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Mucoadhesion on pig vesical mucosa: influence of polycarbophil/calcium interactions

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Abstract

The influence of polycarbophil/calcium interactions on the mucoadhesive properties of polycarbophil has been examined. Polycarbophil dispersions and films with different concentrations of calcium or sodium ions were prepared and the following parameters were measured: detachment force on pig vesical mucosa, zeta potential, pH and viscosity. Polycarbophil detachment force decreased significantly in the presence of calcium but not sodium. Both ions decrease the pH of polycarbophil dispersions. On the other hand, altering the pH of hydrated polycarbophil films in the absence of added ions had an insignificant effect on detachment force. Both ions reduce the absolute values of polycarbophil zeta potential, calcium more efficiently than sodium. We could conclude that decreased mucoadhesion strength of polycarbophil in the presence of calcium is due to the chelation of polycarbophil carboxylic groups by calcium and crosslinking of polymer. The crosslinked polymer chains would be expected to be less flexible, and therefore, interpenetrate to a lesser extent with the glycosaminoglycans of mucus. Additionally, the interactions between functional groups of polycarbophil and mucus glycosaminoglycans are lowered due to the calcium, blocking the carboxylic groups. The mechanism of calcium influence on viscosity of polycarbophil dispersions appears to be different: repulsion between ionised carboxylic groups of polycarbophil prevails over the crosslinking of polycarbophil by calcium.

Keywords: Polycarbophil; Calcium binding; Mucoadhesion; pH; Zeta potential; Viscosity

1. Introduction

Mucoadhesion can be defined as the ability of a synthetic or biological material to adhere to mucosa or mucus for an extended period of time. For mucoadhesion to occur, first an intimate contact must exist between the mucoadhesive polymer and the mucosa. After the contact is established, the most important process is interpenetration of polymer chains with those of the mucus. Secondary chemical bonds can then settle (electrostatic, van der Waals forces, hydrogen and hydrophobic interactions). Primary chemical bonds with their covalent nature are undesirable (Mikos and Peppas, 1986; Duchêne et al., 1988).

Mucus, biological component involved in mucoadhesion, is highly viscous product that covers the mucosa surface. The mucus composition

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varies depending on its source. The most important component of the mucus layer of the urinary bladder are highly anionic glycosaminoglycans (GAG), which are present at bladder luminal surface at very high density (Hurst et al., 1987; Hurst and Zebrowski, 1994). Heparan sulfate, dermatan sulfate and chondroitin sulfate are the most frequently isolated protein-bound GAG from bovine as well as human luminal surface of urinary bladder. Additionally, many GAG and glycoprotein molecules are loosely adherent on the bladder surface. Glycoproteins of the intestinal mucus possess oligosaccharide chains, attached to proteins, which carry negative charge due to sialic acid and sulfates. There are some similarities between mucus layers in urinary bladder and intestine, although they differ in structure and thickness. On the basis of their structure, greater negative charge of urinary bladder mucus in comparison with intestinal could be expected. This could serve as one of the explanations for the lower detachment force of polymer carrying negative charge (Carbopol) on urinary bladder than on intestinal mucosa (Bogataj et al., 1999b). Anyhow, if compared with intestinal mucosa, urinary bladder mucosa is less frequently studied and thus needs to be investigated thoroughly.

Mucoadhesive drug delivery systems are designed to adhere to mucosa of an appropriate organ for a sufficiently long time to improve the extent of release and absorption of the drug. Polycarbophil (BFGoodrich Company, 1998), homopolymer of acrylic acid crosslinked with divinyl glycol, is one of the most potent mucoadhesive polymers in use. The molecular weight of the polymer is in the billions and the pK_a is 6.0 ± 0.5. Polycarbophil does not dissolve in water, but it swells up to 1000 times its original volume to form a gel when exposed to a pH environment above 4.0–6.0. Numerous carboxylic groups on the polymer backbone are the main functional groups of polycarbophil and are responsible for major part of its properties.

