

Supporting information

Scale-Up of the Manufacturing Process To Produce Docetaxel-Loaded mPEG-b-p(HPMA-Bz) Block Copolymer Micelles for Pharmaceutical Applications

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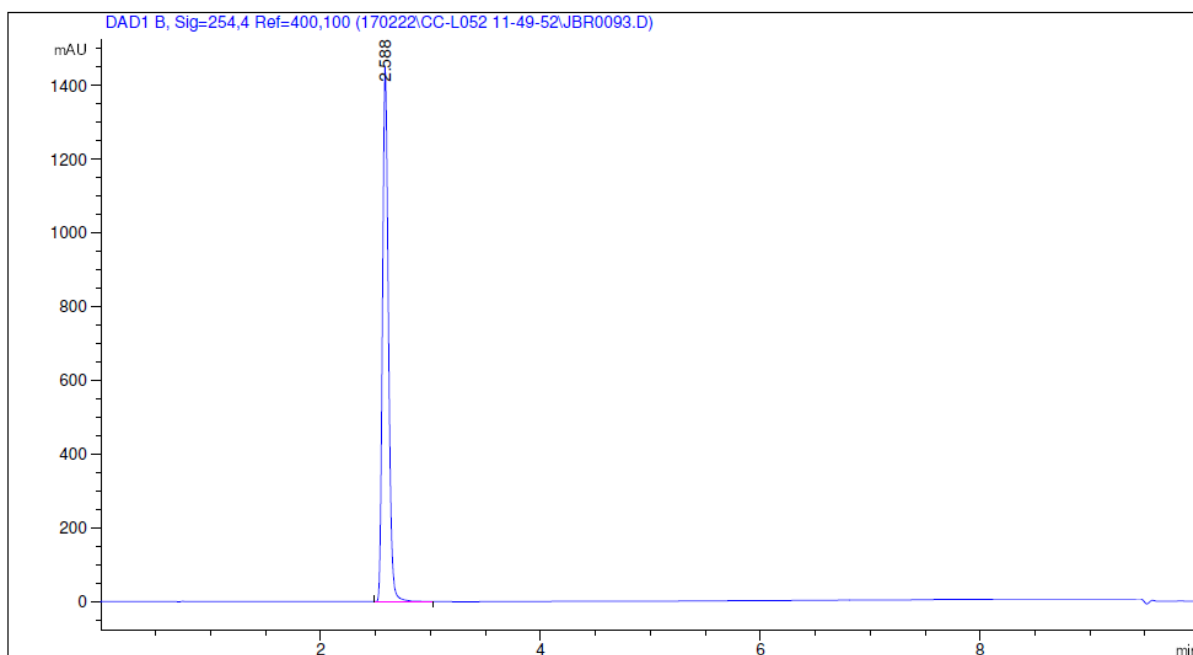


Figure S1. HPLC chromatogram of HPMA (2.6 min) giving a purity of 99.9 %.

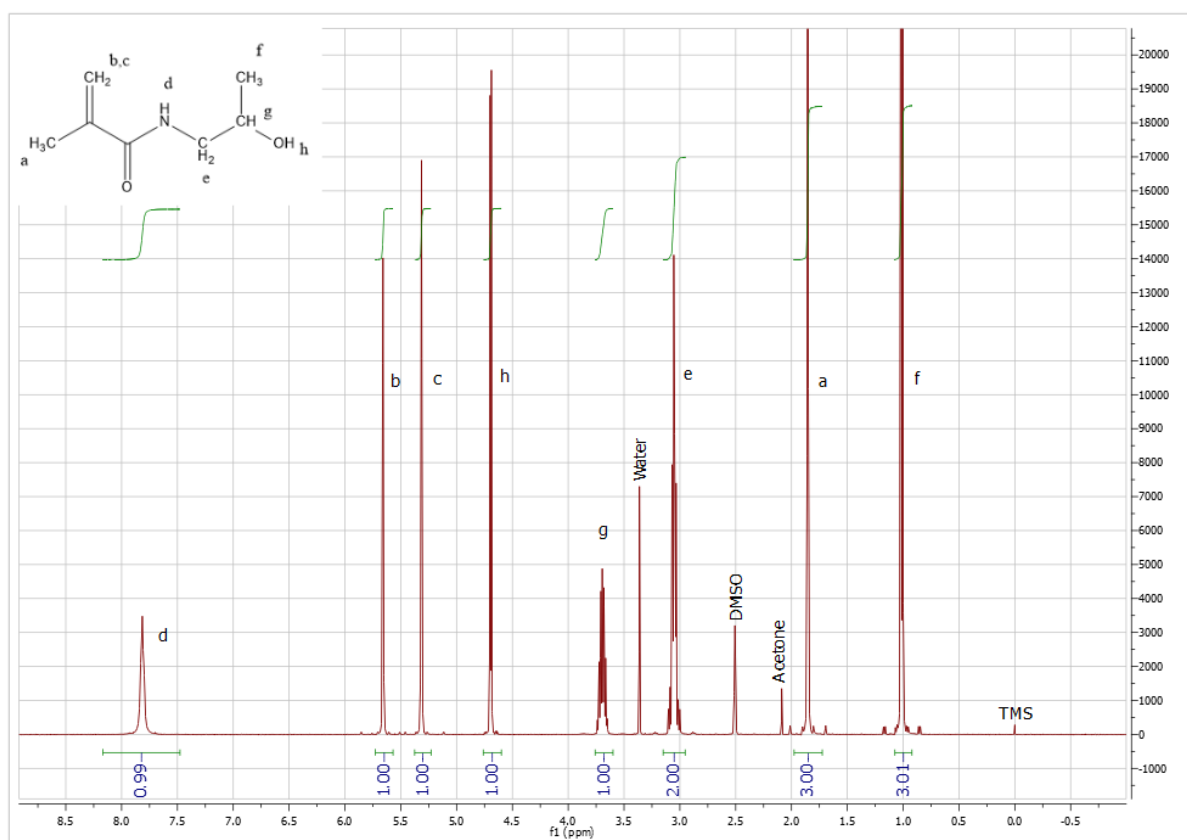


Figure S2. ¹H-NMR spectrum of HPMA.

