Water sorption of glycol-modified cross-linked gelatin-based hydrogels

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The water sorption behaviour of several cross-linked gelatin-based systems were investigated and compared. The systems were gelatin, gelatin/ethyleneglycol, gelatin/polyoxypropylenediamine, and gelatin/polyethylene oxide. For all the systems, an increased water gain was obtained by raising the concentration of the second component, while the swelling was reduced by an increase of the cross-linking density.

1. Introduction

In recent years an increasing interest in biodegradable polymers from renewable sources has been observed in several fields such as agricultural and biomedicine. Controlled-release systems for pesticide in agriculture, or drugs in medicine, are clear examples of their possible applications. Hydrophilicity and water-absorbent characteristics are fundamental requirements when designing absorbent materials, either for biomedical or for other commodity applications. Natural-derived polymers, such as polysaccharides and proteins, represent interesting classes of material obtained from renewable sources that are readily available at low cost. Among them, gelatin represents an interesting material with good hydrophilic characteristic. Its chemical composition allows easy chemical modifications for various properties.

Gelatin, an animal protein, and the degradation product of the structural protein collagen [1] which is the major protein component of mammals, is found in tendons, bones, skin, cornea, etc., where it is fully organized in mechanically strong fibrils with specific structure for each tissue. The basic composition of the backbone protein chain is a sequence of amino-acid monomers, and every protein in the body has a specific and unique sequence of these. The solubility of a protein in water is connected to the nature of the lateral group R. Polar groups like OH, CO, NH_2, interact with water, establishing hydrogen bonds between chains. Polar-charged groups like NH_3^+ or COO^- also influence the hydrophilicity of the chains. The relative amount of charged and uncharged groups varies with pH.

Collagen is unique among the proteins because of its amino acid composition. It contains a large amount of hydroxyproline and is rich in glycine and proline. The pyrolidine rings (imino acids) have no hydrogen atoms on the peptide bond, so the structure is stabilized by means of intramolecular hydrogen bonds. Three individual chains in left-handed helices are wrapped into a triple helix stabilized through interchain hydrogen-bonded and covalent cross-links.

The biological stability and the mechanical strength of the collagen fibres are directly correlated to the number and features of these interactions. The disruption of these highly organized, quasi-crystalline, water-insoluble fibres, leads to a water-soluble system of independent molecules with a much lower degree of internal order than that in collagen. This degradative transformation involves the rupture of the main chain and the interchain covalent cross-links. Hence, gelatin has a lower molecular weight and is rapidly bioresorbable compared with collagen that shows a good resistance to degradation.

Many investigators have studied the possibility to compensate some structural deficiencies of the gelatin through graft polymerization techniques [2–5]. Grafted copolymers with butyl acrylate (BA) in the receiving layer of the transfer blank film, and the photographic dye transfer printing, have been synthesized [6, 7]. The grafting of the gelatin during the polymerization of acrylic monomers (AA, MMA, MA) was studied in order to investigate and manipulate the effective molecular weight and the hydrophilic/hydrophobic properties of these materials [8, 9]. Finally, interpenetrating polymer networks (IPNs) were synthesized in order to improve either the mechanical and the biocompatibility of the gelatin itself [10].

This work reports the study of a series of gelatin-based hydrogels cross-linked with glutaraldehyde (GLA). The effect of some variables, such as concentration of gelatin in the starting solution, the

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amount of cross-linking agent and the presence of a second component, on the physical properties of these systems, were analysed.

2. Experimental procedure

2.1. Gelatin–GLA, system A

Gelatin was dissolved in water at 50 °C. Solutions, with concentrations of 10%, 15% and 20% were obtained. They were then cooled at room temperature on glass plates as 2 mm thick film shapes and then set aside overnight. The gels were then immersed in aqueous solutions of GLA overnight. The amount of GLA added was calculated with respect to the amount of gelatin. 1%, 5% and 10% (wt/wt) were the weight contents of GLA in the final gels. The cross-linked gels were then dried at 25 °C in a vacuum oven for 12 h.

2.2. Gelatin–ethylene glycol–GLA, system B

Water solutions of gelatin with different contents of ethylene glycol were prepared. The gel was then obtained by following the same procedures described for system A. The concentration of gelatin in the initial solution was always 20%, while glycol plus water were 80% (wt/wt).

2.3. Gelatin–Jeffamine–GLA, system C

Polyoxypropylenediamine, marketed by Texaco Chemical Company as Jeffamine D-230 (JD230), was used in addition to GLA to extend the cross-linking chains between the protein macromolecules. JD230 was added to the water before the preparation of the gelatin solution. Calculated amounts of JD230 were 1%, 5% and 10% wt/wt with respect to protein. The gel was cross-linked with 2 mol GLA per mol JD230.

