

# Viscoelastic Properties of Hydrogels Based on Self-Assembled Multi-sticker Polymers Grafted With pH-Responsive Grafts

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## **1) Three steps synthesis of the graft copolymers GxH50**

### *a. Chemical characterization*

*Materials.* 2-hydroxyethyl acrylate (HEA, 96% Aldrich) was purified by liquid-liquid extraction: once with water (1:3 v/v), ten times with cyclohexane (99% Fisher, 3:1 v/v), twice with NaCl solution (200g/L, 1:3 v/v) and once with diethyl ether (99% Aldrich, 3:1 v/v). *n*-butyl acrylate (*n*BA) and *tert*-butyl acrylate (*t*BA) (Acros, 99%) were stirred overnight with hydroquinone (Prolabo) on calcium hydride (Acros, 93%) and distilled under vacuum. N,N'-azobisisobutyronitrile (AIBN, 98%, Merck Chemical ) was recrystallized from 95% methanol (99.99%, Fisher Chemical) before use.  $\alpha$ -bromoisobutyryl bromide (BIBB, Aldrich, 98%), N,N,N',N',N''-Pentamethyldiethylenetriamine (PMDETA, Acros, 99%), copper(II) bromide (CuBr<sub>2</sub>, Acros, 99%), copper(I) bromide (CuBr, Acros, 98%), chloroform (Aldrich, 99.8%), methanol (Aldrich, 99%), trifluoroacetic acid (CF<sub>3</sub>COOH, Acros, 99%), 4-methoxyphenol (99%, Aldrich), tetrahydrofuran (99.9% Aldrich), triethylamine (99% Fisher), toluene (99%

Fisher), dichloromethane (99%, Fischer Chemical) and pentane (99%, Sigma-Aldrich) were used as received.

*Size exclusion chromatography (SEC)* was done with a system consisting of a guard column (5  $\mu\text{m}$ , 50 mm  $\times$  7.5 mm) connected to a PLgel Mixed-D column (5  $\mu\text{m}$ , 300 mm  $\times$  7.5 mm) and a PLgel “individual pore size” column (5  $\mu\text{m}$ , 50 mm  $\times$  7.5 mm). Online light scattering (miniDAWN TREOS from Wyatt) and refractive index (RID10A from Shimadzu) were used for detection. The samples were analyzed in THF at room temperature using a flow rate of 1 mL.min<sup>-1</sup> with a Prominence HPLC system from Shimadzu. After filtration through a 0.2  $\mu\text{m}$  pore size membrane, the polymers were injected at a concentration of 5 mg mL<sup>-1</sup> in THF. The average molar masses were calculated from the light scattering detector, using a specific refractive index increment  $\text{dn/dc} = 0.057 \text{ mL/g}$  that was determined from the integrated refractive index (RI)-signal knowing the polymer concentration.

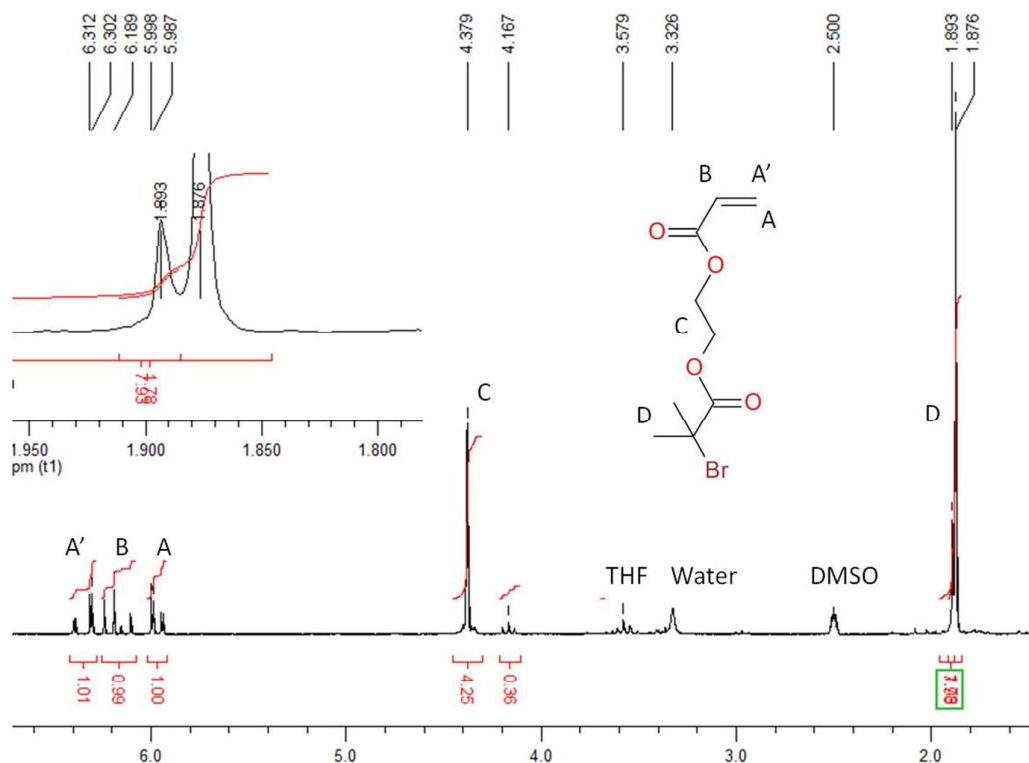
<sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> at 20 °C on a Brüker AC400 (400 MHz) spectrometer.

