TIME DEPENDENCE OF LONG-RANGE ENERGY TRANSFER

By

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This lecture is dedicated to the momory of THEODOR FÖRSTER, whose work has been highly esteemed and continued by scientists throughout the world.

The time dependence of the acceptor fluorescence is governed by Dawson's function if the lifetime of the acceptor is shorter than that of the donor.

Measurements with the system pyrene (donor)/perylene (acceptor) in liquid solutions of various viscosities necessitated a generalization of Förster's theory, taking diffusion into account. New formulae have been derived for the experimental fluorescence characteristics of long range energy transfer combined with spatial diffusion, which are easily applied and shown to be valid under various conditions.

In order to deal with the kinetics of energy transfer in general, the decay functions under excitation by a pulse of negligible duration should first be known. The fluorescence time dependence can then be calculated by convolution for any given time dependence of excitation intensity. The new concept of 'convolution kinetics' can be applied to any mechanism involving energy transfer, whereas the treatment by differential equations is of limited validity.

Introduction

For the rate constant $n_{A\to B}$ of energy transfer from an initially excited donor molecule to an acceptor molecule

$$A^* + B \xrightarrow{n_{A+B}} A + B^*$$

the following expression [1, 2] was derived by Th. Förster in 1947

$$n_{A\to B} = \frac{9000 \cdot \ln 10}{128 \cdot \pi^5 \cdot N_L} \cdot \frac{\varkappa^2 \cdot n_e}{n_m^4 \cdot R^6} \cdot \int F_A(\bar{v}) \cdot \varepsilon_B(\bar{v}) \frac{d\bar{v}}{\bar{v}^4}. \tag{1}$$

Here $F_A(\bar{\nu})$ is the fluorescence quantum spectrum of the donor normalized to unity and $e_B(\bar{\nu})$ is the molar decadic extinction coefficient of the acceptor. n_e denotes the rate constant of spontaneous emission of the donor *i.e.* its reciprocal natural fluorescence lifetime. The relative orientation of donor and acceptor is taken into account by the dimensionless factor κ^2 ; for sufficiently fast Brownian rotation the average value $\kappa^2 = 2/3$ may be used, but another average value applies for random but fixed orientations [3]. N_L denotes Avogadro's number and n_m the refractive index of

the medium. The most important quantity in eq. (1) is the distance R between donor and acceptor on whose sixth power the dipole-dipole interaction depends.

If the donor and acceptor molecules existed in pairs with fixed distance and no transfer between the molecules of different pairs took place, the kinetics of energy transfer could be described as a monomolecular process the rate of which would be given by eq. (1). In fact this case is very rare. In most practical energy transfer systems the molecules are randomly distributed; a correct description of energy transfer thus involves statistical considerations [2, 7]. Generally it is a serious mistake to identify R in eq. (1) with the average distance of acceptor molecules since this gives a wrong dependence of $n_{A\rightarrow B}$ on the acceptor concentration [9]. The result of Förster's ingeniously simple treatment [2] of the statistics of dipole-dipole energy transfer in the case of homogeneous three dimensional distribution is given in eq. (2)

$$f_{A^*} = \frac{[A^*]}{[A^*]_0} = \exp{-(nt + 2\gamma \cdot \sqrt{nt'})}. \tag{2}$$

Here f_{A^*} denotes the ratio of the concentration of excited donor molecules, $[A^*]$, at time t to the initial concentration, provided no further excitation takes place when t>0. f_{A^*} is also the ratio of donor fluorescence intensities at times t and zero. The sum of the rate constants of all processes except energy transfer contributing to the depopulation of A^* is denoted by n, the reciprocal fluorescence decay time of the donor $(n=1/\tau)$.

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is the ratio of the unexcited acceptor concentration [B] to its so-called critical concentration $[B]_k$, which we consider to be merely a function of the constant parameters of the theory defined as follows: Let R_k be the intermolecular distance in an isolated donor-acceptor pair at which the transfer rate $n_{A \to B}$ in eq. (1) is equal to n, the sum of the depopulation constants without energy transfer. The critical radius R_k may be obtained formally by replacing $n_{A \to B}$ by n and n by n by n and n

$$[B]_k = \frac{2}{\sqrt{\pi}} \left[\frac{4\pi \cdot R_k^3}{3} \cdot \frac{N_L}{1000} \right]^{-1} = \frac{1500}{\pi^{1.5} \cdot N_L \cdot R_k^3}.$$
 (4)

We emphasise the formal character of $[B]_k$ and R_k , because ascribing too much significance to these parameters has caused many erroneous work on energy transfer.

