

SUPPLEMENTARY INFORMATION

Shedding the hydrophobic mantel of polymersomes

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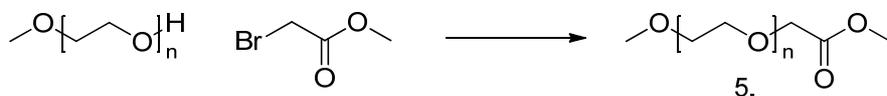
Figure 4: GPC traces of polymer **4** and its constituents.

Experimental section

General note: All reactions are described for the lower molecular weight analogue, polyethylene glycol 1000, indicated with an **A**. The procedures for the polyethylene glycol 2000 analogues, indicated with **B**, are the same, starting with equimolar amounts.

Materials: Sec-butyllithium (ALDRICH 1.4M in hexane), 1,3 butadiene (SIGMA ALDRICH, 99+%), 3-bromo-1-(trimethylsilyl-1-propyne) (ALDRICH, 98%), tetrabutylammonium fluoride (TBAF) (ALDRICH, 1.0M in THF), polyethylene glycol 1000 monomethyl ether (FLUKA), polyethylene glycol 2000 monomethyl ether (FLUKA), methanesulfonyl chloride (MsCl) (JANSSEN CHIMICA, 99%), sodium azide (ACROS ORGANICS, 99% extra pure), sodium hydride (ALDRICH, 60% dispersion in mineral oil), copper bromide (CuBr) (FLUKA, >98%), *N,N,N',N',N''*-penta dimethyldiethylenetriamine (PMDETA) (Aldrich,99%), hydrazine (ALDRICH, 1M in THF) were used as received. Tetrahydrofuran (THF) (ACROS ORGANICS, 99+% extra pure, stabilized with BHT) was distilled under argon from sodium/benzophenone and triethyl amine (TEA) (BAKER) was distilled from calcium hydride under an argon atmosphere prior to use. Polymersome extrusions were performed using 200 nm filters (Acrodisc 13 mm Syringe Filter, 0.2 μ m Nylon membrane) and dialysis was performed using Spectra/Por molecular porous membrane tubing (Spectrum Laboratories, Inc, 12-14.000 MWCO)

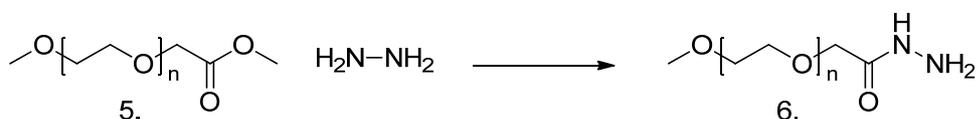
Instrumentation: MilliQ water was obtained from a Labconco water pro PS system. Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F-254 plates (layer thickness 0.25 mm). Compounds were visualized by UV, ninhydrin or permanganate reagent. Column chromatography (CC) was carried out using silica gel, Acros (0.035-0.070 mm, pore diameter ca. 6 nm). Infrared (IR) spectra were obtained using a Thermo Matson IR 300 FTIR spectrometer. Data are presented as the frequency of absorption (cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian Unity Inova 400 FTNMR spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard tetramethylsilane ($\delta=0.00$ ppm). Molecular weight distributions were measured using size exclusion chromatography (SEC) on a Shimadzu (CTO-20A) system equipped with a guard column and a PL gel 5 μ m mixed D column (Polymer Laboratories) with differential refractive index and UV ($\lambda=254$ nm and $\lambda=345$ nm) detection, using tetrahydrofuran (SIGMA ALDRICH chromasolv 99.9%) as an eluent at 1 mL/min and $T = 30$ °C. Particle size distributions were measured on a Malvern instruments Zetasizer Nano-S .

