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ON THE BEHAVIOUR OF 'COUPLED' ION-EXCHANGE MEMBRANE ELECTRODES IN BIOPOLYMERIC SOLUTION

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SUMMARY

A new method is reported for the biochemical and biophysical investigation of solutions of colloidal electrolytes or biopolymers.

This method is recommended whenever the determination of variation in ion activity is difficult. It is particularly suitable for the study of unstable biologically important compounds and of biopolymers which undergo conformational or configurational changes.

Experimental data on the reliability and reproducibility of the method are given as well as an outline of a possible theoretical approach for evaluating electromotive force from such a system.

INTRODUCTION

Many papers have been published[1-3] on the preparation and the electrochemical behaviour of several types of membrane containing fixed charged groups. The interest in this subject is justified mainly for three reasons, namely, applications in electrodialysis and kindred processes, the use of membranes as electrodes to determine ionic activity in solution and the possibility of considering some membranes as models to aid a better understanding of bioelectrical phenomena displayed by biological membranes, such as nerve or muscle membranes. The system of 'coupled' ion-exchange membranes discussed in this work seems to be of some interest from these latter two points of view. In fact some of the possibilities offered by this method—*i.e.* to operate freely in a solution placed between two membranes without involving a liquid junction potential; to vary the chemical composition and the physical state of the interposed macromolecular system; to carry out measurements even in the gel state with no noticeable difficulties—are worthwhile features. This method is particularly useful with macromolecules of highly specific biological activity whose instability and sensitivity to physicochemical changes make their systematic investigation complicated.

The importance of this method, as a starting point for building interesting models to study phenomena related to muscle and nerve problems, is evident. Results obtained[4] on some electrochemical aspects of actomyosin formation seem to confirm this point of view.

EXPERIMENTAL

Membranes

The membranes used in this work were made of sulphonated polystyrene embedded in a collodion matrix. The preparation and the use of such membranes as electrodes are extensively discussed by GREGOR AND SOLLNER[5] and TEORELL[6]. The polystyrene had a molecular weight of about 90 000 and was sulphonated as described by NEIHOF[7]. After purification, the acid value of the polystyrene sulphonic acid was 4.80 mequiv/g, as compared with a theoretical value of 5.43 mequiv/g, calculated for a linear polymer having one sulphonic acid group for each benzene ring.

Membranes were prepared by casting solutions of 4% collodion from a 1:2 mixture of alcohol and ether at various polystyrene sulphonic acid/collodion ratios. Two types of membranes d_i and d_0 with different charge densities, *i.e.* $d_i = 5 \cdot 10^{-4}$ equiv/kg and $d_0 = 5 \cdot 10^{-1}$ equiv/kg, were prepared. Total exchange of the hydrogen ions in a membrane with Na^+ (or K^+) was achieved by equilibrating the membrane in NaCl (or KCl) solution of about 0.5 M. The uptake of NaCl by uniformly charged membranes d_i and d_0 was determined by equilibrating 0.5 g of the membranes with large volumes of NaCl solution according to a procedure described elsewhere[9].

Material

Sodium lauryl sulphate was prepared according to DREGER *et al.*[8]. The critical micelle concentration in aqueous solution at 25±0.01° determined by conductivity measurement, was $8.0 \cdot 10^{-3}$ M.

The sodium polystyrene sulphonate was obtained by neutralizing polystyrene sulphonic acid with NaOH.

All other reagents used were of analytical grade.

Apparatus

Potentiometric measurements were made with a Radiometer pH-meter M4. The activity of Na^+ (or K^+) ions in the solutions was calculated by means of calibration curves which allowed the conversion of electromotive force into activities. The calibration curve was obtained with both membrane electrodes and a couple of sodium-saturated calomel electrodes (Beckman No. 39278). This curve was obtained by gradually diluting a NaCl solution, recording the electromotive force, and plotting these values *versus* the logarithm of Na^+ ion activity at each step; the result was a linear plot.

