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Surface Charges on Membranes

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Summary. The equations of membrane potential developed by Kobatake and coworkers have been applied to the literature data on the resting membrane potential of the crayfish and *Myxicola* axons to derive values for the surface charge density present on the axon membranes. Some shortcomings of the method are briefly discussed. The value for the surface charge density derived for the squid axon membrane agreed with a similar value derived from measurements of shifts in Na and/or potassium conductance-voltage relations following changes in the concentration of calcium in the solutions bathing the axons.

In a recent paper, Begenisich (1975) has described the measurements of shifts in sodium and/or potassium conductance-voltage relations following changes in the concentration of calcium in solutions in which *Myxicola* giant axons are immersed. Values for the density of surface charges present on the membrane of the giant axons have been derived by comparing the measured shifts in conductance-voltage relations with those predicted by an appropriate equation of the Gouy-Chapman theory of the electrical double layer (*see* Delahay, 1965). This followed from the work of Gilbert and Ehrenstein (1969) who for the first time applied the relation between surface potential, charge density, and ionic concentration of the Gouy-Chapman theory to derive values for the surface charges present on the membrane of the squid giant axons, taking into consideration the reaction between calcium ions and the negative sites on the membrane. This type of work has been extended to other membrane systems by a number of investigators (Mozhayeva & Naumov, 1970; 1972*a*, *b*, *c*; McLaughlin, Szabo & Eisenman, 1971; Brismar, 1973; Vogel, 1973; D'Arrigo, 1973; Schauf, 1975; Begenisich, 1975; Hille, Woodhull & Shapiro, 1975).

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In most of the above work, it has been generally considered that the negative surface charges are present near the sodium and potassium channels in the biomembranes. Whether the charge density near the channel region (assumption of membrane inhomogeneity, that is, discrete charge model) indicates also the charge density for the whole membrane (assumption of homogeneity, that is, uniform charge model) requires some experimental proof, although these two models for the distribution of charges on the membrane have been theoretically considered by Cole (1969) and Brown (1974).

The illuminating work of Brown (1974) has shown that the uniform charge and "open portal" (mouth of the channel uncharged) models are better suited for the description of the behavior of the biological membranes than the "charged portal" (existence of point charge at the mouth of the channel) model. Further it has been shown by both Cole (1969) and Brown (1974) that the spacing between charges according to the discrete model is about two-thirds that predicted by the uniform model, despite the prediction by both models of equivalence of affinities to membrane sites of either divalent or monovalent cations. The resolution of these minor differences which, as the brief study of Cole (1969) concluded, may not be of prime importance in the considerations of membrane structure or function, rests on an independent method of estimation of charge density based on the uniform charge model. Such a procedure has been recently described by Lakshminarayanaiah (1975*a*, *b*) who considered the possibility of applying the theories of membrane potential developed by Teorell (1953) and Meyer and Sievers (1936) and by Kobatake and coworkers (*see* Kobatake and Kamo, 1973 for a summary) to estimate charges on the membrane of the barnacle muscle fiber (Lakshminarayanaiah, 1974; 1975*b*). Encouraged by the success of the theory due to Kobatake and coworkers (K-theory), Lakshminarayanaiah and Murayama (1975) have applied this K-theory to the literature data of the resting membrane potential and derived some values for the charge density on the biomembranes of the squid axon, frog and barnacle muscles. At that time the literature data for the resting membrane potential of the crayfish axon measured by Strickholm and Wallin (1967) and of the *Myxicola* axon measured by Goldman (1968) escaped our attention. As these data and those already considered (Lakshminarayanaiah & Murayama, 1975) provide enough material to discuss some possibilities regarding the discrete *vs.* uniform charge models, the calculations for the crayfish and *Myxicola* axons, together with some refinements introduced to calculate the values for $\bar{\phi}$, are presented in this paper.

Results

The equations of the K-theory pertinent to the present purpose are the following:

$$E_{\max}=\frac{RT}{F}\ln\frac{(\mathrm{K})_0}{(\mathrm{K})_i} \tag{1}$$

where $E_{\max}$ is the theoretically possible maximum value for the equilibrium potential determined by the intracellular $(\mathrm{K})_i$ and extracellular $(\mathrm{K})_o$ concentrations of potassium expressed as moles per kilogram water. R, T, and F have their usual meaning.

