

Temperature Effects on the Ethanolic Fractionation of Dilute Gelatin-Sodium Dodecyl Sulfate Solutions

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Introduction

Gelatin is a water-soluble, macromolecular hydrocolloid protein, with a structure that is sensitive to changes in pH and temperature, since these factors influence the net charge of the protein and the strength of the intermolecular interactions. Addition of anionic surfactants such as sodium dodecyl sulfate (SDS) also effects the triple helical structure of gelatin in aqueous solution. SDS associates with gelatin through hydrophobic interactions involving the hydrocarbon tail, and through ionic interactions between the negatively charged headgroup of SDS and positively charged side groups on the gelatin molecule.¹ It can thus be hypothesised that alteration of gelatin structure by changes in temperature and pH of the solution would effect the overall response of the protein-surfactant complex to increasing concentrations of the non-solvent ethanol. The objective of this work was to determine the effect of increasing SDS concentration and temperature change on the response of B225 gelatin to the non-solvent ethanol at pH's below and above the IEP of B225 gelatin, by monitoring the phase behaviour of gelatin-SDS solutions in mixtures of water and ethanol, using turbidity measurements.

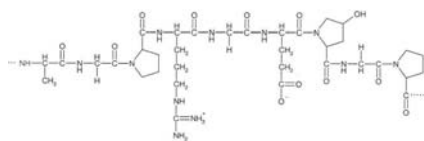


Figure 1: Schematic Diagram of Gelatin

Methodology

Unbuffered solutions of gelatin/SDS containing 0.01% (low), 0.025% (medium) and 0.05% (high) w/w SDS were prepared by heating aqueous gelatin suspensions to 40°C with stirring for 20 minutes, and the pH was adjusted to the desired value (4.5, 5, 6, 7 and 9) accordingly with dilute HCl or NaOH. Increasing volumes of ethanol were added to the gelatine such that the final solutions contained a gelatin concentration of 0.2% w/w and ethanol concentrations from 0 to 80% w/w. All solutions were incubated at three different temperatures (25°C, 37°C and 55°C) for 90 minutes. The ethanol and gelatin/SDS solutions were mixed together and incubated again at their respective temperatures for another 20 minutes. The turbidity of the resulting solutions was then measured by % transmittance using a Shimadzu 1601 UV/Vis spectrophotometer (Shimadzu Corporation, Japan) operated at 600nm. with a Temperature-Controlled Cell Holder set at the respective incubation temperature. The data obtained was subjected to nonlinear regression analysis, using the equation:

$$T = Bottom + \frac{(Top - Bottom)}{1 + 10^{(V_{50} - C) Slope}}$$

where T represents % transmittance, C represents ethanol concentration (% w/w), Top and $Bottom$ are the plateau % transmittance values at the top and bottom of the curve, respectively, and V_{50} is the ethanol concentration at the % transmittance midway between Top and $Bottom$. The changes in V_{50} and $Bottom$ 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} and lower $Bottom$ values being indicative of a greater sensitivity to desolvation.

Results

Figure 2: Effect of SDS on the addition of ethanol to B225 gelatin solutions at pH4.5 at 25°C, 37°C and 55°C.

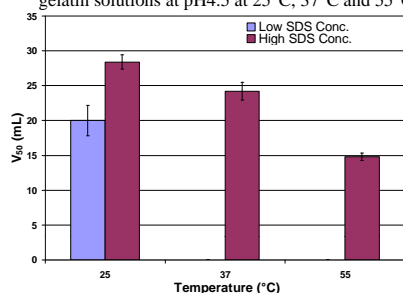


Figure 4: Effect of SDS on the addition of ethanol to B225 gelatin solutions at pH5 at 25°C, 37°C and 55°C.

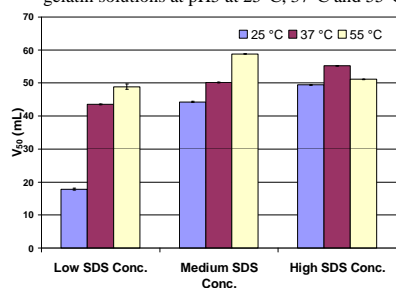


Figure 3: Effect of SDS on the extent of desolvation of B225 gelatin solutions at pH4.5 at 25°C, 37°C and 55°C.

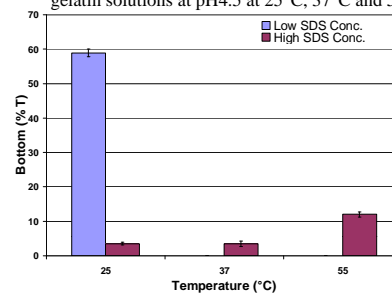
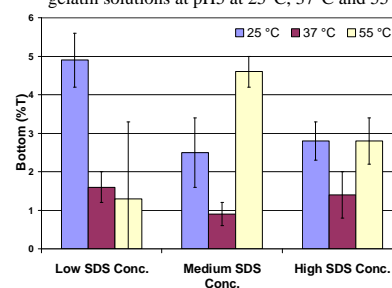


Figure 5: Effect of SDS on the extent of desolvation of B225 gelatin solutions at pH5 at 25°C, 37°C and 55°C.



Conclusions

The prominent trends observed were that a primary desolvation was observed mostly at pH 4.5 and pH 5, while at pH's above the IEP, that is pH's 6, 7 and 9, only secondary desolvation was observed. Similar studies, where no SDS was added to the gelatin solution, showed that the phase behaviour of gelatin is highly dependant on the solution pH.² Unlike solutions with added SDS, gelatin solutions incubated at low and high pH's were both insensitive to the desolvating effect. This is due to the net charge on the gelatin molecule which gives rise to intermolecular repulsive forces that inhibit the aggregation of individual gelatin molecules, as the double layer surrounding the gelatin molecules provides a large enough barrier to inhibit aggregation and hence precipitation, making the process kinetically unfeasible. At high SDS concentration primary desolvation occurred at all temperatures. However, solutions at pH 4.5 in low SDS concentration showed a primary desolvation only at 25°C, while at 37°C and 55°C, no primary desolvation occurred. It can be hypothesised that at very low concentrations of SDS, the hydrophobic gelatin-SDS complex is still formed but the MW increase is not high enough, therefore an increase in temperature makes it entropically favourable for gelatin to remain in solution. At low SDS and pH's close to IEP, the hydrophobic gelatin-SDS complex fails to form. Thus the gelatin molecules are more sensitive to ethanol and precipitate out at an earlier stage. One can conclude that dilute aqueous gelatin-SDS solutions below the IEP show a particular phase behaviour in water-ethanol mixtures since a hydrophobic gelatin-SDS complex is formed which causes the occurrence of primary desolvation. However with increase in ethanol concentration resolution occurs, until an excess of ethanol causes the secondary desolvation. The results obtained also confirm that temperature effects the phase behaviour of gelatin by contributing towards the entropic feasibility of desolvation or solvation events.

References

1. R. Wüstneck, R. Wetzel, E. Buder and H. Hermel, The modification of the triple helical structure of gelatin in aqueous solution I. The influence of anionic surfactants, pH-value and temperature. *Colloid Poly. Sci.* **266** 1061-1067 (1988).
2. Storm J., Farrugia C.A., Sinagra E., The effect of Sodium Dodecyl Sulfate on the ethanolic fractionation of dilute gelatin solutions. In: Proceedings of the 5th World Meeting on Pharmaceuticals, Biopharmaceutics and Pharmaceutical Technology (2006).