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Research paper

In vitro evaluation of the mucoadhesive properties of polysaccharide-based nanoparticulate oral drug delivery systems

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Abstract

Impedance quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) measurements were performed in order to assess the mucoadhesive properties of hydrophobically modified (HM) derivatives of dextran (DEX), with an average molecular weight of 10,000 Da, and of hydroxypropylcellulose (HPC), with an average molecular weight of 80,000 Da. The measurements involved (1) treatment of a hydrophobic surface with bovine submaxillary gland mucin (BSM) under various pH conditions (2.0–8.0) and (2) treatment of the BSM layer with buffer solutions of the amphiphilic polysaccharides (pH 3.0 and 7.0). Control measurements were carried out with DEX, HPC, and chitosan (CH) used as a model mucoadhesive polymer. All HM-polysaccharides were shown to adsorb onto a BSM layer, the extent of adsorption increasing with increasing hydrophobicity of the samples. Under the same conditions, HPC and CH interacted with the BSM layer, but DEX showed no affinity to BSM. All the results suggest that HM-polysaccharide micellar systems have the potential of enhancing the bioavailability of poorly adsorbed drugs in peroral delivery.

Keywords: Mucoadhesion; Polysaccharides; Peroral drug delivery; Polymeric micelles; Quartz crystal microbalance; Surface plasmon resonance; Hydroxypropylcellulose; Dextran; Chitosan

1. Introduction

The concept of mucoadhesive polymers, introduced in the pharmaceutical literature in the 1950s, plays an important part in drug delivery strategy, in particular for oral drug formulations [1]. Mucoadhesive drug delivery systems exploit the attraction between polymers used in drug formulations and the mucus layer that covers epithelial surfaces throughout the body, including the gastrointestinal tract [2,3]. Adhesion of a delivery system to the mucus layer provides localization within a specific body site and prolonged residence time, thus it greatly enhances the bioavailability of drugs, especially peptides and proteins [4]. Mucus occurs in vivo both as a gel layer that adheres strongly to mucosal surfaces and as a luminal soluble or suspended form [5,6]. Mucus gels consist mostly of water which accounts for more than 95% of the gel weight and thoroughly hydrates the other mucus components: lipids, inorganic salts, and mucins. Mucins are O-linked glycoproteins consisting of a protein backbone decorated with oligosaccharide side chains that usually bear sialic acid end groups [7]. The amino acid components, mostly threonine, serine, and proline, account for only 10–30 wt% of the glycoprotein. The oligosaccharide fragments range in size from 1 to 20 monosaccharides assembled in branched and linear sequences [8–12]. Mucins tend to have a blocky architecture: most of the glycans are confined to certain densely glycosylated regions of the chain, separated from each other by "naked" protein stretches bearing only few sugar residues. Mucins are large macromolecules, with molecular weights ranging from 10 to 50×10^6 g/mol [13,14]. They tend to assemble into even larger assemblies, via hydrophobic interactions between amino acid residues, hydrogen bonding among sugar units, or disulfide linkages involving cysteines [15].

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The specific structure and composition of mucins vary substantially depending on their localization in the body [3]. This variability among mucus throughout the body suggests roads towards site-specific delivery of mucoadhesive drug formulations through control, on the molecular level, of the interactions between mucins and mucoadhesive polymers [16]. Such innovative drug delivery strategies exploit the mucoadhesion mechanism, which needs to be unraveled via in vivo and in vitro experiments. Several physicochemical methods have been applied to study mucoadhesion, including the Wilhelmy plate method [17] and tensile force measurements [18–20] using model mucin-coated substrates or direct measurement of the bond strength between a polymeric material and mucosal layers of animal tissues. In the case of polymer colloids, the movement of particles dispersed in a fluid may be monitored as the fluid flows along strips of biological tissues [21] or through channels filled with mucus gels [22]. A number of spectroscopic techniques, such as fluorescence probe measurements [23], infra-red spectroscopy, nuclear magnetic resonance spectroscopy (NMR) and X-ray photoelectron spectroscopy [15], have proven useful to assess the occurrence of H-bonds between specific functional groups of mucin and mucoadhesive polymers.

