XLVI. THE ELECTROPHORETIC VELOCITIES OF GELATIN AND OVALBUMIN IN DIFFERENT CONCENTRATIONS OF THEIR MIXTURES AND THE EFFECT OF ULTRA-VIOLET IRRADIATION.

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OBSERVATIONS of the motions of proteins in an electric field by many different methods have given results which, as shown in a previous paper [1929], are in substantial agreement. In this communication we have described the conditions under which the motion of a boundary, made visible by means of colloidal gold, may be measured in an improved form of U-tube apparatus [1930].

The investigation has now been extended from 0.1 % solutions of proteins to those mentioned in the title of this paper. In these solutions, differences in potential gradient are enhanced, and inconstancies in the motions of the boundaries are more frequent. The causes of these inconstancies, as they affect hydrophobic sols such as those of metals and sulphides, have been considerably elucidated by the work of Mukherjee [1923] and Kruyt [1925; Kruyt and van der Willigen, 1928].

Mukherjee used a U-tube of the type first described by Burton [1906] in which the sol is run in below the sol-free electrolyte. Many workers have found it difficult to set up a sharp boundary in such a manner. The difficulty has been obviated, for example in our apparatus, by separating the sol from the upper solution of electrolyte by means of taps, which are only opened after complete hydrostatic equilibrium has been established between the two limbs. Also disturbances due to diffusion of the products of electrolysis from the electrodes to the moving boundary are encountered when using the Burton type of tube. These were avoided in our apparatus, and in many others, by setting the electrodes in side-tubes. Any outstanding irregularities in Mukherjee's results are probably due to these causes.

This worker measured potential gradients in various sections of the tube by means of side-tubes sealed in at different levels, and found considerable variations when an arsenious sulphide sol containing free HCl and H₂S was introduced below a KCl solution having the same conductivity. When, however, HCl, having a slightly greater conductivity than that of the sol, was placed above the latter, the potential gradients in the sections immediately

above the original position of the boundary remained nearly constant during an experiment. These potential gradients gradually increased from the top section (HCl) to the lowest section (HCl + sol + H₂S). The average velocities in the lower sections, each corrected for their potential gradients, were in good agreement. They were 3.74 and 3.84 μ /sec., v./cm. In the anode limb, where the specifically slower SH⁻ ion follows the faster Cl⁻ ion upwards, the boundary remains sharp, while in the cathode limb where the faster ion follows the slower, the boundary becomes diffuse. Yet the calculated velocities in each limb were found to be nearly equal. These results seem to show that, although ionic diffusing layers may mix up the migrating particles, these do not take part in the ionic motion to the extent of being accelerated by the faster ions, *i.e.* the particles do not behave in this respect like slow ions. In agreement with this, it was shown that equivalent concentrations of sulphuric acid gave the same electrophoretic velocities, although there is considerable difference between the mobilities of Cl⁻ and of $\frac{1}{2}$ SO₄⁼. Although this conclusion does not necessarily hold good for a protein micelle, we may assume that it is approximately true for low velocities of the protein.

The general results of Mukherjee may be summarised:

(a) The velocity of movement of a boundary is dependent on the potential gradient of the section of tube in which it is moving.

(b) This potential gradient is determined by the conductivity of the solution in each section, including any conductivity due to the sol particles themselves.

Krulyt and van der Willigen [1928] also investigated sols of As₂S₃ containing up to 22 g. per litre. On these were superimposed intermicellar liquids obtained by ultra-filtration of the sol through collodion. The conductivities of the sols were 2–3 times those of the intermicellar liquids, partly owing to the decomposition of H₂S in the latter, partly owing to the presence of the negative sol particles in the former. The observed velocities in the “down” and “up” columns (cathode and anode limbs) were divided by the potential gradients in the sol and intermicellar liquid respectively, and agreed well. Thus the velocities of a sol containing 17.5 g. As₂S₃ per litre were 4.18–4.25 μ /sec. v./cm. at 20°. The potential gradients E_1/l_1 and E_2/l_2 in the sol and solution columns may be calculated from the conductivities, since

$$E_1/l_1 : E_2/l_2 = \kappa_2/\kappa_1.$$

Thus, in one case, $E_1/11.5 : E_2/12.1 = 6.21/16.15$

and E_1 and $E_2 = 75$ v.

