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Supporting information

Broadening the applicability of temperature-responsive liquid chromatography (TRLC): Influence of organic modifier on the temperature-responsive effect.

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1. Synthesis and packing procedure

The synthesis performed in this work was performed using the same route as used by Baert M. et al.[1]

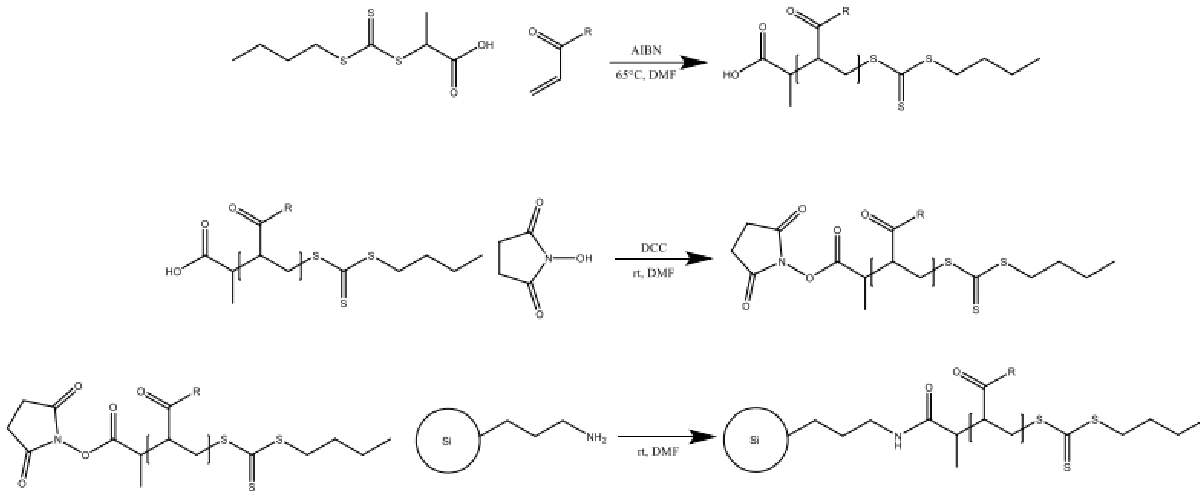

Fig S-1 *Overview of the synthesis route used to perform the temperature responsive material. Figure taken from Baert et al.*[1]

Polymer synthesis: linear homopolymers were synthesized through reversible addition-fragmentation chain-transfer (RAFT) polymerization. For this, 0.1 mol of the respective monomer (N-isopropylacrylamide for PNIPAM and N-n-propylacrylamide for PNNPAM) was dissolved together with 2 mmol of RAFT reagent (2-{[(butylsulfanyl)- carbonothioyl]sulfanyl}propanoic acid) and 0.2 mmol of initiator (AIBN) in dry DMF, ensuring the monomer concentration was 2M. The contents of the flask were then purged with nitrogen for 20 min and subsequently immersed in a preheated oil bath of 65°C to start the reaction. The conversion of monomer and molecular weight of polymer were monitored by GC and SEC respectively. Upon exceeding a conversion of 90%, the polymerisation was terminated by cooling the flask in liquid nitrogen. This yielded polymers with a molecular weight of 7500 for the PNNPAM and 11000 for the PNIPAM polymer respectively.

Polymer activation: After synthesis the polymer was modified by activating the terminal carboxylic group with N-hydroxysuccinimide (NHS). For this, the polymer mixture obtained was cooled to 0°C, subsequently 20 mmol of NHS was added to the mixture and stirred until dissolved. 20 mmol of DCC was dissolved in 10 mL dry DMF and added dropwise to the polymer reaction under vigorous stirring. The flask was then left to stir for 2 hours in an ice bath, after which the stirring was continued at room temperature for another 12 hours. The FMD was evaporated under reduced pressure and the residue was redissolved using a minimal amount of THF. Purification of the activated polymer was performed by means of filtration, followed by a subsequent precipitation in DEE. This yielded the polymers depicted in Figure S-2.

