Tether forces in DNA electrophoresis†

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This *tutorial review* introduces the concepts necessary for understanding the generation of forces on charged objects in solution by externally applied electric fields. We focus on simple, idealized cases and conceptual understanding rather than quantitative modeling. We also discuss experiments in which electrophoretic forces on DNA were directly measured. This review is aimed at readers interested in the fundamentals of electrophoresis in general, as well as those with more specific interests in DNA electrophoresis, nanopores and optical tweezers.

1. Introduction

The separation of DNA molecules by gel electrophoresis—the process of driving the molecules through a dense network of cross-linked polymer with an applied electric field—counts among the most important techniques in biochemistry and molecular biology.^{1–3} Despite the importance of the applications of DNA electrophoresis, however, the fundamental underlying mechanisms responsible for separation have not been fully elucidated,³ and doing so represents a substantial challenge. Conceptually, it requires understanding the interplay between polymer dynamics, electroosmosis, the topology of the gel and, in many cases, specific interactions between the DNA and the gel matrix. Experimentally, elucidating the role of these different contributions requires probing at or near the molecular scale. In addition to the fundamental interest from

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the point of view of polymer science and soft matter physics, present attempts to better understand electrophoresis are motivated by our increasing ability to construct sophisticated fluidic systems for manipulating DNA and other macromolecules.⁴ Such understanding may provide the insight necessary for developing new separation methods capable of outperforming the traditional gel.^{4–6}

Because of its emphasis on quantitative understanding, the contemporary literature on DNA electrophoresis may be difficult to access for researchers from outside the field. In particular, it is often difficult to separate the well-understood basic ideas from further refinements and details aimed at improving quantitative accuracy. It is the aim of this tutorial review to introduce some of the basic ideas while concentrating on conceptual understanding rather than quantitative accuracy. We focus on a single aspect of electrophoresis, the so-called tether force. This is the force that is needed to hold a charged object in place against the action of an externally applied electric field. This situation loosely corresponds to a DNA molecule that is temporarily trapped in a metastable configuration inside a gel. For an overview of gel electrophoresis



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with emphasis on separation and the intricacies of commonly employed gel systems, we refer the reader to recent reviews in the literature.^{1–3}

This tutorial review is organized as follows. We first introduce the key physical concepts in Section 2 using a simple planar geometry and by making several further assumptions. This allows simplifying the calculations to a level that is easily tractable analytically, and we encourage the reader to follow along by performing the calculations him/herself. In Section 3, we move away from the simplifications of Section 2 and discuss some of the additional complexities that arise at high charge densities and in the cylindrical geometry relevant for DNA electrophoresis. This section only summarizes key results rather than providing a full derivation. Finally, Section 4 briefly summarizes experiments that have explicitly addressed electrophoretic forces on DNA.

2. Concepts

In order to focus on the key concepts, we first introduce a system with the very simple geometry illustrated in Fig. 1a. The system consists of a charged surface with a uniform surface charge density σ separated from a second, parallel surface that is electrically neutral. For most systems of interest, σ has a negative value; while this is not assumed in our derivation, the plots in the figures correspond to this case. The charged surface has a surface area A, and the surfaces are separated by a distance d. We assume that the lateral dimensions of the charged surface are sufficiently large that edge effects can be neglected; under these conditions all of the relevant equations become one-dimensional. The volume between the two planes is filled with an electrolyte, which is in diffusive equilibrium with a bulk reservoir. We take the electrolyte as consisting of water containing a number density n_0 of a fully dissociated monovalent salt. A good example of the latter is potassium chloride (KCl), which dissociates into K^+ and Cl^- ions in water. This implies that in bulk solution there is a number density n_0 of both positive ions (also known



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Fig. 1 Charged surface in solution. (a) A negatively charged surface (left) is immersed in an ionic solution and located a distance *d* from a second, uncharged surface (right). A uniform electric field acts on the charged wall and the ions in solution. (b) Potential ϕ as a function of distance *x* from the charged wall with a surface charge $\sigma = 0.001 \text{ C m}^{-2}$ in 20 mM KCl solution. The potential does not change when the uncharged wall is moved from d = 25 nm to d = 50 nm (indicated by the dashed-dotted lines). (c) Distribution of counterions $n_+(x)$ and coions $n_-(x)$ as a function of *x* for d = 25 nm and d = 50 nm. Again the distribution remains the same for both cases. There is a net positive charge at the charged wall near x = 0 which leads to an electroosmotic flow. (d) The velocity v(x) of the fluid *versus* position *x*. This function differs noticeably when the distance of the plates is d = 25 nm (full line) or d = 50 nm (dashed-dotted line), in contrast with (b) and (c).

as *cations*) with charge +e and negative ions (*anions*) with charge -e, where -e is the charge of the electron.[‡] We treat water as a homogeneous medium with permittivity $\varepsilon_w = 80\varepsilon_0$, where ε_0 is the permittivity of free space. A uniform electric field with magnitude *E* is applied parallel to the surface and permeates the region between the surfaces. The direction of this electric field is defined as *z*, while the direction perpendicular to the planes is *x* (with x = 0 corresponding to the position of the charged surface and x = d to that of the

 $[\]ddagger$ Another contribution to n_0 comes from the spontaneous dissociation of water into H⁺ and OH⁻.

neutral surface). We will further assume that σ is "small" and that *d* is "large", as defined more quantitatively in Section 2.1 below.

