

The influence of pH, Temperature and Salt Concentration on the Ethanolic Fractionation of Dilute Gelatin Solutions

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Introduction

Gelatin, the denaturation product of the protein collagen, has been found to be a suitable candidate for the production of nanoparticles for drug delivery systems. The production of these nanoparticles involves the coacervation of the protein by various methods. One such method is the addition of a non-solvent, such as ethanol, to aqueous solutions of the polymer.¹ However, the alteration of the net charge on gelatin in solution, by variation in the solution pH affects the overall response of the protein to the non-solvent. The ionic strength and the temperature of the gelatin solution are also important factors which determine the behaviour of gelatin on addition of ethanol. In order to be able to design a robust method for the preparation of nanoparticles in this way, it is important to fully understand the phase behaviour of gelatin under various solution conditions. The response of lime-cured gelatin to the non-solvent ethanol under different conditions of temperature and pH and dilute sodium chloride solutions was therefore studied.

Methodology

1% unbuffered solutions of 225-bloom, lime-cured gelatin from bovine skin (B225) were prepared by heating aqueous gelatin 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 85% w/w. The mixtures were allowed to equilibrate at constant temperature for another 20 minutes and the turbidity quantified by measuring % transmittance using a UV/Vis spectrophotometer operated at 600nm. The procedure was repeated for temperatures 25°C, 45°C and 55°C, at pHs 4, 5, 6 and 7, and with addition of sodium chloride to concentrations ranging from 0.1 to 0.9 % w/w. 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 1a:
Effect of temperature and pH on V_{50} values in the absence of salt

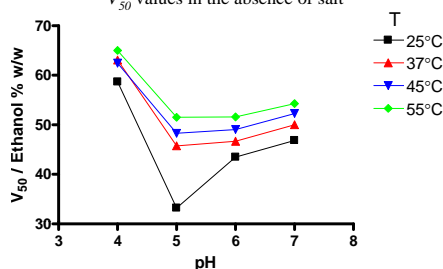


Figure 2a:
 V_{50} vs. Temperature at pH 5 and different NaCl concentrations

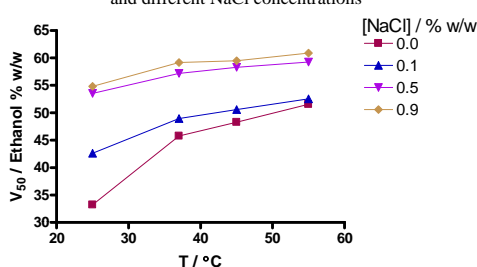


Figure 3a:
Different Temperatures at pH 4 and 0% Salt concentration showing Bottom values above 70%

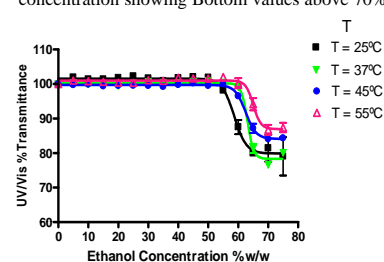


Figure 1b:
 V_{50} vs. pH at 0.1% NaCl concentration and at different Temperatures

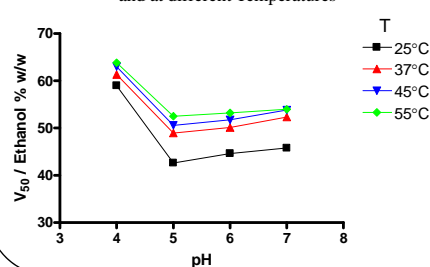


Figure 2b:
 V_{50} vs. Temperature at pH 6 and different NaCl concentrations

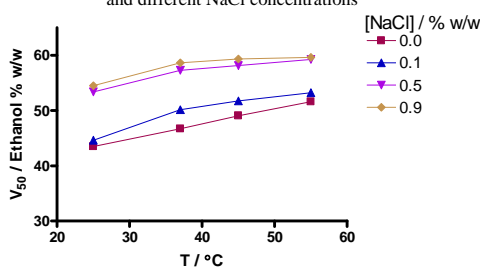
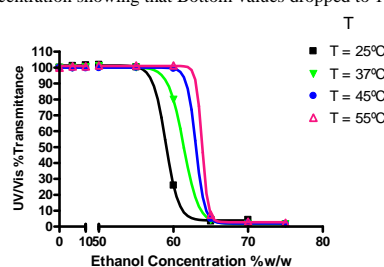


Figure 3b:
Different Temperatures at pH 4 and 0.1% Salt concentration showing that Bottom values dropped to 1%



Conclusions

V_{50} values at all temperatures showed a minimum at pH 5, which is very near the IEP of type B gelatin (pH 4.8 to 5.2). The gelatin has no net charge and intramolecular repulsive interactions are at a minimum at the IEP; the gelatin molecules are coiled up, leading to low solvent interaction. The intermolecular repulsive forces are not strong enough to inhibit aggregation, thus a relatively low concentration of ethanol is required to cause coacervation. V_{50} values at pH 4 were always higher than values obtained at other pHs. Gelatin molecules at pH 4 have a very high net positive charge², hence different gelatin molecules repel each other on close approach; a high concentration of non-solvent, ethanol, was required to cause aggregation. In all other cases the $Bottom$ value was at about 1% transmittance, but the value obtained for pH 4 with no added salt was around 70%, indicating that the gelatin is in a less aggregated state. At pHs 6 and 7 the gelatin has a net negative charge thus the V_{50} 's were higher at these pHs than those at pH 5. The V_{50} 's obtained at pH 4 were higher than those obtained at pH 6 and 7 because the difference in the number of ionised cationic groups to ionised anionic groups at pH 4 is higher than at pHs 6 and 7². Increasing the temperature from 25°C to 55°C increased the V_{50} values at all pHs and salt concentrations. The intermolecular covalent cross-links and intramolecular electrostatic interactions of gelatin are disrupted by increase in thermal energy, causing unfolding of gelatin molecules and better solvent interaction. Salt causes a decrease in solubility due to the decrease in the electrical double layer, hence gelatin molecules can further approach each other; but also causes solubility to increase due to zwitterionic decoupling by shielding the intramolecular attractions of oppositely charged groups. Both effects were seen balanced at 0.9% salt concentration at pH 4; no balancing was observed at other pH's, where gelatin is not as highly charged.

References

1. C.A. Farrugia and M.J. Groves, Gelatin behaviour in dilute aqueous solution: designing a nanoparticle formulation. *J. Pharm. Pharmacol.*, **51** 643-649 (1999).
2. Kowalski, P. (1972), *Applied Photographic Theory*, J. Wiley and Sons: New York.