

Electronic Supplementary Information

A Carbohydrate-Based Hydrogel Containing Vesicles as Responsive Non-Covalent Cross-Linkers

*Sabine Himmelein, Vanessa Lewe, Marc C. A. Stuart and Bart Jan Ravoo**

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General Remarks

All chemicals were purchased from Acros Organics (Schwerte, Germany) or Sigma-Aldrich (Taufkirchen, Germany) and used without further purification, unless otherwise noted. 2-Hydroxyethyl cellulose (HEC) with an average molecular weight of $M_w = 250,000$ g/mol was used. Cyclodextrins (CDs) were kindly donated by Wacker Chemie (Burghausen, Germany). All solvents were dried according to the conventional methods before use. All reactions were carried out in oven-dried round-bottom flasks and stirred magnetically. Analytical TLC was performed on Merck silica gel 60 F-254 plates. Column chromatography was carried out by using silica gel 60 (230–400 mesh). The NMR spectra were recorded on the spectrometer ARX 300 (BRUKER). All measurements were carried out in deuterated solvents. The chemical shift (δ) is recorded in parts per million (ppm) and relative to the residual solvent protons. To analyze the spectra the software MESTRENOVA 8.1 was used. The description of the signals was done as follows: s = singlet, br = broad, d = doublet, t = triplet, q = quartet and m = multiplet. Ultrapure water was used in all analytical measurements. For dialysis tubing made of regenerated cellulose (Spectra/Por® 7) from Spectrum Laboratories (Breda, The Netherlands) with a cut-off of MW = 1000 Da was used. GPC measurements were performed with DMF as eluent containing 0.01% LiBr as additive at a flow rate of 1.0 mL/min at room temperature on a system consisting of a Knauer HPLC pump 64, a set of Shodex gel columns and a Knauer RI detector. Data were analyzed with PSS WinGPC Compact V.7.20 software (Polymer Standards Service) based on calibration curves built upon poly(methyl methacrylate) standards (Polymer Laboratories Poly(methyl methacrylate) Medium MW Calibration Kit M-M-10 to determine the molecular weight of polyketones) with peak molecular weights ranging from 1660 to 1,000,000 g/mol. IR spectra were recorded on a Varian 3100 FT-IR equipped with a MKII Golden Gate Single Reflection ATR unit. To analyze the spectra the software RESOLUTION PRO was used. The description of the signals was done as follows: s = strong, m = medium, w = weak, br = broad.

Synthesis of CD Amphiphiles and Polymer HEC-AD

Cyclodextrin amphiphiles were synthesized from the native α - and β -cyclodextrins in three steps as described before.^[1]

Synthesis of HEC-AD. LiCl (1.00 g, 23.6 mmol) was dissolved in DMF (100 mL) under an argon atmosphere while stirring and heating at 80 °C. 2-Hydroxyethyl cellulose (1.00 g, 4.00 μ M, ~ 5.2 mM glucose units) was added and dissolved. Subsequently, DMAP (0.50 g, 4.10 mM), pyridine (30 μ L), and 1-adamantanecarbonyl chloride (0.40 g, 2.01 mM) were added. The mixture was allowed to stir 5 h at 80 °C and afterwards 18 h at room temperature. Isolation of the functionalized polymer was achieved by precipitation from 2-propanol (1 L). The precipitate was centrifuged washed again with 2-propanol and once again centrifuged. After dissolving the polymer in a minimum amount of water, it was further purified by dialysis against water for 18 h and finally freeze-dried. Yield: 0.80 g, 75 %. The degree of substitution was estimated to 25 % per mole of glucose units by comparison of GPC data of the educt ($M_w = 244,000$ g/mol, PDI = 1.45), and product ($M_w = 298,000$ g/mol, PDI = 1.81). **¹H-NMR** (300 MHz, D₂O, 298 K): $\delta = 4.60 - 2.75$ (153H, br, cellulose backbone), 2.09 - 1.7 (15H, m, CH- and CH₂-adamantyl). **FT-IR** (ATR): $\nu = 3406$ (br, alcohol O-H), 2916 (m, alkyl C-H), 2875 (m, alkyl C-H), 1728 (m, ester C=O), 1238 (m, ester C-O), 1050 (s, ester C-O).

