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Resonance Rayleigh scattering of cyanine dyes in solution

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Every process of light absorption is inherently associated with a resonance scattering of the incident beam regardless of the nature of the absorbing molecules. Both the real and imaginary parts of the refractive index contribute to the resonance scattering, and marked depolarization is expected to be observed as a rule in the light scattered by complex molecules. The resonance and near-resonance Rayleigh scattering has been measured for a number of cyanine dyes: pseudocyanine, orthochrome T, 1,1'-diethyl-2, 2'-pyridyl-quinolyl cyanine, and pinacyanol chloride, in a number of solvents. In no case was the measured scattering intensity weaker than that expected theoretically, and good agreement between theory and experiment was found as a rule at the blue part of the absorption bands. In contrast, the measured scattering intensities exceeded (by up to fourfold) the theoretically expected values at the red parts of the absorption bands. Possible reasons for this discrepancy are discussed.

INTRODUCTION

Rayleigh scattering is expected to undergo significant enhancement as one approaches the absorption bands of scattering molecules. These changes in intensity are due to the fact that the refractive index varies steeply near absorption band, and additionally the imaginary part of the molecular polarizability starts to manifest its independent contribution to the scattering process. Moreover, for molecules undergoing nondegenerate dipolar transitions, marked depolarization effects should show up at or near the absorption bands, whereas at spectral ranges remote from absorption bands depolarization is less pronounced. Various aspects of these phenomena near or at absorption band have been observed experimentally.¹⁻⁶

The theoretical basis of Rayleigh light scattering at absorption band has been worked out by a number of approaches.³⁻¹⁰ All these treatments yield the same or interconvertible results for isotropic scattering particles. Anglister and Steinberg³⁻⁵ and Stanton *et al.*⁶ have included the effect of the large depolarization of the light scattered at absorption bands, an effect which enhances the scattering cross section by a factor as large as 3, and the Rayleigh ratio at 90° to a polarized incident beam by a factor of 2.4.

The process of light absorption by a medium may be viewed as due to destructive interference between the incident beam and the light scattered by particles in the medium in the forward direction at a π phase shift to the incident beam.^{3-5,10} This view may serve to bring out the important point that *all* absorption process are inherently associated with light scattering and that there is a minimum amount of absorbed light that is invariably reemitted (as a resonance Rayleigh scattering) in every absorption process. However, for chromophores in solution the fraction of the absorbed light which is scattered as Rayleigh scattered light is quite small and may be calculated to be of the order of 4×10^{-6} for light of 500 nm and a chromophore of a molar extinction coefficient of 10^5 cm²/mol. The balance of the absorbed energy is dissipated by a multitude of other radiative, as well as nonradiative, processes.

From the theoretical treatments, the intensities of the

resonance Rayleigh scattering have been calculated to be

$$R_{v,v+H}(90^\circ) = (4000\pi^2 n^2 C / \lambda_0^4 N_A) [(\partial n / \partial C)^2 + (\partial k / \partial C)^2] C_v \\ = \frac{(2.303)^2 \cdot 1000 C n}{N_A} \left\{ \frac{\epsilon^2(\lambda_0)}{4\lambda_0^2} + \left[\frac{1}{\pi} \int_0^\infty \frac{\epsilon(\lambda)}{\lambda_0^2 - \lambda^2} d\lambda \right]^2 \right\} C_v, \quad (1)$$

where $R_{v,v+H}$ is the Rayleigh ratio for a vertically polarized incident beam and the sum of vertically and horizontally polarized scattered light at 90° to the plane of polarization of the incident beam, n is the refractive index of the medium, C is the molarity of the solution, λ_0 is the wavelength of the incident and scattered light, N_A is Avogadro's number, $\partial n / \partial C$ and $\partial k / \partial C$ are, respectively, the increments (per 1 M solute concentration) in the real and imaginary components of the refractive index of the solution due to the scattering particles, $\epsilon(\lambda)$ is the molar absorption coefficient at wavelength λ , and C_v is the Cabannes factor which accounts for the enhancement of the intensity of the scattered light when it is depolarized. C_v is given by¹¹

$$C_v = 3(1 + \rho_v) / (3 - 4\rho_v), \quad (2)$$

where ρ_v is the depolarization ratio measured at 90° to the plane of polarization of the incident beam. The integration in Eq. (1) cannot be of course carried out over the whole spectrum. However, the contribution of remote absorption bands falls off steeply as their frequency diverges from the frequency of interest, and the contribution to n of the absorption band overlapping this frequency is dominant, especially so if the absorption band is an intense one. These considerations also lead to the conclusion that also the real part of the molecular polarizability, similarly to the imaginary part, will be highly anisotropic in the vicinity of a strong absorption band involving a nondegenerate dipolar transition. The quantitative aspects of this problem were treated by Stanton *et al.*⁵

Since the resonance Rayleigh scattering of a molecule varies with ϵ^2 , [see Eq. (1)], whereas the light it absorbs varies with ϵ , the fraction of light absorbed which is scattered increases linearly with ϵ . As mentioned above, however, even for a quite intense absorption of dyes in solution of $\epsilon = 10^5$ cm²/mol the fraction of the light scattered is of the order of 4×10^{-6} . Under such

circumstances the scattered light intensity due to the dye will be one-to-two orders of magnitude smaller than the scattered light intensity of the solvent for an optical part of the order of 1 cm, which poses a severe experimental difficulty. This problem has been tackled by measuring the depolarized component of the scattered light. Due to the considerable anisotropy of the solute dye molecules their depolarization ratio may be an order of magnitude larger than that of the solvent,^{4,6} if solvents are chosen which exhibit a low depolarization ratio (2% or less). The scattering of the solvent, which is predominantly polarized, was exploited³ as an internal calibration measure to correct for the attenuation by absorption of the incident and scattered light beams. This attenuation is difficult to evaluate by computation since it depends on geometric factors of the experimental setup. The attenuation is identical for the polarized and depolarized scattered light (since the solution is isotropic) and the former can serve as a standard for the latter.

In the following we present the measurement of the Rayleigh scattering of a series of cyanine dyes at their absorption band in the visible spectrum. These dyes were chosen because of the experimental advantages which they offer. They exhibit intense absorption at their visible absorption band, which enhances their resonance Rayleigh scattering. Furthermore, the transition responsible for this band is dipolar, resulting in a large depolarized light scattering. They offer a choice of compounds which are nonfluorescent and of absorption bands of different location in the visible range, the latter property permitting the investigation of different parts of the absorption bands with a given light source. They are also soluble in polar solvents, such as water and short chain alcohols, which show low depolarization ratios in their scattered light. Reasonable agreement between the measured scattering intensities and the values expected theoretically is generally found in the blue part of the absorption bands, but appreciable deviations are observed in the red part of the bands. Possible causes for this behavior will be considered and discussed.

