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Adsorption behavior of oxidized galactomannans onto amino-terminated surfaces and their interaction with bovine serum albumin

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Abstract

Galactomannans extracted from *Cassia fastuosa* (CF) and *Leucaena leucocephala* (LL) seeds were purified and oxidized with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) to form the uronic acid-containing polysaccharides CFOX with a degree of oxidation (DO) of 0.22 and LLOX with DO of 0.66, respectively. The adsorption behavior of CFOX and LLOX onto amino-terminated surfaces was studied by means of ellipsometric measurements. The influence of pH and ionic strength on the adsorption was investigated. A strong dependence of pH on the adsorbed amount of CFOX and LLOX was observed. At pH 4, there was a maximum in the adsorbed amount caused by strong electrostatic attraction between the substrate and the oxidized galactomannans. On the other hand, no ionic strength effect on the adsorption of CFOX and LLOX was observed. The immobilization of bovine serum albumin onto LLOX and CFOX was studied as a function of pH. LLOX proved to be a more attractive substrate for BSA than CFOX, indicating that the favorable interactions between the carboxylate groups and the BSA segments have driven the adsorption process. At the isoelectric point of BSA a maximum in the adsorbed amount was found for both surfaces.

Keywords: Adsorption; Oxidized galactomannans; Amino-terminated surfaces; Bovine serum albumin; Ellipsometry

1. Introduction

The adsorption of polysaccharides on solid substrates provides an environment for the immobilization of biomolecules (Sackmann, 1996; Wiegand, Jaworek, Wegner & Sackmann, 1997; Charreyre et al., 1997; Siqueira Petri, Choi, Wenz, Bruns, Beyer & Schimmel, 1999) giving rise to conditions for the development of biosensors (Delair, Meunier, Elaissari, Charles & Pichot, 1999) and kits for immunoassays.

Galactomannans (Dea & Morrisson, 1975) occur as endosperm cell wall storage heteroglycans in many leguminous seeds, as well as in those of a few non-leguminous species, where the galactose content generally ranges from ~20–50%. The industrial use (Dey, 1978; Maier, Anderson, Karl, Magnuson & Whistler, 1993) of these renewable materials, particularly those in which the galactosyl substitution is relatively low, as in carob and guar seeds, is mainly due to their ability to form highly viscous solutions at low concentration (Rees, 1982) and to form aqueous gels under appropriate conditions (Robinson, Ross-Murphy & Morris, 1982). In comparison to other polysaccharides such as starch, the galactomannans are particularly interesting because they lead to stereospecific reactions in C2 and C3 mannose units and in C3 and C4 galactose units.

The selective oxidation with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) at CH_2OH-6 of the galactomannan from *Leucaena leucocephala* (LL) seeds (Man,Gal ratio 1:3) gave rise to CO_2H-6 groups (LLOX) (Sierakowski, Milas, Desbrières & Rinaudo, 2000). In this work, the oxidation and characterization of galactomannans extracted from seeds of *Cassia fastuosa* (CF) by the same method to form the uronic-acid containing polysaccharide, CFOX, is presented. The adsorption behavior of the oxidized galactomannans onto amino-terminated surfaces is investigated by means of ellipsometry and contact angle measurements.

The adsorption of bovine serum albumin (BSA) onto mineral surfaces and polymeric surfaces has been extensively studied (do Serpo, Fernandes, Saramago & Norde, 1999; van der Vegt, Norde, van der Mei & Busscher, 1996; Giacomelli & Norde, 2001). However, the interactions between polysaccharide coated surfaces and bovine

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serum albumin (BSA) has been relatively unexplored (Siqueira Petri et al., 1999). The study on the immobilization of BSA onto galactomannan substrates is especially interesting because BSA presents unusual ligand-binding properties (Carter & Ho, 1994), which can provide the basis for the development of biotechnological devices.

2. Experimental

2.1. Extraction, characterization, and oxidation of galactomannan from Cassia fastuosa

Galactomannan extraction was preceded by the treatment of the seeds (40 g) for 20 min with H_2O (1 l) at 96°C to destroy enzymes. They were then milled and extracted exhaustively with H_2O at 25°C. The extracts were treated with EtOH (× 3 vols.), and the precipitated and recovered polysaccharide was dissolved in H_2O to a 1% concentration and the solution centrifuged (16,000 rpm, 25°C, 40 min). The clear supernatant was successively filtered through Millipore membranes of 3.0 and 0.8 μm pore diameter, treated with excess EtOH and the resulting precipitate recovered and dried under vacuum at 25°C. The total carbohydrate in the purified polysaccharide was determined by the phenol-H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956).

The galactomannan was hydrolyzed with 1 M TFA at 100°C for 5 h. The liberated monosaccharides were converted into alditol acetates by successive $NaBH_4$ reduction and acetylation with Ac_2O-pyridine. Gas chromatography–mass spectrometry (GC–MS) analyses were performed using a Finnigan Mat ion trap (model 410) mass spectrometer, incorporating a DB-225 capillary column (30 m × 0.25 mm i.d.), with He as the carrier gas. Injections were carried out at 50°C, the column was then programmed to 230°C at a rate of 4°C min^{-1}, and held until the end of the run. Scans were carried out at *m/z* 40-420 every 2 s at 70 eV.

