

Assembling Alternate Dye–Polyion Molecular Films by Electrostatic Layer-by-Layer Adsorption

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Abstract: Electrostatic alternate adsorption was successfully employed for low-molecular-weight dyes, leading to a large variety of dye–polyion layer-by-layer assemblies. The assembling process of individual dye–polyion layers of Congo Red (CR)–poly(diallyldimethylammonium chloride) (PDDA) was investigated by using a quartz crystal microbalance (QCM). The *in-situ* QCM measurement revealed that the dye adsorption occurred at a rate similar to that of conventional polyion adsorption. Successful assembly of the CR layer was confirmed also by increasing intensities in the UV adsorption. The molecular aggregation within the dye layer was inferred from the observed absorption shift in the case of CR–PDDA. Apparently, stable film formation is promoted by dye aggregation in the adsorbed layer, although the extent of film growth was independent of conceivable aggregation in the solution. Successive frequency decreases due to film growth were observed for other representative dyes under optimized conditions. Comparison of the film thickness estimated from QCM frequency shift with the molecular dimension of individual dyes suggests the formation of well-packed monomolecular dye layers that is affected by size, number of charge, and spatial orientation of dye molecules. The alternate layer-by-layer assembly is now extended from linear polyions to other macroions (proteins and inorganic plates) and charged small molecules. Advantages and disadvantages of the alternate assembly were discussed relative to those of the Langmuir–Blodgett technique.

Introduction

Preparation of organic thin films based on spontaneous molecular assembly is one of the powerful approaches to create novel supramolecular systems.^{1–3} The Langmuir–Blodgett (LB) and self-assembled monolayer (SAM) techniques are among the best-known methods for this purpose. With the LB technique, ultrathin films are formed by transferring monomolecular films of lipids layer-by-layer from a water surface onto a solid substrate. This process produces multilayer films in well-defined layer thickness where the distance between functional groups and ordering of molecules can be controlled with molecular precision in the direction of thickness. Unfortunately, component molecules suitable for the LB preparation are limited, and water-insoluble, surfactant-like properties are usually required. Self-assembled monolayers are prepared by adsorption of dissolved components onto solid substrates. This method is based on strong interaction between the monolayer and substrate, *e.g.*, silanol/glass⁴ and thiol/gold,^{5,6} and supplies physically strong monolayers even on rough surfaces.⁷ It is, however, not good for formation of multilayer structures, unless surface chemical modifications are made.⁴

Recently, Decher and co-workers extended the pioneering work of Iler *et al.* to a new preparative method of organized thin films by layer-by-layer adsorption of linear polyions.^{8–11} Alternate adsorption of a polycation and a polyanion is readily achieved by excessive adsorption of polyelectrolytes on oppositely-charged surfaces. This technique is characterized by several advantages: (1) the preparative procedure is simple and an elaborate apparatus is not required; (2) a large variety of water soluble polyions are used; (3) the individual layer has molecular thickness; (4) any charged surface is employable. Many polymeric materials from biopolymers such as proteins^{12–14} to inorganic macromolecules such as clay^{12e,15,16} have been assembled with this technique. If the alternate assembly can be applied to low-molecular-weight substances, this method will become even more versatile as a means to prepare organized ultrathin films.

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(1) Vögtle, F. *Supramolecular Chemistry*; Wiley: Chichester, 1993.
 (2) Fuhrhop, J.-H.; Koning, J. *Membrane and Molecular Assemblies, The Synthetic Approach*; Royal Society of Chemistry: London, 1994.
 (3) Ulman, A. *An Introduction to Ultrathin Organic Films from Langmuir–Blodgett to Self-Assembly*; Academic Press: New York, 1991; Chapter 3.
 (4) Maoz, R.; Yam, R.; Berkovic, G.; Sagiv, J. In *Thin Films vol. 20, Organic Thin Films and Surfaces: Directions for the Nineties*; Ulman, A., Ed.; Academic Press: New York, 1995; p 41.
 (5) (a) Nuzzo, R. G.; Fusco, F. A.; Allara, D. L. *J. Am. Chem. Soc.* **1987**, *109*, 2358. (b) Nuzzo, R. G.; Dubois, L. H.; Allara, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 558.
 (6) Whitesides, G. M.; Laibins, P. E. *Langmuir* **1990**, *6*, 87.
 (7) Calvert, J. M. In *Thin Films vol. 20, Organic Thin Films and Surfaces: Directions for the Nineties*; Ulman, A., Ed.; Academic Press: New York, 1995; p 109.

(8) Iler, R. K. *J. Colloid Interface Sci.* **1966**, *21*, 569.
 (9) (a) Decher, G.; Hong, J.-D. *Ber. Bunsen-Ges. Phys. Chem.* **1991**, *95*, 1430. (b) Decher, G.; Hong, J.-D.; Schmitt, J. *Thin Solid Films* **1992**, *210/211*, 831. (c) Lvov, Y.; Decher, G.; Möhwald, H. *Langmuir* **1993**, *9*, 481. (d) Korneev, D.; Lvov, Y.; Decher, G.; Schmitt, J.; Yarodaikin, S. *Physica B* **1995**, *213/214*, 954.
 (10) (a) Ferreira, M.; Cheung, J. H.; Rubner, M. F. *Thin Solid Films* **1994**, *244*, 806. (b) Fou, A. C.; Rubner, M. F. *Macromolecules* **1995**, *28*, 7115. (c) Fou, A. C.; Onitsuka, O.; Ferreira, M.; Rubner, M. F.; Hsieh, B. R. *J. Appl. Phys.* **1996**, *79*, 7501.
 (11) Onoda, M.; Yoshino, K. *Jpn. J. Appl. Phys.* **1995**, *34*, L260.
 (12) (a) Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T. *J. Am. Chem. Soc.* **1995**, *117*, 6117. (b) Lvov, Y.; Ariga, K.; Kunitake, T. *Chem. Lett.* **1994**, 2323. (c) Onda, M.; Lvov, Y.; Ariga, K.; Kunitake, T. *Biotechnol. Bioeng.* **1996**, *51*, 163. (d) Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T. *J. Chem. Soc., Chem. Commun.* **1995**, 2312. (e) Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T. *Thin Solid Films*, in press.
 (13) Lvov, Y.; Haas, H.; Decher, G.; Möhwald, H.; Mikhailov, A.; Mtchedlishvily, B.; Morgunova, E.; Valinshtein, B. *Langmuir* **1994**, *10*, 4232.
 (14) Kong, W.; Zhang, X.; Gao, M.; Zhou, H.; Li, W.; Shen, J. *Macromol. Rapid Commun.* **1994**, *15*, 405.
 (15) Kotov, N.; Dekany, I.; Fendler, J. J. *Phys. Chem.* **1995**, *99*, 13065.
 (16) Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T. *Langmuir* **1996**, *12*, 3038.

