Styrene-based polymerised High Internal Phase Emulsions using monomers in the internal phase as co-surfactants for improved liquid chromatography

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Supporting Information

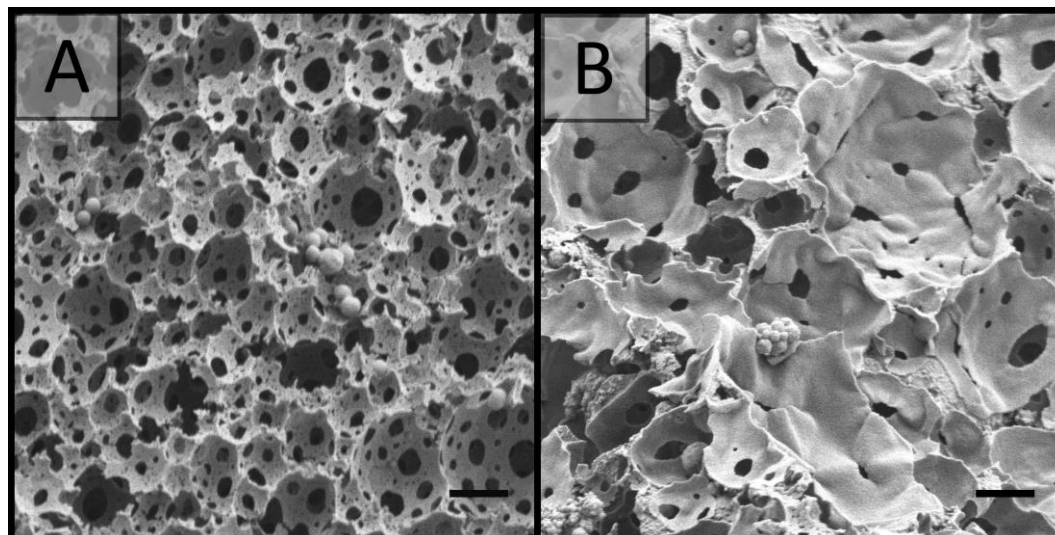## 1. Preparation of poly(HIPE)s grafted with AAm or PEGDA

Fig. S1 SEM images of poly(HIPE)s prepared with different amounts of AAm (w.r.t. internal phase) in the internal phase using KPS as initiator. **A)** 0.1 wt%; **B)** 1 wt%. Scale bar is 2 μm.

Table S1 Elemental analysis for the poly(HIPE)s prepared with different amounts of AAm (w.r.t. internal phase) in the internal phase and using different initiators.

Sample	N / %	C / %	H / %	S / %	AAm [a] / %
0 wt% AAm (KPS)	0.03	90.34	8.16	0.10	0
0.1 wt% AAm (KPS)	0.06	89.49	8.13	0.10	17
1 wt% AAm (KPS)	1.05	86.30	7.86	0.11	58
0 wt% AAm (AIBN)	0.04	90.97	8.14	0.00	0
0.1 wt% AAm (AIBN)	0.06	91.26	8.15	<0.01	11
1 wt% AAm (AIBN)	1.63	86.14	7.99	<0.01	90

[a] Estimate of AAm incorporated into the poly(HIPE)s based on nitrogen content w.r.t. AAm in the internal phase. This was calculated assuming full conversion of monomers and subtracting the nitrogen content in the blank samples (0 wt% AAm).
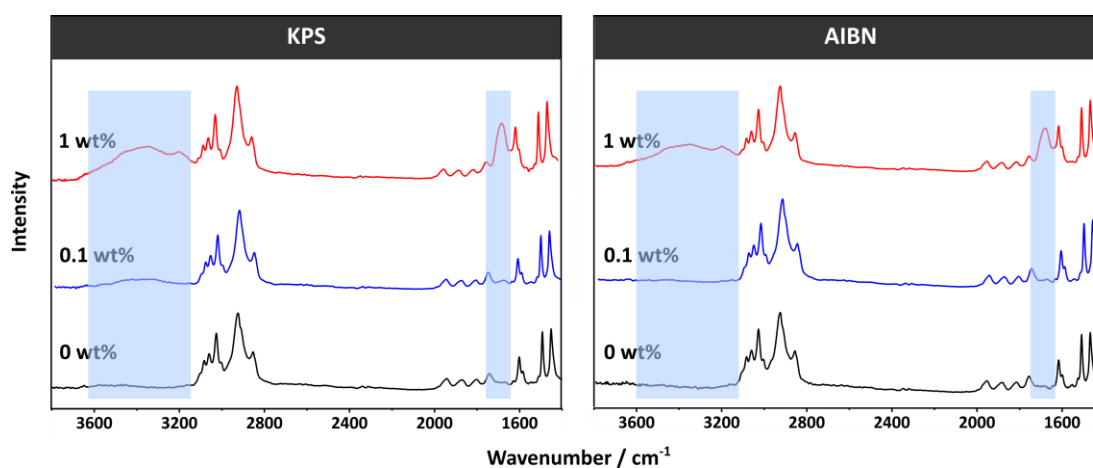

Fig. S2 ATR-IR of poly(HIPE)s prepared with different amounts of AAm (w.r.t. internal phase) in the internal phase and using different initiators.
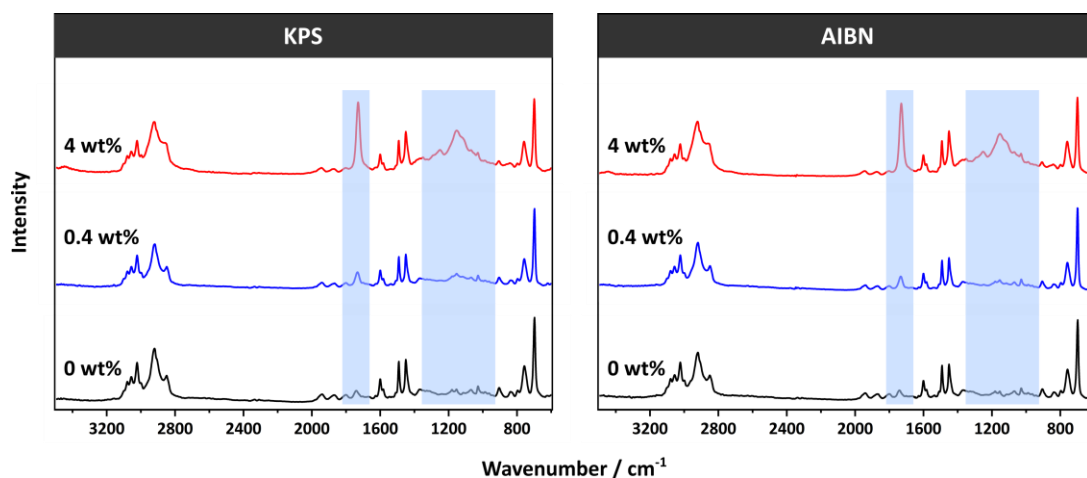

Fig. S3 ATR-IR of poly(HIPE)s prepared with different amounts of PEGDA (w.r.t. internal phase) in the internal phase and using different initiators.

2. Preparation of poly(HIPE)s in capillary format

Table S2 Porous properties of poly(HIPE)s prepared with different amounts of AAm or PEGDA (w.r.t. internal phase) in the internal phase and using different initiators.

