(In)validity of the Constant Field and Constant Currents Assumptions in Theories of Ion Transport

Alexander Syganow and Eberhard von Kitzing

Abteilung Zellphysiologie, Max-Planck-Institut für medizinische Forschung, D-69028 Heidelberg, Germany

ABSTRACT   Constant electric fields and constant ion currents are often considered in theories of ion transport. Therefore, it is important to understand the validity of these helpful concepts. The constant field assumption requires that the charge density of permeant ions and flexible polar groups is virtually voltage independent. We present analytic relations that indicate the conditions under which the constant field approximation applies. Barrier models are frequently fitted to experimental current-voltage curves to describe ion transport. These models are based on three fundamental characteristics: a constant electric field, negligible concerted motions of ions inside the channel (an ion can enter only an empty site), and concentration-independent energy profiles. An analysis of those fundamental assumptions of barrier models shows that those approximations require large barriers because the electrostatic interaction is strong and has a long range. In the constant currents assumption, the current of each permeating ion species is considered to be constant throughout the channel; thus ion pairing is explicitly ignored. In inhomogeneous steady-state systems, the association rate constant determines the strength of ion pairing. Among permeable ions, however, the ion association rate constants are not small, according to modern diffusion-limited reaction rate theories. A mathematical formulation of a constant currents condition indicates that ion pairing very likely has an effect but does not dominate ion transport.

GLOSSARY

The numbers in parentheses refer to the respective equation numbers where the term appears first.

x	Position inside the ion channel along the channel axis
$c(x)$	Concentrations of ions (1)
$p(x)$, $n(x)$	Concentrations of cations or anions, respectively (11)
I_c, I_p, I_n	Electric currents of ions (1), cations, or anions (10)
Z_c	Valencies of permeant ions (1)
D_c, D_p, D_n	Diffusion coefficients of permeant ions (1), cations, or anions (13)
S, L	Channel cross section (1) and length (2)
V	External voltage (2) across the channel length L
$E(x)$	Mean electric field inside the channel (3)
$\langle X \rangle$	Average of X (4), e.g., mean charge density $\langle \rho \rangle$
U_ρ, $U_\mathscr{R}$	Space-charge (5) or pairing voltage (15). The constant field or constant currents approximation applies if the external voltage V is much larger than $U_\rho \ll |V|$ or $U_\mathscr{R} \ll |V|$, respectively.
ℓ_ρ, $\ell_\mathscr{R}$	The space-charge (6) or pairing length (16). The constant field or currents

approximation applies if the channel length is small compared to the space-charge $\ell_\rho \gg L$ or pairing length $\ell_\mathscr{R} \gg L$.

e_0	Protonic charge (7)
$\mathscr{R}(x)$	Pairing intensity (10). The pairing intensity often follows a simple reaction rate equation (11).
k_A, k_D	Ion association and dissociation rates, respectively (11)
p_Q, n_Q	Equilibrium cation and anion concentrations (11) inside the channel, determined by the ionic chemical potential difference between bulk solution and the channel environment
r_A	Effective association radius (14)
ℓ_{Bj}, ℓ_{DH}	Bjerrum distance (13) and Debye length (9 and 14), respectively
ℓ_C	Distance of closest approach between pairing ions (13)

Greek symbols

$\mu_c(x)$	Excess chemical potential of ions (1) divided by RT
$\mu_c^*(x)$	Excess equilibrium chemical potential of ions (2) divided by RT at zero external voltage
$\mu_c^s(x)$	Specific chemical potential of ions (1) divided by RT, representing the short-range interactions with the channel protein
$\phi(x)$	Total electric potential (1) times F/RT
ϕ_{fix}, ϕ_{ion}	Electric potential (2) of fixed and permeant ions times F/RT
$\rho(x)$	The total electric charge density, including permeant as well as permanent charges (3)
ϵ_0	Vacuum permittivity (3)

ϵ_C Effective dielectric constant inside the channel (3)

$\delta_\mu, \Delta\mu_c^s$ Typical spatial distance or energy difference between barrier energy maximum and well energy minimum (8)

Σ Channel conductance (15)

INTRODUCTION

An important class of theories describing ion permeation through biological ion channels starts with the Nernst-Planck equation (Buck, 1984; Cooper et al., 1985; Hille, 1992; Rubinstein, 1990). Charged amino acids lining the pore and permeant ions generate an electric field that is superimposed on the external electric field. Consequently, Gauss' law or the equivalent Poisson equation (Eisenberg, 1996; Jackson, 1962) must be satisfied. In addition, the conservation of the number of ions is generally assumed, leading to a respective number of continuity equations for each ion species. If association and dissociation of oppositely charged ions occur, an ion pairing term is added to the continuity equations (Buck, 1984). Gauss' law and the Nernst-Planck and continuity equations (PNPC; the first P stands for Poisson's equation, which is often used instead of Gauss' law) represent a general framework for explaining ion transport in biological ion channels.

The Smoluchowski equation generalizes the Nernst-Planck equation from purely electrostatic interactions to arbitrary excess chemical potentials $\mu_c(x)$:

$$-I_c = Z_c FSD_c\left(\frac{dc}{dx} + c(x)\frac{d\mu_c}{dx}\right) \qquad (1)$$

where I_c is the electric current of ions with a concentration $c(x)$, a valence of Z_c, and a diffusion constant of D_c. x is the coordinate along the channel axis, F is Faraday's constant, and S is the cross section of the channel. $\mu_c(x)$ represents the excess chemical potential divided by RT seen by a single ion. In general, μ_c depends on the particular channel environment, on the external ion concentrations, and on the external voltage. The ion flux through the channel is often approximated by the one-dimensional form of the Smoluchowski equation.

For practical calculation the excess chemical potential μ_c must be specified. It is useful to distinguish between the specific chemical potential $\mu_c^s(x)$, which includes all short-range interactions of a single ion species with the channel environment, and the electrostatic potential $\phi(x)$, which interacts over long distances with any charge within the system. The specific chemical potential $\mu_c^s(x)$ represents the energy required to transfer an ion of a given species from bulk water into the channel environment (Hille, 1992), such as dielectric effects (Parsegian, 1969, 1975; von Kitzing and Soumpasis, 1996), dehydration, or specific short-range interactions of the ion with polar amino acid side chains inside the channel. This part of the potential is very likely independent of the ion concentrations and the external voltage.

It can be estimated from force-field calculations (Dorman et al., 1996; Furois-Corbin and Pullman, 1991; Pullman, 1987, 1991; Ranatunga et al., 1998; Roux, 1996; Roux et al., 1995; Smith and Sansom, 1997; von Kitzing, 1995; von Kitzing and Soumpasis, 1996).

The potential $\phi(x)$ of the mean electric field $E(x)$ is defined by $d\phi/dx = -FE/RT$. ϕ depends on all charges and dipoles in the system, and on the external voltage V across the membrane. Because electrostatic interactions occur over a long range, charged amino acid side chains and permeant ions in the pore vestibules may also contribute to the electric potential ϕ within the pore. For simplicity, the charges of the channel protein are assumed to be fixed and give rise to the potential $\phi_{fix}(x)$. Furthermore, the channel contains permeant ions that contribute to the potential of the mean electric field by $\phi_{ion}(x)$. In general one should expect the mean orientation and state of protonation of potentially charged amino acid side chains to be concentration and voltage dependent. We include this effect in $\phi_{ion}(x)$.

Several authors assume (Hille, 1992; Levitt, 1986; McGill and Schumaker, 1996; Roux and Woolf, 1997) that the external voltage V drops linearly across the channel and represents the only considerable voltage-dependent contribution to the total electric potential $\phi(x) = \phi_{fix}(x) + \phi_{ion}(x) + (FVx)/(LRT)$, where L is the effective length of the channel. Such a constant field assumption requires that the charge density of the permeant ions and consequently $\phi_{ion}(x)$ are virtually voltage independent. In this case the excess chemical potential $\mu_c(x)$ can be uniquely decomposed into an external contribution $(FVx)/(LRT)$ and a voltage-independent equilibrium excess chemical potential μ_c^*:

$$\mu_c(x) = \mu_c^*(x) + Z_c(FVx)/(LRT)$$
$$\text{with } \mu_c^* = \mu_c^s + Z_c(\phi_{fix} + \phi_{ion}) \qquad (2)$$

Throughout this article, the idea of $\phi_{ion}(x)$ being either negligible or voltage independent is referred to as the *constant field assumption*. ϕ_{ion} is generally concentration dependent. As a result, the equilibrium excess chemical potential $\mu_c^*(x)$ also depends on external ion concentrations.

