

## Ussing pulse Calculations.

### A. Calculation of $V_0$ , $R$ and $C$ .

Try to express the Voltage at time zero in known data.

What are known to start with are the two Voltages at the two blue markers. At time  $t_1$  the voltage is  $V_1$  and at time  $t_2$  the voltage is  $V_2$ . Also the applied current causes a voltage over the epithelial resistance that is equal to the resistance multiplied by the current (see eq 2).

The voltage signal after end of stimulation follows a mono exponential curve that can be expressed as:

$$V(t) = V_0 * e^{-t/(R*C)} \quad (\text{eq 1})$$

where  $V(t)$  is the voltage by time,  $V_0$  the starting voltage,  $t$  is time,  $R$  the epithelial resistance and  $C$  the epithelial capacitance.

At time zero  $t = 0$  and  $V(0) = V_0 * 1 = R * I$ , thus  $R = V_0 / I$  (eq 2)

Calculate  $R*C$ . The two points of the voltage at the blue lines have coordinates

At time  $t_1$  the voltage is  $V_1$ , so  $V_1 = V_0 * e^{-t_1/(R*C)}$

At time  $t_2$  the voltage is  $V_2$ , so  $V_2 = V_0 * e^{-t_2/(R*C)}$

Divide the first expression with the second:

$$\begin{aligned} V_1 / V_2 &= V_0 * e^{-t_1/(R*C)} / V_0 * e^{-t_2/(R*C)} = e^{-t_1/(R*C)} / e^{-t_2/(R*C)} = \\ &= e^{(t_2-t_1)/(R*C)} \end{aligned}$$

Take the natural logarithm of both sides:

$$\ln(V_1 / V_2) = (t_2 - t_1) / (R*C) \text{ or}$$

$$R*C = (t_2 - t_1) / \ln(V_1 / V_2) \quad (\text{eq 3}) \text{ or}$$

$$C = (t_2 - t_1) / (R * \ln(V_1 / V_2)) \quad (\text{eq 4})$$

At time  $t_1$  using equation (1) and then equation (3) we get:

$$V_0 = V_1 * e^{t_1/(R*C)} = V_1 * e^{[\ln(V_1 / V_2) * t_1 / (t_2 - t_1)]} \quad (\text{eq 5}).$$

Conclusion:

1. Calculate  $V_0$  from equation (5).
2. Calculate  $R$  from equation (2).
3. Calculate  $C$  from equation (4).

Note: The exponents in equation (5) doesn't miss an minus sign in the exponent in either expression. Also in equation (5) the expression within square parenthesis [] is in the exponent of the base of the natural logarithm e.

### **B. Correction for a pulse that is too short.**

There is a rule of thumb saying that a capacitor will charge to within one percent after a charging time of five time constants. The stimulating pulse was set to one ms because it was estimated that the epithelial time constant in all cases should be smaller than 0.2 ms but that is not the case. A correction has to be done.

If the epithelial resistance is R and the epithelial capacitance is C then the time constant for the epithelium is  $T_e = R * C$ . If the stimulation pulse duration is  $T_i$ , then the fraction of final charge can be calculated from the expression:

$$F_i = 1 - e^{(-T_i / T_e)} \quad (\text{eq 6})$$

Where  $F_i$  always is larger than zero and smaller than 1.

Example: If the stimulation pulse is one ms, the epithelial resistance is  $R = 390 \text{ ohm}$  and the capacitance is  $C = 1.5 \text{ }\mu\text{F}$  then the epithelial time constant =  $T_e = R * C = 5.85 \cdot 10^{-4}$ . The fraction of charge can then be calculated by  $F_i = (1 - e^{(-5.85 \text{ e-4} / 1.0 \text{ e-3}))} = 0.8190$  that is the capacitor is only charged to 83 % of final value. This will cause a large error in the measured values of R and C.

To correct for stimulation pulses that are too short the program will use a corrected amplitude for the stimulation current. To do that, you really don't have to know R and C. It is enough to know  $T_e = R * C = (t_2 - t_1) / \ln(V_1 / V_2)$  from equation (3) above. Then you can calculate a corrected stimulation pulse  $I_k = I_{\text{stim}} * F_i = I_{\text{stim}} * (1 - e^{(-T_i / T_e)})$  and then use this corrected somewhat smaller stimulation current in the calculation of R above.

### **C. Calculation of resistance in recruited area.**

Certain drugs can affect the tissue so that the measured resistance is decreased and the capacitance is increased. It is not believed that the available tissue cells changes their capacitance and resistance property. Certainly the capacitance is not changed. The change is more probable to be due to areas being opened up and available for measurement by the measuring method used. If so then it is naturally to ask if this recruited area has a resistance that is different from that of the area to start with. This can be studied as follows.

The surface area of the area that has been recruited is not known. So we have to use a measure that is independent of the area and that is the product of resistance and capacitance,  $R * C$ . This can be understood as follows. If you assume that the capacitance is built by the same molecule layer in all measured cells then it is fair to assume that the change in capacitance is a good measure of the change in surface area. If A is the

recruited surface area then the change in resistance is  $R / A$  and the change in capacitance is  $C * A$  where  $R$  and  $C$  are the measures of the factors per area of the recruited surface. Thus the RC product is  $(R / A) * (C * A) = R * C$ .

Assume that the measured resistance control value is  $R_1$  and control capacitance is  $C_1$ . Then assume that the minimum measured resistance value is  $R_2$  and the corresponding measured capacitance value is  $C_2$ . Further assume the corresponding values for the recruited surface is  $C_r$  and  $R_r$ . We want to compare  $C_r * R_r$  with  $C_1 * R_1$ . The capacitance is easy and calculated as follows. The resulting capacitance is the sum of the original and recruited capacitance so:

$$C_2 = C_1 + C_r, \text{ thus } C_r = C_2 - C_1 \quad (\text{eq 1})$$

To figure out the resistance  $R_r$  we apply the formula for resistances in parallel and we get:

$$R_2 = (R_1 * R_r) / (R_1 + R_r), \text{ thus } R_1 * R_2 + R_r * R_2 = R_1 * R_r,$$

$$R_1 * R_2 = R_1 * R_r - R_r * R_2, \text{ thus } R_r = (R_1 * R_2) / (R_1 - R_2) \quad (\text{eq 2})$$

Multiplying (eq 1) with (eq 2) we get:

$$R_r * C_r = (C_2 - C_1) * (R_1 * R_2) / (R_1 - R_2) \quad (\text{eq 3})$$

And this expression should be compared with  $R_1 * C_1$ .

If  $R_r * C_r$  is smaller than  $R_1 * C_1$  then the recruited areas have a more ion leaky property than the area measured in the control situation.