SUPPORTING INFORMATION

Thermodynamics of Multi-Stage Self-Assembly of pH-Sensitive Gradient Copolymers in Aqueous Solutions.

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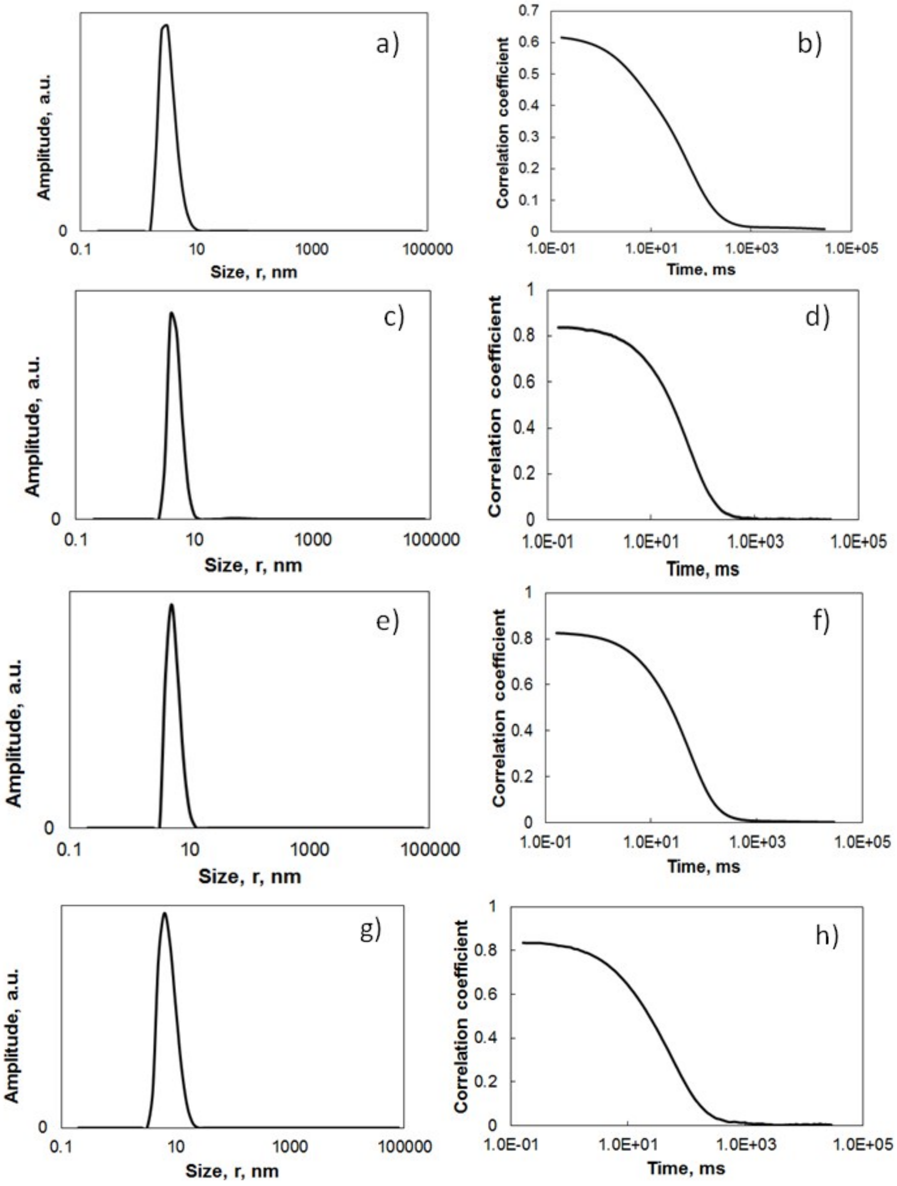


Figure SI 1. Volume-weighted distribution of particles sizes of D1 copolymer in water solution at pH: a) 9.81, c) 7.27, e) 6.59, g) 5.58; and corresponding correlation functions at the same pHs: b) 9.81, d) 7.27, f) 6.59, h) 5.58.

Table SI 1. Hydrodynamic radii pH dependence measured by DLS technique in D1 diblock copolymer 0.1 w% water solution.

pH	R_H, nm	experimental error	pH	R_H, nm	experimental error
12.5	4.9	±0.2	7.19	6.6	±0.4
12.4	4.6	±0.2	7.08	6.7	±0.3
12.2	5.3	±0.2	7.07	5.9	±0.2
11.8	5.4	±0.2	7.04	5.8	±0.5
10.7	4.7	±0.3	7.02	6.8	±0.3
10	4.9	±0.2	7	6.2	±0.5
9.87	4.6	±0.2	6.97	6.5	±0.4
9.79	4.7	±0.2	6.93	6.4	±0.3
9.74	5.1	±0.2	6.88	6.8	±0.5
9.68	4.4	±0.2	6.8	6.4	±0.5
9.62	4.2	±0.3	6.76	6.6	±0.5
9.56	4.9	±0.2	6.59	6.9	±0.5
9.49	4.7	±0.2	6.51	6.6	±0.4
9.42	5.0	±0.2	6.43	6.8	±0.3
9.34	4.9	±0.2	6.35	6.9	±0.3
9.26	4.9	±0.2	6.27	6.5	±0.3
9.17	4.7	±0.2	6.17	7.2	±0.5
8.96	5.0	±0.3	6.08	7.3	±0.4
8.85	5.1	±0.2	6.02	8.3	±0.3
8.73	5.0	±0.2	5.97	9.1	±0.3
8.59	4.7	±0.2	5.93	8.9	±0.4
8.46	4.8	±0.2	5.87	9.1	±0.6
7.95	5.1	±0.2	5.58	11.3	±0.5
7.87	5.2	±0.2	5.4	11.4	±0.4
7.5	5.6	±0.3	5.07	14.4	±0.4
7.49	5.2	±0.2	4.66	15.6	±0.4
7.44	5.4	±0.2	4.56	15.4	±0.5
7.37	5.6	±0.2	4.34	42.0	±0.3
7.31	5.4	±0.2	4.22	40.9	±0.4
7.27	6.0	±0.2	4.1	29.6	±0.4
7.23	5.7	±0.2	3.81	29.9	±0.3
7.22	6.0	±0.3	3.64	54.2	±0.6
7.2	5.6	±0.2	3.47	65.6	±0.7

