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Stuffed brushes: theory and experiment*

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Abstract: The interaction between polymer brushes and mesoscopic particles is investigated both theoretically and experimentally. We present an analytical mean-field theory for a polymer brush (a layer of long polymer chains end-grafted to a substrate) with varying excluded volume interactions between monomer units. This system mimics the reversible adsorption of mesoscopic particles, such as surfactant micelles or proteins, on the grafted chains. The equilibrium structural properties of the brush (the brush thickness and overall degree of complexation) as well as the number of adsorbed particles per unit area, Γ, are analysed as functions of the affinity between particle and chain, grafting density σ and excluded volume interactions. In our model Γ is found to have a maximum as a function of σ. Experimentally the adsorption of BSA on a hydrophobic substrate with grafted PEO chains is measured with reflectometry. In the case of short grafted chains the adsorbed amount of BSA, Γ, decreases continuously with increasing σ, which agrees with previous results and model calculations in the literature. In the case of long PEO chains, however, Γ is found to have a maximum as a function of σ. Qualitatively the experimental dependence of Γ on σ is found to agree with the results of our mean-field model. PEO chains show no affinity for BSA in the bulk, whereas in a grafted conformation an effective attraction is found. Some comments are made on the nature of this affinity, which is not yet fully understood.

1 INTRODUCTION

The prevention of protein adsorption on surfaces in contact with blood is a challenge, both from a medical and a chemical–physical viewpoint. Protein adsorption can result in coagulation of adsorbed blood proteins and subsequent surface-induced thrombosis [1]. Grafting or irreversible adsorption of water-soluble polymers onto the surface is a well-established method to reduce protein adsorption [2–7]. The decrease in the adsorbed amount of protein is generally attributed to steric repulsion between the grafted hydrophilic polymer and the solvated proteins.

Due to its hydrophilicity poly-ethyleneoxide (PEO) is widely used as stabilising agent; a review on the properties of PEO and its biomedical applications is given by Lee *et al*. [2]. PEO is usually end-tethered to the surface through irreversible adsorption of block or triblock copolymers from solution. When a hydrophilic block like PEO is chemically bound to a hydrophobic block, such as poly-propyleneoxide (PPO) or an alkyl chain, the polymer density at the surface is enhanced as the hydrophobic block is prone to adsorb strongly on a hydrophobic surface [8]. Moreover, as desorption of the hydrophobic block from the hydrophobic surface is unfavourable, the surface density remains constant under varying conditions, in contrast to hydrophilic homopolymer that can desorb.

In recent studies the adsorption of several proteins on a hydrophobic surface was measured as a

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function of PEO-PPO-PEO triblock copolymer surface density [3,4]. It was shown that an increase in surface density results in a continuous decrease in protein adsorption. The surface density was concluded to be the main factor in suppressing the protein adsorption, the chain length was found to have little effect [3]. In another study PEO was fixed to an alkyl chain and an increasing PEO length was found to strongly reduce the protein adsorption [7]. The PEO chain length was 128 or less for McPherson *et al*. [3] and Schoën *et al*. [4] and 17 or less units in the case of Prime *et al*. [7].

From a theoretical point of view the interaction between hydrophilic chains grafted onto a surface and mesoscopic particles such as proteins is extremely interesting. Polymer chains grafted at high densities, so-called brushes, have been studied theoretically with various models. An excellent review of theoretical work on brushes has been given by Halperin *et al*. [9], and recently by Szleifer & Carignano [10]. The equilibrium conformation of densely grafted, neutral polymers in a good solvent is the result of two opposing forces: the osmotic pressure between monomer units in the grafted layer induces stretching of the chains normal to the surface, whereas the conformational entropy is reduced by such stretching and thus opposes swelling of the brush layer. The osmotic interactions are also referred to as excluded volume interactions, the conformational entropy as the elasticity of the chains. In the most simple model for a brush the monomer density is assumed uniform throughout the grafted layer, the so-called Alexander–de Gennes model [11,12]. Although several more refined models based on nonuniform density distributions normal to the grafting surface have been presented [13–15], the simple Alexander–de Gennes model has been surprisingly successful in predicting scaling relationships for the brush height and surface pressure as a function of the grafting density and chain length [16]. We refer the reader to the mentioned reviews and references therein for more details [9,10].

Recently the interaction between brushes and colloidal particles in solution has attracted significant attention. Jeon *et al*. suggested a simple model for the interaction between a substrate with terminally attached hydrophilic chains and solvated proteins [17,18]. In this model the proteins are supposed to compress the grafted layer, thus enhancing the excluded volume interactions between monomers. The Van der Waals attraction between proteins and the substrate is compared to the induced steric repulsion of the chains. An important conclusion of this model is that longer chains are more effective in suppressing the adsorption because the Van der Waals interactions between particles and the substrate decrease strongly with increasing distance, viz. increasing brush height. Recently McPherson *et al*. compared experimental studies of PEO-PPO-PEO triblock copolymers and various adsorbing proteins with numerical self-consistent-field (SCF) results [3]. In the SCF model the particles are prone to adsorb on the surface and the excluded volume interactions between the grafted chains and particles are repulsive. They conclude that the blocking of adsorption sites by grafted chains is the main mechanism for reducing protein adsorption, i.e. σ controls the amount of adsorption. The influence of the chain length, however, is reported to be marginal, ill contrast with the model of Jeon *et al*. [17,18].

Although the reduction of the adsorbed amount per unit area, Γ, by the grafting of PEO chains was investigated experimentally, the chain lengths used were 17 or less units in the case of Prime *et al*. [7] and 128 or less for McPherson *et al*. [3]. In recent work by our group dense PEO-brushes containing a small polystyrene (PS) anchoring block were prepared at the air/water interface with a PEO chain length varying from 90 to 700 units [19]. In this paper we show how such PEO brushes are irreversibly adsorbed onto a PS surface using the Langmuir–Blodgett technique. The adsorption of bovine serum albumin (BSA), a blood protein, on PS substrates with grafted PEO chains can be followed by reflectometry. According to the conclusions of Prime *et al*. [7] and the model of Jeon *et al*. [17,18] the considerable length of the PEO chains should be a formidable barrier to proteins. The work of McPherson *et al*., however, suggests the grafting density to be the main factor in determining Γ [3]. Thus measurement of the adsorption of BSA on PS surfaces grafted with PEO of varying length and surface density is an excellent manner to check the theoretical predictions of Jeon *et al*. and McPherson *et al*.

