

Coupled Plasmon-Waveguide Resonators: A New Spectroscopic Tool for Probing Proteolipid Film Structure and Properties

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ABSTRACT A variant of surface plasmon resonance (SPR) spectroscopy has been developed that involves a coupling of plasmon resonances in a thin metal film and waveguide modes in a dielectric overcoating. This new technique is referred to as coupled plasmon-waveguide resonance (CPWR) spectroscopy. It combines a greatly enhanced sensitivity (due to increased electromagnetic field intensities at the dielectric surface) and spectral resolution (due to decreased resonance linewidths), with the ability to directly measure anisotropies in refractive index and optical absorption coefficient in a dielectric film adsorbed onto the surface of the overcoating. Experimental data obtained with an egg phosphatidylcholine bilayer are presented to document these properties.

INTRODUCTION

Over the last decade there has been renewed interest in the application of surface plasmon resonance (SPR) spectroscopy to the study of the optical properties of molecules immobilized at an interface between solid and liquid phases. As has been described in detail elsewhere (Salamon and Tollin, 1997; Salamon et al., 1997), the ability of the SPR phenomenon to provide information on the physical properties of thin dielectric films deposited on a metal layer, including lipid and protein molecules forming proteolipid membranes, is based on two principal characteristics of the effect: 1) The evanescent electromagnetic field generated by the free electron oscillations decays exponentially with penetration distance into an emergent dielectric medium, i.e., the depth of penetration into the dielectric material in contact with a metal layer extends only to a fraction of the light wavelength used to generate the plasmons. This makes the phenomenon sensitive to the optical properties of the metal/dielectric interface, without any interference from the properties of the bulk volume of the dielectric material or any medium that is in contact with it. 2) The angular (or wavelength) position and shape of the resonance curve are very sensitive to the optical properties of both the metal film and the emergent dielectric medium adjacent to the metal surface. As a consequence of these characteristics, SPR is ideally suited to studying both structural and mass changes of thin dielectric films, including lipid membranes, lipid membrane-protein interactions, and interactions between integral membrane proteins and peripheral, water-soluble proteins (Salamon and Tollin, 1997; Salamon et al., 1997).

As an extension of this technology, the present report describes the development of a new thin film design (coupled plasmon-waveguide resonator, CPWR) that couples

surface plasmon and waveguide excitation modes, along with an example of the utilization of this methodology to measure the optical anisotropy in a self-assembled solid-supported lipid membrane system. This design innovation adds several important new properties to those unique features of SPR spectroscopy summarized above. In particular, it generates an optical interface that combines simplicity in assembly and application of SPR devices with protection of the plasmon-generating metallic film against mechanical or chemical deterioration, and provides superior optical properties with greatly enhanced spectroscopic capabilities and sensitivities, thereby allowing a broader spectrum of applications than has heretofore been possible.

PRINCIPLES OF COUPLED PLASMON-WAVEGUIDE RESONATORS AS APPLIED TO THIN MEMBRANE FILMS

General description

In its construction, this new interface design (CPWR) is similar to a conventional SPR device. Thus it contains a metallic (or semiconductor) layer (or layers), typically between 45 and 55 nm thick, formed from either gold or silver (the metals most often used in such devices), which is deposited on either a glass prism or a grating, and which generates the surface plasmon wave. In the designs illustrated in Fig. 1, the silver film is covered with either a single dielectric layer (Fig. 1 A) or two layers (Fig. 1 B), characterized by an appropriate set of values of film thickness (t) and optical parameters (refractive index n , and extinction coefficient k), as described below. Spectroscopic measurements with such a device are based on the resonant excitation of electromagnetic modes of the structure by both TM (transverse magnetic; p) and TE (transverse electric; s) polarized components of cw (continuous wave) laser light (e.g., He-Ne; $\lambda = 632.8$ nm) passing through a glass prism under total internal reflection conditions (see Fig. 1 A).

