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Research paper

Mucoadhesive hydrogels for buccal drug delivery: *In vitro-in vivo* correlation study

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ABSTRACT

Aim: It was the aim of this study to assess *in vitro* methods for the characterization of mucoadhesive hydrogels for their potential to predict the residence time on human buccal mucosa.
Methods: Mixtures of hydrogels comprising hydroxyethyl cellulose (HEC), sodium carboxymethyl cellulose (CMC), xanthan gum (XTGM), hyaluronic acid sodium salt (HA), sodium alginate (ALG), carbopol (CP) as well as polycarbophil (PCP) and porcine mucus were analysed for relative rheological synergism. Furthermore, hydrogels were characterized for their texture and mechanical properties. For the assessment of mucoadhesive strength of formulations tensile studies were performed on porcine buccal mucosa. To facilitate a direct comparability of data the residence time of stained hydrogels was determined *ex vivo* on porcine buccal mucosa and in the oral cavity of volunteers.
Results: The extent of relative rheological synergism was in good agreement with data from *in vivo* residence time studies. Results of tensile studies were further effected by textural properties of hydrogels leading to a restricted correlation with data from the *in vivo* experiment. The resistance towards removal by artificial saliva flow *ex vivo* revealed the highest correlation to the *in vivo* experiment with increasing mucosal residence time in the rank order CP < HEC, HA, ALG, PCP < CMC < XTGM.
Conclusions: This overview of measurement principles to predict the residence time of hydrogels for buccal application in humans may be a potent tool for the development of semisolid intraoral formulations.

1. Introduction

The usage for the treatment of periodontal as well as fungal or inflammatory diseases in the oral cavity gives semisolid dosage forms a vital role in buccoadhesive drug delivery. Besides the more acceptable mouth feel in comparison to solid dosage forms, syringability and ease of placement in the periodontal pocket are key advantages of semisolids. However, the restricted ability to deliver a measured dose of drug in comparison to unit dosage forms and the poor retention on the site of application are major drawbacks of these formulations [1]. In order to prolong the mucosal residence time various mucoadhesive polymers have been incorporated in semisolid delivery systems [2]. The extent of mucoadhesive properties of these formulations, mainly hydrogels, was assessed by means of different measurement principles. Especially rheological as well as tensile studies were employed to establish a ranking of polymers for their mucoadhesive properties [3–6]. The variability of methods including discrepancies in polymer concentration, test parameters, source and type of mucosal tissues or mucin restricts the comparability of studies. Fundamental differences in the underlying measurement principle and validity of results for either the cohesiveness of the gel itself or the interface between mucosa and dosage form need to be taken into consideration [7]. Approaches to correlate results obtained by rheological measurements with varying designs for tensile studies and textural properties of hydrogels showed so far limited success [8,9]. Moreover, a lack of *in vivo* data for correlation makes an interpretation of *in vitro* data difficult. Needleman et al., for instance, performed *ex vivo* adhesion tests on hamster cheek pouch mucosa in organ culture and compared the obtained results with those obtained in humans. The evaluation of three hydrogels revealed the same rank order for adhesion time but strongly deviating absolute residence times for all formulations [10,11].

Aiming to correlate *ex vivo* buccoadhesion with *in vivo* results on cell

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level Kokisch et al. tested four different polymer dispersions for their residence time. Using a direct staining method on extracted human buccal cells bioadhesive properties from *ex vivo* studies could be confirmed *in vivo*. However, the extent of adhesive interactions *in vivo* was not reflected by the *ex vivo* ranking of polymers. Furthermore, discrepancies to previous studies were referred to a lack of sensitivity of the employed staining method [12]. Beyond these limitations, the usage of human buccal cells for *ex vivo* studies may impede the broad application of this method.

The establishment of valid *in vitro* methods for the evaluation of mucoadhesive properties of semisolid formulations on the intraoral mucosa highly correlating with results obtained *in vivo* is therefore urgently needed. In particular, the development of mucoadhesive lozenges and mouthwashes for the treatment of sore throat or alleviation of symptoms of dry mouth syndrome will tremendously benefit from *in vitro* methods of high predictive power for the performance of intraoral formulations in humans [13,14].

Referring to the broad range of established *in vitro* test systems to assess different aspects of mucoadhesion in the oral cavity, the objective of the present study was an evaluation of their predictability for the *in vivo* performance. Apart from the determination of rheological synergism, this work covers the swelling and textural properties of hydrogels comprising polymers of fundamentally different polymeric backbone, charge and molecular mass. Using porcine buccal mucosa, tensile and flow retention studies were optimized for correlation to *in vivo* conditions. Residence time studies in five healthy volunteers served to interpret *in vitro* experiments for their validity for the *in vivo* performance.

2. Materials and methods

2.1. Materials

Polymers employed in this study were kindly donated from the manufacturers. Hydroxyethyl cellulose (HEC) (Natrosol® 250 G PHARM) was a gift from Ashland Industries Netherland B.V., Zwijndrecht, Netherlands, sodium carboxymethyl cellulose (CMC) (Cekol® 4000) from CP Kelco Oy, Äänekoski, Finland, xanthan gum (XTGM) (Xanthan FFOC) from Jungbunzlauer Austria AG, Vienna, Austria, hyaluronic acid sodium salt (HA) (molecular weight 1.50–1.80 MDa) from GfN Herstellung von Naturextrakten GmbH, Wald-Michelbach, Germany, sodium alginate (ALG) (Manucol® LKX MCLLKX) from FMC International, Health and Nutrition, Cork, Ireland, carbopol (CP) (Carbopol® 974P NF) and polycarbophil (PCP) (Noveon® AA-1) from Lubrizol advanced materials Europe BVBA, Westerlo, Belgium. Tromethamine was purchased from Evonik industries AG, Essen, Germany and Brilliant Blue FCF (BB) from Jacobi Decor GmbH, Troisdorf, Germany. All other chemicals and salts were of analytical quality and purchased from Sigma Aldrich, Vienna, Austria. Porcine buccal mucosa and small intestine were kindly donated by a local slaughterhouse.

2.2. Preparation of hydrogels

Hydrogels were prepared by dispersing pre-weighted amounts of each polymer in demineralized water at room temperature. Polyacrylate hydrogels were neutralized by means of tromethamine with a weight ratio of 1:1.3 between poly(acrylic acid) polymers and the base [15]. The dispersion was homogenized and then left for 48 h for complete swelling and equilibration of the polymer at room temperature. To avoid bacterial growth, the non-preserved gels were stored at 4 °C until further use and utilized for maximum 10 days after the manufacturing. To facilitate the determination of *ex vivo* and *in vivo* residence time Brilliant Blue FCF (BB) was added to each formulation in a final concentration of 0.05% (w/v). An overview of all hydrogels used within this study is given in Table 1.

Table 1
Characteristics of employed polymers and hydrogel formulations. Results for complex shear modulus were obtained from frequency sweep measurement at a frequency of 1 Hz (means of at least three experiments ± standard deviation).

