Asymmetric Diffusion through Synthetic Nanopores

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We show that diffusion currents for a membrane containing a single conical nanopore with a fixed surface charge and small enough opening diameter depend on the concentration gradient direction. We interpret the results based on the effect of salt concentration on the thickness of the electrical double layer within the nanopore associated with the nanopore's surface charge and the distribution of electric fields inside the pore. The experimental observations are described by a diffusional model based on the Smoluchowski-Nernst-Planck equation.

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There are a number of examples in biological cells where the transport of a chemical species across the cell membrane is faster in one direction than in the other. Examples include energy-dependent pumps [1,2], which transport species such as K^+ and Na^+ against their concentration gradients across the cell membrane, and voltage-gated ion channels [1], which rectify the ion current flowing across the membrane. Recently, such asymmetric transport phenomena have been observed in synthetic nanopore systems [3,4]. For example, conically shaped nanopores in synthetic polymer membranes can act as rectifiers of ionic current flowing through the nanopore, provided there is excess surface charge on the pore walls [3]. Electrostatic interaction between the ions traversing the conical nanopore and the surface charge at the small-diameter opening (tip) of the pore is the key to observing such asymmetric transport phenomena.

We show here that electrostatic interactions with the surface charge can also produce an asymmetry in the rates of diffusive transport of ions across a polymer membrane containing a single conically shaped nanopore. In this case, ion transport was driven by a concentration gradient across the membrane, by simply placing a higher concentration salt solution on one side of the membrane than on the other side. We define the concentrations of the electrolyte in contact with the nanopore tip and base as c_t and c_b, respectively, (Fig. 1). It is clear that there are two ways to apply the salt concentration gradient—with $c_b < c_t$ [Fig. 1(a)] or with $c_b > c_t$ [Fig. 1(b)]. We have found that the rate of diffusive ion transport through the nanopore is higher when $c_t < c_b$ [Fig. 1(b)], than when $c_t > c_b$. We interpret these results in terms of the effect of the salt concentration on the thickness of the electrical double layer in the solution within the nanopore tip, resulting from the distribution of electric fields inside the pore.

Polyethylene terephthalate (PET) membranes containing a single conically shaped nanopore were prepared by the track-etch method as described previously [5]. In all cases the radius of the base was $r_b \sim 300$ nm, as determined by electron microscopy. The tip radius r_t was varied from 1.5 to about 20 nm (Table I), as determined by a simple electrochemical method [5]. The track-etched PET has surface carboxylate groups [5]. When exposed to solutions having $p\mathrm{H} > 3$, these $-\mathrm{COOH}$ groups are deprotonated to yield negative charge ($-\mathrm{COO}^-$) on both the nanopore walls and membrane faces. The maximum charge density, obtained at high pH, is $1.5e/\mathrm{nm}^2$ of membrane surface area [6].

The membrane sample containing the conical nanopore was mounted between the two halves of a conductivity cell [5], and the half-cells were filled with KCl solutions at the desired concentration. A Ag/AgCl reference electrode placed in each half-cell solution was used to apply a known

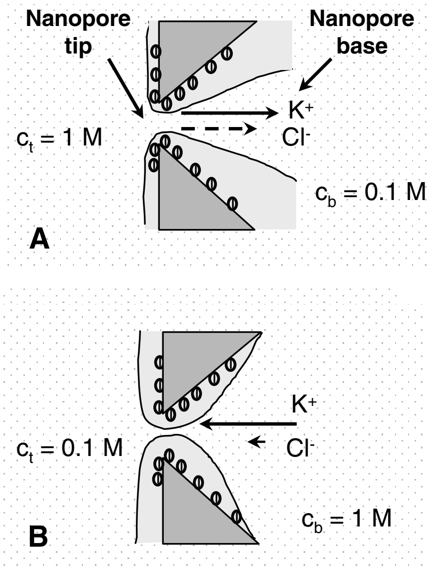

FIG. 1. Two configurations of salt concentration gradient across a conical nanopore together with schematic representation of the double-layer thickness related to the salt concentrations (not in proportions). (a) (b) lower salt-content solution faces the base (the tip) of the nanopore.