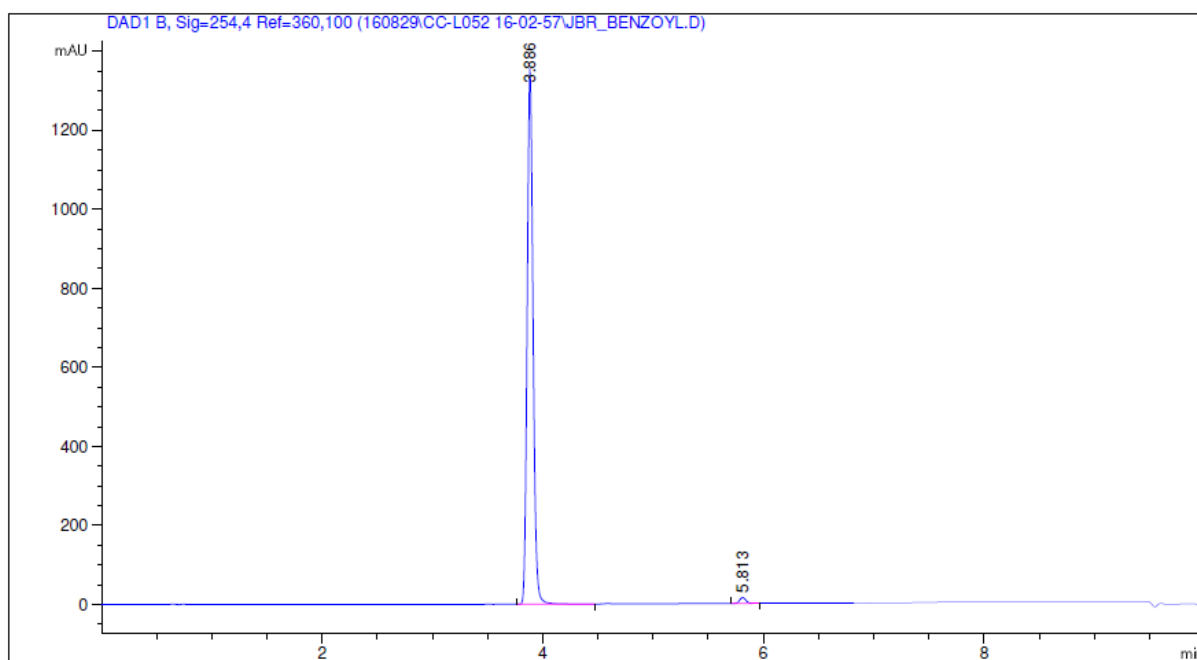


Figure S3. HPLC chromatogram of the benzoyl chloride starting material (3.9 min) which is converted into benzoic acid due to fast reaction with water; at 5.8 min it is already visible that there is also some benzoic anhydride present in the benzoyl chloride or that it is formed during sample preparation.

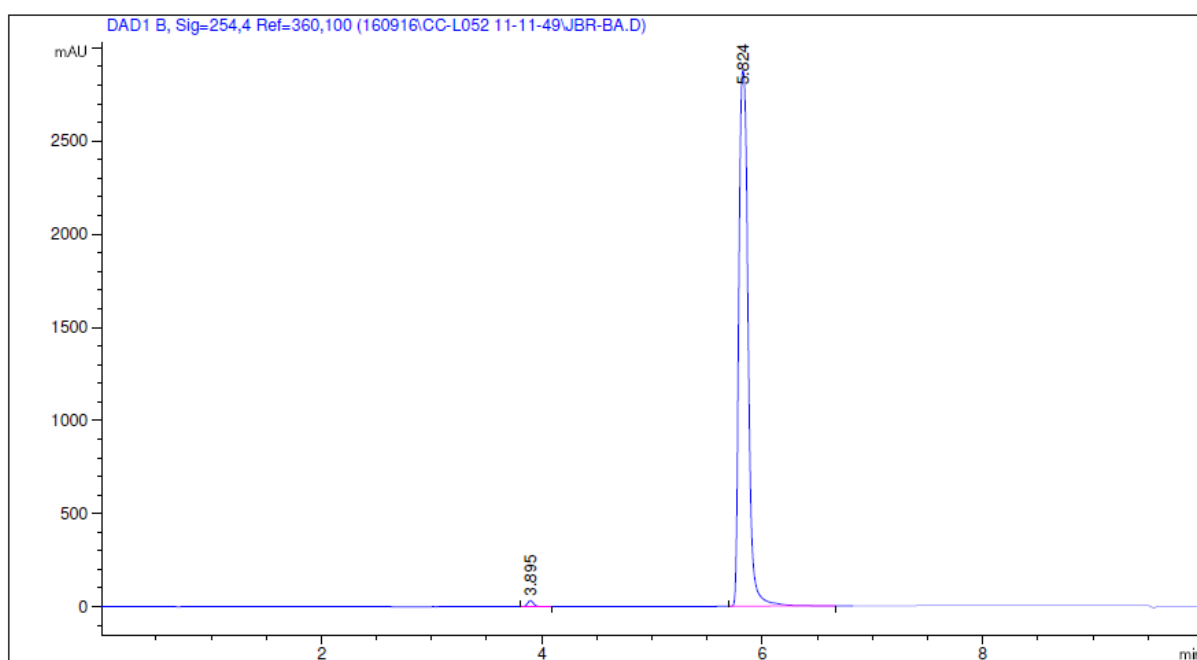


Figure S4. HPLC chromatogram of reference benzoic anhydride (5.8 min); the small peak at 3.9 min is assigned to benzoic acid which is likely formed due to hydrolysis of the anhydride.

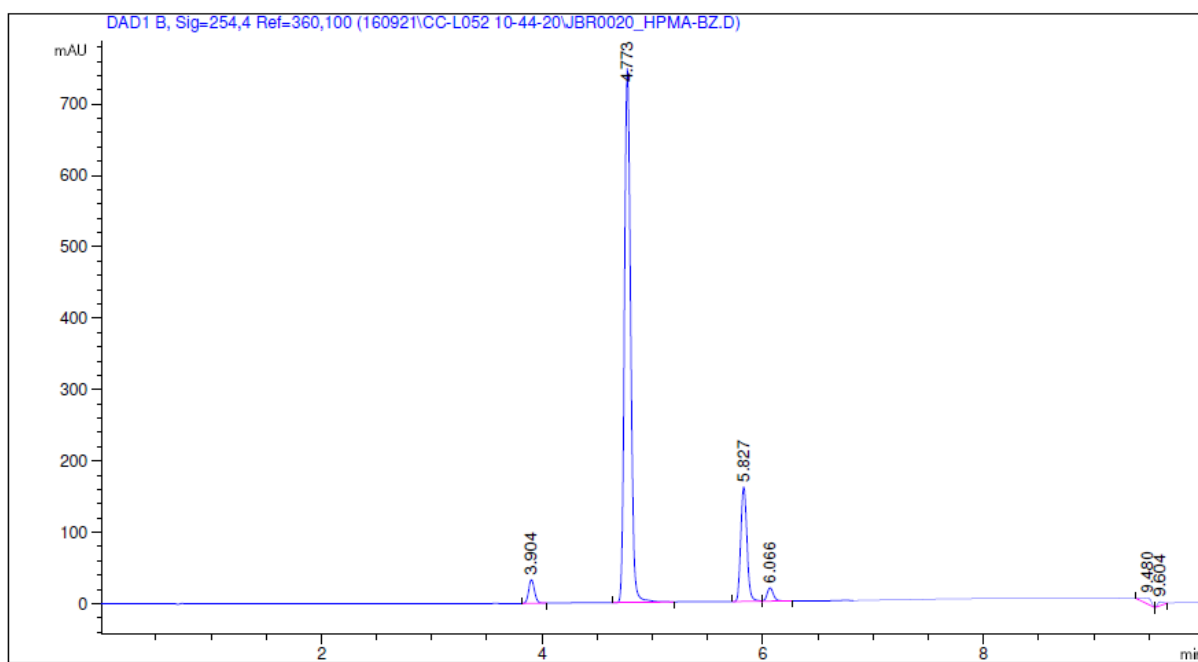


Figure S5. HPLC chromatogram of HPMA-Bz (4.8 min) prior to solid liquid extraction with heptane. The peak at 3.9 min is assigned to benzoic acid and the peak at 5.8 min is assigned to benzoic anhydride.

