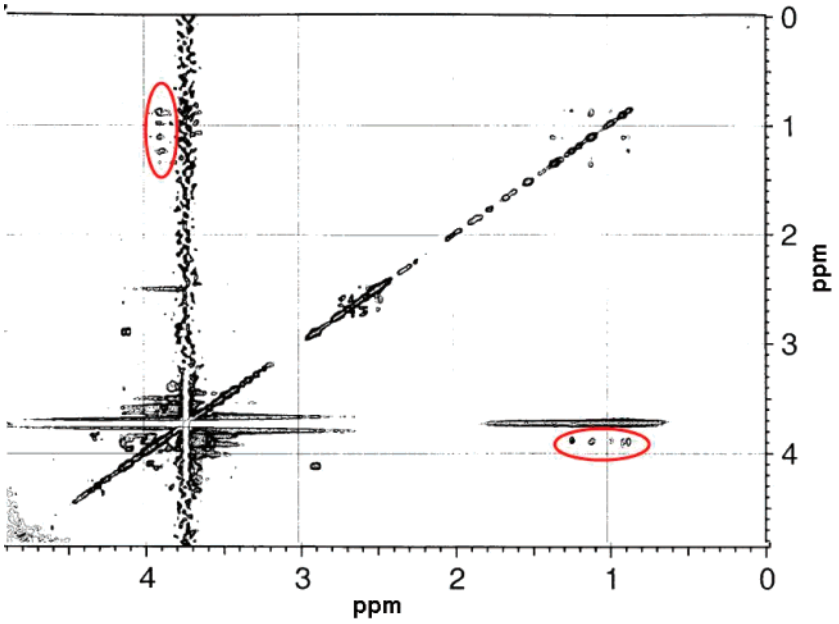


Figure 7. 2D (NOESY) ^{1}H NMR spectrum of a mixed PEG$_8$20K-SA-βCD$_{7.4}$/PEG$_8$20K-SA-chol$_{6.1}$ solution (12 mg /mL) in D_2O. Cross-peaks of interest are indicated by red ovals.

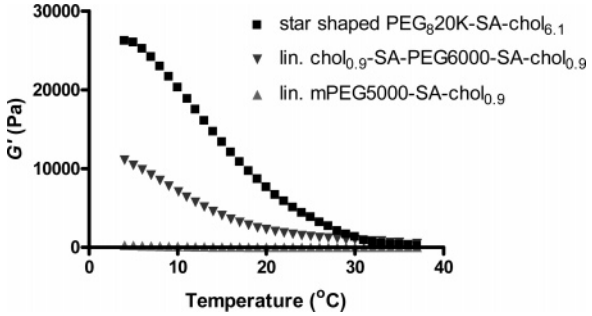


Figure 8. G' of 15% (w/w) hydrogel mixtures as a function of temperature, in which PEG$_8$20K-SA-βCD$_{8.0}$ is combined with either star-shaped PEG$_8$20K-SA-chol$_{6.1}$ (■) or linear mPEG5000-SA-chol$_{0.9}$ (▲) and chol$_{0.9}$-SA-PEG6000-SA-chol$_{0.9}$ (▼).

Cholesterol/β-Cyclodextrin Inclusion Complexes Studied by 2D-NMR Analyses. The formation of cholesterol/β-CD complexes in the hydrogels was further demonstrated by 2D-NMR (NOESY ^{1}H) analyses on solutions of PEG$_8$20K-SA-βCD$_{7.4}$/PEG$_8$20K-SA-chol$_{6.1}$ and PEG$_8$20K-SA-chol$_{6.1}$/PEG$_8$-20K-OH, respectively, in D_2O (12 mg/mL). Cholesterol protons were hardly visible in the spectrum of PEG$_8$20K-SA-chol$_{6.1}$/PEG$_8$20K-OH, likely because the cholesterol units self-assemble in the aqueous environment to form micellar-like structures. In contrast, in the 2D NMR (NOESY ^{1}H) spectrum of the PEG$_8$-20K-SA-βCD$_{7.4}$/PEG$_8$20K-SA-chol$_{6.1}$ mixture in D_2O, cholesterol proton peaks (δ 0.9−1.3 ppm) were detected. Moreover, cross-peaks from cholesterol protons (δ 0.9−1.3 ppm) and protons at δ 3.9 ppm were also observed (Figure 7). At δ 3.9 ppm, both glucosidic β-CD protons and PEG protons resonate. Because the hydrophobic cholesterol moieties and hydrophilic PEG chains are phase-separated in the aqueous environment, it can be concluded that these cross-peaks are the result of cholesterol and glucosidic β-CD protons in an inclusion complex.