TABLE I. Values of r_b and r_t in nm, c_b and c_t in M, $I_{0,b\to t}$ and $I_{0,t\to b}$ in pA, and Q for various nanopores.

r_t	r_b	c_t	c_b	$I_{0,b\to t}$	$I_{0,t\to b}$	Q
18	300	0.1	1.0	0	0	...
7.5	370	0.1	1.0	233	105	2.2
7.5	370	0.01	1.0	107	40	2.7
5.0	320	0.1	1.0	180	101	1.8
2.5	320	0.1	1.0	33	17	1.9
2.5	320	0.01	1.0	60	27	2.2
1.5	315	0.1	0.5	22	12	1.8
1.5	315	0.1	1.0	28	16	1.7

transmembrane potential difference and measure the resulting transmembrane ion current (Fig. 2). Because we are interested in diffusive transport, we focus our attention on the current observed when the transmembrane potential difference was zero, hereafter called the zero-volt current. When the salt concentration on both sides of the membrane is the same, the zero-volt current is zero, as it should be since there is no concentration gradient and thus no driving force for ion transport across the membrane (inset of Fig. 2).

In contrast, with 1.0 M KCl on one side of the membrane and 0.1 M KCl on the other side, the zero-volt current is not zero (Fig. 2). Furthermore, the absolute value of the zero-volt current depends on which side of the membrane the low and high salt-content solutions faced. With $c_b = 1.0$ M and $c_t = 0.1$ M [Fig. 1(b)], the zero-volt current ($I_{0,b\to t}$) was 28 pA for $r_t = 1.5$ nm. With $c_t = 0.1$ M and $c_b = 1.0$ M [Fig. 1(a)], the zero-volt current ($I_{0,t\to b}$) was 16 pA. The magnitude of this asymmetry in the diffusion currents can be quantified by the parameter $Q = I_{0,b\to t}/I_{0,t\to b}$ (Table I).

At the presence of salt concentration gradient, the zero-volt current is nonzero because the nanopore is cation (K^+) selective (cf., Fig. 2). As a result, the rate of transport of K^+ down its concentration gradient across the membrane is higher than the rate of Cl^- transport down its concentration gradient, and this causes the nonzero zero-volt current. As discussed in detail previously for cylindrical pores [7], cation selectivity occurs when the pores are small enough so the electrical double layer associated with the excess negative charge at the pore wall fills a significant part of the pore volume. This is also true for the conical pores used here. However because the radius of the base is so large (~300 nm), whether cation selectivity is observed depends on the relative magnitudes of the double-layer thickness and the radius of the tip of the conical nanopore.

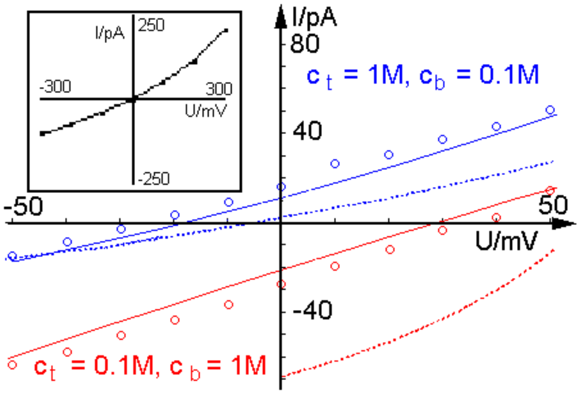

FIG. 2 (color online). $I(V)$ of a single asymmetric PET nanopore (radii $r_t = 1.5$ nm, $r_b = 315$ nm, and length $L = 12$ μm) recorded at pH 8. Open circles are experimental data, dashed lines are current-voltage curves calculated from the bulk model, and solid lines from the fitted model (cf., text). The inset shows $I(V)$ for $c_t = c_b = 0.1$ M.

