

CONCENTRATION EFFECTS IN PIGMENT SOLUTIONS

G.P.Gürinovitch, A.P.Lošev, E.I.Zenkevitch  
Institute of Physics, Academy of Sciences of the By-  
elorussian SSR, Minsk, BSSR/USSR

INTRODUCTION

The investigation of concentration effects in the solutions of luminescent compounds allows us to obtain the detailed information concerning interactions between molecules of dissolved substance. The increasing concentration of dye and pigment solutions is known to lead to the concentration depolarization phenomena (CDF) and the concentration quenching of fluorescence (CQF). At present the overall of these phenomena is rather widely investigated both in theoretical and experimental aspects. Nevertheless the problem of the mutual relation of the concentration depolarization and the concentration fluorescence quenching remains yet unsolved. The improvement of the CDF theory, undertaken in last years, as well the using new methods of investigation, give the real possibility to carry out the comparison of theory and experiment more strictly.

Such comparison permits to determine the mechanism and the regularities of energetic interaction between molecules definitely and to investigate the reasons of CQF more profoundly, and ascertain its influence on CDF.

In present paper the results of experimental investigation of concentration influence on spectral-luminescent characteristics of pigment solutions will be presented and the analysis of obtained data with use of calculations according to modern theories of CDF will be carried out. The second range of questions, investigated in the paper, is connected with the elucidation of possible reasons of CQF and the role of intermolecular interaction in the electronic excitation energy deactivation processes in concentrated solutions and the associates of pigment molecules. From this point of view the regularities of the association and the mixed association of different pigments and the energetics of such systems are investigated as well the investigation of excitation trapping efficiency either by associates or directly in mixed aggregated complexes under variation of their composition is carried out.

#### CONCENTRATION DEPOLARIZATION OF FLUORESCENCE

The modern theories of CDF [4-5] are mainly based on the calculations of fluorescence emission probability  $P$  from the initially excited molecules since every ener-

gy transfer act results practically in full emission depolarization as shown by Galanin [6] and therefore the emission of initially excited molecules brings the cardinal contribution to polarized fluorescence of solution. These theories are based on the following common assumptions: 1) the interacting molecules have not the nongenerated excited states and have been excited to their lowest excited states; 2) the quantum yield of fluorescence doesn't depend on molecule concentration; 3) the distribution of molecules in solution is chaotic and isotropic; 4) the rotational depolarization doesn't occur; 5) the energy migration between molecules is described by the Forster-Galanin inductive resonance theory [7,8]. The theory of CDF developed by Bodunov [5] is also based on these common assumptions but, in contrast with the other theoretical models, stated in works [4-4] and connected with specific assumptions about the character of excitation movement in system. In his theory the most common case is considered with the usage of the Monte Carlo method of calculation. In the case of dipole-dipole interaction between molecules in accordance with the data of work [5] the theoretical values of emission anisotropy within the accuracy up to 2% are packing up the curve, in the range of  $0,1 < \gamma < 10$ , described by the function:

$$\frac{r}{r_0} = \left[ 1 + \frac{\pi}{2\sqrt{2}} \gamma + 0,555 \gamma^2 + 0,035 \gamma^3 \right]^{-1} \quad (1)$$

where  $\gamma = C/C_0$ ;  $r$  is the anisotropy emission of solution with concentration  $C$ ;  $r_0$  is the anisotropy, when  $C \rightarrow 0$ ;  $C_0$  is the critical concentration corresponding to the conditions at which the paired transfer probability is equal to the total probability of all intramolecular deactivation processes,  $C_0 = \left(\frac{4\pi}{3} R_0^3\right)^{-1}$ . In accordance with inductive resonance theory, the value of the critical transfer distance  $R_0$  is calculated with the formulae [7,8]:

$$R_0^6 = \frac{9000 \cdot \epsilon n_{10} \cdot \kappa^2 \cdot B_0}{128 \cdot \pi^3 \cdot n^4 \cdot N} \cdot \int_0^{\infty} f_D(\nu) \cdot \epsilon_A(\nu) \frac{d\nu}{\nu^4} \quad (2)$$

where  $\nu$  is the wavenumber;  $\epsilon_A$  is the molar decimal extinction coefficient of acceptor;  $f_D$  is the spectral distribution of the donor molecule fluorescence (measured in quanta and normalized to 1 in wavenumber scale);  $N$  is the Avagadro number;  $n$  is the refraction index of solvent;  $B_0$  is the donor quantum yield in absence of transfer;  $\kappa^2$  is the orientation factor ( $\kappa^2 = \frac{2}{3}$  for the isotropic solutions of middle viscosity).

