ON MICROELECTRODE IONOPHORESIS

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ABSTRACT An equation for ionophoresis in large tip microelectrodes is derived from Nernst-Planck equations for the general case of a completely dissociated electrolyte. The relation between the release of ions and the applied electric current is mainly determined by two parameters: the transference number of the ions under consideration and the diffusional leak of the microelectrode. Also it is shown how the release of ions is affected by the concentration of the electrolyte within the electrode and that of the external solution. The equation describes the ionophoretic release of polyvalent spermine. In addition, new equations for tip potential and for tip resistance are derived.

INTRODUCTION

In recent years, attempts have been made to give ionophoretic studies with microelectrodes a more quantitative basis (Purves, 1979, 1980*a*). A detailed discussion of the relation between the electric current and the release of ions from microelectrodes has been presented for the case in which all ions under consideration have the same diffusion coefficient and valence (Purves, 1981). Here, ionophoresis in large tip microelectrodes (with internal tip diameters not $\lesssim 1$ μm) will be discussed for the general case of a completely dissociated electrolyte within the framework of Nernst-Planck equations. Large tip microelectrodes are routinely applied not only in the case of extracellular ionophoresis experiments but also for intracellular ionophoretic drug injections, e.g., into large snail neurons (Drouin and Hermann, 1984). Ionophoretic release of polyvalent spermine into artificial seawater will be analyzed with a new equation. The results will be compared with the conventional description by Hittorf's equation.

THEORY

Geometry and Flux Equations

The microelectrode is viewed at the tip as a truncated spherical cone (Fig. 1). Its longitudinal axis is the radius, r, of the sphere. The taper includes the angle, 2θ, and the radius of the sphere at the tip is $r = r_o$. For small angles, the internal tip diameter can be calculated to be

$$2a = 2r_o \sin\theta \simeq 2r_o\theta. \tag{1}$$

Therefore, the cross-sectional area, S, at the tip is

$$S = \pi a^2 \simeq \pi r_o^2 \theta^2. \tag{2}$$

According to thermodynamic convention, the efflux, q, of the electrolyte from the microelectrode is defined by

$$q = -\mathrm{d}n/\mathrm{d}t = \int_S \mathbf{j}\, \mathbf{dS}. \tag{3}$$

The rate, $\mathrm{d}n/\mathrm{d}t$, of moles, n, of electrolyte released from the microelectrode during the time increment, $\mathrm{d}t$, is considered a negative quantity. The flux density, $\mathbf{j}$, is directed towards the center of the spherical cone and is opposite to the direction of the normal vector, $\mathbf{dS}$. Integration of Eq. 3 at any radius, r, leads to

$$q = -j\, r^2\, 2\pi\,(1 - \cos\theta) \tag{4}$$

and for small angles, $\cos\theta \simeq 1 - \theta^2/2$, Eq. 4 becomes

$$q = -j\,\pi\, r^2\, \theta^2. \tag{5}$$

By convention, the direction of the electric current, I, is taken to be the direction in which positive charge flows. Therefore,

$$I = \int_S \mathbf{J}\, \mathbf{dS} = -J\,\pi\, r^2\, \theta^2, \tag{6}$$

where $\mathbf{J}$ is the electric current density. The Nernst-Planck equations are supposed to describe the transport of the completely dissociated electrolyte in the microelectrode. The gradient of electrolyte concentration in the electrode is supposed to rise only in the direction of the longitudinal axis, r. Accordingly, the following expression for the molar flux density of the electrolyte can be derived

$$\mathbf{j} = -D\frac{\mathrm{d}C}{\mathrm{d}r} + \frac{1}{\nu F}\left(\frac{t_1}{z_1} + \frac{t_2}{z_2}\right)\mathbf{J} \tag{7}$$

where D is the diffusion coefficient of the electrolyte; C is the molar concentration of the electrolyte; $\nu = \nu_1 + \nu_2$, the number of moles of cations (1) and anions (2) formed by the dissociated electrolyte; t_1, t_2 are the transference numbers of cations or anions; z_1, z_2 are valences of cations or anions; F is Faraday's constant. The complete integration of Eq. 3 substituted by Eq. 7 results in the equation for the efflux of electrolyte from the microelectrode

$$q = -D\,\pi\, r^2\, \theta^2 \frac{\mathrm{d}C}{\mathrm{d}r} + \frac{1}{\nu F}\left(\frac{t_1}{z_1} + \frac{t_2}{z_2}\right)\cdot I. \tag{8}$$

Distribution of Electrolytes

The condition for steady state efflux of electrolyte requires

$$\mathrm{d}q/\mathrm{d}r = 0. \tag{9}$$

The following assumptions are made. The diffusion coefficient, D, and the transference numbers, t_1 and t_2, do not change within the electrode, the angle, θ, along the taper of the cone remains constant and the current, I, is