EXPERIMENTAL

Method of measurement

As mentioned above, the information about the light scattering was extracted from the data on the depolarization ratio of the solution $\rho_{v,sn}$ and of the solvent $\rho_{v,st}$. The following expression was obtained⁴ for the Rayleigh ratio $(R_{v,H})_d$ for the depolarized scattering by the dye (the incident light is assumed to be vertically polarized) in terms of the Rayleigh ratio for total scattering by the solvent $(R_{v,v+H})_{st}$:

$$\frac{(R_{v,H})_d}{(R_{v,v+H})_{st}} = \frac{\rho_{v,sn} - \rho_{v,st}}{1 - (\rho_{v,sn}/\rho_{v,d})} \cdot \frac{1}{1 + \rho_{v,st}}, \quad (3)$$

$R_{v,H}$ obtained by Eq. (3) is related to $R_{v,v+H}$ [see Eq. (1)] by the identity

$$R_{v,H} = R_{v,v+H} \cdot \rho_v / (1 + \rho_v). \quad (4)$$

Thus, Rayleigh ratio of the dye [Eq. (3)] may be evaluated by the measurement of $\rho_{v,st}$ and $\rho_{v,sn}$. The absolute depolarized scattering intensity of the solvent $I_{1,st}$,

or its Rayleigh ratio, should be known, information which can be obtained from the published literature in conjunction with the measurements on $\rho_{v,st}$. In addition, a knowledge of $\rho_{v,d}$ is required, which was measured previously⁴ and in this study to be very close to 1/3 (except at the blue edge of the absorption band, where $\partial n/\partial C$ may assume negative values),^{4,6} in agreement with the theoretical expectations for the dipolar transitions shown to be involved in the absorption bands studied.^{12,13} More detailed consideration of the value chosen for $\rho_{v,d}$ will be given in the Discussion section.

Instrumentation

The instrument used for the study of light scattering at absorption bands is illustrated in Fig. 1. The light source for the measurements at the spectral range 450–515 nm was an argon-ion laser, (Spectra Physics, 164-09) which has nine lasing lines in the visible with polarization better than 50:1. For the measurements at longer wavelengths a dye laser (Spectra Physics model 375) pumped by a high power argon laser (Spectra Physics model 171-019) was used. The dyes used were coumarin 6 (for the spectral range 517–575 nm) and rhodamine 6G (for the spectral range 565–640 nm). One circular aperture (of 2 mm diameter) was placed in the way of the incident laser beam. The scattered light was measured at 90° to the plane of polarization of the incident light. Three circular apertures, of 3 mm diameter, were placed collinearly 6 cm apart in the viewing optics in order to define a small acceptance angle. Because of the small depolarization of the scattered light from both the solvents and the solutions studied, large acceptance angles may interfere with the measurement of the depolarized scattering.¹⁴ The scattered light was modulated by an elasto-optic modulator (Hinds PEM-3) equipped with two Glan-Thompson polarizers which exhibit an extinction ratio better than 10⁻⁵. The polarizer in front of the elasto-optic element defined the polarization component of the scattered light, whereas the second one was set for total extinction when the modulator was at rest. The modulated scattered light was focused on the entrance slit of a Jarrel-Ash 82-410, 0.25 m double monochromator set to a bandwidth of 3.2 nm (unless otherwise stated), and was detected by an EMI 9863B photomultiplier cooled to -20 °C. The signal was then amplified by a phase sensitive amplifier (Ithaco, Dynatrac 391-A), which received a reference signal from the modulator. Readings were corrected for the different transmission of the monochromator and the different response of the photomultiplier to light of different polarization (see below). The monochromator served to reject Raman and resonance Raman scattering and any possible interference from fluorescence. The maximum intensity of the incident laser beam was 300 mW and measurements were carried out quickly (in less than 60 s) in order to reduce effects of possible bleaching of the dye or turbulence caused by local heating of the solution.

The scattering spectrophotometric cells were cleaned by the method of Dubin *et al.*,¹⁵ and the solutions were cleared of particulate matter by filtering through milli-

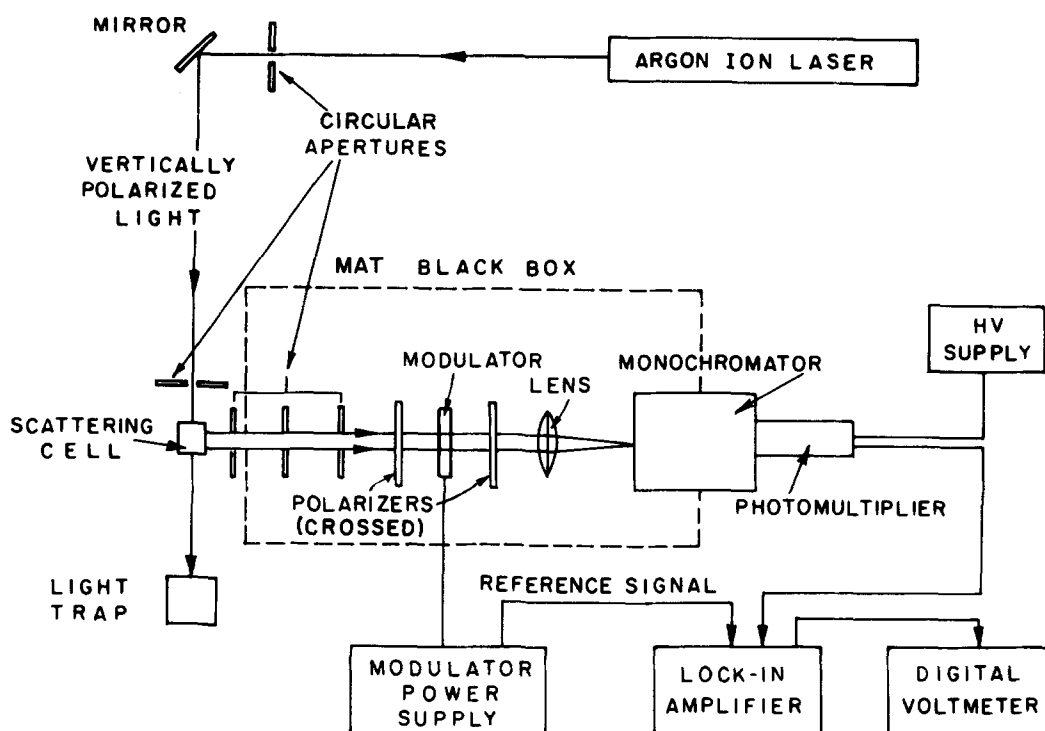


FIG. 1. Block diagram of the instrument used for the measurement of the depolarization ratio of the light scattered from the dye solutions.

pore filters (0.22μ) prior to measurement. All experiments were performed at room temperature ($\sim 23^\circ\text{C}$).

Calibration of the apparatus

The polarization of the system comprising the monochromator and photomultiplier has to be known in order to correct the measured depolarization ratio of the scattered light. The calibration of this system was carried out by measuring the depolarization ratio of completely unpolarized light that is transmitted through the system. We found it to be very convenient to produce such unpolarized light by excitation of a suitable fluorescent dye by a mercury arc lamp. The light of a 200 W high pressure mercury lamp was filtered by a solution of 200 g/l $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in a mixture of 70% ethanol and 30% water containing also 1.0 mol/l HCl (3 cm optical path)¹⁶ and by a colored glass filter UG-11 (Schott) in series. The combination of these filters has very good transmittance in the spectral range 270–370 nm and blocks completely the visible light produced by the mercury lamp. The polarization (I_V/I_H) of the transmitted mercury light was found to be 1.06. The transmitted light was then focused on a fluorescence cell containing 10^{-3} M quinine (Fluka 99%) in 1 N H_2SO_4 . Quinine sulfate has a very broad fluorescence band and enabled us to calibrate the system in the range 390–645 nm. The UV light that passed through the quinine sulfate solution was blocked by 1 cm of 2 M NaNO_2 aqueous solution that was placed in front of the monochromator. The polarization of the fluorescence (I_V/I_H) when excited by fully polarized light was found to be less than 1.02 (due to fast rotatory diffusion during the lifetime of the excited state). For fluorescence detected at 0° to the exciting

mercury light beam, I_V/I_H of the fluorescence is expected to be less than 1.001, and can thus be considered to be practically completely unpolarized. The polarization of the detection system was then calibrated by using this fluorescent light and placing the front polarizers of the modulator in the vertical and horizontal positions.

