Current Voltage Measurements of Electrolyte Filled Microelectrodes with Ohmig and Rectifying Properties

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In Übereinstimmung mit der vorangegangenen, theoretischen Ableitung hängen der Widerstand \( R_0 \) und die Stromspannungs-(i-E)-Kurven von Mikropipetten, die als Elektroden in der Elektrophysiologie verwendet werden, vom Radius \( r_0 \)[cm] und der Konzentration \( c[\text{Äq./cm}^3] \) des Elektrolyten ab. Große Werte von \( r_0 \) c \( [\text{Äq./cm}^2] \) entsprechen Volumenleitung in der Öffnung. \( R_0 \) vol \( \approx 1/ r_0 \) c Abb. 3; die i-E-Kurven sind ohmisch Abb. 2. Kleine Werte von \( r_0 \) c entsprechen Doppelschicht-Leitung. \( R_0 \) dl \( < R_0 \) vol und \( R_0 \) dl ist unabhängig von c und nur wenig abhängig von \( r_0 \) Abb. 3; die i-E-Kurven sind gleichrichtend Abb. 2, 6, 7, 8 and 9. Die oberflächenaktive Substanz Th(NO₃)₄ setzt in geringer Konzentration die Asymmetrie der i-E-Kurve herab Abb. 8 und invertiert die Gleichrichtung vollständig bei hohen Konzentrationen Abb. 9. Die zwei Arten gleichrichtender Mikropipetten haben einander ergänzende Eigenschaften: Beide Ionensorten können in Zellen injiziert werden: Kationen durch normale Gleichrichtung und Anionen durch inverse Gleichrichtung. Bei Verwendung des geeigneten Elektrodentyps kann sowohl Hyper- als auch Depolarisation mit einer niederohmigen Elektrode ausgeführt werden.

It is in the aim of this paper to characterize the resistance of unpolarized micropipettes as a function of the concentration and the radius of the opening. A comparison with concomitantly measured current-voltage-curves should allow to assess the limitations of the validity of the theoretical analysis outlined in the preceding paper (I)¹. The data on current voltage curves of rectifying electrodes should, in addition, give some insight into the nature of the polarization phenomena which occur at the tip of micropipettes.

Methods

Electrode preparation: Glass tubing, outer diameter \( \approx 2 \text{ mm} \) inner diameter \( \approx 1,3 \text{ mm} \) of the ordinary AR laboratory glass type was cleaned with boiling RBS (household-detergent-solution) and subsequently rinsed with deionised and quartzbidistilled water, respectively. Pulling was done with a David Kopf puller ². Fine tips of diameters \(< 0,5 \mu \text{m} \) which cannot be resolved in the microscope were pulled in one stage. Microscopically visible tips of coarser diameter (i.e. \( 0,5 - 5 \mu \text{m} \) were pulled in two stages: the first reduced in the outer diameter to approximately one third of the original value the second yielded the desired tip. The heat of the coil was chosen so low that the force of the solenoid pulled the electrodes apart at the desired diameter. The taper of the tips was frequently found to vary between \( 7 - 20\% \) corresponding to \( \text{tg} \approx 0,035 \) to 0.1, see below. The filling procedure was selected to minimize contamination and aging of the tip: The electrodes were dipped for a few minutes into the chosen electrolyte. This filled the lower part of the tip for a few mm with electrolyte. The electrolyte was then injected with a pipette of o.d. \( \approx 100 \mu \text{m} \). The remaining bubble was removed with a plastic rod. The bubble appeared to be sticking to the rod and both could be withdrawn together. After pulling the electrodes were thus ready for use after \( \approx 5 \) minutes. Solutions were prepared


from reagent grade chemicals (Merck) which were dissolved in quartz-bidistilled water. Solutions were not deaerated. The measurements were carried out at a temperature of 19 – 24 °C.

The electrical circuit diagram for the voltage clamp measurements is shown in fig. 1. The clamp amplifier I clamps the voltage between electrode A and B at the desired level. Amplifier II is wired in a follower configuration in order to exclude loading of the reference electrode. Amplifier III is mounted in the so-called current-to-voltage-conversion mode. The point above \( R_m \) is at virtual ground. The output voltage of the amplifier is \( i \cdot R_m \) where \( i \) is the current through the cell and \( R_m \) is a resistance of known value (for the measurements reported here it was \( 10^7 \, \text{V/A} \)). Amplifier IV is an ordinary adder which delivers the voltage program for voltage clamping. In this case the program consisted in a positive or negative sawtooth of 1 sec duration which was triggered every five seconds. The dc level for zero polarization was also adjusted with the adder by means of a potentiometer. Amplifiers are Philbrick models: II and III Type P 25 A, I and IV Type P 65 A. The wire electrodes were chlorided silver wires of several cm length. The micropipettes were arranged horizontally in a polyethylene cell. The reference electrode in front of amplifier II was omitted because the polarization of the Ag AgCl-wires was negligible for the small currents of 0 to \( \pm 10 \, \text{nA} \).

