Bull. Chem. Soc. Jpn., ***64***, *3115—3120 (1991)*

Transport Studies across the Pericardial Membrane: Evaluation of Fixed Charge Density

Mohammad Ahmad,[†] Hasan Arif,* Haroon Rashid Khan, Mohammad Shoaib, and Mohadi Hasan[††]
Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India
[†]Centre of Cardiology and Cardiovascular Research, Aligarh Muslim University, Aligarh 202002, India
[††]Interdiscipilinary Brain Research Centre, Aligarh Mauslim University, Aligarh 202002, India
(Received September 3, 1990)

The fixed charge density, the permselectivity, and the transport number in several electrolytic solutions of varying concentration were determined across the pericardial membrane of freshly slaughtered buffalo (*Bof. Bubalis*). The irreversible thermodynamic equations originally meant for understanding the behavior of artificial membranes have been found to be equally applicable to those of the biological membranes.

The behavior of membrane systems has extensively been investigated by studying the transport properties of artificial membranes.[1—12] Some of the recent analytical and electronic techniques have added impetus to such investigations. However, in the cases of biological membranes the experimental difficulties have marred the meaningful electrophysiological studies; such as those of the membrane potential, the transport number, the permselectivity, the effective fixed charge density, the conductance, the capacitance, and the impedance.

The present study has, therefore, been undertaken with a view (i) to ascertaining as to whether or not the principle of passive transport is applicable to biological membranes, and (ii) to characterising their thermodynamic properties. Thus, the duramater, the pericardium, and the peritoneum being easily available intact biological membranes may be chosen for the purpose. Also, the transport number, the permselectivity, and the effective fixed charge density of these biological membranes are almost similar in signifying a common pattern in their behavior. In view of these facts the pericardial membrane has been taken as a test case.

Experimental

The pericardial membrane was taken out immediately after slaughtering the buffalo (*Bof. bubalis*) aged between 18—24 months, and immersed in an ice-cold Ringer solution of pH 7.4±0.2 for the preservation of the membrane tissues. The Ringer solution contained (in g dm^{-3}) NaCl: 9.00, KCl; 0.42, $CaCl_2$: 0.24, glucose: 1.00, and $NaHCO_3$: 0.15. The membrane was then washed three times with the deionized water to remove any traces of Ringer solution, prior to recording the membrane potential. The potential was developed by setting up a concentration cell of the type

$$\text{S.C.E.}|\text{Solution}|\text{Membrane}|\text{Solution}|\text{S.C.E.}$$
$$C_1 \qquad\qquad C_2=10C_1$$

in which S.C.E. stands for the saturated calomel electrode employed for measuring the membrane potential. The electrolytes used were the solutions of several concentrations of analytical grade (BDH, India), uni-univalent electrolytes, viz., NaCl, KCl, NH_4Cl, NaF, and KF in deionized water. The same electrolyte with different concentrations was used on both the sides of the pericardial membrane. The dilute side of the solution was taken as negative. Freshly obtained pericardial membranes were used for the potential measurements with each of these electrolytic solutions. The experiments were repeated a number of times with freshly prepared solutions of each of these electrolytes and the maximum potential values thus attained were recorded These measurements were made in a thermostated bath maintained at 25.0±0.1 °C.

Results and Discussion

The values of membrane potential ($\Delta\phi$ or E_m) measured across the pericardial membrane, separating different concentrations of 1 : 1 electrolytes are plotted as a function of $\log(C_1+C_2)/2$, with a fixed ratio of $C_2/C_1=\gamma=10$ (Fig. 1). The membrane potential after an initial increase, has been found to decrease with increase in the value of $\log(C_1+C_2)/2$. Another very important feature observed in the recording of the E_m across the pericardium, provided the concentrated side was hooked up to the positive terminal while the dilute side to that of the negative terminal. Such an observation suggests that the pericardial membrane is positively charged and is anion selective. It may also be noted, however, that for all the electrolytes below 1×10^{-3} M