7.19	6.5	±0.2	3.24	76.6	±0.5
7.08	6.7	±0.2			

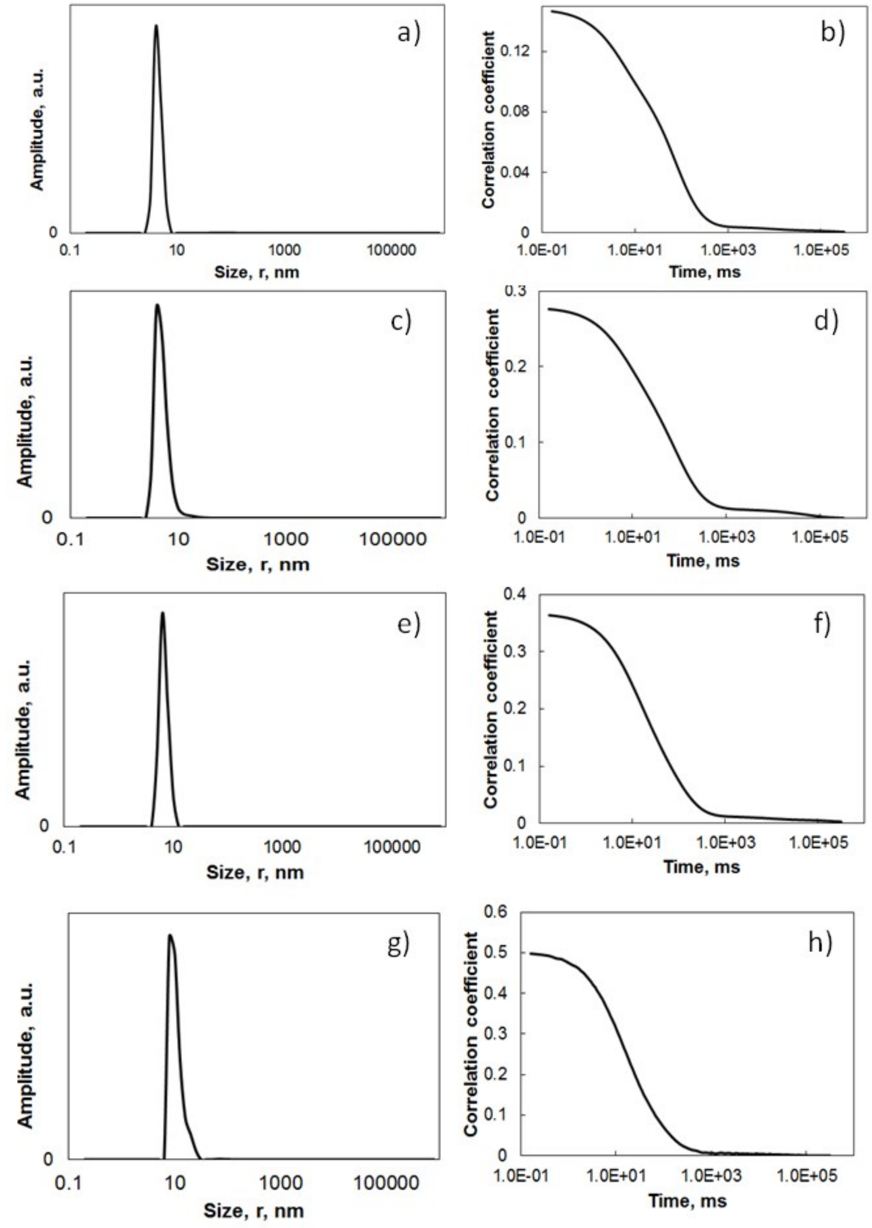


Figure SI 2. Volume-weighted distribution of particles sizes of D2 copolymer in water solution at pH: a) 11.4, c) 7.53, e) 6.77, g) 5.41; and corresponding correlation functions at the same pHs: b) 11.4, d) 7.53, f) 6.77, h) 5.41.

Table SI 2. Hydrodynamic radii pH dependence measured by DLS technique in D2 diblock copolymer 0.1 w% water solution.

pH	R_H, nm	experimental error	pH	R_H, nm	experimental error
12.0	4.0	±0.2	7.53	6.6	±0.3
11.7	4.4	±0.2	7.51	7.4	±0.3
11.4	4.9	±0.2	7.48	7.4	±0.2
10.6	4.8	±0.2	7.4	7.3	±0.3
10.1	4.7	±0.3	7.28	7.2	±0.3
9.84	4.3	±0.2	7.26	7.3	±0.3
9.59	4.0	±0.3	7.2	7.8	±0.4
9.48	4.8	±0.2	7.18	7.8	±0.2
9.35	4.7	±0.2	7.03	7.3	±0.1
9.22	4.3	±0.2	6.77	7.9	±0.4
9.04	4.5	±0.2	6.69	7.6	±0.3
8.85	4.6	±0.3	6.47	7.7	±0.2
8.37	4.9	±0.2	6.28	7.5	±0.3
8.26	5.1	±0.2	6.05	9.7	±0.4
8.08	5.1	±0.2	5.97	8.7	±0.4
8.01	5.3	±0.2	5.89	10.78	±0.4
7.96	4.9	±0.2	5.73	10.4	±0.4
7.9	5.2	±0.2	5.65	11.5	±0.3
7.82	4.9	±0.3	5.41	11.3	±0.3
7.78	5.0	±0.2	5.33	11.3	±0.2
7.74	5.2	±0.2	5.25	13.1	±0.2
7.7	5.0	±0.2	4.45	14.	±0.3
7.68	5.0	±0.2	4.06	15.7	±0.5
7.65	5.8	±0.3	3.98	16.8	±0.3
7.62	6.7	±0.2	3.62	16.4	±0.4