The double-layer thickness depends on the salt content of the solution in contact with the membrane [8]. As a result, the nanopore shows higher cation selectivity, and a higher zero-volt current, when the solutions are arranged as per Fig. 1(b) (Table I). An indirect measure of the ion selectivity is given by the so-called reversal potential E_r, which is the potential at which the current is zero [9]. Figure 2 shows that with the solutions arranged as per Fig. 1(b), the reversal potential is ~36 mV, whereas with the Fig. 1(a) arrangement the E_r is only ~22 mV. Further evidence for these arguments is garnered from analogous current-voltage curves obtained at $p\text{H} = 3$, where there is no excess surface charge. As would be expected, the zero-volt current is zero for both of the solution configurations in Fig. 1. In addition, as shown in Table I, when the tip diameter is large the zero-volt current is again zero regardless of solution configuration.

A similar phenomenon of asymmetry in channel selectivity expressed as E_r has been reported recently for the bacterial porin OmpF [9], which is asymmetric in both diameter and charge. It was found that electrostatic interactions are crucial for the observed effect of E_r asymmetry.

We approached the modeling of asymmetric diffusion currents by calculating the currents for the K^+ and Cl^- ions that result from the known salt concentration gradient across the membrane. The first semiquantitative attempt to describe ion currents through a conical nanopore was based on the Smoluchowski-Nernst-Planck (SNP) equation [10] with a simplified form of electric potential inside the pore. We also based our modeling on the SNP equation, but we attempted to determine the distribution of the electric potential, which would render the geometry and charge of the pores.

The ion currents through the nanopore depend on the potential difference applied across the membrane U, and on the potential of the solution at any point z, within the nanopore $U_e(z)$, that results from this applied potential. The form of $U_e(z)$ is related to the pore's geometry. From Kirchhoff's law, the ratio of U and $U_e(z)$ is equal to the ratio of resistances $\mathcal{R}$ of two cones of the same r_t: the whole cone of length L and base radius r_b, and a shorter cone of length z and base radius $h(z) = r_t + (r_b - r_t)z/L$: $U_e(z) = U\mathcal{R}(z)/\mathcal{R}(L)$. In addition, the ionic currents depend on the potential $V_{\mathrm{int}}(z, r)$ within the pore solution that results from the excess surface charge on the pore wall [4]

$$V_{\mathrm{int}}(z, r) = \frac{1}{4\pi\epsilon} \int_0^L dz' \rho(z') h(z') \int_{-\pi}^{\pi} d\theta' R^{-1}(z, z', r, \theta') e^{-\lambda R(z,z',r,\theta')}, \tag{1}$$

$$R(z, z', r, \theta') = \sqrt{[r - h(z')\cos\theta']^2 + [h(z')\sin\theta']^2 + (z - z')^2}, \tag{2}$$

where ϵ denotes the dielectric constant, $\lambda = 1/l_D$ is the inverse Debye (screening) length, and ρ is the surface charge density. As shown in detail previously, we substitute for $V_{\mathrm{int}}(z, r)$ the corresponding one-dimensional representation $V_{\mathrm{eff}}(z)$ obtained by averaging $V_{\mathrm{int}}(z, r)$ over the area of the pore's cross section at point z [4,11].

The ionic flow (mole s^{-1}) for an ion i of charge Z_i is related to these two potentials, $U_e(z)$ and $V_{\mathrm{eff}}(z)$, via the SNP equation with the solution in the one-dimensional Fick-Jacobs-type approximation [4,11]

$$J_i = \frac{c_{it} - c_{ib} e^{-\beta_i Z_i e U}}{\int_0^L dz e^{\beta_i Z_i e [V_{\mathrm{eff}}(z) - U_e(z)]} / D_i h^2(z)}. \tag{3}$$

D_i is the diffusion coefficient for the ion i, and $\beta_i = \mu_i / D_i$, where μ_i stands for the ion mobility.

