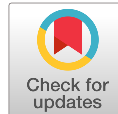

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Research Article

Empirical predictor of conditions that support ideal-filter capillary electrophoresis

Ideal-filter CE (IFCE) is a method for the selection of affinity binders for protein targets from oligonucleotide libraries, for example, random-sequence oligonucleotide libraries and DNA-encoded libraries, in a single step of partitioning. In IFCE, protein–oligonucleotide complexes and unbound oligonucleotides move in the opposite directions, facilitating very high efficiency of their partitioning. For any given protein target and oligonucleotide library, protein–oligonucleotide complexes and unbound oligonucleotides move in the opposite directions only for a limited range of EOF mobilities, which, in turn, corresponds to a limited range of pH and ionic strength values of the running buffer. Rational design of IFCE-based partitioning requires a priori knowledge of this range of pH and ionic strength values, and here we introduce an approach to predict this range for a given type of the running buffer. The approach involves measuring EOF mobilities for a relatively wide range of pH and ionic strength (I) values and finding an empirical predictor function that related the EOF mobility with pH and ionic strength. In this work, we developed a predictor function for a running buffer (Tris-HCl) that is commonly used in CE-based partitioning of affinity binders for protein targets. This predictor function can be immediately used for the rational design of IFCE-based partitioning in this running buffer, while the described approach will be used to develop predictor functions for other types of running buffer if needed.

Keywords:
Electroosmotic flow / Ideal-filter capillary electrophoresis / Ionic strength of running buffer / Mobility of electroosmotic flow / pH of running buffer
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Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

Selection of affinity binders for protein targets from oligonucleotide libraries, for example, random-sequence oligonucleotide libraries and DNA-encoded libraries, can provide leads for the development of diagnostic probes and drugs [1]. For simplicity, such libraries will be called DNA libraries implying that the following consideration is applicable to RNA libraries, DNA-encoded libraries, etc. Abundances of binders in DNA libraries are typically very low, making it very difficult to obtain a pool of high-purity binders in a single step of partitioning [2]. As a result, multiple consecutive rounds of partitioning are typically used for the in vitro selection of affinity binders for protein targets from DNA libraries [3]. Such multiround selection based on low-efficiency partitioning is time consuming and failure prone. We have recently introduced ideal-filter CE (IFCE), a highly efficient partitioning method that appears to facilitate reliable one-step selection [4]. In IFCE, protein–DNA complexes and unbound DNA move inside the capillary in the opposite directions, allowing the efficiency of partitioning to reach a remarkable value of 10^9 (Fig. 1, top). It is 10^4 times higher than the efficiency of classical CE-based partitioning (Fig. 1, bottom) and 10^7 times higher than the efficiency of partitioning on target-immobilized magnetic beads [5]. The opposite migration of protein–DNA complexes and unbound DNA in IFCE is achieved by adjusting the EOF via changing pH and/or ionic strength of the running buffer. Advantageously, IFCE is realized with a near-physiological running buffer making it applicable to select binders intended for use in vivo.

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Abbreviation: **IFCE**, ideal-filter CE

Color online: See article online to view Fig. 1 in color.

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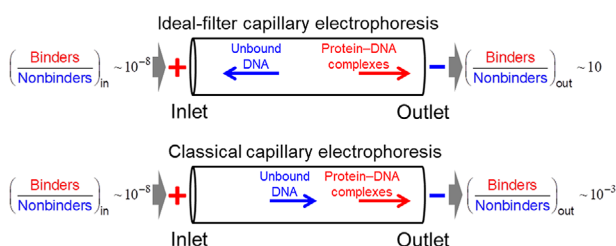

Figure 1. Schematics of the partitioning of protein–DNA complexes from unbound DNA by ideal-filter CE (top) and classical CE (bottom). Binders are protein-bound DNA and nonbinders are unbound DNA. The binder-to-nonbinder ratio at the input (subscript "in") of partitioning indicates binder abundance in the starting library. The binder-to-nonbinder ratio at the output (subscript "out") of partitioning indicates binder abundance in the resulting library.