Polymer	Molecular mass [kDa]	Charge at pH 6.8	Concentration [% w/v]	Complex modulus [Pa]
HEC	300	non-ionic	6.5	60.20 ± 3.86
CMC	450	anionic	3.0	62.04 ± 2.80
XTGM	1000	anionic	2.5	72.67 ± 3.49
HA	1500–1800	anionic	1.5	63.87 ± 11.15
ALG	100–200	anionic	6.0	79.86 ± 10.05
CP	3000	anionic	0.5	638.31 ± 15.27
PCP	3000	anionic	0.5	188.28 ± 5.00

2.3. Intestinal mucus collection and purification

Porcine small intestinal mucus used for this study was collected and purified according to a previously described method [16,17]. Therefore, small intestine from freshly slaughtered pigs was cut in pieces of 15 cm and opened longitudinally. Sections containing chime were removed and discarded. Mucus was collected by gently scraping off the mucosa and stirred (≤ 40 rpm) in 0.1 M sodium chloride (NaCl) for 1 h at 4 °C at a ratio of 1 g of mucus in 5 mL of NaCl solution. The obtained mixture was centrifuged for 2 h at 10,400 g at 10 °C (SIGMA 3-18KS, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) and the supernatant as well as the granular material on the bottom were removed. The resulting pellet was resuspended in NaCl 0.1 M at a ratio of 1 g of mucus to 2.5 mL of NaCl and stirred (≤ 40 rpm) for 1 h at 4 °C. After centrifugation for 1 h at 10,400 g at 10 °C the supernatant was removed and the purified mucus was collected for rheological measurements.

To characterize purified mucus for its water content a gravimetric analysis was performed. Therefore, 500 mg of the resulting mucus was weighted in a pre-dried petri dish, dried under reduced pressure over 42 h (Christ Gamma 1–16 LSC freeze dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and the dry weight of the sample was measured.

2.4. Rheological measurements

To characterize hydrogels for their rheological properties a plate-plate rheometer (Thermo Scientific™ HAAKE™ MARS™ rheometer, Thermo Fisher Scientific, Karlsruhe, Germany; rotor: PP35 Ti, D = 35 mm, gap 0.5 mm) was utilized. The linear viscoelastic region of all formulations and purified mucus was determined by means of initial oscillatory strain sweep measurements at a frequency of 1 Hz at 37 °C. For oscillatory frequency sweep measurements (0.1–20 Hz, 37 °C) a constant shear stress of 0.5 Pa was chosen for all samples. To ensure a sufficient temperature adaption of the samples and relaxation from stress induced by loading an equilibration time of 60 s at 37 °C was allowed after application to the lower plate.

In order to assess interactions between hydrogels and mucus, further oscillatory frequency sweep measurements of a mixture of both components were performed. Mixtures of 500 mg of mucus and 500 mg of hydrogel were carefully homogenized with a spatula and characterized using test parameters as described above for frequency sweep measurements. Mucus mixed with the same amount of demineralized water served as control. Elastic modulus (G′), viscous modulus (G″) and loss tangent (tan δ) of mucus, hydrogels and mixtures with mucus were determined as a function of frequency using HAAKE RheoWin 3 software. In order to obtain rheological parameters for comparisons the mean dynamic moduli were calculated from the 14 points of G′ and G″ detected within the frequency range of 0.1–20 Hz to take the frequency dependence of moduli for linear polymers into consideration [18].

The relative rheological synergism parameter was calculated as

follows [19,20]

$$Relative\ \ \Delta G' = \frac{\Delta G'}{G'} = \frac{G'_{mix} - (G'_{polymer} + G'_{mucus})}{G'_{polymer} + G'_{mucus}}$$

$$Relative\ \ \Delta G'' = \frac{\Delta G''}{G''} = \frac{G''_{mix} - (G''_{polymer} + G''_{mucus})}{G''_{polymer} + G''_{mucus}}$$

$$\Delta tan\ \delta = tan\ \delta_{polymer} - tan\ \delta_{mix}$$

where G'_{mix} is the elastic modulus of the mixture and $G'_{polymer}$ and G'_{mucus} display the elastic modulus of polymer and mucus, respectively. Equivalent abbreviations are employed for the calculation of the viscous modulus (G″) and tan δ.

2.5. Swelling behavior

Swelling behavior of the different hydrogels was determined gravimetrically. In brief, 100 µL of each hydrogel was weighted initially and soaked into 2 mL of artificial saliva comprising potassium chloride (1.2 g/L), sodium chloride (0.85 g/L), magnesium chloride (0.05 g/L), calcium chloride (0.13 g/L) and di-potassium hydrogen orthophosphate (0.13 g/L) [21]. The final pH of artificial saliva was adjusted to 6.8. After an incubation time of 30 min at room temperature, excess of artificial saliva was removed and the weight of samples detected. The following equation was used to calculate the swelling ratio

$$Water\ \ uptake\ \ [\%] = \frac{W_s - W_0}{W_0} \times 100$$

where *Ws* is the weight of the swollen hydrogel after 30 min and W_0 is the initial weight.

2.6. Analysis of gel texture and spreadabiliy

Mechanical properties of hydrogels affecting their application and mucoadhesive performance were analysed with a TA.XT*plus* texture analyser with a 5 kg load cell (Stable Micro Systems Ltd, Surrey, UK) [22]. The impact of swelling and dilution in artificial saliva on textural properties of hydrogels was determined via analysis of binary mixtures comprising different ratios of hydrogel and artificial saliva [23]. In brief, 5 g of each hydrogel or the appropriate mixture with artificial saliva pH 6.8 was weighted in a vial and allowed to rest for 30 min at room temperature. A cylindrical probe of 10 mm diameter (SMSP/10, Stable Micro Systems Ltd) was forced down into each gel sample at a test speed of 2 mm/s to a defined depth of 5 mm and immediately removed with a return speed of 2 mm/s. The study was carried out at room temperature with an acquisition rate of 500 points/s. From the resulting force-distance plots the work of adhesion was calculated by means of Texture Exponent software (Version 6, Stable Micro Systems Ltd) as the negative area under the curve arising from the upwards movement of the probe. This parameter reflects the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe [24].

Spreadability of hydrogel formulations was detected at room temperature using the same instrument equipped with a TTC Spreadability rig (HDP/SR, Stable Micro Systems Ltd). Gel samples were filled in the cone shaped female sample holder and the male probe was lowered downwards forcing the sample to flow outward in an angle of 45°. The probe was lowered with a test speed of 3 mm/s until a slit of 2 mm was arrived and then moved upwards with a return speed of 10 mm/s. The stickiness defined as the peak force required to overcome the forces between the surface of spreaded hydrogel and the probe surface was determined as the maximum negative force. The work of shear reflecting the total force required to perform the shearing process was calculated as the positive area under the force-distance curve [25].

