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# Gelatin film formation at the solid/aqueous interface

M. Thomas [1], I.W. Kellaway [1] and B.E. Jones [2]

*[1] The Welsh School of Pharmacy, University of Wales College, Cardiff CF1 3XF (U.K.) and [2] Eli Lily Co. Ltd, Basingstoke, Hants (U.K.)*

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## Summary

Photon correlation spectroscopy was used to measure the hydrodynamic thickness of gelatin films adsorbed onto latex particles. When prepared in solutions of distilled water acid ossein gelatins showed less dimensional variation over the pH range than limed ossein samples. This difference was not apparent on the introduction of complex ions from a barbitone-acetate buffer. In both systems the average thickness of the film was 30 nm and results suggested that the greatest dimensional contribution was from the loops. Static attraction between the chains leading to multilayer formation, may have been responsible for the greater film thickness measured at alkaline pH values.

## Introduction

It has been demonstrated that the formation of a gelatin film at the air/aqueous interface is dependent on temperature, pH, concentration and nature of the gelatin (Thomas et al., 1991). Under these conditions, the molecules are relatively free to orientate randomly. However, in situations where the film is formed on a solid surface the polymer becomes fixed at particular points, thereby restricting chain configuration. Theories on the conformation of an adsorbed polymer at a solid surface were first put forward in 1954 by Frisch and Simha and led to the more generally accepted proposals by Silberberg (1962a,b, 1968). The underlying principle is that a polymer molecule is attached to the surface by the adsorption of certain groups along the chain described as 'trains'. Free loops extend from the surface whilst the 'tails' flow freely into the bulk solution. The conformation adopted at the surface depends to an extent on polymer concentration. An 'isolated' molecule has space to attach many of its groups, resulting in a flatter configuration. As solute concentration increases the molecules are believed to rearrange, allowing adsorption of a larger number of polymer chains. Fewer segments per chain are able to attach giving rise to larger loops and a more extended conformation. The density of segments is greater adjacent to the solid surface and decreases exponentially with distance from the surface. Certain factors influence the amount of polymer adsorbed at the surface, i.e. solvent, temperature,

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*Correspondence:* M. Thomas, The Welsh School of Pharmacy, University of Wales College Cardiff, Cardiff CF1 3XF, U.K.

molecular weight of the polymer and chain flexibility.

Predictions based on homogeneous polymers also apply to the adsorption of gelatin. However, the polyelectrolytic nature of the molecule leads to two main complications in the theory.

(i) The configuration of the molecule will also be influenced by the overall charge along the chain.

(ii) The adsorption of a charged polymer will depend on the charge at the surface. The preferential adsorption of certain groups will result in tails and loops of varying length (Kragh, 1977). Other considerations include the heterogeneous nature of commercial gelatins, which may also lead to a wider distribution of loops and tail lengths.

Any differences in molecular configuration as a result of varying enviromental conditions should be reflected in the dimensions of the adsorbed film. Therefore, a technique using photon correlation spectroscopy (PCS) was adopted, similar to that of Cohen Stuart et al. (1984), to study gelatin films adsorbed onto latex particles. The diameter of the particles was determined by PCS before and after gelatin adsorption. The difference between the readings was taken as equal to the hydrodynamic thickness of the adsorbed film. This was studied over the pH range 3–9, under different ionic strengths for six gelatins.