The CDF phenomenon for chlorophyll and its analogs in solutions of different nature and properties has been investigated by us [9-11]. For the comparison of theory and experiment the viscous isotropic pigment solution in castor oil were chosen [9], which are corresponding to a best extent common assumption in CDF

theories. In addition the high solubility of chlorophyll and its derivatives in this solvent (up to  $10^{-1}$  m/l ) permits to carry out the comparison of the theory and the experiment in the wide range of concentration. It is worthy to note that in contrast with theoretical consideration supposing the constancy of fluorescence quantum yield, in experimental conditions, CQF is observed for all investigated pigments. Results of determining  $R_0$  according to the Forster-Galanin theory and by fluorescence depolarization with usage of the method stated in [12] are listed in Table 1, where the basic spectral-luminescent parameters of pigments are involved.

As shown in Table 1, the changing of production  $B_0 \times \int_0^{\infty} f_D(\nu) \epsilon_A(\nu) \frac{d\nu}{\nu^4}$  by order doesn't lead to essential deviation of  $R_0^{\text{th}}$  values from  $R_0^{\text{exp}}$  ones. The analogous accordance was observed for these pigments in the detergent micells [10] and in the rigid polyvinylbutyral films [11] investigated by us earlier. These facts can serve as the evidence of the inductive-resonance bonds between pigment molecules in the examined systems.

For the comparison of theoretical calculation of the CDF curve by the formulae (1) with experimental data  $R_0^{\text{theor}}$  value calculated by the formulae (2) must be taken as far as this value is determined by dipole-

TABLE 1

Spectral-Luminescent Characteristics and Transfer Parameters of Chlorophyll and Its Analogs

Pigment pair	Solvent.	$\epsilon_A^{\max}$	$B_{\text{donor}}$	$\int_0^{\infty} f_D \cdot \epsilon_A \frac{d\nu}{\nu^4} \times 10^{13}$	$R_{0A}^{\text{th}}$	$R_{0A}^{\text{exp}}$
Chl <u>b</u> → Chl <u>b</u>	castor oil	47000	0,15	3,3	43	43
Pheo <u>a</u> → → Pheo <u>a</u>	castor oil	50300	0,21	3,2	46	52
4VPChl → → 4VPChl	castor oil	32800	0,11	1,1	34	40
PChl → Chl <u>a</u>	castor oil	32800	0,17	2,7	42	-
Chl <u>b</u> → Chl <u>a</u>	castor oil	47000	0,15	5,6	47	-
Chl <u>a</u> → Chl <u>b</u>	castor oil	77500	0,35	0,9	40	-
Chl <u>a</u> → Chl <u>a</u>	castor oil	77500	0,35	5,5	54	50
Chl <u>a</u> → Chl <u>a</u>	pyridine	77200	0,35	4,5	51	-
Chl <u>a</u> → Chl <u>a</u>	acetone	83500	0,34	5,8	55	-
Chl <u>a</u> → Chl <u>a</u>	ether	9 086	0,32	5,2	53	-

dipole interaction only and is not connected with the other additional assumptions. Really, it should be noted from Table 1, for series of solvents differing through refractive index and influence on the pigment electronic spectra,  $R_{0A}^{\text{theor}}$  values for chlorophyll a differ unessentially. In contrast with this  $R_{0A}^{\text{exp}}$  values found for the same pigment differ essentially, because the determination of  $R_{0A}^{\text{exp}}$  was carried out by

different methods, which suppose beforehand either model of transfer in the limits of inductive bond between molecules.

