

THE EFFECT OF SODIUM CHLORIDE ON THE pH-DEPENDANT ETHANOLIC FRACTIONATION OF GELATIN-SDS MIXTURES

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Introduction

Addition of the non-solvent ethanol to gelatin solutions causes progressive desolvation, coacervation and precipitation of the polymer.¹ This behaviour is highly dependent on pH and ionic strength. B-type gelatin solutions incubated at extremes of pH exhibit decreased aggregation since the molecules carry a net charge that gives rise to intermolecular repulsive forces. The latter are reduced in the presence of added salt due to a decreased electrical double layer thickness. At pH's close to the IEP of the protein, the gelatin molecules carry a reduced net charge; the electrical double layer around each molecule inhibits aggregation poorly and precipitation occurs more readily. Added salt reduces aggregation at these pH's, possibly by disrupting intramolecular attractive forces and causing unfolding of the molecule into a more soluble configuration.¹

Sodium dodecyl sulphate (SDS) associates with gelatin both through hydrophobic and ionic interactions.² It can be hypothesised that alteration of the molecular charge intensity of gelatin by changes in the ionic strength of the solution would influence the degree of SDS-gelatin association, and hence the desolvation behaviour of SDS-gelatin mixtures. The objective of this work was to determine the effect of dilute NaCl concentrations on the response of B225 gelatin-SDS mixtures to 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 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 0.5 or 0.9% w/v sodium chloride, with and without 1.74×10^{-3} mol.dm⁻³ SDS, were also prepared. The mixtures were incubated at 37°C for 20 minutes and

the turbidity measured by % transmittance using a Shimadzu 160 UV/Vis spectrophotometer operated at 600nm. The data 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 $Bottom$ were used to monitor the phase behaviour of gelatin in the various mixtures.

Results and discussion

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, as seen by increasing V_{50} values. (Table 1) The precipitate dissolved with increasing ethanol concentration and a secondary desolvation was subsequently observed. At pH's above the IEP the primary desolvation was not observed and an increased sensitivity to desolvation occurred at the secondary desolvation, as indicated by slightly lower V_{50} values (Table 2).

The addition of salt to native gelatin solutions resulted in increased sensitivity to desolvation at extremes of pH, and reduced sensitivity to desolvation close to the IEP. (Table 2) The co-addition of salt and SDS to gelatin solutions did not appear to have any effect at higher pH values, compared to SDS alone. However, at pH's at or below the IEP, the combined presence of salt and SDS resulted in decreased V_{50} and $Bottom$ values for the primary desolvation, (Table 1) indicating that precipitation of the primary complex occurred more extensively but that resolution of the complex occurred more readily. The secondary desolvation at these pH's exhibited increased resistance to desolvation, as evidenced by increased V_{50} values. (Table 2)

Table 1
 V_{50} and *Bottom* values (mL, mean \pm SEM, n = 2) for gelatin-SDS (primary desolvation) solutions

| Experiment Conditions | pH 4.5 | | pH 5.0 | |
|-----------------------|-------------------|--------------------|-------------------|-------------------|
| | V_{50} | <i>Bottom</i> | V_{50} | <i>Bottom</i> |
| 0% NaCl | 22.3 ± 0.1 | 7.95 ± 0.50 | 21.3 ± 0.1 | 25.4 ± 0.1 |
| 0.5% NaCl | 19.3 ± 0.1 | 4.72 ± 0.14 | 15.7 ± 0.5 | 17.1 ± 0.4 |
| 0.9% NaCl | 16.3 ± 0.5 | 7.38 ± 0.59 | 14.9 ± 0.0 | 13.0 ± 0.4 |