Therefore $E_1/l_1 = 1.75$ and $E_2/l_2 = 4.55$ v./cm.

The observed velocity on the upward side was 1.35/900 cm./sec., therefore the velocity under unit potential gradient was

$$\frac{1.35 \times 10^4}{900 \times 4.55} = 3.3 \mu/\text{sec.}, \text{ v./cm.}$$

On the assumption that the velocity is determined solely by the potential gradient, another formula may be deduced which gives the velocity " v " in terms of the observed velocities v_1 (up) and v_2 (down), *i.e.*

$$v = \frac{v_1 l_1 + v_2 l_2}{E}.$$

We have used this to check some of our results.

It seems probable that the difficulties of determining electrophoretic velocities of hydrophobic colloids have been completely overcome.

As a provisional method of attack and pending a more complete knowledge of the complex protein micelles, it seems justifiable to apply these results to systems containing proteins, bearing in mind that, by virtue of their amphoteric character, these may behave to a greater or less extent as multivalent ions of rather low mobility.

EXPERIMENTAL.

Materials.

Ovalbumin. The whites of eggs, which were not more than 24 hours old, were treated according to the methods developed by Hopkins and Pinkus [1898]. The age of the egg is an important factor, since eggs which are more than 36 hours old do not readily yield a crystalline albumin. The ovalbumin was recrystallised twice and then freed from electrolytes by the dialysis of its solution against distilled water, using a good quality of parchment membrane. A stock solution of 5.14 % concentration was prepared but, owing to the denaturation which could not be prevented, this had to be filtered before use, and the protein content determined by estimating the residue after drying to constant weight at 110°. Toluene was used as a preservative.

Gelatin. Coignet's gold leaf gelatin was purified according to the method of Loeb. A stock solution of 1.85 % concentration was prepared, using toluene-saturated water, and was kept as a gel at 5°. The ash of this material was 0.03 % calculated on the weight of dry gelatin.

Irradiation.

Preliminary experiments on the ultra-violet irradiation of ovalbumin indicated that the molecule underwent a profound change: the possibility that this may play an essential part in the formation of pyrenoids of the Protophyta has already been discussed [Howitt, 1930]. The question of the mechanism of this denaturation is being investigated experimentally by one of us. For the present purpose it will be sufficient to describe the production, by ultra-violet irradiation, of the albumin-gold sol which was to be used for electrophoretic experiments. 300 cc. of a solution containing 0.2 % of pure ovalbumin and 0.02 % of gold (as $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$) were exposed to a mercury vapour lamp in a quartz flask placed about 5" from the source of light. A slow stream of nitrogen was passed through the solution with the twofold purpose of excluding atmospheric oxygen and agitating the liquid. The irradiation

was continued for 22 hours in periods of 2–3 hours, in order to avoid excessive heating. A splendid gold sol was formed by this irradiation, a gold mirror also being deposited on the walls of the flask. One volume of this solution was taken for the electrophoretic experiments, 0.2 *N* acetate buffer was added, and the mixture was diluted to 2 volumes, so that finally it contained 0.1 % of protein, and was 0.02 *N* in acetate. It must be pointed out that the product of irradiation of ovalbumin in presence of gold chloride is not necessarily the same as that prepared in absence of gold salt.

Gelatin. A 0.5 % solution was irradiated in a manner similar to that used for ovalbumin, and for 28 hours. A gold sol was then added. The p_H of the solution rose from 4.85 to 6.55. That the protein had altered was shown by the following observations. Precipitation by half-saturated ammonium sulphate was less evident in the irradiated than in the original material. When a 0.1 % solution was allowed to stand in the presence of 0.01 % gold for some weeks, coagulation occurred in certain cases, *viz.*:

p_H	Appearance
2.07	Clear red colour
2.35	„
2.75	Coagulated, blue
3.38	Purplish red colour
3.56	Light red colour, partly coagulated
5.60	Red colour, clear
7.75	Deep red
8.95	„

Thus there appears to be a loss of protective power in two zones of p_H . The coagulation at p_H 2.8 was irreversible with respect to gold, while that which occurred up to p_H 4.5 was reversible.