Polycarbophil mucoadhesion is greatly influenced by the pH of the medium. The pH-detachment force profiles described in the literature (Ch'ng et al., 1985; Park and Robinson, 1985) differ significantly although both series of experiments were performed on rabbit stomach tissue. All results in the article (Park and Robinson, 1985) were compared with the pK_a value of polyacrylic acid which is 4.75. It was shown that at pH values between 2 and 6 the detachment force of polycarbophil decreases with increasing pH, presumably due to increasing repulsion by carboxylic groups. At pH higher than 6, the charge repulsion dominates and the detachment force becomes insignificant. Another parameter that might influence the mucoadhesion strength is swelling property of polymer. At pH higher than the pK_a of polyacrylic acid, polycarbophil absorbs water to 100–800 times its weight, depending on the ionic strength of the aqueous medium. The authors concluded that the extent of polycarbophil swelling is not the major factor in mucoadhesion. In another study (Ch'ng et al., 1985), also performed on rabbit stomach tissue, the pH-detachment force profile obtained was different. The detachment force increased with increasing pH at pH values between 2 and 6 and maximum mucoadhesion was observed at pH values between 5 and 6. At pH 7, mucoadhesion was significantly reduced as a consequence of the increased negative charge repulsion between mucus and polycarbophil. Up to pH 6 the degree of swelling appeared to be one of the factors affecting the mucoadhesion of polycarbophil. The discrepancy in these two profiles is due to the changes in experimental techniques (Park and Robinson, 1985).

In addition to mucoadhesive properties, polycarbophil also has the ability to enhance the intestinal absorption of drugs (Lehr et al., 1992), which could be explained by the chelation of calcium ions by polycarbophil (Kriwet and Kissel, 1996). Calcium ions have an important role in tight junctions. The removal of extracellular calcium causes the opening of tight junctions that results in enhanced paracellular transport (Lacaz-Vieira, 1997). Polycarbophil may bind calcium and sodium ions but has a higher affinity for divalent than for monovalent ions. The binding of ions depends on the degree of ionisation of the carboxylic groups of polycarbophil (Kriwet and Kissel, 1996).

Thus, it has been already shown that calcium is involved in the drug absorption enhancement caused by polycarbophil. Additionally, Lejoyeux et al. (1989) reported that calcium ions can influence the mucoahesion of poly(acrylic acid) derivatives depending on the mucosa tested. When the experiments were carried out on sublingual mucosa, detachment force and adhesion work decreaed with increasing calcium chloride concentration in the test media. On the other hand, experiments performed on vaginal mucosa showed that calcium has no significant influence on the mucoadhesion.

Calcium ions are present practically everywhere in the human body, where drug delivery systems can be applied. If polycarbophil drug delivery system, i.e. mucoadhesive microspheres, is applied as a suspension into the urinary bladder, polycarbophil can bind calcium, seeing that normal men and women excrete in the urine up to 300 and 250 mg of calcium per day, respectively (Endres and Rude, 1994). Moreover, calcium can be incorporated in a drug delivery system, for example in the medium where mucoadhesive microspheres are suspended before the application. In both cases chelation of calcium by polycarbophil could influence the mucoadhesive properties of a drug delivery system.

Our objective is to develop mucoadhesive microspheres, which can be used in the treatment of urinary bladder infections and superficial bladder cancer. After the application through a urethral catheter microspheres are expected to adhere on mucosa of urinary bladder and release incorporated drug over a certain period of time. Such drug delivery systems would be beneficial for patients, because the side effects of toxic drugs would be lower and the time when drug is present at the site of action would be prolonged and not dependent on the time of urination.

The purpose of this work, where polycarbophil is used as mucoadhesive polymer, is to determine if and how calcium ions influence the mucoadhesive properties of polycarbophil. Different methods were applied to get an insight into mechanism of interactions between mucus, polycarbophil and calcium. The experiments were performed on isolated mucosa of pig urinary bladder.

2. Materials and methods

2.1. Materials

Polycarbophil Noveon AA-1 was gift of BF-Goodrich, Ohio.

The stock solutions were 0.001, 0.1 and 0.4 M NaCl and 0.001, 0.1 and 0.4 M $CaCl_2$ prepared from $CaCO_3$ and HCl.

Phosphate buffer consisted of 0.472 g Na_2HPO_4, 0.095 g KH_2PO_4 and 1.6 g NaCl in 1 l of deionised water (pH 7.4).

Phosphate buffer saline (Ph. Eur. III) consisted of 2.38 g Na_2HPO_4 12 H_2O, 0.19 g KH_2PO_4 and 8 g NaCl in 1 l of deionised water (pH 7.4).

All chemicals used were of analytical grade.

