

THE ELIMINATION OF DISCREPANCIES BETWEEN  
OBSERVED AND CALCULATED P.D. OF PRO-  
TEIN SOLUTIONS NEAR THE ISOELEC-  
TRIC POINT WITH THE AID OF  
BUFFER SOLUTIONS.

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The writer has shown in a series of papers<sup>1</sup> that the P.D. observed between a solution of gelatin chloride or albumin chloride inside a collodion bag and an outside solution free from gelatin could be calculated from the difference in the hydrogen ion concentration between the inside and outside solutions. The agreement between the observed and calculated values was perfect when the solution contained a neutral salt or when the hydrogen ion concentration of the solution was not too close to that of the isoelectric point; the agreement was, however, less satisfactory when the pH was near that of the isoelectric point of gelatin, *i.e.*, near pH 4.7, and no salts were present.<sup>2</sup> The source of this disagreement seemed to lie in the inaccuracy in the measurement of the pH of the aqueous solution free from gelatin (the outside solution) at a pH between 4.0 and 7.0. If this surmise was correct, the disagreement in that region of hydrogen ion concentrations should be caused to disappear by the use of a buffer solution inside and outside.

1 per cent solutions of isoelectric gelatin were made up in M/100 Na acetate solutions containing varying amounts of 1 M acetic acid so that the pH of the gelatin solution varied (at the end of the experiment) between 4.65 (*i.e.*, practically isoelectric gelatin) and 3.34 (Table I.) Collodion bags, of a content of about 50 cc., were filled

<sup>1</sup> Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 667; 1921-22, iv, 351.

<sup>2</sup> Loeb, J., *Proteins and the theory of colloidal behavior*, New York and London, 1922, 138, 156.

with these solutions of gelatin in buffer solutions as described in previous papers. The bags were put into beakers containing 350 cc. of identical solutions of M/100 Na acetate and 1 M acetic acid as those inside the bags, except that the 350 cc. outside solutions contained no gelatin. The temperature was 24°C. After 24 hours the osmotic pressure, the P.D. between inside and outside solutions, and the pH of the inside and outside solutions were measured. From the value pH inside minus pH outside the P.D. was calculated, and Table I shows that the P.D. thus calculated agrees with the observed P.D. The rest of the table needs no explanation.

TABLE I.  
*Influence of pH on P.D. of Solutions of Gelatin Acetate in the Presence of Buffer Solution.*

Cc. 1 M acetic acid in 100 cc. inside and outside solutions.	1.0	1.5	2.0	3.0	4.0	6.0	10.0	15.0	20.0	30.0
Osmotic pressure, in mm. H <sub>2</sub> O.....	21	31	34	43	47	62	83	95	103	108
pH inside.....	4.65	4.52	4.40	4.23	4.14	3.99	3.76	3.61	3.49	3.34
pH outside.....	4.65	4.50	4.37	4.19	4.09	3.92	3.69	3.53	3.39	3.23
pH inside minus pH outside.....	0	0.02	0.03	0.04	0.05	0.07	0.07	0.08	0.10	0.11
P.D. calculated, millivolts.....	0	1.0	2.0	2.5	3.0	4.0	4.0	5.0	5.5	7.0
P.D. observed, millivolts.....	0.5	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.5	6.0

Similar results were obtained, in the case of solutions of edestin, by Dr. Hitchcock, whose paper appears simultaneously with this note.<sup>3</sup>

#### SUMMARY AND CONCLUSIONS.

1. It had been noticed in the previous experiments on the influence of the hydrogen ion concentration on the P.D. between protein solutions inside a collodion bag and aqueous solutions free from protein that the agreement between the observed values and the values calculated on the basis of Donnan's theory was not satisfactory near the

<sup>3</sup> Hitchcock, D. I., *J. Gen. Physiol.*, 1921-22, iv, 597.

isoelectric point of the protein solution. It was suspected that this was due to the uncertainty in the measurements of the pH of the outside aqueous solution near the isoelectric point. This turned out to be correct, since it is shown in this paper that the discrepancy disappears when both the inside and outside solutions contain a buffer salt.

2. This removes the last discrepancy between the observed P.D. and the P. D. calculated on the basis of Donnan's theory of P.D. between membrane equilibria, so that we can state that the P.D. between protein solutions inside collodion bags and outside aqueous solutions free from protein can be calculated from differences in the hydrogen ion concentration on the opposite sides of the membrane, in agreement with Donnan's formula.