The a_{Na^+} values of the solution were calculated from the measured electromotive force by interpolating the calibration curve. This was done by assuming $\gamma_{Na^+} = \gamma_{\pm NaCl}$ in the calibration solution.

Conductivity measurements were performed by means of a WTW model LF3 bridge in a thermostatic bath at 25±0.01°.

In Fig. 1a schematic drawing shows the arrangement of the membranes in a 3-compartment cell. I indicates the layer of the membrane oriented toward the electrode and II the layer oriented toward the other membrane.

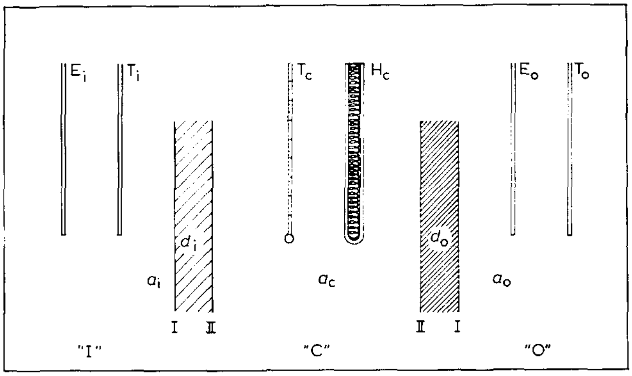


Fig. 1. Schematic arrangement of membranes and electrodes in the cell.

d_i is the membrane having a density of ionizable groups of $5 \cdot 10^{-4}$ equiv/kg.
d_o is the membrane having a density of ionizable groups of $5 \cdot 10^{-1}$ equiv/kg.
a_i is the activity of the solution i, which is in contact with the electrode E_i and the membrane d_i.
a_o is the activity of the solution o which is in contact with the electrode E_o and the membrane d_o.
a_c is the activity of the solution c placed in the central compartment and bathing both membranes d_i and d_o.
E_i is the reference electrode immersed in the solution i.
E_o is the other reference electrode, identical with E_i, and immersed in the solution o. Such electrodes may be either saturated calomel electrodes connected to the solution by means of an agar bridge or Ag/AgCl electrodes.
T_i, T_o, and T_c, are thermometers to show the temperature in the 3 compartments during the thermal treatment.
H_c is a tiny electric heater dipped into solution c.

Procedure

A reference solution (NaCl or KCl $1 \cdot 10^{-2}$ M) of known and constant concentration ($a_i = a_o$) was placed in the two external half cells. The central compartment of the cell contained the solution of a colloidal electrolyte or of a biopolymer. This solution could be stepwise diluted, concentrated, heated or titrated with another solution.

A previous calibration curve was obtained maintaining constant the 2 external solutions (NaCl $1 \cdot 10^{-2}$ M), while varying stepwise the concentration of NaCl in the central compartment of the cell. The electromotive force was recorded at each step (see Table I).

The recorded electromotive force values are compared with those calculated in order to have a calibration curve for the above-mentioned system (see Fig. 2).

RESULTS AND DISCUSSION

The system of 'coupled' ion-exchange membranes previously described may be considered a development of the asymmetric charged membrane studied by LIQUORI AND BOTRÉ[9].