To a solution of the galactomannan (1.25 g = 6.8 mmol of monosaccharide units) in distilled H_2O (1 l), TEMPO (0.0092 g = 0.059 mmol) and NaBr (0.08 g = 0.78 mmol), 15% aq. NaOCl (4.5 ml = 9.37 mmol) at 3 ± 1°C was added under stirring. The pH of the mixture was adjusted with 2 M HCl to 9.45, which was maintained for 100 min by addition of 0.05 M NaOH. $NaBH_4$ (50 mg = 1.3 mmol) was added and the solution was stirred for 1 h. An adjustment to pH 8.0 was then performed by adding 2 M HCl. The solution was treated with 0.17 M NaCl and the oxidized polysaccharide was precipitated three times with EtOH. It was recovered by centrifugation, resuspended several times in pure EtOH, filtered, and dried under vacuum at 25°C to yield 1.12 g.

2.2. Characterization of oxidized galactomannan

As previously described for the galactomannan extracted from the seeds of *Leucaena leucocephala* (Sierakowski et al., 2000), the degree of oxidation (DO) of the polysaccharide was determined by the OH^- consumed, by means of NaOH during the reaction. DO is expressed by the neutralization equivalent in eq g^{-1} = average degree of oxidation (DO) of the galactosyl and mannosyl units.

FTIR spectroscopy (Perkin Elmer 1750) was performed on samples in KBr pellets. ^1H and ^{13}C NMR spectra were obtained with a BRUKER, AVANCE DRX-400 spectrometer (D_2O solutions at 60°C), equipped with a Fourier-Transform System. Chemical shifts are expressed as δ (ppm) relative to the resonance of acetone as internal standard ($\delta = 30.2$, for ^{13}C NMR). Capillary viscometry measurements were performed with an AVS 350 Schott automatic equipment investigating solutions of 0.8–2.5 g l^{-1} at 25.0 ± 0.1°C. At these dilutions, chain entanglement is absent.

2.3. Adsorption experiments

Silicon (100) wafers (Crystec, Berlin, Germany), with a native oxide layer of ~2 nm, were used as substrates. They were rinsed in a standard manner (Petri, Wenz, Schunk & Schimmel, 1999; Motschmann, Stamm & Toprakcioglu, 1991) and the surfaces were then functionalized with aminopropyltrimethoxysilane (Fluka, Switzerland) (Petri et al., 1999). This method provides a flat and homogeneous amino-terminated monolayer covalently bound to the silicon wafers. The pK_b for propylamino groups is reported to be 3.3 at 20.0°C (Hoogendam, de Keizer, Cohen Stuart, Bijsterbosch, Batelaan & van der Horst, 1998; Hoogendam, de Keizer, Cohen Stuart, Bijsterborsch, Smit, et al., 1998). For the adsorption experiments, solutions of CFOX and LLOX at 0.002–5.0 g l^{-1} concentrations were prepared in 0.1, 0.01 and 0.001 M NaCl at a pH range of 3–7. NaCl, NH_4OH, acetate and phosphate buffers, were all of reagent grade (Nuclear, São Paulo, Brazil) and were used without prior treatment. Carboxymethylcellulose (CMC) (MW = 250,000 g/mol and degree of substitution, DS, of 1.8) purchased from Fluka (USA) was dissolved in 0.01 M NaCl solution at pH 4, as described elsewhere (Fujimoto & Petri, 2001).

Ellipsometric measurements were performed using a vertical computer-controlled DRE-EL02 ellipsometer (Ratzeburg, Germany). The angle of incidence was set at 70.0° and the wavelength of the laser was 632.8 nm. The ellipsometric angles Δ and Ψ were measured and recorded at intervals of 4 s. Adsorption from the solution was monitored in situ with a poly(tetrafluoroethylene) cell described elsewhere (Fujimoto & Petri, 2001). The measurements were carried out in an environment controlled at 22–23°C.

From the ellipsometric angles Δ and Ψ and a multilayer model composed of Si, SiO_2, amino-terminated monolayer, polyelectrolyte layer, and the bulk solution, it is possible to determine the thickness of the adsorbed polyelectrolyte, d_{poly}, and its index of refraction n_{poly}, by means of iterative

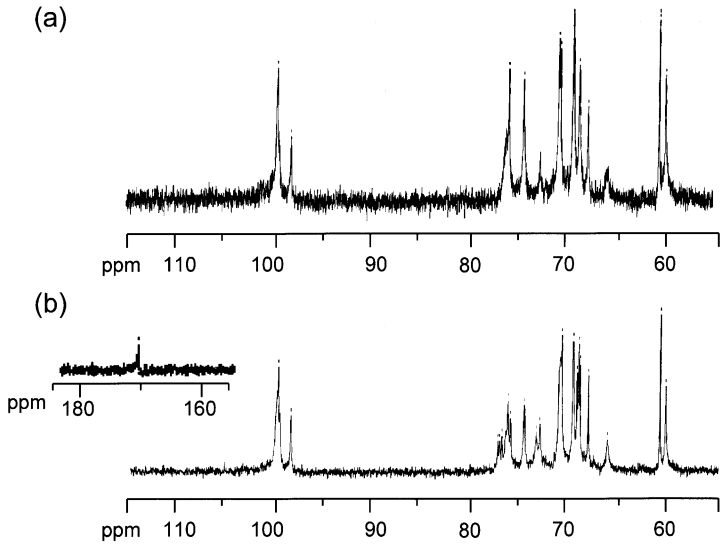

Fig. 1. ^{13}C NMR spectra in D_2O at 60°C (δ in ppm) of (a) galactomannan extracted from *C. fastuosa* seeds, CF, and (b) oxidized galactomannan, CFOX.

calculations with Jones matrices (Motschmann et al., 1991; Fujimoto & Petri, 2001; Azzam & Bashara, 1987). The adsorbed amount A is determined from the equation:

$$A = \frac{d_{\text{poly}}(n_{\text{poly}} - n_0)}{dn/dc} = d_{\text{poly}} c_{\text{poly}} \quad (1)$$

where n_0 is the index of refraction of the bulk solution, as measured with an Abbé refractometer, dn/dc is the increment of refractive index determined with a differential refractometer, and c_{poly}, is the average polymer concentration within the layer. In the polysaccharide systems, n_0 was measured for each concentration and dn/dc was 0.16 ml g^{-1} at 23°C.

