Transforming polyethylenimine into a pH-switchable hydrogel by additional supramolecular interactions

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1 General Information

1.1 General Preparative Details

All solvents were dried according to literature procedures. Et_2O was distilled from sodium with benzophenone as indicator. DCM and DMF were distilled from calcium hydride (in the case of DMF, the distillation was carried out under vacuum). MeOH was distilled before use. Sulfuryl chloride was distilled under vacuum. Water was purified with a TKA MicroPure water system. Where necessary reactions were carried out under argon (99.998%, *Air Liquide*), which was previously dried *via* Silica Gel Orange. Freeze drying of aqueous solutions was carried out with an Alpha 1-4 LD plus device from *Christ*.

Analytical TLC was carried out on aluminium foils ALUGRAM SIL G/UV_{254} and C18 SiO_2 aluminium foils ALUGRAM RP-18 W/UV from Machery-Nagel (40 mm · 80 mm, 0.25 mm). The spots were visualized upon irradiation with UV light of 254 nm or 366 nm respectively. Flash Chromatography was performed using Silica gel 60M with a spherical size of 40-63 µm from *Machery-Nagel*. Organic solvents were distilled prior to use.

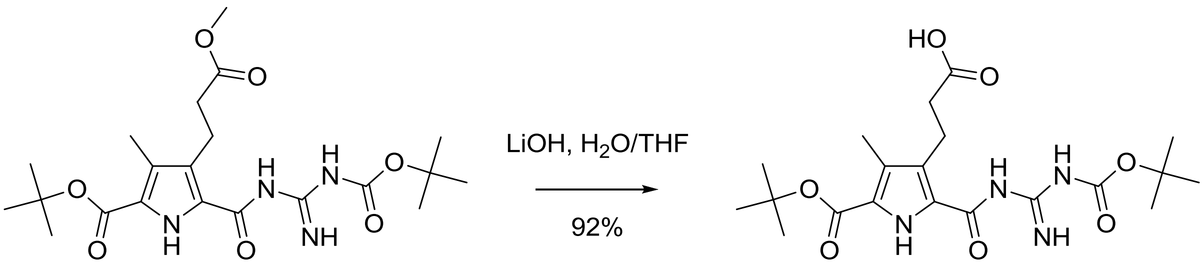
1.2 Analytical Instruments

The ***p*H** was determined with a *p*H-meter 766 Calimatic from *Knick*, which was calibrated with commercial available buffer standards (pH = 4.0 and 7.0). ^{1}H- and ^{13}C-**NMR** spectra were recorded with a DRX 500 and a DMX 300 from *Bruker* at 25 °C. Chemical shifts are reported as δ values in ppm. The spectra were calibrated with the help of the residual peaks of the deuterated solvents as internal standard: DMSO-d_6 (δ [^{1}H] = 2.50 ppm, δ [^{13}C] = 39.52 ppm); $CDCl_3$ (δ [^{1}H] = 7.26 ppm, δ [^{13}C] = 77.16 ppm). For the signal multiplicity the following abbreviations are used: br = broad, s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet. Assignments are based on comparison to literature known compound.[1] **UV/Vis** spectra were recorded with a *JASCO* V-660 spectrophotometer in standard quartz microcuvettes of 1 cm width from *Hellma*. **Atomic Force Microscopy** (AFM) images were recorded in tapping mode with an Innova NanoDrive AFM controller and an Innova Atomic Froce Microscope from *Veeco* with *N*-doped silica cantilevers (AC 160TS *OLYMPUS*). The solutions were spin-coated or drop-casted respectively onto freshly cleaved Mica surface from *Plano*. The data were analyzed with Gwyddion-2.18. **Field Emission Microscopy** (FESEM) images were recorded with a *FEI* Nova NanoSEM-600 equipped with a field-emission gun operating at 10 kV. **Dynamic Light Scattering** (DLS) measurements were performed with a

Zetasizer Nano-ZS from *Malvern Instruments GmbH* and analyzed with the software Dispersion Technology Software (Version 5.03). **Viscosity Measurements** were carried out with a device consisting of the following components: ViscoPump II VZ 8511 and thermostat CT 52 from *Schott Instruments*. The data were analyzed with WinVisco AVS 370. Measurements of storage and loss modulus were performed with a Rheometer Physica MCR301 from *Anton Paar*. ESI **Mass Spectra** were recorded with a BioTOF III device from *Bruker*.

2 Experimental Procedures

2.1 Synthesis of Carboxylic Acid 2.



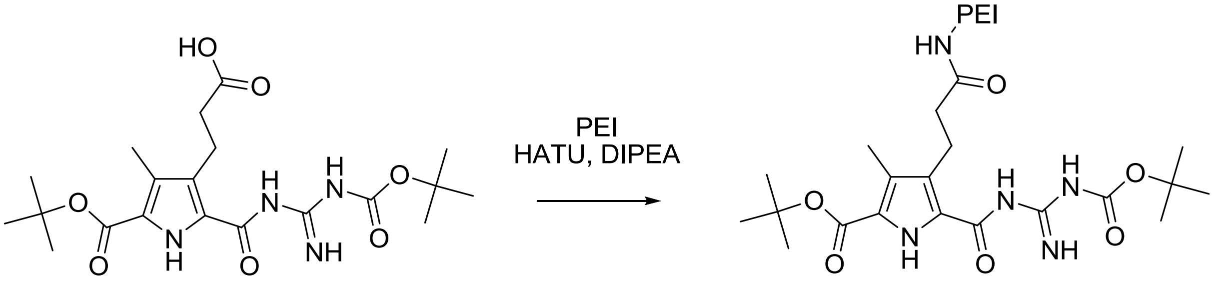
1 **2**

To a solution of **1**[1] (2.6 g, 5.7 mmol) in THF (50 mL) was added LiOH (275.0 mg, 11.4 mmol) in H_2O (15 mL). The reaction mixture was stirred over night and THF was removed *in vacuo*. H_2O (10 mL) and ethyl acetate (25 mL) were added successively. Hydrochlorid acid (5%) was added till the solution appeared to be transparent. The phases were separated and the aqueous phases were extracted with ethyl acetate (3 x 50 mL). The combined organic phases were dried with magnesium sulphate and the solvent was evaporated to give product **2** as a white solid (2.3 g, 5.2 mmol, 92%).

m.p. 124 °C $\mathbf{R_f}$ 0.20 (SiO_2, *n*-hexane/EtOAc 2:1 + 1% AcOH) **^{1}H-NMR** (300 MHz, DMSO-d_6) δ = 1.53 (s, 18 H, -C(C$\underline{H}_3$)$_3$), 2.17 (s, 3 H, Py-CH_3), 2.36 (t, 2 H, Py-C H_2-C$\underline{H}_2$-), 2.91 (t, 2 H, Py-C$\underline{H}_2$-CH_2-), 8.50 (br s, 1 H, NH), 9.39 (br s, 1 H, NH), 10.36 (br s, 1 H, NH), 11.41 (br s, 1 H, NH). **^{13}C-NMR** (75.5 MHz, DMSO-d_6) δ = 10.6 (Py-$\underline{C}H_3$), 20.1 (Py-$\underline{C}H_2$-CH_2-), 28.6

(-C($\underline{C}H_3$)$_3$), 29.9 (-C($\underline{C}H_3$)$_3$), 35.2 (Py-CH_2-$\underline{C}H_2$-), 76.2 (-$\underline{C}(CH_3)_3$), 80.9 (-$\underline{C}(CH_3)_3$), 122.3, 122.8, 125.4, 129.8 ($C_{Py,\ quart.}$), 160.5 (Py-$\underline{C}$OO*t*Bu), 162.9 (-NH-$\underline{C}$OO*t*Bu), 163.8 ($C(NH)_3$), 174.4 (COOH). **HR-MS** (ESI pos.): *m/z* = 439.217 ($M^{\bullet +}$), calc. for $C_{18}H_{19}NO_4^{\bullet +}$: 439.215.

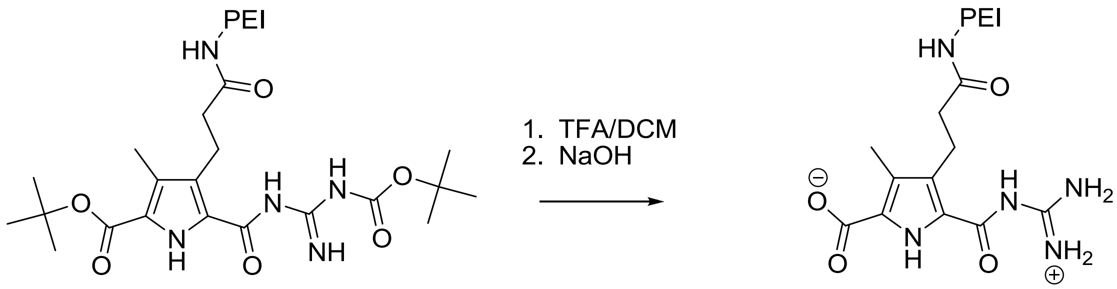
2.2 Coupling of Carboxylic Acid 2 to PEI (3)



2 **3**

2 (500 mg, 1.14 mmol), DIPEA (0.58 mL, 3.40 mmol) and HATU (870 mg, 2.28 mmol) were dissolved in a mixture of DCM/DMF (1:1, 30 ml) and stirred for 2 h at room temperature. A solution of PEI (114 mg, 4.56 µmol) and DIPEA (0.58 mL, 3.40 mmol) in the same solvent mixture was prepared and stirred for 2 h as well. The two solutions were combined, DMAP (cat.) was added and the resulting reaction mixture was stirred over night under reflux. New HATU was added (870 mg, 2.28 mmol) and the solution was stirred for another day under reflux. The solvent was evaporated, water was added (50 mL) and the unsoluble remainings were filtered off. The solution was washed with ethyl acetate (3 x 100 ml) and the water was removed in vacuum. After freeze drying **3** (110 mg) was obtained as a slightly yellow solid.