Förster's derivation of eq. (2) has often been doubted and disparaged though the famous author M. D. Galanin had confirmed [4], and generalized [5] it. A review [2, 4] together with a new derivation based on pair probabilities [6] is given in ref. [7]. A treatment of molecular arrangements of different dimensionalities is given in another lecture on this conference [8].

Fifteen years passed after the first publication of eq. (2) until BENNETT [10] confirmed it directly by measuring the fluorescence decay function of the donor after excitation by a light flash of negligible duration, at various concentrations of the acceptor; the donor was pyrene and the acceptor sevron yellow. According to the

 \sqrt{t} — term in eq. (2) the logarithm of the relative fluorescence intensity does not depend linearly on time but decays much faster at the beginning. MATAGA et al. [11] obtained the same type of decay function with the system donor pyrene, acceptor perylene. Both authors [10, 11] used solid solutions in order to prevent any influence of diffusion.

Energy transfer kinetics of the acceptor

The primary result of energy transfer is the excitation of acceptor molecules B^* irrespective of consecutive fast deactivation processes which may make the experimental proof of transient acceptor excitation extremely difficult. But if the acceptor can fluoresce, energy transfer leads to sensitized acceptor fluorescence *i.e.* a definite transient concentration of excited acceptor $[B^*]$. The calculation of $[B^*]$ (t) is simplified by making use of eq. (2) instead of a new statistical derivation [5]. According to the rules of reaction kinetics we get

$$\frac{d[B^*]}{dt} = -n'[B^*] + \gamma \sqrt{\frac{n}{t}} [A^*]. \tag{5}$$

In the first term on the right hand side n' denotes the sum of the rate constants of all processes deactivating $B^*(n'=1/\tau')$ is the reciprocal acceptor fluorescence decay time); the second term describes the rate of formation of B^* by energy transfer in accordance with eq. (2) which also gives $[A^*]$ as an explicit function of time. The integration of eq. (5) is performed by conventional methods

$$f_{B^*} = \frac{[B^*]}{[A^*]_0} = 2\gamma \cdot \sqrt{n} \cdot \exp\left[(n't) \cdot \int_0^t \exp\left[(n'-n)\vartheta - 2\gamma \cdot \sqrt{n\vartheta}\right] d\vartheta.$$
 (6)

The calculation of the integral is quite different in the cases n' > n and n' < n; the solution of the latter case is straightforward and has been given by BIRKS [12]. The qualitative result may be seen without mathematics, for if the decay of the donor A^* is much faster than that of the acceptor B^* , the formation of B^* is limited to a time interval at the beginning, which will not exceed the lifetime of A^* , and after that the acceptor decay function is simply exponential as if B^* had been directly excited by a light flash. In the case n' > n, which is realized e.g. with the system donor pyrene, acceptor perylene, the problem is more interesting as the integral in eq. (6) diverges. The expression eq. (6) retains its sense, however, due to the exponential function before the integral and can be calculated in closed form with aid of Dawson's function (of the second order). We get

$$f_{B^*} = 2\gamma \sqrt{\frac{n}{n'-n}} \left\{ D\left[\sqrt{(n'-n)t} - \gamma \sqrt{\frac{n}{n'-n}} \right] \cdot f_{A^*} + D\left[\gamma \sqrt{\frac{n}{n'-n}} \right] \cdot \exp(-(n't) \right\}.$$
(7)

Dawson's function [13] is shown in Fig. 1. It is defined as follows

$$D(z) = \exp{-(z^2)} \int_0^z \exp{(p^2)} dp.$$
 (8)

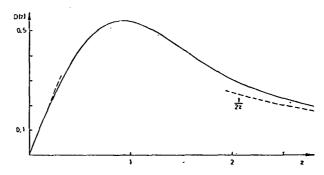


Fig. 1. Dawson's function of the second order cf. eq. (8)

The shape of f_{B^*} depends strongly especially on the parameters n and n'. In Fig. 2 the functions f_{A^*} and f_{B^*} were calculated for $n=6\cdot 10^6 s^{-1}$, $n'=2\cdot 10 s^{8-1}$. The parameter β replacing γ is defined* in eq. (9).

