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Chromatographic separations in a nanocapillary under pressure-driven conditions

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ABSTRACT

We report a unique property of nanocapillaries for chromatographic separations of ionic species. Due to the electric double layer overlap, ions are unevenly distributed inside a nanochannel, with counterions enriched near the wall and co-ions concentrated in the middle of the channel. As a pressure-driven flow is induced, the co-ions will move faster than the counterions. This differential transport results in a chromatographic separation. In this work, we introduce the fundamental mechanism of this separation technology and demonstrate its application for DNA separations. An outstanding feature of this technique is that each separation consumes less than 1 pL sample and generates less than 0.1 nL waste. We also apply this technique for separations of DNA molecules, and efficiencies of more than 100 000 plates per meter are obtained.

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1. Introduction

Current micro- and nano-fabrication technologies enable reliable production of fluidic channels in low micrometer and nanometer scales [1–12]. Electrokinetic transport of liquid and ions in these nanometer-scale channels (nanochannels) has exhibited a series of distinctive effects [1–3], and applications of these effects are being explored for practical uses [4,5,13]. Normally, DNA cannot be separated by capillary zone electrophoresis (CZE), because the electrophoretic mobilities of all DNA molecules are virtually identical in gel-free solutions [14]. Peterson et al. [15] showed that separations of DNA by CZE are possible as long as the channel depth (or capillary diameter) is sufficiently small (e.g. tens of nanometers). Pennathur and Santiago [13,16] studied the fundamental mechanisms of similar separations in nanochannels, and termed this separation technique electrokinetic separations by ion valences (EKSIV). Han et al. [17–21] also demonstrated separations of long DNA molecules in nanochannels containing entropic traps. All these separations were performed under the influence of an external longitudinal electric field.

Interestingly, ionic species can also be separated in a nanocapillary under pressure-driven flow conditions. Fig. 1 illustrates the basic mechanism of this separation. Owing to the electric double layer (EDL) overlap, ions are unevenly distributed inside a nanochannel [1,4,22–24]. For example, inside a silica capillary whose surface is negatively charged, anions are concentrated in the middle of the channel (see Fig. 1a), while cations are enriched near the channel wall (see Fig. 1b) due to the electrostatic repulsive/attractive forces between the ions and the charged surface. When a pressure-driven (Poisseuille) flow is induced (see Fig. 1c and d), the anions will move faster than the cations. A chromatographic separation is thus produced (compare the relative positions of the anion in Fig. 1e and the cation in Fig. 1f). Since this separation mechanism has not been reported before and it dominates the separations of ions in nanocapillaries, we tentatively call this type of separations nanocapillary chromatography (NC). In this work, the nanocapillary refers to a capillary whose radius is in the submicrometer domain.

From the perspective of field-flow fractionation (FFF) [25], NC is similar to electrical FFF (EFFF). In EFFF, an external electric field is applied to force ionic species to reside near a wall of a channel in which a pressure-driven flow is effected [26]. Species with the highest electric charge are driven most forcefully toward the channel surface, and form the most compact layers. These species, confined in the low-flow region, will move more slowly than the low-charge species, and thus have longer retention times. Electric fields have the advantage of being exceedingly powerful, which means they can induce the migration of virtually all (large or small) charged species toward the accumulation wall. The implementation of EFFF, however, has been difficult because of the need to avoid the

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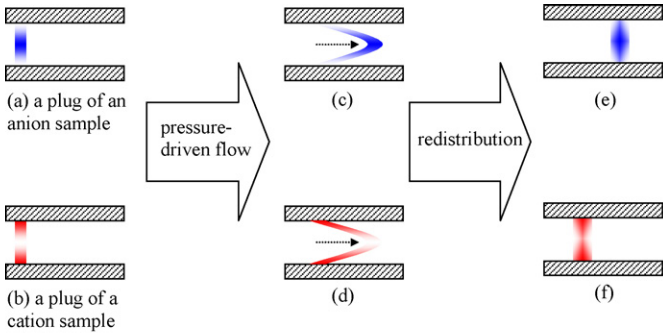

Fig. 1. A schematic presentation of NC separation in a nanocapillary under pressure-driven conditions.

generation of electrolysis products in the channel [27]. NC overcomes this problem, since the electric field comes from the zeta potential at the capillary wall, and an electric field is created without electrolysis.

Because co-ions are somehow "excluded" from the wall (see Fig. 1a) and counterions are "excluded" from the center of the capillary, NC can be considered to be a special format of ion-exclusion chromatography [28]. The solutions in these ion-exclusion regions are equivalent to the occluded liquids in ion-exclusion chromatography. The quotation marks indicate that ions are not absolutely excluded from these regions. [*Note*: Novic and Haddad [29] have recently suggested that ions diffuse to the occluded liquids as well in ion-exclusion chromatography.] Since the volume of such equivalent occluded liquid increases with the net charge on an analyte ion, analytes can be separated according to the volumes of their equivalent occluded liquids. However, there are some unique features for these separations. (1) All occluded liquids are part of the mobile phase; (2) the occluded liquids of co-ions move slower, and those of counterions move faster than the associated mobile phase; and (3) as a consequence of the above feature, all co-ions will have negative retentions (or the retention times of all co-ions are shorter than that of the associated eluent).

