CONCENTRATION POLARIZATION IN NANOCHANNEL DNA ELECTROPHORESIS
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ABSTRACT
We demonstrate that the large field electrophoresis of a single DNA molecule in nanofluidic systems is accompanied by concentration polarization. We illustrate this phenomena by utilizing our electrophoretic simulation tool SIMUL. First we investigate a simple system with univalent strong electrolyte as a bulk solution. Second, a more complicated system with buffering background electrolyte is examined. Concentration polarization in the vicinity of the DNA is observed in both the systems. The practical impact of such observations are also discussed.

KEYWORDS: Concentration polarization, DNA, Electrophoresis, Nanochannel

INTRODUCTION
Concentration polarization (CP) is well-known in nanofluidics in the context of ion migration in the junctions between a perm-selective nanochannel and a microchannel [1] or in channels with spatial wall charge density gradients [2]. Similar to the classical case of CP in ion-exchange-membrane-solution systems [3], also in nanochannels CP stems from a spatially localized imbalance between the mobile and the mobile negative ion charge densities, typically introduced by the formation of Electric-Double-Layer (EDL) in response to a system of fixed charges. For example there is an EDL-induced excess of mobile positively charged counter-ions in a nanochannel with a negatively charged silica wall. When an electric current passes through such a nanochannel, the current is mostly carried by the excess mobile cations, so that cationic flux surpasses the anionic flux. This leads to a depletion of both cations and anions at the anodic junction between the nanochannel and a microchannel and an accumulation of the ions at the cathodic junction side [4]. A similar effect occurs when current passes through an ion-selective membrane where, like in the previous case, the ion-selectivity itself is a result of a fixed charge exposed by the membrane [5].
CP therefore most generally can be regarded as a consequence of the presence of a surplus fixed charge in an electrophoretic system that requires a local excess of mobile counter-ions for electroneutrality. For a long, densely charged negative polyelectrolytes, one can easily predict a similar situation. λ-DNA molecule trapped inside a nanochannel in a stretched conformation can serve as a particular example. The investigation of CP that may accompany DNA electrophoresis may help to comprehend peculiar high field DNA electrophoretic behavior described in the literature [6].

THEORY
A λ-DNA molecule, which has approximately 48kb per 18μm length, is considered. Owing to Manning condensation [7], a significant portion of its bare charge is neutralized by the adsorption of counterions, and following Keyser et al. [8], the resulting net charge density can be approximated as 0.5e− per bp – a value well supported by the prediction by Manning [9]. Thus a λ-DNA molecule has a total charge of 24 000e− per 18μm length, or equivalently 2.5mM concentration of counter-ions in a DNA-containing region of a hypothetical 30x30nm nanochannel (which we choose here as a realistic model situation). This implies a significant excess of mobile counter-ions introduced by the DNA-EDL. The resulting dynamics of the ions is solved numerically by the SIMUL software [10]. The calculation is based on the solution of a set of equations that contain the partial differential equations describing the ion migration as well as set of acid-base equilibrium algebraic equations and the equation that accounts for electroneutrality adjustment in presence of diffusion and/or ionic fluxes due to external potential and pH changes. The diffusion potential is also taken into account appropriately. The SIMUL platform has been previously employed for simulations of a number of complex electrophoretic systems, where excellent agreements between the simulation and experimental results have been obtained, e.g., investigation of desalting during isoelectric trapping separation in a multi-compartment electrophoretic system with ion-permeable buffering membranes [11]. Nevertheless, we do make certain approximations to ensure that the present system is amenable to be studied in SIMUL platform, namely, i) the simulation is strictly one-dimensional. ii) the simulation has a macro-scale resolution, without explicitly accounting for the EDL formation or surface potential distribution, iii) the (continuous) channel wall EDL is not included. iv) electrophoretic movement of the DNA itself is neglected.

SIMULATION DETAILS
Simulation was performed using practically relevant conditions of 150μm long channel with 30x30nm² cross-section and about 200kV.m⁻¹ applied voltage (exact voltage given with data) under constant voltage conditions. The DNA molecule is modeled as a plug of immobilized anions of an effective concentration of 2.46mM occupying 18μm length in the middle of
the nanochannel. The profile of the plug is rectangular with an error-function-like diffusive boundary of approximately 2μm length at both plug sides (for the sake of numerical stability).

Mobility and pKa values supplied to the model are, respectively: 76.2x10⁹ m².s⁻¹.V⁻¹ and 13 for potassium, 79.1x10⁹ m².s⁻¹.V⁻¹ and -2 for chloride, 29.5x10⁹ m².s⁻¹.V⁻¹ and 8.076 for TRIS, and 41x10⁹ m².s⁻¹.V⁻¹ and 9.24 for borate.

Initial conditions for the ionic profiles are: \( c_{\text{co}}(x,t=0) = C \); \( c_{\text{counter}}(x,t=0) = c_{\text{co}}(x,t=0) + c_{\text{DNA}}(x) \), where subscripts co and counter refer to coion and counterion, and C is the bulk BGE concentration (1mM KCl or 20mM TRIS/borate). Boundary conditions are: \( \partial c(x,t)/\partial x \bigg|_{\text{left boundary}} = 0 \) and \( \partial c(x,t)/\partial x \bigg|_{\text{right boundary}} = 0 \). The computed profiles did not reach the boundaries.