The absorption spectra of the dye solutions in the wavelength range 200–700 nm were measured on a Carl-Zeiss PMQ II spectrophotometer. Continuous curves were fitted to the spectra by a computer cubic spline fit program. The integral in Eq. (1) is thus rendered analytic for each spline, provided the borders of the spline do not coincide with λ_0 . The integration over λ was carried out over the range of 200–700 nm and the integral was evaluated for λ_0 values in the same spectral range. The far absorption bands in the UV and IR most probably contribute a relatively small value to n in the region of the visible absorption bands, which are very intense in the cases studied, and this contribution is probably constant in the range of the visible bands. This point will be further discussed below.

Materials

Pseudocyanine (1,1'-diethyl 2,2'-cyanine iodide) (I), an Eastman product, was recrystallized from ethanol. Orthochrome T (II), an Eastman product, of purity not less than 98% was recrystallized twice from *n*-propanol. 1,1'-diethyl 2,2'-pyridyl quinolylcyanine (PQ) (III), a Koch Light product of purity better than 95%, was recrystallized from *n*-propanol. Pinacyanol chloride (1,1'-diethyl 2,2'-carbocyanine iodide) (IV), an Eastman product of purity not less than 98%, was recrystallized

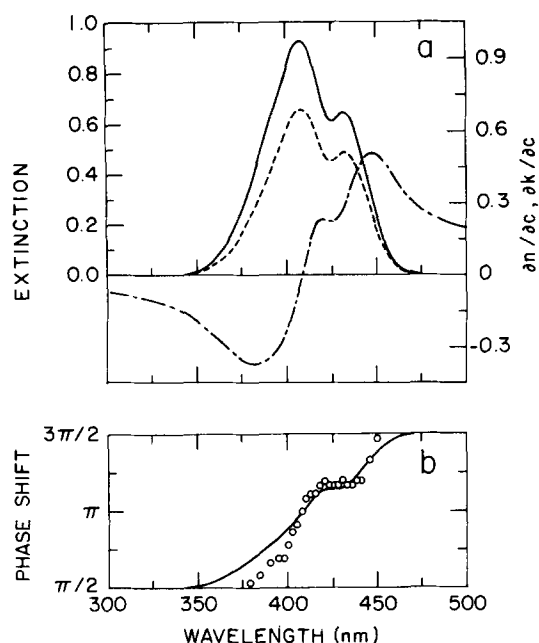
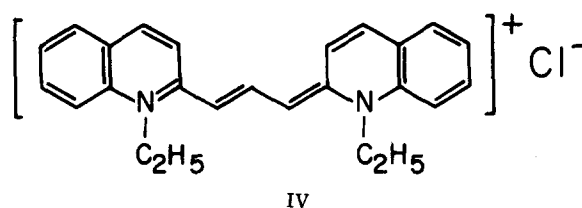
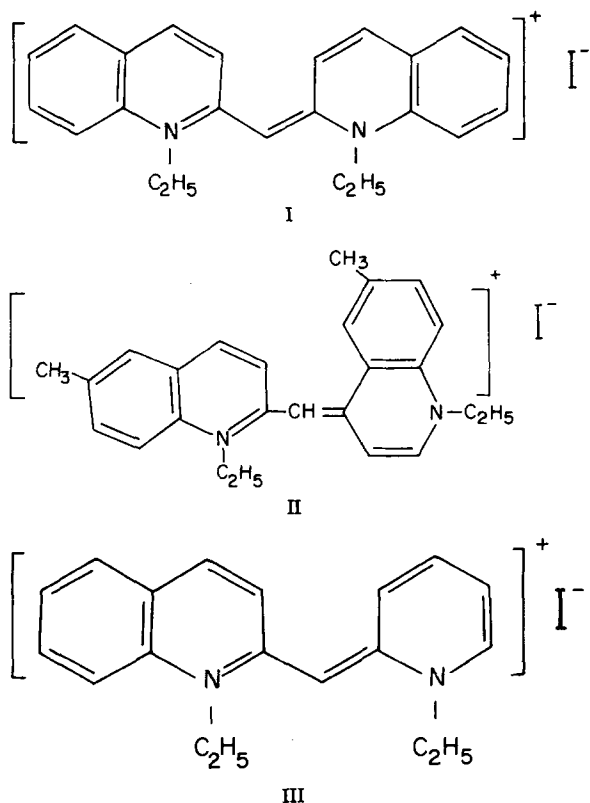


FIG. 2. Comparison between the phase shift of light reflected from a monolayer of NN' distearylthiacyanine measured by Drexhage (Ref. 17) and that evaluated from $\partial n/\partial C$ and $\partial k/\partial C$ of the dye as calculated by us from the absorption spectrum of the dye. a. —, the absorption spectrum of the dye (Ref. 17) (in arbitrary units); - - -, the calculated $\partial n/\partial C$; ··· the calculated $\partial k/\partial C$. b. —, the phase shift calculated from $\partial n/\partial C$ and $\partial k/\partial C$. o, the measured phase shift from a monolayer of the dye (Ref. 17).

from 90% ethanol in water. All dyes were dried in vacuum at 105 °C after recrystallization. Methanol, ethanol, and isopropanol were spectrograde, and water was double distilled.



RESULTS

Light scattering within the absorption bands was studied for a number of cyanine dyes: Pseudocyanine (I), orthochrome T (II), 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine iodide (III), and pinacyanol chloride (IV). These dyes have very high absorption coefficients in their bands in the visible spectrum and, except for pinacyanol chloride, are practically nonfluorescent.

The scattering intensities of the dyes were measured as described above. The results are compared with the theoretically expected values for the Rayleigh scattering from the dyes studied. The expected out-of-phase contributions and in-phase contributions were obtained by Eq. (1), as described in the Experimental section. In the figures to be presented below both the real and imaginary parts of the theoretically calculated refractive index are plotted [in part (a) of the figures], as well as their expected contributions to the Rayleigh ratio [in part (b) of the figures]. The phase shift of the scattered light may be obtained from $\partial n/\partial C$ and $\partial k/\partial C$. Although we have no experimental data about the phase shift, it is nevertheless a measurable quantity, as was demonstrated by Drexhage.¹⁷ A comparison of values of the phase shift calculated from the absorption spectrum of NN' distearylthiacyanine with the data obtained by Drexhage is illustrated in Fig. 2.

A few of the dyes were studied in more than one solvent. It should be noted that there is a limited choice of solvents which can be used for the investigation of solvent effects on light scattering at absorption bands, since the solvent should be a poor scatterer by itself and should exhibit a low depolarization ratio in its scattered light. Otherwise, the solvent scattering may mask the scattering by the solute, since low solute concentrations are mandatory when one studies light scattering at absorption bands by the method described. Suitable solvents in the case of cyanine dyes are methanol, ethanol, and isopropanol (trifluoroethanol and hexafluoroisopropanol are also suitable but were not used). Water can be used for those dyes which do not aggregate in this solvent at the concentration at which the measurements are performed.

