POTENTIOMETRIC STUDIES ON GELATIN SOLUTIONS AND GELATIN NANOPARTICLE DISPERSIONS

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Introduction

Gelatin is the denaturation product of the protein collagen. The denaturation can be performed by acid hydrolysis or base hydrolysis rendering A-type or B-type gelatin respectively. Gelatin molecules have a nonuniform distribution of 18 amino acids. As a typical protein, gelatin carries both positive and negative charges. Table 1 lists the main amino acids present in gelatin that can contribute towards the presence of a charge on a gelatin macromolecule, together with their respective pKa's[1].

Amino Acid	pK_a value
aspartic	3.7
glutamic	4.2
lysine	10.7
arginine	12.1

Aspartic and glutamic amino acid residues are mainly responsible for the presence of negative charges while the positive charges are principally due to arginine and lysine.

At the isoelectric point (IEP), the overall charge on a gelatin molecule is zero, due to equal amounts of negative and positive charges. However, on increasing the pH, gelatin possesses an overall negative charge due to an excess of ionized carboxyl groups. At a pH lower than the isoelectric point, the macromolecule is rendered overall positively charged due to a predominance of protonated amino and guanido groups.

The objective of this study was to the acid-base characteristics of B-type gelatin nanoparticles with those of the parent B-type gelatin.

Experimental Methods

Lime-cured gelatin from bovine skin (type B) of bloom strength 225 was procured from the Sigma Chemical Co., USA.

Production of gelatin nanoparticles

The method of preparation of gelatin nanoparticles was adapted from Marty *et al.*[2]. A 1% gelatin aqueous solution was prepared by weighing 0.1g gelatin in 9.9g distilled water in a sample tube and dissolving it, by heating to 40°C for 20 minutes. Moderate stirring was applied as to avoid vortexing, thus reducing the chance of inclusion of air in the gelatin solution The pH of the solution was adjusted to 7.0 by addition of dilute sodium hydroxide. The sample tube was closed with its screw cap and labeled as *Solution A* .

A solution consisting of 7.0g absolute ethanol and 1.0g water was prepared. The solution was enclosed in a sample tube and labelled as *Solution B.* A third empty airtight sample tube was also prepared and labelled as *Solution C.*

A 35.0g aliquot of absolute ethanol was weighed in a 100mL beaker. 2.0g of 25% w/w glutaraldehyde solution was added and the solution was made up to 50.0g with distilled water. The pH was adjusted to 7.0 by addition of dilute sodium hydroxide. The solution was transferred to an airtight amber sample bottle and labelled as *Solution D.*

The four containers were incubated at 37°C for 1.5 hours. 2.0g of *Solution A* and all (8.0g) of *Solution B* were mixed in the sample tube labelled *Solution C* and incubated at 37°C for 20 minutes.

Using an Eppendorf pipette, 1.5mL of *Solution C* was added dropwise to *Solution D* with moderate stirring. *Solution D* was then incubated at 37°C for two hours.

A 3% aqueous solution of sodium metabisulphite was prepared and 35.0g of this solution was added to *Solution D* and the reaction was allowed to proceed with stirring at 37°C for 10 minutes.

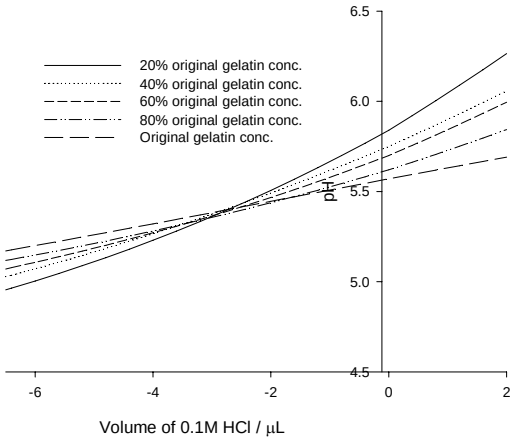
In order to remove unwanted solvents and excess reactants, the prepared gelatin nanoparticle dispersion was subjected to dialysis with four litres of continuously stirred distilled water.

Potentiometric Titrations

Titrations were carried out on gelatin solutions and gelatin nanoparticle dispersions. A 25mL aliquot of the solution under investigation was titrated against 0.1 M hydrochloric acid or 0.1 M potassium hydroxide solution using a micropipette and the pH was monitored with a temperature-compensated pH meter. Readings were made at 25°C and in an air atmosphere. The procedure was repeated for serial dilution of the dispersion or solution.

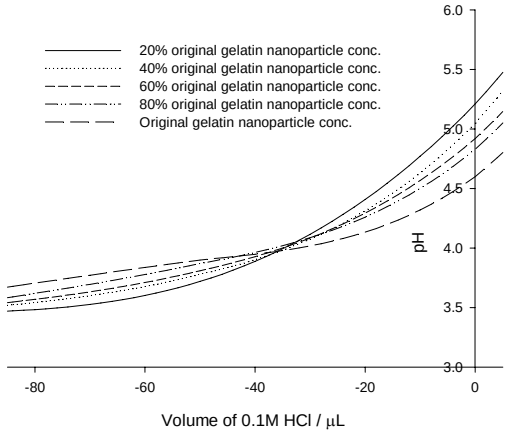
Results and Discussion

Figure 1 shows the titration curves for a series of solutions of B-type gelatin. The curves intersect at a pH value of 5.4. This agrees well with values for the IEP of B-type gelatin[3], suggesting that the cross-over point for the potentiometric titration gives an acceptable indication of the IEP of the dispersed material.



The intersection for titration curves for nanoparticles produced from B type gelatin is seen in Figure 2. In this case the curves all intersect at a pH of 4.0.

This difference in the cross-over point can be attributed to the cross-linking process that the gelatin underwent in nanoparticle formation. The cross-linking process involves the modification of amine groups converting them into aminals[4]. Hence in the case of the nanoparticle dispersion, less amine groups are present for protonation than in the parent gelatin, resulting in a lower IEP. This information is useful in assessing the pH-dependent physical stability of gelatin nanoparticles in vitro and in vivo.



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