

## Supporting information

### Open Channel-based Microchip Electrophoresis Interfaced with Mass Spectrometry via Electrostatic Spray Ionization

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#### 1. Experimental

##### 1.1 Chemicals and Materials

Rhodamin B base (RhB, 99%) and glycerol anhydrous was purchased from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Ciprofloxacin (CIP) was from TCI Deutschland GmbH (Eschborn, Germany), and nalidixic acid (NA) and methanol (99.9%, HPLC grade) from Applichem GmbH (Darmstadt, Germany). Angiotensin I (Ang I, NH<sub>2</sub>-DRVYIHPFHL-COOH, 98%) was obtained from Bachem (Bubendorf, Switzerland). Acetic acid (HAc, 100%) was obtained from Merck (Zug, Switzerland). All the other chemicals, like fluorescein (free acid), formic acid and ethylenediamine (EDA) were analytical reagent grade and obtained from Fluka (Buchs, Switzerland). All solutions were prepared with deionized water produced by an alpha-Q system (Millipore, Zug, Switzerland) unless specified.

Fused silica capillaries (150/363 μm inner/outer diameter (id/od), 50/150 μm id/od, and 25/363 μm id/od) were obtained from BGB Analytik AG (Böckten, Switzerland). The PET substrate (125 μm thick, Biaxially Oriented PET Film) was purchased from GoodFellow Cambridge Limited (Huntingdon, Great Britain). All syringes used were provided by Hamilton (Bonaduz, Switzerland), and syringe pumps by Cole Palmer Instrument Company (Vernon Hills, USA).

The stock solution of RhB was prepared in water and fluorescein in methanol with a concentration of 2 mg/mL each. The mixed solution of CIP and NA for stocking was prepared in 10 mM NaOH with the concentration of 1 mg/mL for each compound. All the stock solutions were diluted with deionized water before use. The background electrolyte (BGE) for microchip electrophoresis was 40 mM EDA (pH 8.0, adjusted by formic acid) with different contents of glycerol (0, 5%, 10%, 15%, 20%, 30% (m/m)). Extraction solution (50% methanol, 49% water, 1% acetic acid (v/v)) was applied for the scanning ESTASI-MS.

##### 1.2 Microchip fabrication

Microchannels (65 mm×0.2 mm×0.075 mm (length×width×depth)) were fabricated in a PET substrate (0.125 mm thick) by photoablation with an ArgonFluoride excimer laser (Lambda Physics LPX 210I, Göttingen, Germany) as described elsewhere [1]. During the fabrication of the simplest linear embodiments, a 10 mm×2 mm mask was employed to obtain the channels of 0.2 mm in width, and the PET substrate was moved at a speed of 0.1 mm/s to produce the channels of approximately 75 μm in depth. The length of channels was always set at 6.5 cm, and 800 pulses of ablation in a static shot mode were applied at one end of the channel to create a rectangular hole (1 mm×0.2 mm). Finally, about 1.4 cm of the channel at the end with rectangular hole was covered with a polyethylene (PE)/PET layer (25/10 μm thick) using a lamination apparatus (Morane Senator, Oxon, UK) at 135 °C and 3 bars, allowing the injection of solutions into the channel. The microchip was afterward cured at 80 °C for one hour to make the lamination stable.

##### 1.3 Microchip electrophoretic separation

Open channel-based microchip electrophoresis was performed as illustrated in Fig. 1a. Taking into consideration both, the separation capacity and the compatibility with MS [2], 40 mM EDA (pH 8.0) with different amount of glycerol was chosen as BGE. Solutions for both, preconditioning and separation, were introduced into the channel at a speed of 90 μL/h by a syringe pump equipped with a 250 μL syringe, which was connected to the channel *via* a fused silica capillary (150/363 μm id/od, 15 cm long). Before each separation, the channel was conditioned with 15 μL 0.1 M NaOH, 15 μL water, and 15 μL BGE. The sample (0.1 μL) was deposited at the position of 1 cm from the open end of the channel by a capillary connected to a 10 μL syringe, which was equipped with another syringe pump, through a fused silica capillary (25/363 μm id/od, 40 cm long). Direct current high voltage (2500 V) provided by an EPS 3501 XL power supply (Amersham Pharmacia Biotech, Sweden) was applied between the needle of the syringe (+) and the open end of the channel (−) through a platinum electrode immersed in a BGE droplet (1.5 μL).