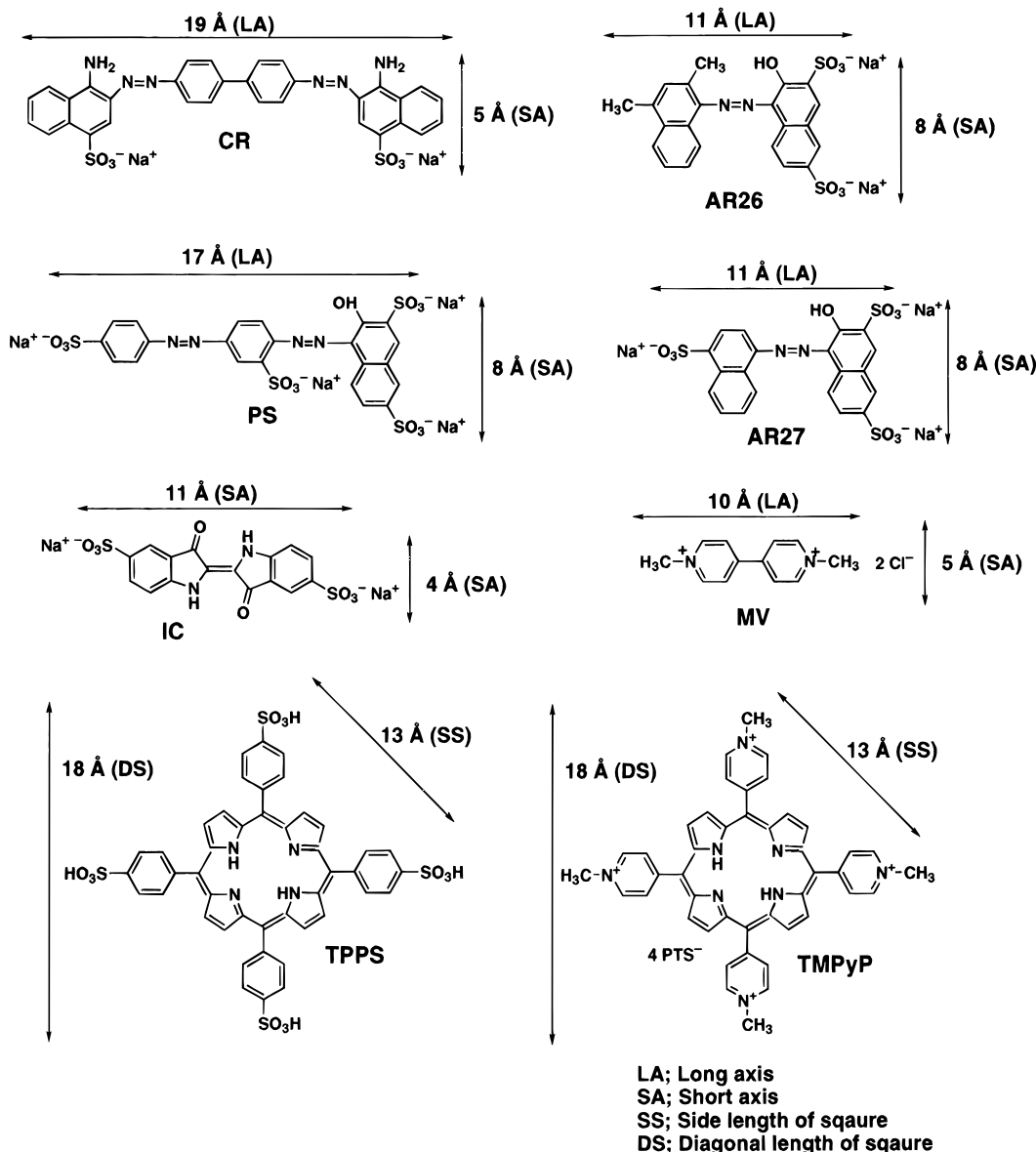


Figure 1. Molecular dimensions estimated by a Chem 3D calculation: LA, long axis; SA, short axis; SS, side length of a square; DS, diagonal length of a square. The Chem 3D Plus calculation is based on the MM2 force field (version 3.1.2, Cambridge Scientific Computing).

Bolaamphiphiles have been used as components of this technique.^{17–20} The layer formation by these bipolar molecules produces charged surfaces upon their adsorption and allows the alternate assembly to continue. Very recently we showed that amphiphile molecules that are capable of spontaneous bilayer assembly in water were suitable as charged components.²¹ Cooper and others demonstrated that anionic Congo Red and copper phthalocyaninetrasulfonic acid could be assembled with cationic poly(L-lysine).²² Rubner *et al.*,²³ Shen *et al.*,¹⁹ and Wrighton *et al.*²⁴ also reported dye-containing layer-by-layer assemblies.

It is important to examine at this stage whether a broad spectrum of charged small molecules are usable as components

of the alternate assembly. As can be seen from the precedents of bolaamphiphiles and bilayer-forming amphiphiles, small molecules that tend to aggregate in water have been assembled as molecular layers in combination with oppositely-charged polyions. It is possible, then, that small molecules that form aggregated layers *only on charged surfaces* (but not necessarily so in bulk water) are generally effective as components of the alternate assembly. In this study, we examined this presumption by studying alternate adsorption of various multicharged dye molecules with linear polyions by a quartz crystal microbalance (QCM) and UV spectroscopy. This methodological extension would enhance the usefulness of the alternate assembly considerably, as a means to prepare ordered molecular films.

Experimental Section

Materials. The molecular structures of the dyes and polyions used are shown in Figure 1. Congo Red (CR), Acid Red 26 (AR26), and

(17) Sellergren, B.; Swietlov, A.; Amembrant, T.; Unger, K. *Anal. Chem.* **1996**, *68*, 402.

(18) (a) Mao, G.; Tsao, Y.; Tirrell, M.; Davis, H. T.; Hessel, V.; Ringsdorf, H. *Langmuir* **1995**, *11*, 942. (b) Mao, G.; Tsao, Y.; Tirrell, M.; Davis, H. T. *Langmuir* **1993**, *9*, 3461.

(19) Zhang, X.; Gao, M.; Kong, X.; Sun, Y.; Shen, J. *J. Chem. Soc., Chem. Commun.* **1994**, 1055.