Another field of polymer–particle interactions is the formation of polymer–micelle complexes between hydrophilic polymers, such as PEO or poly-vinylpyrrolidon (PVP), and anionic surfactants, i.e. SDS. It is well established that at concentrations below the critical micelle concentration (CMC) but above a critical association concentration (CAC) anionic surfactants cooperatively bind to neutral, hydrophilic polymers and form polymer–micelle complexes, also referred to as 'necklaces' [20–22]. The

complexes are separate entities, the degree of coverage of the polymer chain by complexes is determined by the surfactant bulk concentration. The driving force for complex formation is generally attributed to favourable shielding of hydrophobic patches on the micellar surface by the polymer [22]. An extensive review on polymer–surfactant interactions is given by Lindman & Thalberg [23].

The behaviour of such necklaces in a dilute bulk phase has been extensively studied experimentally [20–22] and theoretically [24–27]. However, the interaction between densely grafted polymers and surfactants at a concentration above the CAC has not been considered. Recently we presented an analytical mean-field model for brushes immersed in a surfactant solution above the CAC, using the Alexander–de Gennes model as a starting point [28]. Quantities of interest in the model are the coverage of the grafted chains by micelles, the height of the grafted layer and the total amount of surfactant adsorbed per unit area, Γ. We remark, however, that our model is not only valid for grafted chains in contact with micelles. It can also deal with other (small) particles that can bind to the chain to form a necklace; globular proteins, for instance, are good candidates. We therefore decided to apply our model to examine the binding of BSA on PEO brushes.

The set-up of this paper is the following: in section 2 our analytical model is briefly explained and the results are discussed. The reader is referred to our other paper for a more extensive treatment of the model [28]. In section 3 the preparation of PEO brushes on PS substrates and the adsorption of BSA, followed with reflectometry, are discussed. In section 4 the experimental results are compared to the theoretical. The paper closes in section 5 with some conclusions.

2 THE MODEL

2.1 Theory

We, consider neutral chains of length N end-grafted onto an inert surface with a grafting density σ, defined as number of grafted chains per unit area. The chains are immersed in a good solvent of ionic strength c_s, containing small mesoscopic particles of size R_p and charge q. There is a nonspecified affinity between the polymer and the particles, which drives the adsorption of the polymer onto the particle surface. The adsorbed particles are assumed to be mobile along the adsorbing chain, i.e. they retain translational entropy. The number of polymer segments forming a complex with a particle, N_{ad}, is assumed to be constant, irrespective of the number of adsorbed particles on one chain. In the following all length scales are expressed in the segment unit length, and all energies in kT.

The free energy of a grafted chain carrying particles, Ψ, is written as the sum of four contributions, namely: (i) the adsorption energy due to complex formation between the chain and the particle, (ii) the translational entropy of the complexed particles along the chain, (iii) the configurational entropy (elasticity) of the grafted chain, and (iv) the osmotic interactions in the grafted layer.

$$\Psi = \Psi_{ad} + \Psi_{tr} + \Psi_{elas} + \Psi_{osm} \tag{1}$$

A mean-field model is used, i.e. the interactions in the layer are nonlocal and are expressed in terms of average density and coverage. Moreover, we apply the Alexander–de Gennes model for grafted chains, i.e. the chains are assumed to be uniformly stretched to a height H and thus an uniform density ρ is found throughout the grafted layer [11,12]. It is convenient to define the fraction of polymer segments of the chain complexed with a particle as

$$\theta = \frac{mN_{ad}}{N} \tag{2}$$

where m denotes the number of particles complexed with a chain. If the energy of complex formation, ΔU_{ad}, is expressed as a segmental adsorption energy, i.e. $u \equiv \Delta U_{ad}/N_{ad}$, then the first term in Eqn 1 is simply

$$\Psi_{ad} = N\theta u \tag{3}$$

The origin and values of u are discussed in more detail in section 2.3.

The second term in Eqn 1, Ψ_{tr}, is the translational entropy of the adsorbed particles along the chain. As

the chain consists of N/N_{ad} adsorption sites, the entropy in a mean-field model, expressed in terms of θ, is

$$\Psi_{tr} = N/N_{ad}(\theta \log \theta + (1-\theta)\log(1-\theta)) \tag{4}$$

We, note that such an expression for the translational entropy requires the number of adsorption sites to be large, i.e. N is large compared to N_{ad}.

We now turn towards the third term in Eqn 1, the conformational entropy. The grafted chains are assumed to be Gaussian, irrespective of the degree of loading with particles. The contribution of conformational entropy to the free energy of grafted Gaussian chains is

$$\Psi_{elas} = \frac{3H^2}{2R_0^2} \tag{5}$$

where R_0 denotes the unperturbed radius of gyration of the Gaussian chain. In the case of adsorption of particles R_0 is the *effective* radius of gyration of a 'necklace', i.e. a correction with respect to the radius of gyration of a bare polymer chain must be taken into account. This is illustrated in Fig. 1. The complexed chain consists of $(1-\theta)N$ bare segments and m segments of length $2R_p$. The radius of gyration is therefore

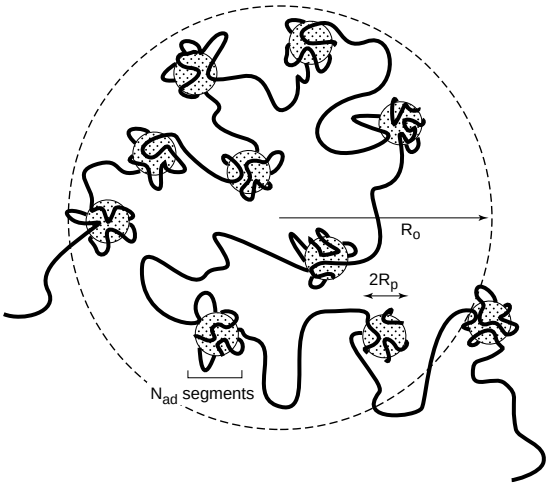

Fig. 1 A polymer necklace.