The addition of a dielectric layer (or layers), with an appropriate set of optical parameters, to a conventional SPR

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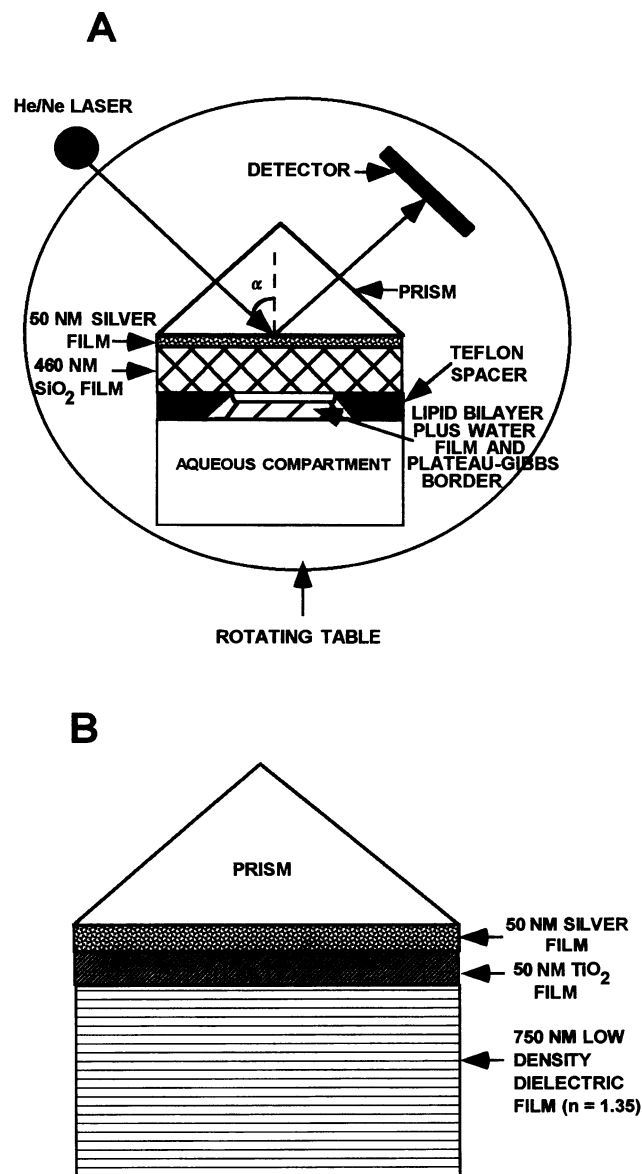


FIGURE 1 Side views of two CPWR coating designs. (A) Glass prism coated with a 50-nm-thick silver layer protected by a 460-nm SiO_2 film. Also shown are a deposited lipid bilayer held in place by a Teflon spacer, and the ATR measuring system. (B) Silver-coated glass prism containing two dielectric layers: 50-nm TiO_2 protecting the silver film, and 750 nm of a low refractive index dielectric material ($n = 1.35$).

arrangement plays the role of both a mechanical and chemical shield for the metal layer and an optical amplifier, which results in an increased sensitivity and enhanced spectroscopic capabilities. In practical terms, there is a large variety of dielectric overcoat film combinations that can be used in particular applications. In Fig. 1 we present two examples of different coating designs, to emphasize two important facts. First, the relative bandwidths of the two resonances (i.e., obtained with either *p*- or *s*-polarized incident light), and therefore the sensitivity of the measurement, can be varied by altering the overcoat film properties. Second, the dielectric overcoat can also be used as a matrix that

adsorbs and immobilizes the sensing material in sensor applications (as is shown in Fig. 1 B). Thus, for example, the dextran layer that is used in a commercial SPR biosensor (BIAcore, Pharmacia Biosensor; Löfås and Johnsson, 1990) for fast and efficient immobilization of ligands can be manipulated to generate resonances with widely varying sensitivities.

There are several ways of understanding the physical basis for the appearance of an *s*-polarized resonance component in a conventional SPR experiment as a consequence of placing a dielectric layer on the metal surface. We will adopt the approach that has been used in earlier descriptions of the SPR phenomenon (Salamon and Tollin, 1997; Salamon et al., 1997), and which is a straightforward consequence of the application of the electromagnetic field theory to thin-film systems (Macleod, 1986). According to the electromagnetic theory, thin-film materials are characterized by a complex dielectric constant, which includes the refractive index n and the extinction coefficient k (i.e., $n - ik$). In the optical region of the electromagnetic spectrum, this parameter is equal to the ratio of light velocity in vacuo (c) to that in a medium (v), and is numerically equivalent to the optical admittance, the latter defined by the ratio of the amplitudes of the electric (B) and magnetic (C) fields of the electromagnetic wave (Macleod, 1986):

$$Y = C/B = c/v = n - ik \quad (1)$$

where Y is the optical admittance divided by the admittance of free space.