Figure 4 shows the CDF theoretical curve calculated in work [5] and the experimental points obtained by us for chlorophylls a and b, pheophytin and 4-vinyl-protochlorophylls (4VPChl). As it followed

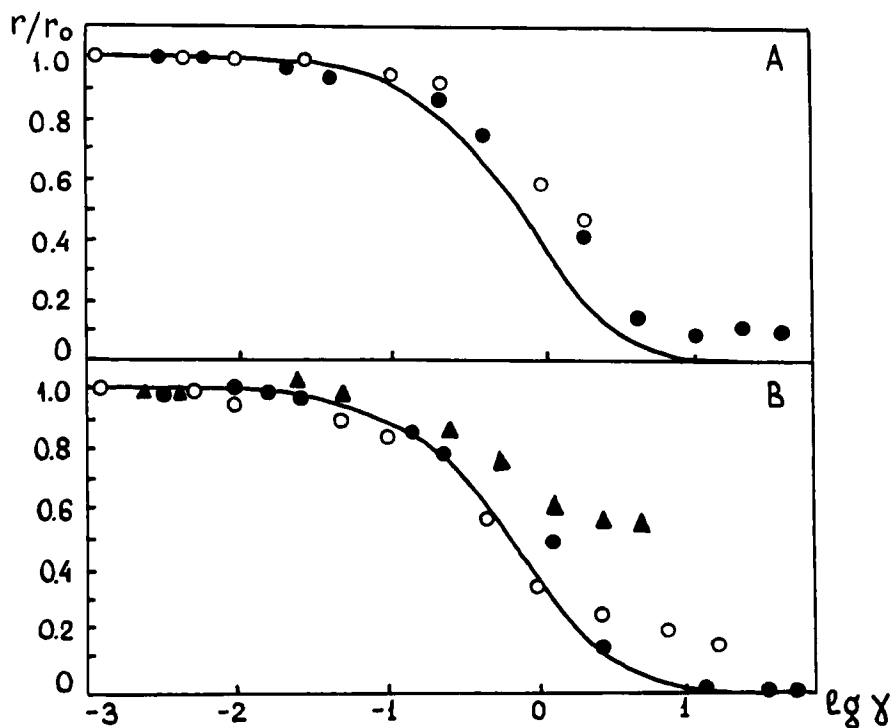


FIG. 4

Comparison of theory and experiment on CDF. Solid curve is theoretical one (equation (4)); A: ● -chlorophyll a; ○ -chlorophyll b. B: ● -pheophytin a; ○ -4VPChl; ▲ -4VPChl+chlorophyll a (1:1). Solvent - castor oil.

from this figure for all pigments the good agreement is observed up to values  $\gamma=0,3\div 0,4$ . At the great values of  $\gamma$  the experimental points lie higher than the theoretical curve. For chlorophyll a and 4VPChl at high concentration range the essential discrepancy of the theory and the experiment is observed. The comparison of these results with the CQF experimental curve for investigated pigments, shown in Figure 2, permits to discover the real relation between CDF and CQF. In-

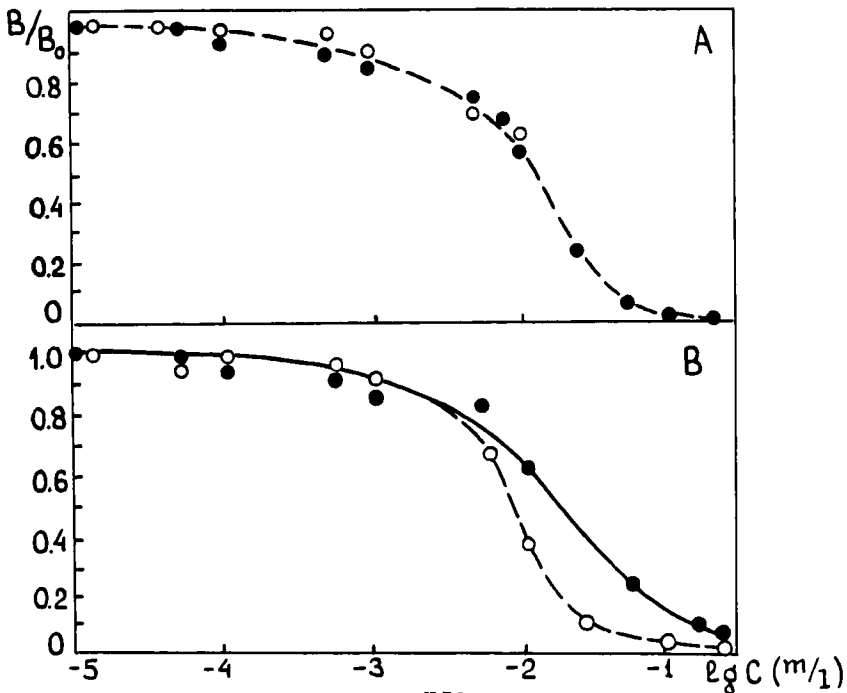


FIG. 2

Relative fluorescence yield of pigments in castor oil as a function of concentration: A: ● -chlorophyll a; ○ -chlorophyll b; B: ● -pheophytin a; ○ -4VPChl.