2.4. Gelatin–PEO–GLA, system D

Polyethylene oxide (PEO, mol. wt 600 000) and gelatin were dissolved in water, keeping constant the total concentration of the two polymers (10% by weight). The films were then prepared using the same procedures as for the above systems. Four compositions of gelatin/PEO systems were studied: 95/5, 90/10, 85/15 and 80/20 by weight. Each blend was treated with GLA to cross-link the gelatin chains and to evaluate the effect of the cross-linking agent on the properties of the blends. The GLA content was calculated with respect to the amount (wt %) of gelatin in each blend.

2.5. Swelling study

The gels were dried under vacuum and weighed until constant weight was maintained. They were then immersed in distilled water, and the percentage of water gain (WG) was calculated as

$$\mathrm{WG}\% = 100\,\frac{W_\infty - W_i}{W_i} \qquad (1)$$

where W_∞ is the weight measured at the equilibrium and W_i the initial weight of dry samples. All the results are the average of four measurements.

3. Results and discussion

Gelatin is dissolved in water between 40 and 50 °C and in this condition the gelatin chain is a random coil. On cooling the solution, the gelatin chains undergo a sol–gel transition that is correlated with a progressive change in the conformation and is coupled with the reformation of inter/intrachain hydrogen bonds [1]. The number and the nature of the hydrogen bonds and the cross-links during this gelation phenomenon are responsible for the stability and physical properties of the gelatin, such as resistance to degradation, mechanical strength, hydrophilicity.

For concentrations of gelatin above 0.5 wt %, at neutral pH and moderate ionic strength, the chains are entangled and the contact points between the molecules could be nucleation sites for the reformation of local aggregations that are responsible for the gelation mechanism. In this aggregation phenomenon, the probability of reconstruction of the three-helix structure is very low. When three different helices are closely entangled with a precise alignment, interactions between the chains could be possible but, because of the difficulty of having three strands in simultaneously the correct position, the "renaturation" of the protein collagen has a very low probability of occurrence [1]. However, the sequence of the polypeptide chain plays an important role in the formation of the triple-helix conformation. It seems that protein in which 66% of the residue is proline, e.g. (Gly–Pro–Pro)*n*, or 33% is proline, e.g. (Gly–Pro–X)*n*, readily form triple helices in the solid state [11].

Cross-linking of gelatin is possible due to the presence of some lateral reactive groups. The model of gelatin is usually –(Gly–X–Pro)*n*– or –(Gly–X–Hypro)*n*, where X is an amino-acid, Gly is the glycin and Pro and Hypro are proline and hydroxyproline (22%). The amino group of the lysine residues (R = -$(CH_2)_4$–NH_2, 33% of the total), can react with the aldehyde group of the GLA to form cross-links between the chains (Fig. 1).

Amino-acid analysis of native and modified gelatin (cross-linked with GLA) have shown that the reaction

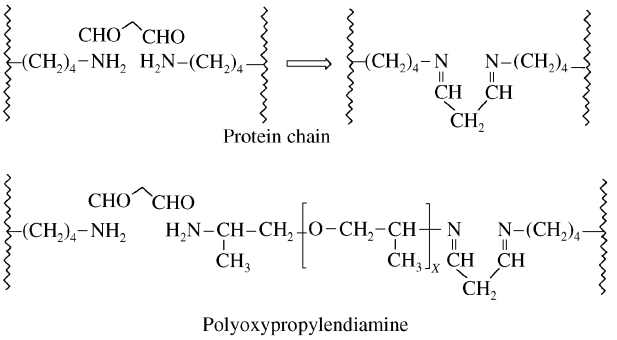

Figure 1 Reaction scheme between gelatin (system A), JD230 (system C) and GLA.

between GLA and gelatin involves only the lysine group of the protein chain [9]. The lysine group can also undergo reactions typical of primary amines producing products such as: (a) amides (reaction with carboxylic acids, esters or anhydrides, etc.); (b) urea (reaction with isocyanates); (c) imine (reaction with aldehyde or ketons); (d) salt (with acids); (e) urethane.

The reaction between gelatin and glutaraldehyde is accompanied by a colour change due to the presence of the aldimine linkage after the cross-link reaction [12]. For the systems investigated, the concentrations of gelatin range from 15%–25% and under these conditions the protein chains can be considered entangled. The increase in gelatin concentration can lead to higher numbers of contact points between the chains and more helices could form. Fig. 2 reports the percentage of water gain versus the percentage of gelatin in the starting solution (wt/wt) for samples cross-linked with different amounts of GLA. As expected, a reduction of the water absorbed can be obtained by increasing both the gelatin concentration and the amount of GLA. The effect of GLA on the swelling properties is more evident, as shown in Fig. 3, where the percentage of the water gain is reported versus the GLA content.