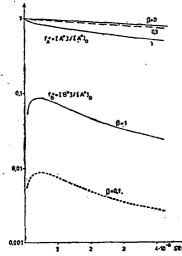


Fig. 2. Calculated time dependences of donor pyrene and acceptor perylene cf. eqs. (2) and (7); $\left(\frac{n_o}{n}\right)^{1/2} \cdot [B]_k = \frac{9 \cdot 10^{-3} \text{ M}}{n}; n = 6 \cdot 10^6 \text{ s}^{-1}; n_o = 1.4 \cdot 10^8 \text{ s}^{-1} \text{ and } n' = 2 \cdot 10^8 \text{ s}^{-1}$

$$\gamma = \sqrt{\frac{n_e}{n}} \cdot \beta. \tag{9}$$

In Fig. 2 we use $n_e = 1.4 \cdot 10^6 \, s^{-1}$; the values of the three chosen parameters correspond to the case pyrene/perylene.

Curves beginning with 1 represent the time dependence of donor fluorescence v.s. excited donor concentration such as were measured by MATAGA[11]. If we compare the acceptor curves with the donor ones we notice a behaviour which is impossible in ordinary reaction kinetics: The concentration of the product B^* changes faster than that of the educt A^* from which it is formed! The reason for this strange feature of energy transfer kinetics can be seen from eq. (5). At t=0 the rate of formation of B^* is extremely fast, formally infinite; this portion decays with a large time constant n' still faster than can be seen from Fig. 2 since the decay is partly compensated by energy transfer. In the course of time the influence of the initial period dec-

^{*} The calculation of the factor of the $\sqrt[n]{t}$ — term in eq. (2) shows that $\sqrt[n]{n}$ cancels and $\sqrt[n]{n_e}$ appears instead of it. Indeed energy transfer does not depend on all the processes contained in n, but it depends physically on n_e which is a measure of donor transition dipole strength. For these reasons we would prefer not to use $[B]_k$ and γ parameters defined more appropriately. However the use of R_k , $[B]_k$ and γ is widespread in literature [15].

reases more and more; the shape of the acceptor curves becomes then similiar to that of the donor curves as is the case in ordinary kinetics, where rate constants do not depend on time.

In experimental tests of eq. (7), precautions must be taken against other mechanisms of energy transfer [14]. Excitation of the acceptor by simple absorption of donor fluorescence can be prevented by thin layer probes and in some cases by a sufficiently high donor concentration, thus limiting the penetration depth of exciting radiation. The prevention of diffusion is a much more difficult problem, see below.

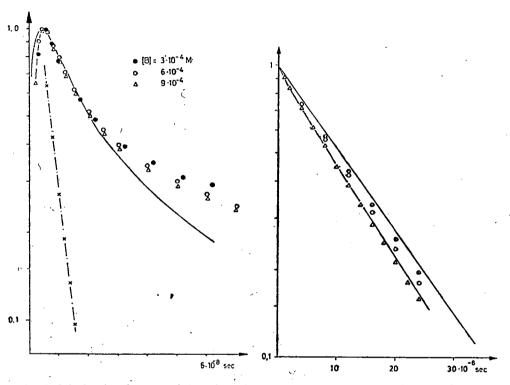


Fig. 3. Calculated and measured time dependence of the acceptor. Parameters are the same as in Fig. 2.

Fig. 4. Calculated and measured time dependence of the donor.

Parameters are the same as in Fig. 2.

Fig. 3 shows the measured time dependence of acceptor perylene with the donor pyrene 10^{-3} M in highly viscous paraffin oil**. The values are normalized to equal ordinates at the maxima. The solid curve was calculated from eq. (6) and similarly normalized. At values of the concentration $[B] \ll [B]_k$ (i.e. at small values of γ or β), the shape of the curves is approximately independent of [B], and β or γ , respectively. The half width of the excitation flash was $2 \cdot 10^{-9} s$ from which result the small

^{**} The influence of pyrene excimer formation was negligible under these conditions (less than 1%).

