

SUPPLEMENTARY MATERIAL

Recycling gradient-elution liquid chromatography for the analysis of chemical-composition distributions of polymers

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S-1 – Procedure for in-house synthesized S/MMA copolymers

Randomly distributed linear styrene/methyl methacrylate (S/MMA) copolymers were synthesized through free radical polymerization with AMBN according to the following procedure.

The initiator solvent was made by dissolving 0.5 g AMBN in 500 mL MEK (250 ppm). The S and MMA monomers were mixed in 5 different ratio's according to **Table S1**. 3 mL initiator solvent was added to each sample. The samples were purged with nitrogen for 1 minute and incubated at 70°C for 3 hours. The samples were purged with compressed air and cooled to 4°C. The cooled samples were precipitated with -20°C, 80/20% (v/v) methanol/water. The samples were then allowed to dry under room temperature for several days to remove the supernatant.

Table S-1: Concentration of the added monomers for each copolymer and their theoretical composition.

S/MMA copolymer sample	Monomer A	Volume (mL)	Monomer B	Volume (mL)
1	S	1.709	MMA	0.363
2	S	1.396	MMA	0.639
3	S	1.080	MMA	0.918
4	S	0.761	MMA	1.200
5	S	0.437	MMA	1.486

To determine the molar mass and polydispersity of the synthesized copolymers, SEC was performed using two Agilent PLgel Mixed B columns, 7.5 × 300 mm with 10 μm particles, and an Agilent 1100 series LC system. 20 μL sample was injected using a flow rate of 1 mL·min⁻¹ of non-stabilized THF. M_w and PDI, determined by using a 12-point calibration curve measured using narrow polystyrene standards, are included in **Table S2**.

Table S-2: Weight-average molecular mass and PDI of the S/MMA copolymers used in this study.

S/MMA copolymer sample	Mw (kDa)	PDI
1	54	2.3
2	64	2.1
3	70	1.9
4	78	1.8
5	83	2.3

S-2 – Signal Alignment

Between individual measurements small differences could be observed in terms of elution composition. On closer inspection this was due to a difference in the time when the gradient eluted from the column (as indicated by the background signal, which was of the same intensity but delayed in certain measurements). This could not be corrected for during the measurement since the cycle timing was kept constant over the entire measurement. However, since the intensity of the background was nearly the same between measurements the data could first be folded after which the intensity of the background signal could be used to shift each cycle. The results of this alignment is illustrated in **Figure S-1**.

