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Adsorption of Hydrophobically Modified 6-Carboxypullulan on a Hydrophobic Surface

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ABSTRACT: New amphiphilic (hydrophobically modified, HM) polymers based on 6-carboxypullulan were synthesized. The reaction chosen was amidation of carboxyl groups via a carbodiimide and was carried out in an organic solvent (DMSO) to ensure random substitution. Four polymers with degrees of substitution of 1, 3, 6, and 12% (per glucose unit) were prepared and characterized by nuclear magnetic resonance (NMR), size exclusion chromatography (SEC), acid/base titration, and dynamic light scattering. We found a very good agreement between the degree of substitution calculated from reaction stoichiometry and as measured by NMR. The kinetics of adsorption and desorption of these HM polymers from water onto polystyrene was studied as a function of pH. The data indicate strong effects due to the hydrophobic groups. We also find evidence for the build up of an electrostatic barrier during adsorption which strongly hampers equilibration.

Introduction

Hydrophobically modified water soluble polymers are a very interesting class of functional polymers.[1] A few hydrophobic groups on the macromolecular chain suffice to give the molecule very pronounced associating properties, leading to either contraction of individual chains or to formation of reversible networks. Because of the latter effect, commercial HM polymers are popular industrial thickeners. In addition, their thickening behavior can be strongly modified by added surfactants. Polymers with a high density of hydrophobic groups have a tendency toward intramolecular association, leading to collapsed chains rather than networks.[2] Because of the tendency to self-associate and form hydrophobic domains,[3] HM polymers are sometimes referred to as “polysoaps”, in particular when association is predominanly intramolecular.[4] However, the term “polysoap” is more commonly used in a more restricted sense as “polymerized surfactants”, thus referring to molecules with a high rather than a low density of alkyl side chains.[1]

In the present paper we are mainly interested in the surface activity of HM polymers.[5] It has been shown that surface activity depends on the way the amphiphile is connected to the backbone: a hydrophilic backbone with pendant apolar chains is very surface active, but when the amphiphiles are attached to the backbone by their apolar end, they are not.[2d,6] Very potent emusifiers are found in the former class of macromolecules. Many HM polymers are also polyelectrolytes. When these are adsorbed on colloidal particles they impart stability not only by a steric effect but also due to the charge they carry. Hence, it should be interesting to know how these chains adsorb, in particular with respect to the density of alkyl groups and charges on the chains. One variable which has turned out to be important but difficult to control is the distribution of the alkyl substituents along the chain. Usually, grafting reactions are carried out in aqueous media in which the parent polymer is obviously soluble but in which the alkyl reagent tends to micellize. As a result, substituents are likely to bind to the chain in “clusters” rather than randomly. This has been shown to affect the associating properties rather dramatically.[7,8] How the substituents are precisely distributed along the chain cannot be determined reliably so that it is difficult to establish correlations between structure and adsorption behavior. Random distributions are simpler in this respect, and these can be achieved in nonaqueous media, provided the polymer dissolves.[9]

From an environmental point of view, polysaccharides should be attractive candidates as raw materials for HM polymers, e.g., as emulsifiers in food systems. We were therefore interested in preparing randomly alkylated polysaccharides and investigate their adsorption behavior on a hydrophobic surface. In this paper we report the preparation of HM polyelectrolytes based on the polysaccharide pullulan. This polymer has been modified in various ways,[10−12] and recently the solution behavior of carboxymethylpullulan carrying hexadecyl groups was studied.[13] We decided to start from carboxypullulan (oxidized pullulan) and to prepare HM polymers carrying various amounts of randomly grafted dodecyl groups. The adsorption of these polymers from aqueous solution onto smooth, hydrophobic polystyrene films was investigated.

Materials and Methods

Surfaces. Polystyrene films were prepared on strips of silicon wafer by spin coating. Polystyrene (BDH, M_w = 100 000, broad distribution) was dissolved in analytical grade toluene to a concentration of 12 g/L. Strips (5 × 1 cm) were cut from a polished monocrystalline silicon wafer (100 face, boron-doped p-type Si). These strips were cleaned and attached to a spin coater, and covered with about 0.5 mL of solution. The strips were then rotated for 30 s at 2500 rpm, and subsequently dried more thoroughly during 2 h at 100 °C. These films are stable when exposed to water up to pH 10. The films were found to be smooth and defect-free when investigated with Atomic Force Microscopy. The thickness and refractive index of the films was established by means of ellipsometry. An average thickness of 56.7 ± 2 nm and a refractive index of 1.577 ± 0.001 were found. The

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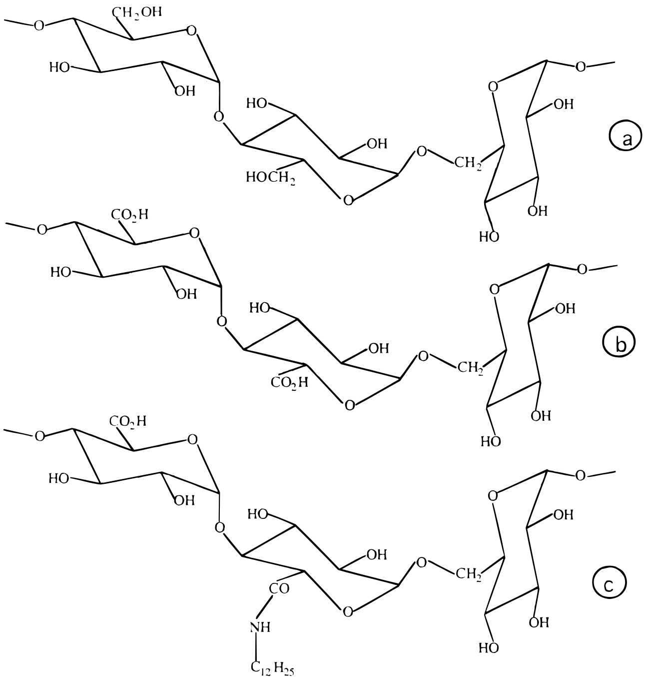

Figure 1. Monomers of pullulan (a), 6-carboxypullulan (b), and hydrophobic-modified carboxypullulan (c).

films are very hydrophobic, with an advancing contact angle for water close to 90°.

