Investigation of Electrostatic Interactions in Polyelectrolyte Multilayer Films: Binding of Anionic Fluorescent Probes to Layers Assembled onto Colloids

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ABSTRACT: The electrostatic binding of two anionic probe molecules, pyrenetetrasulfonic acid (4-PSA) and 6-carboxyfluorescein (6-CF), to poly(allylamine hydrochloride) (PAH)/poly(sodium 4-styrenesulfonate) (PSS) and poly(diallyldimethylammonium chloride) (PDADMAC)/PSS ultrathin multilayer films assembled onto polystyrene (PS) latex particles has been examined using fluorescence spectroscopy. At the probe concentrations studied, binding was observed only when the outermost layer was oppositely (positively) charged to the probe. The amount of probe bound was found to increase linearly with polyelectrolyte layer number up to about 11 layers (15 nm in thickness for the PAH/PSS films), with saturation binding occurring beyond 11 layers. The binding data reveal that a minimum of about 10−30% of the cationic charges of the polycations in the upper region of the multilayer films (where the probe binds) are not directly electrostatically utilized in the multilayer assembly process through ion-pair binding to oppositely charged sites on the polyanions. The removal of probe bound to multilayers of different thicknesses upon subsequent exposure to PSS has also been examined. Evidence that polyelectrolyte multilayer films are long-range electrostatically coupled systems is presented. The results obtained provide some insight into the role and extent of electrostatic interactions in polyelectrolyte multilayers.

Introduction

Ultrathin polyelectrolyte multilayer films are frequently prepared by the sequential adsorption of oppositely charged polyelectrolytes from dilute aqueous solution onto charged solid substrates.[1−3] The driving force for the assembly of the polyelectrolytes is believed to be primarily due to the electrostatic attraction and complex formation between polyanions and polycations. The multilayer assemblies are considered to have the charged monomer units of their polyelectrolytes ionically bound to those of adjacent polyelectrolytes. The vast majority of studies have involved the formation of polyelectrolyte multilayers on macroscopically flat substrates, e.g., silicon, glass, and gold. Among others, the experimental techniques of X-ray reflectivity, UV−vis and IR spectroscopy, cyclic voltammetry, neutron scattering, X-ray photoelectron spectroscopy (XPS), ellipsometry, surface plasmon resonance, and quartz crystal microgravimetry have been used to characterize polyelectrolyte multilayer films.[1−20] These studies have yielded information on the film thickness, film structure, and layer properties. As an extension of the above technique, we recently demonstrated that the stepwise adsorption of polyelectrolytes from solution can also be performed onto charged particles in solution to produce multilayer films.[21−24]

Despite the importance of electrostatic interactions in the formation of polyelectrolyte multilayers, the *binding stoichiometry* between the polycations and polyanions forming pure polyelectrolyte multilayer films is not fully understood. A number of XPS studies on polyelectrolyte multilayers have reported that only trace amounts of small counterions can be detected in the layers.[1,17−20] In some multilayer films where chloride is expected to be present as the counterion, no chloride is observed at all.[17−20] This has prompted the suggestion that pure polyelectrolyte multilayers should have a 1:1 stoichiometry of anionic and cationic groups; that is, every cationic group in a polycation is *bound* to an anionic group of a polyanion.[1,14,20] In contrast, it has recently been reported that free cationic sites of poly(diallyldimethylammonium chloride) (PDADMAC) exist in PDADMAC−SiO_2 multilayer films and that the excess charge on PDADMAC in such films *must* be neutralized by small counterions such as chloride.[25] Although the stoichiometry of the *assembly process* (adsorbed amount) of polyelectrolyte multilayers is often measured, there are little data available concerning the *binding stoichiometry* between the charges of polyelectrolytes (charge compensation) forming multilayer films.

In this work, we report an alternative approach that utilizes fluorescence spectroscopy to investigate the binding stoichiometry between cationic and anionic groups of polyelectrolytes in ultrathin multilayer films. Two fluorescent probes are employed: pyrenetetrasulfonic acid (4-PSA) and 6-carboxyfluorescein (6-CF). The binding of these probes to multilayers of poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS), and PDADMAC and PSS multilayers, formed on polystyrene (PS) latex particles, is examined. In a previous paper we showed that 4-PSA and 6-CF electrostatically bind to the *charged* (cationic) groups of PAH and PDADMAC *in solution* and that the binding behavior of these probes is qualitatively and quantitatively different.[26] In the current work we also investigate the degree of probe removal from multilayer films as a function of multilayer film thickness caused by the subsequent adsorption of PSS. This provides a novel way of examining the effect of polyelectrolyte adsorption on the existing multilayer film structure.

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Experimental Section

Materials. Poly(allylamine hydrochloride) (PAH), M_w 8000–11 000, poly(diallyldimethylammonium chloride) (PDADMAC), M_w <200 000, and poly(sodium 4-styrenesulfonate) (PSS), M_w 70 000, were all obtained from Aldrich. Low M_w PSS (4300) was purchased from Polymer Standards Service, Mainz, Germany. 1,3,6,8-Pyrenetetrasulfonic acid, tetrasodium salt (4-PSA), was obtained from Molecular Probes, Eugene, OR, and 6-carboxyfluorescein (6-CF) from Sigma. All polyelectrolytes and probes were used as received, except for the 70 000 M_w PSS which was dialyzed against Milli-Q water (M_w cutoff 14 000) and lyophilized before use. The negatively charged sulfate-stabilized polystyrene (PS) latex particles (diameter 640 nm) were prepared as described elsewhere.[27] Positively charged homogeneously doped rhodamine B-labeled melamine formaldehyde (RhB-MF) particles of diameter 3.3 μm were purchased from Microparticles GmbH, Berlin, Germany. Sodium chloride (AR grade) was obtained from Merck. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm.

Assembly of Polyelectrolyte Multilayers onto Colloids. A 0.5 mL aliquot of an aqueous 1 mg mL^{-1} PAH or PDADMAC solution containing 0.5 M NaCl was added to 0.2 mL of the negatively charged polystyrene latex particles (approximately 10^{10} particles). (This polyelectrolyte concentration is much larger than that required for saturation coverage of the particles.) The polyelectrolyte was allowed to adsorb for 20 min, during which time the dispersion was occasionally stirred. The dispersion was then centrifuged at 13 500*g* for 15 min, the supernatant removed, water added, and the particles redispersed by gentle shaking. The centrifugation/wash/redispersion cycle was repeated a further three times to ensure removal of free polyelectrolyte in solution. (At this point the particles are coated with polyelectrolyte and exhibit a reversal of ζ-potential, i.e., from negative to positive; see later.) A 0.5 mL aliquot of an aqueous 1 mg mL^{-1} PSS solution containing 0.5 M NaCl was then added to the PAH- or PDADMAC-coated PS latex particles (in 0.5 mL of H_2O) and allowed to interact for 20 min. The method described above was followed for deposition of this PSS layer and for subsequent polyelectrolyte layers to produce polyelectrolyte multilayer films on PS latex particles. Polyelectrolyte multilayers were deposited onto RhB–MF particles also as described above, although the first layer adsorbed was PSS since RhB–MF particles are positively charged.

Electrophoretic Mobility (EPM) Measurements. Electrophoretic mobilities of the bare and coated PS latex particles were measured using a Malvern Zetasizer 4. The mobility u was converted into a ζ-potential using the Smoluchowski relation $\zeta = u\eta/\epsilon$, where η and ϵ are the viscosity and permittivity of the solution, respectively. All ζ-potential measurements were performed without added electrolyte.

Single Particle Light Scattering (SPLS) Measurements. Details of the SPLS experimental system and measurement principle have been described in detail elsewhere.[28,29] Briefly, the dispersion (of either uncoated or polyelectrolyte-coated PS latex particles) is passed through a capillary with a 0.1 mm diameter orifice at the end. Hydrodynamic focusing is applied so that the dispersion stream is directed through a laser beam which is focused to allow only a single particle or aggregate in focus at a particular time. This requires particle concentrations of less than 3×10^8 particles mL^{-1}. The light scattered by the particles moving through the laser focus is recorded in the angular region of 5°–10° in the forward direction. The intensity distributions, obtained with a resolution of 0.5%, are collected by a multichannel analyzer and then stored on a PC. The Rayleigh–Debye–Gans theory is used to obtain a particle size distribution of the dispersion. Using the SPLS technique, it is possible to distinguish between singlets, doublets, and triplets and to determine their respective concentrations. The SPLS technique was also used to determine the particle concentration of each sample since some particles were inevitably lost due to the centrifugation/wash/redispersion cycles when preparing the polyelectrolyte-coated particles.