Sample	V_B [a] / μm	W_B [b] / μm	D_B [c] / μm	V_P [a] / μm	W_P [b] / μm	D_P [c] / μm
0 wt% (KPS)	3.4 ± 0.7	0.8 ± 0.3	3 ± 1	3.4 ± 0.7	0.7 ± 0.3	3 ± 1
0.1 wt% AAm (KPS)	2.3 ± 0.7	0.6 ± 0.2	2 ± 1	8 ± 2	1.8 ± 0.7	4 ± 2
1 wt% AAm (KPS)	2.5 ± 0.9	0.5 ± 0.2	3 ± 1	2.9 ± 0.7	0.8 ± 0.3	1.9 ± 0.9
0.4 wt% PEGDA (KPS)	1.9 ± 0.5	0.5 ± 0.2	2 ± 1	1.9 ± 0.7	0.4 ± 0.2	1.8 ± 0.9
4 wt% PEGDA (KPS)	4 ± 3	0.7 ± 0.3	5 ± 2	3 ± 2	0.4 ± 0.2	5 ± 2
0 wt% AAm (AIBN)	7 ± 2	1.6 ± 0.7	7 ± 3	5 ± 2	1.0 ± 0.5	5 ± 3
0.1 wt% AAm (AIBN)	4 ± 1	1.2 ± 0.4	3 ± 2	4.3 ± 0.7	0.9 ± 0.3	3 ± 1
1 wt% AAm (AIBN)	2.9 ± 0.6	0.7 ± 0.2	3 ± 1	6 ± 2	1.4 ± 0.5	2.2 ± 0.9
0.4 wt% PEGDA (AIBN)	4 ± 2	1.2 ± 0.5	4 ± 2	3 ± 2	0.8 ± 0.3	5 ± 2
4 wt% PEGDA (AIBN)	4 ± 1	1.3 ± 0.4	4 ± 2	4 ± 2	1.6 ± 0.6	3 ± 1

[B] indicates the bulk material. [P] indicates the material passed through 20 cm of capillary. [a] Average void diameter for the poly(HIPE)s as determined from SEM. [b] Average window diameter for the poly(HIPE)s as determined from SEM. [c] Average droplet diameter immediately after preparation, or after being passed through the capillary, for the emulsions.
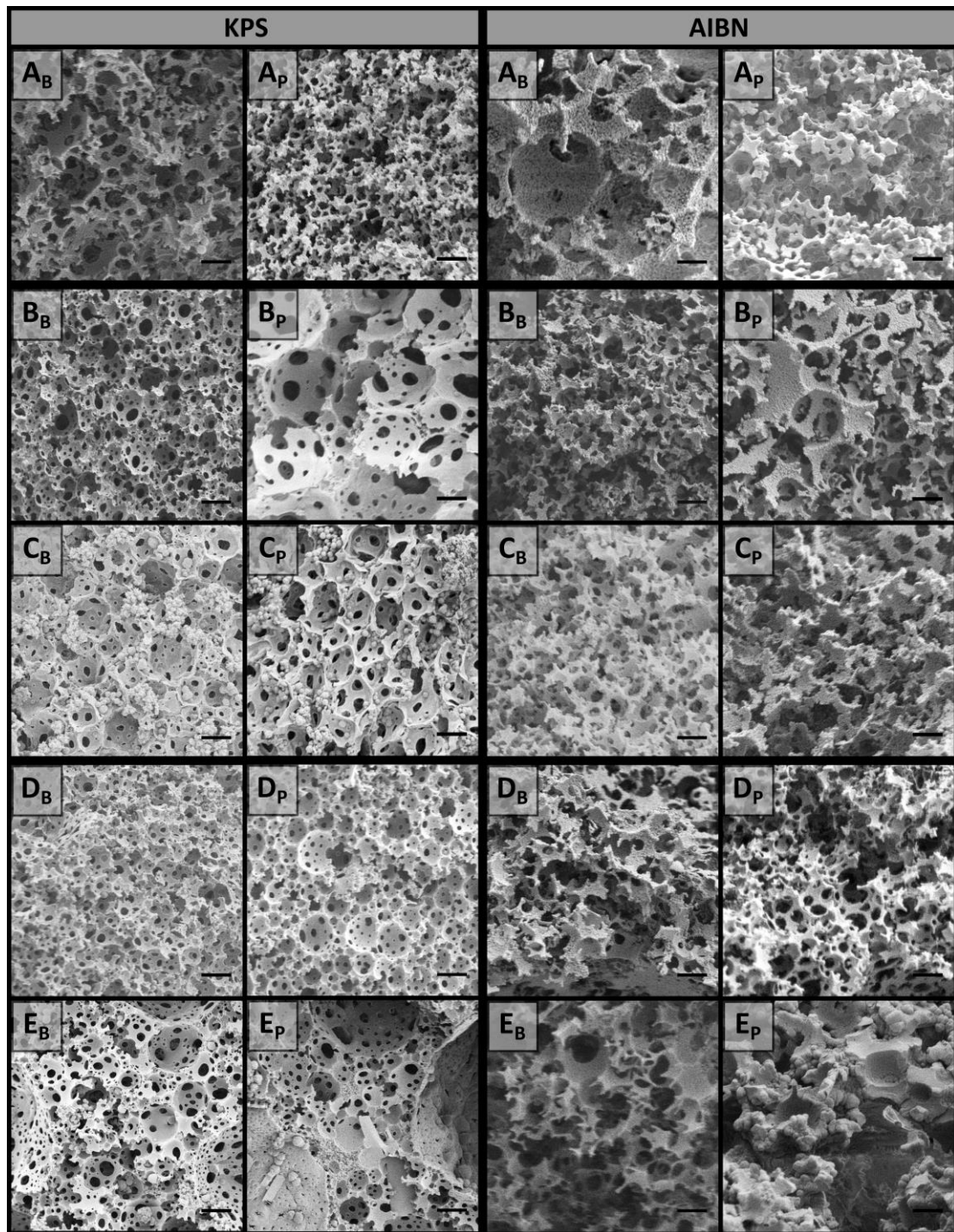
Fig. S4 SEM images of poly(HIPE)s prepared with different amounts of monomer (w.r.t. internal phase) in the internal phase and using different initiators. **A)** 0 wt%; **B)** 0.1 wt% AAm; **C)** 1 wt% AAm; **D)** 0.4 wt% PEGDA; **E)** 4 wt% PEGDA. [B] indicates poly(HIPE)s obtained by curing the bulk emulsions, [P] indicates poly(HIPE)s obtained after curing the emulsions which that had been passed through 20 cm of capillary. Scale bar is 3 μm.
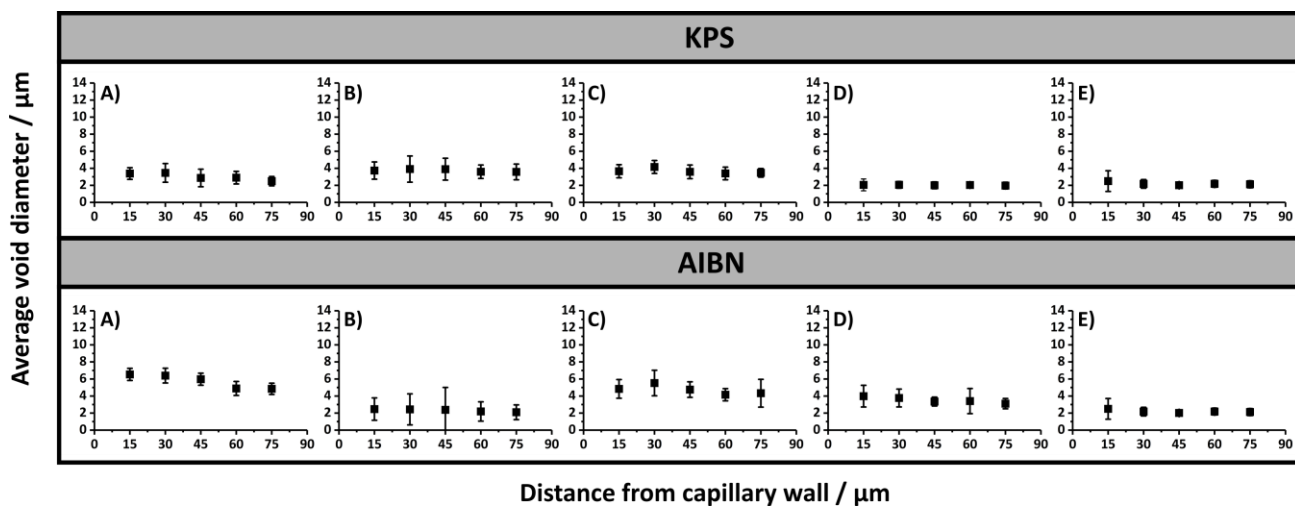
Fig. S5 Plot of average void diameter with increased distance from capillary wall for poly(HIPE)s prepared with different amounts of monomer (w.r.t. internal phase) in the internal phase in 150 μm i.d. capillaries using different initiators. **A)** 0 wt%; **B)** 0.1 wt% AAm; **C)** 1 wt% AAm; **D)** 0.4 wt% PEGDA; **E)** 4 wt% PEGDA.
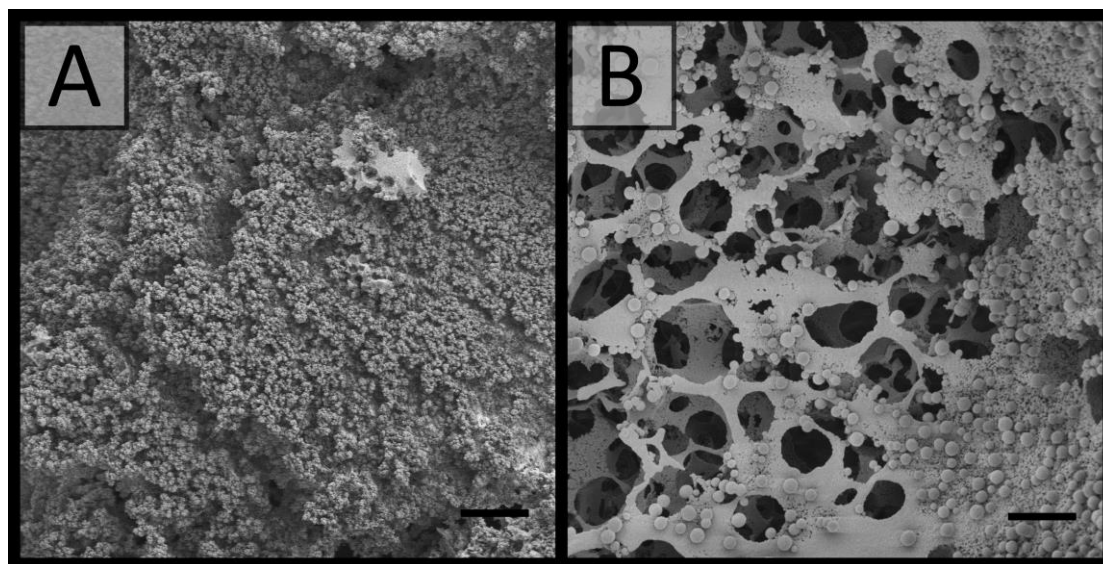

