

Electrophoretic field gradient focusing with on-column detection by fluorescence quenching, Ansell *et al.*

Synthesis of packing material

The preparation of polyacrylamide spheres adapted from the method described by Mosbach *et al.*

The aqueous phase (mixture A) was prepared by mixing 0.8 g of acrylamide (Sigma), 0.2 g of 1,4-diacryloylpiperazine (cross-linker, Lancaster) and 6 ml of buffer solution in a 10 ml glass vial. The buffer solution was made by dissolving 1 tablet of phosphate buffered saline (Oxoid) in 100 ml distilled water. The aqueous phase mixture was sonicated until completely dissolved. 50 mg of F₂₅₄ fluorescence indicator (EM Science) were then added and the mixture shaken so the indicator became uniformly dispersed.

A solution of the initiator (mixture B) was prepared by mixing 10 mg ammonium persulphate (Sigma) and 1ml of above buffer solution in a glass vial. The solution was then sonicated until completely dissolved.

The organic phase (mixture C) was prepared by mixing 29 ml toluene, 11 ml chloroform, 25 μ l *N,N'*-tetramethylethylenediamine (TEMED) and 0.5 g sorbitan sesquioleate (SPAN 83) in a glass tube and sonicated until complete dissolution.

Mixtures A, B and C were placed in the fridge and cooled down for 1 hour. After this time had elapsed, mixtures A and B were combined together in a 10 ml syringe. Mixture C was degassed under a stream of N₂ for 5 minutes. The paddle of an overhead stirrer was then placed inside a 50 ml glass tube containing the organic phase (mixture C) and the system immersed in an ice bath. The mixture of A and B was added into C and stirring started at 500 rpm for approximately 30 seconds. Stirring speed was reduced to 400 rpm and left for 2 hours while passing N₂ through the system.

The suspension was transferred to a Büchner funnel and the majority of the organic solvents filtered away. The polyacrylamide beads were then washed with MeOH under vacuum and placed in a dessicator overnight.

Capillary electrophoresis of HC and HLADH

CE was performed using an Agilent 3^DCE system (Agilent Technologies, Cheshire, UK). The capillary was of fused silica (75 mm i.d., total length 68 cm, distance from inlet to detection window 63 cm). This was conditioned by flushing with 0.05 M NaOH for 3 min, then with deionised H₂O for 3 min. It was then equilibrated by flushing with electrolyte buffer for 3 min. Sample was injected by applying a pressure of 20 mbar for 10 s. A positive polarity 20 kV was applied at the injection end for 30 min. The temperature was maintained at 25 C throughout.

The electrolyte was 5mM Tris base (Aldrich) adjusted to pH 8.6 with phosphoric acid (Aldrich). A mixture of caffeine (neutral EOF marker), HLADH and HC was injected. Observed retention times were caffeine 7.95 min, HLADH 8.2 min, HC 14.65 min. Hence calculated apparent mobilities are caffeine $4.49 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, HLADH $4.35 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, HC $2.44 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.