(20) (a) Saremi, F.; Tieke, B. *Adv. Mater.* **1995**, *7*, 378. (b) Saremi, F.; Maassen, E.; Tieke, B. *Langmuir* **1995**, *11*, 1068.

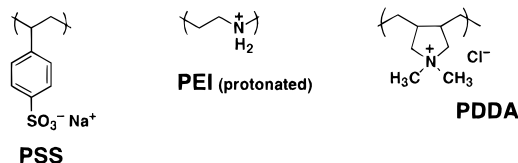
(21) Ichinose, I.; Fujiyoshi, K.; Mizuki, S.; Lvov, Y.; Kunitake, T. *Chem. Lett.* **1996**, 257.

(22) Cooper, T.; Campbell, A.; Crane, R. *Langmuir* **1995**, *11*, 2713.

(23) Yoo, D.; Lee, J.-K.; Rubner, M. F. *Mater. Res. Soc. Symp. Proc.* **1996**, *413*, 395.

(24) Araki, K.; Wagner, M. J.; Wrighton, M. S. *Langmuir* **1996**, *12*, 5393.

$\alpha,\beta,\gamma,\delta$ -tetrakis(1-*N*-methylpyridyl)porphine tetrakis(*p*-toluenesulfonate) (TMPyP) (all from Wako Pure Chemicals), and tetraphenylporphine-tetrasulfonic acid (TPPS), Acid Red 27 (AR27), methylviologen (MV), Ponceau S (PS), and Indigo Carmine (IC) (all from Tokyo Kasei) were used as purchased. Poly(sodium styrenesulfonate) (PSS; MW 70 000, Aldrich), branched poly(ethylenimine) (PEI; MW 70 000, Wako Pure Chemicals), and poly(diallyldimethylammonium chloride) (PDDA; Aldrich) were also commercially available and used without further



purification. All the polyions are expected to be strongly charged at neutral pH (ca. pH 6.5). Ultrapure water obtained by reverse osmosis followed by ion exchange and filtration (Auto Pure WQ500, Yamato, Japan) with a specific resistance of better than 18 $\text{M}\Omega\cdot\text{cm}$ was used.

Alternate Adsorption. The detailed procedure has been published elsewhere.^{12a} Quartz plates ($20 \times 10 \times 1$ mm) and QCM (AT cut, 9 MHz) plates with silver electrodes (0.16 cm^2) were used as substrates. They were washed in alkaline aqueous alcohol with sonication to generate anionic charges on the surface by partial hydrolysis. Prior to dye-polyion assembly three or four PEI-PSS precursor layers were prepared by immersing the substrate in aqueous PEI (35 unit mM) and PSS (15 unit mM) for 20 min alternatively with intermediate water-washing. Dye-polyion layers were prepared on the precursor film with conditions similar to those in the precursor assembly. The immersion time was 20 min unless otherwise mentioned. Sufficient washing of the substrate after dye adsorption was necessary for reproducible QCM and UV measurements.

Measurements. Alternate adsorption films were characterized by the QCM method (USI System, Japan)²⁵⁻²⁷ and by UV spectroscopy (V-570 UV-vis-near-IR spectrometer, JASCO, Japan). The long-term stability (several hours) of the QCM frequency was within ± 2 Hz, and this drift was negligible compared to frequency changes due to adsorption. All measurements were carried out in an air-conditioned room at room temperature (ca. 22 °C). QCM frequencies were measured in air after drying of the film. In the *in-situ* measurement, one side of the QCM plate was kept in contact with solutions, while the upper electrode was open to air. The upper contact wire was insulated from solution by silicone paint (PRX305, Toray Dow Corning Silicone, Japan).^{26b}

Results and Discussion

Optimization of Alternate Adsorption. The QCM frequency decreases in proportion to increasing mass on the electrode and provides nanogram sensitivity in mass measurement. In our system, a frequency decrease of 1 Hz corresponds to a mass increase of 0.9 ng, according to the Sauerbrey equation.²⁵ The QCM method has been found to be suitable for characterization of growth of molecular films such as LB films,^{26a} metal phosphonate multilayers,²⁷ and alternate adsorption films¹² in addition to traditional usage for vapor deposition processes.

Figure 2 contains two examples of alternate adsorption: CR (1 mM)-PDDA (1.9 unit mM) and CR (10 mM)-PEI (35 unit mM). The frequency decreases (mass increases) are plotted against the number of adsorption cycles. In the case of the CR-PDDA film (Figure 2a), a mass increase is observed for both of the CR and PDDA steps, indicating successful film growth

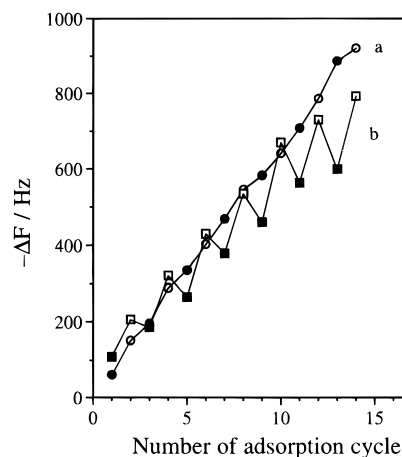


Figure 2. Frequency decrease ($-\Delta F$) of QCM upon dye-polyion film assembly: (a) E, adsorption of 1.0 mM CR; J, adsorption of 1.9 unit mM PDDA, (b) G, adsorption of 10 mM CR; B, adsorption of 35 unit mM PEI. The precursor film, (PSS-PEI)₄, was assembled on the QCM plate prior to dye-polyion assembly.

Table 1. Frequency Change, ΔF , upon Adsorption of Dyes and Polyions

dye (concn, mM)	$-\Delta F,^a$ Hz	polyion (concn, unit mM)	$-\Delta F,^a$ Hz
CR (10)	161 ± 37	PEI (35)	-74 ± 37
CR (10)	84 ± 5	PDDA (19)	2 ± 9
CR (1.0)	84 ± 7	PDDA (19)	46 ± 12
CR (10)	73 ± 12	PDDA (1.9)	43 ± 23
CR (1.0)	77 ± 13	PDDA (1.9)	60 ± 15
CR (0.10)	74 ± 9	PDDA (1.9)	50 ± 15
CR (0.01)	78 ± 10	PDDA (1.9)	46 ± 8
TPPS (10)	378 ± 26	PEI (35)	-347 ± 28
TPPS (10)	176 ± 23	PDDA (19)	-66 ± 12
TPPS (1.0)	211 ± 23	PDDA (19)	-37 ± 31
TPPS (10)	210 ± 20	PDDA (1.9)	2 ± 10
TPPS (1.0)	179 ± 30	PDDA (1.9)	36 ± 19

^a $-\Delta F$ denotes the frequency decrease (and therefore, mass increase) in the adsorption step. Frequency changes for dye and polyion adsorption steps were averaged separately for at least five data in separate adsorption steps. The averaged values are shown with their standard deviations.

for both the dye and polyion. In contrast, the latter film (CR-PEI, Figure 2b) showed a large mass increase for dye adsorption but a mass decrease for polyion adsorption. When CR-adsorbed plates were immersed in the PEI solution in the subsequent step, the color of CR was seen by the naked eye in the aqueous solution. Clearly CR molecules on the film are desorbed into aqueous PEI. This must be reflected by the mass decrease in the PEI adsorption step. Contamination of polyion solutions by desorbed dyes makes it difficult to conduct multilayer assembly simply by alternate immersion.

