

EFFECT OF SOLVENT ON THE ABSORPTION AND FLUORESCENCE SPECTRA OF ALCOHOLIC SOLUTIONS OF TRYPAFLAVINE

By I. KETSKEMÉTY, L. SZALAY and Z. VÁRKONYI

Institute of Experimental Physics, József Attila University, Szeged

(Received 10, January, 1965)

The validity of STEPANOV-relation concerning the connection of absorption and emission spectra has been proved for solutions of tryptaflavine in different alcohols of the homologous series.

1. An equation connecting the absorption spectrum $\varepsilon(\nu)$ and the emission energy spectrum $f_e(\nu)$ has been derived by STEPANOV [1]

$$\frac{f_e(\nu)}{\varepsilon(\nu)} = d\nu^3 \exp(-h\nu/kT), \quad (1)$$

where ν and T denote the frequency and the temperature of the solution, respectively; h and k are PLANCK's and BOLTZMANN's constants, d is a constant independent of frequency. KETSKEMÉTY and co-workers [2] have pointed out, that instead of eq. (1) a modified form of it can be considered more reliable:

$$\frac{f_e(\nu)}{\varepsilon(\nu)} = D\eta^*(\nu)n^2(\nu)\nu^3 \exp(-h\nu/kT), \quad (2)$$

where $\eta^*(\nu)$ and $n(\nu)$ denote the relative yield of fluorescence and the refractive index of the solution, respectively. In case of $\eta^*(\nu) = \text{const}$ and $n(\nu) = \text{const}$ (practically in a smaller spectral region) eq. (2) is identical with eq. (1). Eq. (1) may be rewritten in terms of the wavelength, λ : $F(\lambda) \equiv \log f(\lambda) + 4 \log \lambda - \log \varepsilon(\lambda) = -hc/2,30kT\lambda + \text{const}$; $f(\lambda)$ is the emission quantum spectrum as a function of the wavelength λ . If eq. (1) is valid $F(\lambda)$ is a linear function of λ and the slope of the straight is determined by the temperature T of the solution. The temperature T^* obtained from the slope of the straight was found to be $T^* > T$ is most cases.

STEPANOV [3], ALENTSEV [4], ALENTSEV and PAHOMITCHEVA [5], KRAVTSOV and RUBINOV [6], KETSKEMÉTY and co-workers [2], HEVESI and KOZMA [7], RHAZANOVA and co-workers [8], further BORISSEVICH and GRUZINSKII [9] and GRUZINSKII and BORISSEVICH [10] carried out experiments in order to control the validity of STEPANOV's equation in luminescent solid, liquid and vapour systems. In all the investigated systems eq. (1) — in a given spectral region where $\eta^*(\nu)$ and $n(\nu)$ are approximately constant — has been found to be valid, and temperatures of $T^* \cong T$ were obtained.

There is no doubt temperatures of $T^* > T$ may be derived from the slope of the STEPANOV's straights in many cases, however, the nature of this higher temperature is not clear enough. Though the excess of the exciting energy — the source of the increase of temperature — could easily be degraded in condensed (solid and liquid) systems on account of the strong interaction of solute and solvent, T^* was found to be higher than T even in many of these systems. In order to clear up the nature of this interaction the effect exerted by the solvent on the same solute ought to be known. The aim of the present paper is to contribute to a better understanding of the phenomena in liquid systems.

In order to explain some phenomena found in the polarization of fluorescence of solutions, JABLONSKI [11] suggested a mechanism for degradation of the excess of exciting energy. According to this mechanism a part of the excess-energy is converted into the energy of elastic waves by an "initial shock" and the other part is converted into heat ("slow effect") in the neighbourhood of the excited particles

