Surfactant Effects on the Ethanolic Fractionation of Dilute Gelatin Solutions

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Introduction

Addition of a non-solvent, such as ethanol, to aqueous solutions of the heterogeneous protein, gelatin, causes progressive desolvation of the polymer. Sodium dodecyl sulphate (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; both mechanisms cause unfolding of the protein and yield a hydrophobic complex.[1] Thus, the addition of SDS dramatically alters the desolvation behaviour of gelatin solutions, such that at pH's at and below the IEP, a primary desolvation is observed, the extent of which increases with decreasing pH. The precipitate dissolves with increasing ethanol concentration and a secondary desolvation subsequently occurs. Above the IEP, primary desolvation of SDS-gelatin mixtures is not observed but secondary desolvation occurs to a greater extent than for gelatin solutions alone.[2] It has been suggested that electrostatic binding of SDS to gelatin plays a key role in the primary desolvation event, whilst hydrophobic binding is responsible for the more complete desolvation of SDS above the IEP.[2] It can thus be hypothesised that in the presence of a non-ionic surfactant, such as Tween 20, the primary desolvation effects seen in the presence of an anionic surfactant, such as SDS, will not be observed, but the secondary desolvation effects will still be present.

Methodology

Unbuffered solutions of 225-bloom, lime-cured gelatin from bovine skin (B225) were prepared by heating aqueous gelatin suspensions to 40°C with stirring for 20 minutes, and the pH adjusted using dilute HCl or NaOH. The gelatin solutions were incubated at 37°C for 1.5 hours and mixed with ethanol/H_2O mixtures that had been similarly incubated. The final solutions contained 0.2% w/w gelatin and ethanol concentrations from 0 to 80% w/w. Similar mixtures containing 1.74×10^{-3} mol.dm^{-3} SDS or Tween 20 were also prepared. The three-component systems were incubated at 37°C for a further 20 minutes and the degree of turbidity quantified by measuring percentage transmittance using a Shimadzu 1601 UV/Vis spectrophotometer operated at 600nm. The data obtained was subjected to nonlinear regression analysis, using the equation:

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

T = % transmittance; C = ethanol concentration (% w/w);
Top, *Bottom* = plateau values of T at top and bottom of curve;
V_{50} = value of C 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 1: Effect of SDS and Tween 20 on the addition of ethanol to B225 gelatin solutions.

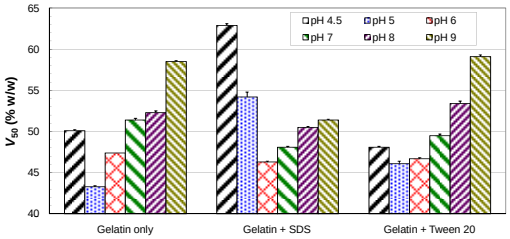


Figure 2: Effect of SDS and Tween 20 on the extent of desolvation of B225 gelatin solutions.

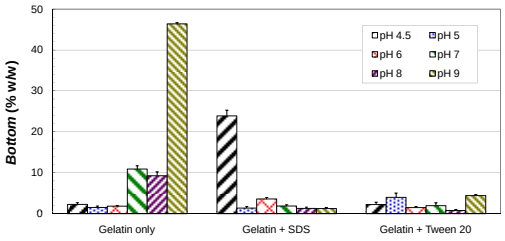


Figure 3: pH-dependent desolvation of gelatin solutions.

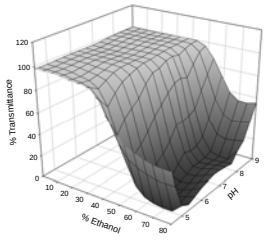


Figure 4: pH-dependent desolvation of gelatin–SDS solutions.

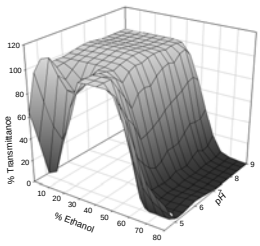
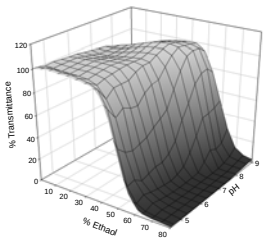


Figure 5: pH-dependent desolvation of gelatin–Tween 20 solutions.



Conclusions

Gelatin-SDS binding is a consequence of both weak hydrophobic interactions and electrostatic interactions between the negatively-charged SDS and the predominantly positively-charged amino groups in gelatin.[1] In the presence of SDS, the initial addition of ethanol resulted in a primary desolvation at pH's at and below the IEP; this primary desolvation has been attributed to gelatin cross-linking by SDS molecules binding electrostatically at one end and hydrophobically at the other end.[2] Tween 20, a nonionic surfactant, possesses no charged groups, precluding electrostatic gelatin-surfactant binding and thus reducing the possibility of gelatin cross-linking. In fact, no primary desolvation was observed in gelatin-Tween 20 mixtures at any pH. The increased resistance of resolvated gelatin-SDS complexes to secondary desolvation compared to native gelatin has been attributed to the unfolded nature of the complex, together with a higher concentration of surface charged groups.[2] The absence of charge on the Tween 20 molecules means that the gelatin-surfactant complex experiences no increase in charge but only an increase in molecular weight, rendering the complex more sensitive to the presence of non-solvent than native gelatin. Thus, at pH's above the IEP, gelatin-Tween 20 systems exhibited a sensitivity to desolvation comparable to that of native gelatin solutions but a degree of precipitation comparable to gelatin- SDS solutions, this being indicative of extensive desolvation. The differences in V_{50} values for gelatin-SDS complexes compared to gelatin-Tween 20 complexes also emphasise the importance of the charged nature of the surfactant SDS in determining the desolvation behaviour of the complex formed with gelatin.

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 Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (2006).