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Thermorheological and thermogravimetric analysis of bioadhesive polymer/mucin mixtures[1]

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Abstract

In this study, the thermal behaviour of bioadhesive polymer hydrogels, a mucin gel and their mixtures, was investigated using thermorheological and thermogravimetric analysis. Three poly(acrylic acid (PAA) polymers with different cross-linking status (Carbopol 974P, Carbopol 971P and Noveon AA-1) were selected on the basis of their good bioadhesive properties. Rheological scans from 10–90°C were performed in the oscillatory mode. A marked increase in elastic modulus (G') with temperature was detected above 50°C in all mucin-containing samples, as opposed to the pure gels which showed a 'flat' response. Thermogravimetric (TG) analysis (from 30–150°C, at a rate of 8°C/min) revealed variations of the pattern of water loss within the gels which corresponded to the rheological properties of the PAA systems. The thermal behaviour of the Noveon AA-1/mucin mixture did not show the same trends on mixing with mucin as did the Carbopol systems, indicating that the type of cross-linking agent (a tetrafunctional entity, as opposite to a bifunctional one in the Carbopols) may have an impact on the polymer interaction with mucin. © 1997 Elsevier Science B.V.

Keywords: Bioadhesion; Carbopol; Mucin; Rheology; Thermogravimetric analysis

1. Introduction

Bioadhesives are materials (mainly hydrophillic polymers) that can bind to a biological membrane and are capable of being retained on that membrane for an extended period of time. If the binding occurs primarily with the mucus, a highly viscous gel which coats the lining of all hollow organs, the process is called mucoadhesion. Bio(muco)adhesion has been studied extensively during the last decade as a promising novel approach in the field of controlled drug delivery [1]. It has proved to be an extremely complex phenomenon, dependent on a large number of variables with respect to the bioadhesive polymer, the mucus and the environment [2].

The interaction of bioadhesives with biological tissues invariably involves the formation of a gel phase, with corresponding interpenetration with the mucus glycoproteins. One possible approach to studying the process of bioadhesion is the evaluation of the polymer/mucin mixtures. Hassan and Gallo [3] were the first to relate the rheological properties of these mixtures to the bioadhesive bond strength, using a continuous flow method. This was performed on the basis that the same forces involved in the sample resistance to flow are responsible for the process of polymer/mucin adhesion; these forces are believed to involve the behaviour of individual chain segments, physical chain entanglements and the non-covalent intermolecular interactions such as electrostatic,

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hydrogen and hydrophobic bonding [3]. The correlation between the rheological parameters of the mixtures and the force of detachment has been further explored by a number of studies using a non-destructive rheological method, oscillatory analysis [4–7].

Thermoanalytical methods have already shown their validity in the structural evaluation of pharmaceutical semisolid systems [8], although little work has been performed to date involving hydrogels. The aim of this investigation was to explore the use of thermal analysis as a novel approach to the understanding of the structure of the polymer/mucin mixes.

2. Experimental

2.1. Materials

Poly(acrylic acid) (PAA) polymers are well-known as good mucoadhesives, being long-chain, high-molecular weight, cross-linked molecules with a large number of COOH groups along the polymer backbone. The three PAA polymers used in this study were Carbopol 974P, Carbopol 971P and Noveon AA-1 (B.F. Goodrich, USA) which differ with regard to their cross-linking status. It is known that Carbopols 974P and 971P have the same bifunctional cross-linker (possibly allylpentaerythritol), with the 971P being considerably less cross-linked [9]. Noveon AA-1 (known as polycarbophil) contains a tetrafunctional cross-linking agent, divinyl glycol [9]. A partly purified porcine gastric mucin (mucin type III, Sigma, UK) was used as a mucus substitute.

2.2. Methods

Hydrogels containing 2.5% (w/w) Carbopol 974P, Carbopol 971P and Noveon AA-1, both unneutralised and neutralised with triethanolamine to pH 6.8–7.2, were prepared in double distilled water. The air entrapped in the gels during preparation was removed by centrifugation at 3000 rpm for 30 min. In order to achieve an approximately isoviscous solution to the unneutralised PAA gels, a 10% (w/w) mucin gel in water was prepared.