#### *b. Synthesis of the functional monomer.*

For the synthesis of the functional monomer, 2-(2-bromoisobutyryloxy)ethyl acrylate (BIEA), 2-hydroxyethyl acrylate (5.52 g, 4.75  $\times 10^{-2}$  mol), triethylamine (5.29 g, 5.23  $\times 10^{-2}$  mol), 4-methoxyphenol (5.9 mg, 4.75  $\times 10^{-4}$  mol), and dry THF (52 g) were mixed in a double-neck flask at 0 °C. A dropping funnel containing  $\alpha$ -bromoisobutyryl bromide (19.8 g, 8.61  $\times 10^{-2}$  mol) and dry THF (20 g) was adjusted on the double-neck flask. A septum was placed on the other neck. The dropping funnel was opened to add dropwise, at 0 °C under stirring, the  $\alpha$ -bromoisobutyryl bromide solution to the 2-hydroxyethyl acrylate solution. The mixture was let reacting for 22 hours. The solution was filtered on a Büchner to remove the insoluble

triethylammonium bromide salt and THF was evaporated under reduced pressure ( $T=40^{\circ}\text{C}$ ). An orange liquid was recovered.

To remove impurities, the liquid was diluted in  $\text{CHCl}_3$  (40 mL) and extracted five times with a saturated solution of  $\text{NaHCO}_3$  (87g/L) to hydrolyze and neutralize the excess of  $\alpha$ -bromoisobutyryl bromide, then twice with salted water (25 mL at 200 g/L) and once with water (10 mL). Finally, the organic phase was dried with  $\text{MgSO}_4$  and  $\text{CHCl}_3$  was removed by rotating evaporation ( $40^{\circ}\text{C}$ ), yielding 11.2 g (yield of 95%) of an orange liquid. Despite our efforts, impurities remained in the product as illustrated by the double peaks at  $\delta=1.9$  ppm in  $^1\text{H}$  NMR, see Figure S1. Our efforts to identify the impurity by liquid chromatography coupled to mass spectrometry proved unsuccessful. However, the  $^1\text{H}$  NMR signature of this impurity is compatible with residual BIBB either in its original or hydrolyzed form. Since neither potential impurity is polymerizable by free radical polymerization, it should not affect the next step consisting in radical copolymerisation and should be efficiently removed by precipitation. BIEA was therefore used further as it was.



**Figure S1.** 200 MHz  $^1\text{H}$  NMR of the 2-(2-bromoisobutryl)ethyl acrylate after purification in DMSO.  $\delta$  (ppm) = 1.9 (s, 2H BIEA+impurity, H-d) ;  $\delta$  (ppm) = 4.4 (m, 4H BIEA, H-c) ;  $\delta$  (ppm) = 6.0 (dd, 1H BIEA, H-a) ;  $\delta$  (ppm) = 6.2 (dd, 1H BIEA, H-b) ;  $\delta$  (ppm) = 6.4 (dd, 1H BIEA, H-a).

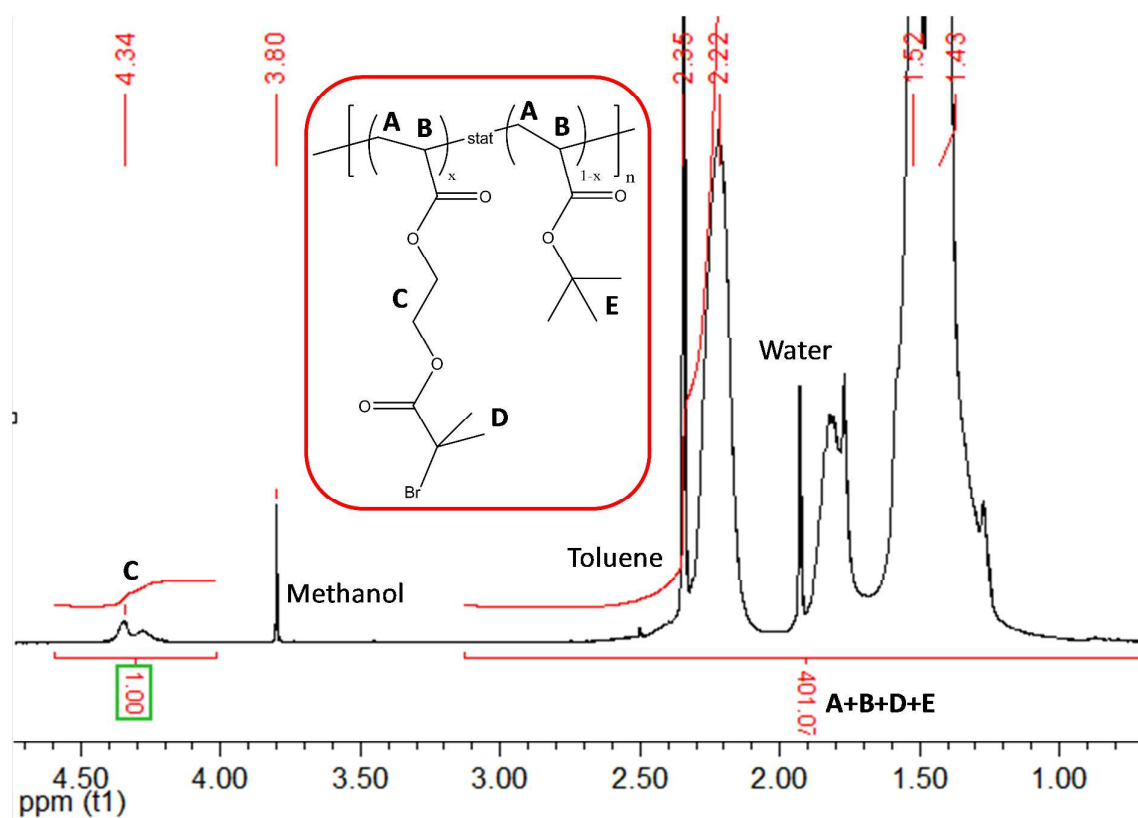
### c. Synthesis of the backbone.

Here, the synthesis of the backbone of G7H50 is detailed. The others backbones were prepared by adjusting the functional monomer to *t*BA ratio.