The wettability of biofilms formed by adsorbed, oxidized galactomannans on to amino-terminated surfaces was determined by contact angle measurements of sessile drops of distilled water at 25°C. Drops of 8 and 4 μl were used for advancing and receding angles, respectively.

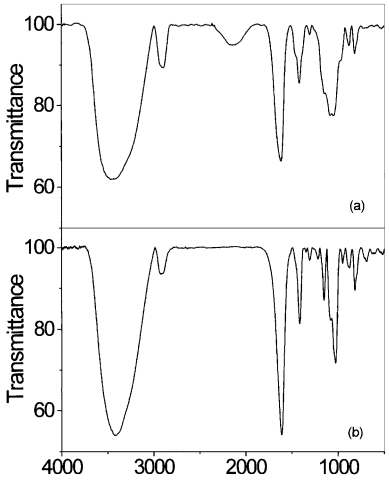

Fig. 2. IR spectra of (a) CFOX and (b) LLOX.

2.4. Immobilization of bovine serum albumin

Polysaccharide-coated substrates were immersed for 4 h at 25°C in 1.0 g l^{-1} aqueous solutions of bovine serum albumin (BSA) (A3803 Sigma, USA) in the pH range of 3–7. The adsorbed amount was determined from the ellipsometric data and Eq. (1). The parameter n_0 was measured for each concentration and dn/dc amounted to 0.16 ml g^{-1} at 23°C.

3. Results and discussion

3.1. Characterization of native and modified galactomannans

The carbohydrate content in the purified polysaccharide (CF) extracted from the *C. fastuosa* seeds was 80%. Hydrolysis of CF showed the presence of 75 mol% of mannose (Man) and 25 mol% of galactose (Gal), as determined by GC–MS of derived alditol acetates. A ratio of Man to Gal close to 3:1 was also calculated from areas of anomeric signals in its ^1H and ^{13}C NMR spectra. The structure of CF was shown by its ^{13}C NMR spectrum [Fig. 1(a)] to have a β-(1 → 4) mannopyranosyl main-chain, which was partially substituted at O-6 (25%) by α-galactopyranosyl units. The three signals at region of δ 76.06–76.80 correspond to C-4 diads of the mannopyranosyl units. The area of the signal at the highest field (δ 76.06) showed the polysaccharide to have a high content of Man-Man diads, not substituted at O-6 (Grasdalen & Painter, 1980; Manzi, Cerezo & Shoolery, 1986; Petkowicz, Sierakowski, Ganter & Reicher, 1998). The single-unit side chains of α-galactopyranose were thus distributed irregularly along the main chain.

The ^{13}C NMR spectrum of CFOX [Fig. 1(b)] showed at

Table 1
Characteristics of polysaccharide samples: Man to Gal ratio, degree of oxidation (DO), intrinsic viscosity [η] measured at pH 3 and (a) 0.001 M NaCl and (b) 0.1 M NaCl

Samples	Abbreviation	Man:Gal	DO	$[\eta]^{(a)}$ (dL/g)	$[\eta]^{(b)}$ (dL/g)
Leucaena leucocephala	LL	1.3	–	1.9	1.6
Cassia fastuosa	CF	3	–	2.7	2.3
Oxidized *Leucaena leucocephala*	LLOX	–	0.62	1.8	1.5
Oxidized *Cassia fastuosa*	CFOX	–	0.22	2.0	1.9

low-field two signals at δ 171.87 and 171.97 corresponding to galacturonic acid and mannuronic acid C=O groups (Keenan, Belton, Matthew & Howson, 1985; Gorin & Mazurek, 1975). A signal at δ 79.07 corresponds to C-4 of α-galactopyranose(uronic acid).The presence of C-4 of β-mannuronic acid was observed at δ 77.69–77.50, and C-3 at δ 73.76 (Gorin & Mazurek, 1975; Grasdalen, Larsen & Smidsrød, 1981). Other internal or anomeric signals had the same shifts as those of CF. The ^{13}C NMR spectra of LL and LLOX have been reported (Sierakowski et al., 2000).