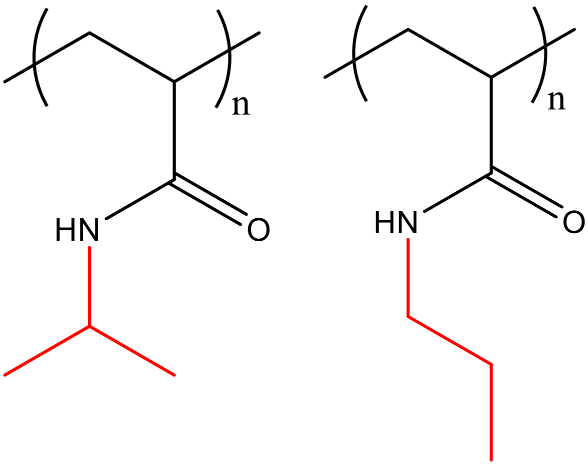

Fig S-2 *PNIPAAm (LCST = 37°C) (left) and PNNPAAm (LCST = 22°C) (right) polymers synthesized and subsequently investigated in this study.*

2. Characterisation of the polymer (Turbidimetry)

Figure S-3.1 and S-3.2 show the results of the turbidimetry curves where the temperature at which 50% transmission occurred for the heating cycles was taken for plotting the cloud point temperature in function of added organic solvent. Upon addition of higher amounts of organic solvent, it is observed that the transmission no longer dropped to 0%, which hints towards a loss in temperature-responsive effect as a fraction of the polymer will always remain soluble in solution.

a. PNNPAM

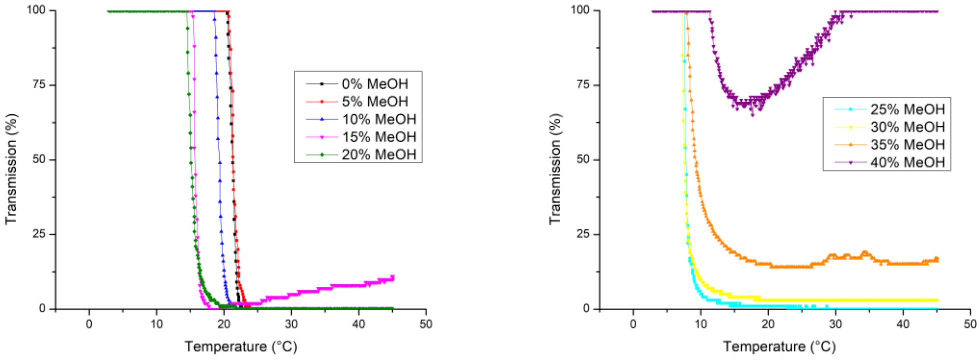

Fig S-3.1 *Results of the turbidimetry measurements (2nd heating cycle) for the poly-N-n-propylacrylamide (PNNPAM) polymer upon addition of methanol.*

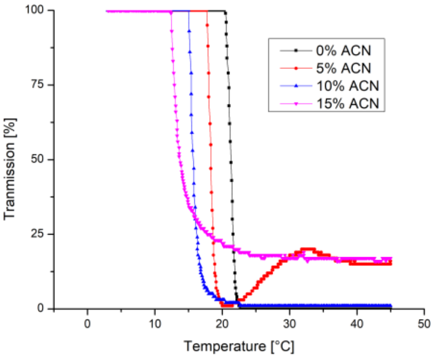

Fig S-3.2 *Results of the turbidimetry measurements (2nd heating cycle) for the poly-N-n-propylacrylamide (PNNPAM) polymer upon addition of acetonitrile.*

Upon addition of over 15% of acetonitrile the same result was observed as for the addition of 40% of methanol (Fig S-2). Therefor, it was determined that the upper limit of acceptable organic solvent for PNNPAM was found to be 15% and 35% for acetonitrile and methanol respectively.