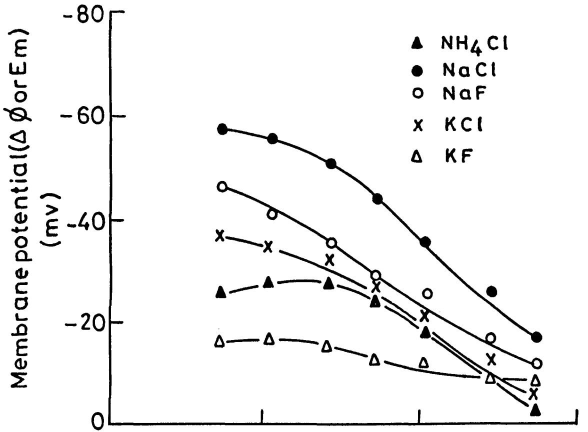

Fig. 1. Plots of observed potentials against log (C_1+C_2)/2 for various electrolytes with pericardium.

concentration (1 M=1 mol dm^{-3}) the number of ions generated matches the number of ions transferred to the other side of the membrane, thereby destroying completely the potential difference generated initially due to slight differences in the concentration of ions. Consequently, no potential differences is observed below the cited dilute concentration.

Starting with the basic flow equations Kobatake et al.[6] obtained the following relations:

$$\Delta\phi = -(RT/F)\left|\frac{1}{\beta}\ln\frac{C_2}{C_1} - \left(1+\frac{1}{\beta}-2\alpha\right)\ln\left(\frac{C_2+\alpha\beta\theta}{C_1+\alpha\beta\theta}\right)\right|, \quad (1)$$

where

$$\alpha = \frac{u}{u+v} \quad (1a)$$

and

$$\beta = 1+\left(\frac{KF\theta}{u}\right), \quad (1b)$$

for the membrane potential ($\Delta\phi$) which arises between two solutions of 1 : 1 electrolytes of different concentration, C_1 and C_2 ($C_1 < C_2$) that are separated by a membrane.

Here, u and v are the mobilities of the positive and the negative ions, respectively, defined in terms of the mass fixed frame of reference, K is a constant, θ is the charge density, and F is the Faraday constant. These parameters are found to be independent of salt concentrations, C_1 and C_2.

For the analysis of the data, Eq. 1 can be used under two sets of conditions, namely, (a) in the dilute range, and (b) in the concentrated range; hence the two limiting forms of the above equation have been obtained as given below: (a) When concentration C_2 becomes sufficiently small, (external dilute range) Eq. 1 can be expanded[6] to given

$$\Delta\phi_r = \frac{1}{\beta}\ln\gamma - \frac{(\gamma-1)}{\alpha\beta\gamma}\left(1+\frac{1}{\beta}-2\alpha\right)\left(\frac{C_2}{\theta}\right) + O\left[\left(\frac{C_2}{\theta}\right)^2\right] \quad (2)$$