$^1\text{H-NMR}$ reveals a ratio of 153 HEC protons per one adamantyl group with 15 protons. An estimation of the degree of substitution based on $^1\text{H-NMR}$ yields one adamantyl on every 8 – 10 glucose unit, depending on the number of hydroxyethyl groups present on each glucose. (153 HEC protons divided by 14 – 18 protons per glucose unit). This corresponds to 10 – 12.5 % per mole of glucose units. These values are lower than results obtained from GPC data. It is assumed that GPC overestimates the number of functionalized sugars and numbers from $^1\text{H-NMR}$ are used for the calculation of the $[\text{AD}]/[\text{CD}]$ ratio.

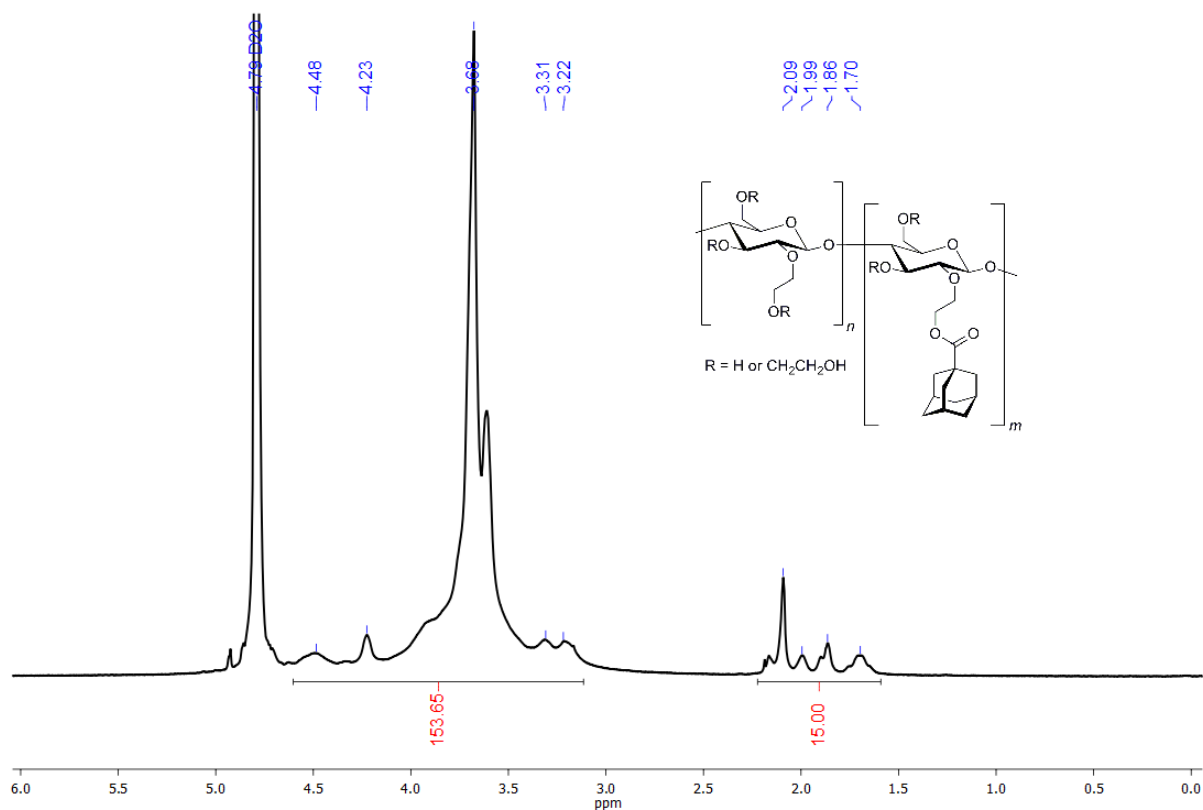


Figure S1: $^1\text{H-NMR}$ (300 MHz, D_2O) of HEC-AD.

