Published in final edited form as:

Anal Chem. 2009 July 1; 81(13): 5146–5154. doi:10.1021/ac801984d.

Reverse current pulse method to restore uniform concentration profiles in ion-selective membranes. Part I: Galvanostatic pulse methods with decreased cycle time

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Abstract

The applications of ion-selective electrodes (ISEs) have been broadened through the introduction of galvanostatic current pulse methods in potentiometric analysis. An important requirement in these applications is the restoration of the uniform equilibrium concentration profiles in the ISE membrane between each measurement. The simplest restoration method is zero current relaxation, in which the membrane relaxes under open-circuit conditions in a diffusion-controlled process. This paper presents a novel restoration method using a reverse current pulse. An analytic model for this restoration method is derived to predict the concentration profiles inside ISE membranes following galvanostatic current pulses. This model allows the calculation of the voltage transients as the membrane voltage relaxes back towards its zero-current equilibrium value. The predicted concentration profiles and voltage transients are confirmed using spectroelectrochemical microscopy (SpECM).

The reverse current restoration method described in this paper reduces the voltage drift and voltage error by 10 to 100 times compared to the zero current restoration method. Therefore, this new method provides faster and more reproducible voltage measurements in most chronopotentiometric ISE applications, such as improving the detection limit and determining concentrations and diffusion coefficients of membrane species. One limitation of the reverse current restoration method is that it cannot be used in a few applications that require background electrolyte loaded membranes without excess of lipophilic cation exchanger.

Introduction

Ion-selective electrodes (ISEs) are widely used as inexpensive, zero-current, potentiometric sensors in a variety of applications.^{1–3} However, non-equilibrium applications have recently gained importance to improve the detection limit of ISEs,^{4–7} to modify the selectivity of membranes and detect large polyions such as protamine and heparin,^{8–15} to determine the concentrations and diffusion coefficients in ISE membranes,^{16–22} and to determine the distance of an ISE tip from the surface in scanning electrochemical microscopy.²³ In these non-equilibrium methods, the sensing membranes of ionophore-based ISEs are perturbed by an applied current or voltage.

The applied current or voltage changes the distribution of species inside the membrane. Following the perturbation of the uniform equilibrium concentration profiles by the applied

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current or voltage, the original concentration profiles have to be restored. During the restoration period the concentration profiles relax towards their original profiles in a diffusion-controlled process, while the membrane potential gradually approaches its equilibrium value. The simplest restoration method is termed as zero current relaxation. In the zero current relaxation method, following the current or voltage pulse, the membrane relaxes under open-circuit conditions as shown in Protocol I of Fig. 1, where t_{relax} is the total relaxation time divided by the initial pulse time (t_1). Unfortunately, the zero current relaxation process is very slow in most ion-selective membranes. For example, following a 5 minute chronoamperometric experiment, a 90 minute relaxation period was needed at zero current to obtain reproducible transients, and even longer times were needed to approach the equilibrium voltage.¹⁷ To accelerate the relaxation processes, Bakker et al. developed a restoration method using a voltage step between each current step.^{8–11, 14}

In this paper, novel membrane restoration protocols based on reversed current polarization are introduced and described theoretically (Protocols II and III in Fig. 1). The quantitative assessment of concentration profiles in current-pulsed ISE membranes requires the extension of our analytic mathematical model published recently.¹⁹ This extended analytic model allows the calculation of concentration profiles in space and time following multiple current steps, as well as the calculation of the time course of the membrane potential. The model is verified (i) by fitting the theory to concentration profiles recorded in ion-selective membranes with spectroelectrochemical microscopy (SpECM) during pulsed potentiometric experiments and (ii) by comparing the predicted and experimentally measured voltages in reverse current pulse experiments. Finally, the model is used to (i) find an optimal restoration current pulse sequence that provides the smallest voltage error following a given relaxation period and (ii) determine the minimum time one must wait to achieve an acceptable level of voltage error or voltage drift. The reverse current method is shown to decrease voltage errors and voltage drift compared to the zero current restoration method. Therefore, with the reverse current method the measurement/restoration cycle length can be reduced, i.e., the frequency of measurements can be increased in current pulse potentiometric applications, such as improving the detection limit^{4–7} and determining concentrations and diffusion coefficients of species inside the membrane.^{16–22}

Theory

A typical cation-selective membrane is composed of ~33% poly(vinyl chloride) (PVC), ~66% plasticizer such as bis(2-ethylhexyl) sebacate (DOS), ~1% ionophore and a small amount of lipophilic cation exchanger (generally between 30 and 85 mol% compared to the ionophore) such as sodium tetrakis[3,5-bis(trifluoromethyl) phenyl] borate (TFPB[–]). The ionophore provides the membrane its selectivity towards a specific ion, while the lipophilic cation exchanger (mobile lipophilic anionic sites), in addition to other benefits,^{24–26} provides the membrane its permselectivity.^{27–29} To reduce the membrane resistance for certain applications, the membrane is also loaded with a lipophilic background electrolyte, such as tetradodecylammonium tetrakis(4-chlorophenyl) borate (ETH 500), or bis(triphenylphosphoranylidene) ammonium tetrakis[3,5bis(trifluoromethyl) phenyl] borate (BTPPATFPB).³⁰ As in our previous work,¹⁹ the following assumptions are made to describe the concentration of species inside of the membrane in space and time: (1) there is electroneutrality inside the membrane, (2) voltage drops occur both at the membrane / solution interfaces and inside the membrane, (3) the membrane/solution interfaces are in thermodynamic equilibrium, (4) the activity coefficients are unity for all species within the membrane, (5) the membrane has the properties of a plane sheet of very large surface area compared to its thickness, (6) the diffusion coefficients are uniform within the membrane but not necessarily identical for all species, (7) ion-pair formation is negligible, (8) the ionophore and lipophilic anions do not leave the membrane phase, and (9) free primary ions do not

contribute to the current inside the membrane. In addition, this work assumes that the membrane contains a lipophilic cation exchanger.