A revival of the study of mucoadhesion occurred with the advent of nanotools, such as the colloid probe technique in atomic force microscopy (AFM) and the surface force apparatus [24–29]. These techniques probe on the nanoscale the interaction between two surfaces of which one is modified with a mucin layer and the other with a mucoadhesive polymer. Other nanoscale techniques used to assess the adsorption of polymers onto mucins include ellipsometry [29] and surface plasmon resonance (SPR) spectroscopy [30], which detects changes in the optical properties of nanocoatings deposited on a gold surface and the quartz crystal microbalance (QCM) [31], which detects changes in resonance frequency of a quartz crystal as a function of changes of its mass. Both SPR and QCM yield information on the kinetics of the adsorption process and provide an estimate of the thickness of the mucin layer before and after contact with a polymer solution.

We present here a study by QCM and SPR of the interactions between a mucin nanocoating and polysaccharide-based nanoparticles designed as vehicles for poorly water soluble drugs administered in oral formulations. The nanoparticles consist of a hydrophobic core surrounded by hydrophilic polysaccharide chains, either hydroxypropyl cellulose (HPC) or dextran (DEX). We have shown previously that the polymers are non-toxic and that in water they form micelles with sizes of ~80 nm for modified HPC and from ~20 nm for modified DEX [32,33]. The micelles are stable in model gastric and intestinal fluids [34]. They solubilize hydrophobic drugs such as cyclosporin-A and facilitate transport of the drug through Caco-2 cell monolayers, employed as model intestinal epithelial cells [35]. In this article, we assess the interactions of bovine submaxilliary mucin (BSM) with dextran- and HPC-based nanoparticles and compare them to the interactions of BSM with unmodified HPC, DEX, and chitosan (CH), a polysaccharide of known mucoadhesive properties [36–38]. We monitor the effect of changes in pH and temperature on the stability of the BSM/nanoparticles binding. Our study demonstrates that QCM is a promising technique for comparing the mucoadhesive properties of biopolymers and that the adsorption of the modified polysaccharides to BSM is greatly enhanced compared to either HPC or dextran, the effect being most pronounced in the case of dextran, which by itself shows little affinity for mucin.

2. Materials and methods

2.1. Materials

Deionized water (resistivity 18.2 MΩ cm) was obtained by purification of house-distilled water with a Milli-Q Gradient System (Millipore, Bedford, MA). 1-Dodecanethiol (98+ %) (DDeT) and hydroxypropyl cellulose (HPC, M_w 80,000) were obtained from Aldrich Chemicals (Milwaukee, WS) and used without further purification. Bovine submaxillary gland mucin (BSM) was purchased from Sigma (St. Louis, MO) and used without further purification. Dextran (DEX, M_w 10,000) was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Chitosan (CH, $M_w = 30{,}000$; 81% degree of deacetylation) was purchased from Wako Pure Chemicals.

The hydrophobically modified polysaccharides (HM-HPC and HM-DEX) were prepared as described previously [32,33]. Briefly, they were synthesized *via* ether formation between a tosylated poly(oxyethylene) methyl ether (POE-C_{16}) and hydroxyl groups of HPC or DEX. As the polymers and POE-C_{16} have similar solubility characteristics, the coupling could be carried out in homogeneous solution. Under these conditions, high levels of hydrophobic modification were achieved with a random distribution of alkyl groups along the polymer chain. The resulting polymers were purified by soxhlet extraction with *n*-hexane to remove free POE-C_{16} residues. The level of POE-C_{16} grafting was determined by ^{1}H NMR spectroscopy measurements carried out with polymer solutions in DMSO-d_6. The characteristics of the polymers are listed in Table 1.

2.2. Surface plasmon resonance (SPR) spectroscopy

2.2.1. Instrumentation

SPR measurements were carried out with a computer-controlled scanning angle instrument (Resonant Probes GmbH, Goslar, Germany) described in detail previously [39]. Kinetic adsorption data ($\Theta_m - t$) were obtained by tracking the angle of minimum reflectivity (Θ_m) over time (t) with a temporal resolution of 20 s. This was done by measuring the reflectivity at three points close to the overall reflectance minimum and fitting these to a parabola, the

Table 1
Characteristics of the polymers used in this study

Polymer	Grafting level[a] (mol%)	Cac[b] (mg L^{-1})	R_h[c] (nm)
HM-HPC	0.9 ± 0.1	75 ± 14	45 ± 3
HM-DEX-6	6.0 ± 0.1	6.5 ± 2	14 ± 1
HM-DEX-9	9.0 ± 0.1	4.3 ± 2	50 ± 3
HM-DEX-16	15.0 ± 0.1	2.9 ± 1	41 ± 3

SD values listed were obtained from three measurements for each sample.
[a] Molar concentration of hydrophobic chain (*n*-hexadecyl) per saccharide unit (in %); from ^{1}H NMR data, see [32,33].
[b] Cac, critical association concentration for the formation of polymeric micelles from fluorescence probe experiments, see [32,33].
[c] R_h, hydrodynamic radius of the polymeric micelles determined by dynamic light scattering measurements, see [32,33].

apex of which was taken as Θ_m. The shift in Θ_m is proportional to the amount of material adsorbed on the surface.