Fig. S6 SEM images of poly(HIPE)s obtained with 4 wt% PEGDA (w.r.t. internal phase) in the internal phase using KPS as initiator. **A)** Poly(HIPE) obtained by passing the emulsion through 20 cm of capillary; **B)** Poly(HIPE) obtained by curing emulsion within capillary. Scale bar is **A)** 20 μm and **B)** 4 μm.
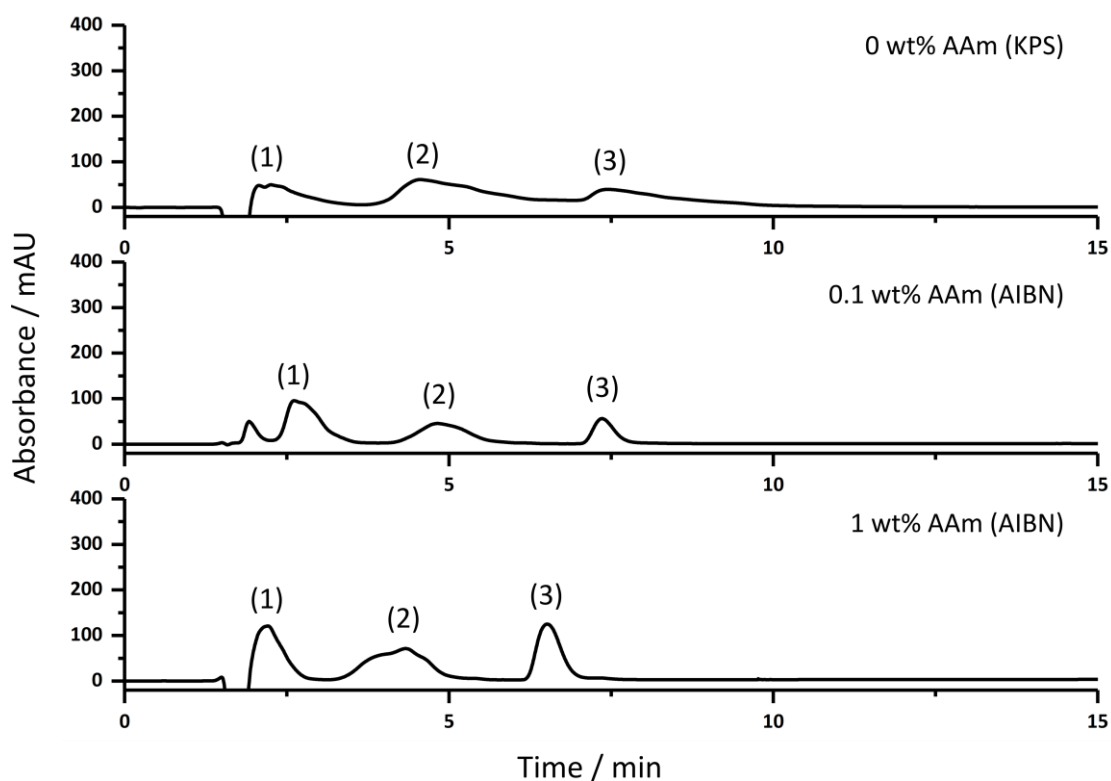
3. Chromatographic performance