The infrared spectra of CFOX [Fig. 2(a)] and LLOX [Fig. 2(b)] exhibited similar bands, namely at 3700–3000 cm^{-1} (OH vibrational stretching), 2924 and 2893 cm^{-1} (symmetric and asymmetric CH stretching), 1415 cm^{-1}

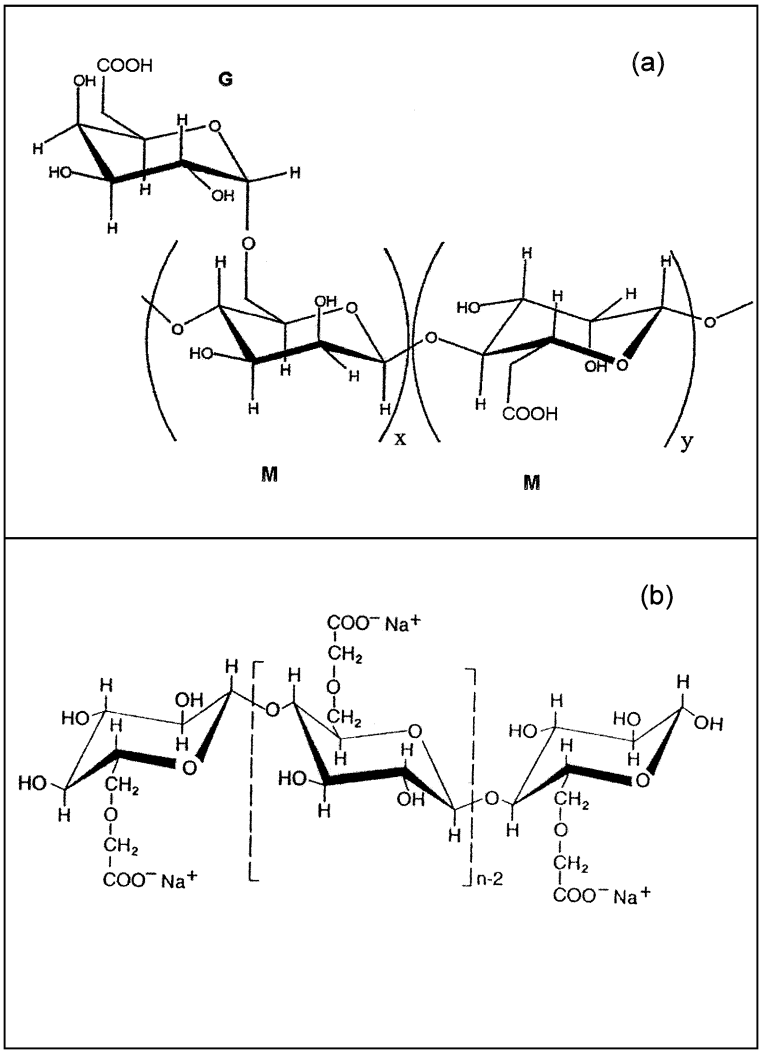

Fig. 3. Schematic representation of the molecular structure of (a) CFOX or LLOX and (b) carboxymethylcellulose. In (a) M and G represent β-D-mannuronic unit and α-D-galacturonic unit, respectively, while x and y represent the number of monomeric units.

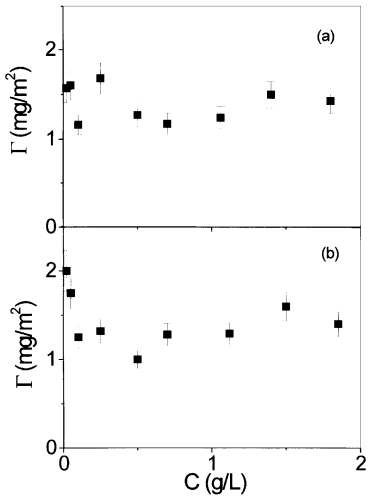

Fig. 4. Adsorption isotherms obtained for (a) LLOX and (b) CFOX at pH 3, ionic strength of 0.001 M NaCl and 22.5 ± 0.5°C onto amino-terminated surfaces.

(OH bending), and 1200–800 cm^{-1} (C—O and C—C stretching vibrations of the hexopyranosyl). The strong absorption at 1615 cm^{-1} was attributed to the C=O stretching absorption of carboxylic acid with an intramolecular hydrogen bond or to a C=O stretching absorption of a carboxylate ion, confirming oxidation of the galactomannans.

The degree of oxidation of CFOX was determined to be 0.22 by OH^- consumption in the reaction, indicating that some C-6 groups of galactosyl and mannosyl units were oxidized by TEMPO. In this work the DO obtained for CFOX is lower than that determined to LLOX. However, the oxidation reaction by TEMPO allows a higher DO to be obtained if required by the final application.

The intrinsic viscosity values, as determined for CF, LL, CFOX and LLOX at pH 3 and an ionic strength of 0.001 M NaCl and 0.1 M NaCl, are shown in Table 1. The native CF and LL chains have a higher hydrodynamic volume than the respective oxidized products, CFOX and LLOX. Although it is not possible to determine their molecular weights, since the Mark–Houwink–Sakurada parameters are not known for the present systems, this reduction in hydrodynamic volume can be attributed to some chain cleavage during the oxidation process. A very weak effect of ionic strength on the hydrodynamic volume was observed.

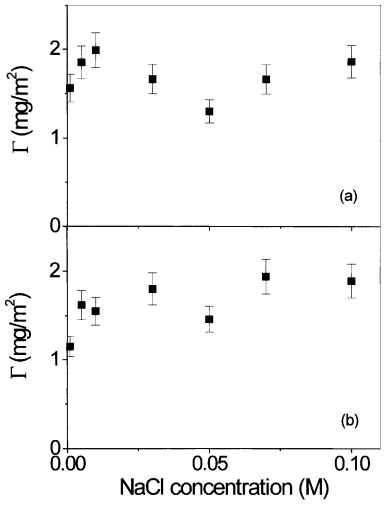

Fig. 5. Plateau values in the adsorption of (a) LLOX and (b) CFOX at pH 3 onto amino-terminated surfaces as a function of ionic strength.