where $\Delta\phi_r$ is the absolute value of reduced potential defined by

$$\Delta\phi_r = \frac{F\Delta\phi}{-RT} \quad (3)$$

and

$$\gamma = \frac{C_2}{C_1}.$$

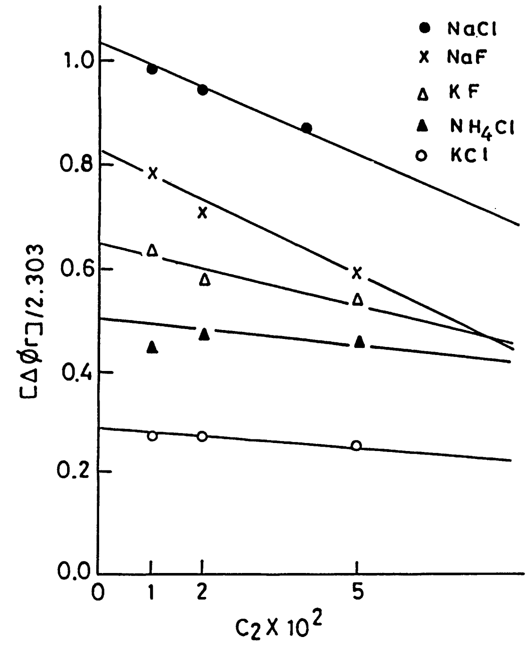

Fig. 2. Plots of $\Delta\phi_r/2.303$ versus $C_2 \times 10^2$ for various electrolyte with pericardium.

Table 1. Calculated Values of the Parameters α and β for Various Electrolytes at $\gamma=10$

Electrolytes	α	β
NaCl	0.56	0.97
NaF	0.55	1.22
KF	0.49	1.55
NH$_4$Cl	0.44	2.00
KCl	0.59	3.51

Equation 2, indicates that the value of β; as well as a relation between α and θ can be obtained by evaluating the intercepts and the initial slope of a plot of $\Delta\phi_r$ against C_2. Figure 2 shows a variation of reduced membrane potential, $\Delta\phi_r$ with variation in concentration in the low concentration range of the electrolyte. With an increase in concentration $\Delta\phi_r$ shows an insignificant decrease. The value may be evaluated from the slope, $1/\beta \ln\gamma$ of the above plot listed in Table 1. (b) It is found experimentally that at a fixed value of γ, the inverse of an apparent transport number, t_{app}, is proportional to the inverse of concentration, C_2, in higher salt concentration range. Here, t_{app} is defined by the relation,

$$\Delta\phi_r = (1-2\,t_{\mathrm{app}})\ln\gamma. \quad (4)$$

The values of the transport number (t_{app}), calculated from the observed membrane potentials using Eq. 4. Figure 3 shows the variation of the inverse of t_{app} of ions with that of the inverse of concentration, C_2. As the inverse of the concentration increases, the inverse of the apparent transference number also increases. This has been observed only in high concentration range. Such an observation, however, has been found to be absent in the dilute concentration range, seemingly due to an inadequate concentration for raising the required polarization.

Kobatake et al.[6] have proposed a simple method using the following approximate equation for the diffusive contribution to the emf of a cell with transport. On substituting Eq. 3 into that of Eq. 4, the following relation between $\Delta\phi$ and t_{app} is obtained:

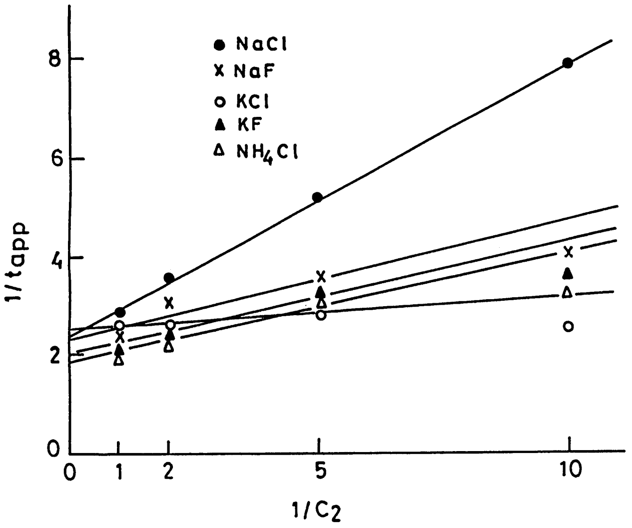

Fig. 3. Plots of $1/t_{app}$ versus $1/C_2$ for various electrolytes with pericardium.