TABLE I

NUMERICAL VALUES OF THE PARAMETERS

$M \times 10^3$	$2a \times 10^3$	$\frac{d_0\gamma_0}{2a_0}$	$\log\left[\frac{d_0\gamma_0}{2a_0} + \left(1 + \frac{d_0^2\gamma_0^2}{4a_0^2}\right)^{\frac{1}{2}}\right]$	ψ_0^{II}	$\Delta\psi_{0\,Donnan}$	$\frac{2a_0}{d_0\gamma_0}$	$1 + \left(\frac{2a_0}{d_0\gamma_0}\right)^2$	$\sqrt{1 + \left(\frac{2a_0}{d_0\gamma_0}\right)^2}$	$U + \sqrt{1 + \left(\frac{2a_0}{d_0\gamma_0}\right)^2}$	$\left[\frac{U + \sqrt{1 + \left(\frac{2a_0^I}{d_0^I\gamma_0^I}\right)^2}}{U + \sqrt{1 + \left(\frac{2a_0^{II}}{d_0^{II}\gamma_0^{II}}\right)^2}}\right] = \mathrm{A}$	log [A]	$\Delta\psi_{0\,diffusion}$
2.0	3.824	5.230	1.023	59.33	35.38	0.191	1.036	1.018	1.888	0.850	−0.071	−3.38
3.0	5.676	3.523	0.857	49.71	25.76	0.284	1.081	1.040	1.910	0.860	−0.066	−3.33
4.0	7.512	2.662	0.741	42.98	19.03	0.376	1.141	1.068	1.938	0.873	−0.059	−2.98
5.0	9.330	2.143	0.655	37.99	14.04	0.467	1.218	1.104	1.974	0.889	−0.051	−2.58
8.0	14.65	1.365	0.485	28.13	4.18	0.733	1.537	1.240	2.110	0.950	−0.022	−1.11
10.0	18.14	1.103	0.413	23.95	0.00	0.907	1.823	1.350	2.220	1.000	0.000	0.00
15.0	26.70	0.750	0.301	17.46	− 6.49	1.335	2.782	1.668	2.538	1.143	+0.058	+2.93
20.0	35.08	0.570	0.234	13.57	−10.38	1.754	4.077	2.019	2.889	1.301	0.114	5.76
25.0	43.36	0.460	0.192	11.14	−12.81	2.169	5.705	2.389	3.259	1.468	0.167	8.43
30.0	51.36	0.390	0.164	9.51	−14.44	2.571	7.610	2.759	3.629	1.635	0.214	10.81
35.0	59.30	0.337	0.142	8.24	−15.71	2.967	9.803	3.131	4.001	1.802	0.256	12.93

$\Delta\psi_{0\,tot}$	$\frac{d_i\gamma_i}{2a_i} \times 10^3$	$\log\left[\frac{d_i\gamma_i}{2a_i} + \left(1 + \frac{d_i^2\gamma_i^2}{4a_i^2}\right)^{\frac{1}{2}}\right] \times 10^4$	ψ_i^{II}	$\Delta\psi_{i\,Donnan}$	$\sqrt{1 + \left(\frac{2a_i^0}{d_i\gamma_i}\right)^2}$	$\left[\frac{U + \sqrt{1 + \left(\frac{2a_i^I}{d_i^I\gamma_i^I}\right)^2}}{U + \sqrt{1 + \left(\frac{2a_i^{II}}{d_i^{II}\gamma_i^{II}}\right)^2}}\right] = \mathrm{B}$	log [B]	$\Delta\psi_{i\,diffusion}$	$\Delta\psi_{i\,tot.}$	$E_{theor.}$	$E_{exp.}$
38.96	3.3	17	0.10	0.08	303	0.212	−0.674	−2.88	2.96	41.92	40.0
29.09	2.2	12	0.07	0.05	455	0.317	−0.499	−2.13	2.18	31.28	30.0
22.01	1.7	9	0.05	0.03	588	0.412	−0.386	−1.65	1.68	23.69	23.5
16.62	1.3	7	0.04	0.02	769	0.539	−0.269	−1.15	1.17	17.79	18.0
5.29	0.9	5	0.03	0.01	1110	0.770	−0.114	−0.49	0.50	5.79	6.5
0.00	0.7	4	0.02	0.00	1428	1.000	0.000	0.00	0.00	0.00	0.8
− 9.42	0.5	3	0.02	−0.001	2000	1.400	+0.146	+0.62	−0.62	−10.04	− 9.2
−16.14	0.4	2	0.01	−0.01	2500	1.750	0.232	0.99	−1.00	−17.14	−15.0
−21.24	0.3	2	0.01	−0.01	3333	2.330	0.367	1.57	−1.58	−22.82	−21.0
−25.25	0.24	1	0.01	−0.01	4166	2.920	0.465	1.99	−2.00	−27.25	−25.0
−28.64	0.21	1	0.01	−0.01	4761	3.334	0.522	2.23	−2.24	−30.88	−28.0

An asymmetric membrane, in fact, is characterized by a sharply different concentration of ionizable groups in the 2 external layers and by a concentration gradient of fixed charges inside the membrane itself going from one layer to the other.