Using Maxwell's equations, one can describe the propagation of the plane, monochromatic, linearly polarized, and homogeneous electromagnetic field within a multilayer thin-film system with the following matrix equation (Macleod, 1986; Salamon and Tollin, 1997; Salamon et al., 1997):

$$\begin{bmatrix} B \\ C \end{bmatrix} = \prod_{j=1}^s \begin{bmatrix} \cos \beta_j & i(\sin \beta_j)/y_j \\ iy_j \sin \beta_j & \cos \beta_j \end{bmatrix} \begin{bmatrix} 1 \\ y_{j+1} \end{bmatrix} \quad (2)$$

where s is the number of layers deposited on the incident medium (glass prism),

$$\beta_j = 2\pi(n_j - ik_j)t_j \cos \alpha_j / \lambda \quad (2a)$$

gives the phase thickness of layer j at the appropriate angle of incidence (α_j) and light wavelength (λ), and

$$y_j = (n - ik)_j / \cos \alpha_j \quad (2b)$$

Equations 2, 2a, and 2b allow examination of the distribution of electromagnetic field amplitudes throughout the thin-film system, as well as calculation of the transmittance, absorbance, and reflectance. It also allows analysis of the resonance phenomena occurring within such thin-multilayer films.

The reflectance of a multilayer system is given by the following relationship involving the optical admittance:

$$R = (y_0 - Y)/(y_0 + Y)^2 \quad (3)$$

where y_0 is the admittance of the incident medium (glass prism). The theory demands that the incident medium should be free from absorption, so that y_0 is real and equals n_0 (see Eq. 1). Equation 3 describes a reflectance spectrum, i.e., reflectance as a function of the incident angle α of a beam of monochromatic light (i.e. constant λ ; this function is used in the present work). Similarly, spectra can be obtained by varying λ at a constant value of α , by varying the thickness of either layer at constant values of the thickness of the other layers, of α , and of λ (an example of the latter is presented in Fig. 2). Analysis of the optical admittance shows that beyond the critical angle, the emergent wave in the final medium is evanescent and the admittance is imaginary—positive imaginary for p -polarized light and negative imaginary for s -polarization. For a surface wave to

be confined to the metal surface, the admittance exhibited by the adjoining medium must be positive imaginary and of a magnitude very close to that of the extinction coefficient k of the metal (i.e., only materials with a small value of the refractive index n and a large value of k , such as silver and gold, will generate a surface wave). For a metallic film, only for p -polarization and a very narrow range of angles of incidence is this condition fulfilled. Coupling of the incident light to the surface wave results in the sharp dip in total internal reflectance characteristic of the resonance effect. For s -polarization the admittance is always negative imaginary, and so there is normally no corresponding resonance. However, in the CPWR device, a dielectric overcoat layer (or system of layers) is used to transform the admittance of the emergent medium, so that the admittance presented to the metal is positive imaginary for both s - and p -polarization. Depending on the details of the admittance matching overcoat (i.e., n , k , and t values), the system can result in a narrowing or a broadening of the range of angles over which the necessary coincidences are achieved, and hence a similar broadening or narrowing of the resonances. Examination of the distribution of electric field amplitudes through the system shows that the admittance matching layers are important components of the resonant system, rather like the cavity layers in narrowband filters or the thin-film waveguides in optical couplers. To distinguish this resonance phenomenon from the conventional unmodified surface plasmon (SPR) resonator, we use the term coupled plasmon-waveguide resonator (CPWR).

As noted above, measurements using CPWR devices are made in the same way as with the conventional SPR technique, i.e., using the ATR method of coupling the light to the deposited thin multilayers, thereby exciting resonances that result in absorption of the incident radiation as a function of either the light incident angle (with a monochromatic light source) or light wavelength (at constant incident angle), with a consequent dip in the reflected light intensity.

