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PHOTOACOUSTIC MEASUREMENT OF OPTICAL AND THERMAL PROPERTIES WITH REFERENCE TO BIOLOGICAL APPLICATIONS

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Since the revival of the photoacoustic (PA) effect as a way to study optical and thermal properties of condensed media, different applications of the photoacoustic spectroscopy (PAS) have been performed in biology and medicine (see ref 1 and 2). That new spectroscopic method is based on the measurement of the heat generated in the sample through the absorption of light. It mainly allows the study of opaque or light-scattering samples, and performs depth - profile analysis and in vivo measurements.

Here we are going to deal with the process according to which PAS enables the measurement of the optical absorption spectra, the quantum yield of fluorescence and the thermal diffusivity and effusivity of thermally thick samples. We will give some experimental results applying particularly to biology. But we will limit our study to thermally thick samples - the thickness of which is significant compared to the thermal diffusion length - in which the absorbed energy is quasi instantaneously-compared to the period of illumination - converted into heat or into fluorescence radiation.

So the amplitude ΔP and phase φ of the photoacoustic signal are given by[3]

$$\Delta P\omega = AI_o\ (k\rho c)^{-1/2}(1 - \phi\ \lambda\lambda_f^{-1})\ \beta\mu\ (1 + (1 + \beta\mu)^2)^{-1/2}$$

$$\varphi = tg^{-1}\ (\beta\mu + 1) - \Pi + \theta$$

where ω is the angular frequency of light modulation, I_o : the intensity of light, λ : its wavelenght. The sample is characterized by : k : its thermal conductivity, ρ : its density, c : its heat capacity, $\mu = (2\alpha/\omega)^{1/2}$ is the thermal diffusion length, α : the thermal diffusivity, ϕ : the fluorescence quantum yield, β : the optical absorption coefficient, λ_f : the mean fluorescence wavelenght. A and θ are independent of the nature of the sample, but depend on its volume and shape. A is expressed in Ref. 3. θ is an instrumental phase shift which is essentially due to the finite dimensions of the gas.

The two parameters, measured by PAS, are :

1) $\beta\mu$ or $\beta(\alpha)^{1/2}$. It can be measured by the direct use of the phase or from the frequency dependence of the amplitude of the PA signal.
2) $(k\rho c)^{-1/2}(1 - \phi\ \lambda\lambda_f^{-1})$. $(k\rho c)^{1/2}$ is the thermal effusivity of the sample, $(\phi\ \lambda\lambda_f^{-1})$ is the photoactivity loss : the part of absorbed energy which is not converted into heat. That parameter can be measured from the saturation value of the PA signal.

The measurement of AI_o and θ, that is the calibration of the spectrometer, can be done with a sample of reference having the same volume and shape as the studied sample. We use a two millimeters thick sample of black India ink which produces a saturated photoacoustic sample : $\beta\mu \gg 1$. That sample is photoinactive and has the thermal properties of water. The phase of the PA signal allows