It should be pointed out that, while we were working on this project, Xuan and Li [30] developed a model that predicts differential retentions of ions under pressure-driven conditions, but no experimental data were shown. In this paper, we introduce the fundamental separation mechanism, and experimentally demonstrate the feasibility of NC for ion separations. We test the effects of key experimental parameters such as buffer concentration and separation pressure on separation efficiency and resolution, and demonstrate the application of NC for separations of DNA molecules.

2. Theory

In a narrow capillary with a negatively charged surface, the potential function has been derived [31], and has the following form,

$$\phi = \phi_0 \frac{I_0(\kappa r)}{I_0(\kappa R)}, \tag{1}$$

where I_0 is the zero-order modified Bessel function of the first kind, κ is the reciprocal of the double-layer thickness (at 25 °C, $\kappa^{-1} \approx 0.3/\sqrt{I}$ nm [23], where I is the ionic strength of the background electrolyte solution), R is the radius of the capillary, r is the independent variable representing the distance to the center of the capillary, and ϕ_0 is the zeta potential (ζ). Once a capillary and a background electrolyte solution are selected, κR is known, and ϕ can be calculated using Eq. (1).

Referring to Fig. 1a and b, when a plug of ionic sample is introduced into the capillary, the concentration profile of an ion inside the capillary can be calculated using Boltzmann equation,

$$C_i = C_0 \cdot \exp \frac{z_i e \phi}{kT}, \tag{2}$$

where C_0 is the concentration of ion i at $\phi = 0$, z_i is the number of unit charge on the ion, e is the charge of the electron, k is the Boltzmann constant, and T is the absolute temperature.

Under a Poisseuille flow condition, the migration of an ion inside a nano-scale capillary can be evaluated from the ion distribution and flow profile. The velocity profile of a Poisseuille flow is expressed as [32],

$$\upsilon = \upsilon_{\max}[1 - (r/R)^2], \tag{3}$$

where $\upsilon_{\max}$ is the maximum velocity in a Poisseuille flow and it is located in the center ($r = 0$) of the capillary. The average flow velocity is,

$$\overline{\upsilon} = \frac{2}{R^2} \cdot \int_0^R \upsilon \cdot r \cdot \mathrm{d}x. \tag{4}$$

Since ions are unevenly distributed across the radius of the capillary, the average velocity of ion i is calculated by the following equation,

$$\overline{\upsilon_i} = \frac{2}{R^2} \cdot \int_0^R C_i \cdot \upsilon \cdot r \cdot \mathrm{d}x. \tag{5}$$

We now define the NC mobility of ion i as

$$\mu_i = \frac{\overline{\upsilon_i}}{\overline{\upsilon}} = \frac{2}{\overline{\upsilon} \cdot R^2} \cdot \int_0^R C_i \cdot \upsilon \cdot r \cdot \mathrm{d}x. \tag{6}$$

As long as the potential function and flow profiles are known, NC mobilities can be calculated based on Eqs. (2), (3) and (6). Fig. 2 presents a typical relationship between the NC mobility and the z value of an ion. For simplicity of the presentation, only the data points from $z = -10$ to 10 are displayed. From this relationship, we draw three conclusions that are basic features of NC.

Feature 1. Co-ions move faster than neutral species, and neutral compounds migrate faster than counterions. In addition, a higher charged co-ion moves faster than a lower charged co-ion, while a higher charged counterion moves slower than a lower charged counterion. Here, co-ions and counterions are relative to the charge on the inner surface of the nanocapillary.

Feature 2. The unit-charge resolution ($\Delta R/\Delta z$) decreases with the absolute value of z. For example, separating X^{-1} and X^{-2} is easier than separating X^{-10} and X^{-11}.

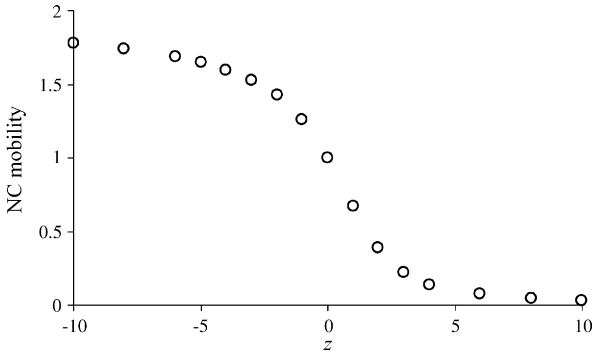

Fig. 2. NC mobility of an ion as a function of its charge. To calculate the data points in this figure, we assumed a capillary radius to be 5λ (where λ is the Derbye thickness of the EDL, $\lambda = \kappa^{-1}$), a zeta potential to be 50 mV, and a velocity profile of a Poisseuille flow. The mobility of a neutral compound (or the bulk solution) was set as 1.