For the backbone synthesis, *tert*-butyl acrylate (39 g,  $3.05 \times 10^{-1}$  mol), BIEA (1.04 g,  $3.9 \times 10^{-3}$  mol), anisole (4.56 g,  $4.2 \times 10^{-2}$  mol), toluene (119 g) and azobisisobutyronitrile (9.2 g,  $5.6 \times 10^{-4}$  mol) were introduced in a 250 mL round-bottom flask. The flask was closed with a screw cap equipped with a septum, degassed by argon bubbling for 30 min and dipped in an oil bath at 60 °C. The mixture was let reacting for 24 hours. The reaction was stopped

by cooling the flask to 0 °C. The polymer was precipitated twice in methanol/water (90/10 vol/vol), yielding a white powder.  $^1\text{H}$  NMR in  $\text{CDCl}_3$  was performed on the final polymer to confirm its composition, see Figure S2. Size exclusion chromatography (SEC) in THF yielded the number ( $M_n$ ) and weight ( $M_w$ ) average molar masses and the dispersity, see Figure S7a. For the three backbones,  $M_n$  was around  $6.0 \times 10^4$  g/mol with  $\text{Đ} \sim 2.7$ .

The  $^1\text{H}$  NMR gave a molar BIEA/*t*BA ratio of 1.48% in agreement with the theoretical value of 1.44%, see Table S1.



**Figure S2.** 200 MHz  $^1\text{H}$  NMR of the polymer backbone of G7H50 in  $\text{CDCl}_3$  after purification.  $\delta$  (ppm) = 1.4-2.5 (m, 21H, H-a-b-d-e);  $\delta$  (ppm) = 3.7 (s, 4H, H-c).

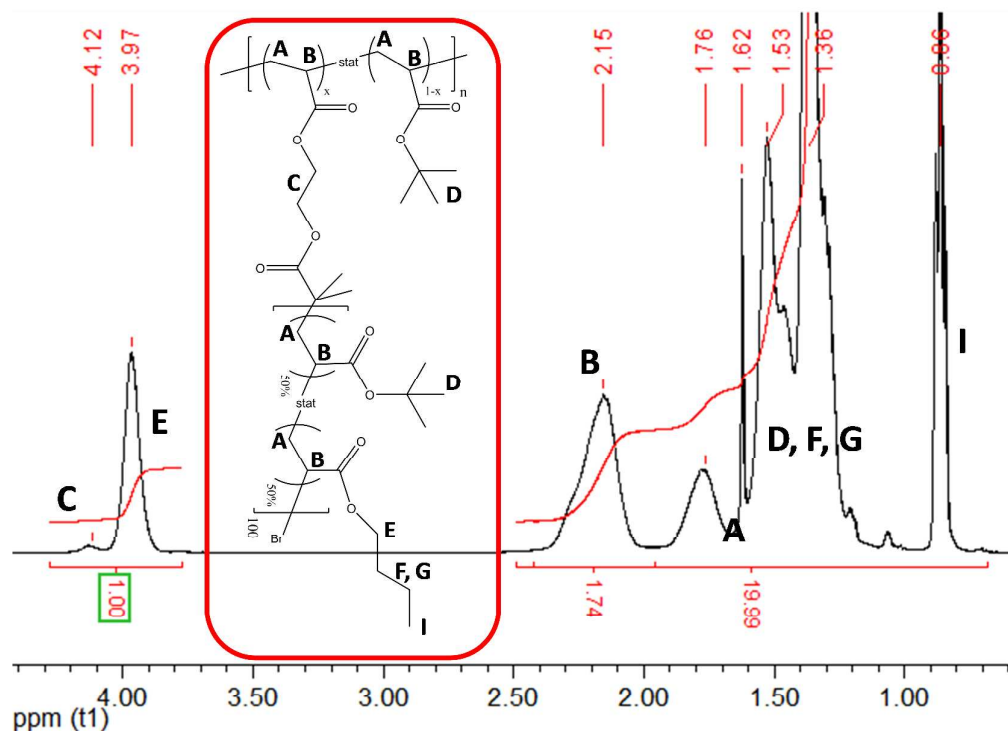
*d. Synthesis of the graft copolymer precursor.*

Here, the synthesis of the graft copolymer precursor of G7H50 is detailed. The others graft copolymers precursors were prepared by adjusting the polymer to monomers ratio.

For the graft copolymer  $P(tBA)_{500}\text{-g-[P}(nBA_{0.5}\text{-stat-}tBA_{0.5})_{100}]_7$ , *tert*-butyl acrylate (41.2 g,  $3.22 \times 10^{-1}$  mol), *n*-butyl acrylate (41.3 g,  $3.23 \times 10^{-1}$  mol),  $Cu(II)Br_2$  (0.038 g,  $1.7 \times 10^{-4}$  mol) and  $P(tBA\text{-stat-BIEA})$  (32.8 g, 1.44% BIEA) were introduced in a 500 mL round-bottom flask. PMDETA (0.62 g,  $3.58 \times 10^{-3}$  mol) and anisole (9.2 g) were introduced in a 50 mL vial. The flask and the vial were closed with screw caps equipped with a septum, and degassed by argon bubbling for 2 hours (a long degassing time was selected because of the viscosity of the polymer solution) and 10 min respectively.  $CuBr$  (0.479 g,  $3.34 \times 10^{-3}$  mol) was introduced in the 500 mL round-bottom flask under a counter-flux of argon and the solution was degassed again after that. The molar ratios were as follows  $[tBA+nBA]:[active\ Br\ in\ P(tBA\text{-stat-BIEA})]:[CuBr]:[PMDETA] = 200:1:1:1.05$ . The PMDETA solution was finally transferred in the flask under argon using a double-tipped needle. A few drops of the solution were taken as sample  $t_0$ , and the flask was dipped in an oil bath at 60 °C. Kinetics were followed by gas chromatography using anisole as internal standard, see Figure S6.  $\ln([M]_0/[M])$  evolved linearly with time for G7H50 and G30H50, whereas it was linear versus  $t^{2/3}$  for G2H50. Conversion of both *t*BA and *n*BA were equal throughout the reaction meaning that statistical grafts were formed as previously observed.<sup>1,2</sup>

The reaction was stopped at 50% conversion. The copper was removed by three liquid-liquid extractions with EDTA solution (1% (w/w) EDTA, 2:1  $NaHCO_3$ /EDTA and 20% (w/w) NaCl) since purification by flash column chromatography on  $SiO_2$  was not possible due to the high viscosity of the polymer solution. Thereafter, the solution was washed three times with water. The polymer was precipitated twice in methanol/water (90/10 vol/vol), yielding a white powder. Size exclusion chromatography (SEC) in THF yielded the number ( $M_n$ ) and

weight ( $M_w$ ) average molar masses and the dispersity, see Figure S7b and Table S1. The  $nBA/tBA$  ratio was measured by  $^1H$  NMR in  $CDCl_3$  and compared to the expected values, see Table S2 and Figure S3.