The apparent transport number of the potassium counterion $\bar{t}_{+(\mathrm{app})}$ referred to the membrane phase is given by (Teorell, 1936; Lakshminarayanaiah, 1969)

$$\bar{t}_{+(\mathrm{app})}=\frac{E_m}{2\,E_{\max}}+0.5 \tag{2}$$

where E_m is the measured resting membrane potential. $\bar{t}_{+(\mathrm{app})}$ has been related to the permselectivity of the membrane (P_s) for the counterion by the relation (Lakshminarayanaiah, 1975*a*, *b*)

$$P_s=\frac{\bar{t}_{+(\mathrm{app})}-t_+}{t_+-\bar{t}_{+(\mathrm{app})}(2t_+-1)} \tag{3}$$

where t_+ is the transport number of potassium cation in the aqueous phase. The membrane parameter P_s is related to the thermodynamically effective fixed charge density ($\bar{\phi}\,\bar{X}$) on the membrane by the equation

$$\xi=\frac{\sqrt{1-P_s^2}}{2P_s} \tag{4}$$

where

$$\bar{\phi}\,\bar{X}=\frac{(\mathrm{K})_o+(\mathrm{K})_i}{2\,\xi}. \tag{5}$$

$\bar{\phi}$ is a constant ($0<\bar{\phi}<1$) and represents the fraction of counterions that are free and $\bar{X}$ is the stoichiometric charge density in the membrane.

The membrane potential data and the results calculated using Eqs. (1)–(5) are given in Tables 1 and 2 for the crayfish and *Myxicola* axons respectively. The values for the effective charge density ($\bar{\phi}\,\bar{X}$) corresponding to the physiological state of the membrane (i.e., ambient concentrations of $(\mathrm{K})_i=235$ and $(\mathrm{K})_o=5.4$ mmole per kg H_2O for the crayfish axon and $(\mathrm{K})_i=322$ and $(\mathrm{K})_o=10.1$ for the *Myxicola* axon) are found to be 0.443 and 0.444 mmoles per kg water for the crayfish and *Myxicola* axons,

Table 1. Membrane potential across giant axon of crayfish membrane at 20 °C (Strickholm & Wallin, 1967)

$(K)_o$ (mmole/kg H_2O)	$(K)_i$[a]	E_m[b] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s[c] [Eq. (3)]	ξ [Eq. (4)]	$\bar{\phi}\bar{X}$ [Eq. (5)] (mole/kg H_2O)
2.5	235	−94.0	−114.4	0.911	0.828	0.339	0.350
5.4	235	−83.0	− 95.0	0.937	0.879	0.271	0.443
10.1	282	−72.1	− 83.9	0.930	0.865	0.290	0.504
16.8	296	−61.1	− 72.3	0.923	0.852	0.307	0.509
25.1	314	−53.1	− 63.6	0.917	0.840	0.323	0.525
40.2	338	−42.3	− 53.6	0.895	0.797	0.379	0.499
75.5	338	−27.8	− 37.8	0.868	0.745	0.448	0.462
100.7	338	−21.0	− 30.5	0.844	0.698	0.513	0.428

[a] The values of $(K)_i$ according to Strickholm and Wallin (1967).

[b] Values of E_m taken from Table 1 (Strickholm & Wallin, 1967).

[c] Value used for t_+ in the evaluation of P_s is 0.49.

Table 2. Membrane potential across giant axon of *Myxicola* membrane at 20–22 °C (Goldman, 1968)

$(K)_o$ (mmole/kg H_2O)	$(K)_i$[a]	E_m[b] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s[c] [Eq. (3)]	ξ [Eq. (4)]	$\bar{\phi}\bar{X}$ [Eq. (5)] (mole/kg H_2O)
10.1	322	−69.3	−87.2	0.897	0.801	0.374	0.444
50.3	322	−44.2	−46.8	0.972	0.946	0.171	1.089
100.7	322	−27.7	−29.3	0.973	0.948	0.168	1.258
201.3	322	−10.1	−11.8	0.928	0.861	0.295	0.887
456.0	322	+ 6.0	+ 8.8	0.841	0.693	0.520	0.748

[a] Value of $(K)_i$ taken from Gilbert and Shaw (1969).