These erratic results indicate that optimization of the assembly process is essential. Therefore, we examined the concentration effect of two dyes (CR and TPPS) on the assembly process. QCM frequency changes in adsorption step are listed in Table 1. PDDA is a polyion component more favorable than PEI, because replacement of PEI by PDDA suppresses the dye desorption for both cases under similar conditions. In addition, the extent of protonation of PEI in an adsorbed film might be different from that in solution, making the situation complex. PDDA is permanently charged and simplifies the interpretation of data. In the subsequent experiment, we fixed the polyion component to PDDA. When CR and TPPS molecules are adsorbed from their high-concentration solutions, they tend to be desorbed in the subsequent step, especially in the case of higher polyion concentrations. For example, mass decreases

(25) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206.

(26) (a) Okahata, Y.; Ariga, K.; Tanaka, K. In *Thin Films vol. 20, Organic Thin Films and Surfaces: Directions for the Nineties*; Ulman, A., Ed.; Academic Press: New York, 1995; p 317. (b) Ebara, Y.; Okahata, Y. *J. Am. Chem. Soc.* **1994**, *116*, 11209.

(27) Yang, H.-C.; Aoki, K.; Hong, H.-G.; Sackett, D. D.; Arent, M. F.; Yau, S.-L.; Bell, C. M.; Mallouk, T. E. *J. Am. Chem. Soc.* **1993**, *115*, 11855.

were observed at 1 and 10 mM TPPS and 19 unit mM PDDA, while film growth was seen at lower concentrations for both processes ([TPPS] = 1 mM and [PDDA] = 1.9 unit mM). It was reported for a polyion–polyion assembly that desorption of polyions was sometimes observed depending on their charge density and ionic strength. This phenomenon was connected to the extent of the charge–charge interaction.²⁸ High polyion concentration might cause formation of ion complexes in solution rather than on the surface, if the dye–polyion interaction is not strong enough at the surface. Constant film growth was observed for the CR–PDDA film at [CR] = 0.01–10 mM and [PDDA] = 1.9–19 unit mM and for TPPS–PDDA at [TPPS] = 1–10 mM and [PDDA] = 1.9 unit mM.

The observed frequency change is independent of the CR concentration at a wide concentration range (10^{-5} to 10^{-2} M). The CMC value of CR in 0.025 M NaCl and KCl is reported to be larger than 10^{-4} M,²⁹ and absorption spectra we measured (data not shown) are against the formation of CR aggregates at 10^{-5} M. We can conclude from these two sets of data that the mode of CR adsorption is independent of its aggregation, if any, in water. The two-dimensional aggregation on the surface may be favored over the bulk three-dimensional aggregation, because of the charge array of the polyion surface.

Adsorption Kinetics. The *in-situ* QCM measurement as described in the Experimental Section provides data on the adsorption kinetics. The time courses of the measured QCM frequency for CR–PDDA and TPPS–PDDA systems are plotted in Figure 3. Both of the CR and PDDA adsorption processes upon each other layer are saturated in 10–20 min (Figure 3A). In the TPPS–PDDA system, very fast adsorption was observed for PDDA, while the frequency change due to TPPS adsorption was much slower and did not display saturation (Figure 3B).

These *in-situ* frequency changes at saturation are apparently larger than frequency shifts by the actual mass change that is measured after drying. Many factors such as swelling,^{30a} the frictional factor at the film surface,^{30b} and overadsorption would contribute to the frequency decrease in water. Therefore, we use here the rate to reach the equilibrium rather than the frequency change at saturation. On the basis of the assumption that the adsorption rate is proportional to the dye concentration (constant) and to the number of remaining unbound sites, the following equation is obtained by converting mass to frequency:

$$\ln\{(F - F_{\infty})/(F_0 - F_{\infty})\} = -kt \quad (1)$$

where t , F , F_0 , F_{∞} , and k are time, the frequency at t , the initial frequency, the saturation frequency, and the pseudo-first-order rate constant ($k = 1/\tau$), respectively. The pseudo data were fitted with eq 1, and the τ value was calculated. In the CR–PDDA system, both CR and PDDA adsorption processes showed straightforward saturation curves with relatively small τ values (2–5 min). This τ value is close to that of the conventional alternate assembly of polyions.³¹

In contrast, the TPPS–PDDA system showed unusual kinetic behavior with slow TPPS adsorption ($\tau = 7$ –9 min) and very fast PDDA adsorption ($\tau = 0.5$ –1 min). In the second run, the frequency change of TPPS adsorption did not give a saturation curve even after 20 min. The slow frequency change does not appear to come solely from TPPS adsorption. In order