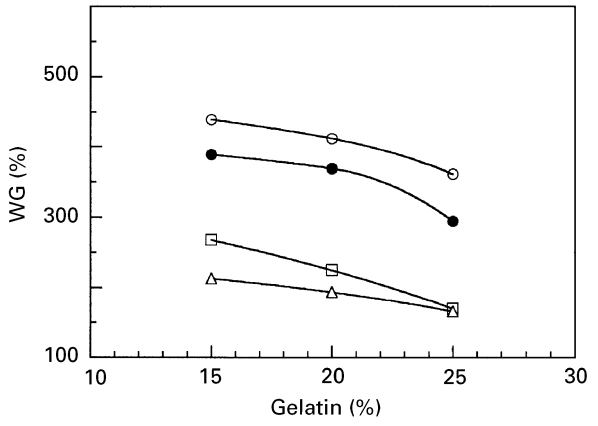

Figure 2 Water gain of samples of system A versus gelatin concentration for different amounts of GLA: (○) 0%, (●) 1%, (□)5%, (Δ)10%.

Gelatin undergoes a progressive hydrolytic degradation when dissolved in aqueous systems [1] and the phenomenon is a function of the temperature, pH, and nature of other solutes present. The rate of hydrolytic degradation proceeds slowly at neutral pH and it is very sensitive to the increase in temperature. One way to detect the degradation of the gelatin chains is to measure the weight change during the absorption of water. Fig. 4 shows the results for uncross-linked systems and for those with 1% GLA after 1 and 5 days immersion in water. Without any cross-links between the chains, the loss of material seems to be faster compared with the other cross-linked samples.

The weight loss could be associated with two concurring phenomena: the hydrolysis and the diffusion of the degradation products from the system. The rate of the overall process is controlled by the slower phenomenon which, in this case, seems to be the diffusion of the materials inside the gel. On increasing the concentration of the gelatin in the starting solution, the amount of entanglement increases and this reduces the extent of swelling of the system. As a consequence, the overall rate of mass loss is lower because the rate of diffusion of low molecular weight fragments decreases in less-swollen materials.

Analogous results were obtained for samples with a higher degree of cross-linking. Materials cross-linked with 5% and 10% GLA, did not show any weight variation after 5 days. They were not completely degraded after 1 month.

Increased water gain was obtained when ethylene glycol was added to the gelatin solution (Fig. 5). This behaviour is related to the influence of the ethylene glycol on the gelation process which affects the final structure of the gelatin. In fact, the presence of the glycolic solvent dilutes the overall solution, and this results in a lower number of entanglements. The formation of well-ordered regions can be restricted and this further influences the swelling properties of the material.

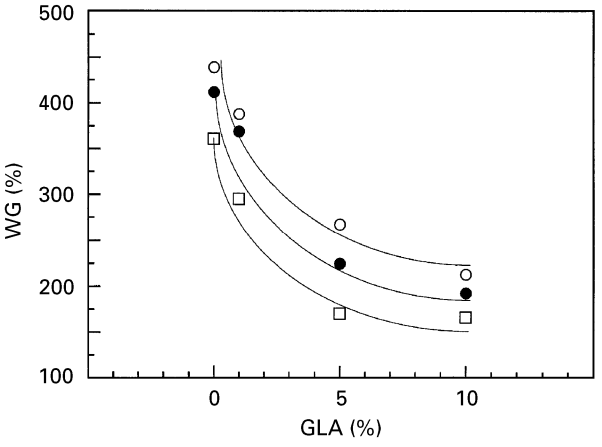

Figure 3 Water gain of samples of system A versus per cent GLA for different gelatin concentrations: (○) 15%, (●) 20%, (□) 25%.

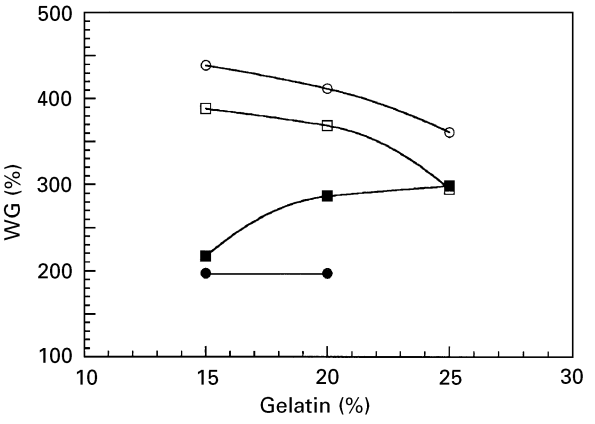

Figure 4 Mass variation of samples of system A after (○,□) 1 and (●,■) 5 days in distilled water. (●,○) 0% GLA, (■,□) 1% GLA.

