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Determination of structural parameters characterizing thin films by optical methods: A comparison between scanning angle reflectometry and optical waveguide lightmode spectroscopy

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We present a comparative study of the structural parameters characterizing thin macromolecular adsorbed films that are obtained from two optical techniques: optical waveguide lightmode spectroscopy (OWLS) and scanning angle reflectometry (SAR). We use polyelectrolyte multilayers and polyelectrolyte multilayers/protein films to perform this study. The comparison between the information obtained with the two methods is possible because the buildup of the polyelectrolyte multilayers is known to become substrate independent after the deposition of the first few polyelectrolyte layers. The analysis of the optical data requires usually to postulate a refractive index profile for the interface. Two profiles have been used: the homogeneous and isotropic monolayer and the bilayer profiles. When the refractive index profile of an adsorbed film is well approximated by a homogeneous and isotropic monolayer, as shown by using an analysis of the deposited films in terms of optical invariants, the two optical techniques lead to similar values for the film thickness and the optical mass. The situation is more complex in the case of the multilayers/protein films for which the calculated parameters can strongly depend upon the refractive index profile that is postulated to analyze the optical data. Whereas the optical mass and, to a lesser extent, the thickness seem fairly model independent for OWLS, they appear to be extremely sensitive to the model for SAR. For proteins deposited on top of the polyelectrolyte film, optical mass and protein thickness were found to be comparable when determined by OWLS and by SAR using the bilayer model. The data analysis of the SAR curves with the monolayer model leads to much larger and even physically unreasonable film thicknesses and optical masses. This was particularly noticeable for proteins having a large size (human serum albumin and fibrinogen), whereas both models lead to similar results for small sized proteins. By means of the different refractive index profiles, we show that great care must be taken in the physicochemical interpretation of the structural parameters determined by these optical techniques. © 2001 American Institute of Physics.
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I. INTRODUCTION

Interactions between macromolecules such as polymers, polyelectrolytes or proteins and solid surfaces are of primary importance in many fields of material sciences as for example in the design of new coatings of biomaterials^{1,2} or in

the stabilization of colloidal solutions. Great effort has thus been devoted to understand and model adsorption kinetics and to follow the structure of the adsorbed films during their buildup. Optical techniques such as surface plasmon resonance,^{3,4} ellipsometry,^{5,6} reflectometry (SAR),⁷⁻⁹ and more recently optical waveguide lightmode spectroscopy (OWLS) (Refs. 10-12) are often used to determine the spatial extension of the adsorbed films and their optical mass. For a review of these techniques in the protein adsorption domain the reader is referred to Ref. 11. All these optical techniques are based on the light reflection properties of the

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surfaces and their responses are complex functions of the refractive index profile of the deposited film. This is best highlighted by the expressions of the reflection coefficients of thin films in terms of optical invariants, which were introduced independently by Lekner,¹³ and by Bedeaux and Vlioger.¹⁴ Unfortunately, no technique allows getting a direct access to the refractive index profile. One merely has to postulate a family of profiles which are characterized by different parameters and one then adjusts these parameters in such a way that they allow to account best for the experimental optical data. The most commonly used profile family is that of homogeneous and isotropic monolayers, which are characterized by a thickness, d_A , and a refractive index, n_A . In the case of macromolecular adsorption, the adsorbed amount is proportional to $(n_A - n_C) \times d_A$, where n_C corresponds to the refractive index of the solution far from the surface. In the following this quantity will be denoted as the “optical mass,” although it has the dimension of a length. This model can, however, usually only constitute a first approximation. Its validity is usually difficult to test and it has only been demonstrated recently for very specific films by making use of optical invariants for SAR, in particular of the invariant denoted by $\mathcal{F}$.^{15,16} This invariant is equal to 0 for a homogeneous and isotropic monolayer and different from 0 when the refractive index profile of the film departs from this model. It has been shown that multilayers of polyelectrolytes obtained by the alternate deposition of polyanions and polycations on a solid surface are characterized by values of $\mathcal{F}$ close to 0,¹⁶ whereas when albumin is adsorbed on these films, the invariant $\mathcal{F}$ departs greatly from 0, indicating that, in this latter case, the homogeneous and isotropic monolayer no longer accounts correctly for the refractive index profile.¹⁶ It is then no longer known what is exactly determined by the different optical techniques, each technique being sensitive to a specific feature of the refractive index profile. This latter point is, again, best seen by making use of optical invariants. They appear naturally as Gibbs excess quantities in the development expansion of the optical properties of a surface in a series of (L_f/λ) , λ being the wavelength of light and L_f corresponding to the characteristic spatial extension of the interface.^{13,14} One can show that there exists one optical invariant of order 1 in (L_f/λ) , usually denoted by J_1 , and two independent optical invariants of order 2. Ellipsometry appears to be mainly sensitive to J_1 . On the other hand, SAR is sensitive to a combination of J_1^2 and of the two second order invariants, the signal being thus sensitive exclusively to second order terms in (L_f/λ) . An analysis of OWLS in terms of optical invariants is currently under way¹⁷ and it appears that OWLS is sensitive to a combination of optical invariants different from the one corresponding to ellipsometry or to SAR. An adsorption process followed by two optical techniques, both being analyzed by making use of the same density profile family for the interpretation of the data, could lead to different results for both the spatial extension of the adsorbed film and the optical mass. Because many of the physicochemical analyses of the systems that were reported in the literature rely closely on the optical mass and on the adlayer thickness, it is of great interest to compare their values for identical systems obtained by different optical tech-

