

Extended Frequency Sweep: A More Realistic Rheological Approach to Investigate the Process of Mucoadhesive Polymer-mucus Gel Chain Interpenetration

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ABSTRACT

Mucoadhesive formulations have been of great interest in recent years. One approach to study mucoadhesion is the technique of dynamic oscillatory rheology (DOR). In this study the use of an extended frequency sweep DOR, instead of the routine limited DOR studies, as a more comprehensive and realistic means of investigating the process of mucoadhesion was investigated. For this purpose polymeric discs were directly placed in contact with natural mucus samples and incubated for various time intervals (15, 60 and 120 min) at 37°C, and then they were rheologically examined across a frequency of 0.0001-10 Hz. Results showed that in the presence of anionic mucoadhesive discs containing Carbopol 934P (C934) and sodium carboxymethyl cellulose (NaCMC), the G' (storage modulus) and to a smaller extent G'' (loss modulus) values of the weakly viscoelastic mucus samples are strengthened much more than the non-ionic polymer hydroxypropyl cellulose and the calcium containing Carbopol EX83, and far less reduced at frequencies below 1Hz. However, in the presence of potassium thiocyanate (hydrogen-bond breaking agent), unlike G'' values, the G' values of particularly the anionic polymers C934 and NaCMC suffered a sharp decline at frequencies below 1Hz. These findings support the theory of mucoadhesive polymer chain interpenetration and formation of hydrogen-bonds with the mucus gel network, and shows that by using an extended frequency sweep rather than a normal limited frequency sweep, a more detailed and realistic approach for studying the process of mucoadhesion and putative mucoadhesive polymers and formulations, could be provided.

Key Words:

bioreheology;
Carbopols;
mucoadhesion;
mucoadhesive polymers;
dynamic oscillatory rheology.

INTRODUCTION

Development of adhesive dosage forms for controlled drug delivery to/or via mucous membranes is of interest with regard to local drug therapy and the systemic administration of peptides and other poorly absorbed drugs [1-5].

The attachment of synthetic or biological macromolecules to a biological tissue is referred to as bioadhesion. When applied to a mucosal epithelium, a bioadhesive system adheres and presumably interacts primarily with the mucus layer, and

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this phenomenon is referred to as mucoadhesion [6,7].

Mucoadhesive materials are generally hydrophilic macromolecules containing numerous hydrogen-bond forming groups, e.g. hydroxyl and carboxyl groups. A number of charged and neutral polymers have been classified as mucoadhesive, since they are known to bind very strongly to mucus via non-covalent bonds. Among these polymers are the well-known polyacrylic acid based Carbopols, such as Carbopol 934P and polycarbophil, as well as the cellulose derivatives such as sodium carboxymethyl cellulose and hydroxypropyl-methyl cellulose [8,9].

Mucus, a weak viscoelastic gel whose major structural component is glycoprotein in nature, is known to be capable of associating with mucoadhesive polymers in the aqueous phase. Chain interpenetration, physical entanglement (chain interlocking) and secondary chemical interactions (such as hydrogen-bonding) at the functional group level between the mucoadhesive polymeric chains and the glycoprotein chains present within the mucus gel network, could result in the formation of mixtures capable of exhibiting rheological synergy [10,11]. This means that the gel-like properties of the mixture are expected to be greatly in excess of when the mucus and polymer gels are examined separately. Hence, strong mucoadhesion would most likely produce changes in the rheological properties of the mucoadhesive polymer-mucus interfacial region, strengthening the weakest component of the adhesive joint. This has led several authors to suggest that the study of the rheological profile of mucoadhesive-polymer mixtures provide an acceptable in-vitro mean of investigating the process of mucoadhesion and determining the mucoadhesive properties of a material. As a result, the rheology of a number of polymer-mucus systems has been investigated [12-18]. Based on these results, a significant strengthening of the mucus gel network on the incorporation of strongly mucoadhesive materials has been observed. Furthermore, it was suggested that the gel strengthening effect observed, was resulted from the formation of both chain interpenetration and secondary chemical bonds between the mucoadhesive polymer and the mucus glycoproteins. Dynamic oscillatory rheology is largely used to perform these studies. For this purpose the mucoadhesive polymer, in gel form, is physically mixed with a sample of natural mucus gel. The rheological behaviour of

the resulting mixture is then examined at a frequency of 10-0.1 Hz. Next, the mean value obtained for the storage modulus (G') and loss modulus (G'') of around 15 data points will be calculated. Results obtained from this method merely show the overall G' and G'' of the polymer-mucus mixture. Hence, only speculations can be made regarding the possibility of polymer-mucus chain interpenetration and interactions.

