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Gelatin film formation at the air/water interface

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Summary

The surface active characteristics of gelatin molecules were studied by measuring the surface tension of dilute limed and acid ossein gelatin solutions. A predicted response to increases in temperature and concentration was obtained. The pH variation revealed behavioural differences between a limed and acid ossein gelatin. Acid ossein gelatins remained relatively unresponsive to changes in solution pH, whereas the rate of change in surface tension (δ) of a limed gelatin solution increased when the molecules were positively charged. A transition in the latter behaviour was observed at approx. pH 5, consistent with the isoelectric point of the limed samples.

Introduction

The term ‘polymer’ describes a realm of complex macromolecules which display a diversity of physico-chemical structures and a breadth of application. Gelatin is a naturally occurring polymer, a polypeptide produced by the denaturation of collagen fibres. Similar to many long chain polymers, gelatin forms a three-dimensional network or gel at concentrations and temperatures conducive to chain entanglement. The ability to form a gel is exploited in pharmaceutical formulation, particularly in capsule manufacture where it supercedes many other naturally occurring or synthetic polymers. In the production of hard capsules, gelation is more aptly referred to as film formation, since it involves the interfacial deposition of a coherent layer of molecules. The appearance of the finished product and stability of the capsule contents are totally dependent on the integrity of the film, which in turn, is related to the molecular properties of the raw material. Unlike many synthetic polymers, gelatin gelation cannot be accurately predicted or controlled. The mechanism has not been fully elucidated but it is generally accepted that in bulk solution, imino residues impose conformational restrictions to the molecule favouring an α-helical configuration and the formation of triple helices. It is believed that these stages represent a partial renaturation to the parent collagen, which suggests that the sequence of amino acids and the orientation of the gelatin molecule are important factors in the setting process. However, as previously stated, film forma-

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tion is an interfacial occurrence where restriction to structural alterations could modify pre-gelation events.

In an attempt to study such affects the air/aqueous interface was considered as an experimental model. The gelatin molecule is composed of alternating hydrophilic and hydrophobic tripeptides which offer moderate surface activity. Adsorption is time dependent, where the initial dynamic surface tension value decreases to a lower static value, over a period of hours. In view of the ampholytic nature of the structure, extremes of pH should increase intra- and inter-chain repulsion, inducing the molecule to adopt a highly extended configuration, thus limiting the numbers adsorbed at the surface towards the isoelectric point, intra-chain charge begins to neutralize and the chain adopts a more compact conformation, thus accommodating a greater number of molecules in the film. At temperatures above 35–40°C gelatin molecules in the bulk are believed to adopt a random coil conformation. At lower temperatures sufficiently concentrated solutions display molecular association preceeding gelation. Molecular association is also believed to occur in very dilute solutions incapable of gelling (Boedkter and Doty, 1954). Sato and Ueberriter (1979a) claim that association in the surface film also occurs to form intra- or inter-molecular helices (depending on the gelatin concentration in solution). The influence of factors such as temperature has been observed by Coopes (1976), who found that the orientation of helical segments in dried films depended on the thermal history and thickness of the film, the helices being more randomly orientated in rapidly dried than slowly dried films.

In order to assess the surface active characteristics of the gelatin molecule under conditions of pH, concentration and temperature, the surface tension of a dilute limed and an acid ossein gelatin was measured using the Wilhemy plate technique.

Materials and Methods

A glass Wilhelmy plate attached to a microforce balance (C.I. Electronics) was used to transmit force fluctuations to a calibrated chart recorder. The output readings were converted to surface tension by the following expression.

$$\delta = \frac{mg}{2(l+b)}$$

where g is the acceleration due to gravity, 9.81 m s^{-2}, m is the mass (kg) and $l+b$ are the dimensions of the plate (m).