α - methoxy ω -methyl ester poly(ethylene glycol), 5

Poly(ethylene glycol) 1000 monomethyl ether, (5 g, 5 mmol), was coevaporated with benzene three times and dissolved in 250 mL freshly distilled THF. 350 mg sodium hydride (60% on mineral oil, 1.75 eq.) was added while an argon atmosphere was applied. While stirring, hydrogen gas was allowed to escape for three hours. The temperature was raised to 60 °C and 3 g of methyl bromoacetate (3.8 eq.) was added via a syringe. The reaction was allowed to proceed for 12 hours after which all THF was removed and the products were dissolved in 25 mL dichloromethane. The solution was poured on a slab of silica gel and flushed with 250 mL dichloromethane. The product was eluted with 10 percent methanol in dichloromethane, yielding 4.5 gram (90%) product. TLC was run in 14 % MeOH in DCM. Staining was performed with MNO_4 , and with ninhydrin as a control (no staining); $R_f = 0,60$

5A) $^1\text{HNMR}$ (CDCl_3): δ 3.38 (s, 3H, CH_3O), 3.65 (m, 90H, $\text{CH}_2\text{CH}_2\text{O}$), 3.75 (s, 3H, COOCH_3), 4.17 (s, 2H, OCH_2COO). **FT- IR:** 2876 cm^{-1} (C-H sat.), 1748 cm^{-1} (C=O). **SEC (THF):** $M_w/M_n = 1.22$

5B) $^1\text{HNMR}$ (CDCl_3): δ 3.38 (s, 3H, CH_3O), 3.65 (m, 180H, $\text{CH}_2\text{CH}_2\text{O}$), 3.75 (s, 3H, COOCH_3), 4.17 (s, 2H, OCH_2COO). **FT- IR:** 2876 cm^{-1} (C-H sat.), 1748 cm^{-1} (C=O). **SEC (THF):** $M_w/M_n = 1.20$

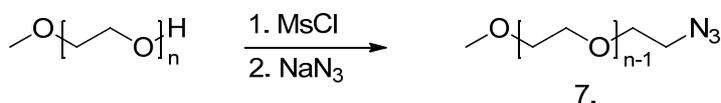
 α - methoxy ω -hydrazide poly(ethylene glycol), 6

1.5 gram of **5A** (1.5 mmol) was coevaporated with benzene three times and dissolved in 50 mL methanol under an argon atmosphere. While stirring, 15 mL of a 1M hydrazine solution (10 eq.) in THF was added at once. The reaction was refluxed overnight after which the mixture was concentrated. The crude product was dissolved in 50 mL of dichloromethane and washed with 1M HCl solution. The organic layer was dried over MgSO_4 and all solvents were removed, yielding 1.2 gram (80%) of product. TLC was performed with 14 % MeOH in DCM as eluent; ninhydrin was used as staining agent ; $R_f = 0,55$

6A) $^1\text{HNMR}$ (CDCl_3) : δ 3.38 (s, 3H, CH_3O), 3.65 (m, 90H, $\text{CH}_2\text{CH}_2\text{O}$), δ 4.14 (s, 2H, OCH_2CON). **FT-IR**: 3400 cm^{-1} (N-H), 1683 cm^{-1} (C=O). **SEC (THF)**: $M_w/M_n = 1.22$, see figure 1

6B) $^1\text{HNMR}$ (CDCl_3) : δ 3.38 (s, 3H, CH_3O), 3.65 (m, 180H, $\text{CH}_2\text{CH}_2\text{O}$), δ 4.14 (s, 2H, OCH_2CON). **FT-IR**: 3400 cm^{-1} (N-H), 1683 cm^{-1} (C=O). **SEC (THF)**: $M_w/M_n = 1.26$, see figure 2

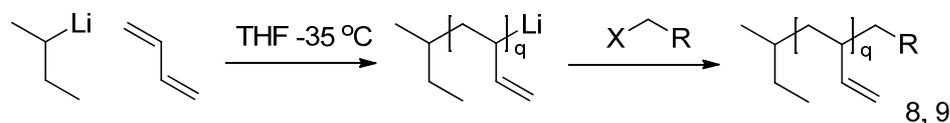
α - azido ω -methoxy poly(ethylene glycol), **7**



