DONNAN POTENTIAL MEASUREMENTS IN EXTENDED HEXAGONAL POLYELECTROLYTE GELS SUCH AS MUSCLE

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ABSTRACT In this paper we reconsider the theoretical and practical aspects of using KCl-filled microelectrodes in extended polyelectrolyte gels such as muscle to measure Donnan potentials, and then calculate protein fixed-charge concentrations. An analytical calculation of the electrical potential function between muscle filaments shows that whether the microelectrode averages the ionic concentration or the local potentials the results are indistinguishable in the practical regime. After consideration of this and other possible sources of error, we conclude that the charge-concentration measurements that have appeared in the literature are legitimate.

INTRODUCTION

During the past decade a number of groups have reported potentials, measured with KCl-filled microelectrodes, from muscle fibers whose membranes have been destroyed or removed by the technique of glycerination (Collins and Edwards, 1971, Pemrick and Edwards, 1974, Elliott et al., 1978) and also by chemical skinning and mechanical skinning (Bartels et al., 1980, Bartels and Elliott, 1980; 1981; Stephenson et al., 1981). Stephenson et al. (1981) used their data largely to work out average ratios between the internal and external concentrations of various ions and other solutes. The other authors have taken the argument one step further, and invoke the principle of electrical neutrality to infer the internal fixed electric charge concentration on the contractile proteins. Bartels and Elliott (1980; 1981), using high-powered light microscopy to observe the position of the microelectrode tip, recorded Donnan potentials from the *A*- and *I*-bands of glycerinated rabbit psoas muscle and mechanically skinned barnacle muscle in rigor and relaxing solutions. They found different *A*- and *I*-band potentials in rigor solutions, and concluded that the fixed-charge concentrations must be different in the two bands under these conditions; in relaxing solutions the *A*- and *I*-band potentials (and therefore the fixed-charge concentrations) were equal.

In making these measurements, all conventional precautions are taken to avoid appreciable tip potentials in the microelectrodes. Attention should be drawn to the effects of KCl diffusion from the microelectrode. This will set up a diffusion (junction) potential to which the modified Nernst equation applies (Geddes, 1972). For 3-M KCl-filled microelectrodes in a solution which is essentially 100 mM KCl this junction potential is ~1.6 mV. Because the internal and external potassium and chloride ion concentrations are similar, the junction potential will be very nearly the same in the internal and external phases (Thomas [1978] remarks that it is both small and stable) and will not affect a measurement of the Donnan potential, because this is the difference between the potentials that the electrode records in the external and internal phases. Kushmerick and Podolsky (1969) have shown that there are no differences of ionic mobility inside and outside the contractile lattice which might make the junction potential inside different from outside. A further effect of KCl diffusion might be to cause an appreciable change in the internal concentrations of the free ions. Fig. 7 in Thomas (1978) shows such an effect, caused by chloride leakage from a KCl-filled microelectrode into a snail neuron, but the time scale (minutes for an appreciable concentration change) is very different from the few seconds which a Donnan measurement requires. Moreover in membraneless systems the internal chloride ion concentration is already approximately 30 times that in a normal cell, so the relative effect of a small leakage should be much reduced. In none of the experiments (see the published time records of Naylor, 1978, and Stephenson et al., 1981) is there any sign of consistent potential drift, on the seconds time scale, which might be caused by bulk diffusion.

Since the distance between muscle filaments is of the order of 40 nm, and the local potential will vary over this distance, while the size of the microelectrode tip used is 100–200 nm (Naylor, 1977), the microelectrode must measure an average potential. The calculation of fixed charge requires a knowledge of how this averaging is done by the microelectrode within the typical dimensions shown in Fig. 2. Our purpose is to reinvestigate this averaging, to

see whether the calculations of charge concentration which have been made in the literature are sensible.

ANALYSIS

Fig. 1, adapted from Alexandrowitz and Katchalsky (1963) represents diagrammatically the extended phase of filaments in a salt solution, in equilibrium with an external salt solution. In the absence of any permselective membrane the small ions are freely diffusible, and in equilibrium. There is a potential minimum midway between the filaments, and a further negative potential (E) between this minimum and the extended potential in the external phase (taken as the zero of potential). For convenience all potentials will be expressed in the reduced form ϕ ($\phi = e\psi/kT$) following the notation of Alexandrowitz and Katchalsky (1963).