2.1 The electrical double layer

A charged surface in solution creates in its vicinity a region containing a net ionic charge that is equal in magnitude and opposite in sign to the charge of the surface itself. This induced charge is said to screen the charge of the surface. The combination of the charged surface and the screening charge is known as the *electrical double layer* and is electrically neutral. As will become clear in subsequent sections, the spatial distribution of charge in the double layer directly influences electrophoresis. We therefore begin our analysis by describing this charge distribution.

The ion distribution in the double layer is most commonly described within the so-called Poisson–Boltzmann formalism. At equilibrium, the average concentration of charged molecules at position x is assumed to follow the Boltzmann distribution,

$$n_{\pm}(x) = n_0 e^{\mp e\phi(x)/kT}.$$
(1)

Here $\phi(x)$ is the local average electrostatic potential, k is the Boltzmann constant, T is the absolute temperature, and n_+ and n_- are the local number densities of cations and anions, respectively. We have also introduced the convention that $\phi(x) = 0$ corresponds to the bulk reservoir far from any charged object, where $n_+ = n_- = n_0$. In this section we will further assume that $\phi(x)$ is small such that the condition

$$|e\phi(x)/kT| \ll 1 \tag{2}$$

holds for all values of x (we further quantify this condition near the end of the calculation, and discuss what happens when it is not satisfied in Section 3). In this case, eqn (1) can be Taylor-expanded to yield a simpler form,

$$n_{\pm}(x) = n_0(1 \mp e\phi(x)/kT).$$
 (3)

In order to determine self-consistently the electrostatic potential $\phi(x)$, eqn (3) is combined with the Poisson equation from electrostatics, $\nabla^2 \phi(r) = -\rho(r)/\varepsilon_w$. Here ε_w is the permittivity of water while $\rho(r) = e[n_+(r) - n_-(r)]$ is the charge density. The result is a differential equation that can be solved for the electrostatic potential $\phi(x)$,

$$\frac{\mathrm{d}^2\phi(x)}{\mathrm{d}x^2} = \frac{kT\varepsilon_w}{2e^2n_0}\phi(x) = \frac{\phi(x)}{\lambda^2}.$$
(4)

In the last step we grouped all of the constants into a single parameter, the so-called Debye screening length, defined as $\lambda = (kT\varepsilon_w/2e^2n_0)^{1/2}$. The Debye length depends on fundamental constants and the salt concentration of the solution. For pure pH 7 water at room temperature (which contains 10^{-7} M of H⁺ and OH⁻ ions), $\lambda \approx 1$ µm. Under typical physiological conditions (approximately 0.1 M of monovalent salt), $\lambda \approx 1$ nm.

Eqn (4) is known as the Debye–Hückel approximation, and has the general solution $\phi(x) = Ae^{-x/\lambda} + Be^{+x/\lambda}$. The corresponding boundary conditions, which are easily derived by applying Gauss' law at x = 0 and at x = d, are

$$\frac{\mathrm{d}\phi(x)}{\mathrm{d}x}\Big|_{x=0} = -\frac{\sigma}{\varepsilon_{\mathrm{w}}}; \frac{\mathrm{d}\phi(x)}{\mathrm{d}x}\Big|_{x=d} = 0.$$
 (5)

Combining these boundary conditions with the general solution for $\phi(x)$ yields the desired expression for the electrostatic potential,

$$\phi(x) = \frac{\sigma\lambda}{\varepsilon_{\rm w}} \left(\frac{e^{-x/\lambda} - e^{-2d/\lambda} e^{x/\lambda}}{1 + e^{-2d/\lambda}} \right).$$

If we restrict ourselves to the most common case, $d \gg \lambda$, the factor $\exp(-2d/\lambda)$ becomes vanishingly small and this expression reduces to the simple form

$$\phi(x) = \frac{\sigma\lambda}{\varepsilon_{\rm w}} e^{-x/\lambda} \, (d \gg \lambda). \tag{6}$$

The neutral surface is then sufficiently far from the charged surface that it no longer influences the charge distribution in the double layer. This situation is illustrated in Fig. 1b. Eqn (6) thus shows that electrostatic interactions decay to zero in electrolytes over a characteristic distance λ . This result also allows specifying the conditions under which the Debye–Hückel approximation is valid: eqn (2) must hold for all values of x, and $\phi(x)$ is greatest at x = 0, yielding the condition $|\sigma| \ll kT\varepsilon_w/e\lambda$. Thus, for a given salt concentration (and its corresponding value of λ), the approximation always holds for sufficiently small values of the surface charge density, σ .