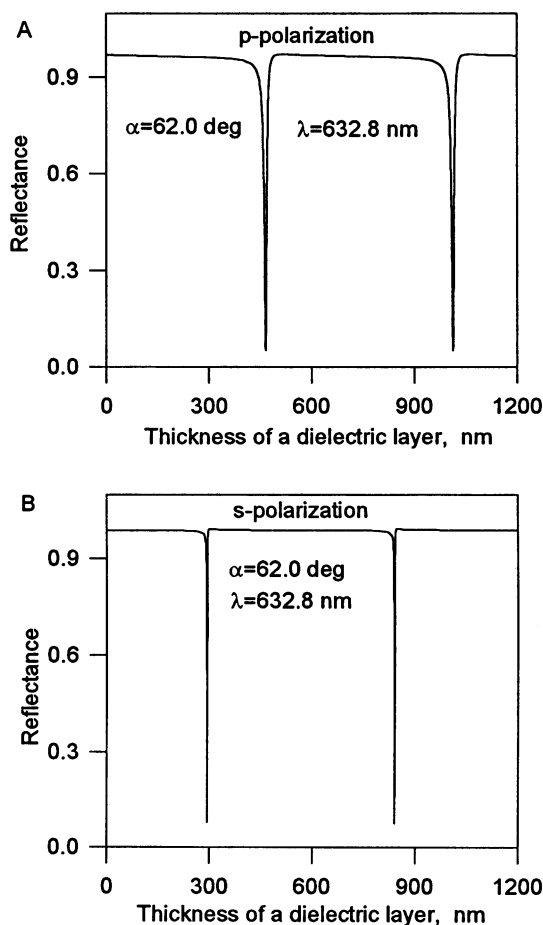


FIGURE 2 Resonance spectra presented as reflected light intensity (calculated from Eq. 3 for the thin film design shown in Fig. 1 A), as a function of the protective SiO_2 layer thickness, with either p - (A) or s - (B) polarized light. The incident angles (α) have been arbitrarily chosen in the range of their usual values. Refractive index of glass prism: 1.5151; refractive index of SiO_2 : 1.4571.

Properties of the resonance spectrum

As described above, under the appropriate experimental conditions, the CPWR can be excited by either p - or s -polarized light to resonantly absorb the incident light energy. Fig. 2 shows such resonances as a function of the dielectric overcoat thickness, obtained with the experimental arrangement shown in Fig. 1 A. Although, as these results indicate, the two resonances are separated and occur at different dielectric thicknesses, it is possible to adjust the thickness of the overcoat layer to obtain both s - and p -resonances with the same device. Fig. 1 presents two examples of such devices that exhibit the resonances shown in Fig. 3. The dashed curve in Fig. 3 A also shows the much broader SPR spectrum obtained with the same silver layer as in Fig. 1, but without the dielectric overcoat. It is therefore clear from these spectra that the dielectric layer adds two very important qualities to the usual SPR resonance