Thermorheological scans (from 10–90°C at a rate of 8°C/min) were performed in the oscillatory mode, using a controlled-stress rheometer (Carri-Med CSL 500, TA Instruments, UK) with the cone-and-plate geometry. A frequency of 1 Hz was employed throughout the measurement, with an angular displacement of 10^{-4} rad (thus, providing measurements within the viscoelastic region). The tests were performed in triplicate, with a coefficient of variation of less than 10% being found. Preliminary isothermal oscillatory runs were carried out at 25°C, using a frequency range from 0.01–10 Hz, under a 1350 Nm torque.

Thermogravimetric analysis was performed by means of TGA 250 Thermogravimetric Analyzer (TA Instruments, UK), with an oxygen-free nitrogen purge under ambient pressure. Aluminium open pans were used as sample holders. The temperature range employed was 30°C to 150°C, with a heating rate of 8°C/min. A hydrogel sample was placed on a pan by means of a plastic syringe in order to keep the sample mass and shape as uniform as possible. The sample mass was in the range of 5 to 7 mg. Measurements were repeated at least three times, with a coefficient of variation of less than 5% found.

Polymer/mucin mixtures, containing 10% (w/w) mucin and 2.5% (w/w) polymer, were then prepared and analysed in the same manner. All samples were evaluated within 48 h of preparation.

3. Results and discussion

Rheological results have been expressed in terms of the elastic (storage) modulus G', as a measure of the energy stored and recovered per cycle of deformation [10]. The elastic modulus is closely related to the connectivity of the polymer network and is known to be directly proportional to the number of entities which can support stress [11], involving both physical entanglement and chemical bonds.

The storage moduli of the unneutralised and neutralised PAA gels and mucin are given in Table 1, these data being obtained from the isothermal runs at a representative frequency; the isothermal rheological properties of Carbopol 974P and Noveon AA-1 gels have been described in greater depth in a previous study [7]. PAA resins are anionic polyelectrolytes and thus have a network structure which is highly dependent on the presence of different ions in the solution. In particular, upon addition of a neutralising agent, the

Table 1
Elastic modulus at the representative frequency of 1.129 Hz for the 2.5% (w/w) unneutralised and triethanolamine-neutralised polymer gels and 10% (w/w) mucin gel

Unneutralised gel	G' (Pa)	Neutralised gel	G' (Pa)		G' (Pa)
Carbopol 974P [a]	9.48±1.79	Carbopol 974P [a]	939.0±12.0	Mucin gel	0.12±0.02
Carbopol 971P [a]	0.60±0.05	Carbopol 971P [a]	61.7±4.2		
Noven AA-1 [a]	3.75±1.01	Noven AA-1 [a]	441.3±18.2		

[a] Data reproduced from [7].

degree of dissociation of COOH groups along the polymer chains is enhanced, leading to the repulsion of negative charges, uncoiling of the macromolecules and further swelling. This, in turn, causes an increase in the elasticity of the neutralised samples. In terms of microstructure, PAA hydrogels are believed to be dispersions of swollen gel particles (clusters) in water [12].

Figs. 1–3 show the thermorheological profiles of the polymer gels and polymer mucin mixtures, along with the mucin gel for comparison. In all cases, PAA hydrogels showed a fairly constant elastic modulus on heating up to 90°C, with the neutralised polymers generally having a higher elasticity. The thermal stability of the PAA hydrogels has already been reported by Barry and Mayer [13,14], who have used isothermal creep and oscillatory runs at 10, 25 and 50°C.

Porcine gastric mucin is a network of mainly linear, flexible and random coiled molecules [3]. Although some interchain disulphide bridges have been observed in porcine gastric mucin [15], it is uncertain to what extent they are present in the partially purified sample. The mucin revealed the lowest network connectivity at 25°C, compared to the PAA samples (Table 1). However, with an increase in temperature above ca. 65°C, a dramatic rise in G' of the mucin gel was seen (Fig. 1); this effect has not been previously reported and the mechanism by which this increase occurs is as yet unknown. It is reasonable