The gold sol was prepared by two methods.

(a) Bredig's method, by passing an arc between two gold wires in 0.0005 *N* HCl [Beans and Eastlack, 1915].

(b) Phosphorus in ether.

No material difference in velocity due to the different preparations was observed.

Electrophoresis.

Our method, as previously described [1929], consisted in the observation of the motion of a boundary between an acetate buffer (0.02 *N* in total acetate) above and a protein-gold sol below (0.02 *N* in acetic acid or total acetate), which had been brought to the same p_H by the addition of dilute acid or alkali. A potential fall of 222 v. was applied at electrodes of zinc in 5 % zinc chloride and 3 % agar, placed in side-tubes far removed from the boundaries.

The sols contained 0.1 % of protein and 0.01 % of colloidal gold. The concentration of sol must always be kept above that point at which mutual precipitation with the gold occurs. It was shown by Gann [1916] that an acid gold sol prepared according to Donau [1905] is precipitated by gelatin up to concentrations of about 0.0001 % of the latter, and by Freundlich and Loening [1921] that an acid solution containing 0.01 to 0.02 % of gelatin does not

flocculate gold, but that at this concentration the gold still moves electrophoretically towards the positive pole, and therefore behaves as (negative) gold. At higher concentrations, however, 0.05 to 0.10 % gelatin, our results and those of other investigators [Reinders and Bendien, 1928] have shown that the gold migrates to the negative pole, and behaves fully as protein; the velocity changing with change of p_H in the manner which is characteristic of the protein without gold. In alkaline solution it is stated that there is no sensitisation or coagulation of the gold, even when the protein is in very low concentration.

We have also noticed sometimes a coagulation of albumin at or near its isoelectric point in the presence of gold. We consider this to be an entirely different effect from that which takes place in acid solution. The isoelectric effect takes place at high protein concentrations, *i.e.* 1 %. The gold seems to be carried down with the protein and is not changed in colour. Such coagula are easily peptised by a trace of alkali, which is insufficient to make any appreciable alteration in p_H , with the production of an apparently unaltered red sol.

Gelatin lowers the velocity of colloidal gold in all sols and reverses it when $p_H \approx 4.2$ and when the gelatin has a concentration of more than 0.005 % [Reinders and Bendien, 1928].

Irregularities in the motion of protein boundaries "up" and "down" are partly due to differences in potential gradients. The ratios of the conductivities of sols to those of buffers are higher when the sols contain higher concentrations (0.5–1.0 %) of gelatin and albumin than when they contain lower concentrations (0.1 %). Therefore the ratios of the potential gradients are also higher for the more concentrated protein sols. In the case of albumin, the differences between up and down velocities were greatly reduced by correcting for the potential gradient.

Potential gradients may also be set up by diffusion potentials, which were measured in several instances and found to be 9 or 17 mv. in different acid solutions of p_H 3.6. If the diffusion potentials extend over a length of 1 mm. the gradients will be negligible compared with those of the main applied potential, which amount to several hundred millivolts per mm. The ionic diffusing layers will be spread out or made more diffuse when the current is carried by a quicker ion which is following a slower ion, and from regions of lower to those of higher conductivity. In the acid solutions, the boundary on the anode side moves downward, the faster Na^+ following the slower gel⁺ cation down, and the faster Cl^- following the slower A^- up. The ionic double layer will on the whole become diffuse after the passage of the current. On the cathode side the ionic double layer will be sharper, the Cl^- ions of the sol moving down and leaving the gel⁺ to move up in a region of higher potential gradient, in which also they may be accelerated by the motion of the Na^+ ions. The very high velocities of 3 μ /sec. or more with which the upward boundary sometimes moved may be due to one or other of these causes. On the other

hand, the protein micelles probably carry with them a considerable proportion of the ions which conduct the current, and this will tend to equalise the potential gradients of both boundaries.