2.3 Preperation of the Zwitterion Functionalized Polymer 4



3 **4**

3 (110 mg) was dissolved in a 1:1 mixture of TFA/DCM (20 mL) and stirred at room temperature over night. The solvent was evaporated and the remaining solid was dissolved in water. Unsoluble remainings were filtered off. 0.1 M NaOH was added to adjust a *p*H of 7.2. The solution was freeze dried to give **4** as a yellow solid (78 mg).

2.4 **Purification of 4**

Purification of **4** was achieved by dialysis. As dialysis membrane a tubing from ZelluTrans V Series (MWCO = 5,000) supplied from *Roth* was used. In a first step it was treated with a mixture of H_2O/EtOH according to manufacturer instructions. Subsequently the tubing was loaded with approximately 10 mL of a solution of the raw product in H_2O/DMF (1:1). The dialysis itself was carried out in the same solvent mixture at room temperature in a 1 L Erlenmeyer flask as external container for the dialysis buffer. The solution was gently stirred with a magnetic stirrer for 3 weeks, during which the solvent was renewed every day. After dialysis the solvent was evaporated, the residue was freeze dried twice and a beige solid was obtained.

3 Images

When left out in the open air hydrogel **5** dried out after a few days. Addition of water to the dried substance regenerated the gel.

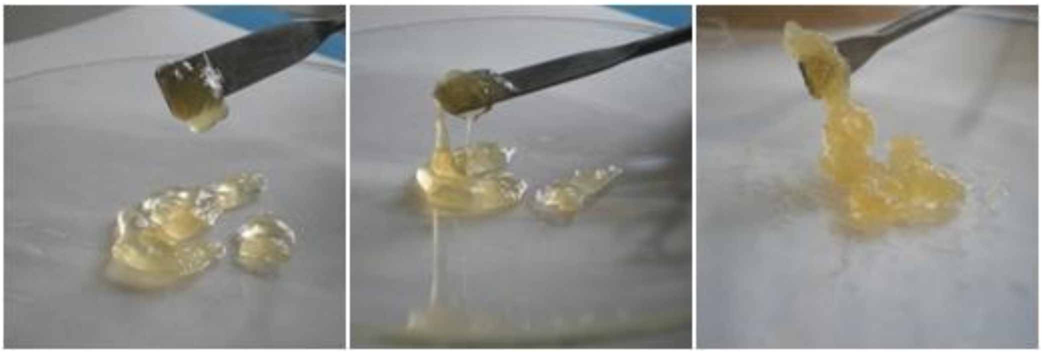


Figure 1 Freshly prepared hydrogel **5** (left and middle) and after 3 days (right).

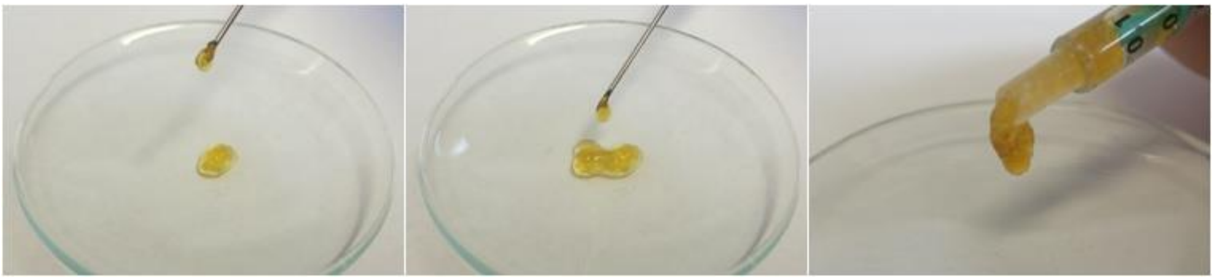


Figure 2 Behavior of the viscosity of hydrogel **5** upon mechanical stress.

4 Atomic Force Microscopy

Preparation conditions: Solution (2.0 mg/mL) of PEI in H_2O was spin-coated on a silica surface at 70 rps for 1 min. All images were recorded in Tapping Mode.

4.1 PEI

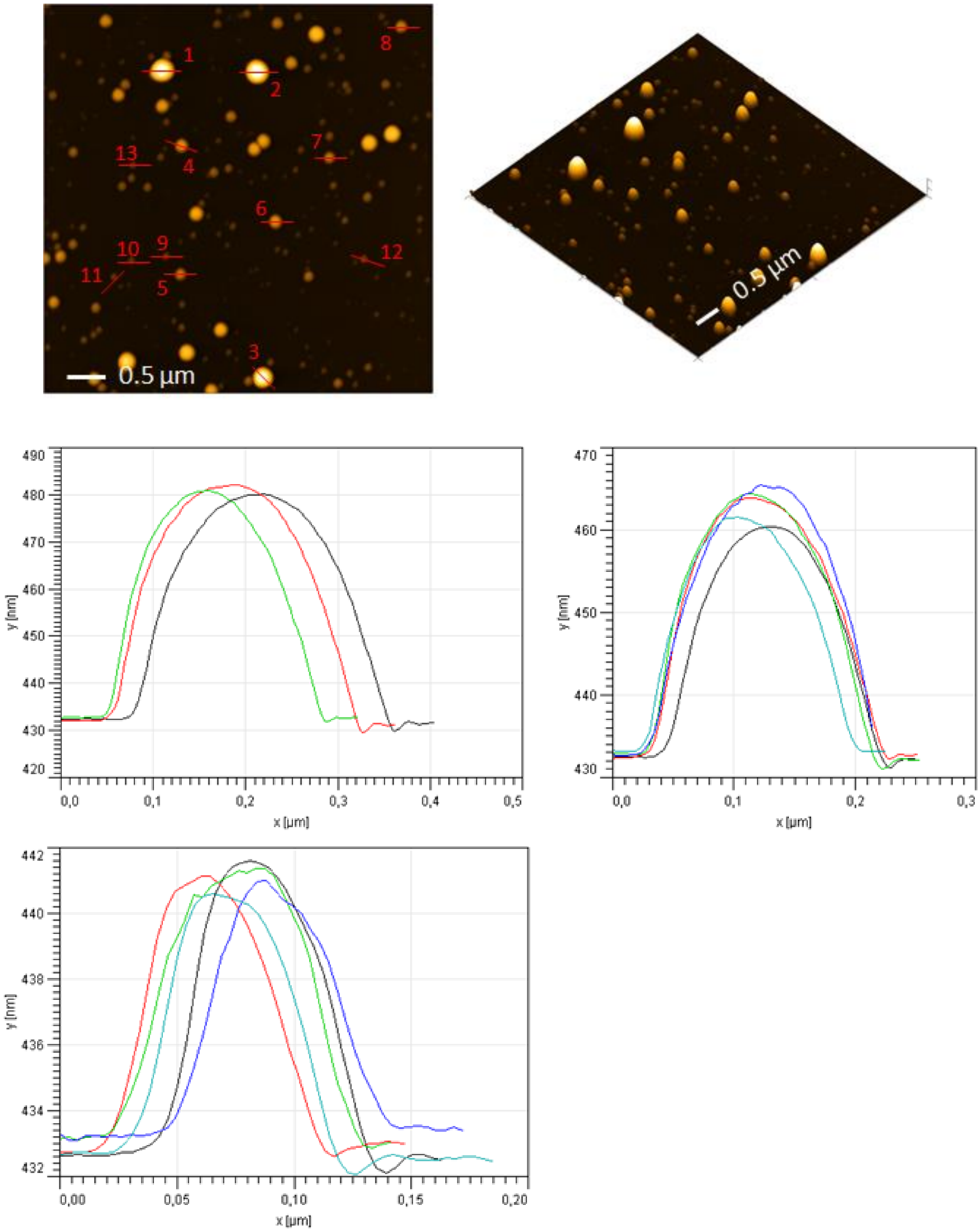


Figure 3 AFM height images of polyethylenimine (top) and corresponding height profiles.

4.2 Hydrogel 5 at neutral pH.

Preparation conditions: Solution (2.0 mg/mL) of hydrogel **5** in H_2O was spin-coated on a silica surface at 70 rps for 1 min. All images were recorded in Tapping Mode.