$$\frac{F\Delta\phi}{RT}=(1-2\,t_{app})\ln\gamma, \tag{5}$$

substituting the value of $\Delta\phi$ from Eq. 1 and expanding the resultant for $1/t_{app}$, in powers of $1/C_2$, we get,

$$\frac{1}{t_{app}}=\frac{1}{1-\alpha}+\frac{(1+\beta-2\alpha\beta)(\gamma-1)\alpha}{2(1-\alpha)^2\ln\gamma}\left(\frac{\theta}{C_2}\right)+0\left[\left(\frac{\theta}{C_2}\right)^2\right]. \tag{6}$$

This indicates that the intercept for a plot of $1/t_{app}$ vs. $1/C_2$, at a fixed value of γ allows the value of α to be determined. The value of the intercept is equal to $1/(1-\alpha)$, from which α may be evaluated. Figure 3 illustrates the plots of $1/t_{app}$ vs. $1/C_2$ for various electrolytes.

For the evaluation of charge density, θ, there are two limiting cases. In the dilute range, the value of θ_d was determined by inserting the values of α and β (Table 1) in the relation between α and θ. Once α and β are known in the manner described above, the charge density θ_c (in the concentrated range), is also evaluated from the initial slope of $1/t_{app}$ against $1/C_2$ plot. The present study reveals that the charge density (θ_d) in dilute as well as in concentrated range (θ_c) agree well with each other (Table 2), thereby confirming the applicability of the Kobatake and co-worker's equation to this biological membrane.

Table 2. Charge Density (mol dm^{-3}) of Pericardial Membrane Obtained by Different Methods at γ=10

Theory	Electrolyte				
	NaCl	NaF	KCl	KF	NH_4Cl
θ_d×10^2 Kobatake's method	20.16	8.84	4.04	13.71	—
θ_c×10^2 Kobatake's method	10.7	4.93	3.94	6.58	6.71
θ_x×10^2 Kobatake's Permselectivity method, P_s vs. $\log(C_1+C_2)/2$	15.14	5.01	—	6.07	8.91
θ_x×10^2 Tasaka's method	4.11	1.29	4.76	1.64	1.90

A general method for the characterization of membranes in terms of permselectivity (P_s), which is applicable to any system, irrespective of ionic species, has been developed by Kamo et al.[9] Kobatake and Kamo[12] derived Eq. 7 for the measurement of permselectivity (P_s) for a positively charged membrane.

$$\frac{1}{(4\,\xi^2+1)^{1/2}}=P_s=\frac{[T_{app}-(1-\alpha)]}{[1-\alpha-(1-2\alpha)T_{app}]}. \tag{7}$$

It may be noted that the P_s value between zero and unity depends on the external salt concentration for the given system of membrane electrolyte pair. Now P_s can be calculated from the data of membrane potential, while the left-hand side of Eq. 7 is a function of the relative concentration $\xi=C/\phi_x$ or $(C_1+C_2)/2\phi_x$. Thus, the value of the right-hand side should be independent of the mobilities of ionic species involved. Equation 7 implies that the plot of P_s vs. $(1+4\,\xi^2)^{-1/2}$ should be a

Table 3. Calculated Values of Permselectivity (P_s), ξ, and an Arbitrary Parameter β'