$$R_0^2 = (1-\theta)N + m(2R_p)^2 = N\left((1-\theta) + \frac{4\theta R_p^2}{N_{ad}}\right) \tag{6}$$

The above equation is easily understood: at zero coverage ($\theta = 0$) it reduces to the radius of gyration of a Gaussian chain, whereas at total coverage ($\theta = 1$) the radius of gyration is that of a Gaussian necklace consisting of N/N_{ad} segments of length $2R_p$. If we introduce a constant $\beta \equiv 4R_p^2/N_{ad} - 1$, we can write the elastic free energy, corrected for complexation, as

$$\Psi_{elas} = \frac{3H^2}{2N(1+\beta\theta)} \tag{7}$$

The last term in Eqn 1 is the osmotic contribution to the free energy, Ψ_{osm}. As we apply a mean-field model, the osmotic interactions are expressed in terms of average segment and complex densities in the grafted layer. The average number density of bare polymer segments and complexes is $N(1-\theta)/V$ and $N\theta/(N_{ad}V)$, respectively, where V denotes the volume that a grafted chain occupies. At low grafting densities V is simply the volume of the complexed coil in a dilute solution, R_0^3, as in this grafting regime no stretching of the chains due to interchain steric repulsion occurs. At high grafting densities V equals H/σ.

We assume that all osmotic interactions in the grafted layer are repulsive, which is reasonable for polymer segments immersed in a good solvent and complexed particles of similar charge. The Van der Waals interactions between complexing particles, chains and grafting surface are thus neglected. Also the

brush is effectively dilute, in which case it is sufficient to only consider binary contributions to Ψ_{osm}. Three binary osmotic interactions are distinguished: noncomplexed segment–segment, complexed particle–particle and noncomplexed segment-complexed particle. The excluded volume coefficients (second virial coefficients) are denoted v_0, $\tilde{v}_1$ and $\tilde{v}_2$, respectively. In our mean-field model the osmotic contribution per chain to the free energy is then

$$\Psi_{osm} = (v_0(N(1-\theta))^2 + \tilde{v}_1 m^2 + \tilde{v}_2 mN(1-\theta))V^{-1} \tag{8}$$

It is instructive to express all osmotic interactions in terms of the degree of complexation, θ, and overall segment density in the grafted layer, N/V. The osmotic free energy is therefore written as

$$\Psi_{osm} = \frac{N^2}{V}(v_0(1-\theta)^2 + v_1\theta^2 + v_2\theta(1-\theta)) \equiv \frac{N^2}{V}v_{eff}(\theta) \tag{9}$$

where the virial coefficients v_1, and v_2 are renormalised with respect to the number of adsorbed segments per complex, i.e. $v_1 = \tilde{v}_1/N^2_{ad}$ and $v_2 = \tilde{v}_2/N_{ad}$. Note that in Eqn 9 the osmotic contribution is written as a function of the overall segment density and an effective excluded volume parameter, $v_{eff}(\theta)$, that depends on the degree of complexation. The excluded volume interactions are therefore annealed, i.e. depend intrinsically on the degree of complexation of the grafted chain. The parameters v_0, v_1, and v_2 are discussed in more detail in section 2.3.

If we sum up Eqns 3, 4, 7 and 9, then the total free energy of a grafted chain is obtained as a function of several parameters. We note that contributions to Ψ due to density variations or end-effects are neglected. However, as the four contributions in Eqn 1 scale linear with N in the brush regime, this approximation is valid for long chains. For a given system (fixed grafting density, adsorption energy, osmotic interaction parameters) the equilibrium conformation of the grafted layer is found by minimizing Ψ with respect to the height, H, and the degree of complexation, θ.

$$\frac{\partial\Psi}{\partial H} = 0 \quad \text{and} \quad \frac{\partial\Psi}{\partial\theta} = 0 \tag{10}$$

The height of the brush and its coverage cannot be varied independently but are both functions of the above mentioned parameters. If the equilibrium conformation is known, then the total number of complexed particles per unit area, Γ, is simply

$$\Gamma = \frac{N\theta\sigma}{N_{ad}} \tag{11}$$

2.2 Grafting regimes

We can distinguish two grafting regimes: a regime at low grafting density consisting of noninteracting grafted coils (mushrooms) and a regime at high grafting density consisting of strongly interacting, stretched chains (brushes). In the *mushroom* regime minimisation of Ψ with respect to H and θ yields

$$H = N^{3/5}((1+\beta\theta)v_{eff})^{1/5} \tag{12}$$

and

$$\log\frac{\theta}{1-\theta} + N_{ad}u + N_{ad}N^{-4/5}\left(\frac{v'_{eff}}{((1+\beta\theta)v_{eff})^{3/5}} - \frac{3\beta v_{eff}^{2/5}}{2(1+\beta\theta)^{8/5}}\right) = 0 \tag{13}$$

where we have defined $v'_{eff} = \partial v_{eff}(\theta)/\partial\theta$ and have omitted the θ-dependence of $v_{eff}(\theta)$ for clarity.

It is evident that no dependence on the grafting density σ is found in the above expressions: at low grafting densities the equilibrium properties should be equal to those of single chains in a dilute bulk phase. If we consider Eqn 13, and take the limit for long chains, i.e. $N >> N_{ad}$, then the last term (viz. the osmotic) in Eqn 13 disappears. The result is a Langmuir-like adsorption relationship, in which the degree of complexation depends solely on the effective adsorption strength:

$$\theta = \frac{1}{1+\exp(N_{ad}u)} \tag{14}$$

The size of the complex is that of a Gaussian coil in a good solvent, $N^{3/5}{v_0}^{1/5}$, but with v_0 replaced by the effective excluded volume parameter and corrected mean segment length $(1+\beta\theta)v_{eff}$.