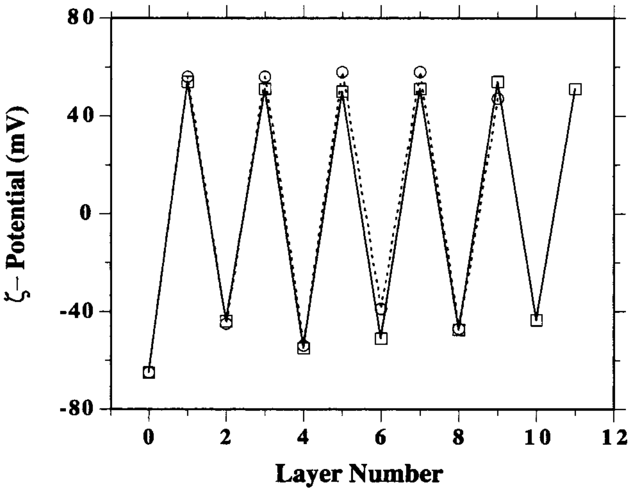

Figure 1. ζ-potential as a function of polyelectrolyte layer number for (circles) PAH/PSS- and (squares) PDADMAC/PSS-coated negatively charged PS latex particles. The odd layer numbers correspond to PAH or PDADMAC deposition and the even layer numbers to PSS deposition.

Steady-State Fluorescence Measurements. Fluorescence measurements were undertaken using a Spex Fluorolog 1680 spectrometer with excitation and emission bandwidths set at 1.0 nm. All measurements were performed on air-equilibrated solutions at 25 °C. Typically, ca. 50 μL of the polyelectrolyte multilayer-coated PS latex suspension was pipetted into 3 mL of water in a fluorimeter cell and the dispersion agitated for 0.5 min. The fluorescence spectrum of this dispersion was recorded and used as a background for subsequently measured spectra. An aliquot of the probe solution was then added, the dispersion stirred for 2 min, and the fluorescence spectrum recorded. This procedure was repeated to cover a range of probe concentrations.

Results and Discussion

Characterization of Polyelectrolyte Multilayers Assembled onto Colloids. Figure 1 shows the ζ-potential as a function of polyelectrolyte layer number for the negatively charged PS latex particles coated with PAH/PSS or PDADMAC/PSS multilayers. The negatively charged (uncoated) PS latex particles yield a ζ-potential of about −65 mV in water. The presence of PAH or PDADMAC (both cationic) causes a reversal in ζ-potential (+60 mV). Subsequent deposition of PSS onto the PAH- or PDADMAC-coated PS latex particles again reverses the ζ-potential. Alternating ζ-potentials (in sign) are observed with the further deposition of each oppositely charged polyelectrolyte, suggesting that stepwise polyelectrolyte multilayer growth occurs on the particles for both PAH/PSS and PDADMAC/PSS systems. No quantitative conclusions are made from the ζ-potential values obtained because the magnitude of the ζ-potential is not proportional to the charge density, since the surface is composed of charges arranged in a layer of finite thickness.[30,31] In addition, the ζ-potential depends on the polyelectrolyte conformation at the surface.[30,31]

SPLS measurements were performed in order to determine the thickness of the polyelectrolyte layers assembled onto the PS latex particles and to assess whether or not the particles aggregate as a result of polyelectrolyte deposition. Normalized SPLS intensity distributions for the bare PS latex particles and those coated with PDADMAC/PSS layers are shown in Figure

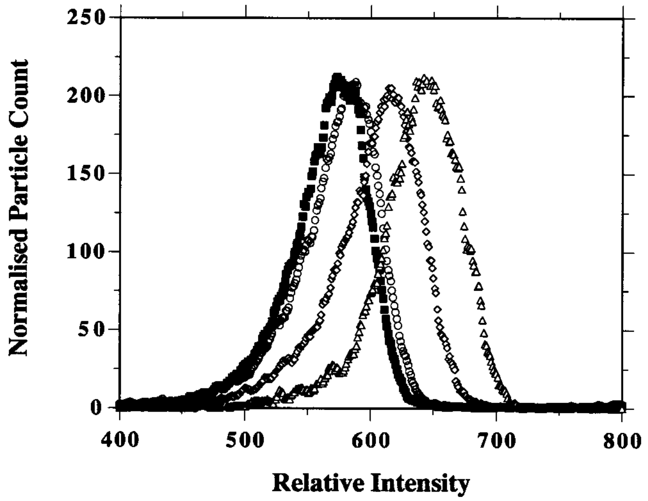

Figure 2. Normalized light scattering intensity distributions of uncoated PS latex particles (filled squares) and PS latex particles with [PDADMAC/(PSS/PDADMAC)$_N$]: $N = 0$, (circles), $N = 2$ (diamonds), and $N = 4$ (triangles), corresponding to 0, 1, 5, and 9 layers, respectively.

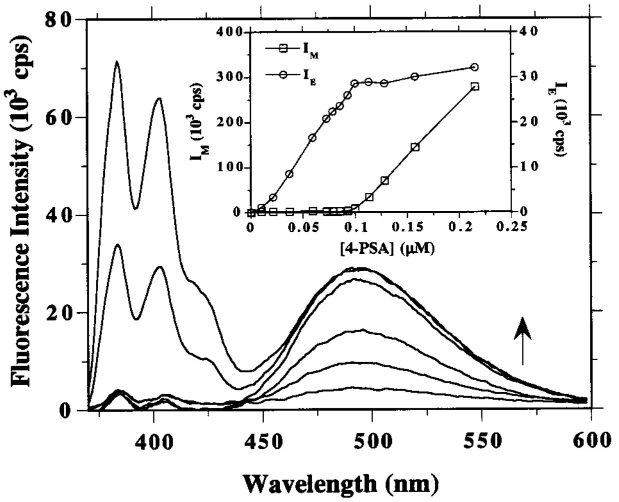

Figure 3. Fluorescence spectra of 4-PSA in the presence of PS latex particles coated with seven polyelectrolyte layers [PAH/(PSS/PAH)$_3$] for different 4-PSA concentrations. Spectra (from bottom to top): 0.02, 0.04, 0.06, 0.09, 0.11, and 0.13 μM 4-PSA. The arrow indicates the direction of increasing 4-PSA concentration. Excitation wavelength = 350 nm. The inset shows I_M (383 nm) and I_E (495 nm) as a function of 4-PSA concentration for 4-PSA in the presence of [PAH/(PSS/PAH)$_3$]-coated PS latex particles.

Table 1. Thickness and Surface Coverage Data for Polyelectrolyte Multilayer Films Assembled onto Negatively Charged Polystyrene Latex Particles

	film thickness, d (nm)[a]		amt adsorbed, Γ (mg m^{-2})[b]	
layers	PAH/PSS	PDADMAC/PSS	PAH/PSS	PDADMAC/PSS
1	1.2	1.4	0.84	0.98
3	3.4	3.3	2.4	2.3
5	5.6	6.6	3.9	4.6
7	8.4	8.5	5.8	5.9
9	11.5	11.7	8.0	8.2
11	15.0		10.5	
15	24.8		17.3	
21	33.9		23.6	

[a] Thicknesses were determined using a refractive index of 1.47. [b] Surface coverages were calculated via de Feijter's equation (see text for details). The error in the values is estimated as 10%.

2. The deposition of the polyelectrolytes onto the particles is manifested as a shift in the SPLS intensity distribution; there is a systematic shift (in the x-axis direction) with increasing polyelectrolyte layer number, confirming layer growth. Similar SPLS intensity distributions were obtained for the PAH/PSS-coated PS latex particles. No intensity peaks were observed at higher intensities in the SPLS intensity distributions, indicating that no significant particle aggregation occurs, not even at higher polyelectrolyte layer numbers.

