Capturing the Sol and Gel States of Thermoresponsive Poly(2-oxazoline) / Poly(2-oxazine) Hydrogels by Ambient and Subambient Solid-State NMR

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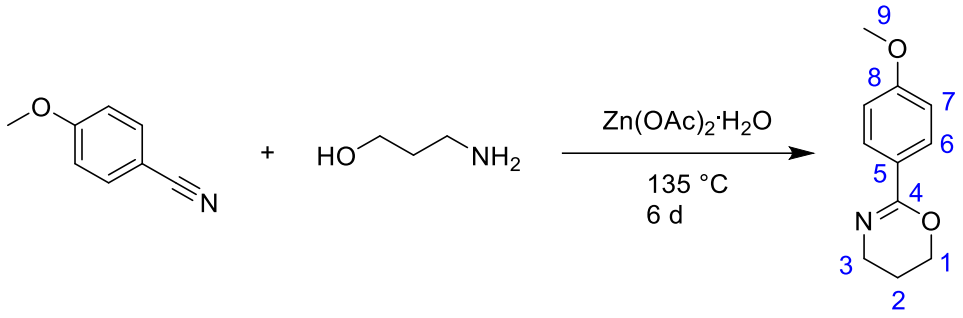
Contents

Monomer Synthesis

Scheme S1: Synthesis of 2-(4-methoxyphenyl)-2-oxazine



1.0 eq. of p-methoxybenzonitrile (15.0 g, 112.7 mmol) was dissolved in 1.2 eq. of 3-amino-propanol (10.3 g, 137.4 mmol). Catalytic amounts of zinc acetate dihydrate (533 mg, 2.65 mmol, 0.02 eq.) were added and the reaction mixture was heated to 135 °C for 6 d until the reaction control, measured by ^{1}H NMR spectroscopy, showed complete conversion of the nitrile reagent. After completion, the reaction mixture was diluted with dichloromethane and washed with brine (3x) and water (2x). The organic phase was dried over magnesium sulphate and the solvent was removed under reduced pressure. Further purification was carried out by column chromatography using a dichloromethane : ethyl acetate, 1 : 2 mixture as eluent. The product obtained was dried over calcium hydride, distilled under reduced pressure and stored under dry and inert conditions. The obtained monomer 2-(4-methoxyphenyl)-2-oxazine was characterized by electrospray ionization mass spectroscopy (ESI-MS), ^{1}H and ^{13}C NMR spectroscopy.

Yield: 11.2 g (58.6 mmol, 52.0 %)

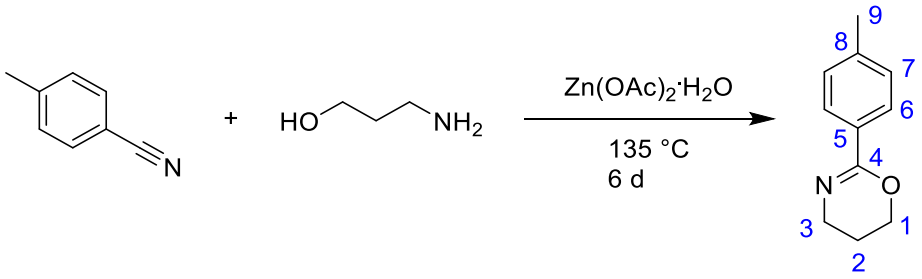
Boiling point: 130 °C (0.001 mbar)

ESI-MS: [M + Na^{+}] 214.1

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, 2H, ^{3}J = 9.0 Hz, H^{6}), 6.87 (d, 2H, ^{3}J = 9.0 Hz, H^{7}), 4.32 (t, 2H, ^{3}J = 5.5 Hz, H^{1}), 3.81 (s, 3H, H^{9}), 3.57 (t, 2H, ^{3}J = 5.8 Hz, H^{3}), 1.95 (tt, 2H, ^{3}J = 5.8 Hz, ^{3}J = 5.5 Hz, H^{2}) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 161.4 (C^{8}), 155.4 (C^{4}), 128.5 (C^{6}), 126.7 (C^{5}), 113.4 (C^{7}), 65.2 (C^{1}), 55.4 (C^{9}), 42.7 (C^{3}), 22.1 (C^{2}) ppm.

Scheme S2: Synthesis of 2-(4-methylphenyl)-2-oxazine



1.0 eq. of p-tolylnitrile (10.0 g, 85.4 mmol) was dissolved in 1.2 eq. of 3-amino-propanol (7.82 g, 104.1 mmol). Catalytic amounts of zinc acetate dihydrate (404 mg, 2.01 mmol, 0.02 eq.) were added and the reaction mixture was heated to 135 °C for 6 d until the reaction control, measured by ^{1}H NMR spectroscopy, showed complete conversion of the nitrile reagent. After completion, the reaction mixture was diluted with dichloromethane and washed with brine (3x) and water (2x). The organic phase was dried over magnesium sulphate and the solvent was removed under reduced pressure. Further purification was carried out by column chromatography using a dichloromethane : ethyl acetate, 1 : 2 mixture as eluent. The product obtained was dried over calcium hydride, distilled under reduced pressure and stored under dry and inert conditions. The obtained monomer 2-(4-methylphenyl)-2-oxazine was characterized by electrospray ionization mass spectroscopy (ESI-MS), ^{1}H and ^{13}C NMR spectroscopy.

Yield: 10.5 g (59.8 mmol, 70.0 %)

Boiling point: 160 °C (0.001 mbar)

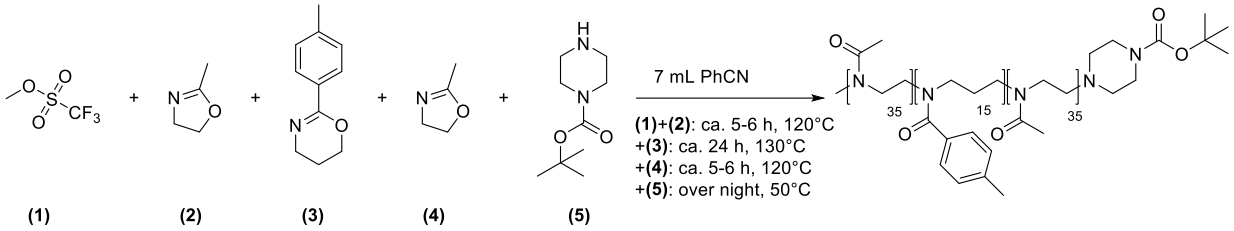
ESI-MS: [M + Na^{+}] 198.1

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, 2H, ^{3}J = 8.2 Hz, H^{6}), 7.17 (d, 2H, ^{3}J = 8.2 Hz, H^{7}), 4.34 (t, 2H, ^{3}J = 5.5 Hz, H^{1}), 3.59 (t, 2H, ^{3}J = 5.8 Hz, H^{3}), 2.36 (s, 3H, H^{9}), 1.96 (tt, 2H, ^{3}J = 5.8 Hz, ^{3}J = 5.5 Hz, H^{2}) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 155.7 (C^{4}), 140.4 (C^{8}), 131.5 (C^{5}), 128.8 (C^{7}), 126.9 (C^{6}), 65.2 (C^{1}), 42.7 (C^{3}), 22.1 (C^{2}), 21.5 (C^{9}) ppm.

Polymer Synthesis

Scheme S3: pMeOx-*b*-pMePheOzi-*b*-pMeOx (A-pMePheOzi-A)



Under dry and inert conditions 1.0 eq. of methyl trifluoromethanesulfonate (59.0 mg, 360 µmol) and 35.0 eq. of 2-methyl-2-oxazine (1.05 g, 12.3 mmol) were added to 7 mL of dry benzonitrile and stirred for three hours at 120 °C. Complete conversion of the monomer and achievement of the desired block length was monitored by ^{1}H NMR measurements. The reaction mixture was cooled to room temperature before 15.0 eq. of 2-(4-methylphenyl)-2-oxazine (1.01 g, 5.74 mmol) were added for the second block to be formed. The reaction mixture was stirred at 130 °C over night until complete conversion of the monomer and achievement of the desired block length was verified by ^{1}H NMR spectroscopy. The reaction mixture was cooled to room temperature before adding another 35.0 eq. of 2-methyl-2-oxazine (1.07 g, 12.6 mmol) and stirring at 120 °C for three hours. After completion of the third block, termination was carried out by adding 5 eq. of N-Boc-piperazine (335 mg, 1.80 mmol) and stirring the reaction mixture at 50 °C for several hours. The solvent was removed under reduced pressure. A viscous yellow residue was dissolved in deionized water, dialyzed against DI water (MWCO = 1 kDa, three days) and subsequently freeze-dried to obtain a colorless powder. The received polymer pMeOx-*b*-pMePheOzi-*b*-pMeOx was characterized by size exclusion chromatography (SEC), ^{1}H and ^{13}C NMR spectroscopy.

Yield: 2.56 (83 %)

$M_{w,\,theor.}$: 8.8 kg/mol

SEC (HFIP): M_n = 1.75 kg/mol; Đ = 1.15

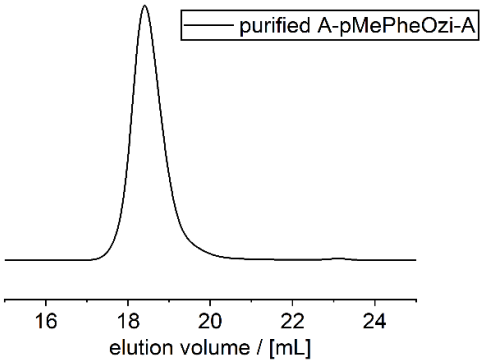


Figure S1: GPC-Trace after purification of pMeOx-*b*-pMePheOzi-*b*-pMeOx.

^{1}H (400 MHz, $CDCl_3$): δ = 7.22 – 6.88 (br.m., 15 x 4H, $H^{G/H}$), 3.48 – 3.10 (br.m., 85x 4H, H^{C}), 2.32 (br.m., 15x 3H, H^{N}), 2.12 – 2.06 (br.m., 70x 3H, H^{A}), 1.75 (br.m., 15x 2H, H^{D}) ppm.

^{13}C (100 MHz, $CDCl_3$): δ = 172.0 (C^{E}), 171.6 / 170.8 (C^{B}), 139.6 (C^{I}), 133.6 (C^{F}), 129.3 (C^{H}), 126.4 (C^{G}), 48.2 – 42.4 (C^{C}), 27.3 (C^{D}), 21.5 (C^{N}), 21.3 (C^{A}) ppm.

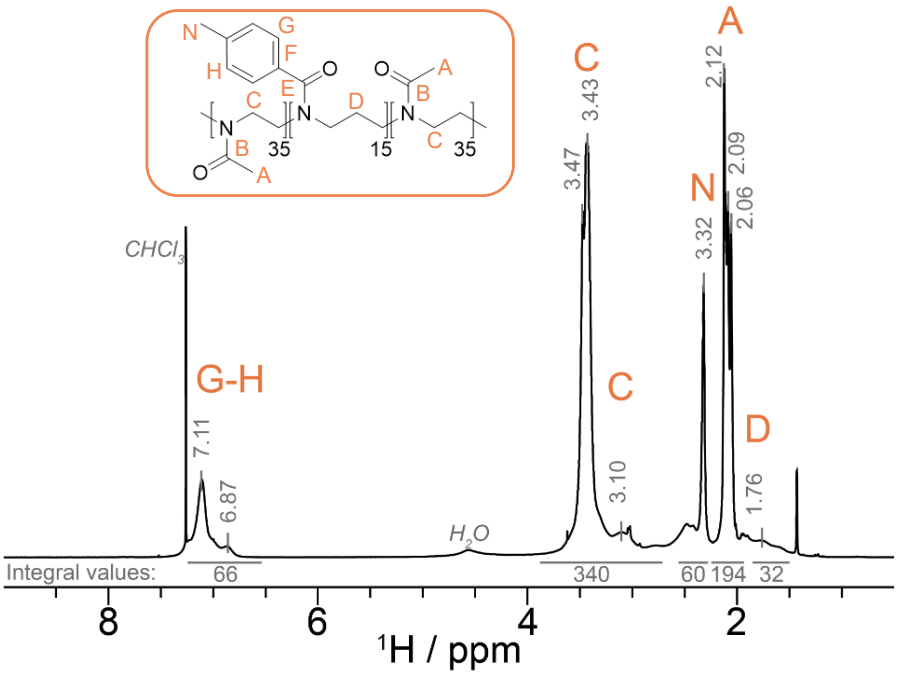


Figure S2: ^{1}H-NMR after purification of pMeOx-*b*-pMePheOzi-*b*-pMeOx in $CDCl_3$. The spectrum was recorded at 400 MHz, with a 30° flip angle, 16 scans, an recycle delay of 0.2 s and without sample spinning.