b. PNIPAM

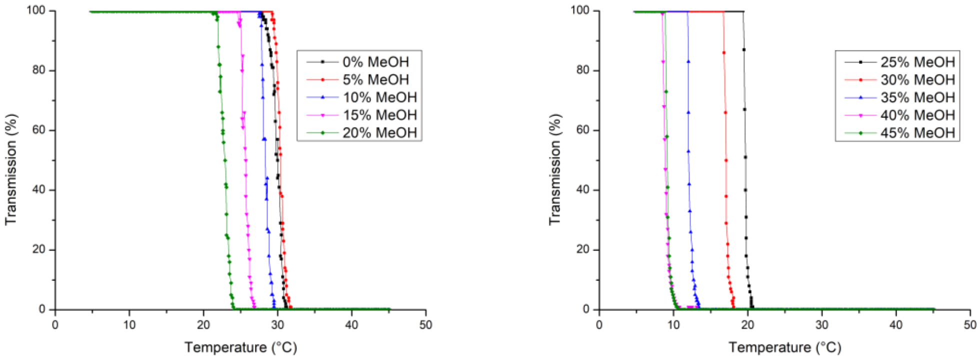

Fig S-4 *Results of the turbidimetry measurements (2nd heating cycle) for the poly-N-isopropylacrylamide (PNIPAM) polymer upon addition of methanol.*

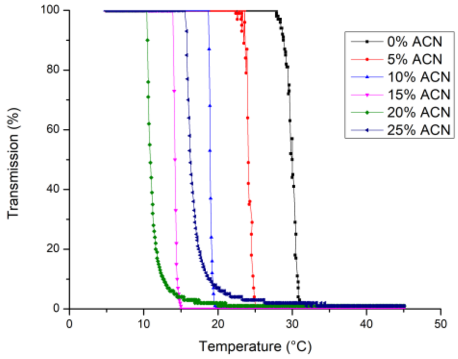

Fig S-5 *Results of the turbidimetry measurements (2nd heating cycle) for the poly-N-isopropylacrylamide (PNIPAM) polymer upon addition of acetonitrile.*

As can be observed from Fig S-4 and S-5, the upper limit was found to be 50%, as it was discovered that transmission was only 40% at 3°C upon addition of 50% of methanol. Before this loss of transmission, however, results did not hint towards a loss of temperature-responsive effect upon addition of higher amounts of organic solvent (at least during turbidimetry measurements).

3. Analysis of the temperature-responsive columns

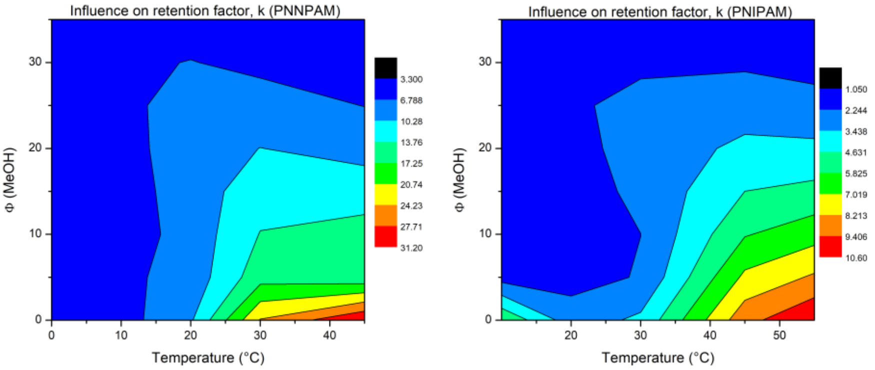

Fig S-6 *Influence of temperature and amount of added methanol on the retention factor k, for butylparaben on the PNNPAM (left) and PNIPAM (right) stationary phases.*

References

1. Baert, M. *et al.* Exploration of the Selectivity and Retention Behavior of Alternative Polyacrylamides in Temperature Responsive Liquid Chromatography. *Anal. Chem.* **92**, 9815–9822 (2020).