The Activity of Chloride Ion in Albumine Solution.

Setsuo OKAMURA and
Itsuro SHIRAISHI
Department of Physiolgy (2nd Institute Director: Prof. G. Kawabata), Yamaguchi University School of Medicine
(Received December 22, 1965)

In the papers, issued previously from this laboratory, we have discussed the liquid junction potential at a tip of microelectrode (1), and it was assumed that the activity of chloride ion in the potassium chloride solution cotaining a protein was the same as that in a simple solution. The reference was the Keynes' results (2) reporting that its activity coefficient in a squid axoplasm had been the same as in the sea water. Whether his result can also be seen in the solution containing bovine albumine or isethionic sodium has re-investigated in the present experiment.

METHODS

The bovine albumine or isethionic sodium ($CH_2OH \cdot CH_2SO_3$–Na) was dissolved, as a test solution, in the potassium chloride (KCl) solution by 7 % or 0.3 molar, respectively. The solution discontaining them served as a control. Sometimes sodium chloride (NaCl) was used instead of KCl.

The test solution or the control solution was placed in a small beaker, into which the silver-silver chloride (Ag-AgCl) electrode was dipped. This electrode was made of a platinum wire coated with silver, and then with chloride by an electrolysis. A calomel electrode was immersed into the 3M (molar) KCl solution in an another beaker, and the 3 M·KCl-bridge was mounted between the two beakers. To the one end of the bridge placed in the breaker containing the test or the control solution, the glass capillary filled with KCl solution of the same concentration was connected. The tip-diameter of the capillary was 3～5 micra in order to avoid the fast mixing of the solution at the boundary.

The potential between the two electrodes was measured with the valve-voltmeter, the details of which had previously been reported (3). If with the 3M·KCl solution as a control, the potential deviated more than 3 mV from the theoretical value (4), the glass capillary was rejected and the Ag-AgCl electrode was cleaned and rechlorided. If the variation of this potential was greater than 0.5 mV. during one series of measurements, the results were discarded. Sometimes the measurement was repeated with the second bridge. The experiments were carried out at the room temperature between 15° and 20°C.

RESULTS and DISCUSSION

The experimental results are represented in the Table 1.

(Table 1)

Concentration, in Molar	0.02	0.05	0.1	0.2	0.5	1.0	1.5	3.0
Control Solution 1	—	50.0	35.0	—	−3.0	−21.3	−29.3	−45.0
Test Solution 1	—	52.0	35.0	—	−2.5	−20.7	−30.0	—
Control S. 2	68.0	46.5	30.5	15.8	−2.8	−18.7	−28.5	−45.0
Test S. 2	71.3	49.3	33.5	16.0	−3.0	−18.7	−27.3	—
Control S. 3	74.0	50.0	34.0	17.3	−2.7	−18.0	−28.0	−44.7
Test S. 3	80.0	53.3	35.6	18.7	−2.7	−17.3	−27.3	—
Control S. 4	71.3	50.0	32.6	16.7	−3.3	−20.0	−29.7	—
Test S. 4	77.4	53.3	35.0	20.0	−3.3	−17.3	−28.0	—

In the table above, the figures in the first row denote the concentrations of KCl or NaCl, in molar, in the control or the test solutions and the others, the measured potentials in millivolts. The test solutions 1 and 2 are the KCl solutions containing bovine albumine by 7 %. Those, 3 and 4 are KCl and NaCl solutions, respectively, in which isethionic sodium is dissolved by 0.3 molar. The negative sign shows the negativity of Ag-AgCl electrode opposite to the solution.

It is well-known that the Nernst's equation is applicable to the present potential: i. e.

$$E = 57.7 \log \frac{a_1}{a_2} \qquad (t : 18^\circ C).$$

where; E is the potential value in millivolts, a_1 and a_2 are the activities of Cl-ion in the Ag-AgCl electrode and in the control or the test solution, respectively. As a_1 can reasonably be assumed to be constant, the relationship between E and the logarithm of a_2 would be linear. The activity coefficient of KCl or NaCl solution is quoted from a monograph of physical chemistry (5) and, assuming that of Cl-ion were the same with it, a_2 in the control solution could be obtained by multipliing it by the concentration. The difference between the molarity and the molality is so small that it can be neglected. These values are listed in the Table 2.

Table 2. Activity Coefficient (γ) and the Activity (a) of the Control Solution.

Concentration (mol.)	0.02	0.05	0.1	0.2	0.5	1.0	1.5	3.0
γ (KCl)	0.88	0.82	0.77	0.73	0.65	0.60	0.59	0.57
a (KCl)	0.018	0.041	0.077	0.147	0.325	0.60	0.88	1.71
γ (NaCl)	0.88	0.82	0.78	0.76	0.68	0.66	0.66	
a (NaCl)	0.018	0.041	0.078	0.152	0.340	0.66	0.99	

In the Figs. 1 and 2, the relationship between the activity of chloride ion (Abscissa, in logarithmic scale) and the measured potential (Ordinate, in millivolts) are shown. The test solution is KCl containing the bovine albumine by 7 % in the Fig. 1 and, in the Fig. 2, it is KCl (solid line) or NaCl solution (broken line) in which isethionic sodium is dissolved by 0.3 molar. The positive or negative sign on the ordinate means the polarity of Ag-AgCl electrode opposite to the test or the control solution. It is clear from the above Nernst's equation that the positivity is resulted when the activity of Cl-ion in the former (a_1) is greater than that in the latter (a_2). In the Fig. 2, the scale of the ordinate for the broken line is displaced by 20 mV. in order to avoid an overlapping.