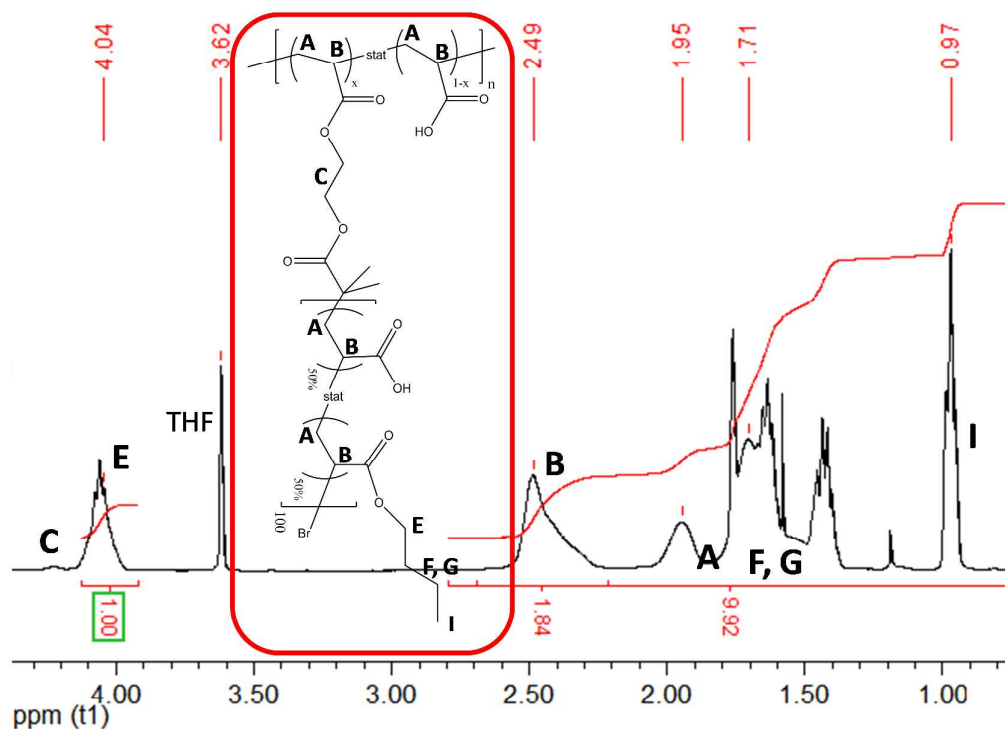


**Figure S3.** 200 MHz  $^1H$  NMR of the G7H50 graft copolymer precursor (unacidolyzed) in  $CDCl_3$  after purification.  $\delta$  (ppm) = 0.96 (t, 3H  $nBA$ , H-i);  $\delta$  (ppm) = 1.2-2.5 (m,  $nBA+tBA$ , H-a+b+d+f+g);  $\delta$  (ppm) = 3.97 (s, 2H  $nBA$ , H-e);  $\delta$  (ppm) = 4.12 (s, 2H-c).

*e. Acidolysis.*

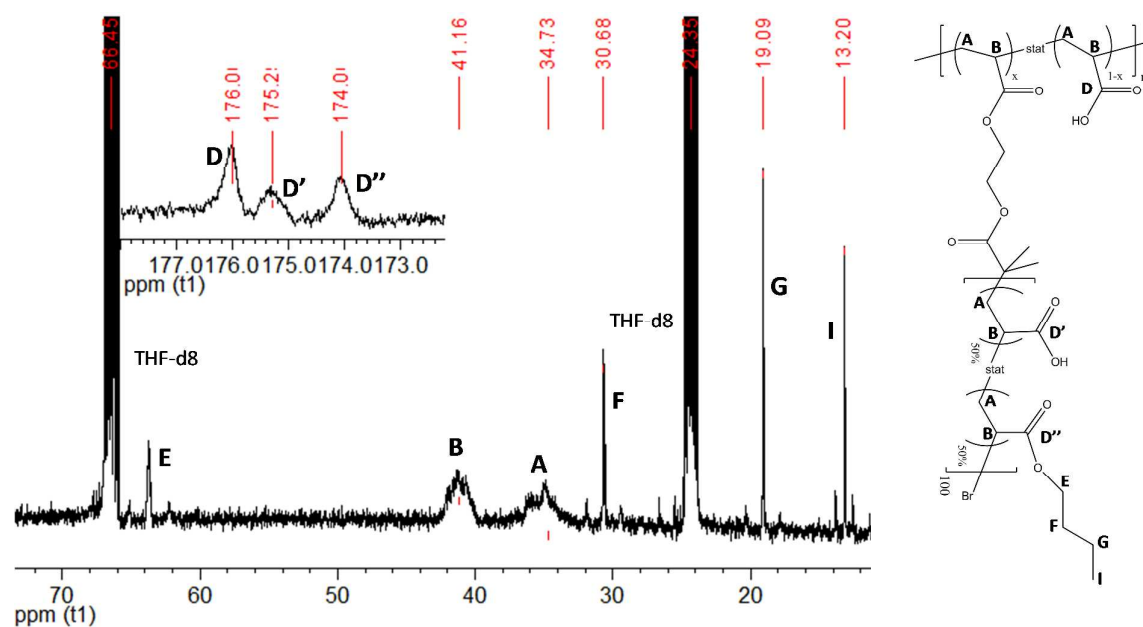
The polymer was dissolved in dichloromethane ( $C \sim 150$  g/L) and stirred at room temperature for at least 24 h with 5 equivalent of trifluoroacetic acid ( $CF_3COOH$ ) relative to the amount of  $tBA$  units, as previously reported.<sup>1-3</sup> The polymer was finally precipitated twice in pentane.