5 g of poly(ethylene glycol) monomethyl ether (5 mmol) was coevaporated with benzene three times and dissolved in 250 mL dry and under argon distilled THF. The flask was cooled on an ice bath and air was replaced by argon before 5 mL freshly distilled triethyl amide was added. The mixture was stirred for 3 hours, after which 1.14 gram (2 eq.) of mesyl chloride in 10 mL THF was added. The mixture was allowed to warm to room temperature and was stirred for 6 hours. All THF was removed and 100 mL methanol containing 3.25 gram (10 eq.) sodium azide was added. The mixture was refluxed overnight after which methanol was removed and 100 mL brine was added. The product was extracted with 5x 100 mL dichloromethane. The combined organic layers were dried over magnesium sulfate and DCM was removed, yielding 4.5 gram (90%) product.

7A) $^1\text{HNMR}$ (CDCl_3) : δ 3.38 (s, 3H, CH_3O), 3.39 (t, 2H, CH_2N_3), 3.65 (m, 90H, $\text{CH}_2\text{CH}_2\text{O}$). **FT-IR**: 2098 cm^{-1} (N_3). **SEC (THF)**: $M_w/M_n = 1.22$, see figure 3

7B) $^1\text{HNMR}$ (CDCl_3) : δ 3.38 (s, 3H, CH_3O), 3.39 (t, 2H, CH_2N_3), 3.65 (m, 180H, $\text{CH}_2\text{CH}_2\text{O}$). **FT-IR**: 2098 cm^{-1} (N_3). **SEC (THF)**: $M_w/M_n = 1.20$, see figure 4

Polybutadiene 8,9

All glassware was extensively cleaned, flushed with 1.7 M butyllithium, rinsed again and flame dried. 10.6 gram 1,3 butadiene (0.19 mol) was condensed into a Schlenk tube and 10 mL freshly distilled THF was added. The solution was stirred and cooled to -35 °C as 2.0 mL 1.4 M sec-butyllithium was added at once. The deep orange reaction mixture was allowed to warm to -10 °C and stirred until the color changed to pale yellow. Another aliquot of 10 mL THF was added and the temperature was lowered to -35 °C. The reaction was either terminated with 3-chloroacetaldehyde diethyl acetal (2eq.) to obtain **8** or with trimethylsilyl propargyl chloride (2 eq.) to obtain **9**.

Aldehyde terminated polybutadiene 8

3-chloroacetaldehyde diethyl acetal (2eq.) was added via a syringe as the reaction was allowed to warm to room temperature. After the yellow color had disappeared 10 mL MilliQ and 5 mL TFA were added. The mixture was heated to reflux overnight, after which all THF was removed. 65 mL 1M NaOH solution was added and the product was extracted with 3x 50 mL DCM. DCM was removed and the product was purified over a silica column (1:1 hexane:DCM, $R_f = 0.9$) to remove the excess of terminator. The product was obtained quantitatively as a colorless viscous oil.

$^1\text{H NMR}$ (CDCl_3): δ 9.72 (d, 1H, CH_2CHO), 5.45 (m, 67H, CHCH_2), 4.94 (m, 134H, CHCH_2), 2.11 (m, 67H, CH_2CH), 1.16 (m, 134H, CH_2CH). **SEC (THF):** $M_w/M_n = 1.14$, see figure 1 and 2. **M_n :** 3.7 kg/mol [1].

Alkyne terminated polybutadiene 9

trimethylsilyl propargyl chloride (2eq.) was added via a syringe as the reaction was allowed to warm to room temperature. After the yellow color had disappeared, 6 mL of a 1M TBAF solution in THF was added and stirred for one hour. The product was extracted by adding 50 mL dichloromethane and washed with two times 25 mL water. The organic layer was reduced and the product was purified over a silica column, eluting with dichloromethane ($R_f = 1$). After removal of all DCM, the product was vacuum dried overnight to yield the product quantitatively as a colorless viscous oil showing two mass distributions in SEC of which the higher mass is not available for click chemistry.

