Mucoadhesion of Latexes. II. Adsorption Isotherms and Desorption Studies

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Adsorption isotherms of poly(styrene) latexes on rat intestinal mucosa were studied under static conditions and analyzed according to different isotherm classifications. Isotherms of latexes with a particle size up to 670 nm had the characteristic shape of a disperse adsorbate on a porous adsorbent. Plateaus were reached at latex concentrations of about 2.5 g/L. The results indicated an increase in adsorption with the size and the hydrophilicity of the latexes. Typically, a surfactant-free carboxylate latex of 230 nm had a plateau of 0.66 g/m^2, and a latex of 320 nm with added sodium dodecyl sulfate had a plateau of 0.881 g/m^2. Surfactant-free carboxylate latex of 2 μm had a Langmuirian isotherm with a plateau level of 2.616 g/m^2, which corresponded to a monolayer of adsorbed particles on the surface of mucosa. Desorption studies showed that the adsorption was irreversible. Adhesion to the mucous gel layer would therefore be limited by the mucus turnover.

KEY WORDS: mucoadhesion; bioadhesion; latex; isotherm; adsorption; nanoparticle.

INTRODUCTION

Determining the mechanisms of adsorption and the relevance of possible influence of various parameters altogether has been shown to be difficult. Parfitt and Rochester (1) proposed, therefore, to establish guiding principles of adsorption by identifying certain critical factors. Applying their theory on mucoadhesion of colloidal systems such as poly(styrene) latexes, one should examine (a) the role of the mucous surface, especially its physicochemical aspects, (b) the nature of the latex, its size and surface properties, (c) the influence of the suspension medium, its electrolyte concentration and pH, and its interactions with the free and the adsorbed latex particles and with the mucous gel, (d) the nature of the interactions between latex and mucosa such as hydrophilic and hydrophobic forces, (e) the structure of the adsorbed layer, depending on the interactions between latex and mucosa and on the lateral interactions between the adsorbed particles, and finally, (f) the effect of temperature and the thermodynamics on adsorption.

Trying to evaluate the interactions between latex particles and mucosa, the time taken to reach equilibrium was suggested to be indicative of the type of interaction, although it may not always be unambiguous (1,2). Therefore, kinetics of latex adsorption to mucosa have been studied under static conditions and using diffusion theories (3). The shape of the adsorption isotherms was also reported to provide information on the nature of the latex–mucus interactions (1,4). Thus, it was the aim of this work to study mucoadhesion of latexes by analyzing the adsorption isotherms and the irreversibility of adsorption.

MATERIALS AND METHODS

Poly(styrene) Latexes

The characteristics of the latexes used in this study are listed in Table I. PCM latexes were supplied by Polysciences, Eppelheim, Germany, and CML-350* and PSL-650* by Polymer Laboratories, Church Stretton, Shropshire, UK (superscript asterisk indicates surfactant-containing latex). Surfactant-free latex CML-350 was prepared by mixed-bed ion-exchange cleaning (5). Briefly, the ion-exchange resins, i.e., Dowex-1 and Dowex-50 (Fluka, Buchs, Switzerland), were cleaned with 3 *N* HCl (cationic resin) or with 3 *N* NaOH (anionic resin) and with deionized water. The diluted latex (1% solids) was then mixed overnight with a fivefold excess of each cleaned resin. The surface groups (carboxy and sulfate groups) on the surfactant-free latexes were determined by potentiometric titration with 0.1 *N* NaOH. The density of surface groups (D_g) describes the milliequivalents (mEq) of base used for titration of a certain latex mass, and the surface density of groups (S) indicates the number of groups per surface unit. S was calculated according to Eq. (1):

$$S = D_g \rho (D_w/6) N_A \tag{1}$$

where ρ is the density of the polymer, i.e., 1.05 g/cm^3 for poly(styrene), D_w is the diameter of the latex particles, and N_A is Avogadro's number. The latexes with surfactant were also titrated. PSL-650* was a plain latex with a fatty acid-type surfactant and its surface properties were, therefore, not very different from the surfactant-free latexes. CML-350* contained sodium dodecyl sulfate (SDS), which was reported to increase the negative charge of latex particles strongly (6).

Adsorption and Desorption Studies

The *ex vivo* mucosa samples from sacrificed male Wistar rats (IFFA CREDO, L' Arbresle, France) were prepared as described earlier (3). For adsorption isotherms, various latex concentrations (g/L) in 1 mL of physiological saline (0.9% NaCl) were put in contact with 2 cm^2 of the mucosa each by means of a special device (3). All experiments were conducted at room temperature. The contact time was fixed for 30 min according to kinetic studies (3). The latexes were then sucked off and the mucosa samples were rinsed with 5 mL of 0.9% NaCl to eliminate nonattached particles.