In the *brush* regime the corresponding expressions for the equilibrium conformation are

$$H = 1/3\ N\sigma^{1/3}((1+\beta\theta)v_{eff})^{1/3} \tag{15}$$

and

$$\log\left(\frac{\theta}{1-\theta}\right) + N_{ad}u + 3^{1/3}N_{ad}\sigma^{2/3}\left(\frac{v'_{eff}}{((1+\beta\theta)v_{eff})^{1/3}} - \frac{\beta v_{eff}^{2/3}}{2(1+\beta\theta)^{4/3}}\right) = 0 \tag{16}$$

We find that the expression for the brush height is that for a 'bare' brush in a good solvent, with again v_0 replaced by $(1+\beta\theta)v_{eff}$, Thus, both the size of the grafted complexed coil as that of the complexed brush is that of its bare conformation, corrected for the change in excluded volume interactions and mean segment length. The degree of complexation in the brush regime, given by Eqn 16, depends strongly on the grafting density, σ, in contrast to single grafted chains. This equation must be solved numerically for given N, σ, v_i and $N_{ad}u$ parameters,

2.3 Estimation of parameters

In section 2.1 several phenomenological parameters were introduced in our model. It is useful to examine these parameters in more detail and where possible to estimate their value. For the sake of illustration we choose the PEO-SDS systems as the experimental reference state. The 'particles' in this case are adsorbed SDS micelles.

The first parameter introduced in our model is the unit length, defined as the segment length of the polymer. A segment of PEO is reported to be approximately 3.5 Å, this value is used throughout the results [29].

The reported radius of a SDS micelle adsorbed on PEO is between 2 and 5 nm, depending on the ionic strength. In some studies the micelle size is concluded to be independent of the degree of complexation, in other a dependence is found [21,30–32]. In the case of complexing colloidal particles (proteins) the dimensions of the particles are similar to those of adsorbed micelles and constant. We therefore choose the radius R_p to be 8 units, corresponding with a particle radius of 3 nm, and independent of θ.

When a polymer adsorbs on the surface of a colloidal particle, it is adsorbed in strands on the surface ('trains') and strands that remain solvated ('loops') [33]. The average size of the loops, denoted as D, is a measure for the strength of adsorption: D decreases with increasing adsorption strength [34]. We assume the affinity of the polymer for the particle surface to be large, so that D is significantly smaller than R_p, The sum of train and loop segments is the number of polymer segments per complex, N_{ad}. When the radius of curvature is significantly larger than the segment length, the curvature of the surface does not affect N_{ad}, and N_{ad} is proportional to the surface, i.e. $N_{ad} \sim {R_p}^2$, The number given in the literature for N_{ad} for PEO-SDS varies between 60 and 120 [21,30]. It is also reported that in bulk there exists a critical length of approximately 100 segments for PEO, below which no complexation is found [35]. We therefore use a constant value of 100 for N_{ad} in the calculations.

The effective segmental adsorption energy, u, is an important parameter in our model. In the case of protein particles and neutral segments it consists solely of a term due to segment–surface interactions, and a term due to loss of conformational entropy of the adsorbed chain. The segment–surface interactions result from shielding of hydrophobic patches by adsorbed polymer, hydrogen bonding, etc. We can sum the two terms into a total contribution, denoted as ΔU_0. In the case of surfactant solutions below the CMC, the formation energy of a micelle of size R_p, or likewise p surfactants, must be taken into account. We can write the difference in chemical potential of a surfactant molecule in the complexed micelle and in solution as $\Delta\mu = \mu_p^0 - (\mu_s^0 + \log\rho_b)$ where μ_p^0 is the standard chemical potential of a surfactant molecule in the complexed micelle, μ_s^0 that in the solvent and ρ_b the bulk surfactant concentration. In the case of complexed micelles u is therefore written as

$$u = \Delta U_0/N_{ad} + p\Delta\mu N_{ad} \tag{17}$$

and the segmental adsorption energy therefore depends on the surfactant concentration. For PEO-SDS the adsorption energy per micelle is estimated of order 10–15 kT [21], viz. 0.1–0.15 kT per adsorbed polymer segment.

We finally consider the second virial coefficients, v_0, v_1, and v_2. The polymer segments are treated as hard spheres, without long–distance interactions. For two hard spheres of radius R_s, the excluded volume is

$$v_0 = \frac{1}{2}\frac{4\pi(2R_s)^3}{3} \tag{18}$$

Since we have defined R_s as the unit length, it is convenient to set v_0 to unity hereafter.

For the particle–particle osmotic interactions the excluded volume parameter not only consists of a geometric contribution (the volume of both particles) but also an electrostatic term. In nearly all cases of polymer–micelle or polymer–protein interactions the particles are charged, for the simple reason that this charge ensures the stability of the particles in an aqueous solution. In the simplest approach we assume that the charged particles exclude each other from a sphere of radius $(R_p + \kappa^{-1})$, where κ^{-1} is the Debye length of the solution. This gives us

$$v_1 \approx \frac{16(R_p + \kappa^{-1})^3}{N_{ad}^2} \tag{19}$$

It is evident that at low ionic strengths the particle–particle excluded volume is mainly due to electrostatics, whereas at high ionic strength the geometric term dominates.

Finally, we must consider the segment–particle excluded volume. As the size of the segments can be neglected with respect to that of the particles, v_2 is determined by R_p. Moreover, as the polymer segments are neutral, electrostatics play no part in the segment–particle excluded volume. It therefore follows that

$$v_2 = \frac{4\pi R_p^3}{3N_{ad}} \tag{20}$$

This is adequate in the limit of total coverage of the particle by polymer. If the adsorbed particle retains bare adsorption sites, an attractive contribution to v_2 must be taken into account. Such a contribution, however, is neglected in our model.