Previous rheological studies on biopolymer gels have used extended (wide range) frequency sweeps, especially frequencies much smaller than 0.1Hz, as a mean of determining the internal structure of these gels [19, 20]. Based on these studies, a gel containing physically mixed polymer chains shows a dramatic decrease in G' and G'' values at frequencies below 1Hz. In contrast, the G' and G'' values of strongly cross-linked polymeric gels will not be altered across the frequency range, even at a very low frequency of oscillation. Based on these findings, it seems possible to examine the internal structural changes occurring within the mucus gel network as a result of having come into contact with mucoadhesive polymers, more accurately. This approach has not yet been used for investigating the process of mucoadhesion. Previous rheological studies on the process of mucoadhesion have merely looked at the overall phenomenon of gel-strengthening and not the internal structural changes occurring. Hence, the main objective and the novelty of this study was to use dynamic oscillatory rheology as a useful tool for obtaining valuable information regarding the internal structural changes which could occur during the process of mucoadhesion. Furthermore, in this study the more realistic and novel approach of placing a polymeric mucoadhesive disc over a sample of natural and freshly removed mucus gel was adopted, instead of in vitro, physically mixing the mucoadhesive polymer with the mucus gel.

EXPERIMENTAL

Materials

Carbopol 934P and Carbopol EX83 were obtained as gifts from B.F. Goodrich, Hounslow, UK. Sodium carboxymethyl cellulose and hydroxypropyl cellulose were purchased from Aldrich Chemical Co. Ltd., Gillingham, UK. Potassium thiocyanate was obtained

from BDH Ltd., Poole, UK.

Methods

Preparation of the Mucus Gel

The crude mucus gel used in this study was obtained by slaughtering healthy young hogs. Stomachs were then removed and longitudinally opened. Finally, the mucus gel present was carefully scraped, using a plastic spatula. Scraped mucus samples were gently blended to ensure homogeneity and then used without further treatment. The percentage dry weight of solids present within the prepared batch of crude mucus gel was determined by leaving a small portion (0.5 g) of mucus in pre-weighed open glass vials at 50 C for 48 h. This was found to be 5.8 – 0.2% (n=5).

Preparation of Polymer Containing Discs

Flat-faced discs with a diameter of 13 mm, and weighing 100 mg were prepared by directly compressing the powder, using a single punch tablet press. Discs containing Carbopol 934P (C934), Carbopol EX83 (CE83) which is calcium polycarboxophil, sodium carboxymethyl cellulose (NaCMC) and hydroxypropyl cellulose (HPC) were prepared. In addition, discs containing these polymers along with potassium thiocyanate (KCNS), at a ratio of 4:1 were also prepared. As control, polymer : lactose discs containing the same ratio of 4 : 1 were also prepared. Discs prepared were kept in airtight containers until use.

Rheological Studies

Discs prepared were individually placed in contact with 300 mg samples of mucus gel, within specially designed enclosed perspex cubical cells (17×17×20 mm (height)). These cells composed of two equal halves, were easily fitted over each other. Cells were then incubated at 37 C for set intervals of 15, 60 and 120 min (in case of polymer-KCNS containing discs, the incubation time was only 120 min). Next, the test discs were carefully lifted and separated from the underlying mucus gel. Following the removal of mucus gel samples, they were individually placed in a Carri-Med CSL 100 rheometer, fitted with a 2 degree stainless steel cone with a diameter of 2 cm. Initial torque sweeps were conducted on test samples in order to determine the linear viscoelastic regions, and to ensure that these values do not fall outside the sensitivity limit of the

rheometer, particularly at very low frequencies.

Next, an extended frequency sweep between 10-0.0001 Hz was conducted on each test sample at room temperature (23–1 C) and the values for storage modulus (G') and loss modulus (G'') were determined across the frequency range. As control, mucus gel samples were placed within the enclosed perspex cells but in the absence of test discs they were also incubated at 37 C for the same time intervals mentioned before and were examined rheologically as above.