For comparing different methods of washout, 1 mL of physiological saline was left in contact with the mucosa for 5 min, 1 min, or 15 sec, respectively. This washout was repeated five times, except for the mucosa samples, which

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Table I. Characteristics of the Latexes

Latex	D_w ± SD (nm)[a]	D_g (mEq/g)[b]	S (groups/nm^2)[c]
PCM-200[d]	230 ± 13	0.40	9.7
CML-350[d]	314 ± 53	0.16	5.3
CML-350*[e]	320 ± 26	0.35	11.8
PSL-650*[f]	670 ± 80	0.05	3.9
PCM-2000[d]	2045 ± 190	0.12	25.9

[a] D_w, weight diameter by PCS (Coulter submicron particle analyzer N4MD, Coultronics, Margency, France).

[b] Density of titrated groups.

[c] Surface density of groups.

[d] Surfactant-free carboxylated latex (–COOH).

[e] SDS as surfactant ($-OSO_3^-$).

[f] Fatty acid-type surfactant on plain latex (–COOH).

were rinsed once with 5 mL of flowing physiological saline. The particles which were washed out were quantified by turbidimetry after denaturation of any detached mucous (3). The amounts of adsorbed latex were measured either by a Fourier transform infrared spectroscopy/attenuated total reflection (FTIR-ATR) technique (3) of by a turbidimetric method (3) and are expressed as the mass of polymer per apparent surface of mucosa (g/m^2).

RESULTS

Isotherms of Adsorption

Figure 1 shows the adsorption isotherms for CML-350* and PSL-650*. Both isotherms followed an initial straight line of increasing adsorption up to a bulk concentration of about 2.5 g/L. After a second branch where both latexes had a plateau, the isotherms increased again at bulk concentrations higher than 15 g/L for PSL-650* and 20 g/L for CML-350*. Figure 2 shows the adsorption isotherms of three surfactant-free latexes: PCM-200, CML-350, and PCM-2000. Again, the initial straight line and the abrupt change to a plateau were observed for PCM-200 and CML-350, whereas PCM-2000 approached the Langmuirian isotherm type.

The plateau levels, the partition coefficients K, and the critical concentrations C^* at the abrupt change to the plateau are listed in Table II. K was calculated by linear regression of the mean adsorption points in the initial straight-line part up to C^*, including an additional point in the origin of the graph. The correlation coefficients were better than 0.97 for all latexes. The critical concentration C^* was situated in the intersection of the linear regression line and the plateau. CML-350* and PSL-650* had significantly higher plateau levels than all surfactant-free latexes (Anova- and Fisher test, $P = 0.05$). All partition coefficients K were about 3 cm^{-1} except for surfactant-free CML-350, which had a K of 2.2 cm^{-1}. The critical concentrations C^* were between 2.2 and 2.8 g/L. For PCM-2000, the Langmuirian isotherm could be described by the following equation (4):

$$y = y_m bC(1 + bC)^{-1} \qquad (2)$$

where y is the amount of latex in the adsorbed monolayer at the equilibrium concentration C, and y_m the adsorbed amount in the completed monolayer. Inversing Eq. (2) and multiplying by C resulted in the following equation (7):

$$C/y = (by_m)^{-1} + C/y_m \qquad (3)$$

The plot of C/y against C resulted in a straight line with $1/y_m$ as the slope and $(by_m)^{-1}$ as the intercept. PCM-2000 therefore had a constant $(by_m)^{-1}$ of 2.2 cm^{-1} ($r > 0.95$) and a plateau level of 2.616 ± 0.341 g/m^2, which was higher than all the other plateaus.

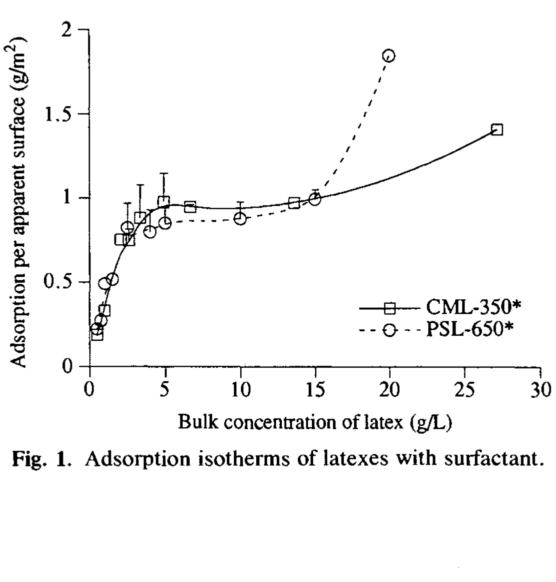

Fig. 1. Adsorption isotherms of latexes with surfactant.