The SPLS technique is sensitive to the amount of polyelectrolyte adsorbed onto particles. Using the Rayleigh–Debye–Gans theory[32] and a layer refractive index (n) of 1.47,[9,22,33] the average thickness of the polyelectrolyte layers (d) can be determined. The calculated average layer thickness increases with the number of polyelectrolyte layers deposited for both the PDADMAC/PSS- and PAH/PSS-coated PS latex particles (see Table 1). The d values for the PAH/PSS multilayers are, within experimental error, the same as those for the PDADMAC/PSS layers. The average thickness (± standard deviation) for a PAH/PSS layer pair is 3.3 ± 1.1 nm (average of 21 layers) and 2.6 ± 0.8 nm (average of nine layers) for a PDADMAC/PSS layer pair. The data obtained are in agreement with those from X-ray reflectivity measurements for PSS/PAH multilayer films formed on silicon substrates under the same ionic strength conditions used in this work (0.5 M NaCl):[14] 16.5 nm for 12 layers or 2.75 nm per layer pair. In the current work, polyelectrolyte layer thicknesses were measured only for films where PAH is the outermost layer, since polyelectrolyte-coated particles with PAH as the outermost layer were subsequently used in a series of fluorescence experiments to quantify the binding of 4-PSA and 6-CF to polyelectrolyte multilayers (see later).

The amount of polyelectrolyte adsorbed (Γ) onto the PS latex particles can be calculated using de Feijter's relation:[34]

$$\Gamma = \frac{d(n_f - n_b)}{dn/dC} \tag{1}$$

where d is the thickness of the polyelectrolyte layer(s), n_f and n_b are the refractive indices of the polyelectrolyte layer(s) and water solution, respectively, and dn/dC is the refractive index increment with concentration of the polyelectrolytes. The value of dn/dC for PAH/PSS and PDADMAC/PSS polyelectrolyte complexes is 0.196 cm^3 g^{-1}.[33] The amount of polyelectrolyte adsorbed onto the particles is similar for both the PAH/PSS and PDADMAC/PSS multilayers (Table 1). The average amount adsorbed per polycation/PSS layer pair is 2.1 ± 0.7 mg m^{-2}. These data are necessary for quantification of the binding stoichiometry in the polyelectrolyte multilayer films (see later).

Binding of 4-PSA and 6-CF to PAH/PSS and PDADMAC/PSS Multilayers. The presence of uncoated PS latex particles in solutions containing the anionic probes 4-PSA or 6-CF had no effect on the probe fluorescence intensity and spectra (when corrected for scattering). No probe adsorption onto the particles was observed (assessed from adsorption experiments). The influence of PS latex particles coated with seven polyelectrolyte layers [PAH/(PSS/PAH)$_3$] on the 4-PSA fluorescence spectrum at different 4-PSA concentrations is shown in Figure 3. The dependence of the monomer (I_M) and excimer (I_E) emission on 4-PSA concentration in the presence of [PAH/(PSS/PAH)$_3$]-coated PS latex particles is displayed in the inset. In the 4-PSA concentration range 0−0.1 μM, I_E steadily increases, whereas I_M remains essentially constant and small. Above ca. 0.1 μM 4-PSA, I_E becomes constant and I_M sharply increases. The above 4-PSA fluorescence−concentration curves can be explained as follows. The polyelectrolyte layers, or more specifically the charged sites of PAH available for probe adsorption, cause extraction of 4-PSA from bulk solution, and binding occurs over the concentration range 0−0.1 μM 4-PSA.[26] At 0.1 μM, 4-PSA saturation binding of the charged sites within the polyelectrolyte multilayer film occurs, and the increase seen in I_M above 0.1 μM is due to the added 4-PSA remaining in bulk solution. Evidence for binding is provided by the 4-PSA monomer fluorescence intensity at 0.1 μM being negligible (<1%), when compared to that in the absence of PAH/PSS-coated PS latex particles. In addition, the rate of monomer intensity increase with 4-PSA concentration above 0.1 μM is identical to that observed for 4-PSA alone in aqueous solution. A series of experiments were also conducted to assess the extent of 4-PSA binding below the saturation binding concentration of 4-PSA. Polyelectrolyte-coated particles were exposed to 4-PSA concentrations over the range 0−0.1 μM, allowed to interact for 2 min, and the particles then centrifuged. The supernatant was analyzed for free (unbound) 4-PSA by fluorescence measurements. At all concentrations studied, the monomer fluorescence intensity from 4-PSA in the supernatant was less than 2% of that measured for 4-PSA/water reference solutions. This confirms that the fluorescence observed at concentrations below that of saturation binding is from *bound* 4-PSA. It is worth noting that excimer emission is observed even at the lowest 4-PSA concentrations (<0.02 μM) studied. This points to an ordered binding process since 4-PSA does not form excimers in aqueous solutions, not even at concentrations greater than 10 μM.[26]

Similar I_M and I_E versus 4-PSA concentration plots to those shown in the inset of Figure 3 were obtained for [PAH/(PSS/PAH)$_N$]-coated PS latex particles, where $N = 0$, 1, 2, 4, 5, 7, and 10 (i.e., 1, 3, 5, 9, 11, 15, and 21 layers) (data not shown). Normalized (for particle concentration) I_E versus 4-PSA concentration curves show that the plateau at which I_E occurs increases with increasing polyelectrolyte layer number. This provides evidence that more 4-PSA is bound by thicker polyelectrolyte films. Quantification of this will be given later.

The influence of PDADMAC/PSS-coated PS latex particles on the fluorescence spectrum of 4-PSA is shown in Figure 4. The corresponding I_M and I_E versus 4-PSA concentration curves are displayed in the inset. In contrast to the fluorescence from 4-PSA in the presence of PAH/PSS-coated PS latex particles (Figure 3), predominantly monomer emission is observed from 4-PSA in the presence of PDADMAC/PSS-coated PS latex particles (spectra shown for nine layers). Both I_M and I_E steadily increase until about 0.3 μM 4-PSA, after which concentration I_M increases due to 4-PSA in solution, and I_E remains constant. The 0.3 μM 4-PSA again corresponds to 4-PSA saturation binding. As for the PAH/PSS-coated particles, 4-PSA monomer fluorescence from the supernatant (after centrifugation of the particles exposed to 4-PSA concentrations below saturation binding conditions) was negligible. This again confirms that essentially all 4-PSA is bound to the multilayer film at 4-PSA concentrations lower than approximately 0.3 μM. Similar fluorescence−concentration curves to those shown in the inset of Figure 4 were obtained for PDADMAC/PSS-coated PS latex particles with 1, 3, 5, and 7 layers. It also should be noted that no interaction between unsubstituted pyrene and the layers assembled onto the particles was observed for all the systems studied.

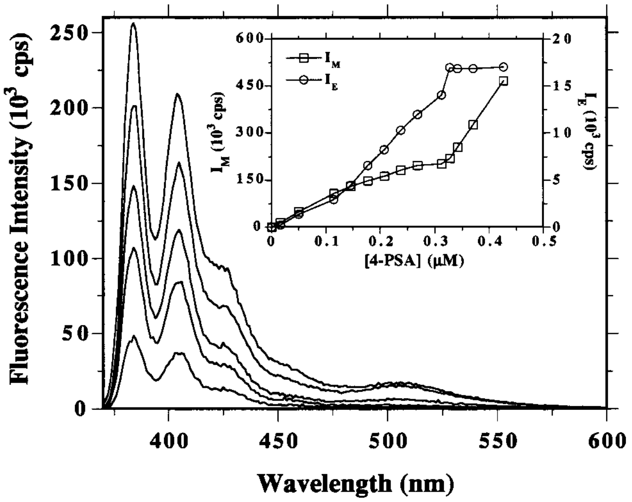

Figure 4. Fluorescence spectra of 4-PSA in the presence of PS latex particles coated with nine polyelectrolyte layers [PDADMAC/(PSS/PDADMAC)$_4$] for different 4-PSA concentrations. Spectra (from bottom to top): 0.05, 0.11, 0.18, 0.31, and 0.34 μM 4-PSA. Excitation wavelength = 350 nm. The inset shows I_M (384 nm) and I_E (500 nm) as a function of 4-PSA concentration for 4-PSA in the presence of [PDADMAC/(PSS/PDADMAC)$_4$]-coated PS latex particles.