RESULTS AND DISCUSSION

As mentioned earlier, in this study mucoadhesive polymer containing discs were directly placed over samples of fresh mucus gel in order to simulate the real situation more closely. Mucus samples left in contact with the test discs were then examined rheologically over an extended frequency sweep. This was done in order to observe the structural changes occurring within the mucus gel, presumably due to the penetration into and interactions of the mucoadhesive polymer chains with the mucus gel network. Different polymers are expected to have varying penetration rates into the mucus gel network. In fact, this is the reason for mucoadhesive polymers showing different adhesions to mucosal surfaces.

As mentioned in the literature [21] mucus is a weak viscoelastic gel, containing around 1-2% glycoprotein as its structure-forming component. This moiety is also responsible for the viscoelastic nature of the mucus gel.

The rheogram (Figure 1) of the mucus gel used in this study also shows the presence of a weakly viscoelastic structure. In here the G' values (representing the elastic or solid-like nature of the material) appear to be higher than their corresponding G'' values (representing the viscous or liquid-like nature of the material). The rheograms of the control mucus samples (not left in contact with the polymeric discs) incubated at 37 C for different time intervals of 15, 60 and 120 min were similar and showed no major difference in G' and G'' values across the frequency range studied. Hence, only the rheogram of the mucus gel incubated for 120 min has been shown in Figure 1. This rheogram clearly shows that at low frequencies, particularly below 1 Hz, a sharp decline in G' values of the mucus

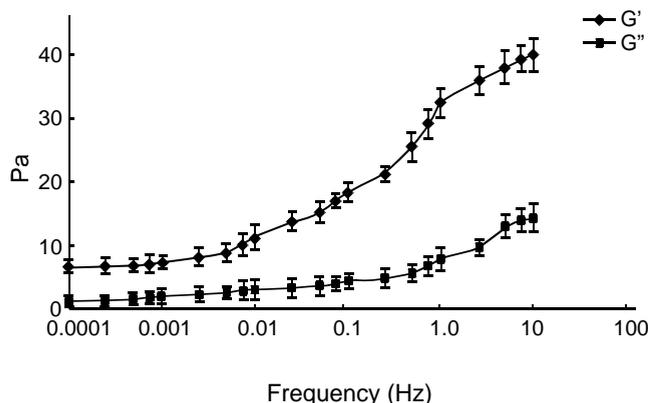


Figure 1. Rheogram of the crude mucus gel incubated for 120 min. at 37°C, showing changes in G' and G'' values over an extended frequency of 0.0001-10 Hz ($n=3$; mean \pm standard deviation).

gel and to a lesser extent G'' values occurs. This finding, as described in other studies carried out on biopolymer gels [19,20], suggests the presence of a physically entangled structure with relatively few inter-chain bonds. In a physically entangled system macromolecules are given time to untangle and move past each other, at low frequencies of oscillation. In fact the elastic part of the material tends to stretch and relax much less and slower as well. This would in turn allow the viscous part of the material to flow easier. Hence, overall resistance of the material for deformation decreases and it behaves more like a viscous liquid. At higher frequencies, the reverse occurs. The elastic part of the material will dominate over the viscous part. This would result in a more cumbersome flow and greater resistance to deformation. Hence, the material behaves more like an elastic solid, with greater G' values. Therefore, it is speculated that within the mucus gel network, long-chained glycoprotein units are largely present as closely entangled structures. These chains can be easily disentangled at low frequencies of oscillation.

In the presence of the well-known anionic mucoadhesive polymers C934 and NaCMC, the G' and to a smaller extent G'' values of the mucus gel are strengthened (Figures 2 and 3). This finding suggests that the elastic or solid-like nature of the mucus is strengthened much more than its viscous or liquid-like characteristics, as a result of coming into contact with the mucoadhesive polymer. This would in turn result in a