The *n*BA/AA ratio was measured by  $^1\text{H}$  NMR, see Table S2.  $^{13}\text{C}$  NMR revealed the absence of significant quantities of residual  $\text{CF}_3\text{COOH}$  (data not shown) and complete acidolysis of the *t*BA units into AA ones as already reported.



**Figure S4.** 200 MHz  $^1\text{H}$  NMR of the G7H50 graft copolymer in  $\text{THF-D}_8$  after purification.  
 $\delta$  (ppm) = 0.97 (t, 3H *n*BA, H-i) ;  $\delta$  (ppm) = 1.2-2.6 (m, *n*BA+*t*BA, H-a+b+f+g);  $\delta$  (ppm) = 4.04 (s, 2H *n*BA, H-e);  $\delta$  (ppm) = 4.1 (s, 2 H-c).





**Figure S5.** 50 MHz  $^{13}\text{C}$  NMR of the G7H50 graft copolymer after purification in THF-D<sub>8</sub>.  $\delta$  (ppm) = 176 (C-d, AA) ;  $\delta$  (ppm) = 175 (C-d', AA);  $\delta$  (ppm) = 174 (C-d'', nBA);  $\delta$  (ppm) = 64 (C-e, nBA);  $\delta$  (ppm) = 41 (C-b, nBA+AA);  $\delta$  (ppm) = 35 (C-a, nBA+AA);  $\delta$  (ppm) = 31 (C-f, nBA);  $\delta$  (ppm) = 19 (C-g, nBA);  $\delta$  (ppm) = 13 (C-i, nBA).

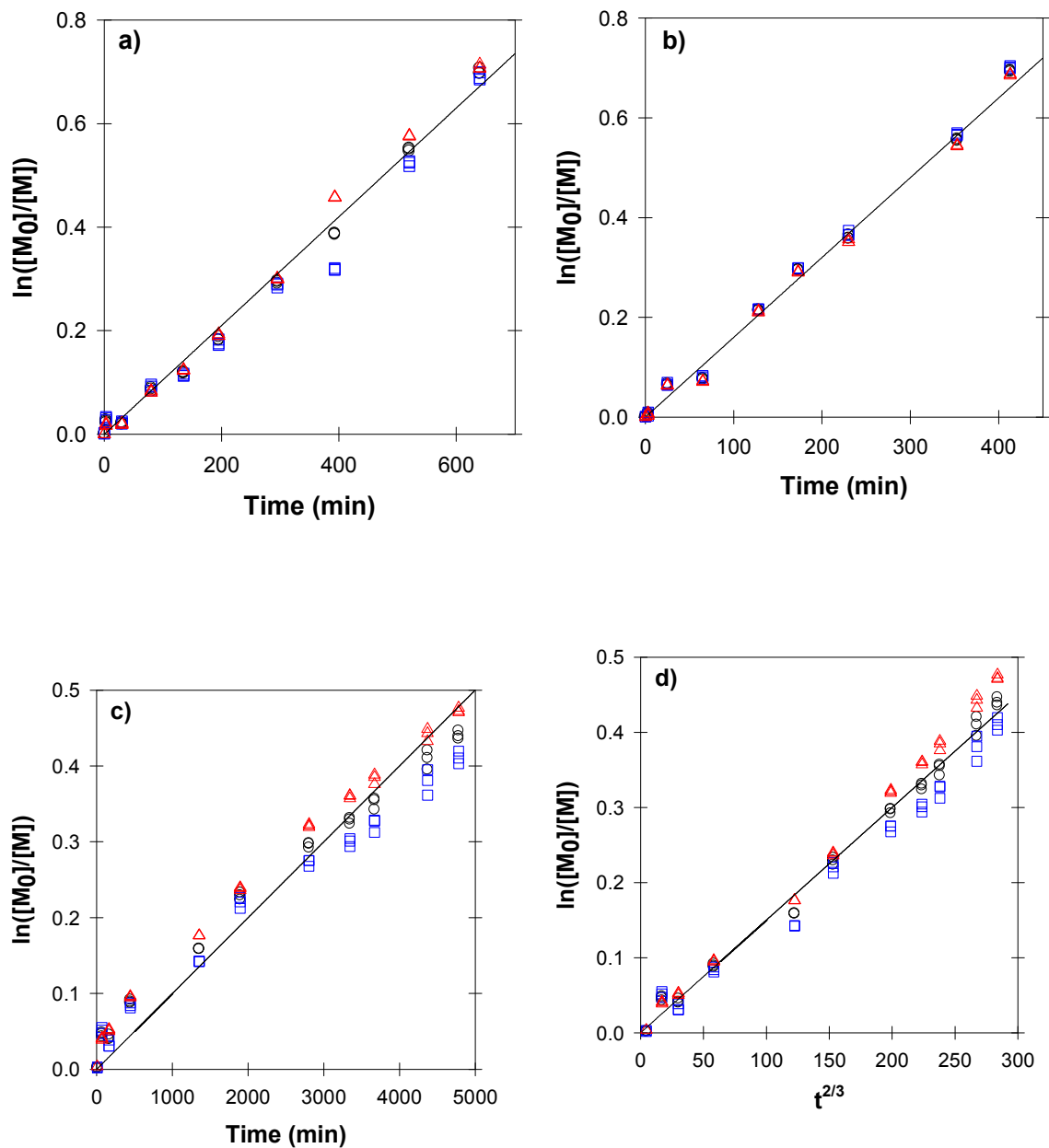
**Table S1.** Characteristics of the GxH50 copolymers synthesized.