The IFCE requirement for protein–DNA complexes and unbound DNA to move in the opposite directions creates some new analytical challenges. For example, it is impossible to detect protein–DNA complexes and unbound DNA in a single IFCE run, which, in turn, makes it impossible to measure the equilibrium constant (K_d) and the rate constant of complex dissociation by IFCE. This problem has been recently addressed by introducing a double-passage approach in which the equilibrium mixture, containing the complexes and unbound DNA, is first moved through the detector by pressure to quantitate both protein-bound and unbound DNA, and then the complexes and unbound DNA are separated by IFCE, and only the complexes pass the detector and get quantitated [6]. Another analytical challenge of IFCE is that the movement of the protein–DNA complexes and unbound DNA in opposite directions can be supported only by a limited range of EOF mobilities. EOF mobility in a fused silica capillary is mainly defined by the pH and ionic strength of the running buffer. Accordingly, IFCE can be supported within a limited range of pH and ionic strength values. The rational design of IFCE-based partitioning requires a priori knowledge of the suitable range of pH and ionic strength values. Currently, there is no approach to predict the range of pH and ionic strength values suitable for IFCE. The goal of this work is to address this methodological problem.

We introduce an approach to predict the range of pH and ionic strength values suitable for IFCE for a given composition of the running buffer. The approach involves two steps. The first step is the measurement of EOF mobility for a relatively wide range of pH and ionic strength values. The second step is finding an empirical predictor function that related the EOF mobility with pH and ionic strength of the running buffer. This function can be used to find a range of pH and ionic strength that can support any given EOF mobility. In this work, we first explain how the range of EOF mobilities that support IFCE can be determined. We then develop the predictor function for a Tris-HCl running buffer that is commonly used in CE-based partitioning of affinity binders for protein targets. There are two major deliverables of this work. The first is the general approach that will facilitate the development of predictor functions for different types of the running buffer. The second is a predictor function for the Tris-HCl, buffer that will be used for rationalizing the design of IFCE-based partitioning in this specific running buffer.

2 Materials and methods

2.1 Chemicals and materials

All chemicals were purchased from Sigma-Aldrich (Oakville, ON) unless otherwise stated. Fused silica capillary with inner and outer diameters of 75 and 360 μm, respectively, was purchased from Polymicro (Phoenix, AZ). DMSO was used as EOF markers for accurate mobility calculations. All solutions were prepared in deionized water filtered through a 0.22 μm Millipore filter membrane (Nepean, ON). All the pH values were measured with Orion 710A+ pH/ISE meter from Thermo (Waltham, MA). Before every measurement of pH, the pH meter was calibrated by the method of three points (pH 4.01, 7.00, and 10.01) pH calibration with standard buffers.

2.2 Capillary electrophoresis

All CE experiments were done with a P/ACE MDQ instrument from Sciex (Brea, CA). UV detection with a wavelength of 214 nm was applied in the experiments for measuring μ_{EOF} of different buffers. An uncoated fused silica capillary of a 48.6 cm total length and a 38.4 cm distance from the inlet to the detection point was used for all CE experiments of measuring μ_{EOF} of different buffers. The EOF marker (2.0 mM DMSO dissolved in the running buffer) was injected into the capillary's end closest to the detector (10.2 cm distance to the detector) by a pressure pulse of 0.5 psi during 10.0 s. All CE runs were carried by an electric field of 61.7 V/cm with a capillary temperature set at 15°C. The short migration distance and the low electric field strength were used on to reduce the negative effect of buffer depletion and joule heating.

3 Results and discussion

3.1 Range of EOF mobility to support IFCE

Our first goal was to explain how a given DNA library and a given protein define the range of IFCE-suitable EOF mobilities. Specifying the protein and the library specifies electrophoretic mobilities of unbound DNA and protein–DNA complexes.

All DNA in the DNA library have the same length and the same negative charge (one unit per nucleotide). Therefore, all unbound DNA molecules are characterized by a single value

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of electrophoretic mobility, μ_{DNA}, which is negative, that is, vector $\boldsymbol{\mu}_{DNA}$ has an opposite direction to the vector of electric field **E** directed from "+" to "−" in Fig. 1.