Pseudocyanine (I)

Light scattering measurements on pseudocyanine were carried out in methanol solution in the spectral range 450–590 nm. The results of both the measured intensities of the depolarized light scattered by this dye and the theoretically expected values are described in Fig. 3. The absorption spectrum $\partial n/\partial C$ and $\partial k/\partial C$ in the spectral range 400–600 nm are presented in Fig. 3(a); the individual contributions of $\partial n/\partial C$ and $\partial k/\partial C$, as

well as their summed contribution, to the scattering are given in Fig. 3(b). A good agreement between the experimental and the theoretically calculated values is observed in the blue part of the absorption band. However, in the red part of the absorption band the measured intensity of the scattered light exceeds by 130% the calculated intensity, the position of the maximum scattering coinciding in the measured and calculated curves. It should be noted that beyond the absorption band towards longer wavelengths the agreement between the measured and theoretical curves becomes excellent.

The Rayleigh scattering of pseudocyanine in water solution at its visible absorption band is described in Fig.

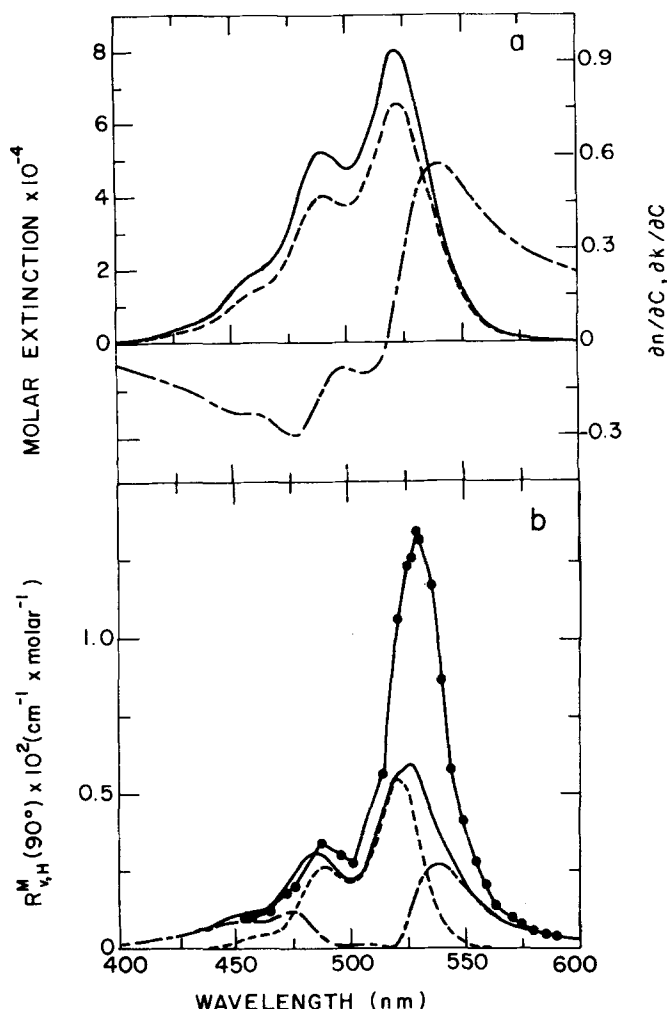


FIG. 3. Spectroscopic and scattering data for pseudocyanine in methanol. Scattering experiments in the wavelength range 450–515 nm were carried out on 4.08×10^{-5} M dye solutions, in the wavelength range 520–545 nm on 2.22×10^{-5} M solutions, and in the wavelength range 550–575 nm on 1.11×10^{-4} M solutions. a. —, the molar extinction of pseudocyanine in methanol; ---, the calculated $\partial n/\partial C$; ---, the calculated $\partial k/\partial C$. b. ●, the experimental Rayleigh ratio $R_{v,H}^M(90^\circ)$ expressed per 1 M concentration of pseudocyanine; ---, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; ---, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

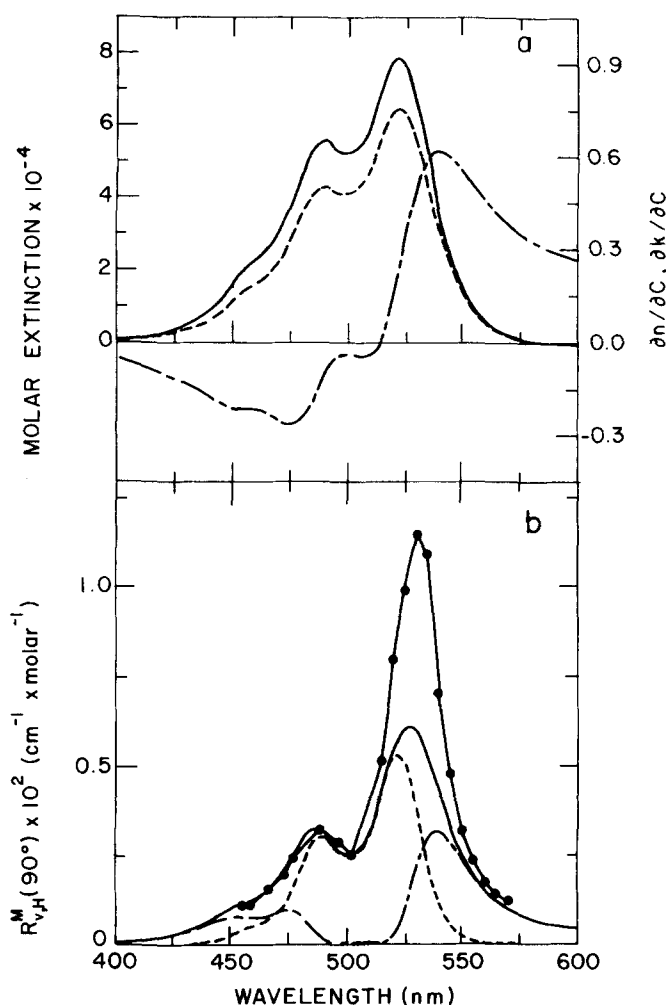


FIG. 4. Spectroscopic and scattering data for pseudocyanine in water. Scattering experiments in the wavelength range 450–515 nm were carried out on 2.2×10^{-5} M dye solutions, in the wavelength range 515–555 nm on 1.51×10^{-5} M solutions, and in the wavelength range 555–570 nm on 3.77×10^{-5} M solutions. a. —, the molar extinction of pseudocyanine in water; ---, the calculated $\partial n/\partial C$; ---, the calculated $\partial k/\partial C$. b. ●, the experimental Rayleigh ratio $R_{v,H}^M(90^\circ)$ expressed per 1 M concentration of pseudocyanine; ---, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; ---, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

4. Also in this case there is a very good agreement between the experimental results and those expected from theory at the blue part of the absorption band as well as at the extreme red edge of the band and outside the absorption band on its long wavelength side. At the red part of the absorption band the experimentally measured scattering intensity exceeds the theoretically calculated values, as was also found in the methanol solution; the deviation is however somewhat smaller in water (100% deviation) than in methanol (130% deviation).