Let us suppose that we have 2 homogeneous membranes, each corresponding to the charge density of the 2 external layers of the asymmetric membrane. If a saline solution of known concentration is placed between these 2 membranes we obtain an arrangement (Fig. 1) of membranes and solutions homogeneous and symmetrical in each part but asymmetrical on the whole.

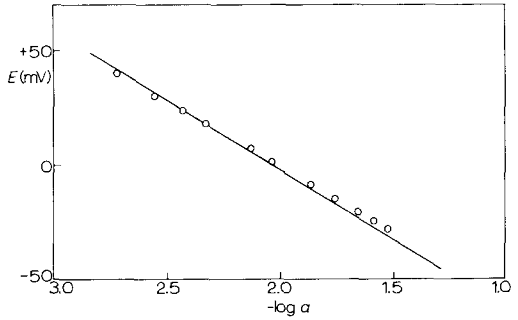


Fig. 2. Electromotive force (in mV) *vs.* logarithm of ionic activity when NaCl solution is diluted stepwise in the central compartment of the cell. Two external compartments were filled with NaCl $1 \cdot 10^{-2}$ M. The electromotive force values were recorded at each step. The points in the figure represent experimental values, the solid line calculated ones.

The use of 2 membranes but with different charge densities was necessary in order to establish a system with a certain degree of asymmetry. This asymmetry allows the use of 2 membranes in an arrangement suitable for measurement. Obviously the use of 2 membranes of the same charge density cannot satisfy this prerequisite of asymmetry. In such an arrangement an electromotive force equal to zero would logically be recorded.

When a permselective membrane is interposed between 2 solutions, i and o, an electromotive force develops. To calculate such an electromotive force 2 different theories have been formulated which lead to an identical expression in limiting conditions.

According to TEORELL[6], the total membrane potential may be calculated by adding, with the proper sign, the potential differences between the faces of the membrane and the solutions in contact with them resulting from the Donnan distributions of the small ions and the diffusion potential. The latter is established through the membrane as a result of the different mobilities of the diffusible ions absorbed in the external layers.

Using the principles of irreversible thermodynamics STAVERMAN[14], GREGOR AND SOLLNER[5], KATCHALSKY AND KEDEM[15] and SPIEGLER[16] formulated a theoretical treatment for membrane phenomena occurring in the steady state. However, in the limiting conditions of highly and uniformly charged membranes (that is, no change in the concentration of fixed ionizable groups in the membrane going from one face to the other and no solvent flow) the following relationship is valid: $\Delta E = \frac{RT}{F} \ln \frac{a_0}{a_i}$.

In fact for an 'ideally ion selective' membrane according to the TEORELL treatment, the diffusion term tends to a minimum, and the Donnan term to a maximum-value. For the same 'ideally ion-selective' membrane according to the irreversible thermodynamic principles the term

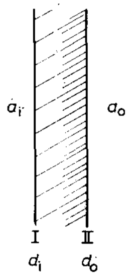
$$\frac{\bar{t}_+ - \bar{t}_-}{\bar{t}_+ + \bar{t}_-}$$

is *plus* or *minus* 1 according to the sign of charged groups in the membrane (where $\bar{t}_+$ and $\bar{t}_-$ are the transference numbers of cations and anions in the membrane).