Because the electrostatic interaction is strong and occurs over a long range, the coupling between the mean electric field, the ion concentrations, and the external potential difference is highly nonlinear (Eisenberg, 1996). We have shown previously (Syganow and von Kitzing, 1995) that the solutions of the Nernst-Planck equation split into three domains: 1) the equilibrium, 2) the drift, and 3) the diffusion domain. Within different regions along the channel axis, different domains dominate. Solutions in the equilibrium domain are characterized by a balance between strong concentration gradients and forces accompanied by small electric currents. In this domain forces and concentrations remain close to their equilibrium values, and linear perturbation methods apply to include the effect of small external voltages. Close to minima or maxima of the concentration, however, the concentration gradients are necessarily small. Here, the electric current becomes proportional

to the driving force, and the concentration profile generally deviates from its form at equilibrium. At voltages above RT/F, these drift domains "destroy" the simple linear relation between the excess chemical potential and the external potential difference assumed in Eq. 2. This was explicitly shown for a model of a synthetic ion channel (Chen et al., 1997) using the Poisson-Nernst-Planck theory. The electric potential along the channel axis could not be decomposed into a linear voltage-dependent part and a voltage-independent part. In the diffusion domain the influence of the excess chemical potential becomes small and the current is determined by strong concentration gradients. This solution also deviates considerably from the equilibrium solutions.

The Smoluchowski equation (Eq. 1), together with the Poisson and the continuity equations, represents a set of coupled, nonlinear differential equations for which a general analytic solution is not available. Consequently, various approximate solutions of the PNPC equations were developed. Those theories differ in the terms neglected in the excess chemical potential μ_c. Goldman, Hodgkin, and Katz (Goldman, 1943; Hille, 1992; Hodgkin and Katz, 1949) assumed a constant electric field and constant ionic currents (GHK theory). The specific chemical potential μ_c^s and the electric field originating form permanent charges ϕ_{fix} are neglected, and ϕ_{ion} is assumed to be small. Thus within the GHK theory the external voltage drops linearly across the channel only if the total charge density inside the channel is small. Today GHK theory is largely discarded as a method for describing ion channels, because the predicted linear conduction-concentration relation is often not found in experiments.

If the excess chemical potential μ_c consists of a few large barriers separated by binding sites, Eq. 1 can be reformulated as a barrier model considered by Kramers (Cooper et al., 1985; Kramers, 1940; Weiss, 1986). In the formally similar absolute rate theory of Eyring (Eyring et al., 1949; Hille, 1992; Laidler and King, 1983), the rate of overcoming barriers applies to ballistic motion of particles in dilute gases and not to diffusion in dense media. A major difference between the Smoluchowski equation and barrier models is that the latter automatically takes care of volume exclusion effects and single filing. Each site can maximally contain a single ion. Incoming ions can enter only unoccupied sites. Barrier models are based on three important characteristics: 1) the external electric field is constant, 2) incoming ions cannot replace or pass resident ions occupying a site, and 3) the free energy profile is independent of the external ion concentrations. These assumptions imply that the chemical forces $d\mu_c^s/dx$ are large compared to the electric forces due to permeant and fixed charges. Cooper (Cooper et al., 1985) concluded from the literature that the barrier height should be at least 5 RT/F.

In the Poisson-Nernst-Planck (PNP) theory (Eisenberg, 1996) the specific chemical potential $\mu_c^s(x)$ is mostly neglected with few exceptions (Chen, 1997; Nonner et al., 1998). The excess chemical potential $\mu_c(x)$ is assumed to be dominated by electrostatic forces. Chen et al. (1997) have

shown that for a particular channel model, the ion distribution and consequently ϕ_{ion} become voltage dependent. Thus the constant field assumption generally does not apply to PNP theories.

The Nernst-Planck and Smoluchowski equations represent mean field theories, often also designated "continuum theories." Modern statistical mechanics (Keizer, 1987; McQuarrie, 1976) show that those continuum theories can be strictly derived from many-particle systems. The first electrolyte theory that accounts for the strong, long-range electrostatic interactions was proposed by Gouy (1910) for one-dimensional systems. Debye and Hückel (1923) extended it to three-dimensional systems. The potential of mean force is replaced by the mean potential (Kirkwood, 1934; Kirkwood and Poirier, 1954; McQuarrie, 1976). This approximation is often referred to as the *Poisson-Boltzmann theory*. In the limit of strong electric fields, e.g., close to a charged wall (Henderson et al., 1979), electrolytes behave as predicted by the Poisson-Boltzmann theory. Recently, this approximation was used to predict the equilibrium ion distribution within atomic models of ion channels (Cheng et al., 1998; Sansom et al., 1997; Weetman et al., 1997). Until today, complex theories have been developed describing experimental equilibrium properties of electrolytes to a high degree of accuracy (McQuarrie, 1976).

Unfortunately, charge transport theories for inhomogeneous steady-state systems are still on a much less sophisticated level compared to equilibrium electrolyte theories. Vlasov (1938) first proposed replacing the electric field seen by a single ion with the mean field produced by all ions within the system. This idea represents the generalization of the Poisson-Boltzmann principle to nonequilibrium systems (Balescu, 1975). This approximation neglects the fact that the concentrations of co- and counterions around a cation at **r** differs from the mean concentrations at **r** as well as from the concentrations around an anion at **r**. Consequently, the local electric field at a specific ion generally differs from the mean electric field at that position. For homogeneous electrolytes the conductivity generally increases sublinearly with increasing ion concentrations. Onsager (1926, 1927) explained this effect by a deformation of the counterion cloud due to the external electric field. The MSA theory (Blum, 1980) accurately describes the conductivities for homogeneous electrolytes up to high concentrations (Bernard et al., 1992; Durand-Vidal et al., 1996).

For inhomogeneous systems, not only does the deformation of the co- and counterion cloud become important, but the interaction of the ion with its own counterion cloud does as well. This "solvation energy" due to the other ions depends on the environment, e.g., the ionic strength, and leads to activity factors differing from unity. When the ion leaves bulk solution to enter the channel, this "self-energy" of the ion changes and leads to different activities in bulk solution and inside the channel. Usually, this effect is neglected in commonly used mean-field theories describing ion permeation. Nonner (Nonner et al., 1998; Nonner and Eisenberg, 1998) introduces chemical potentials into PNP

theory. Levitt (Levitt, 1987) considered two ions within a single channel; however, he neglected the screening of the interaction of those two ions inside the channel due to all other ions in the system. Lehmani (Lehmani et al., 1997) includes the effect of the ion cloud deformation for the diffusion of ions through pores with a diameter large compared to the Debye length.

Another important approximation in most mean-field theories is that the ions can pass each other without interference. This is certainly a good approximation for highly dilute solutions. Hydrodynamic effects in homogeneous electrolytes were introduced some time ago (Fuoss and Onsager, 1964). This generally leads to diffusion constants being functions of the ion concentrations (Hubbard, 1987). The flux coupling between different ions and ions and the solvent was described for ion exchange membranes (Conti and Eisenman, 1966).

There are several articles showing that the electric field within ion channels often is not constant (Eisenberg, 1996; MacGillivray and Hare, 1969); we do not know any article in the field of ion permeation that studies the constant currents assumption. Generally the concentration of ion pairs in water and probably in the channel environment is small (Bockris and Reddy, 1970; Buck, 1984). Consequently, ion pairing is neglected in theories of ion permeation. This argument is based on equilibrium considerations. It relies on the small value of the association equilibrium constant determining the relative concentrations of free ions and ion pairs. As shown in this work, the influence of ion pairing on ion transport is determined by the ion association rate constant k_A and not by the equilibrium constant. According to modern diffusion-controlled reaction rate theories (Calef and Deutch, 1983) and respective Brownian dynamics studies (Sridharan et al., 1989), the association rate constant of oppositely charged ions is not small. Consequently, the constant currents assumption often does not hold, and ion pairing has to be included in theories of ion permeation.

Because the constant field and currents assumptions are widely used in theories of ion transport, it is important to understand the range of their validity. In the present work, we derive analytical constant field and currents conditions. In the Results, we apply these relations to simple practical examples. Finally, we discuss the limitations of those relations and the extent to which our conclusions suffer from the neglect of the ion-cloud self-energy and single filing.

THEORETICAL METHODS

The constant field and constant currents assumptions are widely used in theories of ion permeation. Here we derive the necessary conditions for the applicability of those two assumptions. We further analyze the fundamental suppositions for barrier models and present relations indicating their validity.

Constant field assumption

In general the constant field assumption implies that the electric field originating from permeant ions $-d\phi_{ion}/dx$ is either negligible or only weakly voltage dependent. We show that this is often not the case because electrostatic interactions are strong and occur over a long range. Within this work we particularly analyze the GHK theory and barrier models. PNPC theories with negligible ion pairing have been extensively studied by others (Eisenberg, 1996).