2.4 Results

In Fig. 2 we have plotted the coverage θ in the *mushroom* regime, given by Eqn 13, as a function of the adsorption strength u for three values of v_1, $N = 10\,000$ (default) and $v_2 = 20$. It is evident that at low grafting densities the excluded volume interactions do not play a role in the degree of loading, the loading isotherm is Langmuir-like. This is in agreement with experimental binding isotherms of SDS on PEO in dilute bulk solutions, where the surfactant concentration determines the adsorption strength [21,30].

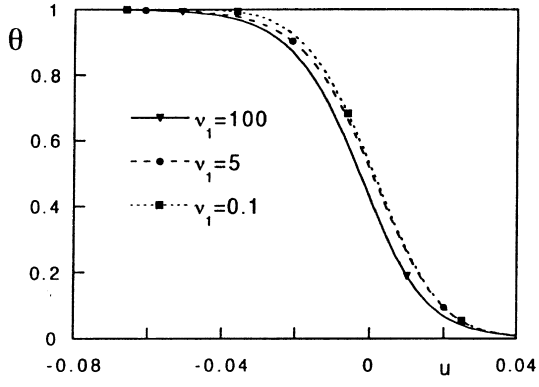

Fig. 2 Coverage θ as a function of segmental adsorption energy u for three values of v_1.

In Fig. 3 the size of the grafted coils with respect to the bare coil size, H/H_0, is given as a function of the adsorption strength for several values of v_1, with $v_2 = 20$. If the particle–particle interactions, denoted by v_2, are the dominant osmotic interactions, the coil swells with increasing coverage. If, however, the monomer–particle interactions, v_2, are large, the coil has a maximum as a function of the coverage. It is also possible that the (renormalised) particle–particle interactions are smaller than the monomer–monomer interactions. In this case the coil shrinks and wraps itself up around the complexing particles. Both the maximum and the increasing coil size have been reported for neutral polymers complexing with anionic surfactants [21,36]; a decrease in size has so far only been reported for polyelectolyte–particle complexes [37].

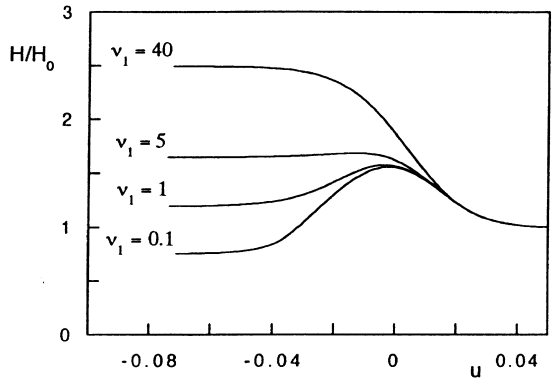

Fig. 3 H/H_0 as a function of u for four values of v_1.

In Fig. 4 the coverage in the *brush* regime, θ, is plotted as a function of the grafting density, σ, for $u = -0.05$, $v_2 = 10$ and for four values of v_1. At low grafting densities the coverage is close to unity. As the grafting density increases the coverage drops monotonically to zero at high grafting densities. The drop in coverage is stronger as the particle–particle interaction parameter is larger. The adsorbed particles are thus 'squeezed' out of the brush by osmotic interactions. This result implies that at high salt concentrations (small v_1) the degree of coverage is larger than at low salt concentration for a given adsorption strength, due to the screened electrostatic interactions.

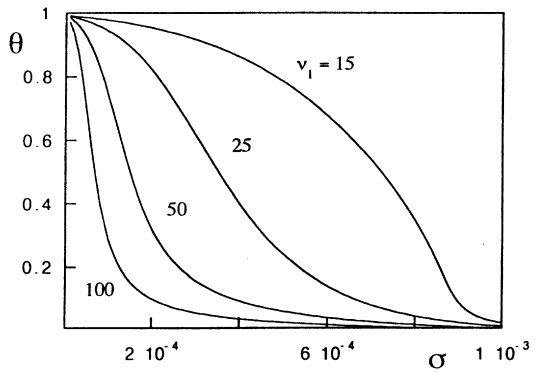

Fig. 4 Coverage θ as a function of σ for four values of v_1.

The height of the brush is plotted for the same parameters in Fig. 5a. The nonmonotonic behaviour of H as a function of σ is clear. At low grafting densities, i.e. high θ, the height increases with increasing σ. If the results are plotted double logarithmically (Fig. 5b) we notice that H scales as $\sigma^{1/3}$, with a corrected excluded volume parameter as prefactor (a line with exponent 1/3 is drawn as an illustration). As σ increases, the coverage θ drops, and the decrease in osmotic interactions results in a decrease in brush height. At high grafting densities the brush, now bare, again scales as $\sigma^{1/3}$ but now as a result of the

increasing monomeric osmotic interactions. In this regime no dependence of H on v_1 is found, the prefactor as expected is v_0. We also notice that the maximum in H increases with decreasing v_1, and is found at higher grafting densities. The explanation is that, although the interactions are less strong, the high coverage is retained up to higher grafting densities (e.g. Fig. 4) and the swelling is strongly enhanced by the grafting density, as is clear in Eqn 15.

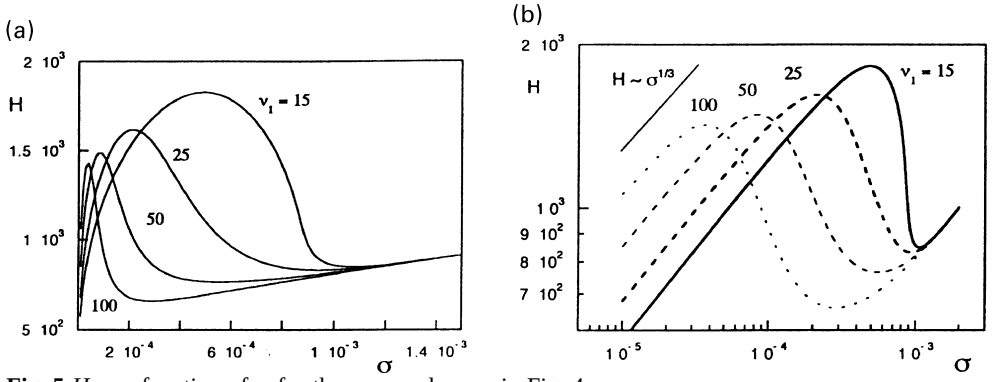

Fig. 5 H as a function of σ for the same values as in Fig. 4.