2.7. Tensile studies

The force required to break the adhesive bond was determined by means of the texture analyser. In brief, porcine buccal mucosa was fixed between two perspex discs as part of a mucoadhesion test rig (A/MUC, Stable Micro Systems Ltd). A circular recess located in the upper disc allowed an interaction between mucosa and the sample. To imitate *in vivo* conditions the uncovered surface of the mucosa (3.8 cm^2) was prehydrated with 200 µL of artificial saliva providing sufficient hydration over the test period. 1.0 mL of each gel formulation was applied to a gel probe (gel mucoadhesion probe A/GMP, Stable Micro Systems Ltd) equipped with concentric grooves encouraging the attachment of semisolid formulations. After loading, samples were placed over the mucosa and then lowered until the gel came in contact with the surface of the mucosa at a pre-test speed of 2 mm/s. After reaching a trigger force of 0.5 N a downward force of 0.05 N was applied for 60 s. Then the probe was moved upwards at a return speed of 0.1 mm/s. The total work of adhesion (TWA), calculated from the positive area under the force-distance curve, maximum detachment force (MDF) obtained as peak force during detachment and deformation to failure were employed to evaluate the mucoadhesive strength of each gel [6]. Deformation to failure was calculated as the distance covered by the probe between incubation on the mucosa and the rupture of the gel string due to upwards movement of the probe.

2.8. Mucosal residence time

2.8.1. Ex vivo

To assess the mucosal residence time of the hydrogel formulations pieces of porcine buccal mucosa (3 × 3 cm) were mounted in an angle of 90° in an incubator with 100% humidity at 37 °C following a previously described method [26]. Immediately before application of hydrogel samples the mucosa was rinsed with artificial saliva pH 6.8 to avoid premature dehydration. 100 µL of each hydrogel was applied in the center of the mucosa and immediately rinsed with artificial saliva at a physiological flow rate of 1.5 mL/min using a peristaltic pump (Ismatec IPC 8, Cole-Parmer GmbH, Wertheim, Germany) [27]. Fractions of artificial saliva were collected every two minutes. The BB concentration in the collected samples was quantified photometrically using a microplate reader (Tecan infinite® M200 spectrophotometer, Tecan, Grödig, Austria) at a wavelength of 630 nm. Buccal mucosa treated in the same way but omitting the application of a sample served as control.

2.8.2. In vivo

The *in vivo* residence time of hydrogel formulations on buccal mucosa was tested on five healthy volunteers (age group 24–32). Before the study, written consent was obtained from all test persons. 100 µL of each hydrogel sample was applied on the buccal mucosa of volunteers beneath the parotid duct using a syringe without needle. During the experiments volunteers were not allowed to eat or drink. For the visual detection of remaining stained hydrogel on the buccal mucosa pictures of the application site were taken every two minutes. The residence time was determined as time interval between application of samples and the time point where no changes in color intensity of BB were detectable at the position of application.

2.9. Statistical data analysis

For statistical data analysis one-way ANOVA was utilized in combination with a Tukey post-hoc test calculated with SigmaPlot 13.0. $P < 0.05$ was set as a minimum level of significance. To assess the correlation between *in vitro* and *in vivo* data Pearson's product moment correlation coefficient was determined. Positive correlation was set as very high for $0.90 < r < 1.00$, high for $0.70 < r < 0.90$ and moderate for $0.50 < r < 0.70$. All results are expressed as means ±

standard deviation of at least three experiments.

3. Results

3.1. Characterization of porcine small intestinal mucus

Following an established protocol for purification, mucus used for rheological studies revealed a water content of 84.08 ± 0.11% [17,28]. Based on the described procedure using 0.1 M NaCl for purification, an approximation of the ion content of mucus to this concentration can be assumed. Moreover, the standardization of purification conditions allows a good comparability of mucus composition and mucin content to previous studies taking fluctuations due to the use of animal tissue into consideration [29,30]. Purified mucus exhibited a mean G′ of 398.15 ± 35.05 Pa, G″ of 138.34 ± 15.95 Pa and tan δ of 0.35 ± 0.01 within the frequency range of 0.1–20 Hz.

3.2. Rheological measurements

In order to characterize a broad range of mucoadhesive polymers with strong variations in the underlying polymeric backbone a rationale for comparisons of hydrogel preparations was needed. Aiming to find a strategy to rank different carbopol derivatives regarding their rheological synergism Hägerström et al. described an adjustment of the rheological properties of gel formulations as a more logical approach than employing equal concentrations of polymers [31]. Therefore, the concentration of gelling agents was adjusted to reach the same level of complex shear modulus (G*) as a quantitative measure for material stiffness and resistance to deformation as shown in Table 1. For the high molecular mass poly(acrylic acid) derivatives, however, an adjustment of complex shear modulus to the range of 60–80 Pa strongly limited further mucoadhesion studies due to insufficient gel strength at the appropriate polymer concentration. A minimum concentration of 0.5 % (w / v) for CP and PCP was observed to form gels of suitable strength for characterization employing the same parameters for all formulations.

The evaluation of structural properties of polymers and structural changes in the polymer network in mixtures with mucus was performed according to numerous previous studies [18,31,32]. The observed frequency dependence of G' for linear polymers and mucus used in this study is displayed in Fig. 1A. Mixtures of hydrogels with porcine mucus imitating the interface between underlying mucosal tissue and dosage form revealed the rheological profile of a weakly crosslinked gel network for all tested formulations (Fig. 1B).

Depending on structural properties of underlying polymers the extent of physical chain entanglement and the formation of non-covalent bonds is reflected by the degree of rheological synergism [18]. To take discrepancies in the initial moduli G′ and G″ of the unmixed materials into consideration, normalized synergism parameters were calculated to compare hydrogel formulations (Fig. 2). The more pronounced increase of G′ with respect to G″ and thus a higher relative ΔG′ than relative ΔG″ reflects the formation of a gel-like structure based on the formation of polymer-mucin interactions. The loss tangent representing the proportion of viscous and elastic components decreased upon mixing both components due to the more pronounced elastic behavior of the mixture [20].

XTGM with its properties of ion sensitivity and temperature induced conformational changes showed the most pronounced interaction with the highest increase in rheological synergism parameters [33]. Though being described as highly mucoadhesive polymers, CP and PCP gels displayed even negative values in rheological synergism. Induced by ions present in mucus the ion sensitivity of poly(acrylic acid) derivatives may contribute to this liquefaction considering the employed low concentrations. In a further examination of time dependent changes in viscosity over a period of 30 min CP and PCP showed an additional decrease of viscosity (data not shown). This may be induced by an approximation to equilibrium distribution within the mixture causing reduced repulsive interactions of carboxylate groups [9].