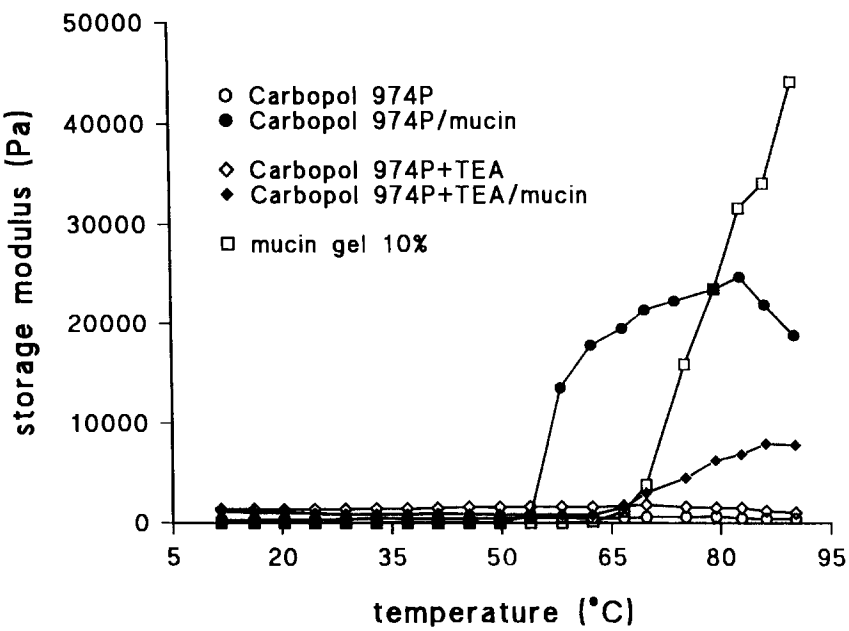

Fig. 1. Thermorheological behaviour of the Carbopol 974P hydrogels (unneutralised and triethanolamine- neutralised), their mixtures with mucin and the mucin gel.

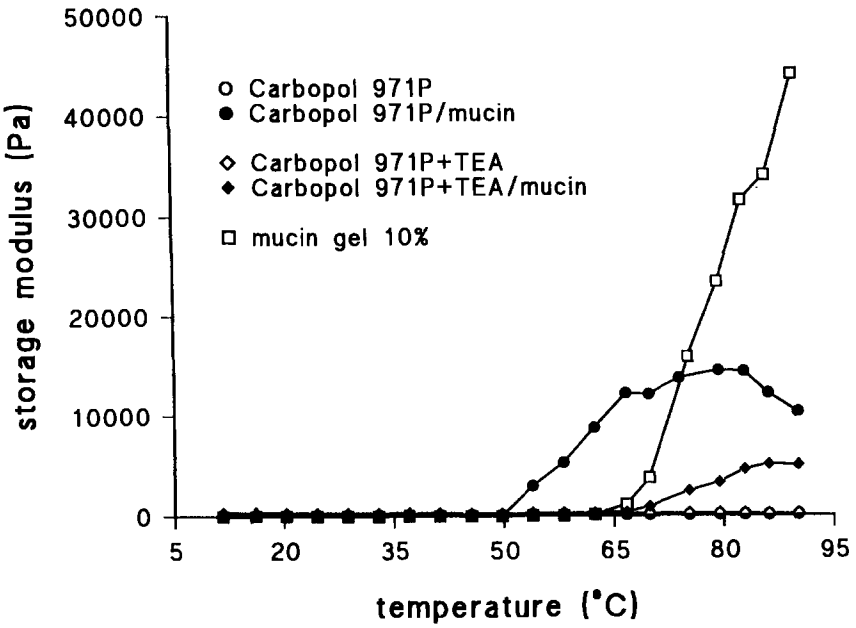

Fig. 2. Thermorheological behaviour of the Carbopol 971P hydrogels (unneutralised and triethanolamine-neutralised), their mixtures with mucin and the mucin gel.