Substituting our result for $\phi(x)$, eqn (6), into eqn (3) yields the ion distribution in the Debye–Hückel approximation,

$$n_{\pm}(x) = n_0 \mp \frac{\sigma}{2e\lambda} e^{-x/\lambda}.$$
 (7)

The screening charge thus consists of two components: an excess of ions with sign opposite to that of σ (commonly called *counterions*) and missing ions with the same sign as σ (*coions*). Both components are localized within a distance of order λ from the charged surface, as shown in Fig. 1c. Interestingly, the total number density of ions, $n_+(x) + n_-(x)$, is independent of *x*; only the ratio of counterions to coions varies, going from 1 : 1 in the bulk to an increasingly asymmetric composition near the charge surface.

Based on the above, the net charge density in the diffuse layer is

$$\rho(x) = -\frac{\sigma}{\lambda} e^{-x/\lambda}.$$
 (8)

Like the potential $\phi(x)$, the charge density $\rho(x)$ drops to zero with increasing distance from the charged surface with a decay length λ . Integration of eqn (8) with respect to x also directly demonstrates that the total charge in the diffuse layer is equal and opposite to that of the surface being screened.

2.2 Electroosmotic flow

In the presence of an electric field *E*, each ion moves with an average drift velocity $v = \mu E$, where μ is a constant known as the mobility. μ has a different value for different species of ions, and it is positive for cations and negative for anions. Ions in a solvent move at constant velocity and do not accelerate

like charges in vacuum because they experience drag from the solvent. By Newton's third law, each ion therefore exerts a reaction force $\pm eE$ on the solvent. Averaged over a microscopic volume of bulk solution large enough to contain several ions, however, the net force averages to zero since the solution is neutral and the reaction forces from cations and anions exactly cancel each other.§

This cancellation does not occur near a charged surface. As indicated by eqn (8) and Fig. 1c, such a surface induces a diffuse layer of charge in its vicinity. An electric field thus exerts a force on the solution in this thin layer near the surface. This force can cause the fluid to move, a phenomenon known as *electroosmotic flow*.

In order to solve the flow problem, we make further approximations. We are interested in describing flows that vary on length scales of microns or less. At these length scales and for typical velocities encountered in electroosmotic flows, the Reynolds number is very small and all flows are expected to be laminar. Furthermore, from symmetry the fluid velocity can only be oriented parallel to the electric field (*z*-direction) and can only vary perpendicular to the surface (*x*-direction). Under these conditions the Navier–Stokes equations that describe fluid motion reduce to the simple form

$$\frac{\mathrm{d}^2 v_z(x)}{\mathrm{d}x^2} + \frac{\rho(x)E}{\eta} = 0, \tag{9}$$

where $\rho(x)E$ is the force per unit volume exerted by the electric field on the solution and η its viscosity. We refer the reader to the literature for a detailed derivation of eqn (9) (see *e.g.* ref. 7).

Given the charge density $\rho(x)$ from eqn (8), eqn (9) can be directly integrated to yield the general solution

$$v(x) = \frac{E\sigma\lambda}{\eta}e^{-x/\lambda} + Bx + C$$

where *B* and *C* are constants. Imposing no-slip boundary conditions for the fluid at the two walls, v(0) = v(d) = 0, yields the desired flow profile,

$$v(x) = -\frac{E\sigma\lambda}{\eta} \left(1 - e^{-x/\lambda} - \frac{x}{d} \right).$$
(10)

This result is plotted in Fig. 1d. The fluid velocity increases steeply from a zero value at the surface to a maximum at a distance of order λ from the surface.¶ It then decays linearly back to zero with increasing x. At positions $x \gg \lambda$, eqn (10) reduces to the simpler form $v(x) = v_0(1 - x/d)$, where $v_0 = -E\sigma\lambda/\eta$. This is the same flow profile as would be obtained if the charged wall was moving at constant velocity v_0 in the absence of an electric field.

2.3 Tether forces in electrophoresis

We now have all of the ingredients required to calculate the tether force F_{mech}^{σ} that must be applied to hold the charged surface in place against the action of the electric field. The relevant forces are illustrated in Fig. 2, where the superscripts





Fig. 2 Force balance. (a) The electric field pulls the negatively charged wall towards the bottom of the image with force F_{bare}^{σ} . The counterions (not shown) experience a force in the opposite direction. This leads to drag forces F_{drag}^{σ} , acting on the charged surface, and F_{drag}^{0} , acting on the uncharged surface. To hold the system in place, a tether force F_{mech}^{σ} (represented by a spring) must be applied to the charged wall, and an equal but opposite force F_{mech}^{0} must be applied to the uncharged wall. (b) When the distance *d* is increased, the drag force on the uncharged wall increases. Correspondingly, the drag force on the uncharged wall F_{drag}^{σ} and the tether force F_{mech}^{0} decrease. This is a direct result of the higher flow velocity of the liquid through the larger gap between the walls (see Fig. 1d).

