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Research paper

Rheological study on mucoadhesivity of some nasal powder formulations

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Abstract

Various powder mixtures were used to administer insulin via the nasal route: a co-spray dried mixture of Amioca® starch and Carbopol® 974 P (1/3), drum dried waxy maize starch and Carbopol® 974 P (9/1), maltodextrin DE38/Carbopol® 974 P (9/1) and pure drum dried waxy maize starch. Oscillatory rheology is performed to study and compare the viscosity, elasticity and mucoadhesivity of these powder formulations. There was no rheological synergism detectable with the co-spray dried mixture of Amioca® starch and Carbopol® 974 P (1/3), drum dried waxy maize starch and Carbopol® 974 P (9/1) and maltodextrin DE38/Carbopol® 974 P (9/1). Interaction due to entanglements was seen with drum dried waxy maize starch (100%). The differences in nasal bioavailability between the different carriers could be explained by differences in G' (storage modulus, elasticity) and G'' (loss modulus, viscosity) values. The formulation giving the highest bioavailability, provided also the highest G' and G'' values.

Keywords: Oscillatory rheology; Elasticity; Viscosity; Mucoadhesion; Bioavailability; Insulin

1. Introduction

The use of bioadhesives for the systemic delivery of peptides via non-invasive routes of administration is becoming increasingly important in recent years. Bioadhesives were used as a strategy to improve the nasal bioavailability of peptides. For bioadhesion to take place, a succession of phenomena is required. The first stage involves an intimate contact between the two surfaces, either from a good wetting of the bioadhesive surface or from the swelling of the bioadhesive. In a second stage, penetration of the bioadhesive into the crevice of the tissue surface or interpenetration of the chains of the bioadhesive with those of the mucus takes place to allow finally the formation of secondary chemical bonds between the surfaces [1]. The mechanism of action of bioadhesives for improving nasal delivery of peptides was suggested to be 2-fold. On the one hand they increased the residence time of drugs in the nasal cavity due to a decrease of the mucociliary clearance and thereby allowing a longer contact time between the drug and the absorbing membrane epithelia [2]. On the other hand a transient widening of the tight junctions occurs between the cells of the nasal mucosa due to the ability of bioadhesives to take up water and to swell giving a temporarily dehydration of the underlying cells [3].

Numerous methods have already been used to study mucoadhesion and were mainly based on the measurement of tensile or shear strength. The disadvantage of these methods was that they did not provide insight in the real mechanism of adhesion at the mucous membrane. Other methods were proposed in order to find an answer on the interaction mechanisms, e.g. the adhesion weight method [4], the fluorescent probe method [5], the mechanical spectroscopic method [6], the viscometric method [7]. Hassan and Gallo [7] used the viscometric method to quantify mucin-polymer bioadhesive strength. The major disadvantage of this procedure is the breakdown of the polymer-mucin network under continuous flow. On the other hand, oscillatory rheology has been used in order to characterise the network structure in a polymer or a polymer mixture [8,9]. The oscillation technique is a non-destructive test which measures both the viscous and elastic behaviour

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of a sample simultaneously and can be used to determine mucoadhesion between polymers and mucin.

Callens and Remon tested different powder mixtures to administer insulin via the nasal route [10]. Bioavailabilities of 17.8, 14.4, 3.8 and 4.4% were obtained with formulations composed of a co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3), a physical mixture of drum dried waxy maize starch and Carbopol® 974P (9/1), a physical mixture of maltodextrin DE 38 and Carbopol® 974P (9/1) and pure drum dried waxy maize starch, respectively. In order to explain those differences in bioavailability, oscillatory rheology based on the method of Ceulemans and Ludwig [8], was performed. The viscosity, elasticity and mucoadhesivity of the powder formulations were determined and compared with each other.

2. Materials and methods

2.1. Materials

The co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) was received from National Starch and Chemical Company (Bridgewater, USA). Drum dried waxy maize starch (DDWM) and maltodextrin DE 38 were obtained from Eridania Béghin-Say, Cerestar (Vilvoorde, Belgium) and Carbopol® 974P from BF Goodrich Co. (Cleveland, OH, USA).

2.2. Preparation of the mucin dispersion

A mucin dispersion (M/S mixture) (pH 4) was prepared by dispersing 8% (g/v) mucin (Mucin type II: Crude from Porcine Stomach (Sigma-Aldrich Chemie, Steinheim, Germany) in simulated nasal fluid (pH 5.7) composed of 7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.32 mg/ml $CaCl_2$ $2H_2O$ [11].

