

Supplementary Information

Combining CROP and ATRP to synthesize pH-responsive poly(2-ethyl-2-oxazoline-*b*-4-vinylpyridine) block copolymers

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1. Experimental conditions for the synthesis of PEtOx and P(EtOx-*b*-P4VP)

Table S1. Experimental conditions, conversion, and yield for the synthesis of PEtOx.

Polymer	Degree of polymerization planned	Volume of TsOMe (μ L)	TsOMe (mmol)	Reaction time (h)	Monomer conversion (%)	Yield (%)
PEtOx ₃₀	30	99.6	0.66	1.69	67	34
PEtOx ₅₁	50	64.0	0.42	3.01	72	48
PEtOx ₅₉	60	52.8	0.35	3.57	70	43
PEtOx ₆₇	70	44.8	0.30	4.13	71	31

Table S2. Experimental conditions, 4VP conversion and yield for the synthesis of P(EtOx-*b*-P4VP).

Polymer	Degree of polymerization planned	Reaction time (h)	PEtOx (mg)	CuCl (mg)	CuCl ₂ (mg)	TPMA (mg)	Monomer conversion ^a (%)
P(EtOx ₃₀ - <i>b</i> -4VP ₇₄)	120	52.89	400	9.7	7.2	40.6	18 (80)
P(EtOx ₅₁ - <i>b</i> -4VP ₉₉)	100	35.75	400	5.8	4.3	24.5	52 (66)
P(EtOx ₅₇ - <i>b</i> -4VP ₈₀)	90	29.75	400	5.1	3.7	21.3	45 (60)
P(EtOx ₆₇ - <i>b</i> -4VP ₄₄)	80	24.83	400	4.5	3.3	18.8	11 (53)

^a The value in parentheses represents the planned conversion for 4VP.

2. $^1\text{H-NMR}$ spectra of PEtOx in the reactional medium

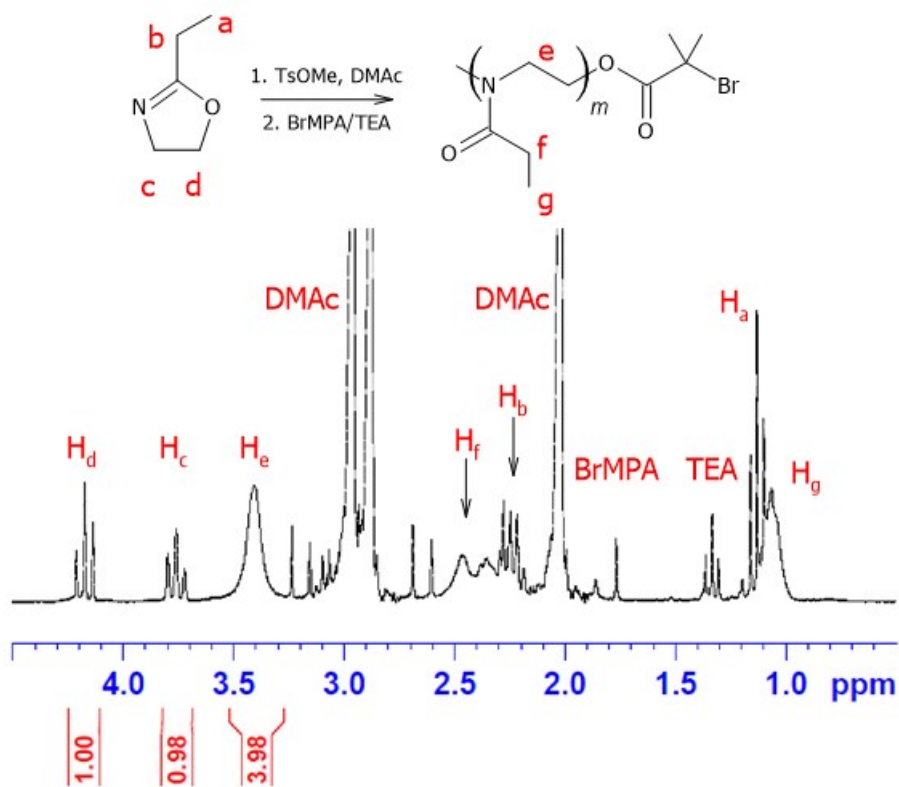


Figure S1. $^1\text{H-NMR}$ spectrum of the reactional medium after the termination reaction for PEtOx₃₀ synthesis.

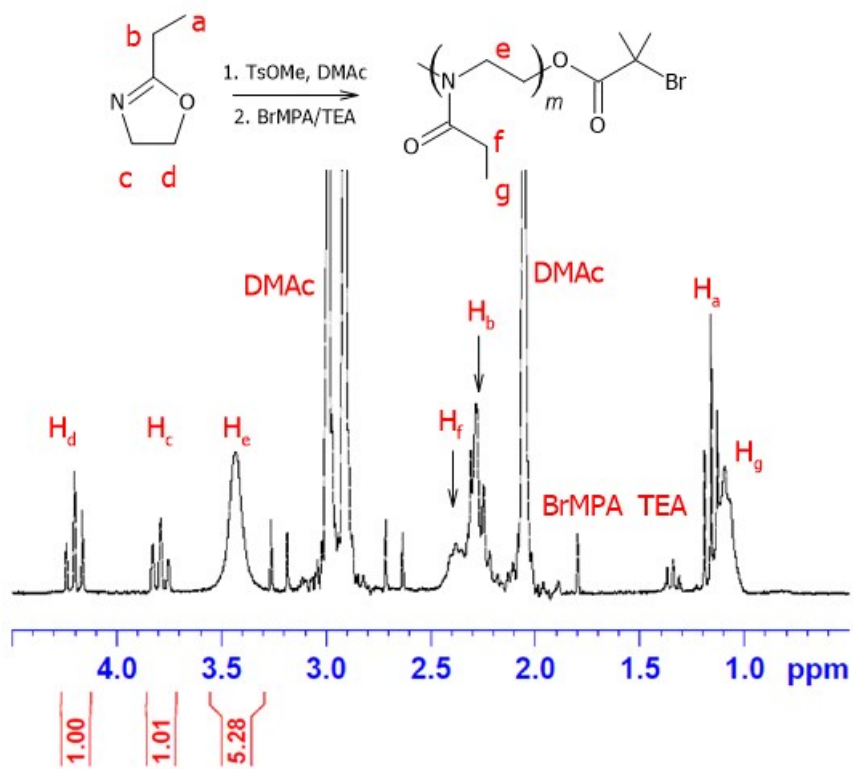


Figure S2. ¹H-NMR spectrum of the reactional medium after the termination reaction for PETox₅₁ synthesis.

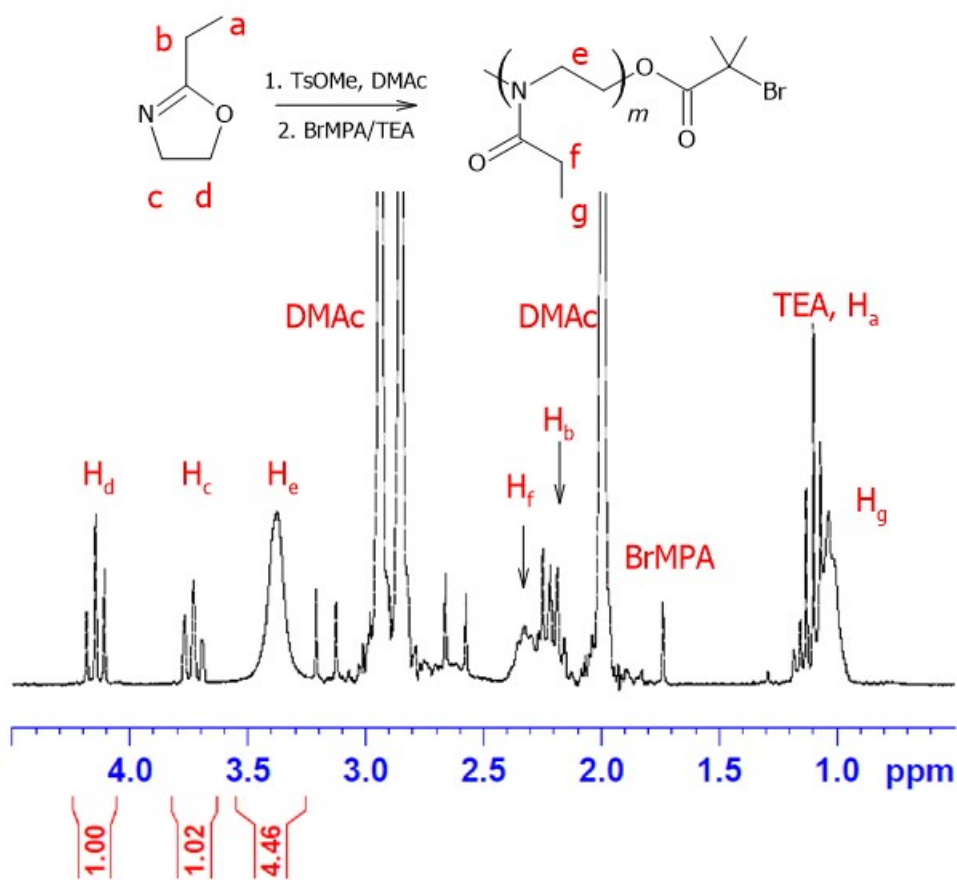


Figure S3. ¹H-NMR spectrum of the reactional medium after the termination reaction for PETox₅₉ synthesis.