deed, as one can see from Figure 2 the concentration emission quenching of solutions is yet insignificant up to concentration of order  $10^{-3} \text{ m/l}$  ( $B/B_0 \approx 0,9 \div 0,95$ ). In this case the good agreement of the theory and the experiment is marked for the CDF phenomena. Subsequent rising of pigment concentration leads to the essential quenching of luminescence quantum yield and correspondingly, to the deviation of experimental results from CDF theoretical curve calculated as has already been noted for the case of constant quantum yield of molecule emission. The decisive importance shouldn't be probably attached to the coincidence of theoretical calculations and experimental data in the case of 4VPChl up to  $C = 10^{-2} \frac{\text{m}}{\text{l}}$  as far as it had been assumed in calculations of  $R_0^{\text{theor}}$  so for that pigment the molar decimal extinction coefficient had been equal to protochlorophyll one which is known from literature [15].

It should be noted that the discovered dependence of the emission depolarization on the concentration change of the luminescence quantum yield in the investigated systems have not full physical interpretation at present, though the influence of fluorescence quenching on CDF was investigated in several works [16- 18]. In work [16] the increase of the fluorescence polarization degree of pigment concentrated solutions is supposed to be conditioned by shortening of the lifetime

of the molecule excited states. But under the same conditions of the intermolecular dipole-dipole interactions and of the high viscosity of solvent the shortening of lifetime due to quenching processes should not change the ratio between the initially excited molecules and the molecule-acceptor of energy. In contrast with this the rising of fluorescence polarization degree in comparison with the theoretical curve observed at  $\chi > 0,4$  signifies that the portion of nonpolarized luminescence in total emission becomes smaller. Such selective luminescence quenching of molecules of second type may be explained as follows: quencher molecules which quantity increases under concentration rise affect the whole system of molecules taking part in energy transfer during the excitation presence in system. At first, by shortening the lifetime of initially excited molecules, the quencher results in decrease of the second-excited molecule portion for which energy migration takes place. But under this condition ratio between the initially and the second-excited molecules retains constant. Then when excitation localizes at second molecule ensemble due to migration the quencher affects the second molecules, deactivating their addition part and therefore, decreasing portion of nonpolarized luminescence in total emission. The experiments with 4VPChl solutions con-

taining the equimolecular concentration of the chlorophyll a as a quencher are the direct confirmation of supposed explanation [12] . As one can see from Figure 4 , experimental points corresponding to CDF of 4VPChl alone are arranged considerably lower than the experimental points obtained for 4VPChl and chlorophyll mixture at the same conditions. The slowing down of experimental CDF in comparison with the theoretical calculations observed, in separated solutions of 4VPChl and chlorophyll a at  $C > 10^{-2}$ , may be also explained by the selective quenching of monomer luminescence due to migration processes to forming pigment aggregated forms [13] . Thus, the difference observed between the theoretical and the experimental results on CDF for pigment solutions at  $\chi > 0,4$  are stipulated by the influence of concentration fluorescence quenching only.

#### CONCENTRATION QUENCHING OF FLUORESCENCE AND INTERMOLECULAR INTERACTION

The concentration quenching of fluorescence is characteristic for chlorophyll a and its derivatives (Figure 2) as for many dyes but it is not always explained by the presence of admixture or associates [9,13,18,20-22] . The fact that the quenching occurs in solvents of high and low viscosity with equal efficiency rules out diffusional processes as a result of quenching. Porter et al. suppose that the mecha-

nism of concentration quenching in chlorophyll solutions is energy transfer between chlorophyll molecules by inductive resonance followed by capture at a non-fluorescent trap. The latter, in polar solvents, is merely a pair of chlorophyll molecules the separation of which, although a pair of the equilibrium statistical distribution, is less than a critical distance  $R_0$ , and the electronic spectra of these quasi-dimers are not different from monomer ones [18,22]. Such interpretation of results is essentially analogous to Vavilov's ideas [23] and the mechanism of excitation deactivation at such quenching in quencher molecules remains unknown as before.