GHK constant field condition

In the GHK theory, the total electric field E is assumed to be constant across the channel and is determined exclusively by the external voltage difference V: $E = V/L$. Thus the electric field created by permeant ions and fixed charges inside the channel must be small compared to this external field. We start our analysis of this constant field approximation with the one-dimensional, differential form of Gauss' law (Jackson, 1962):

$$\frac{dE}{dx} = \frac{\rho(x)}{\epsilon_0 \epsilon_C} \tag{3}$$

where $\rho(x)$ is the charge density, ϵ_0 is the vacuum permittivity, and ϵ_C is the dielectric constant inside the channel. The electric field created by the charge density inside the channel is obtained by integrating Gauss' law (Eq. 3) along the channel's axis:

$$E_o - E_i = \int_{x_i}^{x_o} \frac{\rho(x)}{\epsilon_0 \epsilon_C} dx = \frac{L\langle\rho\rangle}{\epsilon_0 \epsilon_C} \quad \text{with} \quad \langle\rho\rangle = \frac{1}{L}\int_{x_i}^{x_o} \rho(x)dx \tag{4}$$

where $\langle\rho\rangle$ is the average charge density. E_o and E_i are the electric fields at the external and internal entries of the channel at x_o and x_i, respectively, with $L = x_o - x_i$. As long as the field difference is small compared to the external field $|V|/L \gg |E_o - E_i|$, the constant field assumption applies:

$$|V| \gg |U_\rho| \quad \text{with} \quad U_\rho = \frac{L^2\langle\rho\rangle}{\epsilon_0 \epsilon_C} \tag{5}$$

where the space-charge voltage U_ρ indicates nonzero space-charge inside the channel. At sufficiently large external voltages, according to Eq. 5, the electric field becomes practically constant, and the GHK theory applies.

We replace the external voltage V in Eq. 5 with the thermal voltage $RT/F \approx 25$ mV and define a space-charge length ℓ_ρ, which has a form similar to that of the Debye length ℓ_{DH}. To apply the constant field approximation, ℓ_ρ must be large compared to the channel length:

$$L \ll \ell_\rho = \sqrt{\frac{RT\epsilon_0\epsilon_C}{F|\langle\rho\rangle|}} \tag{6}$$

Ion channels, for which Eq. 6 holds, are designated as *short*. In general, the space-charge voltage and length are not

directly accessible by experiment, because they depend on the charge density inside the channel. If one starts to design channel models to explain one's data, however, relations 5 and 6 immediately indicate important physical properties of those channel models and in this way guide the process of model building.

PNPC constant field condition

In PNPC theories, no restrictions are assumed for the charge density inside the channel. The specific chemical forces are either neglected (Eisenberg, 1996) or are assumed not to dominate electrostatic interactions (Chen, 1997; Nonner et al., 1998). If the channel contains permanent charges, the strong electrostatic interaction attracts a corresponding number of counter ions. In this sense, Eq. 6 also applies here because the space-charge length equals or is above the Debye length: $\ell_\rho \geq \ell_{DH}$. The Debye length determines the "flexibility" of the distribution of permeant ions. Thus, if ℓ_{DH} is long compared to the channel length $L \ll \ell_{DH}$, the permeant ion distribution cannot follow the external field, resulting in a constant field. If, however, the channel is long ($L > \ell_{DH}$), the permeant ion distribution adjusts to the external field and consequently becomes voltage dependent. In this case the constant field approximation generally does not apply. In a numerical PNP model of a synthetic ion channel it was explicitly shown (Chen et al., 1997, Fig. 11) that the constant field assumption does not work in this particular case.

Barrier model constant field condition

In this section, we study fundamental suppositions of barrier models. Because of the strong electrostatic interactions among permeant ions, the short-range forces $d\mu_c^s/dx$ must be rather large to dominate the electric forces. If, however, the chemical forces are not dominant, then the basic barrier model assumptions are violated and ion transport must be described by a more general theory.

In barrier models ions are located at specific sites inside the channel. Ions bound to charged amino acid side chains possess a considerable flexibility with respect to their position and orientation (von Kitzing, 1995). If the local electric field E_A created by those dipoles is large compared to the external field, only minor adjustments of the mean orientation of the dipoles will compensate the external field. As a result, the electric potential ϕ_{ion} becomes voltage dependent. This leads to a constant field condition for barrier models in which the resident permeant ions are not tightly fixed:

$$|V| > L|E_A| = \frac{Le_0}{\epsilon_0\epsilon_C S} \qquad (7)$$

The mean orientation and position of bound ions depend on the degree of flexibility, thermal motion, and the external voltage. Thus the electric field of the bound permeant ions becomes voltage dependent; it changes in the same order as the external field, and consequently, the constant field approximation is violated.

At high external concentrations ions frequently attempt to enter an occupied site coming either from a neighboring site or from the external solutions. Let us consider a cation channel with an ion trying to enter an occupied site. If the cation-cation interaction is dominated electrostatically, and the short-range chemical forces keeping the resident ion in place are strong compared to the electrostatic repulsion, the resident ion prevents the incoming ion from entering or passing the site. This mechanism defines one of the basic characteristics of barrier models. Alternatively, if the short-range chemical forces are weak compared to the electrostatic forces, there are two possible outcomes. In the first case, the resident ion may repel the incoming ion, which would also be a "barrier model solution" of this competition. In the second case, the ion, coming from one direction, may push the resident ion in the other direction. This kind of outcome of the competition is not considered in barrier models. The omission of this mechanism is justified, if the height of the barrier $\Delta\mu_c^s$ is large compared to the repulsive energy $FV_\mu/(RT)$ originating from electric interaction of two ions at a typical distance of δ_μ. According to Eq. 5, this potential difference is given by

$$\frac{RT\Delta\mu_c^s}{F} \gg V_\mu = \frac{\delta_\mu e_0}{\epsilon_0\epsilon_C S} \qquad (8)$$

If this condition is violated, the assumption that a resident ion prevents other ions from entering or passing the site is no longer valid.

The frequent mutual electric interactions among incoming and resident ions at high external concentrations lead to a concentration- and voltage-dependent excess chemical potential. The strength of the electrostatic interaction is characterized by the Debye length ℓ_{DH}. If the gradient of the short-range interaction $d\mu_c^s/dx$ (μ_c is given in multiples of RT) is large compared to the inverse Debye length, then the channel is dominated by short-range interactions, and the excess chemical potential $\mu_c(x)$ becomes concentration independent:

$$\ell_{DH}\frac{d\mu_c^s}{dx} \gg 1 \Leftrightarrow \Delta\mu_c^s \gg \frac{\delta_\mu}{\ell_{DH}} \qquad (9)$$

Constant currents assumption

The constant currents assumption is widely used in theories of ion transport through ion exchange membranes (Buck, 1984) and ion channels (Barcilon et al., 1997; Cooper et al., 1985; Eisenberg, 1996; Hille, 1992), where the Nernst-Planck and often Gauss' or Poisson's law are solved, assuming that the flux of each ionic species is constant. The constant currents assumption is also used in barrier models. In contrast to the constant field condition, for the constant

currents assumption the particular theories need not be addressed specifically.

The constant currents assumption has not yet been tested rigorously for any of those cases. It is therefore important to derive constant currents conditions that indicate precisely whether the constant currents assumption holds in a particular case. As shown in this section, modern theories of ion association rate constants (Calef and Deutch, 1983) predict that the dynamic effect of ion pairing often cannot be neglected, even if the concentration of ion pairs is negligible. The question of ion pairing adds to the problems of neglecting the effects of ion clouds and single filing.

Importance of ion pairing

In steady-state systems, ion pairing implies a change in the ion fluxes and the respective electric currents I along the channel axis. This effect is described by the ion pairing intensity $\mathcal{R}(x)$. For a binary monovalent electrolyte, ion pairing leads to the continuity equation:

$$\frac{dI_n}{dx} = -\frac{dI_p}{dx} = FS\mathcal{R}(x) \qquad (10)$$

which is given in its one-dimensional form. I_p and I_n are the cation and anion electric currents, respectively. Of course, the total charge is always conserved. A typical ion pairing intensity $\mathcal{R}$ for a binary monovalent electrolyte has the form of a simple reaction equation:

$$\mathcal{R} = k_A pn - k_D c_P = k_A(pn - p_Q n_Q) \qquad (11)$$

where k_A and k_D are the respective association and dissociation rate constants, p and n are the cation and anion concentrations, and c_P is the concentration of pairs. At equilibrium ion pairing must disappear. Thus we define the equilibrium concentrations p_Q and n_Q. With $k_D c_P = k_A p_Q n_Q$, the pair concentration c_P and the dissociation rate constant k_D are replaced with p_Q and n_Q. These equilibrium concentrations are defined by the difference in the chemical potential between bulk solution and the channel environment. This replacement conveniently simplifies the calculus.

Analogously to the constant field condition, we integrate the continuity equation (Eq. 10), considering only the currents on both sides of the channel:

$$I_{no} - I_{ni} = I_{pi} - I_{po} = FSL\langle\mathcal{R}\rangle = FSLk_A(\langle pn\rangle - \langle p_Q n_Q\rangle)$$

$$\text{with } \langle\mathcal{R}\rangle = \frac{1}{L}\int_{x_i}^{x_o} \mathcal{R}(x)dx \quad \text{and} \quad \langle pn\rangle = \frac{1}{L}\int_{x_i}^{x_o} p(x)n(x)dx$$

$$(12)$$

where I_{no} and I_{ni} are the anion currents at the external and internal channel mouths, and I_{po} and I_{pi} are the respective cation currents.