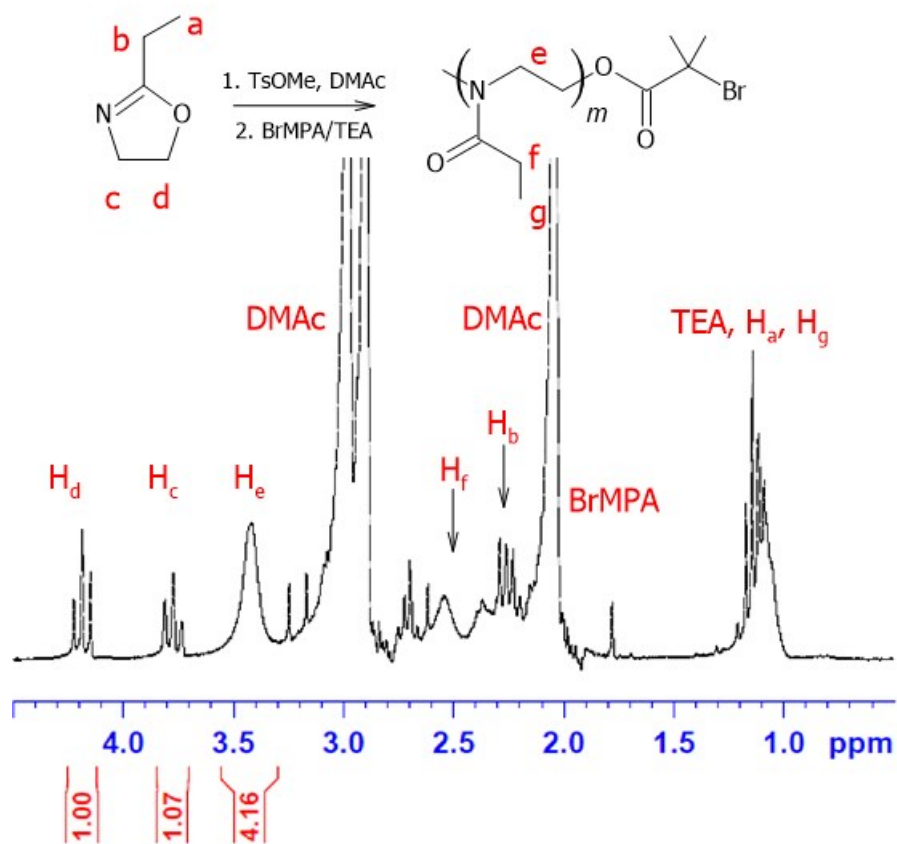


Figure S4. ¹H-NMR spectrum of the reactional medium after the termination reaction for PETox₆₇ synthesis.

3. Monomer Conversion, degree of functionalization of PEtOx and degree of polymerization of PEtOx and P4VP blocks

Conversion (c) for EtOx polymerization was calculated using Equation S1 and $^1\text{H-NMR}$ data from Figures S1-S4. The degree of polymerization of PEtOx (DP_{PEtOx}) was calculated using Equation S2. The degree of functionalization of PEtOx₃₀ was calculated using Equation S3 and $^1\text{H-NMR}$ data from Figures S5-S8. The molar fraction (x_{P4VP}) and the degree of the polymerization of P4VP (DP_{P4VP}) were calculated using Equations S4 and S6, respectively, and $^1\text{H-NMR}$ data from Figures S10-S13.

$$c = \frac{A_{H_e}}{A_{H_c} + A_{H_d} + A_{H_e}}$$

Equation S1

$$DP_{PEtOx} = \frac{[M]_0}{[I]_0} c$$

Equation S2

$$DF = \frac{4A_{H_i}}{6A_{H_e}} \times DP_{PEtOx} \times 100 (\%)$$

Equation S3

$$x_{P4VP} = \frac{0.5 A_{H_p}}{0.25A_{H_e} + 0.5 A_{H_p}}$$

Equation S4

$$DP_{P4VP} = DP_{PEtOx} \times \frac{x_{P4VP}}{(1 - x_{P4VP})}$$

Equation S5

where A_{H_e} , A_{H_c} , A_{H_d} , A_{H_i} and A_{H_p} correspond to the area of hydrogens H_e , H_c , H_d , H_i and H_p in the respective $^1\text{H-NMR}$ spectrum, $[M]_0$ and $[I]_0$ correspond to the initial concentration of monomer and initiator in EtOx polymerization, respectively, DF correspond to the degree of functionalization and x_{P4VP} correspond to P4VP molar fraction in P(EtOx-*b*-4VP).

4. ^1H -NMR spectra of purified PEtOx

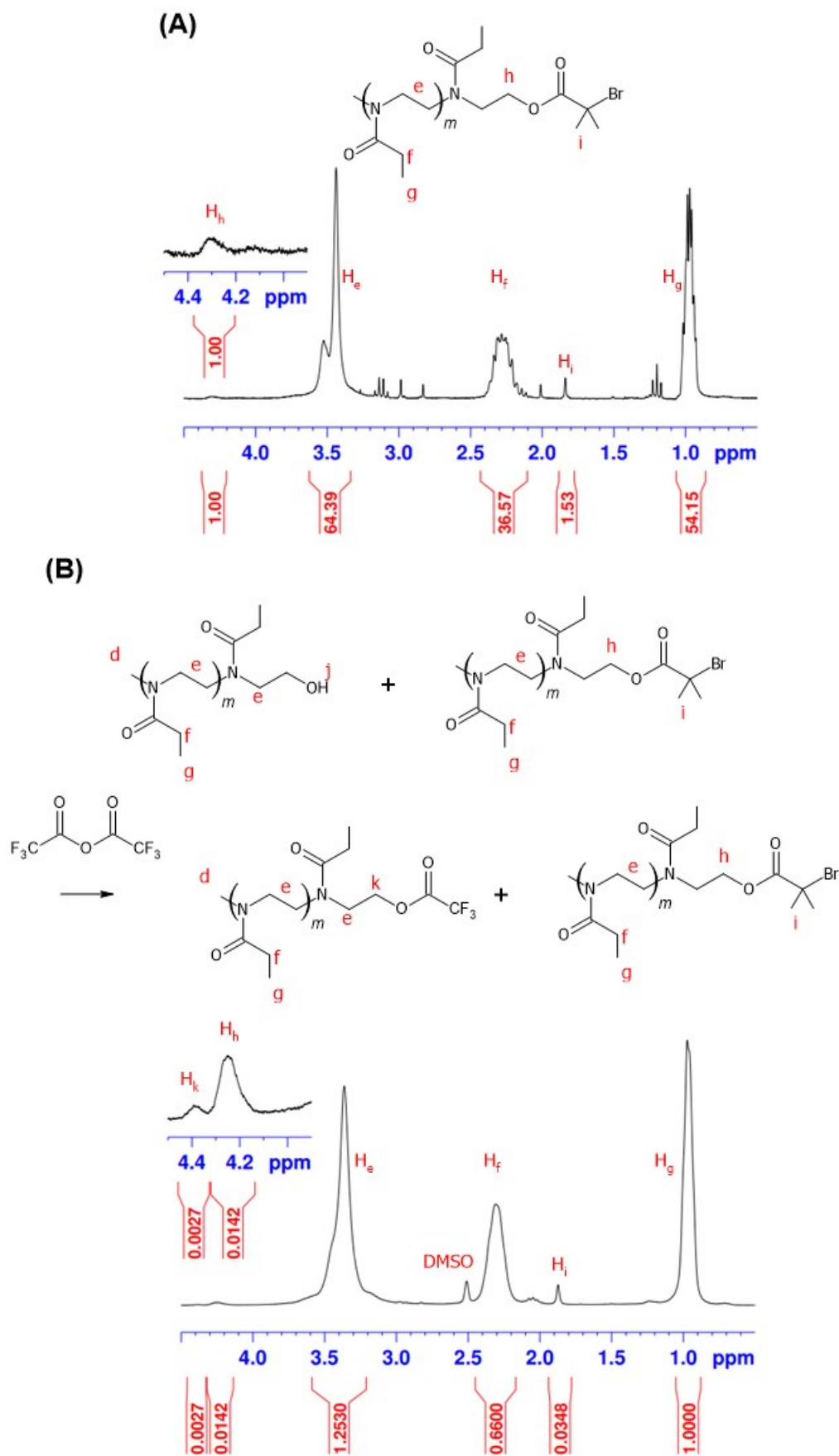


Figure S5. ^1H NMR spectra of purified PEtOx₃₀ in (A) D_2O and (B) DMSO-d_6 after acetylation with trifluoroacetic anhydride.

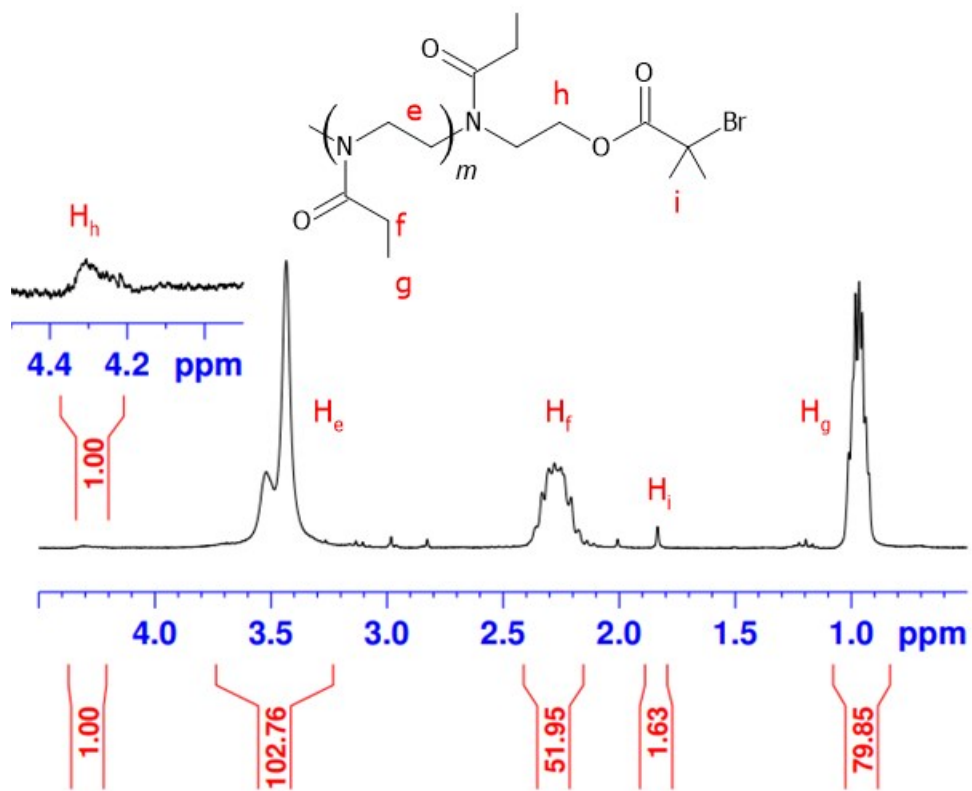


Figure S6. ^1H NMR spectrum of purified PEtOx₅₁ in D₂O.