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

| Experiment Conditions | 0% w/v NaCl | 0.5% w/v NaCl | 0.9% w/v NaCl |
|-----------------------|----------------|----------------|----------------|
| <i>Gelatin</i> | | | |
| pH 4.5 | 50.1 \pm 0.1 | 59.2 \pm 0.1 | 60.3 \pm 0.0 |
| pH 5 | 47.5 \pm 0.1 | 55.9 \pm 0.0 | 59.9 \pm 0.3 |
| pH 6 | 48.6 \pm 0.1 | 58.6 \pm 0.1 | 59.4 \pm 0.1 |
| pH 7 | 51.3 \pm 0.3 | 57.7 \pm 0.0 | 59.7 \pm 0.3 |
| pH 8 | 54.8 \pm 0.2 | 57.8 \pm 0.1 | 60.1 \pm 0.0 |
| pH 9 | 56.8 \pm 0.0 | 59.3 \pm 0.1 | 59.9 \pm 0.1 |
| pH 10 | 65.9 \pm 0.1 | 60.3 \pm 0.2 | 60.7 \pm 0.1 |
| <i>Gelatin-SDS</i> | | | |
| pH 4.5 | 62.0 \pm 0.2 | 63.8 \pm 0.0 | 63.1 \pm 0.4 |
| pH 5 | 51.6 \pm 0.1 | 60.6 \pm 0.0 | 61.3 \pm 0.0 |
| pH 6 | 45.8 \pm 0.2 | 58.7 \pm 0.1 | 60.0 \pm 0.1 |
| pH 7 | 50.7 \pm 0.2 | 56.1 \pm 0.2 | 59.6 \pm 0.2 |
| pH 8 | 51.2 \pm 0.1 | 58.0 \pm 0.1 | 60.5 \pm 0.0 |
| pH 9 | 54.1 \pm 0.1 | 59.9 \pm 0.1 | 60.3 \pm 0.0 |
| pH 10 | 62.0 \pm 0.2 | 59.5 \pm 0.1 | 60.5 \pm 0.7 |

SDS binding to gelatin is initially stabilized by weak hydrophobic interactions,² increasing the number of anionic groups on the gelatin molecule. This decreases the net positive charge of the molecule at pH's below the IEP and reduces intermolecular repulsion. The gelatin structure is also partially unfolded, improving the accessibility of the ionic side groups and favouring electrostatic binding of SDS to gelatin.² The latter occurs markedly at pH's below the IEP, when the cationic amino groups are in excess of the anionic carboxylate groups, and causes further unfolding of the gelatin structure by breaking intramolecular zwitterionic couples.² The increase in molecular weight and hydrophobicity of the gelatin-SDS complex, 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 ethanol concentrations create a solvent mixture which favours dissolution of the hydrophobic complex, resulting in resolution. The presence of NaCl is thought to contribute towards the breaking of zwitterionic couples of gelatin, making more amino groups available for SDS electrostatic binding and leading to more extensive

primary desolvation. However, due to the decrease in double layer thickness, the strength of SDS-gelatin electrostatic binding is probably weaker, and the surfactant dissociates from the gelatin more readily, resulting in a less hydrophobic, and thus more resolubilisable, complex. Excess ethanol causes a secondary desolvation of the molecule. The unfolded nature of the gelatin-SDS complex and the higher concentration of charged groups could account for the secondary desolvation occurring to a lesser extent than in native gelatin solutions. However, the decreased hydrophobicity of the gelatin-SDS complex in the presence of NaCl results in increased resistance to desolvation.

At pH's above the IEP, electrostatic binding of SDS to gelatin is reduced due to electrostatic repulsion as the gelatin molecule acquires a greater net negative charge. Thus the changes in gelatin structure 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. Since electrostatic binding of SDS to gelatin does not appear to play a significant role in the desolvation behaviour at these pH's, the effect of ionic strength in the presence and absence of SDS is comparable.

Conclusion

It appears that in the presence of SDS, gelatin undergoes configurational changes depending on the degree and mechanism of surfactant binding. The effect of added salt in altering the pH-dependent influence of SDS on the response of gelatin to the non-solvent ethanol confirms the role of electrostatic binding as one mechanism in the interaction of SDS with gelatin.

References

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