Theoretical Treatment of Ohmic and Rectifying Properties of Electrolyte Filled Micropipettes

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Electrolyte filled glass-microelectrodes of conical shape with diameters of the opening \( \leq 1 \mu m \) are employed for a variety of electrophysiological experiments:

1. The microelectrodes serve to measure both dc and ac potential changes intracellularly. A separate paper will be concerned with the potential transducing properties of electrolyte filled micropipettes in comparison with metal microelectrodes.

2. The micropipettes are used to pass current into biological tissues. For this application the current frequency has the purpose a) to stimulate excitable tissues b) to deliver polarizing current for membrane impedance measurements (usually in the voltage clamp mode). c) To inject ionic drugs into tissues via iontophoresis. To minimize damage of the impaled tissue the tip diameters are small in comparison with the dimensions of the individual cells. Consequently the current densities in the opening are high since the current serves to supply the whole surface membrane of the cell. In a number of publications it has been reported that current carrying microelectrodes have rectifying properties. It is the objective of this paper to give a more qualitative theory of the physico-chemical nature of the rectification. The resulting consequences for the current carrying applications in electrophysiology will be discussed.

For the derivation of the electrical characteristics of micropipettes the following model is assumed: The electrode consists of a thin glass mantle of conical shape. The characteristic parameters of the electrode are \( \alpha \), the angle between the mantle and the axis and \( r_0 \), the radius of the opening. Common values for \( \alpha \) and \( r_0 \) are 5° to 15° and 0.05 to 5 \( \mu m \), respectively. The electrode is assumed to be filled and surrounded by an ordinary aqueous electrolyte such as KCl. The glass is assumed to be an insulator which carries a negative surface charge of ionic character.

The electrolyte side of the double layer consists of a layer of cations. The charge density of the cations and the extension of the cation layer into the bulk are determined by the surface potential which is closely related to the \( \zeta \) potential. The latter is accessible via electrophoretic measurements. The mobile ions in the interior of the electrode therefore come from two sources:

a) the cations and anions of the volume electrolyte, \( q_{vol} \). 

b) the cations of the electrical double layer of the gas, \( q_{dl} \). All ions are assumed to have the same mobility \( \mu [cm^2/Vsec] \).

The distribution of the two types of ions along the axial distance \( x \) of the electrode can be obtained if the charges are integrated over the whole volume and the total surface of the cone. The ratio of \( q_{dl} [Asec] \) to \( q_{vol} [Asec] \) as a function of \( x \) is:

\[
\frac{q_{dl}}{q_{vol}} \equiv \gamma \equiv \frac{q_{dl}}{q_{vol}} \cdot \frac{3}{\tan \alpha \cdot x} = \frac{3 \, b}{r}.
\] (1)

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Where \( q_{dl}^* = C_{dl} \cdot F \) [Asec/cm²] with \( C_{dl}^* \) [equiv/cm²] the number of ion equivalents per square centimeter of the glass surface; \( F \) [Asec/equiv] the Faraday constant. \( q_{\text{vol}}^* = C_{\text{vol}}^* \cdot F \) [Asec/cm³] with \( C_{\text{vol}}^* \) [equiv/cm³] the concentration in the volume electrolyte. The constant \( b = q_{dl}^* / q_{\text{vol}}^* \) has the dimension of a length [cm]. It is seen from eq. (1) that the ionic composition in the electrode varies with the diameter of the electrode. The constant \( b \) determines for a given diameter whether double layer or volume charges predominate. \( q_{dl}^* \) is not a sensitive function of the concentration \( \gamma \) but \( q_{\text{vol}}^* \) is directly proportional to the concentration. For a given radius the proportion of double layer charges is therefore the higher the lower the concentration of the electrolyte. From the charge distribution one can calculate the resistance contributions of the two types of mobile charges. The specific resistivity of the two phases is given by \( \rho_{\text{vol}} = 1 / \mu q_{\text{vol}}^* [\text{Vcm}/\text{A}] \) and \( \rho_{dl} = 1 / \mu q_{dl}^* [\text{V/A}] \), respectively. For volume conduction the integration over the whole volume of the electrode yields a contribution of volume resistance:

\[
R_{\text{vol}} = \frac{q_{\text{vol}}}{\pi r_a \tan \alpha} [\text{V/A}]. \tag{2}
\]