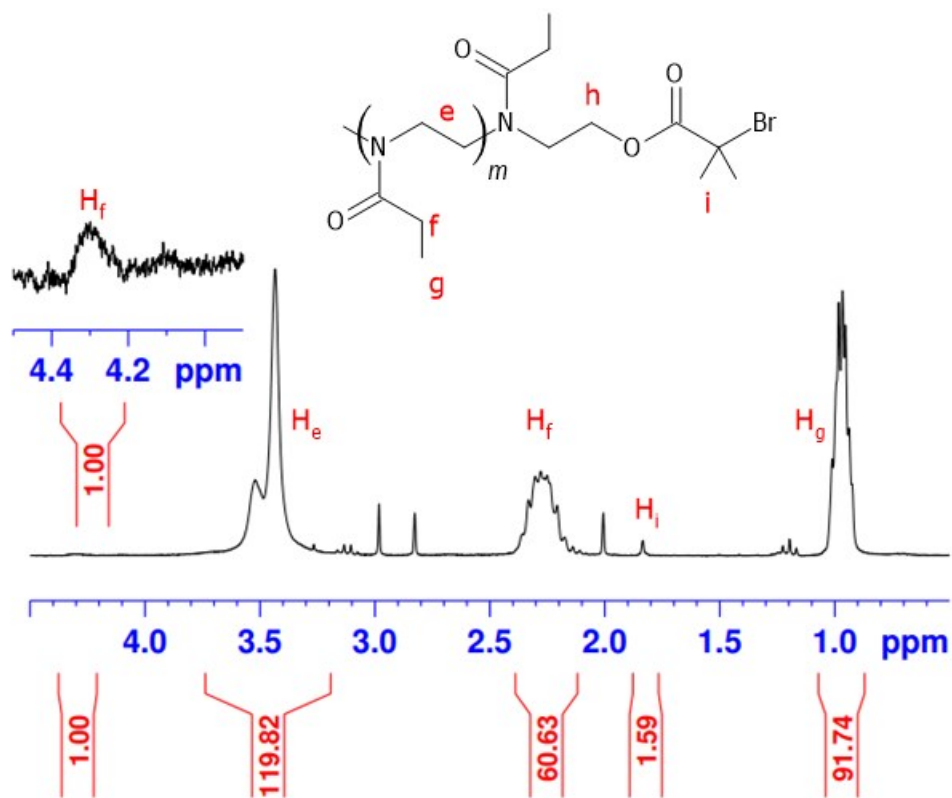


Figure S7. ^1H NMR spectrum of purified PEtOx₅₉ in D_2O .

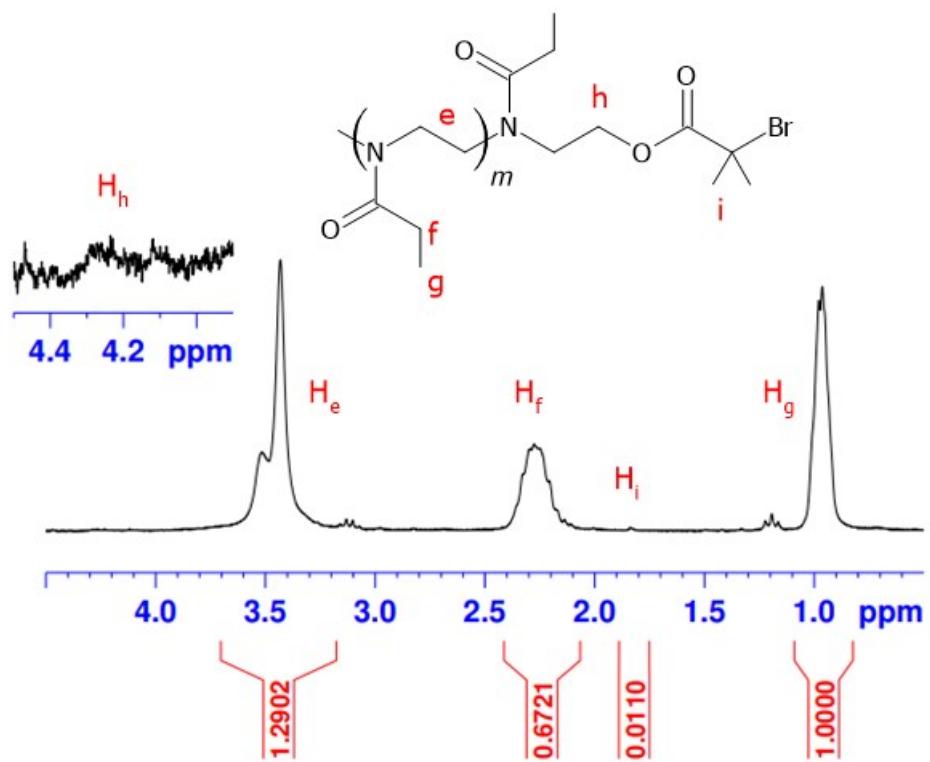


Figure S8. ^1H NMR spectrum of purified PETox₆₇ in D₂O.

5. Kinetic study of 4VP polymerization using PEtOx₅₁ as a macroinitiator.

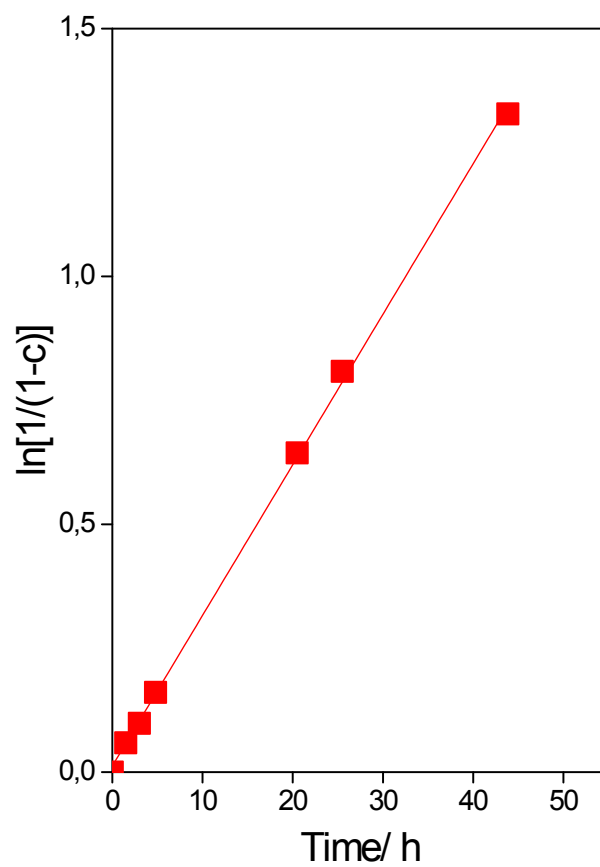


Figure S9. $\ln[1/(1-c)]$ vs. t for the polymerization of 4VP using PEtOx₅₁ as a macroinitiator.

6. ^1H -NMR spectrum of purified P(EtOx-*b*-4VP)

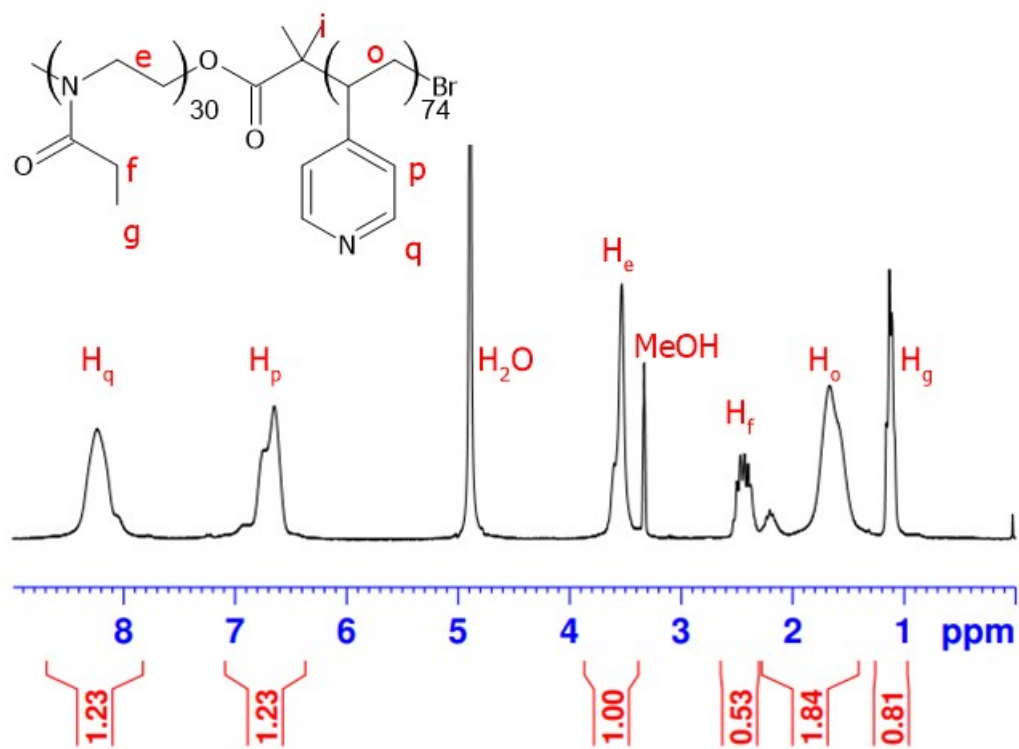


Figure S10. ^1H NMR spectrum of purified P(EtOx₃₀-*b*-4VP₇₄) in MeOD.