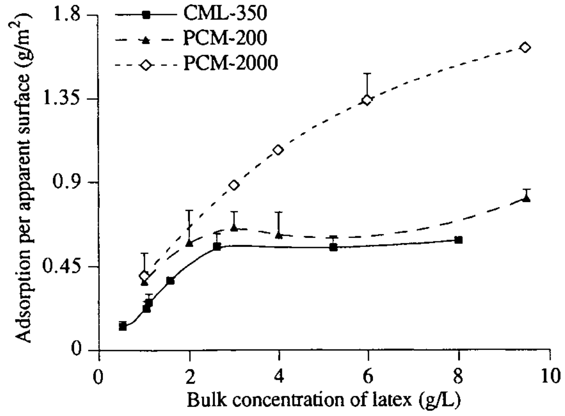

Fig. 2. Adsorption isotherms of surfactant-free latexes.

Desorption Studies

Different methods of washout were compared (Fig. 3). All samples were incubated with PSL-650* during 30 min at

Table II. Latex Adsorption on Rat Intestinal Mucosa

Latex	Plateau ± SD (g/m^2)[a]	Plateau (particles/μm^2)[a]	K (cm^{-1})[b]	C^* (g/L)[c]
PCM-200	0.660 ± 0.154	98.7	2.9 (3)	2.2
CML-350	0.558 ± 0.057	30.1	2.2 (6)	2.5
CML-350*	0.881 ± 0.139	48.9	3.1 (5)	2.8
PSL-650*	0.859 ± 0.125	5.2	3.2 (6)	2.5
PCM-2000	2.616 ± 0.341	0.6	n.d.[d]	n.d.[d]

[a] Values per apparent surface of rat intestinal mucosa.

[b] Partition coefficient for type C isotherms (number of data points).

[c] Critical latex concentration for type C isotherms.

[d] Langmuirian isotherm.

a bulk concentration of 4 g/L, and washouts were done with physiological saline. No differences was found between the different methods of washout. When the samples were rinsed five times during 5 min with 1 mL of physiological saline, the total washouts of CML-350* and PSL-650* were linearly proportional to the equilibrium latex concentrations calculated as the differences between the latex bulk concentration and the adsorbed latex amounts ($r > 0.998$ for CML-350*, $r > 0.999$ for PSL-650*).

DISCUSSION

The isotherms in Figs. 1 and 2 were compared with typical curves in different isotherm classifications. According to the Brunauer, Emmett, and Teller (BET) classification, the experimental adsorption isotherms fit best with a type I isotherm for gas adsorption on highly microporous adsorbents (8,9), except the Langmuirian isotherm of PCM-2000. These isotherms have a much sharper knee than the typical type I (Langmuir) isotherms for monolayers, since micropores are filled up before a monolayer can be established on the apparent surface. According to Giles *et al.* (1,4), who proposed a more extensive classification of isotherms including solid solute adsorption, the experimental isotherms corresponded best to class C. The isotherms of this class occur with microporous adsorbents and are distinguished by an initial linear portion which includes constant partition of the solute between solution and adsorbent. The slope of the initial straight line corresponds to the partition coefficient K. More generally expressed, the class C is consistent with conditions in which the number of adsorption sites remains constant throughout the initial concentration range, since the adsorbates penetrate into the pores and therefore disappear from the apparent surface. This model is valid up to a critical concentration C^* where, provided that all the internal area of adsorbent is saturated, the curve changes abruptly to a horizontal and a second branch which consists in a plateau is reached. Class C isotherms were reported not only for gases (9) and solutes (10) but also for disperse adsorbates such as nonionic disperse dyes, which were adsorbed on ionizable groups containing fibers (10–12).

The second increase in adsorption in the third branch of the isotherms (Fig. 1) seemed to be caused by lateral interactions between the particles, since the distances between the particles in the suspension medium at higher latex concentrations were reduced, i.e., 1.56-μm mean distance between two particles of PSL-650* at the bulk concentration of 15 g/L and 0.58 μm for CML-350* at 20 g/L. At the electrolyte concentration of physiological saline (0.154 mol/L) the energy barrier between particles was considered nonexistent and agglomeration could therefore take place at sufficiently high latex concentrations (2,13).