## Materials and Methods

The following materials were obtained — Limed ossein: Neinburger 06528, Rousselot 32976, Rousselot 32272, Croda, 174. Acid ossein: Croda 163, Rousselot 05759. Distilled filtered water (0.05 mm membrane filter) was used. Gelatin solutions (0.5 mg/ml) were prepared and adjusted to pH with dilute HCl or KOH. An ionic strength of 0.01 was maintained by addition of sodium chloride (NaCl). The above concentration of gelatin represented an equilibrium concentration. This was established by determining the maximum thickness of the adsorbed film under different concentrations of gelatin at pH 7, whilst keeping the concentration of latex constant. A stock dilution of latex particles (267 nm SD ± 0.003) was prepared by adding 25 $\mu$l of the 10% solids suspension to 10 ml of distilled water suitably adjusted to pH and ionic strength. The solutions were equilibrated at 35 ° C and then 0.5 ml of diluted latex suspension incorporated. The solutions were mixed and re-equilibrated for 20 min. A temperature of 35°C was used to minimise aggregatory effects on the coated particles. The final concentration of latex in the gelatin solutions was 1.25 mg/ml. The viscosities of the gelatin solutions were determined between pH 3 and 9 by a 'U' tube viscometer (British Standard size A) and converted to cP by density measurements obtained by a density meter (DMA 60/601 Anton Paar). Over the pH range used, viscosities were between 0.73 and 0.74 cP at 35°C.

An experimental duration of 100 s and a sample time of 10 $\mu$s were used throughout the experiment. The data were accumulated over the first hour of preparation, since it was found that this led to less variation in results. Similar experiments were repeated over several days and the minimum of 30 results averaged before a final figure was taken.

The above work was also performed using barbitone-acetate buffer as the solvent with an ionic strength of 0.118. In this system the equilibrium gelatin concentration was 0.1 mg/ml of solution with the same concentration of latex particles. Viscosities (0.73–0.74 cP) were determined as above. In both experiments the variation of hydrodynamic film thickness was determined over the pH range 3–9, for six gelatin samples.

## Results and Discussion

The configuration adopted by adsorbed molecules at the solid/aqueous interface is difficult to determine. However, one method of study is to measure the thickness of the adsorbed film. At extremes of pH, intrachain repulsion results in an extended molecule, whereas interchain repulsion limits the number adsorbing at the surface, producing an extended but scant film. At the isoelectric point the molecule is able to fold or coil and the lack of overall charge allows maximum adsorption, giving rise to a narrow but dense film. From these considerations it can be appreciated that the actual dimensions of the film measured by various techniques will depend on which part of the chain has the greater influence in determining the thickness. This is one area of polymer adsorption where theories tend to diverge. Scheutjens and Fleer (1980) agree with Hoeve (1966) that the density of segments decreases exponentially with distance from the surface, but unlike the latter they believe that the tails are extremely important in determining the hydrodynamic thickness of the film. Two-thirds of the adsorbed chain are considered to exist as two long dangling tails. They do not rule out the possibility that for very long chains, and hence high molecular weight polymers, there may be only a small contribution from the tails, when the loops determine the film thickness. These proposals were supported by Cohen Stuart et al. (1984) who found that the dominant contribution to the hydrodynamic thickness of polymers adsorbed onto latex particles was from the tails (measurements were determined by PCS up to a molecular weight of 1 290 000).

In solutions of higher ionic strengths, small micro-ions mask the charge on the chains, allowing a less extended conformation and greater adsorption at the surface. At the isoelectric point, intrachain attraction is reduced and a slight extension occurs with a small decrease in adsorption. In systems where the solid surface bears a charge there are similar masking effects that may also influence the degree of adsorption displayed by the polymer. Many of these effects were confirmed by Curme and Natale (1964). Most of the work on gelatin adsorption and subsequent effects of pH and electrostatic forces relate to the weight of polymer adsorbed per unit surface area. However, the changes in the amount adsorbed need not parallel variations in the dimensions of the film. This was reported by Kragh (1977), where a sharp decrease in the amount adsorbed below the isoelectric point (4.8) was not accompanied by a similar decrease in the thickness of the film. The film measured approx. 50 nm and varied by 10 nm, reaching a maximum between pH 4.5 and 6.5.