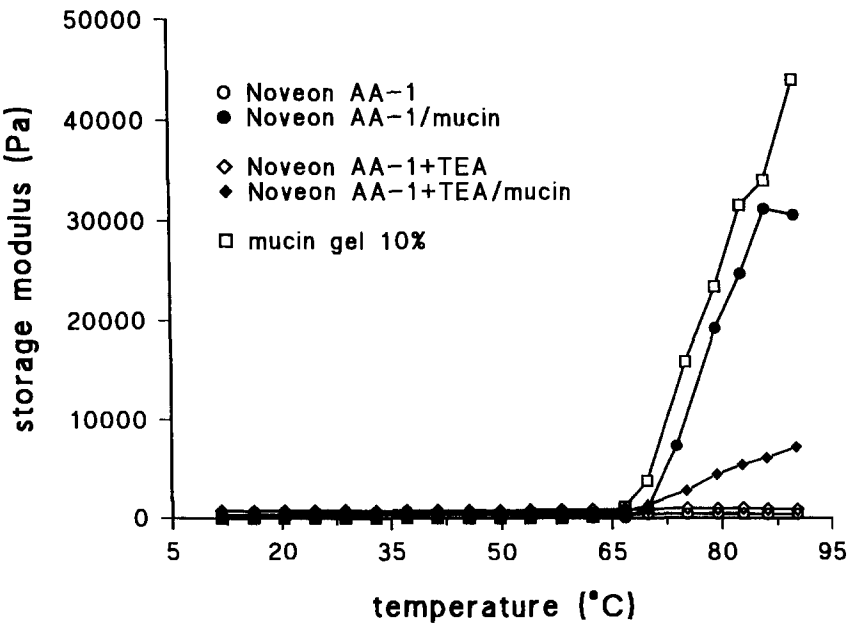

Fig. 3. Thermorheological behaviour of the Noveon AA-1 hydrogels (unneutralised and triethanolamine-neutralised), their mixtures with mucin and the mucin gel.

to suggest that mucin may undergo thermosetting via, for example, a conformational rearrangement process leading to greater interchain interactions. Furthermore, a certain amount of water will inevitably be lost from the sample during the measurement, hence such losses may play a major role in the observed behaviour. However, the TGA results shown below indicate no major discontinuity in the water loss. In addition, the Carbopol gels, which contain a greater proportion of water, show no such increase in G' despite exhibiting water-loss profiles similar to mucin during the TGA studies.

When mixed with mucin, an increase in the elastic modulus at elevated temperatures was detected in all three polymers in both acidic and neutralised states (Figs. 1–3); the effect at lower temperatures was less marked. The thermorheological behaviour of the mixes was found to be polymer-structure dependent, with the two Carbopols showing similar patterns of G' change. In both cases, a substantial increase in storage modulus was observed at ca. 50°C for the unneutralised systems and a smaller increase at 65°C for the neutralised gels. These results should be interpreted carefully, as the larger increase in G' would imply that the unneutralised material undergoes a more intensive polymer/mucin interaction while the similarity between the G' increases – seen for the neutralised samples and mucin – alone would imply that the bioadhesive is not interfering with the thermosetting process. However, our previous studies [7,16] have shown that the unneutralised samples possess much lower bioadhesive ability. This may be a reflection of the weaker structure of the unneutralised gels which, in turn, leads to a lower detachment force. The results presented here, in combination with our previous bioadhesion studies, suggest that the bioadhesive process may be usefully considered to consist of two features, namely the molecular interaction between the polymer and mucin and the interaction between the gel composed of that polymer and mucin. It is the former process that is being observed here, while force of detachment tests may be dominated by the latter. The Noveon AA-1 systems (Fig. 3) indicate that while a larger increase occurs for the unneutralised systems, the temperature at which the increase is seen is similar to that of the neutralised gels. As such, this may indicate that the mechanism of interaction between the unneutralised Noveon AA-1 and mucin differs from the Carbopol systems.

The exact nature of the polymer/mucin interaction is not known. It is believed that chain interlocking, conformational changes and chemical or physical interactions such as hydrogen and van der Waals bonds are likely to occur during interpenetration [17], all having a major impact on the rheological properties of the mixes. It is also proposed that the 'gel strengthening effect' could be due to the formation of hydrogen-bonded intermolecular complexes between the mucoadhesive and the mucus molecules [5]. Oscillatory rheological analysis of the homogenised mucus Carbopol 934 mixes has revealed an intermediate behaviour between that seen with a physically entangled system and a cross-linked system [18], indicating that both physical and chemical bonds take place in the formation of a polymer/mucus network. The only thermorheological data published so far on the polymer/mucin mixture refer to oscillatory scans in the range 5–45°C, with relatively constant elastic and loss moduli obtained [4], which correlates with our results. The results presented here may be a reflection of the miscibility of the bioadhesive polymers with the mucin. The neutralised Carbopols and polycarbophil systems may form separate microregions within the gel, while the unneutralisd Carbopol samples show greater miscibility with the mucin, thereby altering the thermosetting behaviour of the latter.