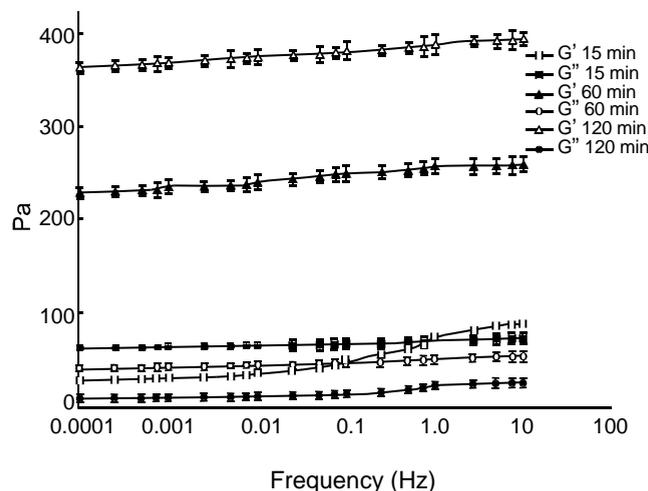


Figure 2. Changes in G' and G'' values of mucus samples placed in contact with C934 discs for various time intervals ($n=3$; mean \pm standard deviation).

greater ability of the adhesive joint formed to resist the environmental stresses encountered, leading to its dislodgment from the adhesion site, in vivo. In addition, the increase in the G' and G'' values observed is time dependent. The greatest increase was found after 120 min contact between the mucus gel and the polymeric disc. Interestingly, the decline in G' and G'' values at frequencies below 1 Hz for mucus samples placed in contact with the polymeric discs for periods longer than 15 min is much less than those contacted for 15 min. Initial contact between the mucus gel and the dry polymeric disc could result in the dehydration

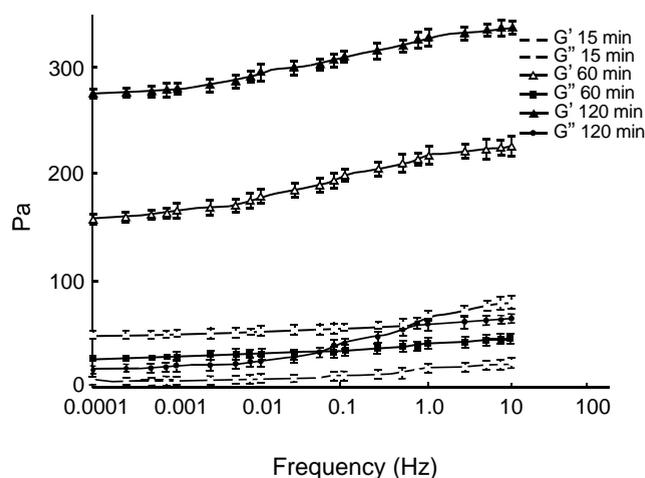


Figure 3. Changes in G' and G'' values of mucus samples placed in contact with NaCMC discs for various time intervals ($n=3$; mean \pm standard deviation).

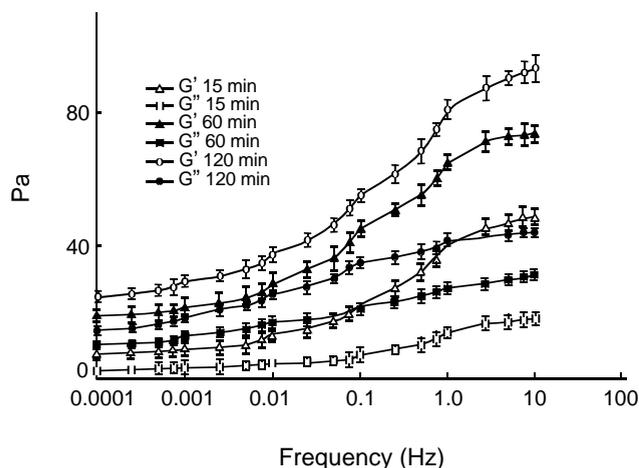


Figure 4. Changes in G' and G'' values of mucus samples placed in contact with CE83 discs for various time intervals ($n=3$; mean \pm standard deviation).