The resistance contribution of the double layer charges is

\[
R_{dl} = \frac{q_{dl}}{2 \pi \tan \alpha} \ln \frac{r_e}{r_a} [\text{V/A}]. \tag{3}
\]

where \( r_e \) is the wider radius of the cone, i.e. the end of the conical section. The contributions of the subsequent cylindrical section of the electrodes are neglected. They may, however, be easily calculated.

The total electrode resistance \( R \) can be described as a parallel switching of \( R_{\text{vol}} \) and \( R_{dl} \). Doing so one obtains with eq. (2) and (3):

\[
R = \frac{R_{\text{vol}}}{2 b} \left( \frac{1}{r_a \ln (r_e/r_a)} + 1 \right) [\text{V/A}]. \tag{4}
\]

\( b \) and \( r \) were already introduced in eq. (1) and (3), respectively.

If an electrical current is passed through the electrode ions move, iontophoresis \(^1\), and liquid flows, electroosmosis \(^4\). The ions move in each cross section of the electrode according to their transport number \( n \) which is determined by the concentration and mobility of the individual ions. Because the mobility of all ions was assumed to be the same the transport numbers \( n^+ \) of the cations and \( n^- \) of the anions as a function of \( x \) are with eq. (1) given by:

\[
n^+ = \frac{\gamma^+}{\gamma^-} + 1; \quad n^- = 1 - n^+. \tag{5}
\]

It is seen that for \( \gamma_q \ll 1 \) \( n^+ = 1/2 \) and for \( \gamma_q \gg 1 \) \( n^+ = 1 \). In other words: In wide openings and at high concentrations cations and anions are both moved in the opening relative to each other. On the contrary in narrow openings or at low concentrations the opening is only passed by cations. The two extreme cases of ion conduction through the opening viz. \( n^+ = 1/2 \) and \( n^+ = 1 \) correspond to ohmic and rectifying properties, respectively and will be discussed separately.

1. **Volume conduction** prevails if \( \gamma_q \ll 1 \). \( n^+ \approx 1/2 \) throughout the electrode. The current contribution of the double layer charges is negligible. The effect of the applied potential \( E[V] \) is iontophoresis and superimposed electroosmosis. The iontophoretic velocity \( w \) is given by

\[
w = \frac{q_{\text{vol}}^i}{\pi r_a^2} \mu = \frac{E \tan \alpha}{r_a} \mu [\text{cm/sec}]. \tag{6}
\]

The electroosmotic flow rate \( v \) is given by:

\[
v = \frac{q_{\text{vol}}^i}{4 \pi \eta_0} D \frac{\zeta}{4 \pi \eta_0} = \frac{E \tan \alpha}{\pi r_a} D \frac{\zeta}{\pi \eta_0} [\text{cm/sec}]. \tag{7}
\]

The expression \( D \zeta / 4 \pi \eta_0 \) has the dimension of a mobility \([\text{cm}^2/\text{Vsec}]\). Its numerical value is in aqueous solutions with \( 7 \times 10^{-4} \text{cm}^2/\text{Vsec} \) similar to \( \mu \) of K\(^+\). \( D = \epsilon \epsilon_0 \) with \( \epsilon \) the dielectric constant and \( \epsilon_0 [\text{Assec/Vcm}] \) the constant of influenita, \( \zeta[V] \) is the zeta potential, \( \eta_0 [\text{VAssec}^2/\text{cm}^3] \) the viscosity.

Under these conditions the current voltage curve of the electrode is linear i.e. ohmic.