The form of Eqs. 11 and 12 indicates that the influence of ion pairing depends on the association rate constant k_A and not on the association equilibrium constant k_A/k_D. It is

therefore important to obtain an estimate for k_A. According to diffusion-controlled reaction rate theory (Calef and Deutch, 1983), the association rate constant, in good agreement with respective Brownian dynamics simulation (Sridharan et al., 1989), has the form

$$k_A = \frac{F^2}{RT}\frac{D_p + D_n}{\epsilon_0\epsilon_C}\frac{r_A}{\ell_{Bj}} \quad \text{with } \frac{\ell_C}{r_A} = \int_1^\infty \frac{e^{-\varphi(\lambda)}}{\lambda^2}d\lambda$$

$$(13)$$

$$\text{and} \quad \ell_{Bj} = \frac{F^2}{4\pi\epsilon_0\epsilon_C N_A RT}$$

where D_p and D_n are the cationic and anionic diffusion constants, and r_A is the effective association radius. N_A is Avogadro's number, $\lambda = r/\ell_C$ is the actual distance between the two ions scaled by the distance of closest approach ℓ_C, and $\varphi(\lambda)$ is the Debye-Hückel potential between the two ions divided by RT. ℓ_{Bj} is the Bjerrum distance of the two ions, i.e., the distance where the interaction energy between the two ions equals thermal energy RT. Even if Eq. 13 is only a crude approximation of the association rate inside the channel, it should predict the correct order of magnitude.

The integral containing the ion-ion interaction term can be solved analytically for two limiting situations:

$$\frac{1}{r_A} \approx \begin{cases} 1/\ell_C \\ \quad \text{for strong screening} \quad \ell_{Bj} \ll \ell_C\left[1 + \dfrac{\ell_C}{\ell_{DH}}\right] \\ \dfrac{1}{\ell_{Bj}} + \dfrac{1}{\ell_C + \ell_{DH}} \\ \quad \text{for weak screening} \quad \ell_{Bj} \gg \ell_C\left[1 + \dfrac{\ell_C}{\ell_{DH}}\right] \end{cases}$$

$$(14)$$

The classic association rate constant proposed by Smoluchowski (1917) with $r_A \approx \ell_C$ is obtained in the limit of strong screening. If the charges are only weakly screened, the effective association radius becomes the Bjerrum length, $r_A \approx \ell_{Bj}$.

Constant currents condition

Now we are in a position to formulate a constant currents condition. The current-voltage curves for many ion channels are often close to linear. One can therefore use a typical channel conductance Σ to approximate these parts of the current-voltage relation: $I \approx \Sigma V$ for $|V| \gg RT/F$. If the effect of ion pairing is small, the difference in the ion currents at both ends of the channel should be small compared to the total current: $|I_{no} - I_{ni}| \ll |I|$. The difference in currents is taken from the integrated form of the continuity equation (Eq. 12) with the association rate constant k_A from Eq. 13. This leads to the global constant currents condition:

$$|V| \gg |U_{\mathcal{R}}|$$

$$(15)$$

$$\text{with } U_{\mathcal{R}} = \frac{F^3 LS}{\epsilon_0\epsilon_C RT}\frac{D_p + D_n}{\Sigma}\frac{r_A}{\ell_{Bj}}|\langle pn\rangle - \langle p_Q n_Q\rangle|$$

This general constant currents condition has a form similar to that of the general constant field condition in Eq. 5. The pairing voltage $U_{\mathscr{R}}$ defines a lower limit for the external voltage, below which the constant currents condition is violated.

Similar to the space-charge length ℓ_ρ defined in Eq. 6, we use Eq. 15 to define a pairing length $\ell_{\mathscr{R}}$. At low external voltages $|V| \lesssim RT/F$, the current for each ion species is constant if the channel is short compared to the pairing length:

$$L \ll \ell_{\mathscr{R}} = \frac{\epsilon_0 \epsilon_C}{F^2 S(D_p + D_n)} \frac{\ell_{Bj}}{r_A} \frac{\Sigma}{|\langle pn \rangle - \langle p_Q n_Q \rangle|} \quad (16)$$

RESULTS

In the analytical part of this article, we derive constant field conditions for the GHK approximation and barrier models. We present constant currents conditions that are valid for all theories. We now apply these rules to some simple, realistic situations. We find that the constant field conditions equations (Eqs. 5–7) in particular are difficult to fulfill and that the basic conditions fundamental for barrier models are often violated.

The validity of the constant field assumption

We formulate constant field conditions for the GHK approximation and barrier models given in Eqs. 5–7. For the latter, conditions for the volume exclusion mechanism and the independence of the energy profile from the external concentrations are also given. To study the practical implications of these conditions, a simplified version of an ion channel is considered. It is assumed to be cylindrical with a length of $L = 1$ nm, a cross section of $S = 1$ nm^2, corresponding to a radius of 0.56 nm, and an internal dielectric constant of $\epsilon_C = 10$. With these numbers the electric field of a single charge becomes $E_s = 1.81$ V/nm = 18.1 MV/cm. With a dielectric constant of 80, this field is still 226 mV/nm = 2.26 MV/cm.

GHK approximations

Equation 5 defines the space-charge voltage U_ρ at which the electric field generated by the internal charge becomes comparable to the external electric field. If this channel contains a single monovalent ion, the space-charge voltage U_ρ as a lower limit for the external voltage becomes

$$|V| \gg |U_\rho| \approx 1.8 \text{ V}$$

Thus, only for external voltages considerably above 1.8 V does the constant field assumption apply for a singly occupied GHK channel. Alternatively, Eq. 6 defines a space-charge length ℓ_ρ at which the external and internal electric fields become comparable. The space-charge length ℓ_ρ of

the studied channel model as an upper limit for the channel length L amounts to

$$L \ll \ell_\rho \approx 1.4 \times 10^{-2} \text{ nm} \quad (17)$$

This length is certainly too short to be relevant for realistic biological ion channels. In an electrically neutral channel, the occupation of the GHK channel should not exceed 1.4×10^{-3}, which corresponds to a concentration of 2.3 mM for the channel parameter given above. Even if the dielectric constant is increased to 80, the space-charge voltage would become 225 mV and the space-charge length 0.1 nm. Thus the GHK approximation implies rather unrealistic assumptions about ion channels.

This extremely short space-charge length ℓ_ρ implies that the constant field assumption does not apply for concentrations above 5 mM and for low energy barriers. If the volume contains a single permanent charge, the space-charge length acts as the Debye length ℓ_{DH}. Of course, such a Debye length is much too short for the Debye-Hückel theory to be relevant. MSA electrolyte theory (Blum, 1980; Klement et al., 1990), however, which is valid up to much higher concentrations, includes the effect of the ion size. For two equal-sized ions with a distance of closest approach of 0.3 nm, the respective MSA lengths gives $\ell_{MSA} = 0.05$ nm or 0.2 nm, using a dielectric constant of 10 or 80, respectively. Thus the constant field assumption becomes invalid as long as the forces inside the channel are not dominated by short-range interactions and $\ell_{DH} \leq \ell_{MSA} \lesssim L$.

Barrier models

One central approximation in barrier models is the constant field assumption. As shown in Eq. 7, this assumption requires tightly fixed permeant ions. Flexible positions and orientations of bound permeant ions lead to a voltage dependence of the distribution of permeant ions inside the channel, resulting in a breakdown of the constant field assumption. The orientational constant field condition becomes

$$|V| > 1.8 \text{ V}$$

Thus the orientation of the resident ion becomes saturated only by voltages above 1.8 V. Very similar results are obtained if one considers a harmonic motion of the resident ion. Interestingly, this result agrees with the constant field condition for a GHK channel.

Another basic assumption of barrier models is that a resident ion at a site prevents incoming ions from entering or passing a site, neglecting concerted ion motions. A test for this assumption is given in Eq. 8. If the channel has two binding sites and a single barrier (or two barriers and a single site), the typical distance between well and barrier peak in our channel model is $\delta_\mu = 0.25$ nm. Thus according to Eq. 8, the barrier height must exceed

$$|\Delta\mu_c^s| \gg 17$$

that concerted motions of ions can be neglected. Even if a dielectric constant of 80 is assumed, the barriers still must be rather large $|\Delta\mu_c^s| \gg 2$ to prevent a second ion from entering an occupied site.

Finally, we analyze the concentration dependence of the energy profile. According to Eq. 9 and a MSA-length of $\ell_{MSA} = 0.05$ nm, the concentration independence of the energy profile implies

$$|\Delta\mu_c^s| \gg 5$$

Thus, also for barrier models, the constant field condition Eq. 7 is extremely difficult to fulfill. But the single occupancy assumption (Eq. 8), as well as the neglect of the concentration dependence of the energy profile (Eq. 9), imposes severe restrictions on the applicability of barrier models.