 σ and 0 refer to the charged and uncharged surfaces, respectively. The total force applied to the charged surface by the electric field, F_{elec}^{σ} , can be decomposed into two components that always act simultaneously. First, the electric field acts directly on the charges of the surface. We refer to this component as the *bare force*, F_{bare}^{σ} , since this is the force that would be experienced by the bare surface if it were suspended in vacuum. This force follows simply from Coulomb's law and is given by

$$F_{\text{bare}}^{\sigma} = A\sigma E. \tag{11}$$

The electric field simultaneously acts on the oppositely charged screening cloud and sets up an electroosmotic flow, as discussed in the previous section. Because this flow causes the fluid to be sheared at the boundary with the charged surface, it exerts a drag force F_{drag}^{σ} on the charged surface whose value is given by Newton's relation as

$$F_{\rm drag}^{\sigma} = A\eta \left| \frac{\mathrm{d}\nu(x)}{\mathrm{d}x} \right|_{x=0} = -AE\sigma \left(1 - \frac{\lambda}{d}\right) = -\left(1 - \frac{\lambda}{d}\right)F_{\rm bare}^{\sigma}.$$
(12)

The drag force is thus comparable in magnitude and opposite in sign to F_{bare}^{σ} .

The mechanical tether force follows directly from the above and is given by

$$F_{\text{mech}}^{\sigma} = -F_{\text{elec}}^{\sigma} = -(F_{\text{bare}}^{\sigma} + F_{\text{drag}}^{\sigma}) = -AE\sigma\frac{\lambda}{d}.$$
 (13)

[§] Note that the forces cancel out even if the cations and anions have different mobilities μ and thus move at different velocities. ¶ The actual maximum occurs at position $x = \lambda \ln d/\lambda$.

This result contrasts strongly with what would be expected in the absence of the drag force induced by the screening charge: in that case we would expect $F_{\text{mech}}^{\sigma} = F_{\text{bare}}^{\sigma} = -AE\sigma$, which is larger than the correct answer by a large factor, d/λ .

Our calculation thus indicates that drag from the counterions, far from being a minor correction, is in fact a dominant factor in determining the magnitude of electrophoretic forces. Furthermore, its influence does not depend solely on local properties in the vicinity of the charged surface, but instead encompasses all aspects of the environment that modify the electroosmotic flow. This is made explicit by the appearance of d, the distance to the far-away uncharged wall, in our equation for F_{mech}^{σ} , eqn (13). It is also manifest in Fig. 1: whereas the potential $\phi(x)$ and the ion distributions $n_{\pm}(x)$ are essentially independent of d (so long as $d \gg \lambda$), the flow profile shown in Fig. 1d is strongly affected by the value of d.

Further insight can be gained by considering the uncharged wall. Although the electric field does not act directly on this wall, the electroosmotic flow does exert a drag force on it since the shear stress is non-vanishing at x = d. Following the same steps as above we have for this force

$$F_{\rm mech}^0 = -A\eta \left| \frac{\mathrm{d}v(x)}{\mathrm{d}x} \right|_{x=d} = AE\sigma \frac{\lambda}{d} = -F_{\rm mech}^{\sigma}.$$
 (14)

The forces on the two walls are thus equal and opposite, which may at first appear surprising. It is however easily understood by considering that *the electric field exerts no net force on the system as a whole* since the force exerted on the charged wall is exactly compensated by the force on its screening charge. Any external mechanical force on the charged wall must therefore be balanced by a second external force. Equivalently, one can consider that the total force on the screening charge, given by $-A\sigma E = -F_{\text{bare}}^{\sigma}$, is transmitted to the two surfaces through shear in the fluid. The fraction that reaches the uncharged surface is λ/d , while the fraction that reaches the charged surface is $(1 - \lambda/d)$. It follows directly that $F_{\text{mech}}^{\sigma} = -(F_{\text{bare}}^{\sigma} + F_{\text{drag}}^{\sigma}) = -F_{\text{mech}}^{0}$.

Eqn (13) predicts that the tether force F_{mech}^{σ} goes to zero as the distance *d* is increased toward infinity, and therefore that there is no tether force on a charged surface far from any boundary. This counterintuitive result is actually an artefact caused by the approximations that we have made. In particular, we have neglected edge effects and essentially treated the charged surface as infinite. For a finite-sized surface, a small but non-zero force remains due to a back flow of fluid on the size scale of the surface itself. More generally, we have neglected inertial effects by using only the Stokes equation to describe the motion of the fluid. On large enough length scales, however, inertia becomes relevant and this approximation breaks down.