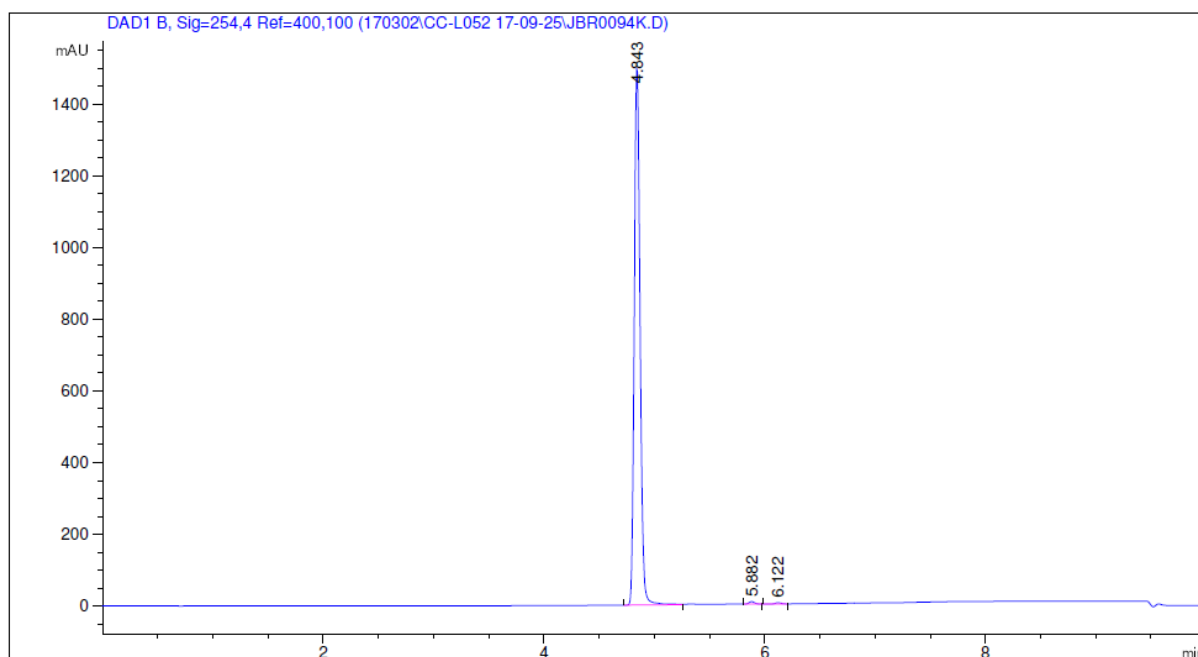


Figure S6. HPLC chromatogram of HPMA-Bz after the solid liquid extraction with heptane; only trace amounts of benzoic anhydride remain, giving a purity of 99.3 %.

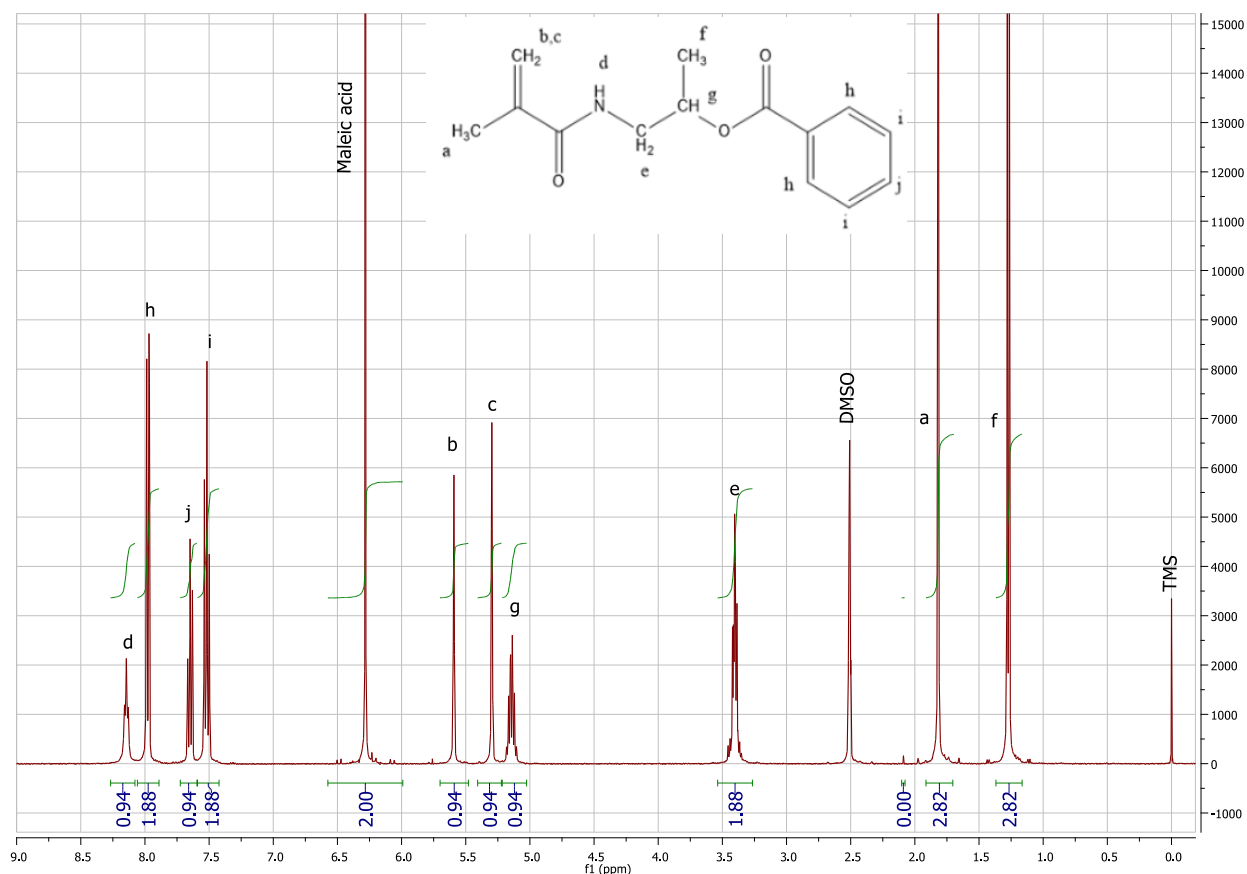


Figure S7. ^1H -NMR spectrum of HPMA-Bz of which the content was determined using maleic acid as internal standard, resulting in a purity of 98.5 % using the equation below.

$$P_x = \frac{I_x * N_{std} * M_x * W_{std}}{I_{std} * N_x * M_{std} * W_x} * P_{std} = \frac{0.94 * 2 * 247.29 \text{ g/mol} * 9.62 \text{ g}}{2 * 1 * 116.072 \text{ g/mol} * 19.55 \text{ g}} * 99.94\% = 98.5 \%$$

Where P_x is the content of the sample (%m/m), P_{std} is the content of the standard (%m/m), I_x is the integration area of the sample at 5.59 ppm, I_{std} is the integration area of the standard at 6.28 ppm, N_x is the number of protons of the integrated peak of the sample, N_{std} is the number of protons of the integrated peak of the standard, M_x is the molecular weight of the sample, M_{std} is the molecular weight of the standard, W_x is what was weighed of the sample (mg) and W_{std} is what was weighed of the standard (mg).