When the theory of TEORELL[6] and MAYER AND SIEVERS[10] for uniformly charged membranes is applied to an asymmetric membrane containing a fixed charged gradient, the total potential difference here may be written as:

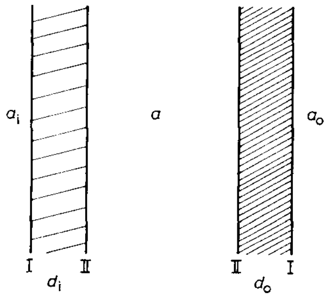
$$\Delta\psi^{A}{}_{\mathrm{tot}} = |\psi_{\mathrm{i}} - \bar{\psi}_{\mathrm{i}}| - |\psi_{\mathrm{o}} - \bar{\psi}_{\mathrm{o}}| - |\bar{\psi}_{\mathrm{i}} - \bar{\psi}_{\mathrm{o}}|$$

where $|\psi_{\mathrm{i}} - \bar{\psi}_{\mathrm{i}}| = \psi_{\mathrm{i}}{}^{\mathrm{I}}$ is the Donnan potential difference between the external layer d_{i} of the membrane and the solution i. $|\psi_{\mathrm{o}} - \bar{\psi}_{\mathrm{o}}| = \psi_0{}^{\mathrm{II}}$ is the Donnan potential difference between the external layer d_0 of the membrane and the solution o $|\bar{\psi}_{\mathrm{i}} - \bar{\psi}_{\mathrm{o}}| = \psi_{\mathrm{oi}}{}^{d}$ is the diffusion potential between the 2 layers of the membrane due to differential diffusion of mobile counterions and co-ions through the membrane. In this connection 2 formally different but substantially similar cases van be considered: (a) asymmetric membranes, (b) coupled symmetric membranes. Schematically the asymmetric membrane may be represented as follows:



$$\Delta\psi^{A}{}_{\mathrm{tot}} = |\psi_{\mathrm{o}}{}^{\mathrm{II}} - \psi_{\mathrm{i}}{}^{\mathrm{I}}| - \psi_{\mathrm{oi}}{}^{d}$$

while the 'coupled' ion permselective membrane system may be represented:



$$\Delta\psi^{C}{}_{\mathrm{tot}} = |\psi_{\mathrm{o}}{}^{\mathrm{II}} - \psi_{\mathrm{o}}{}^{\mathrm{I}}| + |\psi_{\mathrm{i}}{}^{\mathrm{II}} - \psi_{\mathrm{i}}{}^{\mathrm{I}}| - |\psi_{\mathrm{o}}{}^{d} - \psi_{\mathrm{i}}{}^{d}|$$

where $\psi_0{}^{d}$ and $\psi_{\mathrm{i}}{}^{d}$ are, respectively, the diffusion potentials of the membrane d_0 and d_{i}. The contribution of the diffusion term in the asymmetric membrane is higher than that in the coupled system. In fact an electromotive force different from zero develops only in the asymmetric membrane and not in the coupled membrane system when the ionic activities of solutions are the same.

Then $$|\psi_o{}^d - \psi_i{}^d| = |\psi_{oi}{}^d| - |G|$$

where G is a factor which depends on the different distribution of fixed charges and on the different saline uptake on the two layers d_i and d_o of the asymmetric membrane This is true for every asymmetric membrane.

Therefore the total potential difference in the 'coupled' system may also be written:

$$\Delta\psi^C{}_{tot} = |\psi_o{}^{II} - \psi_i{}^I| + |\psi_i{}^{II} - \psi_o{}^I| - |\psi_{oi}{}^d| - |G|$$
$$\Delta\psi^C{}_{tot} = \Delta\psi^A{}_{tot} + |\psi_i{}^{II} - \psi_o{}^I| - |G|$$

In the above-mentioned procedure the activity of the solution is changed only in the central compartment of the cell.