2.2.2. SPR sample cell and substrate preparation

A thin gold layer (~48 nm) was thermally evaporated onto LaSFN9 glass slides at a rate of ~0.1 nm/s and base pressure of $\sim 1 \times 10^{-7}$ Torr using a VE-90 thermal evaporator equipped with a quartz crystal deposition monitor (Thermionics Vacuum Products, Port Townsend, WA). A self-assembled monolayer of DDeT was prepared by incubating the gold-coated slides into a DDeT solution in ethanol (1 mM) for at least 12 h. The backside of the DDeT functionalized Au substrate was optically coupled to the base of a LaSFN9 prism using a Cargille Series B liquid ($n = 1.700$). The prism/Au substrate assembly was fixed against one side of a custom-built Teflon liquid cell (1 mL capacity) fitted with Kalrez O-rings so that the DDeT-Au surface faced the inside of the cell. The other side of the liquid cell was pressed against a microscope glass slide. The prism/Au substrate/liquid cell assembly was mounted onto the goniometer stage so that the center of the Au/glass substrate was in the axis of rotation. The goniometer position at which the back reflected beam overlapped with the incident beam was used to define the angle of incidence of 45° with an accuracy of ±0.01°.

2.2.3. Adsorption protocol

A DDeT surface was exposed first to a Tris buffer saline solution (pH 3, unless stated otherwise) until a stable baseline was obtained. BSM was dissolved in this buffer at a concentration of 0.5 g L^{-1}, a value kept constant throughout the study. BSM solutions of this concentration are clear and sufficiently fluid to allow rapid filling and flushing of the SPR and QCM cell compartments. While more concentrated solutions can be employed if necessary, they may pose technical difficulties. The BSM solutions were injected in the cell. Adsorption was allowed to proceed for 15 min. The cell was rinsed with Tris buffer saline (pH 3), followed by injection of a polymer solution (0.5 g L^{-1}) in Tris buffer saline. Polymer adsorption was allowed to proceed for 15 min, followed by two 5-min rinses with buffer. The thickness of the adsorption layer (see below) was calculated from the data recorded after the final rinse. All experiments were done at room temperature unless otherwise specified.

2.2.4. Data analysis

The Fresnel modeling software provided by Resonant Probes was used to analyze the angular reflectivity curves by a six-layer model (glass prism/gold/thiol/mucin/polymer/aqueous solution) and extract the film thickness (d_{film}) following the adsorption of mucin and of polymer. The refractive index values used were $n = 1.845$ (LaSFN9 prism) [39], 1.335 buffer, 1.5 BSM [28,30], 1.375 HM-polysaccharides [39].

2.3. Impedance quartz crystal microbalance (QCM)

2.3.1. Instrumentation

A KSV QCM-Z500 (KSV Instruments, Helsinki, Finland) was used to monitor polymer adsorption onto gold-coated 5-MHz AT-cut quartz crystals (Quartz Pro, Sweden), cleaned prior to use by dipping in a piranha solution consisting of 70% concentrated sulfuric acid and 30% hydrogen peroxide (*WARNING: Piranha solution is extremely reactive and should be handled with extreme caution*) followed by copious rinsing with deionized water and air-drying. The QCM-Z500 instrument measurement principle is based on impedance analysis. The quartz crystal is swept with potential perturbations of different frequencies close to the quartz crystal resonance frequency. The potential over the crystal and the current flowing through the crystal are recorded. This sweep is done as a function of time, which enables the measurement of mass changes occurring on the quartz crystal electrode surface. The impedance curve recorded at time t can be fitted to an equivalent circuit model, giving the mass and viscoelastic properties of the deposited layers. The frequency changes (ΔF) were measured with a temporal resolution of 15 s at the fundamental frequency (~5 MHz) of the crystal, as well as at the third, fifth, seventh, and ninth harmonics close to 15, 25, 35, and 45 MHz, respectively.