3.2. Adsorption behavior

Theories have been put forward to describe the influence of ionic strength and pH on the adsorption behavior of polyelectrolytes on solid surfaces (Van de Steeg, Cohen Stuart, de Kaizer & Bijsterbosch, 1992; Borukhov, Andelman & Orland, 1998). In the case of polyelectrolytes with low as well as with high segment charge and sufficiently high surface charge density, the adsorbed amount should decrease with increasing salt concentration. This is the so-called *screening-reduced adsorption* regime (Hoogendam et al., 1998). This effect is expected if the attraction between the polyelectrolyte and the surface is mainly electrostatic in nature, since the salt screens not only the segment–segment repulsion, but also the segment–surface attraction. However, when the adsorbed amount increases with increasing salt concentration, the situation gives rise to the *screening-enhanced adsorption* regime (Hoogendam et al., 1998) which has often been found for highly charged polyelectrolytes. The concept is that at high salt concentrations, the strong segment repulsion is screened and the polyelectrolyte chains behave more like uncharged polymers. Hence, they can adopt conformations such as loops and tails, increasing the adsorbed amount. This situation is favored only if there is an attractive interaction between segments and surface, which is not electrostatic in nature.

In the systems studied here, the segment and surface charges are a function of pH. The oxidized galactomannans LLOX and CFOX are ionic polysaccharides, which can behave like polyelectrolytes at appropriate pH values. In comparison with the carboxylic groups present in carboxymethylcellulose, LLOX and CFOX [Fig. 3(a) and (b)], their pK_0 values should be close to 4 (Hoogendam et al., 1998). Therefore, at pH 3 the carboxylic groups are partially dissociated and CFOX and LLOX behave like weak polyelectrolytes. As a reference, the degree of dissociation (α) of carboxymethylcellulose (Hoogendam et al., 1998) is affected by pH and electrolyte concentration. In a 0.01 M NaCl solution, α increases from 0.2 at pH 3.0–0.95 at pH 6.0. The amino-terminated surface is highly protonated at pH < 4, but on increasing the pH value, the surface become uncharged.

Fig. 4(a) and (b) show the adsorption isotherms of LLOX and CFOX, respectively, in 0.001 M NaCl at pH 3, after 4 h of adsorption. Both isotherms present similar features with adsorption plateau close to 1.5 mg m^{-2}. Although LLOX

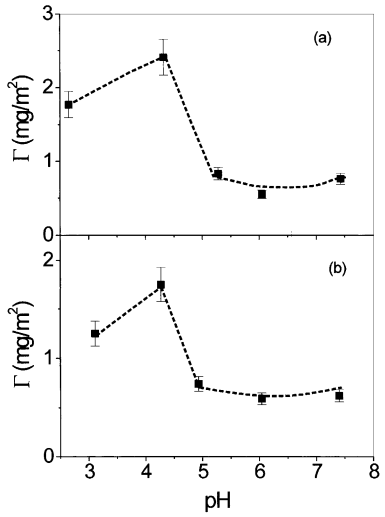

Fig. 6. Plateau values in the adsorption of (a) LLOX and (b) CFOX at ionic strength of 0.001 M NaCl onto amino-terminated surfaces as a function of pH.

has a higher degree of oxidation (DO), this result is not surprising, since Hoogendam et al. (1998) showed that the adsorption of carboxymethylcellulose on mineral surfaces depends neither on DS nor molecular weight.

The influence of ionic strength on the adsorption behavior of LLOX and CFOX on amino-terminated substrates seems to be significant only over a very small range, as shown in Fig. 5. The polymer concentration and pH were set to 1.0 g l^{-1} and 3, respectively. The amount adsorbed is the equilibrium value measured after 4 h of adsorption. An increase in the adsorbed amount was found when the salt concentration was increased from 0.001 to 0.01 M, following the *screening-enhanced adsorption* regime, although LLOX and CFOX are weak polyelectrolytes. Not only electrostatic interactions between LLOX and CFOX segments and the cationic surface might be taking place, but also attractive interactions like hydrogen bonding between amino groups at the surface and the carboxylic or hydroxyl groups belonging to the polysaccharide. For NaCl concentrations higher than 0.01 M, no dependence of the adsorbed amount on the ionic strength could be observed. This is probably due to the low DO of LLOX and CFOX.

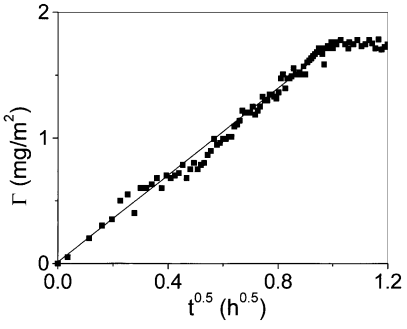

Fig. 7. Adsorption kinetics for a LLOX solution in 0.001 M NaCl at a concentration of 0.002 g l^{-1} at pH 3.

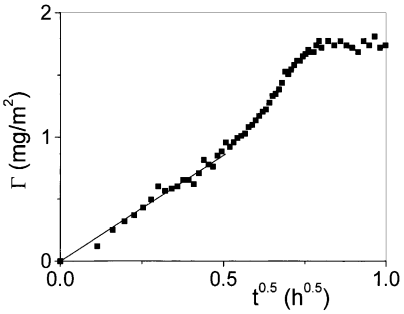

Fig. 8. Adsorption kinetics for a CFOX solution in 0.001 M NaCl at a concentration of 0.002 g l^{-1} at pH 3.