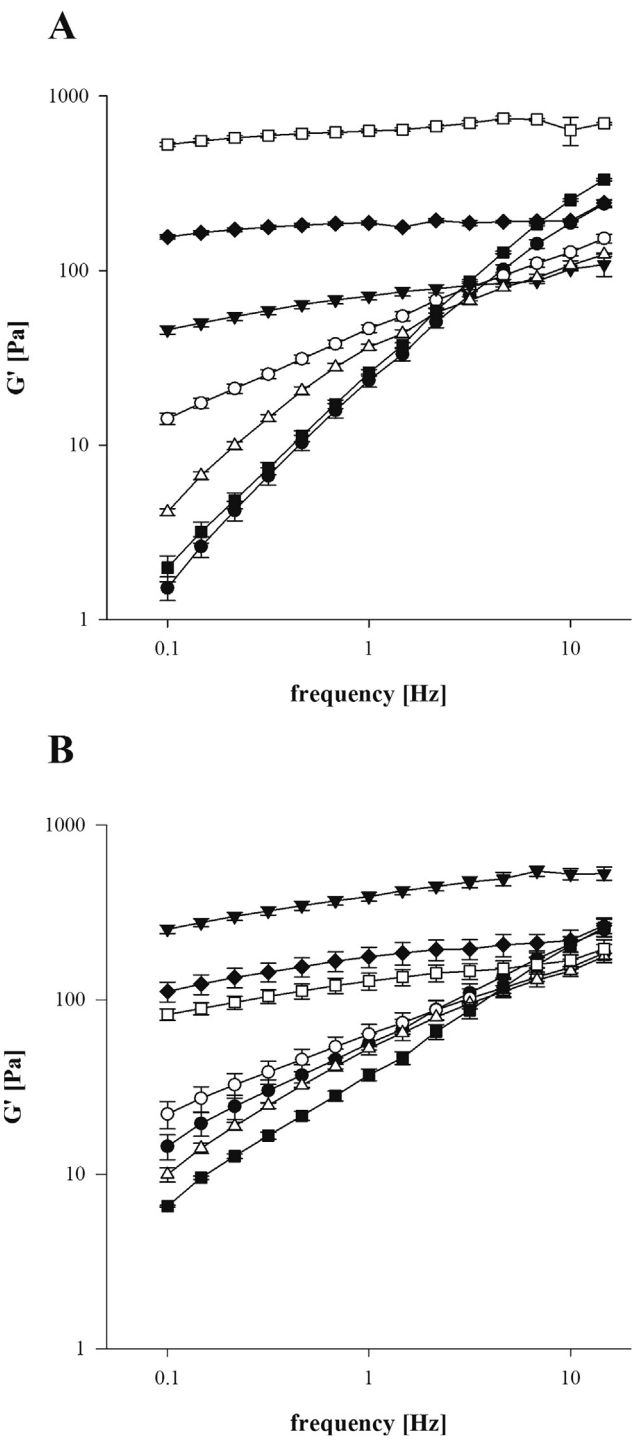

Fig. 1. Frequency dependence of elastic modulus (G′) of hydrogels comprising HEC (●), CMC (○), XTGM (▼), HA (△), ALG (■), CP (□), PCP (◆) and purified mucus (◇) (A) and of corresponding mixtures with mucus (B) at 37 °C and shear rate of 0.5 Pa. Depicted values are means of at least three experiments ± standard variation.

3.3. Swelling behavior

The ability of hydrogels to bind water in the hydrophilic matrix is affected by the underlying chemical structure as well as molecular mass, crosslinking density and charge of polymers. The lack of ionic

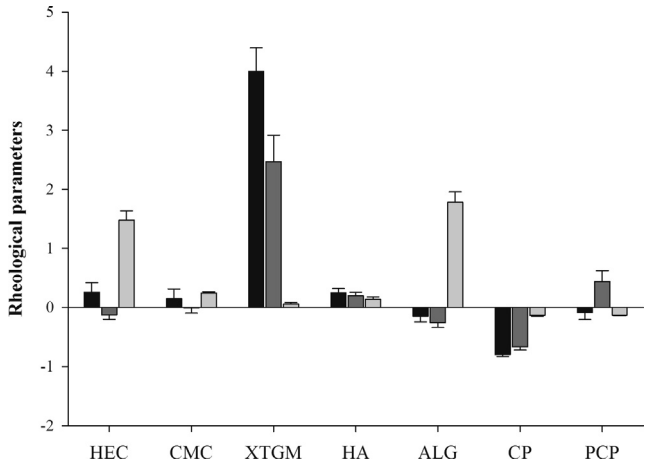

Fig. 2. Relative rheological synergism parameters of hydrogel formulations. Relative ΔG′ (black bars), relative ΔG″ (dark gray bars) and Δtan δ (light gray bars) calculated from means of parameters within a frequency range of 0.1–20 Hz. Displayed values are means of at least three experiments ± standard deviation.

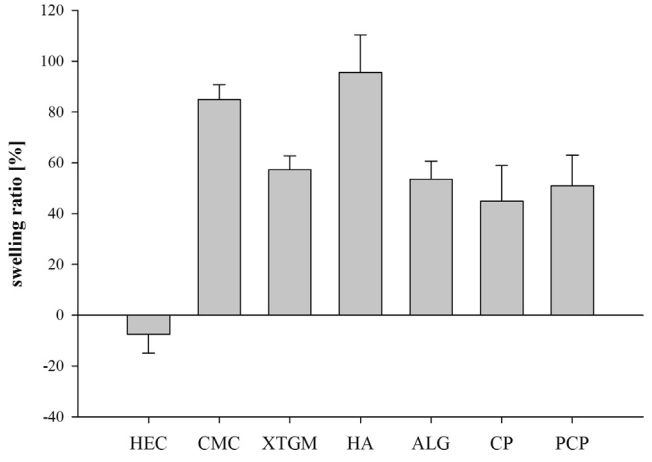

Fig. 3. Swelling behavior of hydrogels immersed in artificial saliva pH 6.8 at room temperature. Values are means of at least three experiments ± standard deviation.

interactions in the network of HEC gels may therefore contribute to its rapid dissolution. The unique water binding capacity of HA was reflected by the highest swelling ratio after 30 min as shown in Fig. 3. Swollen samples of CMC and XTGM remained highly cohesive by contrast to the other hydrogels spreading over the surface of the petri dish.

3.4. Texture analysis

Due to salivary scavenging the residence time of hydrogel preparations in the oral cavity is affected by the mechanical properties of gels being gradually diluted with surrounding saliva. The exposure to increasing percentages of artificial saliva and the resulting work of adhesion of appropriate mixtures is shown in Fig. 4. Though revealing the lowest adhesiveness for the gel itself, dilutions of XTGM with artificial saliva remained adhesive over a broad range of concentrations. As reported by Jones et al. for intraoral hydrogel formulations the adhesiveness determined via texture profile analysis may also serve as a parameter to evaluate mucoadhesion [34].