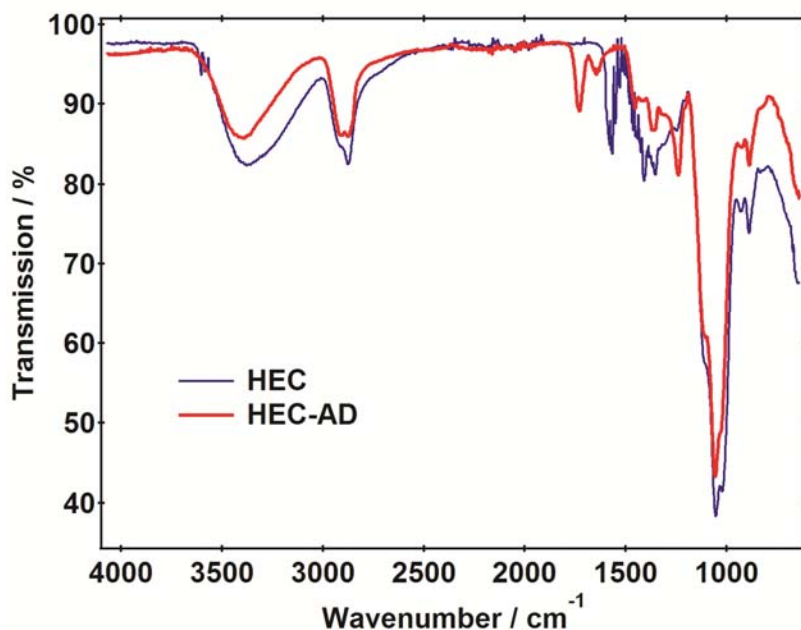


Figure S2: FT-IR spectra of unfunctionalized hydroxyethyl cellulose (HEC) and adamantane functionalized polymer (HEC-AD).

Preparation of CD Vesicles

Unilamellar vesicles of α - and β -CDA were prepared by sonication. Amphiphilic cyclodextrin was dissolved in chloroform, dried while rotating in a flask to obtain a thin film and left under high vacuum for 1 h. Water was added and the mixture was sonicated for 45 min using a Sonorex bath sonicator (Branson) for vesicle formation. Dynamic light scattering (DLS) measurements confirmed the formation of vesicles with an average diameter of 110 nm. DLS measurements were performed with a Malvern Instruments Nano-ZS instrument by using low-volume disposable cuvettes kept at 25 °C. Samples were measured as prepared without further dilution. For data analysis the software ZETASIZER 6.32 was used.

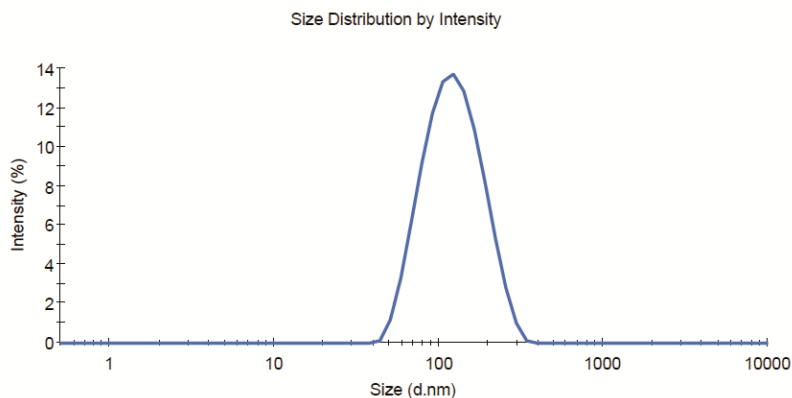


Figure S3: DLS measurement of β -CD vesicles with a concentration of 5 mM.

Preparation of Hydrogels

For preparation of hydrogels the HEC-AD polymer first was dissolved in water with stirring and mild heating. Typically, 20 mg of HEC-AD were dissolved in 1.0 or 0.5 mL of water. The solution was then mixed in a 1:1 ratio with a vesicles dispersion (5 - 10 mM) at room temperature and stirred for about 5 s to obtain the hydrogel. Figure S4 e) shows a gel prepared with β -CDA were 1 % of the amphiphiles were labeled with rhodamine. The gel is consistently colored concluding that the vesicles are homogeneously distributed within the gel network.

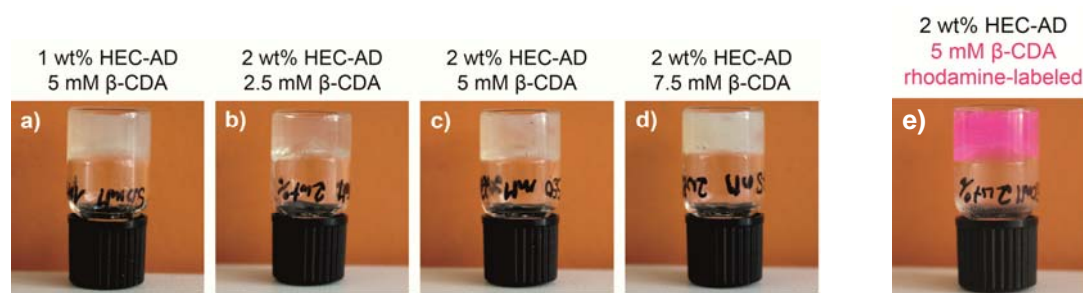


Figure S4: a)-d) Hydrogels prepared with different concentrations of HEC-AD and β -CDV and e) Hydrogel with rhodamine-labeled β -CDA.