2.2. Determination of detachment force

First the dispersions of polycarbophil and calcium or sodium ions were prepared in deionised water. The molar ratio of ions and carboxylic groups of polycarbophil was varied from 0 to 0.2 for calcium and from 0 to 0.25 for sodium. All dispersions had the same concentration of polycarbophil (1% w/v). The films were obtained by pouring 0.505 g of these dispersions on a 4 cm^2 of a glass plate and dried overnight at room temperature. Dry films weighed 1.25 mg/cm^2.

As calcium influences the pH of polycarbophil films, the impact of pH on mucoadhesion strength of polycarbophil in the absence of calcium or sodium ions was evaluated. For that purpose polycarbophil dispersions (1% w/v) in deionised water without calcium or sodium were prepared and their pH was adjusted with 0.1 M HCl so that the hydrated films in the presence or in the absence of calcium had the same pH values. Otherwise polymeric films for determining the influence of pH on polycarbophil mucoadhesion strength were prepared from the dispersions in the same way as the films with calcium. The weight of dry films was also 1.25 mg/cm^2.

The detachment force between polymeric films and pig mucosa was measured by a modified precision balance. The bladders were obtained from a local slaughterhouse and kept in carbogen saturated phosphate buffer saline at room temper-

ature until used. All experiments were performed within 5 h of sacrifice, as scanning electron microscopy examination of urinary bladder showed that bladder surface epithelium remained intact under described experimental conditions within the chosen period of time (Burjak et al., 2000). The mucosa of the middle part of the urinary bladder was used. Before mounting to the lower support of the apparatus, the mucosa was washed for 30 s with phosphate buffer or deionised water. The volume of aqueous medium that remained on the mucosa surface was limited and approximately equal in all experiments. The polymeric film that was prepared on a glass plate was mounted to the upper clamp of the apparatus. After the clamp with the tissue was slowly raised, making contact with dry polycarbophil film, immediately an additional weight of 10 g was added. Two minute after the formation of the contact, the upper support of the apparatus was raised with a constant rate (0.2 N/min) and the detachment force needed for the separation of the two surfaces was determined. Results are means of four to nine experiments.

2.3. Zeta potential measurements

The zeta potential of polycarbophil dispersions containing calcium or sodium ions was measured by Zetasizer 3000 (Malvern Instruments, GB). Each sample was measured at least seven times at 25 °C. One series of polycarbophil dispersions with calcium ions was prepared in phosphate buffer. The other series with calcium, as well as all the dispersions with sodium, were prepared in bidistilled water. The molar ratio of calcium to carboxylic groups of polycarbophil was from 0 to 0.2 and the molar ratio of sodium to carboxylic groups was from 0 to 0.5. All dispersions contained the same concentration of polycarbophil (0.0075% w/v) and they were stirred for 1 h before the zeta potential measurements.

2.4. Viscosity and pH determination

Polycarbophil dispersions were prepared in phosphate buffer or in deionised water. All dispersions had the same concentration of polycarbophil (11.1% w/v). Calcium or sodium ions in phosphate buffer or deionised water were added to obtain molar ratios of calcium to carboxylic groups of polycarbophil from 0 to 0.2 and molar ratios of sodium to carboxylic groups from 0 to 0.4. Polycarbophil concentration (11.1% w/v) was approximately the same as in the films, which hydrate during detachment force determination and was calculated considering the amount of deionised water or phosphate buffer that is available to polycarbophil films during detachment force determination. After stirring the dispersions for 1 h, they were additionally homogenised and left overnight to ensure complete hydration of the polymer.

Viscosity was measured using rotation rheometry (Rheolab MC 100, Paar Physica, Stuttgart, Germany). The apparent viscosity was determined with a cone and plate measuring system KP 22 (diameter 25 mm, α 1°, cone-plate distance 50 μm). The experiments were carried out at constant temperature (20 ± 0.5 °C) and constant shear rate ($10\ s^{-1}$). The apparent viscosity was determined as a function of time.

pH of the dispersions was measured by pH meter MP 225, Mettler, Toledo, Spain.

3. Results and discussion

Polycarbophil is mucoadhesive polymer that can chelate different cations (Kriwet and Kissel, 1996). It is expected that binding of cations will influence the polycarbophil mucoadhesion strength as carboxylic groups of polycarbophil are involved in the interactions with the functional groups of mucus.