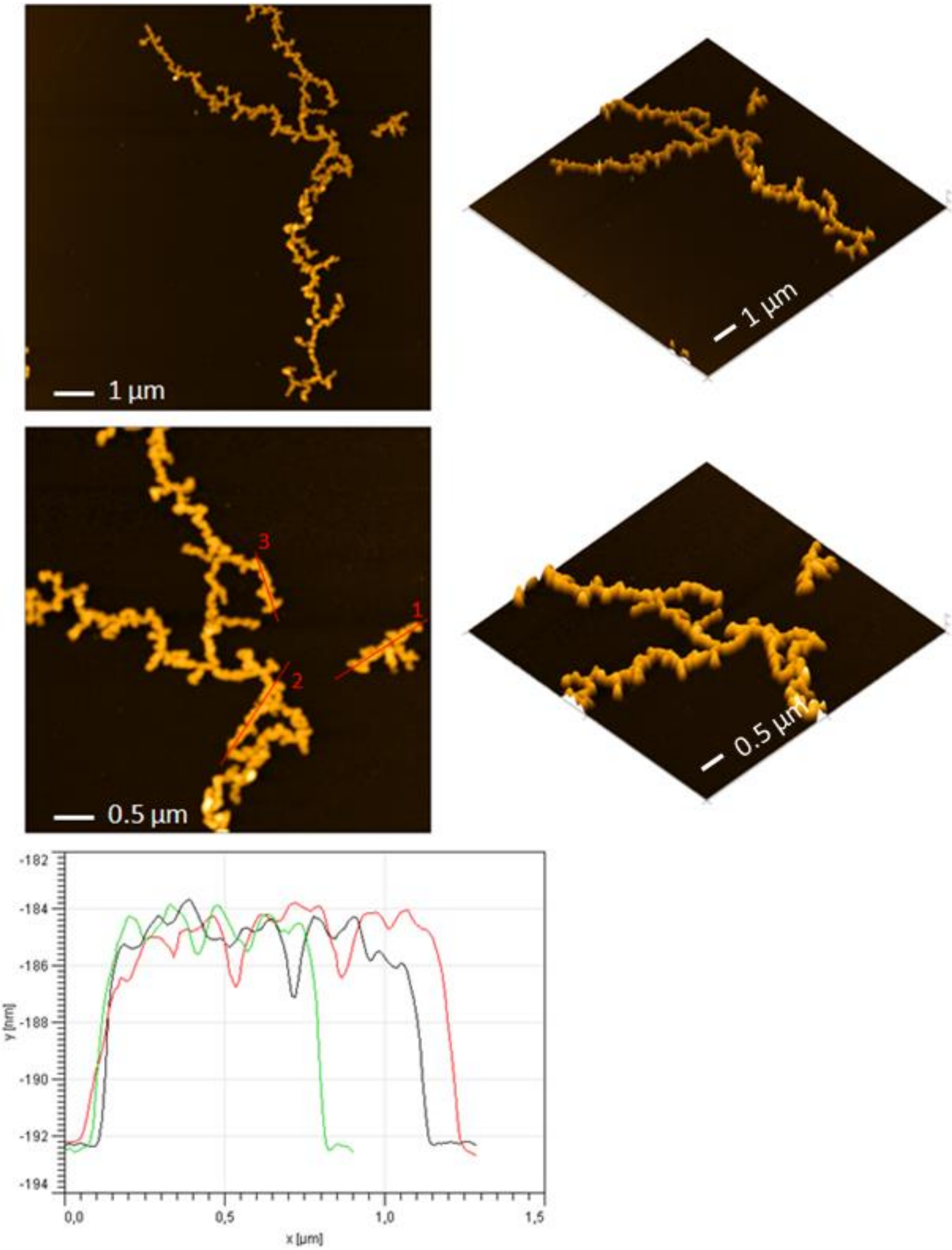


Figure 4 AFM height images of hydrogel **5** and corresponding height profiles.

5 Field Emission Scanning Electron Microscopy

Experimental Details: 20 µL (Sample **I**) and 40 µL (Sample **II**) of a solution of hydrogel **5** (1.7 mg/mL in DMSO/H_2O, 4:1) were drop-cast on a Si(111) surface of ~ 5 mm x 5 mm. Both samples were allowed to dry under ambient conditions for one week.

Observations: Sample **I** (20 µL) showed fractal structures, while sample **II** (40 µL) delivered one dimensional fibers, which appear to be entangled in a three dimensional network.

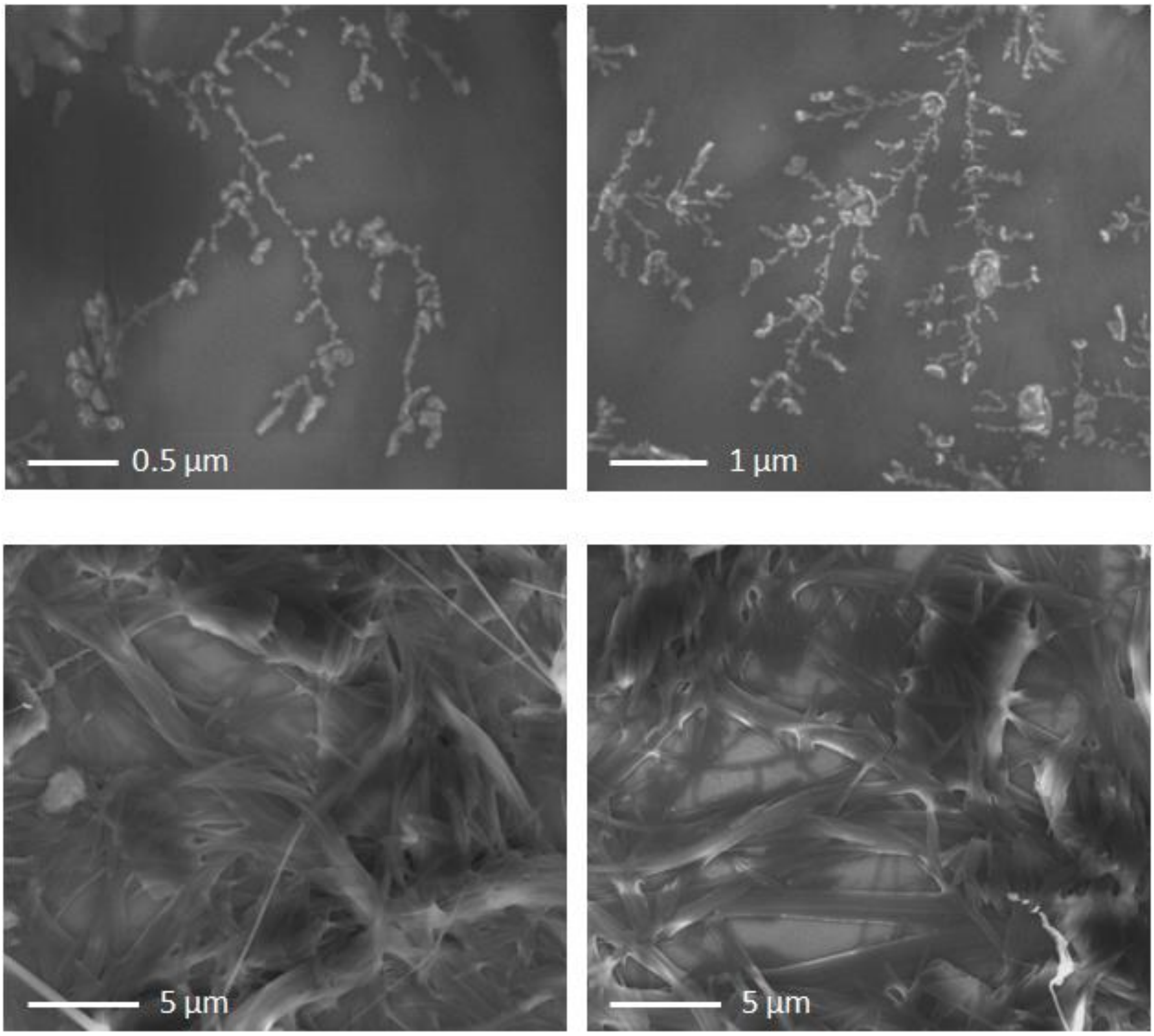


Figure 5 FESEM images of hydrogel **5**. Images obtained from drop-casting with 20 µL (top) and 40 µL (bottom) respectively.

6 Rheology

Experimental Details: Frequency sweep settings: f = 1-100 Hz; Amplitude gamma = 0.1% Strain; Meas. Pts.: 50; Duration: 50 sec; gap = 1 mm. Strain sweep settings: f = 20 Hz; Amplitude gamma = 50-200%; Meas. Pts.: 75; Duration: 5 sec; gap = 1 mm, c = 170 mg/mL.

Concentration dependent measurements of the relative viscosity were carried out in water at room temperature using a Micro-Ubbelohde capillary.

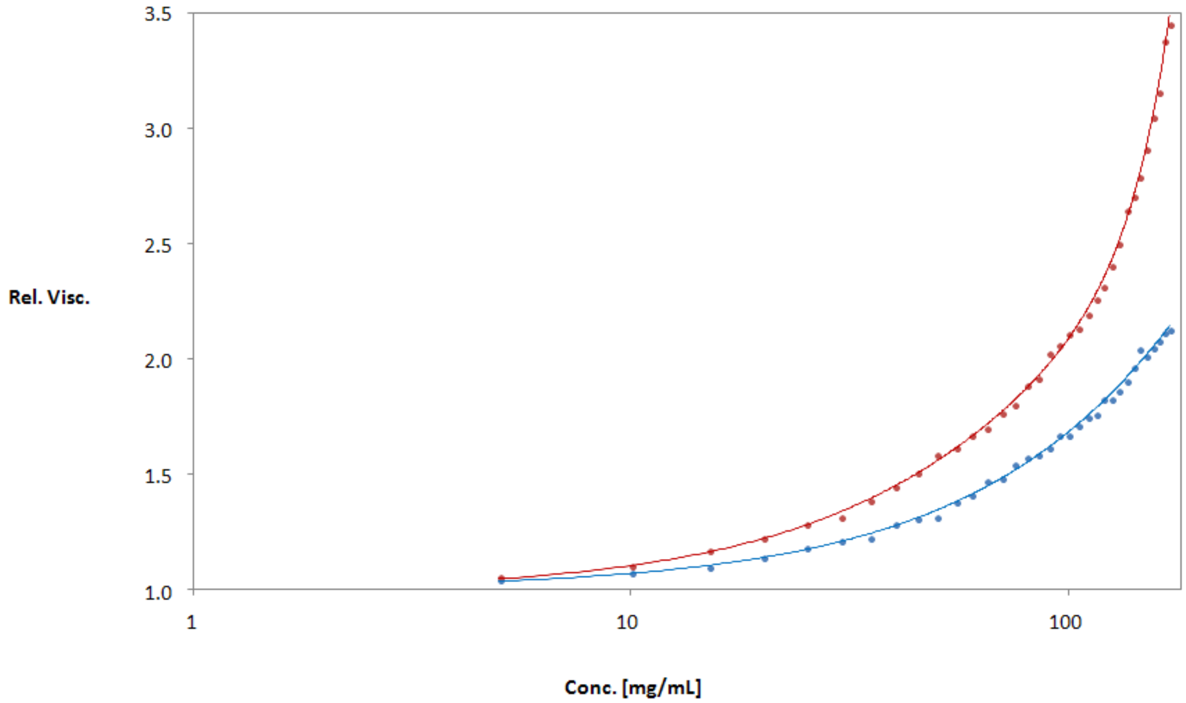


Figure 5 Relative viscosity of polyethylenimin (blue) hydrogel **5** (red) dependent on various concentrations.