Fig. S7 The separation of ribonuclease A (1), lysozyme (2) and α-chymotrypsinogen A (3) under reversed-phase conditions using columns prepared with different amounts of AAm in the internal phase and different initiators. Conditions: 18 cm × 150 μm i.d. columns; eluent A was 0.1 vol% formic acid in Milli-Q H_2O, and eluent B was 0.1 vol% formic acid in ACN; linear gradient 15 to 70% B in 15 min and then isocratic elution at 70% B for 5 min before returning to 15% B in 5 min; flow rate, 4.0 μL/min; injection volume, 1 μL; protein concentration, 0.05 mg/mL; UV detection at 214 nm.
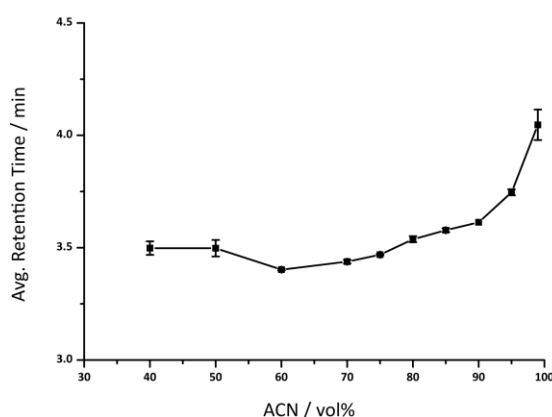
Fig. S8 The effect of ACN content on the retention of guanosine for the 1 wt% AAm (KPS) column. Conditions: 18 cm × 150 μm i.d. column; eluent A was ACN, and eluent B was Milli-Q H_2O; isocratic conditions for 10 min total duration; flow rate, 2.0 μL/min; injection volume, 1 μL; guanosine concentration, 0.05 mg/mL; UV detection at 214 nm.

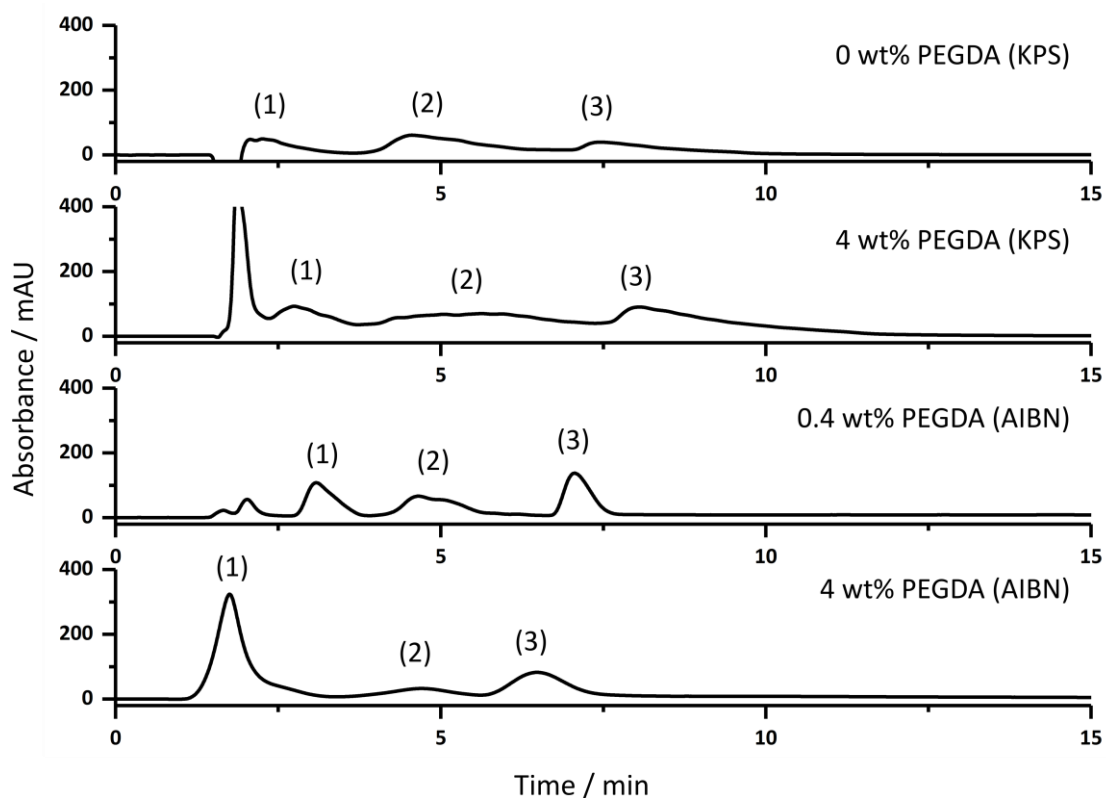**Fig. S9** The separation of ribonuclease A (1), lysozyme (2) and α-chymotrypsinogen A (3) under reversed-phase conditions using columns prepared with different amounts of PEGDA in the internal phase and different initiators. Conditions: 18 cm × 150 μm i.d. columns; eluent A was 0.1 vol% formic acid in Milli-Q H_2O, and eluent B was 0.1 vol% formic acid in ACN; linear gradient 15 to 70% B in 15 min and then isocratic elution at 70% B for 5 min before returning to 15% B in 5 min; flow rate, 4.0 μL/min; injection volume, 1 μL; protein concentration, 0.05 mg/mL, except for 4 wt% PEGDA (KPS) which was 0.1 mg/mL; UV detection at 214 nm.
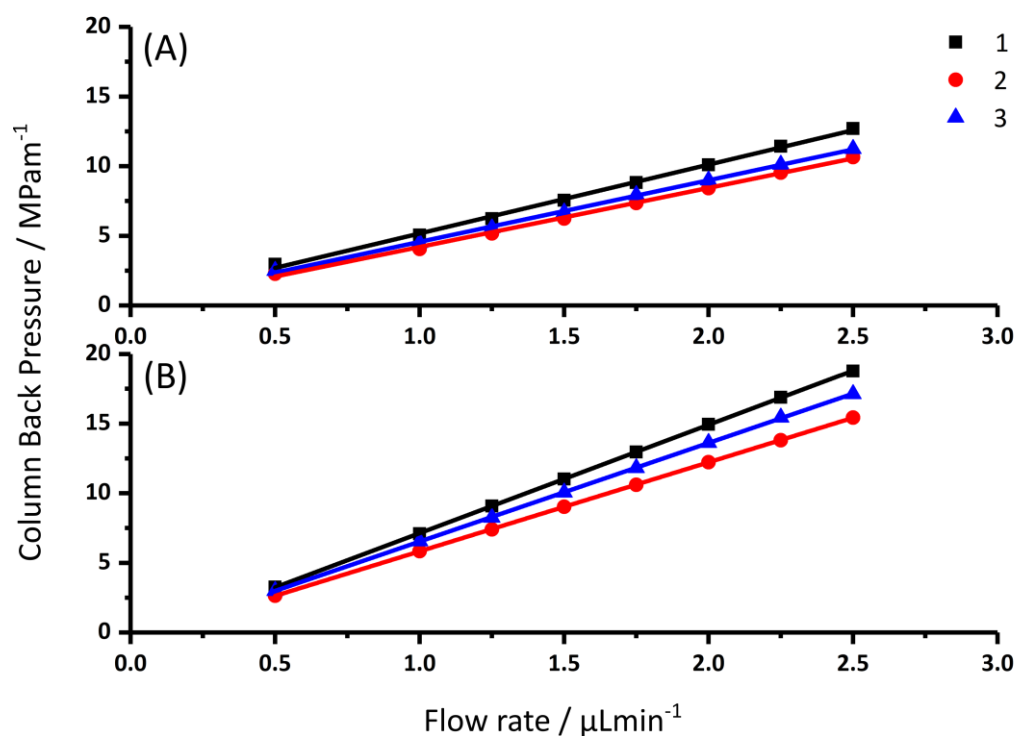
4. Column permeabilities