A further characterization of textural properties revealed the highest resistance to shear for HEC and ALG which was in good agreement with their high stickiness being significantly different to all other gel samples

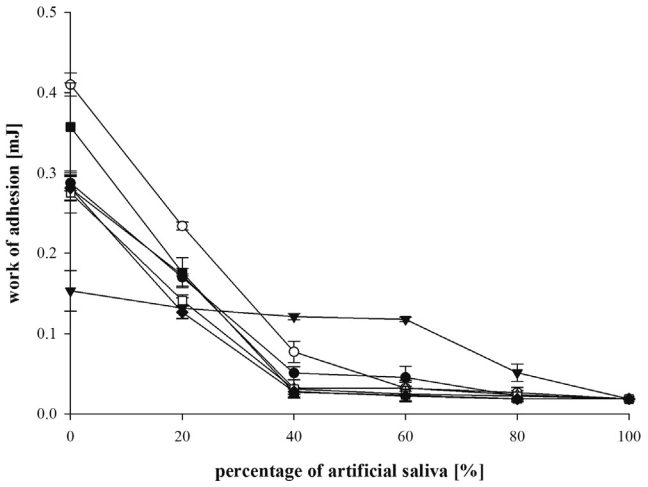

Fig. 4. Results of texture analysis. Work of adhesion of mixtures comprising hydrogels and increasing amounts of artificial saliva measured as the negative area under the force/distance curve. Results for HEC (●), CMC (○), XTGM (▼), HA (△), ALG (■), CP (□) and PCP (♦) with increasing amounts of artificial saliva pH 6.8 at room temperature. Displayed values are means of at least three experiments ± standard deviation.

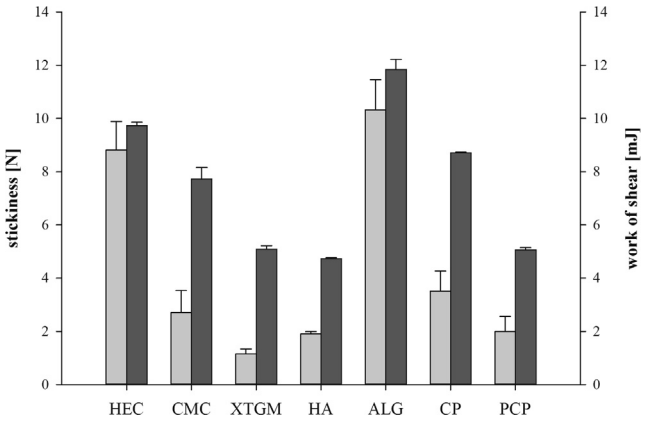

Fig. 5. Results of determination of spreadability. Stickiness (light grey bars) measured as maximum negative force and work of shear (dark grey bars) determined as positive area under the force/distance curve. Values are means of at least three experiments ± standard deviation.

($p < 0.001$) (Fig. 5). The better spreadability over the mucosal surface by hydrogels comprising the other polymers may increase retention under consideration of their adhesiveness when diluted with saliva.

3.5. Tensile studies

Under the employed conditions the rupture of the mucus hydrogel bond occured within the gel itself. The pronounced stickiness observed for ALG hydrogels may contribute to the high resistance of the adhesive bond reflected by the highest MDF of all hydrogels. Poly(acrylic acid) derivatives revealed a liquefication on the mucosal surface due to the present ions in artificial saliva causing the lowest MDF and TWA for CP and PCP. Results for TWA displaying the sum of all established adhesive interactions showed superior adhesive properties for XTGM. MDF and TWA of hydrogel formulations are depicted in Fig. 6A. The formation of an elongated string by the respective hydrogel during the detachment process is a consequence of viscoelastic and cohesive properties of the formulation. The extent of elongation before failure as a measure for the width of the force-deformation, however, did not correlate to the absolute values for MDF and TWA. As shown in Fig. 6B deformation was the highest for CMC and XTGM before rupture of the adhesive

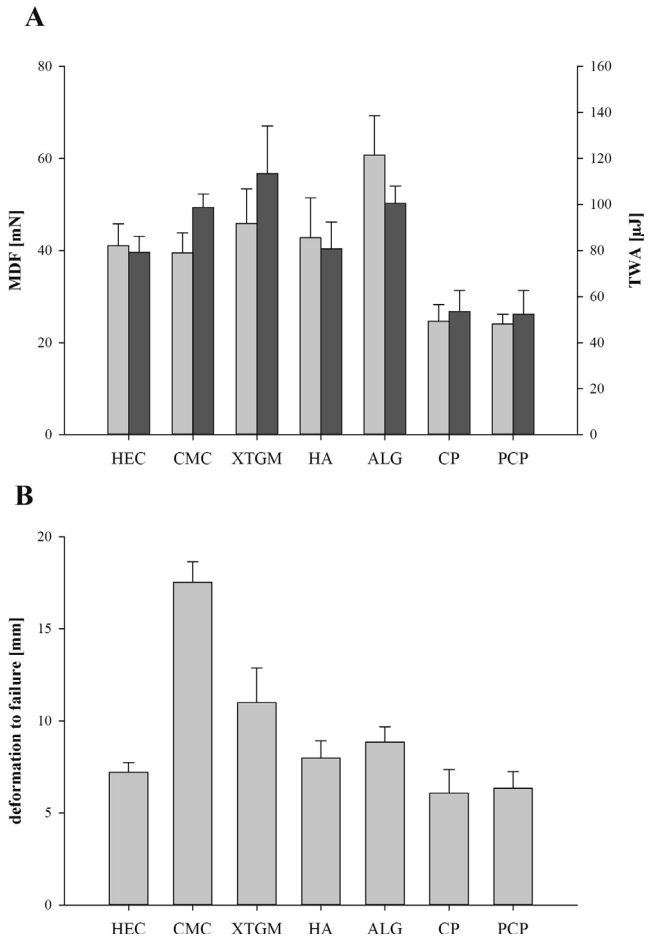

Fig. 6. Results of tensile studies on porcine buccal mucosa. (A) Maximum detachment force (MDF) (light grey bars) and total work of adhesion (TWA) (dark grey bars). (B) Deformation to failure of hydrogel samples. Values are means of at least three experiments ± standard deviation.

bond.

3.6. Mucosal residence time

Brought in contact with the excised buccal mucosa hydrogels showed discrepancies in flow retention as shown in Fig. 7. Due to its *in situ* gelling properties hydrogels comprising XTGM remained cohesive over the measurement period. A gradual wash off was observed for the other polysaccharide hydrogels. After dilution with artificial saliva the retention in the lower part of the mucosa is most pronounced for CMC. CP and PCP were dissolved and thereby removed from the site of application.

Results of healthy volunteers revealed the same rank order of adhesion time as obtained for the percentage of remaining hydrogel from *ex vivo* studies (Fig. 8). Accordingly, XTGM remained significantly longer ($p < 0.05$) than other formulations on human buccal mucosa. A correlation between percentage of remaining hydrogel *ex vivo* and *in vivo* residence time displays a very high correlation with a correlation coefficient of $r = 0.973$. The relationship is displayed in Fig. 9.