2.4 Effective charges

In simple electrostatics, the force on a charged object is proportional to its charge multiplied by the local electric field. As we saw in the previous section, on the other hand, the net tether force on a charged object in an electrolyte is not simply given by its bare surface charge density, and is instead much smaller due to an important contribution from the screening layer. To an observer who is unaware of what is happening in solution and who simply measures the tether force in response to a known electric field, however, it is natural to describe the force as resulting from an effective surface charge density σ_{eff} , such that $F_{\text{mech}}^{\sigma} = -A\sigma_{\text{eff}}E$, with $|\sigma_{\text{eff}}| < |\sigma|$. Comparing to eqn (14) directly yields a value for $|\sigma_{\text{eff}}|$, namely

$$\sigma_{\rm eff} = \sigma \frac{\lambda}{d}.$$
 (15)

The effective charge, as introduced above, is a well-defined quantity, both experimentally and theoretically. Nevertheless, the authors feel that the use of the concept of "effective charge" should usually be discouraged. There are several reasons for this. First, as eqn (15) makes manifest, the effective "charge" does not correspond to a physical charge, but rather includes factors that depend on the geometry of the hydrodynamic environment around the charged object. Second, because the effective charge is not an intrinsic property of the charged object, instead depending on geometry parameters such as d, its value cannot be compared directly between different experimental configurations. Comparisons between tether force and electrophoretic mobility experiments are even more difficult, as the relation between the effective charges that are commonly defined in these two experimental situations is model-dependent. Third, the concept of effective charge is ineffective as a pedagogical tool: it has been our experience that, upon first encountering it, many readers are left with a vague notion that the effective charge consists of the bare charge of the object, minus some countercharge which is physically immobilized on the object. While this sort of complexation can certainly be an important contribution in real systems, thinking about electrophoresis solely in this manner obscures the fact that the tether force is expected to be smaller than $-A\sigma E$ even if there are no immobile countercharges.

3. More complex cases

In the previous section, we made several simplifications so as to keep the mathematics as simple as possible while retaining the relevant physical concepts. In this section we point out the additional complications that arise when some of these simplifications are lifted. We concentrate mostly on a qualitative description rather than a full derivation as in the previous section.

3.1 High charge densities

The calculation in Section 2 holds so long as the surface charge density is sufficiently small that the condition $|\sigma| \ll kT\varepsilon_w/e\lambda$ is satisfied. At higher surface charge densities, the approximation made in eqn (2) breaks down and eqn (1) can no longer be replaced by its linearized version, eqn (3). Combining the full eqn (1) with Poisson's equation yields an alternative to eqn (4) that is valid for arbitrarily large values of σ ,

$$\frac{\mathrm{d}^2\phi(x)}{\mathrm{d}x^2} = \frac{2en_0}{\varepsilon_{\mathrm{w}}}\sinh\left(\frac{e\phi(x)}{kT}\right) \tag{16}$$

This result is known as the Poisson–Boltzmann (PB) equation. Because of its non-linearity, this equation is considerably more difficult to solve than the Debye–Hückel approximation of Section 2. When $d \gg \lambda$, an analytical solution can nonetheless be obtained for the geometry of Fig. 1a,^{8,9}

$$\phi(x) = \frac{2kT}{e} \ln\left(\frac{1 + \gamma e^{-x/\lambda}}{1 - \gamma e^{-x/\lambda}}\right),\tag{17}$$

where $\gamma = -\lambda_{\rm GC}/\lambda + (1 + \lambda_{\rm GC}^2/\lambda^2)^{1/2}$. Here we also introduced a new parameter, $\lambda_{\rm GC} = 2kT\varepsilon_{\rm w}/e|\sigma|$, which is known as the Gouy–Chapman length. Qualitatively, $\lambda_{\rm GC}$ is a measure of the strength of the electrostatic interactions between ions and the surface, with a small $\lambda_{\rm GC}$ corresponding to strong interactions. Readers interested in a more in-depth discussion of solutions to the PB equation, including the derivation of eqn (17), are referred to ref. 8 and 9.

This result for $\phi(x)$ is plotted in Fig. 3 for a range of values of the surface charge σ . Far from the surface, the potential decays exponentially with a characteristic length λ for all values of σ . For large enough σ , however, the potential far from the surface no longer increases with increasing σ . Instead, its value becomes independent of the magnitude of σ and takes on the form $\phi(x) = \pm (4kT/e)\exp(-x/\lambda)$. Correspondingly, for large enough σ the distribution of counter- and coions far from the surface also becomes independent of the magnitude of σ . In this case all of the additional screening charge is located close to the charged surface, as evidenced by the continued increase of $\phi(x)$ with increasing σ in this region. The size of this region is of the order of the Gouy-Chapman length, λ_{GC} . More precisely, at a planar surface and under conditions of low bulk electrolyte concentration ($\lambda_{GC} \ll \lambda$), half of the counterions reside within λ_{GC} from the surface.⁸ In



Fig. 3 Electrostatic potential $\phi(x)$ (in units of kT/e) versus position x (in units of λ) as given by the solution to the PB equation, eqn (17). From bottom to top, the curves correspond to values of the surface charge density σ of 0.001, 0.003, 0.005, 0.01, 0.025, 0.05, 0.1, 0.5 and 1.0 C m⁻² in 20 mM KCl at 300 K. For low values of σ , the curves follow the Debye–Hückel result, eqn (6). Deviations from this linear behavior are evident at low x for the four topmost curves. Inset: counter- and coion distribution for $\sigma = -0.025$ C m⁻² at 20 mM KCl. The coions are depleted in close proximity to the surface. In contrast, the number density of counterions is ten times higher than in bulk solution.

water at room temperature, $\lambda_{\rm GC}$ is only 2.4 Å for a high surface charge density of $\sigma = 0.16$ C m⁻² (corresponding to 1 *e* nm⁻²). Although its value scales inversely with σ , $\lambda_{\rm GC}$ remains a molecular scale length for many charged systems.