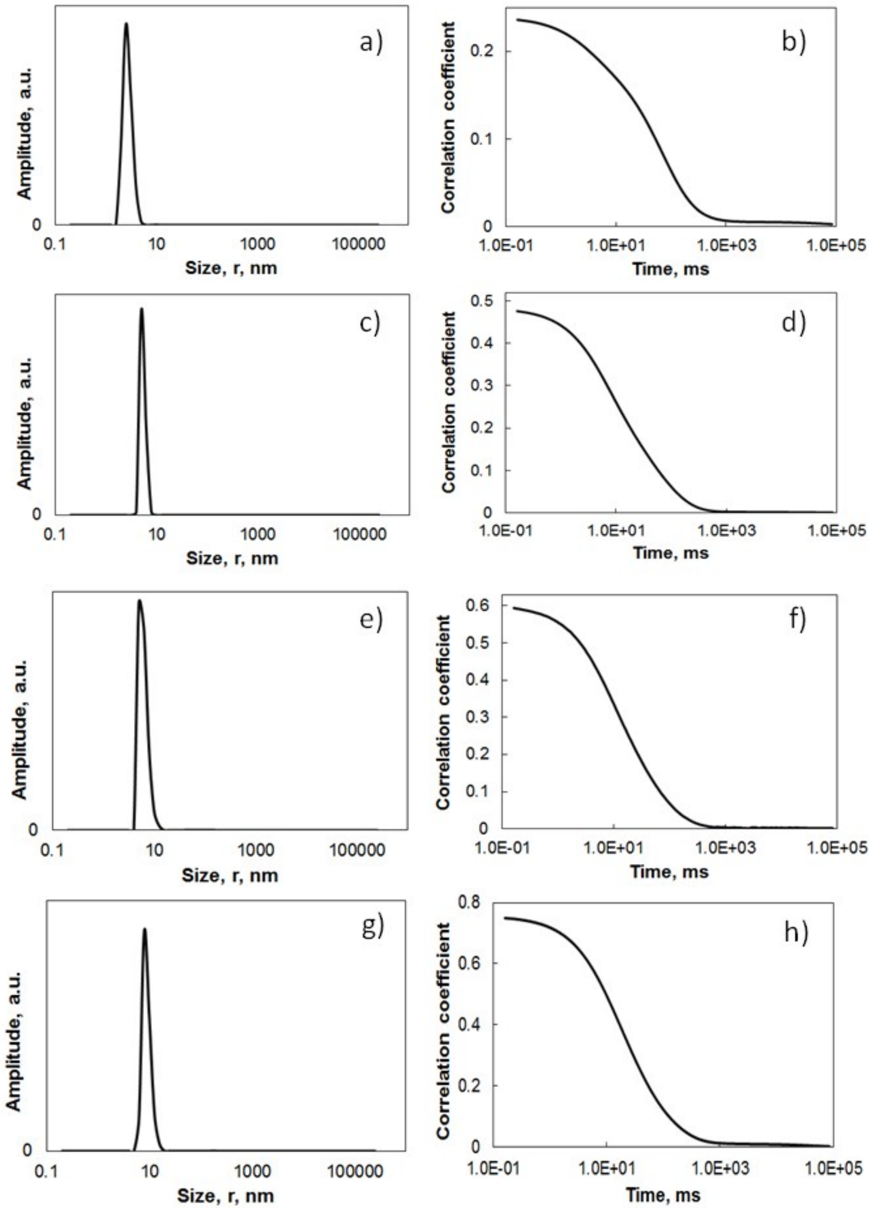
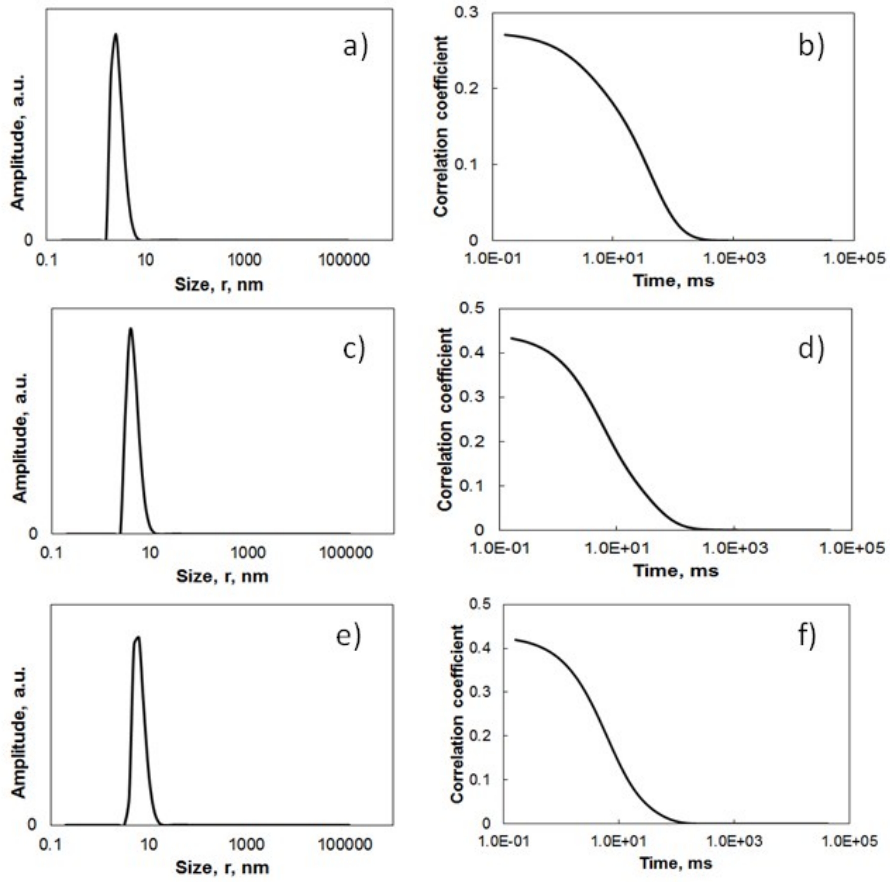


Figure SI 3. Volume-weighted distribution of particles sizes of D3 copolymer in water solution at pH: a) 11.0, c) 6.26, e) 5.88, g) 4.72; and corresponding correlation functions at the same pHs: b) 11.0, d) 6.26, f) 5.88, h) 4.72.

Table SI 3. Hydrodynamic radii pH dependence measured by DLS technique in D3 diblock copolymer 0.1 w% water solution.

pH	R_H, nm	experimental inaccuracy	pH	R_H, nm	experimental inaccuracy
11.7	3.6	±0.2	6.94	4.2	±0.2
11.0	3.4	±0.2	6.91	4.5	±0.2

9.8	3.1	±0.8	6.85	5.1	±0.2
9.45	3.1	±0.2	6.75	5.1	±0.3
9.1	3.3	±0.5	6.61	4.9	±0.2
8.78	3.2	±0.2	6.26	5.7	±0.2
8.66	3.2	±0.2	6.04	6.2	±0.3
8.61	3.4	±0.2	6.0	7.1	±0.4
8.53	3.2	±0.1	5.88	7.2	±0.2
8.35	3.1	±0.2	5.63	7.4	±0.2
8.19	3.4	±0.5	5.39	7.6	±0.2
8.04	3.4	±0.2	5.05	7.2	±0.3
7.89	3.4	±0.2	4.96	7.3	±0.4
7.74	3.8	±0.2	4.85	9.7	±0.2
7.6	3.7	±0.1	4.72	10.0	±0.4
7.47	3.8	±0.2	4.31	12.1	±0.5
7.37	3.9	±0.5	4.11	16.8	±0.6
7.27	4.3	±0.2	4.0	19.2	±0.5
7.18	3.9	±0.2			



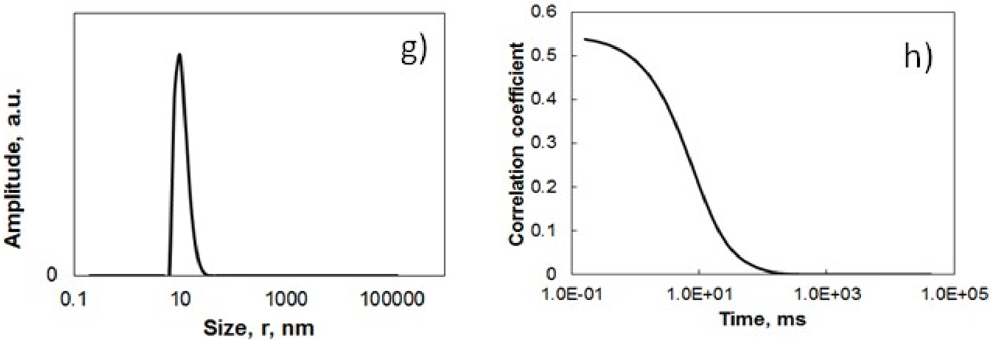


Figure SI 4. Volume-weighted distribution of particles sizes of T1 copolymer in water solution at pH: a) 9.46, c) 6.70, e) 5.39, g) 4.42; and corresponding correlation functions at the same pHs: b) 9.46, d) 6.70, f) 5.39, h) 4.42.

