

## PHYSICOCHEMICAL ASPECTS OF THE HIGHER ORDER STRUCTURE OF GELATIN IN DILUTE AQUEOUS SOLUTION

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The conversion of collagen to gelatin results in a heterogeneous product with a broad molecular weight profile (MWP). Addition of a non-solvent, such as ethanol, to gelatin solutions gradually causes the Florey-Huggins solvent-protein interaction parameter to exceed the critical value for the different molecular weight fractions, causing desolvation of the polymer. When sufficient solvent molecules are removed, the gelatin molecules begin to aggregate, resulting in phase separation, and forming a coacervate. Modification of the net charge of the protein, by adjusting the solution pH to values ranging about the iso-electric point (IEP), influences the degree of interaction between the different molecular weight fractions, and hence the response of the protein to non-solvent.<sup>1</sup>

The objective of this work was to study the response of lime-cured gelatin to the non-solvent ethanol under different conditions of pH and sodium chloride concentration. Dilute gelatin solutions at pH's of 3, 5, 7, 9 or 11 containing 0.2% w/w gelatin and increasing ethanol concentrations (40 to 75% w/w) were incubated at 39°C. Similar mixtures containing 0.1, 0.5 or 0.9% w/v NaCl were also prepared. The turbidity of the solution was measured at 600 nm using a UV/Vis spectrophotometer. The role of salt in influencing the response of gelatin to ethanol was also investigated by analysing the MWP of gelatin-salt mixtures using high pressure size exclusion chromatography.

The behaviour of gelatin solutions with no added salt was observed to be dependent on pH. Gelatin solutions adjusted to pH 3 and 11 were insensitive to the desolvating effect of ethanol, while solutions adjusted to pH 5, 7 and 9 exhibited increased turbidity with increasing ethanol concentration, the solutions at pH 5 being the most sensitive. The effect of added NaCl altered the behaviour of gelatin solutions towards ethanol; gelatin solutions adjusted to pH's 3 and 11 exhibited slight precipitation of gelatin. The opposite effect was observed for gelatin solutions adjusted to pH's 5, 7 and 9, which became less sensitive to increasing ethanol concentration with increasing ionic strength of the system. The MWP of gelatin was greatly influenced by the presence of salt, which resulted in a clear decrease in molecular weight, as evidenced by the shift towards longer retention times, compared to solutions where salt was absent.

In terms of the DLVO theory, gelatin solutions incubated at extremes of pH carry a net charge that gives rise to intermolecular repulsive forces, providing an energy barrier inhibiting aggregation. On the other hand, the proximity of pH 5 to the IEP of B-type gelatins ensured that the gelatin molecules in solution carried a reduced net charge and the electrical double layer surrounding each molecule was not efficient in inhibiting aggregation. The addition of salt to the gelatin solutions where the molecules carried a net charge caused a reduction of the electrical double layer thickness, reducing the energy barrier to aggregation. In solutions where the gelatin carries little or no net charge, the added salt caused dissociation of the gelatin polymers to lower molecular weight species which are more soluble. This is thought to occur by salt decreasing the range of influence of attractive intramolecular forces responsible for protein folding.

### References

1. C.A. Farrugia, M.J. Groves, *J. Pharm. Pharmacol.*, **51** (1999) 643-649.