¹H NMR (CDCl₃): δ 5.45 (m, 67H, CHCH₂), 4.94 (m, 134H, CHCH₂), 2.11 (m, 67H, CH₂CH), 1.16 (m, 134H, CH₂CH). **SEC (THF):** M_w/M_n = 1.65, see figure 3 and 4. **M_n:** 3.7 kg/mol (lower M_n peak) [1].

Polybutadiene-Hz-poly(ethylene glycol) 1, 2

Polymers **6** (80 mg) and **8** (800 mg, 2 eq.) were dissolved in 2 mL dry dichloromethane. The mixture was allowed to react for 24 hours while gently stirring after which the mixture was poured on a short silica column, which was eluted with dichloromethane. After all non reacted **8** was flushed off the product was eluted with 8 percent methanol in dichloromethane. After removal of all solvents 300 mg of the product was obtained in 60 percent yield.

1) **¹H NMR (CDCl₃):** δ 5.45 (m, 67H, CHCH₂), 4.94 (m, 134H, CHCH₂), 3.65 (m, 90H, CH₂CH₂O), 3.38 (s, 3H, CH₃O), 2.11 (m, 67H, CH₂CH), 1.16 (m, 134H, CH₂CH). **SEC (THF):** M_w/M_n = 1.14, see figure 1. **M_n:** 4.7 kg/mol.

2) **¹H NMR (CDCl₃):** δ 5.45 (m, 67H, CHCH₂), 4.94 (m, 134H, CHCH₂), 3.65 (m, 180H, CH₂CH₂O), 3.38 (s, 3H, CH₃O), 2.11 (m, 67H, CH₂CH), 1.16 (m, 134H, CH₂CH). **SEC (THF):** M_w/M_n = 1.17, see figure 2. **M_n:** 5.7 kg/mol.

Polybutadiene-b-poly(ethylene glycol) 3, 4

Polymers **7** (80 mg) and **9** (800 mg, 2 eq.) were dissolved in 10 mL dry tetrahydrofuran under an argon atmosphere. The temperature was raised to 55 °C and 30 mg CuBr and 70 mg PMDETA were added. The reaction was allowed to proceed for 12 hours after which all solvents were removed. The crude product was redissolved in 50 mL dichloromethane and washed three times with 25 mL 0.33 M EDTA. The organic layer was dried over MgSO₄ and poured on a short silica column, which was eluted with dichloromethane. After all non reacted **9** was flushed off the product was eluted with 8 percent methanol in dichloromethane. After removal of all solvents 300 mg of the product was obtained in a 60 percent yield.

3) **¹H NMR (CDCl₃):** δ 5.45 (m, 67H, CHCH₂), 4.94 (m, 134H, CHCH₂), 3.65 (m, 90H, CH₂CH₂O), 3.38 (s, 3H, CH₃O), 2.11 (m, 67H, CH₂CH), 1.16 (m, 134H, CH₂CH). **SEC (THF):** M_w/M_n = 1.16, see figure 3. **M_n:** 4.7 kg/mol

4) $^1\text{H NMR}$ (CDCl_3): δ 5.45 (m, 67H, CHCH_2), 4.94 (m, 134H, CHCH_2), 3.65 (m, 180H, $\text{CH}_2\text{CH}_2\text{O}$), 3.38 (s, 3H, CH_3O), 2.11 (m, 67H, CH_2CH), 1.16 (m, 134H, CH_2CH). **SEC (THF)**: $M_w/M_n = 1.14$, see figure 4. M_n : 5.7 kg/mol.

Full hydrolysis experiments

Vesicles were prepared by dissolving 40 mg polymer **1** or **2** in 0.5 mL THF. While gently stirring, 2 mL of milliQ was added drop wise. The opaque solution was extruded twice through a 200 nm syringe filter and purified over a Sephadex G200 column. To 900 μL of this vesicle solution, 88 μL of a 2 M Na_2HPO_4 solution and 12 μL of a 1M citric acid solution was added (10x concentrated McIlvaine buffer) [2]. After three hours 3 mL THF was added and the solutions were spotted on TLC eluting with 10% methanol in dichloromethane, as were their constituents and the untreated polymers **1** and **2**. Staining was performed with MNO_4 . The acid treated samples showed no trace of polymer **1** or **2** but instead showed spots on the position of PEG and pBD. The untreated samples showed a single spot in between PEG and pBD. Finally under these conditions none of the buffering components moved on TLC. These observations prove the full cleavage of polymer **1** and **2** at pH 4.