Table SI 4. Hydrodynamic radii pH dependence measured by DLS technique in T1 diblock copolymer 0.1 w% water solution.

pH	R_H, nm	experimental inaccuracy	pH	R_H, nm	experimental inaccuracy
10.2	3.7	±0.2	6.83	6.0	±0.2
9.96	3.4	±0.2	6.7	6.3	±0.4
9.76	3.5	±0.4	6.56	6.7	±0.3
9.48	3.5	±0.3	6.4	6.9	±0.1
9.14	3.4	±0.3	6.22	7.1	±0.2
8.57	3.9	±0.2	5.99	6.5	±0.2
7.93	4.2	±0.2	5.71	7.5	±0.6
7.55	4.2	±0.2	5.39	8.9	±0.8
7.36	5.1	±0.4	5.06	9.7	±0.3
7.23	4.8	±0.3	4.74	10.7	±0.4
7.11	4.9	±0.3	4.42	12.4	±0.2
7.01	5.3	±0.4	4.1	15.6	±0.4
6.94	5.6	±0.2	3.76	17.6	±0.5

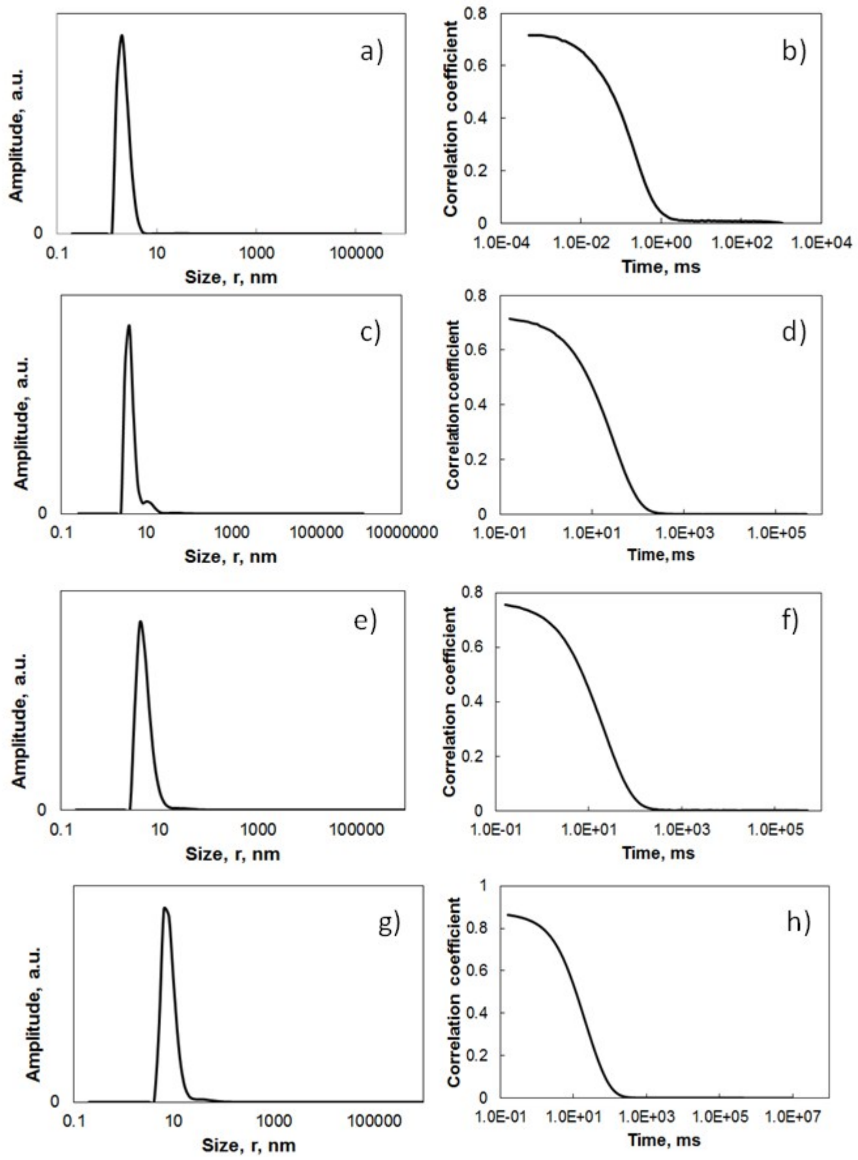


Figure SI 5. Volume-weighted distribution of particles sizes of T2 copolymer in water solution at pH: a) 9.49, c) 6.23, e) 5.21, g) 4.23; and corresponding correlation functions at the same pHs: b) 9.49, d) 6.23, f) 5.21, h) 4.23.

Table SI 5. Hydrodynamic radii pH dependence measured by DLS technique in T2 diblock copolymer 0.1 w% water solution.

pH	R_H, nm	experimental inaccuracy	pH	R_H, nm	experimental inaccuracy
9.49	2.8	±0.1	6.85	3.9	±0.1
9.44	2.7	±0.2	6.39	4.5	±0.2
9.39	2.7	±0.2	6.23	4.9	±0.2
9.25	2.8	±0.1	6.07	7.1	±0.2

9.12	2.7	±0.2	5.91	6.7	±0.2
8.83	2.6	±0.2	5.77	7.4	±0.3
8.79	2.6	±0.2	5.63	7.4	±0.4
8.68	2.7	±0.2	5.46	7.2	±0.4
8.63	2.8	±0.2	5.21	7.9	±0.5
8.62	2.8	±0.1	4.98	8.3	±0.4
8.57	2.8	±0.2	4.7	8.6	±0.3
8.51	2.7	±0.2	4.56	8.5	±0.4
8.18	2.8	±0.2	4.4	10.6	±0.4
7.73	3.1	±0.2	4.23	12.2	±0.5
7.58	3.2	±0.2	4.12	12.2	±0.4
7.01	3.5	±0.2	3.7	18.2	±0.3

