XCVII.—*The Adsorption of Hydrogen Ions and Its Effect on the Swelling and Electrical Charge of Gelatin.*

By Bhupendra Nath Ghosh.

It is well known that gelatin swells more in dilute solutions of acids than in pure water. At p_H 4·8 the swelling is generally a minimum, but it increases with the decrease in p_H, reaches a maximum and then diminishes again. Several theories have been advanced to explain this behaviour.

(1) In acid solutions the particles of the gel adsorb hydrogen ions which impart a positive charge to them. The similarly charged particles repel one another and thereby cause the swelling. At high concentrations of acids, the negative ions tend more and more to

neutralise the electrical charge and so decrease the swelling. This theory was supported by Tolman and Stearn (*J. Amer. Chem. Soc.*, 1918, **40**, 264), who observed that the swelling of blood fibrin and also of gelatin is much greater in solutions of nitric and hydrochloric acids than of sulphuric acid at the same p_H ; the presence of salts in high concentrations also depressed the swelling. They therefore suggest that the SO''_4 ion, because of its bivalent nature, has a much greater effect in neutralising the positive charge, and therefore decreasing the swelling, than the univalent Cl' or NO'_3 ions. Similarly, in presence of salts, the high anion concentration decreases the charge and hence the swelling.

(2) Pauli, Hardy, Loeb and others have suggested that the protein ions are more heavily hydrated than their neutral molecules. According to Pauli (*Biochem. Z.*, 1909, **18**, 368) and Fischer ("Œdema and Nephritis," 1921, pp. 145—151), hydration is responsible for swelling. The increase in volume of gelatin may therefore be due to a greater hydration of its particles with increasing electrical charge.

(3) Gelatin combines with acids forming salts, the kations of which are non-diffusible. This, according to Donnan's principle of membrane equilibrium, brings about a greater concentration and hence greater osmotic pressure of the diffusible ions inside the gel than in the outside solution, and so causes it to swell. This theory was put forward by Procter and Wilson (J., 1916, **109**, 307), and subsequently Wilson and Wilson (*J. Amer. Chem. Soc.*, 1918, **40**, 886) derived an equation connecting swelling with the concentration of a univalent kation inside the gel. The equation agrees fairly with experimental results (compare R. H. Bogue, "The Theory and Application of Colloidal Behaviour," Vol. I, p. 12).

If there be any relation between electrical charge and swelling, they should run parallel to each other. With increasing concentration of hydrogen ions both the electrical charge and the swelling should increase, reach a maximum, and then diminish; also, at the same p_H the electrical charge in solutions of sulphuric acid should be less than in those of hydrochloric or nitric acid. The above considerations should also apply to the case where swelling is attributed to the greater hydration of the gel particles with their increasing electrical charge. A relation is also to be expected between the amounts of hydrogen ion adsorbed and the surface density of the electrical charge, since the latter originates from the adsorption of hydrogen ions. A simultaneous measurement of swelling, electrical charge, and adsorption of hydrogen ions on the same sample of gelatin, was therefore undertaken in order to decide whether there is any relation between the three quantities.

EXPERIMENTAL.

The powdered gelatin was purified by repeated washing with dilute acetic acid at 10° (Loeb, *J. Gen. Physiol.*, 1918, **1**, 45). It was then washed with cold distilled water at 5°, dehydrated by washing with absolute alcohol, and dried in a current of air. The dried sample contained 16·7% water and had an ash content of 0·12%. 0·8 G. portions were placed in two Pyrex-glass beakers and covered with 200 c.c. of the acid solution. The beakers were placed in a thermostat at 17° and their contents stirred from time to time. After 24 hours, the gelatin from one of the beakers was transferred to a filter-paper, drained thoroughly, washed with 20 c.c. of distilled water, again drained, and pressed lightly between filter papers to remove as much liquid as possible. It was then transferred to a measuring cylinder and melted by warming on a water-bath at about 40°. The p_H of the melted gel was measured by the *E.M.F.* method. The p_H of the acid solution before and after adsorption was also measured by the same method, and the amount of acid adsorbed by gelatin, α, calculated in the following way.