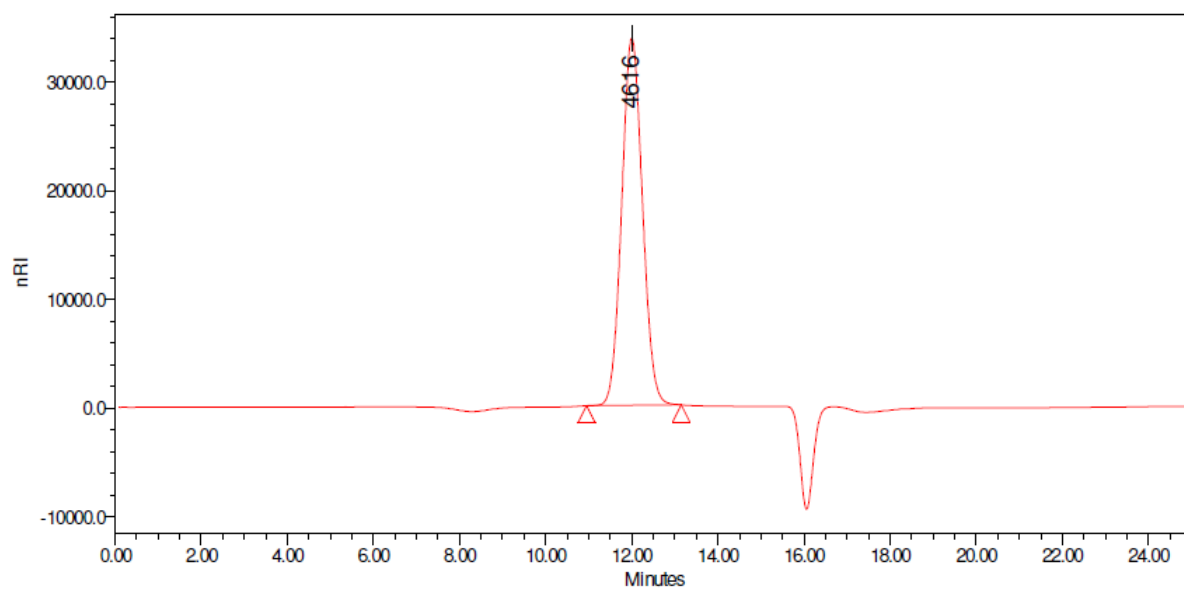


Figure S8. GPC chromatogram of mPEG_{5K}.

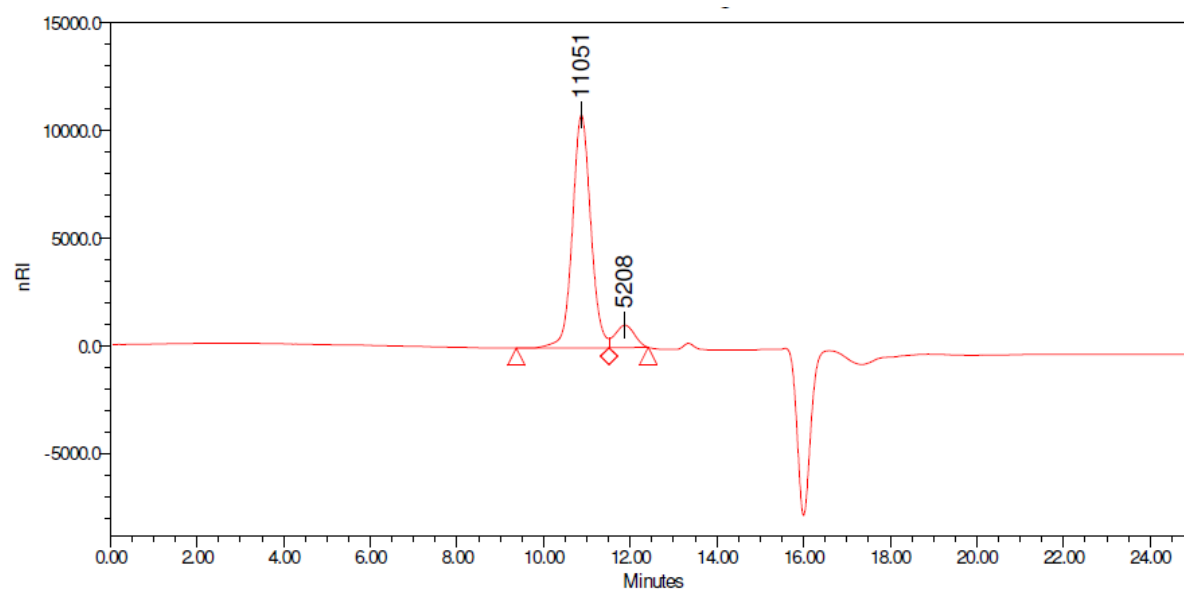


Figure S9. GPC chromatogram of mPEG_{5K}-ABCPA-mPEG_{5K} macro-initiator (peak labelled 11051) with approximately 9 % of free mPEG_{5K} (peak labelled 5208).

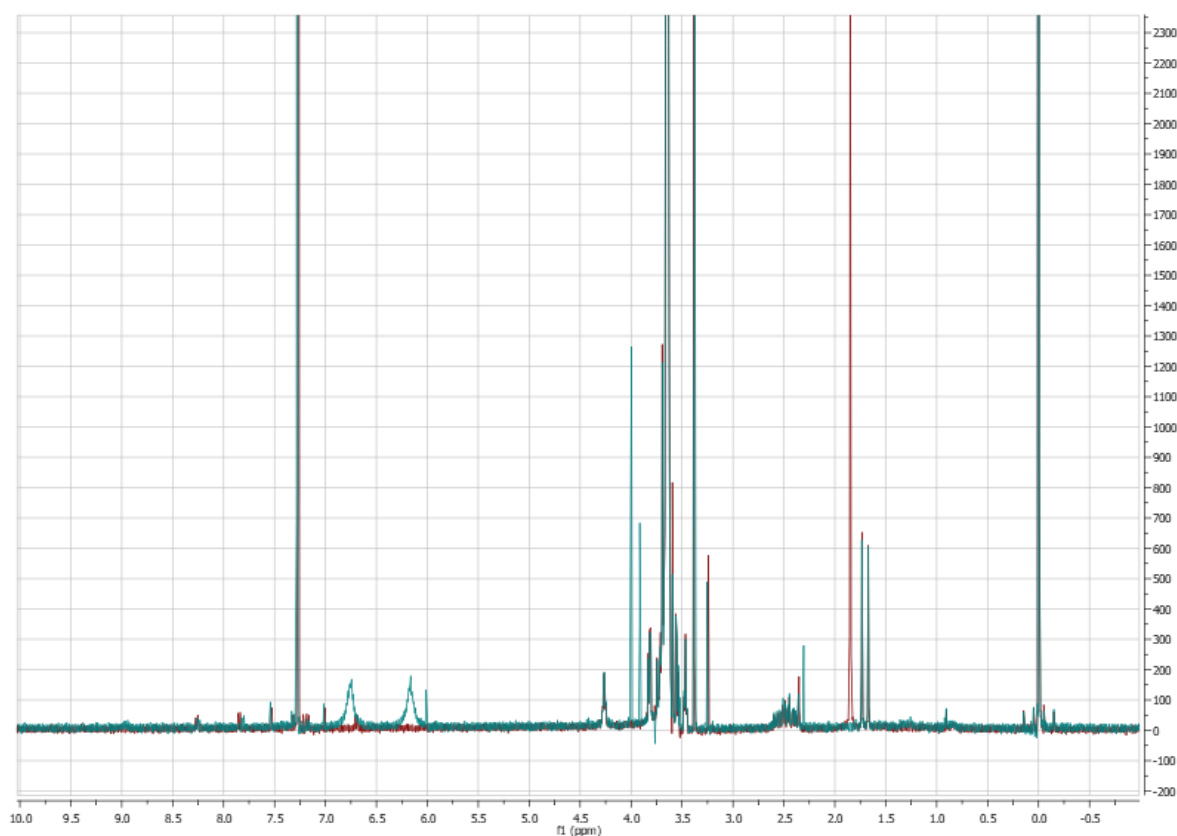


Figure S10. ^1H -NMR spectra of the $\text{mPEG}_{5\text{K}}$ -ABCPA- $\text{mPEG}_{5\text{K}}$ macro-initiator. Red: the spectrum before and Blue: the spectrum after addition of TAIC. The spectra show that the synthesized macroinitiator contains 9 % unreacted mPEG according to the signal of the methylene group neighboring the terminal hydroxyl group shifts from 4.2 to 4.4 ppm.¹

