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TECHNICAL DEVICE FOR OBTAINING RAMAN SPECTRA OF ULTRATHIN FILMS OF PHOSPHOLIPIDS

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ABSTRACT

The association of a sample illumination apparatus for supporting ultra thin films with a microprobe (MOLE) spectral analysis system allowed us to obtain non-resonant Raman spectra of phospholipid films. Films as thin as 75 Å have yielded useful spectra.

INTRODUCTION

To study biological membranes we have developed a Raman spectroscopic technique, the details of which are an extension of our earlier work with thin films (1,2). Takenaka et al. (3) published spectra of absorbing monolayers taking advantage of the resonance Raman effect to attain enhanced signals. Muller et al. (4) using an attenuated total reflection technique (evanescent waves) reported Raman spectra of sample slices about 500 Å thick. In some of these cases, as well as with those involving monolayers adsorbed on metallic electrodes (5,6), the high intensity of observed Raman bands may be due to resonance enhancement. Such is not the case

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here. Our method allows the detection of Raman spectra of phospholipid films (75 to 1425 Å thick) by concentrating the energy density of the excitation source in the film, minimizing the detection of signals originating in the support medium and by collecting as much of the Raman signal from the sample film as possible. To do this, a special sample support configuration and illumination system was developed and coupled to a commercially available Raman microprobe (MOLE) apparatus (7).

II - ILLUMINATION

A four media device (fig. 1) was used in which a high refractive index prism of rutile (n = 2.9) was coated first with a layer of silver (d_2 = 400 Å) and then with the sample of interest of thickness d_3. For this kind of geometry, it is known (2,8) that :

a) with transverse magnetic (TM) polarization of the incident beam and for each value of d_3, there exists one or more values of the incident angle θ (coupling angle) such that the intensity of the reflected beam is minimal and the electric field in the sample film is maximized at a level much higher than in the incident beam,

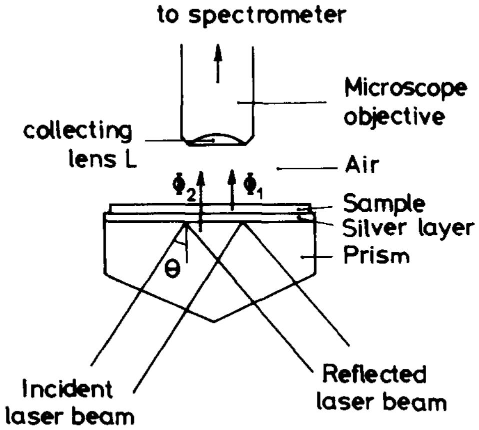

Fig. 1. Experimental device at the level of the incident laser beam and the microscope objective.

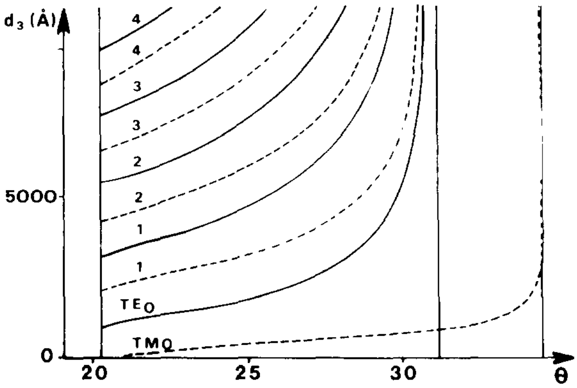

Fig. 2. Coupling angles for to polarization TM and TE versus d_3(Å)

b) with transverse electric (TE) polarization of the incident beam, it is the same, but there is now a minimum value of d_3 below which propagation of plane light waves in the film is impossible and coupling cannot occur (fig. 2).

A plot of film thickness d_3 vs coupling angle θ is shown in figure 2. For a film of d_3 = 1425 Å and $n = 1.5$, the possible coupling angles are 22° and 33° for TE and TM excitation respectively. It was previously shown (2) that for TE excitation, the maximum of the electric field in the film would be 21 times that in the incident beam.

With the sample arrangement of figure 1, the Raman scattered light Φ_1 originating in the sample may be overwhelmed by that originating in the prism Φ_2. The silver layer serves two very useful functions. In addition to its role in the electric field enhancement of the beam in the sample, it attenuates Φ_2 by absorption (∿ 97 %) and reflection but does not affect Φ_1 which originates in the region above the metallic film.

III - OPTIMIZATION OF $\Phi_1/(\Phi_1 + \Phi_2)^{1/2}$

The fluctuations in the total Raman flux $\Phi_T = \Phi_1 + \phi_2$ obey Poisson statistics with a standard derivation given by $\sqrt{\Phi_T}$. For the Raman signals

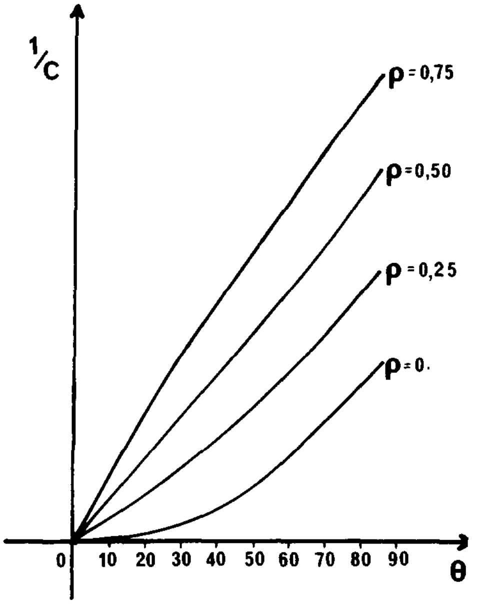

Fig. 3. $1/C = \sqrt{\Phi_T}/\Phi_1$ as a function fo the numerical aperture for different depolarization ratios.