Name	Backbone		Graft copolymer		
	% BIEA (mol/mol) theoretical.	% BIEA (mol/mol) <sup>1</sup> H NMR	M <sub>n,SEC</sub> (g/mol) <sup>a</sup>	Đ <sup>a</sup>	M <sub>n</sub> (g/mol) <sup>b</sup>
G2H50	0.54	0.5(5)	1.1 x10 <sup>5</sup>	2.5	5.6 x10 <sup>4</sup>
G7H50	1.44	1.4(8)	1.7 x10 <sup>5</sup>	2.5	8.6 x10 <sup>4</sup>
G30H50	5.81	5.9(6)	5.2 x10 <sup>5</sup>	2.5	3.4 x10 <sup>5</sup>

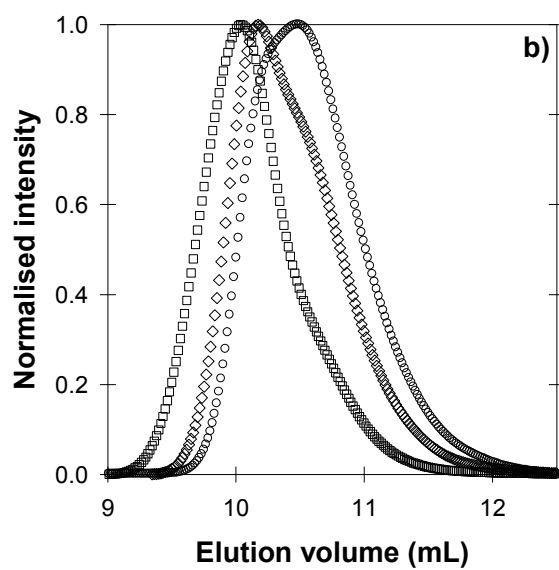
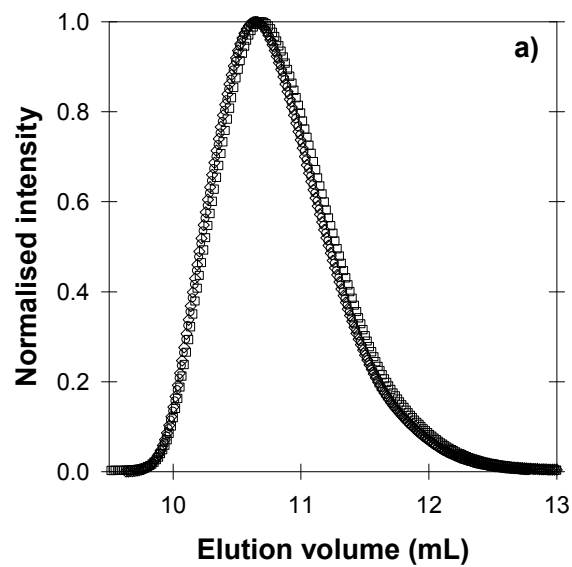
<sup>a</sup> Experimental M<sub>n</sub> and Đ of the unacidolyzed polymers determined by SEC. <sup>b</sup> Theoretical M<sub>n</sub> of the acidolyzed polymers calculated assuming 100% acidolysis of the *t*BA units into AA ones.

**Table S2.** *t*BA/*n*BA and AA/*n*BA ratios of the GxH50 copolymers synthesized.

Name	% <i>t</i> BA (th.)	% <i>t</i> BA (exp.)	% AA (th.)	% AA (exp.)
G2H50	85.0	85.2	85.0	83.4
G7H50	71.0	71.4	71.0	72.8
G30H50	58.0	57.6	58.0	56.1

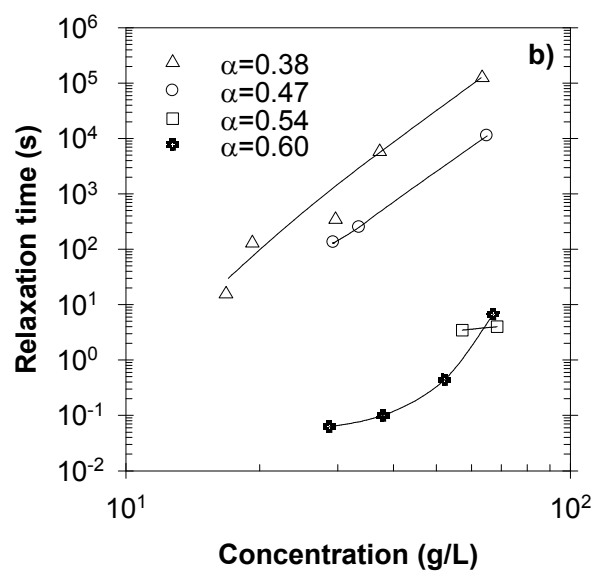
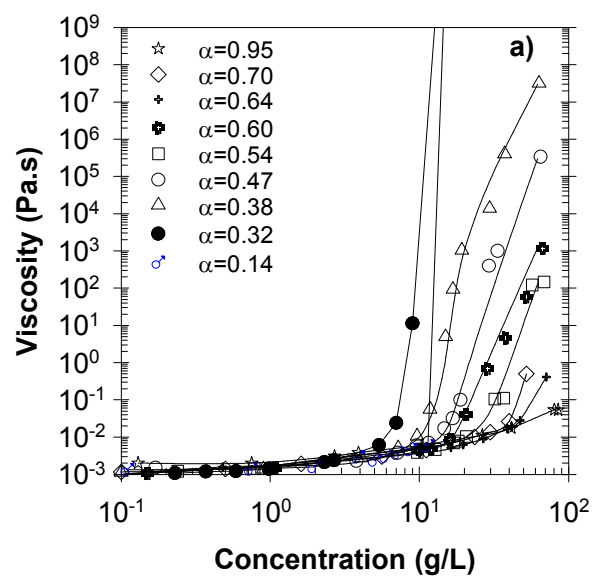