The validity of the constant currents assumption

What is the order of the effect of ion pairing in a simple model? The precise number of the pairing voltage $U_\mathcal{R}$ depends on the particular system parameters. The particular example given below represents a worst-case study. This means we choose parameters that imply large pairing voltages. The anionic diffusion coefficient D_n in Eq. 15 is here assumed to be small compared to that of the cations D_p. With $\Sigma \approx F^2 S D_p p/(LRT)$, Eq. 15 for the pairing voltage $U_\mathcal{R}$ simplifies to

$$U_\mathcal{R} \lesssim \frac{FL^2}{\epsilon_0\epsilon_C} \frac{r_A}{\ell_{Bj}} \frac{|\langle pn \rangle - \langle p_Q n_Q \rangle|}{p} \lesssim \frac{FL^2 p}{\epsilon_0\epsilon_C} \frac{r_A}{\ell_{Bj}}$$

The approximation $|\langle pn \rangle - \langle p_Q n_Q \rangle|/p \approx p$ used in this equation is not justified in general. Here this crude approximation may suffice as a worst-case consideration. For an effective ion radius of 0.5 nm (Ebeling and Scherwinski, 1983; Soumpasis, 1984), including hydration, an ionic concentration of 100 mM and a dielectric constant of 10, the effective association radius, and the Bjerrum length are given by $r_A = 1.1$ nm and $\ell_{Bj} = 5.7$ nm. In this simple approximation the pairing voltage $U_\mathcal{R}$ distinguishes from the space-charge voltage U_ρ mainly by the factor r_A/ℓ_{Bj}. For the given case the upper bound for the pairing voltage and the lower bound for the pairing length approximately yield

$$U_\mathcal{R} \lesssim 21 \text{ mV} \quad \text{and} \quad \ell_\mathcal{R} \gtrsim 1.2 \text{ nm}$$

Obviously, the implications of the constant currents condition in Eq. 15 are less severe than the constant field condition in Eq. 5. Because we made a worst-case analysis, for most channels the pairing voltage is even smaller and the pairing length larger. Nevertheless, for a typical channel length of 1 nm, direct evidence is needed to discount ion pairing.

DISCUSSION

In this work, we present the analytical relations in Eqs. 5, 6, 15, and 16, which estimate the (in)validity of the constant

field and currents approximations. In addition, we analyzed conditions Eqs. 7–9 for the basic assumptions used in barrier models. Because GHK theory and barrier models are frequently used to analyze experimental current-voltage curves, reversal potentials, or blocker positions, it is important to have simple relations to test their validity for practical models.

In this work, we assume a cylindrical channel geometry of a certain length L and cross section S. Such a model is certainly only a caricature of a real ion channel. But the necessary degree of "realism" depends on the kind of questions the model is assumed to answer. Because the electrostatic interaction occurs over a long range, many structural details average out if one considers mean electric fields within channels. Consequently, the cylindrical model of the channel with a single dielectric constant ϵ_C is certainly sufficient for a first guess of the validity of the constant field and currents assumptions. Recently, Nonner and Eisenberg (1998) have shown that homogeneous channel parameters are sufficient to explain the competition between calcium and sodium in the L-type calcium channel. Thus complex forms of selectivity do not always require heterogeneous channel environments. And if any of the conditions 5–9, 15, or 16 is considerably violated for this simple cylindrical model, one should expect the violation of the respective condition in more sophisticated channel models.

Discussing the description of the L-type calcium channel by means of the PNP approximation (Nonner and Eisenberg, 1998), Horn (1998) asks whether the PNP picture of this channel "could produce another set of erroneous insights." A necessary step in avoiding "erroneous insights" is to check the validity of an ion transport model. Fitting a model to a reasonably large set of experimental data results in a set of model parameters. If this model definitely fits the data, one has to check whether fundamental assumptions made to derive this particular ion transport model from first principles are compatible with the found set of parameters. For instance, if the experiments are well reproduced by a barrier model with low barrier heights and high occupancies, such a result must be discarded. As shown in the Results, low barrier heights together with high occupancies leads to a violation of all of the basic barrier model assumptions. In presenting conditions to test the constant field and currents assumptions, this work helps to reduce the likelihood of such "erroneous insights."

The constant field and barrier assumptions

First we analyze the values and validity of the model parameters involved in Eqs. 5–9. Later we discuss those results, using the examples of the acetylcholine receptor channel and the potassium channel.

Ion channel parameters

The application of the constant field condition (Eqs. 5 or 6) and the basic assumption used to establish barrier models

(Eqs. 7–9) require estimates of the channel length L, the channel's cross section S, the dielectric constant ϵ_C, and the influence of the channel vestibules. For the recently resolved potassium channel (Doyle et al., 1998), the length of the narrow pore is given by $L = 1.2$ nm. In the case of the acetylcholine receptor channel (Unwin, 1995), the channel appears to have no "natural boundaries" to define its length precisely. A value of $L = 1$ nm is certainly the correct order of magnitude. In the narrow part the cross section S of the potassium channel is rather small, and the ions are directly solvated by the backbone oxygens. According to classical estimates using organic ions of different sizes (Adams et al., 1980; Hille, 1992), the cross section of the acetylcholine receptor channel is wider. Because the electric field may not be confined only to very narrow pores, the effective "electrostatic" cross section may be wider than the geometric one, and we choose $S \approx 1$ nm^2. Smaller cross sections would make the application of constant field and barrier assumptions even less likely, as presented in the Results.

For the simple channel models such as those used in this article, the dielectric constant ϵ_C can represent only an effective screening constant. In realistic channels, one should expect that the orientation of water molecules and protein side chains results in a complex relation between the three-dimensional electric displacement **D** and the respective electric field **E** (Jackson, 1962; Jordan et al., 1997; Kornyshev, 1985; Kornyshev and Sutmann, 1996; Partenskii et al., 1994). If such an effect would determine ion transport, the constant field assumption would break down, even for empty neutral channels. Molecular dynamic simulations generally indicate a reduced orientational mobility of water molecules inside a channel (Sansom et al., 1997). This would suggest an effective screening parameter below the dielectric constant of bulk water. Jordan (Jordan et al., 1997) proposes a dielectric screening parameter of 4 for the gramicidin channel.

In deriving the constant field and currents conditions, we assume that the external voltage drops completely across the channel. Particularly at low external concentrations, a part of the voltage drops within the channel mouths. This situation reduces the electric field inside the channel, making the conditions defined in Eqs. 5 and 15 even more stringent. Under most "physiological" conditions, however, this effect should not dominate (Muller and Finkelstein, 1972).

Validity of model assumptions

As shown in the Results, the trivial constant field assumption (Eq. 5) applies only at extremely low ion concentrations inside the channel. But this result is based on the mean field approximation, in which the local electric field is replaced with the mean electric field. As long as only a single ion resides inside the channel and all other ions are too distant to interact with this ion, its presence would not lead to nonlinearities in the external electric field. But to avoid any ion-ion interactions, the lifetime of the ion inside the channel must be short, otherwise the co- and counterions in the channel mouths would reorganize to shield this extra charge within the channel, or another ion from bulk solution would try to enter the channel and interact with the resident ion electrostatically. This interaction between incoming and resident ions automatically leads to a violation of the constant field assumption. Because short residence times are equivalent to low concentrations, we obtain the same qualitative result as in Eq. 5.

A quantitative analysis of this kind would decrease the stringency of Eq. 5, but not its major implications. The Vlasov approximation could become critical only at low internal concentrations with $L \lesssim \ell_\rho$. In the crystal structure of the potassium channel (Doyle et al., 1998), two partly occupied sites for ions were found inside the narrow part of the channel. For the acetylcholine receptor channel, the number of potentially charged amino acids is large (Imoto et al., 1988; Konno et al., 1991). These facts suggest a potentially very large ionic strength inside this channel and, as a result, rather short space-charge or Debye lengths. Consequently, GHK theory does not apply to those channels.

Because of the low resolution of the crystal structure of the potassium channel (Doyle et al., 1998), we cannot estimate the flexibility of the positions of the two potassium ions inside the narrow pore. The strong mutual interaction between ions in the two sites makes it unlikely that their occupation remains voltage independent, and thus the generalized constant field assumption does not hold. Because both sites appear to be considerably occupied, one must expect frequent "attempts" of one ion to enter the site of the other one. If both ions are not tightly bound to their sites, these "jumps" would result in a concerted motion of those ions, explicitly excluded in the barrier model. For the development of Eq. 8, the Vlasov approximation is not used. Therefore, it does not suffer from the problem of applying mean fields. On the contrary, the screening from all of the other ions within the system is neglected for this relation. In the crystal structure there is a line of three ions with an approximate distance of 0.8 nm. If the electrostatic interaction would be heavily screened by permeant counterions or by flexible side chains, the right-hand side of Eq. 8 would become smaller, but of course, the necessarily short Debye lengths (only very short local Debye lengths could shield those ions from each other) would imply a concentration-dependent barrier height. Thus the basic assumptions used in barrier models are very likely not fulfilled for this channel. But simple PNP theory also has problems, because it generally predicts an Ussing ratio of unity. Particular terms have to be introduced to obtain Ussing ratios differing from unity (Chen and Eisenberg, 1993). The flux coupling terms proposed by Conti (Conti and Eisenman, 1966) could account for this effect.

