[CONTRIBUTION FROM THE LABORATORIES OF THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

THE MEASUREMENT OF THE ELECTROKINETIC POTENTIAL ON PROTEINS BY THE STREAMING POTENTIAL METHOD[1]

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It has been shown by various investigators that chemically inert particles suspended in a protein solution take on the electrokinetic properties of the protein. Loeb[3] demonstrated this fact with collodion particles suspended in solutions of gelatin and of other proteins. Freundlich and Abramson[4] have recently shown that quartz particles act in the same manner. Svedberg and Tiselius[5] used this phenomenon as a means for measuring cataphoretically the mobility of egg albumin and therefrom its ζ-potential at various hydrogen ion concentrations. Abramson[6] repeated their work, using a simplified method and showed that an extremely dilute solution of the protein (10^{-3} g. per liter or less) could be used and that it was to be preferred to the more concentrated solutions.

The author has shown[7] that the electrokinetic potential, that is, the ζ-potential, may be accurately determined by the streaming potential method upon diaphragms of various materials, for example, of paper pulp, built up between two perforated gold electrodes, provided the specific conductivity of the liquid, as it exists within the diaphragm, is determined. An application of this method for measuring the ζ-potential on proteins at various P_H values seemed desirable because of the ease and accuracy with which such measurements may be made.

Method

It was found that finely ground quartz powder could be made into a diaphragm between the two perforated gold plates in a manner already described for paper pulp.[7] Surface conductance, that is, increased conductivity of the liquid when in contact with the diaphragm material, was found to be relatively unimportant for such a crystalline diaphragm even when conductivity water was used for the interstitial liquid. When dilute electrolyte solutions were present in the diaphragm, surface conductance was found to be negligible and the specific conductivity factor, K,

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[3] Loeb, *J. Gen. Physiol.*, **5**, 395 (1922–23).

[4] Freundlich and Abramson, *Z. physik. Chem.*, **128**, 25 (1927).

[5] Svedberg and Tiselius, THIS JOURNAL, **48**, 2272 (1926).

[6] Abramson, *ibid.*, **50**, 390 (1928).

[7] Briggs, *J. Phys. Chem.*, **32**, 641 (1928).

in the streaming potential formula could be taken as that of the solution in bulk.

Through such a quartz diaphragm could be forced a dilute solution of fairly pure commercial egg albumin from which had been separated the water-insoluble fractions. After an amount of solution which contained approximately 0.2 g. of albumin had passed through the diaphragm (the diaphragm contained 17.5 cc. of quartz powder), the quartz surface was found to be saturated with the protein and the diaphragm then acted as though it were a protein diaphragm. Subsequently, solutions of various hydrogen ion concentrations and containing approximately 10^{-3} g. of the protein per liter were forced through the diaphragm and the ζ-potential was determined in the usual manner. Svedberg and Tiselius used 1% egg albumin solutions of 0.02 *M* buffer mixtures of acetic acid and sodium acetate. Abramson used the very dilute protein solutions in the 0.02 *M* buffer mixtures. Such concentrations of electrolytes cannot be used in streaming potential measurements because the electrical conductivity of such solutions is so great as to prevent entirely the building up of a measurable potential across the ends of the diaphragm. The solutions used in the present work were 0.0004 *M* in hydrochloric acid and lithium chloride, a concentration great enough to eliminate surface conductance in the diaphragm and yet low enough to allow for the formation of a streaming potential which was easily measurable. After 50 cc. of any of the solutions had passed through the diaphragm (requiring about twenty minutes) equilibrium had been attained and the measurements could be taken. The P_H of the solutions used was determined electrometrically.

Experimental

The application of the streaming potential method to the determination of the isoelectric points and the effects of ions upon the ζ-potential on water-soluble proteins, is well illustrated by the data presented in this paper. Egg albumin was used as the protein in order to make a comparison with the data offered by Abramson.[6]

The ζ-potential is calculated according to the formula

$$\zeta = \frac{HK}{P} \cdot \frac{4\pi\eta}{\epsilon}$$

where H is the streaming potential set up across the ends of the diaphragm at pressure P, K is the specific conductivity of the liquid as it exists inside the diaphragm and which, in this case, is equal to that of the liquid in bulk and η and ϵ are, respectively, the viscosity and the dielectric constant of the liquid, which may be taken as those of water for such dilute solutions of electrolytes and protein as are those used. The ζ-potential will be given in volts when H, P and K are given in millivolts,

cm. of mercury, and reciprocal ohms, respectively, and multiplied by the factor 1.0596×10^2.

In Table I are shown the P_H values of a series of 0.0004 M mixtures of hydrochloric acid and lithium chloride, in which was dissolved the egg albumin in a concentration of 10^{-3} g. per liter. Such solutions have no buffer capacity and may vary a few hundredths of a P_H from solution to solution of the same concentration of electrolyte. Because of this variation the P_H was determined individually for every solution used.