[b] Values of E_m read off from Fig. 4 of Goldman (1968).

[c] Value used for t_+ in the evaluation of P_s is 0.49.

respectively. Average values for $\bar{\phi}\bar{X}$ derived earlier (Lakshminarayanaiah & Murayama, 1975) for other systems are 0.300, 0.325, and 0.17 moles per kg water for the squid axon, frog, and barnacle muscles, respectively.

In order to derive values for the stoichiometric charge density $\bar{X}$, the assumption that the values of $\bar{\phi}$ determined for ion exchange membranes containing strong acid and weak acid groups, which are generally considered to be present in the biological membranes (Mozhayeva & Naumov, 1970, 1972*a*, *b*, *c*; Hille, 1973; Woodhull, 1973; Hille *et al.*, 1975), although their exact nature and the organic skeleton to which they are attached are different, are applicable to the biological membrane. In our recent

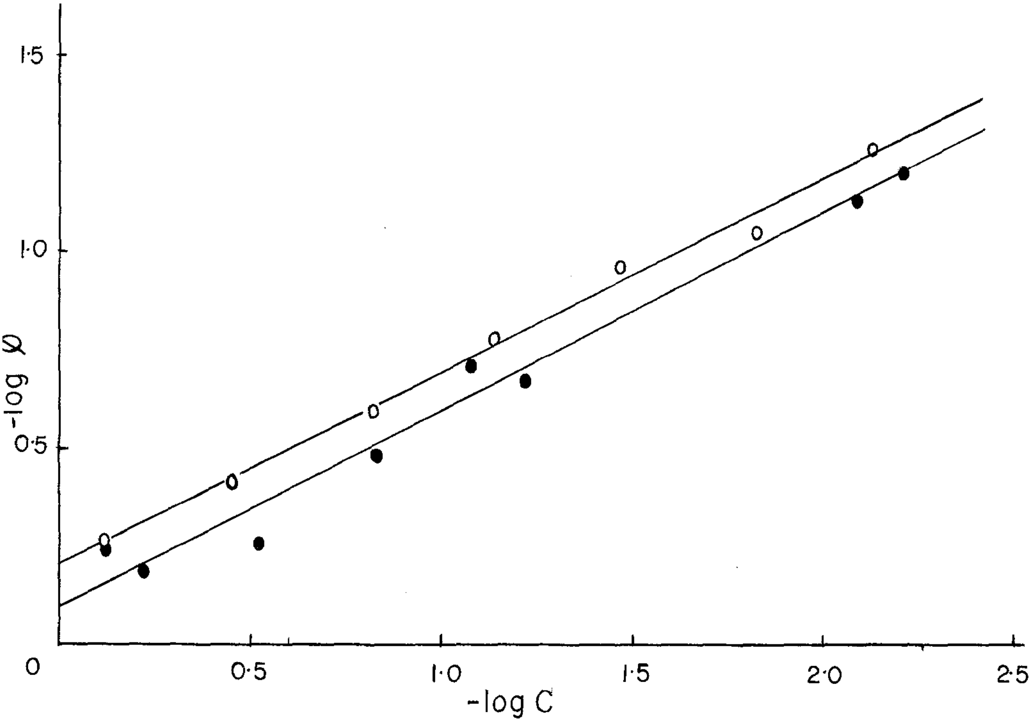

Fig. 1. Logarithm of average ambient concentration $[C=\{(\mathrm{K})_i+(\mathrm{K})_o\}/2]$ of ion exchange membrane plotted against the logarithm of $\bar{\phi}$. Open circles represent the values for the strong acid ion exchange membrane, crosslinked phenolsulfonate. The solid regression line conforms to the equation:

$$-\log\bar{\phi}=0.205-0.492\log C.$$

Closed circles represent the values for the weak acid ion exchange membrane, crosslinked polymethacrylate. The solid regression line conforms to the equation:

$$-\log\bar{\phi}=0.097-0.497\log C$$

paper (Lakshminarayanaiah & Murayama, 1975) very rough estimates of $\bar{\phi}$ pertaining to squid axon, frog, and barnacle muscles were made. Now a more accurate method is used. In Fig. 1, the values of $\bar{\phi}$ determined for the strong and weak acid membranes taken from the paper of Lakshminarayanaiah (1975*a*) are plotted against the concentration C of the ambient electrolyte solutions, i.e., $C=[(\mathrm{K})_i+(\mathrm{K})_o]/2$. The regression lines conform to the equations

$$-\log\bar{\phi}=0.205-0.492\log C \quad \text{for strong acid ion exchange membrane} \tag{6}$$

$$-\log\bar{\phi}=0.097-0.497\log C \quad \text{for weak acid ion exchange membrane.} \tag{7}$$

Table 3. Values derived for the different membrane parameters pertaining to various biolog-
Kobatake and

Membrane system	$\bar{\phi}\bar{X}$ (mole/kg H_2O)	$\bar{\phi}$ for acid group		$\bar{X}$ for acid group	
		strong	weak	strong	weak
				(mole/kg H_2O)	
Squid axon	0.300	0.286	0.364	1.05	0.824
Crayfish axon	0.443	0.220	0.279	2.01	1.59
Myxicola axon	0.444	0.258	0.328	1.72	1.36
Xenopus node	–	–	–	–	–
Frog (nodes of Ranvier)	–	–	–	–	–
Frog muscle (Sartorius and semitendinosus)	0.325	0.169	0.214	1.92	1.52
Barnacle muscle	0.170	0.196	0.248	0.868	0.680

[a] Begenisich (1975) calculations based on the data of Frankenhauser and Hodgkin (1957).
[b] Gilbert und Ehrenstein (1969).
[c] D'Arrigo (1973). Value derived by measurements of shift in theshold for excitation.
[d] Begenisich (1975).
[e] Schauf (1975).
[f] Begenisich (1975). Calculations based on the data of Brismar (1973).
[g] Brismar (1973).
[h] Vogel (1973).
[i] Begenisich (1975). Calculations based on the data of Hille (1968).
[j] Hille, Woodhull and Shapiro (1975).
[k] Mozhayeva and Naumov (1970, 1972*a*, *b*, *c*).

The ambient concentrations (values of C) corresponding to the physiological state of various biological systems substituted in Eqs. (6) and (7) gave values for $\bar{\phi}$. Thus the values for the stoichiometric charge density $\bar{X}$ may be derived. These values derived for various biological membrane systems are given in Table 3.

In order to convert volume charge density to surface charge density (σ), an additional assumption is made and it is (Lakshminarayanaiah, 1974) that the Davson-Danielli model for the biological membrane is valid and the water associated with the polar groups of the membrane that extend into the aqueous phases corresponds to membrane water whose thickness is assumed to be equivalent to the thickness of the electrical

ical systems following the equations of the theory of membrane potential developed by coworkers

(1 e/Å²) Uniform charge model (measurement of resting potential)			Discrete charge model – measurements of shifts in	
strong	weak	Average	g_{Na}	g_K
1/158	1/201	1/180	1/120[a]	1/120[a]
				(1/120–1/280)[b]
1/82	1/105	1/94	(1/43)[c]	
1/97	1/122	1/110	(1/100–1/140)[d]	(1/275–1/350)[d]
			1/77[e]	1/77[e]
		–	1/160[f]	1/290[g]
			1/70[h]	–
		–	1/90[i]	–
			(1/111–1/173)[j]	–
			–	(1/417–1/588)[k]
1/87	1/110	1/99	–	–
1/191	1/242	1/217	–	–

double layer, about 10 Å (Bockris & Reddy, 1970). On this basis, the values for σ, the surface charge density, derived for the various biological membranes are given in Table 3. In the last two columns of Table 3 are also given the values for σ derived by several investigators by the application of the double layer theory to the conductance (g_{Na} and/or g_K)-voltage shifts followed as a function of calcium concentration.