c [mg/mL]	Rel. Visc.	
	PEI	Hydrogel
5,00	1,0422	1,0524
10,00	1,0698	1,1005
15,00	1,0992	1,1682
20,00	1,1408	1,2205
25,00	1,1785	1,2808
30,00	1,2091	1,3118
35,00	1,2255	1,3856
40,00	1,2803	1,4467
45,00	1,3099	1,506
50,00	1,3112	1,5806
55,00	1,3766	1,6119
60,00	1,4115	1,6693
65,00	1,4703	1,7005
70,00	1,4813	1,7635
75,00	1,5444	1,8001
80,00	1,5696	1,8841
85,00	1,5827	1,9155
90,00	1,6118	2,0233
95,00	1,6655	2,0608
100,00	1,6702	2,1055
105,00	1,7122	2,1322
110,00	1,7452	2,1939
115,00	1,761	2,2566
120,00	1,8234	2,311
125,00	1,8266	2,3997
130,00	1,8607	2,4998
135,00	1,9014	2,6438
140,00	1,9653	2,7011
145,00	2,0414	2,7884
150,00	2,0133	2,91
155,00	2,0453	3,0446
160,00	2,0807	3,1577
165,00	2,1116	3,3754
170,00	2,1259	3,45

Table 1 Relative viscosity of polyethylenimin (blue) hydrogel **5** (red) dependent on various concentrations.

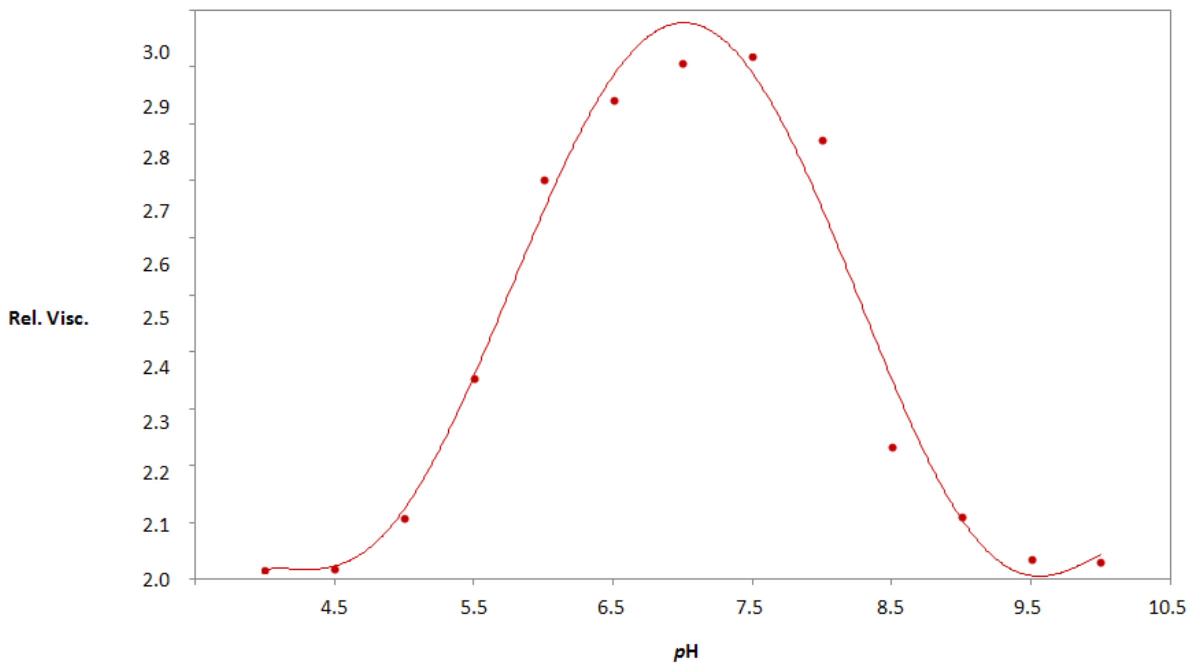


Figure 6 Relative viscosity of hydrogel **5** dependent on *p*H.

*p*H	Rel. Visc. (150 mg/mL)
4	2,0195
4,5	2,0203
5	2,1107
5,5	2,3567
6	2,7055
6,5	2,8443
7	2,9100
7,5	2,9206
8	2,7748
8,5	2,2353
9	2,1115
9,5	2,0386
10	2,0324

Table 2 Relative viscosity of hydrogel **5** dependent on *p*H.

6.1 Measurement of G’ and G’’ (frequency sweep) of polyethylenimin upon temperature changes:

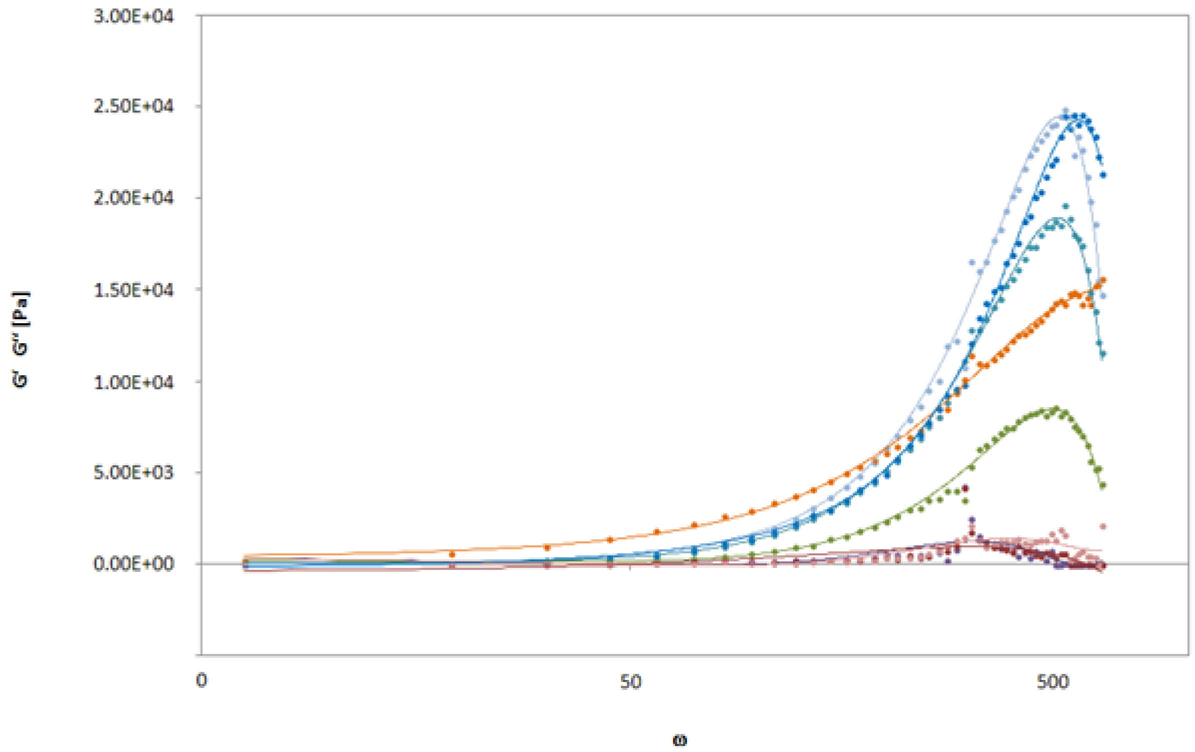


Figure 7 Measurement results for G’’ and G’’ of polyethylenimin (frequency sweep).