4. Discussion

The assessment of mucus-hydrogel interactions plays a vital role in the development of semisolids for mainly local drug delivery. Beyond that, gel formation on the outer surface of solid dosage forms as an inevitable step in the process of mucoadhesion broadens the applicability of aspects found for the performance of hydrogels [3]. However,

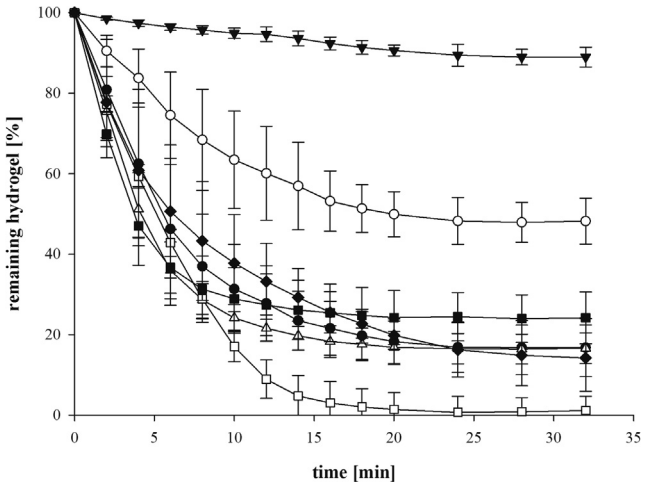

Fig. 7. *Ex vivo* mucosal residence time of HEC (●), CMC (○), XTGM (▼), HA (△), ALG (■), CP (□), PCP (♦) on porcine buccal mucosa mounted in an angle of 90° at 37 °C, 100% humidity. Displayed values are means of at least three experiments ± standard deviation.

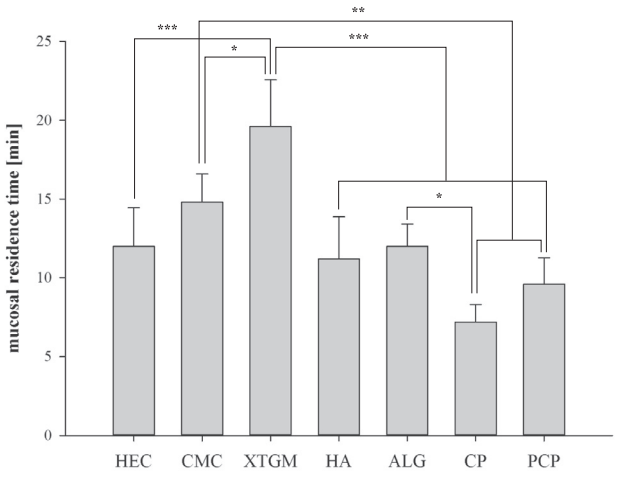

Fig. 8. *In vivo* mucosal residence time of hydrogels on buccal mucosa. Values are means of five volunteers ± standard deviation (*p < 0.05, **p < 0.01, ***p < 0.001).

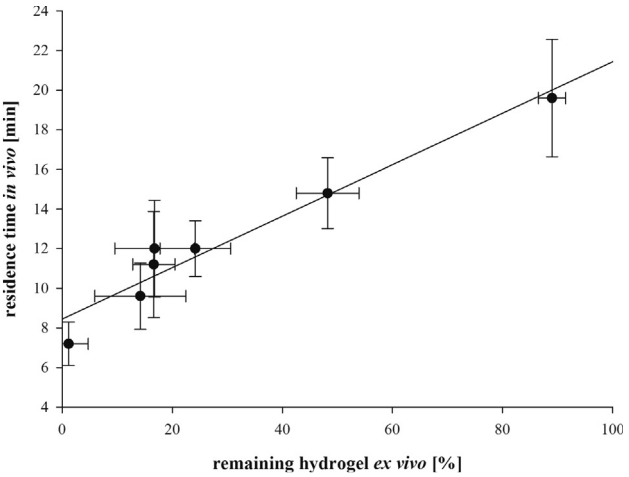

Fig. 9. Correlation between remaining hydrogel on porcine buccal mucosa after 32 min *ex vivo* and residence time on human buccal mucosa. Values are means ± standard deviation of three experiments for *ex vivo* data and means ± standard deviation of five volunteers for the *in vivo* study.

revealing rather weak cohesive properties in comparison to solid dosage forms, the interpenetration layer between mucus and gel is not necessarily stronger than the formulation itself. Therefore, care needs to be taken in the choice and interpretation of methods to evaluate mucoadhesive properties of semisolids, such as hydrogels. To assess established measurement principles for their predictive power of *in vivo* results, test polymers of strongly varying charge density, molecular mass and polymeric backbone were chosen in this study. Unlike previous investigations focusing on the impact of chain flexibility or hydratability of polymers with the same polymeric backbone [20,31,35], this broader range of properties may better prove the applicability of findings from this study. Due to the lack of similar derivatives as references the contribution of single underlying properties of polymers could not be evaluated. However, results of different measurement principles could be correlated to the observed ranking of polymer formulations and compared to *in vivo* data.

As an indirect method to evaluate mucoadhesion focusing on the strength of the interfacial layer the concept of rheological synergism has been introduced by Hassan and Gallo [36]. The usage of different mucin types and processing of extracted mucus as well as varying experimental parameters make comparisons between different studies difficult [6]. Accordingly, the observed negative rheological synergism of poly(acrylic acid) hydrogels being in agreement with previous studies was shown to depend on pH and ion content of different commercially available mucin types [4]. Moreover, rheological synergism was described to occur only in a certain concentration range of polymer depending on the underlying material [18]. Taking these limitations into account, the extent of positive rheological synergism for polysaccharide hydrogels was in agreement with results of the *in vivo* experiment. The ion sensitivity of CP and PCP at the employed low concentration effected their performance throughout this study. By this examination of mucus-polymer interactions no information about the weakest region of the mucoadhesive joint and polymer-surface interactions were obtained making rheology not suitable as a stand alone method to determine mucoadhesion [6,37].

Weakening of the mucoadhesive bond by excessive hydration and therefore a dilution of interacting functional groups plays a fundamental role in the resistance towards removal from the site of application. Equilibrium swelling and moreover the adhesiveness of swollen hydrogels reflected the enhanced interactions in the network of ionic polymers and the superiority of XTGM and CMC *in vivo*. Apart from the extent of adhesiveness of hydrated formulations, high spreadability on the mucosal surface after application increases the area for possible interactions with the underlying mucosa. However, the impact of resistance to shear as well as the stickiness of spreaded formulations did not allow direct correlations to the *in vivo* residence time for the employed formulations.