The filled circles indicated the results obtained with the control solutions and the open ones, with the test solutions. The straight lines were the theoretical ones having

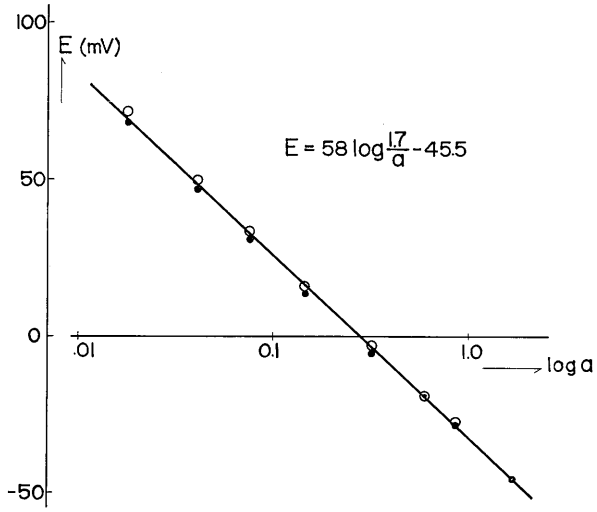

Fig. 1. The relationship between the measured potential (ordinate) and the logarithm of Cl-activity (abscissa) in KCl solution. The straight line is a theoretical one. The test solution contains bovine albumine by 7%. The open circles are the results with the test solution and filled ones, with the control solution (cf. text).

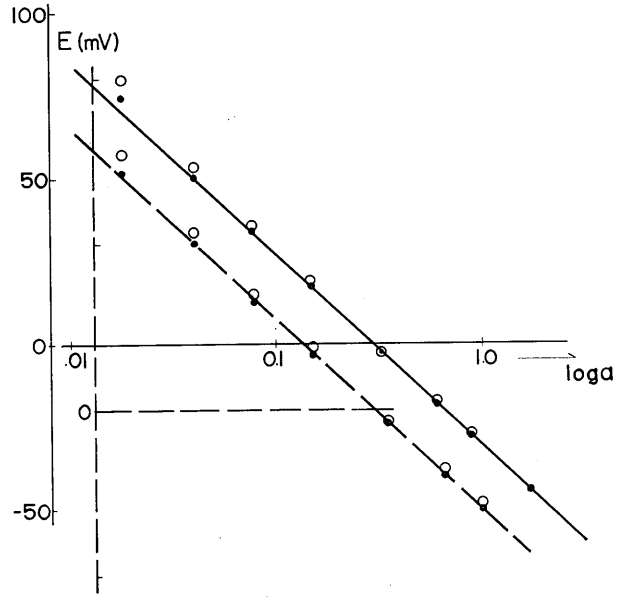

Fig. 2. The same as the Fig. 1. The solid line shows the results obtained with KCl solution and the broken line, with NaCl. In both, the test solutions contain isethionic sodium by 0.3 mol. The co-ordinate for the broken line is displaced in order to aviod an overlapping of the figure.

the slope of minus 58 mV. per ten fold increase of the activity. The co-ordinate of the point which the theoretical line had to cross was, according to Scatchard (loc. cit.), −46.6 mV. versus log 1.7 (activity of 3 mol. Cl-ion). It was reasonable, however, that the physico-chemical characters of calomel and Ag-AgCl electrode were not the same in the individual experiment. These points in the figures were experimentally determined with enough reproducibility (METHODS). Another experimental series, in which the concentration of albumine was 3.5 %, gave the same results as the Fig. 1.

It is well demonstrated in the above figures that the relationship between the measured potential and the logarithm of the activity of Cl-ion in the simple (control) KCl or NaCl solution is strictly linear. As to the test solutions, the potential deviates from the straight line to somewhat high value at the low concentration ranging from 0.02 to 0.1 molar. These phenomena indicate that the activity is decreased in this range, in a small degree; e. g. it is estimated that the activity cofficient of chloride ion in the 0.02 mol. KCl solution containing the bovine albumine by 7% is 0.79 and that it is lower by about 10% than that (0.88) in the simple solution discontaining the protein. In the range between 0.1 and 1.5 molar, on the other hand, the activity does not decrease in accordance with the Keynes' report cited in the beginning.

SUMMARY

An activity of chrolide ion in the KCl or NaCl solution containing bovine albumine (7%) or isethionic sodium (0.3 mol.) has been investigated with a potentiometrie, using a silver-silver chloride electrode. It was compared with that in the simple solution discontaining them. In the former solution, in which the concentration of KCl or NaCl was ranged between 0.02 and 0.1 molar, a little decrease of the activity was observed. On the other hand, when the concentration was between 0.1 and 1.5 molar, it was the same with that in the latter solution.

(Acknowledgment) Grateful thanks are due to Prof. A. M. Tanikado for reading the manuscript.

REFERENCES

1. TANIKUNI, K., K. FUJIMOTO and G. KAWABATA: On the Measurement of Intracellular Potential. Part 3; Liquid Junction Potential of the Protein Solution. *J. Physiol. Soc. Jap.*, **27**: 454-462, 1965 (in Japanese)
2. KEYNES, R. D.: Chloride in the Squid Giant Axon. *J. Physiol.*, **169**: 690-705, 1963
3. SHIRAISHI, I. and S. OKAMURA: On the liquid Junction Potential. *The Bull. Yamaguchi Med. School*, **13**: 67-73, 1966
4. SCATCHARD: *J. A. C. S.* **47**: 641, 1925. (cited from 6)
5. ROBINSON, R. A. and R. H. STOKES: *Electrolyte Solution*: Butterworth Sci. Pub., London, 1955.
6. YOSHIMURA, H.: *The Theory and the Measurement of pH*: Maruzen & Co., Tokyo, 1947. (in Japanese)