Qualitatively, the charge screening a highly-charged surface can thus be thought of as consisting of two components: a diffuse layer, which extends a few Debye lengths into the solution, and a more compact layer very close to the surface. The diffuse layer is composed of more-or-less symmetric distributions of excess counterions and missing coions, whereas the more compact layer is composed predominantly of counterions. The latter results from the non-linearity inherent in eqn (1): while the coion concentration cannot be suppressed below zero, the degree of counterion enrichment can be arbitrarily high. Even for a surface with $\sigma = -0.025$ C m⁻² (corresponding to $e\phi/kT \approx 2$) the ion distributions show an excess of counterions n_+ whereas coions n_- are completely depleted in the screening layer. This is illustrated in the inset of Fig. 3.

3.2 Cylindrical geometry

In describing the ionic screening of charged, stiff biopolymers such as DNA, a common approximation is to treat the molecule as a charged, solid cylinder. Analogous to the discussion of the infinite plane above, we focus here on a charged cylinder with radius a and surface charge density σ positioned in the center of a larger, uncharged cylindrical cavity with radius R. For such a problem with cylindrical symmetry, the PB equation takes the form

$$\frac{1}{r}\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}\phi(r)}{\mathrm{d}r}\right) = \frac{2en_0}{\varepsilon_{\mathrm{w}}}\sinh\left(\frac{e\phi(r)}{kT}\right) \approx \frac{\phi(r)}{\lambda^2},\qquad(18)$$

where the last step corresponds to the Debye–Hückel approximation, $|e\phi(r)/kT| \ll 1$. In this last case and for $R \gg \lambda$, the corresponding Debye–Hückel solution for the potential is

$$\phi(r) = \frac{\sigma\lambda}{\varepsilon_{\rm w}} \frac{K_0(r/\lambda)}{K_1(R/\lambda)},\tag{19}$$

where K_0 and K_1 are the 0th and 1st order modified Bessel functions of the second kind, respectively. Analogous to the planar geometry, the potential decays exponentially away from the charged cylinder.

For a highly-charged surface, the linearization leading to eqn (19) is no longer valid and the full PB equation must be solved. We only note that the resulting solution exhibits the same qualitative features as for the case of a plane, and once again the screening charge can be broken into a diffuse layer, whose size is given by the Debye length, and a compact layer, characterized by the Gouy–Chapman length. The amount of charge in the diffuse layer also saturates at a constant value with increasing σ , while the remaining screening charge resides in the compact layer and consists primarily of counterions.

This separation into two components can be made more precisely in the special case of a line charge (a cylinder

^{||} Unlike ordinary Bessel functions, which are oscillating functions, the modified Bessel functions of the second kind decay exponentially for $r/\lambda \gg 1$.

with $R \rightarrow 0$) with linear charge density ρ and in the absence of supporting salt ($n_0 = 0$). In this case ions accumulate (or *condense*) on the line charge, partly compensating its charge until its value decreases to a magnitude $e/\lambda_{\rm B}$ (where $\lambda_{\rm B} = e^2/4\pi k T \varepsilon_{\rm w}$ is the so-called Bjerrum length**). The linear charge density of the condensed ions is thus ($|\rho| - e/\lambda_{\rm B}$), while the linear density of charge in the diffuse layer is the remaining $e/\lambda_{\rm B}$. This result, known as Manning condensation,^{10,11} often serves as a first approximation for the composition of the double layer in more realistic cases. Double-stranded DNA, for example, has a charge density of 2e per base pair, corresponding to $\rho = 4.2e/\lambda_{\rm B}$. This is well over the critical threshold for counterion condensation to occur, and as a first estimate a fraction 1 - 1/4.2 = 0.76 of the charge can be expected to be screened by condensed counterions.

Because the distribution of screening charge in the vicinity of a charged cylinder is qualitatively very similar to that near a surface, our discussion of electroosmotic flows and tether forces in Section 2 applies directly to the case of a cylinder. In particular, as illustrated in Fig. 4a, here also the tether force is due to a combination of bare electrostatic force and a drag force from the counterions, with the drag force being of comparable magnitude to the bare force. The cylindrical geometry only influences our analysis and conclusions at a quantitative level. For example, Fig. 4b shows the fluid velocity v(r) as a function of radial position r. Far from the charged cylinder, v(r) exhibits a non-linear decay with increasing r, unlike our result for surfaces (Fig. 1d), leading to subtle differences in how the drag force is distributed between the two surfaces. We refer the reader to the original literature^{12–14} for a discussion of these quantitative aspects.