It can be proved experimentally that the $\psi_i{}^{II}$ contribution is negligible (see Table I for NaCl) and the G term constant. All the above-analyzed parameters have been taken into account, the electromotive force of the coupled membrane system previously discussed can be calculated using the following relationship for the Donnan term:

$$\Delta\psi_{\mathrm{Donnan}} = \frac{RT}{F}\left\{\ln\left[\frac{d_i\gamma_i}{2a_i} + \left(1 + \frac{d_i{}^2\gamma_i{}^2}{4a_i{}^2}\right)^{\frac{1}{2}}\right] - \ln\left[\frac{d_o\gamma_o}{2a_o} + \left(1 + \frac{d_o{}^2\gamma_o{}^2}{4a_o{}^2}\right)^{\frac{1}{2}}\right]\right\}$$

where γ_i and γ_o are the activity coefficients of the counterions in the membrane d_i and d_o. These values of γ_i and γ_o were calculated by assuming $\gamma_{Na^+} = \gamma_{\pm NaCl}$. These results were obtained by determining the uptake of NaCl by membranes d_i and d_o equilibrated against salt solutions of various mean activities $a_\pm$ (as elsewhere described[9]) using the relationship:

$$\gamma_\pm = \frac{a_\pm}{\sqrt{m_+m_-}}$$

The value of γ_i, in a membrane (d_i) of very low charge density, was calculated on the basis of this formula and found to be approximately the experimental value of γ_{Na^+} of an aqueous solution of sodium polystyrene sulphonate. This value was regularly 0.25 in a wide range of concentration. The value of γ_o was $4 \cdot 10^{-2}$ as determined in previous work[9].

The diffusion potential, according to TEORELL[6], may be calculated for each membrane on the basis of the following relationship:

$$\Delta\psi_{\mathrm{diffusion}} = U\frac{RT}{F}\left[\ln\frac{d_I}{d_{II}} + \ln\frac{U + \sqrt{1 + \left(\frac{2a_I}{\gamma_I d_I}\right)^2}}{U + \sqrt{1 + \left(\frac{2a_{II}}{\gamma_{II} d_{II}}\right)^2}}\right]$$

where $U = (\bar{u}_+ - \bar{u}_-)/(\bar{u}_+ + \bar{u}_-)$ and $\bar{u}_+$ and $\bar{u}_-$ are the ionic mobilities of the cation and the anion in the membrane. We assume they are constant.

The U term may be evaluated for each membrane by the slope of the plot: electromotive force *vs.* log a. The values for the membranes d_o and d_i were 0.87 and 0.07 respectively, determined with NaCl solutions. The 3 half cells were filled with NaCl solutions. The concentration of NaCl in the 2 external half cells was kept constant ($a_i = a_o = 1 \cdot 10^{-2}$ M) while the concentration of NaCl in the central half cell was changed stepwise and the electromotive force recorded at each step.

In Table I experimental data are compared with those calculated in order to have a calibration curve for the above-mentioned system. In Fig. 2 the points represent experimental data and the line the calculated behaviour. As may be seen, the agreement is good.

If the solution of NaCl in the central half cell is replaced with a solution of colloidal electrolyte (*i.e.* sodium lauryl sulphate), or with a solution of a natural macromolecule, and its concentration is progressively changed, it is possible to determine activity coefficients and to have information about eventual conformational transitions.

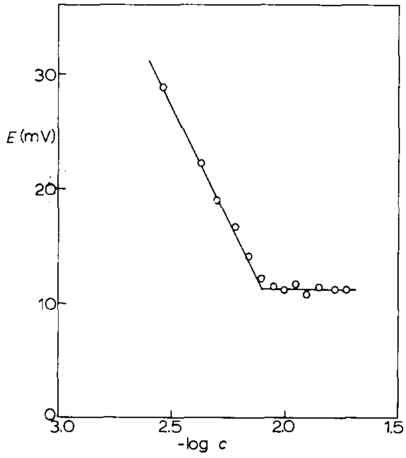


Fig. 3. Electromotive force (in mV) *vs.* logarithm of concentration when sodium lauryl sulphate solution is contained in the central compartment and diluted stepwise or concentrated. The electromotive force values were recorded at each step. The 2 external compartments were filled with NaCl $1 \cdot 10^{-2}$ M. The break marks the critical micelle concentration.