2. **Double layer conduction** prevails in the opening for small values of \( r_a \) i.e. if \( \gamma_q \gg 1 \) eq. (1), \( n^+ = 1 \), eq. (5). The electrolytic conduction \( (n^+ = 0.5) \) which exists outside and in the interior of the electrode is blocked by a plug of exclusive cation conduction in the opening. Current through such a phase boundary cation zone — electrolyte always leads to concentration changes because the condition of electroneutrality requires that the anions which terminate conduction at the phase boundary are neutralized by equivalent amounts of cations. The result of current flow through the cation plug is therefore a concentration of electrolyte on one side i.e. the side where anions move towards the plug and a depletion on the other (anions move away from the
plug). In micropipettes the concentration changes which cause resistance changes do not cancel each other because of an asymmetry of the diffusion resistances on both sides of the plug. The space for diffusion on the inside of the plug is highly restricted, quasionedimensional. The outside space is unrestricted three-dimensional. For equal distance \( x \) from the opening the ratio of the inside to the outside volume is:

\[
\gamma_v = \frac{4}{\tan^2 \alpha}.
\]

With the common value of \( \tan \alpha = 0.05 \) \( \gamma_v \) becomes \( 6 \times 10^{-4} \). An equivalent concentration change at such an electrode would be packed about thousand times denser in the inside than in the outside. The resistance changes by the applied current are therefore much larger for the inside and the contribution of the outside can as a first approximation be neglected. For the assumed model with \( n^+ = 1 \) in the opening positive current i.e. with the inside positive with respect to the outside causes a concentration decrease (resistance increase) and a concentration increase for negative current. A steady state is reached when the applied electrical field is balanced by an opposing osmotic force of the concentration gradient and possibly by a counter e.m.f. of the glass wall (see below). The average velocity \( w' \) of the double layer ions through the opening is given by \( w' = \mu U \) where \( U [V/cm] \) is the longitudinal potential gradient. Since \( U = i \frac{q_\text{dil}}{2 \pi r_0} \), where \( i [A] \) is the current, \( w' \) is given by:

\[
w' = \frac{\frac{q_\text{dil}}{2 \pi r_0}}{\mu} = \frac{E \tan \alpha}{r_0} \frac{1}{\ln(r_c/r_0)} \mu [cm/sec],
\]

if Ohm's law is applied to double layer conduction. Comparison of this velocity \( w' \) of double layer ions with the volume velocity \( w \), eq. (6) shows that \( \frac{w_{\text{vol}}}{\pi r_0^2} \) is replaced by \( \frac{q_\text{dil}}{2 \pi r_0} \) and the velocity of double layer ions appears reduced by the term \( 1/\ln(r_c/r_0) \).

### Discussion

For physiological a.c. potential measurements the resistance of the electrode determines the frequency limit of recording provided the electrode capacity remains constant. It was shown in this paper that the resistance of electrolyte filled micropipettes with wide openings \( (\gamma_q \ll 1) \) and narrow openings \( (\gamma_q \gg 1) \) differ characteristically in their dependence of concentration and radius of the opening. Eq. (2) shows that the resistance of electrodes with volume conduction, \( R_{\text{vol}} \), varies inversely proportional to the radius and the concentration. Eq. (3) gives the value of the double layer conducting electrode. It is seen that \( R_{\text{dil}} \gg \ln 1/r_0 \) i.e. it is much less sensitive to alterations of the tip diameter. Because \( R_{\text{dil}} \) depends on \( q_\text{dil} \) which is rather insensitive to concentration changes \( R_{\text{dil}} \) as a first approximation independent of the concentration.

The transition from volume conduction to double layer conduction takes place if the double layer charges in the cross section predominate.

From eq. (1) it is seen that the ratio of double layer charges to volume charges in a given cross section becomes one if \( r = 3 b = 3 q_{\text{dil}}/q_{\text{vol}} = 3 C_{\text{dil}}/C_{\text{vol}} \). Since \( C_{\text{vol}} = c \), i.e. the concentration of electrolyte, the transition limit can be expressed as

\[
C_{\text{dil}}/r_0 c \gg 1.
\]

With eq. (2) the limit can be more conveniently expressed in terms of resistance values. With \( q_{\text{vol}} = 1/\mu e F \) the limit is

\[
R(C_{\text{dil}} \pi \tan \alpha \mu F) \gg 1.
\]

Relation (11) states that for a given concentration and radius the electrode changes from ohmic to rectifying property if the limit of (11) is passed. The decisive parameter is in (10) and (11) \( C_{\text{dil}} \) i.e. the density of charged ions in the double layer.