In this system, the pH of the solution controls not only the surface charge but also the segment charge. In Fig. 6, the effect of pH on the adsorption behavior of LLOX and CFOX on amino-terminated substrates is shown. The polymer concentration and ionic strength were fixed at 1.0 g l^{-1} and 0.001 M NaCl, respectively. The adsorbed amount again represents the value measured after 4 h of adsorption. The systems containing LLOX and CFOX behaved similarly. At pH values close to 4, there is a maximum in the adsorbed amount, indicating that the negative charges along the polysaccharides were compensating for the positive charges on the substrate. This effect was more pronounced with LLOX than for CFOX, since the DO for the former is 0.62 and the latter 0.22. At this pH, the adsorption might have been driven mainly by electrostatic interactions. At $pH < 4$, there was an excess of positive charges on the surface and just a few carboxyl groups in the adsorbate, reducing the electrostatic attraction and the adsorbed amount. At $pH > 4$, the carboxyl groups were strongly dissociated, resulting in a high negative segment charge. On the other hand, the amino groups on the surface were poorly protonated, making the adsorption process a very weak one.

3.3. Adsorption kinetics

The adsorption kinetics of polymers is well described in the literature (Motschmann et al., 1991; Fujimoto & Petri, 2001; Siqueira, Reiter, Breiner, Stadler & Stamm, 1996; Siqueira, Pitsikalis, Hadjichristidis & Stamm, 1996). At the initial stages the adsorption is controlled by the diffusion of free chains from the bulk solution to the bare substrate. Considering this mass transport as a Fickian diffusion, the diffusion coefficient D can be calculated from the equation:

$$\Gamma(t) = \frac{2}{\sqrt{\pi}} c_{poly} \sqrt{Dt} \tag{2}$$

Fig. 7 shows the adsorption kinetics of LLOX in 0.001 M NaCl at a concentration of 0.002 mg ml^{-1} onto amino-terminated surfaces at pH 3. Considering the slope of the

Table 2
Advancing (θ_a) and receding contact angle (θ_r) and hysteresis in the contact angle ($\Delta\theta = \theta_a - \theta_r$), as measured for water drops on LLOX- and CFOX-covered surfaces

Substrate	θ_a (°)	θ_r (°)	$\Delta\theta$ (°)
Amino-terminated surface	23 ± 3	19 ± 2	4 ± 2
LLOX	39 ± 2	26 ± 2	13 ± 2
CFOX	36 ± 3	18 ± 3	18 ± 3

adsorbed amount Γ of LLOX as a function of $t^{0.5}$ in the initial stages, the diffusion coefficient D was determined to 9×10^{-7} cm^2 s^{-1}. This value conforms with that of a macromolecule (Fujimoto & Petri, 2001; Siqueira et al., 1996a,b). Fig. 8 shows the adsorption kinetics obtained for CFOX in 0.001 M NaCl at a concentration of 0.002 mg ml^{-1} at pH 3.0 on to amino-terminated surfaces. Data treatment gave a diffusion coefficient D of $\sim 1 \times 10^{-6}$ cm^2 s^{-1}, which is close to that obtained for LLOX. These results corroborate the intrinsic viscosity data (Table 1) found for LLOX and CFOX, which are also similar under the current experimental conditions. If the two polymers had very different dimensions, they would have diffused at different rates.

3.4. Contact angle measurements

Contact angle measurements were performed on dried CFOX- and LLOX-covered surfaces (Table 2). The samples were prepared under the experimental conditions corresponding to the adsorption plateau values in Fig. 4(a) and (b). After the adsorption process, the samples were dried under N_2. The advancing angles measured for CFOX and LLOX were 36 ± 3 and 39 ± 2°, respectively. These values show that the galactomannan-covered surfaces are moderately hydrophilic, but more hydrophobic than the amino-terminated ones, which had angles of 23 ± 3° (Petri et al., 1999). The contact angle hysteresis $\Delta\theta$ values indicate that the surfaces are not flat, since flat surfaces should give rise to $\Delta\theta$ values close to zero. An increase in the surface roughness is expected, since on drying the galactomannan chains might form small aggregates on the surface. A similar effect was observed for cellulose monolayers adsorbed onto gold substrates (Siqueira Petri et al., 1999).

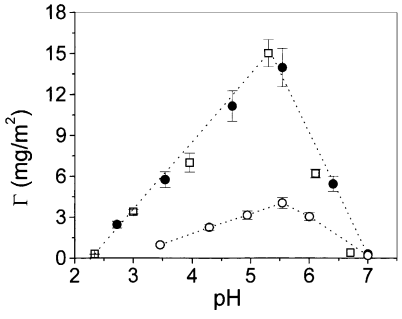

Fig. 9. Adsorbed amount of BSA (c = 1.0 g l^{-1}) onto (□) CMC-, (●) LLOX- and (○) CFOX-covered surfaces as a function of pH.