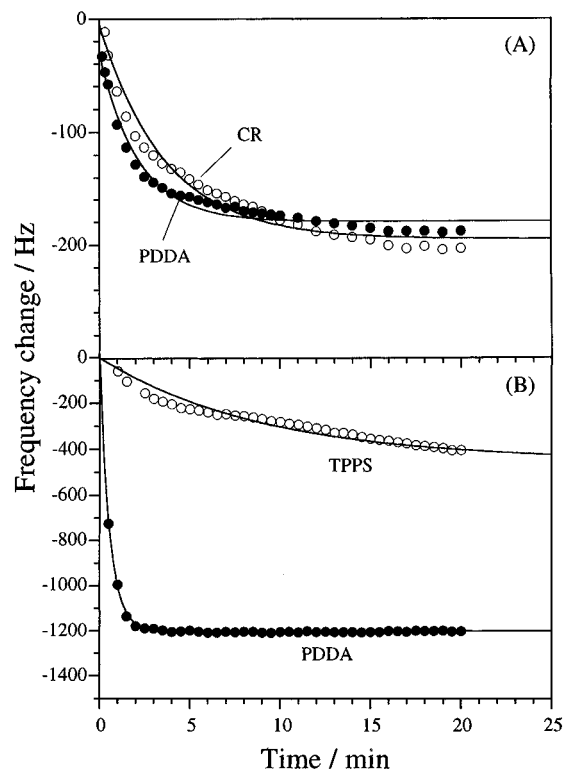


Figure 3. *In-situ* frequency change of the adsorption processes: (A) CR–PDDA film (open circle, the second CR adsorption process; filled circle, the third PDDA adsorption process), (B) TPPS–PDDA film (open circle, the second TPPS adsorption process; filled circle, the third PDDA adsorption process). The data were curve-fitted to eq 1 (see text). Solid curves represent the fitted results with the following τ values: (A) 3.66 min for CR and 2.18 min for PDDA, (B) 9.09 min for TPPS and 0.56 min for PDDA. The precursor film, (PSS–PEI)₄, was assembled on the QCM plate prior to dye–polyion assembly.

to remove the contribution to the frequency change by factors other than mass increase, the actual mass change was measured *in air* after given immersion periods followed by washing and drying. The obtained frequency shifts (data not shown) are almost independent of immersion time from 5 to 30 min, in contrast to the frequency change of the *in-situ* measurement. Presumably, extra TPPS molecules are adsorbed nonspecifically and can be removed readily by washing. Very fast PDDA adsorption observed in combination with slow, nonspecific adsorption of TPPS might be induced by irregular stacking of TPPS on the surface.

Dye Aggregation in Assembled Films. We subsequently employed UV–vis spectroscopy to study the growth process of the dye layer and the mode of dye aggregation. UV spectra of the CR–PDDA and TPPS–PDDA films on a quartz plate with 1–5 dye layers are shown in Figure 4. In all cases the absorbance increases almost in proportion to the number of the dye layer, indicating regular growth of the dye layers. The λ_{\max} value of the main band of the CR–PDDA film is located at 505.5 nm (Figure 4A). Aqueous CR at 10^{-5} M gives λ_{\max} at 499.5 and 494.5 nm with and without PDDA, respectively. Spectra of CR in the film show red shifts, suggesting that CR molecules form J aggregates. As mentioned before, the extent of film growth is independent of probable CR aggregation in solution. Therefore, aggregation of CR molecules must be promoted by adsorption on the charged surface.

In the case of the TPPS–PDDA film, the spectra are very different depending on whether or not the TPPS molecule exists in the outermost layer. The spectra of the film with the outermost TPPS layer have three major peaks at 427.5, 488.0,

(28) Hoogeveen, N. G.; Cohen Stuart, M. A.; Fleer, G. J. *Langmuir* **1996**, *12*, 3675.

(29) Iyer, S. R. S.; Singh, G. S. *Kolloid Z. Z. Polym.* **1970**, *242*, 1196.

(30) (a) Ariga, K.; Okahata, Y. *Langmuir* **1994**, *10*, 2272. (b) Ebara, Y.; Okahata, Y. *Langmuir* **1993**, *9*, 574.

(31) Lvov, Y.; Ariga, K.; Kunitake, T. Manuscript in preparation.

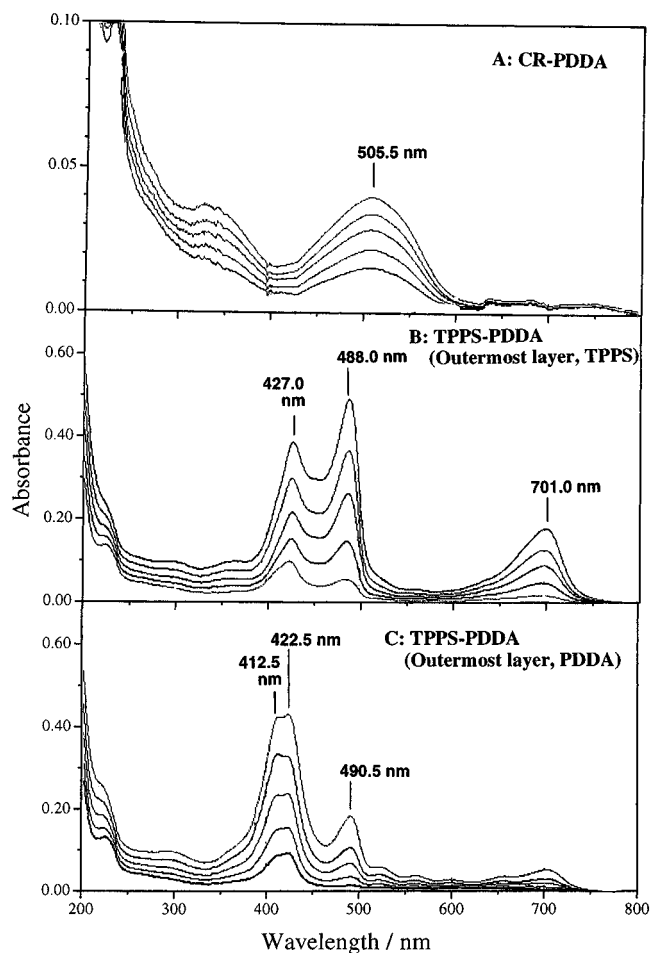


Figure 4. UV spectra of dye-polyion alternate assemblies on quartz plates: (A) $(\text{PEI-PSS})_3(\text{PDDA-CR})_n$; (B) $(\text{PEI-PSS})_3(\text{PDDA-TPPS})_n$; the outermost layer is TPPS; (C) $(\text{PEI-PSS})_3(\text{PDDA-TPPS})_n$; the outermost layer is PDDA. The n values are 1, 2, 3, 4, and 5 from the bottom of each figure. The precursor film, $(\text{PSS-PEI})_4$, was assembled on the quartz plate prior to dye-polyion assembly.

and 701.0 nm (Figure 4B), while those of the film with the outermost PDDA have peak tops at 412.5, 422.5, 490.5, and 703 nm (Figure 4C). Ioki *et al.*³² reported that addition of a polycation to aqueous TPPS (diacid form)³³ solution induced J aggregation with an intensity decrease at 439 nm and an increase at 489 nm relative to the spectra without the polycation. The spectral characteristics of the film with the outermost TPPS indicate that all the TPPS molecules exist as J-aggregated diacid species, because the peak intensity at 488.0 nm significantly increased. The formation of the diacid species is apparently caused by protonation of the TPPS porphyrin ring upon immersion in acidic TPPS solution. In contrast, spectral characteristics of TPPS included in the film with the outermost PDDA are close to those of the free base,³² indicating that the porphyrin ring is deprotonated in the films. The spectral alternation suggests that protonation and deprotonation proceed even for TPPS in inner layers. This fact is consistent with proton permeation reported for the polyion-polyion film by Möhwald *et al.*³⁴