The open channel-based microchip electrophoresis was firstly performed for two fluorescent dyes (0.5 mg/mL fluorescein and 0.1 mg/mL RhB) to determine an optimized experimental conditions. Electrophoresis of antibiotics was carried out afterward with the same protocol for further proof-of-concept.

##### 1.4 *In-situ* ESTASI-MS detection

After electrophoretic separation and solvent evaporation, dried fractions were formed in the open channel. The microchip was attached to an insulating sample stage (PET film, 0.125 mm thick) for ESTASI-MS detection using a linear ion trap mass spectrometer (Thermo LTQ Velos, Thermo Fisher Scientific, San Jose, USA). The ESI source was replaced by a home-designed ESTASI-MS imaging system, which is described in details elsewhere [3, 4]. A pulsed square wave high voltage was produced by amplifying signals from a function generator (TG513, Thurlby Thandar Instruments, Huntingdon, England) with an amplifier (10 HVA24-P1, HVP High Voltage Products GmbH, Mar-tinsried/Planegg, Germany) to induce ESTASI. As schematically illustrated in Fig. 1b, the MS inlet, the electrode for ESTASI, and the wetting capillary were fixed during the analysis, while the sample stage moved horizontally along *x*-axis

by an  $x,y$ -motion system, which was constructed by two perpendicularly oriented MTS50-Z8 motorized translation stages (Thorlabs, Dachau/Munich, Germany). The movement of the sample stage with a defined step size and translation speed was controlled by a program developed in Labview (National Instruments, Austin, TX). The MS inlet was grounded and adapted to an “L” shape, so that the opening of inlet, downward orientated, allowed the scanning of horizontally placed sample stage. The electrode (diameter = 0.1 mm, stainless steel) with upward orientation was placed axially with the MS inlet beneath the sample stage. A fused silica capillary (50/150  $\mu\text{m}$  id/od), named the wetting capillary, was used to wet the sample bands locally by delivering the extraction solution with the internal standard into the channel under the flow rate of 60  $\mu\text{L}/\text{h}$  during ESTASI-MS analysis. It should be noted that the outer diameter of the wetting capillary was smaller than the channel width, so that its tip can touch the bottom of the open channel during ESTASI-MS scanning to extract efficiently any sample present inside.

## 2. Results and discussion

### 2.1. Open channel-based microchip electrophoresis

Open channel-based microchip electrophoresis provides two main advantages in comparison with the closed one. Firstly, the sample injection protocol is greatly simplified, free from complicated microchip design. Additionally, the open channel provides direct access to the sample in the channel by detection devices, *e.g.* MS instruments equipped with an ambient ionization source, for probing separated compounds.

Some problems, however, remain to be solved in open channel-based microchip electrophoresis. First of all, introduction of the solution, *e.g.* the BGE, to the open channel is much more difficult to control than that for a closed channel. Here, the channel was partly closed to simulate the flow injection in standard microfluidic systems, so that the solution flow was firstly introduced into the closed part, and then to the open part. Thanks to the hydrophobic character of the plastic substrate, the solution showed no inclination to flow along the edge of PE/PET cover between the open and closed parts of the same channel. In order to prevent the liquid from running over or accumulating at a certain point of the open channel and keep continuous and stable flow, the injection rate must be well optimized. In our study, the rate was finally set as 90  $\mu\text{L}/\text{h}$ . Furthermore, pre-wetting the channel by a cotton-tipped applicator to obtain a temporarily hydrophilic surface improved the flow reproducibility.

Separation resolution is another core concern in the open channel-based microchip electrophoresis. As described by Tseng K [5], the absence of a portion of the channel wall will decrease the contact between the sample and the inner wall of the channel to lower the diffusion coefficient, leading to an increased resolution compared to the closed channel. In the experiments with fluorescent dyes at high concentrations, the migration and separation of the dyes were visually observed, indicating the feasibility of this open channel-based electrophoresis. Nevertheless, a serious peak broadening existed during the formation of dried bands, because there was no electric field to focus the bands and the solution tended to spread to the position that has been dried. In this work, we introduced glycerol at a concentration of 15% into BGE to eliminate the broadening effect in zone electrophoresis