TABLE I

SHOWING P_H OF MIXTURES OF LITHIUM CHLORIDE AND HYDROCHLORIC ACID WHERE NUMBERS OF CC. INDICATED ARE DISSOLVED IN A LITER OF THE DILUTE PROTEIN SOLUTION

Cc. of N/100 HCl	26.0	16.0	12.0	8.4	6.0	4.0	3.3	2.8	2.0	1.5	1.0	0.0
Cc. of N/100 LiCl	14.0	24.0	28.0	31.6	34.0	36.0	36.7	37.2	38.0	38.5	39.0	40.0
P_H	3.63	3.86	3.99	4.20	4.38	4.65	4.86	5.00	5.31	5.57	5.84	6.21

Table II gives the values of H, P and K, and of the ζ-potential of the albumin in the solutions at various P_H values. Three different quartz diaphragms were prepared and the values obtained on each (A, B and C) were found to check very closely, although the porosity of the different diaphragms was found to vary to some extent, as indicated by the rate at which water flowed through each at a given pressure.

TABLE II

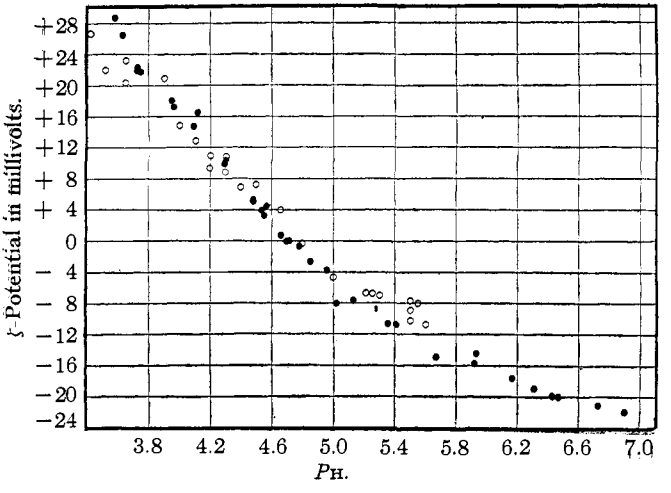
SHOWING CHANGE IN THE ζ-POTENTIAL ON EGG ALBUMIN WITH CHANGE IN THE P_H OF 0.0004 M MIXTURES OF LITHIUM CHLORIDE AND HYDROCHLORIC ACID WITH WHICH THE PROTEIN IS IN CONTACT

P, cm. of Hg	H, mv.	$K \times 10^5$, mho	$\frac{HK}{P} \times 10^5$	ζ, mv.	P_H
		Diaphragm (A)			
38.15	+69	15.00	+27.20	+28.82	3.58
40.42	73	13.70	24.80	26.28	3.63
41.26	75	11.20	20.40	21.62	3.75
40.11	74	8.82	16.30	17.27	3.96
40.24	55	6.72	9.20	9.75	4.29
37.27	+33	5.80	+ 5.14	+ 5.45	4.48
41.01	−4	5.33	− 0.52	− 0.55	4.78
36.89	74	4.98	10.05	10.65	5.36
40.06	60	5.04	7.56	8.01	5.02
36.18	−106	4.62	−13.47	−14.27	5.93
		Diaphragm (B)			
40.19	+70	11.90	+20.72	+21.95	3.73
36.23	68	9.06	17.00	18.01	3.95
41.00	75	7.70	14.10	14.93	4.09
41.15	61	6.54	9.72	10.30	4.30
37.09	31	5.78	4.84	5.13	4.48
40.88	+ 4	5.24	+ 0.51	+ 0.54	4.66
41.61	− 30	5.02	− 3.63	− 3.84	4.96
41.41	61	4.88	7.20	7.63	5.13

TABLE II (*Concluded*)

P, cm. of Hg	H, mv.	$K \times 10^5$, mho	$\frac{HK}{P} \times 10^5$	ζ, mv.	P_H
41.11	86	4.74	9.91	10.50	5.41
37.95	120	4.45	14.10	14.94	5.67
39.98	135	4.38	14.80	15.68	5.94
41.51	154	4.48	16.60	17.59	6.17
39.87	150	4.76	17.90	18.97	6.31
40.48	170	4.48	18.80	19.92	6.43
43.18	180	4.50	18.85	19.97	6.47
39.33	178	4.38	19.85	21.03	6.73
40.78	194	4.35	20.70	21.93	6.90
36.25	−180	4.09	−20.30	−21.51	7.50
40.47	+ 75	11.40	+21.10	+22.36	3.73
		Diaphragm (C)			
37.45	+ 78	7.60	+15.80	+16.74	4.12
38.30	+ 24	6.00	+ 3.77	+ 3.99	4.54
...	0	..	0	0	4.69
38.83	+ 18	6.88	+ 3.19	+ 3.38	4.55
40.30	0	..	0	0	4.70
37.44	− 18	5.15	− 2.48	− 2.63	4.85
37.54	+ 29	5.57	+ 4.30	+ 4.56	4.57
42.12	0	..	0	0	4.72

Fig. 1 shows the values given in Table II in comparison with those obtained by Abramson for the same protein by the cataphoretic measure-



●, By streaming potential method; ○, by cataphoresis, measurements by Abramson.