Alexandrowitz and Katchalsky (1963; Eq. 5) show that the product $(m_+)_r \times (m_-)_r$ [$= (m_0)^2$] is independent of the position vector **r** and that the local chemical potential of the salt is therefore constant throughout the system. This means that the system is (at all points) in Nernst-Donnan equilibrium with the surrounding salt solution. Let us imagine that an infinitely small microelectrode, taking the form of a salt bridge connected to a junction reversible to one of the freely diffusable ions, could be placed at radius r. (We can suppose that the negative ions are chloride, and that a [reversible] Ag/AgCl junction is connected via an [irreversible] KCl bridge.) The electrode would detect the Nernst potential for negative (chloride) ions, $\phi_r = \log \{m_{-r}/m_0\}$ relative to an externally placed reference electrode. (We could write alternatively $\phi_r = -\log \{m_{+r}/m_0\}$ because of the relationship between the ion concentrations mentioned above: Alexandrowitz and Katchalsky Eq. 5). The microelectrode, however, cannot in practice be made much smaller than the situation shown in Fig. 2. Clearly it must record an average potential, and it seems very likely that the effective averaging will be over the electrically neutral sub-volume between the filament surface and the symmetry plane (Figs. 1 and 2).

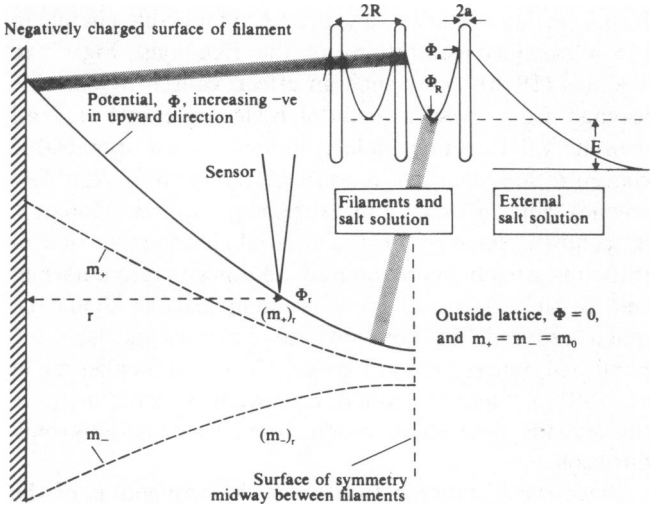

FIGURE 1 The electrical potentials in an extended gel of charged cylindrical filaments, diameter $2a$ and center-to-center separation $2R$, in equilibrium with an external phase containing salt molecules only. The *inset* shows the potential at higher magnification, and the counter and co-ion concentrations, between the filament surface and the sub-volume surface (radius R) where there is a potential minimum between the filaments.

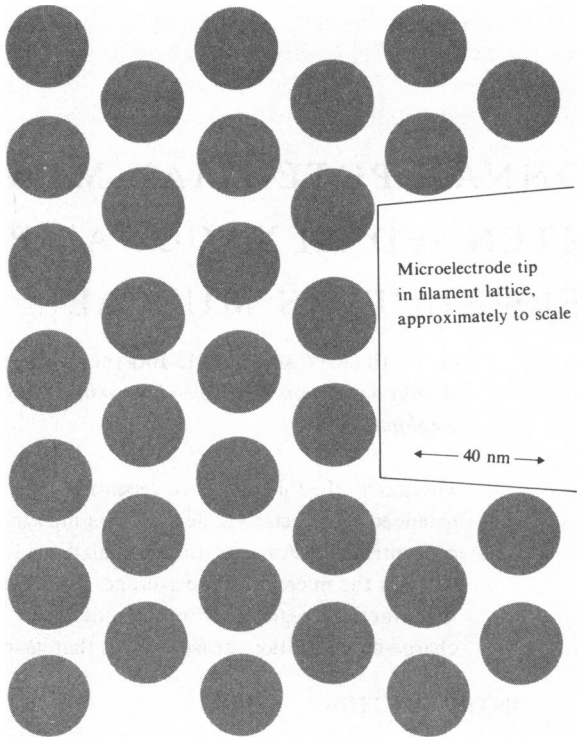

FIGURE 2 A diagramatic cross section of a hexagonal lattice of filaments, radius 15 nm and center-to-center separation 40 nm. The microelectrode tip, ~0.1 μm, is shown to scale.