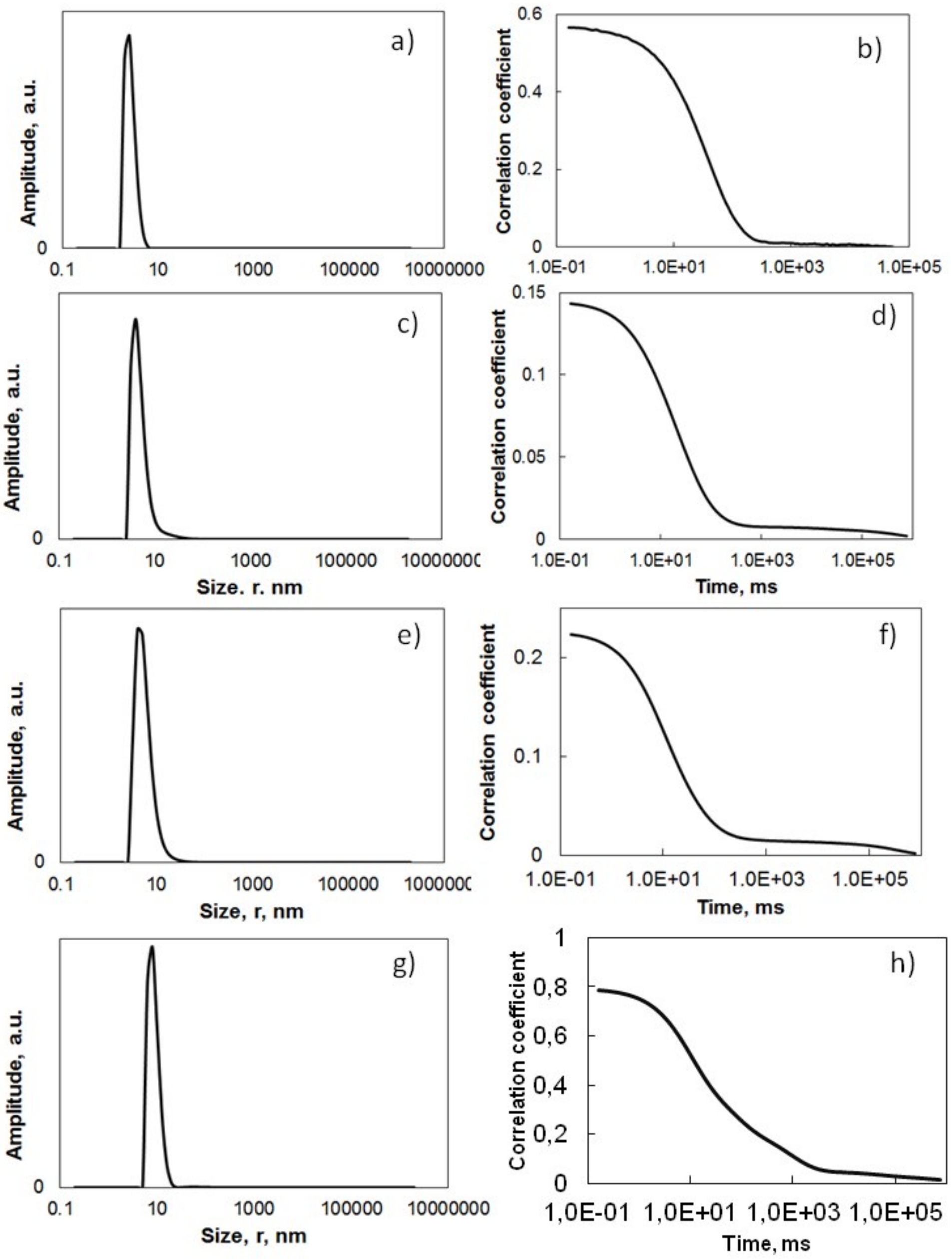


Figure SI 6. Volume-weighted distribution of particles sizes of T3 copolymer in water solution at pH: a) 9.54, c) 5.37, e) 4.4, g) 2.84; and corresponding correlation functions at the same pHs: b) 9.54, d) 5.37, f) 4.4, h) 2.84.

Table SI 6. Hydrodynamic radii pH dependence measured by DLS technique in T3 diblock copolymer 0.1 w% water solution.

pH	R_H, nm	experimental inaccuracy	pH	R_H, nm	experimental inaccuracy
9.54	2.5	±0.1	5.55	4.6	±0.3

8.97	2.6	±0.2	5.54	4.7	±0.3
8.96	2.2	±0.2	5.37	6.2	±0.5
8.4	2.5	±0.2	5.12	6.9	±0.1
8.02	2.3	±0.2	4.4	9.1	±0.2
8.02	2.5	±0.2	4.1	9.9	±0.4
7.71	2.3	±0.4	3.7	10.5	±0.7
7.45	2.4	±0.3	3.35	10.1	±0.8
7.05	2.7	±0.3	3.06	10.0	±0.8
6.68	2.9	±0.3	3.05	10.5	±0.5
6.51	3.0	±0.2	3.04	10.2	±0.9
6.31	3.2	±0.3	2.84	9.9	±0.4
6.12	3.3	±0.3	2.83	10.3	±0.7
5.67	3.9	±0.2			