To ensure correct operation, doubly distilled deionised water was used to check the equipment before the start of each experiment. 200 ml of gelatin solutions 0.5 mg/ml (Rousselot 50720 acid ossein) were prepared and the pH adjusted to 5.8 ± 0.1 by the addition of KOH or HCl. The solutions were held at 50°C for 15 min to destroy thermal history, then 100 ml was transferred to a jacketed beaker maintained at the desired temperature by water circulating from a thermostatically controlled (±1°C) water bath. The beaker was raised by a manually operated jack to a distance of 15 cm above the bench surface ensuring that a constant area of the plate was immersed in the solution during each experiment. Once equilibrium had been achieved, recording of the force fluctuations was commenced and maintained over 4–5 h. Experiments were carried out at temperatures of 25, 30 and 40°C using this procedure. Additionally, the effect of gelatin concentration, i.e. 0.1, 0.15 and 0.25 mg/ml, on the surface tension of solutions at 35°C was measured over similar time periods. To study the effect of pH on surface activity, acid (Rousselot 50720) and limed (Rousselot 32272) ossein gelatin solutions were prepared (0.5 mg/ml) by the above method. Adjustments to pH over the range 3–9 were made with dilute HCl or KOH and surface tension measured at 35°C over 4–5 h. The data obtained was expressed as percentage change in surface tension vs time (min). This eliminated variation arising due to changes in the surface tension of water under the above conditions.

Results and Discussion

Results illustrate the time dependent surface activity of the gelatin molecules. The precise orientation of the molecules in the surface film is unknown but a thermodynamically stable system would require a displacement of the more hydrophobic residues into the air phase, whilst the polar amino acids remain electrostically attracted to the water molecules through their COO^- and NH_3^+ groups. The structure envisaged is one where the apolar residues are in contact with the air and the more polar sequences hang as loops into the aqueous phase. The time-dependent effects of temperature and bulk concentration on the surface tension of gelatin solutions agree with theoretical predictions. Fig. 1 shows that the decrease in surface tension with time occurred at a faster rate and reached a lower static value as the temperature was increased. An input of thermal energy raises the kinetic energy of the molecules and increases the numbers penetrating the surface. Under these conditions surface tension decreases at a faster rate. This effect, illustrated in Fig. 2, is also observed when the bulk gelatin concentration is increased, since a larger number of molecules are available for adsorption. Consequently, the solutions attain lower static values. In contrast to these results, Sato and Ueberreiter (1979b) found an increase in the surface tension of gelatin solutions at higher temperatures. In order to explain this they postulated that a greater adsorption of gelatin molecules at the surface was accompanied by a faster nucleation of hydrophobic segments, rearrangement and ultimately association of the molecules in the film. This served to decrease the actual number of molecules penetrating the surface, hence increasing the surface tension of the solutions.

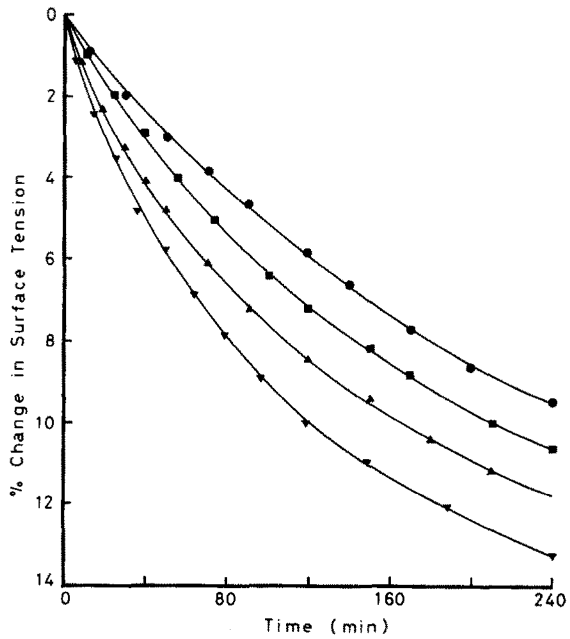

Fig. 1. Percentage change in surface tension vs time at pH 5.8. (Rousselot 50720 acid ossein 0.5 mg/ml): (●) 25°C, (■) 30°C, (▲) 35°C, (▼) 40°C.