Orthochrome T (II)

The absorption spectra of orthochrome T in methanol, ethanol, and isopropanol are presented in Figs. 5(a),

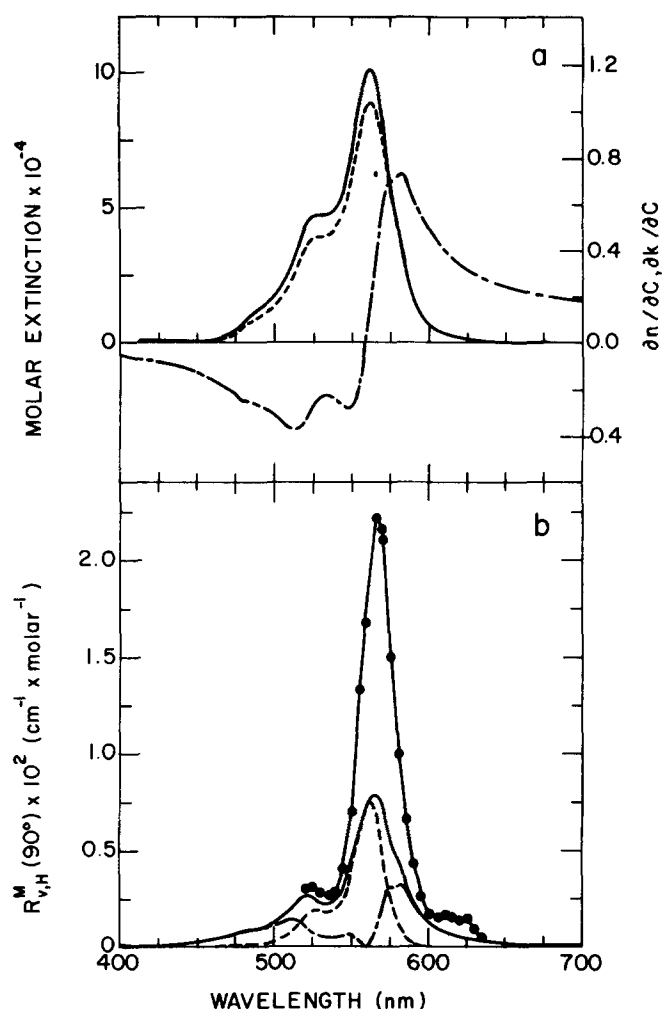


FIG. 5. Spectroscopic and scattering data for orthochrome T in methanol. Scattering experiments in the wavelength range 520–545 nm were carried out on 4.10×10^{-5} M dye solutions, in the wavelength range 550–570 nm on 1.14×10^{-5} M solutions, and in the wavelength range 570–640 nm on 1.75×10^{-5} M solutions. a. —, the molar extinction of orthochrome T in methanol; ---, the calculated $\partial n/\partial C$; - - -, the calculated $\partial k/\partial C$. b. ●, the experimental Rayleigh ratio $R_{\nu,H}^M(90^\circ)$ expressed per 1 M concentration of orthochrome T; ---, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; - - -, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

6(a), and 7(a), respectively, along with the calculated spectra of $\partial n/\partial C$ and $\partial k/\partial C$. The measured molar Rayleigh ratios for depolarized scattered light in the above solvents are given in Figs. 5(b), 6(b), and 7(b). In the same figures are also plotted the contributions of $\partial n/\partial C$ and $\partial k/\partial C$ to the depolarized scattered light as calculated from theory, as well as their summed scattering. In all three solvents, the measured intensities exceed the theoretically calculated ones. At the blue part of the absorption band the deviations are relatively minor (20%–25%), whereas at the red part of the band much higher deviations are observed, which depend significantly on the solvent used (190% in methanol, 280% in ethanol, and 390% in isopropanol). It should be noted that there are almost no differences between the ab-

TABLE I. The depolarization ratio of the scattering from orthochrome T at 570 nm in methanol solution as a function of concentration.

Concentration ($C \times 10^5$) M	Depolarization ratio ($\rho_{\nu,H}$)
0.657	0.0416
0.939	0.0504
1.126	0.0566
1.314	0.0618
1.502	0.0664
1.877	0.0753
2.065	0.0826
2.253	0.08725
2.440	0.0932
2.628	0.0964
2.816	0.0997

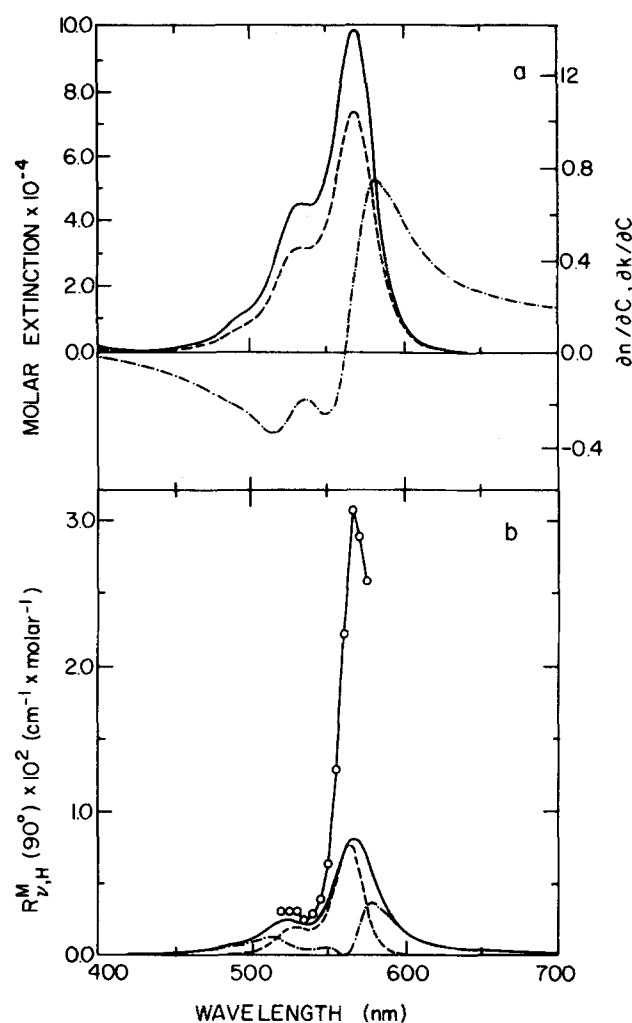


FIG. 6. Spectroscopic and scattering data for orthochrome T in ethanol. Scattering experiments in the wavelength range 515–545 nm were carried out on 5.26×10^{-5} M solutions and in the wavelength range 549–575 nm on 1.58×10^{-5} M solutions. a. —, the molar extinction of orthochrome T in ethanol; ---, the calculated $\partial n/\partial C$; - - -, the calculated $\partial k/\partial C$. b. ○, the experimental Rayleigh ratio $R_{\nu,H}^M(90^\circ)$ expressed per 1 M concentration of orthochrome T; ---, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; - - -, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contribution of $\partial n/\partial C$ and $\partial k/\partial C$.