It has been suggested to us that the microelectrode might form a vacuole round its tip, and the conditions in this vacuole could be different from the original conditions within the lattice. There seems to be no good reason why this should happen. Once the microelectrode is properly inserted, movement generally causes no further potential changes unless the microelectrode tip can be seen to bend under the (high-powered) microscope or is removed from the lattice. A more sensible view is that the microelectrode does indeed average the existing environment within the filament lattice, and does not make any appreciable disturbance in the lattice ahead of its approach.

There are two ways in which the averaging may occur.

(a) If the microelectrode averages so that ϕ_m (measured) $= -\log \langle m_+ \rangle / m_0$, then $\exp(-\phi_m) = \langle m_+ \rangle / m_0$. (The quantity inside the bracket is positive because ϕ_m is negative.) A similar equation can be written for the negative ions.

$$m_0 \{\exp(-\phi_m) - \exp(+\phi_m)\} = \langle m_+ \rangle - \langle m_- \rangle.$$

The right-hand side represents the charge concentration on the filament surface, because the sub-volume must be electrically neutral. This is the charge calculation introduced by Collins and Edwards (1971) and used in its generalized form by Naylor (1977), Elliott et al. (1978) and Bartels and Elliott (1981). More formally,

$$\phi_m = -\log \frac{\frac{1}{V}\int_V m_0 \exp(-\phi_r)\mathrm{d}v}{m_0},$$

i.e., $\exp(-\phi_m) = 1/V\int_V \exp(\phi_r)\,\mathrm{d}v$, where the integral is taken over the sub-volume. Thus,

$$\exp(\phi_m) = \frac{1}{V}\int_V \exp(\phi_r)\,\mathrm{d}v$$

or

$$\exp(\phi_m) = \langle \exp \phi_r \rangle. \tag{1}$$

(*b*) If, on the other hand, the microelectrode averages so that

$$\phi_m = -\left\langle \log\left\{\frac{m_0 \exp(-\phi_r)}{m_0}\right\}\right\rangle,$$

then

$$\phi_m = \frac{-1}{V}\int_V \log[\exp(-\phi_r)]\,\mathrm{d}v$$

(the integral is again taken over the sub-volume)

$$= \frac{1}{V}\int_V \phi_r\,\mathrm{d}v,$$

i.e.,

$$\phi_m = \frac{1}{V}\int_V \phi_r\,\mathrm{d}v$$

$$\phi_m = \langle \phi_r \rangle. \tag{2}$$

Mathematically, Eq. 2 is different from Eq. 1, and might be very different for large values of ϕ_r, so we must ask whether the electrode measures Eq. 1 or 2.

Expanding the exponentials, Eq. 1 can be written

$$1 + \phi_m + (\text{terms in } \phi_m^2, \phi_m^3) = 1 + \langle \phi_r \rangle + (\text{terms in } \langle \phi_r^2 \rangle\ \langle \phi_r^3 \rangle).$$

The bracketed terms on the left-hand side converge rapidly (it is rare that the measured potential exceeds 10–15 mV so $\phi_m \leq 0.6$). As long as $\exp(\phi_r)$ is able to be linearized over the inter-filament space, the terms in brackets on the right-hand side may be neglected and Eq. 1 reduces to

$$\phi_m = \langle \phi_r \rangle$$

which is identical to Eq. 2. Thus to a first approximation Eqs. 1 and 2 are equivalent, as long as ϕ_r can sensibly be regarded as linearizable through the interfilament region. We have therefore calculated the potential ϕ_r using the formal approach of Alexandrowitz and Katchalsky (1963) who matched a linearized (Bessel function) solution of the Poisson-Boltzmann equation for an outer region far from the cylinder surface with a nonlinearized solution for an inner region close to the cylinder surface. The nonlinearized solution was obtained (in trigonometric functions) by Fuoss et al. (1951), who assumed that the co-ion concentration is negligible (within the inner region). The two solutions are matched at a radius chosen to minimize the errors caused by neglecting the co-ion density in the inner region, and by neglecting the nonlinear terms for co- and counter-ion density in the outer region. Both these effects increase towards the match point, which is therefore taken at a radius that makes the effects equal in magnitude (Alexandrowitz and Katchalsky, Eq. 25). Alexandrowitz and Katchalsky state that in the worst possible case (zero salt concentration) this procedure introduces an error of <16% in the value of the charge density, and that the potential calculated by numerical integration is 'practically identical' to the analytical solution.