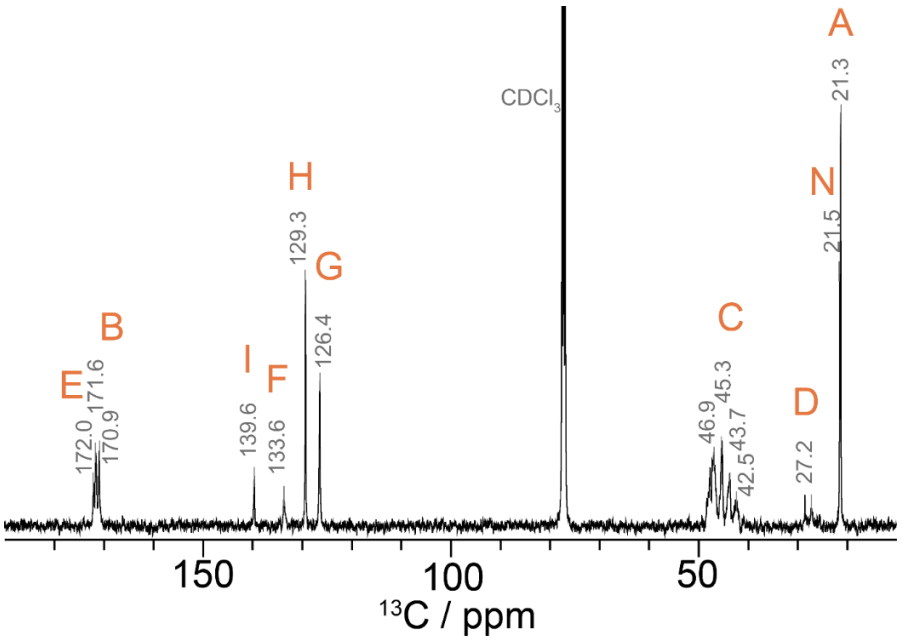
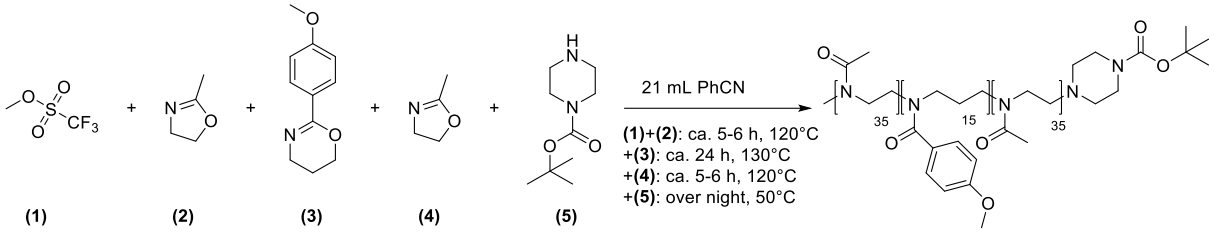


Figure S3: ^{13}C-NMR after purification of pMeOx-*b*-pMePheOzi-*b*-pMeOx in $CDCl_3$. The spectrum was recorded at 100 MHz, with a 30° flip angle and power-gated proton decoupling, 2336 scans, an recycle delay of 0.7 s and without sample spinning.

Scheme S4: pMeOx-*b*-pMeOPheOzi-*b*-pMeOx (A-pMeOPheOzi-A)



Under dry and inert conditions 1.0 eq. of methyl trifluoromethanesulfonate (131 mg,798 µmol) and 35.0 eq. of 2-methyl-2-oxazine (2.31 g, 27.1 mmol) were added to 21 mL of dry benzonitrile and stirred for three hours at 120 °C. Complete conversion of the monomer and achievement of the desired block length was monitored by ^{1}H NMR measurements. The reaction mixture was cooled to room temperature before 15.0 eq. of 2-(4-methoxyphenyl)-2-oxazine (2.29 g, 12.0 mmol) were added for the second block to be formed. The reaction mixture was stirred at 130 °C over night until complete conversion of the monomer and achievement of the desired block length was shown by ^{1}H NMR spectroscopy. The reaction mixture was cooled to room temperature before adding another 35.0 eq. of 2-methyl-2-oxazine (2.38 g, 27.9 mmol) and stirring at 120 °C for three hours. After completion of the third block, termination was carried out by adding 5 eq. of N-Boc-piperazine (743 mg, 3.99 mmol) and stirring the reaction mixture at 50 °C for several hours. The solvent was removed under reduced pressure. A viscous yellow residue was dissolved in deionized water, dialyzed against DI water (MWCO = 1 kDa, three days) and subsequently freeze-dried to obtain a colorless powder. The received polymer pMeOx-*b*-pMeOPheOzi-*b*-pMeOx was characterized by size exclusion chromatography (SEC), ^{1}H and ^{13}C NMR spectroscopy.

Yield: 5.65 (78 %)

$M_{w,\,theor.}$: 9.0 kg/mol

SEC (HFIP): M_n = 1.75 kg/mol; Đ = 1.15

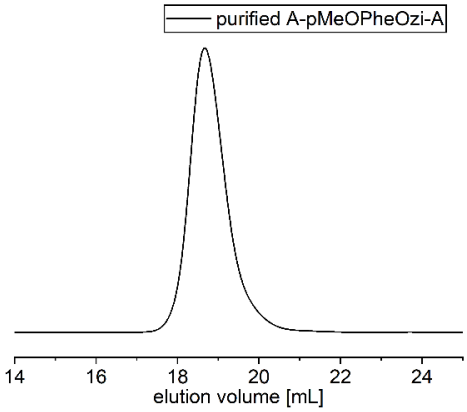


Figure S4: GPC-Trace after purification of pMeOx-*b*-pMeOPheOzi-*b*-pMeOx.

^{1}H (400 MHz, $CDCl_3$): δ = 7.24 – 6.86 (br.m., 15 x 4H, $H^{G/H}$), 3.74 (br.m., 15x 3H, H^{M}), 3.46 – 3.15 (br.m., 85x 4H, H^{C}), 2.11 – 2.04 (br.m., 70x 3H, H^{A}), 1.76 (br.m., 15x 2H, H^{D}) ppm.

^{13}C (100 MHz, $CDCl_3$): δ = 171.8 (C^{E}), 171.5 / 170.8 (C^{B}), 160.5 (C^{I}), 128.3 ($C^{F/G}$), 113.9 (C^{H}), 55.3 (C^{M}), 48.2 – 42.4 (C^{C}), 27.3 (C^{D}), 21.2 (C^{A}) ppm.

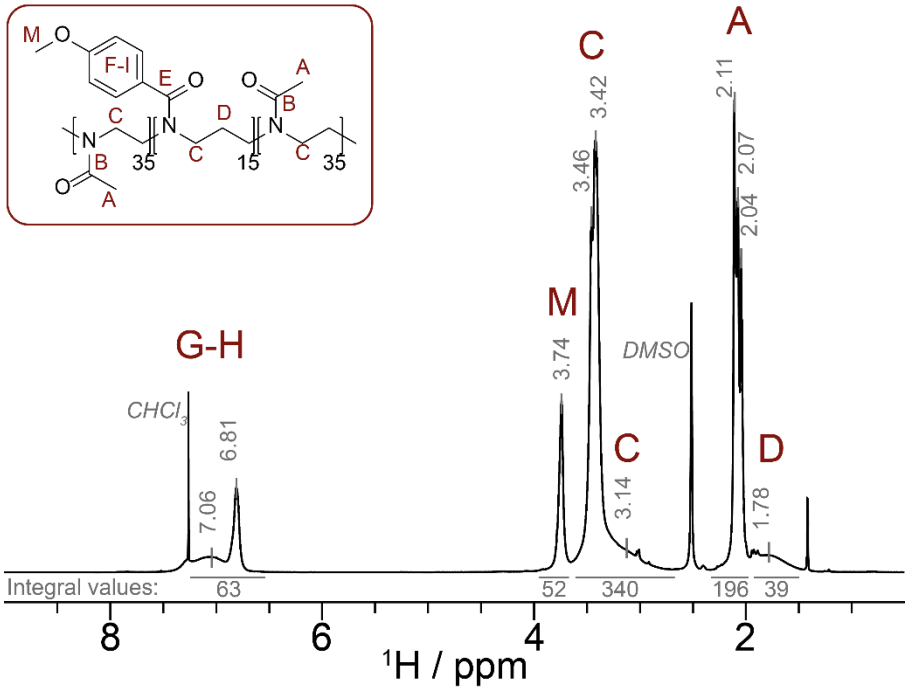


Figure S5: ^{1}H-NMR after purification of pMeOx-*b*-pMeOPheOzi-*b*-pMeOx in $CDCl_3$. The spectrum was recorded at 400 MHz, with a 30° flip angle, 16 scans, an recycle delay of 0.2 s and without sample spinning.

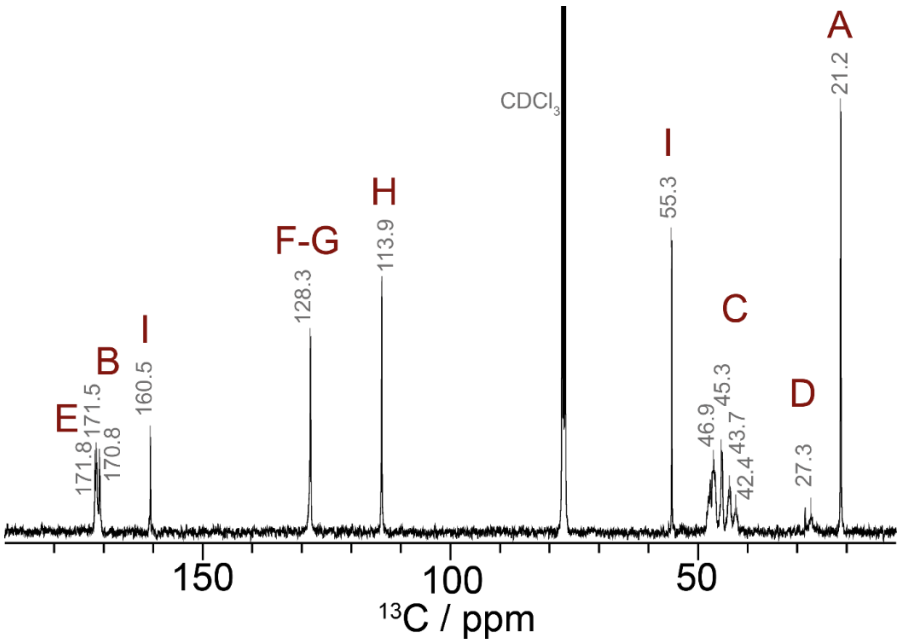


Figure S6: ^{13}C-NMR after purification of pMeOx-*b*-pMePheOzi-*b*-pMeOx in $CDCl_3$. The spectrum was recorded at 100 MHz, with a 30° flip angle and power-gated proton decoupling, 1408 scans, an recycle delay of 0.7 s and without sample spinning.

Tube Inversion Test

a)  b) 

Figure S7: Pictures of 20 wt.% aqueous solutions of a) A-pMeOPheOzi-A before heating to 105 °C (left) and after heating to 105°C (right) and of b) A-pMePheOzi-A before heating to 105 °C (left) and after heating to 105°C (right).

DSC measurements

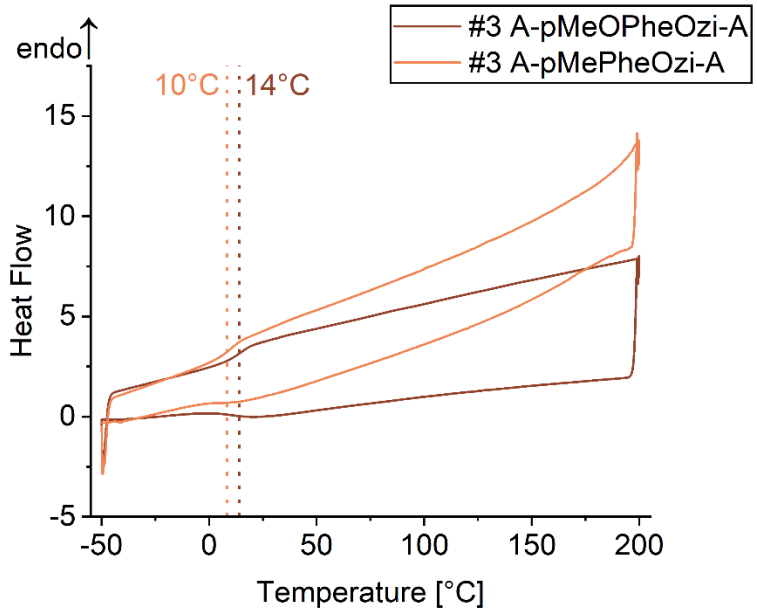


Figure S8: Third DSC heating and cooling cycle of dried A-MeOPheOzi-A and A-MePheOzi-A samples measured from -50 °C to 200 °C. The T_g was defined as the turning points of the stages in the heating curves characteristic of glass transitions.