The value of I_E/I_M at binding saturation for the PDADMAC/PSS multilayers ($I_E/I_M \sim 0.05-0.2$) is significantly smaller than that for the PAH/PSS multilayers ($I_E/I_M \sim 2-10$). (The I_E/I_M values systematically increase as the film thickness increases.) The I_E/I_M values at saturation binding for 4-PSA with PDADMAC in aqueous solution were also found to be considerably smaller (2−3 orders of magnitude) than those for 4-PSA/PAH in aqueous solution.[26] In the solution studies, this difference was attributed to the different flexibility and local structure of the polyelectrolytes.[26] The presence of PAH clearly facilitates excimer formation of 4-PSA to a greater extent than does PDADMAC, whether assembled in multilayer films or in solution. The degree of 4-PSA quenching in the PDADMAC/PSS multilayer films is significantly less than that for 4-PSA with PDADMAC in solution. The extent of 4-PSA quenching in the PDADMAC/PSS multilayer films, as well as in the PAH/PSS films, will be quantitatively assessed in a subsequent publication.[35]

Figure 5 displays the fluorescence spectra of 6-CF in the presence of [PAH/(PSS/PAH)$_2$]-coated PS latex particles for various 6-CF concentrations. Spectra a and b correspond to 6-CF concentrations below that required to saturate all available binding sites in the polyelectrolyte multilayer film, whereas spectrum c is for a 6-CF concentration exceeding saturation binding. At low levels of 6-CF (spectrum a), all the probe is bound to the multilayer film, and the fluorescence maximum occurs at ca. 520 nm. At higher concentrations, more 6-CF binds and the fluorescence maximum is still at ca. 520 nm, but there is a reduction in the fluorescence intensity due to increased probe−probe interaction (via 6-CF self-quenching) as a result of increased filling of

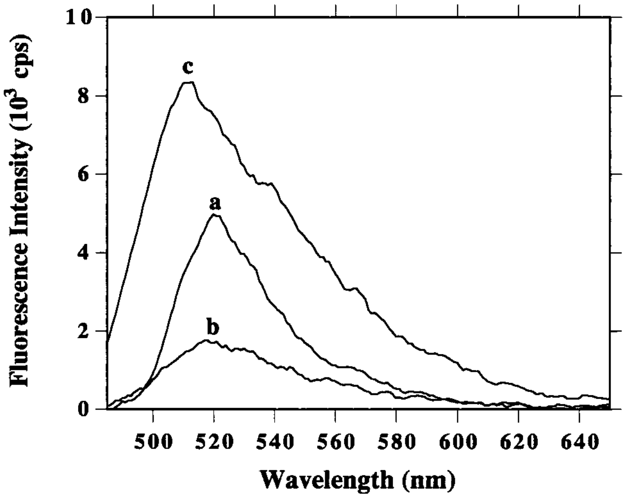

Figure 5. Fluorescence spectra of 6-CF in the presence of PS latex particles coated with five polyelectrolyte layers [PAH/(PSS/PAH)$_2$] for various 6-CF concentrations: (a) 0.02, (b) 0.20, and (c) 0.36 μM. Excitation wavelength = 450 nm.

the binding sites (spectrum b). Beyond the concentration where the maximum number of polyelectrolyte binding sites are filled, 6-CF remains in solution, and a noticeable blue shift (from 520 to ca. 512 nm) in its fluorescence spectrum occurs (spectrum c). This spectrum is, of course, a composite of that of 6-CF bound to the multilayer film and that of 6-CF in solution. (It should be noted that the contribution to spectrum c from 6-CF in solution is significantly larger than that due to bound 6-CF.) Similar shifts in the maximum wavelength of 6-CF fluorescence spectra were obtained for 6-CF interacting with PAH or PDADMAC in aqueous solution: 520 nm (6-CF in bound state) to 512 nm (6-CF in bulk solution).[26]

The fluorescence intensity (at λ_{max}) of 6-CF in the presence of PAH/PSS and PDADMAC/PSS multilayer-coated PS latex particles is plotted as a function of 6-CF concentration in Figure 6. All the fluorescence–concentration curves show an initial fluorescence intensity increase at low 6-CF concentrations, followed by a decrease, and thereafter a sharp increase at higher concentrations. As mentioned above, the decrease in fluorescence intensity is brought about by 6-CF self-quenching. The 6-CF concentration at which maximum binding to the multilayer film occurs is taken as that where a minimum in 6-CF fluorescence intensity results, just prior to added 6-CF remaining in solution.[26] The rate of increase in fluorescence intensity with 6-CF concentration for 6-CF in the presence of the PDADMAC/PSS-coated PS latex particles after saturation binding is the same as that for 6-CF in pure water. However, this rate is somewhat lower in the presence of PAH/PSS-coated particles. The reason for this is most probably due to the lower number of compensated positive charges by 6-CF in the PAH/PSS multilayer film, compared to the PDADMAC/PSS film (see later). Differences in the rate of increase in fluorescence intensity with 6-CF concentration after saturation binding for cationic polyelectrolytes in solution have been attributed to the formation of a diffuse counterion layer by the 6-CF molecules, since only about 50% of the total charged sites of the polyelectrolyte are compensated in solution.[26]

Evaluation of Probe Binding Data. SPLS enabled determination of the number of polyelectrolyte-coated PS latex particles in each sample. Using these data and the probe concentrations at which saturation binding occurs, the number of 4-PSA or 6-CF molecules bound per polyelectrolyte multilayer-coated PS latex particle (N_{probe}) (at saturation binding) for the polyelectrolyte multilayers was determined for films consisting of up to 21 layers (Figure 7). Figure 7a shows that a linear relationship is observed between the amount of probe bound and polyelectrolyte layer number over the layer number range 1–11 (thickness range 1–15 nm), for both probes and both PAH/PSS and PDADMAC/PSS multilayer films. The amount of bound probe was found to saturate when the layer number exceeded about 11 for the PAH/PSS multilayer films (Figure 7b). The linearity observed clearly shows that the number of available binding sites for the probes increases with the deposition of each subsequent polycation layer (whether PAH or PDADMAC) and suggests that not all charges in the polyelectrolyte multilayer films for about the first 11 layers are involved in the assembly process by forming ion pairs (see later). The surface area increase with increasing multilayer film thickness on the PS latex particles is negligible (<5%) and therefore cannot explain the linear increase of N_{probe} per polyelectrolyte multilayer-coated PS latex particle with polyelectrolyte layer number observed. The linear relationship also suggests that the probe is distributed throughout the entire film (where the linear range occurs). The saturation of probe binding above 11 layers for the PAH/PSS

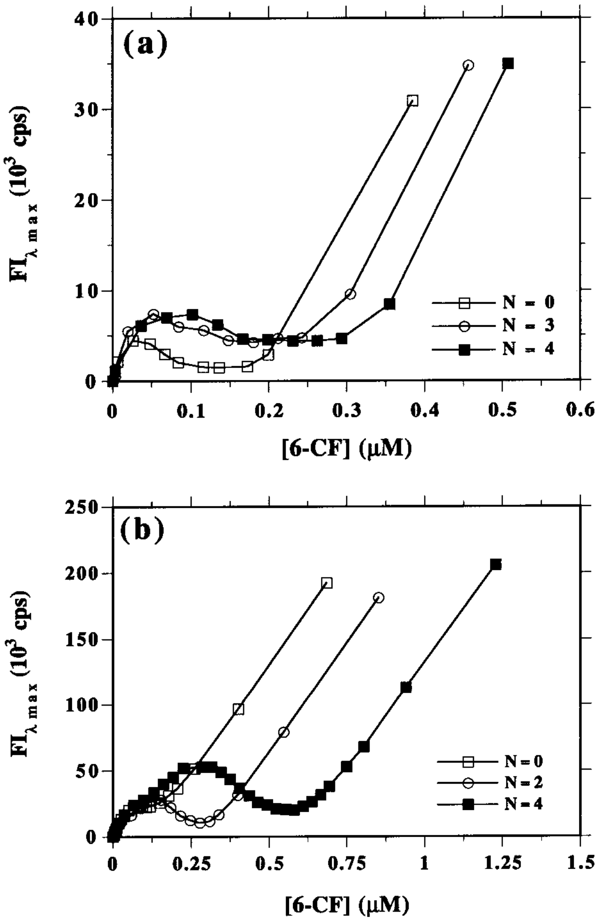

Figure 6. Fluorescence intensity (at λ_{max}) as a function of 6-CF concentration for 6-CF in the presence of (a) [PAH/(PSS/PAH)$_N$]-coated PS latex particles with N = 0, 3 and 4 and (b) [PDADMAC/(PSS/PDADMAC)$_N$]-coated PS latex particles with N = 0, 2, and 4. Excitation wavelength = 450 nm for both sets of data.