2.3. Preparation of the powder formulations and test samples

Dispersions were prepared by adding distilled water to the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (Amioca/CP), a physical mixture of drum dried waxy maize starch and Carbopol® 974P (9/1) (DDWM/CP), maltodextrin DE 38 and Carbopol® 974P (9/1) (MD/CP) and pure drum dried waxy maize starch (DDWM). After neutralisation of the dispersion with NaOH 2M (except for DDWM), the dispersions were lyophilised in vials using an Amsco-Finn Aqua GT4 freeze-dryer (Amsco, Germany) and sieved (63 μm). The fraction below 63 μm was stored in a desiccator at 4–8°C until use.

For the experiments, the powders were dissolved in a concentration of 10% (g/v) in the simulated nasal fluid (P/S mixture) or in the mucus dispersion (P/M mixture) prepared as mentioned above. The pH of the P/S mixtures of Amioca/CP, DDWM/CP, MD/CP and DDWM were 5.7, 6.4, 6.4 and 6.7, respectively. This of the P/M mixtures were 4.1, 5.5, 5.5 and 6.5, respectively.

2.4. Rheological measurements

The rheological analysis was carried out using a Carri-Med CSL 100 rheometer (TA Instruments Ltd., Leatherhead, England) fitted with a 4 cm cone (with an angle of 1.59°) for the P/S and P/M mixtures and with a double concentric cylinder with radii R_1: 20, R_2: 20.38, R_3: 21.96 and R_4: 22.38 mm for the M/S mixture. The test samples were individually loaded on the Peltier plate after which a pre-shear stress was applied on the sample. The samples were allowed to equilibrate at 32 ± 0.5°C for 10 min to relax from the pre-shear stress. All the rheological studies were conducted at 32 ± 0.5°C to mimic the temperature of the nasal cavity [12]. A solvent trap covering the geometry, was used to prevent evaporation, because sample dehydration would seriously affect the rheological properties of the sample [13].

Two types of oscillatory measurements were performed. On the one hand the stress sweep analyses in which G' and G'' were recorded at a constant frequency and increasing stress amplitudes. On the other hand the frequency sweep analyses in which the stress amplitude was kept constant and the frequency was varied.

2.5. Interpretation of the data

The interpretation of the stress and frequency sweep analyses was based on the method used by Ceulemans and Ludwig [8].

The storage (elastic) modulus G' and the loss (viscous) modulus G'' were determined as a function of stress or frequency. The storage modulus is a measure of energy stored and recovered per cycle of deformation and reflects the solid-like component of a viscoelastic material. The storage modulus will be large if a sample is predominantly elastic or highly structured. The loss modulus is a measure of the energy lost per cycle and reflects the liquid-like component [14]. The loss modulus will be large, if a sample is predominantly viscous.

In the stress sweep analyses, the structure of the sample is progressively destroyed by applying oscillations with an increasing stress amplitude at a fixed frequency. The linear viscoelastic region is determined by the maximum stress which can be applied without affecting G' and G''. Furthermore, the relative magnitude of the moduli is a qualitative indication for the structure in the sample. Two different situations can occur: $G' > G''$ for a network consisting of secondary bonds and $G' \leq G''$ for a physically entangled polymer solution.

Frequency sweep tests were performed in the viscoelastic region of each sample, keeping the structure of the system intact during the measurement. By performing such small

stress amplitude oscillations at a whole range of frequencies, the type of network structure can be revealed. The main difference between a network of secondary bonds and one of physical entanglements is located in the low frequency range: in an entangled network the polymers can disentangle if the available time is long enough (low frequency). In a network with secondary bonds the bonds are fixed irrespective of the time scale. This results for an entangled solution in a limiting slope of 2 for G′ and 1 for G″ at low frequency in a log-log plot of moduli versus frequency, while at intermediate frequency a plateau develops. For a network of secondary bonds an almost constant value of G′ and G″ is observed over the whole frequency range, with the value of G′ exceeding that of G″ [15].

3. Results

3.1. Drum dried waxy maize starch (DDWM)

Frequency sweep tests could not be performed since no linear region was present in the case of drum dried waxy maize starch.

G″ was higher than G′ for both P/M and P/S mixtures, indicating the presence of a physically entangled system (Fig. 1). The moduli of P/M are higher than the moduli of the P/S and M/S mixtures, pointing to rheological synergism. This means that physical entanglements were formed between mucin and drum dried waxy maize starch.