2.3.2. Adsorption protocol

All measurements were performed at 25.0 ± 0.1 °C, unless otherwise indicated. To prepare hydrophobic surfaces, a clean gold-coated quartz crystal was kept in a solution of DDeT (1 mM) in ethanol for at least 12 h. It was rinsed with ethanol and water, dried with filtered nitrogen in a laminar flow, and mounted in the QCM sample cell. The crystal was exposed to a Tris buffer saline until a stable baseline was obtained. A solution of BSM (0.5 g L^{-1}) in the same Tris buffer was injected in the sample compartment. After 15 min, the cell was rinsed twice with buffer saline solution (15 min), followed by injection of a polymer solution (2 mL, 0.5 g L^{-1}) in Tris buffer saline solution. After 30 min, the cell was subjected to two consecutive rinses (15 min) with Tris buffer saline. The mass/area values reported in Table 2 were calculated from the ΔF values recorded at the end of the second rinse.

Table 2
Surface concentration and optical thickness of various HM-polysaccharides adsorbed onto a BSM layer deposited on a hydrophobic surface (pH 3.0)

Polymer	Polymer surface concentration (mg/m^2)[a]	Optical thickness[b] (nm)
HPC	1.1 ± 0.1	0.6 ± 0.1
HM-HPC	10.9 ± 0.1	2.6 ± 0.1
DEX	0	0
HM-DEX6	2.5 ± 0.1	1.5 ± 0.1
HM-DEX9	4.3 ± 0.1	3.1 ± 0.1
HM-DEX15	5.3 ± 0.1	4.7 ± 0.1

All measurements performed in triplicate.
[a] From analysis of QCM experimental data using the Sauerbrey model.
[b] From analysis of SPR experimental data.

To assess the influence of the crystal surface modification, a solution of HM-HPC (0.5 g L^{-1}) in a pH 3 buffer solution was injected in the QCM cell fitted with a DDeT-coated crystal. Adsorption equilibrium was reached after 15 min. The sample cell was rinsed twice with a pH 3 buffer saline allowing for an equilibration time of at least 5 min between rinses.

2.3.3. Data analysis

The mass of the adsorbed layer, assumed to be a thin and rigid film, was calculated from an analysis of the $\Delta F - t$ curves using the Sauerbrey model using the software provided by the manufacturer [40]. The density of the layers was assumed to be 1 g/mL.

3. Results and discussion

3.1. Mucin adsorption on a DDeT monolayer

Since the aim of the study was to assess the mucoadhesive properties of several biopolymers, it was necessary, as a first step to prepare mucin-saturated surfaces amenable to QCM and SPR analysis. This was achieved by adsorption of BSM onto a gold surface rendered hydrophobic with a DDeT monolayer. The adsorption of BSM on this lipidic monolayer was monitored by QCM measurements. Adsorption time courses following injection of a 0.5 g/L BSM solution in buffers of constant ionic strength (0.15 M) are presented in Fig. 1 for solutions ranging in pH from 2.0 to 8.0. Upon exposure of the hydrophobic surface to a BSM solution (pH 2.0–7.0), rapid mass uptake occurred reaching a constant plateau value after a contact time of ~5 min or less. After a contact time of 15 min, the BSM solution was rinsed from the cell by addition of a buffer solution of identical pH. The rinsing step was repeated once more to ensure complete removal of unbound mucin. The BSM solution of pH 8.0 did not adhere to the hydrophobic surface. The mass of BSM adsorbed on the lipidic surface was estimated using the Sauerbrey equation. Three adsorption patterns emerge, depending on the pH of the BSM solutions. The mass of BSM adsorbed (5.25 ± 0.56 mg/m^2) was the highest in the case of BSM

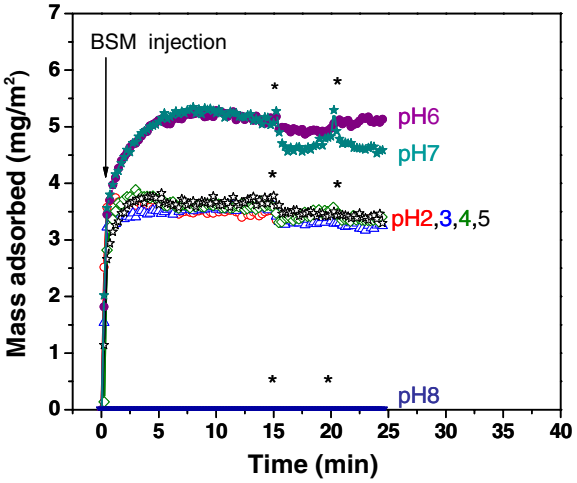

Fig. 1. Plot of mass uptake as a function of time monitored by QCM for the adsorption of BSM (0.5 g/L) onto a DDeT coated gold surface; pH of the BSM solution: 2.0, 3.0, 4.0, 5.0, 7.0 and 8.0; [NaCl], 0.15 M; the stars indicate the rinsing steps with a buffer of corresponding pH.