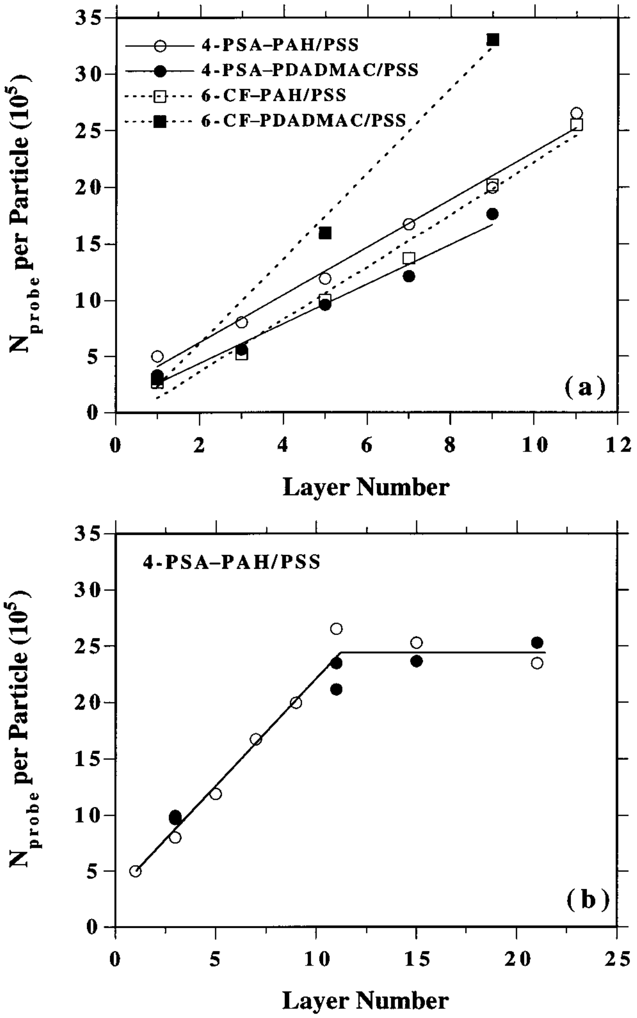

Figure 7. (a) Number of probe molecules (N_{probe}) bound per PAH/PSS- and PDADMAC/PSS-coated PS latex particle (at saturation binding) as a function of polyelectrolyte layer number (1−11) in the multilayers. (b) N_{probe} bound per PAH/PSS-coated PS latex particle (at saturation binding) as a function of polyelectrolyte layer number (1−21) in the multilayers. Each data symbol represents a separate series of experiments.

multilayers indicates that the equivalent of 11 layers still bind probe, even if the layer number increases. Evidence that it is the upper layers which bind probe is provided later. Polycation−polyanion association is much stronger than that of the polycation with 4-PSA or 6-CF (as evidenced by the removal of prebound probe by subsequent polyanion adsorption; see later). Hence, breaking of electrostatic interactions (ion pairs) between the oppositely charged polyelectrolytes forming the multilayer films upon probe binding can be excluded. The probe molecules bind to positively charged sites which are not utilized in the assembly process.

The results presented above all correspond to polyelectrolyte-coated particles where the outermost layer is oppositely charged to the probe molecules; that is, positively charged (PAH or PDADMAC), as revealed by the ζ-potential data. Experiments were also conducted on PS latex particles coated with PAH/PSS or PDADMAC/PSS multilayers and with the outermost layer negatively charged (PSS). PS latex particles coated with PAH/PSS (two layers) had no effect on the fluorescence spectrum and intensity of 4-PSA. Similarly, thicker films [$(\mathrm{PAH/PSS})_4$] (eight layers) and films of $(\mathrm{PDADMAC/PSS})_N$ ($N = 1$ or 4) on PS latex particles also had no influence on the fluorescence properties of 4-PSA. This was also observed for 6-CF for both polycation/PSS systems when the outermost layer was PSS. The simple view that the outermost layer (which is negatively charged) prevents diffusion of the probe into the film as a result of an electrostatic potential barrier was ruled out by performing experiments where probe was exposed to positively charged RhB−MF particles coated with PSS/PAH multilayers of varying thickness (1, 3, and 5 layers) and with an outermost negative (PSS) layer. (Alternating ζ-potential values with polyelectrolyte layer number are observed for PSS/PAH multilayers on positively charged RhB−MF particles, and the magnitude of the ζ-potential is similar to those measured for PAH/PSS-coated latex particles.[21]) 4-PSA binding to the RhB−MF particles was observed (determined by adsorption experiments) for all three multilayers (1, 3, and 5 layers) examined. In addition, our recent work has shown that polyelectrolyte layers assembled onto RhB−MF particles are readily permeable to probes such as 6-CF when the outermost layer is negatively charged—this was assessed by Förster resonance energy transfer experiments—and that energy transfer occurred as a result of probe adsorption onto the positively charged particle surface.[21] Hence, such evidence for probe binding to the RhB−MF particles shows that electrostatics does not prevent probe penetration into layers. This strongly suggests that (i) there are either no accessible sites in the film for the probes to bind to when the outermost layer of polyelectrolyte multilayers is negatively charged or (ii) that the binding constant is so small that binding occurs only at very high probe concentrations. (This is elaborated on in more detail below.) If situation (ii) were to occur, the binding would not be detectable on the background of the intensity of the free/unbound probe fluorescence.

The cationic groups of PAH and PDADMAC polyelectrolytes have been identified as the sites for 4-PSA and 6-CF binding.[26] Using the surface coverage data (Table 1) for the polyelectrolyte layers and the concentrations at which probe saturation binding of the multilayer films occurs, the percentage of cationic sites in the films compensated by probe can be calculated. In this calculation, it is assumed that equimolar amounts of the polycation and polyanion are deposited with each step (2.1 mg m^{-2} per layer pair or 1.0 mg m^{-2} per layer). The percentage of cationic sites in the polyelectrolyte multilayer films compensated by 4-PSA and 6-CF is displayed as a function of polyelectrolyte layer number up to 11 layers in Figures 8 and 9, respectively. With the exception of the 6-CF−PDADMAC/PSS system, the percentage of cationic sites compensated is essentially constant with increasing film thickness up to 15 nm (11 layers for the PAH/PSS system). There are, however, slightly more cationic sites compensated for the first layer of the PAH/PSS multilayer film by 4-PSA. It is known for planar surfaces that a few layers are required before regular stepwise polyelectrolyte growth occurs.[13,14,16] This may also apply to particle surfaces. The number of layers required before regular growth is obtained depends on the nature of the surface, the polyelectrolytes, and the adsorption conditions.[6−9,16]

The percentage of cationic sites compensated by 4-PSA for PAH/PSS and PDADMAC/PSS multilayers comprising of three or more layers is approximately 20% and 25−30%, respectively. 6-CF compensates approxi-

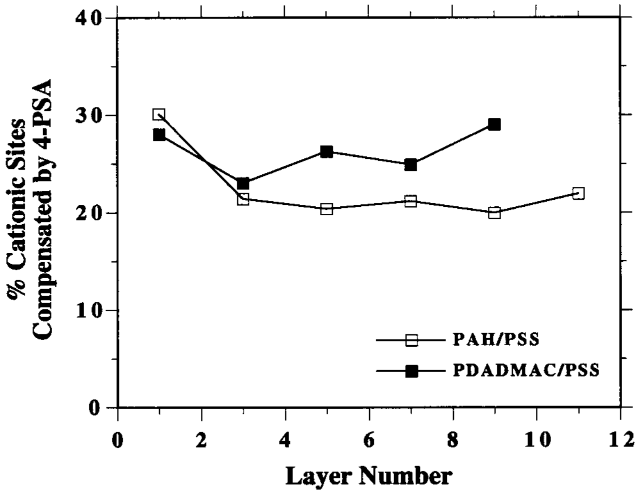

Figure 8. Percentage of cationic sites in PAH/PSS and PDADMAC/PSS multilayer films assembled onto PS latex particles compensated by 4-PSA as a function of polyelectrolyte layer number.