3.2. Maltodextrin DE38/Carbopol® 974P 9/1

The stress sweep analyses demonstrated a network structure with secondary bonds in the P/S mixture because $G'_{P/S}$ (e.g. 19.61 ± 1.82 Pa at oscillation stress of 0.16 Pa) > $G''_{P/S}$ (e.g. 1.83 ± 0.54 Pa at oscillation stress of 0.16 Pa). From a certain stress on, the storage modulus G′ decreased while the behaviour of G″ was not straightforward. This indicated that a network with secondary bonds was present in the different polymers, as due to breaking up bonds some extra energy was dissipated (increase of G″) while afterwards the frictional losses decreased due to alignment (decrease of G″) [16]. This formulation contained neutralised Carbopol® 974P, which is a high molecular weight homopolymer of acrylic acid crosslinked with allylsucrose, and maltodextrin DE38, partially hydrolysed maize starch with a dextrose equivalent of 38. Due to many functional groups (carboxyl and hydroxyl groups), secondary bonds can be expected inside the polymers.

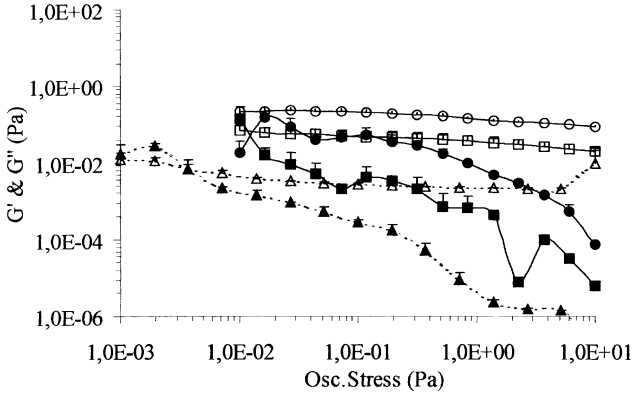

Fig. 1. Dynamic stress sweep curve of the formulation DDWM. G′ (P/M): ●, G″ (P/M): ○, G′ (P/S): ■, G″ (P/S): □, G′ (M/S): ▲, G″ (M/S): △ (data presented as the mean ± SD, $n = 3$).

Contrary to the P/S mixture, the G′ of the P/M mixture (e.g. 0.88 ± 0.08 Pa at oscillation stress of 0.16 Pa) was lower than the G″ (e.g. 1.90 ± 0.17 Pa at oscillation stress of 0.16 Pa) which is an indication for physical entanglements. The increase of G″ followed by a decrease was not observed. Mixing maltodextrin DE38/Carbopol® 974P 9/1 with mucin did not result in a synergistic effect but in a decrease of the secondary bonds between the functional groups of the formulation. The lower G′ values and the smaller linear viscoelastic region for the P/M mixtures compared to the P/S mixtures were also due to a decrease of secondary bonds.

The slope of log G′ versus log frequency for the P/S mixture (0.03) was lower than for the P/M mixture (0.29) which confirmed the higher elasticity of the P/S mixture.

3.3. DDWM/Carbopol® 974P 9/1

G′ was higher than G″ for both P/M and P/S mixtures suggesting a network with secondary bonds (Fig. 2a). G″ increased while G′ was decreasing, which is typical for this type of network. $G'_{P/S}$ was higher than $G'_{P/M}$ and a smaller linear viscoelastic region was obtained for the P/M mixtures compared to the P/S mixtures. This indicated that a decrease of the elasticity (thus no rheological synergism) was also observed when comparing P/M with P/S. The same viscosity was obtained for P/M as for P/S.

The slope of log G′ versus log frequency was lower for the P/S mixture (0.1) than for the P/M mixture (0.2) which demonstrated the higher elasticity of the P/S mixture (Fig. 2b).

3.4. Co-spray dried mixture of Amioca® starch and Carbopol® 974 P 1/3

As observed for DDWM/Carbopol® 974P 9/1, G′ was higher than the G″ for both P/M and P/S mixtures (e.g. $G'_{P/M} = 1633 \pm 111$ Pa, $G''_{P/M} = 281 \pm 48$ Pa, $G'_{P/S} = 3412 \pm 252$ Pa, $G''_{P/S} = 372 \pm 43$ Pa at oscillation stress of 1.93 Pa), which means that secondary bonds were present in the mixtures. $G'_{P/S}$ was higher than $G'_{P/M}$ with a smaller linear viscoelastic region for the P/M mixtures and a similar viscosity between the two mixtures. This indicated that there was again a decrease of the elasticity when P/M was compared with P/S.