3.3 Limitations of the model

Several properties of real systems are not captured by the formalism that we have introduced here. Some of the more flagrant problems include:

• The PB equation ignores the finite size of ions, instead treating them as point particles. It predicts that, near a highly-charged surface, a significant fraction of the counterions accumulates within a length λ_{GC} of the surface. For highly-charged surfaces, however, the calculated λ_{GC} is comparable to or even smaller than the size of an ion (a few Å). Neither these steric clashes nor the effect of crowding of the ions near the charged surface are included in the model.

• The PB equation only includes electrostatic interactions between ions and the average electrostatic potential, but ions can also have affinity for particular sites on the charged surface. Such specificity modifies the composition of the electrical double layer, and in particular its compact part, compared to the purely electrostatic case. This can be investigated by molecular dynamics simulations¹⁵ or X-ray diffraction on DNA in solution.¹⁶

• The calculations deal with perfectly smooth surfaces with uniform charge densities, but matter is intrinsically rough and charges are discrete on the molecular scale. To what extent this affects the validity of using continuum equations at this length



Fig. 4 Force balance for DNA in the center of a nanopore with radius *R*. (a) Left: the balance of forces is qualitatively identical to the case of the surfaces in Fig. 2. Briefly, the electric field pulls the negatively charged DNA towards the bottom with a force F_{bare}^{σ} , whereas the counterions experience a force in the opposite direction. This leads to drag forces F_{drag}^{σ} , acting on the DNA, and F_{drag}^{0} , acting on the nanopore wall. The latter force is ultimately transferred to the rest of the experimental setup to which the pore is rigidly attached. The DNA is stalled by a tether force F_{mech}^{σ} acts on the uncharged nanopore. Right: when the nanopore radius *R* is increased, the drag force on the DNA also increases. Correspondingly, the drag force on the nanopore F_{drag}^{0} and the tether force F_{mech}^{σ} decrease. (b) The flow velocity v(r), calculated by numerically solving eqn (18) and combining the result with the Stokes equation.¹⁴ The maximum flow velocity depends on the nanopore radius *R*.

scale remains somewhat unclear. Recent molecular dynamics simulations observe an effect of the roughness on the tether forces. $^{17}\,$

Ultimately, most of these difficulties come down to our limited ability to describe ionic systems on the molecular length scale. Although substantial work has been carried out toward resolving them (see *e.g.* ref. 18), no consensus exists at this time on how best to address these issues in an analytical theory. It is likely that this situation will not change significantly until more microscopic experimental evidence can be brought to bear.

4. Experiments

The considerations above strongly suggest that hydrodynamic interactions—and their modification by the local environment inside a complex environment such as a gel—represent one of

^{**} The Bjerrum length represents the distance between two ions at which the electrostatic energy equals kT.

the major ingredients for understanding electrophoresis. In particular, although great care is usually taken in gel or capillary electrophoresis applications to minimize large-scale hydrodynamic flows, we have seen that the forces experienced by a molecule undergoing electrophoresis are intrinsically linked to the geometry of its hydrodynamic surroundings. But while there exists a vast array of measurements of the mobility of macromolecules in gel electrophoresis (see *e.g.* the recent review by Stellwagen and Stellwagen³), the electrophoretic forces on the molecules cannot be directly extracted.

One clever solution to measure forces in bulk solution is the use of steady-state electrophoresis.^{19–23} Here, charged biomolecules are driven against a semi-permeable membrane by an electrical field. The membrane lets ions pass but is impenetrable for anything larger. The macromolecules therefore become concentrated near the membrane until a steady state is reached in which diffusion away from the membrane exactly cancels the driving force. In this case the distribution of the molecules as a function of distance from the membrane yields the force. Laue *et al.* also used this technique to measure electrophoretic mobility in bulk solution.²⁰ This is probably one of the best techniques to study the free-solution electrophoretic properties of charged molecules. To our knowledge, however, this approach has not so far been applied inside gels.

Conceptually, a much simpler alternative for experimentally testing the theoretical ideas described above is to directly measure the tether force. In doing so one faces two main challenges: (i) a means must exist for detecting the small forces involved, and (ii) the geometry of the microscopic environment near the molecule should be controlled or at least well characterized. Advances in single-molecule techniques over the last two decades provide the ideal tools for performing this sort of measurement and probing the mechanisms of electrophoresis in confined geometries.

It is instructive to first estimate the magnitude of the forces expected during the experiments. This requires knowledge about the polyelectrolyte molecule to be investigated. Double-stranded (ds) DNA is the ideal candidate due to its long persistence length of 50 nm in physiological conditions, well-characterized structure, high charge density, broad availability, and the existence of an extensive molecular biology toolkit for manipulating it. The diameter of dsDNA in its B-form is about 2 nm, and the distance between base pairs is l = 0.34 nm. At pH = 8.0 the phosphate backbones are fully deprotonated, yielding two elementary charges per base pair. As noted above, this corresponds to a linear charge density of $4.2e/\lambda_{\rm B}$. The typical potential difference ΔV used for the electrophoretic translocation of DNA through a membrane is 0.1 V. The corresponding maximum electrophoretic force (ignoring all drag contributions) is thus $F_{\text{bare}}^{\sigma} = 2e\Delta V/l = 100 \text{ pN}$ for $\Delta V = 0.1$ V applied across a straight segment of DNA.²⁴

The optimal experimental tools for measuring forces of this magnitude are optical tweezers. These have a typical spatial resolution in the single nanometre range and pN or even sub-pN force sensitivity.^{25,26} Optical tweezers use tightly focused laser light to trap and manipulate dielectric particles in aqueous solution. The most common force transducers are antibody-coated polystyrene microspheres which are

commercially available. dsDNA with one receptor molecule can be assembled by standard biochemical techniques and a single dsDNA is then easily attached to such a colloid. A commonly used receptor–ligand pair is streptavidin–biotin.