These uncertainties seem to be inherent; however we considered that the most important condition is the constancy of p_H throughout the liquid. Differences in p_H not only greatly affect the velocities but also may give much higher diffusion potentials than those observed, which are due to unequal concentrations and mobilities of Na^+ , Cl^- and CH_3COO^- . It is possible, while maintaining p_H constant, to equalise also the conductivities in the two solutions by adding a neutral salt such as KCl to one or both. This device was successfully employed by Hardy [1905] who thus equalised the up and down movements of methylene blue. Kruyt [1925] found however that a gelatin sol had different velocities (*a*) when sol and solution were at the same p_H , (*b*) when the conductivities were made the same by addition of neutral salt to the solution. This result was confirmed by special experiments which are described in the series which deals with 1.0 % gelatin.

On account of the possible effect of the addition of neutral salts upon the proteins we have decided not to equalise the conductivities in this way. But, since the correction for potential gradients greatly improves the agreement between the "up" and "down" values, we have determined separately the conductivities of the buffer and sol containing 1 % albumin, and hence have determined the best form in which a mean correction can be made (fourth column of Table I). From the conductivities of the buffer and sol are determined the potential gradients in these parts, as described in the example (below). The means of all the buffer potential gradients are taken, and likewise the mean of all the sols. The mean of these means is used as a divisor of the mean (up and down) velocities at each p_H . The result (fifth column of Table I) agrees in most cases with the values in the fourth column.

Specimen calculations: albumin 1 %. At p_H 4.09.

$$v = +18.5 \text{ (down)} - 31.5 \text{ (up) in mm./hour.}$$

$$= 5.15 \text{ (down)} - 8.75 \text{ (up) in } \mu/\text{sec.}$$

κ_1 (sol) and κ_2 (buffer) are proportional to 0.001136 and 0.000538 respectively.

$$l_1 \text{ (sol)} = 20 \text{ cm.}, l_2 \text{ (buffer)} = 34 \text{ cm.}$$

$$\frac{E_1}{l_1} = \frac{E\kappa_2}{\kappa_2 l_1 + \kappa_1 l_2} = 2.35 = \text{potential gradient in sol,}$$

$$\frac{E_2}{l_2} = \frac{E\kappa_1}{\kappa_2 l_1 + \kappa_1 l_2} = 5.15 = \text{potential gradient in buffer,}$$

$$v_1 = \frac{v \text{ (down)}}{E_1/l_1} = 2.2, \quad v_2 = \frac{v \text{ (up)}}{E_2/l_2} = 1.7.$$

$$\frac{v_1 + v_2}{2} = 2.0 \text{ at } 16.0 \quad \frac{2.0 \times \eta_{16}}{\eta_{18}} = 2.09 \text{ at } 18^\circ.$$

$$\text{Mean uncorrected "v"} = \frac{5.15 + 8.75}{2} = 6.79.$$

$$\text{Mean "v" under unit potential gradient at } 18^\circ = \frac{6.9 \times \eta_{16}}{3.8 \times \eta_{18}} = 1.93.$$

The agreement is not always so good as this. Thus at p_H 2.90, v_1 ($\mu/\text{sec.}$) = 1.67, v_2 = 19.1, E_1/l_1 = 0.83, E_2/l_2 = 6.0. Therefore, at unit potential gradient, v_1 = 1.98, v_2 = 3.2, mean = 2.60 at 17°. In the middle part of the series, from about p_H 4.5 to 8.0, the conductivities of sol and buffer do not differ much from one another, and the "up" and "down" values are in fair agreement, which is usually improved by the correction for potential gradient. There are occasional exceptions such as the velocities at p_H 5.95 which are v_1 = 0.85 and v_2 = 2.0 respectively.

Gelatin 1 % at 28° and p_H 3.63.

v $\mu/\text{sec.}$ + electrode 2.5 (down) – electrode 7.65 (up).

κ_1 and κ_2 proportional to 4.41×10^{-4} and 2.26×10^{-4} respectively (sol and buffer).