deviations at the very beginning. Until $2 \cdot 10^{-8}s$ after the excitation the measured values fit well to the theoretical curve; the obvious deviations are caused by the influence of diffusion which inevitably must be taken into account in the case of acceptor fluorescence even at a viscosity of approximately 10 poise. As can be seen in Fig. 4, the deviations are negligible in the case of the donor. The average contribution of energy transfer to the deactivation of the donor at $[B]=9 \cdot 10^{-4} \,\mathrm{M}$ is about 10% without diffusion and approximately 12% with diffusion. The visible influence of diffusion in Fig. 4 is therefore only $\approx 2\%$ (from the curve according to eq. (2),) but in Fig. 3 approximately 20%. In spite of the low efficiency of energy transfer in the case of Fig. 3 and 4, the fluorescence intensities of pyrene and perylene are of the same magnitude at $[B]=9 \cdot 10^{-4} \,\mathrm{M}$, because pyrene is strongly quenched by dissolved oxygen and perylene is not. (This can not be seen, of course, from the normalized curves). The dash-dot line in Fig. 3 corresponds to the decay of directly excited perylene; there is no influence of this type on the measured values.

Energy transfer combined with diffusion

The investigation of acceptor time dependence in particular showed that even in very highly viscous solvents the intermolecular distances are not constant in energy transfer. Several authors have dealt with this problem, see refs. [16, 17]. The two phenomena do not act like parallel processes but form a dynamic combination. Our treatment is based upon an *ab initio* statistical model using pair probabilities; the calculations were published elsewhere [17] in detail. Here we report on our first applications of the theoretical results.

The fundamental result is again the expression for the time dependence of the excited donor

$$f_{A^*} = \frac{[A^*]}{[A^*]_0} = \exp{-\left[(n + \theta \cdot n_a[B])t + 2\gamma \cdot \sqrt{nt}\right]}.$$
 (10)

The only difference to eq. (2) is the term $\theta \cdot n_a[B]$ which is added to n. If diffusion contributed to the deactivation of the donor like an independent process of 'collisional energy transfer' [18] we would expect a term $n_a[B]$, like in ordinary Stern-Volmer kinetics. The content of the theory is found in the dimensionless entity

$$\theta = \frac{r_F}{r_{AB}} > 1. \tag{11}$$

 r_{AB} denotes the collision radius of A^* and B which in many cases may be assumed to be 5...6 Å, and

$$r_F = 0.676 \sqrt[4]{\frac{\overline{n \cdot R_k^6}}{D}},\tag{12}$$

must be calculated from the known critical radius R_k , n of the donor and the mutual diffusion coefficient D. If $r_F/r_{AB} < 1$ the theory is not valid because diffusion then predominates and other theories should be applied [19]. Having reached this stage

we can calculate the time dependence of the acceptor in the same manner as above, cf. eqs. (2), (5) and (6). Analogously to eq. (5) we find

$$\frac{d[B^*]}{dt} = -\{n' - \theta \cdot n_a[B]\}[B^*] + \gamma \sqrt{\frac{n}{t}}[A^*]$$
 (13)

omitting the expression corresponding to eq. (6) we finally obtain

$$f_{B^*} = \frac{1}{a} \left\{ 2\gamma \sqrt{\frac{n}{a}} (n'-n) \left[D\left(\sqrt{at} - \gamma \sqrt{\frac{n}{a}}\right) \cdot f_{A^*} + D\left(\gamma \sqrt{\frac{n}{a}}\right) \cdot \exp(-(n't)\right] + \theta \cdot n_a[B] \cdot [f_{A^*} - \exp(-(n't)] \right\}$$
(14)

with $a=n'-n-\theta \cdot n_a[B] > 0$

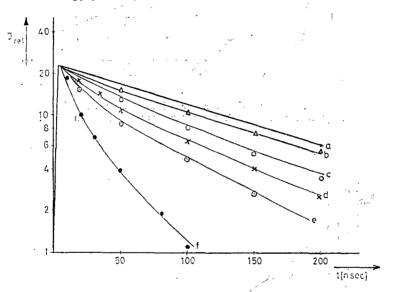


Fig. 5. Measured and calculated time dependence of the donor cf. eq. (10). Solvent paraffin oil with $D = 10^{-7} \, \text{cm}^2 \, s^{-1}; n_a = 5 \cdot 10^7 \, \text{M}^{-1} \, s^{-1}$. $\left(\frac{n_e}{n}\right)^{1/2} [B]_k = 8 \cdot 10^{-3} \, \text{M}$. Concentrations of the unexcited acceptor a) 0, b) 1, c) 2, d) 4, e) 8, f) $20 \cdot 10^{-3} \, \text{M}$. $n = 7 \cdot 10^6 \, s^{-1}$, $n' = 2 \cdot 10^8 \, s^{-1}$

As can be seen in Figs. 5—8, the measured values fit well to the calculated curves. The values of the parameters used for the calculation are mentioned with the figures. The diffusion controlled rate constant n_a was obtained from measured viscosity and proved with the aid of pyrene excimer kinetics [23]. In Figs. 7 and 8, where the viscosity is ten times lower than in Figs. 5 and 6, the curves are similar to ordinary excited state kinetics [20], where the curves consist of simple exponential functions with time-independent rate constants, cf. the 'second row term' in eq. (14). Nevertheless there is no collision transfer [18] what can be seen from the large average rates of transfer in Figs. 7 and 8.