At equilibrium, the concentration profiles of the free ionophore (L), the ion-ionophore complex (IL_k^{n+}) and the lipophilic anion (R^-) in ionophore-based ion-selective membranes are horizontal (uniform). However, when a current is applied to the membrane, the concentration profiles of all species are perturbed, and the membrane potential changes as described by Eq. 1:¹⁹

$$\Delta V = \frac{RT}{nF} \ln \left(\left(\frac{C_L(-d)}{C_L(d)} \right)^k \left(\frac{C_{IL_k^{n+}}(d)}{C_{IL_k^{n+}}(-d)} \right)^\lambda \frac{\tilde{C}_{R^-}(-d)}{\tilde{C}_{R^-}(d)} \right) - I_{\text{appl}} R_{\text{ohm}} \quad (1)$$

where R, T, and F have their usual meanings, ΔV is the total voltage drop across the membrane, k is the ionophore:ion stoichiometry, n is the primary ion charge, C_L and $C_{IL_k^{n+}}$ are the concentrations of the free ionophore and ion-ionophore complex, respectively, $(-d)$ and (d) indicate the two boundaries of the membrane having a thickness of $2 \times d$, tildes indicate solution concentrations, I_{appl} is the applied current, and R_{ohm} is the resistance between two reference electrodes placed on the opposite sides of the membrane (i.e., the total resistance of the membrane and solution, which is assumed to be constant in this work). The exponent λ is equal to one for membranes without migration (i.e., membranes with a large concentration of dissociated background electrolyte), and $\lambda = 2 \times t_-$ for membranes with migration, where t_- is

the transference number of the anions: $t_- = \frac{D_{R^-}}{nD_{IL_k^{n+}} + D_{R^-}}$, and $D_{IL_k^{n+}}$ and D_{R^-} are the ion-ionophore complex and lipophilic anion diffusion coefficients, respectively.

At large current densities and long times, the free ionophore or the ion-ionophore complex concentration can approach zero at one of the membrane-solution boundaries. At this time, $\tau_{1,L}$ or $\tau_{1,IL_k^{n+}}$, respectively, a characteristic drop in the voltage (a breakpoint) appears in the chronopotentiometric transients.^{16–18, 20, 21} The theory proposed in this work assumes that the applied currents and times are sufficiently small that a breakpoint does not occur in the chronopotentiometric transients, i.e., the duration of the applied current pulse is shorter than the breakpoint times.

Analytic model for multiple current steps

To calculate the concentrations in space and time in ion-selective membranes perturbed with multiple current steps, like for the protocols shown in Fig. 1, we derived an extension of our previously published model aimed for a single current step.¹⁹ The derivation is provided in the supplementary information. It assumes that the ion- and perm-selectivity of the membrane are preserved during the experiment, i.e., no interfering cations or anions enter the membrane. The concentration profile of the free ionophore in the membrane following multiple current steps can be calculated as the sum of changes in the concentration profiles induced by the individual current steps:

$$C_L(x, t) = C_L^0 + \sum_{j=1}^q \Delta C_{L,j}(x, t) \quad (2)$$

where $C_L(x, t)$ is the free ionophore concentration in the membrane as a function of location and time, C_L^0 is the initial, uniform concentration of the free ionophore in the membrane, q is

the number of current steps, and $\Delta C_{L,j}(x,t)$ is the concentration profile change due to individual current steps for $j = 1$ to q :

$$\Delta C_{L,j}(x,t) = \frac{k(I_j - I_{j-1})}{nAF} \frac{u_{t_j}(t)}{\sqrt{D_L}} \sum_{m=0}^{\infty} (-1)^m \left[\begin{aligned} &2\sqrt{\frac{t-t_{j-1}}{\pi}} \exp\left(-\frac{[(2m+1)d-x]^2}{4D_L(t-t_{j-1})}\right) - \frac{(2m+1)d-x}{\sqrt{D_L}} \operatorname{erfc}\left(\frac{(2m+1)d-x}{2\sqrt{D_L(t-t_{j-1})}}\right) \\ &-2\sqrt{\frac{t-t_{j-1}}{\pi}} \exp\left(-\frac{[(2m+1)d+x]^2}{4D_L(t-t_{j-1})}\right) + \frac{(2m+1)d+x}{\sqrt{D_L}} \operatorname{erfc}\left(\frac{(2m+1)d+x}{2\sqrt{D_L(t-t_{j-1})}}\right) \end{aligned} \right] \quad (3)$$

where I_j is the current applied between times t_{j-1} and t_j , $u_{t_j}(t)$ is the unit step function (equal to zero before time t_i and one after time t_i), A is the membrane surface area, D_L is the diffusion coefficient of the free ionophore, C_L^0 and $C_{\text{IL}_k}^0$ are the equilibrium concentrations of the free ionophore and ion-ionophore complex, respectively, and $2 \times d$ is the membrane thickness. Concentrations were calculated using a script written in Matlab. The error function solutions were used in this work rather than the theta functions described previously^{19, 31} because the error functions give smaller errors for short applied current times (i.e. on the order of seconds or minutes). Values from $m = 0$ to 4 were used in the summation terms, which gave less than 0.01% error in the boundary concentration changes for $t < 10000$ s.