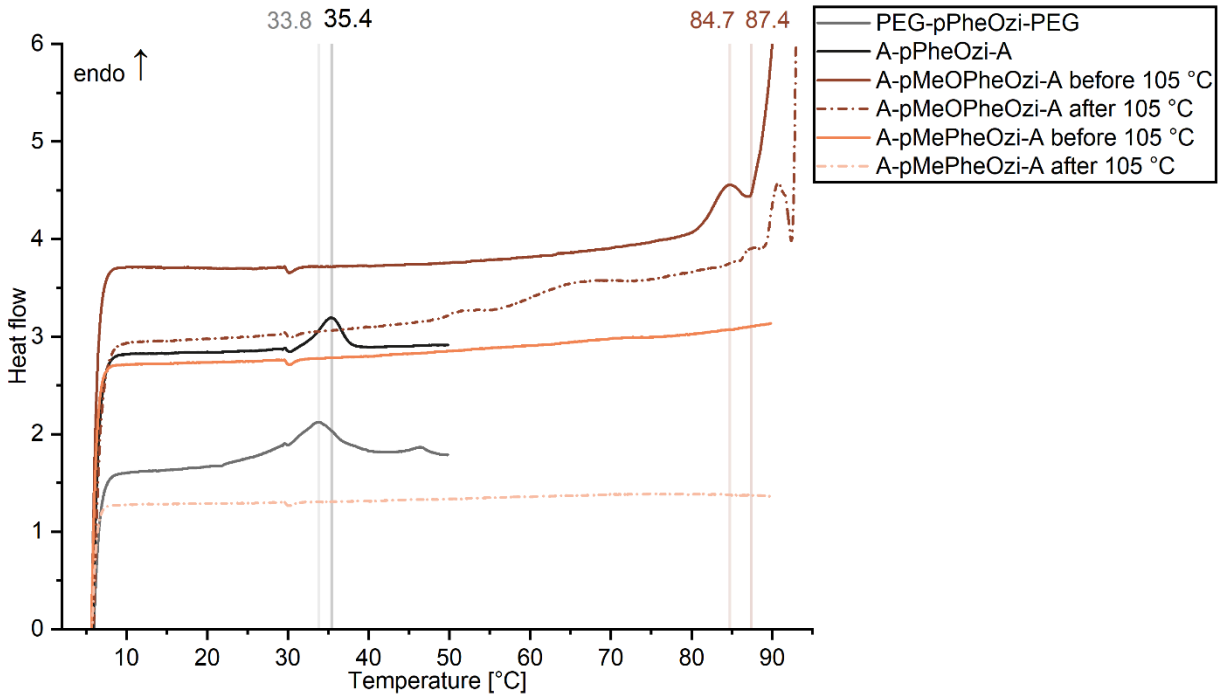


Figure S9: DSC heating curves of the 20 wt.% aqueous samples of A-pPheOzi-A, PEG-pPheOzi-PEG (5 °C – 50 °C), A-pMePheOzi-A (5 °C – 90 °C) and A-pMeOPheOzi-A (5 °C – 93 °C) for gel-sol transition temperature ($T_{gel \rightarrow sol}$) determination. All samples were stored at 5 °C for at least 24 h before measurement. A-pMeOPheOzi-A and A-pMePheOzi-A were measured before and after heating to 105 °C for several minutes and cooling back to 5 °C for 24 h.

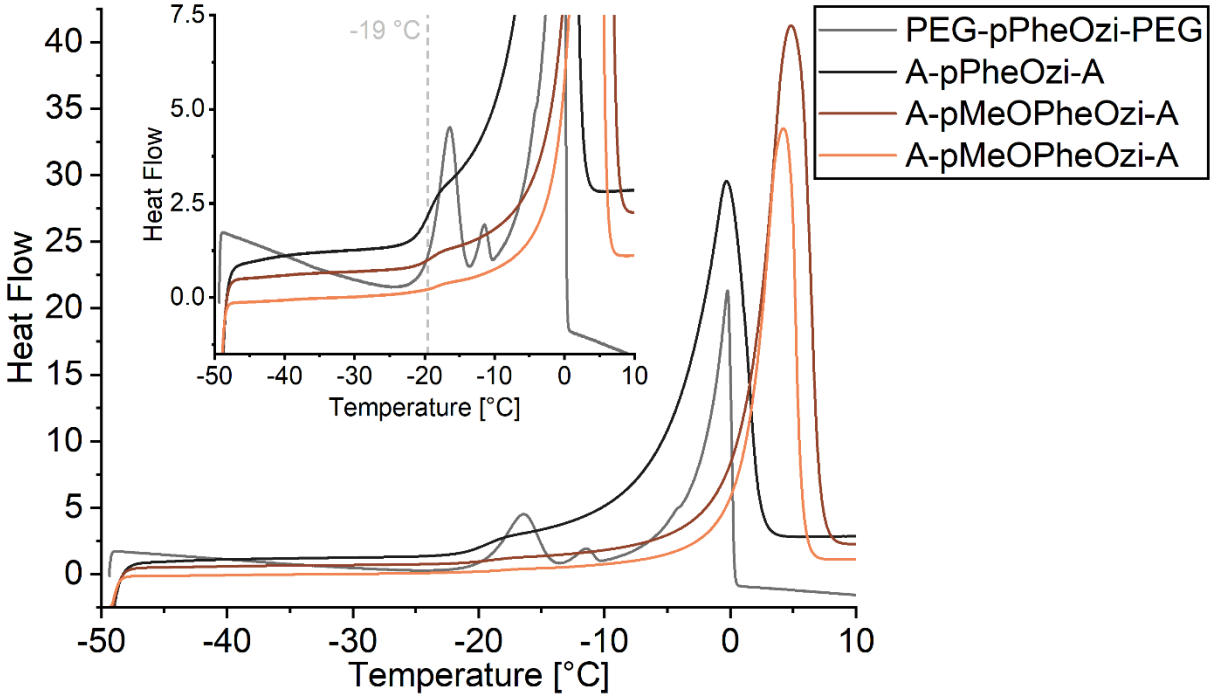


Figure S10: DSC heating curve from -50 °C to 10 °C with a rate of 5 °C/min of 20 wt.% aqueous solutions of the polymers, which were frozen with a rate of 20 °C/min starting from 5 °C.

Rheology

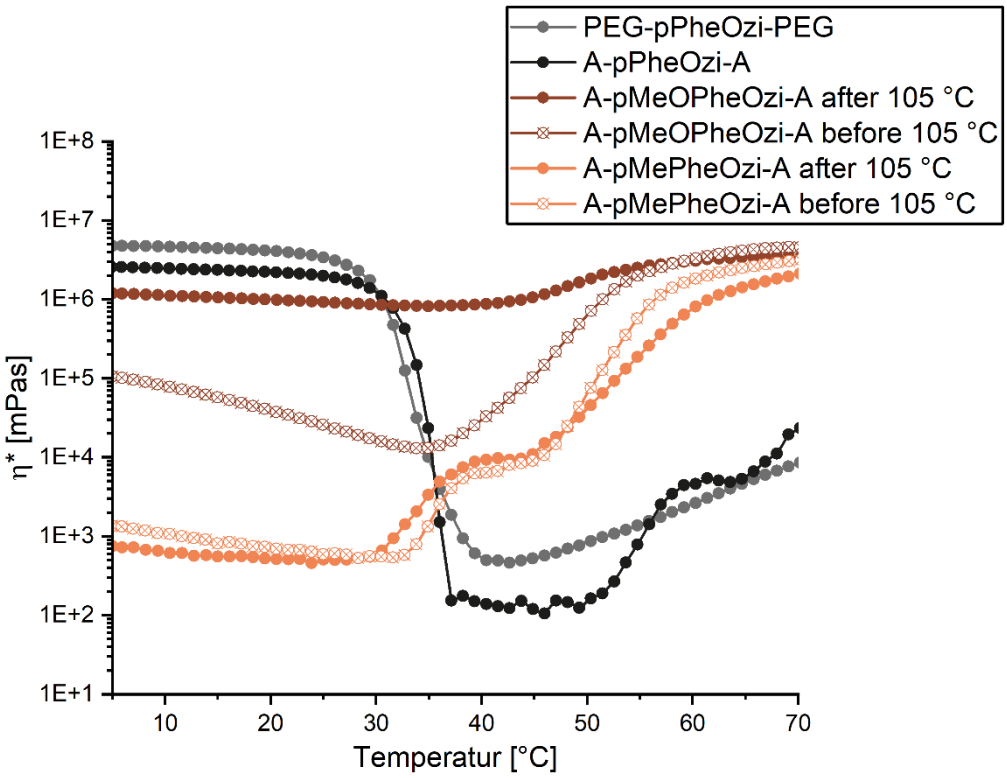


Figure S11: Temperature sweeps for determination of the complex viscosity (η*) measured from 5 °C – 70 °C with a heating rate of 0.5 °C/min of 15 wt.% aqueous samples of A-pPheOzi-A, PEG-pPheOzi-PEG, A-pMeOPheOzi-A and A-pMePheOzi-A. All samples were stored at 5 °C for at least 24 h before measurement. A-pMeOPheOzi-A and A-pMePheOzi-A were measured before and after heating to 105 °C for several minutes and cooling back to 5 °C for 24 h.

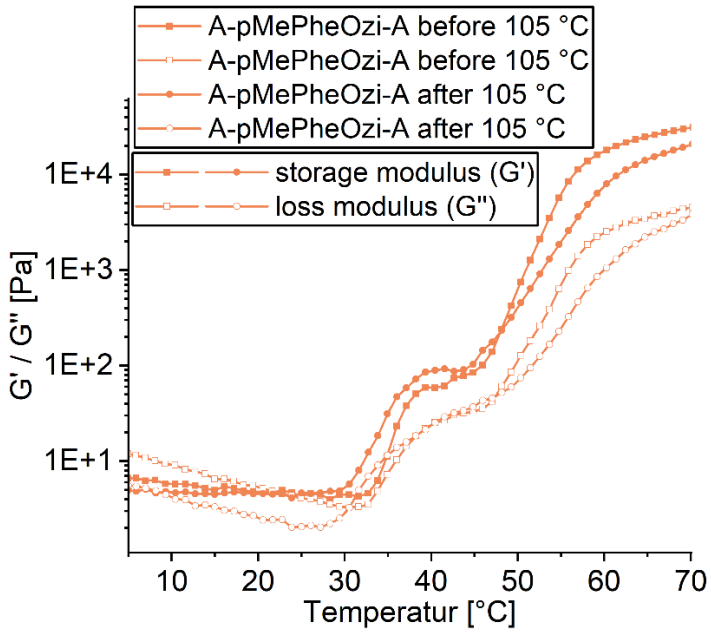


Figure S12: Temperature sweeps for determination of the storage (G´) and loss modulus (G´´) measured from 5 °C – 70 °C with a heating rate of 0.5 °C/min of 15 wt.% aqueous samples of A-pMePheOzi-A before and after heating to 105 °C for several minutes and cooling back to 5 °C for 24 h.

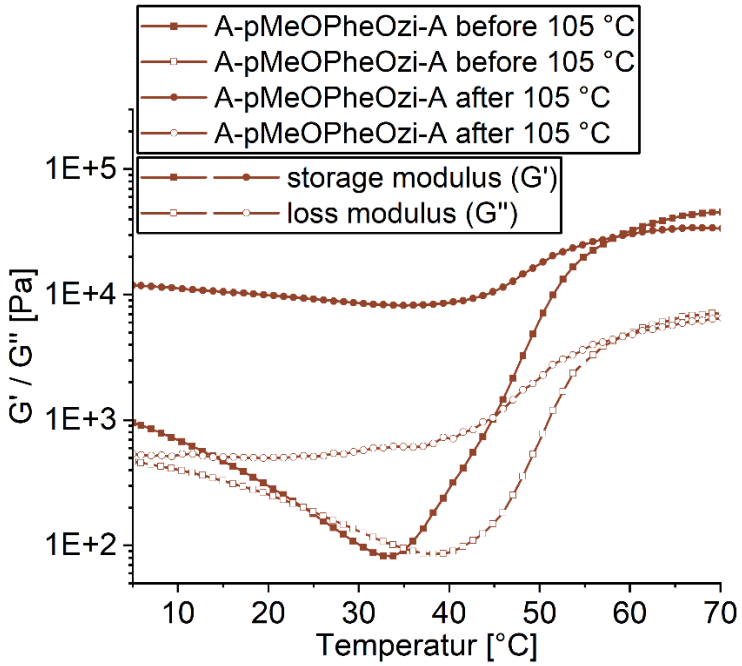


Figure S13: Temperature sweeps for determination of the storage (G´) and loss modulus (G´´) measured from 5 °C – 70 °C with a heating rate of 0.5 °C/min of 15 wt.% aqueous samples of A-pMeOPheOzi-A before and after heating to 105 °C for several minutes and cooling back to 5 °C for 24 h.