niques. This goal was very difficult to reach up to now since different optical techniques require working with different solid substrates as adsorption surfaces and adsorption processes are usually very sensitive to the nature of the adsorbing surface. The development of polyelectrolyte multilayers¹⁸ offers now a unique opportunity to reach this goal. Indeed, the buildup of polyelectrolyte multilayers appears to become surface independent after the adsorption of the first few layers, as it was theoretically predicted by Castelnovo and Joanny.¹⁹ The goal of this article is to compare the structural parameters obtained by SAR and by OWLS and related to polyelectrolyte multilayers and to multilayers on which proteins have been adsorbed. The polyelectrolyte multilayers should behave almost as homogeneous and isotropic monolayers whereas the multilayers with proteins are expected to have more complex refractive index profiles. Two refractive index profiles, the homogeneous and isotropic monolayer and the bilayer model will be applied to SAR and OWLS data. Such a bilayer analysis is developed for the first time for OWLS data which have been, up to now, usually examined with a simplified homogeneous and isotropic^{10,11,20} or anisotropic^{21,22} monolayer model.

II. DESCRIPTION OF THE OPTICAL TECHNIQUES

A. Optical waveguide lightmode spectroscopy (OWLS)

OWLS has been extensively described^{10,11} and will only be summarized here. A laser beam is directed on the diffraction grating incorporated in a planar optical waveguide. The guiding layer (F) of thickness d_F is deposited on a planar glass substrate (S) and is in contact, on the other side, with a solution contained in a holder supported by the waveguide. The adlayer (A), in our case polyelectrolyte multilayers, can be deposited on the waveguide surface. The waveguide sealed in the sample holder is mounted on a goniometer allowing the variation of the incidence angle θ_i between the laser beam and the diffraction grating. At certain discrete values of θ_i between the laser beam and the normal to the grating, the beam diffracted from the grating matches a possible guided mode (TE or TM) and incoupling takes place. To each incidence angle corresponds an effective refractive index N given by

$$N = \sin \theta_i + l\lambda/\Lambda, \quad (1)$$

where l represents the diffraction order, equal to 1 in our experimental condition, λ corresponds to the wavelength of the light and Λ is the grating constant. The power coupled into the waveguide is measured with a photodiode situated on both ends of the waveguide. The values of these matching angles θ_i correspond to effective refractive indexes $N(\text{TE})$ and $N(\text{TM})$ which are the solutions of the phase equation for both the transverse electric (TE) and the transverse magnetic (TM) polarizations,

$$2k_{z,F}d_F + \Phi_{S,F} + \Phi_{F,A} = 2\pi m, \quad (2)$$

where m is the order of the guide, all the quantities entering in this equation being functions of N . All the guides used in our experiments are monomode guides so that m can only take the value 0. $\Phi_{S,F}$ and $\Phi_{F,A}$ represent the phase shifts

between the incident and the reflected beams relative to the substrate/waveguide (S,F) interface and to the waveguide/film (F,A) interface respectively. $k_{z,F}$ ($k_{z,F}=k_0(n_F^2-N^2)^{1/2}$) represents the component perpendicular to the interface of the wave vector in the guide. The values of $N(\text{TE})$ and $N(\text{TM})$ depend on the refractive index profile of the film deposited on the oxide layer. The method used for calculating these phase shifts is given in Appendix A.

B. Scanning angle reflectometry (SAR)

SAR is an optical technique in which one measures the reflected intensity of a TM-wave around the Brewster angle. The method has been extensively described elsewhere^{7,8} and will only be summarized here. For an interface characterized by an abrupt change of the refractive index (Fresnel interface), the reflected intensity at the Brewster angle vanishes totally. Once an adlayer is deposited on the interface, the reflectivity coefficients of the interface change. One observes, in particular, a nonvanishing reflected intensity at the Brewster angle and, in addition, a change in the shape of the reflectivity curves around the Brewster angle. By measuring these changes one gets structural information about the adlayer. This constitutes the principles of SAR. More precisely, one determines the reflected intensity around the Brewster angle before adsorption of a film. This leads to a reflectivity curve corresponding to a perfectly abrupt interface with the reflected intensity following the relation:

$$I(\theta)=I_0+\alpha R(\theta), \quad (3)$$

where $R(\theta)$ represents the intensity reflectivity coefficient of a TM-wave, given by the Fresnel law. The curve measured before adsorption is used to determine the apparatus parameters I_0 and α and the refractive index of the transmission medium, n_C . Following the determination of these parameters the adsorption process can take place. Then, one determines again the reflectivity curve after the film deposition, $R(\theta)$ appearing in Eq. (3), corresponding now to the intensity reflectivity coefficient of the interface in the presence of the film. We used both a monolayer and a bilayer model to evaluate $R(\theta)$. Its calculation procedure is given in Appendix A. The coefficient $R(\theta)$ is a function of the parameters defining the refractive index profile (adlayer thickness, refractive index, ...). The refractive index profile describing best the reflectivity curves is obtained by minimizing the function χ^2 characterizing the quality of the fit with respect to these parameters. This function is defined by²³

$$\chi^2=\frac{1}{N_a}\sum_i\left(\frac{I_{\text{exp}}(\theta_i)-I_{\text{mod}}(\theta_i)}{\sigma_0+0.01I_{\text{exp}}(\theta_i)}\right)^2, \quad (4)$$

where σ_0 corresponds to the background noise and $I_{\text{exp}}(\theta_i)$ and $I_{\text{mod}}(\theta_i)$ represent the measured and the predicted reflected intensities at the incidence angle θ_i , respectively. The sum runs over the N_a incidence angles for which the reflected light intensity was measured.