In Fig. 3 the electromotive force recorded with sodium lauryl sulphate solutions is plotted as a function of the logarithm of molarity of sodium lauryl sulphate, the concentration of NaCl in the 2 external half cells being kept constant and equal to $1 \cdot 10^{-2}$ M.

The break marks the critical micelle concentration and coincides with concentration $8 \cdot 10^{-3}$ M (ref. 12). This is also in agreement with conductivity measurements.

The reliability of this method is therefore evident since this result is in agreement with those obtained by other authors through generally accepted means. Further supporting the reliability of this system is the fact that we can easily compare the shape of the plot with another one obtained with a completely different technique[11] based on a determination of the activity of the lauryl sulphate anions by means of an electrode Hg/Hg_2(lauryl sulphate)$_2$.

Even small amounts of solution may be enough (0.5 ml), since the distance between the 2 membranes can be reduced to 1–2 mm. Another advantage of this system is the absence of errors generally involved in potentiometric measurements and due to the liquid-junction potential E_j. This potential is generally lowered using liquid or agar bridges containing high salt concentration and choosing salts that dissociate into ions (*e.g.* KCl or NH_4NO_3) with very nearly equivalent conductance. It is some-times impossible to use such bridges in colloidal electrolytes or in natural macromolecular solutions since precipitation or denaturation may take place at the tip of the electrodes. On the other hand, in the above-mentioned system of membranes, the E_j contribution to the total electromotive force of the system vanishes since the 2 identical saturated calomel electrodes, or alternatively 2 identical Ag/AgCl electrodes, are dipped in 2 identical solutions.

Structural changes were also studied in aqueous solution as a function of temperature and, even if in a semiquantitative way, useful information may be drawn from this arrangement of membranes and electrodes. In Fig. 4 the variation of γ_{Na^+} as a function of temperature for sodium lauryl sulphate is reported for different concentrations. The dotted line refers to a solution $5 \cdot 10^{-3}$ M of NaCl. As may be seen, a transition is evident only when concentrations higher than $8 \cdot 10^{-3}$ M are present in solution.

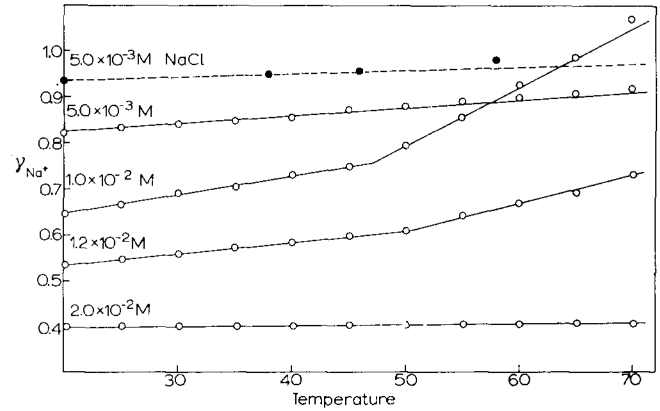


Fig. 4. γ_{Na^+} *vs.* temperature (°C) for several concentrations (as indicated) of sodium lauryl sulphate (○—○). ●— — —●, refers to a solution of NaCl $5 \cdot 10^{-3}$ M.

It is to be emphasized that, when the concentration is progressively increased, the transition is progressively shifted toward higher temperatures. However this transition may no longer be detectable in the range of temperatures considered if the concentration becomes very high ($2 \cdot 10^{-2}$ M).

We might add that, the biological membrane being asymmetric[13] and taking into consideration only this asymmetry of charge distrubition, it might be possible to try to reproduce this asymmetry in a model based on the above-mentioned system.

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