The remainder of the solution was analyzed by GPC as depicted in figure 2b. After the acid treatment the SEC trace clearly shifted back to the original position of uncoupled pBD. Because of the addition of the buffering components, eluting right after PEG, it was not possible to obtain an undisturbed signal of the free PEG. Since the visualization of the shift also was better observed by concentrating on the pBD region this section was evaluated only.

Vesicle preparation

In general polymeric vesicles were prepared by dissolving 10 mg block copolymer (**1**, **2**, **3**, **4** or the desired combination) in 0.5 mL THF. While gently stirring, 2 mL MilliQ was added drop wise. The opaque solution was extruded twice through a 200 nm syringe filter and purified over a Sephadex G200 column and all vesicle containing fractions were combined (DLS). The combined vesicle size distribution was measured by DLS.

Stability studies

All buffers were prepared according the method of McIlvaine [2] which combines a 0.2 M Na_2HPO_4 and 0.1 citric acid solution to the desired pH in milliQ water. Vesicles were prepared as described above in MilliQ. For each measurement, 200 μL of vesicle solution was added to 800 μL of buffer. The pH was checked and the size distribution was monitored in time by dynamic light scattering.

Notes and References

[1] Determined by HNMR at two time points. First, after the anionic polymerization the terminal CH₃ of the initiator can be distinguished from the bulk and secondly after the block copolymer is assembled, by using the PEG as a standard. In both cases the HNMR calculated length is in good agreement with the theoretical length based on the reaction stoichiometry.

[2] T. C. McIlvaine, *Journal of Biological Chemistry*, 1921, **49**, 183-186

Figures

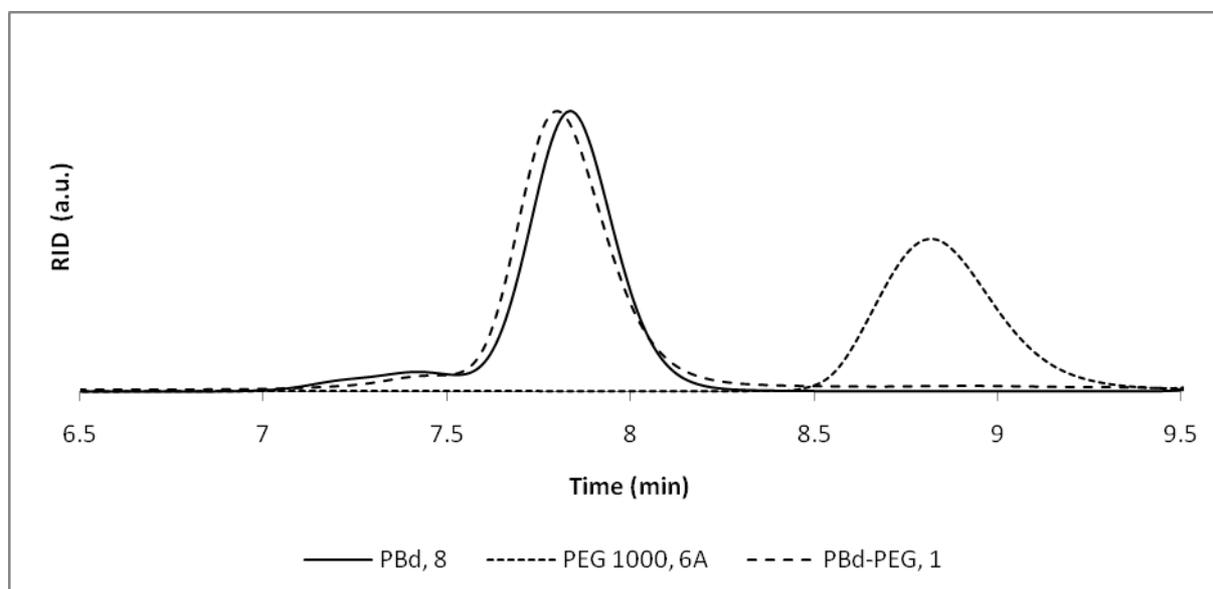


Figure 1: SEC traces of PBd-PEG (**1**) and its constituents PBd (**8**) and PEG 1000 (**6A**).