III. MATERIALS AND METHODS

A. Materials

1. Polyelectrolyte solutions

Anionic poly(sodium 4-styrenesulfonate) (PSS, MW = 60 000), cationic poly(allylamine hydrochloride) (PAH, MW = 70 000), and cationic poly(ethyleneimine) (PEI, MW = 60 000) were purchased from Aldrich. Sodium chloride (purity $\geq 99.5\%$) was purchased from Fluka, and Tris(hydroxymethylaminomethane) (Tris) from Sigma. All the chemicals of commercial origin were used without further purification. Ultrapure water (Milli-Q-plus system, Millipore) was used for buffer and solution preparation, as well as in the different cleaning steps. The resistivity of the water was approximately 18.2 M Ω cm. All the buffer solutions were degassed under vacuum and filtered before use. The polyelectrolytes were either dissolved in 0.9 M NaCl in water, or in 10^{-2} M Tris (pH 7.35) in the presence of 0.15 M NaCl at a concentration of 5 mg/ml.

2. Proteins

Proteins adsorption experiments were performed at pH 7.35 in 10^{-2} M Tris containing 0.15 M NaCl. α -lactalbumin (α -LA, L6010, MW = 14 200 Da, $pI=4.3$), human fibrinogen (FIB, F4883, MW = 340 000 Da, $pI=5.8$) and lysozyme (LSZ, L6876, MW = 14 600 Da, $pI=11.1$) were purchased from Sigma. Human serum albumin (HSA, MW = 69 000 Da, $pI=4.6$) was provided in concentrated solutions (20 g/100 ml) for intravenous injection (prepared and purified by the Cohn's method²⁴) from the Center de Transfusion Sanguine of Strasbourg (France); its purity was controlled by gel electrophoresis. All proteins were dissolved without further purification.

Protein solutions were always deposited either on a positive terminating film, e.g., PEI-(PSS-PAH)₃, or on a negative terminating film, e.g., PEI-(PSS-PAH)₃-PSS, at concentrations ranging from 12 to 25 mg/100 ml. After uptake, the adsorbed layer was rinsed with a pure buffer solution of the same ionic strength (0.15 M NaCl) as the adsorption solution.

B. Experimental setup

1. OWLS experiments

The core of the homebuilt experimental setup is an input-grating sensor whose principle has been described previously.^{11,21} The linearly polarized light of a 5 mW He-Ne laser (Melles-Griot, Boulder, USA), wavelength of 632.8 nm, is incoupled into a $48\times 16\text{ nm}^2$ planar monomode waveguide chip made from Si_{0.8}Ti_{0.2}O₂ (ASI-2400, Artificial Sensing Instruments, Switzerland) at 45° with respect to the normal of the waveguide, in order to excite simultaneously the zeroth transverse electric (TE) and transverse magnetic (TM) guided modes. The coupled light is collected at both ends of the waveguide by photodiodes (Hamamatsu, Bridgewater, USA). The incidence angle is varied by mechanical rotation of the waveguide achieved by means of a computer-

controlled, high precision translation stepped motor (0.1 μm resolution) (Microcontrôle, Evry, France), leading to an angular precision of 4×10^{-5} deg.

The waveguiding layer of thickness $d_F \approx 170$ nm and with a refractive index $n_F \approx 1.77$ is supported by a glass substrate (refractive index $n_S = 1.52578$). On this waveguiding film of high refractive index, one adsorbs in a first step an adlayer corresponding to a polyelectrolyte multilayer (refractive index n_A , thickness d_A). In a second step, an additional adlayer of proteins is deposited (refractive index n_B , thickness d_B) on the top of the polyelectrolyte multilayer. The presence of these adlayers perturbs the evanescent field at the guide/solution interface and leads to a highly sensitive change of the effective refractive indices $N(\text{TE})$ and $N(\text{TM})$ of the guided modes.

Each experiment is preceded by a cleaning procedure of the waveguides by dipping them first in a 10^{-2} M sodium lauryl sulfate (SDS) solution during 15 min, followed by a second dipping (15 min) in 10^{-1} N, HCl both in a boiling water bath. This cleaning procedure ends on an extensive ultrapure water rinse and a drying with a nitrogen stream. The waveguide is then introduced in its holder and connected to a three holes sealed cover (injection port, buffer entrance port, and buffer exit port tubes). The measuring cell is tightly sealed to the chip by a circular perfluorinated O-ring ("Kalrez," Du Pont, Wilmington, USA) and has an internal volume of 37 μl . Buffer is flushed through the cell at a constant flow rate (10 ml/h) with a syringe pusher. The temperature is recorded by a sensor embedded in the waveguide holder. The temperature variations (smaller than $\pm 0.2^\circ\text{C}$) do not lead to significant refractive index changes (data not shown). Data collection is pursued in the presence of buffer until an absolute variation of less than 10^{-5} on the values of $N(\text{TE})$ and $N(\text{TM})$ is observed. These baselines are used to calculate the waveguide parameters n_F and d_F with the three-layer (substrate, guide, buffer) mode equations,¹⁰ knowing the buffer refractive index, n_C , which is measured with an RFM 340 refractometer (Bellingham-Stanley, Kent, UK).