Polymer Synthesis. Pullulan is a bacterial polysaccharide consisting essentially of maltotriose units. The structure of this unit is presented in Figure 1; as can be seen it carries two primary alcohol groups per monomer unit. A few maltotetraose units, which carry three primary alcohol groups each, may occur.[14] Pullulan is very flexible,[15] with a characteristic ratio of 4.4 at 25 °C and a persistence length of 1.2−1.9 nm. It is quite soluble in water.[16] The pullulan we used was supplied by Hayashibara Biochemical Laboratories, Okayama, Japan. Its molar mass was established by means of SEC in DMSO: $M_w = 110$ kg/mol, $M_n = 63$, and $M_w/M_n = 1.8$.

The primary alcohol groups were first selectively oxidized in aqueous solution using a method developed by Nooy et al.[17] based on the use of tetramethyl-1-piperidinyloxy (TEMPO) as a catalyst. After excess oxidant was reduced, this solution was dialyzed against water in a cold room and subsequently freeze-dried. An acid ion-exchange resin was added in order to obtain the polymer in its acid form. The oxydized polymer was again analyzed by SEC: $M_w = 95$ kg/mol, $M_n = 65$ kg/mol, and $M_w/M_n = 1.4$. This is somewhat lower than the initial masses, which we ascribe to (very minor) degradation. Short chains may have been removed during dialysis, which probably explains the lower polydispersity index.

For the grafting reaction, oxydized pullulan was dissolved into DMSO to a concentration of 30 g/L. To be soluble, the polymer has to be strictly in its acid form. The solution was stirred for 8 h at a temperature of 60 °C, and the required amount of dodecylamine, dissolved in warm DMSO (70 °C), was added under stirring. Finally, dicyclohexylcarbodiimide (DCC),[18] dissolved in warm DMSO, was added dropwise up to a mole ratio of 1.2 with respect to the amine, under vigorous stirring. After adding DCC, the reaction mixture was stirred strongly for another 15 min and then more gently during 18 h, meanwhile keeping the temprature at 60 °C. The reaction mixture was then allowed to cool to room temperature.

Attempts to precipitate the polymer with organic solvents were not successful. We tried methanol, ethanol, dioxane, THF, and acetone. With acetone, clouding occurs, but no precipitate is formed. However, adding a small amount of salt is very effective, as the salt form of the polymer is much less soluble due to the well-known ′ionomer effect′.[19] Thus, 20 mg of NaCl (0.3 mmol) is first added to the solution. The solution is then filtered over sintered glass and subsequently poured into excess (250 mL) acetone. The polymer then precipitated and could be collected over a filter and dried. Finally, the polymer was purified by redissolving it into water and precipitating into acetone. Overall yield: 85%.

Polymer Characterization. The degree of oxidation of the 6-carboxypullulan was determined with a specific colorimetric test.[20] The method was calibrated with solutions of D-glucuronic acid of known concentrations. We found a value of 74% with respect to the initial number of primary alcohols, implying that about three out of four primary alcohols had been oxidized. The degree of oxidation with respect to the total number of glucosidic units is 49%.

^{1}H NMR in D_2O at 200 MHz (AC-E 200 spectrometer, Bruker) was also used to check the degree of substitution of the polymers. A small amount of the sodium salt of 3-(trimethylsilyl)propionic acid-d_4 was used as an internal reference. The concentration of the polymer was 10 g/L.

Molecular weights were determined by size exclusion chromatography (SEC). The SEC system was composed of a column Styragel HT 6E (Waters Chromatography), a Sodex RI SE−51 detector, and a Mini-Dawn light scattering detector (Wyatt Technology Corporation). A mixed solvent DMSO/water (90/10, in volume) was used (temperature, 50 °C; flow rate, 0.8 mL/min). In this solvent, we expect no intramolecular association between the macromolecular chains. A series of pullulan standards (Polymer Laboratories) was used to calibrate the setup.

Convential automated potentiometric acid−base titration was used to determine the number and nature of the dissociating groups. The samples were titrated under nitrogen with 1 M KOH and 1 M HCl as titrants and 10^{-3} M KNO_3 as background electrolyte.

Diffusion coefficients were measured by means of dynamic light scattering in dilute aqueous solution using an ALV 125 setup, with a 150 mW Ar ion laser tuned at 514.5 nm as the light source, and an ALV-500 digital correlator. Samples were thoroughly filtered and centrifuged at 15000*g* for 1 h. The polymer concentration was kept constant at 0.5 g/L and the temperature was 25 °C. Without added electrolyte, even at pH 2, scattering was very low due to the strong electrostatic interaction between molecules (high $d\pi/dc$). Addition of 10^{-3}M NaCl dramatically increased the scattered intensity. The scattering angle was scanned from 30 to 120° with steps of 10°, and each measurement was repeated three times. The intensity autocorrelation function was recorded for each angle, and analyzed with the multiexponential fit program CONTIN [21] to give the distribution of diffusion coefficients, D_c. The distribution of the hydrodynamic radius, R_h, was then calculated using the Stokes−Einstein equation

$$R_h = \frac{k_B T}{6\pi\eta D_c} \qquad (1)$$

where k_B, T, and η are respectively the Boltzmann constant, the temperature, and the viscosity of the solvent.

The specific refractive index increment of the polymers dn/dc, needed to convert measured reflectometry signals to an adsorbed amount (see below), was measured (at various degrees of dissociation) with a (white light) differential refractometer (Model 1107, Laboratory Data Control). The temperature in the measurement cell was kept constant at 24.0 ± 0.1 °C with a thermostat bath (Julabo F10). Calibration was done with sucrose solutions of known concentrations.[22] The presence of water in the solid polymers (10.2% and 5.3% of the total weight, respectively for OxPu and OxPu-12/12) was taken into account when making up solutions.