TG analysis showed loss of water with temperature for the unneutralised and neutralised samples (Figs. 4 and 5); first derivative curves have not been included as, given the high water content of these systems, the overall water-loss profile is the feature of interest in the present study. The unneutralised samples showed marked differences among the various PAA gels, with the Carbopol 974P gels showing the highest temperature at which water loss was still evident and Carbopol 971P the lowest. This trend correlates with the storage modulus data in that the systems with the highest values of G' also showed the highest temperature of maximal water loss, suggesting that this rank order may be a reflection of the ease of diffusivity of water through the gels. However, the mucin gels showed intermediate water-loss curves, despite having the lowest G' values; hence, the relationship is clearly material dependent. It is conceivable that, as water is

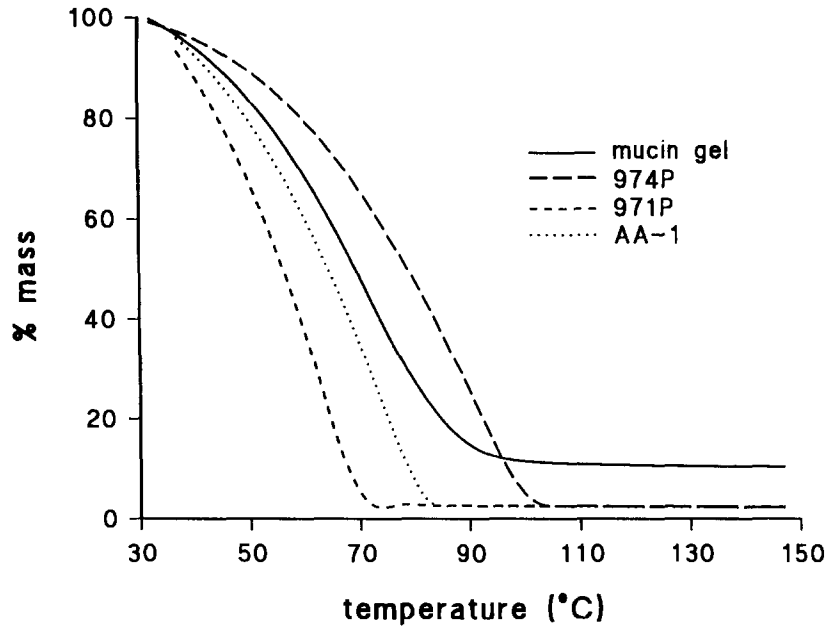

Fig. 4. TGA profiles of different PAA hydrogels in the unneutralised state and the mucin gel.

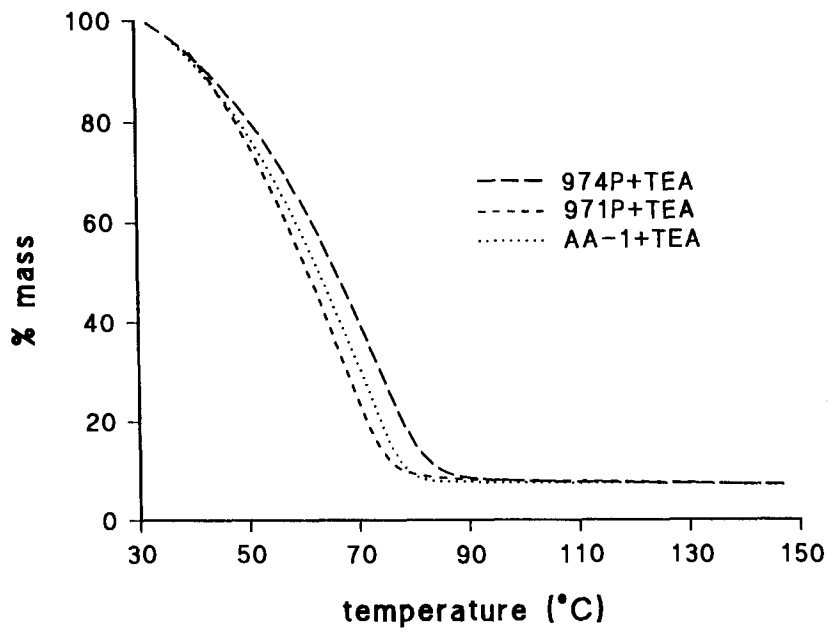

Fig. 5. TGA profiles of different PAA hydrogels neutralised with triethanolamine.