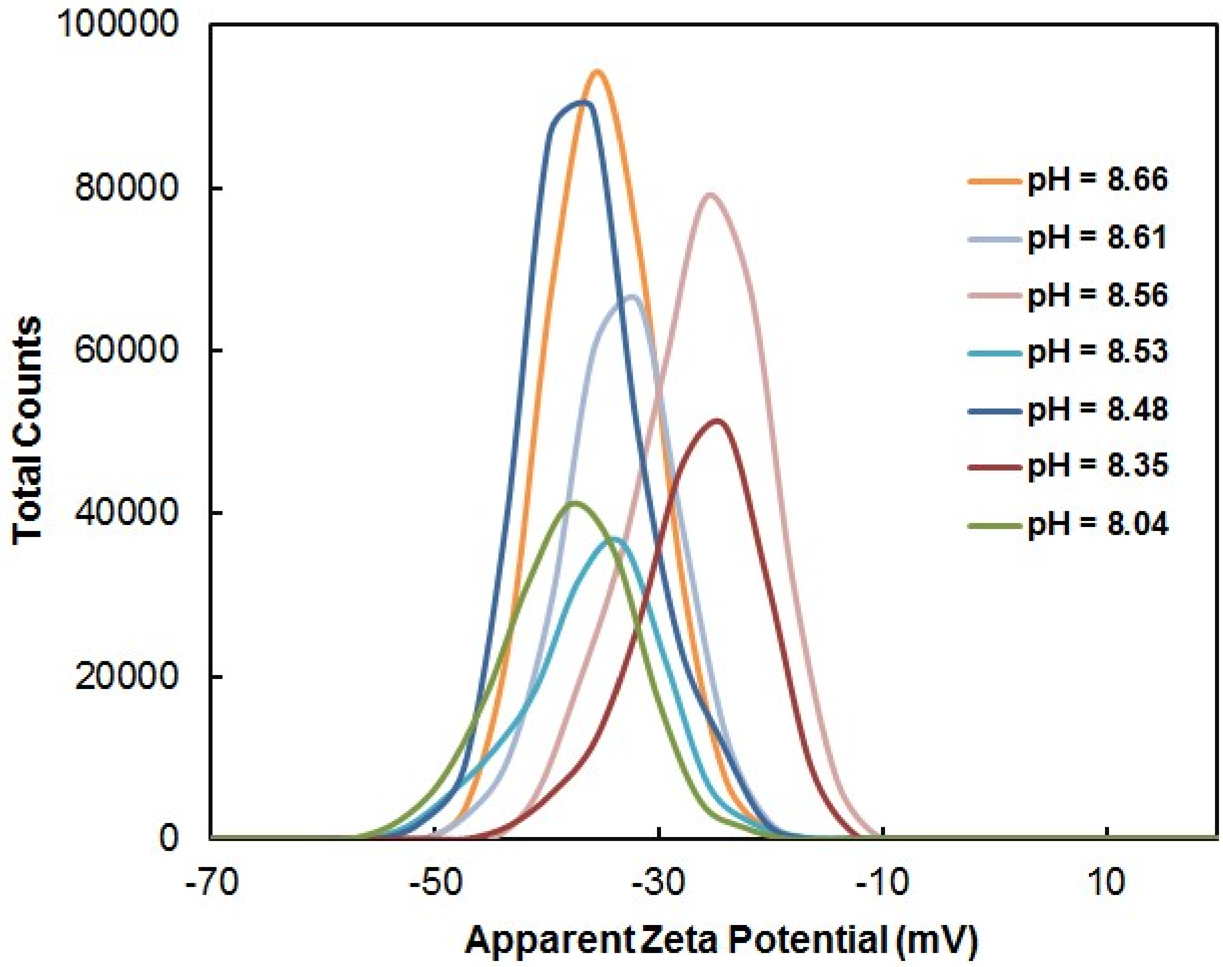


Figure SI 7. Zeta potential measurements performed on D3 copolymer 0.1w% water solution in the range of pH values from 8.7 to 8

Table SI 7. Zeta potential values measured on D3 copolymer 0.1w% water solution in the range of pH values from 11.4 to 8.

pH	Zeta Potential	Zeta Deviation	Mobility	Conductivity
	mV	mV	µmcm/Vs	mS/cm
11.4	-35.7	4.6	-2.70	1.12
9.45	-36.3	4.9	-2.73	0.963
8.66	-35.2	4.9	-2.66	1.03
8.61	-33.4	5.1	-2.52	1.04
8.56	-26.3	5.9	-1.99	1.05
8.53	-35.8	6.1	-2.70	1.06
8.48	-36.6	5.4	-2.76	1.07
8.35	-26.5	5.6	-2.00	1.09
8.04	-38	5.9	-2.89	1.14

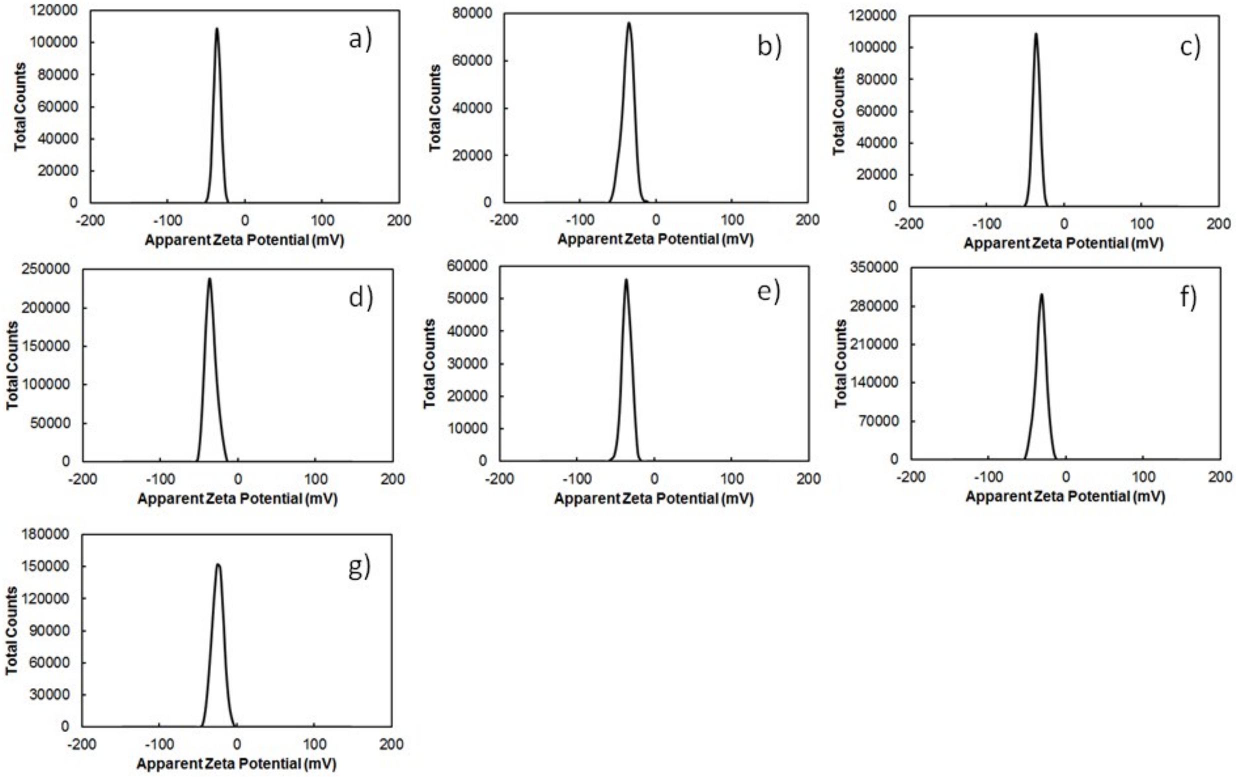


Figure SI 8. Zeta potential measurements performed on D3 copolymer 0.1w% water solution at pH: a) 11.5, b) 9.45, c) 7.89, d) 6.46, e) 4.85, f) 4.47, g) 4.0.

