DEPENDENCE OF THE BEFORE-RELAXATION ENERGY TRANSFER IN MIXED SOLUTIONS OF DYES ON THE OVERLAP OF THEIR ABSORPTION SPECTRA

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The change of fluorescence emission anisotropy (EA) due to concentration depolarization was measured for two component solutions with increasing magnitude of the overlap of absorption spectra. In the case of negligible overlap the experimental results agree well with the predictions of JABŁOŃSKI's theory regardless the excitation wavelength. If, however, the overlap of the donor and acceptor spectra increases markedly the measured EA depends on the excitation wavelenght. This may be a result of before-relaxation back-transfer.

Introduction

The study of concentration depolarization is a method to gain information about energy transfer mechanism. The majority of concentration depolarization theories and experiments deal with the simplified case of pure one-component solutions. In cases interesting for biophysicist such simple solutions do not occur at all.

JABŁOŃSKI's "active sphere" theory of concentration depolarization is specially suitable to be developed for the case of multicomponent solutions. Treating this case for a solution containing only dye I and dye II Jabłoński comes to the equation [1]:

$$r = r_0 \frac{\bar{F}_D}{\bar{F}_D + \bar{F}_A + \bar{F}'_A}$$

where r_0 is the limiting value of the emission anisotropy (EA) r of dye I for concentrations approaching zero, and $\bar{F}_D$, $\bar{F}_A$, $\bar{F}'_A$, are the total probabilities that the light is emitted in solution by the donor I, and an acceptor I excited by transfer from the donor I or from a molecule of dye II, respectively [2]. In solutions with two dyes having overlaping absorption spectra one excites both dyes and transfer may occure from dye II (with lower 0—0 transition frequency) to dye I only before relaxation to a Boltzmann distribution of the first excited electronic state of dye II. Thus, if one wants to calculate r numerically from Eq. (1) one must take into account not only the retransfer itself but also its before-relaxation mechanism [3]. For a two component solution a numerical calculation of r according to Eq (1) is very troublesome and computer-time consuming.

The aim of this paper was to measure the influence of the concentration depolarization of the fluorescence on the excitation wavelength for two-component solution with increasing absorption spectra overlap.

Experimental

Three two-component dye solutions were chosen:
1. trypaflavine plus rhodamine B, 2. uranine plus rhodamine B, 3. rhodamine 6 G plus rhodamine B. The donors were, trypaflavine, uranine and rhodamine 6 G, respectively. The solvent was a glycerol-alcohol mixture experimentally selected to give a possibly low dimer formation (which in the worst case did not exceed 2%). The optical density of the mixed solutions was equal to the sum of the optical densities of the pure components.

The absorption and emission spectra were carefully measured to find the 0—0 transition wavelengths, which for the donor 1, 2, 3 were 476.5 nm, 510 nm, 550 nm, respectively. The excitation of two-component solution with their appropriate 0—0 transition wavelength eliminates before-relaxation effects.

The fluorescence polarization of pure and mixed solution were measured by a method described earlier [4]. However, in addition to an excitation monochromator we used a second one in the emission path, which make possible an accurate selection of the wavelength of the emission maximum. This is necessary since there is a strong overlap of the emission spectra of the donor and acceptor and a special technique of polarization measurements must be applied to calculate the EA of the donor itself. This technique is described in [5]. During the polarization measurements an observation of the front surface of the cuvette illuminated under a small angle, took place. The thikness of the cuvette was chosen so as to obtaint a maximum optical density of 0.1.

The results of the polarization measurements are plotted in Figs. 1—5, together with the absorption spectra of the two dyes forming the mixtures 1, 2 and 3. The general conclusion is that the concentration depolarization of the mixed solutions (upper curves) is always less pronounced than the one for pure solutions. The solution 1 has a negligible absorption spectra overlap and the mutuall position of the two

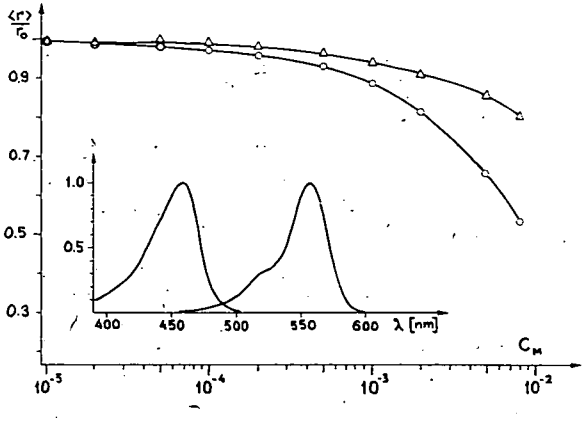

Fig. 1. The dependence of the EA on concentration of pure trypaflavine and its equimolar mixture with rhodamine B. (Inserted are their absorption spectra). Excitation wavelength λ=476, nm