The value of \( C_{\text{dil}} \) for solid electrolyte interfaces can be estimated from electrokinetic measurements (via the \( z \) potential), and from electrochemical measurements (via electrocapillarity, differential capacity and other measurements). Common values range from \( 10^{-12} \) to \( 10^{-10} \) eq/cm². If a variation of the surface roughness factor of 1 to 10 is allowed \( C_{\text{dil}} \) is between \( 10^{-12} \) and \( 10^{-9} \) eq/cm². With the usual electrophysiological value of \( c = 0.1 \) eq/l the limiting radius of relation (10) would be \( 10^{-8} \) to \( 10^{-5} \) cm. The latter value of \( r_0 \) is frequently encountered at electrophysiological microelectrodes.

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The resistance limit of relation (11) as calculated with \( \tan \alpha = 0.05 \), \( \mu = 7 \times 10^{-4} \text{cm}^2/\text{Vsec} \) is \( 10^{14} \) to \( 10^8 \) [V/A]. The value of 100 MegOhms is fairly close to the usual resistance values of electrophysiological microelectrodes if it is taken into account that the calculation was based on 0.1 molar solution whereas the usual filling is 3 molar KCl.

In current carrying micropipettes as they are used in impedance measurements, stimulation and ion injection both ohmic (volume conducting) and rectifying (double layer conducting) electrodes have in common the direction of the flow. If the inside of the electrode is made positive with respect to the outside (reverse polarization) the ohmic electrodes yield electroosmotic efflux and rectifying electrodes expell cations from the tip. Intracellular depolarization (i.e. making the inside more positive) is therefore for the usual micropipette of negative surface charge always coupled with the injection of electrolyte into the cell. Hyperpolarization extracts electrolyte.

The ionic composition of the injected or extracted electrolyte differs for ohmic and rectifying electrodes: For volume conducting (ohmic) electrodes cations are concomitantly moved by iontophoresis and electroosmosis whereas anions only flow with electroosmosis together with the cations. Iontophoresis moves them the opposite direction. The movement of the ions relative to the wall of the opening therefore depends for volume conducting electrodes on the mobilities of the ions. For the assumed model anions would be standing still if the iontophoretic and the electroosmotic mobilities contained in \( w \) and \( v \), eq. (6) and (7) were the same. For rectifying electrode behavior, which is observed if \( r_0 < b \), eq. (1), the current has to pass the cation plug in the opening. Cations are therefore the only current carriers through the opening of electrodes with a perfect cation plug.

If current is passed through electrodes with \( r_0 < b \) the current-voltage relation stays linear only if the current \( i \ll RT/r_0F \) where \( RT/F \) is the thermal voltage equivalent (\( \approx 25 \text{mV} \) at room temperature) and \( R_0 \) the electrode resistance at rest. Polarizing currents which exceed this value cause the mentioned depletion (with the inside positive). Because the charge density \( q_{dl} \) and the extension of the double layer both increase somewhat with decreasing concentration of electrolyte the phase boundary plug-electrolyte is moved towards the interior of the electrode. Fig. 1 demonstrates schematically the increase of the plug length for the case that anodic current doubled the extension of the double layer. Cathodic current (inside negative) does the reverse: it diminishes the length of the plug. This feedback effect of current which causes an increase of \( C_{dl}/r_0 \) for anodic polarization and a decreases for cathodic current ensures rectifying properties for values of \( C_{dl}/r_0 \) smaller than one, relation (10).

![Fig. 1. Effect of anodic polarization on the cation zone length of a micropipette: The polarization doubled the extension of the double layer in the electrolyte leading to a fourfold increase of the cation zone length.](image)

Rectifying current-microelectrodes deserve therefore particular attention for electrophysiological applications:

a) The current delivering capability for efflux is limited by the resistance increase due to polarization.

b) The ionic composition of the current is dependent on the sign of the polarization.