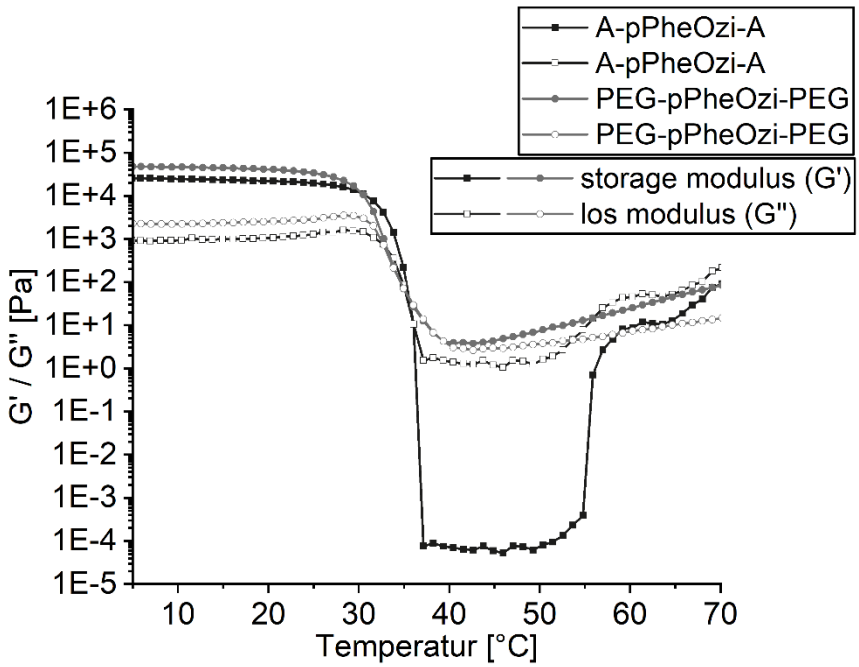


Figure S14: Temperature sweeps for determination of the storage (G´) and loss modulus (G´´) measured from 5 °C – 70 °C with a heating rate of 0.5 °C/min of 15 wt.% aqueous samples of A-pPheOzi-A and PEG-pPheOzi-PEG after storage at 5 °C for 24 h.

AFM and Negative Stain TEM Images

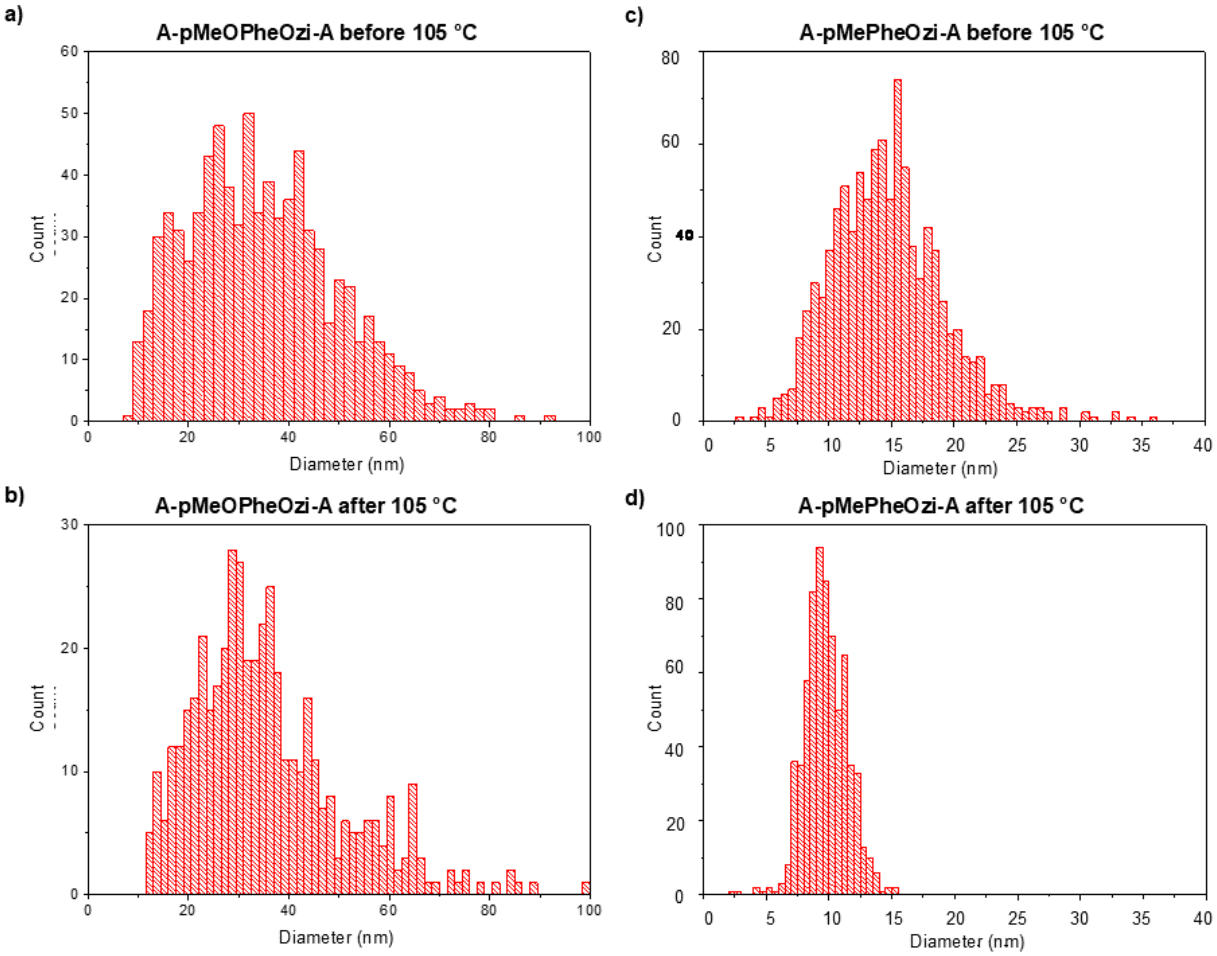


Figure S15: Histogram: a) length of the rod-like aggregates of A-pMeOPheOzi-A before heating to 105 °C b) length of the rod-like aggregates of A-pMeOPheOzi-A after heating to 105 °C c) diameter of the spherical aggregates of A-pMePheOzi-A before heating to 105 °C d) diameter of the spherical aggregates of A-pMePheOzi-A after heating to 105 °C

The length dispersity $Đ_L$ was calculated by $L_w/L_n = Đ_L$. The number-averaged length (L_n) and weight-averaged length (L_w) were calculated according to equation (1) and (2) where L = length of rods and N = number:

$$(S1)\ \boldsymbol{L_n} = \frac{\sum_i^n N_i L_i}{\sum_i^n N_i} \qquad (S2)\ \boldsymbol{L_w} = \frac{\sum_i^n N_i L_i^2}{\sum_i^n N_i L_i}$$

a) The rod-like aggregates of A-pMeOPheOzi-A before heating to 105 °C exhibit the low length-dispersity of $Đ_L$ = 1.18. The number-averaged length (L_n) and weight-averaged length (L_w) are calculated to be L_n = 34.7 nm, L_w = 41.1 nm.

b) The aggregates of A-pMeOPheOzi-A after heating to 105 °C exhibit the low length-dispersity of $Đ_L$ = 1.18. The number-averaged length (L_n) and weight-averaged length (L_w) are calculated to be L_n = 35.4 nm, L_w = 41.6 nm.

The diameter dispersity $Đ_D$ was calculated by $D_w/D_n = Đ_D$. The number-averaged diameter (D_n) and weight-averaged diameter (D_w) were calculated according to equation (3) and (4) where D = diameter of particles and N = number:

$$(S3) D_n = \frac{\sum_i^n N_i D_i}{\sum_i^n N_i} \qquad (S4)\ D_w = \frac{\sum_i^n N_i D_i^2}{\sum_i^n N_i D_i}$$

c) The particles of A-pMePheOzi-A before heating to 105 °C exhibit the low diameter-dispersity of $Đ_D$ = 1.10. The number-averaged diameter (D_n) and weight-averaged diameter (D_w) are calculated to be D_n = 14.6 nm, D_w = 16.0 nm.

d) The particles of A-pMePheOzi-A after heating to 105 °C exhibit the low diameter-dispersity of $Đ_D$ = 1.03. The number-averaged diameter (D_n) and weight-averaged diameter (D_w) are calculated to be D_n = 9.7 nm, D_w = 10.0 nm.

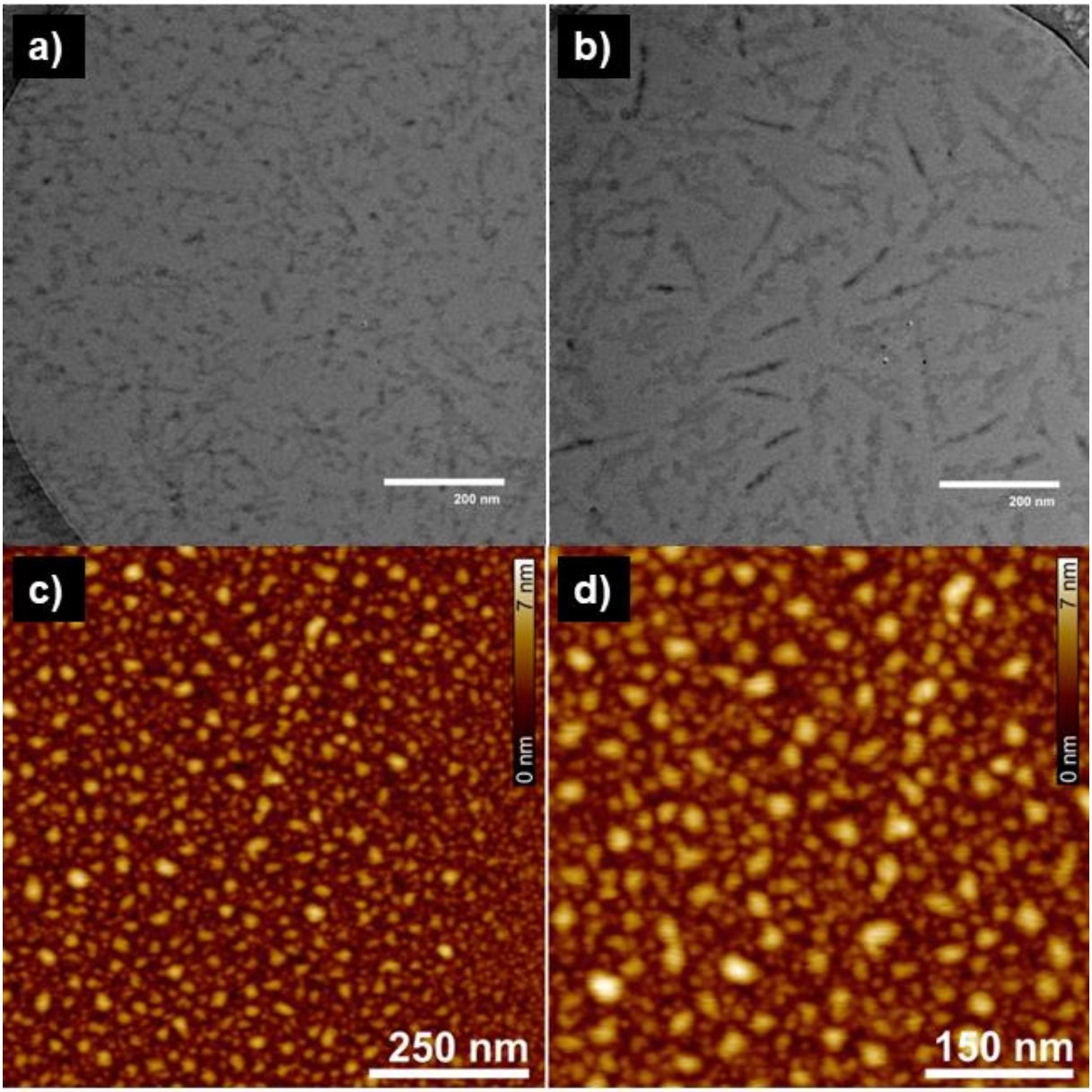


Figure S16: Morphologies of the diluted (~1 wt.%) aqueous samples of A-pMeOPheOzi-A before (a) and after heating to 105 °C (b) measured as negative-stain TEM image. Morphologies of the diluted (~1 wt.%) aqueous samples of PEG-pPheOzi-PEG measured by AFM (c / d). Height AFM images of spin-coated samples on SiO_x. Z scale is 12 nm. Probably due to interactions of PEG with the matrix no organized morphologies could be observed.