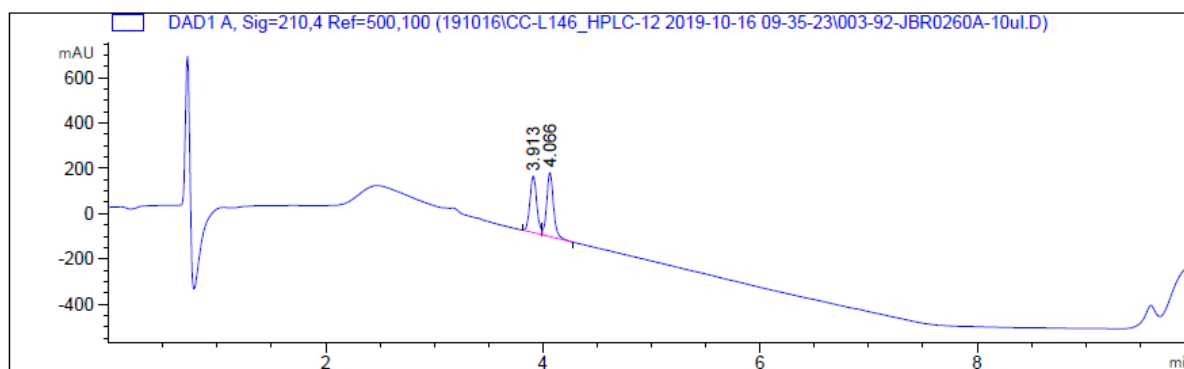


Figure S11. HPLC chromatogram of ABCPA.

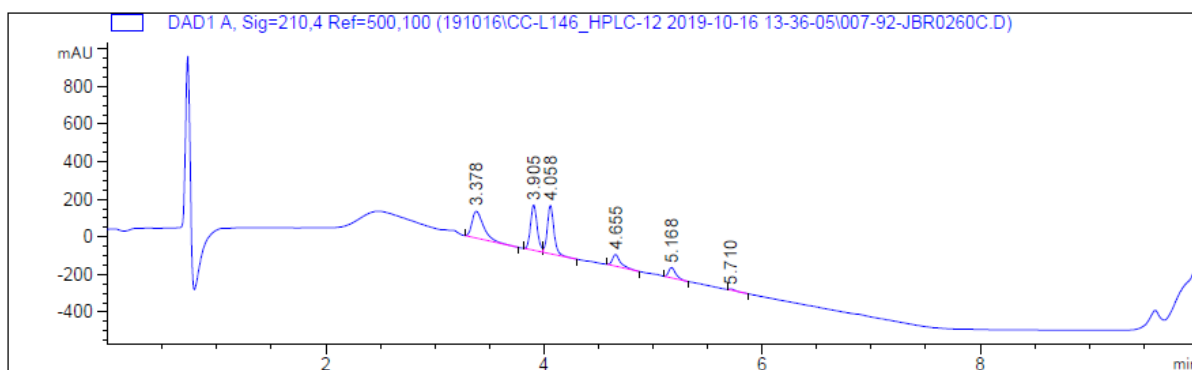


Figure S12. HPLC chromatogram of macro-initiator spiked with ABCPA.

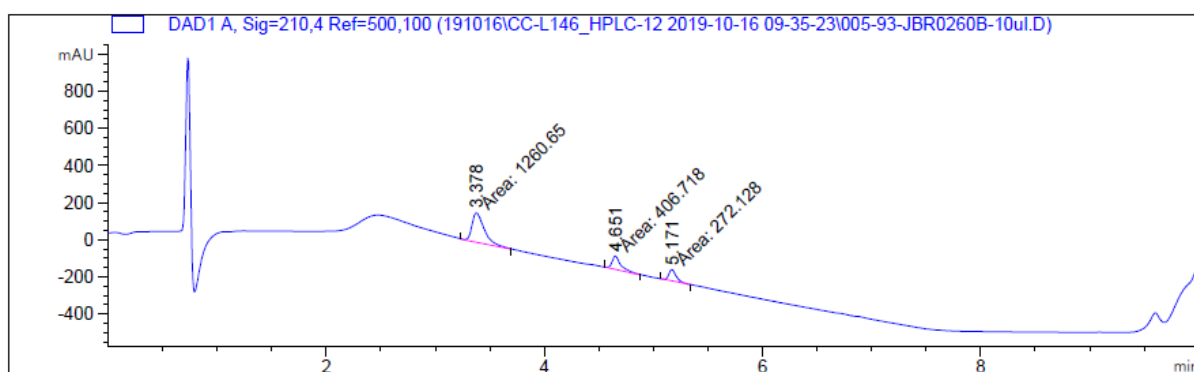


Figure S13. HPLC chromatogram of the macro-initiator. When compared with the spiked sample (Figure S12), no ABCPA could be detected.

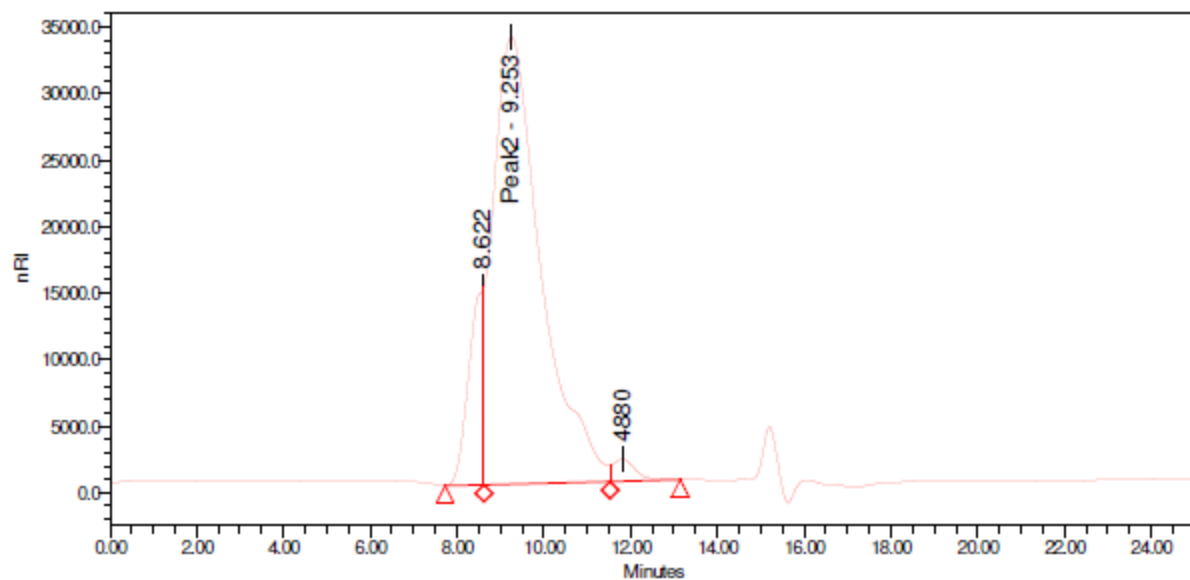


Figure S14. GPC chromatogram of mPEG-b-p(HPMA-Bz) prior to homopolymer removal.

