Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Integrated microreaction system for optical resolution of racemic amino acids

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Design of the microchannels

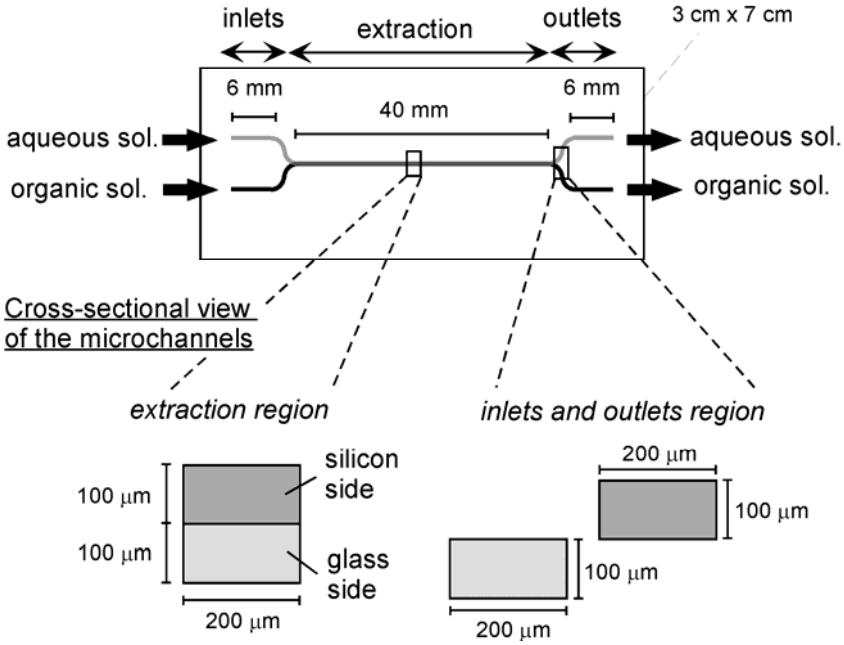

Figure S1 Design of the microchannels in the microextractor.

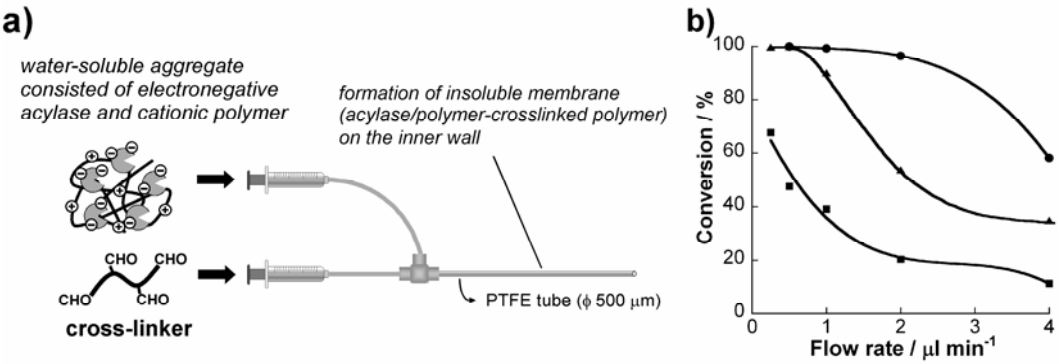

Figure S2 a) Preparation of insoluble enzyme-membrane on the inner wall of a PTFE tube. Cross-linker reagent with aldehyde group and a water-soluble aggregate consisting of anionic acylase and cationic poly-Lys were each charged into a 1-ml syringe. The solutions were supplied to a PTFE tube using a syringe pump at 4°C. Consequently, an insoluble acylase-containing membrane was formed cylindrically on the tube's inner wall. b) Hydrolysis of acetyl-D,L-Phe in a microreactor. A solution of substrate in Tris buffer (pH 8.0) was charged into a tubing microreactor (inner volume = 24 μl) with acylase/polyLys-crosslinked membrane at various flow rates. The reactions were carried out at 37°C at three concentrations (1 mM (●), 4 mM (▲), and 20 mM (■)). Products were analyzed using RP-HPLC. This acylase-based reaction converted L-body only.

Enzymatic conversion of acetyl-L-Phe to L-Phe (%) = 100 × (moles of L-Phe produced)/(moles of acetyl-L-Phe fed).

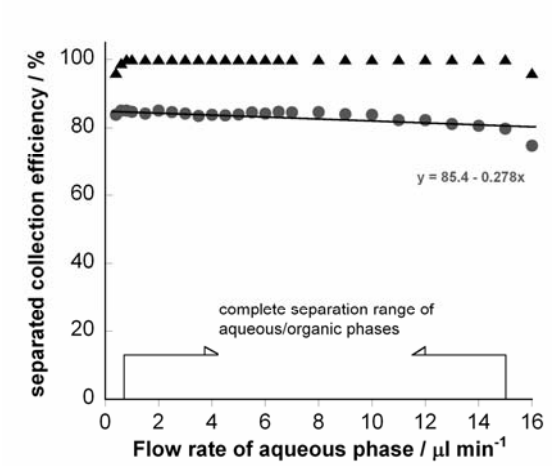

Figure S3 Extraction efficiency of acetyl-D-Phe (●) from the aqueous phase and residual ratio of L-Phe (▲) in aqueous phase. The ratio of flow rates between aqueous and organic phase was constant at 1:2. The horizontal axis shows the flow rate of aqueous phase.

For greater improvement of processing efficiency, we examined the relationship between residence time and extraction efficiency. A substrate solution used an equimolar L-Phe and Ac-D-Phe mixture corresponding to complete hydrolysis, instead of Ac-D,L-Phe solution. The ratio of aqueous and organic flow rates was kept constant at 1:2. Each phase was separated completely at aqueous flow rates of 0.8 – 15 μl min^{-1}. In the fastest flow rate corresponding to 3.2 s of

residence time of the aqueous phase, the extraction efficiency of Ac-D-Phe achieved 80%. When using 1 mM substrate, the extraction yield (6.00 nmol min^{-1}) of Ac-D-Phe per unit time increased to about seven times that of the yield (0.87 nmol min^{-1}) at a 1 µl min^{-1} aqueous flow rate. Acceleration of optical separation was feasible if complete hydrolysis is achieved at a faster flow rate, for example, by mutually joining several CEMs.

Table S1 Kinetic parameters of various amino acid derivatives in hydrolysis assay using acylase-CEM.

Amino acids	K_m (mM)[a]	V_{max} (mM min^{-1})[b]
Thi	0.387	0.120
Phe	0.065	0.115
(*p*-F)Phe	0.188	0.126
Cha	0.710	0.106
(F_5)Phe	0.233	0.081
Bpa	0.064	0.027
Nal	0.130	0.063

[a] The K_m and V_{max} were computed by fitting the data to the Michaelis-Menten equation using a public domain program: GNUPLOT.