In the experiments where detachment forces were measured polycarbophil films were used, although the aim of our work was to develop mucoadhesive microspheres. Polycarbophil, which is the only component of the films, is also the main component of the microspheres responsible for mucoadhesion. Besides that, changes in the mucoadhesion strength produced by calcium ions are probably the consequence of its interactions solely with polycarbophil.

The influence of molar ratio of calcium to carboxylic groups of polycarbophil on the detachment force is shown in Fig. 1.

A large reduction in the detachment force in the presence of calcium ions can be seen in both phosphate buffer and deionised water. At lower molar ratios of calcium to carboxylic groups the dependence of detachment force on calcium is markedly greater than at higher ratios. Thus, at molar ratio varying from 0.06 to 0.2, calcium ions significantly decrease the detachment force of polycarbophil in phosphate buffer as well as in deionised water (t-test, $P < 0.05$) if compared with the detachment force of polycarbophil films without calcium ions.

With the purpose to obtain controlled ionic composition on the mucosa surface (i.e. mucus), mucosa was washed by phosphate buffer or deionised water before the determination of detachment force. The medium did not have significant influence on the detachment force (t-test, $P > 0.05$) under the described conditions (Fig. 1).

It is obvious from Fig. 1 that detachment force of polycarbophil films is lowered in the presence of calcium what could be explained in different ways. Within the scope of this work four of them were considered. First, chelation of polycarbophil carboxylic groups by calcium may occur. This would be expected to decrease the ability of polycarbophil to interact with the mucus. Second, calcium ions also lower the pH of polycarbophil dispersions and may affect the interaction in this way. Third, consequence of calcium chelation could be to reduce repulsion between negatively charged mucus and polycarbophil. At last, the viscosity of hydrated polycarbophil films could also play an important role in mucoadhesion.

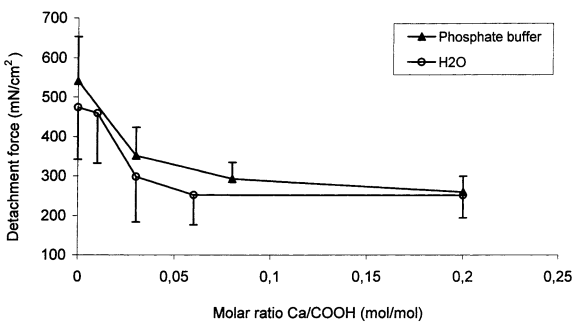

Fig. 1. The influence of molar ratio of calcium ions to carboxylic groups of polycarbophil on detachment force when mucosa was washed by phosphate buffer or deionised water (mean ± S.D., $n = 4–5$).

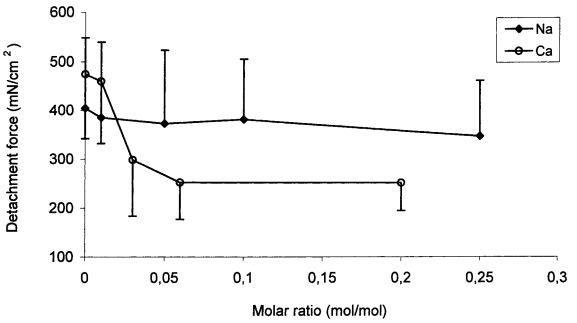

Fig. 2. The influence of molar ratio of sodium or calcium ions to carboxylic groups of polycarbophil on detachment force. Mucosa was washed by deionised water (mean ± S.D., $n = 5–9$).

3.1. The influence of calcium chelation of carboxylic groups

Within the interval from 0 to 0.2 molar ratio of calcium to polycarbophil carboxylic groups, the amount of calcium, bound to polycarbophil, increases almost linearly with the increasing molar ratio (Bogataj et al., 1999a). Similar results were found by Kriwet and Kissel (1996). With increasing calcium concentration fewer carboxylic groups are free for the formation of interactions with the mucus.

The influence of sodium on detachment force compared with calcium is shown in Fig. 2. In both cases mucosa was washed by deionised water. Compared with the strong influence of calcium on mucoadhesive properties of polycarbophil, sodium ions do not significantly influence the detachment force of polycarbophil films (t-test, $P > 0.05$ for all molar ratios). Divalent cations can bind intra or inter molecularly and thus chelate the carboxylic groups of polycarbophil, but may also crosslink the polymer. The crosslinked polymer chains would be expected to be less flexible and therefore reduce the interpenetration of the polymer chains with the GAG of mucus. Additionally, the extent of the interactions between functional groups of polymer and GAG is lowered due to the calcium, blocking the car-

boxylic groups. Sodium as monovalent cation is not able to crosslink the polymer and, because of this, has a much smaller effect on mucoadhesive properties of polycarbophil. Polycarbophil has also lower affinity for monovalent cations (Kriwet and Kissel, 1996) and thus divalent cation (i.e. calcium) prevents the interactions much more efficiently than sodium.