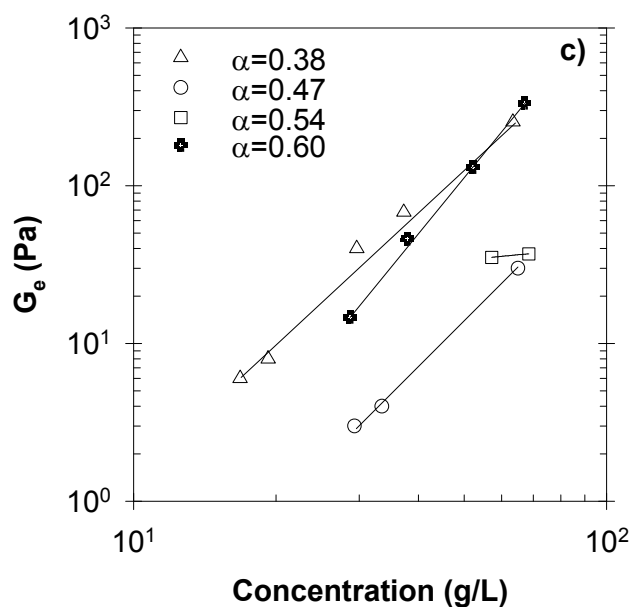
**Figure S6.** Kinetics of the polymerisation for the synthesis of the  $P(nBA_{50\%}\text{-stat-}tBA_{50\%})_{100}$  grafts from the  $PtBA$  functional backbones (step 3 in Figure 1 of the manuscript) with  $[tBA+nBA]:[P(tBA\text{-stat-BIEA})]:[CuBr]:[PMDETA]$  ratios of 200:1:1:1.05 for G7H50 (a), 200:1:1:1.05 for G30H50 (b) and 245:1:1:1.05 for G2H50 (c+d). The monomer consumption of  $tBA$  ( $\Delta$ ),  $nBA$  ( $\square$ ) and total ( $\circ$ ) were measured by gas chromatography.<sup>4</sup>



**Figure S7.** Size exclusion chromatograms for the backbones of G2H50 ( $\diamond$ ), G7H50 ( $\circ$ ) and G30H50 ( $\square$ ) (a) for the unacidolyzed graft copolymers G2H50 ( $\diamond$ ), G7H50 ( $\circ$ ) and G30H50 ( $\square$ ) (b). The light scattering detector was used.

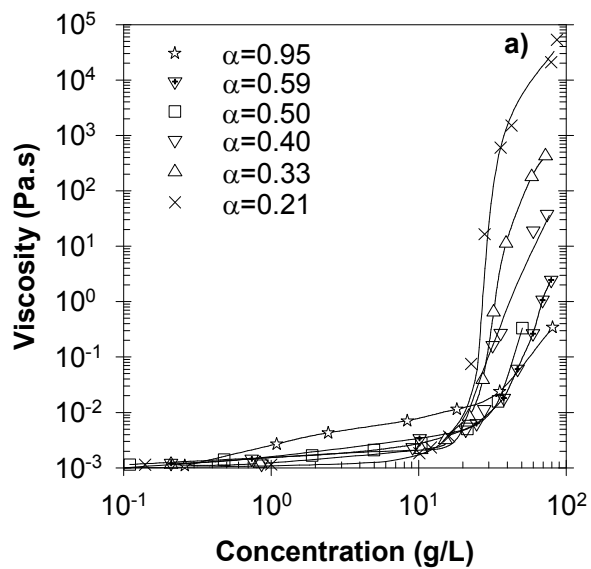
## 2) Viscoelastic properties of G2H50

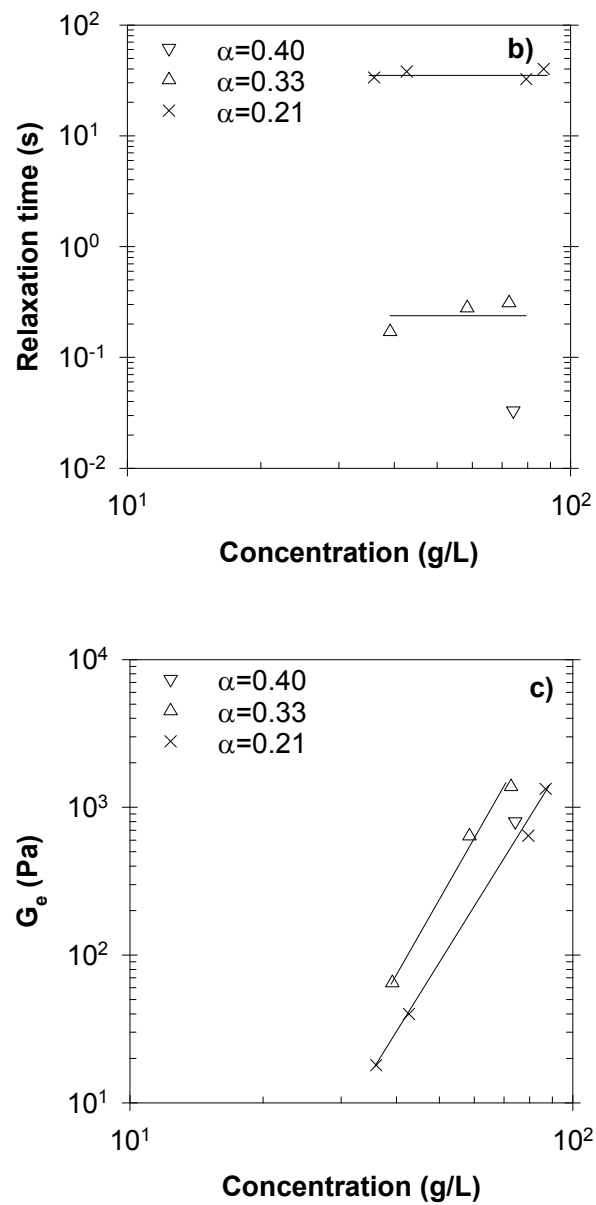




**Figure S8.** (a) viscosity (b) relaxation time and (c) elastic moduli as a function of the concentration for G2H50 at different ionization degrees. The solid lines are guides to the eye.