Reflectometry. Adsorption experiments were carried out with the reflectrometric technique described by Dijt et al.,[23] i.e., with an impinging jet flow cell. The principle and setup of this technique has been extensively discussed elsewhere. The reflectometer signal can be shown to depend linearly on the adsorbed amount and can be calibrated using standard optical reflection theory. For the calculation of the calibration factor, optical constants (refractive index, layer thickness) of the substrate assembly consisting of Si with an oxide and a polystyrene layer are required, as well as the specific refractive index increment dn/dc of the adsorbate. For our substrate the calibration factor satisfies the empirical equation: $Q = 5.82\ (\mathrm{d}n/\mathrm{d}c)^{-0.98}$, with Q in mg·m^{-2} and dn/dc in mL·g^{-1}.

The adsorption in and around the stagnation point arising from the wall jet is followed as a function of time. It has been shown that the rate of adsorption dΓ/dt in the impinging jet flow cell can be quite generally described by[23]

$$J = \kappa\, D_c^{2/3}(c_b - c_s) \qquad (2)$$

where D_c is the diffusion coefficient, c_b the bulk concentration, and c_s the subsurface concentration of the adsorbate. The factor κ depends on the hydrodynamic conditions (flow rate) and geometric properties of the flow cell. The limiting adsorption rate J_0 is obtained for the "perfect sink" condition $c_s = 0$. By calibration with poly(ethylene oxide) with a known D_c, we determined an experimental value for κ of 6.56 m$^{-1/3}$ s$^{-1/3}$, which is in reasonable agreement with the theoretical value.

In all adsorption experiments, the concentration of the polymer was 100 mg/L, unless stated otherwise. No extra salt was added to the solutions, and the pH was adjusted with concentrated chlorhydric acid (HCl, 1 M) or concentrated sodium hydroxide (NaOH, 1M). The solutions were stored at 5 °C and used within 2 weeks. It was not possible to control the temperature in the measuring cell directly but the room was thermostated at 24 ± 2 °C. All adsorption experiments were repeated at least twice, and the results given are the average value of the different measurements.

Results and Discussion

Polymer Characteristics. Four hydrophobically modified samples were prepared with different levels of dodecyl groups (see Table 1). In the following, we will refer to the different polymers with the abbreviations OxPu for the C6-carboxypullulan and OxPu-12/t (t, percent of dodecyl groups) for the hydrophobically modified oxypullulans.

Table 1. Percentage of Carboxyl Groups, *p*, and of Dodecyl Groups, *t*, for the Five Different Polymers[a]

	OxPu	OxPu-12/1	OxPu-12/3	OxPu-12/6	OxPu-12/12
t(%)		1.3[b]	3.3[bh]	6.5[b]	13.1[b]
		0.9[c]	3.1[c]	6.2[c]	12.2[c]
p(%)	48.7[c]/49.3[d]	48.1[e]	45.9[e]	42.8[e]	36.8[e]

[a] With respect to the number of glucosidic units. [b] Calculated, supposing that the reaction is complete. [c] Measured by ^{1}H NMR. [d] Measured with a specific uronic acid test. [e] Obtained by subtraction: $p = 49 - t$.

A representative NMR spectrum is given in Figure 2. Spectrum a is for 6-carboxypullulan. This shows a number of overlapping resonances due to the glucose ring in the ranges 3.5–4.4, 5.0, and 5.35–5.55 ppm. This latter peak is quite broad and a shoulder is clearly seen corresponding to a nonoxidized glucose. The integration of the peaks 3.5–4.4 ppm and 5.35–5.55 ppm allowed to estimate the degree of oxidation. Spectrum b is for 10% modified carboxypullulan. Similar chemical shifts were found for the hydrophobically modified oxypullulans. The presence of alkyl groups is proven by a broad resonance between 0.75 and 2.1 ppm which corresponds to 23 hydrogens of the aliphatic chain. In addition, there is a third shoulder on the peak at 5.5 ppm. By integration of the 0.75–2.1 ppm region we obtained the degrees of substitution of the different polymers, summarized in Table 1, in percent with respect to the number of *glucosidic* units. Note that for the five polymers, 26% of primary alcohol groups are still present. The results clearly show the good agreement between the degree of modification as calculated supposing that the reaction is complete and as measured by ^{1}H NMR. The small discrepancy observed for the highest level (OxPu-12/12) may come from a broadening of the peaks and a less acurate integration, certainly due to an increase of the viscosity.

The SEC results are summarized in Table 2. The molar masses of oxidized pullulan were found to be slightly lower than for the native polymer, indicating some (but very minor) degradation. Short chains may have been removed during dialysis, which probably explains the lower polydispersity indices. We should realize, however, that the light scattering detector becomes rather insensitive toward the lower molar mass end of the chromatogram, so that M_n may be lower, and the polydispersity indices higher than given here. The result found for OxPu-12/12 (Table 2) essentially shows that the chemical reaction is nondegrading.

Using the measured number average molar mass M_n and the percentage of dodecyl groups t on the chain, we calculated the average number of glucosidic units N and the average number of dodecyl groups per chain ν. We also give the average number of glucosidic units μ between dodecyl substituents, see Table 3. We note that these values may have been somewhat overestimated, again because of the overestimation of M_n.