Results

A typical set of current voltage curves of a micropipette of tip diameter \( \approx 0.5 \, \mu\text{m} \) in KCl solutions of varying concentration is shown in fig. 2. Starting from the lowest concentration the curves for the higher concentrations were obtained by replacing the electrolyte on both sides of the tip, applying vacuum of \( \approx 15 \, \text{mm Hg} \) to the inside for \( \approx 1 \, \text{minute} \) and allowing to equilibrate for 15 minutes prior to the next measurement. For more details see fig. caption.

It is seen that the transition from ohmic behavior (straight line) to rectifying properties (curved line) occurs between \( 10^{-1} \) and \( 10^{-2} \) molar solutions. It is also seen that the current densities which can be reached for a given voltage are strongly concentration dependent at high concentrations and are practically independent changing from \( 10^{-3} \) to \( 10^{-4} \) molar.

To check whether leakage through the thin glass wall of the tip is responsible for the rectification a few micropipettes were made which had about the same inside dimensions as the usual ones but which had an outer “tip” diameter of approximately 1 mm. The rectification was about the same. A rigorous comparison was impossible because of the difficulties in manufacturing the pipettes reproducibly in the same shape.

Fig. 3 represents a double logarithmic plot of the electrode resistance at zero polarization \( R_0 \) for electrodes of tip diameter 0.5 \( \mu\text{m} \) and 2 \( \mu\text{m} \), respectively, against the concentration. The bars are the standard deviations for three different electrodes. Dashed curve: diameter of the openings \( \approx 0.5 \, \mu\text{m} \), solid curve 2 \( \mu\text{m} \). For the method of electrolyte exchange in the electrodes, see text.
solutions usually yielded about the same resistance values as for 10^{-4} molar solutions. In the later experiments this concentration was therefore omitted.

The slope for the two curves d log R_{0}/d log c starts to deviate from the value -1 at concentrations < 10^{-2} M. It can be seen from this plot that the begin of the rectification, figs. 2, 4, 5 coincides with a deviation of R_{0} from the straight line with slope -1.

The electrode resistances of a polarized electrode for various concentrations are shown in fig. 4. The curves represent the resistance i.e. the slope dE/di of the polarized electrode in fig. 2. The upper curve is the resistances under anodic, the middle under zero and the lower under cathodic polarization. It is seen that positive polarization increases the resistance and negative polarization decreases it.

The relative alterations of the polarized resistances as a function of concentration are seen more clearly in fig. 5: The ratio of the polarized to the unpolarized electrode-resistance is plotted double logarithmically against the concentration. The coincidence of the curves at high concentrations demonstrates the ohmic behavior.

It is seen that the concentration at which the rectification begins is quite different. The product of R_{0} c, which is marked in the figure, differs, however, not very much. R/R_{0} becomes independent of concentration at a concentration which is about one order of magnitude smaller than the concentration of the begin of rectification.

Fig. 4. Resistance of a microelectrode of diameter ≈ 0.5 μm, under polarization at various concentrations of KCl: double logarithmic plot. R_{an} = slope dE/di of the current-voltage-curve of a positively (anodic) polarized electrode; R_{0} = (dE/di)_{i=0}; R_{cath} = dE/di of negatively polarized electrode. The current-voltage-curves were measured as described in fig. 2.

Fig. 5. The ratio of the polarized resistance, R, to the resistance at zero polarization R_{0}, as a function of KCl concentration for two electrodes; double logarithmic plot. Solid curve tip diameter 0.5 μm, dashed curve tip diameter 2 μm. The arrows indicate the magnitude of the product of the radius r_{0} times the concentration c [mol/cm^2] for the particular electrode and concentration. The current-voltage-curves were measured as described in fig. 2.

Fig. 6 demonstrates that electrodes of quite different tip diameters: solid curve 0.1 μm (estimated) dashed 0.5 μm, dot-dashed 2 μm can have relatively similar current-voltage-curves if appropriate concentrations: 5·10^{-2}, 10^{-3} and 10^{-4} molar are chosen.