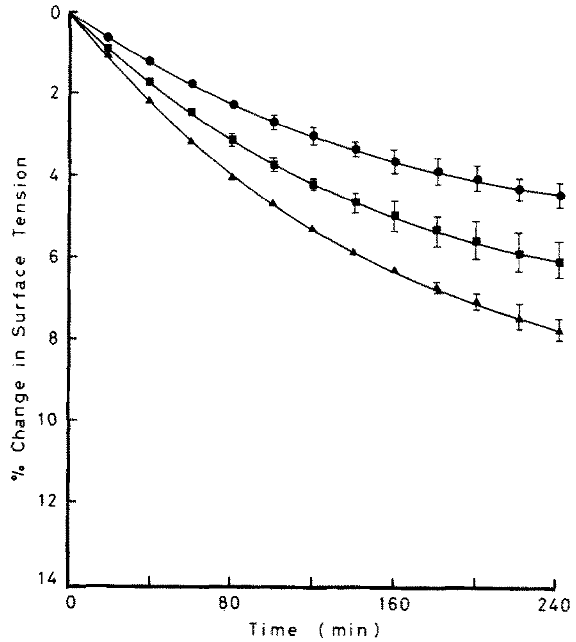

Fig. 2. Percentage change in surface tension vs time at 35°C, pH 5.8 (Rousselot 50720 acid ossein): (●) 0.10 mg/ml, (■) 0.15 mg/ml, (▲) 0.25 mg/ml.

Fig. 3 shows that for a limed ossein gelatin, the rate of decrease in surface tension is faster at pH values below the isoelectric point (IEP) than above. However, for an acid ossein gelatin the rate of decrease in surface tension is virtually the same over the pH range (Fig. 4).Theoretical predictions on the influence of pH on the orientational and adsorption characteristics of the gelatin molecule do not adequately explain results obtained in this work. Possible errors arising from pH drift over the extended observation period were unavoidable, since the introduction of a system that buffers over pH 3–9 means the addition of large complex ions, some of which reportedly possess time-dependent surface-active properties (Sato and Ueberreiter 1979a). Consequently, this may have complicated and/or influenced the behavioural characteristics of the gelatin molecules. Solutions of limed ossein gelatins showed a faster decrease in surface tension when bearing a net positive charge, thus implying that an extended conformation of the molecule favours surface adsorption. This may be the result of a thermodynamic driving force, since in the bulk an extended molecule fully exposes its hydrophobic groups to an aqueous enviroment, causing an increase in surface energy. At the surface, structural rearrangement can remove these residues into the air phase, resulting in a more stable system. Surface adsorption appears to decrease as the molecule becomes electrically neutral. From a configurational aspect surface tension would be expected to fall, since there is less spatial restriction to the number of

