

The Influence of pH, Temperature and Salt on the Ethanolic Desolvation of Dilute Cold Water Fish Skin Gelatin from Aqueous Solution

C. Farrugia, M. G. Schembri, E. Sinagra

Department of Chemistry, University of Malta, Msida MSD2080, Malta



Introduction

Gelatin is a heterogeneous mixture of water-soluble proteins of high molecular weight. It is the degradation product of the protein collagen, a long triple-stranded helical rod which is stabilized by non-covalent interactions and covalent crosslinking. A number of studies have been carried on the desolvation of various types of gelatin of mammalian origin. Cold-water fish skin gelatin (CWFSG) has a different imino acid composition to mammalian gelatin. The number of proline and hydroxyproline groups in CWFSG is lower and this means that there is less hydrogen bonding. This leads to a different secondary structure. Moreover, the differences in the primary structure of the protein would also be expected to cause differences in other physical properties fish gelatin may exhibit. The objective of this work was to determine the response of CWFSG in aqueous solution, to the non-solvent ethanol under different conditions of pH, temperature and concentration.

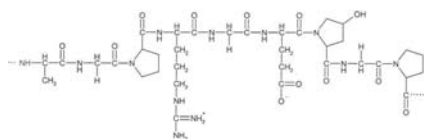


Figure 1: Schematic Diagram of Gelatin

Methodology

Unbuffered solutions of CWFSG were prepared by stirring aqueous suspensions of the gelatin at 25°C, with stirring for 20 minutes. The pH was adjusted to 4, 5, 6, 7, 8, 9 or 10 by adding dilute HCl or dilute NaOH. The gelatin solutions prepared above were incubated at 15°C, 25°C or 39°C for 90 minutes 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 (0 to 80% w/w). Similar mixtures containing 0.9% w/w sodium chloride were also prepared for the gelatin solutions and incubated at 15°C. 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 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 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 Temperature and pH on addition of ethanol to CWFSG

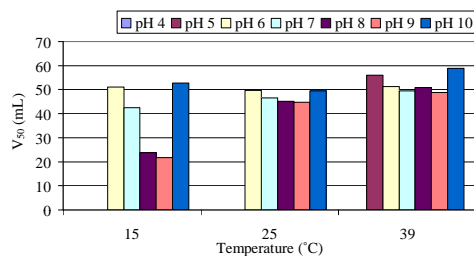


Figure 3: Effect of Temperature and pH on addition of ethanol to CWFSG: Solutions containing 0.5% NaCl

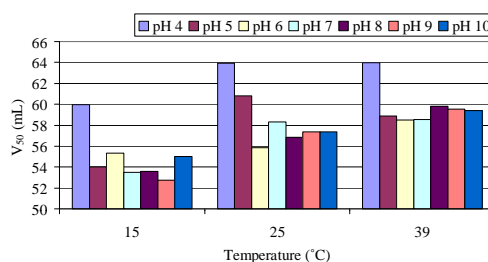
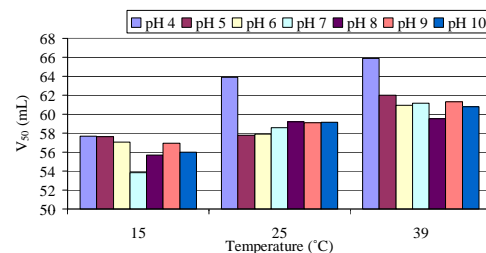


Figure 4: Effect of Temperature and pH on addition of ethanol to CWFSG: Solutions containing 0.9% NaCl



The behaviour of fish gelatin solutions (with no added salt) was observed to be highly dependent both on the temperature and the pH of the setup. Gelatin solutions adjusted to pH 4 and at the three temperatures at which the solution was investigated were shown to be insensitive to the desolvating effect of ethanol. This insensitivity was also observed from similar solutions adjusted to pH 5 at 15°C and at 25°C. Solutions adjusted to pH 6, 7, 8 and 9 exhibited increased turbidity with increased ethanol concentration; the V_{50} values decreased as the pH increase from 6 to 9; the lowest values being obtained at pH 9. This trend was observed at all temperatures at which the investigation was carried out (Figure 2).

In the studies on mammalian gelatin, the lowest values of V_{50} are observed near the IEP of the gelatin. It is understood that at this point the low net charge on gelatin molecules leads to closer approach between the gelatin molecules and thus less non-solvent is required to cause coagervation of the gelatin in solution. The results here would imply that the IEP of the CWFSG used in this study is at or at least near pH 9. This makes the CWFSG closer in nature to acid cured mammalian gelatin rather than the lime cured variety gelatin. It can also be noted that, along with such trends, the lowest V_{50} values were obtained for the lowest temperature, which was 15°C; these values increased as the temperature was increased. Such increase across temperature was more pronounced at pH 8 and 9. Figures 3 and 4 show the influence of salt, NaCl on the desolvation of CWFSG by ethanol. With the addition of salt desolvation is also induced at the lower pH values of 4 and 5.

Conclusions

It appears that in the absence of salt, the response of the protein to non-solvent is dependent on pH, with the lowest requirement of ethanol for desolvation being pH 9. Such response, arising from CWFSG, was found to be similar to that of mammalian gelatin, however the pH for minimum ethanol required for desolvation would suggest that the CWFSG has an IEP of about 9. In this respect the system is more like the A-type gelatin.

It was also noted that temperature affects the sensitivity of CWFSG towards desolvation in the presence of non-solvent. It has been shown that increasing temperature shifts the molecular weight profile to lower molecular weights which in turn leads to better interaction with solvent; the solution thus would require more ethanol to desolvate the molecule. Addition of salt results in the possibility of causing coagervation of CWFSG over a wider pH range. This can be understood in terms of the DLVO theory since the salt would reduce the repulsion even between molecules with a net charge and hence these molecules can approach each other more closely and eventual aggregation may occur.

References

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