Discussion

There are a number of objections that may be raised in regard to the method described herein for the estimation of surface charges in biological membranes. First, how valid are the values used for $\bar{\phi}$? In the present state of our knowledge we do not know what these values are although, as pointed out already, the nature of the ionogenic groups are known to some extent. The break-through in this direction, it is believed, can come only from model system analysis which has been attempted in our work. When better model membranes, which mimic the network structure of biological membranes and at the same time become amenable for analytical analysis, become available, one can derive more reliable values for $\bar{\phi}$. Until then, the reliability of the values of $\bar{\phi}$ chosen in this work can be gauged only indirectly by comparing the results of ultimate analysis (for example, the values of σ) with those derived by other independent methods (*see* Table 3 for comparison of σ values).

A second question that may be raised is: is the K-theory developed for a situation in which only one cationic and one anionic species are present applicable to a situation in which more than one cation is present and E_m is governed by the concentrations and permeabilities of all cations and anions? The answer to this question is in the affirmative because the parameter essential to the calculation of charge density is permselectivity which, as defined by Eq. (3), is based on the measured value of E_m which takes into account the concentrations and permeabilities of all ions existing on either side of the membrane, although $\bar{t}_{+\,(\mathrm{app})}$ and $\bar{t}_+$ are referred only to potassium, the critical ion that controls to a significant level the value of E_m in the resting state of the membrane.

As developed in this paper, a third point of objection could be posed and it is: is the K-theory a uniform charge model? The answer to this question rests on the kind of model visualized for the biological membrane. If the membrane is considered a mosaic of both lipids and proteins where the ions permeate only through the regions occupied by the latter, then it is not a uniform model. On the other hand if the membrane is considered as a lipo-protein barrier with no discreteness attached to the distribution of lipids and proteins in the membrane (*see* Robertson, 1964; Stoeckenius & Engelman, 1969; Vanderkooi & Green, 1970; for various membrane models), it is a uniform model in that permeation of ions can occur through charged sites on the protein as well as on the lipids, at least in the resting state. Our present knowledge about the location of proteins and lipids in the membrane structure and their involvement in controlling at least the resting properties of the membrane does not permit any definite conclusions to be drawn in regard to discrete *vs.* uniform charge models.

To add to the foregoing complexities, a comment about the assumption concerning membrane water may be made. This assumption implies ignoring the water that may be present in the voids between the hydrocarbon chains of the lipid bilayer. Its magnitude although unknown may be negligible compared to the quantity of water associated with the polar groups. Equating the thickness of this water to that of the electrical double layer is based on the concepts concerning the structure of the double layer (really triple layer) at metal-aqueous solution surfaces (Bockris & Reddy, 1970). Whether this double layer is a multilayer because of the presence of water clusters (Horne, Day, Young & Yu, 1968) is a possibility that must be recognized. Further possibility that overlapping of double layers due to criss-crossing of biopolymeric chains in the neighborhood of the membranes surface cannot be ignored. In any case, what these possibilities would do to the thickness of the double layer remain unknown and so have been ignored.

A number of observations can be made about the values of σ given in Table 3. Application of the same method (conductance-voltage shifts) to the same preparation (*Myxicola* axon and *Xenopus* node) by two different investigators gave different values for σ. Whether these differences are significant or not is difficult to assess. Application of different methods, viz. the homogeneous model (the subject of this paper) and the measurement of threshold for excitation at appropriate concentrations of di- and trivalent cations, for the same preparation (crayfish axon) gave different results. Here again, it is difficult to assess the significance of these differences.

The method of analysis, based on what we believe to be a uniform charge model, indicates that the charge density on the membrane of the barnacle muscle is low compared to that on the frog muscle. In the case of the squid axon, the agreement in the values of σ derived by the application of the methods of uniform and discrete charge models is satisfactory. On the basis of all these results taken together, the following conclusion may be drawn: the uniform charge model analysis applicable to ion exchange membrane systems, on extrapolation to biological membrane systems, leads to results that show that, for the purposes of estimation of surface charge densities in biomembranes, both the homogeneous and discrete model approaches give almost identical results.

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