Fig. S10 Plot of column back pressure (MPa/m) against flow rate (µL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 µm i.d. silica capillaries from the same batch with 0 wt% monomer (w.r.t. internal phase) in the internal phase using KPS as initiator using: **A)** MeOH; **B)** H_2O.
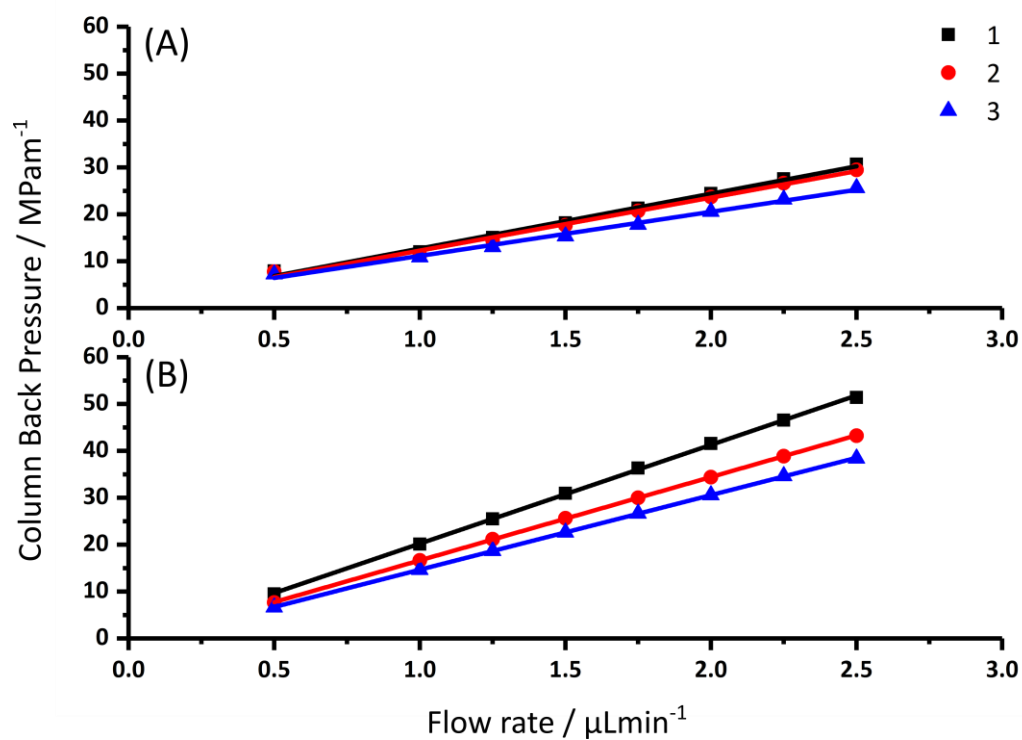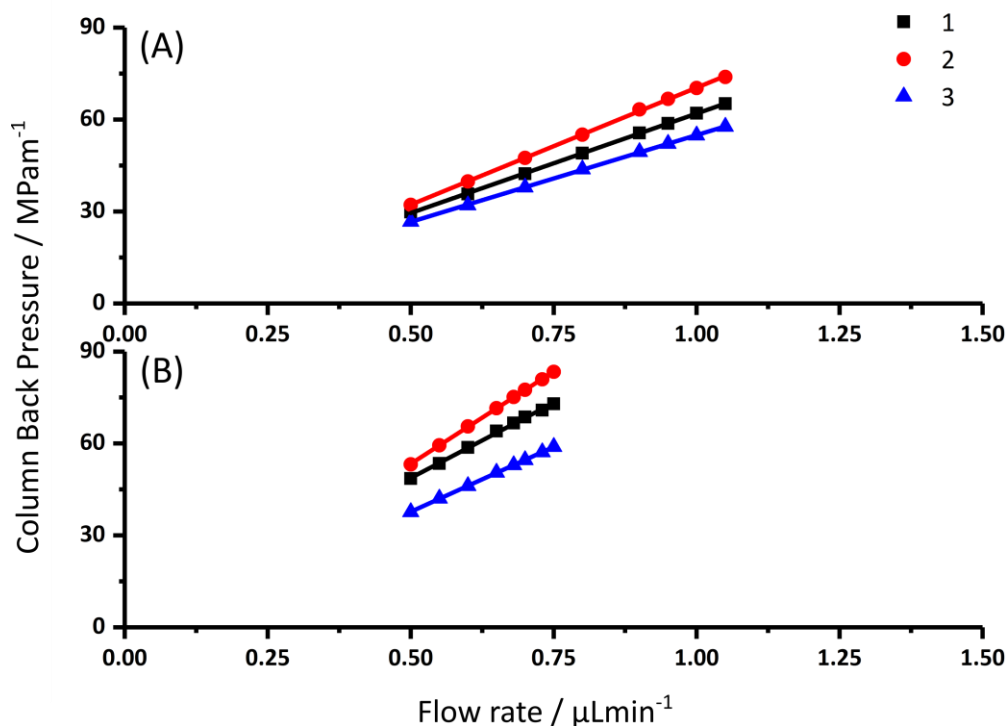