Any protein induces a significant change in electrophoretic mobility of DNA by binding to it. A protein is extremely unlikely to have a positive charge of magnitude greater than the negative charge of DNA used for aptamer selection. Accordingly, all protein–DNA complexes for a single protein and a single library have identical negative charges and are characterized by a single value of negative electrophoretic mobility $\boldsymbol{\mu}_{P-DNA}$. The vector $\boldsymbol{\mu}_{P-DNA}$ is codirected with $\boldsymbol{\mu}_{DNA}$ and counterdirected with **E**. Further, the hydrodynamic size of the protein–DNA complex is always greater than that of the DNA. Thus, protein–DNA complexes experience a greater friction force in electrophoresis than unbound DNA. As a result, the magnitude of electrophoretic mobility of protein–DNA complexes is lower than that of unbound DNA: $|\mu_{P-DNA}| < |\mu_{DNA}|$.

If we consider a 1D system of coordinates with axis x codirected with **E**, then IFCE is possible when the velocity of protein–DNA complex is positive and the velocity of unbound DNA is negative:

$$v_{P-DNA} > 0 > v_{DNA} \tag{1}$$

These two velocities depend on **E** as well as on $\boldsymbol{\mu}_{P-DNA}$, $\boldsymbol{\mu}_{DNA}$, and the mobility vector of EOF, $\boldsymbol{\mu}_{EOF}$:

$$\begin{aligned} v_{P-DNA} &= \mathbf{E} \cdot (\boldsymbol{\mu}_{EOF} + \boldsymbol{\mu}_{P-DNA}) \\ v_{DNA} &= \mathbf{E} \cdot (\boldsymbol{\mu}_{EOF} + \boldsymbol{\mu}_{DNA}) \end{aligned} \tag{2}$$

E and $\boldsymbol{\mu}_{EOF}$ are co-directed in an uncoated capillary except for very acidic pH. Therefore, the velocity requirement of IFCE (Eq. 1) can be realized when the mobilities are related as:

$$|\mu_{DNA}| > |\mu_{EOF}| > |\mu_{P-DNA}| \tag{3}$$

This simple inequality defines the sought range of EOF mobilities in relation to electrophoretic mobilities of DNA and protein–DNA complex.

Electrophoretic mobility of DNA is proportional to the ratio between its charge and hydrodynamic size. The charge and the size of the DNA molecule do not change significantly with changing pH and ionic strength of the environment. Accordingly, the electrophoretic mobility of DNA remains near invariant when pH and the ionic strength change within one unit and one order of magnitude in the physiological range [5]:

$$\mu_{DNA} \approx -(20\text{–}25)\ \text{mm}^2/\text{kV/s} \tag{4}$$

The mobility of protein–DNA complex is proportional to the ratio between charge and the hydrodynamic size of the complex. Both the charge and the size depend on the molecular weight of the protein and the number of nucleotides in DNA. Accordingly, μ_{P-DNA} is unique for every combination of a DNA library and a protein. The value of μ_{P-DNA} can be determined experimentally if the bulk affinity between the library and the protein is high so that complexes can be formed in detectable amounts upon a saturating concentration of the protein [7]. When the experimental approach is inapplicable, μ_{P-DNA} can be predicted with three major mobility models developed in the past few years. Two models are applicable to double-strand DNA and unstructured single-strand DNA attached to the protein in a single point [8]. The third model predicts μ_{P-DNA} for structured single-strand DNA (e.g., an aptamer) tightly linked to the protein through a number of bonds [9]. Thus, the values of μ_{DNA} and μ_{P-DNA} can be considered as known and can, thus, serve to determine the range of IFCE-suitable μ_{EOF} according to Eq. (3).

3.2 Link of EOF mobility to pH and ionic strength

Now, when it is clear how the range of IFCE-suitable μ_{EOF} can be determined, we can consider how conditions supporting this range can be found. The mobility of EOF depends on the ζ-potential of the inner capillary wall [10]:

$$\mu_{EOF} = \varepsilon\zeta/(4\pi\eta) \tag{5}$$

Here, ϵ and η are the dielectric constant and the dynamic viscosity of the running buffer. The ζ-potential increases with increasing pH and decreasing ionic strength (I) of the running buffer, suggesting a means of controlling μ_{EOF}. In the proof-of-principle work on IFCE, we adjusted μ_{EOF} via varying the ionic strength at a constant pH [5]. There is no explicit function that would link μ_{EOF} with pH and/or ionic strength of the running buffer because μ_{EOF} also depends on the types of ions that the running buffer is composed of [11]. However, for a fixed composition of the running buffer and fixed pH, the dependency of μ_{EOF} on the ionic strength (I) has been proposed as [12]:

$$1/\mu_{EOF} = a + b\sqrt{I} \tag{6}$$

Regarding the dependence of μ_{EOF} on pH, we could not find a mathematical model describing this dependence in the literature. On the other hand, the positive correlation between μ_{EOF} and pH is often suggested by experimental results [13–15]. As our studied pH range (7.0−7.8) was narrow, we presumed that such dependence could be assumed to be linear in this range. Two possible mathematical models could, then, be applied in the narrow range of pH: (1) μ_{EOF} linearly depends on pH and (2) $1/\mu_{EOF}$ linearly depends on 1/pH. Preliminary evaluation of these models revealed that $1/\mu_{EOF} \sim 1/\text{pH}$ was found to be more reliable and suitable to fit into a concise model for the final predictor function (Supporting Information). As a result, the dependence of μ_{EOF} on pH is hypothesized to be:

$$1/\mu_{EOF} = c + d/\text{pH} \tag{7}$$

Therefore, by combining Eqs. (6) and (7), the hypothetical dependence of μ_{EOF} on two parameters—the ionic strength (I) and pH—will be:

$$1/\mu_{EOF} = (a + b\sqrt{I})(c + d/\text{pH}) \tag{8}$$

or

$$\mu_{EOF} = [(a + b\sqrt{I})(c + d/\text{pH})]^{-1} \tag{9}$$

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Here, *a*, *b*, *c*, and *d* are empirical parameters that depend on a set of conditions: the type of ions in the running buffer, type of the material the capillary is made of, temperature, etc. The empiric parameters *a*, *b*, *c*, and *d* should be found experimentally for every unique set of the above-mentioned conditions in order to facilitate the use of Eq. (9) for finding pH and I which satisfy the desired value of μ_{EOF}. Equation (9) may be potentially robust to changes of some of the additional (to pH and I) conditions; however, this robustness cannot be assumed a priori but should be tested and confirmed experimentally.

3.3 Experimental determination of μ_{EOF} as a function of pH and ionic strength of the running buffer

We chose 50 mM Tris-HCl as the basis of our CE running buffer; the choice was justified by the suitability of this running buffer for CE-based aptamer selection and the ability to maintain biomolecular interactions. The ranges of pH (7.0–7.8) and I (80–180 mM) were chosen to be near the physiological condition (pH 7.4, $I = 164$ mM). Thus, near-physiological buffers can be found by the developed empirical predictor for the IFCE partitioning. As a result, the binders selected in these buffers could still bind tightly to the protein target in physiological environments for the intended use. The ionic strengths of 50 mM Tris-HCl depend on pH and are the following: $I = 46$, 44, 41, 38, and 33 mM for pH 7.0, 7.2 7.4, 7.6, and 7.8, respectively. With different pH, the concentrations of NaCl required to reach different ionic strengths are shown in Supporting Information Table 3. Based on these calculations, NaCl was added to the running buffer to increase its ionic strength to 80–180 mM; the value of pH was adjusted after adding NaCl. Thus, 30 buffers with different combinations of pH and I in the designated ranges were studied. EOF was tracked by CE propagation of a plug of DMSO (2.0 mM dissolved in a corresponding running buffer) at an electric field of 61.7 V/cm. Three sets of experiments were conducted for each of the 30 running buffers with different combinations of pH and I with 3 repeats in each set. The total number of runs was thus 270. Fresh running buffers were prepared for each set of experiments.