### 2.2 Effect of glycerol on electrophoresis and ESTASI-MS

For the electrophoretic separation of the fluorescence dyes, the current was observed to decrease at 4.6 min and reach 0 A at 4.7 min (Fig. S1a) attributed to the evaporation of BGE solvent. As a consequence, the electrophoretic bands were prone to broaden axially and overlap with each other quickly, because there was no electric field to focus the bands and the solution tended to spread to the position that has been dried. It has been reported to use a heat gun to accelerate the solvent evaporation, so that a complete evaporation could happen shortly after the current breakdown to minimize the peak broadening [6], which, however, is a protocol largely relying on experience and not well controllable. Glycerol, which acted as a replacement of aqueous anti-convective gels in capillary isoelectric focusing (CIEF) owing to its high viscosity [7], was employed here to reduce the longitudinal diffusion of electrophoretic bands. Additionally, it has been previously proved that the introduction of glycerol to sample solutions affects little the intensity of ESI-MS signal even at a level up to 43% (w/w) [8]. In our study, no remarkable suppression in the ESTASI-MS signal of fluorescein was found, when a BGE containing up to 30% glycerol was used for electrophoresis, followed with direct ESTASI-MS analysis, indicating the high tolerance to glycerol of ESTASI-MS. During the scanning ESTASI-MS, samples were extracted by the aqueous extraction solution delivered by the wetting capillary for ionization and MS analysis, so that only water-soluble molecules were sprayed to the MS inlet. Thus, no contamination of the MS inlet was observed. The concentration of glycerol in BGE was optimized only for high separation resolution of the microchip electrophoresis.

As depicted in Fig. S1a, the initial current in electrophoresis decreases, when the glycerol in BGE increases from 0 to 30%, as the organic solvent decreases the conductivity of the BGE. On the other hand, the decay of the electrophoresis current is slower with the incremental change of glycerol concentration, and the current does not fail completely after the evaporation of all the aqueous solvent, because glycerol has a low vapor pressure, leaving a non-aqueous BGE filled in the open channel for a long term. Thus, a higher current could be obtained, when the higher portion of glycerol was added into BGE after the complete evaporation of water, as indicated in Fig. S1a. Because of the electrophoretic current and the increasing viscosity of BGE during water evaporation, the broadening effect can be obviously suppressed in the presence of glycerol.

Due to the high viscosity of glycerol, the electrophoretic bands could keep in invariant positions after electrophoresis and complete evaporation of the water. The separated fluorescence dyes were imaged by a digital camera under UV excitation (254 nm), as displayed in Fig. S1b. When no glycerol was added into BGE, the sample bands extended rapidly, and the fluorescein filled almost the whole channel. Such a situation was improved a lot by using BGE containing 5% glycerol, where two bands of dyes were observed but still largely overlapped. Further increase of glycerol content brought better separation until the concentration of glycerol was up to 15%. Higher concentrations of glycerol gave slightly narrower sample bands, but influenced negatively the re-dissolving of the sample in the extraction solution during ESTASI-MS analysis, since the sample was partly buried inside the glycerol. Moreover, the presence of

glycerol strongly reduced EOF, as indicated in the last three images in Fig. S1b, which is another big advantage. Similar results were not found when the glycerol content ranged from 0 to 10% because of the backflow and the diffusion of sample bands. Finally, 40 mM EDA (pH 8.0) with 15% glycerol was selected as BGE for open channel-based microchip electrophoretic separation followed by ESTASI-MS analysis.

### 2.3 Antibiotics analysis

A mixture of two antibiotics (0.05 mg/mL NA and 0.05 mg/mL CIP) was also analyzed with the newly developed method. Extracted ion electropherogram displayed in Fig. S2a indicates an acceptable separation resolution, as well as greatly decreased peak widths comparing to Fig. 2a. Mass spectra of the two antibiotics are shown in Fig. S2b.

Additionally, the sensitivity of this offline hyphenation was investigated by taking NA and CIP as model samples. A mixed solution (5 µg/mL for each compound) was processed in the same protocol, obtaining S/N 2.5 and 6.6 for NA and CIP, respectively, on mass spectra. Therefore, the platform for *in-situ* detection by combining open channel-based microchip electrophoresis and ESTASI-MS provided LODs of 6.0 µg/mL for NA and 2.3 µg/mL for CIP.