E_1/l_1 = 2.58, E_2/l_2 = 5.0.

$v_1 = \frac{2.5}{2.58} = 0.97$, $v_2 = \frac{7.65}{5.0} = 1.53$.

$\frac{v_1 + v_2}{2} = 1.25$ at 28° $\times \frac{\eta_{28}}{\eta_{18}} = 0.99$ at 18°.

Mean uncorrected v = 5.07.

Mean v under unit potential gradient at 18° = $\frac{5.07 \times \eta_{28}}{3.8 \times \eta_{18}} = 1.05$.

The correction for viscosity. A series of measurements of the relative viscosities of albumin, gelatin and their mixtures, at various values of p_H , made in the Ostwald viscosimeter, will be the subject of a further communication. Great differences are found between the viscosities of 0.5 % gelatin and albumin on the one hand and 0.5 % and 0.25 % gelatin on the other. Since the velocities of the 1.0 % gelatin and albumin and of the 1.0 and 0.1 % gelatin respectively agree in the main, and a correction on the supposition that the values of " v " are inversely proportional to these viscosity figures, *i.e.* under low rates of shear, would give the curves quite a different character, in the equation

$$v = \frac{1}{4\pi} \frac{HD\xi}{\eta},$$

it has been assumed that the viscosity η is really that under high shearing stresses, *i.e.* from 400 mm. Hg upwards [Abramson, 1928]. It has also been observed by Freundlich, Abramson and others that the electrophoretic velocities of red blood corpuscles are almost unaffected by great variations in the viscosities of media containing serum, gelatin *etc.*, even by the very high values which are found after gelation has occurred.

Since electrophoresis is due to movements of ionic double layers, it is generally considered that the viscosity is that which affects and varies inversely as the conductivity. It has been found that the addition of a neutral substance, such as starch, in amounts sufficient to produce a very high viscosity has only a slight effect on conductivity [Hardy, 1905].

Since also the temperature coefficient of viscosity is the inverse of and nearly the same as that of conductivity, the correction, *e.g.* to 18°, may be introduced

either as κ_{18}/κ_t or η_t/η_{18} . It is known that the electrophoretic velocities of silver sols, determined at different temperatures, become constant when corrected to a constant temperature [Burton, Physical properties of colloidal solutions]. Abramson [1929] assumes a temperature coefficient of electrophoretic velocity of 2 % per 1°.

We have thought it best to correct the results simply for the alteration in viscosity of water. In our former paper, the velocities were referred to those at 15° as standard. The present series have been referred to 18°, so that the former values are multiplied throughout by the factor η_{15}/η_{18} for comparison.

Table I. *Albumin 1 %*.

p_H	Temperature	v in $\mu/\text{sec.}$ Mean	$v/3.8$ Mean velocity divided by mean potential gradient and corrected to viscosity at 18°	$\frac{v_1 l_1/E_1 + v_2 l_2/E_2}{2}$ Mean of "up" and "down" velocities each divided by its potential gradient and corrected to viscosity at 18°
2.50	18.0°	6.70	+1.76	+2.25
2.90	16.5	10.38	2.83	2.70
3.56	18.5	7.80	2.05	2.56
4.09	16.0	6.90	1.90	2.10
4.44	18.5	4.45	1.16	1.15
5.42	16.0	1.65	-0.45	-0.41
5.95	19.0	4.95	1.26	1.33*
6.48	18.5	4.50	1.17	1.14
6.58	17.0	4.03	1.08	1.02
7.03	19.5	6.40	1.62	1.59
8.24	20.0	6.30	1.57	1.53
9.38	19.5	7.55	1.91	1.92

* Not plotted in Fig. 1.

Fig. 1.

—○— 1.0 % ovalbumin. -+- 1.0 % gelatin.

Comparison of 1 % gelatin and albumin.