Electrolytes	Concn C_2/C_1	P_s[a)]	ξ	$\beta'=n\beta$
NaCl	1/1×10^{-1}	0.178	3.66	0.863
	5×10^{-1}/5×10^{-2}	0.344	1.83	0.800
	2×10^{-1}/2×10^{-2}	0.533	0.73	1.102
	1×10^{-1}/1×10^{-2}	0.689	0.36	1.784
	5×10^{-2}/5×10^{-3}	0.837	0.183	2.830
	2×10^{-2}/2×10^{-3}	0.919	0.007	8.703
	1×10^{-2}/1×10^{-3}	0.982	0.0029	10.109
NaF	1/1×10^{-1}	0.09	11.00	0.778
	5×10^{-1}/5×10^{-2}	0.22	5.50	0.630
	2×10^{-1}/2×10^{-2}	0.34	2.20	0.716
	1×10^{-1}/1×10^{-2}	0.42	1.10	0.983
	5×10^{-2}/5×10^{-3}	0.53	0.55	1.601
	2×10^{-2}/2×10^{-3}	0.65	0.22	4.868
	1×10^{-2}/1×10^{-3}	0.75	0.11	12.173
KF	1/1×10^{-1}	0.11	9.16	0.758
	5×10^{-1}/5×10^{-2}	0.23	4.58	0.662
	2×10^{-1}/2×10^{-2}	0.38	1.83	0.725
	1×10^{-1}/1×10^{-2}	0.46	0.91	1.033
	5×10^{-2}/5×10^{-3}	0.56	0.45	1.954
	2×10^{-2}/2×10^{-3}	0.59	0.18	8.659
	1×10^{-2}/1×10^{-3}	0.66	0.09	26.062
NH_4Cl	1/1×10^{-1}	0.09	6.87	0.918
	5×10^{-1}/5×10^{-2}	0.28	3.43	0.657
	2×10^{-1}/2×10^{-2}	0.43	1.37	0.788
	1×10^{-1}/1×10^{-2}	0.51	0.68	1.286
	5×10^{-2}/5×10^{-3}	0.56	0.34	3.051
	2×10^{-2}/2×10^{-3}	0.55	0.13	18.278
	1×10^{-2}/1×10^{-3}	0.55	0.059	83.629

a) P_s values were obtained using the relation, $P_s=\dfrac{T_{app}-(1-\alpha)}{1-\alpha-(1-2\alpha)T_{app}}$, $\xi=\dfrac{c}{\phi_x}$, β' or $n\beta$ values were computed using the relation $P_s=[1/\sqrt{1+4\xi^2}]^{\beta}$.

straight line of unit slope provided the charge density is independent of salt concentration. The various values of permselectivity (Table 3) were calculated by substituting the values of α and t_{app} for pericardium. Another method of determining the charge density, based on permselectivity as developed by Kamo et al.[9] is also used here. The calculated values of P_s of pericardial membrane were plotted against log $(C_1+C_2)/2$. The curves thus obtained with various electrolytes are shown in Fig. 4. When the average concentration, $(C_1+C_2)/2$, becomes equal to the effective fixed charge density ϕ_x, the value of ξ becomes unity, i.e. $C/\phi_x=1$. Substituting this value of $\xi=1$ in $P_s=(1+4\ \xi^2)^{-1/2}$, the value of P_s becomes equal to 0.448. At this particular value (0.448), the corresponding concentration was obtained from the P_s versus log C plot. It is apparent from Fig. 4 that this value of concentration should be equal to a fixed charge density for various electrolytes. The values of charge density obtained with various electrolytes are given in Table 2. Plots of P_s vs. $(1+4\ \xi^2)^{-1/2}$ using different electrolyte is shown in Fig. 5.

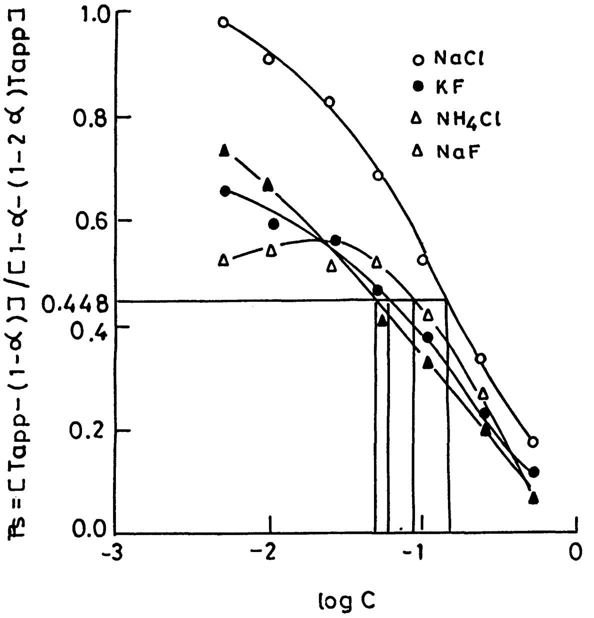

Fig. 4. Plots of P_s versus log $(C_1+C_2)/2$ for various electrolyte with pericardium.

