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A mechanistic based approach for enhancing buccal mucoadhesion of chitosan

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ABSTRACT

Mucoadhesive buccal drug delivery systems can enhance rapid drug absorption by providing an increased retention time at the site of absorption and a steep concentration gradient. An understanding of the mechanisms behind mucoadhesion of polymers, e.g. chitosan, is necessary for improving the mucoadhesiveness of buccal formulations. The interaction between chitosan of different chain lengths and porcine gastric mucin (PGM) was studied using a complex coacervation model (CCM), isothermal titration calorimetry (ITC) and a tensile detachment model (TDM). The effect of pH was assessed in all three models and the approach to add a buffer to chitosan based drug delivery systems is a means to optimize and enhance buccal drug absorption. The CCM demonstrated optimal interactions between chitosan and PGM at pH 5.2. The ITC experiments showed a significantly increase in affinity between chitosan and PGM at pH 5.2 compared to pH 6.3 and that the interactions were entropy driven. The TDM showed a significantly increase in strength of adhesion between chitosan discs and an artificial mucosal surface at pH 5.2 compared to pH 6.8, addition of PGM increased the total work of adhesion by a factor of 10 as compared to the wetted surface without PGM. These findings suggest that chitosan and PGM are able to interact by electrostatic interactions and by improving the conditions for electrostatic interactions, the adhesion between chitosan and PGM becomes stronger. Also, the three complementary methods were utilized to conclude the pH dependency on mucoadhesiveness.

1. Introduction

Buccal drug administration has attracted attention as a means to achieve a fast onset and systemic delivery of drugs susceptible to the gastro intestinal or hepatic first pass metabolism (Salamat-Miller et al., 2005). However, the continuous flow of saliva and swallowing reduces the amount of drug actually absorbed in the oral cavity, which is a limitation of this route of administration (Patel et al., 2012). Use of a buccal mucoadhesive formulation is an attractive approach to alleviate this limitation as provision of a prolonged high local concentration of the active pharmaceutical ingredient, will decrease the loss and even further facilitate fast absorption due to a steep concentration gradient (Haas and Lehr, 2002). Thus, lower and more available doses can be administered by the use of mucoadhesive formulations. The term mucoadhesion refers to the specific phenomenon of adhesion between a polymer and a mucus layer[1–5]. The mucus layer comprise of a viscous loosely adherent layer mainly composed of mucins and water, it lines mucosal membranes, such as those found in the oral cavity (Andrews et al., 2009; Phillipson et al., 2008). The mucus layer lubricates, moistures and protects the epithelium from physical and chemical insults (Salamat-Miller et al., 2005; Schenkels et al., 1995). The ability of a polymer to attach to the mucus layer is dependent on several factors other than the formation of chemical bonding (Duchene et al., 1988). Swelling, molecular weight, and flexibility of polymer chains are all factors that have great influence on the strength and duration of adhesion (Gaserod et al., 1998; Hagesaether et al., 2009; Salamat-Miller et al., 2005).

In the oral cavity, mucins are epithelial surface bound as well as solvated of salivary origin. Mucins are glycoproteins with a peptide backbone and oligosaccharide side chains, which often terminate in sialic acid residues (Salamat-Miller et al., 2005). With an isoelectric point of 2–3 (Lee et al., 2005), mucins will thus be overall negatively charged in the oral cavity, as pH is around 6.8. Chitosan is a non-toxic, biocompatible and biodegradable polysaccharide

Abbreviations: PGM, porcine gastric mucin; CCM, complex coacervation model; ITC, isothermal titration calorimetry; TDM, tensile detachment model; CS, CHITOPHARM® S; CM, CHITOPHARM® M; CL, CHITOPHARM® L; MES, 2-(N-morpholino)ethanesulfonic acid; HWS, human whole saliva.

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consisting of alternating glucosamine and N-acetyl-glucosamine units with a pK_a of 6.5 (Illum, 1998; Liu et al., 2005; Sogias et al., 2008). Chitosan has received interest as a bioadhesive excipient due to its ability to interact with mucins in the mucus layer by electrostatic, hydrophobic, hydrophilic and by hydrogen bonding (Sogias et al., 2008).

The following stepwise process of mucoadhesion has been proposed (Duchene et al., 1988): The initial contact stage of polymer and mucus, facilitating dehydration of the mucosa while hydration of polymer, forming a swelled polymeric network. The second step is the consolidation stage where polymer and mucin chains interdiffuse and entangle, thus facilitating non-covalent bonding and electrostatic interactions (Duchene et al., 1988; Smart, 2005). Mechanistic studies of chitosan and mucin have shown that the electrostatic interaction between the positively charged amines on the polymer chains and the negative sialic acid residues on the mucin glycoprotein play a role in the mucoadhesive properties of chitosan (He et al., 1998; Salamat-Miller et al., 2005; Sogias et al., 2008). The electrostatically driven reaction between chitosan and mucin can be interpreted as the process of complex coacervation. Complex coacervation is a process, where electrostatic interactions between a colloid suspension of a polymer and a protein will lead to the phase separation into a polymer-protein high concentration phase and polymer-protein low concentration phase (de Kruif et al., 2004). The formation of a protein-polymer rich phase will give rise to a sharp increase in turbidity of the solution. Typically, this reaction will take place in the pH range between the pK_a value of the polymer and the isoelectric point of the protein with an optimum at the pH, where the molecules carry the same numeric charge (Liu et al., 2010). Thus, the maximal electrostatic interactions between chitosan and mucin should occur in the pH-range of 2–6.5. The process of complex coacervation is influenced by a number of factors, one of which is the ionic strength of the solution (Liu et al., 2010). The ionic strength influences the overall charge of the protein and polymer due to the screening effect of counter ions (Burgess, 1990). In this study, the interaction between chitosan and mucin was examined in a buffer solution resembling human saliva with regards to ionic composition and ionic strength.

