

THE EFFECT OF SODIUM DODECYL SULFATE ON THE ETHANOLIC FRACTIONATION OF DILUTE GELATIN SOLUTIONS

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Introduction

Gelatin is a heterogeneous protein with a broad molecular weight profile (MWP), which determines its behaviour in solution. Addition of a non-solvent, such as ethanol, to aqueous gelatin solutions causes 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.¹ 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.² It can be hypothesised that addition of SDS to dilute gelatin solutions will affect their desolvation behaviour, depending on the degree of binding of SDS to gelatin at different pH's. The objective of this work was to determine the effect of dilute SDS concentrations on the response of B225 gelatin to the non-solvent ethanol at different pH's.

Experimental Methods

Unbuffered solutions of lime-cured gelatin from bovine skin (Type B), bloom strength 225, 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, at which temperature there is practically no change in the MWP of the gelatin solution,¹ for 1.5 hours and mixed with ethanol/H₂O 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 8.68×10⁻⁴ mol.dm⁻³ (Low SDS) or 1.74×10⁻³ mol.dm⁻³ (High SDS) SDS were also prepared. The three-component systems were incubated at 37°C for a further 20 minutes and their turbidity measured by percentage transmittance using a Shimadzu 160 UV/Vis spectrophotometer (Shimadzu Corporation, Japan) operated at 600nm.

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

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

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 and discussion

The behaviour of gelatin solutions with no added SDS was highly dependent on the solution pH. Gelatin solutions at pH 5 were most sensitive to the presence of ethanol, while solutions of increasingly higher pH values exhibited decreasing sensitivity, as exhibited by increasing *V*₅₀ and *Bottom* values. (Tables 1 and 2) Gelatin solutions incubated at extremes of pH carry a net charge that gives rise to intermolecular repulsive forces, providing an energy barrier which inhibits aggregation, while the proximity of pH 5 to the IEP of B-type gelatins ensures that the gelatin molecules in solution carry a reduced net charge; the electrical double layer surrounding each molecule is not efficient in inhibiting aggregation, and precipitation results.

Added SDS dramatically altered the behaviour of gelatin solutions towards ethanol. At pH's at and below the IEP, the initial addition of ethanol to SDS-gelatin mixtures resulted in a primary desolvation, the extent of which increased with decreasing pH and increasing SDS concentration, as seen by increasing *V*₅₀ and decreasing *Bottom* values. (Table 3) The precipitate dissolved with increasing ethanol concentration and a secondary desolvation was subsequently observed, occurring at higher *V*₅₀ values than that of gelatin without SDS. At pH's

above the IEP, however, the primary desolvation was not observed, and the secondary desolvation, while occurring at similar ethanol concentrations as gelatin without SDS, appeared to result in more complete desolvation, based on the decreased *Bottom* values.

Table 1

V_{50} values (mL, mean \pm SEM, n=2) for gelatin and gelatin-SDS (secondary desolvation) solutions

Experiment Conditions	Gelatin only	Gelatin + Low SDS	Gelatin + High SDS
pH 4.5	50.1 \pm 0.1	55.6 \pm 0.0	62.0 \pm 0.2
pH 5	47.5 \pm 0.1	46.4 \pm 0.1	51.6 \pm 0.1
pH 6	48.6 \pm 0.1	46.2 \pm 0.1	45.8 \pm 0.2
pH 7	51.3 \pm 0.3	49.6 \pm 0.1	50.7 \pm 0.2
pH 8	54.8 \pm 0.2	53.0 \pm 0.0	51.2 \pm 0.1
pH 9	56.8 \pm 0.0	56.8 \pm 0.0	54.1 \pm 0.1

Table 2

Bottom values (mL, mean \pm SEM, n=2) for gelatin and gelatin-SDS (secondary desolvation) solutions

Experiment Conditions	Gelatin only	Gelatin + Low SDS	Gelatin + High SDS
pH 4.5	2.25 \pm 0.41	1.05 \pm 0.19	29.3 \pm 1.6
pH 5	1.82 \pm 0.58	5.97 \pm 0.74	3.15 \pm 0.68
pH 6	3.78 \pm 0.75	1.35 \pm 0.43	4.02 \pm 0.81
pH 7	5.23 \pm 1.61	1.08 \pm 0.56	2.52 \pm 1.18
pH 8	19.8 \pm 1.0	1.42 \pm 0.24	1.73 \pm 0.61
pH 9	53.6 \pm 0.2	5.23 \pm 0.32	0.88 \pm 0.29

Table 3

V_{50} and *Bottom* values (mL, mean \pm SEM, n = 2) for gelatin-SDS (primary desolvation) solutions

Experiment Conditions	Low SDS		High SDS	
	V_{50}	<i>Bottom</i>	V_{50}	<i>Bottom</i>
pH 4.5	13.3 \pm 0.1	27.2 \pm 0.3	22.3 \pm 0.1	7.95 \pm 0.50
pH 5.0	-	-	21.3 \pm 0.1	25.4 \pm 0.1

The first step in SDS binding to gelatin is stabilized by weak hydrophobic interactions,² probably increasing the number of hydrophilic negatively charged groups on the gelatin molecule. This decreases the net positive charge of the molecule observed at pH's below the IEP and reduces the repulsion between adjacent gelatin molecules. This interaction is also assumed to cause a partial unfolding of the gelatin structure, improving the accessibility of the ionic groups of the gelatin chains and favouring electrostatic binding of SDS to gelatin.² The latter occurs markedly at pH's below the IEP, when the positively charged amino side groups are in excess of the negatively charged carboxylate side groups, and results in further

unfolding of the gelatin structure by breaking zwitterionic couples of the gelatin.² The increase in molecular weight and hydrophobicity of the gelatin-SDS complex causes its dissolution to be a more entropically unfavourable event, which, together with the possibility of gelatin cross-linking due to SDS molecules binding electrostatically at one end and hydrophobically at the other, leads to precipitation. Increasing concentrations of ethanol create a solvent mixture which is more favourable to dissolution of the more hydrophobic complex than water alone, resulting in resolution. However, excess ethanol eventually causes a complete secondary desolvation of the molecule. The hypothesized unfolded nature of the gelatin-SDS complex, together with the higher concentration of surface charged groups could be responsible for the secondary desolvation occurring to a lesser extent than in native gelatin solutions.

At pH's above the IEP, electrostatic binding of SDS to gelatin is progressively reduced due to electrostatic repulsion as the gelatin molecule gradually acquires a greater net negative charge. Thus the changes in gelatin structure, particularly gelatin cross-linking, due to electrostatic binding of SDS are no longer seen and the primary desolvation does not occur. However the increase in molecular weight and hydrophobicity of the gelatin-SDS complex due to hydrophobic binding results in more complete precipitation when desolvation occurs at high ethanol concentrations.

Conclusion

It appears that in the presence of SDS, gelatin undergoes configurational changes depending on the degree and mechanism of surfactant binding. The latter are in turn affected by the pH of the solution, particularly at and below the IEP. The combined effect of surfactant binding and configurational changes appears to influence the pH-dependent response of gelatin to the non-solvent ethanol.

References

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