Figs 1 and 2 illustrate hydrodynamic film thickness vs pH, for six gelatin samples. The average thickness of the gelatin film over the pH range studied, measured 30 nm with a maximum SD of ±3 and 3.5 nm in the unbuffered and buffered systems, respectively. In the unbuffered system the acid ossein gelatins were less sensitive to dimensional variation over the pH range, i.e. 3 nm for the acid (cf. 10 nm for the limed) samples. The limed ossein film appeared more extended around pH 6–8 where the molecule bears a negative charge. The acid ossein film is also thinner than the limed sample which may be the result of the more compact structure claimed for the acid ossein gelatins (Veis, 1964). Evidence suggests that an alkali precursor gelatin contains a high proportion of multi-chain structures, whereas the acid precursor variety has a high percentage of single-chain species, (John and Courts, 1977). Veis and Cohen (1957) showed that an acid precursor gelatin displayed small configurational transitions only when studied by light scattering and viscosity measurements. In addition, the intrinsic viscosity of acid precursor gelatins has been shown by Veis and Cohen (1957) to be lower than a limed precursor sample of the same molecular weight, indicating a more compact structure. Veis (1964)

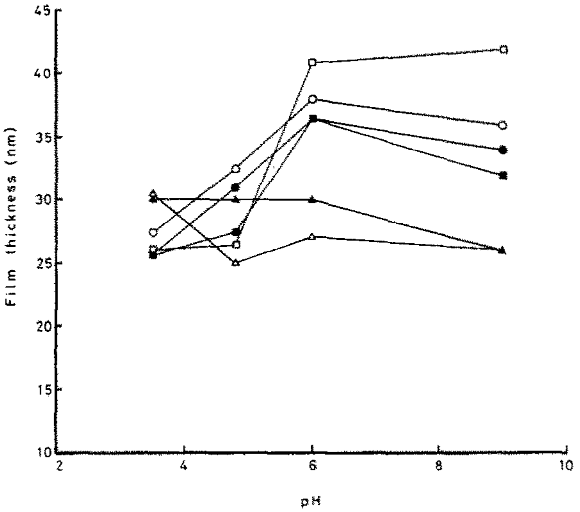

Fig. 1. Hydrodynamic film thickness vs pH (distilled water). Rousselot 32272 (●), Rousselot 32976 (○), Croda 174 (■), Neinburger 06528 (□), Croda 163 (▲), Rousselot 05759 (△).

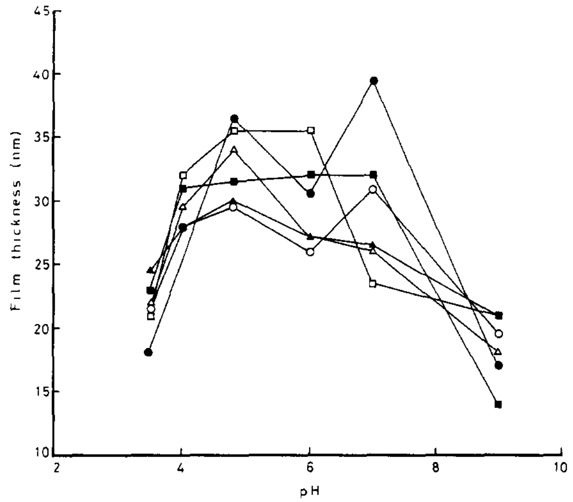

Fig. 2. Hydrodynamic film thickness vs pH (barbitone-acetate buffer). Rousselot 32272 (●), Rousselot 32976 (○), Croda 174 (■), Neinburger 06528 (□), Croda 163 (▲), Rousselot 05759 (△).