Solution Properties. All the polymers were readily soluble in water up to concentrations well into the semidilute regime (40 g/L). No gel appeared, even though the viscosity of the solutions was high, especially for OxPu-12/12. We take the good solubility of the polymers in water as evidence of the random distribution of the alkyl groups on the macromolecular chain. When the pH decreased, all modified polymers remained

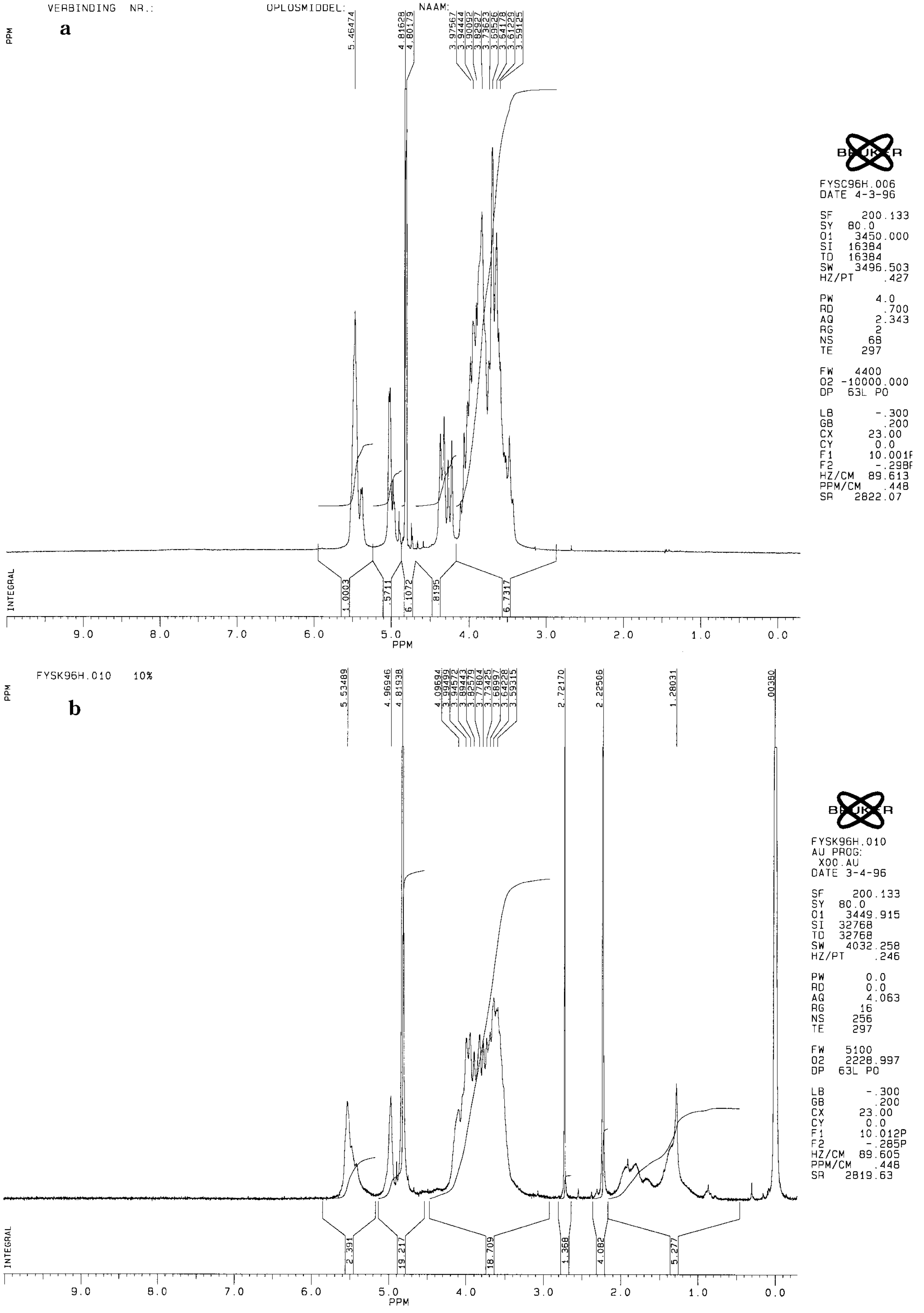

Figure 2. NMR spectra of OxPu (a) and OxPu-12/10 (b).

Table 2. Weight Average Molar Mass, M_w, and Polydispersity Index, I, Determined by SEC

	pullulan	oxidized pullulan OxPu	modified pullulan OxPu-12/12
M_w (kg/mol)	120	95	105
$I = M_w/M_n$	1.85	1.45	1.32

Table 3. Average Numbers of Dodecyl Groups ν, and of Glucosidic Units Between Dodecyl Groups μ

	OxPu-12/1	OxPu-12/3	OxPu-12/6	OxPu-12/12
μ	83	29	15	7
ν	4	13	26	51

Table 4. Average Hydrodynamic Radius Measured with DLS, in nm

	OxPu	OxPu-12/6	OxPu-12/12
pH = 2	7.5 ± 0.5	8.2 ± 0.6	6.8 ± 0.4[a]
pH = 3	6.2 ± 0.6	10.5 ± 1.2	8.8 ± 0.7

[a] Measured at 286 K.

soluble except OxPu-12/12 which gave at 0.5 g/L a hazy solution at low pH; a 0.1 g/L solution gave a clear and homogeneous phase. At room temperature the pH of transition, as determined by visual observation, was 2.3. The clouded solution returned clear when it was cooled indicating a (pH dependent) LCST behavior, as shown by many water soluble polymers. The clouding point at pH 2.3 was approximatively determined to be 289 K. Whereas the polymer responds rapidly to pH changes, the response to changes in temperature is much slower, especially once an aggregated phase has formed.

Figure 3 gives the degree of dissociation α as inferred from titration data.[24] As can be seen, the behavior is rather similar for all samples. Dissociation starts at rather low pH, indicating a relatively strongly acidic character of the 6-carboxypullulan. The apparent pK is on the order of 3.5 which is one unit stronger than acetic acid. We tend to ascribe this to the presence of several oxygen atoms in the vicinity of the carboxyl group. The apparent pK increases only slightly with pH, indicating that the electrostatic interaction between neighboring carboxyl groups is not very important, which is expected because of the relatively large distance between them. As a result, full dissociation is achieved at about pH 5.5. Minor differences are seen in the low pH range between the titration curves for the unmodified and the modified carboxypullulans, suggesting a stronger acidity and a somewhat stronger interaction between charged groups. However, the differences are hardly significant in view of inevitable experimental errors at these low pH values.