In the acetylcholine receptor channel, the flexible potentially charged side chains close to the narrow part of the pore reorient in the external field, resulting in a violation of the constant field assumption. Because the charge of those side chains must be screened by protons or permeant ions, the strong charge-charge interactions imply a correlated

motion (von Kitzing, 1995). Because of the larger pore compared to the potassium channel, the energy profile very likely is concentration dependent. Also here, the Vlasov approximation influences the quantitative results, but not the qualitative outcome. For a first estimate the unmodified form of Eqs. 7–9 is certainly sufficient. Only in questionable cases, for which those relations are only slightly violated, a more careful analysis becomes necessary. At least for those two channels, we should expect a violation of basic assumptions for GHK theory and barrier models. For both channels flux coupling between ions and solvent would be necessary to account for the streaming potential.

Vlasov's mean field approximation

The strong, long-range electrostatic interaction creates considerable difficulties for mean field theories. Even the most accurate equilibrium electrolyte theories contain certain approximations (McQuarrie, 1976). Cooper (Cooper et al., 1985) addressed the problem of the mean field approximation by comparing mean field results with Brownian dynamics simulations. In that study an uncharged channel was considered, and only cations were allowed to enter that channel. Comparing PNP and Brownian dynamics (BD), they found considerable differences. The PNP concentrations were much lower, indicating a stronger repulsion between the ions in the mean field theory.

Unfortunately, the electrostatic interactions in the PNP and the BD channels were not equivalent. On the one hand, a PNP theory with one-dimensional electrostatics was used, i.e., the electric field of a single ion was assumed to focus within the pore of the channel. On the other hand, the electrostatic interactions in the BD simulations consisted of three-dimensional Coulomb forces. Because one-dimensional electrostatic repulsion at long distances is stronger than the three-dimensional repulsion, qualitatively the difference found by Cooper should have been expected. Therefore, at least a part of the differences in concentrations should be attributed to the use of different electrostatic interactions between permeant ions.

Another important aspect of that study is that the neglect of the coion cloud should be particularly strong for a neutral channel that only cations may enter. In contrast, most important biological ion channels contain permanent charges (Imoto et al., 1988; Konno et al., 1991). In such cases, the neglect of the counterion cloud can influence the ion concentrations to a lesser degree, because the neutralization of these charges inside the channel dominates the behavior of the ions. In addition, the electric field of the permanent charges creates a "high field" situation for which the mean field theory becomes nearly exact (Henderson et al., 1979).

Consequently, we should consider Vlasov's mean field approximation as a useful tool for analyzing ion permeation. Its inherent limitations for inhomogeneous nonequilibrium systems still remain to be analyzed. The recent increased interest in formulating improved theories for such systems (Bernard et al., 1992; Durand-Vidal et al., 1996; Lehmani et

al., 1997; Turq et al., 1992) may rapidly improve our understanding in this field. A preliminary way to account for the neglected activity factors is to include different chemical potentials for different ions (Nonner et al., 1998; Nonner and Eisenberg, 1998; see also below).

Ion pairing in biological ion channels

The validity of the constant field approximation has been questioned in earlier publications. MacGillivray (MacGillivray and Hare, 1969) provides a first-order correction for GHK theory assuming a small electric field. Eisenberg (1996) discusses the impact of the electric field in the framework of PNP theory. We are not aware, however, of studies on the validity of the constant currents assumption in the field of biological ion channels.

The importance of ion pairing

The constant currents assumption states that ions are neither destroyed nor created during ion transport. For steady state this assumption means that the number of ions that enter a certain volume element must equal the number of ions leaving this volume element; otherwise the number of ions inside the volume would change within time. As a consequence, the flow of each ion species must be the same through the channel cross section at any position inside the channel. The situation changes if ions form pairs. Now two ions of opposite charge may enter a volume element, form a pair, and leave it as a pair. The number of free ions has decreased and the number of pairs increased in the considered volume. Although the number of free ions inside a volume element may change in cases of ion pairing, the total charge does not change, and the total current is conserved.

In water and similar media with high dielectric constants ϵ_C, the concentration of ion pairs is extremely low (Bockris and Reddy, 1970). Consequently, the effect of ion pairing on ion transport is generally assumed to be small in those media (Buck, 1984). If, however, ions pass a boundary where their excess chemical potential changes, close to the boundary the balance between association or dissociation does no longer hold, leading to $dI_p/dx \equiv -dI_n/dx \neq 0$. As shown in Eq. 12, this dynamic effect, in contrast to the situation in homogeneous media, depends on the association rate constant k_A. Thus, even if the association equilibrium constant is extremely small, ion pairing may become crucial for the ion dynamics in heterogeneous nonequilibrium situations for sufficiently large association rate constants k_A. According to modern theories of diffusion-controlled reaction rates (Calef and Deutch, 1983), the association rate constant of oppositely charged ions is not small.

Fig. 1 gives a simple model of how substantial ion pairing may determine ion transport. For instance, the acetylcholine receptor channel contains a considerable number of potentially charged side chains close to the narrow constriction of the pore (Konno et al., 1991; Villarroel and Sakmann,
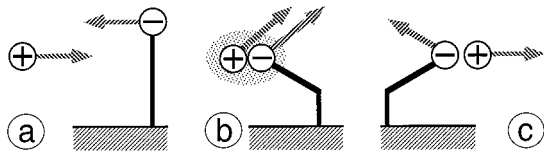
FIGURE 1 Binding to flexible charged side chains may play an important role in ion transport, for instance, in the acetylcholine receptor channel. The electric field is assumed to drag a cation from the left to the right side. (*a*) The incoming cation attracts a flexible anionic side chain. (*b*) The cation binds to the side chain, and together they diffuse a short length. (*c*) The cation is released and travels to the right-hand side, and the cationic side chain follows the electric field and moves toward the left-hand side. Because pairing occurs predominantly on the left-hand side and release on the right-hand side, on average pairs diffuse from the left to the right.

1992). A molecular mechanics model study (von Kitzing, 1995) indicates that the side chains are sufficiently flexible to migrate a short length together with cations in the constriction of the channel.

Ion channel parameters and pairing

To apply the constant currents conditions (Eq. 15 or 16), we have to know the parameters required for the constant field condition (see above). But furthermore, we need the channel conductance Σ, the ion diffusion coefficients D_p and D_n, the effective association radius r_A, the Bjerrum length ℓ_{Bj}, and the ion concentrations inside the channel p and n to calculate $\langle pn \rangle$, and the equilibrium concentrations p_Q and n_Q. How can we get reasonable estimates for all of these parameters?

Because the Bjerrum length is only a function of the effective dielectric constant, it will be very similar in most ion channels: $\ell_{Bj} \approx 5$ nm if the dielectric constant stays around 10. Except for ion concentrations as low as 0.1 mM, the Debye length ℓ_{DH} generally is much smaller than this Bjerrum radius. Thus, inside the channel we have the strongly screened case, and the effective association radius r_A becomes the distance of closest approach ℓ_C between the ion pair. Estimates of the distance of closest approach ℓ_C for hydrated alkali-halide ions from fitting thermodynamic properties of these electrolytes using the MSA theory (Blum, 1980) are in the range of 0.3–0.8 nm (Ebeling and Scherwinski, 1983; Soumpasis, 1984; Triolo et al., 1976). The radii proposed by Soumpasis (1984) for hydrated alkali metal ions are in good agreement with the radii obtained for the acetylcholine receptor channel projecting their permeability ratio on the curve obtained from organic cations where the hydration shell is assumed to be negligible (Adams et al., 1980, Fig. 4). Thus the order of magnitude of the quotient becomes $r_A/\ell_{Bj} \approx 1/10$ for hydrated ions and $r_A/\ell_{Bj} \approx 1/50$ for bare ions.

Because the channel conductance Σ can be directly obtained from measured current-voltage relations, this quantity is easily accessible for experimental studies. For theoretical model studies, the channel's conductance can be approximated by the relation of $\Sigma \approx F^2 S(D_p p_E + D_n n_E)/$

(*LRT*), were p_E and n_E are the cation and anion concentrations close to the channel constriction. The estimate of the diffusion coefficients D_p and D_n is certainly difficult. From molecular dynamic simulations (Roux and Karplus, 1994) and from experimental studies (Macias and Starzak, 1993, 1994) an ionic diffusion coefficient inside the gramicidin channel is obtained, which is below the diffusion coefficient of the respective ions in bulk solution by a factor of 10. In cases, where the cation diffusion coefficient D_p dominates the other, this problem disappears, because in this case $F^2 S(D_p + D_n)/(RT\Sigma) \approx L/p_E$, but the cation concentration p_E close to the channel constriction still remains to be estimated.

The influence of ion pairing is strongest where the actual concentrations p and n deviate considerably from the respective equilibrium concentrations. Thus one should expect the influence of ion pairing, when the product of the internal equilibrium ion concentrations $p_Q n_Q$ differs considerably from that of the solution $p_S n_S$. If this product remains constant, ion pairing is negligible. Thus ion pairing requires a considerable difference in the chemical potentials μ_p and μ_n of the ions between bulk solution and the channel interior. Such excess chemical potential difference may originate from electrostatic repulsion due to the low membrane dielectric constant (Parsegian, 1969, 1975; von Kitzing and Soumpasis, 1996) or dehydration of the ions (Hille, 1992).