Gel Properties as a Function of Solid Content, Star PEG Molecular Weight, and after Combining Star-Shaped PEG$_8$-SA-βCD with Linear PEG-SA-Cholesterol. Figure 8 shows the G' of two different 15% (w/w) mixtures of linear bifunctional chol$_{0.9}$-SA-PEG6000-SA-chol$_{0.9}$ or linear monofunctional mPEG5000-SA-chol$_{0.9}$ with star-shaped PEG$_8$20K-SA-βCD$_{8.0}$ (molar ratio β-CD/chol was 1/1) as a function of temperature. In line with expectations, the addition of monofunctional mPEG5000-SA-chol$_{0.9}$ did not result in the formation of a viscoelastic hydrogel. On the contrary, the bifunctional chol$_{0.9}$-SA-PEG6000-SA-chol$_{0.9}$ mixed with star shaped PEG$_8$20K-SA-βCD$_{8.0}$ resulted in the formation of a hydrogel. The resulting gel strength (e.g., at 4 °C, $G' = 13 \pm 2$ kPa ($n = 4$)), however, was considerably lower than that of a hydrogel composed of star-shaped polymers ($G' = 29 \pm 2$ kPa). This might be explained by the lower cross-link density in gels composed of star PEG$_8$20K-SA-βCD$_{8.0}$ and linear bifunctional chol$_{0.9}$-SA-

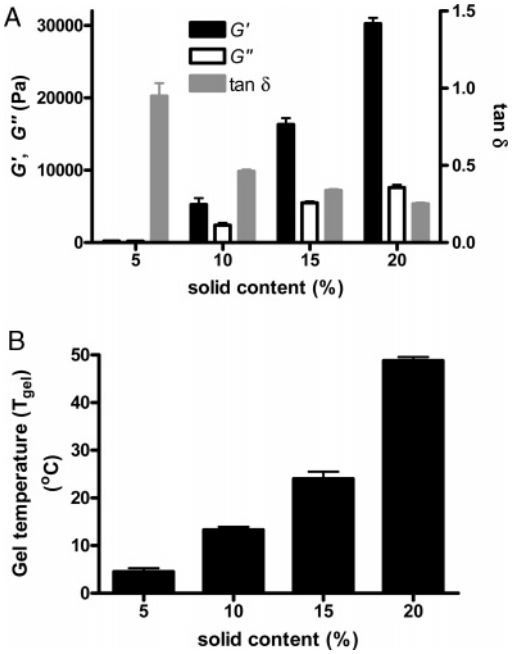


Figure 9. (A) PEG$_8$20K-SA-βCD$_{7.1}$/PEG$_8$20K-SA-chol$_{5.1}$ hydrogels; gel properties (4 °C) as a function of the percentage solid content. Left axis: G' and G''; right axis: tan δ. The data are shown as average and SEM, $n = 3$. (B) T_{gel} of hydrated PEG$_8$20K-SA-βCD$_{7.1}$/PEG$_8$20K-SA-chol$_{5.1}$ mixtures as a function of the percentage solid content. Data are shown as average and SEM, $n = 3$.

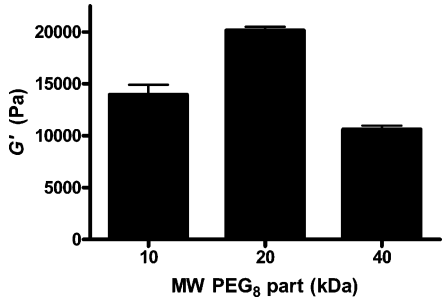


Figure 10. G' (Pa) as a function of the molecular weight of the PEG moieties in PEG_8-SA-$\beta CD_{7.0\pm0.1}$ and PEG_8-SA-$chol_{6.2\pm0.2}$. 15% (w/w) hydrogels (molar ratio chol/βCD = 1) were prepared and G' was measured at 4 °C. Data are shown as average and SEM, $n = 3$.