of the mucus gel [22,23]. As a result of dehydrating the mucus gel, polymer chains of the dry mucoadhesive disc will be hydrated and uncoiled to some extent. This would in turn allow the process of mucoadhesive polymer chain interpenetration into the mucus gel network to take place. Hence, the initial increase in the G' and G'' values of the mucus gel is expected to be merely due to this mucus dehydration process. This explains the reason for the sharper decline in G' and G'' values observed after 15 min contact of the mucus gel with the polymeric discs, compared with longer contact times. At longer contact times, beside the process of mucus dehydration by the polymeric discs, the process of polymer chain interpenetration into the glycoprotein structure of the mucus gel, could take place. This stage could then be followed by the formation of secondary chemical bonds (mainly hydrogen-bonds) between the polymer-mucus glycoprotein chains. Hence, it appears that the process of polymer-mucus chain interpenetration and interaction is a time dependent and not an immediate process. In fact this process results in the formation of a physically entangled macromolecular complex between the mucoadhesive polymer and the mucus gel, which is stabilized by the formation of secondary chemical bonds. This could explain the reason for not observing the sharp declines in the G' and G'' values at frequencies below 1 Hz for mucus samples placed in contact with the polymeric discs for periods longer than 15 min.

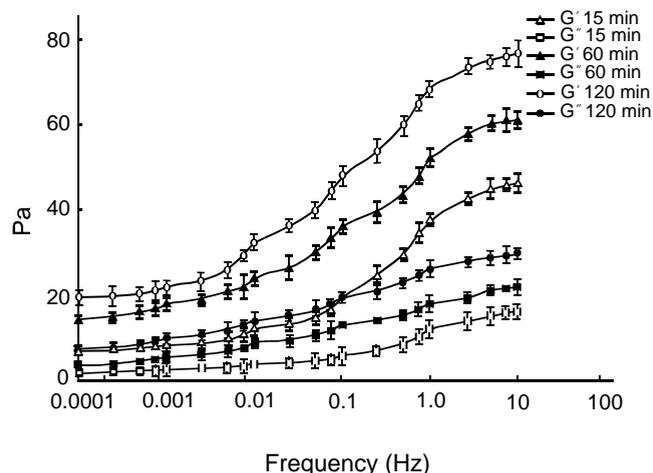


Figure 5. Changes in G' and G'' values of the mucus samples placed in contact with HPC discs for various time intervals ($n=3$; mean \pm standard deviation).

It should also be noted that overall, C934 has been able to strengthen the mucus gel network more than NaCMC. This is evident from the greater G' and G'' values obtained from mucus samples placed in contact with C934 discs (Figure 2). Furthermore, it appears that the C934 polymer chains have been able to penetrate into and interact with the mucus gel network better than NaCMC polymeric chains. This is because at frequencies below 1 Hz, the decline in G' and G'' values of mucus samples left in contact with C934 discs is less than that of NaCMC. This could explain the better efficacy of C934 than NaCMC as a putative mucoadhesive material [24,25].

With CE83, which is a polyacrylic acid based polymer containing calcium, the overall increase in the G' and G'' values of mucus gel (Figure 4) is much less than C934 (Figure 2). In addition, at frequencies below 1 Hz, the decline in G' and G'' values is much greater than the mucus samples placed in contact with C934 discs. Presence of calcium within the polymeric structure of CE83 could act as a counter-ion for ionized carboxyl groups present within the polyacrylic acid structure of CE83. This means that the polymer chains present within CE83 will not be able to hydrate and expand as well as the C934 polymer chains. The divalent calcium ions neutralize the ionized carboxyl groups. This could then lead to the formation of cross-links between the polymer chains, not allowing them the freedom of movement and flexibility required for penetration into the mucus gel network.

Finally, with the non-ionic polymer HPC, the ability to strengthen the mucus gel network appears to be much less than the anionic polymers C934 and NaCMC. In the literature [6, 8, 25] HPC is ranked as a weak mucoadhesive material. Based on the results obtained (Figure 5), it is clear that HPC cannot increase the elastic nature (G') of mucus gel as well as the anionic polymers C934 and NaCMC (Figures 2 and 3). Furthermore, it is clear that the extent of increase observed in G' and G'' values of the mucus gel, as a result of increasing the HPC-mucus contact time, is far less than that observed with C934 and NaCMC discs. However, the decline obtained in the G' and G'' values of mucus samples placed in contact with HPC discs at frequencies below 1 Hz is much greater than those placed in contact with C934 and NaCMC. These findings suggest that the non-ionic polymer HPC cannot penetrate as well as the anionic polymers studied (C934 and NaCMC) into the mucus gel network. Hence, the extent of interactions formed between HPC and mucus is poor, resulting in its relatively weak mucoadhesive nature.