Temperature-Dependent NMR Measurements in Solution

Experiments were performed at a Bruker Avance III HD 600 spectrometer (Karlsruhe, Germany) operating at 14.1 T equipped with a BBFO 5 mm probe using a BCU-02 temperature control unit. ^{1}H NMR experiments of 20 wt.% hydrogel samples in D_2O were acquired with a 90° flip angle, 8 scans, 25 s relaxation delay and without sample spinning. A series of variable temperature experiments was performed in the range from 5 °C to 40 °C (PEG-pPheOzi-PEG) or 90 °C (A-pMeOPheOzi-A, A-pMePheOzi-A) in 5 °C steps. The sample was kept at the desired temperature for 10 minutes prior to each measurement. Temperature calibration was done using 4 % MeOH in MeOD and 80 % ethylene glycol in DMSO-d_6. All recorded spectra were referenced using the temperature dependent HDO signal. The longitudinal (T_1) relaxation times of the polymer protons was measured using an Inversion Recovery pulse sequence, 16 scans, 16 time increments τ ranging from 1 ms – 5 s and a relaxation delay of 20 s. The obtained build-up curves were transferred to OriginLab and fitted using a monoexpontial function (eq. S1).

$$I(\tau) = I_0[1 - 2Aexp^{\left(-\frac{\tau}{T_1}\right)}] \quad \text{(S5)}$$

The obtained integral value I is plotted against the respective delay time τ with the maximum integral defined as I_0, A being an intensity prefactor and T_1 is the desired longitudinal relaxation time.

^{1}H NMR Measurements in Solution

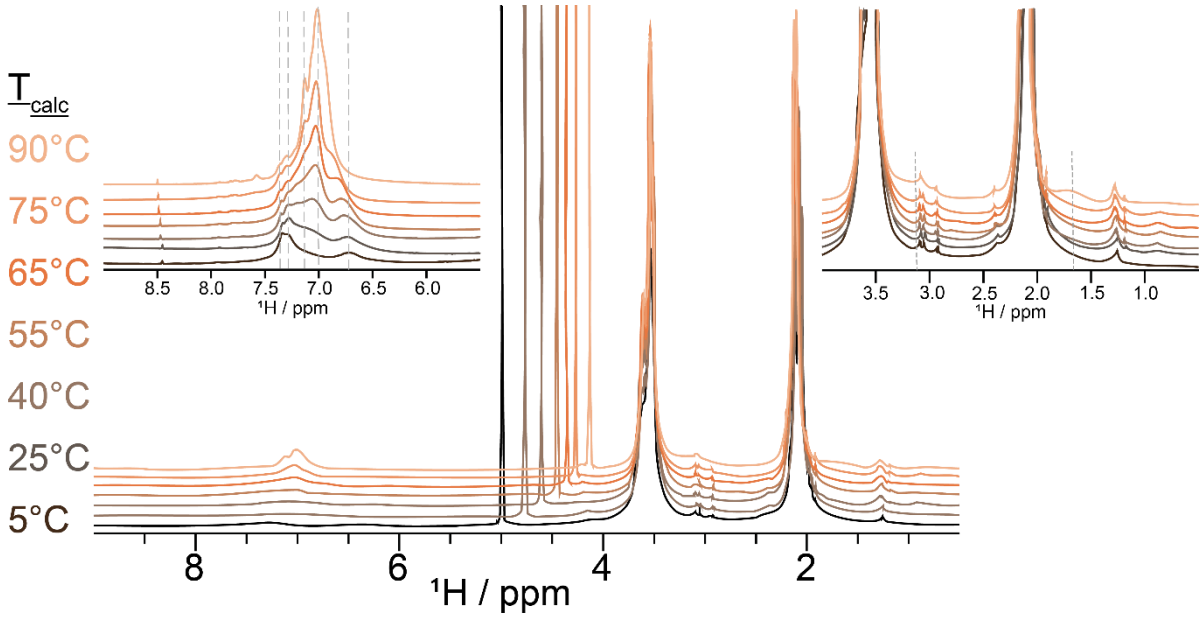


Figure S17: ^{1}H NMR spectra of a 20 wt.% sample of A-MePheOzi-A in D_2O measured at different temperatures between 5 °C – 90 °C. The peaks between 7.5 – 6.5 ppm refer to the phenyl group, between 4.0 – 3.0 ppm to the methylene backbone unit neighboring the amide function, between 2.5 – 2.0 ppm to the methyl group of the pMeOx side-chains, and between 1.7 – 1.5 ppm to the central methylene unit of the pPheOzi backbone.

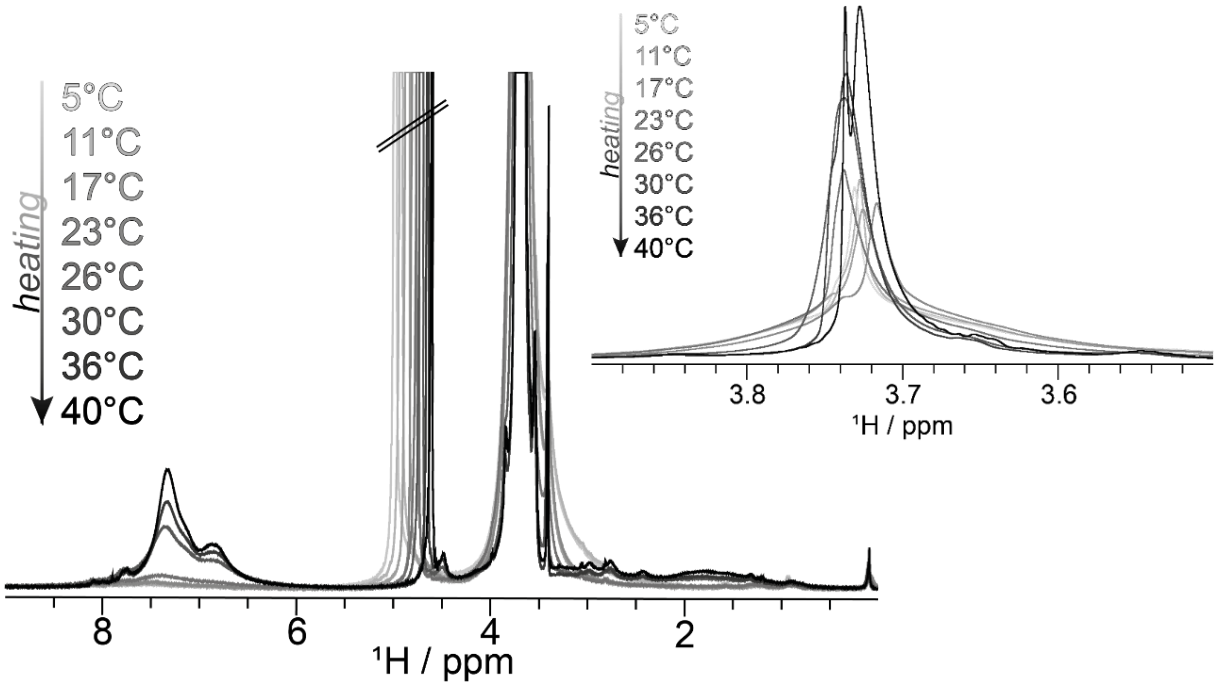


Figure S18: ^{1}H NMR spectra of a 20 wt.% sample of PEG-pPheOzi-PEG in D_2O measured at different temperatures between 5 °C – 40 °C. The peaks between 8.0 – 6.0 ppm refer to the phenyl group, between 4.0 – 3.6 ppm to the PEG and B-block methylene backbone units, and between 1.7 – 1.5 ppm to the central methylene unit of the B-block backbone.

T_1 Relaxation Times of Polymer Protons Measured with NMR in Solution

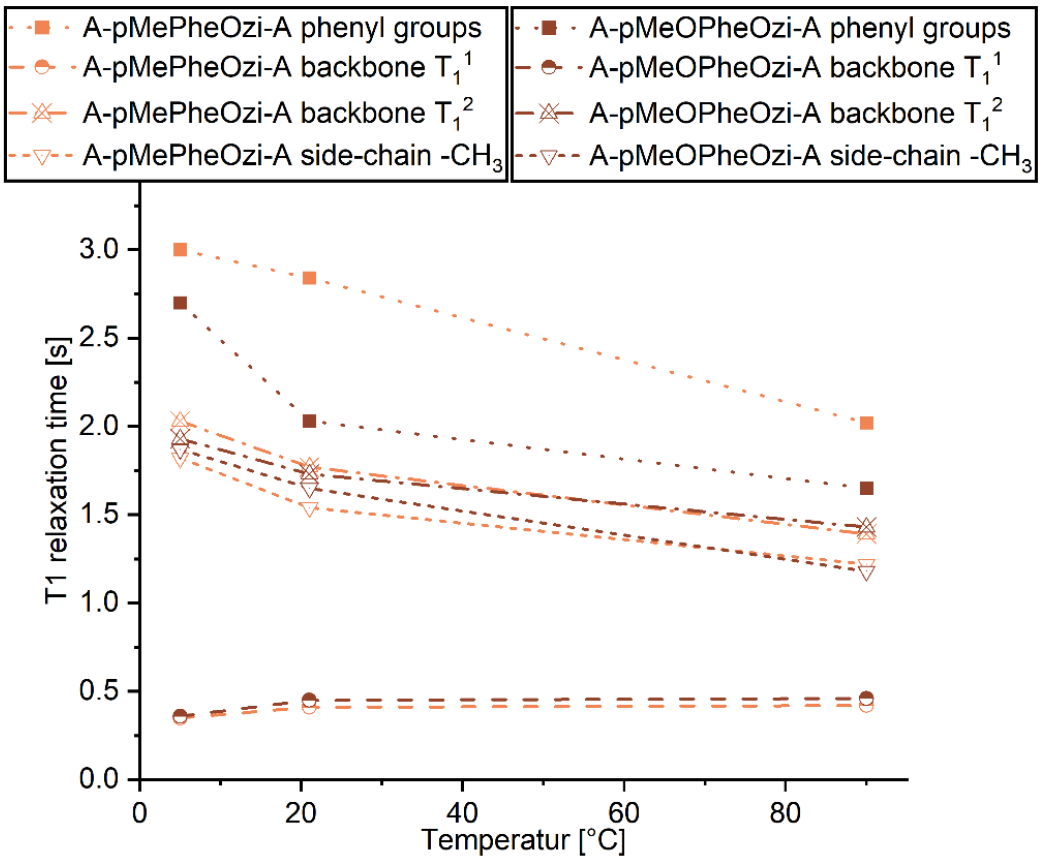


Figure S19: T_1 relaxation times of 20 wt.% aqueous A-pMeOPheOzi-A and A-pMePheOzi-A proton signals measured with NMR in solution.

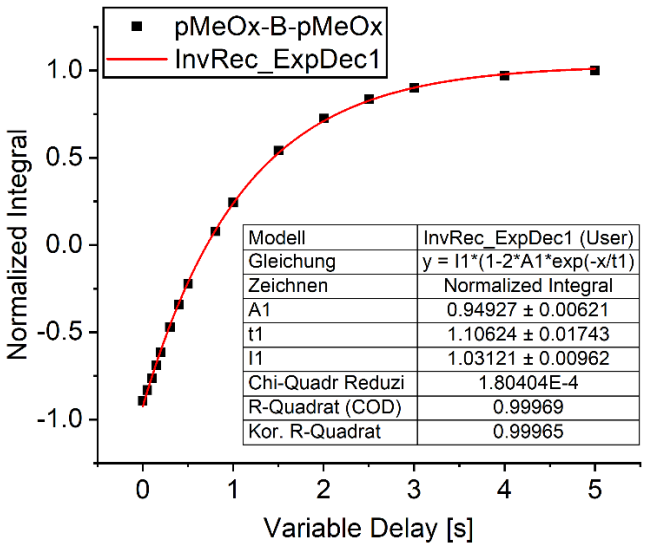


Figure S20: Exemplary T_1 build-up curve of the A-pPheOzi-A side-chain CH_3 proton signals measured with a ^1H inversion recovery experiment and fitted by a monoexponential function.