According to Eq. (6), the experimental data are presented as $1/\mu_{\mathrm{EOF}}$ versus $\sqrt{I}$ (Fig. 2), and the dependencies are statistically proved to be linear (Supporting Information). Experimental dependencies of $1/\mu_{\mathrm{EOF}}$ on 1/pH for different values of ionic strength are shown in Fig. 3 and are linear as suggested in Eq. (7). Based on the results shown in Figs. 2 and 3, we could infer that Eq. (9) was a suitable mathematical model for describing the relationship between pH, I, and μ_{EOF} in narrow ranges of pH and I (pH: 7.0–7.8, I: 80–180 mM) for Tris-HCl buffer. Finally, with the use of "Nonlinear Surface Fit" function in the software of OriginPro 8.6, we fit the experimental data into Eq. (9) with *a*, *b*, *c*, and *d* as fitting parameters; the values of *a*, *b*, *c*, and *d* were determined to be −0.319, 4.00, 0.0425, and 0.0164, respectively. As a result, the

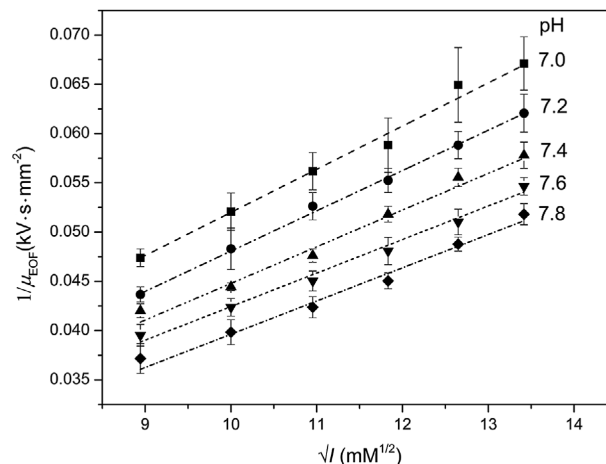

Figure 2. Experimental dependencies of $1/\mu_{\mathrm{EOF}}$ on the square root of ionic strength ($\sqrt{I}$) of running buffer (50 mM Tris-HCl) for different pH values. Thirty data points were collected in total. The measurement for each combination of pH and ionic strength was carried out in triplicates.

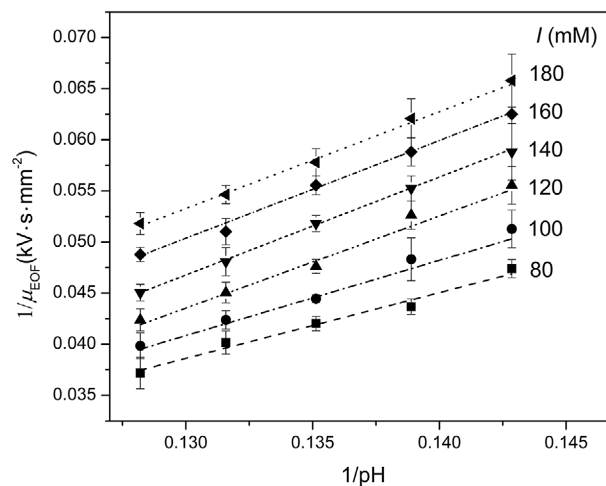

Figure 3. Linear relationship between $1/\mu_{\mathrm{EOF}}$ and 1/pH for Tris-HCl buffer with different ionic strengths achieved by adding NaCl. pH values were 7.0, 7.2, 7.4, 7.6, and 7.8.

empirical predictor function for the 50 mM Tris-HCl buffer supplemented with NaCl was determined to be:

$$\mu_{\mathrm{EOF}} = [(-0.319 + 4.00/\mathrm{pH})(0.0425 + 0.0164\sqrt{I})]^{-1} \qquad (10)$$

The acceptable tolerance of a predictor function can be set to be 5%. For checking if the determined predictor function has this value of tolerance, the experimental values of pH and I were plugged back into Eq. (10), and the corresponding values of μ_{EOF} determined by the empirical function were calculated. The detailed comparison between the experimental and predicted values of μ_{EOF} for the entire ranges of pH and I are shown in Supporting Information Table 4. According to these results, the deviation between the experimental and predicted values did not exceed 5%. Hence, we can conclude that the empirical function of Eq. (10) fits the experimental

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data well and can be used to quickly find pH and *I* for a required value of μ_{EOF}.

4 Concluding remarks

In this study, we performed a series of experiments and conducted a mathematical analysis that allowed us to derive a predictor function that links μ_{EOF} with pH and *I*. This function can be applied to predict the pH and ionic strength ranges for the Tris-HCl running buffer to support IFCE. With a similar experimental and analytical approach, this method can be expanded to find the relationship between μ_{EOF} and pH and *I* for other CE running buffers.

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The authors have declared no conflict of interest.

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