Fig. 6. Comparison of current-voltage-curves measured under voltage controlled conditions like in fig. 2 of electrodes of different tip diameter, see figure. The concentrations were: 5·10^{-2} for the electrode of tip diameter 0.1 μm, 10^{-3} for 0.5 μm and 10^{-4} for 2 μm.

Fig. 7 represents a current voltage curve which was measured with voltage sweeps of varying duration. Starting in the lower branch at +1 V the potential was swept within 1 second to -2 V and without interruption swept back within 100 milliseconds. It is seen that the fast sweep, upper branch, has at zero volts a much lower resistance than the curve measured with the slow sweep (lower branch).
Fig. 7. Hysteresis of a current-voltage-curve of an electrode of tip diameter 0.5 μm, measured dynamically in 10^{-2} M KCl solution. The cycle was triggered automatically every 5 seconds. The slow sweep started at $\approx +1$ V, continued to $\approx -2$ V and was followed immediately by the fast reset.

The effect of alteration of the $\zeta$ potential of the glass wall on the polarization phenomena of micropipettes is shown in fig. 8. One curve was measured with 10^{-3} M KCl inside and outside of the electrode, the other with the same electrode but after replacement of the solution by 10^{-2} M KCl which contained 10^{-5} M Th(NO_3)_4. It is seen that the small amount of added Th(NO_3)_4 reduces the asymmetry of the current voltage curve considerably. $R_{\text{mol}}/R_{\text{oth}}$ is 11.3 for KCl and 3.2 for KCl + Th^{4+}.

The normal current-voltage-curve, is completely reversed in polarity if KCl is replaced by 10^{-2} M Th(NO_3)_4, solid curve fig. 9.

The hysteresis of dynamically measured current-voltage-curves was for 10^{-2} M Th(NO_3)_4 like in fig. 7 but with reversed polarities.

In one experiment Th(NO_3)_4 was again replaced by 10^{-2} M KCl. The polarity of the rectification was not inverted again. This indicates an irreversible adsorption of Th^{4+} in the tip. This point was, however, not studied in detail.

**Discussion**

The resistance of unpolarized micropipettes has a dependence of the electrolyte concentration as it is expected from (I). Fig. 3 shows that for concentrations $\geq 10^{-2} M$ $d \log R_0/d \log c = -1$, eq. (2), (I). This is an indication that volume conduction prevails in the opening. The vertical distance (double arrow) of the curves measured for $r_0=0.25$ and 1 μm is found 0.65 decade (log 4.5) compared with 0.6 decade (log 4) calculated with eq. (2). At 0.1 M KCl, $\tan \alpha = 0.05 R_{\text{vol}}$ is calculated 4.6 and 18.4 MegOhms for electrodes of diameter 2 and 0.5 μm, respectively. The experimental values in fig. 3 are 6.5 and 26 MegOhms, respectively. In view of the correct slope and vertical distance of the curves this quantitative discrepancy appears insignificant.

At concentrations $\leq 10^{-2} M$ the resistances of fig. 3 are smaller than calculated for volume conduction. The diminution of the vertical distance of the resistance vs concentration curves and the independence of the resistance from the concentration, horizontal part of the curves, indicate double layer conduction of the electrode for concentrations $\leq 10^{-3} M$. With $\tan \alpha = 0.05$, $r_e = 0.1$ cm, $C_\parallel = 5 \cdot 10^{-10} \text{eq/cm}^2$, $\mu = 7 \cdot 10^{-4} \text{cm}^2/\text{Vsec}$ one calculates $R_\parallel$ 720 and 860 MegOhms, eq. (3) (I), for tip diameter 2 and
0.5 μm, respectively. The agreement with the experimental values of $R_{di}$ in fig. 3 is satisfactory. It has to be pointed out, however, that $C_{di}^{*} = 5 \times 10^{-10}$ eq/cm² is a rather high value. $\varrho_{di}$ [Ohm] would be calculated 3·10⁷ Ohms if all surface charges would participate in conduction with the mobility of K⁺ in the volume, a value which is about one order of magnitude below the usual values of $\varrho_{di}$ ⁵. Even ordinary metal surfaces with an interfacial condenser of 50 μF/cm² and a surface potential of 0.1 V would only have a $C_{di}^{*}$ of 5·10⁻¹¹ eq/cm². Since glass surfaces probably have a smaller interfacial condenser⁶ the observed value of $C_{di}$ appears unusually high. May be pores or cracks or a smollen gel covers the surface in the opening and causes this unusually high surface charge. The experiments of this paper are insufficient for a detailed explanation.