In the final part of this study the effect of KCNS on the rheological properties of the adhesive joint formed between the mucoadhesive polymer and the mucus gel was investigated. KCNS is known to be a hydrogen-bond breaking agent [26]. Results obtained are shown in Figures 6 and 7. At frequencies less than 1 Hz, the presence of KCNS has resulted in a reasonably sharp

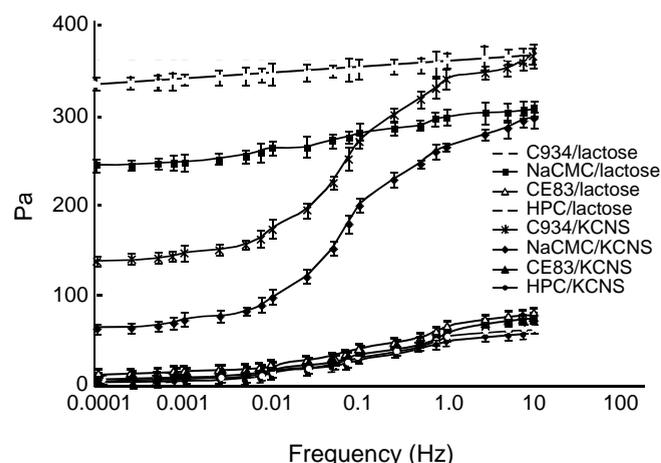


Figure 6. Changes in G' and G'' values of the mucus samples placed in contact with various polymeric discs containing KCNS (4:1 (KCNS) ratio) as well as control discs containing lactose, for a period of 120 min at 37°C ($n=3$; mean \pm standard deviation).

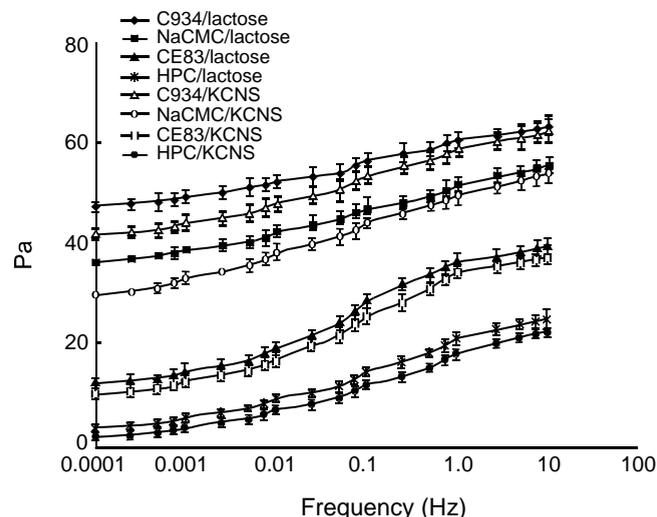


Figure 7. Changes in G'' values of the mucus samples placed in contact with various polymeric discs containing KCNS (4:1 (KCNS) ratio) as well as control discs containing lactose, for a period of 120 min at 37°C ($n=3$; mean \pm standard deviation).

decline in the G' values (Figure 6) of the mucus samples. Among the polymers investigated, this effect was more noticeable with mucus samples incubated with C934 and NaCMC discs. On the other hand the G'' values (Figure 7) of the mucus samples placed in contact with discs containing KCNS is almost similar to the control mucus samples. This finding suggests that the formation of hydrogen-bonds between the mucoadhesive polymer and the mucus gel is critical for obtaining a strong and elastic adhesive joint. This is required to enable the formulation to resist deformation and quick removal from the site of adhesion.

CONCLUSION

In this study the use of an extended frequency sweep, covering frequencies lower than 1 Hz, for examining the process of mucoadhesion, was investigated. This method seems to be superior over the ordinary limited frequency sweep dynamic oscillatory rheological studies, which do not cover very low frequencies. In fact, an extended frequency sweep study could provide more detailed and accurate data regarding the structural changes and interactions occurring within the mucus gel network, when placed in contact with mucoadhesive polymers. In limited frequency sweep studies a mean G' and G'' value is calculated, only showing the

overall changes in the viscoelastic nature of the mucus gel. However, by performing an extended frequency sweep study, beside observing the overall changes occurred in the viscoelastic nature of the mucus gel, one could gain valuable information regarding the nature of interactions occurring between the mucoadhesive polymer and the mucus gel. This information could then be used in the development of more effective and durable mucoadhesive formulations.

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