Adsorption Behavior of Various Dye Molecules. It is essential to see whether the alternate assembly method is

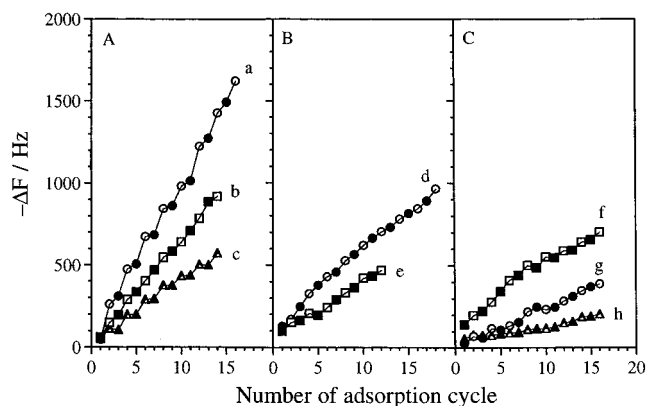


Figure 5. Frequency decrease ($-\Delta F$) of QCM upon dye-polyion film assembly (open plot, dye adsorption step; filled plot, polyion adsorption step): (A) a, TPPS-PDDA; b, CR-PDDA; c, TMPyP-PSS; (B) d, AR27-PDDA; e, AR26-PDDA; (C) f, IC-PDDA; g, PS-PDDA; h, MV-PSS. [dye] = 1.0 mM, [PDDA] = 1.9 unit mM, and [PSS] = 1.5 unit mM. The precursor film, $(\text{PSS-PEI})_4$, was assembled on the QCM plate prior to dye-polyion assembly.

applicable to a wide class of dye molecules. We thus tested the assembly of other representative dyes (TMPyP, AR27, AR26, IC, PS, and MV) under conditions that resulted in successful assembly of CR-PDDA and TPPS-PDDA: [dye] = 1.0 mM, [PDDA] = 1.9 unit mM, and [PSS] = 1.5 unit mM.

The results obtained are summarized in Figure 5, together with those for CR and TPPS. All the combinations except for MV-PSS showed successful film growth. The frequency change upon immersion of the plate into aqueous MV (9 Hz) is comparable to the standard deviation (9 Hz) of repeated measurements and cannot reflect film growth. Apparently, MV molecules are only weakly adsorbed and are desorbed completely in the next PSS step, as discussed for terphenyl-*p*-disulfonic acid by Decher *et al.*³⁵

In other cases, each dye molecule showed characteristic film growth. Generally, adsorption of large molecules causes large frequency changes (77 Hz for CR (MW 697)), and small dye molecules show smaller film growth (56 Hz for IC (MW 466)). However, the molecular size is not necessarily the predominant factor that determines the adsorbed mass. For example, AR26 and AR27 have the identical molecular dimension with a long axis of 11 Å and short axis of 8 Å, but they show different film growth (46 and 64 Hz for AR26 and AR27, respectively). Major structural difference between these two dyes is the presence of a third sulfonate group at the left side in the AR27 molecule. The additional sulfonate would produce a different mode of interaction with the precursor polycation surface.

The frequency change upon PDDA adsorption on the CR layer is 60 Hz, while the corresponding value for PS-PDDA film is only 13 Hz. Apparently, the charge density and structure of preadsorbed dye layer influence the subsequent polyion adsorption.

The surface roughness of QCM plates, if any, requires modification of the Sauerbrey equation.²⁵ In a previous study,^{12a} we analyzed several protein-polyion films by QCM (frequency change) as well as by X-ray diffraction (actual thickness), and obtained the following relationship between film thickness (d) and frequency shift ($-\Delta F$):

$$d (\text{Å}) = -0.16\Delta F (\text{Hz}) \quad (2)$$

(32) Ioki, M.; Igarashi, S.; Yotsuyanagi, T. *Anal. Sci.* **1995**, *11*, 123.

(33) TPPS is reported to be converted from free base to diacid at pH 4.8: Fleischer, E. B.; Palmer, T. S.; Srivastava, T. S.; Chatterjee, A. *J. Am. Chem. Soc.* **1980**, *100*, 6077.

(34) Klitzing, R. v.; Möhwald, H. *Langmuir* **1995**, *11*, 3554.

(35) (a) Decher, G.; Hong, J.-D. *Makromol. Chem. Macromol. Symp.* **1991**, *46*, 321. (b) Decher, G. In *Comprehensive Supramolecular Chemistry*, Vol. 9; Sauvage, J.-P., Hosseini, M. W., Eds.; Pergamon Press: Oxford, 1996; p 507.

Table 2. QCM Characteristics of Dye–Polyion Adsorption

dye–polyion ^a	$-\Delta F(\text{dye}),^b$ Hz	$-\Delta F(\text{polyion}),^b$ Hz	thickness of dye layer, $d,$ ^c Å	dye molecule	
				MW	molecular dimensions, ^d Å
CR–PDDA	77 ± 13	60 ± 15	12 ± 2	697	SA, 5; LA, 19
TPPS–PDDA	179 ± 30	36 ± 19	29 ± 5	935	SS, 12; DS, 18
AR26–PDDA	46 ± 8	30 ± 13	7 ± 1	480	SA, 8; LA, 11
AR27–PDDA	64 ± 12	43 ± 17	10 ± 2	604	SA, 8; LA, 11
PS–PDDA	36 ± 7	13 ± 6	6 ± 1	761	SA, 8; LA, 17
IC–PDDA	56 ± 9	17 ± 12	9 ± 1	466	SA, 4; LA, 11
TMPyP–PSS	75 ± 13	17 ± 4	12 ± 2	1363	SS, 13; DS, 18
MV–PSS	9 ± 9	5 ± 4	– ^e	257	SA, 5; LA, 10

^a Conditions of alternate assembly: [dye] = 1.0 mM, [PDDA] = 1.9 unit mM, [PSS] = 1.5 unit mM. ^b $-\Delta F$ represents averaged frequency changes due to dye (polyion) adsorption for 6–8 runs. The values are shown with their standard deviations. ^c The d value was estimated by eq 2. See the text. ^d The molecular dimensions were estimated by Chem 3D calculation (LA, long axis; SA, short axis; SS, side length of a square; DS, diagonal length of a square). ^e The d value cannot be estimated.

