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Integrating microflow reactions with subsequent continuous product separation in a single microfluidic chip

Stefan Jezierski , Vivian Tehsmer, Stefan Nagl and Detlev Belder*

*Institut für Analytische Chemie, Universität Leipzig, Linnéstr. 3, D-04103 Leipzig.
E-mail:belder@uni-leipzig.de

Supplementary Information

Chemicals and materials

Polyethyleneglycol diacrylate (PEG-DA, MW 258), 2,2-dimethoxy-2-phenylacetophenone (DMPA), 3-(trichlorosilyl)propyl methacrylate (TPM), trichloromethane, PEG(20)sorbitan monolaurate (Tween 20), ortho-phthaldialdehyde (OPA), mercaptoethanol, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), rhodamine B, (hydroxypropyl)methyl cellulose (HPMC), alanine, arginine, aspartic acid and glutamic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium chloride and sodium tetraborate decahydrate were acquired from Merck (Darmstadt, Germany) and fluorescein sodium salt, ethanol, methanol, and n-heptane from Roth (Karlsruhe, Germany).

Chip production

Fluidic contacts holes were powder blasted into microscope glass slides (Menzel, Braunschweig, Germany) at the designated positions for inlets and outlets. To obtain covalent bonding between the polymer and glass slides a surface modification based on the protocols by Revzin *et al.*[1] and Brzoska *et al.*[2] were performed. Therefore the cleaned and dried glass slides were treated with 5 mM TPM in n-heptane and trichloromethane (3:1) for 2 min. Finally, the slides were rinsed with n-heptane and deionized water.

Photopolymerization was carried out on a MJB4 laboratory mask aligner (Süss MicroTec, Munich, Germany) equipped with a mercury arc lamp (15 mW * cm^{-2} at 365 nm). For that purpose 80 µL of 99 % PEG-DA and 1 % DMPA were distributed onto a TPM-treated slide. A prepared lid was lowered carefully onto the polymer, avoiding air bubbles. Subsequently, the light-impermeable mask (offset print, 3600 DPI, DTP-System-Studio, Leipzig, Germany) was aligned onto the lid. The chip was placed in the mask aligner and was illuminated for 1.2 s. Afterwards the uncured prepolymer was removed by reduced pressure. The microfluidic system was flushed with ethanol to remove PEG-DA residues in the channels and separation bed. To ensure complete polymerization, the dried chip was re-exposed to UV light for 30 s.

Microfluidic setup

Microfluidic connectors consisted of short silicone tubes glued onto the lid holes with Elastosil E43 (Wacker, München, Germany). Tubes from syringe pumps were directly attached to the connectors. For electrical contact external electrodes were used.[3] For the flow in separation area neMESYS syringe pumps (cetoni, Korbussen, Germany) in combination with 1 mL glass syringes (ILS, Stuetzerbach, Germany) were applied. The flow in the electrode channels was generated by a PHD 22/2000 (Harvard Apparatus, March-Hugstetten, Germany) syringe pump using 5 mL Omnifix syringes (B. Braun, Melsungen, Germany). A HCL 35-6500 power supply (FuG Elektronik, Rosenheim, Germany) was used for application of an electric field.

Microscopic imaging

Fluorescence imaging was performed with a Nikon D90 digital camera mounted on an IX 71 inverted fluorescence microscope (Olympus, Hamburg, Germany) with mercury lamp excitation. For the experiments with fluorescein and rhodamine, a multispectral filter set (FITC Cy3 Cy5, λ_{exc} = 470–500/550– 570/630–660 nm, λ_{em} = 510–530/580–620/680–740 nm, LOT-Oriel, Darmstadt, Germany) was applied. For reaction monitoring and subsequent continuous µFFE separation a near UV filter set (U-MWU2, λ_{ex} = 330-385 nm, λ_{em} > 420 nm, Olympus) was used. Line scan evaluation was performed with ImageJ (version 1.42q, US National Institutes of Health, Bethesda, MD, USA).

Reaction conditions

For reaction of OPA and mercaptoethanol with Ala and Glu (Fig. 2c. d) one analyte channel was filled with 4.8 mM OPA and 9.6 mM mercaptoethanol in methanol with 36% 40 mM borate buffer (pH 9). The second analyte channel was filled with Ala and Glu (8 mM each) in 40 mM borate buffer. For reaction of OPA and mercaptoethanol in molar excess with amino acids first analyte channel was filled with 21 mM OPA and 42 mM mercaptoethanol in 72 % borate buffer, 28 % methanol. The second channel was filled with Arg, Ala, and Asp (6 mM each) in 40 mM borate buffer. The flow rates for each channel in the flow reactor were 200 nL * min^{-1} in all experiments.

Free-flow electrophoresis

The reaction mixture was directly introduced into the separation area with flanking electrolyte streams consisting of aqueous 40 mM CHES (pH 9) as buffer with additions of 0.2 % HPMC and 0.1 % Tween 20 for dynamic surface coating of the separation bed. For reaction of OPA and mercaptoethanol with Ala and Glu (Fig. 3b) the linear velocity in the FFE chamber was 1 mm * s^{-1}, the residence time 7.5 s, the electrode buffer 50 mM KCl and the applied voltage 330 V * cm^{-1} (900 µA). For reaction of OPA and mercaptoethanol in molar excess with Arg, Ala and Asp (Fig. 3c) the applied electric field was 295 V * cm^{-1} (800 µA) and 100 mM KCl was used as electrode buffer, the residence time was 5.6 s.

Supplementary references:

1 Revzin, A.; Russell, R. J.; Yadavalli, V. K.; Koh, W.-G.; Deister, C.; Hile, D. D.; Mellott, M. B.; Pishko, M. V. *Langmuir* **2001**, *17*, 5440–5447.

2 Brzoska, J. B.; Azouz, I. B.; Rondelez, F. *Langmuir* **1994**, *10*, 4367–4373.

3 Koehler, S.; Benz, C.; Becker, H.; Beckert, E.; Beushausen, V.; Belder, D. *RSC Adv.* **2012**, *2*, 520-525.