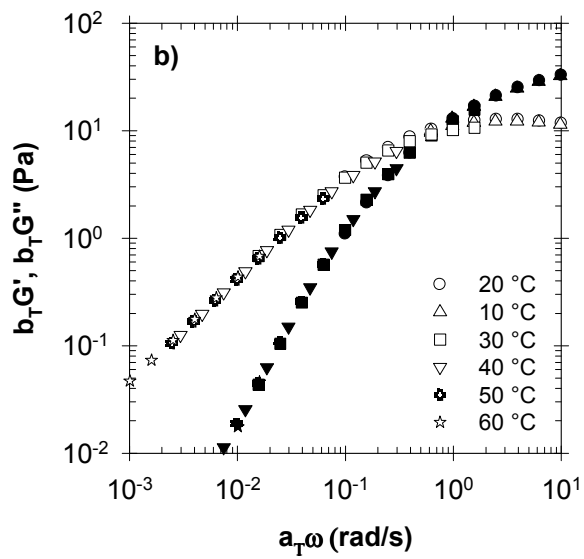
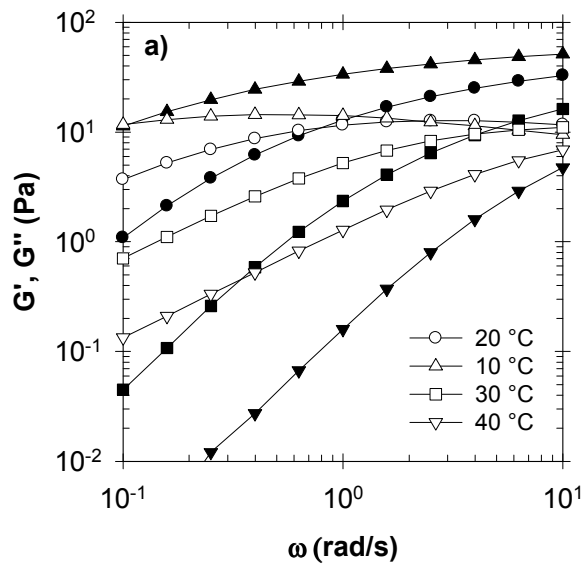
### 3) Viscoelastic properties of G30H50





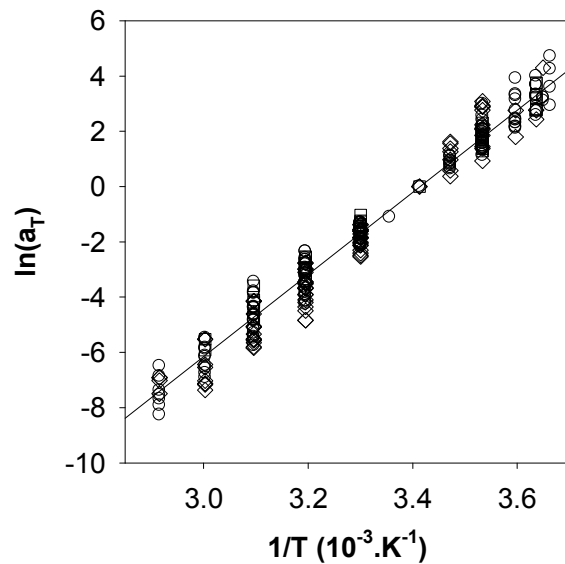
**Figure S9.** (a) viscosity (b) relaxation time and (c) elastic moduli as function of the concentration for G30H50 at different ionization degree. The solid lines are guides to the eye.

#### 4) Example of frequency-temperature superposition at $T_{\text{ref}}=20^\circ\text{C}$



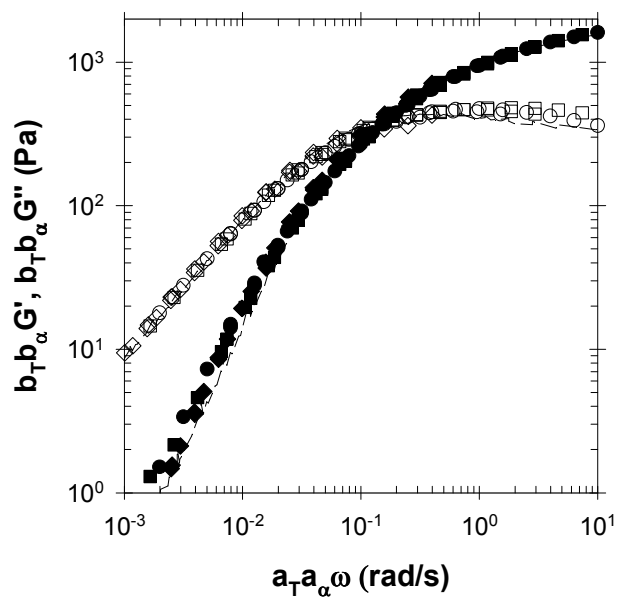
**Figure S10.** (a) Frequency dependence of the storage (open symbols) and loss (filled symbols) shear moduli of G2H50 at different temperatures for  $C = 20$  g/L and  $\alpha = 0.60$ . (b) Same data as in (a) after frequency-temperature superposition at  $T_{\text{ref}} = 20$  °C.





**Figure S11.** Arrhenius representation of the temperature dependence of the vertical shift factor at several ionization degrees and concentrations for G2H50 ( $\diamond$ ), G7H50 ( $\circ$ ) and G30H50 ( $\square$ ). The solid line represents  $E_a = 120$  kJ/mol.

##### 5) Master curves of G2H50, G7H50, G30H50 and TH50



**Figure S12.** Comparison of master curves of the elastic modulus (closed symbols) and the loss modulus (open symbols) obtained for G2H50 ( $\diamond$ ), G7H50 ( $\circ$ ) and G30H50 ( $\square$ ). The master curves were obtained by combining frequency–temperature and frequency– $\alpha$  superposition with  $\alpha_{\text{ref}}=0.60$  for G2H50,  $\alpha_{\text{ref}}=0.45$  for G7H50 and  $\alpha_{\text{ref}}=0.21$  for G30H50 at  $T_{\text{ref}}=20^{\circ}\text{C}$ . The dashed lines represent TH50.

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