Parallel investigation of CQF and the formation of pigment triplet states in solution can give an additional information in this plan. First measurements of such sort were undertaken in work [24] in which it was shown that up to  $C = 1 \cdot 10^{-2} \text{ m/l}$  for chlorophyll a in cholesterol the parallelism was observed in the course of fluorescence quenching and triplet state formation. But it is impossible to disseminate this conclusion to the most interesting concentration range  $10^{-2} \div 1 \cdot 10^{-1} \text{ m/l}$ , where average intermolecular distances change from  $34 \text{ \AA}$  to  $15 \text{ \AA}$  and the interaction between molecules can essentially influence on the energetics of intramolecular transitions. Experimental results of measurement

of fluorescence and triplet state formation relative quantum yields depending on pigment concentration (chlorophyll a, pheophytin a), which we obtained in wide concentration range [13,25,26], are represented in Figure 3. As is seen from Fig.3 at  $C > 10^{-2} \text{ m/l}$  the  $B/B_0$  curve for chlorophyll stretches lower than the curve of relative changing of triplet state formation quantum yield  $\gamma/\gamma_0$ . Analogous differences are observed for Al-Cl phthalocyanine in castor oil - dimethylformamide mixture (1:1) [27]. Neither changes of electronic spectra, showing to molecular association, are discovered for all pigments up to  $C = 1 \cdot 10^{-2} \text{ m/l}$ . Therefore, the coincidence of  $B/B_0$  and  $\gamma/\gamma_0$  curves, observed up to  $C = 1 \cdot 10^{-2}$  testifies the basic channel of excitation deactivation in this concentration range is the pro-

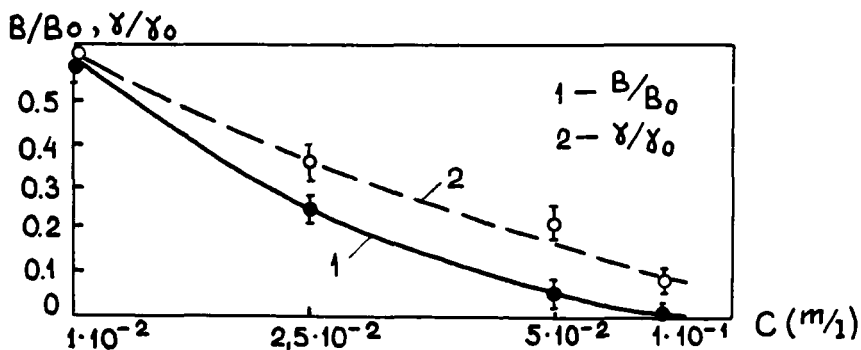


FIG.3

Concentration dependence of relative quantum yields of fluorescence and triplet state formation of chlorophyll a solutions in castor oil.

cess, caused by internal conversion increasing from singlet excited level. Probably, the excitation migration between molecules increases quenching action of such centres.

The analysis of possible reasons, resulting in the difference of  $B/B_0$  and  $\delta/\delta_0$  curves at  $C > 1 \cdot 10^{-2} \frac{m}{l}$ , carried out in [25-27], permits to conclude that it can be connected with the increasing of intersystem crossing probability which for chlorophyll a at  $C = 1 \cdot 10^{-4} \frac{m}{l}$  is 5 times greater than analogous value in low concentration solution. The changing of intramolecular transition probabilities in pigment molecules at high concentrations is probably conditioned by intermolecular interaction which does'nt yet result in the formation of true associates but as a perturbing factor influences on the characteristics of intramolecular transitions. Thus, data obtained point out again the formation of specific centres in the concentrated pigment solutions which have sharply decreased fluorescence quantum yield due to increasing of intersystem crossing and internal conversion probabilities. It is important to note that the intermolecular interaction does be the principal reason of such center formation. For 4VPChl and pheophvtin a such interaction results in formation of true aggregates, presence of which is readily observed at  $C > 10^{-2}$  by the changing of absor-

ption and luminescence spectra [13,28]. At the same time these centres for chlorophyll a don't practically distinguish from monomers by its spectral properties.