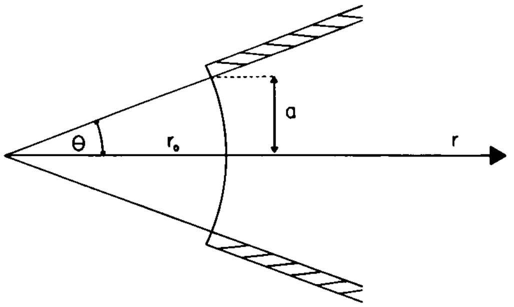

FIGURE 1 Microelectrode viewed at the tip as a truncated spherical cone; radius of the sphere at the tip, r_o; longitudinal axis of the microelectrode, r; taper included angle, 2 θ, and internal tip diameter, $2a$.

kept constant experimentally. The remaining problem is the determination of the electrolyte concentration, C, at any point within the electrode according to the differential equation

$$\frac{d}{dr}\left(r^2\frac{dC}{dr}\right) = 0. \tag{10}$$

Suppose that at the tip of the electrode, at $r = r_o$, the electrolyte concentration, C, is equal to the initial equivalent concentration

$$C_o^* = \sum_k |z_k| C_k \tag{11}$$

of an external mixture of k dissociated electrolytes with molar concentrations, C_k, and absolute valences, $|z_k|$. Further that at $r = \infty$, C is equal to the initial equivalent concentration

$$C_o = |z_s| C_{os} \tag{12}$$

of the internal completely dissociated electrolyte with initial molar concentration, C_{os}, and absolute valence, $|z_s|$. Then Eq. 10 can be solved to describe the distribution of the eqivalent concentration of electrolytes

$$C_{eq} = C_o^* \left[w + (1 - w)\, r_o/r\right] = C_o^* f = |z_s| C_s. \tag{13}$$

C_s is the molar concentration of the internal electrolyte. The following substitutions are used

$$w = C_o/C_o^*. \tag{14}$$

and

$$f = w + (1 - w)\, r_o/r. \tag{15}$$

The assumed boundary conditions are valid for small angles, θ. At the tip, the equivalent concentration, C_o, of the internal electrolyte solution is reduced by the factor, $\theta^2/4$, which is the ratio of the small solid angle, $\pi\theta^2$, of the cone of the microelectrode and the large solid angle, 4π, of the external spherical space (see Appendix). However, the concentration of electrolyte has to remain finite at the tip. Therefore, the initial equivalent concentration, C_o^*, of the external electrolyte is taken as the appropriate boundary electrolyte concentration.

Diffusional Leak of Electrolyte

If no electric current is applied to the electrode ($I = 0$), the molar diffusional efflux, q_D, of the electrolyte according to Eq. 8 is

$$q_D = -D\, \pi\, r^2\, \theta^2\, (dC/dr). \tag{16}$$

Suppose that at the tip of the electrode, at $r = r_o$, and for small angles, θ, the concentration of the filling electrolyte solution is ~0: $C_s = 0$, and that at $r = \infty$, C_s is equal to the initial concentration, $C_{os} = C_o/|z_s|$, of the internal filling solution, then integration of Eq. 16 results in

$$q_D = -D\, \pi\, r_o\, \theta^2\, C_{os} = -D\, \pi\, r_o\, \theta^2\, w C_o^*/|z_s|, \tag{17}$$

using Eq. 14. In case the initial equivalent concentrations of internal and external electrolytes are equal, that is $w = 1$, or

$$C_o = |z_s| C_{os} = \sum_k |z_k| C_k = C_o^*, \tag{18}$$

the diffusional leak of the microelectrode becomes

$$q_D^* = -D\, \pi\, a\, \theta\, C_o^*/|z_s|, \tag{19}$$

using Eq. 1.

Tip Potential and Tip Resistance

The following equation for the electric current density can be derived from Nernst-Planck equations for the transport of cations and anions of one dissociated electrolyte. The gradient of the electrostatic potential, ϕ, is supposed to rise only in the direction of the longitudinal axis of the electrode. Then

$$\mathbf{J} = -\sigma\left[\left(\frac{t_1}{z_1} + \frac{t_2}{z_2}\right)\frac{RT}{F}\frac{d \ln C}{dr} + \frac{d\varphi}{dr}\right], \tag{20}$$

where R is the gas constant and T is the absolute temperature. The specific electric conductivity, σ, of the electrolyte is related to the concentration of the electrolyte by

$$\sigma = C\, (\nu_1\, z_1)^2\, DF^2/\nu\, t_1\, t_2\, RT. \tag{21}$$

It is a function of distance along the tip of the electrode, as can be seen when Eq. 13 is inserted into Eq. 21. Eq. 20 is integrated according to Eq. 6 for any radius, r, of the spherical cone. Then the voltage difference, E, at the tip of the microelectrode during passage of a constant electric current, I, can be calculated by integration between the boundaries $r = r_o$, $\varphi = \varphi_o$ and $r = \infty$, $\varphi = \varphi_\infty$, with, $E = \varphi_\infty - \varphi_o$ as

$$E = E_t + R_t\, I. \tag{22}$$

The diffusion potential, E_t, at the tip of the microelectrode is generated by the transport of the internal electrolyte, with different transference numbers and valences of cations and anions, in its internal concentration gradient. Initially, the concentration of the filling electrolyte in the solution outside the electrode is 0. The small concentration of the internal electrolyte at the tip, at $r = r_o$, is calculated according to Eq. A5 (see Appendix) and $C_s = C'_s$, and is related to the filling concentration, $C_s = C_{os}$ at $r = \infty$. The diffusion potential at the tip is then

$$E_t = -\left(\frac{t_1}{z_1} + \frac{t_2}{z_2}\right)\frac{RT}{F}\ln\left(1 + \frac{4}{\theta^2}\right). \tag{23}$$