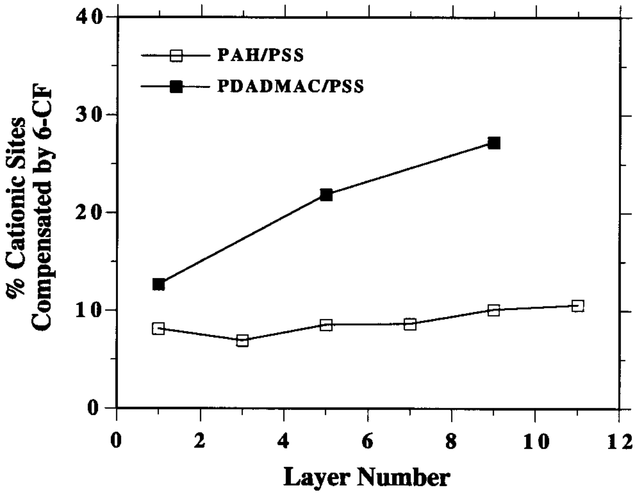

Figure 9. Percentage of cationic sites in PAH/PSS and PDADMAC/PSS multilayer films assembled onto PS latex particles compensated by 6-CF as a function of polyelectrolyte layer number.

mately 10% of the cationic sites in the PAH/PSS multilayer films and between 10 and 30% of the cationic sites in the PDADMAC/PSS multilayer films. In contrast to the PAH/PSS data, an increase in the number of cationic sites in the PDADMAC/PSS multilayer films compensated by 6-CF with polyelectrolyte layer number is seen in Figure 9; this may reflect differences in the binding characteristics of 6-CF to the two multilayer films. The degree of charge compensation by probe is larger when PDADMAC is used as the polycationic species, rather than PAH, for both the 4-PSA and 6-CF probes. This can be explained by the more flexible PAH being able to more efficiently form ion pairs with the neighboring polyanionic PSS. From the above data it can be concluded that there are cationic sites within about the top 11 layers of the films which do not form ion pairs. In the next section it will be shown that it is the upper region to which the probe is bound.

Removal of 4-PSA Bound to PAH/PSS Multilayer Films by PSS Adsorption. PS latex particles coated with PAH/PSS multilayers of varying thickness (1−34 nm) were exposed to 4-PSA concentrations so that the amount of 4-PSA bound was approximately 90% of that required for saturation binding. PSS was then added from a solution containing 0.5 M NaCl—this electrolyte concentration is used when forming pure polyelectrolyte multilayers in this work—and 4-PSA removal was monitored by recording its fluorescence intensity and quantified by comparing it to fluorescence calibration curves obtained for 4-PSA in pure water. Figure 10 displays the percentage of 4-PSA remaining (relative to the amount initially bound to the film) as a function of film thickness after the addition of PSS of different molecular weight. The final PSS concentration was ca. 6 μg mL^{-1} (and [NaCl]$_{\mathrm{final}}$ $\sim$ 3 mM). Increasing the amount of PSS added to the system did not cause further removal of 4-PSA. Addition of only NaCl, to give a NaCl concentration in the sample equal to that when the PSS/NaCl solution was added ($\sim$3 mM), resulted in only less than 5% of the probe being displaced from the multilayer films. Therefore, the probe removal observed is due to PSS binding to the polycation in the multilayer films rather than due to the added NaCl; that is, the probe is replaced by the direct interaction of the cationic groups of PAH and the and anionic groups of PSS. Similar data are obtained for both the 70 000 and 4300 M_w PSS. The subsequent adsorption of PSS onto PS latex particles coated with only one PAH layer and loaded with 4-PSA displaces all of the bound 4-PSA. For a thicker film (ca. 3 nm or three layers), there is only a slight reduction in the amount of 4-PSA removed, with about 5% remaining in the film, and for a 6 nm thick film (five layers), about 20% of 4-PSA remains. There is a further decrease in the amount displaced by PSS when the film is 8 nm thick (seven layers), and above this thickness the percentage of 4-PSA remaining ($\sim$50%) is essentially constant with film thickness (up to 34 nm). These findings are fully consistent with the view that polyelectrolytes in multilayer films do not form strictly stratified layers but show an overlap (or interpenetration) over about four neighboring layers.[1,19] Taking this view, the top layer would extend at least four layers downward into the film, while the seventh layer would contact the fourth layer from the top at

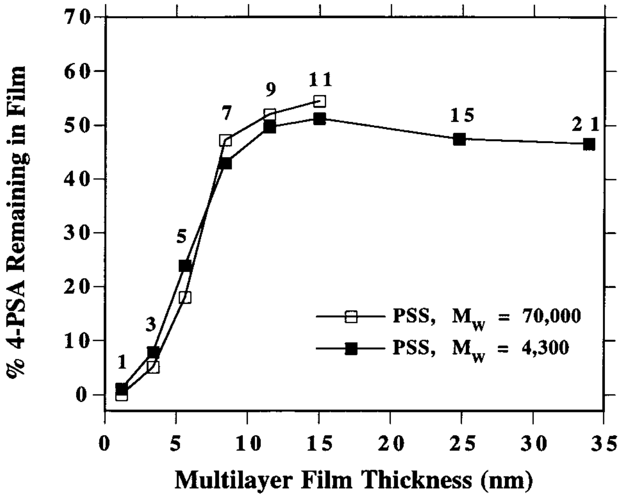

Figure 10. Percentage of 4-PSA remaining bound to PAH/PSS multilayers on PS latex particles as a function of multilayer film thickness after exposure to PSS of 70 000 or 4300 molecular weight. The numbers of polyelectrolyte layer numbers corresponding to film thickness are indicated. Each data point represents a separate multilayer film with different thickness. The amount of 4-PSA initially bound was 90% of that required for saturation probe binding before PSS was added.

some points. Hence, the incoming top layer (PSS) would influence probe bound as low as 7−8 layers into the film from the top. This most probably explains why the probe removal amount stays constant after about seven layers have been deposited. The constant amount of probe removed after seven layers further indicates that it is the interaction between the incoming PSS and the sites to which the probe is bound within the multilayer film which causes probe removal. Furthermore, it provides evidence that the probe binds to the upper region of the multilayer films.

The effect of the addition of sodium chloride (with no added PSS) to the same PAH/PSS multilayers with bound 4-PSA investigated above was also examined. The amount of probe displaced increases with increasing concentrations of NaCl. At 0.14 M NaCl approximately 60% of the probe was removed. This is most likely due to a combination of (i) an electrostatic shielding effect of the salt on the attraction between the cationic sites of the polycations in the multilayer film and the anionic 4-PSA probe and (ii) any changes occurring within the multilayer film upon exposure to high concentrations of NaCl; PSS/PAH multilayer films have been observed to undergo structural changes, i.e., swelling, when immersed in NaCl solutions of concentration above 0.02 M.[14]

Electrostatic Interactions in Polyelectrolyte Multilayer Films. To arrive at a model to describe the electrostatic interactions in polyelectrolyte multilayer films, the following main experimental observations from the probe binding data need to be considered: (i) Binding of negatively charged probes only occurs if the outermost layer is positive (over the probe concentration range studied). (ii) Binding does not only occur in the outermost layer or in neighboring positive layers, but throughout the multilayer film up to a depth of 11 layers. This is concluded from the probe binding experiments, where it is shown that the amount of probe bound at saturation increases linearly with the layer number in the multilayer film up to a total of 11 polyelectrolyte layers (PAH/PSS films) and thereafter saturates (Figure 7b). (iii) The probe removal experiments (Figure 10) show that not all of the probe bound is removed upon deposition of a subsequent negative layer (PSS) if the multilayer film comprises more than one polyelectrolyte layer. (iv) The ζ-potential decreases from ca. +50 mV (PAH or PDADMAC outermost layer) to small positive values (0−5 mV) at conditions of probe saturation for 4-PSA but *does not* demonstrate overcharging of the polyelectrolyte multilayer on the particle surface. For 6-CF saturation, there is a counterintuitive small increase toward more positive ζ-potential values.