The size of the polymer molecules in solution was studied by dynamic light scattering. For most samples, the autocorrelation function showed two decay times, one of them corresponding to a large particle size, with a hydrodynamic radius higher than 50 nm. The appearance of a slow mode in a system of higly charged polyelectrolyte and low ionic strengh has been previously reported.[25] Nevertheless, this higher decay time clearly disappeared when the scattering angle increased and it seems likely that a few aggregates persisted in the solution, as is often the case with polysaccharides. The diffusion coefficient D_c, corresponding to the short decay time, was independent of the scattering angle and was considered to represent free molecules in solution. The average value of D_c was calculated and the corresponding hydrodynamic radius, R_h, calculated with eq 1, is given in Table 4.

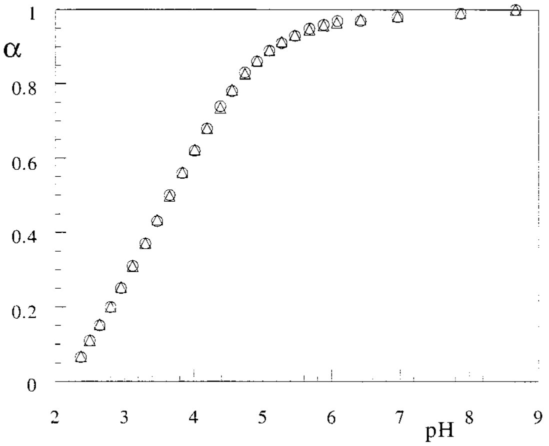

Figure 3. Degree of dissociation as a function of pH: (○) OxPu and (△) OxPu-12/12.

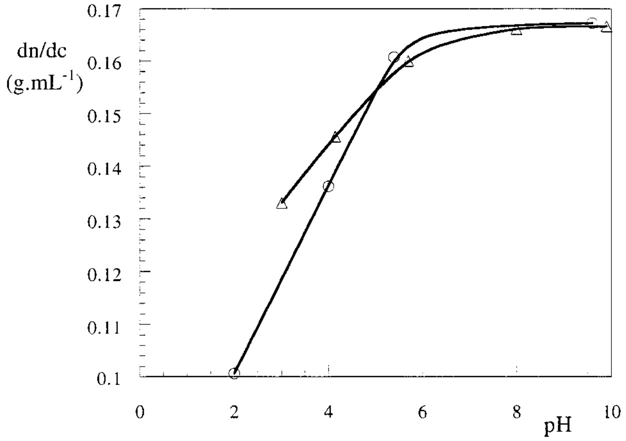

Figure 4. Specific refractive index increment dn/dc as a function of pH: (○) OxPu and (△) OxPu-12/12.

At pH = 2, the polymers are essentially uncharged and their configuration is certainly very close to the configuration of pullulan in water. According to ref 16a, R_h for pullulan is 8.7 nm (respectively 9.1 nm) for M_w = 95 kg/mol (respectively 105 kg/mol). At pH 2 and 3 we found a somewhat lower value for the oxidized pullulan. This may be due to intermolecular interactions.[25,26] (It would have been better to determine R_h from measurements at various concentrations and extrapolating to zero concentration C. However, this is practically excluded because of lack of sensitivity at low C.) Alternatively, the result may reflect the slight degradation of the polymer during oxidation.

The R_h values found for OxPu-12/6 are closer to the values for pullulan of the same molecular weight. When the pH is increased from 2 to 3, R_h increases from 8.2 to 10.5, which is consistent with a coil expansion of the macromolecule. Molecules of OxPu-12/12 are clearly smaller that those of OxPu-12/6. Moreover, diffusion coefficients inferred from the initial rate of adsorption are consistent with those obtained from light scattering. It is thus likely that this result indeed reflects the (slight) contraction of the polymer under the influence of the hydrophobic groups.

The specific refractive index increment data for two polymers, OxPu and OxPu-12/12, as a function of pH are given in Figure 4. As can be seen, the differences between the two samples are minor; both exhibit a pronounced increase with pH. The dn/dc for the other HM polymers was calculated assuming it to be a linear

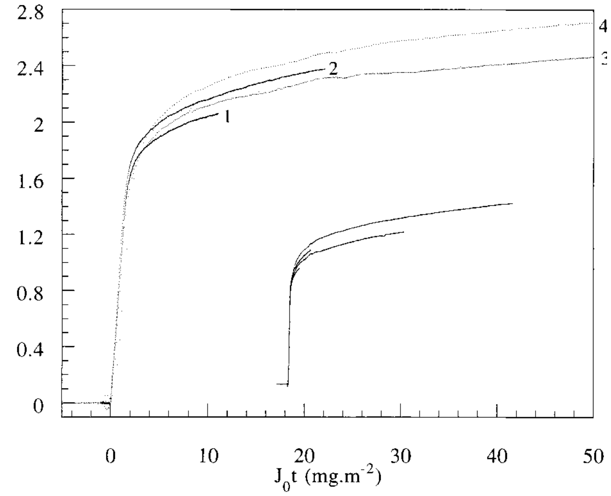

Figure 5. Adsorption of OxPu-12/12 as a function of time (normalized with respect to the limiting flux J_0 for four different concentrations: (1) 5, (2) 10, (3) 50, and (4) 100 mg/L. The inset shows the adsorption curve for J_0t between 0 and 240 mg.m^{-2}. The pH was fixed at 2.

function of the composition of the polymer expressed in terms of monomeric weight fractions of pullulan, carboxypullulan, and dodecyluronamide[22] (see Figure 1). For native pullulan, dn/dc was taken to be 0.15 mL/g independent of pH.[16b]