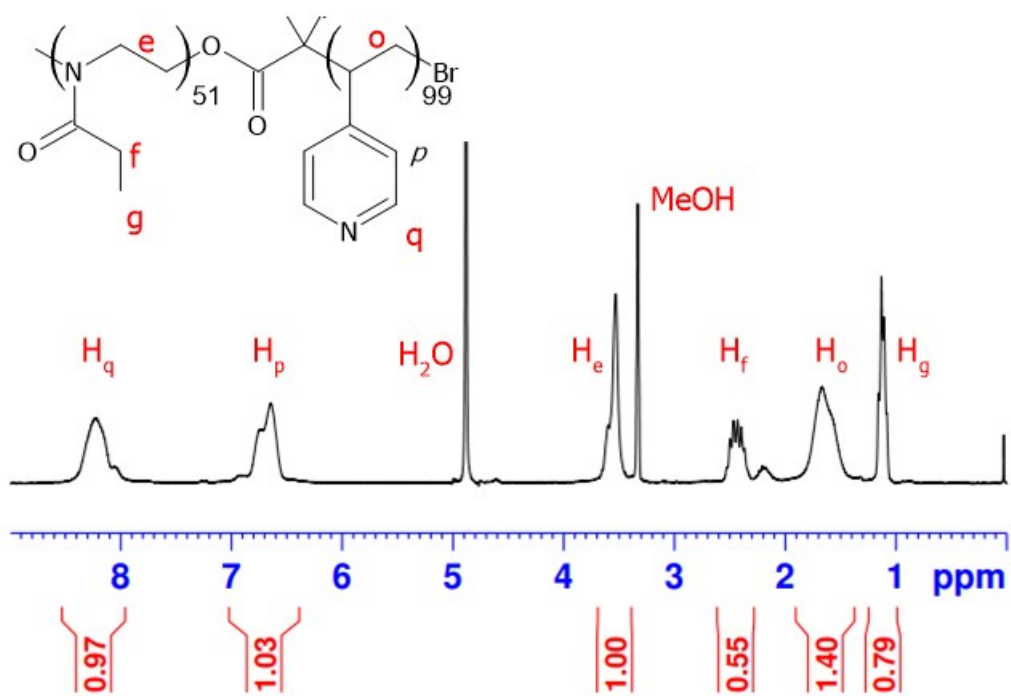


Figure S11. ¹H NMR spectrum of purified P(EtOx₅₁-*b*-4VP₉₉) in MeOD.

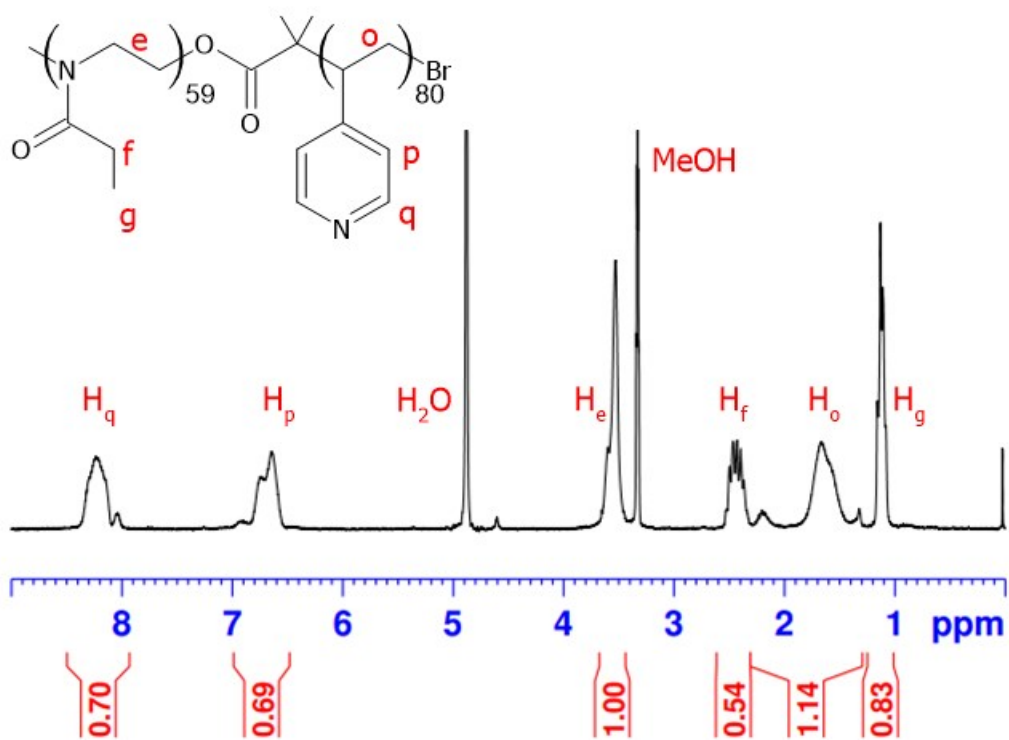


Figure S12. ¹H NMR spectrum of purified P(EtOx₅₉-*b*-4VP₈₀) in MeOD.

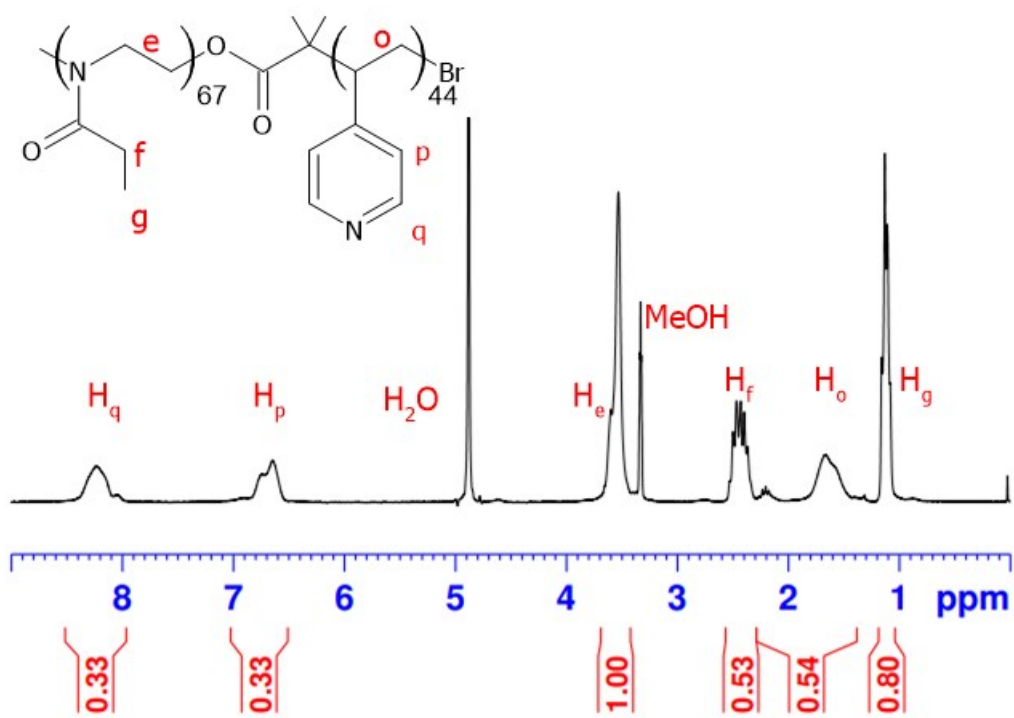


Figure S13. ^1H NMR spectrum of purified $\text{P}(\text{EtOx}_{67}\text{-}b\text{-}4\text{VP}_{44})$ in MeOD .