Solid-State NMR of Aqueous Polymer Samples

Solid-state NMR (ssNMR) measurements on aqueous polymer samples were performed using a 4 mm double-channel HX probe and a Bruker Avance Neo spectrometer operating at 9.4 T with 5 - 8 kHz magic angle spinning (MAS). All samples were measured in Kel-F inserts to prevent leaking of the aqueous samples and at 273 K to ensure a persistent and stable gel (if possible) throughout the measurements. For the ^{13}C CP MAS experiment, a 2 ms ramp (50 to 100 %) on the ^{1}H channel was used during the cross-polarization (CP) contact time for all samples. ^{13}C NMR spectra with direct excitation were recorded with short interscan delays of 1 s to detect predominantly mobile components. ^{1}H-^{13}C HETCOR MAS spectrum were acquired with 80 - 180 t_1 FID increments using a contact time of 2 ms, 1.3 s relaxation delay and 512 -1024 scans. For heteronuclear decoupling during acquisition, SPINAL64 was employed with a 100 kHz nutation frequency (^{1}H). The chemical shifts were referenced using adamantane by subsequent adjustment of the magnetic field.

^{1}H NMR Spectra Measured with Increasing MAS Rate by Solid-State NMR

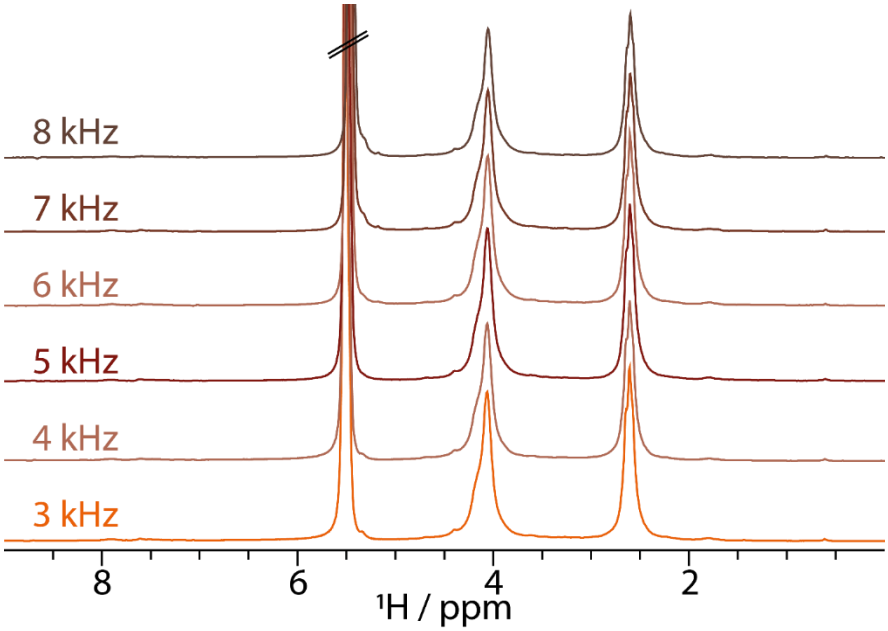


Figure S21: ^{1}H NMR spectra of 20 wt.% aqueous solutions of A-pMeOPheOzi-A measured with increasing MAS rate at a solid-state NMR probe.

^{1}H-^{13}C HETCOR of the Hydrogels Measured with Solid-State NMR

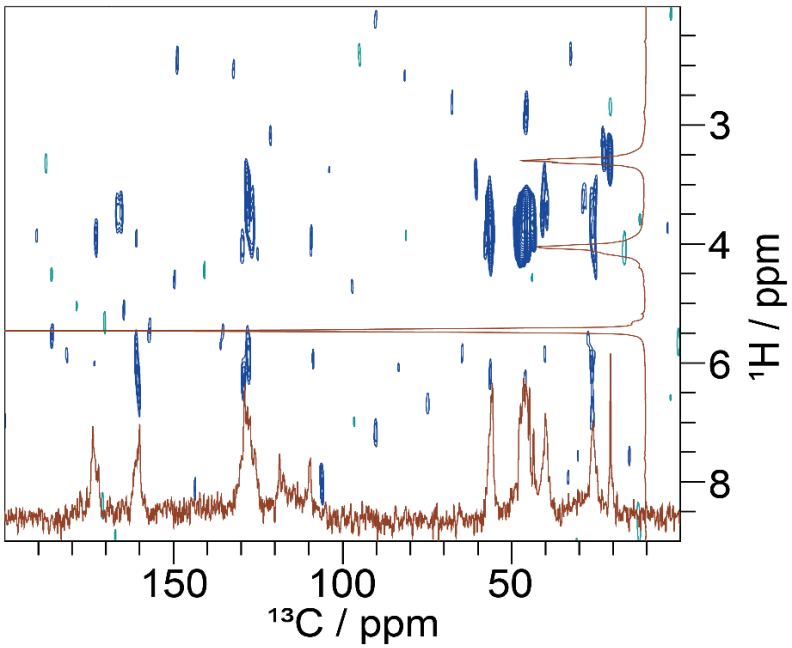


Figure S22: 20 wt.% sample of A-pMeOPheOzi-A in D_2O: Overlay of the ^{13}C NMR spectra using CP MAS with 2 ms contact time (horizontal brown 1D spectrum) and the ^{1}H onepulse spectrum (vertical brown 1D spectrum). ^{1}H-^{13}C HETCOR MAS spectrum using a contact time of 2 ms. 58 t1 FID increments were acquired using a recycle delay of 1.5 s, each with 800 co-added transients. All spectra were recorded at 400 MHz and a MAS rate of 8 kHz.

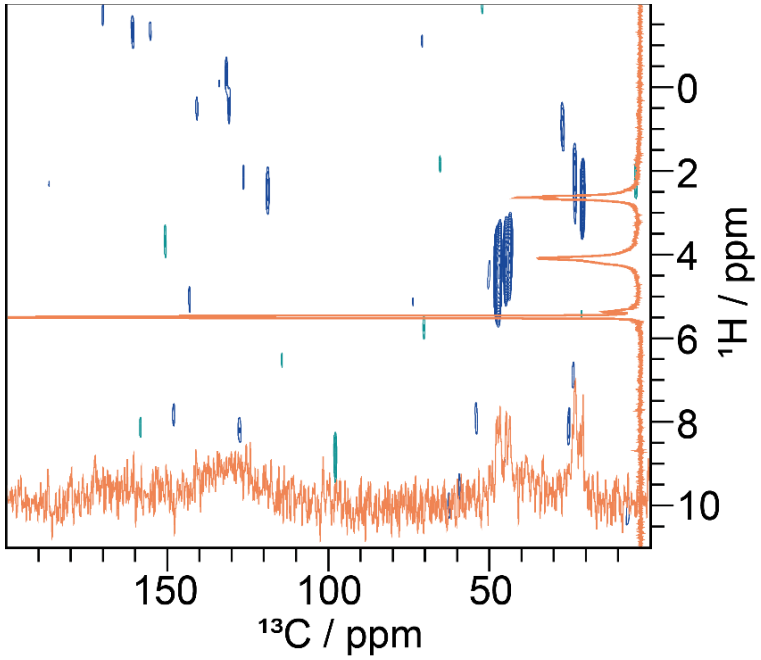


Figure S23: 20 wt.% sample of A-pMePheOzi-A in D_2O: Overlay of the ^{13}C NMR spectra using CP MAS with 2 ms contact time (horizontal orange 1D spectrum) and the ^{1}H onepulse spectrum (vertical orange 1D spectrum). ^{1}H-^{13}C HETCOR MAS spectrum using a contact time of 2 ms. 72 t1 FID increments were

acquired using a recycle delay of 3 s, each with 512 co-added transients. All spectra were recorded at 400 MHz and a MAS rate of 8 kHz.

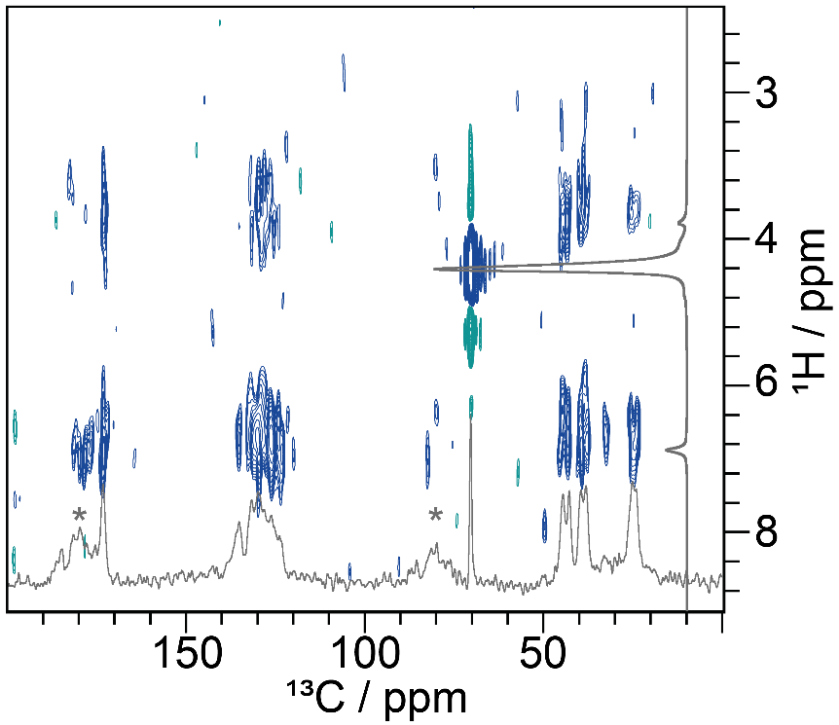


Figure S24: 20 wt.% sample of PEG-pPheOzi-PEG in D_2O: Overlay of the ^{13}C NMR spectra using CP MAS with 2 ms contact time (horizontal orange 1D spectrum) and the ^{1}H onepulse spectrum (vertical orange 1D spectrum). ^{1}H-^{13}C HETCOR MAS spectrum using a contact time of 2 ms. 59 t1 FID increments were acquired using a recycle delay of 1 s, each with 1024 co-added transients. All spectra were recorded at 400 MHz and a MAS rate of 8 kHz.

Solid-State NMR of Frozen Aqueous Polymer Samples

ssNMR measurements on frozen samples were performed using a 3.2 mm double-channel probe and a Bruker Avance III HD spectrometer operating at 14.1 T with 7 kHz magic angle spinning (MAS). The samples were cooled to 243 K to obtain a completely frozen sample throughout the measurements, although temperature calibration demonstrated an increase in temperature about +10 °C due to frictional heating. For the ^{13}C CP MAS experiment, a 1 ms or 2 ms ramp (90 to 100 %) on the ^{1}H channel was used during the cross-polarization (CP) contact time for all samples. ^{13}C NMR spectra with direct excitation were recorded with interscan delays of 2 s. ^{1}H-^{13}C HETCOR MAS spectrum were acquired with 80 - 180 t1 FID increments using a contact time of 2 ms, 1.3 s relaxation delay and 512 -1024 scans. For heteronuclear decoupling during acquisition, SPINAL64 was employed with a 100 kHz nutation frequency (^{1}H). The magic angle was calibrated with KBr and α-glycine was used to set the ^{1}H 90° pulse. The chemical shifts were referenced using adamantane by subsequent adjustment of the magnetic field. ^{13}C T_1 relaxation time measurements were recorded using a Torchia pulse sequence. Spectra were acquired with 96 - 300 scans, a relaxation delay of 3 s, 15 variable delay increments τ ranging from 50 ms to 60 s. The obtained curves were transferred to OriginLab and fitted using a monoexpontial function.

$$I(\tau) = I_0 + Aexp^{\left(-\frac{\tau}{T_1}\right)} \quad \text{(S6)}$$

The obtained integral value I is plotted against the respective delay time τ with the maximum integral defined as I_0, A being an intensity prefactor and T_1 is the desired longitudinal relaxation time.

^{13}C T_1 Torchia NMR measurements

Table S1: ^{13}C T_1 relaxation times measured at the frozen aqueous polymer samples. The different carbon moieties are defined as CO (carbonyl groups), PHE (phenyl groups), pOx BB (backbone protons adjacent to the amide nitrogen), SC CH_3 (side-chain CH_3 group of hydrophilic A-blocks) and pOzi (central methylene unit within a polyoxazine backbone).