Adsorption. By way of example, we plot in Figure 5 a set of four reflectometry curves for OxPu-12/12 from water at pH 2; each curve corresponds to a different concentration in solution. As can be seen, the adsorption initially rises linearly with time (dΓ/dt constant). For various uncharged polymers, it has been shown that this corresponds quite well to the limiting rate. Plotting the initial slopes as a function of c^b gives a straight line in agreement with eq 2. It is therefore adequate to plot the adsorption curves as a function of J_0t rather than t, so that curves for various concentrations coincide at low t. Beyond $J_0t = 1$ mg/m^2, at a coverage of about 1.6 mg/m^2, the curves begin to level off. Here, the curves do not exactly coincide, but there is no systematic effect of the polymer concentration on the adsorbed amounts reached, which seems to exclude slow spreading effects.[27]

In parts a and b of Figure 6, we present adsorption curves for the various polymer samples, all taken at 100 mg/L and pH 3.5. The differences are very pronounced, OxPu-12/12 showing the strongest adsorption and OxPu the lowest. The initial slopes are not very different; we discuss these below. The adsorption of OxPu levels off to a very stable value after 100 s, but all the other, hydrophobically modified polymers retain a substantial adsorption rate up to at least 1800 s. It is interesting to note that the adsorption rate in this “slow regime” (e.g., >800 s) is nearly the same for all HM pullulans. For OxPu-12/1 it is even almost constant between 300 and 1800 s. In terms of eq 2, this implies that c^s is also constant, but markedly different from zero. If we may assume (as is often done) that c^s is determined by a *local* equilibrium between adsorbed layer and free polymer in solution,[28] our result implies that for OxPu-12/1 the adsorbed amount doubles between 300 and 1800 s, without a notable change in the corresponding concentration. In other words, the isotherm would have a steeply rising part at finite concentration, as is often found for associating molecules such as surfactants. At 1800 s, the supply of polymer was stopped, but the shape of the curves suggest that it is very unlikely that saturation had been reached by this time.

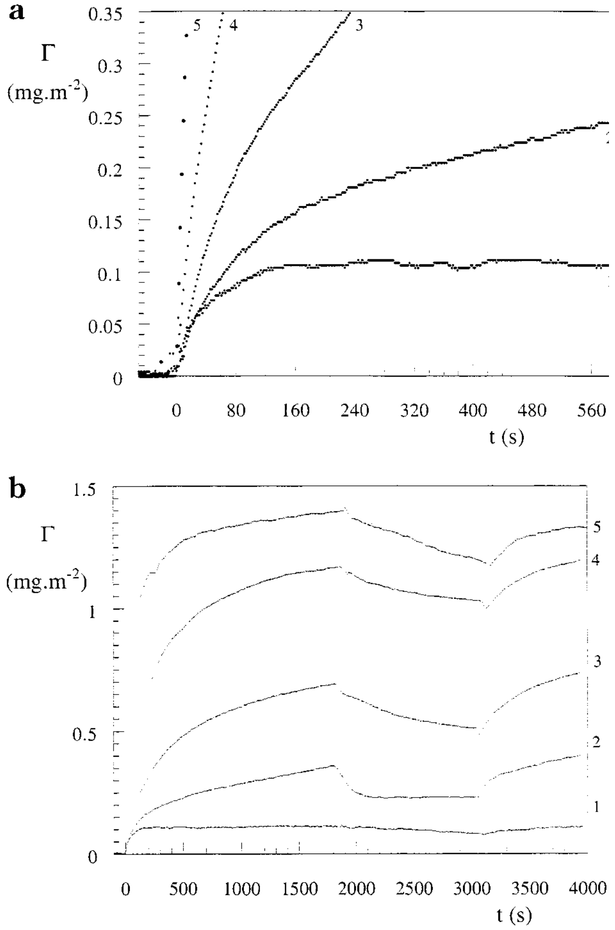

Figure 6. Adsorption as a function of time for five polymers: (1) OxPu, (2) OxPu-12/1, (3) OxPu-12/3, (4) OxPu-12/6, and (5) OxPu-12/12. The pH was initially 3.5 and the polymer concentration was 100 mg/L. At 1800 s, the surface was rinsed with demineralized water. Supply of polymer solution was resumed at 3000 s. Key: (a) curves up to 560 s; (b) experimental curves up to 4000 s.

The polymer-covered surface was then rinsed with demineralized water. This induces no detectable desorption in the case of OxPu, but for the other samples, there is substantial desorption. For OxPu-12/1, a stable value is reached after 200 s, but the more hydrophobic samples continue to desorb up to 3000 s, when supply of polymer is resumed, and the adsorption rises again. The desorption curves are very similar for OxPu-12/3, 12/6, and 12/12, showing first a minor rapid desorption (~0.05 mg/m^2) and then a slow decay at roughly fixed rate. OxPu-12/1 shows a larger rapid desorption step (0.15 mg/m^2), but this is not followed by slow further desorption. The data suggests that there is a substantial fraction of the adsorbed chains which has a low affinity and is therefore able to detach rather easily. This contrasts sharply with the behavior of simple homopolymers, where desorption is usually extremely small (if detectable at all) and the desorption follows a logarithmic law.[29] Similar effects are found for block copolymers adsorbed from a selective solvent in which association of insoluble blocks occurs. This might indicate that part of the adsorbed molecules is primarily adsorbed by association with other molecules rather than by direct attachment to the substrate.