Table S1 provides the molar ratio of AD/ β -CD for the hydrogel samples shown in Figure 2 and S4 a)-d). The molar ratios were calculated assuming that 11.25 ± 1.25 % of the glucose units are functionalized with AD. Consequently 1 wt % and 2 wt % of the polymer HEC-AD correspond to approximately 3.7 ± 0.5 mM and 7.4 ± 1.0 mM of AD groups present, respectively.

Table S1: Molar ratio of AD/CD for hydrogel samples shown in Figure 2 and S4.

Hydrogel sample	Molar ratio AD/CD
b) 2wt % HEC-AD + 2.5 mM β -CDA	3.0 ± 0.3
c) 2wt % HEC-AD + 5.0 mM β -CDA	1.5 ± 0.2
d) 2wt % HEC-AD + 7.5 mM β -CDA	1.0 ± 0.1
a) 1wt % HEC-AD + 5.0 mM β -CDA	0.7 ± 0.1

Rheology

Rheological measurements were performed using a shear rheometer (MCR101, Anton Paar) equipped with a CP25-2 cone-plate (radius = 12.5 mm, cone angle = 2° , sample volume = 0.16 mL). The measurement temperature was fixed at 20 °C unless stated otherwise. Strain sweep measurements were performed at a frequency of 10 rad/s and frequency sweep measurements were performed at a 5% strain amplitude. Furthermore, a temperature sweep

was performed on a temperature ramp from 10 to 50 °C at a rate of 2 °C/min and performed at 0.5% strain and 10 rad/s. Starting from a temperature of 50 °C evaporation of water from the hydrogel material became an issue. Within the measured temperature range the complex viscosity as well as storage and loss moduli decreased slightly due to a loss in structural strength. This is also reflected in an increase of the damping factor $\tan \delta = G''/G'$ from 0.25 to 0.36. The gel character is retained throughout this temperature range.