PEG6000-SA-$chol_{0.9}$, compared to gels consisting entirely of the star-shaped functionalized polymers.

Figure 9A shows the G' at 4 °C of $PEG_8$20K-SA-$\beta CD_{7.1}$/$PEG_8$20K-SA-$chol_{5.1}$ hydrogels (molar ratio chol/β-CD = 1) with increasing polymer content (5−20% (w/w)). With increasing solid content of the hydrogels, G' significantly increased and tan δ decreased. Logically, an increase of polymer concentration and thus concentration of cholesterol and β-CD results in a higher cross-link density. Further, Figure 9B shows that in line with expectations T_{gel} increases with increasing solid content as well; at high polymer concentration (20% (w/w)), T_{gel} was 49 ± 1 °C and thus exceeded body temperature. This finding shows that this hydrogel system is formed under physiological conditions, which makes it possible to use it for pharmaceutical applications, like protein release purposes.

Figure 10 shows the effect of the molecular weight of the used PEG stars on the G' of PEG_8-SA-$\beta CD_{7.0\pm0.1}$/PEG_8-SA-$chol_{6.2\pm0.2}$ hydrogel mixtures. This figure shows that the highest G' values were observed with the star-shaped $PEG_8$20K. The functionalized $PEG_8$40K products resulted in weaker hydrogel, likely because of a lower cross-link density (less β-CD and cholesterol groups per volume). Figure 10 also shows that hydrogels composed of $PEG_8$10K had a lower G' compared to the $PEG_8$20K hydrogels. This is probably caused by a low solubility of the hydrogel component $PEG_8$10K-SA-$chol_{6.3}$.

4. Conclusions

In this paper we report on a novel hydrogel system, based on star-shaped 8-arm PEG modified with either β-cyclodextrin or cholesterol moieties. Hydrogel formation is based on β-cyclodextrin/cholesterol inclusion complexes as demonstrated by rheological and NMR analyses. The resulting hydrogels are thermoreversible, and gel characteristics can be tailored by changing % solid content, β-CD/cholesterol stoichiometry, 8-arm PEG molar mass, or the addition of telechelic PEG-cholesterol. Other ways to tailor gel properties, such as combining β-CD- or cholesterol-modified 8-arm PEG with low-molecular-weight multifunctional cross-linking agents or using an 8-arm PEG with β-CD- and cholesterol moieties in the same polymer molecule, are currently under investigation. Star-shaped polymers exhibit smaller hydrodynamic radii and a lower viscosity compared with linear polymers of the same molar mass,[58] which is beneficial for future pharmaceutical applications, such as injectable in-situ gelling devices. PEG is a biocompatible, nonimmunogenic polymer, and a wide variety of PEG-containing products have been approved by the US Food and Drug Administration.[59] Consequently, this hydrogel system based on the formation of β-cyclodextrin/cholesterol inclusion complexes is a very attractive candidate for further pharmaceutical and biomedical applications.

Acknowledgment. Dr. J. Kemmink is gratefully acknowledged for his assistance with the 2D-NMR (NOESY) measurements. This research was financially supported by a grant of the Ministry of Economic Affairs, The Netherlands (Senter-Novem IS042016).