Similar equations can be derived for the concentration of the ion-ionophore complex. If the role of migration is insignificant in the membrane, $\Delta C_{\text{IL}_k,j}(x,t)$ can be calculated using Eq. 3 by replacing the diffusion coefficients of the ionophore (D_L) with the diffusion coefficients of the ion-ionophore complex (D_{IL_k}) dividing by k , and using the opposite sign for the concentration changes. Migration is expected to be small in membranes loaded with a high concentration of background electrolyte. However, if the role of migration is not negligible, then Eq. 3 can be modified as follows: (1) D_L should be replaced by D_S and (2) the right side

$$D_S = \frac{(n+1)D_{R^-}D_{\text{IL}_k}}{n(D_{\text{IL}_k} + D_{R^-})}$$

should be multiplied by $-t_-/k$, where D_S is the salt diffusion coefficient:

In the next chapter, the dependence of the voltage error on the relative concentration error is shown. Since the relative concentration error is independent of the diffusion coefficients, the results discussed in this paper are valid both for the free ionophore and for the ion-ionophore complex with and without migration.

Converting concentration changes to voltage changes

In pulsed current methods, the voltage is usually the analytic signal. This voltage is logarithmically dependent on the concentrations of the free ionophore and the ion-ionophore complex at both membrane boundaries (Eq. 1). During the relaxation period (t_{relax} in Fig. 1), the boundary concentrations decay back towards their original equilibrium concentrations, so that the voltage also decays back towards the equilibrium voltage. The difference between the measured voltage and the equilibrium voltage is the voltage error (V_{err}). The voltage error can be estimated from the boundary concentration errors ($C_{\text{err}} = C(\pm d) - C^0$), i.e., the deviations in the boundary concentrations from their equilibrium concentrations. As long as the concentration errors at the phase boundaries are small compared to the absolute concentrations, the related voltage error has a close to linear dependence on these concentration errors. Assuming the concentration errors are small ($C_{\text{err}}/\Delta C < 0.3$) and the applied current time t_1 is less than the breakpoint time τ_1 , the relative error in concentration change ($C_{\text{err}}/\Delta C$) can be converted to a voltage error due to changes at both membrane boundaries using $\Delta C/C^0 = (t_1/\tau_1)^{1/2}$ and Eq. 1:

$$V_{\text{err}} \approx \frac{2\gamma RT}{nF} \frac{C_{\text{err}}}{\Delta C} \sqrt{\frac{t_1}{\tau_1}} \approx \frac{C_{\text{err}}}{\Delta C} t_1^{1/2} \left. \frac{dV}{dt^{1/2}} \right|_{t=+0} \quad (4)$$

where τ_1 is the breakpoint time (the time when the concentration of the free ionophore ($\tau_{1,L}$) or the ion-ionophore complex reaches ($\tau_{1,IL}^{n+}$) zero at the boundary),^{16–18, 20, 21} ΔC is the boundary concentration change during the initial applied current pulse, and $\gamma=k$ for the free ionophore and $\gamma=\lambda$ for the ion-ionophore complex. Similarly, the voltage drift can be described as:

$$\frac{dV}{dt} \approx \frac{2\gamma RT}{nF\Delta C} \frac{dC_{\text{err}}}{dt} \sqrt{\frac{t_1}{\tau_1}} \approx \frac{dC_{\text{err}}}{\Delta C dt} t_1^{1/2} \left. \frac{dV}{dt^{1/2}} \right|_{t=+0} \quad (5)$$

As shown in Eqs. 4 and 5, the voltage error or the voltage drift can be calculated either from the breakpoint times ($\tau_{1,L}$ and $\tau_{1,IL}^{n+}$) or from the initial voltage transient during the applied current step.

When V_{err} and dV/dt are calculated from the breakpoint times, $C_{\text{err}}/\Delta C$ and $dC_{\text{err}}/(dt\Delta C)$ are the same for the free ionophore and the ion-ionophore complex, since these ratios are independent of the absolute concentrations and the diffusion coefficients, but the breakpoint times for the free ionophore ($\tau_{1,L}$) and ion-ionophore complex ($\tau_{1,IL}^{n+}$) will generally be different. The breakpoint times can be determined either theoretically (from prior knowledge of the concentrations and diffusion coefficients in the membrane and the surface area of the membrane) or experimentally.^{16–18, 20, 21} The experimental determination of the breakpoint times of both the free ionophore and ion-ionophore complex from a single voltage transient will be discussed in a future paper. When the voltage error is calculated from the breakpoint times, the total voltage error will be the sum of the voltage errors of the free ionophore and ion-ionophore complex.

A simpler and often more accurate method for determining V_{err} and dV/dt uses the voltage transient triggered by the initial applied current pulse (see the second part of Eqs. 4 and 5). It requires only the measurement of the voltage change induced by the applied current. This voltage change should be a linear function of the square root of time ($t^{1/2}$) for short times, i.e., when the logarithmic term in Eq. 1 can be linearized. Therefore, the total change in boundary concentrations during the current pulse, as well as the voltage transients during membrane restoration, can be calculated by multiplying the slope of the ΔV vs. $t^{1/2}$ function by $t_1^{1/2}$.