Implications of ion pairing

On the one hand, the fact that the space-charge or Debye lengths are generally much smaller than the pairing lengths $\ell_{DH} \lesssim \ell_\rho \ll \ell_{\mathcal{R}}$ indicates that the interaction inside such channels is dominated by electrostatic interactions and not by pairing. On the other hand, the pairing length is on the order of the channel length $L \approx \ell_{\mathcal{R}}$. This indicates that ion pairing is not strong but is also not negligible, which would require $\ell_{\mathcal{R}} \gg L$. To make sure that one does not "produce another set of erroneous insights" (Horn, 1998), the validity of the constant currents assumption must be tested for particular models of ion permeation.

The values of the equilibrium concentrations p_Q and n_Q are closely related to the respective chemical potentials. If μ_p and μ_n are the differences in the chemical potential for cations and anions, respectively, between bulk solution and the channel interior (divided by RT), bulk and equilibrium concentrations are related by $p_Q/p_S = e^{-\mu_p}$ and $n_Q/n_S = e^{-\mu_n}$. If the external concentration of the dominant ion species (e.g., cations) is larger than the internal concentration, the current-voltage curves become superlinear, particularly for voltages above RT/F, and sublinear in the opposite case (Syganow, manuscript in preparation). The strongest effects of ion pairing should occur for triionic solutions, where two ions compete for binding partners or sites (Nonner et al., 1998). Because ion pairing adds to the complexity of standard PNP theory, it is not yet clear which would be the most obvious indication of ion pairing. We would expect

clear effects from ion pairing for mixtures of mono- and divalent ions.

CONCLUSIONS

In this article we present necessary conditions for the trivial constant field approximation in Eq. 5, where the total electric field is assumed to remain close to the external field. Using some typical ion channel parameters, this condition implies that GHK theory works only at extremely low ion concentrations inside the channel.

To analyze barrier models, a more elaborate constant field assumption is required; the change in the electric field of the permeant ions (and mobile permanent charged amino acid side chains) must be small compared to the change of the external field as a function of the external voltage. For barrier models the constant field condition Eq. 7 requires very immobile fixed charges, which is unlikely for ion channels such as the potassium, as well as the acetylcholine receptor channel. Another basic assumption of barrier models is that incoming permeant ions cannot replace or pass resident permeant ions, i.e., the correlation of the motion of permeant ions inside the channel is negligible. This requires that the barrier height is large compared to the mutual electrostatic interaction between permeant ions. According to this condition (Eq. 8), the barrier height must be rather large. Furthermore, the condition (Eq. 9) that the energy profile is practically concentration independent requires large barriers.

This analysis of the barrier model suggests that for most biological ion channels one has to use PNPC theories that include the electrostatic interactions self-consistently. This result agrees with the consideration of electrodynamics of macroscopic media (Pekar, 1941; Robinson, 1973). The error made in Vlasov's mean field approximation for the strong long-range interaction between permeant ions has not yet been systematically studied. For charged channels its impact should be small, because of the "high field" situation in which the mean field comes close to the local electric field. The inclusion of single filing into PNPC theories is important for predicting streaming potentials and Ussing ratios deviating considerably from unity.

We also calculate a constant currents condition (see Eq. 15). If this condition holds, ion pairing becomes negligible. In an inhomogeneous steady-state system, the dynamics of ion permeation is determined by the association rate constant k_A, which is not small, according to theories of diffusion-controlled reactions (Calef and Deutch, 1983). Clear effects from pairing should be expected for solutions containing mixtures of mono- and divalent ions.

REFERENCES

Adams, D. J., T. M. Dwyer, and B. Hille. 1980. The permeability of endplate channels to monovalent and divalent metal cations. *J. Gen. Physiol.* 75:493–510.

Balescu, R. 1975. Equilibrium and Non-Equilibrium Statistical Mechanics. John Wiley and Sons, New York, London, Sydney, Toronto.

Barcilon, V., D. P. Chen, R. S. Eisenberg, and J. W. Jerome. 1997. Qualitative properties of steady-state Poisson-Nernst-Planck systems—perturbation and simulation study. *SIAM J. Appl. Math.* 57:631–648.

Bernard, O., W. Kunz, P. Turq, and L. Blum. 1992. Conductance in electrolyte solutions using the mean spherical approximation. *J. Phys. Chem.* 96:3833–3840.

Blum, L. 1980. Primitive electrolytes in the mean spherical approximation. *In* Theoretical Chemistry: Advances and Perspectives, Vol. 5. Academic Press, San Diego, 1–66.

Bockris, J. O. M., and A. K. N. Reddy. 1970. Modern Electrochemistry. Plenum Press, New York.

Buck, R. P. 1984. Kinetics of bulk and interfacial ionic motion: microscopic bases and limits for the Nernst-Planck equation applied to membrane systems. *J. Membr. Sci.* 17:1–62.

Calef, D. F., and J. M. Deutch. 1983. Diffusion-controlled reactions. *Annu. Rev. Phys. Chem.* 34:493–524.

Chen, D. P. 1997. Non-equilibrium thermodynamics of transport in ion channels. *In* Towards Molecular Physics of Ion Channels. Progress in Cell Research, Vol. 6. M. Sokabe, A. Auerbach, and F. Sigworth, editors. Elsevier, Amsterdam. 269–277.

Chen, D. P., and R. S. Eisenberg. 1993. Flux, coupling, and selectivity in ionic channels of one conformation. *Biophys. J.* 65:727–746.

Chen, D., J. Lear, and B. Eisenberg. 1997. Permeation through an open-channel—Poisson-Nernst-Planck theory of a synthetic ionic channel. *Biophys. J.* 72:97–116.

Cheng, W., C. X. Wang, W. Z. Chen, Y. W. Xu, and Y. Y. Shi. 1998. Investigating the dielectric effects of channel pore water on the electrostatic barriers of the permeation ion by the finite-difference Poisson-Boltzmann method. *Eur. Biophys. J.* 27:105–112.

Conti, F., and G. Eisenman. 1966. The steady-state properties of an ion exchange membrane with mobile sites. *Biophys. J.* 6:227–246.

Cooper, K., E. Jakobsson, and P. Wolynes. 1985. The theory of ion transport through membrane channels. *Prog. Biophys. Mol. Biol.* 46:51–96.

Debye, P., and E. Hückel. 1923. Zur Theorie der Elektrolyte: (1) Gefrierpunktserniedrigung und verwandte Erscheinungen. *Phys. Z.* 24:185–205.

Dorman, V., M. B. Partenskii, and P. C. Jordan. 1996. A semimicroscopic Monte Carlo study of permeation energetics in a gramicidin-like channel—the origin of cation selectivity. *Biophys. J.* 70:121–134.

Doyle, D. A., J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science.* 280:69–77.

Durand-Vidal, S., P. Turq, O. Bernard, C. Treiner, and L. Blum. 1996. New perspectives in transport phenomena in electrolytes. *Physica A.* 231:123–143.

Ebeling, W., and K. Scherwinski. 1983. On the estimation of theoretical individual activity coefficients of electrolytes. *Z. Phys. Chem. Leipzig.* 264:1–14.

Eisenberg, R. S. 1996. Computing the field in proteins and channels. *J. Membr. Biol.* 150:1–25.

Eyring, H., R. Lumry, and J. W. Woodbury. 1949. Some applications of modern rate theory to physiological systems. *Rec. Chem. Prog.* 10:100–114.

Fuoss, R. M., and L. Onsager. 1964. The conductance of symmetrical electrolytes. (4) Hydrodynamic and osmotic terms in the relaxation field. *J. Phys. Chem.* 68:1–8.

Furois-Corbin, S., and A. Pullman. 1991. The effect of point mutations on energy profiles in a model of the nicotinic acetylcholine receptor channel. *Biophys. Chem.* 39:153–159.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27:37–60.

Gouy, G. 1910. Sur la constitution de la charge électrique a la surface d'un électrolyte. *J. Phys. Theor. Appl.* 9:455–468.

Henderson, D., L. Blum, and J. L. Lebowitz. 1979. An exact formula for the contact value of the density profile of a system of charged hard spheres near a charged wall. *J. Electroanal. Chem.* 102:315–319.

Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.).* 108:37–77.

Horn, R. 1998. Run, don't hop, through the nearest calcium channel. *Biophys. J.* 75:1142–1143.

Hubbard, J. B. 1987. Non-equilibrium theories of electrolyte solutions. *In* The Physics and Chemistry of Aqueous Ionic Solutions. Mathematical and Physical Sciences, Vol. 205. M.-C. Bellissent-Funel, editor. D. Reidel, Dortrecht, the Netherlands. 95–128.

Imoto, K., C. Busch, B. Sakmann, M. Mishina, T. Konno, J. Nakai, H. Bujo, Y. Mori, K. Fukuda, and S. Numa. 1988. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature.* 335:645–648.

Jackson, J. D. 1962. Classical Electrodynamics. John Wiley, New York.

Jordan, P. C., M. B. Partenskii, and V. Dorman. 1997. Electrostatic influence on ion-water correlations in ion channels. *In* Towards Molecular Biophysics of Ion Channels. Progress in Cell Research, Vol. 6. M. Sokabe, A. Auerbach, and F. Sigworth, editors. Elsevier, Amsterdam. 279–293.