Our previous results, in 0.1 solution, which are included in Table III, seem to show a close agreement between the electrophoresis of the two proteins. A comparison is more difficult in the case of the 1 % solutions on account of the irregularities on the acid side, and the necessity of carrying

Table II. *Gelatin 1 % at 28°.*

p_H	v in mm./hr. Mean at 28°	" v " in μ /sec. v./cm. at 18°
2.37	19.0	+1.09
2.80	27.5	1.58
3.06	31.7	1.94*
3.63	18.2	1.04
3.63	27.0	1.67†
4.19	9.5	+0.55
4.97	4.0	+0.23
5.77	3.0	-0.19‡
6.48	11.0	0.63
7.15	9.0	0.51
7.83	11.0	0.63
8.70	11.0	0.63
9.30	14.0	0.80

* 1 % gelatin at 25.0.

† 0.8 % gelatin at 25.0. The conductivities of sol and buffer were made nearly the same by the addition of KCl to the buffer.

‡ 1 % at 25°. Both buffer and sol were 0.1 N with respect to KCl, which nearly equalised the conductivities.

Table III. *Gelatin, ovalbumin and their mixtures, 0.1 % in total protein.*

All velocities expressed as in previous tables.

Albumin 0.1 %				Gelatin 0.1 %				Albumin 0.05 %, gelatin 0.05 %			
p_H	t	v in mm./hr. at t°	v in μ /sec. at 18°	p_H	t	v in mm./hr. at t°	v in μ /sec. at 18°	p_H	t	v in mm./hr. at t°	v in μ /sec. at 18°
2.35	15°	5	+0.36 _s	2.75	15.0°	+40.0	+3.15	2.77	16.5°	±0.0	0.0
3.56	"	22	1.61	3.70	15.0	16.2	1.25	3.17	16.5	0.0	0.0
3.95	"	16	1.17	4.60	15.0	5.0	+0.39	—	—	—	—
4.45	"	8	+0.58	4.95	16.0	0.0	±0.00	3.90	15.0	+4.0	+0.29
5.56	"	12	-0.88	5.45	15.0	4.5	-0.35	4.22	19.0	3.5	0.25 _s
5.95	"	13	0.95	5.93	17.0	8.2	0.63	4.72	19.0	2.5	0.18
6.55	"	16.2	1.18	6.60	20.0	8.0	0.55	4.66	16.5	2.0	0.15
7.00	"	26	1.90	7.15	15.0	6.7	0.52	4.94	16.5	1.2 _s	0.10
7.65	"	24.2	1.86	8.02	21.0	10.0	0.67	5.55	15.0	2.8	0.39
				9.27	20.0	11.0	0.86	6.38	15.0	10.0	0.78
								6.98	15.0	8.0	0.63
								7.70	15.0	9.0	0.78
								8.66	16.0	10.5	0.81
								9.55	16.0	10.5	0.81

The velocities in mm./hour are the means of "up" and "down" values, exclusive of those which had diffused. The velocities in μ /sec. are calculated for unit potential gradient.Table IV. *Albumin 0.1 % and gelatin 0.1 % after irradiation.*

Albumin				Gelatin			
p_H	t	v in mm./hr. at t° Mean	v in μ /sec. v./cm. at 18°	p_H	t	v in mm./hr. at t° Mean	v in μ /sec. v./cm. at 18°
2.63	15.0°	15.2	+1.2	2.07	17.0°	38.0	+2.84
3.19	16.0	21.0	1.6	2.35	18.0	37.5	2.74
3.90	17.0	21.2	1.6	2.84	20.0	32.7 _s	2.27
4.55	17.5	7.8	+0.6	3.38	19.5	19.5	1.37
5.72	15.0	12.5	-1.0	3.56	20.5	18.2 _s	+1.24
6.30	18.0	15.0	1.1	5.60	20.5	22.2 _s	-1.52
7.54	19.0	22.5	1.6	7.75	20.0	22.2 _s	1.54
9.30	19.5	27.7	2.0	8.95	19.5	21.0	1.47
				9.34	19.0	27.2 _s	1.93

out the gelatin experiments at a higher temperature, 25° or 28°. On the whole it appears that the velocities at each p_H are lower in the case of gelatin. This may be connected with the much higher viscosities of gelatin solutions which we hope to investigate further by another method.