3.2. The influence of pH

We wanted to establish if the reduced detachment force in the presence of calcium is due to decreased pH, which is a consequence of calcium binding to the carboxylic groups of polycarbophil. Polycarbophil swelling and the charge repulsion between polycarbophil and mucus are both influenced by the pH of the medium. In this way pH influences the mucoadhesion strength of polycarbophil (Ch'ng et al., 1985; Park and Robinson, 1985).

The influence of calcium and sodium ions on the pH of polycarbophil dispersions prepared in deionised water or phosphate buffer is shown in Fig. 3. Increasing amount of calcium in the polycarbophil dispersions decreases the pH of the dispersions. The same is true for sodium, but here the decrease of pH is smaller compared with calcium. The decrease of pH caused by calcium was similar if the dispersions were prepared in deionised water or phosphate buffer due to the low capacity of phosphate buffer.

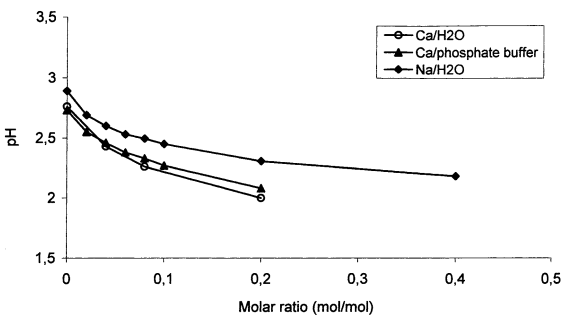

Fig. 3. The influence of added calcium and sodium ions on the pH of polycarbophil dispersions. Dispersions were prepared in phosphate buffer or deionised water.

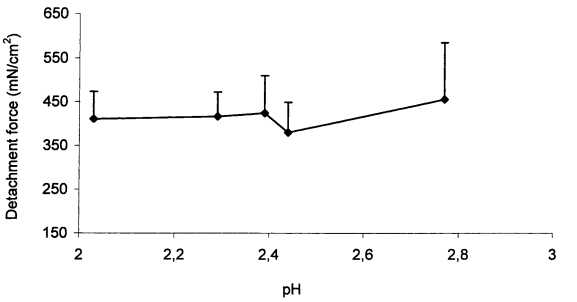

Fig. 4. Detachment force as a function of pH of polycarbophil films in the absence of calcium and sodium ions (mean ± S.D., $n = 6$). Mucosa was washed by deionised water.

In the polycarbophil dispersion in aqueous medium there is an equilibrium between ionised and nonionised carboxylic groups. Addition of calcium or sodium ions to the dispersion results in the partial displacement of hydrogen ions on the carboxylic groups. Calcium as a divalent cation has the ability to replace twice as many hydrogen ions as sodium. Polycarbophil has also lower affinity for binding monovalent ions (Kriwet and Kissel, 1996). Both can explain the smaller decrease of pH in the presence of sodium.

It is known (Ch'ng et al., 1985; Park and Robinson, 1985) that mucoadhesion strength is influenced by the pH of the medium, although the data are contradictory. Ch'ng et al. (1985) showed that up to pH 6 mucoadhesion strength between polycarbophil and rabbit stomach mucosa increases with increasing pH while Park and Robinson (1985) reported that it decreases. We, therefore, examined the detachment force between mucosa and polycarbophil films in the absence of calcium and sodium as a function of pH (Fig. 4). The pH of the polymeric films within the pH range 2–2.8 has no significant influence on detachment force (t-test, $P > 0.05$) under applied conditions. This means that other parameters have much greater influence on mucoadhesion strength than lowering of pH by calcium.

Sodium ions also lower pH of the polycarbophil dispersions (Fig. 3) but have no significant effect on detachment force (Fig. 2). This is further evidence for the minor importance of the pH decrease caused by calcium and sodium on mucoadhesion strength of polycarbophil.