in buffers of pH 6 and 7. No adsorption was detectable for BSM in a pH 8 buffer. An intermediate value (3.3 ± 0.33 mg/m^2) was recorded in all other cases (pH 2, 3, 4 and 5). These values are of the same order of magnitude, although somewhat higher, as values of BSM saturated surface concentration previously reported (2.3–3.0 mg/m^2) for BSM deposited on various hydrophobic surfaces [22].

The identical adsorption protocol was performed within the flow cell of an SPR apparatus. A typical adsorption time profile recorded upon treatment of a DDeT monolayer with a BSM solution of pH 3.0 and subsequent rinses is represented in Fig. 2. The optical thickness of the layer, 1.8 ± 0.1 nm, was estimated from the shift of the resonance angle upon BSM adsorption and subsequent rinses.

The adsorption of mucins onto hydrophobic surfaces is believed to involve primarily hydrophobic interactions between the surface and the unglycosylated polypeptide backbone segments acting as binding sites. In a solution of pH 2, the tertiary structure of BSM is such that hydrophobic segments are exposed to the water, triggering adsorption of BSM through multiple sites per macromolecule [25]. In solutions of pH 6–7, in contrast, BSM adopts a more compact tertiary structure in which the hydrophobic peptide residues are protected from the aqueous environment by negatively charged hydrophilic chains [25]. Hydrophobic sections are exposed only as they encounter a hydrophobic surface. Under these conditions BSM molecules can pack more densely at the solid/liquid interface, due to the smaller area required for adsorption of a single BSM macromolecule. As the pH of the solution is increased further (pH > 7), the protein becomes highly charged and the hydrophobic sections, buried towards the core of the BSM tertiary structure, are unable to interact with the hydrophobic surface. There is no driving force for adsorption, the mucin remains in the solvent.

3.2. Adsorption of HM-polysaccharide micelles onto BSM-modified surfaces

The HM-polysaccharides selected for this study were obtained by conjugation of *n*-hexadecyl terminated poly(ethylene glycol) (PEG) chains to either dextran (10,000 g/mol) or HPC (80,000 g/mol) [32–34]. We studied here three HM-DEX samples differing in terms of grafting level i.e. the number of hydrophobic substituents linked to the chain, and a sample of HM-HPC also modified with *n*-hexadecyl-terminated PEG chain, but with a lower grafting density (Table 1). In water, all polymers form micelles above a concentration ranging from 5 to 75 mg/L depending on the polymer. The average size of the micelles expressed in terms of their hydrodynamic radii derived from dynamic light scattering measurements is ~80 nm in the case of HM-HPC samples and ~20 nm for HM-DEX (Table 1). All adsorption studies described below were performed with solutions of concentration such that the polymers were in the micellar form to the exclusion of detectable amounts of free polymer chains.

3.2.1. Dextran and hydrophobically modified dextrans

An SPR profile recorded during the adsorption of a micellar solution of HM-DEX6 (pH 3.0) onto the BSM-coated substrate is presented in Fig. 2 (open circles). The thickness of the polymer layer (1.5 nm), estimated from the shift in resonance angle upon treatment of the BSM layer with the polymer solution, is significantly less than the hydrodynamic radius of the corresponding micelles in solution (14 nm), indicating a reorganization of the micelles as they come into contact with the mucin layer. Moreover, it may indicate that the HM-polymer chain interpenetrates within the BSM layer [24]. Similar measurements were carried out with solutions of HM-DEX9 and HM-DEX15. The HM-DEX layer thickness increases with the hydrophobe content of the polymer (Table 2), which can be taken as an indication of the prevalence of hydrophobic interactions as driving force for adsorption.