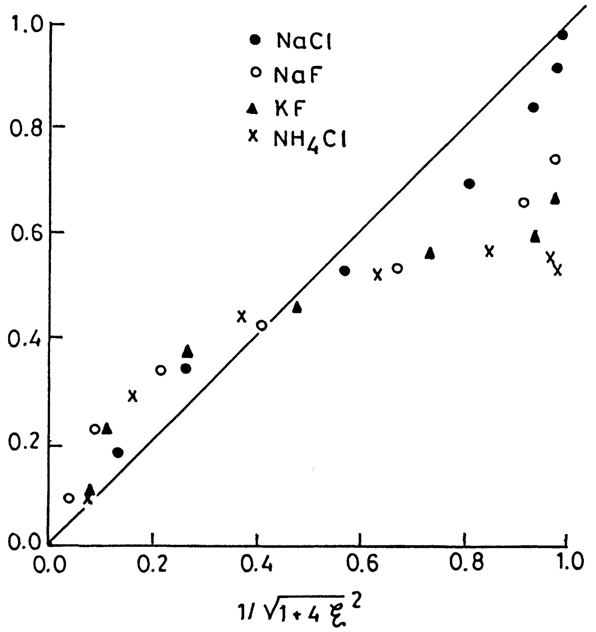

Fig. 5. Plots of P_s versus $1/\sqrt{1+4\ \xi^2}$ for various electrolytes with pericardium.

An apparent straight line of slope close to unity is the theoretical line with the assumption that the values of ϕ_x are independent of salt concentration as seemingly found for the NaCl case unlike those of the other electrolytes for which ϕ_x seems to be a function of salt concentration as apparent from the limiting values of P_s versus $(1+4\ \xi^2)^{-1/2}$ plots. An examination of Table 2 suggests that the extent to which the values of ϕ_x depend on the salt concentration is different in the cases of NaF, NaCl, KF, KCl, and NH_4Cl in which such a dependence is quite marked. Such a behavior may consequently be understood in terms of the nature as well as the concentration of the ionic species present across the pericardial membrane. Consequently, an attempt has been made to express the P_s values in terms of an arbitrary parameter, β', which turns out to be a sort of measure of the extent of deviation from linearity and is equal to $n\beta$ where n is an arbitrary parameter. This has been achieved by accounting for $(1+4\ \xi^2)^{-1/2}$ term contributing to P_s to different extent $(n\beta)$ depending upon the interaction of the solute ions with the solvent. Thus, P_s is expressed as

$$P_s=\left[\frac{1}{(1+4\ \xi^2)^{-1/2}}\right]^{\beta'}=\left[\frac{1}{(1+4\ \xi^2)^{-1/2}}\right]^{n\beta}=\frac{[T_{\text{app}}-(1-\alpha)]}{[1-\alpha-(1-2\alpha)\ T_{\text{app}}]}. \tag{8}$$

It is worthwhile to note that the proposed Eq. 8 is expected to be applicable to any type of biological membrane irrespective of the extent of deviation shown in the behavior of P_s versus $1/\sqrt{1+4\ \xi^2}$ plots. The β' values for NaCl, NaF, KF, and NH_4Cl solutions were computed (Table 3) using the rearranged form of the above equation, $\beta'=-2\log P_s/\log(1+4\ \xi^2)]$. Finally the plots of P_s against $[1/(1+4\ \xi^2)^{1/2}]^{n\beta}$ (Fig. 6) supports the above equation in which the extent of deviation has already been accounted for in terms of $n\beta$.