states that the proposed phenomenon of ion binding by acid processed gelatins supports this more compact structure. He predicts a network structure of compensating charged groups on adjacent segments of laterally aggregated chains. Whether this arrangement renders the acid precursor gelatin configurationally less responsive to pH changes compared to a limed sample or it is merely the result of the wider distribution of components with different isoionic points is debatable. Gelatin is molecularly heterodispersed which could result in the preferential adsorption of the higher molecular weight species from solution onto the solid surface. Acid ossein gelatins have wider molecular weight distributions and this could be another contributing factor to the difference in adsorption characteristics between a limed and acid ossein gelatin. The actual situation is probably a combination of many of the prementioned factors. The variation of 10 nm for limed ossein gelatins over the pH range compares with the results reported by Kragh (1977). On the basis of work by Curme and Natale (1964), who studied the variation in amount of gelatin adsorbed over the pH range, a maximum film thickness would be expected at the isoelectric point, i.e. 4.8–5.0. However, in the present work the film appeared more extended between pH 6 and 8 where the molecule is negatively charged. It is unlikely that this is the result of intrachain repulsion effecting an extended chain, since the film appears thinnest at extremes of the pH range. Therefore, the situation is difficult to explain without a knowledge of which groups are responsible for adsorption or the nature of the forces involved. It is assumed that a charged surface will promote the number of groups adsorbed of an oppositely charged polymer (Kragh, 1977). However, anomalies have been reported (for example, Zimkin and Klyuchevitch, 1968). In the case of polystyrene latex, it is more likely that the hydrophobic residues of the gelatin molecule are responsible for adsorption and influenced by the distribution of charge along the chain. A tentative explanation of the results would suggest that at low pH values, a gelatin molecule approaching the surface is in an extended state due to charge repulsion along the chain. However, interchain repulsion results in fewer molecules at the surface, therefore allowing the gelatin molecules to adsorb in a flatter conformation, thus giving rise to a thin or narrow film. At the isoelectric point, repulsive forces are reduced and more molecules are adsorbed. According to the theories, they will be adsorbed by fewer groups per chain, resulting in large loops and long tails and consequently under these conditions the hydrodynamic thickness will be greater. As pH increases a return to the situation observed at acid pH values would be expected. However, the film appears thickest between pH 6 and 8. Although there is no evidence of multilayer formation, Silberberg (1972) proposed that molecules in the bulk could associate with the adsorbed film without themselves being attached to the surface. Therefore, there is a likelihood that gelatin molecules could interact by hydrogen bonding or electrostatic forces, possibly giving rise to more than one layer and greater film thicknesses. It is possible that this is occurring here and in support of this theory Sato and Ueberreiter (1979) found that association of gelatin molecules in aqueous film greatest over the pH range 6–8.

A buffered system was originally used in this work to prevent any drift in pH. However, few cover such a wide pH range and are usually composed of large complex ions. Barbitone-acetate buffer is mentioned in the literature as a buffer system for gelatin (Veis, 1964) and was therefore chosen for this study. The increase in ionic strength resulted in the predicted increase in adsorption, and the requirement of a higher equilibrium concentration. The pH range of maximum film thickness was slightly shifted to between 5 and 7 and there was less distinction between acid and limed gelatins. The very thin film thicknesses obtained at extremes of pH were not the effect expected from an increased ionic strength. The overall results suggest that some interaction between ion/ions in the buffer and the gelatin molecule might be taking place. Ion binding is principally a phenomenon of acid process gelatins, however, Bello et al. (1956) proposed that an interaction between the non-polar groups in gelatin and organic ions is possible. In this situation it is possible that the binding of a bulky barbitone ion could modify the configuration of the gelatin molecule at the solid surface.

## Conclusion

Photon light scattering proved a useful technique for studying the adsorption of gelatin molecules at the solid/aqueous interface. The above work illustrates that whilst theories on polymer adsorption are applicable to gelatin, consideration must be given to the complex polyelectrolytic nature of the molecules. When prepared in solutions of distilled water, acid ossein gelatins showed less dimensional variation over the pH range than limed ossein samples. However, this was not apparent in barbitone-acetate buffer, suggesting that some complexation of ions with the gelatin chains may have been occurring. Theoretically, a greater film thickness was expected at the isoelectric point, but this did not explain the expansion of the film at low alkaline pH. It is possible that some multilayer formation was taking place as the result of static attraction between the adsorbed and free chains. Over the pH range studied the average hydrodynamic film thickness measured 30 nm. Existing theories conflict on whether the tails or the loops make the largest contribution to the film. However, in the systems studied, results suggested that the loops were more important in determining film thickness.

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