After one reaches a stable baseline with the buffer flowing through the cell, the buildup of the polyelectrolyte multilayers film is performed as follows:

- (i) The buffer flow is stopped and 100 μl of the PEI solution is directly injected in the cell through the injection port. $N(\text{TE})$ and $N(\text{TM})$ values increase and reach a plateau after about 10 min. When a stable adsorption signal was obtained, the buffer flow is restarted for 15–20 min to rinse the excess material from the cell.
- (ii) In the same way we continue with the alternate adsorption of negatively charged PSS and positively charged PAH on the waveguide. Thus, progressively PEI-PSS, PEI-(PSS-PAH)₁, PEI-(PSS-PAH)₁-PSS, ... layers are deposited. The adlayer obtained after the deposition of the n th pair of layers is noted PEI-(PSS-PAH) _{n} .
- (iii) The buildup of the film is stopped either after the deposition of a positively charged PEI-(PSS-PAH)₃ or

a negatively charged PEI-(PSS-PAH)₃-PSS layer to be in the same conditions as for the SAR experiments (see Sec. III B 2). On the so-formed multilayer film, proteins are further adsorbed under a continuous flow in the measuring cell (10 ml/h) for about 2 h. The solution is then replaced by the buffer solution for 2 additional hours. These durations are sufficient to ensure that the adsorption process leveled off and that a plateau is reached at rinsing.

2. SAR experiments

The scanning angle reflectometer used in this study has been described in detail elsewhere.²³ Briefly, the light from a He-Ne laser is polarized in the incidence plane (TM-polarized) before entering nearly perpendicularly through one face of a prism and reflecting on its hypotenuse. The light beam leaves then the prism through another face, again almost perpendicularly. A further polarizer selects the TM-wave, and the resulting beam passes through a 200- μm pinhole to fall onto a photodetector. The prism rotates with a high precision goniometer, changing the incidence angle, while the position of the detector is adjusted to follow the reflected beam. The reflectivity is measured at a series of discrete incident angles, typically spaced by 0.025° intervals, over a range of 1° around the Brewster angle. At this angle, approximately 42.5° for the silica/water interface, the reflectivity of an abrupt, flat interface is zero. The signal around the Brewster angle is thus most sensitive to the presence of interfacial films.

Each experiment is preceded by a cleaning step of the silica prism during 15 min at 100°C in a 10^{-2} M SDS solution. This procedure is followed by an extensive ultrapure water rinse. The prism is then brought in contact during 15 min at 100°C with 10^{-1} M HCl and finally extensively rinsed with ultrapure water.

The silica surface is first brought in contact with the buffer solution and the reflectivity curve corresponding to the Fresnel (abrupt density profile) silica/buffer solution interface is measured for calibrating the apparatus and for determining the refractive index of the buffer solution (n_C). The buildup of the polyelectrolyte multilayers film is then realized as follows:

- (i) First 50 ml of a PEI solution is flowed for 20 min. The flow is then stopped, and after 30 min of contact with the silica surface, the PEI solution is replaced by 50 ml of buffer solution, which stays in contact with the prism for 20 min.
- (ii) Afterward, PSS and PAH are alternatively adsorbed on the PEI coated silica surface by using the same procedure as described for PEI. The subsequent interaction time between the polyelectrolyte solution and the silica surface are reduced to 15 min (instead of 30 min in the PEI case). Thus, progressively PEI-PSS, PEI-(PSS-PAH)₁, PEI-(PSS-PAH)₁-PSS, ... layers are deposited.
- (iii) For protein adsorption, the buildup of the film is stopped either after the deposition of a positively charged PEI-(PSS-PAH)₃ or a negatively charged

FIG. 1. Raw $N(TM)$ signal obtained during the *in situ* buildup of a polyelectrolyte PEI-(PSS-PAH)₃-PSS multilayer film as measured by the OWLS technique, as a function of the time. The polyelectrolytes PEI, PSS, and PAH were used at a concentration of 5 mg/mL in a 10^{-2} M Tris buffer containing 0.15 M NaCl. The alternate deposition process was performed for 15 min adsorption and 15 min rinsing steps. The baselines (used for the calculation of the waveguide parameters) and the successive rinsing of the layers were achieved under flowing buffer at 10 mL/h, $T=24.5^\circ\text{C}$. Arrows indicate the rinsing with buffer.

PEI-(PSS-PAH)₃-PSS film. At this stage, reflectivity curves are determined for about 16 h allowing the film to reach full equilibrium. The choice to stop the buildup of the polyelectrolyte film after 7 or 8 layers (including the initial PEI layer) is due to the fact that typically only few layers, depending on the precise buildup conditions, are required to obtain a good reproducibility from one preparation to another.²³

- (iv) The protein solution is then flowed over the polyelectrolyte film and kept in its contact for several hours. During this time period the reflectivity curve is continuously acquired in order to follow the adsorption of proteins on the film. Once the adsorption process levels off, the protein solution is replaced by the buffer solution. Reflectivity curves are again continuously determined for several hours.