7. GPC chromatograms of PEtOx and P(EtOx-*b*-4VP)

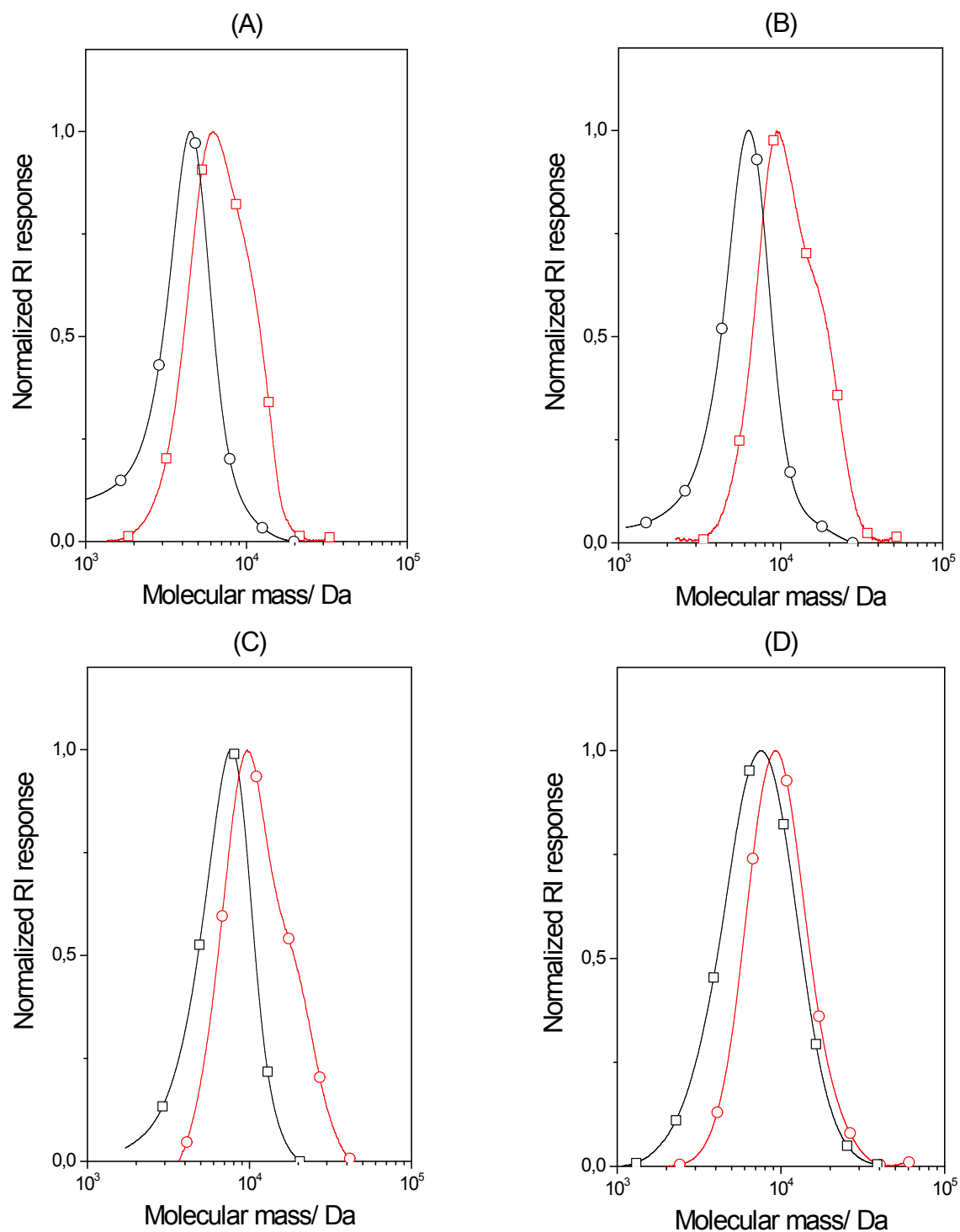


Figure S14. GPC Chromatograms of **(A)** PEtOx₃₀ (□) and P(EtOx₃₀-*b*-4VP₇₄) (○), **(B)** PEtOx₅₁ (□) and P(EtOx₅₁-*b*-4VP₉₉) (○), **(C)** PEtOx₅₉ (□) and P(EtOx₅₉-*b*-4VP₈₀) (○) and **(D)** PEtOx₆₇ (□) and P(EtOx₆₇-*b*-4VP₄₄) (○) in THF.

8. Are P(EtOx-*b*-4VP) copolymer free of P4VP and PEtOx homopolymers?

The copolymers were purified by dialysis. The material released by the dialysis membrane was freeze-dried, solubilized in D₂O, acidified with 20 μ L of concentrated hydrochloric acid, and analyzed by ¹H-NMR. The GPC chromatograms of the reactional medium and purified P(EtOx₃₀-*b*-4VP₇₄) (Figure S15A) showed that the polymer chains with lower molar mass were removed from the reactional medium by dialysis. On the other hand, the ¹H-NMR spectrum of the released material in the dialysis (Figure S15B) proved that PEtOx and residual 4VP monomer were removed by dialysis.

In order to evaluate the presence of residual homopolymeric P4VP in the P(EtOx-*b*-4VP), aqueous solutions of the P4VP, P(EtOx₅₁-*b*-4VP₉₉) and mixtures of PEtOx/P4VP and P(EtOx₅₁-*b*-4VP₉₉)/P4VP were prepared at pH = 2.5 and pH was further adjusted to 8. P4VP was synthesized using the same procedure adopted for the block copolymers, however, propargyl 2-bromoisbutyrate (25 mg, 0.124 mmol, according to Tsarevsky et. al ¹) was used as initiator and the reaction time was 5 h. The number average molar mass, M_n, estimated by GPC using the same procedure adopted for the block copolymers, was 5.3 kDa.

Images of the solutions/dispersions at different pH are shown in Figure S16. Solutions of P4VP and its mixtures with PEtOx and P(EtOx₅₁-*b*-4VP₉₉) were transparent at pH=2.5, because PEtOx is a water-soluble polymer as well as P4VP in the protonated state. However, the increase in the pH to 8 resulted in the precipitation of P4VP in the mixtures. On the other hand, the copolymer solution became hazy and bluish with the increase in the pH and no precipitate was observed. The bluish aspect was observed for P(EtOx₅₁-*b*-4VP₉₉)/P4VP mixtures too, besides the precipitate (Figures S16C and S16D). In the self-assembly experiments described in our study no precipitate was observed.

DLS analyses of the crude solutions/mixtures revealed one population of the scattering objects with hydrodynamic diameter, D_h, higher than 600 nm and 400 nm for the P4VP and PEtOx/P4VP solutions at pH = 8, respectively (Figures S17A and S17B). A population of particles with average D_h lower than 100 nm was observed for the copolymer solution at pH = 8 (Figure S17E), while the D_h distributions by number of the supernatant of the aqueous mixtures of P4VP and P(EtOx₅₁-*b*-4VP₉₉)

presented two populations of particles with average D_h lower than 100 nm and higher 100 nm (Figures S17C and S17D) attributed to copolymer and P4VP aggregates, respectively.

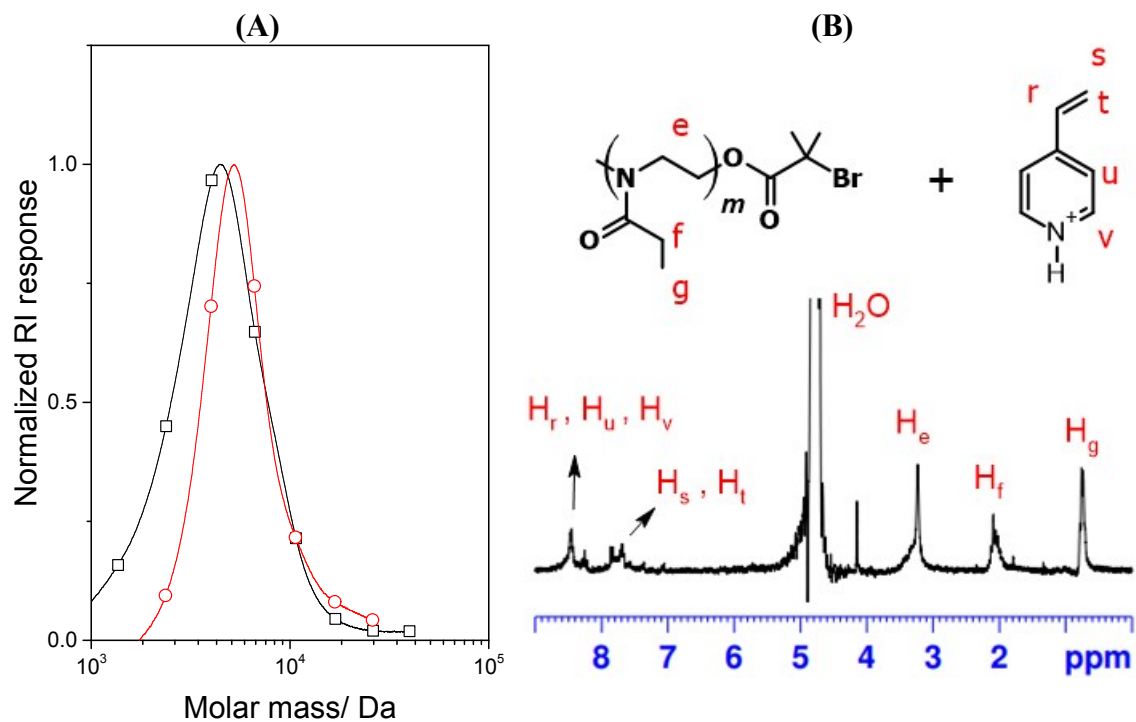


Figure S15. (A) GPC Chromatograms of P(EtO_x₃₀-b-4VP₇₄) reactional medium before (□) and after 1 day dialysis (●). (B) ¹H-NMR spectrum in D₂O of the released material in the dialysis.