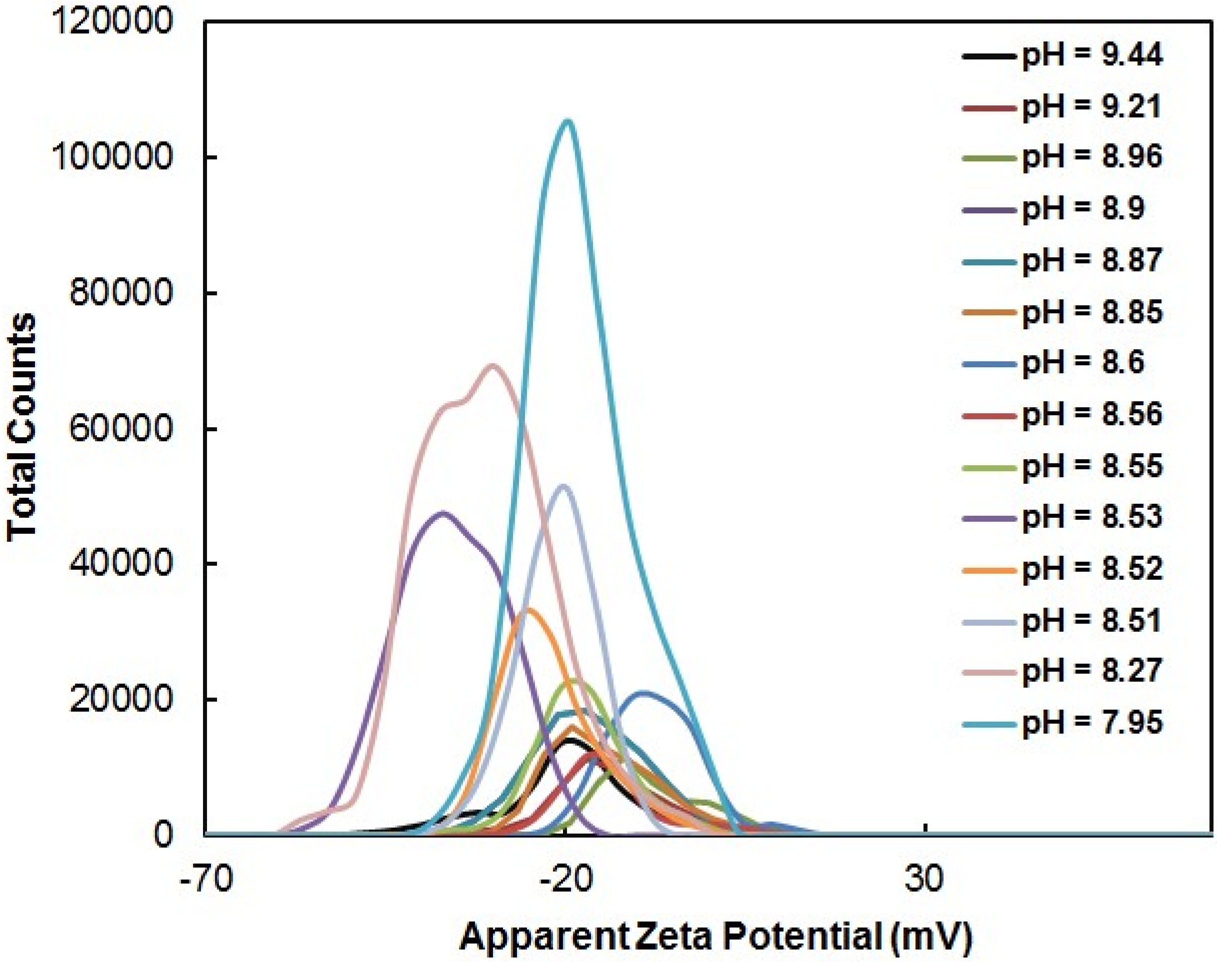


Figure SI 9. Zeta potential measurements performed on T2 copolymer 0.1w% water solution in the range of pH values from 9.5 to 8.

Table SI 8. Zeta potential values measured on T2 copolymer 0.1w% water solution in the range of pH values from 9.5 to 8.

pH	Zeta Potential	Zeta Deviation	Mobility	Conductivity
	mV	mV	µmcm/Vs	mS/cm
9.44	-19.8	10.3	-1.50	1.75
9.21	-13.8	8.5	-1.04	1.8
8.96	-11.3	7.1	-0.86	1.85
8.9	-9.69	7.6	-0.73	1.86
8.87	-16.7	7.6	-1.26	1.87
8.85	-14.8	7.6	-1.11	1.87
8.6	-7.81	6.4	-0.589	2.04
8.56	-13.3	11.1	-1.00	2.1

8.55	-17.8	6.7	-1.34	2.11
8.54	-8.53	7.7	-0.64	2.13
8.53	-35.7	7.6	-2.69	2.36
8.52	-21.2	7.0	-1.60	2.31
8.51	-31.2	5.6	-2.35	2.78
8.27	-31.1	9.4	-2.35	2.69
7.95	-18.2	7.6	-1.37	1.42

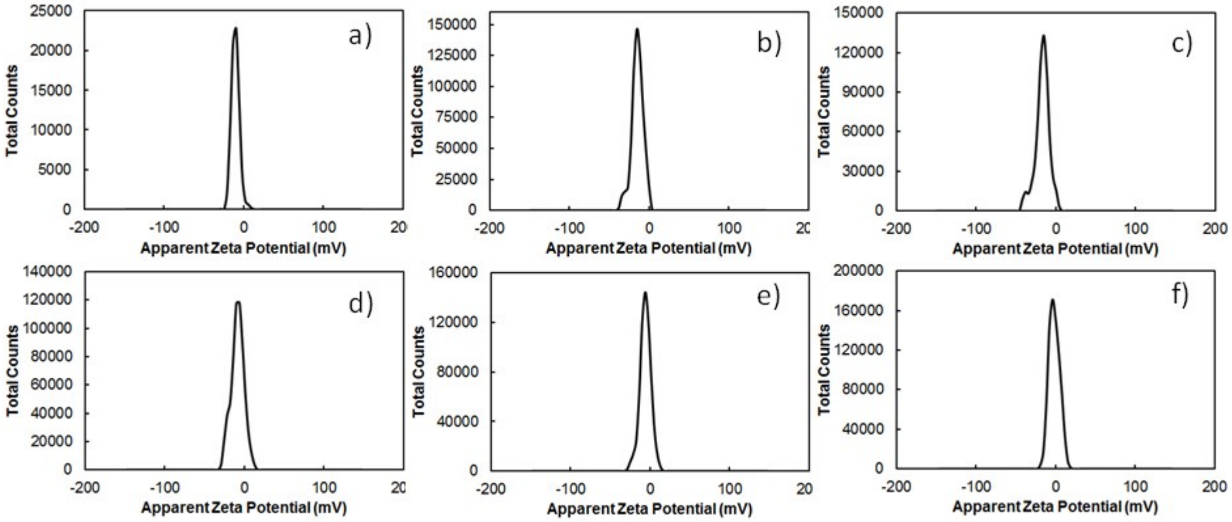


Figure SI 10. Zeta potential measurements performed on D3 copolymer 0.1w% water solution at pH: a) 10.3, b) 7.29, c) 7.08, d) 6.63, e) 4.84, f) 3.7.

Method of treatment of experimental ITC curve by one set of identical sites model

Analysis of ITC data is based on two general and fundamental equations, Gibbs free energy equation and Arrhenius equation, which are the following:

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

$$\Delta G = -RT\ln K \tag{2}$$

where K is equilibrium constant of reaction. The nature of those equations is such, that they might be applied to various systems: chemical reactions[1], protein-ligand binding[2] or micelle formation mechanism[3,4]. Basic of ITC method suggests that we titrate one solution by another and account signal. That signal represent a heat flow, that observed after elemental act of mixing (Figure SI 11). Since measurement by ITC set-up performed at constant temperature, heat flow after every act of mixing can be considered as apparent enthalpy of reaction. However, during the titration process the amount of one component (titrated substance) is continuously decreased upon addition of second component (titrant) and formation of resulting product.

$$n_1A + n_2B \rightarrow nAB \tag{3}$$

Thus the heat flow and consecutive apparent enthalpy are also decreased, although the enthalpy of reaction is still preserved to be constant.

$$Q_i = n_{1,i}\,\Delta H \tag{4}$$

Following to this way of understanding, typical ITC curve of elemental process, after integration of individual mixing heats, should be presented as sigmodal curve with plateau at the beginning and at the end of titration. The inflection point on such curve corresponds to the case, then amount of first component and second are equal, while the slope of the transition corresponds to the equilibrium constant (or $[\mathrm{cmc}]^{-1}$ in case of micelle formation mechanism). Now, when two parameters ΔH and K are known, others parameters can be evaluated from equations 1 and 2.