For small angles, θ, the diffusion potential at the tip can obtain large values. In the case when a finite concentration, C_{se}, of the internal filling solution is found in the external solution, then the equation for the diffusion potential at the tip changes into the form

$$E_t = -\left(\frac{t_1}{z_1} + \frac{t_2}{z_2}\right)\frac{RT}{F}\ln\frac{\left(1 + \dfrac{4}{\theta^2}\right)}{\left(1 + \dfrac{4\, C_{se}}{\theta^2\, C_{os}}\right)}. \tag{24}$$

When the external medium and the internal filling solution consist of the same electrolyte and have equal concentrations, for $C_{se} = C_{os}$, the diffusion potential at the tip becomes 0.

The electric resistance, R_t, at the tip of the microelectrode is derived with the approximation for small angles, θ, corresponding to the electrolyte concentrations, $C_s = C_o^*/|z_s|$ at $r = r_o$, and $C_s = C_o/|z_s|$ at $r = \infty$. The result is

$$R_t = \nu\, t_1 t_2\, RT \ln w/(\nu_1 z_1)^2 F^2 q_D^* (w - 1), \tag{25}$$

using the definition of Eq. 19. The measured resistance of a microelectrode includes the tip resistance, the shank resistance, and the convergence resistance to current flow in the external medium. Although the shank resistance of a microelectrode may not be negligible compared with the tip resistance (e.g., Snell, 1969; Sakmann and Neher, 1983), the required addition of the convergence resistance is usually negligibly small (Firth and De Felice, 1971).

Ionophoretic Release

Basic transformations of Nernst-Planck equations lead to the following expression for the molar flux density of the ions ($i = 1, 2$)

$$\mathbf{j}_i = -D\frac{\mathrm{d}C_i}{\mathrm{d}r} + \frac{t_i C_i}{z_i F \nu_i C} \cdot \mathbf{J}. \tag{26}$$

If ions are to be ionophoretically released, Eq. 26 has to be adjusted to the geometry of the microelectrode

$$q_i = \int_S \mathbf{j}_i\, \mathbf{dS}. \tag{27}$$

Integration of the ion flux density, $\mathbf{j}_i$, with respect to the direction of the spherical cone area element, $\mathbf{dS}$, yields

$$q_i = -D\pi r^2 \theta^2 (\mathrm{d}C_i/\mathrm{d}r) + t_i I C_i |z_s| / z_i F \nu_i C_o^* f, \tag{28}$$

using Eq. 13.

Rearranging and substitution of Eq. 28 with Eq. 19 gives

$$q_i/D\pi\theta^2 = -r^2 (\mathrm{d}C_i/\mathrm{d}r) - t_i I r_o C_i / z_i F \nu_i q_D^* f. \tag{29}$$

The condition for steady state efflux of ions requires

$$\mathrm{d}q_i/\mathrm{d}r = 0 \tag{30}$$

or

$$\frac{\mathrm{d}}{\mathrm{d}r}\left(r^2 \frac{\mathrm{d}C_i}{\mathrm{d}r} + \frac{H r_o \cdot C_i}{f}\right) = 0 \tag{31}$$

with the substitution

$$H = t_i I / z_i F \nu_i q_D^*. \tag{32}$$

The problem left, is the determination of the ion concentration, C_i, at any point in the electrode according to the differential equation (Eq. 31). Suppose that at the tip of the electrode, at $r = r_o$, and for small angles, θ, the ion concentration is approximately $C_i = 0$, and that at $r = \infty$, $C_i = \nu_i C_{os} = \nu_i C_o/|z_s| = \nu_i w C_o^*/|z_s|$, then Eq. 31 can be solved (see Appendix) to be

$$C_i = \frac{\nu_i C_o^* w \{f - f^{[H/(1-w)]}\}}{|z_s| \{w - w^{[H/(1-w)]}\}} \tag{A19}$$

using Eqs. 14, 15, and 32. According to Eq. 28, the efflux of ions at the tip of the electrode at $r = r_o$ and $C_i = 0$ is

$$q_i = -D\pi r_o^2 \theta^2 (\mathrm{d}C_i/\mathrm{d}r)_{r_o}. \tag{33}$$

Differentiation of Eq. A19 at $r = r_o$ gives

$$\left(\frac{\mathrm{d}C_i}{\mathrm{d}r}\right)_{r_o} = -\frac{\nu_i C_o^* w (1 - w)\left(1 - \frac{H}{1 - w}\right)}{|z_s| r_o (w - w^{[H/(1-w)]})} \tag{34}$$