Finally, we consider the behaviour of the total adsorbed amount per unit area, Γ, as a function of the grafting density for a given adsorption strength and varying particle–particle osmotic interactions. In Fig. 6, Γ is plotted as a function of σ. We notice that Γ has a maximum, the position and height of which depend on the value of v_1. The maximum in Γ is similar to that in the brush height: it results from a balance between more adsorption sites with increasing σ, and increasing osmotic interactions favouring desorption. The height of the maximum in Γ therefore increases with decreasing strength of particle–particle interactions, and the maximum is found at higher grafting densities, in occordance with the maximum in H.

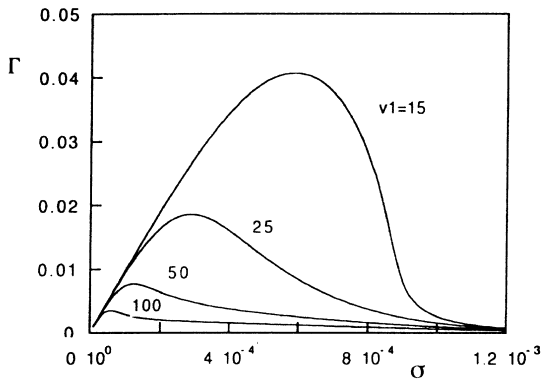

Fig. 6 Γ as a function of σ, for the same values as in Fig. 4.

3 PEO-BSA

In this section we describe the preparation of PEO brushes using the LB technique, and the subsequent measurement of BSA adsorption on brush-covered substrates. The protein used, BSA, adsorbs easily on the hydrophobic PS substrate; the variance in adsorption properties with varying PEO grafting density and chain length are thus followed by preparing films of different block copolymers and deposition densities. We stress that the grafted layers are not formed by block copolymer adsorption from solution. This method is often employed, but does not allow to achieve a known and controlled grafting density. It therefore requires determination of the grafting density after adsorption, which is not always reliable. The LB-technique, however, yields stable films of controlled and known density. In the next section the materials and techniques used are discussed, after which the adsorption results are presented.

3.1 Materials and Methods

The hydrophobic substrates were made from silicon wafers with a natural silica layer of approximately 2 nm, as determined by ellipsometry. On this surface a layer of poly-vinylpyridine-polystyrene (PVP-PS) block copolymer (MW 21400/20 700, Polymer Source Inc.) was adsorbed from a 100 p.p.m. chloroform solution. After adsorption the wafers were rinsed for 10 s in chloroform to wash away excess polymer, and dried under nitrogen. The PVP-PS layer thus formed was approximately 3 nm thick and stabilised the PS layer spincoated on top. The PS layer (MW 184k) was spincoated from a 0.1% solution in chloroform at 3000 r.p.m. The thickness of the PS layer, including the PVP-PS layer, was approximately 8 nm. Reflectometry showed the bare PS layers to be stable in water.

The PS-PEO block copolymers were received from the group of Dr G. Riess in Mulhouse. The weight of the PS-block is 4000 (38 segments), that of the PEO-blocks 6500, 19 600 and 23 000, corresponding with 148, 445 and 700 segments. The reported polydispersities are respectively 1.15, 1.12 and 1.25. For dynamic light-scattering experiments a PEO homopolymer (MW 445k, Pol. Lab. ltd) was used. The protein used for adsorption measurements was Bovine Serum Albumin (BSA), from Sigma Chemical Co. The molecular weight of BSA is approximately 66k, its iso-electric point 4.8. BSA is reported to be cigar shaped, with a radius of 3 nm and a length of 14 nm [38].

Monolayers of PS-PEO block copolymers were spread on an air/water interface in a Langmuir trough from a chloroform solution, 1 g/L. The small hydrophobic PS blocks anchor the long hydrophilic PEO blocks to the interface. The monolayers were compressed until the desired surface density was obtained. The surface pressure was measured with a Wilhelmy plate. The Si-PS wafers, prepared as described above, were dipped through the interface (air → water) in the usual fashion of′ LB-preparation. The deposition on the wafer proceeded readily, a transfer ratio of 1 was usually obtained. After retraction, the LB sample was washed with water in order to remove any polymer not taken up in the brush. In this manner we obtained a single PS-PEO layer adsorbed on PS. Due to the strong hydrophobicity of the PS a stable layer is formed. continuous washing with water did not desorb the PS-PEO monolayer, as indicated by the stable base line in the reflectometer.

The adsorption of BSA on PS surfaces with grafted PEO was subsequently followed with reflectometry. A detailed description of a reflectometer is given by Dijt *et al*. [39]. In short, this method exploits the change in polarisation of a polarised laser beam upon reflection on a substrate, in order to construct a signal that varies linearly with the increase in mass of a surface layer. Calibration can be carried out using the standard theory of reflection in thin films. The measurement is carried out using an impinging jet flow cell, constructed such that the reflection spot of the optical beam is centered around the stagnation point formed by the impinging jet. This allows precise control over the flux of molecules towards the surface.

3.2 Results

In Fig. 7 the surface pressure isotherms of PS-PEO block copolymers at the air/water interface are shown. The interpretation of these isotherms is discussed elsewhere [16]. Here, we merely note that for surface pressures above 10 mN/m dense PEO-brushes are formed at the air/water interface. At pressures below 10 mN/m the PEO is adsorbed in a flat conformation ('pancake') due to the affinity of the PEO chain for the air/water interface. It should be mentioned that the isotherms are reversible, i.e. there is no loss of material into the bulk phase during compression/expansion cycles.

The interactions of PEO and proteins in the bulk have been extensively studied [2]. The current opinion is that no attractive interactions between PEO and proteins are present, which facilitates for instance the use of PEO as a protein excluder. The hydrodynamic radius of PEO (MW 450k) in salt-free BSA solutions ranging from 0 to 500 p.p.m. was measured by us. No significant change with respect to the radius of gyration in pure water was found, indicating that in a dilute bulk solution the interactions between PEO and BSA, if present, are weak.