Materials/Methods

For ISE membrane casting, poly(vinyl chloride) high molecular weight (PVC) (Fluka-81392), bis(2-ethylhexyl) sebacate (DOS) (Fluka-84818), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate (NaTFPB) (Dojindo Laboratories), the H^+ -selective chromoionophore (ETH 5294, 9-(Diethylamino)-5-octadecanoylimino-5H-benzo[a]phenoxazine) (Fluka 27086), and tetrahydrofuran (THF) from Sigma were used. The spacer ring membranes used in the SpECM studies^{18, 32–34} were cast from polyurethane (Tecoflex SG85A, Thermedics Polymer Products, Woburn, MA) and plasticized with 2-nitrophenyl octyl ether (o-NPOE) (Fluka 73732).

SpECM Concentration Profiles

In the spectroelectrochemical microscopy (SpECM) experiments, a Gamry Reference 600 potentiostat/galvanostat was used with a homemade spectroelectrochemical cell.^{18, 32–34}

SpECM allows imaging of the concentration profiles of the free and complexed ionophores in current-polarized membranes with simultaneous recording of the voltage transients during the chronopotentiometric measurements. SpECM experiments and ion-selective membrane preparations were performed as described previously³² using a Nikon Eclipse E600 microscope (Southern Micro Instruments, Atlanta, GA, <http://www.southernmicro.com>) connected to a PARISS® (LightForm, Inc., Hillsborough, NJ, <http://www.lightforminc.com>) spectroscopic imaging spectrometer. Briefly, the microscope was focused onto a cross-section of a ~320 μm -wide membrane strip, with spectra collected at 240 pixels. A 10 $\times$ objective was used, so that the membrane strip covered ~90% of the field of view and the pixel width was 1.7 μm . A complete spectrum between 400 and 800 nm was collected for each pixel.

The membrane contained a 1:2 ratio of PVC and DOS, 1.2 mM ionophore ETH 5294, and 39 mol% NaTFPB relative to the ionophore. The estimated membrane width ($2 \times d$) was 320 ± 12 μm , and the membrane thickness was approximately 160 μm . The spacer ring membranes used in the thin layer spectroelectrochemical cell³² were made from 41 wt. % o-NPOE and 59 wt. % of Tecoflex SG85A. The 283 mg of the polyurethane/o-NPOE mixture was dissolved in 2 mL THF, and poured into a glass cylinder (i.d. 30 mm) fixed on a glass substrate. The membrane thickness obtained after THF evaporation was also approximately 160 μm . The transient absorbance profiles were recorded in the following experiments: A current of 10 nA was applied for 240, 210, or 180 s. This initial current pulse was followed by a reverse current pulse of -23.83 nA for 100.70, 88.124, or 75.535 s, respectively, and finally by a zero current relaxation period until the end of the experiment. The spectra were obtained (1) at equilibrium before the constant current polarization, (2) every 30 s during the first seven minutes of the experiment, (3) every 60 s between 8 and 15 min, and (4) at 17 and 20 min.

The absorbance profiles were converted to concentration profiles using the absorbance values measured at wavelengths of 502 and 660 nm. These wavelengths were chosen for the unprotonated and protonated chromoionophores, respectively, in order to have large absorptivities with minimal overlap. The residual interference related to the partial overlap of the spectra of the protonated and unprotonated ionophores was compensated using the absorbance values measured at 502 and 660 nm in membrane segments in which only the unprotonated or protonated ionophore was present. This could be achieved by polarizing the membrane with large currents as described previously.¹⁸ From these measurements, the following molar absorption coefficient (ϵ) ratios were determined: $\epsilon_{\text{unprot.,502}}/\epsilon_{\text{prot.,502}} = 5.6$, $\epsilon_{\text{prot.,660}}/\epsilon_{\text{unprot.,660}} = 51$, and $\epsilon_{\text{prot.,660}}/\epsilon_{\text{unprot.,502}} = 2.7$. To eliminate some of the noise from the measurements, the concentration profile recorded at time zero was subtracted from the subsequent concentration profiles. Also, to minimize errors due to changes in light source intensity and due to leakage or decomposition of the ionophore, the offset in the overall average concentration after time zero was eliminated by setting the average concentration to zero.

Next, Eqs. 2 and 3 were fitted to the concentration profiles recorded after time zero for the unprotonated ionophore by simultaneously varying the membrane boundary positions, the initial current density (I_1/A), and the unprotonated ionophore diffusion coefficient (D_L) (e.g., see Fig. 2(a)). Even though the total applied current was known, the current density was estimated by this fitting protocol due to uncertainty in the membrane surface area. After finding I_1/A , D_L , and the locations of the membrane boundaries, the values of $D_{\text{IL}^{\text{n+}}}$ and D_S were simultaneously determined by fitting Eqs. 2 and 3 (modified for the ion-ionophore complex as described above) to the concentration profiles after time zero for the protonated ionophore (see Fig. 2(b)). When the concentration profiles during the forward, reverse, and zero current steps are fitted simultaneously, each of the fitted parameters is independent of the others.