Thus, the model of one set of identical sits describes the elemental process, which characterized by only one constant rate of reaction. Moreover, some analytical treatment should be implemented to take into account technical aspects of experiment (dilution of titrated substance, overflow of experimental cell). They are thoroughly described in ref. 2 and 5.

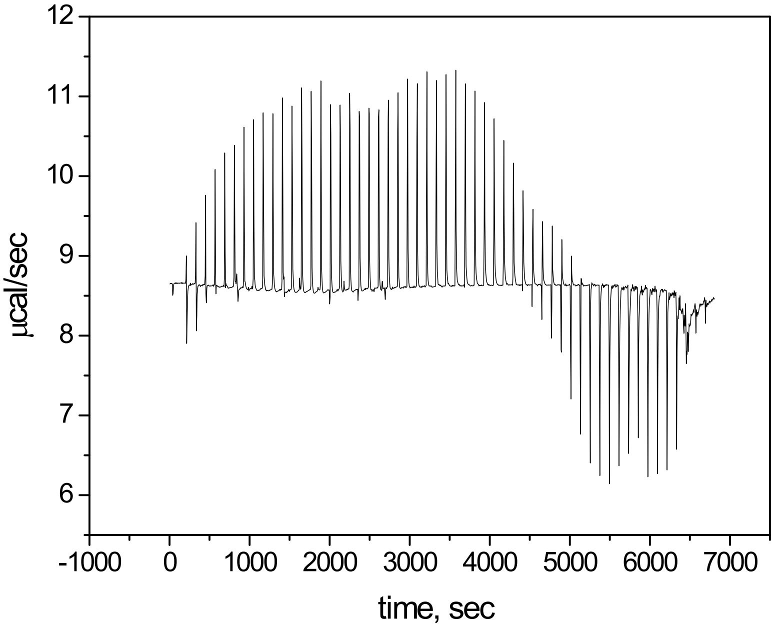


Figure SI 11. Heat flow (the power of the heater) vs. time. Raw date for polymer D2, directly obtained from ITC set-up before treatment.

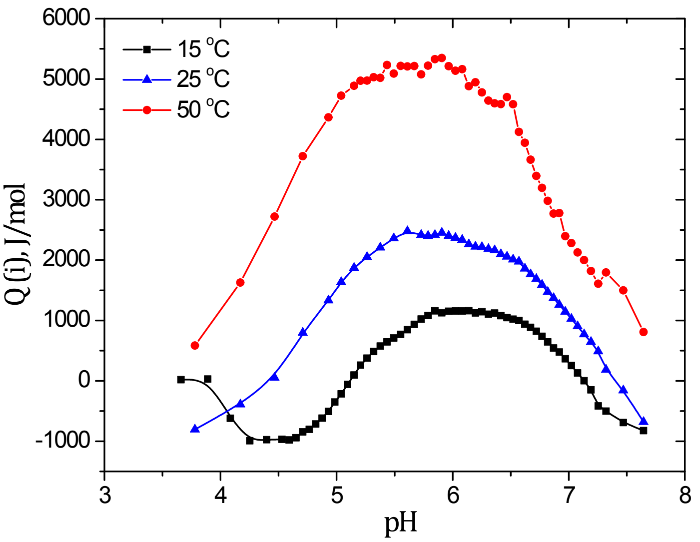


Figure SI 12. The isotherms of D1 di-block copolymer in water solution at different temperatures

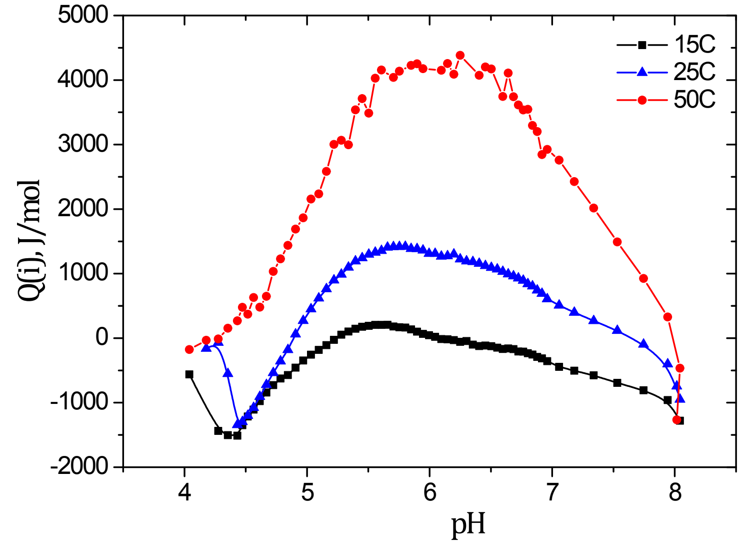


Figure SI 13. The isotherms of D3 di-block copolymer in water solution at different temperatures

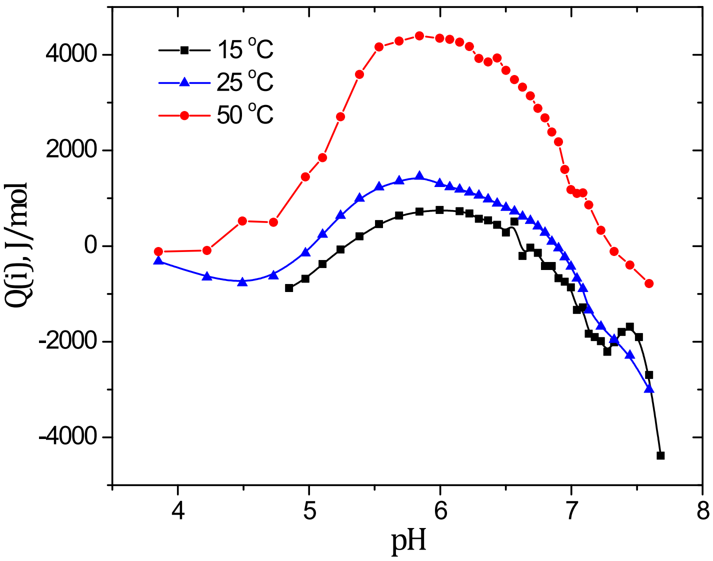


Figure SI 14. The isotherms of T1 tri-block copolymer in water solution at different temperatures

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