The aim of this study was to examine the pH-dependent interactions between chitosan polymers of increasing molecular weight and porcine gastric mucin (PGM) applying simulated oromucosal conditions. The mechanism of chitosan mucoadhesive properties was studied using complex coacervation model (CCM), isothermal titration calorimetry (ITC) and tensile detachment model (TDM).

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grade and were used as received unless otherwise described. Acetic acid (glacial), calcium chloride dihydrate, sodium chloride, sodium hydrogen carbonate and sodium hydroxide of analytical grade were purchased from Merck (Darmstadt, Germany). CHITOPHARM® L (CL) (MW 500–5000 kDa), CHITOPHARM® M (CM) (MW 100–2000 kDa), and CHITOPHARM® S (CS) (MW 50–1000 kDa) were provided as a free sample from Cognis GmbH (Monheim, Germany). 2-(N-morpholino)ethanesulfonic acid (MES), potassium chloride, sodium phosphate dibasic, sodium phosphate monobasic and Type II porcine gastric mucin (PGM) purified from porcine stomach mucosa were all purchased from Sigma–Aldrich (St. Louis, MO). Deionized water was obtained from Millipore Milli-Q Ultrapure Water purification system (Billeria, MA).

2.2. Complex coacervation model (CCM)

The electrostatic interactions between chitosan (S, M and L) and PGM were studied by complex coacervate formation. A buffered electrolyte solution (pH 6.5) mimicking human whole saliva (HWS buffer) was prepared in accordance to (Gaserod et al., 1998):210 mg/L $NaHCO_3$, 430 mg/L NaCl, 750 mg/L KCl, 220 mg/L $CaCl_2$ $2H_2O$ and 910 mg/L NaH_2PO_4 in deionized water. A stock solution of 2 mg/mL PGM was prepared by solvating PGM in HWS buffer at 5 °C overnight to ensure complete solvation. Chitosan (CS, CM and CL) stock solutions of 1 mg/mL were prepared by dissolving chitosan in HWS buffer. Test solutions containing 0.5 mg/mL PGM in HWS buffer with 0.15 mg/mL CS, CM, or CL was prepared by mixing chitosan stock solutions and PGM stock solution and diluting with HWS. The control solution contained 0.5 mg/mL PGM in HWS buffer. The test solution (200 mL) was titrated at room temperature with 0.2 M HCl solution using a 842 Titrando titrator fitted with a Unitrode pH electrode with Pt1000 temperature sensor, a Dosino 10 mL dosing unit and a rod stirrer with a 20 mm wide propeller (Metrohm AG, Herisau, Switzerland). Tiamo software version 1.1 was used for controlling the titration as well as acquiring and analyzing the results. The titrations were performed in the pH range 6.5–2.7 and a sample (2.0 mL) was withdrawn for each 0.2 pH interval after reaching pH equilibrium. Sample turbidity was determined at a wavelength of 400 nm (Cary 100 UV, Agilent Technologies Denmark, Horsholm, Denmark) using the WinUV software. All samples were measured using disposable 1.5 mL plastic cuvettes from Brandtech Scientific Inc. (Essex, CT). The absorbance was zeroed against HWS buffer and the dilution effect of the titration was taken into account.

2.3. Isothermal titration calorimetry (ITC)

The binding interactions involved in the mucoadhesive process were studied using ITC. To ensure uniform ionic strength, all test solutions were dialyzed. The test solutions consisted of 0.500 mg/mL PGM at pH 5.2 (0.0125 M acetic acid) and pH 6.3 (0.016 M MES). The solutions were dialyzed using dialysis bags with a cut-off of 12 kDa (Sigma, St. Louis) and were dialyzed against an excess of either acetic acid or MES buffer. The two different buffer concentrations ensured similar ionic strength of the buffer solutions. The dialysis fluid was changed seven times during approximately 20 h and was stable at the desired pH values after the fourth change of dialysis fluid. Before titration analysis, all samples were thoroughly degassed in vacuum, for a minimum of 30 min, under rapid magnetic stirring. Titration of 0.500 mg/mL PGM at pH 5.2 and pH 6.3 with 0.600 mg/mL CM were conducted on a NanoITC 2G, TA Instruments (Centennial Park, United Kingdom). Initially the reaction cell was rinsed thoroughly by flushing with degassed deionized water followed by PGM test solution. The CM solution to be tested was loaded in a 250 μL syringe, which was carefully evacuated of air. The propeller was gently wiped clean of residual CM solution. The syringe was set to a propeller speed of 250 rpm, and the temperature in the reaction cell was 25.000° C. Each titration consisted of initial 3.15 μL (i.e. lowest injection volume to omit residual air) and followed by 37 consecutive 5.15 μL injections. The peak resulting from the first injection was subsequently ignored. All ITC data were analyzed using the NanoAnalyze Data Analysis software, version 2.3.6 (TA Instruments) by splitting the area of the heat peaks into two distinct curves and fitting the area of the heat peak of each curve to an independent site model, deriving a value for the affinity constant (K), the binding stoichiometry (n) and the enthalpy change (ΔH). The amount of single sugar units of CM was calculated and used as molar concentration, assuming a degree of deacetylation of 70%. The molecular