RESULTS AND DISCUSSION

The computer simulation shows an extensive and rapid (time scale of 1 ms) ion depletion/accumulation at the DNA edges under the conditions of 30x30nm² channel cross-section, a 1mM KCl solution and 200 kV.m⁻¹ applied electric field (Figure 1: Left and Table 1). Focusing on the depletion zone, CP is seen to cause almost complete ion depletion at the anodic side of the DNA when a strong electrolyte is used as a bulk solution. This subsequently makes hydroxonium important in current transport and leads to significant changes in pH in the vicinity of the DNA.

![Figure 1: Left: Concentration profiles after 1ms of 200 kV.m⁻¹ applied voltage in a 1 mM KCl solution (potassium in red, chlorides in green). Right: Concentration profiles after 20ms of 180 kV.m⁻¹ applied voltage in a 20 mM TRIS/borate buffer (TRIS in red, borate in green). Dashed zoom-in inset: Partial concentration profiles of TRIS⁺ (in red), TRIS⁻ (in magenta), borate⁹⁻ (in green) and borate¹⁻ (in yellow). Both: local electric field (in brown) and pH (in magenta).](image)

| Table 1. Values in the depletion zone at the point of maximal depletion. BGE: 1mM KCl |
| Time [ms] | K⁺ [mM] | Cl⁻ [mM] | pH     |
| 0         | 1       | 1        | 7.0    |
| 1         | 1.4x10⁻⁵| 0.7x10⁻⁵| 3.5 - 10.4 |

| Table 2. Values in the depletion zone at the point of maximal depletion. BGE: 20mM TRIS/borate |
| Time [sec] | TRIS [mM] | TRIS⁺ [mM] | TRIS⁻ [mM] | Borate [mM] | Borate⁹⁻ [mM] | Borate¹⁻ [mM] | pH     |
| 0          | 20.0      | 15.8      | 4.2        | 20.0       | 15.9         | 4.1           | 8.7    |
| 20         | 10x10⁻³   | 0.2x10⁻³  | 9.8x10⁻³   | 3.035      | 3.031        | 4x10⁻³        | 6.4    |

With buffering bulk solution (TRIS/borate, concentration 20 mM), the system behavior is more complex (Figure1: Right and Table 2). The positively charged TRIS undergoes depletion while a portion of borate becomes neutral by the buffering action. Thus in the depletion zone there is a tiny concentration of TRIS⁺ and a corresponding concentration of borate⁻, but a high concentration of borate⁹⁻. After a certain time the bulk solution is no longer able to buffer further and the pH starts dropping while the (neutral) borate molecules still remain at a relatively high concentration level.

We additionally altered the channel cross-section (data not published), which effectively alters the DNA plug concentration. Remarkably, the extent of CP depends mainly on bulk solute concentration to DNA effective negative charge concentration ratio (as that dictates the extent of imbalance of the mobile counterions), and hence in smaller channels the CP effect is...
even more enhanced (characterized by an even smaller time of the ion depletion/accumulation). Similar enhancement is also witnessed in case the electric field is increased (with the depletion/accumulation time scaling as $E^β$, where $β < -1$).

Such CP effects will become important in DNA electrophoresis only when the time scale of the depletion is substantially smaller than the DNA electrophoresis time scale (DNA length divided by DNA velocity), or otherwise DNA electrophoresis will disturb the CP zones formation – in this light the above finding is of significant relevance, making the CP effect important at large fields as DNA electrophoresis time scales as $E^{-3}$. Note, however, that although it is reasonable to model the DNA-EDL as a plug of immobilized ions/mobile counter-ions within the DNA region, it is rather tricky for the small regions at the left- and right-hand side borders of the DNA, where the CP actually occurs. The EDL (re)formation in these regions can lead to a local electroneutrality disruption (and creation of space charge) counterbalancing (and thus possibly smoothing out) the electric potential peak observed in our simulation (ref. Figures 1, left and right, brown line) [12]

The one-dimensionality of the model is important for the CP effect. Only in 1-D systems, can the CP neutralizing diffusion fluxes (induced by the CP-induced concentration gradients) vanish at long time limits, ensuring no hindrance to the CP effect [13]. On the other hand for a 3-D system (typically a single DNA molecule in a microchannel or bulk solution), such diffusion currents never vanish completely and will always remain finite and suppress the CP effect [13]. We consider the 1D approximation realistic in the case of nano-confined highly stretched DNA but the situation should be regarded as appreciably different in micro-channel or bulk DNA electrophoresis, namely due to: i) less effective charge concentration introduced by the DNA, ii) 3D diffusion flux, iii) preferable coiled conformation of the DNA. Thus we infer that such a significant manifestation of CP-induced ion depletion and accumulation will only be observed in highly confined nanochannel-DNA systems.

CONCLUSION
A DNA molecule confined in a stretched conformation inside a nanochannel can cause CP in its vicinity in presence of applied axial electric field. What results is the formation of ion depleted/enriched zones at the front/rear ends of the electrophoretic DNA molecule, which may significantly alter the DNA effective electrophoretic mobility. This effect, predominant for thin nanochannels, is assumed to be considerably sensitive to system setup, namely BGE concentration and composition, channel dimension and electric field. Computer simulation results of DNA-caused CP in both strong and buffering BGEs are provided. We believe that more detailed investigation of this phenomenon can lead to better understanding of some exceptional high field DNA electrophoresis behavior observed in nanofluidic systems.

REFERENCES

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