IV. RESULTS AND DISCUSSION

A. Films with an expected homogeneous and isotropic monolayer refractive index profile

The buildup of PEI-(PSS/PAH)_n multilayers was followed by OWLS. The evolution of the values of the effective refractive index $N(TM)$ is represented in Fig. 1. As the adsorption process is fast and as the determination of a reflectivity curve by SAR takes about 4 min, we could not follow the adsorption process by SAR and then determined the reflectivity curves only after 15 min of adsorption of each polyelectrolyte (30 min for PEI). Both OWLS and SAR data were analyzed by assuming that the polyelectrolyte multilayers behave as homogeneous and isotropic monolayers. Typical evolutions of the thickness d_A and of the optical mass $(n_A - n_C) \times d_A$ of the film during a buildup process are shown in Fig. 2. The thickness and mass corresponding to multilayers films up to PEI-(PSS-PAH)₂-PSS and determined by SAR were very scattered. Some of the points do even not appear on the graph since they are out of scale. This is due to the fact that the reflectivity curves for these multilayers were

FIG. 2. Comparison of an OWLS and a SAR experiment for a PEI-(PSS-PAH)₅ polyelectrolyte multilayer film buildup prepared in 0.9 M NaCl with polyelectrolytes at 5 mg/mL concentration. Data were analyzed by means of the homogenous and isotropic monolayer model which allows the refractive index n_A and the adlayer thickness d_A to be determined. The optical mass $(n_A - n_C) \times d_A$ is directly calculated from these parameters. (A) Evolution of the polyelectrolyte film thickness d_A during the different steps of the PEI-(PSS-PAH)₅ film buildup as obtained by SAR (●) and by OWLS (□) as a function of the number of layers deposited on PEI: “1” means PEI-PSS, “2” means PEI-(PSS-PAH), “3” means PEI-(PSS-PAH)-PSS, ..., “10” means PEI-(PSS-PAH)₅. (B) Evolution of the optical mass $(n_A - n_C) \times d_A$ for the same buildup obtained by SAR (●) and by OWLS (□) (waveguide parameters: $n_F = 1.7732$, $d_F = 177.73$, $n_C = 1.3418$).

only very slightly different from the Fresnel reflectivity curves. Their analysis becomes thus very difficult, the variations of the reflected intensities being practically not distinguishable from the noise. The weakness of the signal corresponding to these first few layers can be explained by the fact that the variation of the reflectivity coefficient around the Brewster angle due to adsorption is proportional to $(L_f/\lambda)^2$ and not to (L_f/λ) itself.^{13,14} In addition, the refractive index of the deposited layers is very close to that of the silica of the prism ($n_S = 1.45718$) used as the adsorption substrate. If the refractive index of the deposited layers would be exactly equal to that of the prism, no signal variation at all would be observed. In striking contrast to SAR, OWLS allows to follow the whole build up process accurately already after the deposition of the first polyelectrolyte layer.^{25,26}

After the deposition of the PEI-(PSS-PAH)₂-PSS multilayer, the thickness and optical mass of additional PAH and PSS layers appear to vary linearly, whether measured with one or the other of the two techniques, as was already suggested for this system by others.^{21,27} The layer thicknesses determined by both techniques become almost identical, whereas the optical mass differs by a constant quantity over the whole buildup process. This constant difference can be due to the fact that the adsorption process of the first deposited layers depends on the deposition substrate which is different for both techniques, the following deposition steps

TABLE I. Determination of the optical parameters characterizing polyelectrolyte multilayers built *in situ*. The notation PEI-(PSS-PAH)_n means that *n* pairs of PSS/PAH layers have been deposited on a precursor PEI film. The analysis of the data obtained using SAR or OWLS, by means of the homogeneous and isotropic monolayer model (see Appendix A), leads to the polyelectrolyte film thickness d_A and refractive index n_A , and to the corresponding optical mass $(n_A - n_C) \times d_A$ (mean $\pm$ standard error). Indicated are also the ionic strength [NaCl] and the number of experiments $\mathcal{N}$.

Film architecture		[NaCl] (M)	$\mathcal{N}$	n_A	d_A (nm)	$(n_A - n_C) \times d_A$ (nm)
PEI-(PSS-PAH) ₃	SAR	0.15	16	1.486 ± 0.002	17.40 ± 0.80	2.65 ± 0.14
	OWLS	0.15	7	1.507 ± 0.008	18.23 ± 0.72	2.98 ± 0.15
PEI-(PSS-PAH) ₃ -PSS	SAR	0.15	7	1.489 ± 0.002	20.40 ± 0.70	3.17 ± 0.12
	OWLS	0.15	5	1.502 ± 0.005	21.30 ± 0.92	3.55 ± 0.18
PEI-(PSS-PAH) ₅	SAR	0.9	1	1.494	43.74	6.72
	OWLS	0.9	1	1.480	44.02	6.05

being independent of the nature of the adsorbing surface, as has been shown theoretically.¹⁹ The comparison of the structural parameters relative to different multilayers and determined by both techniques is summarized in Table I. The average values (computed on the basis of $\mathcal{N}$ experiments) of the layer thickness, the refractive index and the optical mass obtained from OWLS and SAR show good agreement. The thicknesses agree within 6% and the adsorbed amounts within 12% for the different types of polyelectrolyte multilayers films. As to the refractive indices, the values lie in the range 1.480–1.519 for OWLS compared to 1.480–1.490 for SAR.

An analysis of the SAR curves by making use of the optical invariants allows to determine the evolution of the invariant $\mathcal{F}$ (see Appendix B for a brief description of this quantity) over the buildup process (Fig. 3). Disregarding the first points corresponding to the multilayers up to PEI-(PSS-PAH)₂-PSS, which are unreliable due to the small signal difference with the Fresnel curve, one observes that $\mathcal{F}$ becomes close to 0 after the deposition of a few polyelectrolyte layers indicating that the model of an homogeneous and isotropic monolayer should be fairly valid for these systems. For this reason, it is not surprising that both SAR and OWLS give similar values for the structural parameters of these

films. As a further step, it is also of interest to compare the structural parameters determined on films that are expected to have a more complex refractive index profile.