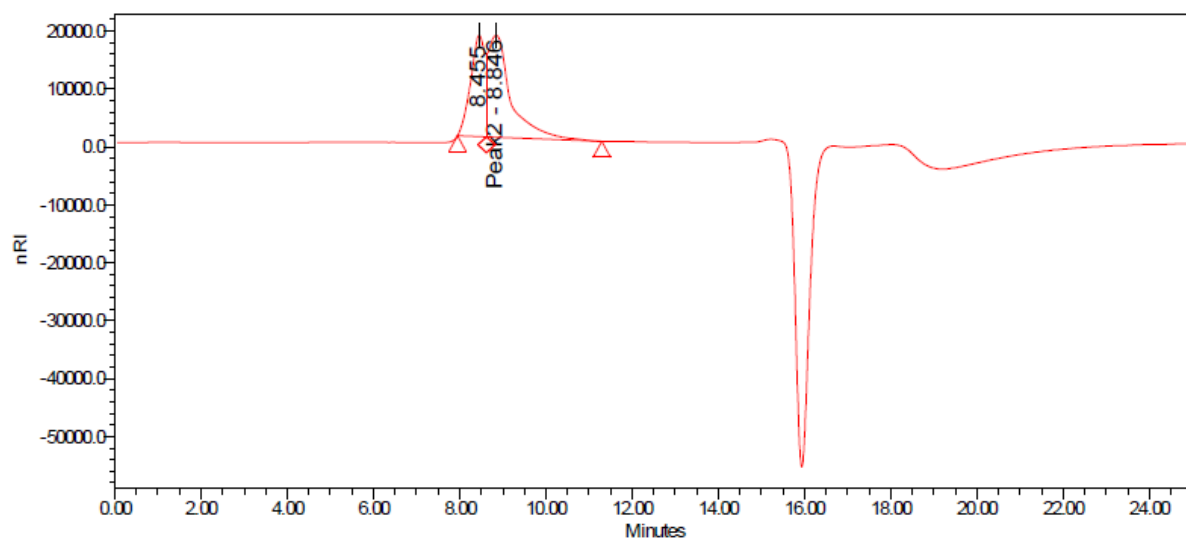


Figure S15. GPC chromatogram of the precipitate obtained after centrifugation and drying in the vacuum oven; it is thought to be mainly the removed p(HPMA-Bz) homopolymer with also a bit of the block copolymer itself in the mixture. More evidence can be found in the NMR analysis (Figure S15).

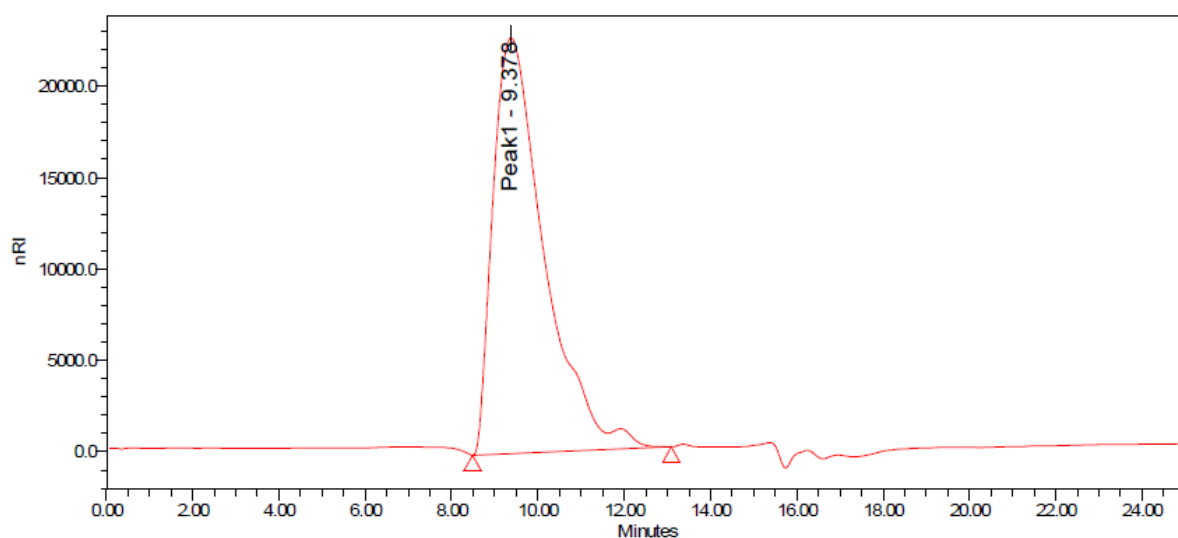


Figure S16. GPC chromatogram of the mPEG-*b*-p(HPMA-Bz) block copolymer after removal of homopolymer (M_n : 19.3 kDa and M_w : 21.6 kDa). The shoulder at ~12 minutes is assigned to free mPEG_{5K}.