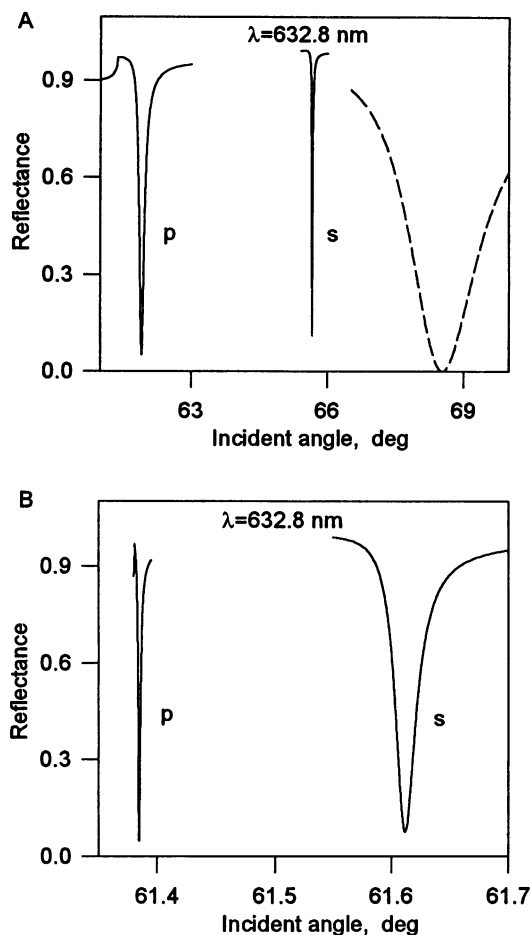


FIGURE 3 Resonance spectra presented as reflected light intensity versus incident angle, calculated from Eq. 3 for the design given in either Fig. 1 A or Fig. 1 B. The dashed curve in A demonstrates the surface plasmon resonance spectrum obtained with a 50-nm-thick silver layer without a protecting dielectric film.

properties: 1) an additional spectroscopic dimension, by generating a second type of resonance with different polarization (*s*-polarized component); and 2) an increased sensitivity, as a consequence of greatly decreasing the half-width of both *s*- and *p*-polarized resonances. Furthermore, the resonance linewidth, and therefore the spectral sensitivity, can be adjusted by choosing the appropriate overcoating layers according to the experimental needs, as indicated by the two sets of results shown in Fig. 3. These spectra result from two different dielectric layer designs (see Fig. 1), for one of which the *s*-spectrum (Fig. 3 A) is narrower than the *p*-spectrum, whereas the opposite is true for the other design (Fig. 3 B).

The overall sensitivity of such a device includes the sensitivity of the shift of the resonance minimum angle, defined in principle by the refractive index and thickness of the sensing layer (which is deposited on the surface of the dielectric overcoat; e.g., a lipid bilayer) and the sensitivity to the change in the shape of the resonance spectrum, which is dependent mainly on the light absorption (and/or scatter-

ing) properties of the sensing layer. Both of these parameters (i.e., the minimum resonance angle and the shape of the spectrum as defined by its depth and width) are dependent upon the form of the quasi-modes of the electromagnetic field generated in the design device. Fig. 4 shows the electric field distributions for *p*- and *s*-polarizations obtained with the interface presented in Fig. 1 A. The electromagnetic field at this outer interface of the device is higher by a factor of ~ 50 for the *s*-component and ~ 25 for the *p*-component, in comparison with that at the entrance interface between the glass and metal layers (Fig. 4). This translates into such a high sensitivity that the three parameters describing the resonance spectrum, i.e., thickness of the sensing layer (*t*), refractive index (*n*), and extinction coefficient (*k*), can be obtained with accuracies better than 1 Å, 0.001, and 0.002, respectively, for a sensing layer whose thickness is only 5 nm, a value comparable to the thickness of a lipid membrane (Salamon et al., 1996; Salamon and Tollin, 1996a,b). In practical terms, this means that, in many cases, the limitation of accuracy will result not from the measuring technique itself, but from the ability to generate a thin sensing film in a reproducible manner.

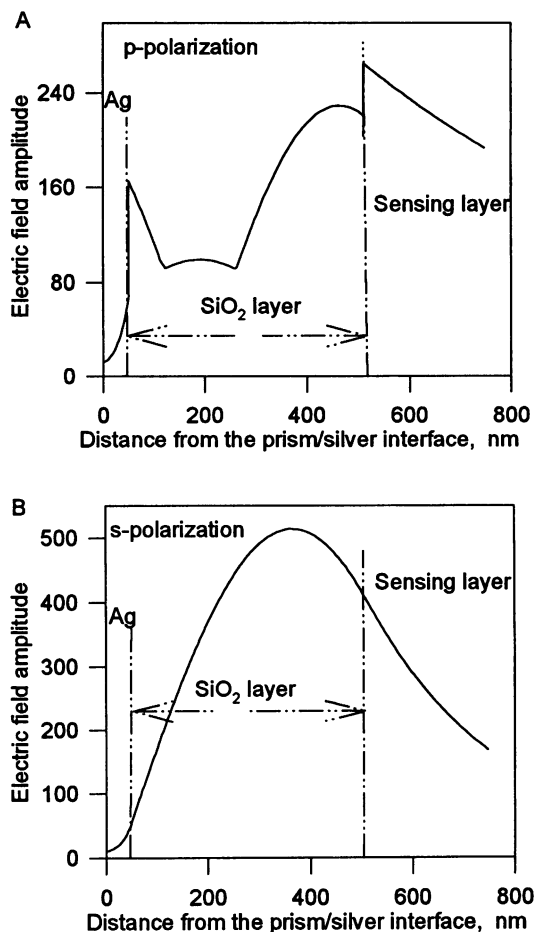


FIGURE 4 Amplitude of the electric fields within a silver layer, an SiO_2 film, and a sensing layer for *p*- (A) and *s*- (B) polarized light, as a function of the distance from the glass-metal interface, for the design presented in Fig. 1 A.