B. Films expected to have more complex refractive index profiles

In order to obtain films with more complex refractive index profiles, we adsorbed different proteins on the polyelectrolyte multilayers films. We used two proteins of small size, the α -LA ($3.7 \times 3.2 \times 3.5 \text{ nm}^3$)²⁵ and LSZ ($4.6 \times 3.0 \times 3.0 \text{ nm}^3$)²⁵, a protein of intermediate size, the HSA ($12 \times 2.7 \times 2.7 \text{ nm}^3$)²⁵ and a large size protein, the FIB ($45 \times 9 \times 9 \text{ nm}^3$)²⁸. Moreover, the different conformations of the proteins, i.e., the globular HSA, or the fibrous FIB are expected to have a significant influence on the structure of the adsorbed protein layer.²⁶ The structure of the multilayers/protein films were then analyzed using the homogeneous and isotropic monolayer and the bilayer models. In this latter case, the refractive index and the thickness of the first layer constituting the bilayer, which corresponds to the polyelectrolyte multilayer, were fixed to the values found before protein adsorption, i.e., n_A and d_A , respectively.

Optical parameters defining the protein film [index n_B , optical thickness d_B , and optical mass $(n_B - n_C) \times d_B$] are gathered in Table II. The thickness and the optical mass for the adsorbed proteins as determined by using the monolayer model correspond to the differences of the corresponding amounts estimated after and before protein adsorption. One can first observe that the adsorbed quantities determined by OWLS are similar for both models. The optical thicknesses derived by the two models remain also comparable for the smaller sized proteins whereas they can vary by up to 40% for the largest sized ones (HSA, FIB). In this latter case, a finer analysis shows that the monolayer model leads to thicknesses smaller than those derived with the bilayer model, whereas the refractive indices are higher. Nevertheless, both models give values which are physically reasonable, taking the characteristic size of the proteins into account.

When considering SAR data, the optical thicknesses and masses are systematically larger when the monolayer model is used, as compared to the bilayer model, except for the LSZ where very similar values are obtained. The differences even increase with the size of the protein and can reach 350% for

FIG. 3. Evolution of the optical invariant $\mathcal{F}$ calculated from SAR reflectivity intensity curves during the buildup of a PEI-(PSS-PAH)₅ multilayer film in the presence of 0.9 M NaCl (same conditions as in Fig. 2).

TABLE II. Optical thickness (d_B), refractive index (n_B), and optical mass $(n_B - n_C) \times d_B$ for four proteins: α -lactalbumin (α -LA), lysozyme (LSZ), human serum albumin (HSA), and fibrinogen (FIB) adsorbed onto PEI-(PSS-PAH)₃ or PEI-(PSS-PAH)₃-PSS multilayers, measured by SAR and by OWLS. Negatively charged α -LA and FIB were adsorbed on a positive PAH terminating film. LSZ and HSA were adsorbed on a negative PSS terminating film. The optical parameters (d_A, n_A) for the polyelectrolyte precursor film are also given for each experiment. The optical parameters for the protein film ($d_B, n_B, (n_B - n_C) \times d_B$) were calculated both with the homogeneous and the isotropic monolayer (*M*, data in *italics*) and with the bilayer model (*B*).

	Protein concentration (mg/100 mL)		n_A		d_A (nm)		Model	n_B		d_B (nm)		$(n_B - n_C) \times d_B$ (nm)	
	SAR	OWLS	SAR	OWLS	SAR	OWLS		SAR	OWLS	SAR	OWLS	SAR	OWLS
α -LA	20.1	18.1	1.487	1.519	14.50	18.33	B	1.469	1.463	10.66	11.71	1.43	1.52
							<i>M</i>	<i>1.476</i>	<i>1.506</i>	<i>15.79</i>	<i>10.67</i>	<i>2.10</i>	<i>1.56</i>
	21.4	23.06	1.486	1.515	15.74	15.06	B	1.468	1.440	10.85	12.52	1.44	1.33
							<i>M</i>	<i>1.476</i>	<i>1.495</i>	<i>16.36</i>	<i>10.50</i>	<i>2.16</i>	<i>1.37</i>
LSZ	24.4	20.3	1.490	1.491	17.64	20.40	B	1.489	1.449	3.74	5.09	0.58	0.58
							<i>M</i>	<i>1.490</i>	<i>1.486</i>	<i>3.64</i>	<i>4.61</i>	<i>0.56</i>	<i>0.58</i>
	24.9	25.6	1.487	1.500	18.70	24.44	B	1.484	1.489	3.95	3.40	0.60	0.53
							<i>M</i>	<i>1.487</i>	<i>1.500</i>	<i>4.06</i>	<i>3.33</i>	<i>0.60</i>	<i>0.50</i>
HSA	22.74	26.5	1.488	1.494	21.60	20.02	B	1.434	1.437	8.52	4.40	0.86	0.43
							<i>M</i>	<i>1.471</i>	<i>1.489</i>	<i>23.71</i>	<i>3.70</i>	<i>2.89</i>	<i>0.45</i>
		25.6		1.518		19.28	B		1.399		6.01		0.36
							<i>M</i>		<i>1.508</i>		<i>3.49</i>		<i>0.38</i>
FIB	11.05	12.50	1.486	1.500	16.48	17.80	B	1.456	1.415	29.20	28.07	3.55	2.27
							<i>M</i>	<i>1.464</i>	<i>1.449</i>	<i>61.80</i>	<i>22.94</i>	<i>7.71</i>	<i>2.23</i>

the thickness and mass (HSA and FIB) found with the monolayer model if compared to the bilayer model.