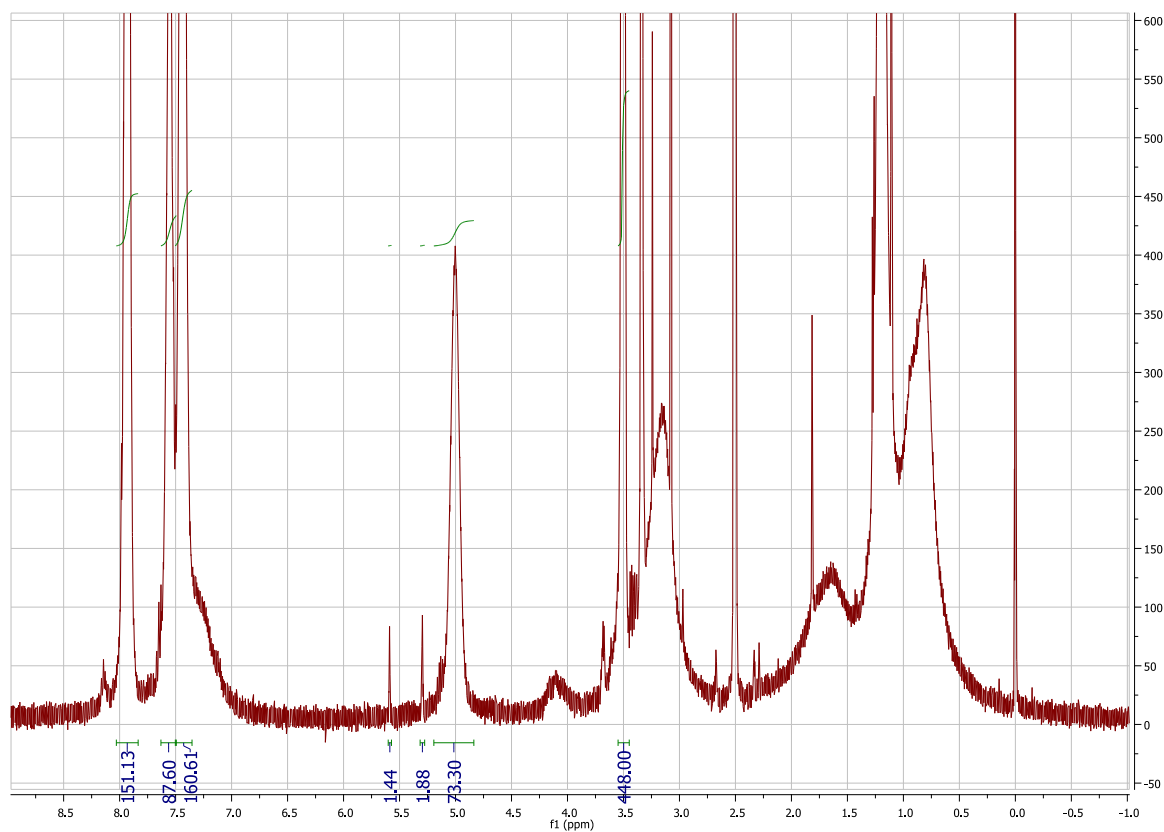


Figure S17. ^1H -NMR of mPEG-b-p(HPMA-Bz) prior to homopolymer removal with an M_n of 23.7 kDa and some trace amounts of monomer still present (5.6 ppm and 5.3 ppm).

$$\begin{aligned}
 M_n &= (\text{integral at } 8.0 \text{ ppm} / 2 * \text{molecular weight of HPMA} - \text{Bz}) + 5000 \text{ g/mol} \\
 &= (151 / 2 * 247.29 \text{ g/mol}) + 5000 \text{ g/mol} = 23.7 \text{ kDa}
 \end{aligned}$$

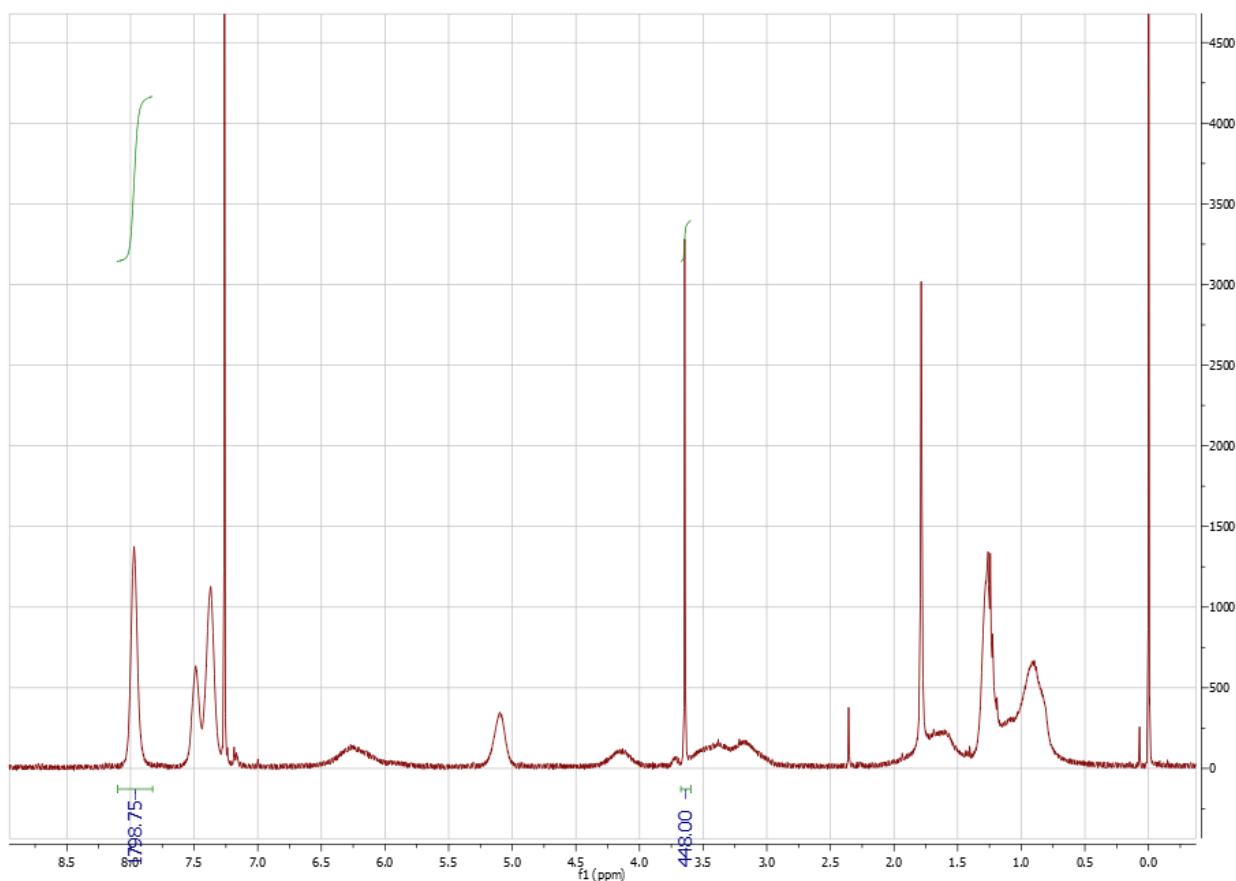


Figure S18. ^1H -NMR of precipitate obtained after centrifugation and drying in the vacuum oven; The NMR data provides insight in the composition of the isolated precipitate. It is clear that the peaks in the aromatic domain (between 7.25 and 8.25 ppm) are a lot bigger than the peak from mPEG (3.6 ppm). When the M_n is calculated, as if it were a normally synthesized block copolymer, a value of 222.3 kDa is obtained. This gives a weight fraction of PEG of only 2 %. This is 10 times smaller than envisioned for the block copolymer and therefore contributes to the suspicion that the precipitate mainly exists of p(HPMA-Bz) homopolymer.

$$\begin{aligned}
 M_n &= (\text{integral at } 8.0 \text{ ppm} / 2 * \text{molecular weight of HPMA - Bz}) + 5000 \text{ g/mol} \\
 &= (1798 / 2 * 247.29 \text{ g/mol}) + 5000 \text{ g/mol} = 222.3 \text{ kDa}
 \end{aligned}$$