Φ_1 of interest, there is always a contribution from Φ_2 and in general, $\Phi_2 >> \Phi_1$. Therefore, the relative error $C = \sqrt{\Phi_T}/\Phi_1 \simeq \sqrt{\Phi_2}/\Phi_1$ in measuring Φ_1 must be minimized. It can be demonstrated (8) that C is a decreasing function of the numerical aperture of the collecting lens L (fig. 1) and the results are shown in figure 3. With an increasing aperture both Φ_1 and Φ_2 increase but the marginal rays of Φ_2 are significantly attenuated because of the longer optical path through the silver film. This is not the case for Φ_1. In addition, a large aperture reduces the depth of field and thus contributions to the signal from Φ_2. Matching the dimensions of the illuminated image to the optical system of the spectrometer increases Φ_1 more than $\Phi_T^{1/2}$. Details will be published in due course (9).

IV - EXPERIMENTAL SECTION

Films of various thichnesses (d_3 = 1425, 425, 125 and 75 Å) of calcium L β γ dipalmitoyl phosphatidate were deposited in (2n + 1) layers using a variation (10) of the Langmuir-Blodgett technique on to an evaporated silver film of thickness d_2 = 400 Å. Light (4880 Å) from an Argon ion laser (TM or TE) was used. Using a spherical lens, the illuminated area on the sample was elliptical (50 x 25 μ). Spectra were recorded with two different optical systems. In the first, a Coderg PH01 spectrophotometer with f/6 light collecting fore-optics and direct current detection was used with a spectral band pass of 10 cm^{-1}. In the second, a microprobe (MOLE, Jobin Yvon) was employed. This is a commercial instrument in which a microscope (magnification = 100, numerical aperture 0.9) is coupled to a double grating spectrophotometer. In our experiment, however, the sample illumination is from below (fig. 1) rather than the more normal axial illumination used in the MOLE. The spectral band pass was 9 cm^{-1} and a photon counting system was used.

V - RESULTS AND DISCUSSION

Using the Coderg instrument, TM excitation of a sample with d_3 = 1425 Å exhibit prominent bands due to C-H stretching vibrations of the phospholipid. In good agreement with theoretical predictions coupling angles were found for both TM and TE excitation. When $d_3 < 1425$ Å, only TM excitation was possible as expected, and furthermore, for such very thin films, spectra were not rigorously reproducible.

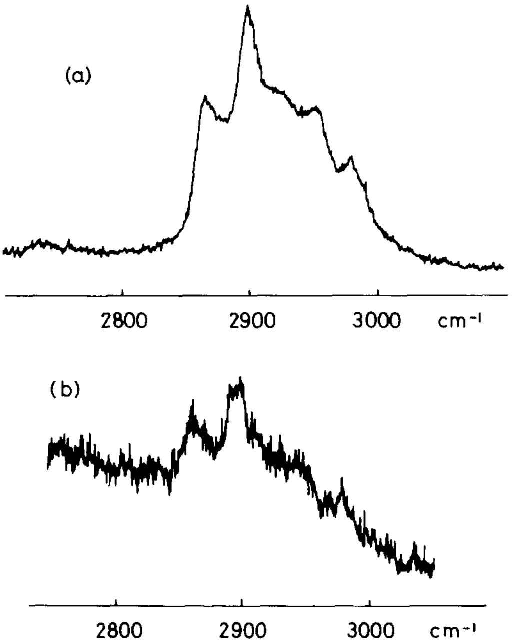

Fig. 4. a) Spectrum of a film thickness : 1425 Å - Pol TM. Scan speed : 20 cm^{-1}/mm (20 cm^{-1}/cm), counting time : 2 s, entrance slit = 9 cm^{-1}, laser power : 300 mw.
b) Spectrum of a film thickness : 75 Å - Pol TM. Scan speed : 5 cm^{-1}/mm (20 cm^{-1}/cm), counting time : 7 s, entrance slit = 9 cm^{-1}, laser power : 70 mw.

In 4 A and B, Raman spectra of films with d_3 = 1425 Å and 75 Å taken with the MOLE are shown (TM excitation). The advantage of this experimental arrangement are clear, for the spectrum of the 75 Å film is roughly equivalent to the one obtained with the CODERG instrument with d_3 = 1425Å. Thus a signal enhancement of about 20 times was realized. Calculations for a point source and the two optical gathering systems suggested that a factor of almost 300 was to be expected. That we do not have a point source is evident and this contributes significantly to the difference. Moreover, optimized coupling of the microscope objective with the sample and the spectrometer has not been completed at this time for our special sample configuration and therefore further improvements are to be anticipated.

Although current results are promising even without requiring resonance signal enhancement processes, it is impossible at the moment to determine accurately the film temperature under excitation conditions and for biological samples, this is an important consideration.

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