#	f [Hz]	35 °C		45 °C		55 °C		65 °C	
		Storage	Loss	Storage	Loss	Storage	Loss	Storage	Loss
1	6,28	1,93E+02	8,74E+00	1,09E+01	6,11E+00	4,57E+00	4,21E+00	1,02E+01	7,86E+00
2	19	5,78E+02	2,41E+01	7,05E+01	1,55E+01	1,01E+02	1,17E+01	7,95E+01	4,01E+01
3	31,7	9,58E+02	3,59E+01	1,69E+02	2,12E+01	2,36E+02	1,60E+01	1,95E+02	8,72E+01
4	44,4	1,37E+03	7,37E+01	3,05E+02	5,24E+01	3,64E+02	4,50E+01	3,71E+02	1,48E+02
5	57,1	1,79E+03	9,96E+01	5,03E+02	8,20E+01	5,76E+02	7,41E+01	6,09E+02	2,49E+02
6	69,8	2,21E+03	1,52E+02	7,38E+02	1,12E+02	8,35E+02	1,01E+02	8,98E+02	3,54E+02
7	82,5	2,62E+03	1,95E+02	1,01E+03	1,52E+02	1,12E+03	1,47E+02	1,24E+03	4,86E+02
8	95,1	2,94E+03	1,42E+02	1,31E+03	9,91E+01	1,44E+03	8,54E+01	1,63E+03	6,23E+02
9	108	3,35E+03	1,84E+02	1,66E+03	1,15E+02	1,79E+03	9,64E+01	2,05E+03	7,87E+02
10	121	3,74E+03	2,28E+02	2,07E+03	1,96E+02	2,22E+03	1,19E+02	2,52E+03	9,99E+02
11	133	4,12E+03	1,43E+02	2,50E+03	1,94E+02	2,71E+03	1,44E+02	3,15E+03	1,05E+03
12	146	4,56E+03	2,14E+02	2,96E+03	2,05E+02	3,06E+03	2,53E+02	3,68E+03	1,40E+03
13	159	4,97E+03	2,38E+02	3,41E+03	2,80E+02	3,52E+03	2,08E+02	4,31E+03	1,58E+03
14	171	5,36E+03	3,12E+02	4,02E+03	2,18E+02	4,11E+03	3,22E+02	4,90E+03	1,85E+03
15	184	5,70E+03	3,96E+02	4,53E+03	2,96E+02	4,67E+03	2,72E+02	5,59E+03	2,08E+03
16	197	6,03E+03	5,91E+02	5,15E+03	3,76E+02	4,96E+03	3,87E+02	6,42E+03	2,40E+03
17	209	6,45E+03	5,11E+02	5,72E+03	2,67E+02	5,84E+03	3,97E+02	7,04E+03	2,67E+03
18	222	6,91E+03	4,37E+02	6,34E+03	4,90E+02	6,53E+03	4,26E+02	7,96E+03	3,01E+03
19	235	7,34E+03	5,21E+02	6,93E+03	3,66E+02	7,13E+03	4,72E+02	8,69E+03	3,15E+03
20	247	7,71E+03	5,32E+02	7,57E+03	4,51E+02	7,79E+03	6,40E+02	9,57E+03	3,55E+03
21	260	8,08E+03	7,01E+02	8,09E+03	7,91E+02	8,54E+03	7,82E+02	1,01E+04	3,61E+03
22	273	8,52E+03	2,26E+02	8,91E+03	7,26E+02	9,25E+03	1,09E+03	1,20E+04	4,06E+03
23	286	9,33E+03	8,50E+02	9,55E+03	9,88E+02	9,61E+03	1,38E+03	1,23E+04	4,06E+03
24	298	1,01E+04	4,27E+03	1,12E+04	4,16E+03	9,85E+03	1,53E+03	1,08E+04	3,54E+03
25	311	1,14E+04	2,51E+03	1,29E+04	1,75E+03	1,21E+04	2,16E+03	1,66E+04	5,36E+03
26	324	1,10E+04	1,53E+03	1,29E+04	1,29E+03	1,35E+04	1,33E+03	1,61E+04	6,34E+03
27	336	1,09E+04	1,23E+03	1,35E+04	1,24E+03	1,43E+04	1,01E+03	1,66E+04	6,53E+03
28	349	1,12E+04	9,80E+02	1,41E+04	9,51E+02	1,50E+04	1,19E+03	1,78E+04	6,90E+03
29	362	1,15E+04	9,32E+02	1,46E+04	9,00E+02	1,52E+04	1,28E+03	1,84E+04	7,25E+03
30	374	1,18E+04	9,25E+02	1,53E+04	7,64E+02	1,65E+04	1,30E+03	1,94E+04	7,53E+03
31	387	1,22E+04	7,34E+02	1,57E+04	6,31E+02	1,70E+04	1,28E+03	2,02E+04	7,55E+03
32	400	1,25E+04	4,83E+02	1,62E+04	9,88E+02	1,76E+04	1,46E+03	2,06E+04	7,85E+03
33	413	1,26E+04	7,11E+02	1,68E+04	7,03E+02	1,88E+04	1,19E+03	2,17E+04	8,07E+03
34	425	1,28E+04	3,86E+02	1,74E+04	7,12E+02	1,91E+04	1,15E+03	2,24E+04	8,28E+03
35	438	1,31E+04	7,55E+02	1,74E+04	5,28E+02	2,01E+04	1,29E+03	2,28E+04	8,29E+03
36	451	1,33E+04	6,18E+02	1,81E+04	4,51E+02	2,04E+04	1,38E+03	2,32E+04	8,48E+03
37	463	1,37E+04	2,35E+02	1,85E+04	6,30E+02	2,12E+04	1,32E+03	2,36E+04	8,17E+03
38	476	1,40E+04	7,19E+02	1,85E+04	5,28E+02	2,19E+04	1,74E+03	2,40E+04	8,43E+03
39	489	1,43E+04	0	1,88E+04	3,14E+02	2,22E+04	1,33E+03	2,41E+04	8,62E+03
40	501	1,44E+04	0	1,86E+04	5,93E+02	2,34E+04	1,94E+03	2,45E+04	8,19E+03
41	514	1,42E+04	1,08E+02	1,97E+04	5,90E+02	2,45E+04	1,63E+03	2,49E+04	8,40E+03
42	527	1,48E+04	0	1,90E+04	0	2,39E+04	2,51E+02	2,45E+04	8,02E+03
43	539	1,49E+04	0	1,81E+04	0	2,46E+04	3,80E+02	2,24E+04	7,59E+03
44	552	1,47E+04	0	1,79E+04	0	2,41E+04	6,20E+02	2,34E+04	7,36E+03
45	565	1,42E+04	0	1,75E+04	0	2,46E+04	7,91E+02	2,27E+04	7,10E+03
46	578	1,46E+04	0	1,62E+04	0	2,43E+04	4,96E+02	2,12E+04	6,59E+03
47	590	1,42E+04	0	1,49E+04	0	2,39E+04	5,06E+02	1,99E+04	5,68E+03
48	603	1,52E+04	0	1,39E+04	0	2,34E+04	3,75E+02	1,87E+04	5,23E+03
49	616	1,53E+04	0	1,22E+04	0	2,23E+04	0	1,56E+04	5,35E+03
50	628	1,56E+04	0	1,16E+04	0	2,14E+04	2,15E+03	1,48E+04	4,41E+03

Table 3 Measurement results for G’ and G’’ of polyethylenimin upon various temperatures (frequency sweep).

6.2 G’ and G’’ (frequency sweep) of hydrogel 5 upon temperature changes:

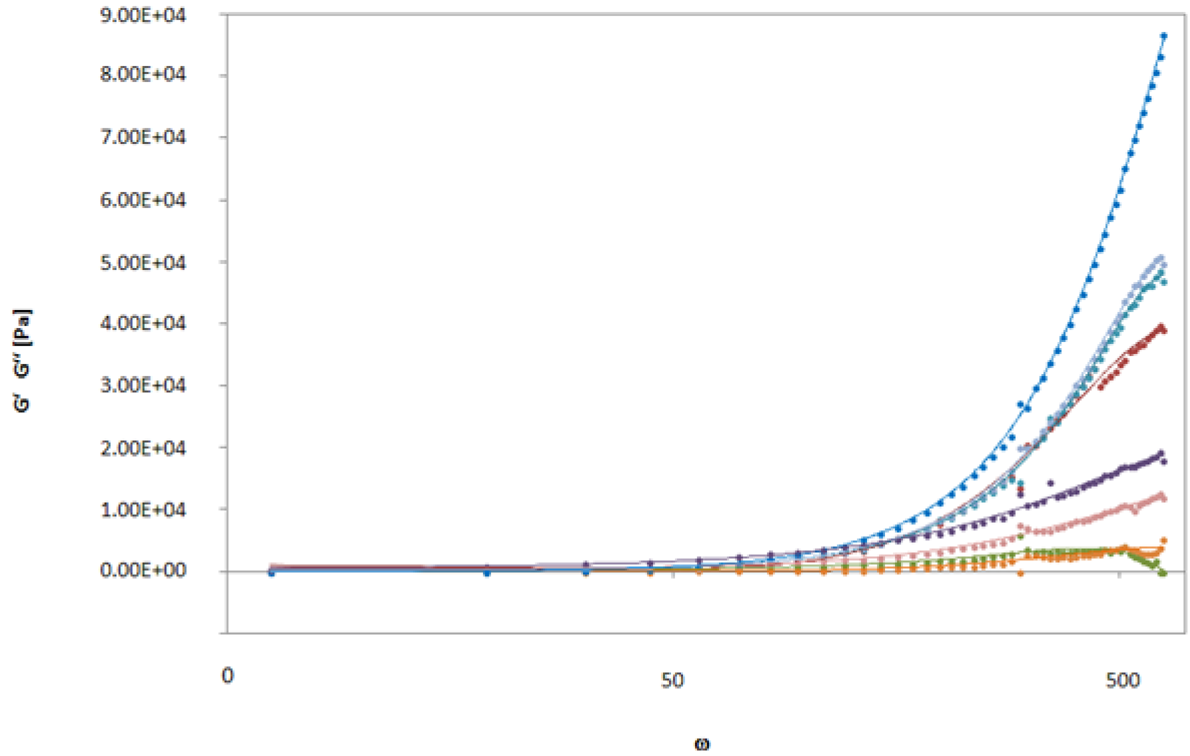


Figure 7 Measurement results for G’’ and G’’ of hydrogel **5** (frequency sweep).