A crude estimate of the efflux current limit can be obtained if it is assumed that an upper limit exists for the build up of a longitudinal potential gradient \( U [\text{V/cm}] \). Above such a limit of \( U = U_{\text{lim}} \) a counter e.m.f. of the glass wall most likely prevents a further increase of \( U \). Since \( U = q_{dl} i/2 \pi r_0 [\text{V/cm}] \) the current limit \( i_{\text{lim}} \) would be

\[
 i_{\text{lim}} = \frac{2 \pi U_{\text{lim}}}{q_{dl}} \frac{R_0}{r_0} \equiv \varepsilon r_0 [\text{A}] .
\]  

For a given radius of the opening the efflux current limit can therefore be calculated if the constant \( \varepsilon [\text{A/cm}] \) is known. If \( U_{\text{lim}} \) were \( 10^4 \) to \( 10^6 \) V/cm and \( q_{dl} = 10^9 \) V/A \( \varepsilon \) would be \( 6 \times 10^{-5} \) to \( 6 \times 10^{-3} \) A/cm. For an electrode of tip diameter \( 2 \mu \text{m} \) the efflux current limit is calculated 6 to 600 nA.

The assumption of this particular model implies crude simplifications. One is the assumption of only one mobility for the ions in the double layer and
the volume. A second one is the assumption of a **uniform parameter** $Q_{dl}$ of the glass surface within the tip.

Most likely $Q_{dl}$ is not the same as that of ordinary glass surfaces (Carl Wagner, personal communication). A quantitative check of the theory will therefore be difficult.

A third one is the assumption of a **perfectly insulating glass mantle**. Fine tips have so thin glass walls that leakage through the pores of the glass may become significant. Being aware of these problems it was not attempted to give a quantitative treatment of the polarization phenomena.

The analysis showed, however, that the phenomenon of rectification can be explained with the well established physicochemical effects of the interaction of surface and volume conduction. The following generalization can be deduced:

The electrical properties of micropipettes of different size and a filling of varying concentration include a phase transition problem. If the current is flowing in one phase only the current-voltage-curve is ohmic. Once the contribution of a second phase i.e. the surface phase in the opening becomes significant current-voltage-curves are measured which are typical for polarized phase boundaries. Examples for two phase systems with similar rectification are p-n junctions, the Germanium-electrolyte interface and the interface Ag/AgCl-KCl solution.

The parameter which is responsible for the transition from ohmic to rectifying current-voltage-curves is $Q_{vol}/Q_{dl}$, the ratio of the specific resistances of the two phases. The conduction in the less restricted volume electrolyte prevails if the dimensions such as the radius of the opening exceed this length. If, on the contrary, the opening is smaller than $Q_{vol}/Q_{dl}$ the conduction goes through the better conducting surface phase.

An analogous phase transition problem represents the passage of current from a volume electrolyte phase through a metal surface phase in electrolytic cells of varying geometry. Wagner, 1951, showed that the current density distribution is determined by the ratio of a characteristic linear dimension $A$ of the cell to a length constant $K_e$ which is given by the ratio of the characteristic resistances of the two phases, $Q_{surf}/Q_{vol}$. For $A \ll K_e$ the current density distribution is homogenous i.e. conduction in the volume phase of the transition region is preferred. If $A \gg K_e$ the current passes the surface phase preferentially in selected areas (peaks). Part of the conduction of the transition region occurs in the metal phase.

It appears plausible that whenever the passage of current involves two phases the ratio of the characteristic resistivities of the two phases (which always has the dimension of a length) to the characteristic dimension of the transition region decides the electrical behavior. At porous membranes a critical point would be reached if the diameter of the pores comes close to the magnitude of the length constant $Q_{vol}/Q_{dl}$. The fact that in biological membranes pores are usually not detectable leads to the conclusion that the observed electrical properties are governed by double layer type polarization phenomena.

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11 C. Wagner, J. electrochem. Soc. 98, 116 [1951].