Inasmuch as some of the equations are transcendental in form, we programmed a computer with the calculation

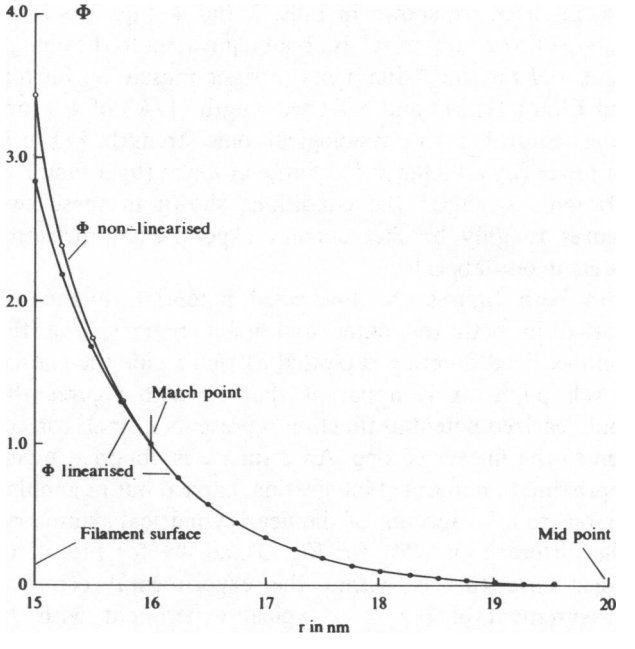

FIGURE 3 The two potential functions, linearized and nonlinearized (expressed in units of $e\psi/kT$) calculated for $K = 1\ \mathrm{nm}^{-1}$, $a = 15$ nm, $R = 20$ nm and charge per unit length = 57 e/nm. The difference between the two functions (cylindrically integrated, see text) is ~5%. Note that in this diagram the zero of potential has been shifted for convenience of plotting so that it is at the sub-volume surface potential minimum. The potential difference E between this minimum and the external phase is 0.0345 in these potential units (i.e., ~0.86 mV).

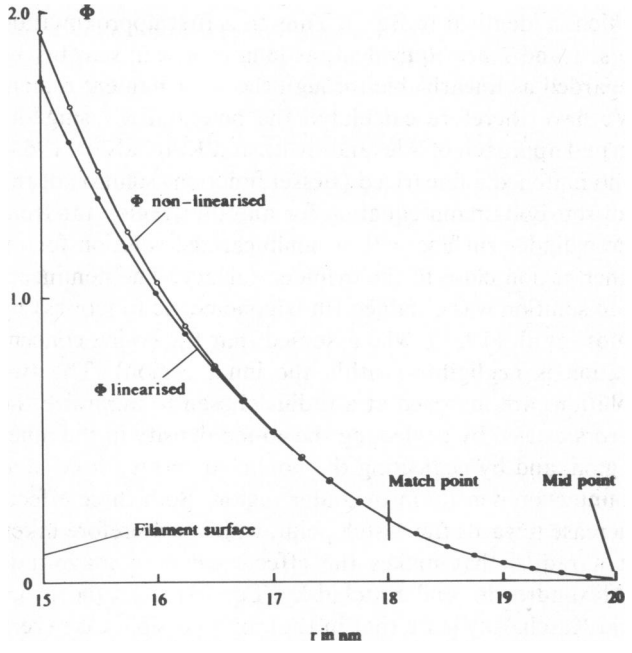

FIGURE 4 As Fig. 3, but with $K = 0.33$ nm^{-1} and charge per unit length = 12 e/nm. The cylindrically integrated difference is ~4%. Here again the zero of potential is at the mid-point for convenience of plotting; $E = 0.94$ or 23.5 mV.

using the (full) procedure suggested in Alexandrowitz and Katchalsky (III.3, p. 3240).[1] Calculated potentials for two typical cases are shown in Figs. 3 and 4. Fig. 3 is for a charge of 57 e/nm, which is about equivalent to the charge measured for the A-filaments in rigor muscle by Bartels and Elliott (1981) and a Debye length ($1/K$) of 1.0 nm, about equivalent to physiological ionic strength. Fig. 4 is for lower (by a factor of 5) charge at lower (by a factor of 10) ionic strength; the conditions shown in these two figures roughly bracket current experimental measurements in our laboratory.

In both figures the linearized potential function is plotted in both the outer and inner regions, and the nonlinearized function is plotted as well inside the chosen match point. It is apparent that in both figures the nonlinearized potential function represents a small correction to the linearized one. An estimate is obtained by an approximate numerical integration, carried out in annular regions to take account of the near-cylindrical symmetry. The difference is ~5% for Fig. 3 and 4% for Fig. 4. In either case this is within the experimental error of measurement of ϕ_m; in a typical experiment with 25 observations the standard error is usually 5–10%. In all cases the integrated potential derived from the linearized potential function is lower than that derived from the nonlinearized analytical solution. Further calculations show that at physiological ionic strength the difference is ~10% if the charge is twice as great (~120 e/nm) and is ~20% if the charge is six times as great (~360 e/nm).