#	f [Hz]	35 °C		45 °C		55 °C		65 °C	
		Storage	Loss	Storage	Loss	Storage	Loss	Storage	Loss
1	6,28	0	6,29E+01	1,63E+01	1,57E+01	1,32E+01	1,25E+02	1,57E+01	2,73E+02
2	19	1,21E+02	1,72E+02	9,54E+01	4,88E+01	9,97E+01	3,37E+02	1,26E+02	7,07E+02
3	31,7	2,89E+02	2,76E+02	2,51E+02	7,69E+01	2,49E+02	5,43E+02	3,24E+02	1,14E+03
4	44,4	5,18E+02	4,00E+02	4,63E+02	1,31E+02	4,64E+02	7,66E+02	6,13E+02	1,57E+03
5	57,1	8,26E+02	5,19E+02	7,54E+02	1,86E+02	7,59E+02	9,81E+02	1,01E+03	1,99E+03
6	69,8	1,20E+03	6,45E+02	1,10E+03	2,42E+02	1,12E+03	1,20E+03	1,50E+03	2,41E+03
7	82,5	1,63E+03	7,61E+02	1,52E+03	3,12E+02	1,55E+03	1,42E+03	2,07E+03	2,82E+03
8	95,1	2,11E+03	8,33E+02	1,97E+03	2,90E+02	2,00E+03	1,54E+03	2,73E+03	3,15E+03
9	108	2,66E+03	9,20E+02	2,51E+03	3,23E+02	2,56E+03	1,76E+03	3,48E+03	3,54E+03
10	121	3,28E+03	9,92E+02	3,10E+03	3,64E+02	3,25E+03	1,97E+03	4,24E+03	3,91E+03
11	133	3,41E+03	1,35E+03	3,70E+03	2,99E+02	3,90E+03	2,16E+03	5,34E+03	4,31E+03
12	146	4,60E+03	1,11E+03	4,52E+03	5,72E+02	4,56E+03	2,30E+03	6,25E+03	4,77E+03
13	159	5,44E+03	1,32E+03	5,29E+03	5,57E+02	5,46E+03	2,57E+03	7,27E+03	5,11E+03
14	171	6,31E+03	1,43E+03	6,06E+03	6,85E+02	6,22E+03	2,85E+03	8,53E+03	5,49E+03
15	184	7,19E+03	1,58E+03	6,97E+03	7,56E+02	7,19E+03	3,06E+03	9,80E+03	5,84E+03
16	197	7,89E+03	1,78E+03	8,44E+03	1,09E+03	8,34E+03	3,16E+03	1,14E+04	6,21E+03
17	209	9,18E+03	1,80E+03	8,86E+03	9,20E+02	9,10E+03	3,59E+03	1,27E+04	6,62E+03
18	222	9,98E+03	1,82E+03	9,89E+03	9,90E+02	1,02E+04	3,83E+03	1,40E+04	7,17E+03
19	235	1,11E+04	2,00E+03	1,08E+04	1,09E+03	1,13E+04	4,03E+03	1,58E+04	7,54E+03

20	247	1,23E+04	2,19E+03	1,20E+04	1,23E+03	1,25E+04	4,30E+03	1,73E+04	7,95E+03
21	260	1,35E+04	2,44E+03	1,30E+04	1,41E+03	1,35E+04	4,70E+03	1,89E+04	8,60E+03
22	273	1,49E+04	2,74E+03	1,40E+04	1,43E+03	1,48E+04	4,94E+03	2,04E+04	8,62E+03
23	286	1,54E+04	3,02E+03	1,50E+04	1,85E+03	1,63E+04	5,48E+03	2,21E+04	9,46E+03
24	298	1,36E+04	5,95E+03	1,45E+04	0	2,01E+04	7,63E+03	2,73E+04	1,25E+04
25	311	2,05E+04	3,57E+03	2,01E+04	2,94E+03	2,00E+04	7,18E+03	2,68E+04	1,07E+04
26	324	2,05E+04	3,09E+03	2,08E+04	2,93E+03	2,13E+04	6,71E+03	2,99E+04	1,09E+04
27	336	2,20E+04	3,40E+03	2,18E+04	2,60E+03	2,28E+04	6,71E+03	3,16E+04	1,14E+04
28	349	2,34E+04	3,20E+03	2,49E+04	2,42E+03	2,42E+04	6,77E+03	3,38E+04	1,45E+04
29	362	2,48E+04	3,32E+03	2,44E+04	2,47E+03	2,56E+04	7,14E+03	3,60E+04	1,21E+04
30	374	2,57E+04	3,23E+03	2,59E+04	2,51E+03	2,71E+04	7,40E+03	3,80E+04	1,24E+04
31	387	2,72E+04	3,27E+03	2,74E+04	2,38E+03	2,85E+04	7,73E+03	4,02E+04	1,28E+04
32	400	2,87E+04	3,51E+03	2,89E+04	2,68E+03	3,02E+04	8,32E+03	4,26E+04	1,31E+04
33	413	3,01E+04	3,46E+03	3,01E+04	2,89E+03	3,13E+04	8,24E+03	4,51E+04	1,37E+04
34	425	3,16E+04	3,32E+03	3,14E+04	2,94E+03	3,28E+04	8,45E+03	4,75E+04	1,42E+04
35	438	3,29E+04	3,46E+03	3,29E+04	3,04E+03	3,45E+04	8,96E+03	4,98E+04	1,45E+04
36	451	3,00E+04	3,63E+03	3,44E+04	3,06E+03	3,59E+04	9,13E+03	5,24E+04	1,49E+04
37	463	3,09E+04	3,62E+03	3,61E+04	3,85E+03	3,69E+04	9,72E+03	5,47E+04	1,55E+04
38	476	3,16E+04	3,23E+03	3,75E+04	3,72E+03	3,88E+04	1,00E+04	5,74E+04	1,56E+04
39	489	3,23E+04	3,75E+03	3,87E+04	3,82E+03	4,05E+04	1,02E+04	5,95E+04	1,61E+04
40	501	3,35E+04	3,42E+03	3,96E+04	3,93E+03	4,14E+04	1,06E+04	6,20E+04	1,67E+04
41	514	3,43E+04	3,99E+03	4,17E+04	4,23E+03	4,37E+04	1,09E+04	6,54E+04	1,71E+04
42	527	3,56E+04	2,94E+03	4,28E+04	3,71E+03	4,48E+04	1,06E+04	6,78E+04	1,69E+04
43	539	3,59E+04	2,44E+03	4,32E+04	3,77E+03	4,63E+04	1,00E+04	7,01E+04	1,69E+04
44	552	3,66E+04	2,33E+03	4,45E+04	3,34E+03	4,65E+04	1,08E+04	7,24E+04	1,75E+04
45	565	3,69E+04	1,87E+03	4,58E+04	3,05E+03	4,80E+04	1,14E+04	7,45E+04	1,76E+04
46	578	3,77E+04	1,64E+03	4,63E+04	3,11E+03	4,88E+04	1,15E+04	7,68E+04	1,80E+04
47	590	3,83E+04	1,11E+03	4,63E+04	3,06E+03	4,96E+04	1,20E+04	7,89E+04	1,84E+04
48	603	3,91E+04	1,69E+03	4,76E+04	3,23E+03	5,05E+04	1,22E+04	8,09E+04	1,86E+04
49	616	3,98E+04	2,78E+01	4,86E+04	4,00E+03	5,09E+04	1,27E+04	8,35E+04	1,93E+04
50	628	3,92E+04	0	4,69E+04	5,38E+03	4,98E+04	1,21E+04	8,69E+04	1,80E+04

Table 4 Measurement results for G’ and G’’ of hydrogel **5** upon various temperatures (frequency sweep).

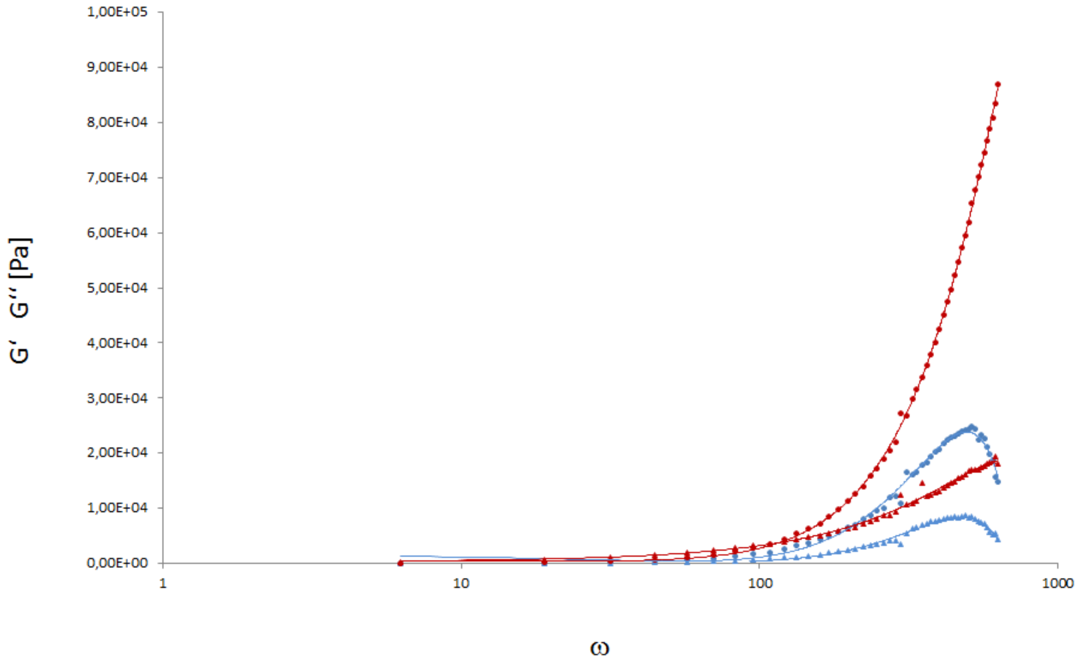


Figure 8 Comparison of storage and loss moduli between PEI and hydrogel 5 at 65 °C.