Combining Eqs. 33 and 34, substituting with Eq. 19 and inserting Eq. 32, results in the general equation that is expected to describe ionophoresis experiments with large tip microelectrodes (Drouin, 1983)

$$q_i = \frac{\frac{t_i I}{z_i F} + \nu_i q_D^* (w - 1)}{1 - \exp\left\{-\left[1 + \frac{t_i I}{z_i F \nu_i q_D^* (w - 1)}\right] \ln w\right\}}. \tag{35}$$

Fig. 2 represents a three-dimensional graph of Eq. 35. For ion ejection, I/z_i takes positive values and in the limit towards increasing positive values, Hittorf's equation emerges

$$\lim_{I/z_i \to \infty} q_i = t_i I / z_i F, \tag{36}$$

In the case for $I = 0$, the diffusional leak of the ions is described by

$$q_i = \nu_i q_D^* w = -D\pi a \theta \nu_i C_{os}, \tag{37}$$

using Eqs. 12, 14, and 19. In the limit towards increasing negative values of I/z_i, the efflux of ions vanishes

$$\lim_{I/z_i \to -\infty} q_i = 0. \tag{38}$$

MATERIALS AND METHODS

Microelectrodes

Microelectrodes were pulled from glass capillaries (Hilgenberg Glas, Maisfeld, Federal Republic of Germany; outer diameter: 1.2 mm, wall

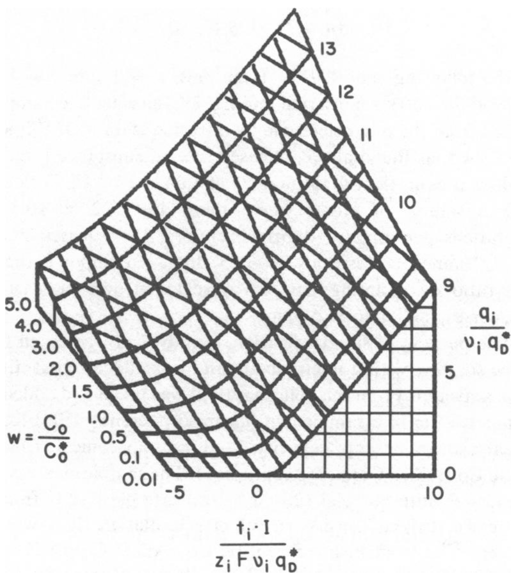

FIGURE 2 A three-dimensional graph of normalized ion efflux, $q_i/\nu_i q_D^*$, from microelectrode, normalized electric current, $I\, t_i/z_i F \nu_i q_D^*$, and ratio of equivalent concentrations, $w = C_o/C_o^*$, of initial internal and external electrolyte solutions as calculated from Eq. 35.

thickness: 0.25 mm; the capillaries contained strands of fiber glass to facilitate filling; outer diameter of fiber glass: 0.11 mm). Microelectrodes were pulled in two stages using a vertical puller (model 700 C; David Kopf Instruments, Tujunga, CA) and standard nichrome heating wire (diameter: 1.2 mm). Heating coils were fabricated with three and one-half turns, a length of 4.5 mm and an outer width of 6.5 mm. The heating coil was placed 13 mm beneath the upper fixed holder. 70 mm long glass capillaries were placed into the puller. The first pull was made at 17A heater current setting with a pulling length of 7.5 mm. The thinner part of the capillary was then recentered with respect to the heating coil by an upward shift of ~4.5 mm. Then in the second pull, the thinner part broke producing two electrodes, an upper and a lower one.

To gain an estimate of the geometry, the external taper of the microelectrode was measured from the tip up to a length of 500 μm under a usual light microscope. It was found that the ratios of inner and outer diameters of a pulled glass capillary remained approximately constant over a wide range of measured values at different lengths. Indeed, electron microscopic studies with fine tip microelectrodes showed that the ratio of inner and outer diameters at the tip was approximately the same as that of the not pulled capillary (Bils and Lavallée, 1964). Therefore, it was assumed that this relation also holds near the tip of large tip electrodes. From linear least-squares interpolations of the inner radii of the taper at different lengths, from the tip up to 60 μm, the radius, a, and the semiangle, θ, at the tip of the microelectrode were determined.

Spermine

Measurements of the diffusional leak of spermine from microelectrodes were to be included in the ionophoresis experiments. ^{3}H-labeled spermine tetrahydrochloride, $(H_2NC^3H_2CH_2CH_2NHCH_2CH_2\text{-})_2\,4\,HCl$; 348.2 mol wt; with a specific activity of 17.0 Ci/mmol was lyophilized in a combi-vial. The activity was 1.0 mCi in 0.020 mg (New England Nuclear, Boston, MA). Another solution of 10 mM cold spermine (Sigma Chemical Co., St. Louis, MO) was adjusted to pH = 7.3 with KOH. 200 μl of this spermine solution were added into the combi-vial. 100 μl (500 μCi) were stored as reference solution; the other 100 μl (500 μCi) were used for filling of microelectrodes. The ratio of concentrations of unlabeled to labeled spermine was 34.8.