A lower slope of logG′/log frequency for P/S (0.05) than

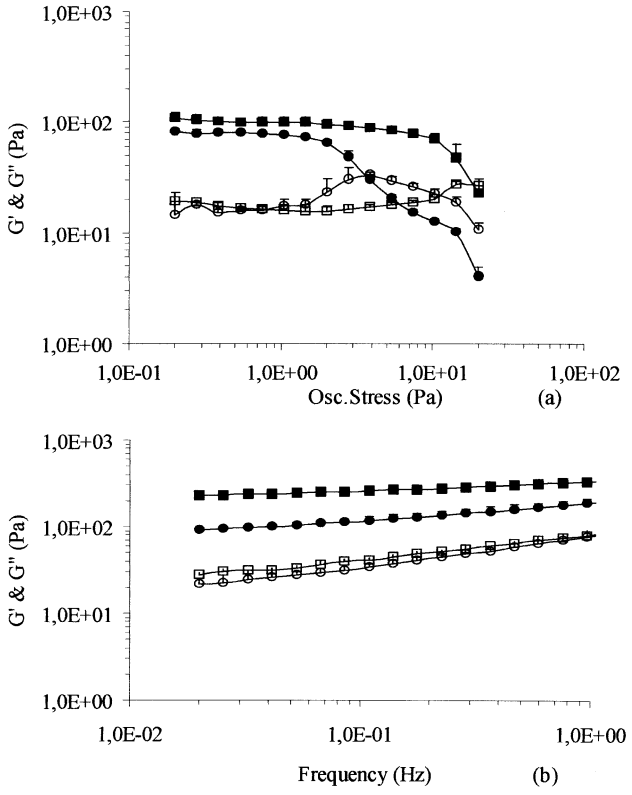

Fig. 2. Dynamic stress (a); and frequency sweep curves (b) of the formulation composed of drum dried waxy maize starch and Carbopol® 974P (9/1). G′ (P/M): ●, G″ (P/M): ○, G′ (P/S): ■, G″ (P/S): □ (data presented as the mean ± SD, $n = 3$).

for P/M (0.1) was obtained during the frequency measurements. No rheological synergism or mucoadhesion were detected with the co-spray dried mixture of Amioca® starch and Carbopol® 974 P 1/3.

4. Discussion

4.1. Interpretation of rheological synergism

Using the stress sweep and frequency sweep measurements it was impossible to detect rheological synergism between the mucin and the formulations consisting of maltodextrin DE38/Carbopol® 974P 9/1, DDWM/Carbopol® 974P 9/1 and the co-spray dried mixture of Amioca® starch and Carbopol® 974 P 1/3 (Amioca/CP). Only for drum dried waxy maize starch an increase of the elasticity and viscosity was obtained when comparing P/M with P/S mixtures.

The high bioavailability values for insulin using the formulations DDWM/Carbopol® 974P 9/1 (14.4%) and Amioca/CP (17.8%) could not be explained on the basis of rheological synergism phenomena.

Important to take into account are the pH differences between the P/M and the P/S mixtures as the viscoelastic properties of Carbopol® 974P are pH dependent. An increase of the pH causes an increase of the ionisation of carboxyl groups and uncoiling of the structure, releasing the non-ionised groups responsible for the formation of secondary bonds. The formation of secondary bonds results in an increase of the elasticity [15]. The viscosity increases with increasing pH until a plateau is reached from pH ± 6 to ±9 (Carbopol® product information 1995). As this plateau becomes broader at higher Carbopol® 974P concentrations [17], the influence of pH is discussable.

Carbopol® 974P contains ionised and non-ionised carboxyl groups at a pH near his pKa value. Repulsion between the negative charge of the carboxyl groups and the negative charges of the mucus can also be the reason for the lack of rheological synergism with the formulations maltodextrin DE38/Carbopol® 974P 9/1, DDWM/Carbopol® 974P 9/1 and Amioca/CP.