The experimental data provide unambiguous evidence that the upper layers of the PAH/PSS (11 layers) and PDADMAC/PSS (at least nine layers) multilayer films contain positive binding sites (represented by the cationic groups of the polycations) which do not take part in the ion-pair formation between oppositely charged polyions. Therefore, these sites present themselves as potential probe binding sites. Evidence for this is provided from the fact that the polyanion−polycation interaction is much stronger than the probe−polycation binding: addition of polyanion to *multilayer films* (or to polycationic species *in solution*) with prebound probe causes removal of the probe due to complexation of the polyelectrolyte species. Hence, not all polyelectrolyte charges are utilized for ion-pair formation in the film buildup. Further, the polyelectrolyte multilayer bound anionic probe species can also be removed by adding salt; the salt concentration necessary for probe removal is much higher than the probe concentration required and is specific for the anion species employed. This supports the view that polyelectrolyte multilayers contain small ions (in the upper region of the films; approximately 15 nm in this work) which compensate part of the polyelectrolyte charges. These findings, coupled with the changes in the fluorescence emission spectra and degree of excimer formation (see above), provide overwhelming evidence that the 4-PSA and 6-CF probes do not screen uncompensated polycation fixed charges by an unspecific double-layer formation but that they electrostatically bind to sites of PAH or PDADMAC which do not directly form ion-pairs with PSS. The presence of only trace amounts of salt ions (sodium, chloride, etc.) in multilayer films, as determined by XPS, can be attributed to their removal (replacement by H^+ and OH^- ions) as a result of the numerous water washings which are commonly employed prior to sample analysis.[1,17−20]

An intriguing finding is that binding of the anionic probes occurs only if the outermost layer is positively charged. As mentioned earlier, polyelectrolyte multilayers are permeable to anionic probes when the outermost layer is negatively charged.[21] Hence, the argument that a negative outermost layer may form an electrostatic barrier for negative anions and thus prevent them permeating into and through the multilayer film can be ruled out. An existing energy barrier formed by an accumulation of negative charges in the outermost layer should have a kinetic effect only, resulting in slowing down the diffusive flow. It should *not* have a direct effect upon the binding equilibrium. Further, in a recent study it was shown that diffusion of the probes rhodamine and tetramethyl-4-piperidinol 1-oxide through PAH/PSS multilayer films is *independent* of the charge on the outer layer.[36] Moreover, at the probe concentrations employed the Debye length is of the order of 300 nm, thus being sufficiently large to average out any local charge variations in the layer.[37,38] The probes (ions) sense an average potential and hence the existence of pores,[21] as well as the oscillating nature of the polyelectrolyte charge density, will not affect the distribution of the mobile probes in the diffuse part of the double layer. Thus, an electrostatic barrier does not have an effect on diffusion.[37,38]

In this context it has to also be considered that the binding constants for the interaction of the probe molecules with cationic polyelectrolytes in solution are not necessarily the same as those for polyelectrolytes in multilayers. Taking into account that the mean electric potential difference between the layer and the bulk phase, $\Delta\varphi$ (which is negative if PSS is the outermost layer), contributes to the net free energy change upon binding, the binding constant has to be multiplied by $\exp(n_c e_0 \Delta\varphi / kT)$ where n_c is the number of charges of the probe, e_0 the elementary charge, k the Boltzmann constant, and T the absolute temperature. The potential difference between the interior of the film and bulk is larger than approximately −50 mV (ζ-potential) if the outermost layer is PSS. For charged layers the ζ-potential is in most cases a lower estimate for the total potential difference.[30,31] With $n_c = 4$ (4-PSA) and $\zeta = -50$ mV, the apparent binding constant is expected to be a factor of 4.2×10^{-4} smaller than in solution. If the potential difference is taken as 100 mV, the factor becomes 1.7×10^{-7}. While these numbers alone, at least for 4-PSA, cannot fully explain the absence of binding observed when the outermost layer is negative (in the concentration range studied), they nonetheless do provide a considerable (if not a major) contribution. (It should be mentioned, however, that binding may be observed if significantly higher concentrations of probe are employed.) The experimental observation not consistent with the above forwarded idea of a purely electrostatic equilibrium nature of the observed binding asymmetry (i.e., binding when outer layer is positive but not when it is negative) is that of the incomplete removal of probe bound deep within the multilayer film with subsequent PSS deposition (Figure 10). Within the framework of equilibrium thermodynamics, the final state of the system should be independent of the pathway to attain it.

To understand the incomplete removal of probe, we consider the nature of the binding sites in more detail. The *first* possibility discussed is that in which there are no cationic groups for probe binding in the film since they are all utilized in the assembly process by ion-pair formation with the anionic sites of the polyanion when the outermost layer is negative and that some of these groups are dissociated when the next layer (polycation) is adsorbed. Here, potential binding sites are those that are created as a result of ion-pair "breaking" due to polycation adsorption. The adsorption of the polycation layer can only result in a direct interaction with the polyanion layer(s) residing in the outer region of the film.[1] Therefore, a mechanism that takes into account changes in the ion pairs between polycations and polyanions in the interior of the film upon adsorption of a polycation is required to explain the above observations.

A mechanism consistent with the above findings is one that involves the long-range coupling of structural and electrostatic changes. Such changes are introduced at the outer surface by polyelectrolyte deposition, subsequently producing changes in the electrostatics and structure in the interior of the multilayer film. These subsequent changes are to occur throughout the multilayer film up to a depth of approximately 11 layers (e.g., for PAH/PSS films); otherwise, the linear increase in the bound probe amount with polyelectrolyte layer number cannot be explained. When the polycation is deposited onto the existing polyanion forming the outermost layer, certain anionic groups that formed ion pairs with the underneath cationic layer may rearrange and take part in the binding with the cationic sites of the polycation forming the new outermost layer. Consequently, the polycationic layer ("third layer" from the top) now has extra uncompensated positive charges which in turn interact with the polyanion underneath it. This further continues until the rearrangement leading to "free" cationic groups inside the multilayer has propagated perpendicularly throughout the film (up to ca. 15 nm). Such a mechanism would be in agreement with free energy considerations. The minimum of free energy of a charged polyelectrolyte layer is reached if the fixed uncompensated charges are distributed evenly over the whole layer.[31,37] A local accumulation of fixed charges, either positive or negative, would give rise to strong electric fields capable of influencing the layer arrangement by interacting with the charged polyelectrolyte groups. This would finally lead to an even distribution together with the tendency of expanding the top region of the film, provided the net charge composed of the sum of positive and negative polyelectrolyte charges is not zero. The above is in agreement with two recent studies which report that rearrangement of the internal film structure (underlying layers) of polyelectrolyte multilayers occurs with the subsequent deposition of polycation layers.[39,40]

Although the proposed coupling model can explain the creation of cationic binding sites inside the film and the linear increase of the amount of probe bound with polyelectrolyte layer number up to 11 layers, it cannot explain the incomplete removal of probe upon PSS adsorption. Since the ion pairs between the cationic and anionic groups of the polyelectrolytes do not constitute potential binding sites and thus prevent any probe from binding (their interaction is stronger than probe–polyelectrolyte binding), it is difficult to understand why the adsorption of PSS to multilayers with prebound probe does not lead to total probe removal, as should be the case for a system obeying thermodynamic equilibrium. This points to a kinetically nonequilibrium system when the probe is bound, with the probe most probably able to arrest structural changes in the multilayer film.

Another possibility for the nature of the binding sites is that where cationic groups in the film are *not* utilized in the assembly process by ion-pair formation: these groups are *not* accessible to the probe for binding if the outermost layer is negative, but do become accessible if the outermost layer is positive (when polycation is adsorbed). In this case the local accessibility of the cationic groups not utilized in the layer formation may be controlled by the outermost layer charge density. When the outermost layer is negative, the net fixed charge density of the layer system together with its negative substrate also becomes negative. When the outermost layer is positive, the net charge density of the whole system will be either positive or at least close to zero (since we have a negative surface). It is well established that polyelectrolyte multilayer films do not have strictly well separated layers; interpenetration of individual polyelectrolytes up to approximately four layers has been reported.[1] Thus, various topological constraints may be responsible for only part of the polyelectrolyte charged groups contributing to the layer formation by directly forming ion pairs. Local groups of charges of one sign may occur. This situation is depicted in Figure 11, where these groups of charges are shown for the two cases of either excess negative or excess positive charge density, as determined by the charge of the outermost layer. As discussed above, the strong electric fields acting in such highly charged systems will try to dilute the charged groups with the same sign as that of the excess charge density. A quantitative treatment of this (in connection with the electrostatic interaction of surfaces) is available elsewhere.[37] It has to be pointed out that it is specifically the electric field and not the electric potential which gives rise to the suggested structural changes of the polyelectrolyte multilayer system. Since the penetration depth of the electric double layer into the multilayer system is given by the Debye length, it is conceivable that only the outer region of a multilayer film experiences a strong electric field produced by the net surface charge of the system. The multilayer system further down may be described as a Donnan system with a