For an estimate of the order of magnitude of an expected diffusional leak, Eq. 17 is combined with Eqs. 1 and 3

$$\mathrm{d}n = D\,\pi a\,\theta\,C_{\infty}\,\mathrm{d}t. \tag{39}$$

When the following values: $D = 1\ \mu\mathrm{m}^2/\mathrm{ms}$, $a = 1\ \mu\mathrm{m}$, $\theta = 1°$; $C_{\infty} = 10\mathrm{mM}$, and $\mathrm{d}t = 60\ \mathrm{s}$ are inserted into Eq. 39, the calculated amount that is released from the microelectrode by diffusion is $\mathrm{d}n = 32.9$ fmol. With the ratio, 34.8, as found above, released labeled substance is $\mathrm{d}n^* = 0.94$ fmol, which is equivalent to the activity of 1.64×10^{-11}Ci. Per definition, 1 Ci is a sample of atoms where there are 2.22×10^{12} nuclear disintegrations per minute (dpm). Therefore, the released amount of labeled substance corresponds to 3.65 dpm. To improve the signal-to-noise ratio for counting, samples were taken over at least 10-min periods, corresponding to 36.5 dpm.

For the purpose of standardization, exactly 2 μl was taken from the reference solution with a micropipette and was added to exactly 500-μl artificial seawater. From this solution, 10 μl was taken and added to 1-ml artificial seawater in a sample cup (clear polysterene FISHERbrand$^{\text{TM}}$, auto analysis sample cup, capacity 2 ml, bottom conical, from Fisher Scientific Co., Allied Corp., Pittsburg, PA). The sample cup was emptied into a glass scintillation vial (20-ml borosilicate glass vials from Fisher Scientific Co., Allied Corp.). 10 ml of scintillation fluid were added (ScintiVerse$^{\text{TM}}$II from Fisher Scientific Co., Allied Corp.). The sample was counted for 20 min in a liquid scintillation spectrometer (model 3330; Packard Instrument Co., Inc., United Technologies, Downers Grove, IL). It was found that 1,000 cpm corresponded to 5.52 ± 0.26 pmol total spermine (mean ± SD of two samples).

Labeled spermine solution was sucked into a thinned glass capillary that was connected by a plastic tubing and a three-way stopcock (one way to the outer air) to a syringe (20 ml). Then the thinned part of the capillary was inserted into the microelectrode, and labeled spermine solution was ejected by applying pressure onto the syringe. Silver wire was inserted into the filled microelectrode. The upper open end was sealed by sticky wax (Deiberit 502 from Ludwig Böhme K.-G., Bad Sachsa/Harz, Federal Republic of Germany). The microelectrode was placed onto a holder that was kept vertical by a micromanipulator. In a 6-mm distance from the microelectrode, the holder also kept another silver wire having the same length (30 to 40 mm) as the microelectrode. Both wires were connected to an ionophoresis unit capable of passing 1,000 nA through 100 MΩ (micro-iontophoresis programmer, model 160, W-P Instruments, Inc., New Haven, CT). On lowering the holder, spermine was either released by diffusion or ionophoresed for a period of 5 min into 1-ml samples of artificial seawater in polysterene cups at different current values (I is between 20 and 1,000 nA) at room temperature (23 to 25°C). The composition of the artificial seawater was in millimoles per liter: 500 NaCl, 10 KCl, 10 $CaCl_2$, 50 $MgCl_2$, 10 Tris HCl at pH = 7.3; the equivalent concentration was, $C_o^* = 0.64$ eq/l. Spermine cations, $SPM^{4+} = H_3^+N(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$, bear four proton charges ($z_1 = 4$) at pH = 7.3, and the mole number, $\nu_1 = 1$. Therefore, the equivalent concentration was, $C_o = 0.04$ eq/l. Release of spermine, q_{SPM} in picomoles per second, was measured by counting the activity of diffusionally or ionophoretically released tritiated spermine. Values were corrected for background activity. The measured standard was used to transform cpm into picomoles.

EXPERIMENTAL RESULTS

Release of Spermine from Microelectrodes

Diffusional leak and ionophoretic release of spermine were measured. The diffusional leak, $q_D = 0.9 \pm 0.5$ fmol/s (mean ± SD), was found with 4 microelectrodes in 4 different experiments and a total of 15 samples, when diffusion of spermine into normal artificial seawater was studied. The average diffusional leak, $q_D = 1.7 \pm 1.1$ fmol/s (mean ± SD) from 4 microelectrodes, was found in 10 different diffusion experiments and a total of 37 samples, when in addition values of diffusion into diluted artificial seawater (0.32 and 0.064 eq/l), and into solutions consisting of 10 and 29 mM KCl were taken into account. The transference number, $t_1 = 0.39 + 0.10$ (mean ± SD), was found with 4 microelectrodes for ionophoresis of spermine into normal artificial seawater. The transference number of spermine, $t_1 = 0.42 \pm 0.07$ (mean ± SD), was determined with 4 microelectrodes from 10 different ionophoresis experiments that included ionophoresis of spermine into normal artificial seawater, into diluted artificial seawater (0.32 and 0.064 eq/l), and into solutions consisting of 10 and 29 mM KCl.