Although there are some alternative techniques for the detection and measurement of small optical changes based on optical waveguides (for a review see Lukasz, 1991), the present technique possesses two significant advantages over these. First, the coupling arrangements are much simpler and more convenient in the CPWR devices. Second, the geometric arrangement in CPWR spectroscopy is characterized by a complete isolation of the optical probe from the system under investigation, as is also the case in conventional SPR spectroscopy.

The three optical parameters (n , k , t) characterizing a deposited dielectric film can be evaluated for both polarizations, at different angles of light incidence, and by using different light wavelengths, thereby producing enough experimental data to characterize all of the structural parameters of thin films, i.e., thickness, mass distribution within the film, orientation of molecules (by measuring the anisotropy in n), and the orientation of chromophores attached to the molecules within the sensing layer (by measuring the anisotropy of k). All of these characterizations can be obtained using a single device covered with a sensing layer, and using a measurement method that involves only a determination of reflected light intensity under total internal reflection conditions (for details of experimental techniques employed to measure the resonance spectrum, see recent reviews: Salamon and Tollin, 1997; Salamon et al., 1997). Furthermore, because the electromagnetic field decays exponentially within the emerging medium (see Fig. 4), the measurement is sensitive only to the interface region between the dielectric overcoat and the emerging medium, and is not interfered with by the bulk properties of the medium. In addition, there is no limitation on the dielectric material that can be used in such coatings (as long as the optical characteristics are favorable), and therefore the dielectric film can be formed from any number of layers designed and optimized for different uses. This might be especially important in various sensor applications, where the dielectric overcoat can also be designed to adsorb and immobilize the sensing material, either on its surface or within its interior. Such a combination of properties in one interface permits the construction of a durable sensor device with the highest sensitivity and an expanded dynamic range of measurements.

Although all of the above-mentioned features of the resonance spectrum can be employed in a variety of different ways, one of the most fruitful applications lies in biophysical and biochemical studies of the structural properties of proteolipid assemblies. Studies of the microscopic structure of lipid membranes and interacting lipid-protein films represent a technically difficult challenge for two major reasons: 1) these are very thin layers comprising only one or two monolayers; 2) they contain relatively small amounts of material located at the interface between two immiscible phases, and may be labile and structurally heterogeneous. As a result, only a limited number of studies have been dedicated to the problem of lipid and/or protein orientation in molecular films. Here we will demonstrate the capabilities of the above-described new technique by employing it

to obtain information about structural anisotropy in a self-assembled solid-supported lipid bilayer.

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine (PC) was obtained in solid form from Avanti Polar Lipids (Alabaster, AL) and was used in a membrane-forming solution as described below.

Preparation of lipid membranes on solid supports

In the present study, self-assembled solid supported lipid membranes have been used (Salamon et al., 1997). The method of preparation employs the principles that govern the spontaneous formation of a freely suspended lipid bilayer membrane (Mueller et al., 1962) and involves spreading a small amount of lipid bilayer-forming solution ($\sim 2\text{--}4\ \mu\text{l}$) across an orifice ($\sim 4\ \text{mm}$ in diameter) in a Teflon sheet that separates a dielectric thin film from the aqueous phase (see Fig. 1 A) (Salamon et al., 1997). The hydrophilic surface of the dielectric (hydrated SiO_2 in the present case) attracts the polar groups of the lipid molecules, thus forming an adsorbed lipid monolayer with the hydrocarbon chains oriented toward the bulk lipid phase. Subsequent to the first step of lipid membrane formation, the main body of the sample cell is filled with the appropriate aqueous solution. This initiates the second step, which involves a thinning process, i.e., formation of both the second monolayer and a plateau-Gibbs border that anchors the bilayer film to the Teflon spacer, allowing the excess of lipid and solvent to move out of the Teflon orifice (see Fig. 1 A) (Salamon et al., 1997). In previous work from this laboratory involving both SPR and electrochemical measurements (Salamon et al., 1993, 1996; Salamon and Tollin, 1996a,b) with different protein molecules, we have demonstrated that this technique generates a membrane that provides a biocompatible medium for binding and immobilizing both peripheral and integral membrane proteins. In the present experiments, the lipid films were formed on an SiO_2 surface from solutions containing 7 mg/ml egg PC in squalene (Fluka)/butanol (0.15:10, v/v), using thin film coatings prepared at the Tucson Optical Research Co. (Tucson, AZ) by vacuum deposition.