Experimental Voltage Transients

The experimental voltage transients were collected using the Gamry Reference 600 potentiostat/galvanostat with the membrane spanned into a Philips electrode body (IS-561, Moller Glasblaserei, Zurich, Switzerland). The membrane contained PVC and DOS in 1:2 ratio, 4.8 mM ionophore ETH 5294, and 2.4 mM NaTFPB. The current pulses were applied to the studied membranes in a three-electrode setup in which the Ag/AgCl wire inside the Philips electrode body served as working electrode, a coiled platinum wire served as the counter electrode, and a double-junction Ag/AgCl electrode served as the reference electrode (Orion model 900200 with Orion 900002 inner filling solution and 1 mM HCl outer solution). The H^+ -selective membrane was conditioned with 1 mM HCl for more than 2 days prior to the experiment. Data were collected every 1 ms during the forward and reverse current pulses, and every 0.1 s during the zero current relaxation. Three replicates were performed for each experiment and averaged. Membranes were allowed to relax at zero current for at least 15 min between experiments to reestablish a stable voltage before the next experiment. To find the voltage-time slope, dV/dt was calculated using a moving slope technique to reduce the noise in the derivative. Specifically, a second order polynomial has been fitted to the 19 points surrounding of each point (± 0.9 s) using the Matlab script "Movingslope.m", which is freely available at <http://www.mathworks.de/matlabcentral/fileexchange/>.

Results

Reverse Current Restoration Protocols: Simulations and Limitations

Using the theoretical models developed in this paper, the efficacy of the restoration protocols II and III in Fig. 1 were compared first, since they have not been tested previously. The efficacy of the restoration protocols were quantified by the concentration errors ($C_{err} = C(\pm d) - C^0$), i.e., the deviations in the boundary concentrations from their equilibrium concentrations. In protocol III, between 3 and 9 alternating pulses were used, with 1) decreasing current magnitudes and constant pulse times, 2) decreasing pulse times and constant currents, or 3) decreasing both currents and times. These simulations showed that the most efficient program is a single very short current pulse with the opposite polarity of the initial current pulse, but with similar total charge, followed by zero current relaxation (protocol II). Protocol III, in which multiple alternating current pulses are applied with varying times and magnitudes, did not perform better than the single reverse current pulse in protocol II, regardless of the sequence of current magnitudes and times. Since the efficacy of protocol III was not better than protocol II and protocol III is also more complicated, protocol III is not examined further in this paper, and only protocols I and II are compared.

Theory predicts that the fastest restoration can be achieved with an infinitely short reverse current pulse that contains the same total charge as the initial current pulse. However, the application of an infinitely short pulse is not feasible experimentally, and the application of very large currents is not ideal for several reasons. First, a very large reverse current requires that the potentiostat uses a large current range. However, a large current range is associated with inadequate current resolution, i.e., the initial current magnitude cannot be specified accurately. Second, the limited time resolution of the potentiostat may cause significant errors because very short pulse times cannot be specified with adequate accuracy. Third, the RC time constants of the instrumentation or membrane can be significant at very short times. Finally, and often most importantly, the utilization of very large currents is impractical because if the reverse current pulse is too large, the boundary concentration of the free ionophore or ion-ionophore complex will approach zero.¹⁸ When the free ionophore concentration approaches zero, a breakpoint appears in the voltage transients and interfering ions can enter the membrane, which is not desirable during membrane restoration. Also, when either species approaches zero concentration at the phase boundary, the assumptions of our theory are no longer valid.

Consequently, an optimized experimental setting utilizes a large reverse current that does not permit a breakpoint to occur on the chronopotentiometric transients. The breakpoint time for a given reverse current setting can be calculated theoretically from the ordinary chronopotentiometric breakpoint time (τ_1), which has been determined experimentally upon the application of an adequate current step in an independent experiment. The calculations are provided in the supporting information.

In many applications, instead of utilizing an experimentally determined breakpoint time τ_1 to calculate the optimal reverse current pulse width (t_2) and magnitude (I_2) without a breakpoint, it may be sufficient to use a conservative estimate for I_2 as $I_2 = -2.383 \times I_1$. This estimate is obtained by solving Eq. S3(b) in the supporting information with $t_1 = \tau_1$ and $f_{\text{charge}} = 1$, where f_{charge} is the fraction of the charge forced out of the membrane in the reverse current pulse compared to the charge delivered in the forward current pulse:

$$f_{\text{charge}} = -I_2 \frac{(t_2 - t_1)}{I_1 \cdot t_1} \quad (6)$$

With $I_2 = -2.383 \times I_1$, no breakpoint is expected during the restoration pulse, assuming that no breakpoint appeared during the forward current pulse, i.e., the forward current pulse width t_1 is less than the breakpoint time τ_1 . This conservative value for I_2 is used in both the simulated and experimental results of this paper to give an upper limit on the expected errors. An infinitely large and short current pulse may decrease the voltage error by about 30% compared to using $I_2 = -2.383 \times I_1$. Although an infinite pulse is not feasible experimentally, the errors can be reduced for experiments with $t_1 \ll \tau_1$ by selecting the reverse current pulse width and amplitude from the experimentally determined breakpoint time τ_1 and using the equations for τ_2 in the supporting information. Certainly, the instrumental limitations discussed above for short pulse times should also be kept in mind.