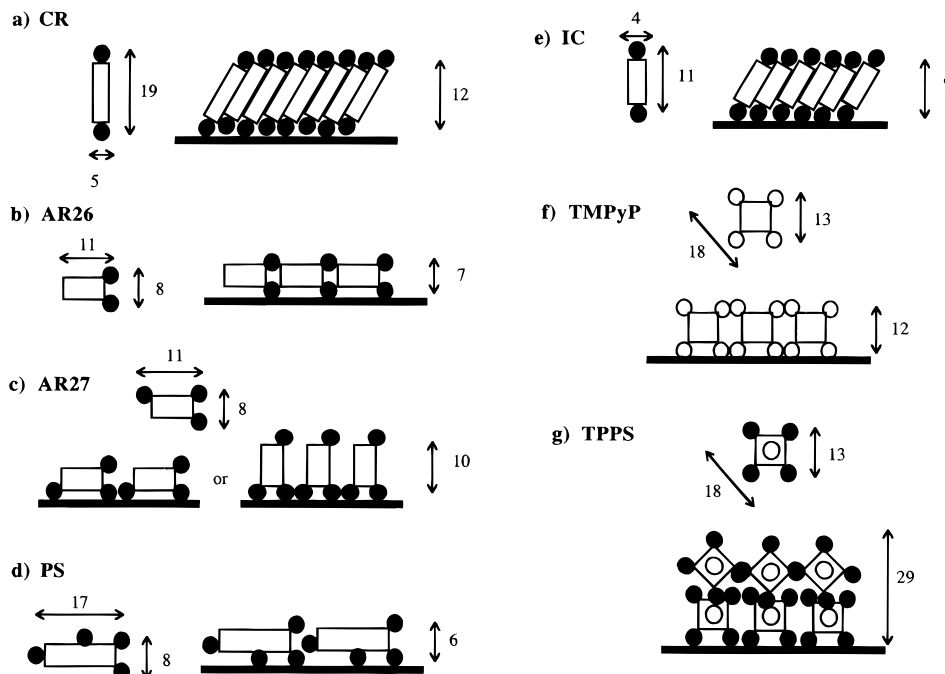


Figure 6. Schematic adsorption mode of dyes based on QCM and UV evidence. The filled circle, open circle, and rectangle represent the negatively-charged group, positively-charged group, and backbone of the dyes, respectively. Arabic numerals represent the corresponding length in angstroms.

The thickness of adsorbed dye layers may be estimated from this relation because the densities of dyes and proteins are close ($1.3 \text{ mg}\cdot\text{mL}^{-1}$ is estimated). It is given in Table 2. The table also lists molecular dimensions of the dyes, as estimated by Chem 3D Plus (MM2 calculation with the standard parameters). In the case of TPPS, the estimated thickness d is significantly larger than the calculated molecular dimensions. This suggests multilayered adsorption of TPPS as described later. In contrast, the observed thickness is close to either one of the molecular dimensions in all other cases. This agreement implies formation of regular dye layers.

Spatial Arrangement of Dye Molecules in Adsorbed Layers. The layer thickness estimated from QCM for individual dyes are explicable on the basis of adsorption modes illustrated in Figure 6. The measured thickness for the CR layer, 12 Å , is smaller than the long axis of the molecule (19 Å), but is significantly larger than the short axis (5 Å). The red shift in UV spectra suggests that CR molecules form J aggregates. These two sets of data are compatible with tilted aggregation of CR molecules in the regularly packed layer, as given in Figure 6a. The QCM frequency change for the PDDA layer in this assembly is -60 Hz , corresponding to a 10 Å thickness. The combined thickness of the CR–PDDA double layer ($12 + 10 = 22 \text{ Å}$) coincides with the ellipsometric data (20 Å) of a related

layer of CR–poly(L-lysine) reported by Cooper *et al.*²⁰ This agreement endorses the validity of eq 2 for thickness estimation of the CR layer.

AR26 gives a rather thin layer of 7 Å thickness. This value is consistent with the side-on orientation as shown in Figure 6b. This orientation allows the contact of two sulfonate substituents with the two PDDA layers above and below. The layer thickness for AR27 is estimated to be 10 Å , which is in between the lengths of the short and long axes (Figure 6c). Therefore, two types of the orientation mode or their mixture are conceivable. The other, related orientation where the sulfonate substituent is richer in the upper rim is also possible; however, we assume here that maximal pairing of the sulfonate with the preceding cationic surface is favored. In either case, the sulfonate substituent is located in the upper and lower faces of the dye layer in the first two cases, and satisfies the mechanism of the alternate adsorption.

The layer thickness observed for PS, 6 Å , suggests that this dye molecule is adsorbed in the side-on mode of Figure 6d. It is also possible that layer coverage is not complete due to lesser π -stacking and/or high charge density, thus giving rise to a seemingly thinner layer than actually is. IC gives a thicker layer of 9 Å . Its stronger stacking capability upon the lateral hydrogen bridge may give the molecular packing illustrated in Figure 6e,

in which IC molecules form a stacked layer with the end-on attachment. Other molecular arrangements do not agree with the experimentally determined layer thickness. The layer thickness observed for TMPyP, 13 Å, is very close to the calculated dimension (side length of a square). Therefore, TMPyP molecules stand on the PDDA surface with the packed card mode (Figure 6f). Again, the strong stacking of this molecule appears to provide a molecular layer with less deficiency.

Only in the case of TPPS, the film thickness is significantly larger than the molecular dimension. As shown by UV data, its porphyrin ring becomes protonated when the film is immersed in aqueous TPPS. Therefore, ion-pairing between the sulfonate of the incoming TPPS and the protonated porphyrin ring of the adsorbed TPPS can occur at the film surface, inducing multilayer formation (ca. two-layer thickness). Since the multilayer formation was observed only for TPPS, the ion-pairing is a major factor on the multilayer formation. A regular layer thickness was observed after water-washing, although nonsaturating kinetic behavior was observed in the *in-situ* QCM measurement. A TPPS molecule has two positive charges in the center and four negative charges in the side chains; thus, the ion-pairing cannot quench all the charges. The remaining excess negative charges will satisfy the charge resaturation condition at a certain thickness. It would determine the thickness of a stable multilayer which is not readily removed by water-washing.