Let V_1 be the original volume of the acid solution, V_2 its final volume and V the volume of water imbibed by the gelatin. Also let C_1 and C_2 be the initial and final concentration of hydrogen ions in the solution and C_3 the concentration of free hydrogen ions inside the gel. Then

$$\alpha = V_1C_1 - V_2C_2 - VC_3 \quad . \quad . \quad . \quad . \quad (1)$$

But, neglecting the slight change in the total volume of the solution and gelatin, we have $V_2 = V_1 - V$, and substitution in (1) then gives $\alpha = V_1C_1 - (V_1 - V)C_2 - VC_3 = V_1(C_1 - C_2) + V(C_2 - C_3)$.

In applying this equation, V is taken to be equal to the volume of the melted gels; any slight error thus introduced causes a relatively much smaller error in α.

The gelatin contained in the second beaker was transferred to a measuring cylinder and allowed to settle in the thermostat for 3 hours. The volume was noted. The substance was then removed on a filter-paper, and washed two or three times with a solution having the same p_H as the melted gel. It was again transferred to the measuring cylinder and melted by warming to about 40°; a few c.c. of acid solution of the same p_H as the gel were added to make the total volume always 26 c.c., and 23 c.c. of this were transferred to a U-tube and allowed to set for 24 hours.

The electroendosmotic method was used to measure the variation of electrical charge with hydrogen-ion concentration. The arrangement was an improvement on that previously described by the author (J., 1926, 2605), in that a definite potential (indicated by a voltmeter) was applied between the electrodes. The potential could be maintained constant within ± 1 volt by means of an adjustable resistance. The potential across the diaphragm was determined in the following way. The total resistance between the electrodes, consisting of the diaphragm and the acid solution, was measured by the conductivity method. The electrodes were then lowered so as just to touch the diaphragm, and the resistance was measured again. If T and D be the total and the diaphragm resistances, respectively, then the potential across the diaphragm is given by the expression DE/T, where E is the potential indicated by the voltmeter when the electrodes were in their original position (*i.e.*, when the total resistance between them was T). In order to maintain a constant potential across the diaphragm, it is necessary that E as well as the ratio D/T should remain constant. E was kept constant during the experiment by means of the adjustable resistance, and resistances D and T, as measured immediately before and after the experiment, always showed a variation of less than 3% in D/T. The mean was used in calculating the potential across the diaphragm. The U-tube was always filled with a solution having the same p_H as the gel constituting the diaphragm immediately before the experiment, which lasted about ½ hour. The electroendosmotic experiments were carried out at 16°. The distance moved by the air bubble in a definite time at constant potential gradient across the diaphragm is proportional to the surface density of the electrical charge of the gel, which was found to be zero at p_H 4·8—5·0. The results are in the table.

$(p_H)_1$, $(p_H)_2$, and $(p_H)_g$ respectively represent the p_H of the solution before and after adsorption, and of the melted gel; α is given in terms of c.c. of N-acid adsorbed; "Vol." is the volume (in c.c.) of the gel; V the approximate volume of the melted gel; and β the velocity of the air bubble in cm. per min.

In the above experiments the potential across the diaphragm was maintained constant at 80 volts. The calculated values of α were derived from the formula $\alpha = K_3x/(1 + K_4x)$ (see p. 717), where K_3 and K_4 are constants and x is the concentration of hydrogen ions inside the gel. For hydrochloric, nitric, and trichloroacetic acids, the values of K_3 and K_4 used were 1240 and 1800, respectively. They are somewhat different in the case of sulphuric acid, *viz.*, $K_3 = 1420$ and $K_4 = 2240$. All the acids have been assumed to be completely dissociated within the range of concentrations investigated. If the low value of the dissociation of sulphuric acid be taken into consideration, the amount of adsorption will be a little higher than that given in the table.