Tasaka et al.[13] gave two limiting forms of expressions for the membrane potential, (i) in very low concentrations, it was found that

$$\Delta\phi_r=\ln\gamma, \tag{9}$$

where $\Delta\phi_r$ is the reduced membrane potential, and (ii) at high concentration of electrolyte Eq. 10 was found to be applicable.

$$-\Delta\phi/\frac{\gamma-1}{\gamma}=\frac{RT}{F}(\phi_x/2)\frac{1}{C_1}, \tag{10}$$

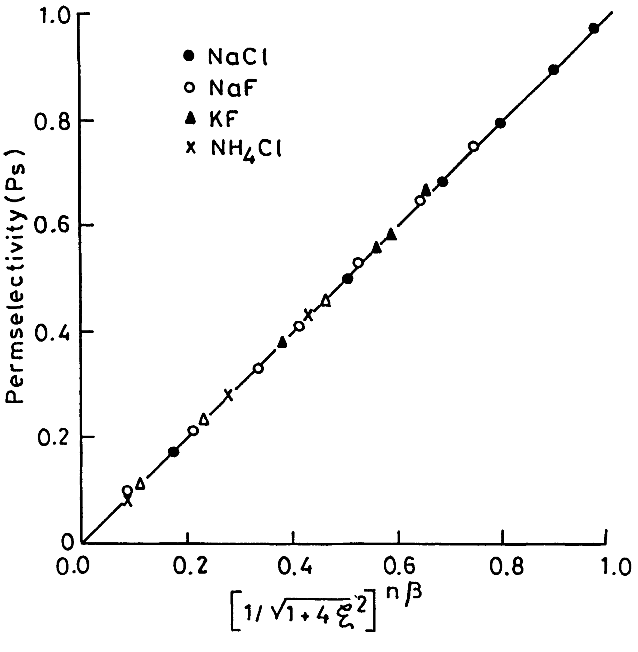

Fig. 6. Plots of P_s versus $[1/\sqrt{1+4\,\xi^2}]^{n\beta}$ for various electrolytes with pericardium.

when α=0.5, i.e. in ordinary electrolyte solutions, Eq. 10 leads to

$$\Delta\phi_r = \frac{(2\alpha-1)}{2(1-\alpha)}\ln\gamma + \frac{(\gamma-1)\phi_x}{2(1-\alpha)\,2\gamma\,C_1}, \qquad (11)$$

In extremely high concentrations, Eq. 10 predicts a linear relationship between $\Delta\phi/[(\gamma-1)/\gamma]$ and $1/C_1$ from which the charge density (ϕ_x) can be determined. The different values of ϕ_x, derived from the initial slope of various curves of Fig. 7 are given in Table 2.

The results of these investigations show that the membrane potential can be determined with some reasonable accuracy. Also, when the pericardium separates concentrated solutions, the values of potential generated across the pericardial membrane are negative. This means that the membrane is anion selective and is positively charged. It may be noted that the anion selectivity decreases as the concentration across the membrane is increased. Again it was also observed that the apparant transport number values for the different electrolytes increases by increasing the concentration of the electrolytes across the pericardium. Furthermore, it is noted from Table 2 that the charge density values of the membrane electrolyte system are low.

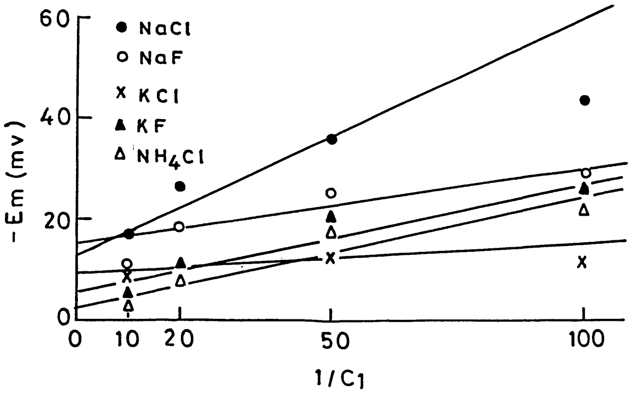

Fig. 7. Plots of E_m versus $1/C_1$ for various electrolytes with pericardium.