Optical tweezers can be used to measure the electric force acting on this molecule through the displacement of the trapped sphere from its equilibrium trapping position. For small displacements Δx , the optical trap behaves like an ideal spring following Hooke's law, $F = k\Delta x$, where k is the stiffness of the optical trap. In other words, the trapped colloid provides the mechanical handle which will be used to insert the dsDNA into a desired environment and to measure the force on the molecule when an electric field is applied.

The second requirement for any successful experimental test of the above models is the precise control over the experimental geometry the DNA is residing in. One obvious choice is the gel matrix used for electrophoretic separation. Bustamante *et al.* realized this by developing a sample cell in which DNA bound to a colloid could be electrophoretically stretched either in open solution or in a gel.²⁷ In these experiments it was shown that the force on DNA *increases* by a factor of four when the molecule is in the gel compared to open solution. This indicates that hydrodynamic interactions affect the force on the molecule substantially, as the force increases along with the level of hydrodynamic coupling, as expected from the analysis above. A quantitative interpretation of these data is however complicated by the complex structure of the gel matrix.²⁷

An unambiguous comparison between the model and experimental measurements requires confining the DNA in a simple geometry with precise control of the dimensions on the nanometre scale. One way to achieve this is by employing state-of-the-art nanotechnology to machine solid-state nanopores, holes with radii of sub-nm to tens of nanometres in ultra-thin (20 nm) insulating membranes using focused ion²⁸ or electron beams.²⁹ In combination with the optical tweezers, these solid-state nanopores provide a highly controlled platform to test the influence of hydrodynamic interactions on a single charged polyelectrolyte molecule in a well-defined geometry.

Using this combination, the tether force on dsDNA in a nanopore was recently measured.^{14,24,30} In these experiments a single dsDNA molecule was inserted into a nanopore with an independently determined radius. The tether force was measured in pores having radii between 3 nm and 50 nm. These experiments showed that the tether force on dsDNA is a factor of two larger in a small pore with radius 3 nm compared with the force in a pore with radius 50 nm, while keeping the salt concentration and pH value of the solution constant. These results confirm the importance of hydrodynamic interactions unambiguously and on the single-molecule level. They also directly show the limitations of the effective charge for parameterizing electrophoretic forces since its value is explicitly found to depend on the experimental configuration.

We further note that nanopore translocation experiments illustrate another profound result that follows from the non-linearity of the PB equation. The voltage difference applied over a nanopore to drive the dsDNA through the pore also gives rise to an ionic current that can be independently measured. At high salt concentrations, inserting a dsDNA molecule through a nanopore causes the ionic current to decrease. This can be easily understood by considering that the DNA molecule occupies a finite volume and therefore expels some salt ions from the nanopore, causing the latter's ionic conductance to decrease. Counterintuitively, however, at salt concentrations below 300 mM, a dsDNA molecule threading through the nanopore causes the current to *increase*.^{31–34} This additional current is believed to be carried by the excess counterions that screen a highly-charged object, as discussed in Section 3.1. The inset of Fig. 3 shows the distribution of co- and counterions for a surface with $\sigma = -0.025 \text{ Cm}^{-2}$. The clear asymmetry between the two ionic species leads to an effective increase of the ion concentration in the double layer.

In practice, only the high charge density of dsDNA enables the detection of a single molecule in nanopores with > 50 nm radii.³⁵ The reason for this limitation is that a macromolecule with a low charge density (below the critical threshold for non-linear counterion accumulation) would be expected to exhibit only very weak enhanced conductance, if any, since in that case the co- and counterion distributions are symmetrical (Fig. 1c) and the number of charge carriers thus remains constant.†† The latter prediction has to our knowledge not been probed experimentally, however.

5. Closing remarks

This tutorial review has provided an overview of the concepts behind the generation of forces in electrophoresis, and in particular of the central role played by hydrodynamics. These concepts have long been well-understood, yet their surprisingly subtle interplay can often lead to important misconceptions in interpreting new experiments. Developing an accurate intuition of the forces at work in electrophoresis represents an important prerequisite for developing a full understanding of the complexities of practical gel systems. More tentatively but also perhaps more excitingly—it may provide the spark that leads to new electrophoresis-based science and technology when combined with advances in nanofluidic techniques.^{4,5}

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^{††} This is however not true if the counterions have a mobility that is significantly higher than that of the coions.