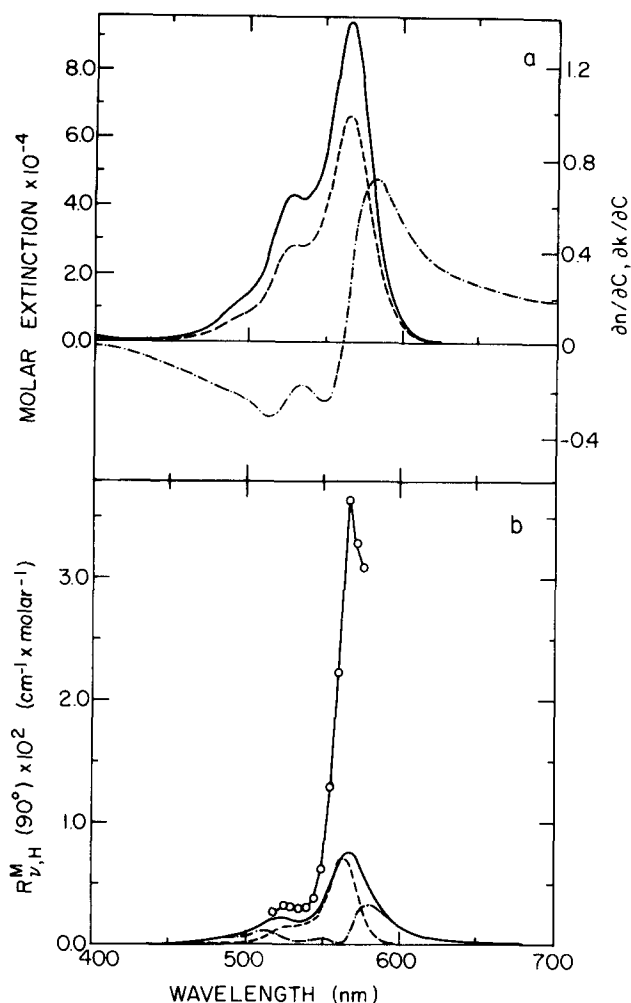


FIG. 7. Spectroscopic and scattering data for orthochrome T in isopropanol. Scattering experiments were carried out on 1.0×10^{-5} M dye solutions in wavelength range 517.6–545 nm and on 1.64×10^{-5} M solutions in the wavelength range 549–575 nm. a. —, the molar extinction of orthochrome T in isopropanol; ---, the calculated $\partial n/\partial C$; - - -, the calculated $\partial k/\partial C$. b. o, the experimental Rayleigh ratio $R_{v,H}^M(90^\circ)$ expressed per 1 M concentration of orthochrome T; - - -, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; ---, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

sorption spectra of orthochrome T in these alcoholic solvents. It may be noted that the resonance scattering of 1,1'-diethyl 2,4'-cyanine iodide (which is similar in structure to orthochrome T except that it lacks the methyl groups on the quinoline rings) in methanol, was also measured. The spectrum of the scattering intensities and the extent of fit to the theoretically calculated intensities were similar to those of orthochrome T in the same solvent.

The depolarization ratio of orthochrome T was measured by the method described previously.⁴ By this method the depolarization ratio of the dye is evaluated by a reiteration procedure from the measured depolarization ratios of dye solutions at various solute concentrations. The measured depolarization ratios of orthochrome T in methanol at 570 nm are presented in

Table I. From the data presented in this table a value of 0.325 ± 0.025 was obtained for the depolarization ratio of orthochrome T at the above experimental conditions. This value is identical within experimental error with the value of $1/3$ which is expected for a dipolar transition.

1,1'-Diethyl-2,2'-pyridyl-quinolyl-cyanine (III)

The absorption spectra of compound (III) in the spectral range of 400–600 nm in aqueous and methanol solutions are presented in Figs. 8(a) and 9(a), respectively. The spectral shape of the visible absorption band is quite similar in both solvents, but the absorption inten-

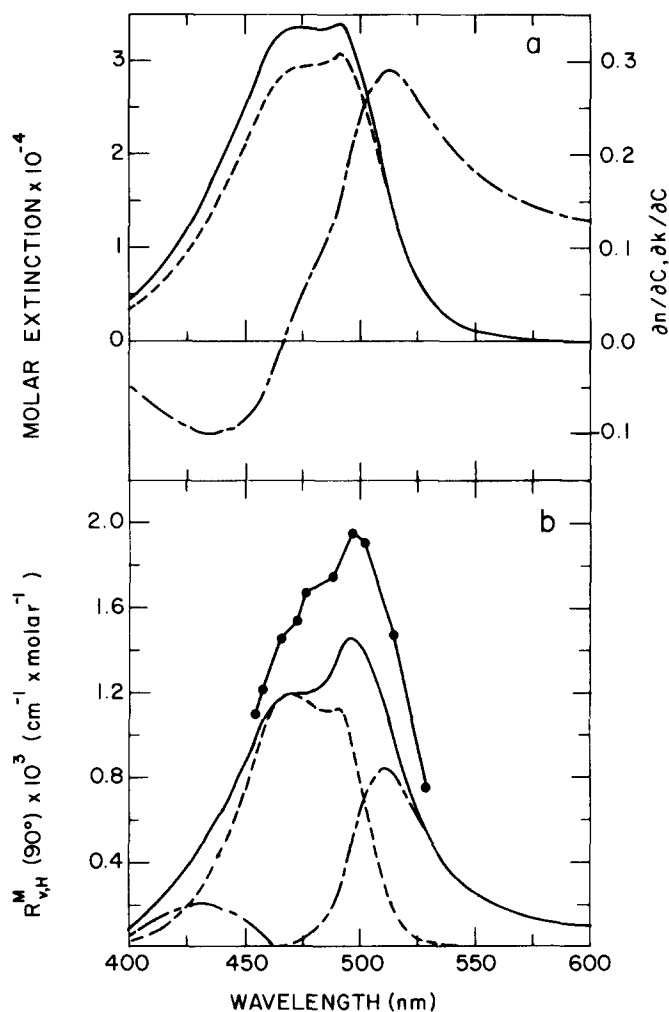


FIG. 8. Spectroscopic and scattering data for 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine in methanol. Scattering experiments in the wavelength range 454–515 nm were carried out on 8.59×10^{-5} M dye solutions, in the wavelength range 520–541 nm on 9.74×10^{-5} M solutions, and in the wavelength range 545–570 nm on 3.25×10^{-4} M solutions. a. —, the molar extinction of 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine in methanol. ---, the calculated $\partial n/\partial C$; - - -, the calculated $\partial k/\partial C$. b. ●, the experimental Rayleigh ratio $R_{v,H}^M(90^\circ)$ expressed per 1 M concentration of 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine; - - -, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; ---, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

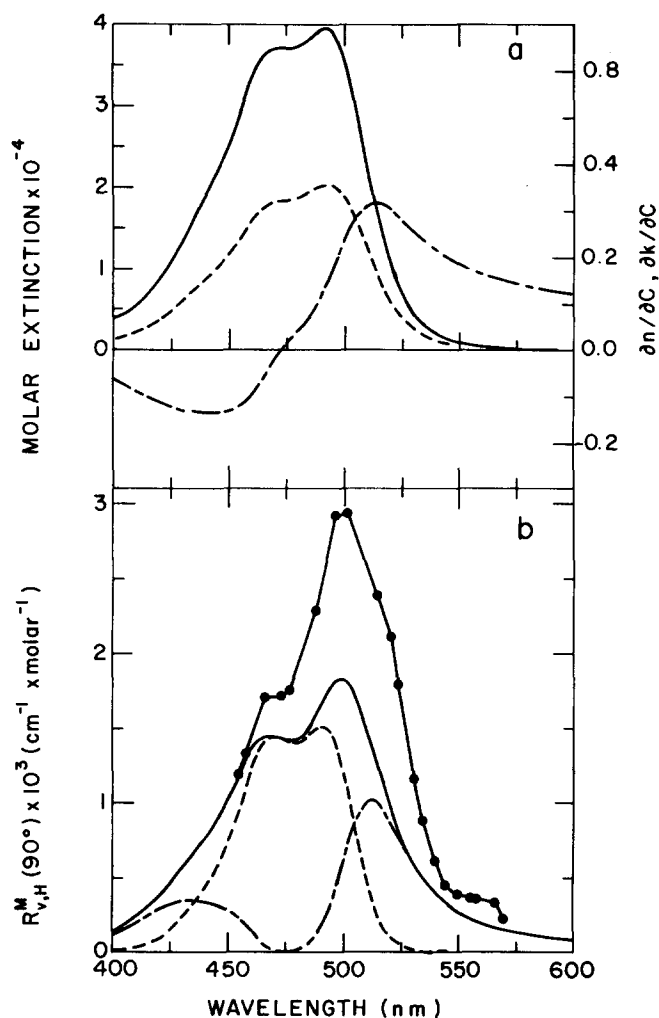


FIG. 9. Spectroscopic and scattering data for 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine in water. Scattering experiments were carried out at the concentration of 6.13×10^{-5} M. a. —, the molar extinction of 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine in water; ---, the calculated $\partial n/\partial C$; ---, the calculated $\partial k/\partial C$. b. ●, the experimental Rayleigh ratio $R_{v,H}^M(90^\circ)$ expressed per 1 M concentration of 1,1'-diethyl 2,2'-pyridyl quinolyl cyanine; ---, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; ---, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