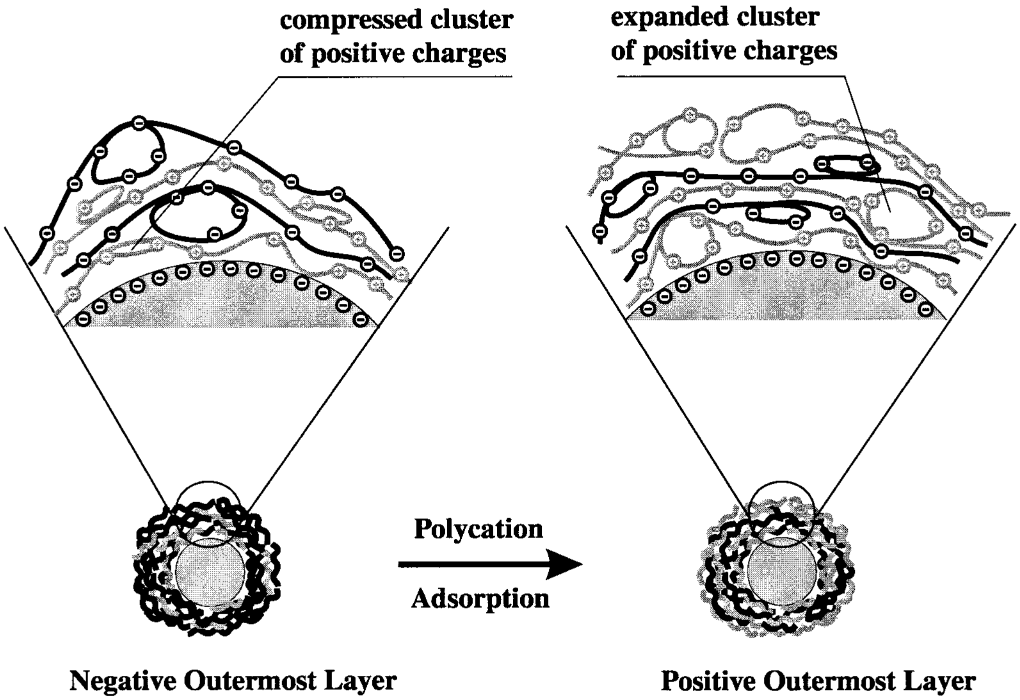

Figure 11. Schematic illustration of the electrostatic interactions between polycations and polyanions in polyelectrolyte multilayer films. Layer structural changes are introduced by polyelectrolyte deposition. The regions of positive and negative charges capable of forming potential binding sites for multivalent charged probes are determined by the charge of the outermost polyelectrolyte layer.

constant potential. Hence, the stress acting on the multilayer system, produced by the electric field, is present only in the outer region of the system. This may explain why in thicker films the amount of probe bound levels off. In this context it is important to note that an increase of salt would reduce the expanding electric field strength. This in turn would reduce the number of available binding sites for the probe leading to a release of the bound probe by an indirect interaction of the salt with the polyelectrolyte multilayer. It is conceivable that this mechanism contributes to the observed effect of probe removal by the increase of salt concentration. However, as shown above, the observed specificity of the salt does not permit explanation of this effect by the Debye length change alone.

The probe molecules are large compared with the size of a monomer unit in the polyelectrolyte. This may readily explain why there is not enough volume available for the probes to occupy binding sites in the layer system, although potential cationic binding sites are still present when the outermost layer is negative. Such accessibility constraints are equivalent to a further reduction of the energy of interaction between the probe and the potential binding site.

Thus, the probe binding situation can be described as follows. If the outermost layer is positive, local clusters of positively charged groups in the top region of the multilayer film are expanded and made accessible to the probe. The probe itself compensates the positive charge. When the next negative layer is deposited, the probe in deeper layers will *not* be totally replaced since the cationic sites with bound probe cannot be accessed by the polyanion (i.e., no binding competition for these sites with the anionic polyelectrolyte occurs). The suggested compression−dilation model is in agreement with the finding that the addition of salt causes multilayer films to swell.[14] In such a situation long-range electrostatic forces are screened, only ion pairs remain strongly attracted, and due to the entropic forces of the polyelectrolyte chains the layer system as a whole will relax.

The ζ-potential changes observed provide further evidence that charges not taking part in ion-pair formation exist in the upper region of the polyelectrolyte multilayer. When 4-PSA was used as the probe, the ζ-potential decreased to approximately 0−5 mV at saturation binding. This situation is very similar to what is observed in aqueous solutions of polyelectrolytes, where 4-PSA compensates 85% of the polyelectrolyte charge.[26] Approximately the same degree of compensation seems to occur in multilayers, although the degree of excimer formation is less in the multilayers. The latter finding indicates that the binding entities consist of chain segments that are not capable of being ordered to the same extent as PAH or PDADMAC in solution under the influence of the cross-linking 4-PSA molecules.[26] The polyelectrolytes in the multilayers may not have sufficient degrees of freedom for 4-PSA to significantly structure the layers within them upon its binding.

When the binding sites of the multilayer films were saturated with 6-CF, a small increase in the ζ-potential was observed (value ca. 55 mV), even though more negative charges are introduced into the layers. To explain this finding, we need to remember that 6-CF compensates approximately 50% of the charge of cationic polyelectrolyte in solution: this was attributed to the lack of an intra- and intermolecular cross-linking capacity of 6-CF.[26] The same effect may also occur for the multilayers. To understand the increase in ζ-potential, we take into account that 6-CF binding inside the multilayer removes a large part of the net surface conductance by both excluding small and very mobile ion species such as OH^- and net surface charge from the layer. On the other hand, it is well-known that the large surface conductance necessarily present in highly charged surface systems is a major factor for the apparent reduction of the electrophoretic mobility in low ionic strength solutions. Hence, it is probable that a surface conductance decrease together with the nonlinear dependence of mobility on charge density outweighs the 50% surface charge compensation introduced by 6-CF binding.

It is further worth mentioning that in all cases the number of cationic charges in the multilayer films compensated by probe is larger when PDADMAC is employed as the cationic polymer species, compared with the case of PAH. Again, the more flexible nature of PAH may give rise to *smaller* topological constraints; such constraints have been identified as the major cause of polyelectrolyte charges not taking part in ion-pair formation. Since not all charges may be titrated, for example 4-PSA may not bind if only one or two fixed charges are available in the local vicinity of the probe, the number of titrated groups in Figures 8 and 9 can only be interpreted as a first and lower estimate. Hence, it can be concluded that a minimum of 10−30% of cationic charges of PAH and PDADMAC are not directly involved in ion-pair formation in the multilayer assemblies.

This work also shows that polyelectrolyte layers may be loaded with charged probe molecules by the adsorption of probe after their formation: this provides an alternative method of incorporating probe in multilayer films, thereby avoiding problems such as probe desorption commonly associated with constructing polyelectrolyte−probe multilayer structures by the stepwise adsorption of the two species.[41,42] In addition, the amount of probe incorporated can be controlled by altering the polyelectrolyte multilayer film thickness (up to 15 nm for the PAH/PSS multilayers in this work).

Conclusions

Binding of the anionic probes 4-PSA and 6-CF to polyelectrolyte multilayers of PAH/PSS and PDADMAC/PSS assembled onto PS latex particles has been demonstrated. At the probe concentrations studied (0−1 μM), binding was observed only when the outermost layer was oppositely charged to the probe molecule. The amount of probe bound is linearly dependent on the number of polyelectrolyte layers deposited up to a film thickness of 15 nm (PAH/PSS system) and thereafter remains constant. A minimum of about 10−30% of the cationic sites of PAH and PDADMAC are *not* utilized in ion-pair formation with adjacent PSS layers in PAH/PSS and PDADMAC/PSS multilayer films. For films where PSS was the outermost layer, no probe binding was detected in the probe concentration range employed. The absence of probe binding when the outermost layer is negative, and the incomplete removal of probe prebound to the polyelectrolyte multilayers by subsequent PSS adsorption is attributed to a combined effect of the electrostatic influence on the apparent binding constant and topological constraints existing in polyelectrolyte multilayer films. In summary, the results obtained reveal that not all charges of polyelectrolytes forming PAH/PSS and PDADMAC/PSS multilayers are utilized in ion-pair formation in the upper region of the films (about 15 nm) and that polyelectrolytes deposited by stepwise adsorption to form multilayer films do not form well-separated layers, but rather there is high overlap between individual layers in the multilayer films. To further understand the role of electrostatic interactions in polyelectrolyte multilayer films, we are currently investigating differences in the binding stoichiometry between multilayers deposited from salt-containing and salt-free solutions.

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