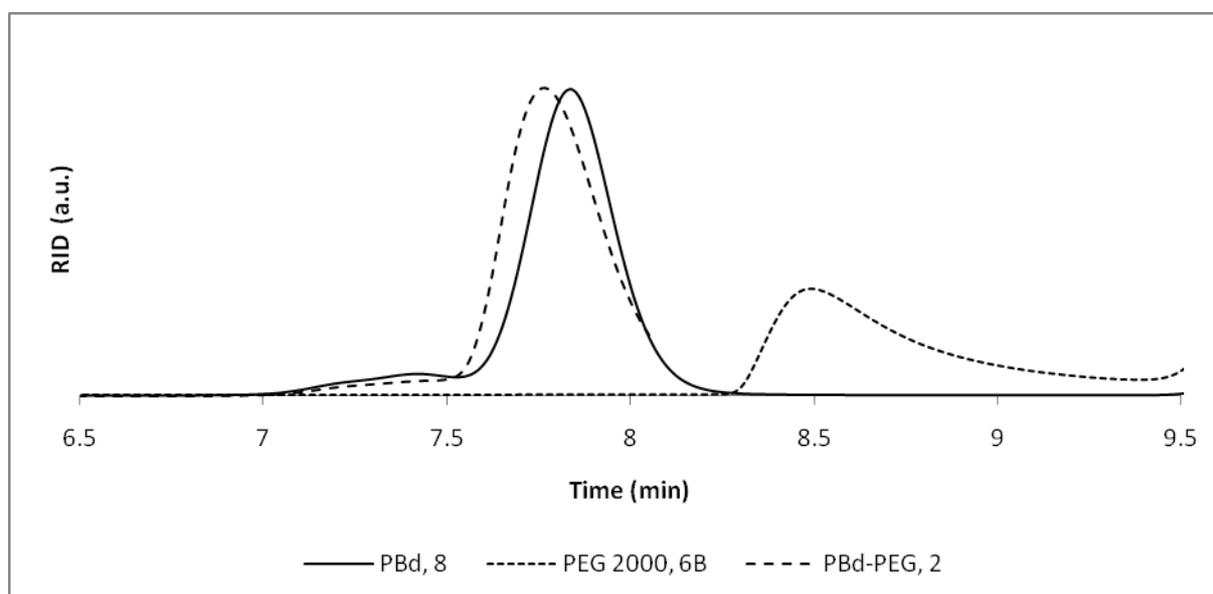


Figure 2: SEC traces of PBd-PEG (**2**) and its constituents PBd (**8**) and PEG 2000 (**6B**).