sity is somewhat higher in the methanol solution. The calculated spectra of $\partial n/\partial C$ and $\partial k/\partial C$ are also included. The measured depolarized scattering intensity is described in Figs. 8(b) and 9(b), along with the expected intensities as computed by the theoretical treatment presented above (the individual contributions of $\partial n/\partial C$ and $\partial k/\partial C$ to the scattering intensity are also included in Figs. 8(b) and 9(b)). The agreement between the measured and expected scattering intensities is reasonably good in the blue part of the absorption band, but deviations are observed in the red part of the band. The observed scattering intensity at the scattering peak exceeds the theoretical one by about 35% in water and 65% in methanol. In both cases, however, the positions of the maximum in the experimental and theoretical scattering spectra practically coincide.

Pinacyanol chloride (IV)

Unlike the above dyes, pinacyanol is fluorescent. Fluorescence was found to strongly interfere with the scattering measurements above 600 nm, but was negligible below 580 nm. Interference by fluorescence was detected by shifting the wavelength of transmittance of the monochromator away from the wavelength of the incident light towards the red. If fluorescence was present, the measured intensity increased upon doing so. In contrast, if the maximum of the scattered light coincided in wavelength with the incident light, interference by fluorescence was considered to be small.

The absorption $\partial n/\partial C$ and $\partial k/\partial C$ of pinacyanol chloride in methanol solution are presented in Fig. 10(a). The measured light scattering intensities are presented in Fig. 10(b), along with the theoretically expected intensities and the individual contributions of $\partial n/\partial C$ and $\partial k/\partial C$ to the scattering. Reasonable agreement can be

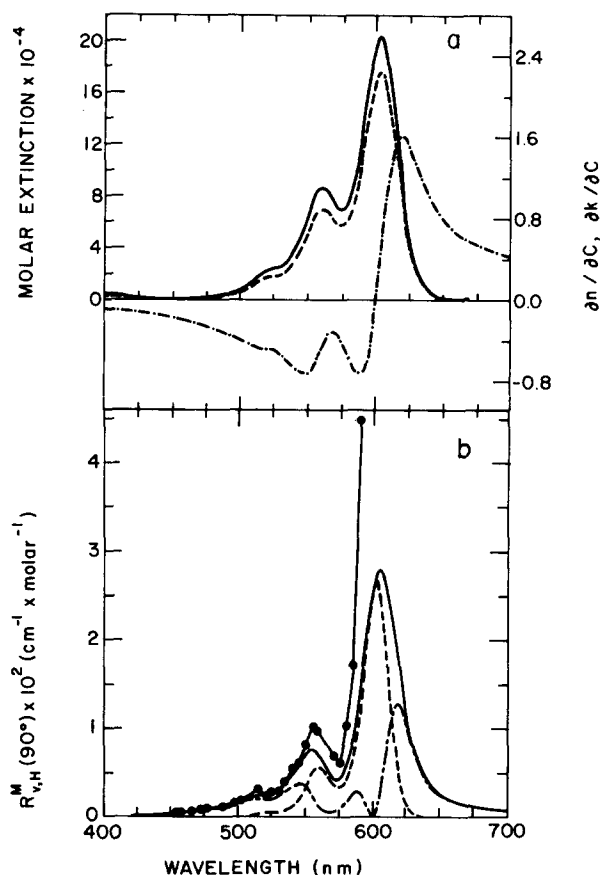


FIG. 10. Spectroscopic and scattering data for pinacyanol chloride in methanol. Measurements in the wavelength range 450–490 nm were carried out on 2.87×10^{-4} M dye solutions, in the wavelength range 490–515 nm on 7.17×10^{-5} M solutions, in the wavelength range 520–541 nm on 4.37×10^{-6} M solutions, in the wavelength range 545–570 nm on 1.31×10^{-5} M solutions, and in the wavelength range 570–640 nm on 1.16×10^{-5} M solutions. a. —, the molar extinction of pinacyanol chloride in methanol; ---, the calculated $\partial n/\partial C$; ---, the calculated $\partial k/\partial C$. b. ●, the experimental Rayleigh ratio $R_{v,H}^M(90^\circ)$ expressed per 1 M concentration of pinacyanol chloride; ---, the calculated contribution of $\partial n/\partial C$ to the molar Rayleigh ratio; ---, the calculated contribution of $\partial k/\partial C$ to the molar Rayleigh ratio; —, the sum of the calculated contributions of $\partial n/\partial C$ and $\partial k/\partial C$.

seen between the measured and theoretically expected light scattering intensities at the blue part of the absorption band between 540 to 580 nm, and the agreement is excellent below 540 nm. Above 580 nm the contribution of fluorescence to the observed intensity becomes very high, the measured intensities becoming 30-fold higher than the expected ones for scattering above 600 nm.

DISCUSSION

Absorption of light by molecules is invariably associated with elastic scattering regardless of the nature of the absorbing molecules. It is instructive to calculate the fractional amount thus scattered relative to the light absorption. As shown above, the scattering intensity varies with ϵ^2 , whereas the light energy absorbed per unit time varies linearly with ϵ for a given incident light intensity; the fraction of absorbed light which is scattered elastically is thus proportional to ϵ . The highest values attainable for ϵ of molecules in solution are of the order of $10^5 \text{ M}^{-1} \text{ cm}^{-1}$. For such a value of ϵ , the fraction of light absorbed which is scattered elastically is about 4×10^{-6} (at $\lambda = 514 \text{ nm}$), a small quantity, but which is invariably present. The balance of the absorbed energy is dissipated by a variety of other emissive, as well as by nonemissive, processes in a way characteristically dependent on the nature of the absorbing molecules and their physical and chemical environment.

The light scattered by a few cyanine dyes at the wavelength of the incident beam was measured in a few solvents. In no case was the intensity of the scattered light found to be smaller than that calculated theoretically. This is in accord with the above statements that the elastic scattering is inherently associated with light absorption and illustrates experimentally the consequences of the optical theorem. Furthermore, at the blue part of the absorption bands the agreement between the measured scattered intensity and the theoretically expected intensity is generally good and sometimes excellent. It should be noted that no adjustable parameters were used in the comparison between the experimental and theoretical data. In contrast to the findings at the blue part of the spectrum, the measured scattering intensity at the red part of the absorption band appreciably exceeded the theoretically expected values in all cases studied. The discrepancy varied from 35% to 400% depending on the particular system in question. The cause of the above discrepancy has not been pinpointed down with any certainty, but the following considerations may serve to narrow down the range of possible causes of the above behavior.