Fig. 3 shows one example for the analysis of an ionophoresis experiment. From optical measurements of the outer diameters of this electrode with an usual laboratory microscope, the inner radius, $a = 0.7\ \mu$m at the tip, was estimated using the ratio, 0.583, of inner and outer diameters of the capillary. The value, $\theta = 1.22°$, was found for the inner semiangle of the taper of this electrode. Figs. 3 *a–d* show one experiment of ionophoretic spermine release at two different scalings. The squares represent measurements of the release of tritiated spermine at different values of ionophoretic current. In Figs. 3 *a*, *b*, the

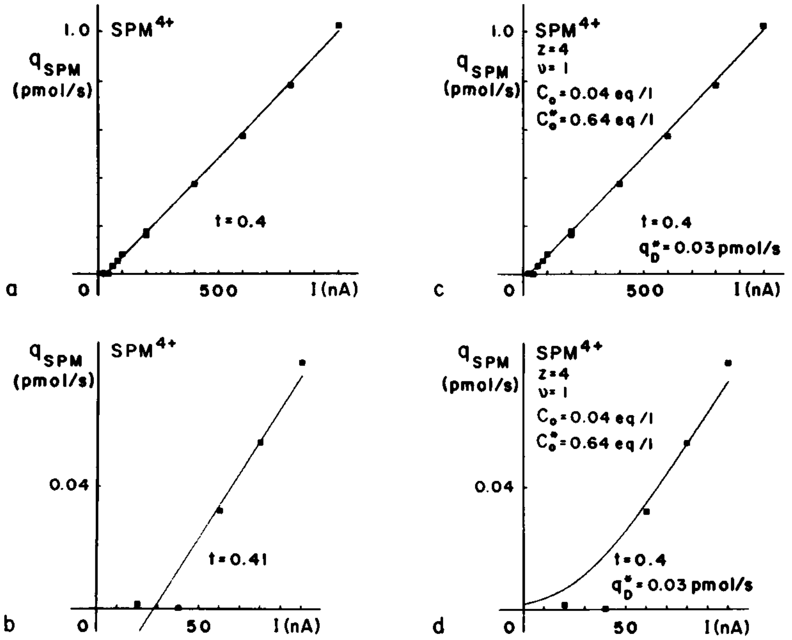

FIGURE 3 Microelectrode ionophoresis of spermine into artificial seawater. The same experiment is shown at two different scalings (*a*, *c* and *b*, *d*). *a* and *b* show linear approximations by Hittorf's equation. *c* and *d* represent descriptions by Eq. 35 of the text. The subscript, 1, for cations has been dropped. For a confidence level of 95%, the proportional error of counts is 0.92% at 1,000 nA and increases to 4.7% at 60 nA, but has values of 98% at 40 nA and 22.2% at 20 nA.

drawn lines are calculated according to the linear approximation of Hittorf's equation. In Figs. 3 *c*, *d*, the lines are calculated according to Eq. 35. At high current values (I = 100 to 1,000 nA), the linear approximation of Hittorf's equation (in Fig. 3 *a*) and Eq. 35 (in Fig. 3 *c*) lead to the same transference number for spermine, $t_1 = 0.4$. However, as indicated in Fig. 3 *c*, Eq. 35 provides additional information for the leak of the microelectrode, $q_D^* = 0.03$ pmol/s. At low current values (I = 0 to 100 nA), Figs. 3 *b*, *d* show completely different characteristics. The nonlinear relationship between spermine release and applied current is described in Fig. 3 *d*. The efflux of spermine approaches 0 with decreasing currents. The same parameters as in Fig. 3 *c* are inserted.

DISCUSSION

Hittorf's equation has been widely used in the ionophoretic literature (e.g., Curtis, 1964; Chowdhury, 1969; Krnjević, 1971; Kelly, 1975), although it may give false predictions when applied to microelectrodes (Purves, 1980*b*, 1981). As shown above, Hittorf's equation emerges as a limiting equation for increasing values of the ratio of ionophoretic current and valency. It appears to be independent of the parameters imposed by the geometry of the microelectrode and the external electrolyte solution. However, the straight line approximation in Fig. 3 *b* predicts negative values of spermine release. Clearly, this is a wrong prediction, because spermine was released from the microelectrode into the external seawater and not the reverse. Therefore, a better description of the ionophoresis experiment is made possible by Eq. 35. The limit of validity for the linear approximation of Hittorf's equation is marked by the onset of the nonlinearity of the characteristics described by Eq. 35. The general equation for microelectrode ionophoresis derived here describes experimental results more completely. Information is given not only for the transference number of the ions under consideration but also for the leak of the microelectrode. For any experimental situation, both parameters permit calculation of tip resistance. Thus the presented theory provides a more thorough analysis of microelectrode ionophoresis than could be achieved by applying Hittorf's equation only.

Main assumptions in the derivation of the equation for ionophoresis in large tip microelectrodes are the following: (*a*) complete dissociation of the internal electrolyte; (*b*) condition of electroneutrality in the electrolyte solution; (*c*) validity of Nernst-Planck equation; (*d*) conical geometry of the tip, and (*e*) condition of steady state.