3.3. The influence of polycarbophil/mucus functional groups repulsion

Zeta potential is a measure of the net surface charge on a particle in a dispersed phase. It serves as an important parameter in determination of the electrostatic interactions between particles in dispersed systems and can characterize the properties of the dispersion affected by this electrical phenomenon (Müller et al., 1996; Li and Tian, 1997). Fig. 5 shows the zeta potential of the polycarbophil dispersions with calcium or sodium ions as a function of molar ratio between these ions and carboxylic groups of polycarbophil. The dispersions were prepared in bidistilled water or phosphate buffer. With increasing amount of sodium or calcium ions the zeta potential of dispersions in bidistilled water becomes decreasingly negative due to the compensation of polycarbophil negative charges. Calcium decreases the negative zeta potential more efficiently than sodium. The explanation for this could be in different valency of sodium and calcium ions and different affinity of polycarbophil for these two ions. The added calcium did not influence the zeta potential if the polycarbophil dispersions were prepared in phosphate buffer. This is due to the very much lower concentrations of added calcium compared to the buffer ions.

As polycarbophil concentration in the dispersions prepared for zeta potential measurements was low (0.0075% w/v), also calcium concentrations used to obtain desired molar ratios were

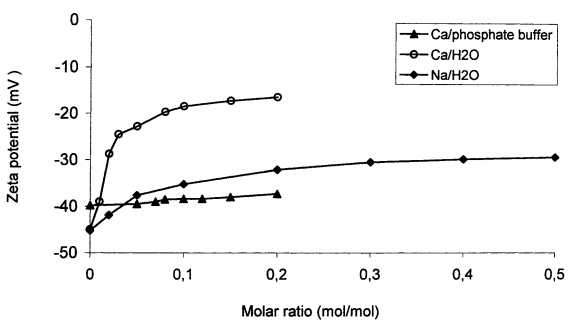

Fig. 5. The influence of molar ratio of sodium or calcium ions to carboxylic groups of polycarbophil on zeta potential of polycarbophil dispersions. The dispersions were prepared in phosphate buffer or bidistilled water.

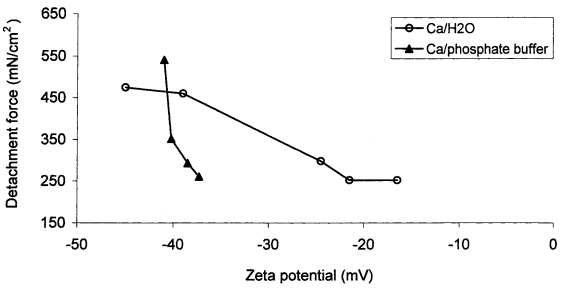

Fig. 6. The correlation between zeta potential and detachment force for polycarbophil dispersions containing calcium. The dispersions were prepared in bidistilled/deionised water or phosphate buffer and both parameters were measured at the same molar ratios of calcium to carboxylic groups of polycarbophil.

low. There was a theoretical possibility of calcium phosphate precipitation in dispersions prepared in phosphate buffer, but all dispersions were found by visible examination completely clear.

The correlation between zeta potential and detachment force for polycarbophil systems with calcium is shown in Fig. 6. Both parameters were measured at the same molar ratios of calcium and carboxylic groups of polycarbophil. The dispersions were prepared in bidistilled/deionised water or phosphate buffer. Takeuchi et al. (2001) reported that positively charged liposomes show a higher mucoadhesion to the rat intestine mucus layer than the negatively charged ones. This was attributed to the negative charge of the mucus layer. Also in our case it was expected that a less negative zeta potential of polycarbophil dispersions would result in lower repulsion of polycarbophil with negatively charged mucus and therefore the detachment force would increase. But as seen from Fig. 6 this correlation is, for dispersions in water, just opposite to what is expected. Additionally, the absolute zeta potential values of polycarbophil dispersions in the presence of calcium decline in bidistilled water much more strongly than in phosphate buffer (Fig. 5), but the decrease in detachment force is similar in both cases (Fig. 1). These confirm the minor contribution of the lowered repulsion between polycarbophil and mucus to mucoadhesion strength in the presence of calcium.