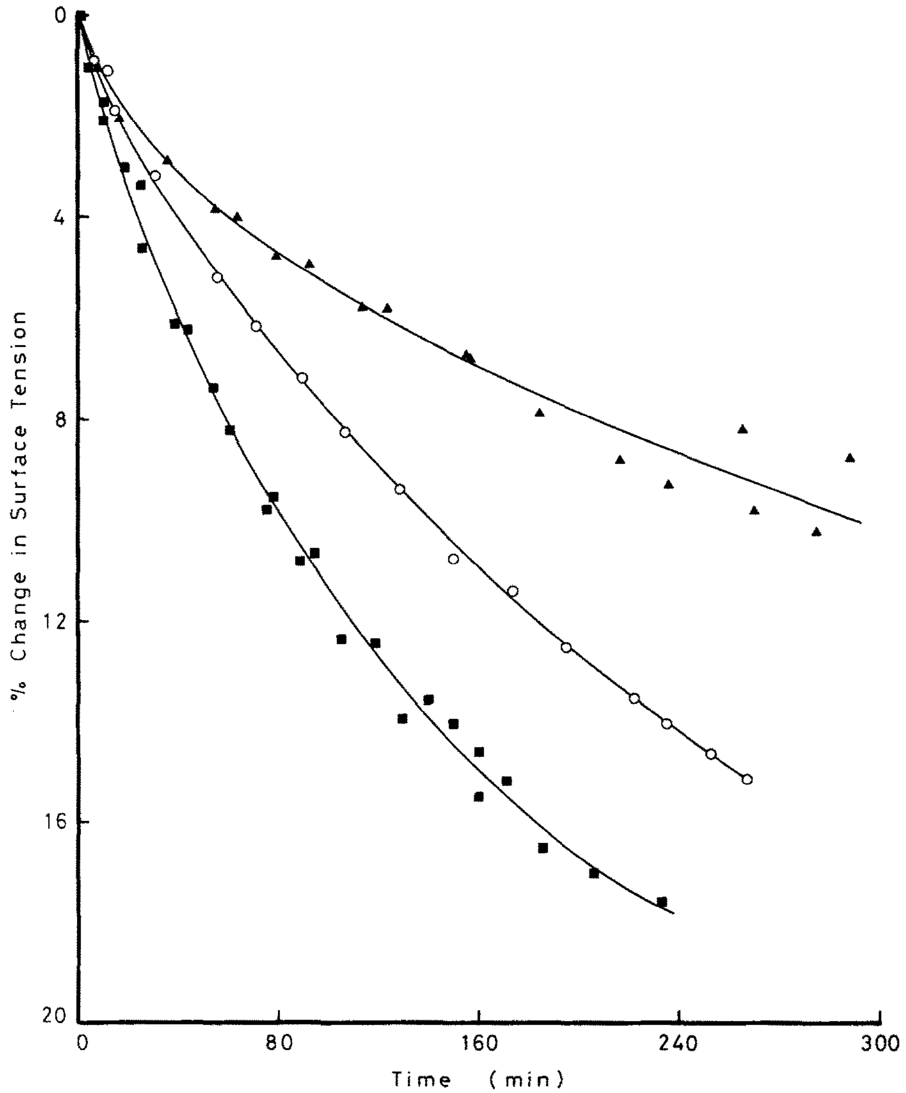

Fig. 3. Percentage change in surface tension vs time at 35°C (Rousselot 32272 limed ossein 0.5 mg/ml): (▲) pH 9, (○) pH 4.9, (■) pH 4.0.

molecules collecting in the film. Therefore, it appears, that a coiled conformation which shields the hydrophobic groups from the aqueous enviroment, reduces the thermodynamic drive towards adsorption.

The above theories do not explain occurrences at alkaline pH. The tendency for surface adsorption markedly decreases when the molecules bear a negative charge. This implies that at concentrations of 0.5 mg/ml, molecular conformation is not critical in controlling adsorption, but the nature of the charge may be more important. Electrical charge involving the attraction between ionisable groups and water may impede the surface adsorption of the gelatin molecules. In this instance, a negative charge appears to have a greater affinity for water. It is also possible that an alkaline pH may promote molecular association in the film by a mechanism unrevealed in this work. These theoretical points should be compared with the proposals of Sato and Ueberreiter (1979a,b). They suggest that a positive charge would give the molecule a good affinity for water and reduce its tendency to adsorb at the surface. However, they also proposed that an extended conformation adsorbs more easily at the surface, to fully orientate its hydrophobic and hydrophilic regions. This arrangement they believe encourages association through the exposed hydrophobic regions particularly between pH 6.6 and 8 as indicated from their work. However, it is further argued that the aggregates are rendered more hydrophilic and are able to desorb into the bulk causing the suface tension value of the solution to rise. A compact form of the molecule would shield the hydrophobic regions from the aqueous enviroment and