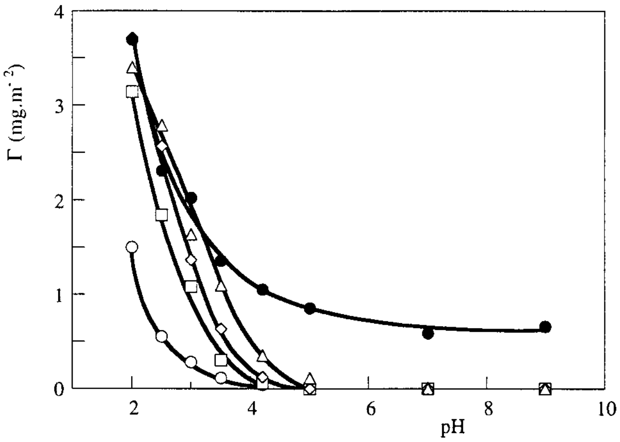

Figure 7. Adsorbed amount as a function of pH: (○) OxPu, (□) OxPu-12/1; (◇) OxPu-12/3; (▽) OxPu-12/6, (●) OxPu-12/12. The adsorbed amount was measured after 1800 s, and the polymer concentration was 100 mg/L.

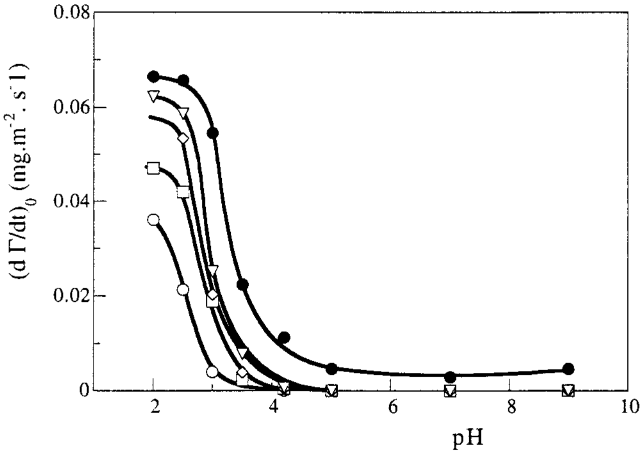

Figure 8. Initial adsorption rate (d/dt)$_0$ as a function of pH: (○) OxPu, (□) OxPu-12/1, (◇) OxPu-12/3, (▽) OxPu-12/6, (●) OxPu-12/12. The polymer concentration was 100 mg/L.

In Figure 7 we give the pH dependence of the adsorbed amounts from 100 mg/L solutions as determined after 1800s of adsorption in the flow cell. Again, we see considerable effects of the degree of alkylation. At pH 2, the carboxyl groups are nearly uncharged. At this pH, all samples exhibit considerable adsorption, the highest adsorbed amount being found for OxPu-12/6 (3.7 mg/m^2). Unmodified carboxypullulan, even though it lacks any hydrophobic groups, still adsorbs up to 1.5 mg/m^2. This amount is quite comparable with the (pH-independent) adsorption of native pullulan without any carboxyl groups.[30] Apparently, the polysaccharide ring interacts sufficiently strongly (presumably by London–van der Waals forces) with the hydrophobic surface to induce adsorption.

Between pH 2 and pH 6, the adsorbed amounts drops continuously. This is exactly the range where the carboxyl groups are titrated. Hence, we conclude that the reduction in adsorption is primarily due to dissociation of these groups. With the exception of OxPu-12/12, the adsorption even goes to zero for all samples. The fact that the desorption goes to completion implies that the buildup of charge does not only lead to unfavorable lateral interaction (which has only consequences at finite adsorbed amounts) but also to a repulsion between the polymer and the substrate. Since the small ions (notably cations) will not adsorb on polystyrene, adsorption of the polyelectrolyte would confine the counterions to a smaller volume than in the bulk solution, which is entropically unfavorable. This appears to be sufficient to suppress the adsorption, even for samples with a substantial amount of dodecyl groups. Only OxPu-12/12 appears to adhere more strongly so that, at high pH, its adsorption drops to a constant value of 0.7 mg/m^2. Similar results were found with HM poly(acrylic acid).[31] An alternative explanation is that the polystyrene surface is negatively charged due to adsorption of anions, and thus repels negatively charged polymers. As yet we cannot easily discriminate between these two explanations.

Figure 8 presents the initial slopes for various pH's and alkylation levels. Again, we notice the effects of alkylation. At pH 2, the initial rate increases somewhat: OxPu-12/12 adsorbs about twice as rapidly as OxPu; other samples give intermediate rates. This means that at pH 2 the modified samples diffuse somewhat faster than the unmodified ones. This rules out the possibility that intermolecular association occurs. Instead, we have to conclude that alkylation leads to a modest shrinking of the chains. This conclusion is supported by our light scattering results which are also indicative of some contraction of the molecules at low pH. Taking the initial slopes as a measure of the diffusion coefficient, we find that the hydrodynamic radii of the modified samples are all smaller than the unmodified OxPu by factors varying between 1.5 (for OxPu-12/1) and 2.5 (for OxPu-12/12). Our light scattering data confirm this in a qualitative sense. Quantitative agreement is not obtained, however; in particular for OxPu the molecular size as obtained from light scattering (7.5 nm) is sustantially smaller than that derived from the initial rate of adsorption (17 nm). This may be due to the lack of an extended linear regime in the adsorption curve of this polymer and the concomitant underestimation of the adsorption rate, see Figure 6a.

At pH's higher than 2, the rate of adsorption drops gradually, as does the adsorbed amount after 1800 s. This agrees with our interpretation that a repulsive electrostatic barrier builds up when the chain is progressively charged.[32] The general trend that the adsorption rate is enhanced by increased levels of alkylation, however, remains.

It has been found for various systems that adsorption of polyelectrolytes shows hysteresis in pH cycles: the adsorbed amount is not uniquely defined by the pH but depends on the pH history of the sample. Hysteresis tends to be particularly pronounced when strong non-electrostatic interactions of short-range bind a polymer to a surface. We have therefore examined, for the various polymers, the fraction desorbed upon increasing the pH from a given initial value (at which adsorption took place) to pH 6. We have seen that beyond pH 6 adsorption did not occur, except for OxPu-12/12. Hence, if hysteresis is absent, complete desorption should occur upon increasing the pH to 6.