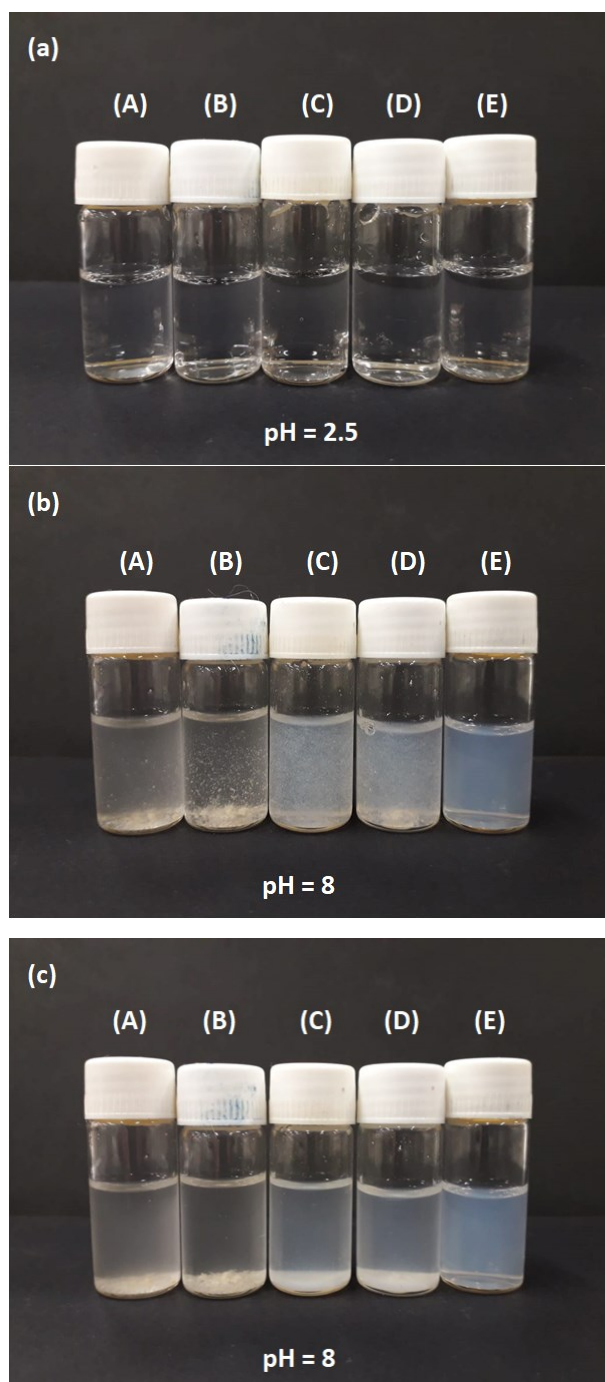


Figure S16. Images of aqueous solutions of the **(A)** P4VP, **(B)** P_{EtOx}/P4VP homopolymers mixture (50 % m/m of P4VP), **(C)** P(EtOx₅₁-*b*-4VP₉₉)/P4VP mixture (50 % m/m of P4VP), **(D)** P(EtOx₅₁-*b*-4VP₉₉)/P4VP mixture (10 % m/m of P4VP) and **(E)** P(EtOx₅₁-*b*-4VP₉₉). **(a)** pH = 2.5; **(b)** just after pH adjustment to 8 and **(c)** after 60 min at pH = 8.

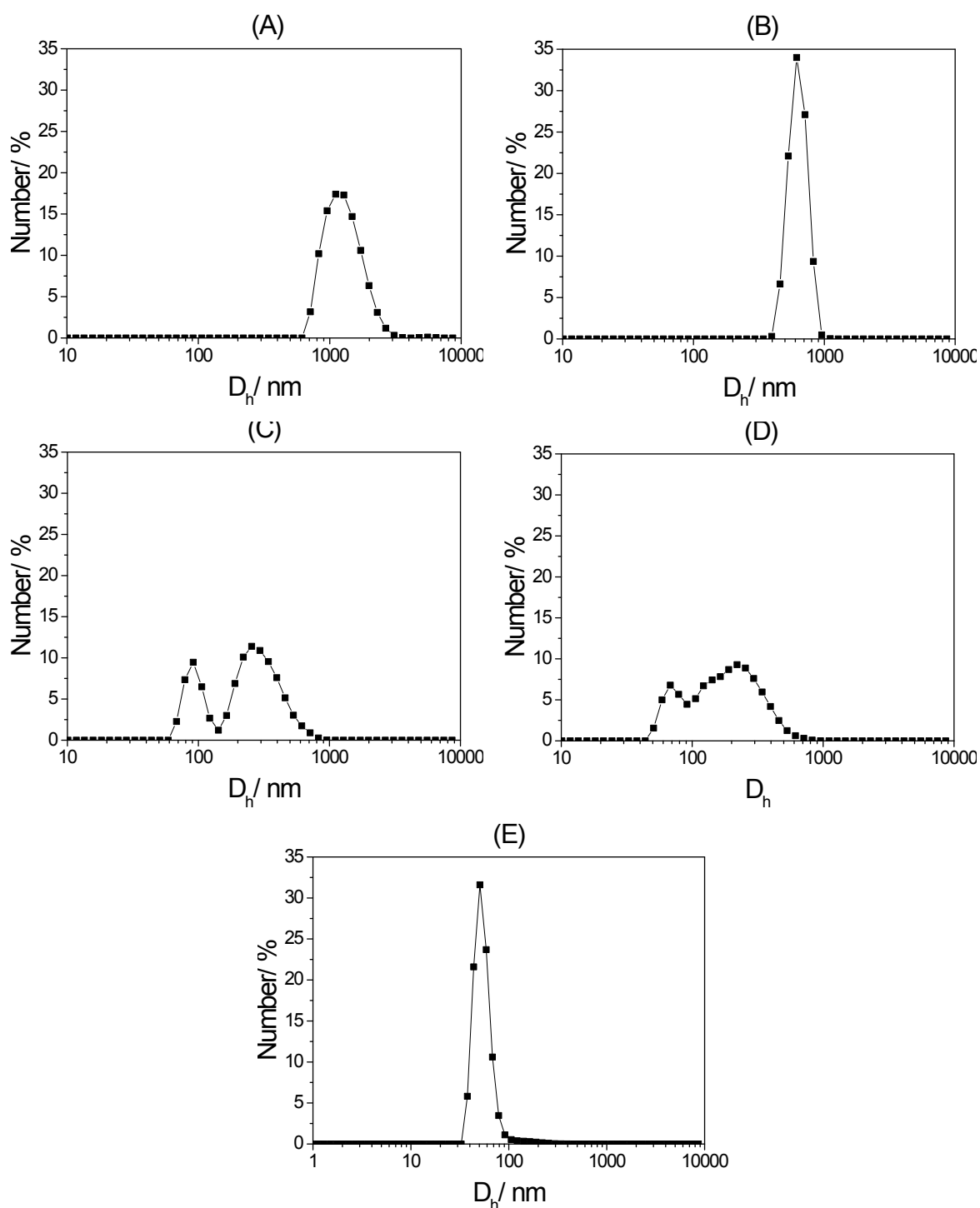


Figure S17. Size distribution curves (in terms of hydrodynamic diameter, D_h) of the aggregates in the aqueous solutions of the **(A)** P4VP, **(B)** P4VP/P4VP homopolymers mixture (50 % m/m of P4VP), **(C)** P4VP/P4VP mixture (50 % m/m of P4VP), **(D)** P4VP/P4VP mixture (10 % m/m of P4VP) and **(E)** P4VP at pH = 8.

9. DOSY spectrum of PEtOx in D₂O/MeOD/4VP

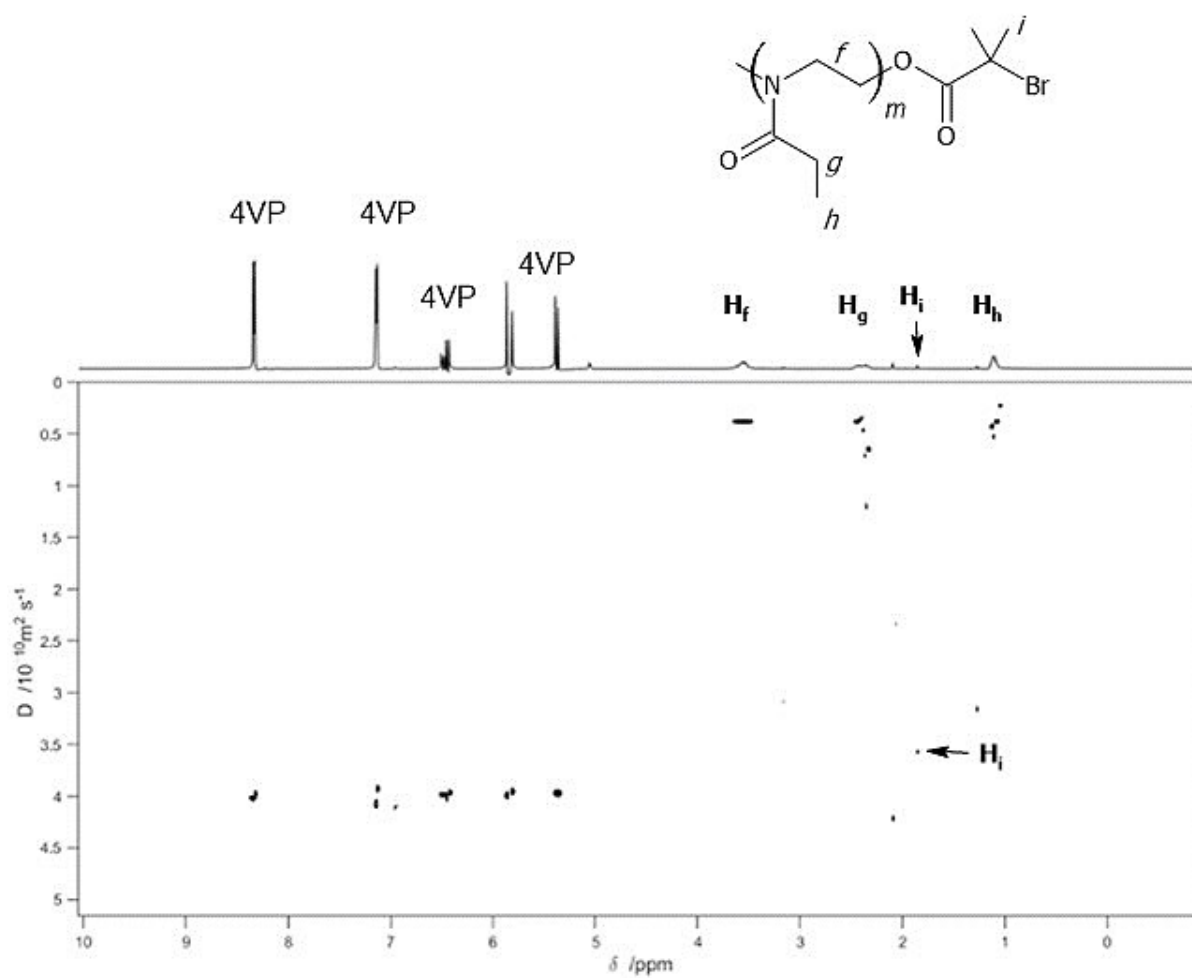


Figure S18. DOSY spectrum of PEtOx₃₀-BrMP in D₂O/MeOD/4VP.