3.5. Adsorption of BSA on to oxidized galactomannans

CFOX- and LLOX-covered surfaces were tested as substrates for the adsorption of bovine serum albumin (BSA) at pH values of 3–7. For comparison, the adsorption of BSA onto carboxymethylcellulose (CMC) covered substrates (Fujimoto & Petri, 2001) was also measured. The adsorbed amount of BSA varied as a function of pH for all substrates in a bell-shaped fashion (Fig. 9). A preferential adsorption of BSA on to LLOX is evidenced by the high values of Γ with a maximum at pH ~5.5, which coincides with the isoelectric point of BSA (Carter & Ho, 1994). In the case of CFOX, a maximum can also be observed, but the Γ values are much lower than those for LLOX. This different adsorption behavior can be attributed to the degree of oxidation, LLOX having a much higher DO than CFOX. When LLOX is adsorbed on to amino-terminated surfaces, a greater number of carboxylate groups remains free, leading to a higher adsorption process than in the case of CFOX. This is often observed in the formation of polyelectrolyte multilayers (Decher, 1997; Ladam, Schaaf, Cuisinier, Decher & Voegel, 2001). The adsorption behavior of proteins is even more complex than that of polyelectrolytes. Considering that proteins are very complex macromolecules with polar, hydrophobic, and charged regions, three modes of interaction can occur between proteins and solid substrates, namely electrostatic, hydrophobic, and hydrogen bonding. At pH 5.5, the net charge of BSA is zero and the carboxylic groups are partly dissociated, indicating the absence of electrostatic interactions. The net charge of the complex formed by substrate and BSA is very low or close to zero. The CFOX- and LLOX-covered surfaces were hydrophilic, as shown by the contact angle measurements. It follows that hydrophobic interactions between BSA and the substrates are very unlikely. By a process of elimination, it appears that hydrogen bonding between BSA and CFOX or LLOX should have been the most probable interaction, and favored in systems where more carboxylic groups are present, as in the case of LLOX surfaces. However, it has been shown that the enthalpy change involved in the adsorption of proteins is often positive, whatever the substrate (Ladam et al., 2001). It was proposed that the adhesion of proteins on to polymeric surfaces might be driven by structural rearrangements within the protein molecule with breakdown of secondary and tertiary structures on adsorption, leading to an entropy gain, which overcomes the positive enthalpy values. In this way, the Gibbs energy of adsorption becomes negative and the adsorption process is spontaneous. Comparing the values of adsorbed amounts of albumin onto uncharged, positively and negatively charged substrates (van der Vegt et al., 1996; Haynes, Sliwinsky & Norde, 1994; Norde & Lyklema, 1989; Cohen Stuart, Fleer, Lyklema, Norde & Scheutjens, 1991) with those of BSA on

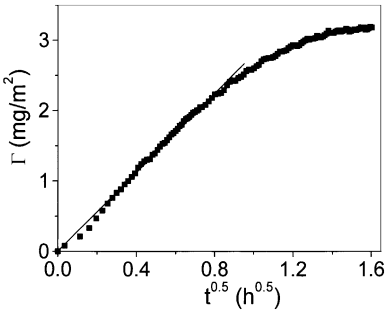

Fig. 10. Typical curve of adsorbed amount Γ of BSA onto LLOX as a function of $t^{0.5}$.

to CFOX and LLOX, it was found that they were similar in the case of CFOX. However, the adhesion of BSA on LLOX is much stronger than that reported for other substrates, whatever the pH. The adsorbed amount of BSA onto LLOX is comparable to that observed for CMC films, albeit the latter presented a much higher substitution degree. It indicates that the preferential adsorption of BSA onto LLOX is driven by the favorable interactions between the polar BSA segments and the carboxyl groups. Moreover, one could speculate about the possibility of the adsorption on LLOX has been also favored by the presence of stereospecific units in its structure. This is an interesting finding, because LLOX seems to provide an excellent substrate for the development of immunoassays or biosensors.

At pH higher and lower than 5.5, the adsorbed amount of BSA on to CFOX and LLOX decreased. On increasing the pH, the net electrostatic repulsion between the protein and carboxyl groups on the surface became greater. At pH 7 there was no adsorption, since the surfaces and the proteins were negatively charged. Decreasing the pH, BSA suffered a native-to-apo transition in solution and thus carried a net positive charge (Norde and Lyklema, 1989), while the substrate became protonated. In the absence of an electrostatic attraction force, the adsorption is likely to have become driven by hydrogen bonding between BSA peptide residues and carboxyl groups on the surface.

The adsorption kinetics of BSA onto CFOX and LLOX covered substrates was investigated at pH 5.5 (isoelectric point) at the concentration of 0.005 g l^{-1}. The kinetic behavior was similar for both galactomannan surfaces. Fig. 10 shows a typical curve of adsorbed amount Γ of BSA onto LLOX as a function of $t^{0.5}$. From the initial slope, the diffusion coefficient of BSA was determined to be 7.5×10^{-7} cm^2 s^{-1}. This value is in agreement with values reported in the literature (Carter & Ho, 1994; Hadly & Andrade, 1988) for single proteins using scattering techniques. It indicates that isolated BSA macromolecules diffuse to the CFOX or LLOX substrates during the adsorption process.

4. Conclusions

CFOX and LLOX, obtained from natural and renewable resources, are attractive substrates for the adsorption of BSA, especially when employed close to its isoelectric point. In comparison to other substrates, the immobilization of albumins onto biofilms of LLOX was very extensive, reaching an adsorbed amount of 13.5 mg m^{-2} at the isoelectric point. This is potentially useful for the development of biotechnological devices. The adhesion of BSA to CFOX surfaces is comparable with that reported for other substrates (Haynes et al., 1994; Norde et al., 1989; Cohen Stuart et al., 1991). The preferential BSA adsorption onto LLOX might be attributed to its higher DO. At pH 7, there was no adsorption, due to electrostatic repulsion. This is also pertinent for the development of biomaterials, where blood clotting should be avoided.

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References

Azzam, R. M. A., & Bashara, N. M. (1987). *Ellipsometry and polarized light*, Amsterdam: North Holland Publication.