Supporting Information Available: Additional GPC, ^{1}H NMR, and polarimetry data of the polymers used in this study; synthesis protocol for linear mono- and bifunctional PEG-SA-cholesterol polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27−46.
(2) Hoffman, A. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 3−12.
(3) Kopeček, J. *Biomaterials* **2007**, *28*, 5185−5192.
(4) Hennink, W. E.; van Nostrum, C. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 13−36.
(5) van Dijk-Wolthuis, W. N. E.; Hoogeboom, J. A. M.; van Steenbergen, M. J.; Tsang, S. K. Y.; Hennink, W. E. *Macromolecules* **1997**, *30*, 4639−4645.
(6) van Dijk-Wolthuis, W. N. E.; Kettenes-van den Bosch, J. J.; van der Kerk-van Hoof, A.; Hennink, W. E. *Macromolecules* **1997**, *30*, 3411−3413.
(7) Oral, E.; Peppas, N. A. *J. Biomed. Mater. Res., Part A* **2004**, *68A*, 439−447.
(8) Van Tomme, S. R.; van Steenbergen, M. J.; De Smedt, S. C.; van Nostrum, C. F.; Hennink, W. E. *Biomaterials* **2005**, *26*, 2129−2135.
(9) Lee, K. Y.; Rowley, J. A.; Eiselt, P.; Moy, E. M.; Bouhadir, K. H.; Mooney, D. J. *Macromolecules* **2000**, *33*, 4291−4294.
(10) Vermonden, T.; Besseling, N. A. M.; van Steenbergen, M. J.; Hennink, W. E. *Langmuir* **2006**, *22*, 10180−10184.
(11) Noble, L.; Gray, A. I.; Sadiq, L.; Uchegbu, I. F. *Int. J. Pharm.* **1999**, *192*, 173−182.
(12) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature (London)* **1997**, *388*, 860−862.
(13) Haglund, B. O.; Josi, R.; Himmelstein, K. J. *J. Controlled Release* **1996**, *41*, 229−235.
(14) Nagahara, S.; Matsuda, T. *Polym. Gels Networks* **1996**, *4*, 111−127.
(15) de Jong, S. J.; van Dijk-Wolthuis, W. N. E.; Kettenes-van den Bosch, J. J.; Schuyl, P. J. W.; Hennink, W. E. *Macromolecules* **1998**, *31*, 6397−6402.
(16) de Jong, S. J.; De Smedt, S. C.; Wahls, M. W. C.; Demeester, J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Macromolecules* **2000**, *33*, 3680−3686.
(17) Hiemstra, C.; Zhong, Z. Y.; Dijkstra, P.; Feijen, J. *Macromol. Symp.* **2005**, *224*, 119−131.
(18) Miyata, T.; Asami, N.; Uragami, T. *Nature (London)* **1999**, *399*, 766−769.
(19) Yamaguchi, N.; Zhang, L.; Chae, B. S.; Palla, C. S.; Furst, E. M.; Kiick, K. L. *J. Am. Chem. Soc.* **2007**, *129*, 3040−3041.
(20) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389−392.
(21) Veerman, C.; Rajagopal, K.; Palla, C. S.; Pochan, D. J.; Schneider, J. P.; Furst, E. M. *Macromolecules* **2006**, *39*, 6608−6614.
(22) Wang, C.; Stewart, R. J.; Kopecek, J. *Nature (London)* **1999**, *397*, 417−420.
(23) Packhaeuser, C. B.; Schnieders, J.; Oster, C. G.; Kissel, T. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 445−455.
(24) Hatefi, A.; Amsden, B. *J. Controlled Release* **2002**, *80*, 9−28.
(25) Drury, J. L.; Mooney, D. J. *Biomaterials* **2003**, *24*, 4337−4351.
(26) Loftsson, T.; Duchene, D. *Int. J. Pharm.* **2007**, *329*, 1−11.
(27) Challa, R.; Ahuja, A.; Ali, J.; Khar, R. K. *AAPS PharmSciTech* **2005**, *6*, E329−E357.
(28) Liu, L.; Guo, Q. X. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *42*, 1−14.
(29) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.
(30) Harries, D.; Rau, D. C.; Parsegian, V. A. *J. Am. Chem. Soc.* **2005**, *127*, 2184−2190.
(31) Loftsson, T.; Magnusdottir, A.; Masson, M.; Sigurjonsdottir, J. F. *J. Pharm. Sci.* **2002**, *91*, 2307−2316.
(32) Somogyi, G.; Posta, J.; Buris, L.; Varga, M. *Pharmazie* **2006**, *61*, 154−156.
(33) Siemoneit, U.; Schmitt, C.; Alvarez-Lorenzo, C.; Luzardo, A.; Otero-Espinar, F.; Concheiro, A.; Blanco-Mendez, J. *Int. J. Pharm.* **2006**, *312*, 66−74.
(34) Ramirez, H. L.; Valdivia, A.; Cao, R.; Torres-Labandeira, J. J.; Fragoso, A.; Villalonga, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1499−1501.