Nevertheless, a contribution of textural properties to findings from tensile studies could be observed. This is in accordance with a previous study on buccal hydrogels for local delivery of benzydamine hydrochloride [38]. In literature different measurement configurations for tensile studies with hydrogels were described reflecting varying perspectives for the assessment of the mucoadhesive joint. Most studies employed a large volume of hydrogel and measured the MDF and TWA of a mucosa with defined dimensions being moved downwards onto the surface of the bulk gel [8,38]. Hägerström et al. compared results of this set-up to an arrangement where a small amount of hydrogel was placed between two mucosal surfaces. They concluded, that the large volume configuration rather reflects the properties of the gel formulation and expected the small volume set-up to provide more valid results for the interaction between mucosa and formulation [5]. Tensile experiments on the basis of a horizontally aligned mucosa allowed a further variation in the humidification and therefore a tool to adapt to *in vivo* conditions. Higher quantities of artificial saliva, for instance, resulted in comparable MDF for all hydrogels allowing a differentiation only by means of TWA (data not shown). This phenomenon of a lower sensitivity of TWA in comparison to MDF towards changing parameters in tensile tests was also described by Bassi da Silva et al. [39].

Though being the most widely employed approach in bioadhesion studies, the measurement principle of a vertically applied tensile force does not reflect the shear stress by salivary scavenging in the oral cavity. To evaluate the impact of mechanisms contributing to the elimination from the site of application a flow retention study was found to be most suitable for the employed test formulations. Being hydrated by the artificial saliva flow the downwards movement on buccal mucosa and reattachment on lower parts of the tissue contributed to the observed discrepancies between hydrogel samples. This phenomenon could not be reflected by other experiments employed in this study. For an adaptation to *in vivo* conditions a high inclination of the buccal mucosa compared to a previous study as well as a flow rate of 1.5 mL/min was chosen [40]. The consideration of hydration, spreading and interaction with the mucosal surface makes this measurement principle also attractive for particulate formulations allowing to measure adhesion without prior compression to a disc [41].

Due to the widespread use of tensile experiments in the field of bioadhesion the approach to replace biological tissues revealing an inherent variability has emerged [42]. Bassi da Silva et al. compared a synthetic alternative to animal tissue composed of N-acryloylglucosamine and 2-hydroxyethylmethacrylate to porcine buccal mucosa in tensile and flow retention experiments. Significant differences in MDF and TWA were found for two of four tested formulations when comparing biological and synthetic material [32]. The usage of porcine buccal mucosa as a reference could further be confirmed by the very high correlation between the *in vitro* model and *in vivo* data in this study. Moreover, the contribution of polymer entanglements with the surface as well as the formation of non-covalent interactions to adhesion were previously shown by the usage of materials with strongly varying properties in the flow retention set-up [32].

These direct methods for prediction of mucoadhesion *in vivo* have been stressed in literature for solid and semisolid drug delivery systems. The ability of previous studies to correlate results of static set-ups for mechanical force determination and of dynamic methods under imitation of shear forces to *in vivo* data showed strong variations. Besides varying dosage forms, the diversity of parameters and experimental set-ups may contribute to this heterogeneity of results [43,44]. Displaying a comparison of similar measurement principles the correlation of *in vivo* residence time studies with flow retention set-ups may be more suitable.

5. Conclusion

The interpretation of studies focusing on different processes in the context of mucoadhesion is an important tool for the development of novel semisolid dosage forms. For a better understanding various methods to evaluate mucoadhesive properties of different model hydrogels were analysed for their predictability of the residence time in the oral cavity of human volunteers.

Imitating the interfacial joint rheological studies and the determination of normalized rheological synergism parameters allowed to draw conclusions about the *in vivo* performance. The assessment of the weakest region of the mucoadhesive bond by tensile studies revealed a fracture in the gel itself and a reflection of textural properties of hydrated gels by MDF and TWA. Optimized conditions for the employed flow retention experiment displayed a very high correlation to *in vivo* residence time and therefore a promising tool for formulation development of semisolids.

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Declaration of Competing Interest

None.

References

[1] E. Russo, F. Selmin, S. Baldassari, C.G.M. Gennari, G. Caviglioli, F. Cilurzo, P. Minghetti, B. Parodi, A focus on mucoadhesive polymers and their application in buccal dosage forms, J. Drug Delivery Sci. Technol. 32 (2016) 113–125.

[2] V.F. Patel, F. Liu, M.B. Brown, Advances in oral transmucosal drug delivery, J. Control. Release 153 (2011) 106–116.

[3] S. Tamburic, D.Q.M. Craig, A comparison of different in vitro methods for measuring mucoadhesive performance, Eur. J. Pharm. Biopharm. 44 (1997) 159–167.

[4] S. Rossi, M.C. Bonferoni, G. Lippoli, M. Bertoni, F. Ferrari, C. Caramella, U. Conte, Influence of mucin type on polymer-mucin rheological interactions, Biomaterials 16 (1995) 1073–1079.

[5] H. Hägerström, C.A. Bergström, K. Edsman, The importance of gel properties for mucoadhesion measurements: a multivariate data analysis approach, J. Pharm. Pharmacol. 56 (2004) 161–168.

[6] H. Hägerström, K. Edsman, Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method, J. Pharm. Pharmacol. 53 (2001) 1589–1599.

[7] K. Edsman, H. Hägerström, Pharmaceutical applications of mucoadhesion for the non-oral routes, J. Pharm. Pharmacol. 57 (2005) 3–22.

[8] J. das Neves, M.H. Amaral, M.F. Bahia, Performance of an in vitro mucoadhesion testing method for vaginal semisolids: influence of different testing conditions and instrumental parameters, Eur. J. Pharmaceut. Biopharmaceut. 69 (2008) 622–632.

[9] J. Bassi da Silva, S.B.d.S. Ferreira, A.V. Reis, M.T. Cook, M.L. Bruschi, Assessing mucoadhesion in polymer gels: the effect of method type and instrument variables, Polymers 10 (2018) 254.

[10] I.G. Needleman, G.P. Martin, F.C. Smales, Characterisation of bioadhesives for periodontal and oral mucosal drug delivery, J. Clin. Periodontol. 25 (1998) 74–82.

[11] I.G. Needleman, F.C. Smales, In vitro assessment of bioadhesion for periodontal and buccal drug delivery, Biomaterials 16 (1995) 617–624.

[12] S. Kockisch, G.D. Rees, S.A. Young, J. Tsibouklis, J.D. Smart, A direct-staining method to evaluate the mucoadhesion of polymers from aqueous dispersion, J. Control. Release 77 (2001) 1–6.

[13] K. Tietz, S.I. Gutknecht, S. Klein, Predicting local drug availability of locally acting lozenges: From method design to a linear level A IVIVC, Eur. J. Pharm. Biopharm. 133 (2018) 269–276.

[14] A. Partenhauser, A. Bernkop-Schnürch, Mucoadhesive polymers in the treatment of dry X syndrome, Drug Discov. Today 21 (2016) 1051–1062.

[15] S. Tamburic, D.Q.M. Craig, An investigation into the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems, J. Control. Release 37 (1995) 59–68.

[16] I. Pereira de Sousa, B. Cattoz, M.D. Wilcox, P.C. Griffiths, R. Dalgliesh, S. Rogers, A. Bernkop-Schnürch, Nanoparticles decorated with proteolytic enzymes, a promising strategy to overcome the mucus barrier, Eur. J. Pharm. Biopharm. 97 (2015) 257–264.