In the case of an incompletely dissociated electrolyte, an appreciable fraction of the transport of solute may occur as a result of the motion of ion pairs or larger aggregates; in the extreme case of a weak electrolyte, the covalent molecular form is the predominate diffusing entity compared with the transport of dissociated ions.

The condition of electroneutrality implies that the net charge in any macroscopic volume of solution is 0. Also the concentration of possible surface charges at the wall of the electrode constriction is negligibly small, relative to the concentrations of external or internal electrolyte solutions. This view is supported by experimental and theoretical investigations of electrolyte transport through large pore membranes consisting of quartz powder or sintered glass and having pore diameters in the range from 0.1 to 10 μm (Drouin, 1969*a*, *b*). Therefore, the presented theory is expected to hold in the case of large tip microelectrodes with internal tip diameters not smaller than ~1 μm. Indeed, the theory receives strong support from the quantitative analysis of microelectrode ionophoresis in experiments with tritiated spermine, as is illustrated by Fig. 3. However, in the case of fine tip microelectrodes with internal tip diameters below 0.5 μm, the presence of surface charges and space charges need to be considered. The effects of surface charges in these electrodes are inversely related to the internal tip diameter and add to the potential at the tip (e.g., Snell, 1969).

The Nernst-Planck equation comprises the processes of diffusion and conduction. Consistent with the assumption of electroneutrality, each ionic species migrates independently of every other one. This statement neglects the presence of ionic atmospheres resulting from ion-ion interactions. Therefore, the Nernst-Planck equation is considered as an ideal law of ionic transport, strictly valid only in the limit of highly diluted electrolyte solutions.

The microelectrode is considered to affect ion transport only by the geometric dimensions of its tip as a truncated spherical cone. The calculations are based on the approximations for small angles of the taper. No assumptions are made about the chemical or electrical nature of the microelectrode material. For large tip microelectrodes, internal tip diameter and taper angle can be determined from optical measurements (e.g., Sakmann and Neher, 1983). Fabrication of microelectrodes with microprocessor control is a way to reproduce the same geometry in narrow limits without any user intervention (Bertrand et al., 1983).

The condition of steady state presumes that the initial concentrations of the internal electrolyte and that of the external electrolyte remain constant. Therefore, the condition for a steady state efflux can be approximated during an ionophoresis experiment if the loss of electrolyte from the microelectrode is made small compared with the quantity stored in the whole volume. This requirement supports the validity of the assumed boundary conditions at the tip of the electrode, stating that the electrolyte concentration is equal to the initial concentration of the external solution, and that the ion concentration of the filling solution is vanishingly small at the tip. Obviously, these boundary conditions are expected to hold particularly well in the case $w < 1$, where the equivalent concentration of the internal electrolyte is smaller than that of the external solution. However, in the case $w >> 1$, where the equivalent concentration of the internal electrolyte is made much higher than that of the external solution, an unstirred region of internal electrolyte might develop outside the tip of the electrode. Clearly then the assumed boundary conditions are violated, and the theory has to be extended to include ion transport through the unstirred region (see extended treatment by Purves, 1979). The validity of the assumed boundary conditions has to be tested experimentally for the range $w >> 1$.

Volume flow through the tip of the electrode is not considered in this paper. However, volume flow can be caused by a hydrostatic pressure gradient resulting from gravity (Purves, 1981) when exceeding capillary forces, by an osmotic pressure gradient between internal and external solutions, and by electroosmosis, when surface charges at the wall of the electrode generate an electric double layer at the interface to the internal electrolyte solution (Firth and DeFelice, 1971). Volume flow has to be considered when a pressure gradient is applied over the microelectrode (Firth and DeFelice, 1971). Then additional nonlinearities of the characteristics and instabilities of the boundary between internal and external electrolytes are to be expected, which may be similar to those found in porous membranes (e.g., Drouin 1969*a*, *b*; Meares and Page, 1972; Langer et al., 1981; and recent review by Meares, 1983).

APPENDIX

Diffusion of Electrolyte at the Tip

Continuity of concentration, C^{I}_s, of internal electrolyte at the tip requires

$$C^{\mathrm{I}}_s = C^{\mathrm{II}}_s \text{ at } r = r_o, \tag{A1}$$

if C^{II}_s denotes the concentration of filling electrolyte outside the microelectrode. Continuity of diffusional flux from the microelectrode into external spherical space is described by

$$-D\pi r_o^2 \theta^2 (\mathrm{d}C^{\mathrm{I}}_s/\mathrm{d}r) = D\,4\pi r_o^2 (\mathrm{d}C^{\mathrm{II}}_s/\mathrm{d}r) \text{ at } r = r_o. \tag{A2}$$

