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# Using contemporary liquid chromatography theory and technology to improve capillary gradient ion-exchange separations

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ABSTRACT

The gradient-performance limits of capillary ion chromatography have been assessed at maximum system pressure (34.5 MPa) using capillary columns packed with 4.1 μm macroporous anion-exchange particles coated with 65 nm positively-charged nanobeads. In analogy to the van-Deemter curve, the gradient performance was assessed applying different flow rates, while decreasing the gradient time inversely proportional to the increase in flow rate in order to maintain the same retention properties. The gradient kinetic-performance limits were determined at maximum system pressure, applying $t_G/t_0 = 5$, 10, and 20. In addition, the effect of retention on peak width was assessed in gradient mode for mono-, di-, and trivalent inorganic anions. The peak width of late-eluting ions can be significantly reduced by using concave gradient, resulting in better detection sensitivity. A signal enhancement factor of 8 was measured for a late-eluting ion when applying a concave instead of a linear gradient. For the analysis of a complex anion mixture, a coupled column with a total length of 1.05 m was operated at the kinetic-performance limit applying a linear 250 min gradient ($t_G/t_0 = 10$). The peak capacity varied between 200 and 380 depending on analyte retention, and hence on charge and size of the ion.

## 1. Introduction

A landmark paper published in 1975 by Small et al., describing a novel stationary phase for the separation of inorganic ions using suppression technology prior to conductivity detection [1], laid the basis for the emergence of ion chromatography (IC). Nowadays, IC has become a well-established analytical technique and is widely used for the separation of inorganic ions, small organic acids, and aliphatic amines [2–4] with dedicated instrumentation and column technology. The application of capillary IC columns with internal diameters ranging between 100 and 500 μm has been reported since the early 1980s [5,6]. However, only in recent years dedicated instrumentation has become commercially available. The most important advantages of capillary IC over conventional systems with analytical scale columns (2–4 mm i.d.) include the possibility to analyze minute amounts of (life-science) samples and the improved flow rate compatibility with detectors such as electrospray ionization mass spectrometry (ESI-MS) [7,8].

Different modes of IC can be distinguished; (i) ion-exchange chromatography for the separation of anions and cations based on Coulomb interactions with the stationary phase carrying functionalities of opposite charge, (ii) ion-exclusion chromatography for the separation of weak acids and bases, and (iii) ion-pair chromatography for the separation of polarizable hydrophilic compounds [9–11]. Selectivity tuning in IC is most commonly performed by the application of different stationary phases. Both silica- and polymer-based materials are widely used in IC, the latter being stable over a wide pH range and therefore more broadly applicable [12,13]. Much effort has been directed toward the development of advanced polymer-based stationary-phase chemistries and architectures [14–17]. To create high capacity (30–150 μEquiv./mL) ion-exchange materials, wide-pore polymer resins have been developed that are electrostatically coated with polymer nanobeads [14]. To further enhance the capacity and minimize intra-particle mass transfer, shielded resins can be created. These resins are grafted with polymer strands containing ionizable moieties projected from the substrate surface, which subsequently can be functionalized with nanobeads [15]. To get better control over the selectivity, IC resins have been shielded with an amine copolymer *via* electrostatic interactions [16]. Branching

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points can be introduced *via* a step-growth polymerization, that can be conducted in repetitive steps. In this way, different layers of mixed-mode chemistries have been created to tune the selectivity [17].

Whereas in the field of HPLC significant efforts are conducted to improve the kinetic performance, *i.e.*, by employing short columns packed with small (sub-2-micron) particles to increase separation speed [18] and by the application of column-coupling technology to increase the efficiency [19,20], while applying ultra-high-pressure conditions, the developments in IC have lagged behind. This was mainly due to the low rupture pressure of the membrane in the eluent generator [21]. Another limiting factor is the low pressure resistance of polyether ether ketone (PEEK) components. Only recently capillary columns with 4 μm particles have become commercially available for IC separations [22,23]. To assess the performance limits of capillary IC in isocratic mode contemporary LC theory, originally proposed by Giddings [24], was applied to visualize the time required to obtain a specific number of plates (or peak capacity), while applying the maximum available operating pressure and taking into account both the separation efficiency and the column permeability [25,26]. In gradient mode, Causon et al. predicted the gradient-performance limits based on isocratic data and an empirical peak-capacity calculation [27]. In a next step, Causon demonstrated that the predictions (peak capacity of 82) match very well with a gradient experiment. Separating a test mixture of 14 anions using a 120 min gradient yielded a peak capacity of 84 on a conventional coupled-column system with of total length of 1.25 m.

In the present contribution the effect of gradient parameters, including flow rate, column temperature, and gradient steepness on performance limits were experimentally investigated using capillary columns packed with 4.1 μm anion-exchange particles. The separation performance in gradient mode is visualized *via* kinetic plots depicting the time required to achieve a certain peak capacity while operating columns at maximum system pressure. To account for the effect of gradient retention factor on peak width, the average peak capacity has been estimated by discretization of the gradient window. Gradient separations are performed applying linear and multistep concave gradients. Finally, the potential of high-efficiency ion separations is demonstrated for the separation of a complex mixture applying a 1.05 m long coupled column.