In the case of both proteins, velocities seem to reach maxima at p_H 3.0–3.5, then to fall off, in the case of gelatin, very rapidly, so that they even become zero at still lower p_H values. In this connection it may be noted that the combination curve of 1 % gelatin (p_H plotted against x cc. of HCl added) seems to show that the maximum amount is combined at about p_H 2.5, but the acid blank correction is large here so that the amount actually combined is rather uncertain [Atkin and Douglas, 1924].

The isoelectric point of gelatin determined graphically seems to lie above p_H 5.0. On account however of the low values of $\Delta v/\Delta p_H$ on either side of this point, a small alteration in the somewhat irregular velocities of positively charged gelatin would make a large difference in the point at which the line cuts the axis of zero velocity.

On the alkaline side, the velocities “up” and “down” are in good agreement, and as previously found in the case of 0.1 % solutions, become nearly constant at about 0.5–0.7 μ /sec. between p_H 6.0 and 6.5. Referring again to the combination curve of gelatin we find, after p_H 6.0, a rapid increase of p_H with added alkali, showing that the formation of alkali gelatin, or, according to the newer view, the displacement of the weaker set of basic groups from combination with OH, has reached a maximum. Therefore, no additional charge on the micelle is to be expected until higher values of p_H are reached. In the curve of 1 % gelatin [Atkin and Douglas, 1924], buffer action increases again at about p_H 9.5. This is also about the point where in our results an increase in negative velocity reappears.

In the case of 0.1 % solutions of albumin, the negative velocities appear

to increase with increasing p_H at about the same rate as that at which the positive velocities increase with diminishing p_H . The velocities on the alkaline side were more irregular in the case of the 1.0 % solutions. They increased with p_H up to $1.0 \mu/\text{sec.}$ or more, at p_H 6, and at higher values of p_H , continued to increase, although more slowly. They remained considerably above the velocities of 1 % gelatin at the same p_H .

Mixtures of 0.05 % gelatin and 0.05 % albumin.

The mixtures contained as usual 0.01 % of gold sol and were adjusted by addition of acid and alkali to the p_H of the 0.02*N* acetate buffers. The results in Table III are compared with those of the 0.1 % proteins measured separately. It might be expected that the motion would be intermediate between those of the separate proteins, or that it would be the same as that which has the greater velocity at each p_H , since each protein should carry with it sufficient gold to make the margin visible. Neither expectation was realised.

On the alkaline side, the protein behaves almost exactly like gelatin, which has the lower velocity. The tendency of the albumin to combine with increasing OH^- concentrations, giving an increasingly negative micelle, is somehow masked by the gelatin, which hardly increases its negative charge in this range of p_H .

On the acid side, the mixture does not behave like either protein. The velocities become somewhat irregular—a negative value having been registered at the isoelectric point 4.7, which according to our results in 0.1 % solution should have given not a zero, but a positive velocity. At p_H 4.0 a slight positive velocity was noted, but at lower p_H values the velocities fell to zero. It appears as if the mixture forms a more stable amphoteric ion, which is not appreciably opened up by the addition of acids. The viscosities of the mixtures, which will be the subject of a further communication, are to some extent intermediate but nearer to those of albumin.

The irradiation does not appear to have made any material alteration in the electrophoretic velocities of either protein. On account of the coagulation, velocities cannot be observed so close to the isoelectric point of the irradiated gelatin. A small portion has undoubtedly been rendered extremely sensitive to oxidation by the gold chloride, and has been destroyed, but the remainder seems to retain the same positive or negative charges.

SUMMARY.

1. The mean velocities of albumin are higher than those of gelatin both in acid and in alkaline solutions.
2. Neither the curve of gelatin nor that of albumin is symmetrical on the acid and alkaline side of the isoelectric point.
3. On the acid side the velocities increase rapidly with diminishing p_H to a maximum, and then fall off.

4. On the alkaline side the velocities increase up to p_H 6-7, then fall off and reach a constant or nearly constant value, which is lower and more nearly constant in the case of gelatin.

5. Mixtures of gelatin and albumin behave anomalously.

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