Fig. S11 Plot of column back pressure (MPa/m) against flow rate (µL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 µm i.d. silica capillaries from the same batch with 0.1 wt% AAm (w.r.t internal phase) in the internal phase using KPS as initiator using: **A)** MeOH; **B)** H_2O.
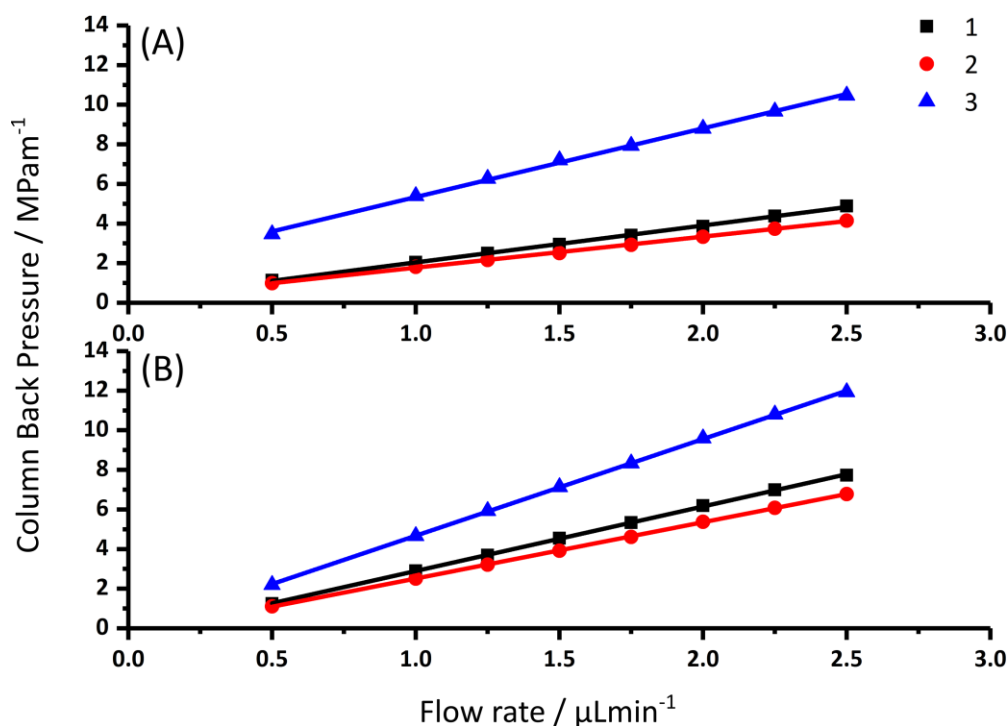
Fig. S12 Plot of column back pressure (MPa/m) against flow rate (μL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 μm i.d. silica capillaries from the same batch with 1 wt% AAm (w.r.t internal phase) in the internal phase using KPS as initiator using: **A)** MeOH; **B)** H_2O.
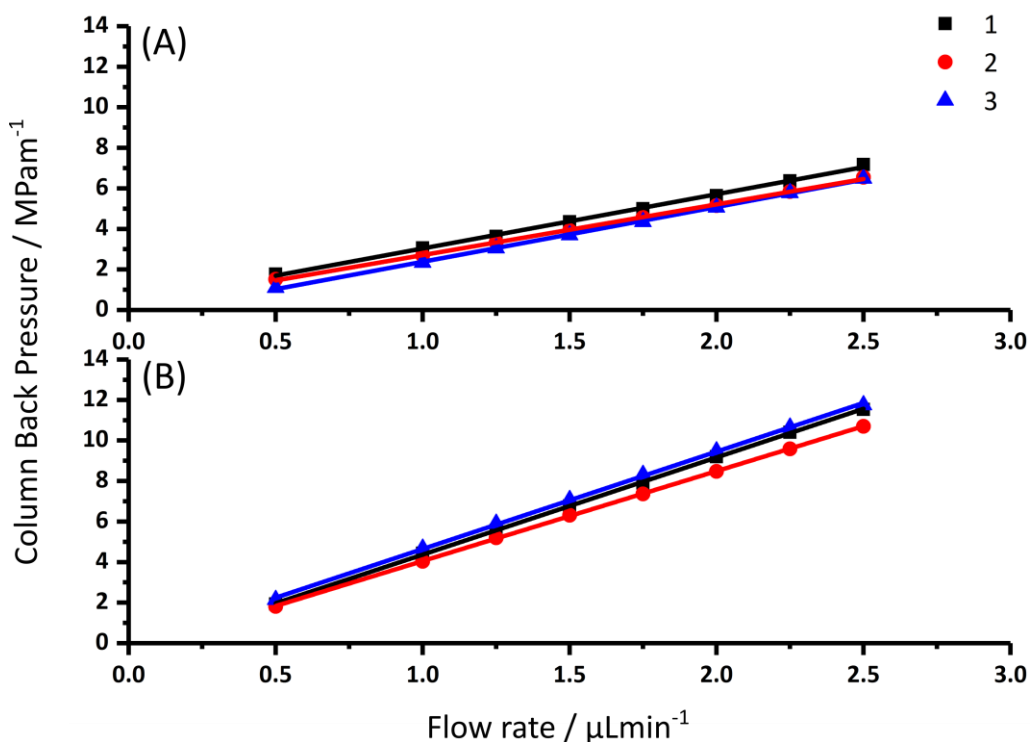

Fig. S13 Plot of column back pressure (MPa/m) against flow rate (μL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 μm i.d. silica capillaries from the same batch with 0.1 wt% AAm (w.r.t internal phase) in the internal phase using AIBN as initiator using: **A)** MeOH; **B)** H_2O.
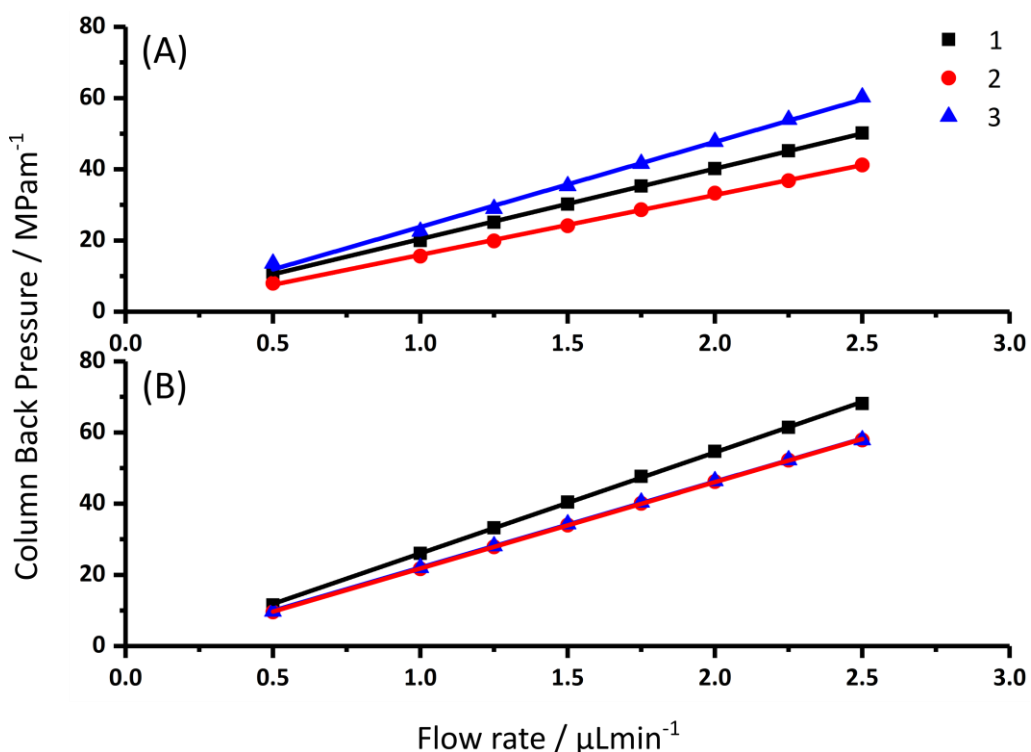
Fig. S14 Plot of column back pressure (MPa/m) against flow rate (μL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 μm i.d. silica capillaries from the same batch with 1 wt% AAm (w.r.t internal phase) in the internal phase using AIBN as initiator using: **A)** MeOH; **B)** H_2O.