## 2. Materials and methods

### 2.1. Chemicals and reagents

A 7-anion standard containing fluoride, chloride, nitrite, nitrate, bromide, sulfate, and phosphate was purchased from Thermo Fisher Scientific (Amsterdam, The Netherlands). Propionic acid (for synthesis), butyric acid (for synthesis), 2-methylbutyric acid (for synthesis), and formic acid (98–100%, for analysis) were purchased from Merck (Darmstadt, Germany). Trichloroacetic acid (98%), sodium arsenate (dibasic: heptahydrate), and phenylacetic acid were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). Trifluoroacetic acid (HPLC grade), maleic acid, and fumaric acid (99%) were purchased from Biosolve (Valkenswaard, The Netherlands), Fluka (Steinheim, Germany), and Alfa Aesar (Ward Hill, MA, USA), respectively. Isobutyric acid, 2-keto-D-gluconic acid, malic acid, D-tartaric acid, potassium bromide, sodium molybdate, sodium thiosulfate, sodium valerate, sodium iodide, oxalic acid, and trisodium citrate were obtained from Thermo Fisher Scientific (Breda, The Netherlands).

The 7-anion standard was 1:100 (v/v)% diluted in Milli-Q water (18.2 MΩ cm) obtained from a Milli-Q Gradient A10 system (Millipore, Molsheim, France), resulting in final concentrations of 0.2 mg/L fluoride, 1 mg/L chloride, nitrite, bromide, nitrate, and sulfate, and 2 mg/L phosphate. Complex anion mixtures were prepared by mixing the ions listed in Table S1 of the Supplementary information, yielding final concentrations ranging between 0.2 and 2.0 mg/L.

150 mm × 400 μm i.d. and 250 mm × 400 μm i.d. capillary columns packed with anion-exchange AS18 particles were provided by Thermo Fisher Scientific (Sunnyvale, CA, USA). The 4.1 μm anion-exchange resin are sulphonated poly(ethylvinylbenzene-*co*-divinylbenzene) macro-porous particles (1500 Å) coated with 65 nm nanobeads containing alkanol quaternary ammonium functionalities (8% crosslinked). Column coupling was performed by removing one of the end fittings of the column and mounting the column outlet onto an end fitting of the next column. The coupled column was placed in helical formation in the thermostatted column compartment.

### 2.2. IC instrumentation and gradient conditions

Ion chromatography experiments were performed on a capillary ICS-5000 system from Thermo Fisher Scientific (Sunnyvale, CA, USA), composed of an autosampler, a capillary IC dual-piston pump, an eluent-generation module, a temperature-controlled IC cube module (integrating the capillary EG degasser, an injection valve equipped with a 400 nL loop, the separation column, an anion capillary electrolytic suppressor, and a capillary conductivity detector with a 20 nL flow cell). Chromeleon software (version 6.80, SR11) was used for system operation and data evaluation.

The sample was delivered from the autosampler to a 4-port injection valve for a full-loop injection. Capillary IC separations were performed in gradient mode, applying linear and concave gradients. Gradient IC separations were performed with a gradient window tuned according to the slope, and the retention factors of the components in the mixture. For the separation of the 7-anion mixture applying linear gradients with $t_G/t_0 = 5$, 10, and 20, the $[OH^-]$ ranged between 4 and 38 mM. Separations of the complex anion mixtures were performed after optimizing the gradient composition, such that the whole gradient span was covered with peaks. The potassium hydroxide mobile-phase was generated online from Milli-Q water (18.2 MΩ·cm) using an eluent generator equipped with a potassium-hydroxide cartridge.

## 3. Results and discussion

### 3.1. Column technology and performance evaluation

When applying an ion strength of 30 mM, the minimum plate height ($H_{\min}$) was determined in isocratic mode for small inorganic anions to be 10 μm at a linear velocity of approximately 0.7 mm/s, see Fig. S1 in the Supplementary Information. The reduced plate height ($h = H/d_p$) of 2.5 indicates that the 4.1 μm particulate capillary columns were well packed and extra-column band broadening introduced by the connection tubing and suppressor module prior to conductivity detection was relatively low. The magnitude of the A-, B-, and C-term contributions was estimated (for nitrate) applying non-linear regression by minimizing the sum of squares of deviation between observed and expected LC values. The values were determined at $A = 5.5$ μm, $B = 1.9 \times 10^6$ μm$^2$/s, and $C = 4.0$ ms, respectively. These values match with those reported in literature for a column operated in HPLC mode [28]. The column permeability ($K_v$) was determined to be $2.9 \times 10^{-14}$ m$^2$. The suitability of this material for the IC separation of small ions may be explained by the absence of a significant stationary phase mass-transfer ($C_s$) contribution to band broadening in the stagnant mesopores (pores with a diameter between 2 and 50 nm). Apparently, analytes