Keizer, J. 1987. Statistical Thermodynamics of Nonequilibrium Processes. Springer-Verlag, Heidelberg.

Kirkwood, J. G. 1934. On the theory of strong electrolyte solutions. *J. Chem. Phys.* 2:767–781.

Kirkwood, J. G., and J. C. Poirier. 1954. The statistical mechanical basis of the Debye-Hückel theory of strong electrolytes. *J. Phys. Chem.* 58:591–596.

Klement, R., D. M. Soumpasis, E. von Kitzing, and T. M. Jovin. 1990. Inclusion of ionic interactions in force field calculations of charged biomolecules—DNA structural transitions. *Biopolymers* 29:1089–1103.

Konno, T., C. Busch, E. von Kitzing, K. Imoto, F. Wang, J. Nakai, M. Mishina, S. Numa, and B. Sakmann. 1991. Rings of anionic amino acids as structural determinants of ion selectivity in the acetylcholine receptor channel. *Proc. R. Soc. Lond. B.* 244:69–79.

Kornyshev, A. A. 1985. Nonlocal electrostatics of solvation. *In* The Chemical Physics of Solvation. Studies in Physical and Theoretical Chemistry, Vol. 38. R. R. Dogonadze, E. Kalman, A. A. Kornyshev, and J. Ulstrup, editors. Elsevier, Amsterdam. 77–118.

Kornyshev, A. A., and G. Sutmann. 1996. The shape of the nonlocal dielectric function of polar liquids and the implications for thermodynamic properties of electrolytes—a comparative study. *J. Chem. Phys.* 104:1524–1544.

Kramers, H. A. 1940. Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica.* 7:284–304.

Laidler, K. J., and M. C. King. 1983. The development of transition-state theory. *J. Phys. Chem.* 87:2657–2664.

Lehmani, A., O. Bernard, and P. Turq. 1997. Transport of ions and solvent in confined media. *J. Stat. Phys.* 89:379–402.

Levitt, D. G. 1986. Interpretation of biological ion channel flux data: reaction rate versus continuum theory. *Annu. Rev. Biophys. Biophys. Chem.* 15:29–57.

Levitt, D. G. 1987. Exact continuum solution for a channel that can be occupied by two ions. *Biophys. J.* 52:455–466.

MacGillivray, A. D., and D. Hare. 1969. Applicability of Goldman's constant field assumption to biological systems. *J. Theor. Biol.* 25:113–126.

Macias, F., and M. E. Starzak. 1993. Ion velocity distributions in gramicidin channels determined with laser-Doppler velocimetry. *Biochim. Biophys. Acta.* 1153:331–334.

Macias, F., and M. E. Starzak. 1994. Laser-doppler scattering for the determination of ionic velocity distributions in channels and membranes. *Adv. Chem. Ser.* 235:401–413.

McGill, P., and M. F. Schumaker. 1996. Boundary conditions for single-ion diffusion. *Biophys. J.* 71:1723–1742.

McQuarrie, D. A. 1976. Statistical Mechanics. Harper and Row, New York, Evanston, San Francisco, London.

Muller, R. U., and A. Finkelstein. 1972. The effect of surface charge on the voltage-dependent conductance induced in thin lipid membranes by monazomycin. *J. Gen. Physiol.* 60:285–306.

Nonner, W., D. P. Chen, and B. Eisenberg. 1998. Anomalous mole fraction effect, electrostatics, and binding in ionic channels. *Biophys. J.* 74:2327–2334.

Nonner, W., and B. Eisenberg. 1998. Ion permeation and glutamate residues linked by Poisson-Nernst-Planck theory in L-type calcium channels. *Biophys. J.* 75:1287–1305.

Onsager, L. 1926. Zur Theorie der Elektrolyte (1). *Phys. Z.* 27:388–392.

Onsager, L. 1927. Zur Theorie der Elektrolyte (2). *Phys. Z.* 28:277–298.

Parsegian, V. A. 1969. Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature.* 221:844–846.

Parsegian, V. A. 1975. Ion-membrane interactions and structural forces. *Ann. N.Y. Acad. Sci.* 264:161–174.

Partenskii, M. B., V. Dorman, and P. C. Jordan. 1994. Influence of a channel-forming peptide on energy barriers to ion permeation, viewed from a continuum dielectric perspective. *Biophys. J.* 67:1429–1438.

Pekar, S. I. 1941. The electric conductivity of thin layers of semiconductors and the pool effect (Russian). *Zh. Eksp. Teor. Fiz.* 11:708–716.

Pullman, A. 1987. Energy profiles in the gramicidin A channel. *Q. Rev. Biophys.* 20:173–200.

Pullman, A. 1991. Contribution of theoretical chemistry to the study of ion transport through membranes. *Chem. Rev.* 91:793–812.

Ranatunga, K. M., I. D. Kerr, C. Adcock, G. R. Smith, and M. S. P. Sansom. 1998. Protein-water-ion interactions in a model of the pore domain of a potassium channel: a simulation study. *Biochim. Biophys. Acta.* 1370:1–7.

Robinson, F. N. H. 1973. Macroscopic Electromagnetism. Pergamon, Oxford.

Roux, B. 1996. Valence selectivity of the gramicidin channel—a molecular-dynamics free-energy perturbation study. *Biophys. J.* 71:3177–3185.

Roux, B., and M. Karplus. 1994. Molecular-dynamics simulations of the gramicidin channel. *Annu. Rev. Biophys. Biomol. Struct.* 23:731–761.

Roux, B., B. Prodhom, and M. Karplus. 1995. Ion-transport in the gramicidin channel—molecular-dynamics study of single and double occupancy. *Biophys. J.* 68:876–892.

Roux, B., and T. B. Woolf. 1997. Influence of small perturbations on the conductance of an ion channel. *In* Towards Molecular Physics of Ion Channels. Progress in Cell Research, Vol. 6. M. Sokabe, A. Auerbach, and F. Sigworth, editors. Elsevier, Amsterdam. 261–268.

Rubinstein, I. 1990. Electro-Diffusion of Ions. SIAM, Philadelphia.

Sansom, M. S. P., G. R. Smith, C. Adcock, and P. C. Biggin. 1997. The dielectric properties of water within model transbilayer pores. *Biophys. J.* 73:2404–2415.

Smith, G. R., and M. S. P. Sansom. 1997. Molecular-dynamics study of water and Na^+ ions in models of the pore region of the nicotinic acetylcholine-receptor. *Biophys. J.* 73:1364–1381.

Smoluchowski, M. V. 1917. Versuch einer mathematischen Theorie der Koagulation kolloider Lösungen. *Z. Phys. Chem. Leipzig.* 92:129–147.

Soumpasis, D. M. 1984. Statistical mechanics of the B-Z transition of DNA: contribution of diffuse ionic interactions. *Proc. Natl. Acad. Sci. USA.* 81:5116–5120.

Sridharan, S., J. A. McCammon, and J. B. Hubbard. 1989. Diffusion-controlled reactions of ions in fluctuating ionic atmospheres. *J. Chem. Phys.* 90:237–240.

Syganow, A. N., and E. von Kitzing. 1995. The integral weak diffusion and diffusion approximations applied to ion transport through biological ion channels. *J. Phys. Chem.* 99:12030–12040.

Triolo, R., J. R. Grigeria, and L. Blum. 1976. Simple electrolytes in the mean spherical approximation. *J. Chem. Phys.* 80:1858–1861.

Turq, P., J. M. G. Barthel, and M. Chemla. 1992. Transport, Relaxation, and Kinetic Processes in Electrolyte Solutions. Springer Verlag, Heidelberg.

Unwin, N. 1995. Acetylcholine-receptor channel imaged in the open state. *Nature.* 373:37–43.

Villarroel, A., and B. Sakmann. 1992. Threonine in the selectivity filter of the acetylcholine receptor channel. *Biophys. J.* 62:196–208.

Vlasov, A. A. 1938. About the vibrational properties of an electron gas. *Zh. Eksp. Teor. Fiz.* 8:291–318.

von Kitzing, E. 1995. Structure modeling of the acetylcholine receptor channel and related ligand gated channels. *In* Modelling of Biomolecular Structures and Mechanisms. The Jerusalem Symposia on Quantum Chemistry and Biochemistry, Vol. 27. A. Pullman, J. Jortner, and B. Pullman, editors. Kluwer Academic Publishers, Dortrecht. The Netherlands. 39–57.

von Kitzing, E., and D. M. Soumpasis. 1996. Electrostatics of a simple membrane model using the Green's functions formalism. *Biophys. J.* 71:795–810.

Weetman, P., S. Goldman, and C. G. Gray. 1997. Use of the Poisson-Boltzmann equation to estimate the electrostatic free-energy barrier for dielectric models of biological ion channels. *J. Phys. Chem. B.* 101: 6073–6078.

Weiss, G. H. 1986. Overview of theoretical models for reaction rates. *J. Stat. Phys.* 42:3–36.