Resonance measurements

Details of the experimental procedures for resonance spectra measurement and data analysis are the same as those employed previously with SPR studies and have been described elsewhere (Salamon and Tollin, 1997; Salamon et al., 1997).

RESULTS AND DISCUSSION

Fig. 5 shows a typical example of the resonance spectra obtained with the device described in Fig. 1 A, in which a bare SiO_2 film (*left curve*) is modified by depositing an egg PC membrane (*right curve*). The measurements with this system have been made in two different ways. First, the resonance spectra generated by both *s*- and *p*-polarizations have been taken with the same device and a single lipid membrane. Second, two different devices (varying in the metal and dielectric coating layer parameters) with two separately formed lipid bilayers have been used to measure the resonance spectrum generated by each polarization. This has been done to evaluate the experimental errors produced by the formation of new lipid membranes and by using

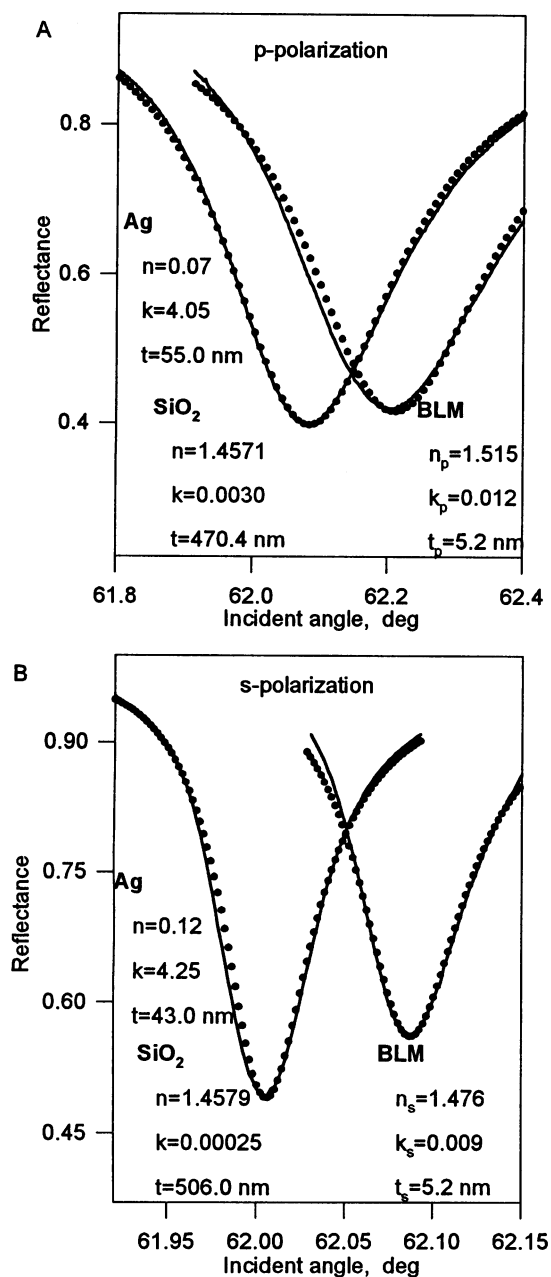


FIGURE 5 Experimental resonances (line) and theoretical fits (points) obtained with the design presented in Fig. 1 A, either with *p*- (A) or *s*- (B) polarized light, before and after an egg phosphatidylcholine bilayer was deposited on the SiO₂ surface. Optical parameters for the theoretical fits are given in the figure.

different thin film coatings. The resonance spectra shown in Fig. 5 have been obtained with the second procedure.