By comparing SAR and OWLS, the bilayer model gives the most similar values for all the protein thicknesses. The best agreement is found for the two smaller sized proteins (LSZ and α -LA) for which the same optical thicknesses and masses are found with the bilayer model applied to SAR and to OWLS data. Again, there are some discrepancies for the two larger proteins (HSA and FIB) with up to 100% higher masses determined by SAR as compared to OWLS.

The fact that the bilayer model leads to the most similar optical parameter values, whether applied to OWLS or SAR experimental data, tends to prove that it represents a first reasonable approximation for the analysis of the optical properties of a complex architecture film, such as a polyelectrolyte multilayers/protein assembly.

In order to better understand these results, we determined the evolution of the invariant $\mathcal{F}$ during the adsorption process. Typical examples for the adsorption of α -LA, LSZ, HSA, and FIB are shown in Fig. 4. One observes that $\mathcal{F}$ remains close to 0 for the α -LA and LSZ, whereas $\mathcal{F}$ reaches values of one order of magnitude higher for the intermediate case of HSA and two orders of magnitude higher for FIB. Moreover, the sign of $\mathcal{F}$ is different for HSA and FIB. We are, however, unable to relate the sign of $\mathcal{F}$ to structural characteristics of the film. The fact that $\mathcal{F}$ greatly departs from 0 indicates that the refractive index profile for the largest sized protein deviates strongly from the homogeneous and isotropic monolayer model. This result could have been expected since FIB is a molecule with an elongated shaped, which can adsorb in “end-on” or in “side-on” configurations. As a consequence, for such a complex deposition process, even a bilayer model seems oversimplified. One can also notice that FIB is a protein for which the discrepancies in the optical parameters obtained by the monolayer and bilayer models are the most striking, especially when deter-

mined by SAR. On the other hand, $\mathcal{F}$ departs only slightly from 0 for α -LA. This should explain why, for this protein, the predictions of the monolayer and the bilayer models are of the same order of magnitude for both experimental techniques.

V. CONCLUSION

As a conclusion, it can be underlined that great care must be taken when using structural parameters determined from optical techniques for a fine physicochemical analysis of adsorption processes. When the refractive index profile of the adsorbed film is well approximated by an homogeneous and isotropic monolayer, different optical techniques should lead to similar conclusions. This is the case for the buildup of

FIG. 4. Evolution of the optical invariant $\mathcal{F}$, as a function of the deposition duration, calculated from SAR reflectivity intensity curves during the deposition of four proteins, two large sized proteins (FIB, $\blacktriangledown$; HSA ∇) and two small sized proteins (α -LA, $\bullet$; LSZ, $\circ$). FIB and α -LA were adsorbed on top of a PEI-(PSS-PAH)₃ multilayer film, whereas HSA and LSZ were adsorbed on a PEI-(PSS-PAH)₃-PSS multilayer film, in 10^{-2} M Tris buffer with 0.15 M NaCl.

polyelectrolyte multilayers films which can be well depicted by a single layer with defined optical properties using the monolayer model in SAR and OWLS. When the profile becomes more complex, the experimentally determined parameters characterizing the film can strongly depend on the model postulated for the analysis. Our results suggest that the optical parameters determined by OWLS are fairly model independent, both models giving masses and thicknesses of the same order of magnitude. This is not the case for SAR which is extremely model sensitive, particularly when the deposition of large sized proteins on top of the polyelectrolyte films is analyzed. For the small proteins, the monolayer and bilayer models lead to predictions of the structural parameters which are of the same order of magnitude for both experimental techniques. In contrast, when the monolayer model is not appropriate, based on an invariant analysis, notably for large proteins, OWLS and SAR lead again to similar structural parameters when the bilayer model is used.

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APPENDIX A

Both SAR and OWLS require to calculate the amplitude reflectivity coefficients of the interface on which the multilayers are deposited. In SAR only the coefficient for the TM wave is needed, whereas for OWLS the coefficients are needed for both the TE and the TM waves. These reflectivity coefficients are defined by

$$r = \frac{E_r}{E_i}, \quad (\text{A1})$$

where E_i and E_r represent, respectively, the amplitude of the electric field of the incident and of the reflected waves. The amplitude reflection coefficient is a complex number and can be written under the form,

$$r = |r| \exp(i\Phi). \quad (\text{A2})$$

In SAR, one measures $|r|^2$ as a function of the incidence angle around the Brewster angle, and the phase shift Φ is the quantity that enters in Eq. (2) for OWLS. In this latter case, because the reflection takes place under total reflection conditions, $|r| = 1$.

In order to calculate r one usually postulates a refractive index profile. Such a profile can be approximated as a sequence of ν homogeneous and isotropic adlayers, each adlayer i ($1 \leq i \leq \nu$) being characterized by a refractive index n_i and a thickness d_i . The refractive index of the incidence medium (oxide layer for OWLS, silica for SAR) will be denoted by n_F and that of the solution in contact with the adlayer by n_C . The reflectivity coefficients can be calculated

by a matrix method developed by Born and Wolf.^{29,30} In this method each layer i is characterized by two matrices,

$$\mathbf{I}_i = \begin{pmatrix} 1 & 1 \\ \frac{k_i}{n_i^{2\rho}} & -\frac{k_i}{n_i^{2\rho}} \end{pmatrix} \quad (\text{A3})$$

and

$$\mathbf{L}_i = \begin{pmatrix} \exp(ik_i d_i) & 0 \\ 0 & \exp(-ik_i d_i) \end{pmatrix}, \quad (\text{A4})$$