Adsorption time profiles recorded via QCM upon injection onto a pH 3 BSM substrate and subsequent rinses of the three HM-DEX samples are presented in Fig. 3, together with data obtained by treatment of the BSM layer with a solution of unmodified dextran. Rapid mass uptake occurred upon exposure of the BSM layer to micellar HM-DEX solutions. This fast adsorption process was followed by a slower continuous mass increase, which was most pronounced in the case of HM-DEX15, the sample with the highest level of hydrophobic group incorporation. Replacement of the micellar solution with buffer resulted in some weight loss, corresponding to the removal of loosely adsorbed excess polymer. The saturated surface concentration extracted from the QCM data, listed in Table 2, more than doubles as the grafting level passes from 6 to 15 mol%. Note that exposure of the BSM layer to a solution of unmodified dextran triggered no mass change, confirming previous reports on the poor mucoadhesive characteristics of dextran [41]. The results described above correspond to measurements performed at room temperature. Identical adsorption profiles were recorded upon treatment of a mucin layer kept at 37 °C with a solution of HM-DEX (pH 3.0) warmed to 37 °C. Given the lack of significant temperature effect on polymer adsorption, at least within the limits of physiological conditions, all other measurements were performed on systems kept at 25 °C in order to avoid lengthy equilibration times.

To assess the effect of solution pH on the adsorption of HM-DEX on BSM, we carried out a series of QCM measurements consisting of (1) treating a DDeT-coated gold crystal with a BSM solution of specific pH (between 2 and 6) and (2) placing the rinsed BSM-coated substrate in contact with a solution of HM-DEX6 in the corresponding

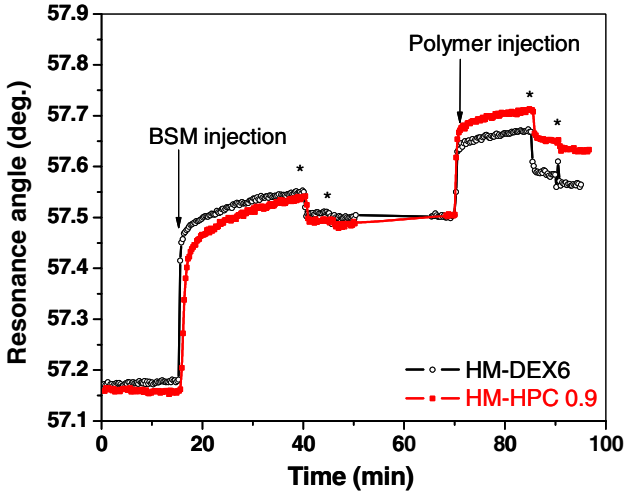

Fig. 2. Changes in SPR resonance angle as a function of time following injection of a BSM solution (0.5 g/L, pH 3.0, [NaCl] 0.15 M) and subsequent buffer rinses (indicated by the stars) followed by exposure to a solution of HM-HPC (0.5 g/L) (full squares) or HM-DEX6 (0.5 g/L) (open circles).

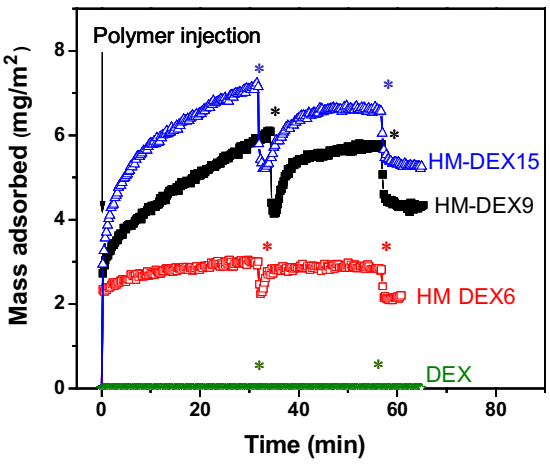

Fig. 3. Plot of mass uptake as a function of time monitored by QCM for the adsorption of three HM-DEX samples (0.5 g/L) and of DEX (0.5 g/L) onto a BSM layer coated on a DDeT modified gold surface; pH. 3.0; [NaCl], 0.15 M; the stars indicate the rinsing steps with a buffer of pH 3.