Borukhov, I., Andelman, D., & Orland, H. (1998). *Macromolecules*, *31*, 1665–1673.

Carter, D. C., & Ho, J. X. (1994). *Adv. Protein Chem.*, *45*, 153–203.

Charreyre, M. -T., Tcherkasskaya, O., Winnik, M. A., Hiver, A., Delair, T., Cros, P., & Pichot, C. (1997). *Langmuir*, *13*, 3103–3110.

Cohen Stuart, M. A., Fleer, G. J., Lyklema, J., Norde, W., & Scheutjens, J. M. H. M. (1991). *Adv. Colloid Interface Sci.*, *34*, 477–535.

Dea, I. C. M., & Morrisson, A. (1975). *Adv. Carbohydr. Chem. Biochem.*, *31*, 241–312.

Decher, G. (1997). *Science*, *277*, 1232–1237.

Delair, T., Meunier, F., Elaissari, A., Charles, M. -H., & Pichot, C. (1999). Colloids Surf: A Physiochem. *Eng. Aspects*, *153*, 341–353.

Dey, P. M. (1978). *Adv. Carbohydr. Chem. Biochem.*, *35*, 341–376.

Do Serpo, A. P. V. A., Fernandes, A. C., Saramago, B. D. V., & Norde, W. (1999). *J. Biom. Mater. Res.*, *46*, 376–381.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). *Anal. Chem.*, *28*, 350–356.

Fujimoto, J., & Petri, D. F. S. (2001). *Langmuir*, *17*, 56–60.

Giacomelli, C. E., & Norde, W. (2001). *J. Colloid Interface Sci.*, *233*, 234–240.

Gorin, P., & Mazurek, M. (1975). *Can. J. Chem.*, *53*, 1212–1223.

Grasdalen, H., & Painter, T. (1980). *Carbohydr. Res.*, *81*, 59–66.

Grasdalen, H., Larsen, B., & Smidsrød, O. (1981). *Carbohydr. Res.*, *89*, 179–191.

Hadly, V., & Andrade, J. D. (1988). *Colloids Surfaces*, *32*, 359–365.

Haynes, C. A., Sliwinsky, E., & Norde, W. (1994). *J. Colloid Interface Sci.*, *164*, 394–409.

Hoogendam, C. W., de Keizer, A., Cohen Stuart, M. A., Bijsterbosch, B. H., Batelaan, J. G., & van der Horst, P. M. (1998). *Langmuir*, *14*, 3825–3839.

Hoogendam, C. W., de Keizer, A., Cohen Stuart, M. A., Bijsterbosch, B. H.,

Smit, J. A. M., van Dijk, J. A. P. P., van der Horst, P. M., & Batelaan, J. G. (1998). *Macromolecules*, *31*, 6297–6309.

Keenan, M. H. J., Belton, P. S., Matthew, J. A., & Howson, S. J. (1985). *Carbohydr. Res.*, *138*, 168–170.

Ladam, G., Schaaf, P., Cuisinier, F. J. G., Decher, G., & Voegel, J. C. (2001). *Langmuir*, *17*, 878–882.

Maier, H., Anderson, M., Karl, C., Magnuson, K., & Whistler, R. L. (1993). In R. L. Whistler & J. N. BeMiller, *Industrial gums* (pp. 181–227). Chapter: Guar, locust bean, tara and senugreek gum. Academic Press, New York.

Manzi, A., Cerezo, A. S., & Shoolery, J. N. (1986). *Carbohydr. Res*, *148*, 189–197.

Motschmann, H., Stamm, M., & Toprakcioglu, C. (1991). *Macromolecules*, *24*, 3681–3688.

Norde, W., & Lyklema, J. (1989). *Colloid Surf.*, *38*, 1–13.

Petkowicz, C. L. O., Sierakowski, M. -R., Ganter, J. L. M. S., & Reicher, F. (1998). *Phytochemistry*, *49*, 737–743.

Petri, D. F. S., Wenz, G., Schunk, P., & Schimmel, T. (1999). *Langmuir*, *15*, 4520–4523.

Rees, D. A. (1982). *Biochem. J.*, *126*, 257–273.

Robinson, G., Ross-Murphy, S. B., & Morris, E. R. (1982). *Carbohydr. Res.*, *107*, 17–32.

Sackmann, E. (1996). *Science*, *271*, 43–48.

Sierakowski, M. -R., Milas, M., Desbrières, J., & Rinaudo, M. (2000). *Carbohydr. Polym*, *42*, 51–57.

Siqueira, D. F., Pitsikalis, M., Hadjichristidis, N., & Stamm, M. (1996a). *Langmuir*, *12*, 1631–1637.

Siqueira, D. F., Reiter, J., Breiner, U., Stadler, R., & Stamm, M. (1996b). *Langmuir*, *12*, 972–979.

Petri, D. F. S., Choi, S. W., Beyer, H., Schimmel, Th., Bruns, M., Wenz, G. (1999). *Polymer*, *40*, 1593–1601.

van de Steeg, H. G. M., Cohen Stuart, M. A., de Kaizer, A., & Bijsterbosch, B. H. (1992). *Langmuir*, *8*, 2538–2546.

van der Vegt, W., Norde, W., van der Mei, H. C., & Busscher, H. J. (1996). *Colloid Polym. Sci.*, *274*, 27–33.

Wiegand, G., Jaworek, T., Wegner, G., & Sackmann, E. (1997). *Langmuir*, *13*, 3563–3569.