[17] M.D. Wilcox, L.K. Van Rooij, P.I. Chater, I. Pereira de Sousa, J.P. Pearson, The effect of nanoparticle permeation on the bulk rheological properties of mucus from the small intestine, Eur. J. Pharm. Biopharm. 96 (2015) 484–487.

[18] F. Madsen, K. Eberth, J.D. Smart, A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration, J. Control. Release 50 (1998) 167–178.

[19] S. Rossi, B. Vigani, M.C. Bonferoni, G. Sandri, C. Caramella, F. Ferrari, Rheological analysis and mucoadhesion: A 30 year-old and still active combination, J. Pharm. Biomed. Anal. 156 (2018) 232–238.

[20] S. Rossi, M.C. Bonferoni, F. Ferrari, M. Bertoni, C. Caramella, Characterization of mucin interaction with three viscosity grades of sodium carboxymethylcellulose. Comparison between rheological and tensile testing, Eur. J. Pharmaceut. Sci. 4 (1996) 189–196.

[21] A. Preetha, R. Banerjee, Comparison of artificial saliva substitutes, Trends Biomater. Artif. Organs 18 (2005) 178–186.

[22] A. Tai, R. Bianchini, J. Jachowicz, Texture analysis of cosmetic/pharmaceutical raw materials and formulations, Int. J. Cosmet. Sci. 36 (2014) 291–304.

[23] I.D. Rupenthal, C.R. Green, R.G. Alany, Comparison of ion-activated in situ gelling systems for ocular drug delivery. Part 1: physicochemical characterisation and in vitro release, Int. J. Pharm. 411 (2011) 69–77.

[24] R.R. Pereira, J.S. Ribeiro Godoy, T.I. Stivalet Svidzinski, M.L. Bruschi, Preparation and characterization of mucoadhesive thermoresponsive systems containing propolis for the treatment of vulvovaginal candidiasis, J. Pharm. Sci. 102 (2013) 1222–1234.

[25] M. Javanmard, N.L. Chin, S.H. Mirhosseini, J. Endan, Characteristics of gelling agent substituted fruit jam: studies on the textural, optical, physicochemical and sensory properties, Int. J. Food Sci. Technol. 47 (2012) 1808–1818.

[26] M. Ijaz, M. Ahmad, N. Akhtar, F. Laffleur, A. Bernkop-Schnürch, Thiolated α-cyclodextrin: the invisible choice to prolong ocular drug residence time, J. Pharm. Sci. 105 (2016) 2848–2854.

[27] Y. Sudhakar, K. Kuotsu, A.K. Bandyopadhyay, Buccal bioadhesive drug delivery–a promising option for orally less efficient drugs, J. Control. Release 114 (2006) 15–40.

[28] I. Pereira de Sousa, C. Steiner, M. Schmutzler, M.D. Wilcox, G.J. Veldhuis, J.P. Pearson, C.W. Huck, W. Salvenmoser, A. Bernkop-Schnürch, Mucus permeating carriers: formulation and characterization of highly densely charged nanoparticles, Eur. J. Pharm. Biopharm. 97 (2015) 273–279.

[29] H.T. Lam, G. Leonaviciute, O. Zupančič, A. Bernkop-Schnürch, Thiomers: Impact of in situ cross-linkers on mucoadhesive properties, Eur. J. Pharm. Sci. 106 (2017) 41–48.

[30] I. Pereira de Sousa, W. Suchaoin, O. Zupančič, C. Leichner, A. Bernkop-Schnürch, Totally S-protected hyaluronic acid: Evaluation of stability and mucoadhesive properties as liquid dosage form, Carbohydr. Polym. 152 (2016) 632–638.

[31] H. Hägerström, K. Edsman, Limitations of the rheological mucoadhesion method: The effect of the choice of conditions and the rheological synergism parameter, Eur. J. Pharm. Sci. 18 (2003) 349–357.

[32] J.B. da Silva, V.V. Khutoryanskiy, M.L. Bruschi, M.T. Cook, A mucosa-mimetic material for the mucoadhesion testing of thermogelling semi-solids, Int. J. Pharm. 528 (2017) 586–594.

[33] C.-E. Brunchi, M. Bercea, S. Morariu, M. Dascalu, Some properties of xanthan gum in aqueous solutions: effect of temperature and pH, J. Polym. Res. 23 (2016) 123.

[34] D.S. Jones, A.D. Woolfson, J. Djokic, W.A. Coulter, Development and mechanical characterization of bioadhesive semi-solid, polymeric systems containing tetracycline for the treatment of periodontal diseases, Pharm. Res. 13 (1996) 1734–1738.

[35] F. Ferrari, S. Rossi, M.C. Bonferoni, C. Caramella, J. Karlsen, Characterization of rheological and mucoadhesive properties of three grades of chitosan hydrochloride, Farmaco (Societa chimica italiana) 52 (1997) (1989) 493–497.

[36] E.E. Hassan, J.M. Gallo, A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength, Pharm. Res. 7 (1990) 491–495.

[37] F. Madsen, K. Eberth, J.D. Smart, A rheological assessment of the nature of interactions between mucoadhesive polymers and a homogenised mucus gel, Biomaterials 19 (1998) 1083–1092.

[38] S.Y. Karavana, P. Guneri, G. Ertan, Benzydamine hydrochloride buccal bioadhesive gels designed for oral ulcers: preparation, rheological, textural, mucoadhesive and release properties, Pharm. Dev. Technol. 14 (2009) 623–631.

[39] J. Bassi da Silva, S. Ferreira, A. Valim Reis, M. Cook, M. Bruschi, Assessing Mucoadhesion in Polymer Gels: The Effect of Method Type and Instrument Variables, Polymers, 2018.

[40] S. Bonengel, S. Hauptstein, G. Leonaviciute, J. Griessinger, A. Bernkop-Schnürch, Thiolated alkyl-modified carbomers: Novel excipients for mucoadhesive emulsions, Eur. J. Pharm. Sci. 75 (2015) 123–130.

[41] K.D. Madsen, C. Sander, S. Baldursdottir, A.M.L. Pedersen, J. Jacobsen, Development of an ex vivo retention model simulating bioadhesion in the oral cavity using human saliva and physiologically relevant irrigation media, Int. J. Pharm. 448 (2013) 373–381.

[42] D. Caccavo, G. Lamberti, S. Cascone, A.A. Barba, A. Larsson, Understanding the adhesion phenomena in carbohydrate-hydrogel-based systems: Water up-take, swelling and elastic detachment, Carbohydr. Polym. 131 (2015) 41–49.

[43] M. Davidovich-Pinhas, H. Bianco-Peled, Mucoadhesion: a review of characterization techniques, Expert Opin. Drug Deliv. 7 (2010) 259–271.

[44] C. Woertz, M. Preis, J. Breitkreutz, P. Kleinebudde, Assessment of test methods evaluating mucoadhesive polymers and dosage forms: an overview, Eur. J. Pharm. Biopharm. 85 (2013) 843–853.