Fig. 1.—The variation of the ζ-potential with P_H on egg albumin.

ments. It will be noted that through the range of P_H between 3.8 and 5.2 there is shown a remarkable agreement between the values of the ζ-

potential as obtained by the two methods. Through this range the ζ-potential is influenced almost entirely by the hydrogen and hydroxyl ion concentrations of the solution. Beyond this range on either the alkaline or acid side, the values obtained in the 0.0004 *M* solutions diverge to a greater extent from zero potential than those measured in 0.02 *M* solutions. The higher concentrations of the other ions begin to show their effect toward reducing the ζ-potential soon after that function moves away from the zero value in either direction, but this effect is very small compared to that of the H^+ and OH^- ions until the hydrogen ion concentration has moved out of the above mentioned range. While this divergence between the two curves is primarily a concentration effect, it is not entirely unlikely that the kind of ion used may also have some influence. Recent work by the author[8] has shown that the lithium ion reduces the ζ-potential on a negatively charged surface to a less extent, at any given concentration, than does the sodium or the potassium ion. It was for this reason that lithium chloride was used in the present experiment instead of the more common alkali chlorides. The chloride ion, likewise, should have a greater effect toward lowering the ζ-potential at a positively charged surface than the acetate ion, which may account for the fact that the two curves lie together for a longer range of P_H on the acid side of the isoelectric point than they do on the alkaline side.

Incidentally, the streaming potential method for measuring the ζ-potential offers an accurate means for determining the isoelectric point on a water-soluble protein. The solution, which is being streamed through the diaphragm covered with the protein, may be adjusted to a hydrogen ion concentration such that there is no streaming potential set up. The P_H of this solution can then be accurately determined, this P_H being the isoelectric point of the protein. By such a procedure it is possible to determine this point to within the experimental error of the P_H determination.

Further work is being done both upon proteins and upon other colloidal materials which are peptized in water or in dilute acid, alkali or alcohol solutions, and which have a marked effect upon lowering the interfacial tension, such as saponin and lecithin. It is not improbable that the electrokinetic properties of many dyestuffs and other organic compounds may be similarly studied.

Summary

1. The streaming potential method of measuring electrokinetic potentials has been applied in a study of egg albumin by utilizing the property of the protein to adsorb onto a quartz surface so as to give that surface the properties of the protein.

[8] Briggs, *J. Phys. Chem.*, **32**, October, 1928.

2. Data, upon comparison with the work of Abramson with the same protein on quartz surfaces but by the cataphoresis method, show excellent agreement between the values of the ζ-potential obtained by the two methods between P_H 3.8 and P_H 5.2. Divergence of the values of ζ-potential beyond this range is explained by the differences in the concentration of the electrolyte utilized in the two experiments and probably, to some extent, by the differing effects of the ions used.

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DIPHENYLCARBAZIDE AS A TEST FOR CHROMIUM

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A search of the literature has revealed a number of organic reagents which have been used for the detection and estimation of chromium when present as chromate or dichromate. Of these organic compounds, diphenylcarbazide, $CO(NHNHC_6H_5)_2$, seemed very promising. Cazeneuve[1] used this reagent to detect chromic acid in a dilution of 1:1,000,000. Brandt[2] proposed the use of diphenylcarbazide as an inside indicator in the titration of iron with potassium dichromate, while more recently Snoddy[3] has made it the basis of a colorimetric method for estimating small amounts of chromium in fats. Scott[4] mentions the use of this reagent in detecting chromium as chromate.

Since the test is very simple to carry out and the reagent produces a distinctive violet or reddish-violet color with exceedingly small amounts of dichromate, it seemed desirable to compare the sensitivity of diphenylcarbazide with that of the ether—hydrogen peroxide test usually employed in qualitative analysis and to determine the best conditions under which the test might be applied.

Experimental Work

I. Tests with Potassium Dichromate

Solutions of potassium dichromate containing varying amounts of chromium were first tested with diphenylcarbazide and also by the ether–hydrogen peroxide method in order to determine which method was the more sensitive. Further, the tests with diphenylcarbazide were carried out in solutions acidified with different acids, namely, sulfuric acid, acetic acid and citric acid.

[1] Cazeneuve, *Bull. soc. chim.*, [3] **23**, 592, 701, 769 (1900).

[2] Brandt, *Z. anal. Chem.*, **45**, 95 (1906).

[3] Snoddy, *J. Oil Fat Ind.*, **2**, 20 (1925).

[4] Scott, "Standard Methods of Chemical Analysis," D. Van Nostrand Co., New York. 1918, p. 132.