CLXXII.—*The Alleged Second Isoelectric Point of Gelatin.*

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THE observation of a minimum in the viscosity curve of gelatin at p_H 7·7 at 40° by Davis and Oakes (*J. Amer. Chem. Soc.*, 1922, **44**, 464) and of a similar minimum in swelling by Wilson and Kern (*ibid.*, p. 3633; 1923, **45**, 3139) led to the suggestion that gelatin has a second isoelectric point at this p_H. This was further supported by Matthews (*ibid.*, 1924, **46**, 852), who noticed two minima in the ultra-violet absorption spectrum of gelatin at p_H 4·7 and p_H 7·65, corresponding to the two isoelectric points. Wilson and Kern tried to explain these properties by assuming that gelatin can exist in two forms, the " gel " and the " sol " form, the former having an isoelectric point at p_H 4·7 and the latter at p_H 7·7. Increase of temperature or of p_H can transform the " gel " form to the " sol " form. Kraemer (*J. Physical Chem.*, 1925, **29**, 411) and Kraemer and Fanselow (*ibid.*, p. 1169) have further investigated the subject. They have discussed the different views regarding the existence of the two forms of gelatin, *i.e.*, the " sol " and " gel " forms of Wilson and Kern, and the keto-enol transformation suggested by Lloyd (*Biochem. J.*, 1920, **14**, 147), and have concluded that such assumptions are not justifiable and do not lead to useful results. They believe that the properties of gelatin can in many respects be explained from our knowledge of the behaviour of such colloids as ferric hydroxide sol. In Kraemer's opinion, the second isoelectric point at p_H 7·7 corresponds with the neutralisation of the negative charge of gelatin by positive ions in the alkaline solution.

Bogue (*J. Amer. Chem. Soc.*, 1925, **47**, 1194) has recently measured the optical rotation of gelatin sol at different hydrogen-ion concentrations, and concludes that gelatin has no second isoelectric point at p_H 7·7. In view of these controversies, it seemed desirable to measure the electrical charge of gelatin over a range of hydrogen-ion concentrations covering the two isoelectric points. The electro-endosmotic method of measurement has been chosen as being very suitable for measuring the charge of colloidal gels. Moreover, this method gives an idea of the resultant charge per unit area of the surface, and hence, even if gelatin contains particles having opposite charges, then, assuming that the amount of charge carried by each particle is the same, the rate of flow of water gives an indication as to which type of particle is in excess.

EXPERIMENTAL.

The gelatin used was purified by repeated washing with dilute acetic acid solution as suggested by Loeb (*J. Gen. Physiol.*, 1918, **1**, 45). The purified sample was dehydrated by washing it with absolute alcohol and then dried in air. Its ash content was 0·23%. 1·5 G. of the substance were placed in a Pyrex glass bottle containing 300 c.c. of a dilute sodium hydroxide and phosphoric acid mixture at 20° and left in contact with the solution for 24 hours. The p_H of the solution in equilibrium with the substance was determined by comparison with a set of standards as recommended by Clark and Lubs. The swollen masses of gelatin were then transferred to a U-tube, melted by warming in a water-bath, and then allowed to set at 18° for nearly 4 hours. The U-tube was then filled with the solution with which the jelly had previously been left in contact. The other experimental arrangements were exactly the same as described by the author in a previous paper (J., 1926, 2609). The experiments were carried out at 18° and the results are given in Table I, in which *b* represents the distance (in cm.) moved by the air bubble in 3 minutes. The plus and minus signs in the *b* columns indicate the charge of the set gel.

TABLE I.

p_H.	*b*.	p_H.	*b*.	p_H.	*b*.
3·6	0·8(+)	6·0	0·6(−)	8·0	1·6 (−)
4·2	0·3(+)	6·6	0·9(−)	8·8	1·85(−)
4·8	0·0	7·0	1·2(−)		
5·2	0·2(−)	7·4	1·4(−)		

The above results show that gelatin has only one isoelectric point, in the neighbourhood of p_H 4·8. Below that it is positively charged and the charge increases as the p_H diminishes. Above p_H 4·8 the negative charge on the diaphragm increases with the rise in p_H within the range investigated. There is thus no indication of neutralisation of the negative charge of the substance at p_H 7·7. In view of the recent observations of Kruyt and Tendeloo (*J. Physical Chem.*, 1925, **29**, 1303), it might be thought that the multivalent phosphate ion had some influence on the negative charge of gelatin. Experiments were therefore performed using sodium hydroxide solution alone. The results are given in Table II; they, too, show no indication of an inversion in the electrokinetic potential of gelatin at p_H 7·7.

TABLE II.

p_H	7·0	7·6	8·6
b	1·0(–)	1·3(–)	1·7(–)

The above experiments also throw light on the structure of gelatin gels. There are different views regarding their structure : (1) Procter, Wilson, Katz, and others believe that gels form a sort of homogeneous solid solution; (2) Wo. Ostwald and others consider them to be two-phase liquid–liquid systems; (3) many others think that gels are two-phase solid–liquid systems and have an open network-like structure. Hatschek (Faraday Soc. Discussion, October, 1921) has shown that the mechanical properties of gels cannot be explained on the basis of the two-phase liquid–liquid theory of structure. In our experiments the occurrence of electro-endosmotic flow of water through a gelatin gel when it is used as the diaphragm material has been definitely established. This fact seems inexplicable on the solid-solution theory. It points to the conclusion that gelatin gel must have a porous capillary-like structure. The gelatin forms the solid walls of the capillaries and the pores are filled with the electrolyte solution. When an electric field is applied across a diaphragm of the gel, the liquid flows through the zig-zag system of capillaries. The above experiments therefore furnish strong evidence in favour of the third view of gel structure.

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