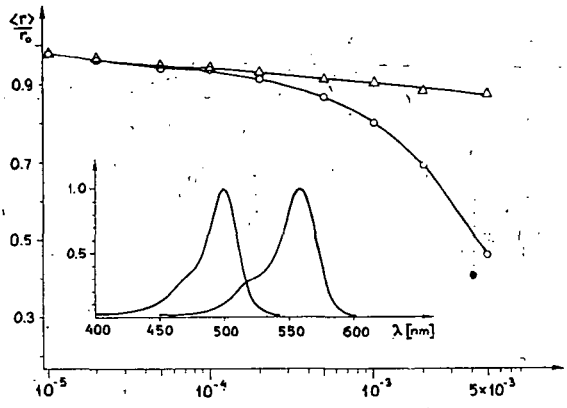

Fig. 2. The dependence of the EA on concentration of pure uranine and its equimolar mixture with rhodamine B. (Inserted are their absorption spectra.) Excitation wavelength $\lambda = 510$ nm

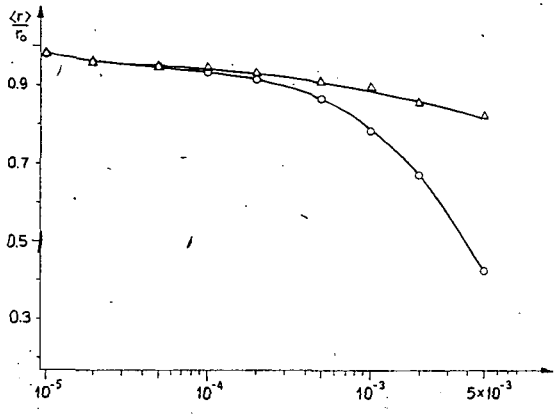

Fig. 3. The dependence of the EA on concentration of pure uranine and its equimolar mixture with rhodamine B. Excitation wavelength $\lambda = 460$ nm

curves in Fig. 1 does not depend on excitation wavelength. The dependence of EA on concentration of pure rhodamine 6 G or or the mixed dyes combination 3 is illustrated in Fig. 4 and 5. Then, although the polarization of the donor in mixed solution is alaways higher as compared with the one of the pure solution, this difference is smaller for shorter excitation wavelength (500 nm) than for the 0—0 transition wavelength 550 nm. As we see in Fig. 2 and 3, which represent the experimental results for the dye combination 2 (with smaller overlap), there is also a difference between the upper and lower curves due to different excitation wavelength. This λ_{exc} dependent difference is however smaller than the respective value for the dye combination 3.

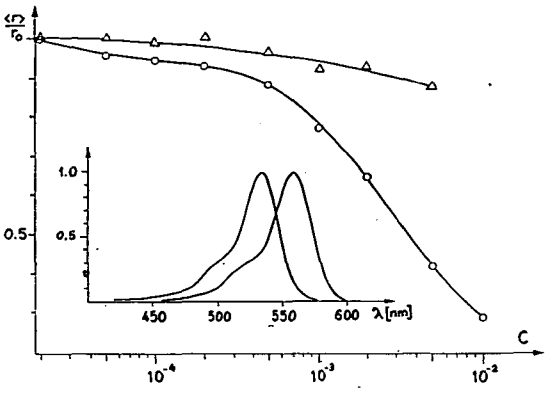

Fig. 4. The dependence of the EA on concentration of pure rhodamine 6 G and its equimolar mixture with rhodamine. B. (Inserted are their absorption spectra). Excitation wavelength $\lambda = 550$ nm

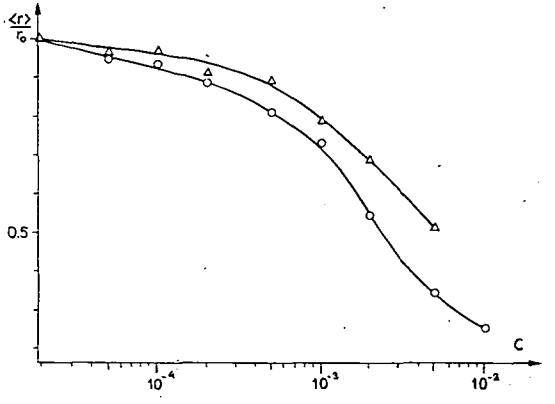

Fig. 5. The dependence of the EA on concentration of pure rhodamine 6 G and its equimolar mixture with rhodamine. B. Excitation wavelength $\lambda = 550$ nm