Thus even if the microelectrodes do average as in Eq. 2, no significant error will be introduced under our experimental conditions by treating the data as if the averaging were done in Eq. 1, and calculating the fixed-charge concentration as described in Collins and Edwards (1971) and Elliott et al. (1978).

DISCUSSION

Although it seems to us intuitively sensible that the microelectrode will average the ion concentrations as in Eq. 1, it has been suggested that a significant number of electrophysiologists would disagree and assume Eq. 2 (averaging the local potentials) to be correct. We have demonstrated that the two are equivalent when the potential function can be approximated by linearization, and that this is the case within the experimental regimes used in our laboratory and in Edwards's.

Naylor (1982) has calculated the interfilament potentials independently, and also concludes that the interfilament space is a regime in which the potentials may be treated as simple Donnan averages.

In the course of calculations of the interfilament potential, we have confirmed that this approach predicts the charge saturation effect, which Millman and Nickel (1980) first pointed out, and Naylor (1982) has also shown. For example, at a Debye length of 1 nm the calculations show that however great the filament charge, the potential difference (E) between the potential minimum and the surrounding phase cannot be increased beyond 1.25 mV. This potential, at a constant Debye length, is directly related to the swelling pressure of the gel. For these small values of E the swelling pressure is proportional to E^2 (Alexandrowitz and Katchalsky, 1963; Eq. 14). A similar conclusion was derived by Bell and Levine (1958); see Elliott (1968). Using this relationship, a comparison with the calculations of Millman and Nickel (1980) was made with the cooperation of Dr. B. M. Millman. At a charge density of 100 e/nm, with a Debye length of 1.2 nm, their calculations using numerical integration give a swelling pressure of 3 Torr and the present calculation gives a swelling pressure of 6.5 Torr. Either value is in reasonable agreement with their experimental curves, and the theoretical accord is pleasing considering the different approaches adopted. The value taken for the cylinder radius in this work, 15 nm, is about twice the electron-microscope myosin (thick-filament) backbone radius of ~7 nm. Both Millman and Nickel (1980) and Naylor (1982) have found it necessary to depart from this radius to get reasonable agreement between experimental results and theory. We have calculated the swelling pressure near charge saturation for a cylinder radius of 7.5 nm, and separation of 20 nm, and find it about 10^{-6} times that with 15-nm radius. A similar comparison can be obtained

[1]There are a couple of minor errors in that paragraph, in line nine the second expression should read q/B, and the bracketed expression in the final term of Eq. 33′ should be squared.

by extrapolating Fig. 6 of Millman and Nickel (1980), the factor from their work is $\sim 2 \times 10^{-5}$; once again the agreement is reasonable.

In this paper we have followed Alexandrowitz and Katchalsky (1963) and used ion concentrations rather than ion activities in our equations for the Nernst-Donnan potential. Our justification for this is largely experimental. The experiments of Hinke and Gayton (1971), who measured activities using ion selective microelectrodes and compared these with chemical measurements of the ion concentrations, establish that at least for the major monovalent ions the activity coefficients are about equal inside and outside and thus cancel out in the equations. See for example Figs. 1 and 2 of Hinke and Gayton (1971), where the experimental points for K^+ activity and concentration ratios, both measured, are very similar. There are differences between the measured concentration and activity data for the Cl^- ions, but Hinke and Gayton attribute these to chloride binding to the contractile proteins. We have come to the same conclusion from our microelectrode studies and have discussed it elsewhere (Elliott, 1980).

For barnacle muscle, the internal ion concentrations and fixed charge concentrations have also been measured independently by isotope distribution (Hinke, 1980) and by membrane incorporation (Caillé, 1981). The accord with our Donnan measurements on the same muscle (Bartels and Elliott, 1981) is very reasonable.

CONCLUSION

After a reexamination of the theoretical and practical implications of Donnan potential measurements using KCl-filled microelectrodes in extended hexagonal gels such as muscle, without effective permselective boundary membranes, we conclude that these potential measurements can indeed be interpreted to give the fixed electric charge on the protein filament lattice in the manner introduced by Collins and Edwards (1971) and modified by Elliott et al. (1978).

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