At this point of view it is interesting to estimate excitation deactivation rates in systems with sharply changed transition probabilities due to the mutual influence of molecules against each other. Whether it is probable considerable increasing of intersystem crossing probability and correspondingly, decreasing of luminescence quantum yield in associated pigment complexes? Really, results of the spectral-luminescent investigation of energetics of chlorophyll and bacteriochlorophyll in dry petroleum ether showed that the association of molecules results in decreasing of intersystem crossing probability (in 3-10 times) at invariable radiative deactivation probability [29]. The principal channel of singlet excitation energy deactivation in such systems are nonradiation transitions by internal and intersystem crossing conversion. It is also observed (for associated pigment complexes in water-dioxane mixtures) the essential shortening of excited state lifetime and the decreasing of fluorescence quantum yield up to values  $10^{-2} \div 10^{-3}$ , that is the excitation deactivation probability corresponds to  $10^{10} \div 10^{11} \text{ sec}^{-1}$  [30,31].

But even with such high excitation deactivation probabilities intermolecular interaction in associates of chlorophyll and its derivatives can result in fluorescence quenching. The direct experimental confirmation is the discovering of intracomplex energy transfer in mixed pigment associates from weakfluorescent or nonfluorescent donors (quantum yield of which is  $B \ll 10^{-5}$ ) and also for systems with weak overlapping spectra [30]. Estimates obtained with using the spectral-luminescent characteristics of donor-acceptor pairs (including circular dichroism spectra) showed that the excitation transfer probability in mixed pigment aggregates account for  $F \approx 3,5 \cdot 10^{12} \div 1,2 \cdot 10^{13} \text{sec}^{-1}$  and upper limit of interaction energy corresponds to  $V_{12} \approx 75 \text{ cm}^{-1}$  [31]. In that case as long as the migration process probability in complex is comparable with the vibration relaxation probability in complex organic molecules ( $W \approx 10^{12} \div 10^{13} \text{sec}^{-1}$ ) in such systems it is possible to discover phenomena which take place before or during the process of Boltzmann distribution of vibrational energy in the excited electronic states of interacting molecules.

The before-relaxation electronic excitation energy transfer which was discovered in mixed associates containing donor component (protochlorophyll



or 4VPChl) and increasing acceptor quantity (chlorophyll a; Pd-pheophytin a; Cu-pheophytin a) [32,33] is believed to such processes. It was shown (in our experiments) that under similar conditions (ratio of donor and acceptor components, resonance conditions;  $S_D^* - S_A^* = \Delta E \gg KT$ ) the quenching of protochlorophyll luminescence by Cu-pheophytin a molecules in mixed aggregate excels in order the emission quenching of this pigment by chlorophyll a and Pd-pheophytin a molecules (Fig.4). Listed estimates of excitation migration probability values for complex and available experimental facts permitted to conclude that in systems protochlorophyll - chlorophyll a and protochlorophyll -

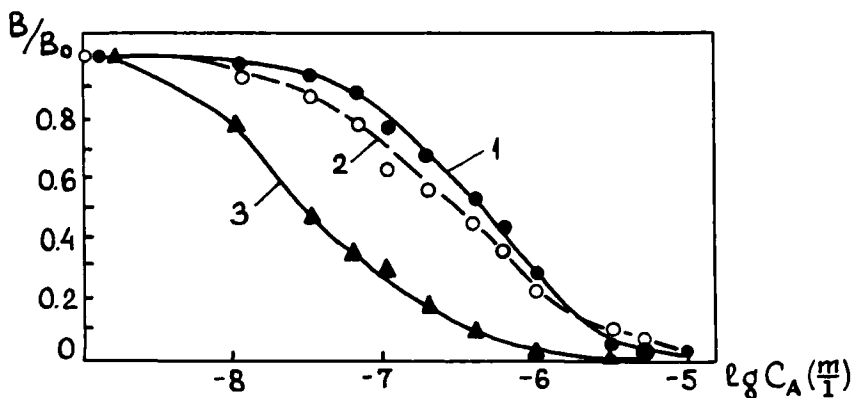


FIG.4

Changing of protochlorophyll luminescence relative quantum yield in mixed associates under increasing of relative concentration of different acceptors (1 - chlorophyll a; 2 - Pd-pheophytin a; 3 - Cu-pheophytin a) in water-dioxane (4:1) mixtures.

-Pd-pheophytin a back before-relaxation energy transfer took place. It occurs before or during the process of establishment of thermal equilibrium among vibration levels of acceptor excited states. Excitation migration from protochlorophyll to Cu-pheophytin a in such conditions is essentially irreversible due to the high excitation deactivation probability in acceptor which is not lower than  $10^{13} \text{ s}^{-1}$  according to our estimations.