Experimental and fitted concentration profiles in ISE membranes during reverse current restoration experiments—

In order to verify our theoretical description of the reverse current restoration method, concentration profiles of the H^+ ion-selective chromoionophore ETH 5294 were measured using SpECM during the forward current, reverse current, and zero current steps. Then, Eqs. 2 and 3 were fitted to the experimental concentration profiles, as shown in Fig. 2, by varying the membrane boundary locations, the applied current density, and the diffusion coefficients of the free ionophore, ion-ionophore complex, and lipophilic anion inside the membrane, as described in the Methods section. For the membrane used in this work, the best fits were found for $D_L = 2.48 \pm 0.09 \times 10^{-8} \text{ cm}^2/\text{s}$,

$D_{\text{LH}^+} = 1.79 \pm 0.10 \times 10^{-8} \text{ cm}^2/\text{s}$, $D_S = 1.28 \pm 0.05 \times 10^{-8} \text{ cm}^2/\text{s}$, $D^{\text{R}^-} = 1.00 \pm 0.06 \times 10^{-8} \text{ cm}^2/\text{s}$, and $t_- = 0.36 \pm 0.02$ ($n=3$). The mean concentration errors of the fits were $0.025 \pm 0.002 \text{ mM}$ ($3.4 \pm 0.3 \%$) and $0.005 \pm 0.001 \text{ mM}$ ($1.1 \pm 0.2 \%$) for the free ionophore and ion-ionophore complex, respectively. It should be noted that these errors depend more on noise in the measurement than on the accuracy of the fit.

The concentration profiles in an ion-selective membrane at equilibrium are approximately horizontal. As shown in Fig. 2, this horizontal profile is disturbed in pulsed-current potentiometric experiments. During the initial current pulse, hydrogen ions are forced into the left side of the membrane, causing the free ionophore concentration to decrease and the ionophore complex concentration to increase, as shown previously.^{18, 19} Then, when the reverse current is applied, the boundary concentrations change in the opposite direction because ions are driven in the opposite direction. Finally, during the zero current relaxation step, the concentrations relax back towards their original flat equilibrium concentrations. During these

steps, opposite but symmetric processes are occurring on the other, right hand side of the membrane.

Fig. 2 demonstrates that the fitted curves match very well the experimentally recorded concentration profiles of the free ionophore. The fit for the ion-ionophore complex is slightly worse during the reverse current step. Although the reason for this difference in the quality of the fits is not entirely clear, the concentration profiles during the reverse current are highly dependent on the calculated position of the membrane/solution boundary. Identifying these boundaries is not trivial, because the membrane-solution boundaries are generally not perfectly planar as assumed in the theoretical derivation. Also, it is difficult to align the optics of the system perfectly. In addition, ion-pairing may affect the concentration profiles, although it should be minimal at these concentrations.³⁵ Even with these deviations, the fit for the ion-ionophore is quite good, and the theory appears to be adequate.

Boundary concentration and voltage transients during the zero-current relaxation step in different restoration protocols—In pulsed potentiometric methods, it is essential to know the maximum frequency of measurements, i.e., the time one must wait to have the voltage drift (dV/dt) below a certain value or to have the measured voltage approach the equilibrium voltage with certain accuracy (V_{err}). The voltage drift and voltage error are primarily dependent on the boundary concentrations, because at zero current the voltage is usually determined mostly by the voltage drops across the membrane boundaries, as described by Eq. 1. Eqs. 4 and 5 describe how to calculate the voltage error from boundary concentration errors, and how to calculate the voltage drift from boundary concentration transient slopes, respectively.

The boundary concentration transients during the restoration protocols were calculated using Eqs. 2 and 3. It was found that $C_{\text{err}}/\Delta C$ and $dC/(dt_{\text{relax}}\Delta C)$ were independent of most experimental parameters, including the initial current pulse width and amplitude, the diffusion coefficients, and the membrane thickness, except for very long times when $t > d^2/D$. Therefore, the percent error of concentration depends only on the relative relaxation time $t_{\text{relax}} = (t - t_1)/t_1$, and the relative charge utilized in the reverse current pulse, f_{charge} . For $f_{\text{charge}} = 1$ (i.e., equal charge in forward and reverse current pulses), a plot of $\log(C_{\text{err}}/\Delta C)$ vs. $\log(t_{\text{relax}})$ is approximately linear for $t_{\text{relax}} > 2$, so that $C_{\text{err}}/\Delta C = -0.164 \times (t_{\text{relax}})^{-1.48}$. From simulations performed using Eqs. 2, 3, and 6 with many different values of f_{charge} , it was found that this equation can be generalized for all f_{charge} as:

$$\kappa = a \cdot (t_{\text{relax}})^b \cdot \left(1 - (1 - f_{\text{charge}}) \cdot (c \cdot t_{\text{relax}} + d)\right) \quad (8)$$

where κ , a , b , c , and d are given in Table 1. Similar equations were found for the slopes of the concentration-time profiles, for which $dC/(dt_{\text{relax}}\Delta C) = 0.240 \times (t_{\text{relax}})^{-2.48}$ for $f_{\text{charge}} = 1$ and $t_{\text{relax}} > 2$, which can be generalized for all f_{charge} as described in Eq. 8 and Table 1. If no reverse current pulse is used (i.e., protocol I in Fig. 1), then Eq. 8 can be used with $f_{\text{charge}} = 0$ and the parameters listed for the zero current method in Table 1.