Fig. S15 Plot of column back pressure (MPa/m) against flow rate (μL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 μm i.d. silica capillaries from the same batch with 0.4 wt% PEGDA (w.r.t internal phase) in the internal phase using KPS as initiator using: **A)** MeOH; **B)** H_2O.
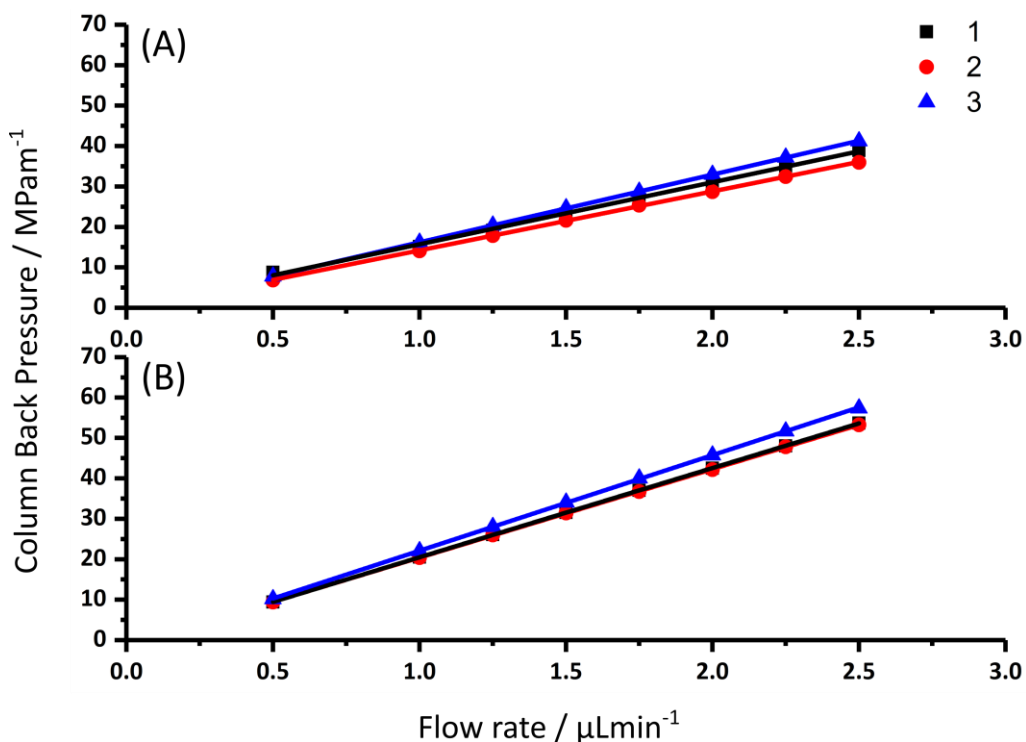
Fig. S16 Plot of column back pressure (MPa/m) against flow rate (µL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 µm i.d. silica capillaries from the same batch with 4 wt% PEGDA (w.r.t internal phase) in the internal phase using KPS as initiator using: **A)** MeOH; **B)** H_2O.
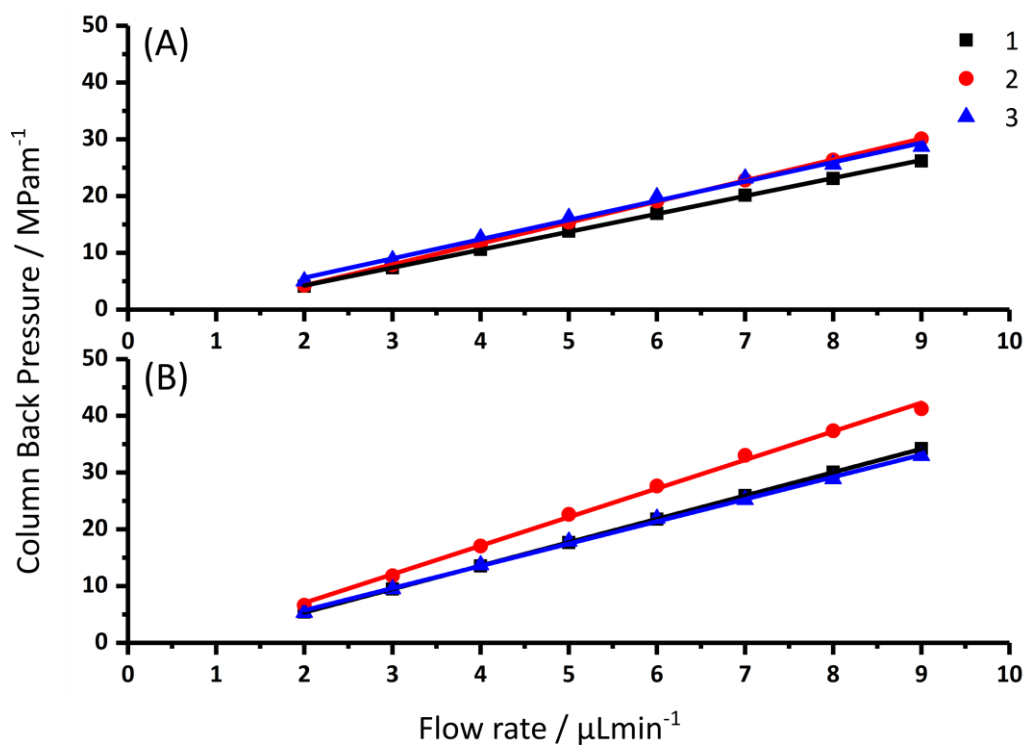