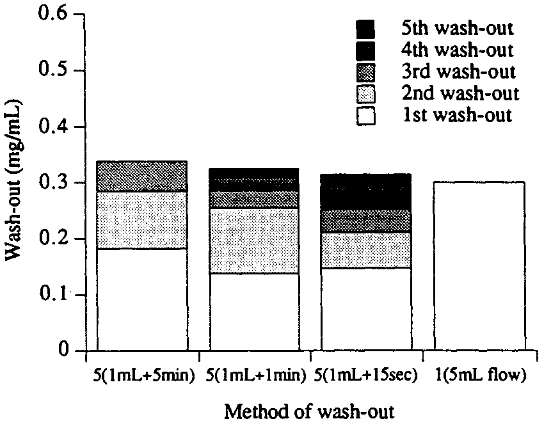

Fig. 3. Comparison of different methods of washout, after incubation for 30 min with PSL-650* at a bulk concentration of 4 g/L.

The significantly increased plateaus of CML-350* and PSL-650* (Table II) were probably due to the presence of the surfactants, as well as the higher K of CML-350* compared with CML-350. The hydrophilicity (S in Table I) of CML-350 was increased with SDS. The slightly higher plateau value of PCM-200 compared with CML-350 may also be explained with its higher hydrophilicity. The dependence of the adsorption on the hydrophilicity of the surfaces was in agreement with another study, where mucin was adsorbed on polymer films under physiological conditions, i.e., physiological saline at pH 7.2, and where the adsorption data were related to the wetting behavior of the polymer surfaces (14). The mucin adsorption increased with the increase in poly(ethylene) surface oxidation, especially for surface densities of oxidized sites higher than 15 sites/nm^2 (15).

PSL-650* had a significantly higher plateau level than PCM-200 and CML-350 but significantly lower than PCM-2000. Thus, it was deduced that the plateau level increased with the particle size. The size dependence of adsorption was approached using the following theoretical model: On a smooth surface, the amount of adsorption increases with the particle size, i.e., with the thickness of the monolayer. This is also the case for a porous adsorbent if the pores are much larger than the particles but there is an upper limit of particle size for penetrating into the pores, as found for PCM-2000. For multilayers of adsorbed particles, as is the case for the smaller latexes (see the number of adsorbed particles in Table II), the amount of adsorption also depends on the packing, and the packing depends on the surface properties of the particles. On the one hand, the lateral interactions, such as repulsion forces between adsorbed particles, may be more important for smaller particles because of their higher specific surface, and consequently the adsorption may increase with the particle size. On the other hand, latex penetration into the adsorbent might increase with reduced particle size. In the present study, the concurrence of the last two phenomena may best describe the adsorption of the latexes, except for PCM-2000.

For PCM-2000, the Langmuirian isotherm shape suggested a monolayer of adsorbed particles. In fact, the saturation value corresponded to 1.5 monolayers of adsorbed particles when referred to the apparent surface of the mucosa, i.e., 0.6 particle/μm^2. On a microscopic scale, the surface of the mucous gel was not smooth and the organization of the adsorbed PCM-2000 latex particles corresponded therefore quite well to a monolayer on the "real" surface. When latexes are larger than PCM-2000, the saturation value of adsorption might still increase with the particle size, but the increase in adsorption with the particle size will certainly have an upper limit due to the hydrodynamic desorption forces. These forces which intervene, especially during the washout process, increase with the particle size (16).

Desorption Studies

Adsorption of polyelectrolytes such as proteins often appears irreversible on an ordinary time scale, although it is not necessarily thermodynamically irreversible (17). From a microscopic viewpoint, desorption of the polyelectrolyte is unlikely, because a polymer, which is attached with several segments, can be desorbed only if all adsorbed segments are loosened simultaneously (18). Thus, it was of interest to know whether the latex adsorption on a network of polyelectrolyte molecules, such as the mucous gel, was irreversible or not. Since the amount of washout did not depend on the method (Fig. 3), the eliminated particles probably did not originate from time-dependent desorption phenomena but very probably from remaining droplets of latex when the bulk suspension was sucked off from the mucosa. If the washout was controlled by latex desorption from the mucosa, the amount of eliminated particles would depend on the total time of washout and therefore be higher after 25 min (5 × 5 min) than after 75 sec (5 × 15 sec). This was not the case. If the washout was controlled by diluting the remaining droplets of the supernatant, there must have been a linear dependence of the eliminated latex amounts on the equilibrium concentration of the latex suspensions, as observed in this study. Since the adsorbed latexes were not detached during the washout process, which took up to 25 min, at least not in an essential amount, it was assumed that the adsorption was irreversible. The limiting factor for the time period of adhesion of latexes to the mucous gel layer would therefore be the mucous turnover. The estimated time for complete renewal of the intestinal mucous gel layer in the rat *in situ* loop was reported to range between 47 and 270 min (19).

CONCLUSION

Mucoadhesion of nanoparticles has been studied by means of adsorption isotherms. Their shape indicated that the mucous gel layer behaved like a porous adsorbent. The amounts of adsorbed particles appeared high enough to consider colloidal systems as drug carriers in oral administration.

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