10. Determination of c.m.c by fluorescence spectroscopy

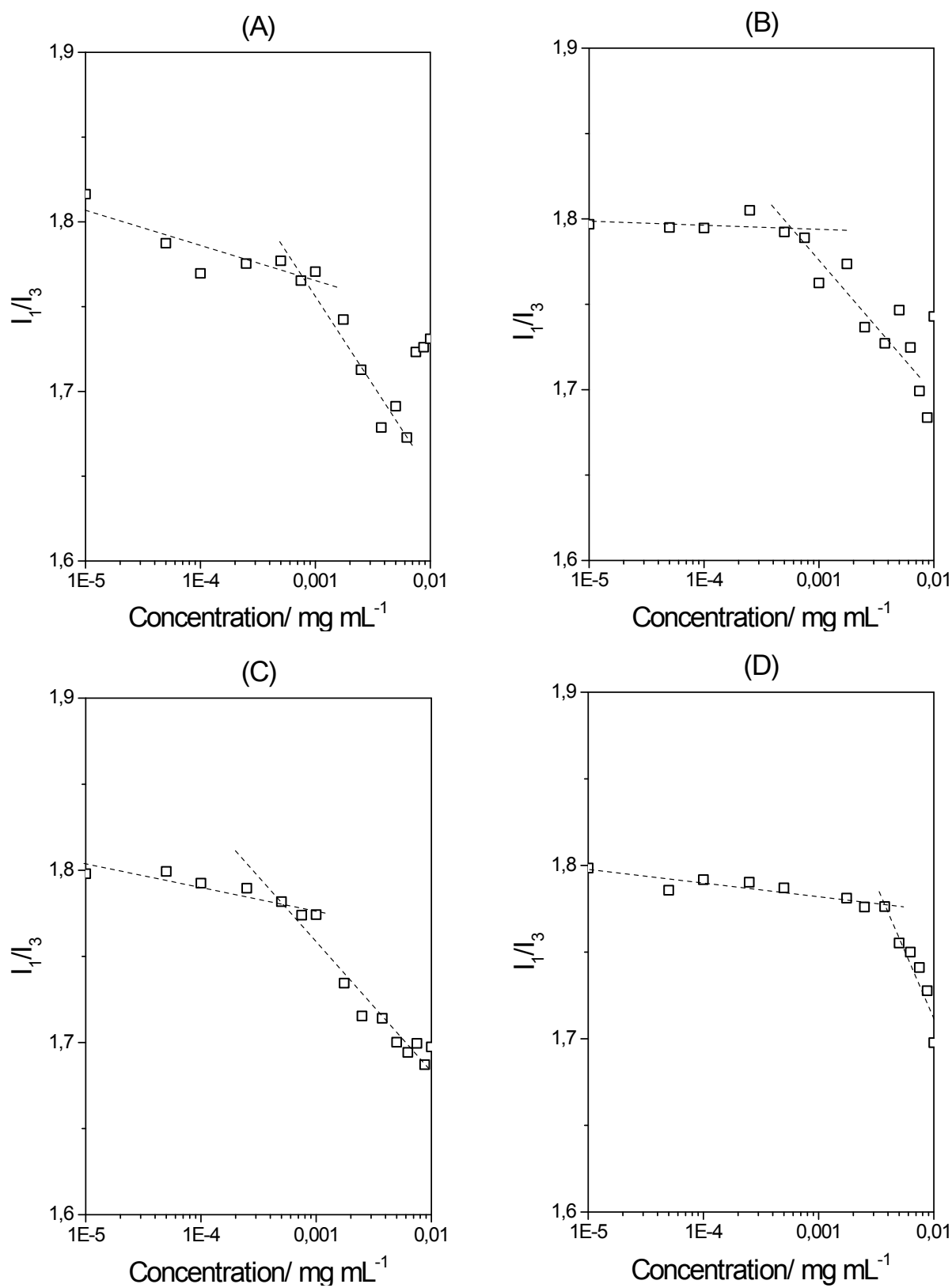


Figure S19. I_1/I_3 vs concentration for **(A)** P(EtOx₃₀-*b*-VP₇₄), **(B)** P(EtOx₅₁-*b*-VP₉₉), **(C)** P(EtOx₅₇-*b*-VP₈₀) and **(D)** P(EtOx₇₀-*b*-VP₄₄) solutions.

11. Transmittance vs. water content for P(EtOx-*b*-4VP) in water/DMF solution

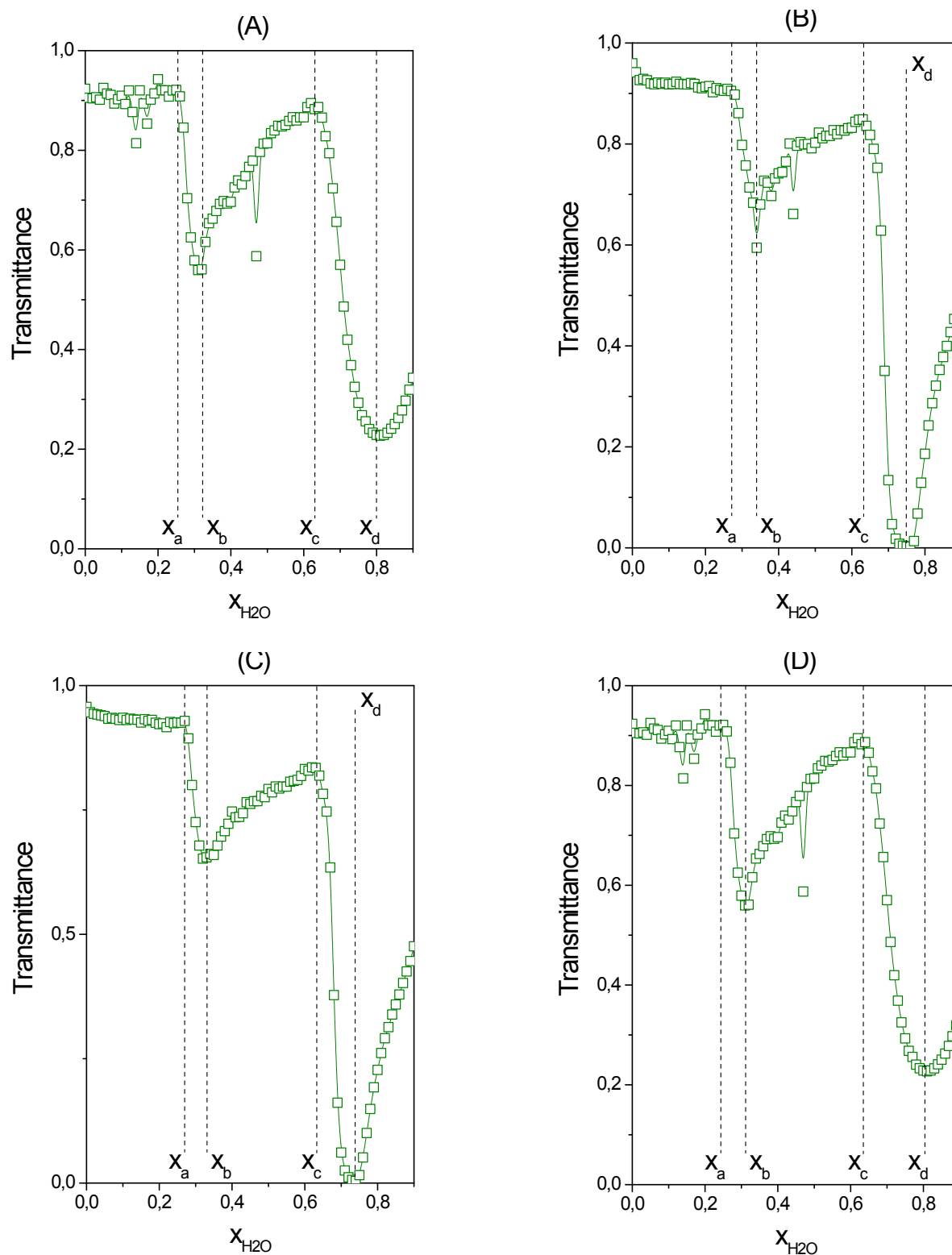


Figure S20. Transmittance at 600 nm of **(A)** P(EtOx₃₀-*b*-VP₇₄), **(B)** P(EtOx₅₁-*b*-VP₉₉), **(C)** P(EtOx₅₇-*b*-VP₈₀) and **(D)** P(EtOx₇₀-*b*-VP₄₄) in DMF/water mixtures as a function of water mass fraction (x_{H_2O}).