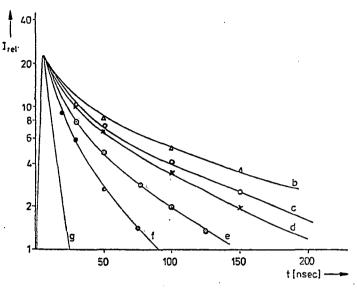


Fig. 6. Measured and calculated time dependence of the acceptor cf. eq. (14). Parameters are the same as in Fig. 5.

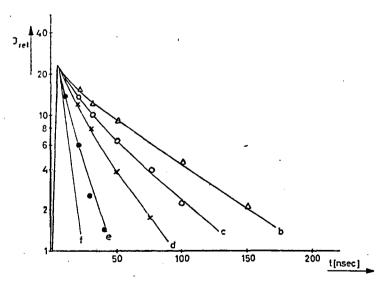


Fig. 7. Measured and calculated time dependence of the donor cf. eq. (10). Solvent 70% paraffin oil/30% 1-Methylnaphthalin with $D=10^{-6}$ cm³ s⁻¹ $n_o=5\cdot 10^8~M^{-1}$ s⁻¹. a) 0, b) 1.5, c) 3, d) 6, e) $20\cdot 10^{-8}$ M. $n=12\cdot 10^8$ s⁻¹, $n'=2\cdot 10^8~s^{-1}\cdot \left(\frac{n_o}{n}\right)^{1/2}=8\cdot 10^{-3}$ M

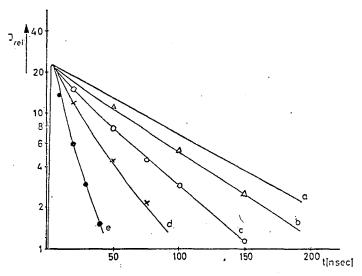


Fig. 8. Measured and calculated time dependence of the acceptor. Parameters are the same as in Fig. 7.

The influence of the time dependence of excitation and the treatment of energy transfer in multiprocess mechanisms

The excitation intensity may be constant or time dependent, but in most experiments on fluorescence and related phenomena it cannot be taken as a δ -function. Thus excitation and deactivation happen at the same time. In the ordinary kinetics of excited states, where the rate constants do not depend on time, the excitation energy $I_a(t)$ may be dealt with as 'inhomogeneity' in the kinetic differential equations. This procedure has proved valid for example in excimer kinetics [16] but it is easily shown that it is not applicable to energy transfer kinetics. From eq. (2) follows by differentiation

$$\frac{d[A^*]}{dt} = -n[A^*] - \gamma \cdot \sqrt{\frac{n}{t}} [A^*]. \tag{14}$$

If we add a term $I_a = I_{a0} = \text{const.}$ at the right hand side and try to calculate $[A^*]_{\infty}$ in the photostationary state by allowing $t \to \infty$, we get the absurd result, that energy transfer plays no role at all (!) To derive the right result we remember first that eq. (2) has proved valid for excitation by a light flash of negligible duration, a δ -function. The time dependent intensity $I_a(t)$ may be thought of as continuous sequence of δ -pulses $I_a(t) \cdot dt$, each of which generates an increment of excited molecules decaying with the function of, say, eq. (2) in our case (and with the appropriate δ -response function in the general case), cf. Fig. 9.

We are interested in the total amount of excited molecules $[A^*]$ as a function of time. This can be calculated by 'convolution':

$$[A^*] = \int_{\theta=0}^{t} I_a(t-\theta) \cdot f_{A^*}(\theta) d\theta \equiv I_a * f_{A^*} = f_{A^*} * I_a.$$
 (15)

(We also introduce the usual symbol '*' for convolution and using this symbol we express that convolution is commutative.)