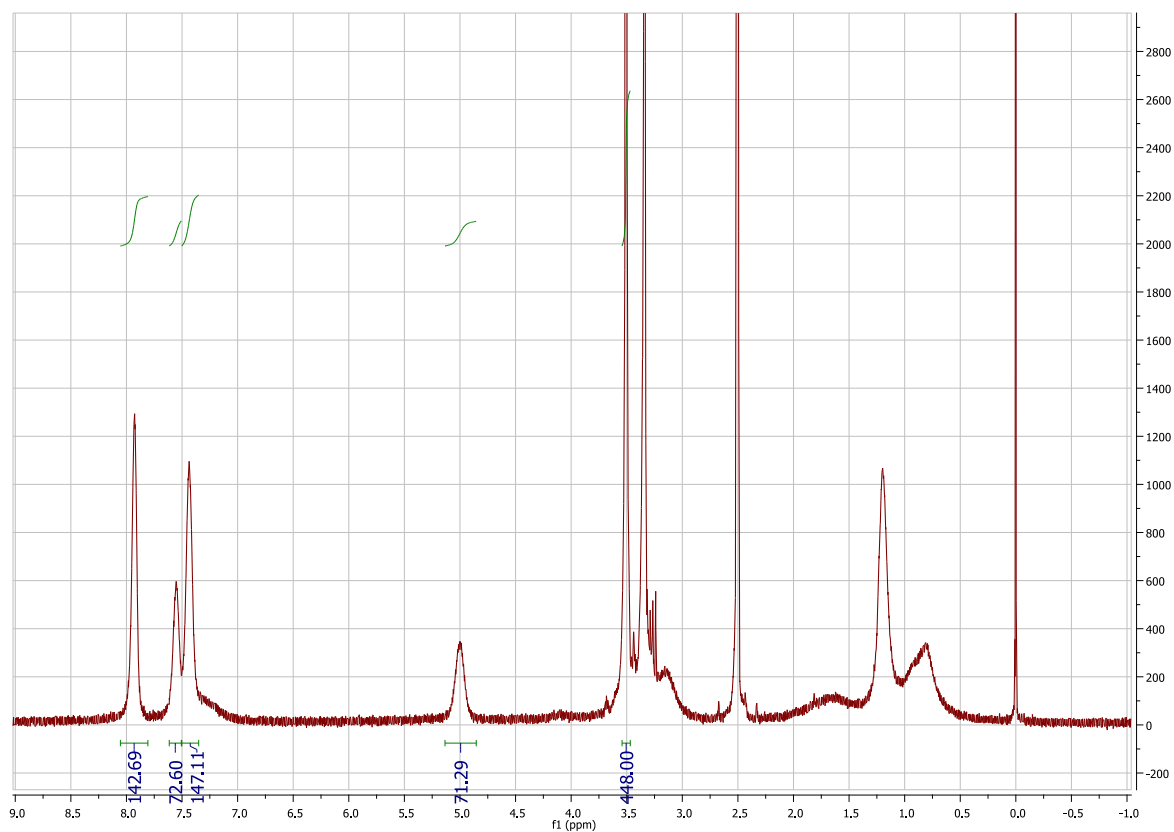


Figure S19. ^1H -NMR spectrum of the mPEG-*b*-p(HPMA-Bz) block copolymer, after homopolymer removal, which gives M_n : 22.5 kDa. The PEG integral at 3.4-3.6 is put at 448 for the average number of protons it contains. The M_n is calculated using the following formula:

$$\begin{aligned}
 M_n &= (\text{integral at } 8.0 \text{ ppm} / 2 * \text{molecular weight of HPMA - Bz}) + 5000 \text{ g/mol} \\
 &= (142 / 2 * 247.29 \text{ g/mol}) + 5000 \text{ g/mol} = 22.5 \text{ kDa}
 \end{aligned}$$

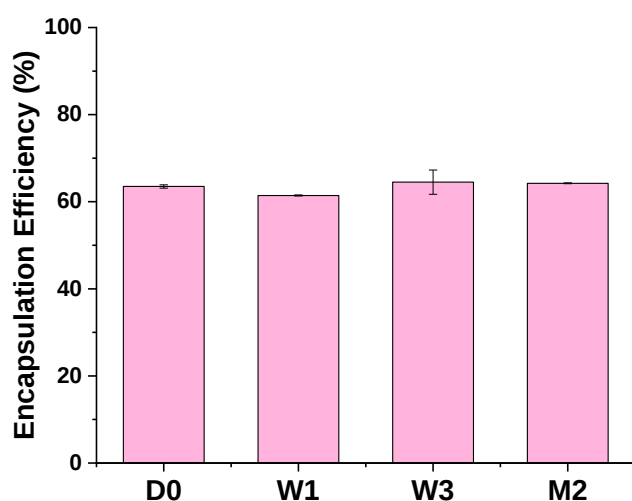


Figure S20. Stability of the DTX loaded micelles in water at room temperature that were produced in batch mode and via dialysis work up (polymer concentration: 20 mg/mL). Time points are starting point (D0), after 1 week (W1), after 3 weeks (W3) and after 2 months (M2).

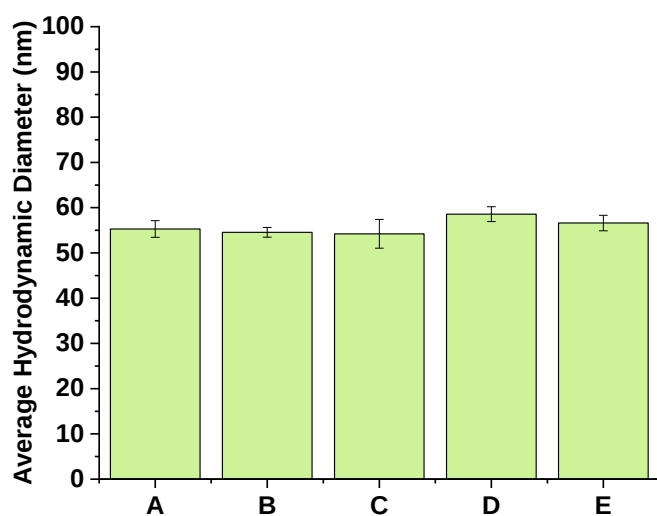


Figure S21. Average hydrodynamic diameters as measured by DLS. A: micelles prepared using the nanoprecipitation method, followed by solvent evaporation, B: micelles prepared using the nanoprecipitation method followed by dialysis, C: micelles prepared using flow formation and TFF, D: DTX loaded micelles prepared using the nanoprecipitation method and evaporation, E: DTX loaded micelles prepared using flow formation and TFF.

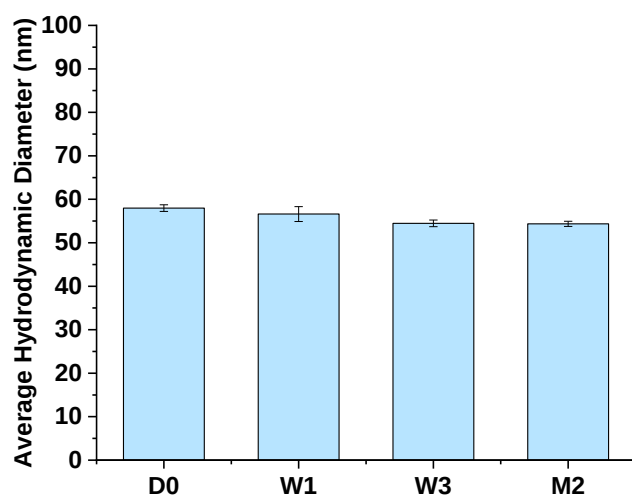


Figure S22. Average hydrodynamic diameters as measured by DLS during the stability study at room temperature for the DTX-loaded micelles that were produced in continuous flow. Time points are starting point (D0), after 1 week (W1), after 3 weeks (W3) and after 2 months (M2).

REFERENCES

- (1) R. De Vos; E.J. Goethals. End Group Analysis of Commercial Poly(Ethylene Glycol) Monomethyl Ether's. *Polym. Bull.* **1986**, *15*, 547–549.