THE EFFECT OF SODIUM CHLORIDE ON ETHANOLIC FRACTIONATION OF DILUTE GELATIN SOLUTIONS

A. Aquilina[1], C. Farrugia[2] and E. Sinagra[3]

[1] Malta Standards Authority, Evans Building, Merchants Street, Valletta VLT03, Malta
[2] Chemistry Department, Junior College, University of Malta, Msida MSD06, Malta
[3] Department of Chemistry, University of Malta, Msida MSD06, Malta

Introduction

Gelatin is the denaturation product of the protein collagen, the main constituent of animal skin, bone, and connective tissue. The conversion of collagen to gelatin results in a heterogeneous product with a broad molecular weight profile (MWP), which is important in determining the behaviour of the protein in solution. Addition of successive increments of a non-solvent, such as ethanol, to gelatin solutions causes the Florey-Huggins solvent-protein interaction parameter, χ, of the system to successively exceed the critical value for the different molecular weight fractions, causing progressive desolvation of the polymer. When sufficient solvent molecules are removed, the gelatin molecules begin to aggregate, resulting in phase separation, and forming a coacervate or, if sufficient desolvation occurs, a precipitate.

Modification of the net charge of the protein molecules, 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.[1] It can be hypothesised that alteration of the molecular charge intensity by changes in the ionic strength of the solution would affect the overall response of the protein. The objective of this work was to determine the effect of dilute NaCl concentrations on the response of B225 and B75 gelatins to the non-solvent ethanol at different pH's.

Experimental Methods

Lime-cured gelatins from bovine skin (Type B) of bloom strengths 75 and 225 were purchased from Sigma Chemical Co., USA. Unbuffered solutions of gelatin 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 by adding dilute HCl or dilute NaOH.

The method used was that of Farrugia and Groves (1999).[1] The gelatin solutions prepared above were 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 increasing ethanol concentrations (40 to 75% w/w). Similar mixtures containing 0.1, 0.5 or 0.9% w/v sodium chloride were also prepared for the gelatin solutions incubated at 39°C, at which temperature there is practically no change in the MWP of the gelatin solution.[1] The three-component systems were incubated at the same temperature for a further 20 minutes and the turbidity of the solutions measured by percentage transmittance using a Shimadzu 160 UV/Vis spectrophotometer (Shimadzu Corporation, Japan) operated at 600nm.

The data obtained from the desolvation experiments was subjected to nonlinear regression analysis, using the equation:

$$T = Bottom + \frac{(Top - Bottom)}{1 + e^{\frac{V_{50} - C}{Slope}}}$$

where T represents % transmittance, C represents ethanol concentration (% w/w), Top is the plateau % transmittance value at the top of the curve, $Bottom$ is the plateau % transmittance value at the bottom of the curve, and V_{50} is the ethanol concentration at the % transmittance midway between Top and $Bottom$. The changes in V_{50} with changes in experimental conditions were used to monitor the effects of the various experimental conditions on the phase behaviour of gelatin in solution, lower V_{50} values being indicative of a greater sensitivity to desolvation. A 2-way analysis of variance (ANOVA) was used to determine statistically significant changes in V_{50}.

Results and discussion

The behaviour of gelatin solutions with no added salt 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. (Table 1) 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. Solutions at pH's 7 and 9 had V_{50} values indicative of intermediate degrees of intermolecular repulsion and hence sensitivity to desolvation.

Table 1
Effect of temperature and pH on addition of ethanol to B75 and B225 gelatin solutions

Experiment Conditions	V_{50} (mL, mean ± SEM, n = 3)		
	20°C	39°C	56°C
B225 gelatin			
pH 5	44.0 ± 29	48.6 ± 0.5	52.7 ± 0.2
pH 7	48.1 ± 1.9	54.2 ± 0.7	53.6 ± 0.6
pH 9	57.2 ± 0.1	60.3 ± 0.2	65.8 ± 0.1
B75 gelatin			
pH 5	26.6 ± 23	46.9 ± 0.4	51.0 ± 0.5
pH 7	57.2 ± 0.4	58.2 ± 0.4	58.2 ± 0.3
pH 9	65.6 ± 1.1	65.0 ± 0.2	70.6 ± 0.3

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

The effect of added NaCl dramatically altered the behaviour of gelatin solutions towards ethanol; gelatin solutions adjusted to pH's 3 and 11 exhibited slight precipitation of gelatin with ethanol, as opposed to no precipitation in solutions not containing any salt. The opposite effect was observed for gelatin solutions adjusted to pH's 5, 7 and 9, which became progressively less sensitive to increasing ethanol concentration with increasing ionic strength of the system. (Table 2) In terms of the DLVO theory, the addition of salt to the gelatin solutions where the molecules carried a net charge caused a reduction of the electrical double layer thickness, thus reducing the energy barrier to

aggregation. However, in solutions carrying little or no net charge, the added salt reduced the ease of aggregation, aiding the solubility of gelatin in ethanol. This may be explained by noting that although the net charge on the gelatin molecules is reduced, charged groups still exist in regions along the molecule, resulting in attractive intramolecular forces that cause the molecules to fold. Addition of salt could result in a reduction in these forces, and hence an increase in molecular extension, yielding a more soluble entity than the coiled structure.

Table 2
Effect of added salt and pH on addition of ethanol to B75 and B225 gelatin solutions

Experiment Conditions	V_{50} (mL, mean ± SEM, n = 3)		
	0.1% w/v NaCl	0.5% w/v NaCl	0.9% w/v NaCl
B225 gelatin			
pH 5	55.9 ± 0.1	62.2 ± 0.0	64.2 ± 0.2
pH 7	55.9 ± 0.1	60.5 ± 0.0	63.9 ± 0.1
pH 9	57.1 ± 0.1	62.3 ± 0.1	63.4 ± 0.1
B75 gelatin			
pH 5	55.2 ± 0.3	62.6 ± 0.0	63.8 ± 0.1
pH 7	56.9 ± 0.1	61.4 ± 0.1	62.2 ± 0.0
pH 9	58.8 ± 0.2	62.1 ± 0.1	63.5 ± 0.1

The V_{50} values of both B225 and B75 gelatins were insensitive to changes in pH (F_{B225}=0.95, p>0.05; F_{B75}=0.81, p>0.05). However, both gelatin types were sensitive to the concentration of added salt (F_{B225}=82.1, p<0.01; F_{B75}=29.3, p<0.01). Thus, the effect of salt was superimposed on the changes in precipitability caused by changing pH.

Conclusion

It appears that in the absence of salt, the solution pH affects the net charge of the protein, altering interchain interactions and the response of the protein to non-solvent. The presence of salt affects the electrical double layer surrounding localised charges and alters the response of the protein to a greater extent than that due to the solution pH.

References

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