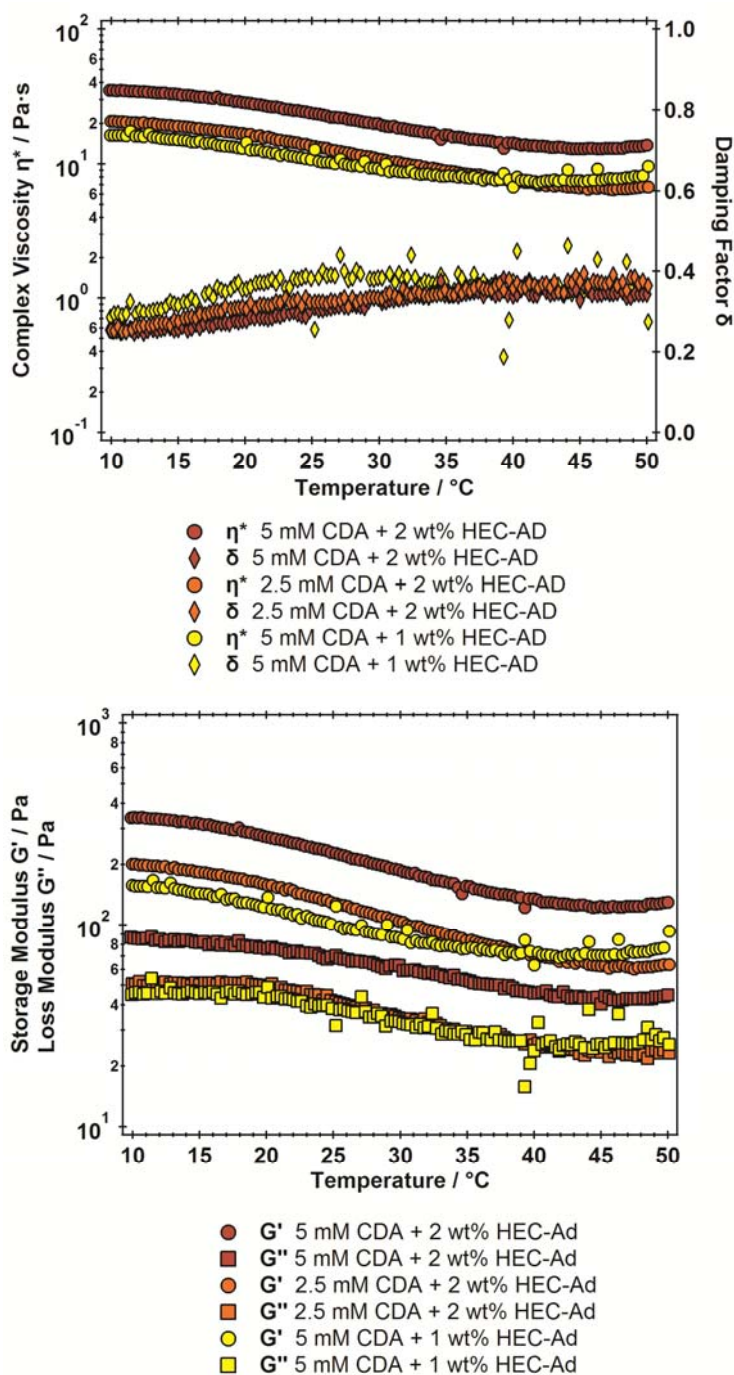


Figure S5: Dynamic oscillatory temperature sweep displaying thermal stability of the hydrogels.

Cryo-TEM Measurements

For cryogenic transmission electron microscopy (Cryo-TEM) a hydrogel sample containing 5 mM β -CDA and 2 wt% HEC-AD was diluted about 10-fold with water to allow the sample preparation. Then a few microliter of this solution were placed on glow-discharged holey carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting away the excess liquid at 100% humidity and 22 °C the grids were quickly vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands). The frozen-hydrated specimens were mounted in a liquid nitrogen-cooled Gatan 626 cryo-holder (Gatan Inc., Pleasanton, CA) and inserted in the electron microscope. Low-dose images were recorded with a Gatan 4K slow-scan CCD camera (Pleasanton, CA) on a Philips CM 120 electron microscope (FEI, Eindhoven, The Netherlands) equipped with a LaB₆ tip operated at 120 kV.

SAXS Measurements

Small-angle X-ray scattering (SAXS) experiments were performed at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, at the high brilliance beamline ID02. An X-ray energy of 12.46 keV and two sample-to detector distances of 1.5 m and 10 m were used to cover a q -range of $0.0089 < q < 4.486 \text{ nm}^{-1}$, with q being the magnitude of the scattering wave vector. Measurements of the hydrogel sample was performed at only 1.5 m detector distance covering a q -range of $0.07 < q < 4.486 \text{ nm}^{-1}$.

The samples were injected into a polycarbonate or glass capillary (for the vesicle dispersion or the hydrogel, respectively) at a temperature of 20 °C. The scattering data were corrected for background scattering, detector response and primary beam intensity fluctuations. The instrument scattering vector was calibrated using a silver behenate standard. Peak positions were determined by taking the midpoint between the local extremes in the first derivative of the scattering intensities.

Video

A video is attached showing the simple injection experiment of a hydrogel with 2 wt% HEC-AD and 5 mM β -CDA concentration through a 1mL syringe with a 26 G needle (outer diameter = 0.45 mm).

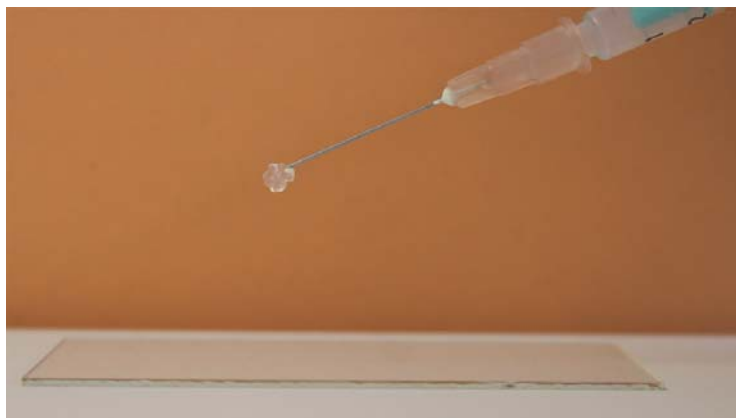


Figure S6: Snapshot of the video demonstrating that the hydrogel material is injectable.

Reference

- [1] P. Falvey, C. W. Lim, R. Darcy, T. Revermann, U. Karst, M. Giesbers, A. T. M. Marcelis, A. Lazar, A. W. Coleman, D. N. Reinhoudt, et al., *Chem. – Eur. J.* **2005**, *11*, 1171–1180.