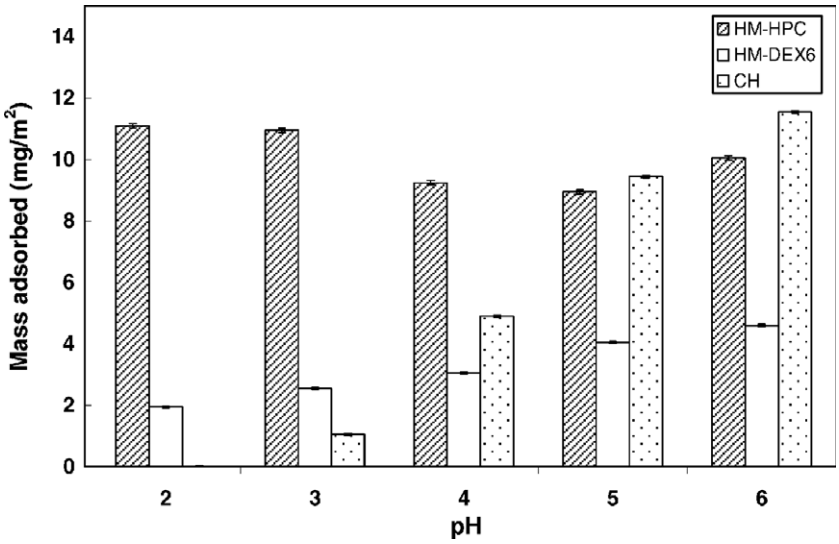

Fig. 4. Plot of mass uptake as a function of pH determined by QCM for the adsorption of HM-HPC, HM-DEX6, and CH solutions (0.5 g/L) onto a BSM layer coated on a DDeT modified gold surface; [NaCl], 0.15 M.

buffer. The mass of polymer adsorbed increased moderately with increasing pH, from ~2 mg/m^2 (pH 2.0) to ~4.6 mg/m^2 (pH 6.0) (Fig. 4). As indicated in Section 3.1, the conformation adopted by the adsorbed BSM is pH-dependent. The adsorption trends exhibited by the neutral HM-DEX6 may be a consequence of the enhanced availability of BSM hydrophobic sites on substrates obtained under near neutral conditions.

3.2.2. *Hydroxypropylcellulose and hydrophobically modified hydroxypropylcellulose*

We carried out the same adsorption protocols starting with a micellar solution of HM-HPC and with a solution of HPC. The QCM and SPR adsorption profiles of HM-HPC onto a BSM layer (pH 3.0) [Figs. 5 and 2 (full squares), respectively] present the same overall features as in the case of HM-DEX: rapid mass uptake immediately upon injection of the polymer, followed by a continuous small uptake and a slight desorption upon rinsing. We note however (Fig. 5) that, contrary to the case of dextran, injection of the unmodified polymer (HPC) results in a small mass increase and even after surface rinsing with a buffer solution, a significant amount of HPC remains adsorbed on the BSM layer. This observation confirms previous reports on the mucoadhesive and bioadhesive characteristics of HPC [5]. Another difference between HM-DEX and HM-HPC was revealed by recording pH-dependent adsorption profiles under the conditions described in Section 3.2.1. Unlike the case of HM-DEX6, the adsorption of HM-HPC onto BSM was unaffected by pH (Fig. 4). This observation can be taken as an indication that the mucoadhesive properties of HM-HPC are due not only to hydrophobic interactions, but also to other weak forces such as hydrogen bonds which are responsible for the mucoadhesive characteristics of unmodified HPC.

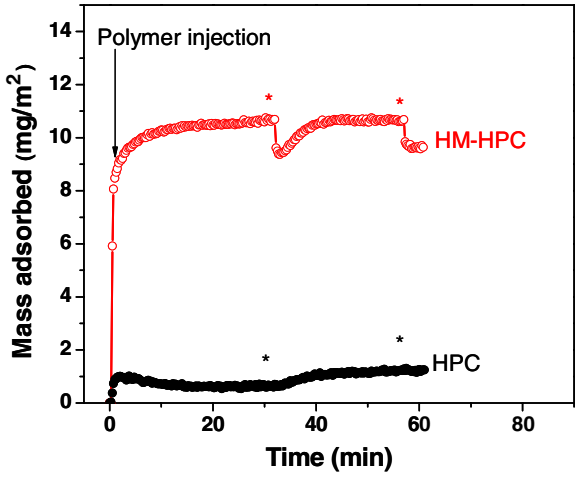

Fig. 5. Plot of mass uptake as a function of time monitored by QCM for the adsorption of HM-HPC (0.5 g/L, open circles) and of HPC (0.5 g/L, black circles) onto a BSM layer coated on DDeT modified gold; pH, 3.0; [NaCl], 0.15 M; the stars indicate the rinsing steps with a buffer of pH 3.