(35) Tang, S. W.; Kong, L.; Ou, J. J.; Liu, Y. Q.; Li, X.; Zou, H. F. *J. Mol. Recognit.* **2006**, *19*, 39−48.
(36) Ohga, K.; Takashima, Y.; Takahashi, H.; Kawaguchi, Y.; Yamaguchi, H.; Harada, A. *Macromolecules* **2005**, *38*, 5897−5904.
(37) Miyauchi, M.; Kawaguchi, Y.; Harada, A. *J. Inclusion Phenom. Macrocyclic Chem.* **2004**, *50*, 57−62.
(38) Hashimoto, H. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *44*, 57−62.
(39) Kawaguchi, Y.; Nishiyama, T.; Okada, M.; Kamachi, M.; Harada, A. *Macromolecules* **2000**, *33*, 4472−4477.
(40) Li, J.; Ni, X. P.; Leong, K. W. *J. Biomed. Mater. Res., Part A* **2003**, *65A*, 196−202.
(41) Huh, K. M.; Ooya, T.; Lee, W. K.; Sasaki, S.; Kwon, I. C.; Jeong, S. Y.; Yui, N. *Macromolecules* **2001**, *34*, 8657−8662.
(42) Huh, K. M.; Cho, Y. W.; Chung, H.; Kwon, I. C.; Jeong, S. Y.; Ooya, T.; Lee, W. K.; Sasaki, S.; Yui, N. *Macromol. Biosci.* **2004**, *4*, 92−99.
(43) Sabadini, E.; Cosgrove, T. *Langmuir* **2003**, *19*, 9680−9683.
(44) Li, J.; Li, X.; Ni, X. P.; Wang, X.; Li, H. Z.; Leong, K. W. *Biomaterials* **2006**, *27*, 4132−4140.
(45) Zhao, S. P.; Zhang, L. M.; Ma, D. *J. Phys. Chem. B* **2006**, *110*, 12225−12229.
(46) Li, J.; Li, X.; Zhou, Z. H.; Ni, X. P.; Leong, K. W. *Macromolecules* **2001**, *34*, 7236−7237.
(47) Choi, H. S.; Kontani, K.; Huh, K. M.; Sasaki, S.; Ooya, T.; Lee, W. K.; Yui, N. *Macromol. Biosci.* **2002**, *2*, 298−303.
(48) Weickenmeier, M.; Wenz, G.; Huff, J. *Macromol. Rapid Commun.* **1997**, *18*, 1117−1123.
(49) Hashidzume, A.; Tomatsu, I.; Harada, A. *Polymer* **2006**, *47*, 6011−6027.
(50) Kretschmann, O.; Choi, S. W.; Miyauchi, M.; Tomatsu, I.; Harada, A.; Ritter, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 4361−4365.
(51) Soltes, L.; Mendichi, R.; Kogan, G.; Mach, M. *Chem. Biodiversity* **2004**, *1*, 468−472.
(52) Nishijo, J.; Moriyama, S.; Shiota, S. *Chem. Pharm. Bull.* **2003**, *51*, 1253−1257.
(53) Loccufier, J.; Vanbos, M.; Schacht, E. *Polym. Bull. (Berlin)* **1991**, *27*, 201−204.
(54) Winter, H. H. *Polym. Eng. Sci.* **1987**, *27*, 1698−1702.
(55) Weickenmeier, M.; Wenz, G. *Macromol. Rapid Commun.* **1996**, *17*, 731−736.
(56) Shiotani, K.; Uehata, K.; Irie, T.; Uekama, K.; Thompson, D. O.; Stella, V. J. *Pharm. Res.* **1995**, *12*, 78−84.
(57) Akiyoshi, K.; Sasaki, Y.; Kuroda, K.; Sunamoto, J. *Chem. Lett.* **1998**, 93−94.
(58) Maglio, G.; Nese, G.; Nuzzo, M.; Palumbo, R. *Macromol. Rapid Commun.* **2004**, *25*, 1139−1144.
(59) Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. *Adv. Mater.* **2006**, *18*, 1345−1360.

MA702607R