			a,					
$(p_H)_1$.	$(p_H)_2$.	$(p_H)_g$.	Found.	Calc.	Vol.	V.	β.	β/a.
			Hydrochloric acid.					
1·81	1·89	2·17	0·64*	0·63	29	20	0·81	1·27
2·02	2·14	2·52	0·55	0·58	31	21	0·68	1·24
2·10	2·24	2·70	0·52	0·54	32·5	22	0·63	1·25
2·40	2·62	3·22	0·36*	0·36	35	24	0·47	1·30
2·68	2·99	3·60	0·24	0·22	27·5	20·5	0·33	1·37
			Nitric acid.					
1·82	1·90	2·19	0·63	0·63	29	20·5	0·79	1·25
1·94	2·04	2·38	0·58	0·60	30·5	21	0·70	1·21
2·09	2·22	2·68	0·57	0·54	32	22·5	0·63	1·23
2·42	2·66	3·23	0·36	0·36	34·5	24	0·50	1·39
2·72	3·05	3·62	0·23	0·22	27·5	20	0·31	1·35
			Trichloroacetic acid.					
1·77	1·84	2·10	0·64	0·64	28·5	19·5	0·80	1·25
1·92	2·02	2·38	0·60	0·60	30·5	21	0·72	1·20
2·07	2·20	2·68	0·53	0·54	32	22	0·65	1·23
2·34	2·53	3·19	0·38	0·37	34	24	0·54	1·31
2·67	2·99	3·60	0·24	0·22	27	21·5	0·33	1·37
			Sulphuric acid.					
1·77	1·84	2·10	0·60*	0·60	15	13	0·62	1·03
1·88	1·97	2·30	0·57	0·58	16	14	0·59	1·03
2·12	2·26	2·74	0·47	0·50	18·5	15	0·55	1·17
2·36	2·55	3·26	0·33*	0·35	20·5	16·5	0·46	1·31
2·60	2·90	3·58	0·25	0·23	16·5	14	0·34	1·37

* The values thus marked were used in calculating K_3 and K_4 (see p. 717).

Discussion.

It will be seen from the above table that, at first, with increasing concentration of hydrogen ion the swelling, electrical charge, and adsorption of hydrogen ions, all increase. At a certain p_H (about 2·6) the swelling reaches its maximum value and then begins to diminish, whilst the electrical charge and adsorption of hydrogen ions continue to increase. Had the swelling been due to the electrostatic repulsion between the similarly charged particles of the gel, it could not have diminished so long as the electrical charge was increasing. With sulphuric acid, swelling and electrical charge are both less than with hydrochloric, nitric, and trichloroacetic acids, but this fact alone does not justify one in inferring any relation between them. Here also, as with the other acids, we observe that whilst the electrical charge continues to increase with diminishing p_H, the swelling attains its maximum value at about p_H 2·6 and then decreases. Furthermore, at low hydrogen-ion concentration the electrical charge with sulphuric acid is the same as with the other acids, whilst the swelling is distinctly less. The above results therefore lead definitely to the conclusion that there is no relation between electrical charge and swelling in the case of gelatin. For the same reasons the swelling of gelatin in acid solutions cannot be due to the increasing hydration of its particles with increasing electrical charge.

It has already been mentioned that as the adsorption of hydrogen ions imparts a positive charge to the gel there should be a close relation between the two. It will appear from the above results that there is a direct proportionality between the surface density of electrical charge and the amount of hydrogen ions adsorbed. The ratio (β/α) of the distance moved by the air bubble to the amount of hydrogen ions adsorbed is, within the range investigated, approximately constant for the first three acids, indicating that only few univalent anions are electrically adsorbed. This is possible when the thickness of the electrically adsorbed layer is great (compare Mukherjee, Faraday Soc. Discussion, 1920—1921, **16**, Appendix, p. 105). With increasing surface density of electrical charge, however, the electrical attraction on the anions will increase more and more, and so at high concentrations the number of anions electrically adsorbed will become appreciable and the ratio β/α will tend to decrease. This will be observed in the case of sulphuric acid. At low hydrogen-ion concentrations the ratio is almost the same as with the other acids (about 1·35), but as the concentration of sulphuric acid increases, .the ratio gradually decreases. The SO_4'' ion carries two units of electrical charge and hence the force tending to bind it to the surface is much greater than in the case of the univalent anions:

There are different views regarding the interaction of acids with gelatin. Some, *e.g.*, Procter and Wilson, hold that acids combine chemically with gelatin forming salts of the type GHA, where GH represents the kation and A the anion. Many others, however, consider the process as one of adsorption (Wilson and Wilson, *loc cit.*). It is difficult to believe that the colloid particles in the sol and gel of gelatin are molecules and not aggregates of a large number of molecules. The so-called molecular weight of gelatin, as obtained by different workers, varies from 10,000 to 20,000 or more. A molecular weight much smaller than this would generally give a particle of colloidal size, with a large solid–liquid interface. Besides the molecular weight, other properties of gelatin vary with its source and its previous treatment. Recently Kraemer and Dexter (*J. Physical Chem.*, 1927, **31**, 764) have observed that even the isoelectric point of gelatin, as revealed by measurements on the scattering of light, varies with its origin. Kruyt and Tendeloo (*ibid.*, 1925, **29**, 1303) have shown that ions other than hydrogen, such as ferrocyanide, affect the viscosity and electrical charge of gelatin. In such cases the same valency effects of the ions make themselves felt as are observed with the hydrophobic colloids. In view of these facts, it is better to consider the interaction of gelatin with hydrogen ions as one of adsorption. Rinde (*Phil. Mag.*, 1926, **1**, 32) has shown from Loeb's data that the amount of hydrogen ions adsorbed by gelatin can be calculated with fair accuracy from an equation similar to that which gave satisfactory results in the present instance (see tables and below).

It will now be shown that the assumption that hydrogen ions are adsorbed by gelatin also leads to the derivation of an equation for swelling similar to that obtained by Wilson and Wilson (*loc. cit.*).

Let it be assumed that 1 g. of gelatin is immersed in a dilute solution of hydrochloric acid and sufficient time allowed for the attainment of equilibrium. If N be the total number of active points on the surface of the gel, the rate at which hydrogen ions are adsorbed $= K_1N(1 - \theta)x$, where x is the equilibrium concentration of hydrogen ions inside the gel, θ the fraction of the total surface covered, and K_1 a constant. The rate at which these ions escape from the surface $= K_2N\theta$. When equilibrium is attained we have (compare Langmuir's equations)

$$K_1N(1 - \theta)x = K_2N\theta$$

or $$xN = N\theta(x + K_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $K_0 = K_2/K_1$, or, putting $N/K_0 = K_3$, and $1/K_0 = K_4$,

$$N\theta = K_3x/(1 + K_4x) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)^*$$

Corresponding to the $N\theta$ hydrogen ions adsorbed there must be in the neighbourhood of the surface an equal number of chlorine ions, of which a fraction a may be electrically adsorbed. The number of free anions is then equal to $(1 - a)N\theta$. The value of a, however, depends on the concentration of the chlorine ions and also on the thickness of the double layer (compare Mukherjee, *loc. cit.*). The electroendosmotic experiments show that a is negligible in the case of hydrochloric, nitric, and trichloroacetic acids below about 0·02 normal. These free anions should behave normally so far as their activity is concerned. The concentration of free chlorine ions inside the gel $= N\theta(1 - a)/(V + V_0)$ where V_0 is the initial volume and V the increase in volume of the gel (see p. 713). The equilibrium concentration of hydrogen ions inside the gel is x, and corresponding to this we have a concentration x of chlorine ions in the gel. Hence the concentration of total chlorine ions inside the gel $= N\theta(1 - a)/(V + V_0) + x$. If y be the equilibrium concentration of hydrogen