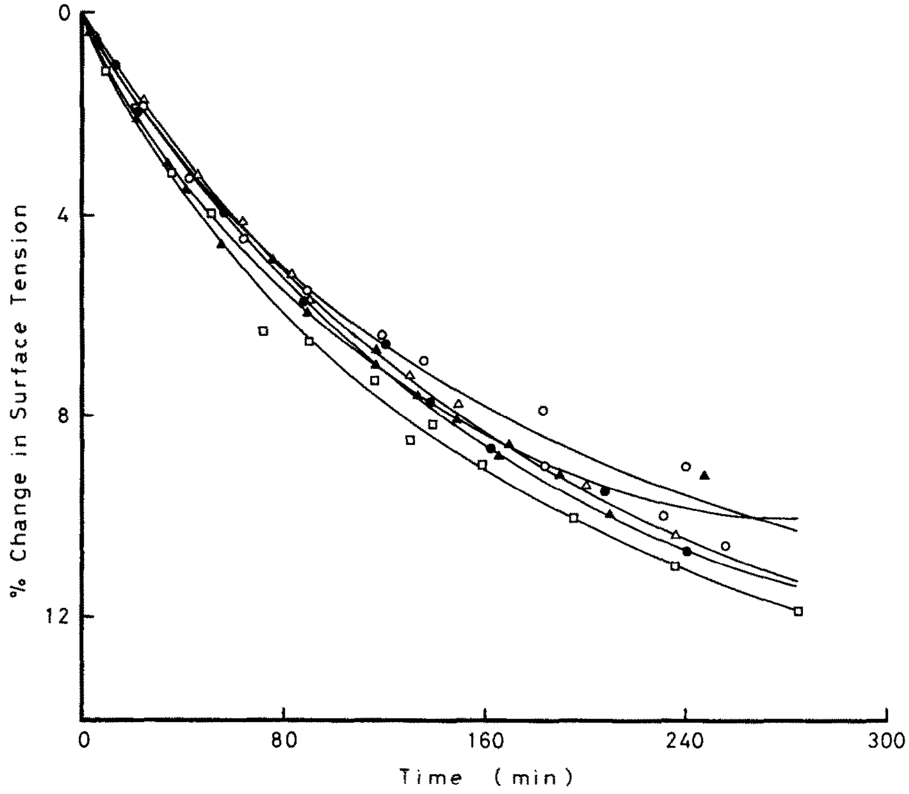

Fig. 4. Percentage change in surface tension vs time at 35°C (Rousselot 50720 acid ossein 0.5 mg/ml): (○) pH 9.1, (▲) pH 8.1, (□) pH 5.6, (●) pH 4.8, (△) pH 3.8.

lose some of the driving force for surface adsorption. The poor orientation displayed by this form does not favour association in the film.

A transition in the behavioural pattern of the molecule around pH 5, is consistent with the well defined IEPs of alkaline precursor gelatins. Acid precursor gelatins have broader IEPs, which is claimed (Veis, 1964) to be the result of the binding of alkaline microions at specific sites along the chain. This phenomenon may render the molecule less sensitive to changes in enviromental pH and account for the similar surface-active properties of the acid ossein gelatin over the pH range. The most likely binding site for small ions on the gelatin molecule appears to be the peptide bond (Bello et al. 1956). The complexing of ions to these regions may prevent the formation of hydrogen and similar bonds, thus preventing the formations necessary for helix formation. Similarly, the binding at peptide bonds could hinder any changes in conformation that could be otherwise induced by pH changes.

Maxey and Palmer (1974) and Todd (1983), using the technique of isoelectric focusing found the isoionic point (PI) range in acid processed wider than that in alkali processed gelatins. The former workers attributed this to the acid processed samples bearing a greater range of molecules with different numbers of ionisable acidic groups. Additionally, if the titration curve of an acid processed gelatin is considered (Kenchington and Ward, 1954), then the ionisable acidic groups are on the part of the curve where a small change in the ratio of acidic to basic groups have a large affect on the isoionic point.

Conclusion

This work of gelatin film formation at the molecular level revealed different adsorption characteristics between a limed and an acid ossein gelatin as a function of pH but lacked the sensitivity required to discriminate further between gelatins. In a similar context to gelation, interfacial behaviour is influenced by polydispersity, the resultant charge on the molecule and also by the flexibility of the chain. However, it appears that an acid processed gelatin is a far more complicated structure than a limed sample of similar molecular weight. A more compact molecular conformation, the phenomenon of ion binding and the wider molecular weight disribution displayed by the acid processed variety could contribute towards the different interfacial properties observed.

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