- ● G’ (hydrogel **5**)
- ▲ G‘‘ (hydrogel **5**)
- ● G’ (PEI)
- ▲ G‘‘ (PEI)

6.3 G' and G'' (strain sweep) of hydrogel 5 upon temperature changes:

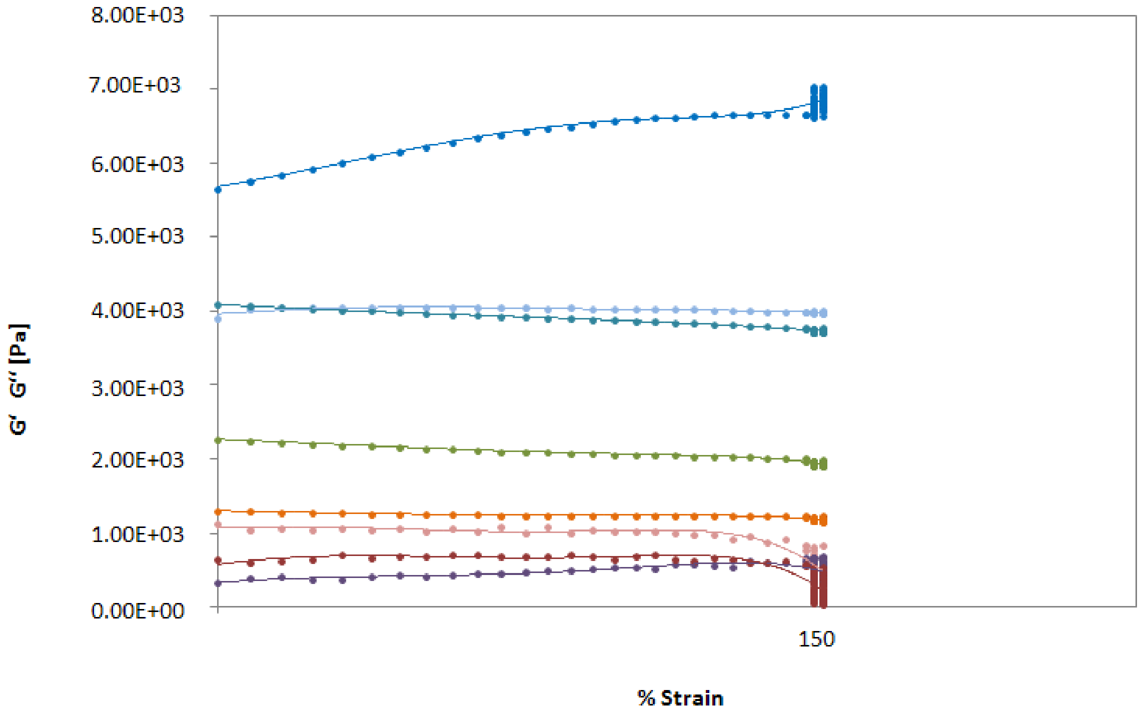


Figure 9 G'' and G'' of hydrogel **5** (strain sweep).

#	Strain [%]	35 °C		45 °C		55 °C		65 °C	
		Storage	Loss	Storage	Loss	Storage	Loss	Storage	Loss
1	5,00E+01	2,27E+03	0	4,09E+03	6,37E+02	3,91E+03	1,13E+03	5,66E+03	1,30E+03
2	5,20E+01	2,25E+03	0	4,06E+03	6,03E+02	4,03E+03	1,05E+03	5,75E+03	1,29E+03
3	5,40E+01	2,23E+03	0	4,04E+03	6,13E+02	4,04E+03	1,06E+03	5,85E+03	1,28E+03
4	5,60E+01	2,21E+03	0	4,03E+03	6,53E+02	4,04E+03	1,05E+03	5,93E+03	1,28E+03
5	5,80E+01	2,19E+03	0	4,01E+03	6,97E+02	4,04E+03	1,06E+03	6,02E+03	1,27E+03
6	6,01E+01	2,18E+03	0	4,00E+03	6,66E+02	4,05E+03	1,04E+03	6,09E+03	1,26E+03
7	6,21E+01	2,16E+03	1,16E+01	3,98E+03	6,75E+02	4,04E+03	1,07E+03	6,16E+03	1,26E+03
8	6,41E+01	2,15E+03	4,42E+01	3,97E+03	6,87E+02	4,04E+03	1,03E+03	6,23E+03	1,25E+03
9	6,62E+01	2,14E+03	5,48E+01	3,95E+03	6,97E+02	4,04E+03	1,07E+03	6,28E+03	1,25E+03
10	6,82E+01	2,13E+03	7,39E+01	3,94E+03	6,97E+02	4,04E+03	1,02E+03	6,34E+03	1,25E+03
11	7,02E+01	2,11E+03	9,84E+01	3,93E+03	6,86E+02	4,04E+03	1,08E+03	6,38E+03	1,24E+03
12	7,22E+01	2,10E+03	1,16E+02	3,92E+03	6,78E+02	4,04E+03	1,01E+03	6,43E+03	1,24E+03
13	7,42E+01	2,10E+03	1,26E+02	3,91E+03	6,94E+02	4,03E+03	1,08E+03	6,47E+03	1,24E+03
14	7,63E+01	2,09E+03	1,51E+02	3,90E+03	7,06E+02	4,04E+03	1,01E+03	6,50E+03	1,24E+03
15	7,83E+01	2,08E+03	1,69E+02	3,88E+03	6,76E+02	4,03E+03	1,05E+03	6,54E+03	1,24E+03
16	8,03E+01	2,07E+03	1,75E+02	3,87E+03	6,52E+02	4,03E+03	1,02E+03	6,57E+03	1,24E+03
17	8,24E+01	2,06E+03	1,94E+02	3,86E+03	6,89E+02	4,03E+03	1,03E+03	6,59E+03	1,24E+03
18	8,44E+01	2,06E+03	2,22E+02	3,85E+03	6,98E+02	4,02E+03	1,03E+03	6,61E+03	1,24E+03
19	8,64E+01	2,05E+03	2,20E+02	3,84E+03	6,42E+02	4,02E+03	1,01E+03	6,63E+03	1,24E+03
20	8,84E+01	2,04E+03	2,29E+02	3,83E+03	6,13E+02	4,02E+03	9,80E+02	6,64E+03	1,24E+03
21	9,05E+01	2,04E+03	2,48E+02	3,82E+03	6,70E+02	4,01E+03	9,86E+02	6,66E+03	1,24E+03
22	9,25E+01	2,03E+03	2,70E+02	3,81E+03	6,40E+02	4,00E+03	9,30E+02	6,67E+03	1,24E+03
23	9,45E+01	2,03E+03	2,58E+02	3,80E+03	5,94E+02	4,00E+03	9,54E+02	6,67E+03	1,23E+03
24	9,65E+01	2,02E+03	2,72E+02	3,79E+03	6,06E+02	3,99E+03	8,72E+02	6,67E+03	1,23E+03
25	9,86E+01	2,01E+03	2,88E+02	3,78E+03	6,32E+02	3,99E+03	9,12E+02	6,66E+03	1,23E+03