3.4. The influence of viscosity

When measuring the detachment force, the separation of the contact between mucosa and polymeric film can occur on their interface or inside the mucus, but separation as a result of disruption of the polymeric hydrogel is also possible. In the latter case the influence of viscosity on detachment force would be very important: with decreasing viscosity a decrease in detachment force would be expected. In our experiments however it was observed that separation occurred mainly at the polymer-mucosa interface and the influence of viscosity would therefore be of minor importance. In spite of that small residues of polymer, which could not be observed macroscopically, can rest on the surface of mucosa and the part of the contact can be fractured inside the polycarbophil film. Additionally, we expected that the results of viscosity measurements may give us insight into the processes occurring during mucoadhesion.

It is known from the literature (BFGoodrich Company, 1998) that cations as well as pH, influence the viscosity of the different dispersions of Carbopol, which have very similar structure as polycarbophil. In Fig. 7 the viscosity of polycarbophil dispersions prepared in phosphate buffer or deionised water, is shown as a function of molar ratio of ions and counterions. The viscosity is not significantly influenced by the medium of the dispersions. With increasing concentration of calcium or sodium ions the viscosity of polycarbophil dispersions decreases. This is probably due to decrease of the charge repulsion between carboxylic groups of polycarbophil. Additional parameter, which could also influence the viscosity of polycarbophil dispersions, is the ability of calcium ions to crosslink the polymer. As there are no meaningful differences in polycarbophil viscosity in the presence of calcium or sodium ions at measured molar ratios, crosslinking of polycarbophil could not be considered as the major factor influencing the viscosity in our system. However, it is reported in the literature (BFGoodrich Company, 1998) that at higher weight ratios of sodium or calcium ions to Carbopol the differences in the Carbopol viscosity appear. Our results are consistent with those of Charman et al. (1991) who showed that addition of calcium to aqueous dispersions of Carbopol 934P causes a decrease in the viscosity of the dispersions. The authors concluded that with increasing concentrations of calcium the viscosity decreases due to the decrease in the electrostatic repulsion between ionised carboxylic groups and to a decrease in polymer hydration. Moreover, they also reported that the viscosity of Carbopol 934P dispersions is highly pH dependent. At pH lower than 6 the viscosity decreases with decreasing pH. At low pH values the viscosity of other Carbopol dispersions also decreases with decreasing pH (BFGoodrich Company, 1998). In our system beside electrostatic interactions between ionised carboxylic groups of polycarbophil also pH and polycarbophil hydration could influence the viscosity of polycarbophil dispersions in the presence of calcium or sodium.

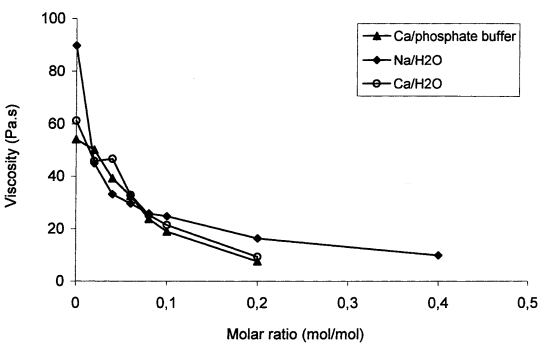

Fig. 7. Viscosity of polycarbophil dispersions prepared in deionised water or phosphate buffer as a function of molar ratio of sodium or calcium ions to carboxylic groups of polycarbophil.

It would be expected that interactions between polycarbophil and mucus are comparable with those inside the polymeric film. Consequently, mucoadhesion strength could be predicted from much more simple viscosity measurements. We have confirmed that at measured molar ratios crosslinking of polycarbophil by calcium has much more important influence on polycarbophil mucoadhesion strength than on its viscosity. On the other hand repulsion is probably the most important parameter for viscosity. Determination of viscosity alone is, therefore, not of itself a reliable method for predicting the polycarbophil

mucoadhesive properties in the presence of calcium.

4. Conclusion

We can conclude that binding of calcium by polycarbophil results in lower detachment force whereas sodium has no such influence. We have shown that calcium does not influence the detachment force of polycarbophil by lowering the pH of the hydrated polymeric films or by lowering the electrostatic repulsion between carboxylic groups of polycarbophil and negatively charged GAG of vesical mucus. The decreased mucoadhesion strength of polycarbophil in the presence of calcium is due to the chelation of calcium by polycarbophil carboxylic groups and crosslinking of polymer. The extent of interactions between functional groups of polycarbophil and mucus GAG is lowered due to the calcium, blocking the carboxylic groups of polymer. Additionally, the crosslinked polymer chains would be expected to be less flexible and to interpenetrate to a lesser extent with the GAG of mucus.

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