Calculations of the electric fields throughout the thin-film system consisting of the CWPR device and the deposited lipid bilayer (cf., for example, Fig. 4) show that for *p*-polarized light the amplitude of the component of the electric field normal to the film boundaries is an order of magnitude larger than that parallel to the surface. In *s*-polarized excitation the field is completely parallel to the boundaries. Therefore, a good first approximation is to

assume that in the *p*-polarization case the electric vector is completely normal to the surface. As a consequence, the optical parameters obtained with these two modes of excitation refer to the parallel and perpendicular directions within the lipid film.

Table 1 presents the average values obtained for the optical parameters of the lipid film, together with the experimental errors resulting from the different measurement procedures described above. These results clearly indicate that, although the thickness values obtained with both polarizations are the same within the experimental error, and compare well with those obtained previously with the conventional SPR technique (Salamon et al., 1996; Salamon and Tollin, 1996a,b), there are significant differences in both the n and k parameter values.

In this type of measurement there are two possible contributions to the anisotropy in n , one from the two-dimensional nature of the film itself, and another from the ordering of anisotropic molecules within the two-dimensional structure. Simple calculations show that the difference between n_p and n_s obtained here cannot be explained by a form birefringence; the difference between the two indices is too large. Thus the source of the anisotropy in n must be the anisotropic character of the lipid molecules comprising the bilayer structure. Because in this experiment the lipid does not absorb the exciting light (the wavelength of the laser excitation, 632.8 nm, is far removed from absorption band of PC), a k value other than zero reflects a diminution of measured light intensity due only to scattering processes that result from imperfections in the lipid film. It is expected that in anisotropic films the two polarized components of light will be scattered differently, thereby producing a scattering anisotropy; this effect will not be discussed further in the present work.

The value obtained here for the refractive index n_p agrees rather well with those obtained in our previous measurements from SPR with a bare silver layer (Salamon et al., 1996; Salamon and Tollin, 1996a,b). Although with a complex mixture such as egg PC we are not able to calculate theoretical polarizabilities and refractive indices for comparison, the average experimental values of n_p and n_s presented in Table 1 are in very good agreement with theoretical values of these indices calculated for five different lipid molecules containing saturated fatty acid side chains (the average values are $n_p = 1.559$ and $n_s = 1.483$), assuming the additivity principle in tetrahedral aliphatic hydrocarbons

TABLE 1 Average values of the thickness (t), refractive index (n), and extinction coefficient (k) of a self-assembled solid-supported egg phosphatidylcholine bilayer, obtained by measuring CPWR resonance spectra excited either by *p*- or *s*-polarized light

	<i>p</i>	<i>s</i>
t (nm)	5.2 ± 0.1	5.2 ± 0.1
n	1.52 ± 0.01	1.47 ± 0.01
k	0.10 ± 0.01	0.020 ± 0.002

(den Engelsen, 1976) and ellipsometrically measured values of various phosphatidylcholines (Ducharme et al., 1990). This similarity clearly indicates a high degree of ordering of the egg PC molecules in the solid-supported self-assembled lipid bilayer system used here. The refractive index anisotropy obtained in the present measurements seems to be larger than that calculated from ellipsometric determinations on freely suspended black lipid membranes of lecithin, where values of $n_p = 1.47$ and $n_s = 1.45$ were obtained (den Engelsen, 1976), indicating a higher degree of ordering of the PC molecules in the solid-supported membrane. This is not unexpected.

CONCLUSIONS

The results described herein clearly demonstrate that CPWR spectroscopy provides a useful new technique for obtaining information about molecular assemblies that can be immobilized at a dielectric/water interface. Three major improvements over conventional SPR methodologies have been documented: increased spectral resolution, improved sensitivity, and the ability to measure anisotropy in both n and k . Furthermore, the method is applicable to a wide range of materials, including, but not limited to, lipid membranes that have either integral membrane proteins incorporated into them or peripheral membrane proteins bound to their surface. Such extensions of the present measurements are under way.

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