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Design and performance evaluation of a microfluidic ion-suppression module for anion-exchange chromatography

Sam Wouters[a], Bert Wouters[a], Sander Jespers[a], Gert Desmet[a], Hamed Eghbali[a], Cees Bruggink[b], Sebastiaan Eeltink[a,*]

[a] Vrije Universiteit Brussel, Department of Chemical Engineering, Pleinlaan 2, B-1050 Brussels, Belgium
[b] Leiden University Medical Center, Center for Proteomics and Metabolomics, PO box 9600, 2300 RC Leiden, The Netherlands

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ABSTRACT

A microfluidic membrane suppressor has been constructed to suppress ions of alkaline mobile-phases *via* an acid–base reaction across a sulfonated poly(tetrafluoroethylene)-based membrane and was evaluated for anion-exchange separations using conductivity detection. The membrane was clamped between two chip substrates, accommodating rectangular microchannels for the eluent and regenerant flow, respectively. Additionally, a clamp-on chip holder has been constructed which allows the alignment and stacking of different chip modules. The response and efficacy of the microfluidic chip suppressor was assessed for a wide range of eluent (KOH) concentrations, using 127 and 183 μm thick membranes, while optimizing the flow rate and concentration of the regenerant solution (H_2SO_4). The optimal operating eluent flow rate was determined at 5 μL/min, corresponding to the optimal van-Deemter flow velocity of commercially-available column technology, *i.e.* a 0.4 mm i.d. × 250 mm long column packed with 7.5 μm anion-exchange particles. When equilibrated at 10 mM KOH, a 99% decrease in conductivity signal could be obtained within 5 min when applying 10 mM H_2SO_4 regenerant at 75 μL/min. A background signal as low as 1.2 μS/cm was obtained, which equals the performance of a commercially-available electrolytic hollow-fiber suppressor. When increasing the temperature of the membrane suppressor from 15 to 20 °C, ion suppression was significantly improved allowing the application of 75 mM KOH. The applicability of the chip suppressor has been demonstrated with an isocratic baseline separation of a mixture of seven inorganic ions, yielding plate numbers between 5300 and 10,600 and with a gradient separation of a complex ion mixture.

1. Introduction

Ion chromatography (IC) constitutes an analytical technique applied for the separation of inorganic ions and ionizable analytes including small organic acids, aliphatic amines, and carbohydrates [1–3]. Given the nature of the analytes, conductivity detection is the most applied detection method, being used in approximately 60% of published articles on IC [4]. The signal intensity is dependent on the conductivity of the solution in the detection cell, as governed by the Kohlrausch equation:

$$\kappa = 10^{-3}\sum_i \lambda_i c_i \quad (1)$$

where κ is the solution conductivity (μS/cm), λ the equivalent conductivity (μS/cm meq) and c the equivalent concentration (eq/L). The application of eluent suppressor technology prior to conductivity detection is indispensable as this provides two main benefits over non-suppressed conductivity detection: (i) lowering the mobile-phase background signal and (ii) amplifying the analyte signal. Suppressor technology was first introduced by Small et al., laying the basis for the emergence and breakthrough of ion chromatography as analytical separation technology [5]. Early suppressors or "strippers" consisted of large i.d. columns packed with ion-exchange resins, and had limited capacity [6]. Counter ions in the mobile phase (such as K^+ in anion-exchange separations with KOH as the mobile phase) are replaced by protons. To overcome the need for off-line regeneration, suppressor technology in the form of a bundle of sulfonated polystyrene hollow-fibers and single Nafion fibers was explored [7,8]. Problems with mechanical stability of the fiber and with establishing interfaces with other system components (column outlet and inlet of the conductivity detector) led

* Corresponding author. Tel.: +32 0 2 629 3324; fax: +32 0 2 629 3248.
E-mail address: seeltink@vub.ac.be (S. Eeltink).

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to the development of membrane suppressors based on flat sheets of ion-exchange material [9]. Moreover, the suppression capacity improved due to a higher surface area available for the acid–base reaction. Ion transport across the membrane was further enhanced by a factor of 4 by packing the suppressor channel with inert particles, reducing concentration polarization on the membrane surface [10]. The latest advancement in suppressor technology has been the introduction of an electrolytic membrane suppressor [11,12]. The main benefits are the generation of counter ions by electrolysis of water, overcoming the need to supply a regenerant solution, and the enhancement of ion transfer due to the application of an electrical field across the membrane. A drawback of this design is the local generation of heat, affecting the noise level depending on operating conditions [13,14].

In the last two decades high-performance liquid chromatography (HPLC) technology has undergone an evolution, *i.e.* better kinetic-performance limits in terms of efficiency and analysis times were enabled by the higher pressure rating of instrumentation [15,16] Furthermore, reduction of the solvent consumption and increased detection sensitivity has been achieved *via* miniaturization [17,18]. Chip technology dedicated to proteomics LC–MS applications has become commercially available [19,20]. The developments in ion chromatography have lagged behind, mainly since most efforts were directed to the development of new stationary phases providing unique separation selectivity [21,22]. Only recently capillary high-pressure IC instrumentation complemented with 0.4 mm i.d. capillary columns was introduced [23,24]. However, to analyze minute amounts of sample mixtures as for example encountered in life-science studies, and maintaining high separation efficiencies, the development of integrated chip-based ion analyzers minimizing extra-column volume connections is required.