12. Size distribution curves of the structures formed by P(EtOx-*b*-VP) in water/DMF solutions

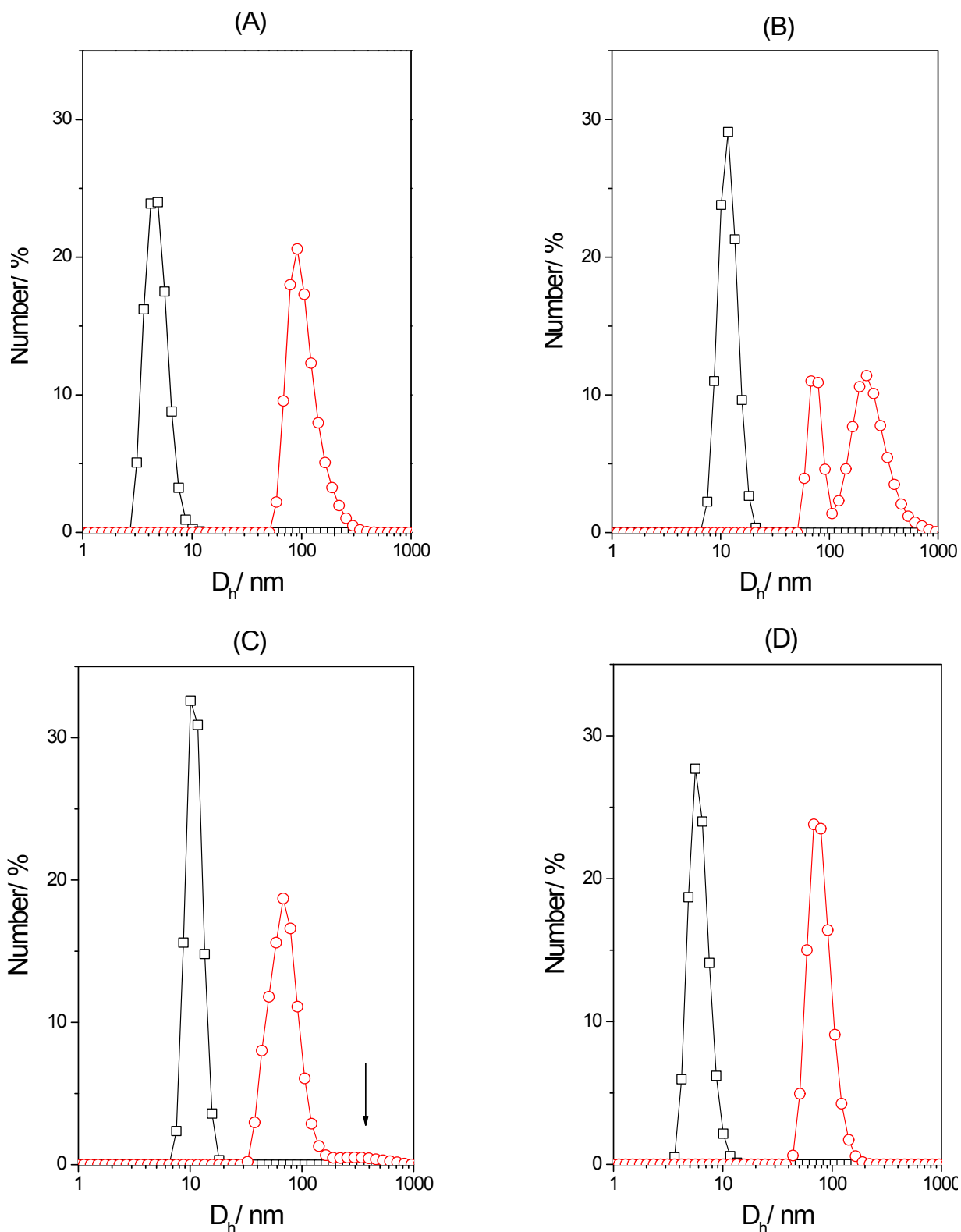


Figure S21. Size distribution curves (in terms of hydrodynamic diameter, D_h) of the structures formed by **(A)** P(EtOx₃₀-*b*-VP₇₄), **(B)** P(EtOx₅₁-*b*-VP₉₉), **(C)** P(EtOx₅₇-*b*-VP₈₀) and **(D)** P(EtOx₇₀-*b*-VP₄₄) in water/DMF solutions with $x_{H_2O} = 0.6$ (□) e 0.9 (○).

13. Size distribution curves of the structures formed by P(EtOx-*b*-VP) at pH \approx 2.5

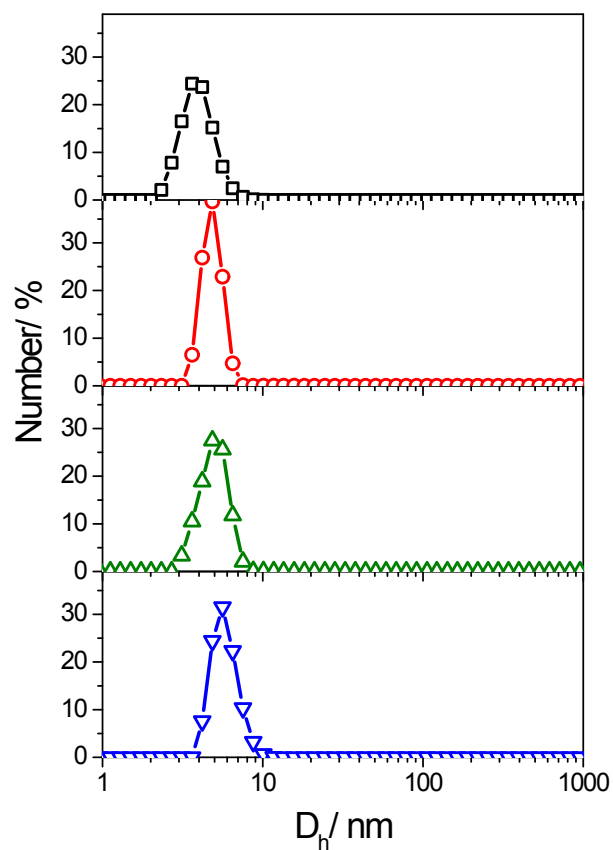


Figure 22. Size distribution curves (in terms of hydrodynamic diameter, D_h) of the structures formed by P(EtOx₃₀-*b*-VP₇₄) (●), P(EtOx₅₁-*b*-VP₉₉) (⊗), P(EtOx₅₇-*b*-VP₈₀) (◻) and P(EtOx₇₀-*b*-VP₄₄) (◊) in aqueous medium at pH \approx 2.5.

14. DLS results - Size distribution curves of the structures formed by P(EtOx-*b*-VP) at pH = 8 after 30 days

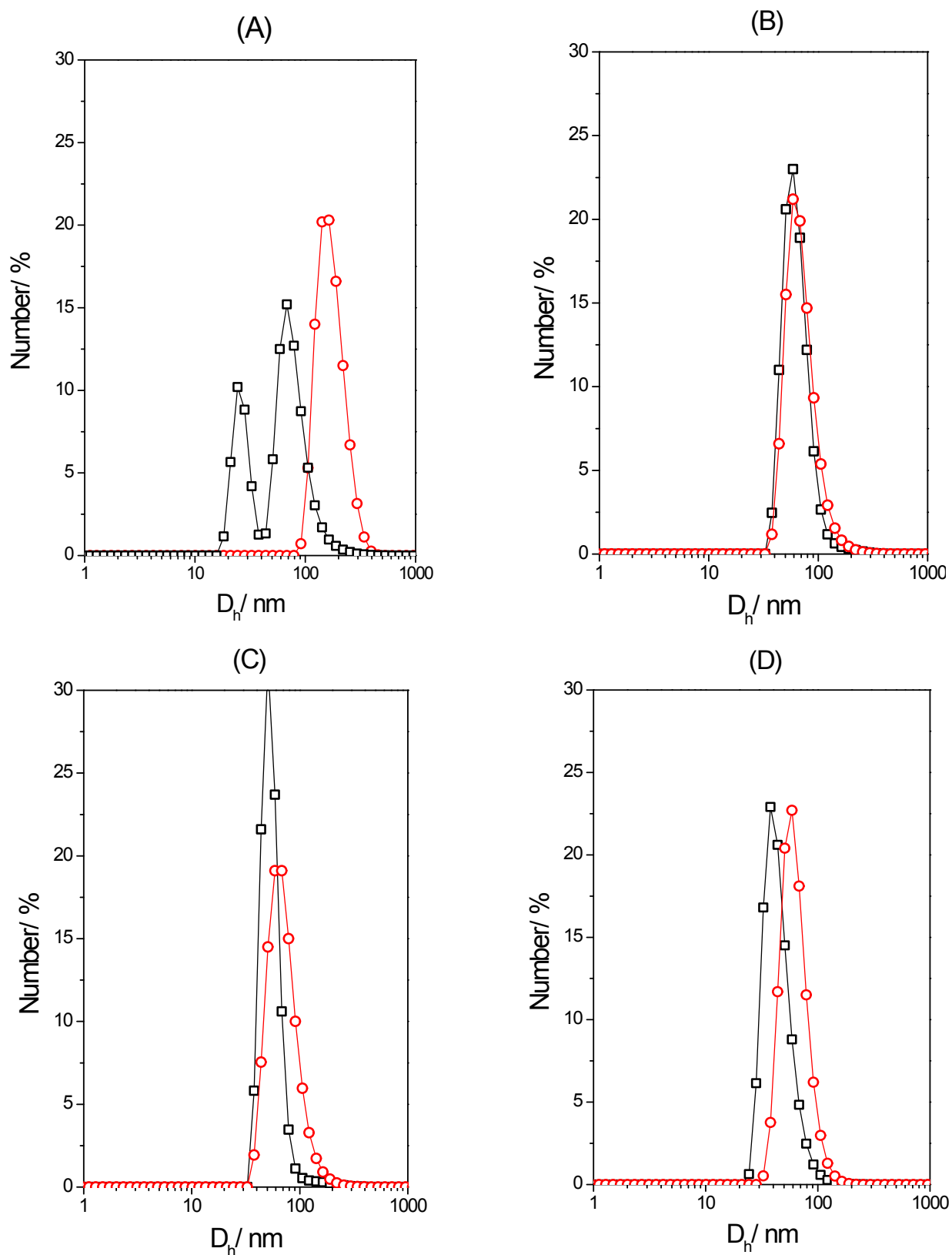


Figure S23. Size distribution curves (in terms of hydrodynamic diameter, D_h) of the structures formed by **(A)** P(EtOx₃₀-*b*-VP₇₄), **(B)** P(EtOx₅₁-*b*-4VP₉₉), **(C)** P(EtOx₅₉-*b*-4VP₈₀) and **(D)** P(EtOx₆₇-*b*-4VP₄₄) after the preparation (●) and after 30 days (○).

15. References

- 1 N. V. Tsarevsky, B. S. Sumerlin and K. Matyjaszewski, *Macromolecules*, 2005, **38**, 3558–3561.