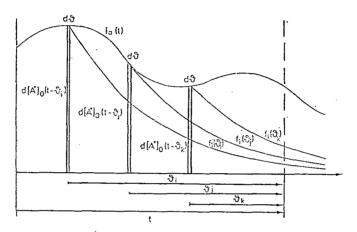


Fig. 9. Explanation of the 'convolution kinetics'

Treating the problem of the photostationary state with the method of eq. (15) we get from eq. (2) and with $I_a = I_{a0} = \text{const.}$

$$[A^*]_0 = I_{a0} * f_{A^*} = \frac{I_{a0}}{n} [1 - \sqrt{\pi} \cdot \gamma \cdot \exp(\gamma^2) \cdot erfc(\gamma)]$$
 (16)

in accordance with Förster's result [21], which was derived from other considerations.

In our derivation of convolution kinetics we made no restriction on a certain decay law. If the new kinetics is correct it must be generally applicable. Indeed, with all problems without time dependent rate constants the differential-equation-method gives the same results as the convolution kinetics. But only the convolution kinetics is claimed to be universely applicable. Convolution in the case where the excitation cannot be taken as a δ -function was probably introduced to photophysics by Bennett [22]. Moreover, if a species B is not excited by irradiation but is formed by a chemical or physical process such as energy transfer, we get $[B^*](t)$ by convolution of its 'ideal decay function', i.e. the response on δ -excitation, with the rate function of its formation. Thus the expression for f_{B^*} in eq. (6) can also be derived as follows

$$[B^*] = \exp(-n't) * \sqrt{\frac{n}{t}} [A^*].$$

Here exp (-n't) is the ideal decay function of B^* and the rate of formation function can be taken as explicitly given function of time, using eq. (2). In such case a solving eq. (5) involves the same integral as the convolution. Generally this is not the case and convolution must be applied.

Finally let us look at examples of how energy transfer can be dealt with in multiprocess mechanisms.

$$\xrightarrow{I_a = \delta(0)} A^* \xrightarrow[n'_r]{n_r} B^* \xrightarrow{n_{B+C}} C^*.$$

First we neglect the process with rate constant n_r' . The species A^* is formed by δ -flash excitation (at time t=0) and will decay simply exponential if all its depopulation processes have time-independent rate constants the sum of which is n. One of these processes is the formation fo B^* with the rate constant n_r . The depopulation of B^* happens both analogously to A^* with the sum of rate constants n' and by energy transfer $n_{B\to C}$. — With the decay function of A^* which is $g_A = \exp(-(nt))$ the time dependence of B^* can easily be calculated using eq. (2) for simplicity

$$g_{B^*} = n_r \exp -(nt) * \exp -(n't + 2\gamma' \cdot \sqrt{n't}).$$

Now we ask for the expressions corresponding to g_{A^*} and g_{B^*} if the back reaction from B^* to A^* with rate constant n', takes place additionally. We get two simultaneous integral equations

$$h_{A^*} = \exp{-(nt) * [n_r \cdot h_{B^*} + \delta(0)]}$$

 $h_{B^*} = \exp{-(n't + 2\gamma' \cdot \sqrt{n't}) * n_r \cdot h_{A^*}}$

because the time function h_{A^*} cannot be calculated without the knowledge of h_{B^*} and vice versa. — The simpler one of our examples can be realized with an excimer system without excimer dissociation (e.g. pyrene) and the more complicated case with most other excimer systems; in both cases the excimer has to play the role of the donor in energytransfer to a convenient acceptor (e.g. one of the various rhodamines if the pyrene excimer is the donor).

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ВРЕМЕННАЯ ЗАВИСИМОСТЬ ДАЛЬНОДЕЙСТВУЮЧЕГО ПЕРЕНОСА ЭНЕРГИИ

М. Хаузер, Р. Фрец, У. К. А. Клени и У. Гёзеле

Если время затухания флуоресценции акцептора меньше чем у донора, тогда зависимость времени флуоресценции акцептора описывается функцией ДАВСОНА.

При исследовании системы пирен (донор) и перилен (акцептор) в водных растворах при различных вязкостях стало необходимым обобшение теории ФЕРСТЕРА, и нужно было учитывать диффузное движение по объему.

При описании кинетики переноса энергии применен метод конволюционой кинетики. Новую теорию можно более широко использовать, чем общепринятую, по методу дифференциальных уравнений.