LI. NOTE ON THE PRODUCTION OF A CONTRACTING CLOT IN A GEL OF GELATIN AT THE ISO-ELECTRIC POINT.

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With Plate XI.

In a recent paper published in this *Journal* [Jordan Lloyd, 1920] the theory was developed that gelatin gels consist of a solid framework of pure uncombined gelatin, with an interstitial fluid which consists of a solution of gelatin salts, either acidic or basic. Equilibrium in the system was held to be the resultant of two opposing sets of forces, the elastic forces of the solid framework which tend to make the gel contract, and the osmotic forces due to the dissolved salts in the interstitial fluid which tend to keep it expanded. The special case of a hot solution of pure gelatin cooling at a reaction of $P_H = 4.6$ was considered, and the following deduction drawn from the theory:

"The iso-electric gelatin will be precipitated at numerous crystallisation centres, the solid drops will run together to form a framework, but since there can be no osmotic forces in the system... the framework will contract under the action of its own surface forces and the internal phase will be squeezed out." This condition of the formation of a contracting clot in a gelatin gel has now been realised in the laboratory and is shown in Plate XI, fig. 1. The gel was prepared by the following method: sheets of Coignet's Gold Label gelatin were dialysed against two changes of $N/1000$ hydrochloric acid, then against three changes of distilled water, and finally against dilute hydrochloric acid at $P_H = 4.6$. The dialysing acid was tested after twenty-four hours for change of reaction and was replaced daily by fresh acid at $P_H = 4.6$ until no change of reaction after twenty-four hours' dialysis could be detected by the colorimetric method. The indicator used was methyl red, and the colour was standardised by comparison with that obtained by adding an equivalent amount of indicator to a solution consisting of 50 cc. $0.2M$ acid potassium phthalate + 13 cc. $0.2M$ caustic soda in a total volume of 200 cc. [Clark and Lubs, 1917]. The whole course of dialysis lasted about a month.

The soaked sheets of gelatin were then removed from the dialysing fluid, dried, melted in an evaporating basin and filtered hot into a large volume of 97 % alcohol. A litre of alcohol was allowed to every 100 cc. of melted gelatin. The gelatin was precipitated as a white fibrous mass possessing marked elasticity. It was allowed to stand in the alcohol twenty-four hours,

Fig. 1

Fig. 2

Fig. 3

then in three changes of strong alcohol, each for twenty-four hours, and then in three changes of absolute alcohol. Finally the white fibrous mass was washed with ether and dried in a desiccator. This method of preparing pure gelatin differs from that employed by Dheré [1913] and by Dheré and Gorgolewski [1910] in the reaction of the dialysing fluid, but is otherwise similar to that described by these workers. The pure gelatin is snow-white and quite friable. The ash content was 0.1 %. A full account of its properties will be given in a subsequent paper. The three gels made from this gelatin and shown in Plate XI are all of a 1 % concentration. They differ in their content of acid, hydrochloric acid being present in tubes 2 and 3.

Tube 1 (fig. 1). The gelatin was dissolved in distilled water by heating, and cooled to 18°. The reaction of the solution at this temperature was $P_H = 6.09$. The solution set on standing to an opaque white gel. The contraction of the opaque gel was visible in twenty-four hours. The photograph reproduced was taken after standing for fourteen days. The reaction of the expressed clear fluid was taken again and was found to be $P_H = 4.42$. The fluid gave a faint positive biuret reaction.

A portion of the clot was stained with methylene blue and examined under a $\frac{1}{12}$ " oil immersion lens, with compensating eye-piece $\times 12$.

The crushed gelatin clot appeared to consist of numerous small spheres similar to the spherites described by Bradford [1920]. They were however very uniform in size, being about 0.5μ in diameter. No formation of filaments was detected. This, however, may have been due to the crushing of the clot under the cover-glass.

Tube 2 (fig. 2). The system contained 1 % of gelatin and HCl to a concentration of 0.005 *N*. The reaction of the freshly prepared solution at 18° was $P_H = 3.43$. The solution set after two hours to a clear colourless gel, which became faintly opalescent after forty-eight hours' standing. No further change occurred during the following fortnight, *i.e.* till the end of the experiment.

Tube 3 (fig. 3). The system consisted of 1 % of gelatin in 0.01 *N* HCl. The reaction of the freshly prepared solution after cooling to 18° was $P_H = 2.53$. The solution set to a glassy clear gel which showed no changes in transparency on standing.

These observations demonstrate that gelatin gels are unstable at the iso-electric point. Small quantities of caustic soda added to the system have the same stabilising influence as small quantities of hydrochloric acid. The formation of stable gels therefore is only possible in the presence of electrolytes.

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