Inspection of the current-voltage-curves shows that the same electrode may have ohmic or rectifying properties, depending on the concentration of electrolyte, figs. 2, 4 and 5. The polarity of all rectifying current-voltage-curves in KCl solutions, figs. 2, 6, 7, 8 and 9, can be explained with the model of a negatively charged glass surface which originates a cation plug in the opening (1). For positive polarization of the inside (anodic) with respect to the outside cations are ejected from the electrode. Simultaneously the interior is depleted of electrolyte. Fig. 7 represents an indirect proof for these current-induced concentration changes: During the slow cathodic sweep electrolyte is concentrated in the tip. This low ohmic tip survives the quick reversal of polarization for a short period of time and yields a transient low ohmic anode, upper branch in fig. 7.

An indicaton for the concentration at which for one electrode transition from ohmic to rectifying behavior takes place can be obtained from fig. 5. It is seen that the electrode of tip diameter 0.5 μm begins to rectify at a higher concentration than the electrode of tip diameter 2 μm. This is a confirmation of (1) that the magnitude of the parameter $C_{di}^{*}/r_0 c$ decides whether the electrode rectifies or not. With the numerical value of $C_{di}^{*}$ obtained from fig. 3 one evaluates $C_{di}^{*}/r_0 c$ for the begin of the rectification in fig. 5 approximately 0.2 and 0.5 for the electrodes of tip diameter 0.5 and 2 μm, respectively. These values and the corresponding resistance values $R_0$ in the transition region of fig. 3, which are 30 and 50 MegOhms, respectively, are in fair agreement with the transition limits 10 and 11 of (1). In fig. 6 the current-voltage-curve of an ordinary electrophysiological microelectrode of an estimated tip diameter of 0.1 μm, solid curve, is compared with the current-voltage-curves of two other electrodes for which the pulling process was especially selected to yield electrodes of wider openings. For each electrode the concentration of KCl was chosen so that $C_{di}/r_0 c > 1$. It is seen that the different electrodes have relatively similar current-voltage-curves.

All rectifying current-voltage-curves, figs. 2, 6, 7, 8 and 9 shows a characteristic resistance increase at approximate efflux current values of 0.5 to 5 nA i.e. positive currents in KCl and negative currents in Th(NO₃)₄.

Fig. 8 shows that the rectification is diminished if small amounts of the surface active substance

Th(NO₃)₄ are added to the KCl solution. The surfactant decreases the negative surface charge of the glass i.e. it reduces $C_{di}^\ast$.

For the solid curve of fig. 9 the concentration of the surfactant Th⁴⁺ is so high ($10^{-2}$ M) that the surface charge of the glass is positive. The electrolyte-side of the double layer is in this case made up of anions. Consequently polarization leads to opposite ion movements than in the normal case of negatively charged glass and the polarity of the rectification is also inverted. The similarity of the two current-voltage-curves of fig. 9 indicates that in the two cases $C_{di}^\ast$ i.e. the number of charged sites in the tip is of comparable magnitude.

For a quantitative discussion of the parameters of the current-voltage-curves, in particular the resistance values under positive and negative polarization a more quantitative investigation of the steady state and dynamic polarization phenomena would be required. For the lack of these data we restrict this paper to this qualitative discussion.

**Conclusions**

The experiments gave a qualitative confirmation of the theory which was developed in (I). It was shown that for practical purposes the parameter $C_{di}$ in the tip of the electrode which can be measured via the resistance at low concentrations, $R_{di}$ eq. (2) (I) is of eminent importance. The observed high value of $C_{di}$ is possibly not typical for all microelectrodes. Most likely electrodes of other glass types and other methods of pulling have different values of $C_{di}$. If, however, $C_{di}^\ast$ is known the critical radius of the opening for the transition from ohmic to rectifying behavior can be calculated, eq. (10) (I). The current limitation for efflux also depends on $C_{di}$, eq. (12) (I).

Particular attention deserves the inverted rectification. With electrodes of such rectification it is possible to inject anions into biological cells which is impossible with electrodes of normal rectification. The two types of electrodes very usefully complement each other: Both types of ions may be injected or extracted and hyper- and depolarization of cells for impedance measurements and stimulation can be carried out with a low ohmic electrode.

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