Fig. 3 compares the dynamic moduli of the stress sweep analyses of the P/M mixtures obtained for the different polymers. Making a relationship between the dynamic moduli and bioavailability was possible. The highest G′ and G″ and bioavailability values were obtained with Amioca/CP which contains a high Carbopol® 974P concentration (75%). DDWM/Carbopol® 974P 9/1 contains only 10% Carbopol® 974P resulting in lower moduli than those of Amioca/CP. Significantly ($P < 0.05$, one-way analysis of variance) lower bioavailabilities and dynamic moduli were obtained for maltodextrin DE38/Carbopol® 974P 9/1 compared to DDWM/Carbopol® 974P 9/1. These formulations contain both 10% Carbopol® 974P but differ in the type of starch. Maltodextrine DE 38 is a partially hydrolysed maize starch with much lower molecular weight (M_n: 552, M_w: 7846) than drum dried waxy maize starch [18]. Pure DDWM did not contain any Carbopol® 974P and showed also the lowest G′ and G″ value.

4.2. Nasal bioavailability and rheological synergism

No rheological synergism occurred using the formu-

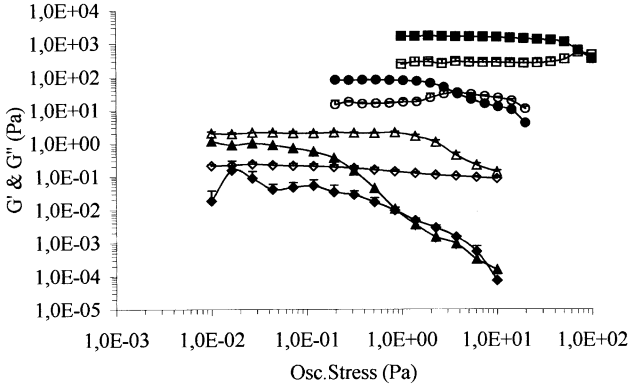

Fig. 3. Comparison of G′ (filled symbols) and G″ (open symbols) of the P/M mixtures of the formulations DDWM (diamonds), maltodextrin DE38/Carbopol® 974P 9/1 (triangles), DDWM/Carbopol® 974P 9/1 (circles) and the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (squares) (data presented as the mean ± SD, $n = 3$).

lations Amioca/CP, DDWM/Carbopol® 974P 9/1 or maltodextrin DE38/Carbopol® 974P 9/1. Nevertheless, high bioavailabilities for insulin were obtained with the formulations Amioca/CP and DDWM/Carbopol® 974P 9/1. These formulations showed the highest G′ and G″ values so elasticity and viscosity can be considered as important factors in relation to bioavailability. Illum et al. [2] pointed already out that increasing the viscosity of a formulation resulted in a decrease of the mucociliary clearance and a prolongation of the residence time of the formulation and absorption time of the drug in the nasal cavity. Additionally, these high bioavailability values could also be explained by the water absorbing capacity of the formulations, giving a temporarily dehydration of the mucosa and an opening of the tight junctions [3]. Amioca/CP provided the highest insulin bioavailability and contained the highest amount of neutralised Carbopol® 974P. The differences of the pH between P/S and P/M have also to be taken into consideration in order to avoid wrong conclusions.

It should be mentioned that the lack of any kind of rheological synergism might be due to the 'overhydration' state of the powders during the in vitro tests. Chen and Cyr [19] pointed out that when some polymers are 'overhydrated' by an excess of water, mucoadhesion may vanish spontaneously. Also Madsen et al. [13] demonstrated that polymers, which loose some of their mucoadhesive strength upon overhydration, produced only limited gel strengthening when introduced to the mucus gel. This might explain the absence of rheological synergism in this study. Effectively when tablets made from these powders are brought into contact with mucosa, strong mucoadhesion occurred (unpublished results). By moistening the tablets, mucoadhesion completely disappeared before sticking them on the mucosa.

Another explanation for the absence of mucoadhesion between the polymer and mucin can be due to the protease inhibiting capacity of Carbopol®, the high concentration of polymers in the P/M mixtures or the high viscosity of the P/M mixtures. In highly concentrated solution, the polymer molecules become solvent-poor, take the dimensions of the unperturbed state and the chain length available for interfacial interpenetration decreases [20]. The high viscosity will decrease the chain flexibility and mobility necessary for interpenetration in the mucus [21].

5. Conclusion

For the formulations composed of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3), drum dried waxy maize starch and Carbopol® 974P (9/1) and maltodextrin DE 38 and Carbopol® 974P (9/1) no interactions (no rheological synergism) with the mucus components were detected. Entanglements with mucus were observed using the drum dried waxy maize starch formulation. Nasal bioavailability differences seen for insulin using the different powder formulations as an administration platform could be explained by the different values of the dynamic moduli. The highest G′ and G″ values were obtained with the formulations giving the highest bioavailabilities. High elasticity and viscosity of the powder formulations seemed to be the important factors for a good bioavailability.

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