With the boundary conditions at the tip, $r = r_o$, $C_s = C^{\mathrm{I}}_s$, and within the electrode, $r = \infty$, $C_s = C_{os}$, integration of Eq. 10 gives

$$C_s = C_{os} - (C_{os} - C^{\mathrm{I}}_s)\, r_o/r. \tag{A3}$$

With the boundary conditions at the tip, $r = r_o$, $C_s = C^{\mathrm{II}}_s$, and the spherical external space, $r = \infty$, $C_s = 0$, integration of Eq. 10 results in

$$C_s = C^{\mathrm{II}}_s\, r_o/r. \tag{A4}$$

Differentiation of Eqs. A3 and A4 and insertion into Eq. A2 leads with Eq. A1 to the concentration of internal electrolyte at the tip

$$C^{\mathrm{I}}_s = C_{os}/(1 + 4/\theta^2). \tag{A5}$$

For small angles, θ, the approximation

$$C^{\mathrm{I}}_s/C_{os} \simeq \theta^2/4 = \pi\theta^2\,(\mathrm{rad})^2/4\pi\,(\mathrm{rad})^2 \simeq 0, \tag{A6}$$

is valid. The ratio of internal electrolyte concentrations at the tip and that of the initial filling solution is equal to the ratio of solid angles of the cone of the microelectrode and that of the external spherical space, and is, therefore, vanishingly small. The approximation (Eq. A6) is valid for angles, $\theta \leqq 5°$, even when electric current is applied (Purves, 1979). Eq. A6, with Eqs. A1 and 14, also implies that

$$w = C_o/C_o^* = |z_s|\,C_{os}/C_o^* \ll |z_s|\,C_{os}/|z_s|\,C_s = 4/\theta^2. \tag{A7}$$

The ratio of initial equivalent concentrations of internal and external electrolyte solutions is supposed to always be smaller than the ratio of equivalent concentrations of initial internal filling solution and that which is released at the tip. From Eq. A7 follows

$$|z_s| C_s^{\text{I}} \ll C_o^*. \tag{A8}$$

At the tip, the equivalent concentration of released filling electrolyte is negligibly small compared with the initial equivalent concentration of the external electrolyte. Therefore, C_o^* is the appropriate boundary electrolyte concentration at the tip.

Distribution of Ion Concentration in the Tip

The differential equation (Eq. 31) can be transformed into a first-order linear differential equation

$$(\mathrm{d}C_i/\mathrm{d}r) + H\, r_o\, C_i/r^2 f = A/r^2, \tag{A9}$$

with, A, being an undefined integration constant. The homogeneous differential equation related to Eq. A9 is

$$(\mathrm{d}C_h/\mathrm{d}r) + H\, r_o\, C_h/r^2 f = 0. \tag{A10}$$

Substitution of Eq. A10 with the derivative, $\mathrm{d}f/\mathrm{d}r$, of Eq. 15 gives

$$(\mathrm{d}C_h/\mathrm{d}r) - (\mathrm{d}f/\mathrm{d}r)\, H\, C_h/(1-w) f = 0. \tag{A11}$$

Further calculation leads to the solution of Eq. A11 in the form

$$C_h = K f^{H/(1-w)}, \tag{A12}$$

with K being an undefined integration constant. A solution of the nonhomogeneous differential equation can be found with the solution of the homogeneous differential equation in the form

$$C_i = C_h X, \tag{A13}$$

and its derivative

$$\mathrm{d}C_i/\mathrm{d}r = C_h\, (\mathrm{d}X/\mathrm{d}r) + (\mathrm{d}C_h/\mathrm{d}r)\, X. \tag{A14}$$

The unknown function is now X. Substitution of Eqs. A13 and A14 into Eq. A9 and using Eq. A11 results in

$$\mathrm{d}X/\mathrm{d}r = A/r^2\, C_h. \tag{A15}$$

Substitution of Eq. A15 with the derivative, $\mathrm{d}f/\mathrm{d}r$, of Eq. 15 and inserting Eq. A12 leads to the determination of the unknown function

$$X = B - \frac{A \cdot f^{1-[H/(1-w)]}}{K\, r_o\, (1-w)\left(1 - \dfrac{H}{1-w}\right)} \tag{A16}$$

with B being an undefined integration constant. Combining Eqs. A12, A13 and A16 gives the general solution of the nonhomogeneous differential equation (Eq. A9)

$$C_i = K \cdot B \cdot f^{[H/(1-w)]} - \frac{A \cdot f}{r_o\, (1-w)\left(1 - \dfrac{H}{1-w}\right)}. \tag{A17}$$

Eq. A17 has to be adjusted to the appropriate boundary conditions. (*a*) At the tip of the microelectrode, at $r = r_o$ and for small angles, θ, the ion concentration is approximately, $C_i = 0$ and $f = 1$; this condition transforms Eq. A17 into

$$C_i = \frac{A\, \{f^{[H/(1-w)]} - f\}}{r_o\, (1-w)\left(1 - \dfrac{H}{1-w}\right)}. \tag{A18}$$

(*b*) At $r = \infty$, the ion concentration is $C_i = \nu_i C_\infty = \nu_i C_o/|z_s| = \nu_i w C_o^*/|z_s|$ and $f = w$; this condition transforms Eq. A18 into the boundary adjusted solution of Eq. A9

$$C_i = \frac{\nu_i\, C_o^*\, w\, \{f - f^{[H/(1-w)]}\}}{|z_s|\, \{w - w^{[H/(1-w)]}\}}. \tag{A19}$$

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