One would expect that the thermodynamic properties of biological membranes and those of artificial ones would be very different from each other because of the fact that the biological membranes are hitherto believed to function by active process (Ussing[14–16]), while artificial membranes are governed by the principle of passive transport. However, as stated above the present findings tend to support the contention that the laws of irreversible thermodynamics meant for passive transport are equally good for biomembranes.

The phenomenon of passive transport observed in cases of artificial membranes and that of active transport in those of biological membranes depend essentially on chemical nature of the membrane as well as on the electromotive forces developed across the cell membranes. Such a consideration has led us to examine their applicability to the biological membranes also. Consequently, it was found that the said models originally meant for explaining the behavior of artificial membranes, were equally applicable to biological membrane under investigation. As is well known, the purpose of active transport is to regulate the transfer of ions to suit the requirements of the cell. This cannot be achieved by passive transport. It seems likely that under diseased states, changes in electrolytic concentration, emotional factors etc., active transport may be inhibited and passive transport may become the dominant phenomenon. Such a situation will influence cell nutrition and its longevity because transport of essential materials and prevention of entry of toxic substances into the cell depend on the integrity of membrane function.

Thanks are due to Aligarh Muslim University, Aligarh for providing necessary facilities and to the Indian council of Medical Research, New Delhi for providing financial assistance. The cooperation and support of Professor S. A. A. Zaidi Chairman, Department of Chemistry is appreciated, We are highly indebted to Professor Nural Islam, Professor of Physical Chemistry, A.M.U. Aligarh for the help rendered in revising the manuscript.

References

1) N. Lakshminarayanaiah and F. A. Siddiqui, *Biophys. J.*, **11**, 603 (1971).

2) N. Lakshminarayanaiah and F. A. Siddiqui, *Biophys. J.*, **12**, 540 (1972).

3) S. Sheela Santhakumari and P. Radhakrishnamurty, *Bull. Electrochem.*, **1**(3), May-June, 337 (1985).

4) Y. Kobatake, *J. Phys. Chem.,* **28**, 146 (1958).

5) M. Nagasawa and Y. Kobatake, *J. Phys. Chem.,* **56**, 1017 (1952).

6) Y. Kobatake, N. Takeguchi, Y. Toyoshima, and H. Fujita, *J. Phys. Chem.,* **69**, 3981 (1965).

7) Y. Toyoshima, M. Yussa, Y. Kobatake, and H. Fujita, *Trans. Faraday Soc.,* **63**, 2803, 2814 (1967).

8) N. Kamo, Y. Toyoshima, and Y. Kobatake, *Kolloid Z. Z. Polym.,* **249**, 1061 (1971).

9) N. Kamo, Y. Toyoshima, H. Nozaki, and Y. Kobatake, *Kolloid Z. Z. Polym.,* **248**, 914 (1971).

10) N. Kamo, M. Oikawa, and Y. Kobatake, *J. Phys. Chem.,* **77**, 92 (1973); *ibid.,* **77**, 2995 (1973).

11) M. Yussa, Y. Kobatake, and H. Fujita, *J. Phys. Chem.,* **72**, 2871 (1968).

12) Y. Kobatake and N. Kamo, *Prog. Polym. Sci. Jpn.,* **5**, 257 (1972).

13) M. Tasaka, N. Aoki, Y. Konda, and M. Nagasawa, *J. Phys. Chem.,* **79**, 1307 (1975).

14) H. H. Ussing, Ion Transport Mechanism, Symp. Biol. Approaches to Cancer Chemotherapy, Louvain, June 1960.

15) H. H. Ussing, *Acta Physiol. Scand.,* **17**, 1 (1949).

16) H. H. Ussing and K. Zerhn, *Acta Physiol. Scand.,* **23**, 110 (1951).