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* This formula was used in the calculation of the adsorption of hydrogen ions (see tables on p. 715).

and chlorine ions outside the gel, we have from Donnan's principle of membrane equilibrium

$$\left[\frac{N\theta(1-a)}{(V+V_0)}+x\right].x=y^2 \quad . \quad . \quad . \quad . \quad (4)$$

It follows mathematically from this that the total concentration of ions inside the gel is greater than that in the surrounding solution. If e denotes this excess then

$$e=2x+\frac{N\theta(1-a)}{(V+V_0)}-2y \quad . \quad . \quad . \quad . \quad (5)$$

From (4) and (5) we get

$$y=x+\sqrt{ex} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

and from (5) and (6)

$$N\theta(1-a)/(V+V_0)=e+2\sqrt{ex} \quad . \quad . \quad . \quad (7)$$

As the concentration of ions inside the gel is greater than that outside, the osmotic pressure inside will be greater, and so the gel tends to swell.

It is now assumed that $e = HV$, where H is a constant containing the modulus of bulk elasticity (compare Wilson and Wilson, *loc. cit.*, and Scarth, *J. Physical Chem.*, 1925, **29**, 1019). Substituting this value of e in (7) we have

$$N\theta=(V+V_0)(HV+2\sqrt{HVx})/(1-a) \quad . \quad . \quad (8)$$

Putting the above value of $N\theta$ in (2), and neglecting V_0, since it is small compared with V, we obtain

$$V(K_0+x)(HV+2\sqrt{HVx})=xN(1-a) \quad . \quad . \quad (9)$$

It has already been mentioned that a is negligible at low concentrations of the monobasic acids, so equation (9) reduces to the form

$$V(K_0+x)(HV+2\sqrt{HVx})=xN \quad . \quad . \quad . \quad (10)$$

The equation obtained by Wilson and Wilson, when one starts with 1 g. and not 1 millimol. of gelatin, assumes the form

$$MV(K_0+x)(HV+2\sqrt{HVx})=x$$

where M is the molecular weight of gelatin, and thus resembles (10).

In deducing their equation, Wilson and Wilsòn had to assume (1) that the active mass of gelatin is equal to the number of g. mols. of gelatin divided by the volume of the gel, and (2) that the kation of the salt GHA does not exert any osmotic pressure (compare Procter, Faraday Soc. Discussion, 1920—1921, **16**, Appendix, p. 41). These assumptions do not enter into the deduction of equation (10); moreover, the value of M for gelatin is uncertain. The value of N

in equation (10) is given by the maximum number of hydrogen ions which 1 g. of gelatin can adsorb and may be easily determined experimentally for any particular sample.

It follows from equation (9) that when x is constant any factor which will tend to increase (a) will decrease the swelling. The depression in swelling caused by the addition of alcohol and similar substances is probably due to a diminution in the dielectric constant of the medium. This will tend to increase (a) and hence depress the swelling.

Summary.

(1) Simultaneous measurements of electrical charge and swelling at different values of p_H indicate that electrostatic repulsion between the similarly charged particles of the gel cannot be the cause of the swelling. They also show that swelling is not due to the increasing hydration of the gel particles with increasing electrical charge.

(2) (*a*) The amount of hydrogen ions taken up by gelatin can be calculated with fair accuracy from Langmuir's adsorption equation.

(*b*) On this conception, an equation for swelling similar to that of Wilson and Wilson has been derived.

(3) A proportionality between the amount of hydrogen ions adsorbed and the electrical charge has been observed. For hydrochloric, nitric, and trichloroacetic acids the ratio of the electrical charge to the amount of hydrogen ions adsorbed is nearly constant. For sulphuric acid the ratio diminishes with increasing hydrogen-ion concentration.

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