Conclusions

The above reported experimental results can not be explained on the basis of FÖRSTER'S "very weak" interaction case with the assumption that excitation energy transfer takes place after a Boltzmann distribution of vibrational energy has been established. The question arises whether it is enough to assume that in the case of "very weak" interaction excitation energy transfer takes place before a vibrational energy relaxation or whether we have to assume a "weak" interaction.

This problem was discussed in a number of papers [5—9]. Recently KENKRE and KNOX [10, 11] as well as PAILLOTIN [2] published theories expressing a different

point of view on possible intermediate intereactions between molecules in solutions from which it follows that the rate of transfer between two isolated molecules must not be strictly R^{-6} (very weak interaction) or R^{-3} (weak interaction) distance dependent.

Without numerical calculation on the basis of the "active sphere" concentration depolarization theory (which we aim to performe) our experimental results do not answer the question what kind of mechanism is responsible for "before-relaxation" excitation energy transfer. The interpretation of our results leads, however, to the conclusion that such mechanism exists. In Fig. 6 three level diagrams are drawn

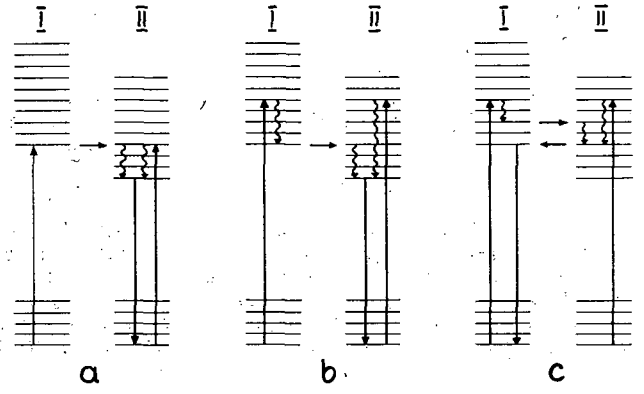

Fig. 6. Level diagrams for two molecules with different 0—0 transitions: *a* Excitation to the 0—0 level of I. *b* Excitation to higher vibrational levels. Energy transfer is of the "after-relaxation" kind. *c* Excitation to higher vibrational levels of the both molecules, "before-relaxation" transfer and retransfer is possible

illustrating possible excitation energy paths in and between two molecules with different 0—0 transition. The diagram "*a*" illustrates the case of 0—0 transition excitation of the donor. Any kind of transfer from molecule II to molecule I is not possible. The energy transfer from molecule I to molecule II causes that the efficiency and the decay time of the fluorescence of molecule I decrease, thus lowering also the concentration depolarization. The case "*b*" represent the situation where "before-relaxation" energy transfer is forbidden; also here any transfer from II to I is not possible. In that case the polarization of the fluorescence emitted from molecule I can not depend on overlap of absorption spectra or on λ_{exc}. Finally in the Fig. 6*c* the excitation energy-paths for "before-relaxation" transfer are illustrated. The excitation energy transfer to molecule I may be a retransfer (changing only the efficiency) or a normal transfer of energy from the primarly excited molecule II (this means that molecule I emits unpolarized sensitized fluorescence). Both effects decrease the polarization of the donor fluorescence.

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ЗАВИСИМОСТЬ ПРЕДРЕЛАКСАЦИОННОГО ПЕРЕНОСА ЭНЕРГИИ В СМЕШАННЫХ РАСТВОРАХ КРАСИТЕЛЕЙ ОТ ПЕРЕКРЫТИЯ СПЕКТРОВ ПОГЛОЩЕНИЯ КОМПОНЕНТОВ

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Измерялось изменение анизотропии флуоресцентной эмиссии (АЭ), обсуловленное концентрационной деполяризацией при увеличении величины перекрытия спектров поглощения. В случае пренебрежимого перектырия экспериментальные данные хорошо совпадают с предсказыванием теории Яблонского, независимо от длины волны возбуждения. Однако, если перектрытие спектра донора и акцептора значительно увеличится, измеренная АЭ зависит от длины волны возбуждения. Это может быть результатом предрелаксационного обратного переноса.