Control QCM profiles were recorded upon injection of a micellar solution of HM-HPC into a cell fitted with a DDeT-coated gold substrate that had not been treated with BSM. The HM-HPC uptake was 18.1 ± 0.1 mg/m^2, a value twice as high as the value recorded in the case of a BSM-coated surface (10.9 ± 0.1 mg/m^2). The strong adsorption of HM-HPC onto the lipidic monolayer may be ascribed to the insertion of the polymer-linked hydrophobes into the monolayer which provides a strong driving force for adsorption.

3.3. *Chitosan*

Mucin, which carries sialic acid residues, has a pK_a of 2.6 and a pI of around 3 [27,28,42]. It is neutral or slightly negatively charged at pH 2. As the pH value increases the

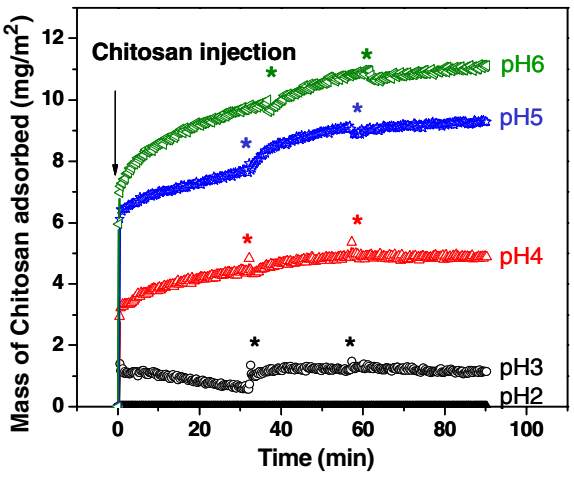

Fig. 6. Plot of mass uptake as a function of time for the adsorption of CH samples (0.5 g/L) onto a BSM layer coated on a DDeT modified gold surface at different pH monitored by QCM, [NaCl], 0.15 M; the stars indicate the rinsing steps with a buffer of corresponding pH.

amount of ionized sialic acid residues increases, favoring interactions with polycations. To test the effect of electrostatic interactions on mucoadhesion, we recorded QCM adsorption profiles for CH, a polymer known to exhibit mucoadhesive properties [36–38]. CH is a linear polysaccharide composed of β-(1 → 4) linked 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose. It has a pK_a value of ~6.5 and is soluble only in acidic media, where it exists in its ionic form and behaves as a polycation. The adsorption of CH onto BSM was monitored as a function of solution pH following the experimental protocol described in Section 3.2.1. The mass/area values determined are presented in Fig. 4 and the adsorption profiles are depicted in Fig. 6. As anticipated, no adsorption occurred under very acidic conditions (pH 2). The amount of chitosan adsorbed onto BSM increased sharply with increasing solution pH, reaching a value of 11.5 mg/m^2 for solutions of pH 6, the conditions for which the electrostatic interactions between BSM and CH are most favorable. Similar results have been reported recently by Sigurdsson et al., who have suggested that in this pH range, the interaction of CH with BSM involves mostly H-bonds via the unionized amine groups of CH [43]. We note that the mass of CH adsorbed onto BSM under these conditions is of the same order of magnitude as the mass of HM-HPC bound to BSM (Fig. 4). This observation suggests that the synergistic effect of hydrophobic forces and hydrogen bond formation enhances the adsorption of neutral polymers to BSM, bringing it to a level comparable to that reached in cases where electrostatic interactions prevail.

4. Conclusions

Mucin readily adsorbs onto hydrophobic surfaces under neutral or acidic conditions yielding an interface which, in this study, has been used as a model surface for the mucous layer covering the gastrointestinal tract. Such mucin layers readily associate with hydrophobically modified polysaccharides forming a firm layer that resists rinsing with buffer. The kinetics of absorption of HM-polymers onto the mucin layer involve two steps, a rapid uptake followed by a slower process during which interface reorganization may occur. The uptake of HM-polysaccharide by the mucin layer is enhanced with increased hydrophobicity of the polymer. On the basis of the SPR-determined thickness of the adsorbed HM-polysaccharides and by comparison with the hydrodynamic radius of the HM-polysaccharides in solution, we propose a mechanism of adsorption in which polymeric micelles unwrap upon contact with the BSM layer, a process permitting intimate contact of the hydrophobic sections of BSM and the polymer-linked hydrophobes and promoting the interpenetration of the polymer within the BSM layer. Our work demonstrates that QCM is a sensitive technique to detect mucoadhesive properties of biopolymers. It is particularly useful if one wants to compare the mucoadhesive properties of various materials under specific conditions.

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