In Fig. 3 and Fig 4, the concentration errors and concentration-time slopes are plotted vs. t_{relax} for the zero current method and for the reverse current method with several values of f_{charge} . These plots are independent of the magnitudes of the concentrations and the initial current pulse width and magnitude. Fig. 3(a) shows that the boundary concentration error (and therefore the voltage error) approaches zero much more quickly with the reverse current method compared to the zero current method. In Fig. 3(b), the asymptotic section of Fig. 3(a) is magnified to demonstrate the effect of f_{charge} on the concentration error during the reverse current method. It is apparent that the error at very long relaxation times is smallest for

$f_{\text{charge}}=1$, because with $f_{\text{charge}}<1$ only a fraction of the charge introduced during the initial current pulse is removed during the reverse current pulse, so that the ratio $C_{\text{err}}/\Delta C$ overshoots zero. However, the error can be reduced at shorter relaxation times by using $f_{\text{charge}}<1$.

Fig. 4 shows the changes in the slopes of the concentration-time profiles as a function of time during the restoration period. Similar to the concentration errors in Fig. 3, the slopes of the concentration-time profiles approach their equilibrium values faster when using the reverse current restoration method compared to the zero current method. However, compared to the concentration errors, the overshoots of the transients in Fig. 4 are less significant for $f_{\text{charge}}<1$. Therefore, if minimizing the voltage drift at short times is most important, and a small voltage drift can be tolerated at longer times, then $f_{\text{charge}}<1$ may be the best choice. On the other hand, if the accurate restoration of the original equilibrium voltage is most important, then a restoration pulse with an f_{charge} of one or very close to one may be the best approach. In practice, at long relaxation times it may not be possible to distinguish between similar values of f_{charge} , but the reverse current method is clearly better than the zero current method at all times.

To verify the theoretical predictions of Fig. 3 and Fig 4, current pulse experiments were performed with membranes containing 4.8 mM ETH 5294 and 2.4 mM (50 mol%) TFPB⁻. In these experiments, a 600 nA current pulse was passed through the membrane for 1 s, resulting

in $t_1^{1/2} \frac{dV}{dt^{1/2}} \Big|_{t=+0} = 187.8 \text{ mV}$ (the $dV/dt^{1/2}$ vs. $t^{1/2}$ plot was linear from $t=+0$ to $t=0.9$ s). Following the initial current pulse and a reverse current pulse with $f_{\text{charge}}=1$ or 0.95 or no reverse current pulse (i.e., $f_{\text{charge}}=0$), the relaxing voltages were measured at zero current. In Fig. 5, the experimental voltage errors and the theoretically predicted voltage errors are compared. From Fig. 5(a), it is clear that in agreement with the theory, the voltage relaxes much slower with the zero current method compared to the reverse current method. From Fig. 5, it is also clear that the theory predicts the experimental errors quite well for all restoration methods, although the agreement is best for the zero current method. For the reverse current method, the experimental errors decay slightly less quickly than predicted theoretically. Interestingly, the experimental errors for $f_{\text{charge}}=0.95$ are very similar to the theoretical errors for $f_{\text{charge}}=1$, and the experimental errors for $f_{\text{charge}}=1$ are very similar to the theoretical errors for $f_{\text{charge}}=1.05$. Very similar results are observed for the voltage drift, as shown in Fig. 6. The reason for this difference between theory and experiment is unclear, but it appears that $f_{\text{charge}}=0.95$ is probably close to optimal to minimize the experimental voltage errors and voltage drift. One possible cause of this difference is the asymmetry of the inner and outer surface areas of the membrane in the Philips electrode body.

The experimental voltage transients are reasonably reproducible, with the standard deviation becoming smaller as time increases. For the zero current restoration method, the standard deviation of V_{err} is less than 0.25 mV for $t_{\text{relax}}>1$ (n=3). For the reverse current methods, the standard deviations are larger at short times ($t_{\text{relax}}<5$). However, longer times are usually more important for practical measurements, and the standard deviations for the reverse current methods decay from 0.5 mV to less than 0.1 mV from $t_{\text{relax}}=5$ to 100 (n=3).

Discussion

The theoretical simulations in this paper for calculating the concentration profiles in ion-selective membranes during the reverse current restoration protocol are based on a few assumptions that are important for interpreting the results. First, it is assumed that membrane selectivity is maintained, so no interfering ions are in the membrane. A second assumption is that mobile lipophilic anionic sites from the incorporated lipophilic ion exchanger are present in the membrane. In addition, relatively short pulse times were used in the simulations, so that the membrane thickness did not have a significant effect on the results. However, processes at

one side of the membrane can influence concentrations on the opposite side when 1) the membrane thickness is very small, 2) diffusion coefficients are very large, or 3) measurement/restoration times are long. A few simulations were run with longer pulse times, and, in general, slightly smaller errors were obtained compared to those summarized in this paper. If the dimensionless time constant $D \times t / d^2 \ll 1$, the membrane can be assumed to be infinitely thick. This condition is usually satisfied for current pulse experiments. However, if the membrane is thin (e.g., thin Celgard® polypropylene membranes¹¹), the diffusion coefficients are large, or experimental times are long, then smaller errors than those in this work could probably be obtained.

