Brief Research Report

ETHANOLIC FRACTIONATION OF DILUTE GELATIN SOLUTIONS *

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Gelatin is a heterogeneous protein with a broad molecular weight profile (MWP). Addition of a non-solvent to gelatin solutions causes progressive desolvation and aggregation of the polymer. 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 [1]. The objective of this work was to determine the response of gelatins of different bloom strengths, and hence with different MWP's, to the non-solvent ethanol at different pH's.

Unbuffered gelatin solutions were prepared by heating aqueous suspensions of undissolved gelatin to 40°C with stirring for 20 minutes. The pH was adjusted to 3, 5, 7, 9 or 11. The gelatin solutions were then incubated at 20°C, 39°C or 56°C for 1.5 hours and mixed with ethanol/water mixtures that had been similarly incubated such that the final solutions contained 0.2% w/w gelatin and ethanol concentrations from 40 to 75% w/w. The three-component systems were incubated for a further 20 minutes and the turbidity of the solutions measured by percent transmittance using a Shimadzu 160 UV/Vis spectrophotometer operated at 600nm. The data obtained was subjected to nonlinear regression analysis and the parameter V_{50} (the ethanol concentration at the % transmittance midway between the initial and final values) was used to monitor the effects of the experimental conditions on the phase behaviour of gelatin in solution, lower V_{50} values being indicative of a greater sensitivity to desolvation.

The behaviour of the gelatin solutions was observed to be highly dependent on the solution 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, with the solutions adjusted to pH 5 being the most sensitive. In terms of the DLVO theory, gelatin solutions incubated at extremes of pH carry a net charge that gives rise to intermolecular repulsive forces and to a double layer around the gelatin molecules, which provided 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. Thus, the electrical double layer surrounding each molecule was not efficient in inhibiting aggregation, and precipitation resulted. The V_{50} values of B225 type gelatin solutions were sensitive to both changes in temperature (F=16.9, p<0.05) and pH (F = 49.1, p<0.01), while those of B75 type gelatins were sensitive to changes in pH (F=10.0, p<0.05) but not in temperature (F=1.59, p>0.05).

Earlier studies have shown that factors altering the MWP of gelatin in solution affect the phase behaviour of gelatin solutions in the presence of a desolvating agent such as ethanol [1]. Thus, increasing temperature causes a shift in the MWP to lower molecular weights, accounting for the above observations. Lower bloom strength gelatins already have a MWP that is shifted towards lower

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molecular weights [2], accounting for the lack of temperature effects with B75 gelatin.

References:

[1] C.A. Farrugia and M.J. Groves, *J. Pharm. Pharmacol.*, **51** (1999) 643.

[2] C.A. Farrugia *et al.*, *Pharm. Pharmacol. Commun.*, **4** (1998) 1.