Polymer		T_{frozen}	T_1 [s] CO	T_1 [s] PHE	T_1 [s] pOx BB	T_1 [s] SC CH_3	T_1 [s] PEG	T_1 [s] pOzi BB
PEG-pPheOzi-PEG		5 °C	-	10	9.9	-	0.5	11.4
A-pPheOzi-A		40 °C	11.4	16.1	7.7	6.3		-
A-pPheOzi-A		5 °C	25.4	17.3	8.9	7.1		24.4
A-pMePheOzi-A		5 °C	13.2	26.3	9.6	6.4		-
A-pMeOPheOzi-A		90 °C	16.9	-	10.1	9.3		-
A-pMeOPheOzi-A	before 105 °C	5 °C	-	-	10.9	6.3		-
A-pMeOPheOzi-A	after 105 °C	5 °C	17.1	18.9	7.6	5.6		22

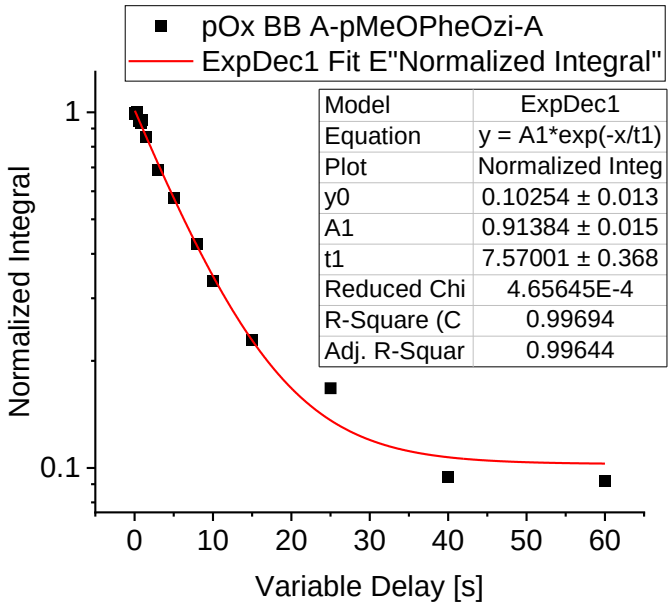


Figure S25: Exemplary T_1 decay curve of the A-pMeOPheOzi-A backbone carbon signal measured with a ^{13}C Torchia experiment and fitted by a monoexponential function.

^{13}C NMR Spectra Comparison

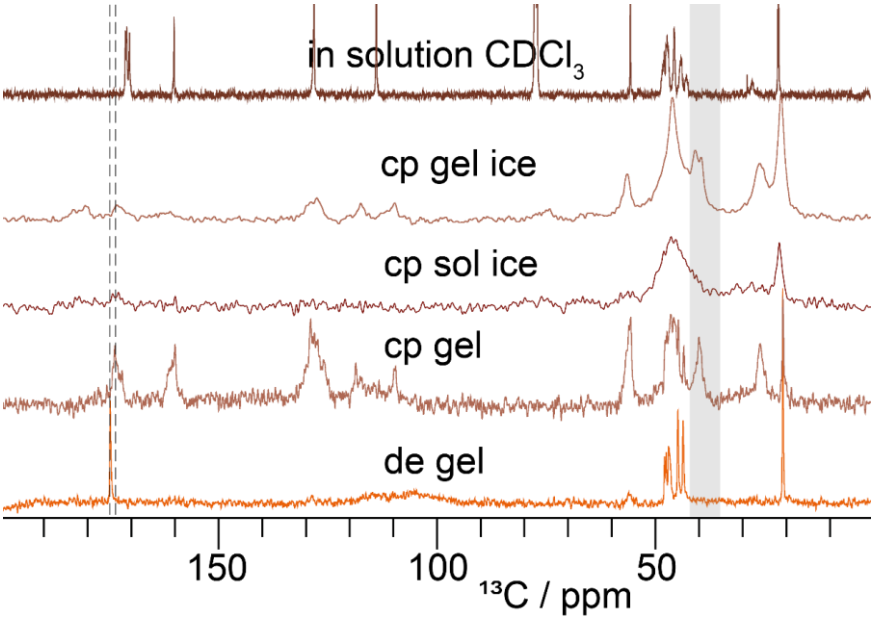


Figure S26: Overlay of the ^{13}C NMR spectra of A-pMeOPheOzi-A measured in $CDCl_3$ with NMR in solution or measured as a 20 wt.% aqueous solution with DE or CP of the unfrozen gel or frozen sol and gel samples by solid-state NMR.

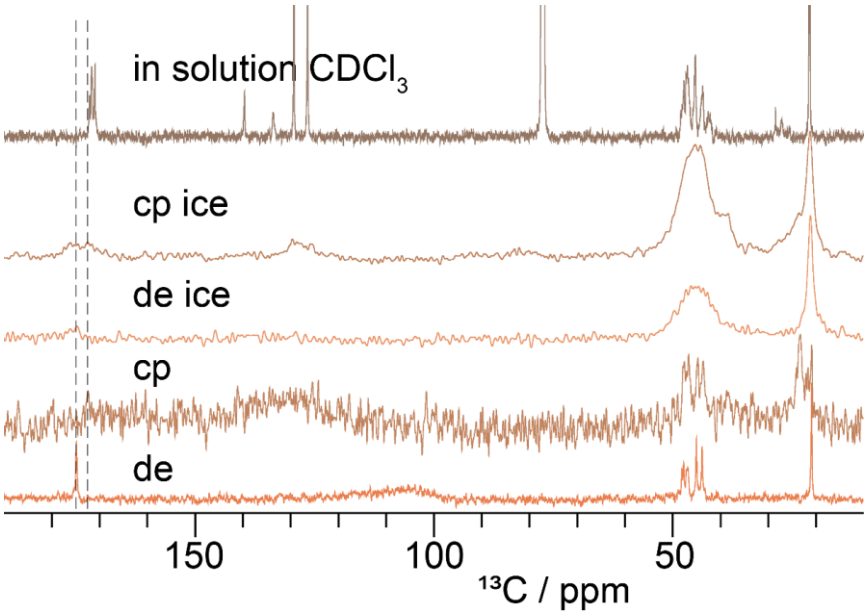


Figure S27: Overlay of the ^{13}C NMR spectra of A-pMePheOzi-A measured in $CDCl_3$ with NMR in solution or measured with DE or CP of frozen or unfrozen 20 wt.% samples in D_2O by solid-state NMR.

FT-IR Spectra

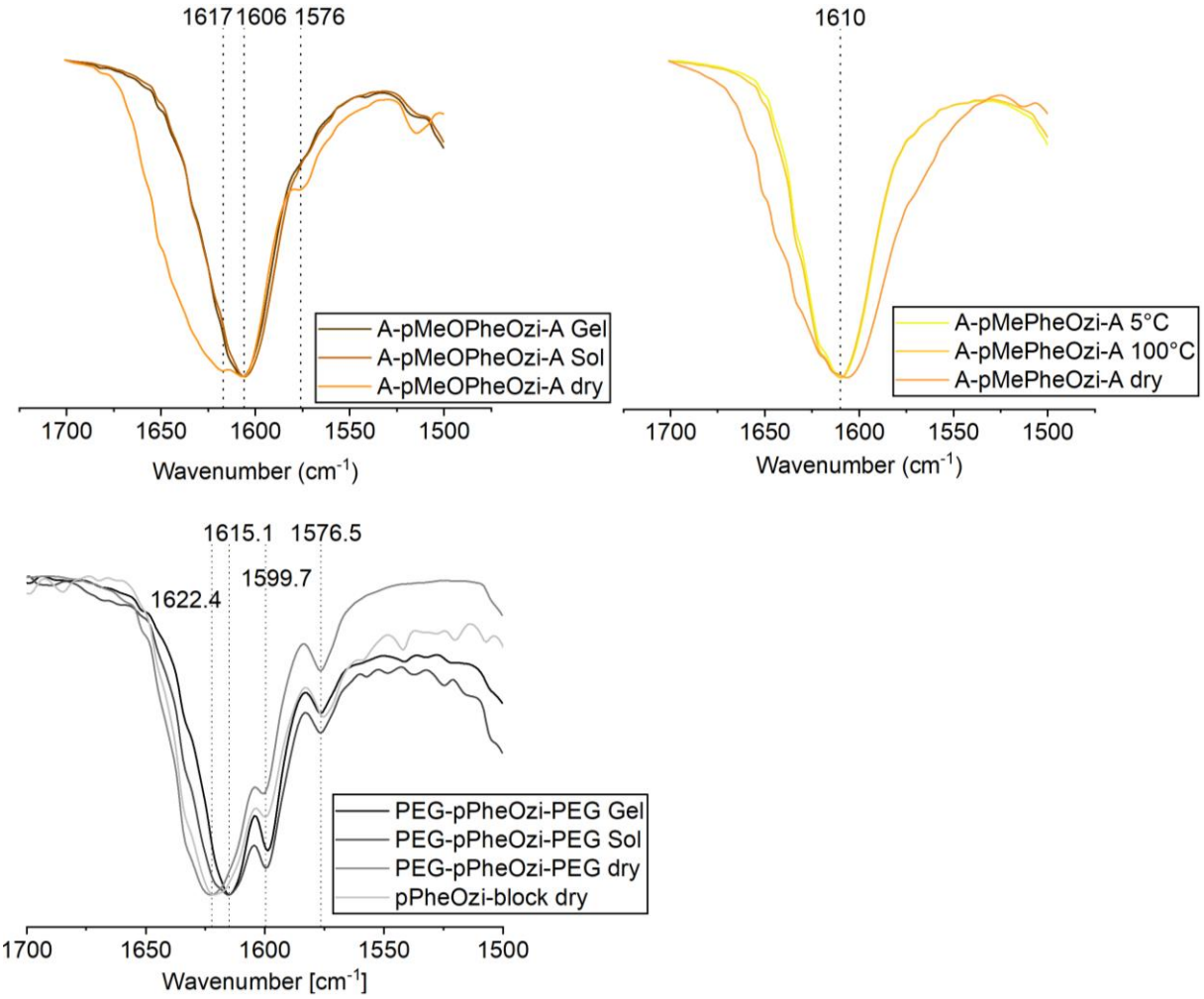


Figure S28: IR spectra of the dried polymer powders as well as 20 wt.% polymer solution in D_2O were measured with IR at different temperatures. D_2O was used instead of H_2O to avoid signal overlap with the carbonyl band of the respective polymer. The normalized transmittance of the carbonyl bands is shown.

Preparation of Guest-loaded Polymer Formulations

Guest-loaded polymer formulations were prepared according to the thin-film method.[21] A 30 g/L ethanolic stock solution of A-pPheOzi-A and a 5 g/L or 8 g/L ethanolic stock suspension of the guest molecule were prepared. For higher accuracy, the amount of ethanol was added by weight rather than volumetrically. The curcumin stock suspension and the polymer stock solution were then mixed by weight in a specified ratio to obtain the desired guest:polymer ratio. The exact weights and mixing ratios of the prepared samples can be found below. The combined ethanolic solutions were shaken in a ThermoMixer™ C (Eppendorf, Hamburg, Germany) (750-1000 rpm) at 50 °C for 60 min. Subsequently, ethanol was removed by a constant stream of nitrogen and heating at 50 °C to obtain a thin film at the container wall. High vacuum was used for further removal of ethanol. The dried thin-film was hydrated by volumetric addition of water to obtain a total of 80 wt.% water in the sample. The hydrated sample was shaken (750-1250 rpm) at 50 °C for 60 min. For complete removal of residual ethanol, the hydrated sample was freeze-dried and subsequently rehydrated again by volumetric addition of water to obtain a total of 80 wt.% water in the sample. The rehydrated sample was shaken again (750-1250 rpm) at 50 °C for 60 min. Precipitated guest molecules/drug was removed by centrifugation at 4400-9000 rpm for 5 min. After rehydration the samples were stored at 5 °C. The theoretical loading was calculated as follows:

$$guest - loading\ [wt.\%] = \frac{m(guest)}{m(polymer)} = \frac{w(StockGuest)\cdot m(StockGuest)}{w(StockPol)\cdot m(StockPol)} \quad \text{(S6)}$$

The theoretical guest loading is calculated according to eq. (S6) where w(StockGuest) is the mass fraction of the guest in the prepared guest stock suspension, w(StockPol) is the mass fraction of polymer in the prepared polymer stock solution, m(StockGuest) is the mass taken from the guest stock solution and m(StockPol) is the mass taken from the polymer stock solution.

pMeOx-b-pPheOzi-b-pMeOx

Polymer stock solution: c(StockPol) = 25.60 g/L; w(StockPol) = 3.14 wt.%
Curcumin stock solution #1: c_1(StockCur) = 5.05 g/L; w_1(StockCur) = 0.64 wt.%
Curcumin stock solution #2: c_2(StockCur) = 8.10 g/L; w_2(StockCur) = 1.02 wt.%

Table S2: Exact mass taken from the polymer stock solution m(StockPol) and the curcumin stock suspension m(StockCur) for the preparation of the P1_Cur formulations and the theoretically achieved loading in wt.%.