26	1,01E+02	2,01E+03	3,03E+02	3,78E+03	5,83E+02	3,99E+03	8,30E+02	6,66E+03	1,23E+03
27	1,03E+02	2,00E+03	2,96E+02	3,77E+03	5,47E+02	3,96E+03	8,37E+02	6,64E+03	1,23E+03
28	1,02E+02	1,96E+03	2,98E+02	3,73E+03	5,47E+02	3,98E+03	8,14E+02	6,61E+03	1,18E+03
29	1,01E+02	1,98E+03	2,97E+02	3,75E+03	5,50E+02	3,98E+03	7,75E+02	6,66E+03	1,21E+03
30	1,02E+02	1,98E+03	2,91E+02	3,75E+03	5,36E+02	3,98E+03	7,63E+02	6,67E+03	1,21E+03
31	1,02E+02	1,98E+03	2,82E+02	3,75E+03	5,18E+02	3,98E+03	7,14E+02	6,68E+03	1,21E+03
32	1,02E+02	1,98E+03	2,74E+02	3,75E+03	5,02E+02	3,98E+03	7,12E+02	6,68E+03	1,21E+03
33	1,02E+02	1,97E+03	2,67E+02	3,74E+03	4,90E+02	3,98E+03	6,68E+02	6,69E+03	1,20E+03
34	1,03E+02	1,97E+03	2,59E+02	3,74E+03	4,73E+02	3,98E+03	6,57E+02	6,70E+03	1,21E+03
35	1,02E+02	1,96E+03	2,53E+02	3,73E+03	4,71E+02	3,96E+03	6,11E+02	6,71E+03	1,19E+03
36	1,02E+02	1,97E+03	2,39E+02	3,74E+03	4,49E+02	3,99E+03	5,98E+02	6,73E+03	1,20E+03
37	1,03E+02	1,97E+03	2,31E+02	3,74E+03	4,27E+02	3,97E+03	5,55E+02	6,74E+03	1,20E+03
38	1,02E+02	1,95E+03	2,22E+02	3,73E+03	4,31E+02	3,99E+03	5,35E+02	6,74E+03	1,18E+03
39	1,02E+02	1,96E+03	2,07E+02	3,74E+03	4,07E+02	3,99E+03	5,07E+02	6,76E+03	1,20E+03
40	1,03E+02	1,94E+03	1,98E+02	3,73E+03	3,84E+02	3,99E+03	4,86E+02	6,75E+03	1,19E+03
41	1,02E+02	1,95E+03	1,87E+02	3,73E+03	3,87E+02	3,99E+03	4,60E+02	6,76E+03	1,19E+03
42	1,03E+02	1,96E+03	1,76E+02	3,74E+03	3,61E+02	3,99E+03	4,41E+02	6,78E+03	1,20E+03
43	1,02E+02	1,94E+03	1,67E+02	3,73E+03	3,47E+02	3,99E+03	4,18E+02	6,77E+03	1,18E+03
44	1,03E+02	1,95E+03	1,52E+02	3,72E+03	3,27E+02	3,99E+03	3,99E+02	6,79E+03	1,20E+03
45	1,02E+02	1,94E+03	1,44E+02	3,73E+03	3,24E+02	3,99E+03	3,79E+02	6,79E+03	1,18E+03
46	1,03E+02	1,95E+03	1,33E+02	3,74E+03	2,99E+02	3,99E+03	3,61E+02	6,81E+03	1,20E+03
47	1,02E+02	1,93E+03	1,25E+02	3,72E+03	3,02E+02	3,99E+03	3,43E+02	6,80E+03	1,18E+03
48	1,03E+02	1,95E+03	1,12E+02	3,74E+03	2,77E+02	3,99E+03	3,27E+02	6,82E+03	1,20E+03
49	1,02E+02	1,93E+03	1,04E+02	3,72E+03	2,73E+02	4,00E+03	3,11E+02	6,81E+03	1,17E+03
50	1,03E+02	1,95E+03	9,34E+01	3,74E+03	2,50E+02	4,00E+03	2,96E+02	6,83E+03	1,20E+03
51	1,02E+02	1,93E+03	8,62E+01	3,72E+03	2,51E+02	0	0	6,82E+03	1,17E+03
52	1,03E+02	1,95E+03	7,43E+01	3,74E+03	2,27E+02	0	0	6,84E+03	1,20E+03
53	1,02E+02	1,93E+03	6,71E+01	3,72E+03	2,28E+02	0	0	6,83E+03	1,17E+03
54	1,03E+02	1,95E+03	5,71E+01	3,74E+03	2,04E+02	0	0	6,85E+03	1,20E+03
55	1,02E+02	1,93E+03	5,09E+01	3,72E+03	2,06E+02	0	0	6,84E+03	1,17E+03
56	1,03E+02	1,95E+03	4,01E+01	3,74E+03	1,83E+02	0	0	6,86E+03	1,20E+03
57	1,02E+02	1,92E+03	3,37E+01	3,72E+03	1,86E+02	0	0	6,85E+03	1,17E+03
58	1,03E+02	1,95E+03	2,48E+01	3,74E+03	1,62E+02	0	0	6,86E+03	1,19E+03
59	1,02E+02	1,92E+03	1,92E+01	3,72E+03	1,67E+02	0	0	6,86E+03	1,17E+03
60	1,03E+02	1,94E+03	1,02E+01	3,74E+03	1,43E+02	0	0	6,89E+03	1,19E+03
61	1,02E+02	1,93E+03	4,54E+00	3,73E+03	1,50E+02	0	0	6,89E+03	1,17E+03
62	1,03E+02	1,94E+03	0	3,74E+03	1,25E+02	0	0	6,91E+03	1,19E+03
63	1,02E+02	1,93E+03	0	3,73E+03	1,33E+02	0	0	6,91E+03	1,18E+03
64	1,03E+02	1,93E+03	0	3,74E+03	1,07E+02	0	0	6,91E+03	1,18E+03
65	1,02E+02	1,93E+03	0	3,74E+03	1,16E+02	0	0	6,90E+03	1,18E+03
66	1,03E+02	1,92E+03	0	3,73E+03	9,17E+01	0	0	6,93E+03	1,17E+03
67	1,02E+02	1,93E+03	0	3,74E+03	9,99E+01	0	0	6,97E+03	1,18E+03
68	1,03E+02	1,91E+03	0	3,72E+03	7,87E+01	0	0	6,96E+03	1,16E+03
69	1,02E+02	1,94E+03	0	3,75E+03	8,17E+01	0	0	7,00E+03	1,19E+03
70	1,02E+02	1,92E+03	0	3,72E+03	7,00E+01	0	0	6,98E+03	1,17E+03
71	1,03E+02	1,92E+03	0	3,73E+03	5,79E+01	0	0	7,00E+03	1,17E+03
72	1,02E+02	1,94E+03	0	3,74E+03	6,53E+01	0	0	7,02E+03	1,19E+03
73	1,03E+02	1,91E+03	0	3,72E+03	4,86E+01	0	0	7,00E+03	1,16E+03
74	1,03E+02	1,93E+03	0	3,75E+03	4,01E+01	0	0	7,04E+03	1,18E+03
75	1,02E+02	1,94E+03	0	3,74E+03	4,87E+01	0	0	7,03E+03	1,19E+03

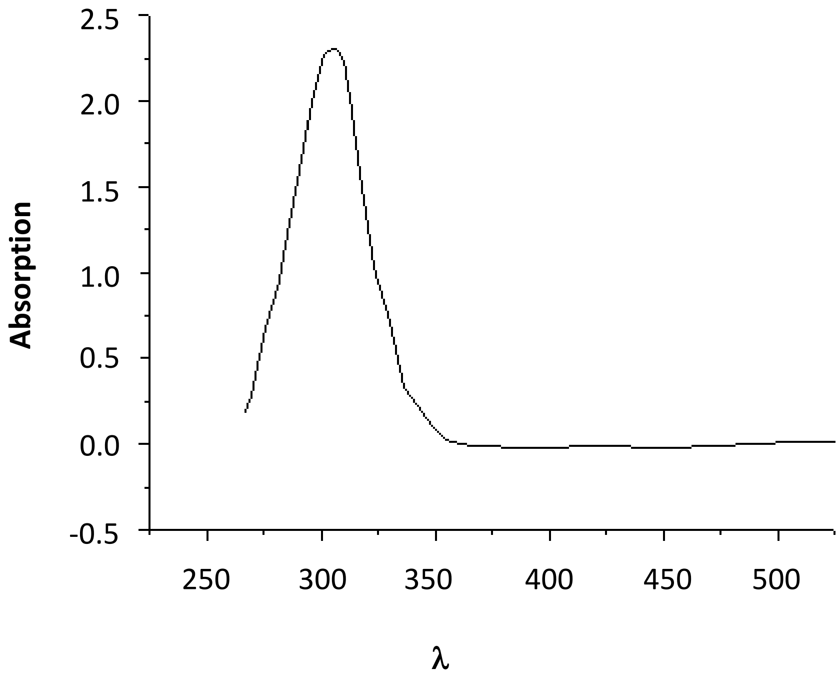
Table 5 G' and G'' of hydrogel **5** for various temperatures.

6.4 GPC

GPC Measurements of polyethylenimine and hydrogel **5** were carried out in THF as eluent at 20 °C. The injection volume was 40 µL for each sample and the concentrations were adjusted to 1 g/L. The GPC device consisted of a pump (Waters 590), a detector (Shodex RI-71) and a column (MZ SD plus), which was designed for a molar mass range of 1000-70000 g/mol. The flow was 1 mL/min. For the evaluation the software PSS WinGPC 6.2 was used.

6.5 UV/Vis Spectroscopy

The loading of the polyethylenimin with GCP was determined by concentration dependent UV measurements of the protected version of GCP. Measurements were carried out at 20 °C in standard quartz microcuvettes of 1 cm width. Stock solutions were prepared at neutral pH in a concentration of $3*10^{-5}$ mol/L in DMSO.



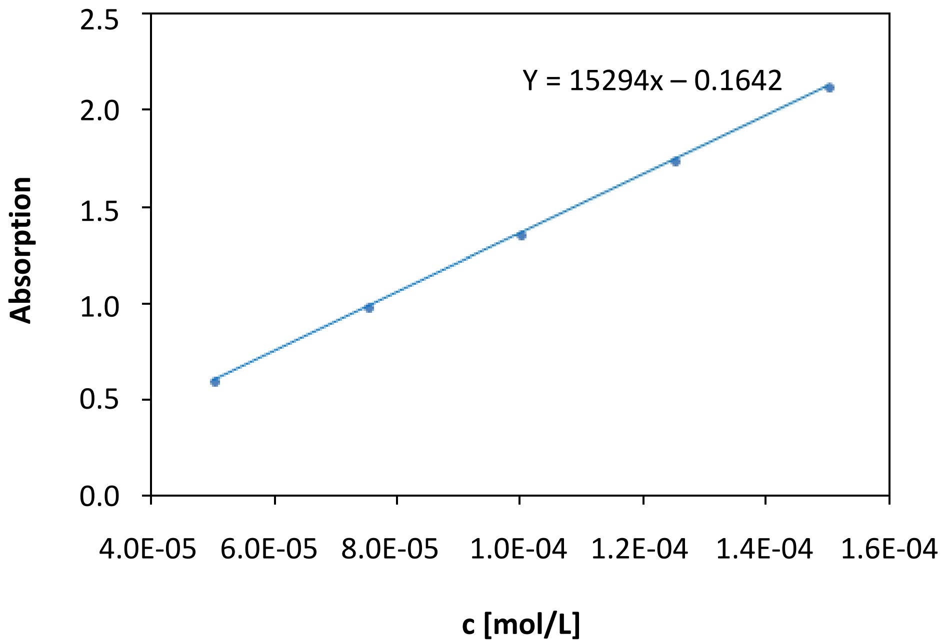


Figure 10 Single UV spectra (3 x 10^{-5} mol/L in DMSO) and concentration dependent absorption of the protected version of GCP in DMSO.

#	Conc. [mol/L]	Abs.
1	0,000050	0,600410
2	0,000075	0,982720
3	0,000100	1,365503
4	0,000125	1,747350
5	0,000150	2,129790

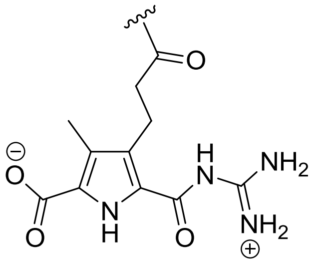
Table 6 Concentration dependent absorption of the protected version of GCP.

The molar extinction coefficient ε was determined from the linear regression. By taking the stochiometric ratio of polyethylenimine and GCP into account a loading of 40 % is obtained.

Mw (polyethylenimine, measured): 22724 g/mol

Mw (hydrogel, measured): 46112 g/mol

Mw (hydrogel) – Mw (polyethylenimine) = 46112 g/mol – 22724 g/mol = 23388 g/mol

 M = 265 g/mol

23388 g/mol / 265 g/mol ≈ 88

Number of terminal amino groups of polyethylenimine: ca 220

88 / 220 * 100 % = 40 %