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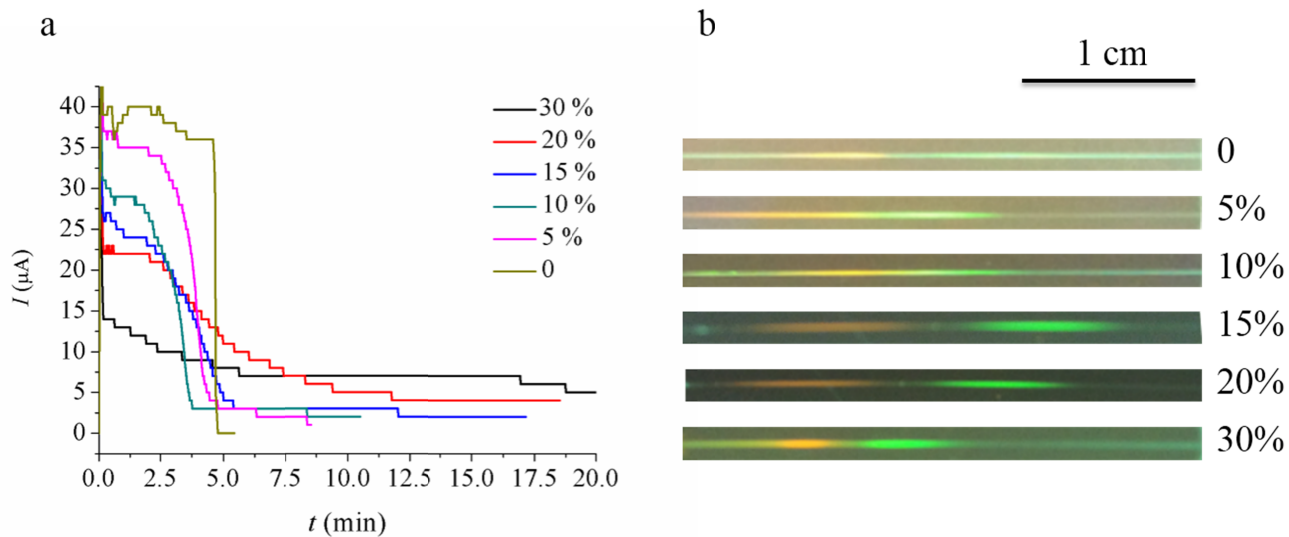


Fig. S1 Effect of glycerol concentration on the (a) current and (b) separation resolution of open channel-based microchip electrophoresis. The photographs were taken under UV excitation at 254 nm after the evaporation of aqueous solvent.



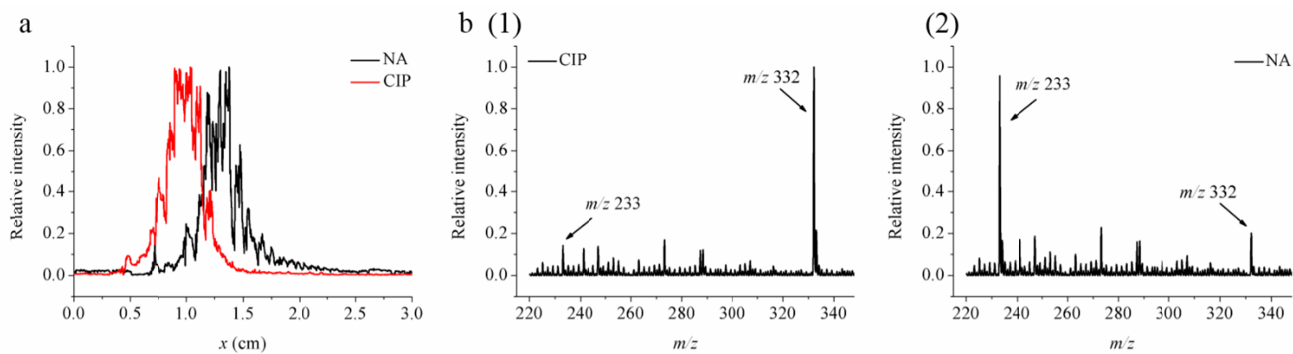


Fig. S2 (a) Extracted ion electropherogram of antibiotics (0.05 mg/mL for NA and CIP respectively) by combining open-channel-based microchip electrophoretic separation with *in-situ* ESTASI-MS detection. (b) Mass spectra of CIP obtained at  $x=0.98$  cm (1) and NA at  $x=1.30$  cm (2).