It may be considered that aggregates of complex molecules are the intermediate link between crystal state and solutions, so far as there is definite order in the structure of such systems [34], and the intermolecular interaction energy can correspond to the case of localized exciton. Unfortunately, the general and strict theory of such interactions in associated systems is absent and this is the serious obstacle for full interpretation of the whole complex of experimental facts. It may be hoped that solution of this problem permits to consider the regularities of different concentration effects more definitely and differentially, and also to take into account their mutual influence.

#### REFERENCES

1. F.W.Crawley, R.S.Knox. *Mol.Phys.*, 22, 385, 1971.
2. C.Bojarski, *J.Luminescence*, 5, 413, 1972.
3. I.M.Rozman, R.Sh.Sichinava, *Izv. AN SSSR, ser.fiz.*, 29, 1863, 1975.
4. E.N.Bodunov, *Optika i Spekr.*, 41, 990, 1976.

5. E.N.Bodunov. Zh.Prikl.Spektr., 26, II23, 1977.
6. M.D.Galanin. Trudy FIAN, 5, 341, 1950.
7. Th.Forster. Fluorezcenz organischer Verbindungen. Vandenhoeck und Ruprecht in Gottingen, 1951.
8. M.D.Galanin. Trudy FIAN, 12, 3, 1960.
9. A.P.Losev, E.I.Zenkevitch. Zh.Prikl.Spektr., 9, I44, 1968.
10. E.I.Zenkevitch, A.P.Losev, G.P.Gurinovitch. Mol. biol., 824, 1972.
11. E.I.Zenkevitch, A.P.Losev, G.P.Gurinovitch. Izv. AN SSSR, ser.fiz., 36, 979, 1972.
12. C.Bojarski, A.Kawski, Ann. der Phys., 5, 31, 1959.
13. E.I.Zenkevitch. Avtoreferat kand.diss., Minsk, 1973.
14. L.Szalay, Acta Phys.Pol., 26, 5II, 1964.
15. V.M.Koski, J.H.C.Smith, J.Amer.Chem.Soc., 70, 3558, 1942.
16. P.P.Feofilov. Poljarizovannaja lyuminestsentsija atomov, molekul i kristallov, Fizmatgiz, M., 1959, str.166.
17. L.Szalay, B.Sarkany, E.Tombacz, Acta Phys.Chem., Szeged, 11, 2I, 1965.
18. G.S.Beddard, G.Porter, Nature, 260, 366, 1976.
19. E.I.Zenkevitch, A.P.Losev. Izv. AN SSSR, ser.fiz., 39, 1845, 1975.
20. W.F.Watson, R.Livingston, J.Chem.Phys., 18, 802, 1950.
21. A.G.Tweet, G.L.Gains, W.D.Bellamy, J.Chem.Phys., 41, 1008, 1964.
22. A.R.Kelly, L.K.Patterson, Proc.Roy.Soc., A324, II7, 1971
23. S.I.Vavilov, Sobr.soch, t.2, Izd-vo AN SSSR, 1952.
24. G.Porter, G.Strauss, Proc.Roy.Soc., I, 295, 1966.
25. A.P.Losev, Int.Seminar on Energy Transfer in Condensed Phase, Prague, June 29-July 2, 1976 (in print).
26. A.P.Losev, E.I.Zenkevitch, E.I.Sagun. Zh.Prikl. Spektr., 27, 244, 1977.
27. E.I.Sagun. Avtoreferat kand.diss., Minsk, 1977.
28. G.P.Gurinovitch, E.I.Zenkevitch, A.P.Losev. V sborn. "Chlorofill", Izd. "Nauka i Technika", Minsk, 1974, st74.
29. G.P.Gurinovitch, A.P.Losev, E.I.Sagun, Zh.Prikl.Spektr., 26, 1028, 1977.
30. E.I.Zenkevitch, A.P.Losev, G.P.Gurinovitch, Mol. biol., 9, 516, 1975.
31. E.I.Zenkevitch, G.A.Kochubeev, A.P.Losev., G.P.Gurinovitch, Mol. biol., II, 1039, 1977.
32. E.I.Zenkevitch, A.P.Losev., G.A.Kochubeev, G.P.Gurinovitch, Abstracts of XIII European Congress on molecular Spectroscopy, Wroclaw, Poland, 1977, p.383.
33. E.I.Zenkevitch, A.P.Losev, G.A.Kochubeev, G.P.Gurinovitch, Izv. AN SSSR, ser.fiz., 1978 (in print).