In the present study, the possibilities and limitations to design and apply a microfluidic ion-suppression module for anion-exchange chromatography have been explored. Therefore, a chip holder was designed allowing to stack different chip modules, including a membrane chip suppressor utilizing 127 and 183 μm thick sulfonated poly(tetrafluoroethylene) polymer sheets. The performance of the chip suppressor was evaluated taking into account eluent and regenerant flow rates, and concentrations. Furthermore, the effect of temperature on ion transport across the membrane and hence on the resulting background conductivity signal was evaluated. The isocratic performance of the microfluidic chip suppressor is compared with that of a commercially-available capillary electrolytic hollow-fiber suppressor. Finally, the potential of the microfluidic ion-suppression module is demonstrated with the separation of a complex 14 anion mixture in gradient mode.

2. Materials and methods

2.1. Materials and chemicals

Cyclic olefin copolymer (COC, grades 8007 and 6017) was purchased from Kunststoff-Zentrum (Leipzig, Germany). Dupont Nafion sheets (grades N115: 127 μm and N117: 183 μm, with total acid capacities of 0.95 to 1.01 meq/g) were obtained from IonPower (Munich, Germany). Capillary poly(etheretherketone) (PEEK) tubing (50 and 150 μm i.d., 360 μm o.d.) was purchased from Achrom (Zulte, Belgium). PEEK foil was obtained from Victrex (Lancashire, UK).

Water was purified (18.2 MΩ cm) using a Milli-Q Reference water purification system by Millipore (Billerica, USA). A 7-anion standard was obtained from Thermo Fisher Scientific (Sunnyvale, CA, USA), containing fluoride (20 mg/L), bromide (100 mg/L), chloride (100 mg/L), nitrate (100 mg/L), nitrite (100 mg/L), phosphate (150 mg/L), and sulfate (150 mg/L). A complex anion mixture was prepared by diluting the 7 anion standard 1:100 and adding: formate (0.6 mg/L), phenylacetate (1.5 mg/L), arsenate (1.5 mg/L), oxalate (1.5 mg/L), thiosulfate (1 mg/L), iodide (1.5 mg/L), and citrate (1.5 mg/L). Formic acid, phenylacetic acid, sulfuric acid (97%) and sodium arsenate were purchased from Sigma-Aldrich (Bornem, Belgium). Oxalic acid, sodium thiosulfate, sodium iodide, and trisodium citrate were kindly donated by Thermo Fisher Scientific.

2.2. Fabrication of microfluidic devices and chip holder

The channel layouts of the separation and suppressor modules were designed using AutoCAD software and micromilled in COC polymer substrate (machined to 70 × 20 × 2 mm plates) using a Datron M7 Compact computer-numerically-controlled micro-milling robot (Datron AG, Mühltal, Germany), allowing the fabrication of channel widths of 100 μm and larger. The bottom plate (grade 6017 COC, T_g = 178 °C) of the separation chip containing a 0.5 × 0.5 × 40 mm ($w \times d \times l$) separation channel was irreversibly bonded to a top plate (grade 8007 COC, T_g = 78 °C) using solvent-vapor-assisted bonding with cyclohexane at room temperature [25]. For the suppressor module, the cation-exchange membranes were hydrated, cut to size and clamped between top and bottom COC plates (grade 8007), accommodating the eluent (0.3 × 0.1 × 40 mm) and the regenerant channel (0.3 × 0.2 × 50 mm), respectively.

The module was clamped together using a custom-made holder with adjustable clasp locks. This holder was machined from aluminum and the inside lined with PEEK foil. To connect the microfluidic device with other instruments, commercially-available flat-bottom headless Nanoport connections from Upchurch Scientific (Oak Harbor, USA) were used, compatible with 360 μm o.d. capillary PEEK tubing. For this purpose, flat-bottom tapped receiving ports were micromachined in the top and bottom plate of the holder.

2.3. Suppressor characterization

The suppressor performance was characterized using an ICS-5000 instrument from Thermo Fisher Scientific. The system was equipped with a capillary pump, electrolytic eluent (potassium hydroxide) generation module, autosampler, injection valve, capillary electrochemical suppressor, and a capillary conductivity detector. Chromatography experiments were conducted employing a 250 mm × 400 μm i.d. capillary column packed with 7.5 μm super macroporous sulfonated poly(ethylvinylbenzene-*co*-divinylbenzene) particles coated with 65 nm positively-charged nanobeads (IonPac AS18 column, Thermo Fisher Scientific). For our purpose, the prototype microfluidic suppressor was placed between the capillary column and the conductivity detector, and connected with two 200 mm long capillary PEEK tubings (50 μm i.d.), fitted with 1/16 inch sleeves, bolts and nuts, to match the ports of the commercial system.

The eluent flow, with flow rates ranging between 5 and 15 μL/min, was provided by the capillary ICS-5000 pump. The regenerant for the suppressor was delivered in counter-flow direction with respect to the eluent flow, using a LPG3000 loading pump equipped with an online degasser (Thermo Fisher Scientific) while applying flow rates of 25 and 75 μL/min. The column temperature was maintained at 30 °C in isocratic mode and 60 °C in gradient mode. The chip suppressor was operated at 15 and 20 °C, respectively, and the temperature of the conductivity cell was maintained at 35 °C. Injection of sample occurred in full-loop mode (V_{inj} = 400 nL). Isocratic separations of the 7-anion mixture (diluted 1:100 from standard stock solution, see materials and chemicals)