Figure 9 gives the fraction desorbed upon increasing the pH to 6, plotted as a function of the initial pH. Clearly, desorption is far from complete. The desorption of OxPu responds most strongly to the initial pH; almost 90% is desorbed when adsorption is carried out at pH 2, and this fraction falls more or less linearly with the adsorption pH. At an initial pH of 4.2, the (absolute) adsorbed amount is already very small; here, hardly any

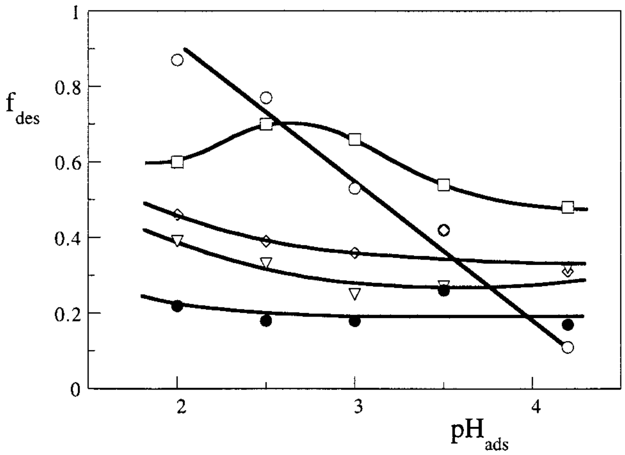

Figure 9. Fraction f_{des} of desorbed polymer after increasing the pH to 6, as a function of the pH of adsorption pH_{ads}: (○) OxPu, (□) OxPu-12/1, (◇) OxPu-12/3, (▽) OxPu-12/6, (●) OxPu-12/12. See text for the definition of f_{des}.

desorption occurs at all, and it is difficult to determine the desorbed fraction reliably. However, it is certainly very small.

The hydrophobically modified samples behave rather differently, each showing more or less constant desorbed fractions (i.e. the desorbed amount is proportional to the initial adsorption). The higher the degree of alkylation, the smaller the desorbed fraction, hence the stronger the hysteresis. The modified polymers have adsorbed amounts of about 3.4 mg/m^2 at pH 2; bringing the pH to 6 causes desorption of 60%, 46%, 40% and 22%, respectively, for OxPu-12/1, -12/3, -12/6, and -12/12. In absolute amounts, this ranges between 0.7 and 2 mg/m^2. Clearly, the higher the alkyl content of the polymer, the more tenacious its attachment to the substrate and the more pronounced the hysteresis.

From the data discussed so far, it is clear that the dodecyl groups attached to OxPu affect its adsorption behavior substantially, both with respect to kinetics and with respect to the adsorbed amount. In solution, the modified molecules appear to be somewhat contracted due to the formation of hydrophobic domains by the dodecyl groups. As a result, the molecules diffuse slightly faster to the surface, where they first attach and then spread. Depending on pH, each molecule brings a certain amount of negative charge to the surface, so that patches of negative potential are formed which repel incoming molecules. In this stage, short range polymer−surface attractions compete with counterion entropy losses; too much charge on the molecule can preclude its adsorption, unless this is compensated by sufficient hydrophobicity (OxPu-12/12 at pH > 6). Once the surface is largely covered with charged patches, adsorbing molecules must overcome a repulsive potential barrier which slows down their adsorption; since no salt was added in these experiments, such a barrier is substantial.[32] As soon as the barrier becomes sufficiently high, adsorption may stop altogether, even though equilibrium (in a thermodynamic sense) is not reached. This seems to occur at low adsorption for OxPu.

As is borne out by the data in Figure 6, the dodecyl groups postpone the formation of such a barrier. Since the HM polymers possess almost as much charge as OxPu (see Figure 3) one expects a similar barrier for the same coverage, in contrast with what the data show. It is tempting to conclude that the modified molecules tend to retain their compactness to some extent, forming smaller patches and leaving larger open spaces for incoming molecules. As a consequence, the rate of adsorption decreases less abruptly. An intriguing finding is that the rate of adsorption reaches a new stable level after some time. This means that the attachment rate is again insensitive for the amount of polymer (and charge) per unit area. If one supposes that the barrier is mainly of electrostatic origin this implies that the surface potential remains constant, despite the accumulation of charged groups. The most likely explanation is that the local potential at the surface has become so high that protons are taken up to the extent that further accumulation of charge stops. The rate at which this uptake occurs seems to control the adsorption rate; it is more or less the same for all samples at a given pH.

If we accept the idea of an electrostatic barrier, we can qualitatively account for the hysteresis effects as substantiated by the data in Figure 9: adsorption is kinetically limited, but can be enhanced by removing or lowering the barrier. Once a molecule is attached, the short-range forces can contribute to stabilizing the adsorbed layer at a high coverage.

Conclusions

New and interesting hydrophobically modified water soluble polymers can be prepared from pullulan. This polymer is first carboxylated by selective oxidation of primary alcohol groups and then reacted with alkylamines to alkylamides. Carrying out the reaction in nonaqueous medium (DMSO) leads to randomly substituted polymers which remain water soluble up to remarkably high degrees of substitution. These molecules do not aggregate but contract, probably due to the formation of hydrophobic domains within them.

At low pH, the modified polymers adsorb much more strongly to a hydrophobic polystyrene surface than the parent pullulan and carboxypullulan. At high pH, adsorption occurs only for the highest degree of alkylation. Adsorption at low pH, followed by an increase in pH, leads to incomplete desorption. This kind of hysteresis in the adsorbed amount is interpreted in terms of an electrostatic barrier repelling incoming molecules during the adsorption process. The adsorption kinetics displays for all the modified pullulans a characteristic pattern which can also qualitatively be explained on the basis of this repulsive barrier.

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