where $\rho=0$ for a TE wave and $\rho=1$ for a TM wave. The incident medium (resp. solution) is characterized by one matrix $\mathbf{I}_F$ (resp. $\mathbf{I}_C$), where the index i in expression (A3) is replaced by F (resp. C). The quantity k_i represent the coordinate of the wave vector in the i th layer perpendicularly to the interface. It satisfies the relation

$$k_i^2 + k_0^2 N^2 = n_i^2 k_0^2, \quad (\text{A5})$$

where $k_0 = 2\pi/\lambda$, λ being the wavelength of light and N representing the effective refractive index. In the case of SAR, N is given by $N = n_F \sin \theta_F$ and k_i is given by

$$k_i = k_0(n_i^2 - N^2)^{1/2}. \quad (\text{A6})$$

In the case of OWLS, $N = n_F \sin \theta_F + \lambda/\Lambda$ and

$$k_i = ik_0(N^2 - n_i^2)^{1/2}. \quad (\text{A7})$$

In this latter case, at least one of the k_i 's is imaginary since the reflection takes place under total reflection conditions. The amplitudes of the incident and reflected electric fields are then obtained by

$$\begin{pmatrix} E_i \\ E_r \end{pmatrix} \propto \mathbf{I}_F^{-1} \cdot \mathbf{I}_1 \cdot \mathbf{L}_1 \cdot \mathbf{I}_1^{-1} \cdot \mathbf{I}_2 \cdot \mathbf{L}_2 \cdot \mathbf{I}_2^{-1} \cdots \mathbf{I}_\nu \cdot \mathbf{L}_\nu \cdot \mathbf{I}_\nu^{-1} \cdot \mathbf{I}_C \begin{pmatrix} 1 \\ 0 \end{pmatrix}. \quad (\text{A8})$$

From this matrix multiplication one directly deduces a vector proportional to $\begin{pmatrix} E_i \\ E_r \end{pmatrix}$. This allows the amplitude reflection coefficient r to be determined. In the case of a perfectly abrupt interface (Fresnel interface), as for instance in SAR before the layer deposition, and in OWLS for the silica/waveguide interface, relation (A8) becomes

$$\begin{pmatrix} E_i \\ E_r \end{pmatrix} \propto \mathbf{I}_F^{-1} \cdot \mathbf{I}_C \cdot \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \quad (\text{A9})$$

which directly leads to the Fresnel laws of reflection.

APPENDIX B

Usually, one analyzes optical data relative to thin films by assuming a refractive index profile. It would, however, be of interest to characterize the adsorbed film by parameters which are independent of the model used to analyze the experimental data and which do not depend on the optical method used for the investigation. Such parameters exist and are called optical invariants. The optical invariants are excess quantities in the sense of Gibbs. These parameters are the ones that characterize optically the interface and that are independent of the location of the Gibbs dividing surface. They have been introduced independently by Lekner¹³ for stratified media and by Bedeaux and Vlieger¹⁴ in their most gen-

eral form. The optical invariants emerge out of a (L_f/λ) expansion of the reflection properties of an interface, L_f being the characteristic extension of the interface and λ the wavelength of light.

For an interface of spatial extension L_f small compared to the wavelength of light, the intensity reflection coefficient for TM waves can be written in the general form,

$$R_{\text{TM}} = r_0^2 - \frac{8Q_F Q_C (\epsilon_C - \epsilon_F) k_0^2}{\epsilon_F \epsilon_C (Q_F + Q_C)^4} \left\{ \left[1 - \epsilon_F \frac{\epsilon_F + \epsilon_C}{\epsilon_C \epsilon_F} \sin^2 \theta_F \right] \times \left[J_{23} + \left(\frac{\epsilon_F \epsilon_C}{\epsilon_F + \epsilon_C} - \epsilon_F \sin^2 \theta_F \right) J_{22} \right] - \frac{\epsilon_F^2 \sin^4 \theta_F}{2\epsilon_F \epsilon_C (\epsilon_F - \epsilon_C)} J_1^2 \right\}, \quad (\text{B1})$$

where r_0 corresponds to the Fresnel amplitude reflection coefficient in the absence of an adsorbed layer. The index F

(respectively, C) has been chosen for the incidence (respectively, transmission) medium, ϵ_i representing the relative dielectric constant of the medium i . The dielectric constant ϵ_i is related to the refractive index n_i by $\epsilon_i = n_i^2$ and Q_i is given by

$$Q_i = k_0 \cos \theta_i / n_i. \quad (\text{B2})$$

The parameters J_1 , J_{22} , and J_{23} are the so-called optical invariants. J_1 is an optical invariant of first order in (L_f/λ) , whereas J_{22} and J_{23} are second order invariants. Using the expressions of the invariants in terms of the refractive index profile, Mann *et al.*¹⁵ have constructed a new invariant denoted by $\mathcal{F}$, which is equal to zero when the refractive index profile of the interface is identical to a monolayer profile. The expression of $\mathcal{F}$ is given by

$$\mathcal{F} = 1 - \frac{(\epsilon_F - \epsilon_C) \left\{ 2J_{23} - \frac{\epsilon_F(\epsilon_F - \epsilon_C)}{\epsilon_F + \epsilon_C} J_{22} \right\} \left\{ 2J_{23} + \frac{\epsilon_F(\epsilon_F - \epsilon_C)}{\epsilon_F + \epsilon_C} J_{22} \right\}}{2J_1^2 \left\{ J_{23} + \frac{\epsilon_F \epsilon_C}{\epsilon_F + \epsilon_C} J_{22} \right\}}. \quad (\text{B3})$$

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