Fig. S17 Plot of column back pressure (MPa/m) against flow rate (µL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 µm i.d. silica capillaries from the same batch with 0.4 wt% PEGDA (w.r.t internal phase) in the internal phase using AIBN as initiator using: **A)** MeOH; **B)** H_2O.
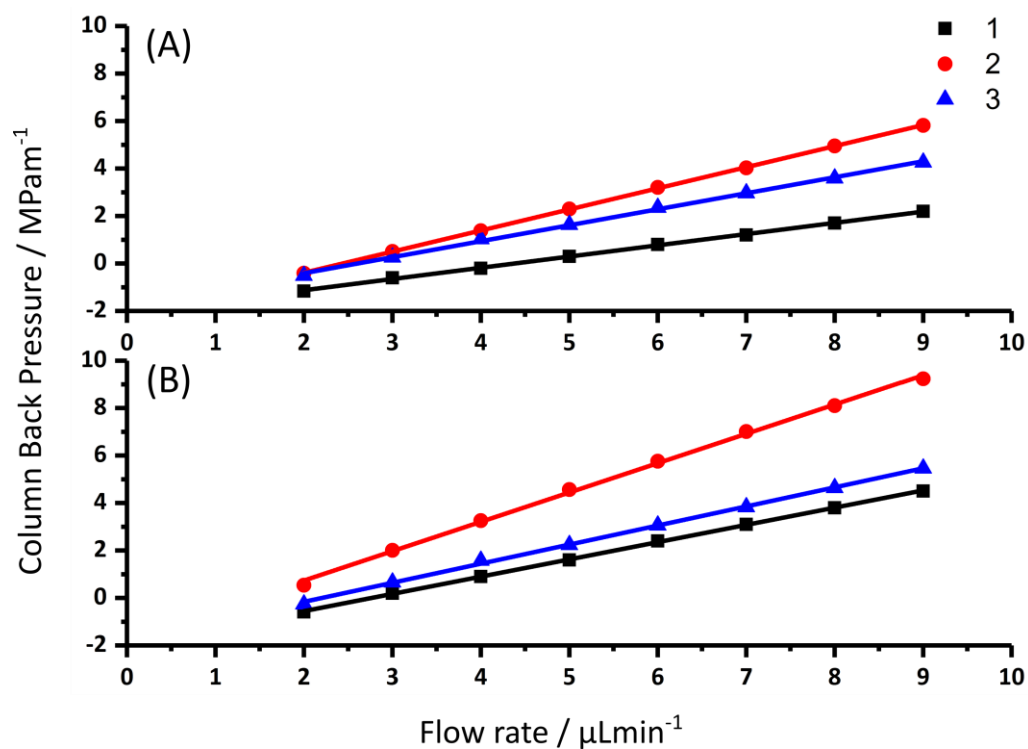
Fig. S18 Plot of column back pressure (MPa/m) against flow rate (μL/min) for three poly(Sty-*co*-DVB) poly(HIPE)s prepared in 150 μm i.d. silica capillaries from the same batch with 4 wt% PEGDA (w.r.t internal phase) in the internal phase using AIBN as initiator using: **A)** MeOH; **B)** H_2O.
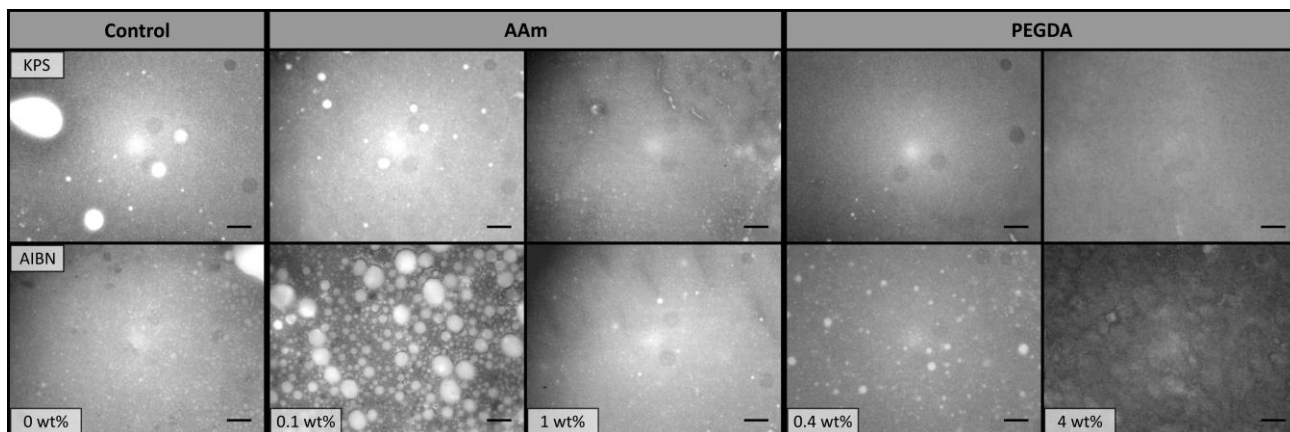
5. Optical Microscopy

Fig. S19 Optical microscopy images of emulsions prepared with different amounts of monomer (w.r.t. internal phase) in the internal phase immediately after preparation. Scale bar is 50 μm.
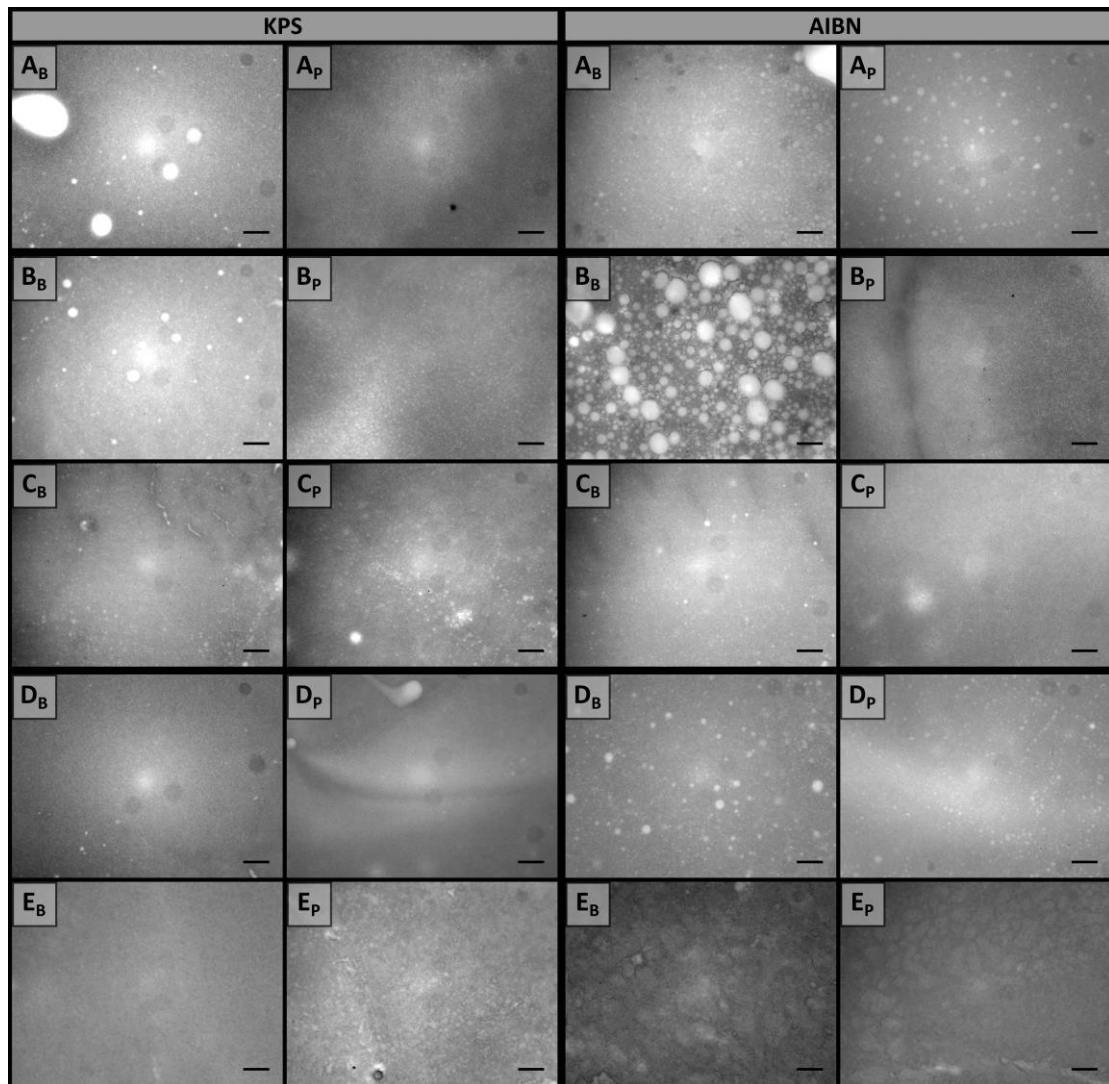
Fig. S20 Optical microscopy images of emulsions prepared with different amounts of monomer (w.r.t. internal phase) in the internal phase and using different initiators. **A)** 0 wt%; **B)** 0.1 wt% AAm; **C)** 1 wt% AAm; **D)** 0.4 wt% PEGDA; **E)** 4 wt% PEGDA. [B] indicates the bulk emulsions immediately after preparation, [P] indicates emulsions after being passed through 20 cm of capillary. Scale bar is 50 μm.