In the evaluation of the Rayleigh ratio for light scattering at absorption bands by the method used by us, prior knowledge of the depolarization ratio $\rho_{v,a}$ of the solute dye is required [see Eqs. (3) and (4)]. From the nature of the molecules studied and the intense absorption involved it is plausible to deduce that the corresponding electronic transitions are dipolar. The diagonalized tensor of the imaginary part of the molecular polarizability thus contains only one nonvanishing term, and for that part of scattered light which is due to the imaginary part of the polarizability one expects $\rho_{v,a}$ to be 1/3. The situation is somewhat more complicated as

regards the depolarization ratio of the scattered light due to the real part of the molecular polarizability. While the absorption band under discussion has a dominant contribution to the real part of the molecular polarizability at the spectral range of this band, the effects of the contributions of remote absorption bands on $\rho_{v,a}$ may not be negligible at wavelengths just outside the absorption band.^{4,6} Stanton *et al.*⁶ have treated this problem theoretically in detail for Gaussian absorption bands and have evaluated the expected values for $\rho_{v,a}$ at a wide spectral range assuming the value of $\rho_{v,a}$ at the red edge of the band is known. The latter quantity contains information on the relative contributions of the visible band and the spectrally removed bands to the real part of the molecular anisotropy. These calculations show that if the value of $\rho_{v,a}$ at the red edge of the band is close to 1/3 it stays nearly constant through the band except at wavelengths at the extreme blue side of the band, where $\rho_{v,a}$ increases steeply. (This steep rise in $\rho_{v,a}$ was actually observed experimentally⁴.) The depolarization ratio at the red edge of the absorption band was measured by us directly in a few cases, i.e., β -carotene,⁴ lycopene,⁴ and orthochrome T (this study) and was found to have a value very close to 1/3. We may thus conclude that, except possibly at the high energy side of the absorption bands, the value of 1/3 employed by us for $\rho_{v,a}$ throughout the absorption bands is plausible.

The real part of the refractive index of the solute $\partial n / \partial C$ contributes significantly to the light scattering intensity, in addition to the contribution of the imaginary part. Unfortunately, $\partial n / \partial C$ could not be measured directly at the absorption band since the light is practically blocked even at low solute concentrations. Resort was therefore taken to the use of the Kramers-Kronig equation [see Eq. (1)] for the evaluation of $\partial n / \partial C$; but for practical reasons the integration was carried out over the limited spectral range of 200–700 nm. It is felt, however, that this restricted integration, which ignores contributions from far removed bands, does not affect the calculated scattering intensity significantly. Strong experimental support for this comes from the measurements performed at the extreme red edge of the absorption bands at which only $\partial n / \partial C$ contributes to the scattering. In some cases nearly perfect agreement was obtained between the measured and theoretically expected intensities (see, for example, Figs. 3 and 4 of this paper for pseudocyanine, and Fig. 1 of Ref. 5 for lycopene). Furthermore, as discussed above, the values obtained for $\rho_{v,a}$ at the red edge of the absorption bands, which were very close to 1/3, also indicate that contributions from far removed bands to $\partial n / \partial C$ are small compared to the contribution of the visible band under consideration.

The discrepancies observed between the measured and calculated intensities of light scattering at the red part of the absorption band cannot be attributed to the presence of aggregates of the solute dye molecules. Such aggregates, if present, could in principle account for the discrepancies since they have a high contribution to the scattering intensity and may have absorption spectra which are different from the monomeric spectra. However, in the range of concentrations studied (10^{-4}

10^{-5} M) the experimentally determined Rayleigh ratios varied linearly with solute concentrations. Incidentally, the fraction of scattered light was not dependent on the intensity of the incident beam in the range studied (100–400 mW) which indicates that effects due to nonlinear behavior or induced emission do not play a role in the experiments performed.

It is difficult to eliminate completely the presence of contributions from fluorescence to the measured scattering intensities. However, such a contribution, if present, does not seem to be large. As stated in the Results section, the measured scattered intensity drops at wavelengths at the immediate vicinity, but differing from that of the incident beam, for all dyes measured except for pinacyanol chloride above 580 nm. This is a very strong indication against the presence of significant contributions of fluorescence to the scattering of all the dyes considered nonfluorescent. Similarly, the effect of temperature on the scattering intensity seems to indicate that it is not fluorescence which is responsible for the discrepancies found between experiment and theory at the red part of the absorption bands. Thus, the discrepancies found for orthochrome T at room temperature and 59 °C were practically the same within experimental error, whereas Gillbro and Sundström¹⁸ have reported that such a temperature rise produced a marked drop (by a factor of 2) in the lifetime of a fluorescent cyanine dye, pinacyanol, which means a marked drop in quantum yield. For orthochrome T the above discrepancies increase on changing the solvents from methanol to ethanol or propanol with respective viscosities of 0.6, 1.7, and 2.4 cp at 20 °C. As studied with fluorescent cyanine dyes,¹⁹ an increase in viscosity increases the fluorescence intensity by reducing the degree of quenching. In contrast, for β -carotene the discrepancies at the red part of the absorption bands are not significantly different in the two solvents pentane and cyclohexane⁹ although their viscosities differ by nearly a factor of 4. Thus, fluorescence cannot be invoked as the explanation for the effects of the solvents on the above discrepancies.

As was explained above, in all the experiments performed we used a bandpass of 3.2 nm for the monochromator at the detection side of the scattered light. This was necessary in order to include all the depolarized scattered light of the solvent, which served as an internal standard to compensate for the marked absorption losses in the medium. Under such circumstances some inelastically or incoherent scattered light may interfere with the measurements of the Rayleigh scattering. To account for the observed deviations between the measured and theoretically expected scattering intensities in these terms one will have to assume that for a reason as yet unexplained there is more inelastic scattering when the incident beam coincides in wavelength with the red part than with the blue part of the absorption spectrum. It should be noted that the possibility exists that the observed light scattering is in fact elastic scattering, but that the discrepancies between the experimental and theoretically expected results, whenever observed, is only apparent, and is due to the momentary heterogeneity of the solute molecules as regards their

extinction coefficient at the wavelength of the incident beam. Since the scattering intensity depends on $\langle\epsilon^2\rangle$, whereas the absorption intensity varies as $\langle\epsilon\rangle$, it is obvious that in heterogeneous systems the scattering intensity will exceed the theoretical intensity evaluated from $\langle\epsilon\rangle^2$, since $\langle\epsilon^2\rangle$ is greater than $\langle\epsilon\rangle^2$ under such circumstances. Various molecules may at any given instant assume different values for ϵ due to differences in conformation or solvation. A special source of heterogeneity in ϵ may occur at the red edge of the absorption band, where transitions occur from thermally excited molecules in the electronic ground state. Obviously not all molecules are equally thermally excited at any given instant since the excited population is expected to follow a Boltzmann distribution; a heterogeneity in ϵ at the red edge of the band thus seems likely. This will explain the marked excesses in scattering intensities observed at the red part of the absorption bands in all the cases studied.

Besides the intrinsic interest in the scattering behavior of molecules at their absorption bands, such studies may be applied to a variety of problems. Thus, the degree of aggregation of dye molecules in solution should be readily obtainable from the intensities of their light scattering since the scattering intensity depends on the molar extinction coefficient, the molecular weight being that of the aggregate. The extent of binding of chromophores to macromolecules, stained cells, etc., may be similarly obtained. In such cases, the marked enhancement of the scattering at absorption bands is advantageous in adding selectivity towards the absorbing molecules in the measurements. Moreover, the extent of depolarization of the scattered light may shed light on the structure of the aggregates, since ρ depends on the spacial orientations of the individual molecules relative to one another in the aggregate. In the extreme case of a completely isotropic packing the scattered light is expected to be negligibly depolarized.

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