The current I_i is related to the diffusion flow via $I_i = Z_i F J_i$ and the total current is the sum of the contributions from K^+ and Cl^-. We calculated the total current for the two different solution configurations represented in Fig. 1 [12]. In order to calculate these currents, we must have values for the various parameters in Eq. (3). One approach is to use values for bulk solution [4,10]: $D_{K^+} \approx D_{Cl^-} \approx 2 \times 10^{-9}$ m^2/s [13], a linear profile of KCl concentration, and approximation of β_i by the Einstein relation $\beta_i = \beta = 1/kT$. At room temperature $\beta e = 38.9$ V^{-1} and the Debye length depends on KCl concentration c as $l_D \approx 0.307/\sqrt{c}$ nm [8]. When these bulk-solution values are used the dashed curves in Fig. 2 are obtained. These curves show that the theory semiquantitatively predicts the trends observed in the experimental data, as has been previously demonstrated for symmetric electrolyte conditions [4]. However, because the bulk-solution values undoubtedly do not apply to the solution confined in the nanopore [9], the quantitative agreement is poor.

An alternative approach is to adjust the various parameters so that the calculated currents fit the experimental values, shown in Fig. 2. We fitted $D_{K^+} = 1.8 \times 10^{-9}$, $D_{Cl^-} = 0.5 \times 10^{-9}$ m^2/s, added the contribution from the surface currents [4,14] in pA $I_s = 0.35U$, as well as changed the screening, i.e.—the V_{eff} values by a factor of 0.2 for K^+ and 4 for Cl^- [15]. While the fit is good, it is important to emphasize that this fit is not unique—other sets of parameters can do even better. We chose this set because it has some heuristic background: (i) it was shown recently that D_i for ions and neutral molecules are smaller inside nanopores than in the bulk [9,15]; (ii) it is known that for cation selective pores $D_{Cl^-} < D_{K^+}$ [9]; (iii) Eq. (1) suggests that V_{int} (and therefore V_{eff}), decreases with increasing $\lambda \sim \sqrt{c}$, and vice versa. There are, however, no independent data on how ion screening changes inside a nanopore, and how it is influenced by strongly different concentrations of anions and cations.

Using bulk and fitted models we calculated values for the asymmetry factor Q as a function of r_t, assuming $r_b = 300$ nm (Fig. 3). Both models predict Q to be relatively insensitive to tip diameter for $r_t < 10$ nm, and this is reflected in the experimental data as well (Table I). That Q is independent of r_t for low values of r_t is not surprising, because as long as r_t is comparable to the Debye screening length, most of the coions are depleted inside the entire volume element of solution within the conical pore tip. Because of the curvature of the pore's surface, one also expects that the thickness of the electrical double layer inside a nanopore with excess surface charge is larger than at the flat charged surface, which also influences the distribution of cations and anions inside the pore. For $r_t > 15$ nm, the effect of asymmetric diffusion currents almost vanishes ($Q \rightarrow 1$), which again is in agreement with experimental data (Table I).

To understand the origin of the observed diffusion asymmetry one needs to consider how $V_{\mathrm{eff}}(z)$, the electric fields $E_{\mathrm{eff}} = -dV_{\mathrm{eff}}/dz$, and the ions' concentrations vary with distance z along the axis of the conical nanopore; calculated values are shown in semilog fashion in Fig. 4. The

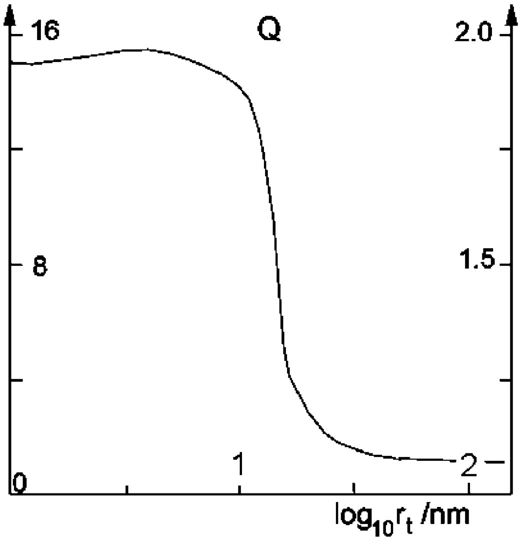

FIG. 3. Calculated asymmetry factor Q as a function of the tip diameter for a cone of length $L = 12$ μm and $r_b = 300$ nm, according to the bulk model (left axis) and fitted model (right axis) with parameters as in Fig. 2.