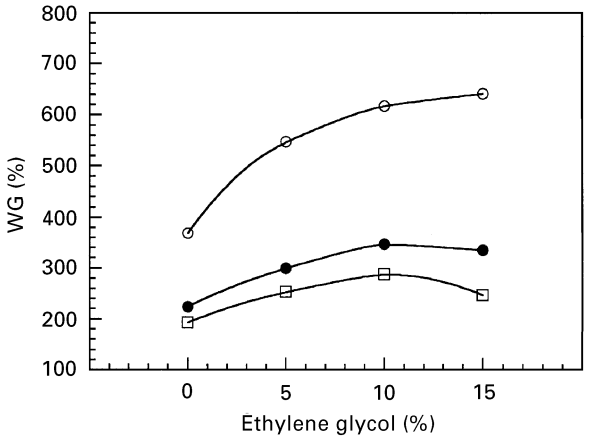

Figure 5 Water gain of samples of system B versus glycol content. GLA (○) 1%, (●) 5% and (□) 10%. Gelatin concentration 20%; water + glycol 80%.

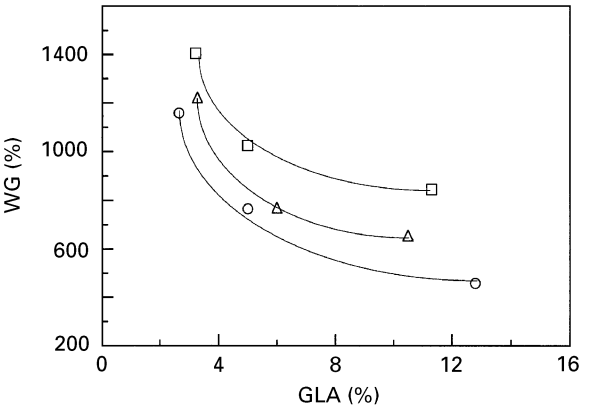

Figure 7 Water gain versus GLA content of gelatin/PEO blends containing (○) 5%, (Δ) 15% and (□) 20% PEO.

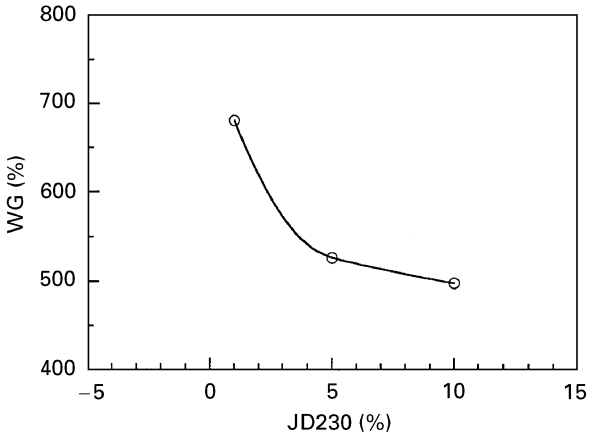

Figure 6 Water gain of samples of system C versus JD230 (% by weight).

Analogous results were obtained by using polyoxypropylenediamine as a chain extender for the cross-linking reactions (system C). The swelling behaviour is shown in Fig. 6. JD230 was supposed to react with gelatin chains and GLA following the scheme in Fig. 1. However, this is a simplified scheme, compared to the actual reactions that take place in the system, because GLA can react with protein chains without reacting with JD230. As a consequence, the diamine could react with one molecule of GLA forming a long lateral chain. The formation of ordered regions, during the gelation process, is therefore limited and, also in this case, the swelling properties are affected.

According to the results observed for the above systems, the presence of another water-soluble polymer, such as PEO, influences the network structure of the gelatin with immediate consequence on the water-sorption characteristics. Fig. 7 shows the swelling behaviour of this system as a function of PEO content, for different amounts of cross-linking agent. As expected, an increased water gain can be obtained by either increasing the PEO content and/or decreasing the amount of GLA added.

4. Conclusion

Several systems with uncross-linked and cross-linked gelatin were investigated. The cross-linking reactions, involving the amine group of the lysine residue, lead to less-swellable materials that show brittle behaviour in the swollen state. The swelling characteristics of the gelatin can be improved by adding an organic solvent such as ethylene glycol, by extending the chain length of the cross-linking molecules, or by adding a synthetic water-soluble polymer, such as polyethylene oxide. The hydrophilic behaviour of these gelatin-based systems is related to the extent and amount of ordered regions that could form during the gelation process, and that are influenced by the presence of a second component, either as low molecular weight material (ethylene glycol) or high molecular weight polymer (polyethylene oxide).

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Received 23 July
and accepted 17 September 1996