lost from the gels, the network steadily collapses; hence, it is the architecture of the collapsed gel that may determine the subsequent rate of water loss. The relationship between the isothermal storage modulus and the water-loss profile for the PAA systems may therefore not necessarily be a causal one; the degree and type of cross-linking within the PAA gels may determine both the rheology and the way in which the gel collapses on

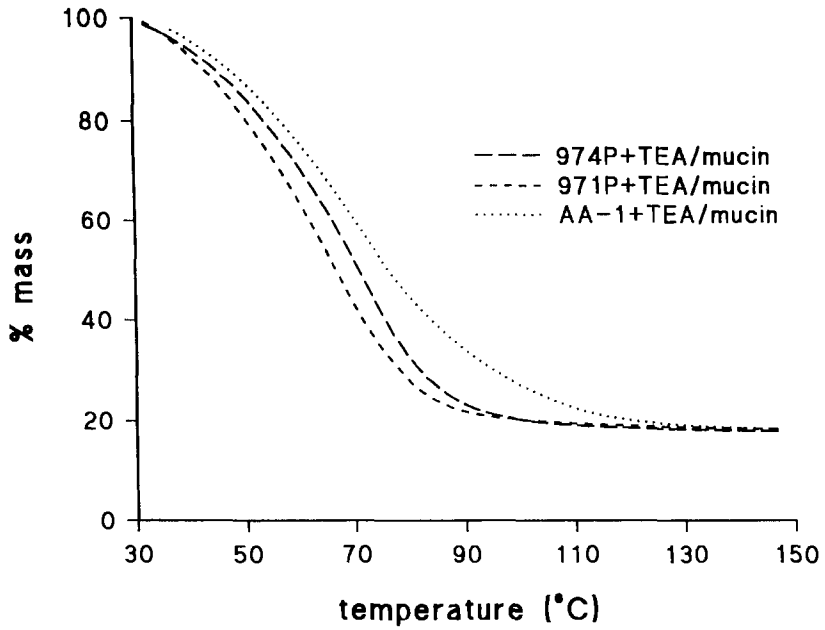

Fig. 6. TGA profiles of the polymer/mucin mixtures.

dehydration. The differences in the final masses between the PAA systems and mucin are a reflection of the different initial concentrations of the gels.

The water-loss profiles for the neutralised systems (Fig. 5) are similar for the three PAA gels, although the rank order of water loss remains unchanged. This greater uniformity in response may be a direct reflection of the ionised state of the PAA molecules or, alternatively, as these molecules are theoretically uncoiled due to the presence of the neutralising agent, the collapse behaviour may be less dependent on cross-linking characteristics.

TG analysis of the neutral PAA/mucin mixtures was performed and the data is shown in Fig. 6. Given the higher level of non-aqueous material in the mixed gels, the shapes of the profiles are relatively similar to the neutralised systems alone. However, the rank order of the polymers had altered, with Noveon AA-1 (polycarbophil) showing the greatest temperature at which water loss was evident (up to 130°C), indicating either a marked change in the collapse architecture on mixing with mucin which was not seen with the other polymers or, alternatively, a greater degree of water binding.

4. Conclusion

Thermorheological and thermogravimetric analyses used in conjunction have proved to be useful in characterising polymer hydrogels and polymer/mucin complexes, and therefore present a promising new approach to studying the nature of the mucoadhesive bond. In particular, use of the two techniques has indicated that there is a thermally induced structural change in mucin which may be altered by the addition of PAA. If one regards the extent of this alteration as a reflection of the degree of molecular interaction with mucin, then the unneutralised gels appear to have a greater interaction than do the neutralised ones. However, anomalous behaviour was observed with Noveon AA-1 in both the thermorheological and the TGA studies; hence, more work is required to clarify the mechanisms involved for this particular polymer. Thermal analysis has been almost totally unused in the bioadhesion field. However, these studies indicate that there is a positive role for thermal methods in attempting to understand the interaction between bioadhesive molecules and mucin.

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