In Fig. 8 the adsorbed amount of BSA is given as a function of the number of molecules per nm^2, σ, for the three PEO blocks. Curves are drawn through the data points to guide the eye. The amount of BSA adsorbed on bare PS is approximately 0.5 mg/m^2. In the case of grafted PEO chains of 148 segments, the adsorbed amount drops continuously with increasing grafting density. However, if long PEO chains are

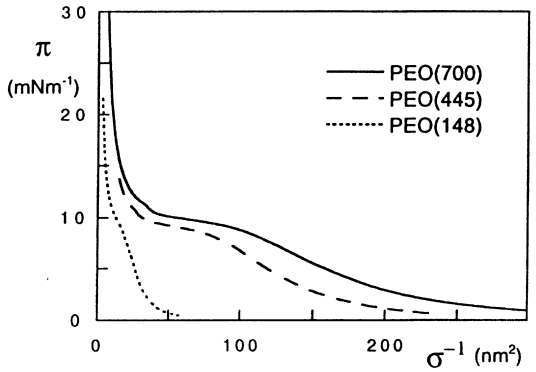

Fig. 7 Surface pressure isotherms of the PS-PEO block copolymers.

grafted (445 and 700 segments), the adsorbed amount first increases at low grafting densities, and then decreases at high grafting densities. The maximum adsorbed amount seems to increase with increasing chain length.

In Fig. 9 the results for the BSA adsorption on LB-films made of short PEO-chains (148 segments) are

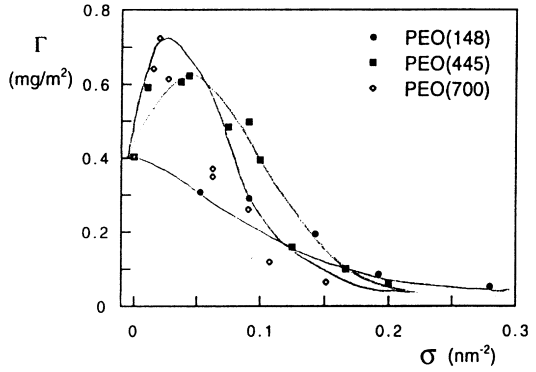

Fig. 8 Γ, as a function of σ for the three PEO lengths.

renormalised with respect to the adsorbed amount on bare PS, Γ/Γ_0. These data are compared with the same renormalised amount as found for the adsorption of lysosome on PEO-PPO-PEO triblock copolymers by McPherson *et al*. [3]. The PEO chain length of the triblock copolymer of McPherson is 128 segments and comparable to our short block. A curve is drawn through the data points to guide the eye.

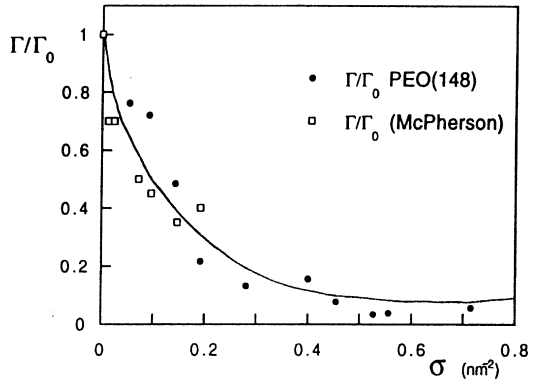

Fig. 9 Γ/Γ_0, of our results and PEO(128) from McPherson *et al*. [3].

It is clear that the decrease in protein adsorption with increasing σ is qualitatively equivalent in both studies. Our results for the short chains are thus shown to agree with previously reported results. The results with long chains are qualitatively different though, and at variance with currently held opinions.

4 DISCUSSION

The intriguing result in the BSA adsorption presented in Fig. 8 is that there is a continuous decrease in Γ with increasing σ for short chains but a distinct maximum in Γ for long chains. This is a novel result: so far, all experimental studies of protein adsorption on surfaces with grafted PEO have used chain lengths of at most 128 segments, and all studies report a continuous decrease of Γ with increasing σ. Moreover, theoretical models so far have emphasised the repulsive nature of protein–PEO interactions, so that they cannot cope with the maximum that we find.

It is instructive to compare the maximum in the adsorbed amount of BSA (Fig. 8) with the maximum predicted by our model for grafted chains interacting with mesoscopic particles (Fig. 6). Qualitatively, the adsorption isotherms show the same trend. We can therefore compare both the analytical and experimental results. One feature present in the case of the PEO-BSA system, but not considered in our model, is adsorption of particles on the hydrophobic substrate. As argued by McPherson *et al*. the main mechanism for the decrease of adsorption with increasing σ is the decrease of accessible substrate surface. We can implement this in a simple way in our model by assuming a linear relationship between adsorption on the substrate, Γ_s, and the grafting density,

$$\Gamma_s = \begin{cases} \Gamma_0(1 - a\sigma) & \text{for } a\sigma \leq 1 \\ \Gamma_s = 0 & \text{for } a\sigma \geq 1 \end{cases}$$

where Γ_0 is the amount adsorbed on the bare hydrophobic substrate and the prefactor a is a measure for the size of the adsorbing particles. If the area per grafted chain, σ^{-1}, is equal to a then no substrate adsorption takes place. The total adsorption, Γ, now is the sum of the substrate adsorption, Γ_s, and adsorption on the brush, Γ_b. In Fig. 10 the experimental data for BSA adsorption for the longest PEO chains (dots) are plotted together with $\Gamma_s + \Gamma_b$ as calculated theoretically (solid line). The values used are $a = 6\,\text{nm}^2$, $v_1 = 20$, $N = 700$, other values as in Fig. 4. Although the values of the excluded volume parameters and adsorption strength have no direct quantitative relevance, it is evident that both the experimental and the calculated adsorption follow the same trend.