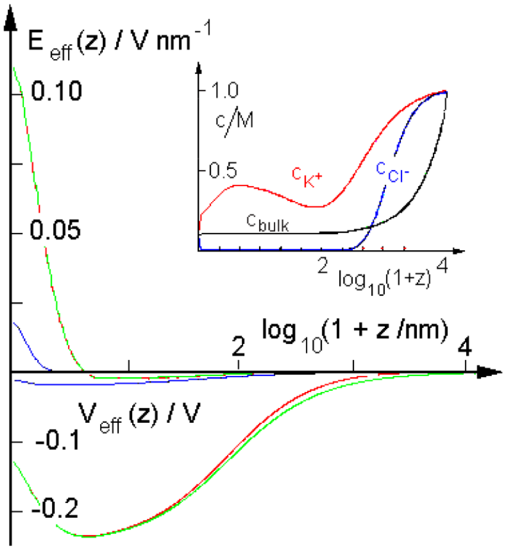

FIG. 4 (color online). Calculated effective electric fields $E_{\rm eff}$ (upper scale) and effective potentials $V_{\rm eff}$ (lower scale) for: $c_t = c_b = 0.1$ M (most upward and bottom curves, green), $c_t = 0.1$ M, $c_b = 1.0$ M (second curves from top and bottom, red), and $c_t = 1.0$ M, $c_b = 1.0$ M (third curves counted from top and bottom, blue). Parameters of the bulk model were used for calculations; scaling the values by 0.2 for cations, and by 4 for anions leads to values of the fitted model. Inset shows concentration profiles of cations, anions, and of bulk-type (linear) ion distribution for $c_t = 1.0$ M, $c_b = 1.0$ M (with parameters from the fitted model).

ratchet-type [16] shape of the $V_{\rm eff}(z)$ plot is expected for conical pores with fixed surface charge [3]. When $c_t > c_b$, the absolute value of $V_{\rm eff}$ is much smaller (the third curve from the bottom, blue) than when $c_b > c_t$ (the second curve, red). $V_{\rm eff}$ values for $c_b > c_t$ are comparable to the values when $c_t = c_b = 0.1$ M (the first curve from the bottom, green). This is reinforced by the corresponding plots of electric field $E_{\rm eff}$ vs z (Fig. 4), where we see that the curve for $c_b > c_t$ is nearly identical to that for $c_b = c_t$. The electric field acting on cations and drawing them inside the pore is therefore much higher for the salt concentration arrangement shown in Fig. 1(b) than in the situation in Fig. 1(a). $E_{\rm eff}$ causes the enhancement of the cation concentration and depletion of anions inside the pore (Fig. 4, inset), observed as nonzero E_r in Fig. 2.

We have demonstrated that the diffusion currents for a membrane containing a conical nanopore with fixed surface charge and appropriate r_b and r_t depend on whether the concentration gradient is applied such that $c_b > c_t$ or $c_b < c_t$. We interpret this result in terms of the effect of c_b and c_t on the thickness of the electrical double layer within the nanopore, associated with the nanopore's surface charge, and with the distribution of electric fields inside the pore. These results further reinforce the concept that the rate of ion transport in a charged nanopore depends critically on the extent of interaction between the ion and the surface charge on the pore wall [3,4,14].

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[12] The standard procedure (cf., e.g., [11]) is to calculate $V_{\rm int}$, $V_{\rm eff}$, and J_i from the assumed zeroth approximation for the concentration gradient $c(z, r)$, then to calculate $c_i(z, r)$ from the above functions, and so on. However, the above procedure is not unique—the calculation of $V_{\rm int}$ uses the bulk-type local value of the Debye length $l_D \sim 1/\sqrt{c}$, which is almost certainly wrong inside the charged nanopore [9], whereas the correct form is unknown, especially when $c_{\rm cation}(z, r) \neq c_{\rm anion}(z, r)$.

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