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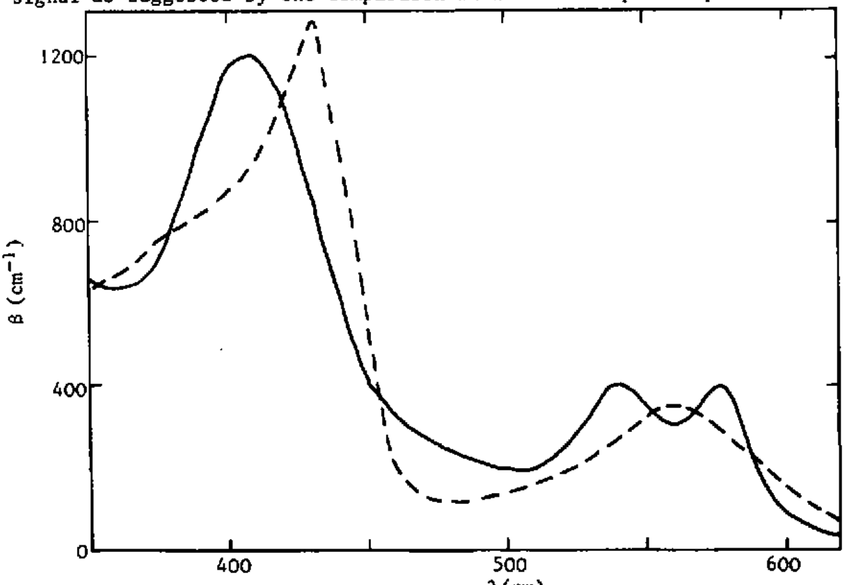
direct measurement of $\beta(\alpha)^{1/2}$. The variations of the amplitude of the PA signal as a function of the frequency are fitted with the theoretical results through the least squares method using $\beta(\alpha)^{1/2}$ and $(k\rho c)^{-1/2}$ $(1 - \phi\, \lambda\lambda_f^{-1})$ as parameters. As far as the thermal diffusivity and effusivity of the sample are known, both β and $(1 - \phi\, \lambda\lambda_f^{-1})$ can be deduced from PAS.

We are going to describe different experiments where β and ϕ of dyes in different solvents are measured. For instance the measured optical absorption coefficient of methylene blue in water at 600 nm is 1020 $\pm$ 70 cm^{-1} with the phase and 980 $\pm$ 10 cm^{-1} with the amplitude of the PA signal. The optical absorption coefficient of methylene blue in methanol at 600 nm measured from the amplitude : 650 $\pm$ 10 cm^{-1} is close to the measured value by transmission spectroscopy : 620 cm^{-1}. The quantum yield of fluorescence of proflavine 10^{-3} M in water, an acridine used as fluorescent probe in molecular biology, measured at 440 nm is 0.25 $\pm$ 0.03 corresponding to a photoactivity loss of 0.21 and a mean fluorescence wavelength of 520 nm.

Quantitative absorption spectra can be deduced from a PA spectra. In the event of non fluorescent samples, the ratio of the PA spectrum of the studied sample to the PA spectrum of the reference, is equal to the ratio of the saturation values of the signals (measured from their frequency dependences) multiplied by $\beta\mu\ (1 + (1 + \beta\mu)^2)^{-1/2}$. $\beta(\alpha)^{1/2}$ and β can be calculated. Different spectra will be presented, as examples the spectra of blood red cells, in the oxyded and reduced forms, are presented. The samples are plasma depleted, heparinized blood to prevent sedimentation in the cell. The deoxygenated red cells are anaerobically introduced in the PA cell filled with nitrogen. The light scattering by the red cells does not seem to change the frequency dependence of the PA signal as suggested by the comparison with the absorption spectra.



BLOOD : RED CELLS OXY (——) AND DEOXY (---)

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The thermal diffusivity α and the thermal effusivity $(k\rho c)^{1/2}$ can also be measured from PA experiments. If the sample is photoinactive $(k\rho c)^{1/2}$ has to be measured from the saturation value of the PA signal. But a discrepancy has been observed between the measured values of the thermal effusivity and the known ones(4).If the optical absorption coefficient of the sample can be measured by another way, the thermal diffusivity can be deduced from the measurement of $\beta(\alpha)^{1/2}$. For instance the measured value of the thermal diffusivity of methanol: 1.21 $10^{-3}cm^2/s$ is close to the known value : 1.1 $10^{-3}cm^2/s$.

Photoacoustic spectroscopy is an efficient tool to measure optical and thermal properties of opaque and light scattering samples. A precise calibration of the spectrometer and an adequate theoretical model enable the measurement of optical absorption coefficient or spectra, quantum yield of fluorescence and thermal diffusivity. On the contrary, measurements of thermal effusivity by PAS have not succeeded at the time being.

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