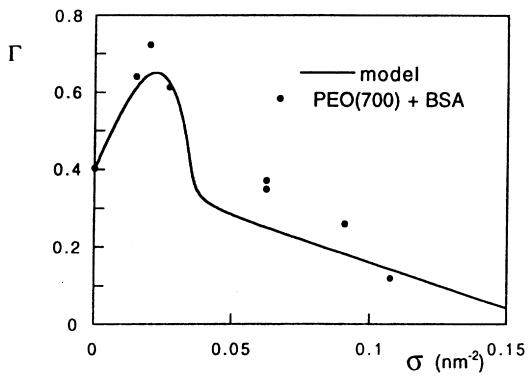

Fig. 10 Total adsorbed amount of PEO(700) and model calculations.

The idea that grafted PEO chains can have affinity for proteins is confirmed by considering the results for the short grafted chains, both ours and those of McPherson *et al*. At high grafting densities the net protein adsorption is expected to be zero, as the steric hindrance of the grafted chains is too high for the proteins to reach the substrate. This conclusion is validated by the Single-Chain Mean-Field-Theory calculations in their paper. Experimentally, however, a considerable residual adsorption is found at high grafting densities, as is shown in Fig. 9. McPherson *et al*. attribute the adsorption at high grafting densities to inhomogeneities in the coverage of the substrate by polymer and dismiss any attractive interactions

between PEO and the proteins [3]. However, the existence of such inhomogeneities is not substantiated. In addition, the maximum in Γ in the case of long chains, clearly contradicts the notion that the sole interaction between the grafted chains and proteins is steric repulsion.

One may wonder what the nature of attractive interactions between the grafted PEO and adsorbing BSA is. Our dynamic light-scattering experiments suggest that there are no attractive interactions between PEO and BSA in a dilute solution of free chains, but grafted chains appear to bind BSA. The surface of BSA consists of hydrophobic and hydrophilic (charged) areas, similar to that of a micelle. It is therefore reasonable to suggest that the same enthalpic interactions are present between PEO and BSA as in the case of PEO-SDS complexes, stemming from a (weak) attractive hydrophobic interaction between PEO and the micellar surface. In a bulk solution this attractive interaction must be compared to the loss in conformational entropy of the polymer upon adsorption: this cost can prove too large for appreciable adsorption to occur. However, if the PEO chains are grafted, the conformational entropy of a chain is significantly lessened by the presence of the grafting substrate and neighbouring chains. Therefore complex formation can be favourable for such restricted chains, resulting in a maximum in Γ as a function of σ.

A different possibility is that there are no attractive interactions between PEO and BSA but the proteins diffuse into the grafted layer and are 'trapped' there. This would explain the length dependence in an elegant manner. Also, since the proteins are less prone to diffuse into a densely grafted layer and are more easily trapped by long chains, this argument explains the maximum in Γ and its length dependence. However, if the proteins are really kinetically trapped within the layer, then the adsorbed amount, whilst washing with pure solvent, should decrease in time. This was checked by adsorbing BSA at a grafting density near the maximum and washing the cell with pure water. The signal, and thus adsorbed amount, remained constant during washing for several hours. This indicates that the interactions between grafted PEO and BSA are attractive in nature, and that the proteins are not merely trapped in the PEO brush.

A final remark is concerned with biomedical applications of long grafted PEO chains. Although it has been shown that short chains offer a better reduction in protein adsorption, Γ is not expected to reach zero at either low or high grafting densities, as is clearly shown in Fig. 9. Moreover, the fact that the protein adsorption is significantly stronger in the case of long chains does not necessarily imply that the protection offered against protein coagulation by long chains is therefore inferior to that of short chains. It is not yet clear whether the proteins adsorbed in the PEO layer tend to coagulate: evidence of polymer–micelles complexes shows that the interactions between the adsorbed particles are repulsive, resulting in an increase in coil size of complexed chains [21,30]. Moreover, the long PEO chains form layers of order 10–30 nm thick [19]. It is possible that such thick layers offer a better protection against adsorption, for instance, bacteria. The choice of chain length and grafting density will depend on the properties of the system in which grafted chains are applied. Such matters need to be investigated in future work.

5 CONCLUSIONS

We have presented a mean-field analytical model for the interactions of grafted polymers with adsorbing mesoscopic particles. It is shown that at low grafting densities (the 'mushroom' regime) the adsorption isotherm is Langmuir-like, and does not depend on the osmotic interactions in the polymer–particle complex. The size of the coil does depend strongly on these interactions: the coil swells with increasing coverage if the particle–particle interactions are dominant and repulsive, has a maximum if the monomer particle interactions are dominant, and decreases in size if the particle–particle interactions are weak compared to the monomer–monomer repulsion.

At high grafting-densities (the brush regime) the degree of complexation, for a given adsorption strength, is found to decrease with increasing grafting density, due to the increasing osmotic interactions in the grafted layer. If the particle–particle osmotic interactions increase in strength, the decrease in degree of complexation is stronger. The brush height is predicted to have a local maximum as a function of the grafting density. The adsorbed amount per unit area, Γ, has a maximum as a function of the grafting density, resulting from a balance between an increasing amount of adsorption sites and a decreasing degree of coverage.

This model appears adequate to describe in a qualitative manner the experimental data of adsorption of BSA on a hydrophobic substrate with grafted PEO chains. It is found that in the case of short PEO chains (148 segments) Γ decreases continuously with increasing σ. This result agrees with previous experimental studies and calculations from analytical and numerical models. In the case of long chains (445 and 700 segments) a maximum in Γ is found as a function of σ. This maximum indicates attractive interactions between the grafted PEO and BSA. The BSA adsorption at high σ does not become zero, but retains a finite value for all chain lengths, also indicating attractive interactions.

Our new model naturally explains the maximum in Γ that is experimentally found for long, grafted PEO chains, and this brings us to the conclusion that there is a weak attraction between PEO and BSA. The exact nature of the attractive interactions between grafted PEO and BSA is unclear, as BSA and PEO in bulk do not seem to form complexes. One possible explanation is that the conformational entropy of the grafted chain plays a decisive role in determining whether the interactions are attractive or repulsive.

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