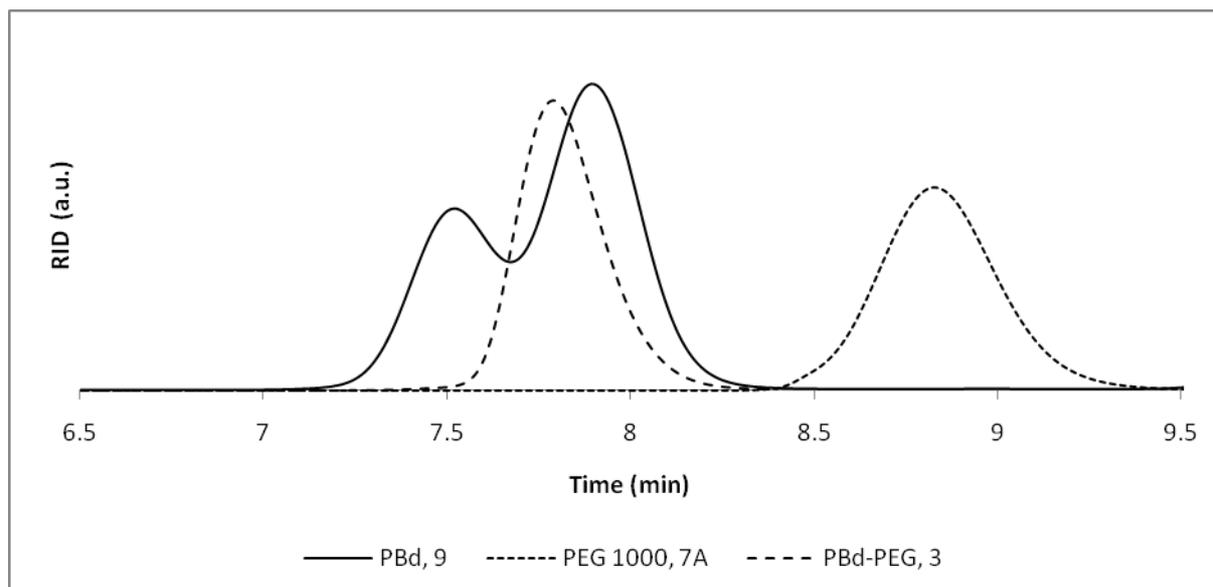


Figure 3: SEC traces of PBd-PEG (**3**) and its constituents PBd (**9**) and PEG 1000 (**7A**).

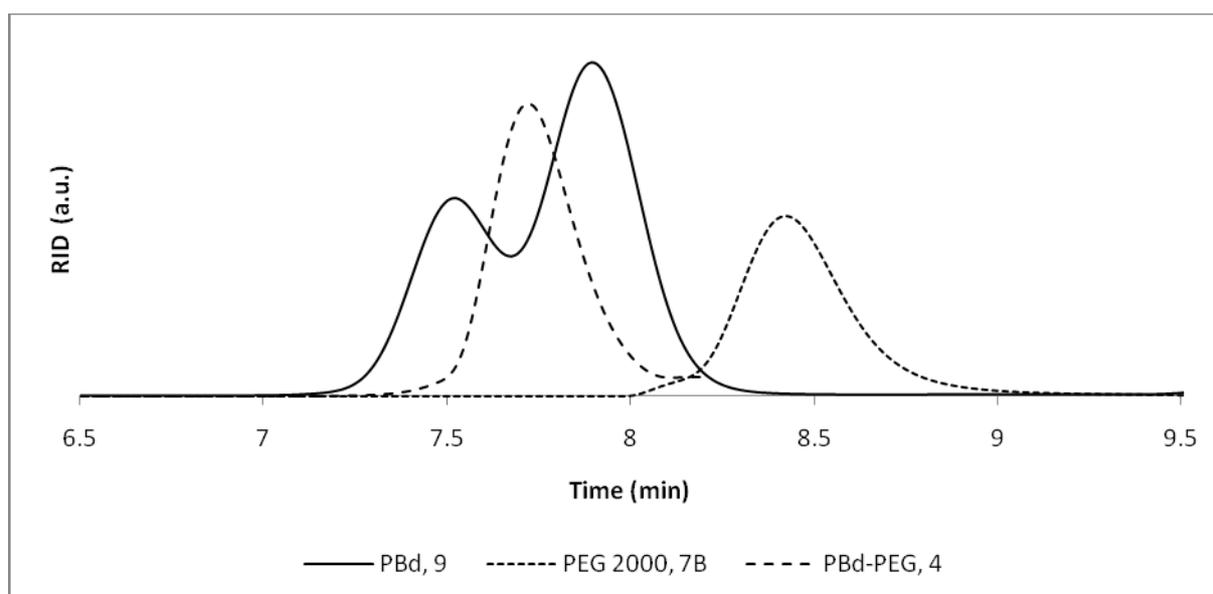


Figure 4: SEC traces of PBd-PEG (**4**) and its constituents PBd (**9**) and PEG 1000 (**7B**).