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The Isoelectric Point of Gelatin in Relation to its "Minimum Physical Properties."

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Most investigators are of the opinion that the physical properties of gelatin (viscosity, conductivity, osmotic pressure) are at a minimum at its isoelectric point and that its opacity is a maximum at this point. This erroneous conception is partly due to the notion that ash-free gelatin is necessarily isoelectric. Gelatin, whose high degree of purity is indicated by an exceptionally low conductivity when it is free from diffusible electrolytes is not isoelectric. Cataphoresis experiments show that purified gelatin derived from lime conditioned stock migrates to the anode in an electrical field, while gelatin from acid conditioned stock migrates toward the cathode. The former must therefore be a weak acid while the latter is a weak base. The physical properties of gelatin are at a minimum in pure gelatin and not in isoelectric gelatin, which is a salt of gelatin and more highly ionized than pure gelatin.

Pure gelatin from any source should have the formula $HONH_3\,X\,COOH$ (where X represents the main gelatin radical and the other groups are representative of its acid and basic groups) and should accordingly furnish the ions $HO^-\,NH_3^+\,X\,COOH^+$. In the case of gelatin, which is a weak acid, the number of ionized groups furnishing H^+ would be greater and in the case of gelatin which is a weak base the number of groups furnishing OH^- ions would be greater, so that the 2 types of ionized gelatin may be represented by $(HONH_3\,X\,COO)^-\,H^+$ and $HO^-(NH_3\,X\,COOH)^-$ respectively.

It can be seen readily that the addition of an acid (HCl) to the type of gelatin $(HONH_3\,X\,COO)^-\,H^+$ will partly suppress its ionization and react with some of the OH groups of the molecule to form a chloride of gelatin $ClNH_3\,X\,COOH$ which would dissociate to furnish the more highly ionized $Cl^-\,NH_3^+\,X\,COO^-\,H^+$, just as the addition of HCl to NH_4OH forms the more highly ionized NH_4Cl.

If the proper amount of acid is added to pure gelatin, a point must be reached where the $\bar{Cl}$ ions equal the H^+ ions in number. (More correctly the $\bar{Cl} + \overline{OH}$ ions equal the H^+ ions.) At that point the gelatin radicle would have equal positive and negative charges and be, according to the correct interpretation of the term, isoelectric. Such an isoelectric, ionized salt of gelatin must necessarily have an osmotic pressure, viscosity and conductivity which is higher than that of pure gelatin.

Pure gelatin from acid conditioned stock furnishes the ions $HO^- NH_3^+ X COOH^+$. The addition of a base (NaOH) would suppress this ionization and at the same time form a salt $HO NH_3 X COO Na$ furnishing the ions $HO^- NH_3^+ X COO^- Na^+$. If the proper amount of base is added, a point must be reached where the positive and negative charges on the gelatin radicle are equal, and it is isoelectric.

It would seem evident then that the isoelectric point of gelatin is still an unknown quantity, and there are, at present, no methods for its exact determination. Any attempt to study the migration of gelatin in a gelatin solution would result in a destruction of the balance of diffusible ionic groups necessary to maintain the isoelectric point. Any attempt to study the migration of gel particles of gelatin by direct observation are open to the objection that the electric charge on such particles is partly due to selective adsorption and not entirely to ionization.

The purified gelatin which Loeb[1] prepared at a pH of 4.7 for his classic experiments may have been somewhat nearly isoelectric gelatin but its physical properties were not at a minimum. Dhere's criticism[2] that this gelatin was not free from ionic impurities is justified. Northrup and Kunitz,[3] who have recently criticized Dhere's point of view have apparently misinterpreted Dhere's criticism of Loeb's gelatin. In another part of Dhere's paper he states clearly the difference between isoelectric gelatin which is a compound containing "foreign ions", and "free gelatin" which is gelatin free from diffusible ionic impurities, although he does not state clearly the exact nature of isoelectric gelatin. His contention is that Loeb's gelatin was not free from diffusible ions, and that gelatin free of such ionic impurities is not isoelectric because it is negatively charged. In other words Loeb's gelatin could not be isoelectric and free of diffusible ionic impurities at the same time. It must not be overlooked that isoelectric gelatin is in part a salt of gelatin and is more highly ionized than pure gelatin but that the number of positive charges contained in the gelatin radicle are equal to the number of negative charges which it contains. The assumption sometimes made that isoelectric gelatin is not ionized at all is erroneous.

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1 Loeb, J., Proteins and Theory of Colloidal Behavior, 1922, McGraw-Hill.

2 Dhéré, C., *Kolloid Zeit.*, 1927, xli, 315.

3 Northrop, J., and Kunitz, M., *J. Gen. Physiol.*, 1928, xi, 478.

Northrup and Kunitz definitely appreciate the fact that isoelectric gelatin must have a diffusible anion present (and it should be added "or cation" if the gelatin is, by any chance, of acid conditioned stock). It would seem, however, that they, as well as Hitchcock,[4] who uses their method of purification, render their gelatin free of a part of the acetic acid necessary to maintain it at its isoelectric point when they precipitate it and wash it with alcohol and ether. At any rate, the pH for the new isoelectric point which Hitchcock recently gave for ash free gelatin is more nearly that of pure gelatin, free from diffusible ionic impurities, and the conductivity of this gelatin is also that of Cooper's purified, electrolyte-free gelatin which was recently purified electrolytically by the method described by the writer some years ago.

This new concept of isoelectric gelatin and its relation to the physical properties of gelatin in no way diminishes the validity or importance of Loeb's work or of that of many other investigators who have helped to develop the concept that proteins are amphoteric electrolytes which react with acids and bases in a perfectly normal manner. It helps rather to emphasize this point of view and to clarify the confusion which now exists because of the variable pH of different proteins and of different concentrations of proteins. This critical point of hydrogen ion concentration, where proteins have these "minimum physical properties," previously referred to as the isoelectric point, now becomes the point where the protein in its purest form exists as an acid or a base, and has a pH which is a function of the ionization constants of the protein in question.

Data and references in support of some of the contentions which have been made will be given in detail later.

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Effect of Feeding Whale Oil on the Depot Fat of the White Rat.

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Forty-three female white rats of average weight 154 gm. were fed for 6 weeks on a diet of 62.5% whole wheat flour, 15% casein, 15% skim milk powder, 1.5% precipitated chalk, 1% NaCl, 5%

[4] Hitchcock, David, *J. Gen. Physiol.*, 1929, xii, 495.