Sample Code	m(StockPol) [mg]	StockCur	m(StockCur) [mg]	Cur-load. [wt.%]	V(water) [µL]
Cur2-0	334.1	#1	31.9	1.93	44.0
Cur2-1	322.0	#1	31.8	2.00	41.9
Cur4-0	306.7	#1	62.9	4.14	39.5
Cur4-1	323.6	#1	60.5	3.78	39.7
Cur6-0	319.4	#1	92.9	5.88	39.2
Cur6-1	320.5	#1	90.6	5.71	39.7
Cur8-0	315.0	#1	122.7	7.87	40.3
Cur8-1	314.4	#1	122.2	7.85	40.1
Cur15-0	319.0	#1	229.3	14.52	39.8
Cur15-1	315.2	#1	236.2	15.14	41.2
Cur20-0	313.4	#1	311.3	20.07	39.9
Cur20-1	320.0	#1	304.1	19.20	44.2

Cur25-0	320.1	#1	382.0	24.11	39.6
Cur25-1	319.4	#1	380.3	24.06	39.8
Cur35-0	306.3	#1	532.2	35.10	40.6
Cur35-1	329.4	#1	534.8	32.80	39.9
Cur50-0	314.4	#1	771.4	49.57	39.6
Cur50-1	317.6	#1	767.9	48.85	39.9
Cur75-0	313.2	#1	1167.3	75.30	38.8
Cur75-1	311.2	#2	715.0	74.18	39.0
Cur100-0	320.0	#2	990.3	99.92	40.0
Cur100-1	324.2	#2	999.3	99.52	39.2
Cur125-0	315.0	#2	1200.0	122.99	39.6
Cur125-1	116.0	#2	1199.1	333.74	39.6

Polymer stock solution: c(StockPol) = 30.00 g/L; w(StockPol) = 3.80 wt.%
Danazol stock solution: c(StockDana) = 4.57 g/L; w(StockDana) = 0.58 wt.%
Felodipin stock solution: c(StockFelo) = 5.07 g/L; w(StockFelo) = 0.64 wt.%
Efavirenz stock solution: c(StockEfa) = 5.78 g/L; w(StockEfa) = 0.73 wt.%
Dolutegravir stock solution: c(StockDolu) = 5.23 g/L; w(StockDolu) = 0.66 wt.%

Table S3: Exact mass taken from the polymer stock solution m(StockPol) and the guest stock suspension m(StockGuest) for the preparation of the formulations and the theoretically achieved loading in wt.%.

Sample Code	m(StockPol) [mg]	m(StockGuest) [mg]	theo. guest-load. [wt.%]	V(water) [µL]	Measured guest-load. [wt%]
Dana	523.4	206.0	5.99	84.4	1.43
Felo	515.0	184.6	6.06	83.0	5.76
Efa	512.7	160.4	6.02	82.7	6.68
Dolu	588.2	202.5	6.00	94.8	2.06

For the determination of the actual guest content of drug-loaded A-pPheOzi-A, a qNMR approach was chosen, as UV-vis spectroscopy as fur Cur was not applicable. An exact weight of the formulation is diluted with DMSO-d_6 and measured with a proton zg pulse sequence with 128 scans, a relaxation delay of 20 s and a calibrated pulse of 7.8 µs. A reference sample prepared of 0.888 mg Felodipine, 48.38 mg water and 596.9 mg DMSO-d_6 was measured under identical conditions. The obtained proton signal at 5.30 ppm of the felodipine reference was integrated and used for calculation of the guest loading in the actual samples.

Table S4: Exact mass taken from the tested formulation (sample mass) and from DMSO-d_6 used for dilution. The measured sample integral in relation to the reference integral is given and the derived calculated guest-loading in wt.%.

Sample Code	Sample mass [mg]	DMSO-d^6 mass[mg]	Reference Integral	Sample Integral	Measured guest-load. [wt%]
Dana	48.29	610.81	1	1.0185	1.43
Felo	65.06	439.91	1	0.2041	5.76
Efa	62.79	609.00	1	0.8565	6.68
Dolu	47.56	610.06	1	0.1439	2.06

Ultraviolet-visible (UV-vis) Spectroscopy

A JASCO V-770 UV-vis spectrometer equipped with a PAC-743R Peltier for temperature control and conventional quartz cells with 10 mm light path were used to verify the curcumin-loading of the prepared formulations. To enable quantification, a calibration curve was recorded. Five curcumin concentrations in the range of 0.94 mg/mL to 15.4 mg/mL were measured with absorbances of 0.14 a.u. to 2.3 a.u., which is the area of high data validity for the instrument used here. The obtained calibration curve is shown in the SI. To verify curcumin loading, an exact weight of the formulation is diluted with ethanol to be within the calibrated concentration range.

Calibration Curve

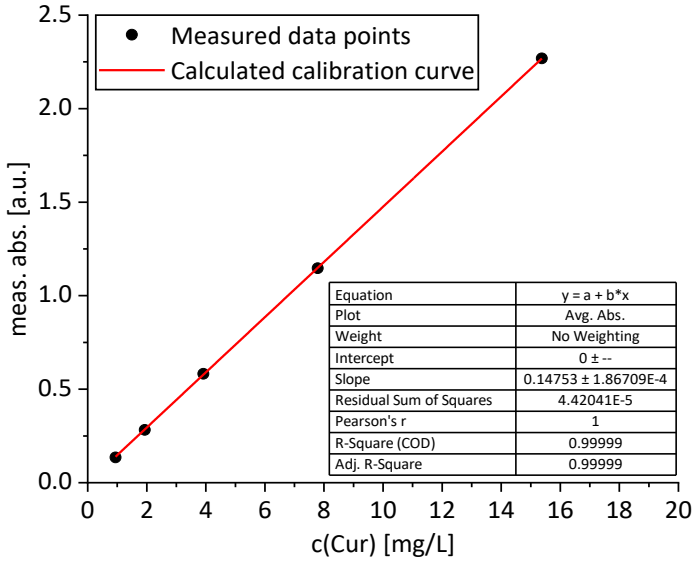


Figure S29: Plotted calibration curve for the determination of a curcumin concentration c(Cur) calculated from the measured absorbance meas. abs.

Table S5: Concentrations of the five prepared ethanolic curcumin solutions c(Cur) and the respective measured absorbances meas. abs.

Data point	1	2	3	4	5
c(Cur) [mg/L]	0.942	1.932	3.918	7.788	15.375
meas. abs. [a.u.]	0.135	0.282	0.582	1.147	2.269

Table S6: Exact mass taken from the tested A-pPheOzi-A Cur formulation m(form.) and from ethanol m(EtOH) used for dilution. The measured absorption is given and the derived calculated curcumin mass m(Cur) and Cur-loading in wt.%.

sample code	m(form.) [mg]	m(EtOH) [g]	meas. abs. [a.u.]	m(Cur) [µg]	Cur-load. [wt.%]
Cur2-0	2.39	3.93	0.271	9.14	1.91%
Cur2-1	2.4	3.92	0.247	8.31	1.73%
Cur4-0	2.67	3.91	0.583	19.5	3.66%
Cur4-1	2.13	3.90	0.415	13.9	3.26%
Cur6-0	2.08	7.84	0.400	26.9	6.48%
Cur6-1	1.98	7.86	0.305	20.6	5.21%

Cur8-0	2.36	7.84	0.608	40.9	8.66%
Cur8-1	4.19	7.90	1.064	72.1	8.61%
Cur15-0	2.28	7.78	0.892	59.6	13.1%
Cur15-1	3.2	8.45	0.729	97.6	15.2%
Cur20-0	1.29	7.76	0.837	55.8	21.6%
Cur20-1	1.85	7.81	0.939	63.0	17.0%
Cur25-0	1.6	7.92	0.275	66.2	20.7%
Cur25-1	2.44	7.94	0.686	102.5	21.0%
Cur35-0	2.39	7.83	0.354	118.9	24.9%
Cur35-1	2.56	7.82	0.541	158.8	31.0%
Cur50-0	2.8	7.67	0.557	220.8	39.4%
Cur50-1	1.19	7.88	0.404	102.1	42.9%
Cur75-0	1.71	7.83	0.273	211.0	61.7%
Cur75-1	1.5	7.76	0.772	193.8	64.6%
Cur100-0	3.53	7.79	0.358	136.4	19.3%
Cur100-1	1.07	7.81	0.541	144.5	67.5%
Cur125-0	0.36	7.81	0.765	51.3	71.3%
Cur125-1	0.82	7.85	0.308	52.3	31.9%

DSC measurements

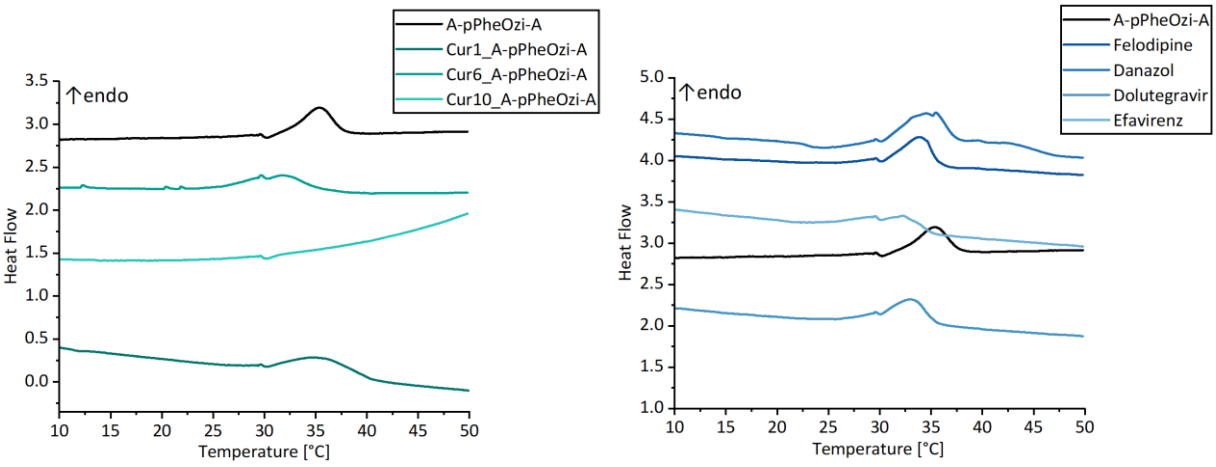


Figure S30: DSC heating curves (5 °C – 50 °C) of the 20 wt.% aqueous samples of A-pPheOzi-A loaded with 1 wt.%, 6 wt.% and 10 wt.% Cur (left) or 6 wt.% of felodipine, danazol, dolutegravir and efavirenz (right). All samples were stored at 5 °C for at least 24 h before measurement. A gel-sol transition is observed at ≈35°C for each sample, except for the 10 wt.% Cur-loaded A-pPheOz-A, which is the only sample that does not form a hydrogel.

Solid-state NMR of 1 wt.% Cur-loaded A-pPheOzi-A

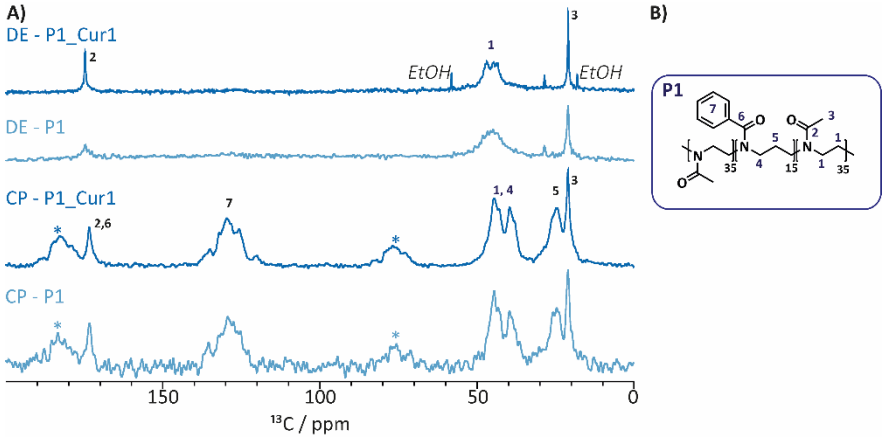


Figure S31: a) Solid-state NMR spectra of the Cur1 formulation and the pure 20 wt.% hydrogel. All samples were frozen inside the NMR spectrometer by cooling the rotor down to -28 °C. All NMR spectra were recorded at 14.1 T at -28 °C, which refers to an actual measurement temperature of -23 °C within the sample. ^{13}C DE NMR spectra were acquired with 8 kHz MAS and a short interscan delay of 2 s. ^{13}C CP MAS NMR spectra were acquired with 8 kHz MAS and with 2 ms contact time. Spectra were scaled to matching peak intensities for best comparability. b) Chemical structure of P1 with numbering scheme for spectral assignment.