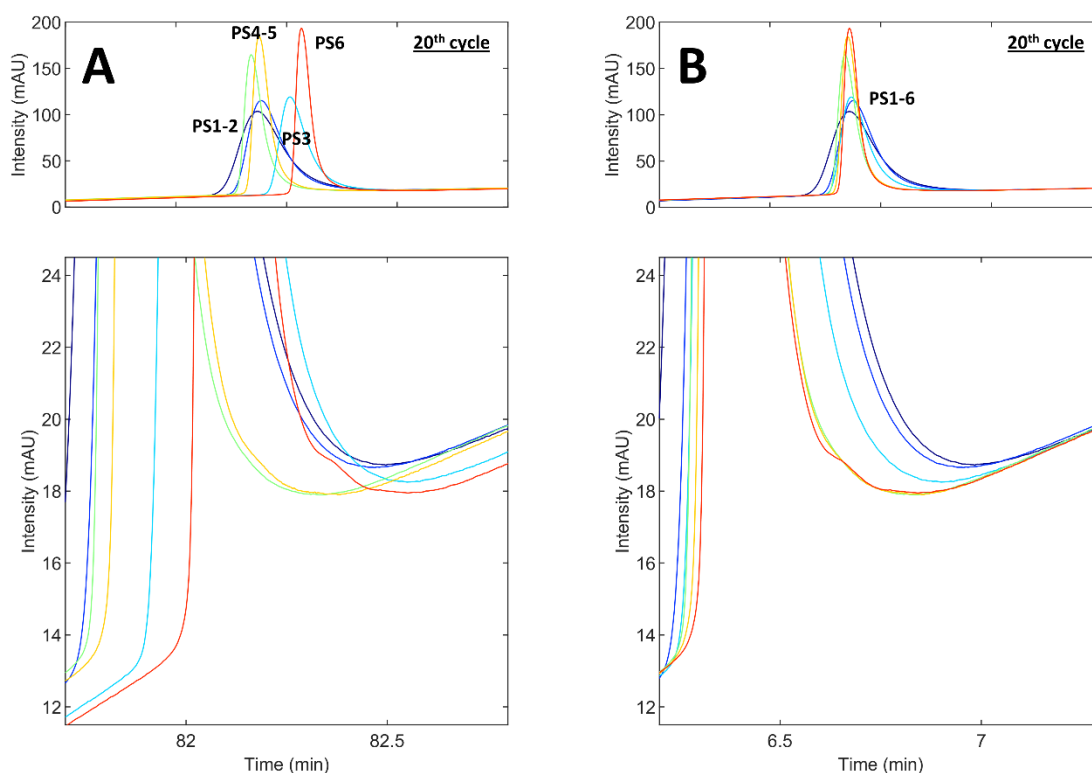


Figure S-1: Illustration of LCPLC experiments performed on the non-porous C18 particle column. A) (top) 20th cycle before alignment, (bottom) zoom-in of the region from 81.7 to 82.7 min, before alignment; B) same as in A but after alignment based on the background signal.

S-3 – Investigation of changes in peak width with cycle number

A short investigation of changes in peak width with an increase in the number of cycles for a broad and narrow polystyrene standard was performed. Parameters checked included the flowrate and change in modifier fraction. The data allows to distinguish between chromatographic peak broadening and the decrease of the influence of the molecular weight distribution. This is illustrated in **Figure S-2**.

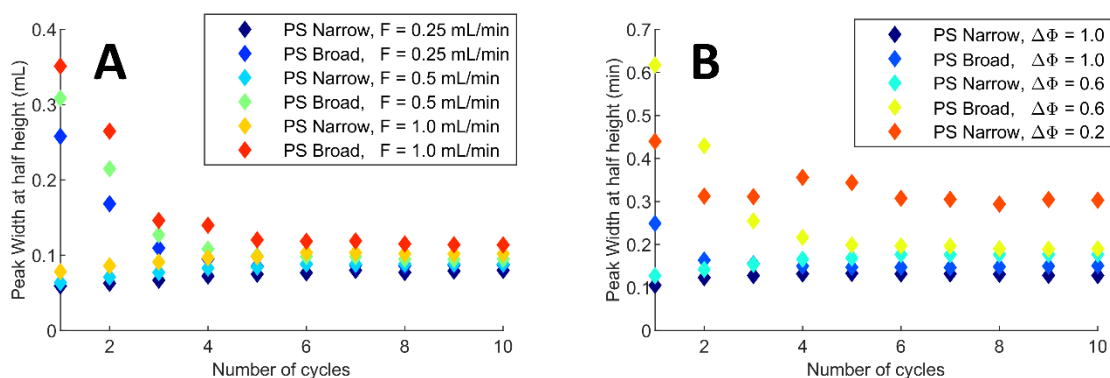


Figure S-2: Peak width at half height for PS narrow ($M_p = 32$ kDa, PDI = 1.07) and PS broad ($M_p = 22$ kDa, PDI = 2.2) as measured on the 4000-Å C18 columns. A) Peak width obtained at different flowrates for a gradient from 0 to 100% THF in ACN. Gradient duration was adjusted for constant gradient steepness, B) Peak width obtained for different changes in modifier fraction ($\Delta\phi$), gradient duration was 5 min, at a flowrate of $0.5 \text{ mL}\cdot\text{min}^{-1}$.