The extent of polyion adsorption shows interesting dependency on the kind of dye molecules. Larger $-\Delta F(\text{polyion})$ values were obtained for CR-PDDA and AR27-PDDA systems, while the $-\Delta F(\text{polyion})$ value in AR26-PDDA and PS-PDDA systems is small. At the dye aggregation mode of Figure 6, adsorption layers of CR and AR27 produce higher charge densities than those of the other two dyes. Therefore, the amount of polyion adsorption might be determined by the charge density of the preceding dye layer. However, the situation is not necessarily simple as in the case of IC-PDDA (small $-\Delta F(\text{polyion})$ and high charge density).

These results reveal that there is a set of general rules for dye adsorption. The dye exposes its charged side to the polyion surface by maintaining at least one charged site to the outer solution phase. This adsorption mode satisfies charge neutralization on polyion surfaces and avoids unfavorable contact of the hydrophobic side of dye molecules with water. Regular repetition of the assembly process as evidenced by QCM data indicates formation of uniform layers for both the dye and polyion. The agreement of the thickness of the dye layers with one of the molecular dimensions of the respective dye is consistent with regular assembly of adsorbed dye molecules within the layer. Dye molecules may not be fully packed all over the surface, even if they are regularly packed. Recently, Dähne³⁶ reported a thin-layer crystallization method to produce large-scale thin layers of oriented polymethine dyes by modified spin-coating. According to their results, the population of highly oriented aggregates depends on the aggregation tendency of a dye. In our system, the regular orientation of adsorbed dyes may be optimized to obtain highly oriented dye films, by selecting appropriate conditions.

Can the Alternate Assembly Replace the Langmuir-Blodgett Technique? We examined in this paper electrostatic, alternate adsorption of representative water-soluble dyes and linear polyions by the QCM method and UV spectroscopy. Although there are a few exceptions, most of the dyes tested here can be assembled alternately with oppositely-charged

polyions under experimental conditions similar to those of the conventional polyion-polyion assembly. The dye layers formed possess molecular thicknesses that are mainly defined by the size and shape of a dye molecule and the number and distribution of charges in the molecule. The layer thickness was estimated in this study from the adsorbed mass on the basis of the QCM frequency change. An implicit assumption is made here that the dye layer is uniform. This is a debatable problem. If the dye layer is composed of patches of aggregated dye, the thickness of the individual dye patches should be greater than the average QCM thickness. Therefore, the observed thickness value corresponds to the minimal thickness of the actual layer. That means if the observed thickness agrees with the longest axis of dye molecules, the layer must be composed of dye molecules with uniform packing. The cases of AR27, IC, and TMPyP may be included in this category. The formation of uniform layers is also probable in other cases. CR dyes probably form a uniform layer, considering the presence of the tilted molecular stacking as evidenced by UV spectral shifts.

It is appropriate at this time to discuss advantages and disadvantages of the alternate assembly technique in relation to the LB technique. The LB technique supplies organized thin films by transferring monomolecular films of lipids layer-by-layer from the water surface onto a solid substrate. The component molecules in monomolecular layers become ordered by compression. Substances used for this technique are usually limited to water-insoluble, surfactant-like molecules unless we use a newly developed channel-flow-type trough.³⁷ Water-soluble molecules may be useful, if they are modified with long alkyl chains either covalently or noncovalently. Proteins have been used through adsorption to lipid monolayers³⁸ or by mixing with lipid components.³⁹ Unfortunately, these modifications do not always give successful results. The alternate adsorption method supplies well-defined layer-by-layer structures through the charge reversal mechanism. It has been developed for water-soluble polymeric ions.⁹⁻¹¹ However, more recent studies demonstrate that other types of charged macromolecules such as proteins¹²⁻¹⁴ and inorganic clays^{12e,15,16} can be assembled with an oppositely-charged polyion. Charged bolaamphiphiles¹⁷⁻²⁰ and bilayer membranes²⁷ can also be assembled alternately with oppositely-charged polyions. The present paper shows that the alternate assembly is applicable even to molecularly dispersed small molecules. The assembly need not be electrostatically driven. Formation of a metal-ligand bond^{27,40,41} and strong biospecific interactions^{12d,42} can substitute for the electrostatic interaction. It is clear now that the alternate adsorption has become a quite general technique for buildup of molecularly defined layers.

The LB technique is characterized by transfer of insoluble monolayers. Regular packing of monolayer components is required for stabilization, and therefore, attachment of long alkyl chains to monolayer components becomes essential in most cases. Very low through-put of formation and transfer of monolayers compounded with rather expensive instrumentation seems to hinder practical applications of the LB technique. Some of these problems are absent in the alternate assembly. Uniform adsorption in the alternate assembly can replace monolayer

(37) Nitsch, W.; Kurthen, C. *Thin Solid Films* **1989**, *178*, 145.

(38) Fromherz, P. *Biochim. Biophys. Acta* **1971**, *225*, 382.

(39) (a) Okahata, Y.; Turuta, T.; Ijio, K.; Ariga, K. *Thin Solid Films* **1989**, *180*, 65. (b) Okahata, Y.; Turuta, T.; Ijio, K.; Ariga, K. *Langmuir* **1988**, *4*, 1373.

(40) Watanabe, S.; Regen, S. *J. Am. Chem. Soc.* **1994**, *116*, 8655.

(41) Keller, S. W.; Kim, H.-N.; Mallouk, T. E. *J. Am. Chem. Soc.* **1994**, *116*, 8817.

(42) Hong, D.-J.; Lowack, K.; Schmitt, J.; Decher, G. *Prog. Colloid Polym. Sci.* **1993**, *93*, 98.

(36) Dähne, L. *J. Am. Chem. Soc.* **1995**, *117*, 12855.

formation in the LB technique. Molecules need not be aligned in advance in the former technique, and this factor eliminates limitations in the kind of molecules used. Experimental procedures are inexpensive and fast in the alternate assembly, since the adsorption process is usually over in less than 10 min, and simple dipping is good enough, once the adsorption conditions are fixed. One of the unsolved problems in the alternate assembly would be structural regularity of assembled films. In spite of X-ray diffraction data, the two-dimensional regularity of the individual layer has not been confirmed at the level of molecular precision. Direct determination of molecular organization in the film is indispensable. Such data have been accumulated extensively in the case of LB films. Improvements

in the adsorption technique and further structural elucidation are imminent, if we consider recent explosion of interest in the alternate assembly. Atomic force microscopic (AFM) studies of the dye-polyion films are essential for this purpose. Tieke *et al.*²⁰ and Tirrell *et al.*¹⁸ already discussed molecular orientation and film morphology of polymerizable bolaamphiphile assemblies on the basis of AFM observation. The former group reported a fairly regular coverage with small island domains, and the latter group discussed the effect of ionic strength and polymerization on film regularity. We are also working on AFM examination of the present samples.

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