Conclusion

In this work, a novel reverse current method is proposed for pulsed potentiometric experiments to restore the original uniform concentration profiles in ISE membranes more quickly. An analytic theory is developed that accurately predicts the concentration profiles during consecutive forward, reverse, and zero current steps. The theory also predicts the experimental voltage error and voltage drift as they decay over time, thereby allowing one to estimate the time necessary to achieve a voltage drift or voltage error smaller than a threshold value. Interestingly, although the theory predicts that the reverse current pulse should contain 98–100% of the charge of the initial pulse, experimentally 95% of the charge gives similar results to what is theoretically predicted for 100%. Although the reasons for this difference are unclear, it appears that the experimental voltage error and voltage drift can be minimized with a reverse pulse containing about 95% of the charge of the initial pulse. Regardless of the amount of charge in the reverse pulse, the reverse current method is proven to be a very effective new restoration method for membranes containing a lipophilic cation exchanger. It reduces the voltage error and voltage drift by 10 to 100 times compared to the zero current method. Using the results of this work, more efficient protocols can be devised for current pulse experiments aimed at improving the detection limit of ISEs, determining concentrations and diffusion coefficients of membrane species, and calculating the distance of an ISE SECM tip from a surface. A subsequent paper will demonstrate the advantages of the reverse current method compared to the commonly used applied voltage restoration method during many consecutive measurement cycles.³⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work has been supported by the NIH/NHLBI #1 RO1 HL079147-01 and the NSF #0202207 grants.

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Fig. 1. Schematics of voltage transients (dashed blue lines) upon the application of different membrane restoration protocols using current steps (thick red lines): (I) Zero current relaxation, (II) Reverse current relaxation, and (III) Multiple current pulses controlled relaxation. t_{relax} is the total relaxation time divided by the initial pulse time (t_1).

Fig. 2.

Experimental SpECM (thin solid lines) and theoretical fitted (thick dotted lines) concentration profiles of the free ionophore (a) and the ion-ionophore complex (b) in ion-selective membranes during an initial current pulse and upon the application of the single reverse current pulse restoration protocol (II in Fig. 1). Equations 2 and 3 were used for the fitting. For each time instance, the same colors are used for the experimental and theoretical profiles. For clarity, only the left third of the membrane is shown, with arrows indicating the directions of the concentration changes at the boundary during the forward, reverse, and zero current steps. The membrane contained 1.2 mM ETH 5294 and ~39 mol% TFPB⁻ relative to the ionophore. The applied current parameters were: $I_1=10\text{ nA}=500\pm30\text{ nA/cm}^2$, $I_2=-23.83\text{ nA}$, $t_1=210\text{ s}$, $t_2=88.12\text{ s}$, and $f_{charge}=1$. The individual concentration profiles shown in the figures were recorded during the initial current pulse at times $t=0, 0.5, 1.5, 2.5, 3.5\text{ min}$; the reverse current pulse at times $t=4, 4.5, 5\text{ min}$; and the zero current relaxation period at times $t=5.5, 7, 9, \text{ and } 20\text{ min}$. Although additional concentration profiles were recorded (at $t=1, 2, 3, 6, 6.5, 8, 10, 11, 12, 13, 14, 15, \text{ and } 17\text{ min}$) and fitted, they are not shown for clarity.

Fig. 3.

(a) Comparison of the relative concentration errors over time from simulations using Eq.8 for the zero current (protocol I) and reverse current (protocol II) restoration methods. (Note that the zero current values were multiplied by -1 for comparison). (b) Magnification of part (a) to compare the relative concentration errors for the reverse current method with $f_{charge}=1$, 0.98, 0.96, 0.94, and 0.92.

Fig. 4.

(a) Comparison of the relative slopes of the concentration-time profiles over time from simulations using Eq. 8 for the zero current (protocol I) and reverse current (protocol II) restoration methods. (Note that the zero current values were multiplied by -1 for comparison). (b) Magnification of part (a) to compare the relative slopes of the concentration-time profiles for the reverse current method with $f_{\text{charge}} = 1, 0.98, 0.96, 0.94$, and 0.92 .

Fig. 5.

(a) Comparison of the experimental (dotted) and theoretical (solid, from Eq. 8) voltage errors over time for the zero current (blue) and reverse current restoration methods with $f_{charge}=1$ (black), 0.95 (red), and 1.05 (solid grey, theoretical only). (Note that the zero current values were multiplied by -1 for comparison). In (b), the plot is magnified for clarity. For the averaged experimental curves ($n=3$), an initial current of $0.6 \mu\text{A}$ was applied for $t_1=1$ s, followed by $-1.43 \mu\text{A}$ for 0.3987 s ($f_{charge}=0.95$) or 0.4196 s ($f_{charge}=1$) for the reverse current methods, followed by zero current. The membrane contained 4.8 mM ETH 5294 and 2.4 mM NaTFPB. For the theoretical curves, the second part of Eq. 4 was used with the experimental $dV/dt^{1/2}=187.8$ mV.

Fig. 6.

(a) Comparison of experimental (dotted) and theoretical (solid) voltage-time slopes over time for the zero current (blue) and reverse current restoration methods with $f_{\text{charge}}=1$ (black), 0.95 (red), and 1.05 (solid grey, theoretical only). (Note that the zero current values were multiplied by -1 for comparison). In (b), the plot is magnified for clarity. Experimental and theoretical parameters are the same as Fig. 5.

Table 1
Parameters of Eq. 8 to calculate the concentration errors and the slopes of the concentration-time profiles

Method	κ	a	b	c	d
Reverse Current	$C_{\text{err}}/\Delta C$	-0.164	-1.48	2.82	1.39
Reverse Current	$dC/(dt_{\text{relax}}\Delta C)$	0.240	-2.48	0.939	1.12
Zero Current	$C_{\text{err}}/\Delta C$	-0.164	-1.48	2.82	0.0741
Zero Current	$dC/(dt_{\text{relax}}\Delta C)$	0.240	-2.48	0.940	0.215