S-4 – Calibration on chemical composition

Using the calibration approach described in the manuscript the critical compositions of several MMA/BMA copolymers were calculated. These predictions are illustrated in **Figure S-3**, using $\varphi_{\text{crit,PMMA}} = 0.09$; $\varphi_{\text{crit,PBMA}} = 0.47$; and $\varphi_{\text{crit,MB5}} = 0.34$ to determine $\frac{p_A}{p_B}$.

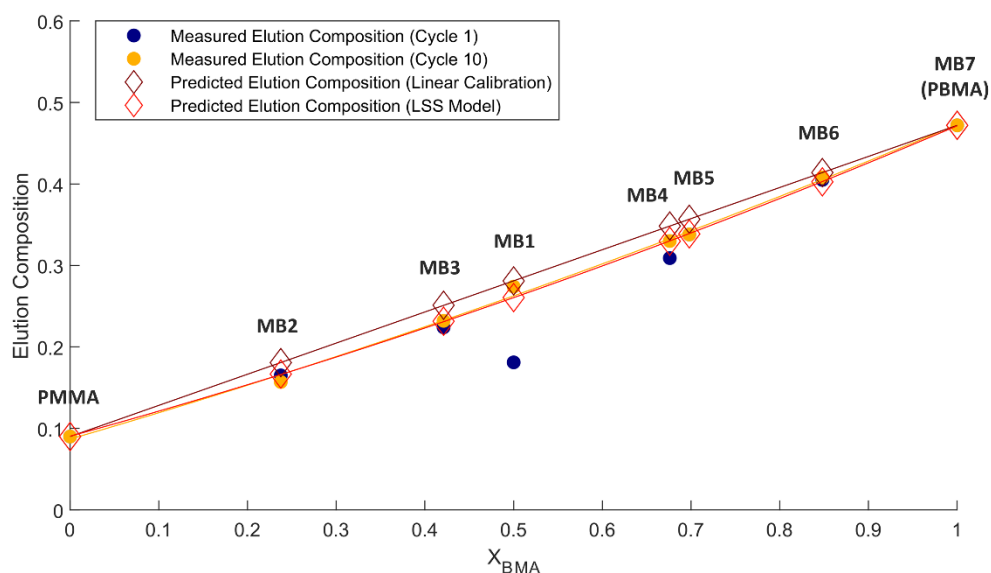


Figure S-3: Measured elution compositions from an LC \cup LC experiment of MMA/BMA copolymers MB1-7 using non-porous C18 particles and a gradient of 0-60% THF in ACN in 3 min at a flowrate of 0.4 mL \cdot min $^{-1}$. Orange diamonds indicate the predicted critical compositions using the LSS model. Dark red diamonds indicate the critical compositions calculated by assuming a linear dependence between the critical composition of the homopolymers. Blue and yellow circles indicate the measured elution composition before and after recycling. Average MMA/BMA compositions (as determined by $^1\text{H-NMR}$) and M_w : MB1, 50/50 (4.2 kDa); MB2, 76/24 (80 kDa); MB3, 58/42 (20 kDa); MB4, 32/68 (15 kDa); MB5, 30/70 (50 kDa); MB6, 85/15 (100 kDa); MB7, 0/100 (160 kDa).

As shown in **Figure S-3** the measured elution compositions for the lowest-molecular-weight analytes (MB1 and MB4) differ strongly from the predicted critical compositions before the gradient is recycled (indicated in blue). After recycling of the gradient the difference between the measured elution compositions (indicated in yellow) and those calculated using the approach described above (in orange) are much smaller for all copolymers, as indicated by the yellow and the orange line which nearly completely overlap. Calculating the critical compositions based solely on the critical compositions of the homopolymers and assuming a linear relation between the critical composition and the mass fraction of BMA (shown in dark red) led to an overestimation of the critical composition in all cases, illustrating the value of our approach.