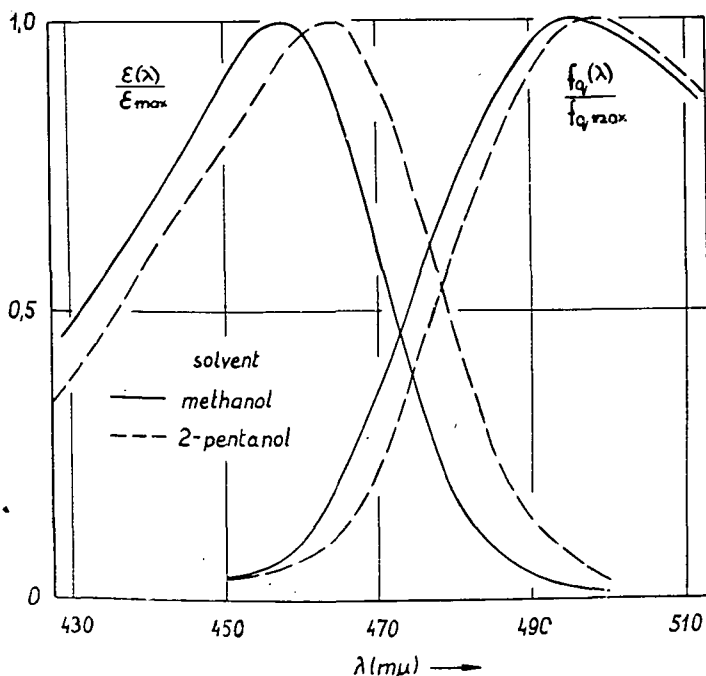


Fig. 1

thus causing a depolarization of fluorescence. JABLONSKI's investigations seem to furnish further reasons to carry out experiments referring to the problem of T^* .

2. Trypaflavine purified by repeated crystallization was dissolved in different monohydric alcohols (see Table 1) of spectroscopic purity¹ in a concentration of $c_M = 3 \cdot 10^{-4}$ mole/l. The absorption and emission spectra have been determined

¹ Authors are indebted to dr. J. Hires for making available some purified solvents.

by means of a spectrophotometer (Optica Milano CF4) at a temperature of $T = 293 \pm 2^\circ \text{K}$. Fig. 1 exhibits two examples of these spectra, the main characteristics of absorption and emission for all solutions investigated are shown in Table I ($k(\lambda)_{\max} = 2,3026 \cdot c_M \cdot \varepsilon(\lambda)_{\max}$).

Table I

Solvent	2		3	4		5		6
	absorption		$k(\lambda)_{\max}$	emission		T^* obtained from spectra corrected to		
	$\lambda_{\max} (m\mu)$			$\lambda_{\max} (m\mu)$		reabsorption	secondary fluor.	
methanol	457,6		14,40	496,9		352,3		324,0
ethanol	462,0		19,35	493,8		356,8		326,6
1-propanol	462,8		15,05	495,0		375,0		336,7
2-propanol	463,0		12,99	495,4		380,1		338,1
1-butanol	463,2		14,14	495,4		390,5		345,2
2-pentanol	463,8		13,62	498,6		445,9		373,9

The excitation was carried out at a wavelength of $436 m\mu$ with a high pressure mercury lamp. The directions of excitation and observation were approximately parallel. The layer thickness of solutions was $l=0,1$ cm. The spectral influence of reabsorption (a) of primary fluorescence and the spectral influence of secondary fluorescence (b) on emission spectra were eliminated according to a method given by BUDÓ and KETSKEMÉTY [12]. Both the emission spectra corrected taking into account of effect (a) and those corrected taking the effect (b) into consideration were used for the determination of T^* in all solutions; the results are shown in Table I. Some examples of the fulfilment of eq. (1) is to be seen in Fig. 2. (The straight lines were constructed by the method of "least squares".)

As for the experimental results, the following remarks should be made. The elimination of influence (b) (of secondary fluorescence) seems to be very important, because the temperatures T^* obtained from the emission spectra corrected taking into account reabsorption (*i. e.* influence (a)) only are much greater than those obtained from spectra corrected taking the effect (b) into consideration (see Table I, columns 5 and 6)². The temperature T^* increases when we go over to a higher alcohol. This phenomenon is similar to that found by HEVESI and KOZMA [7] in case of fluorescein dissolved in glycerol-water mixtures. In both systems T^* increases with the viscosity.

3. A rather rough model — as an alternative of that given in [13] — can be constructed for an illustration of the processes taking place after excitation — provided there exists a microregion of a temperature $T^* > T$. The excess of the

² Ketskeméty and co-workers give a value of $T^* = 321^\circ \text{K}$ for an ethanolic solution of tryptophan in a good accordance with the present value of $326,6^\circ \text{K}$ obtained from an emission spectrum corrected to secondary fluorescence. According to Alentsev and Pahomitcheva [4] effect (b) should cause an increase of T^* . As a matter of fact, the spectral influence of secondary fluorescence depends on the conditions of excitation and observation and on the exciting wavelength as well and may change (either increase or decrease) the value T^* .

exciting energy is converted into heat during the process of excitation and is distributed in a sphere of radius r' and it causes a local temperature of $T_0^* > T$. The temperature difference may be estimated as follows:

$$T_0^* - T = \frac{3k'h(v_a - v_{e \max})}{4\pi r'^3 \rho c}, \quad (3)$$

where $k' = 2,39 \cdot 10^{-8}$ cal/erg, $h(v_a - v_{e \max}) = 5,4 \cdot 10^{-13}$ erg (the excess of the exciting energy estimated from the frequencies of excitation and emission maximum),

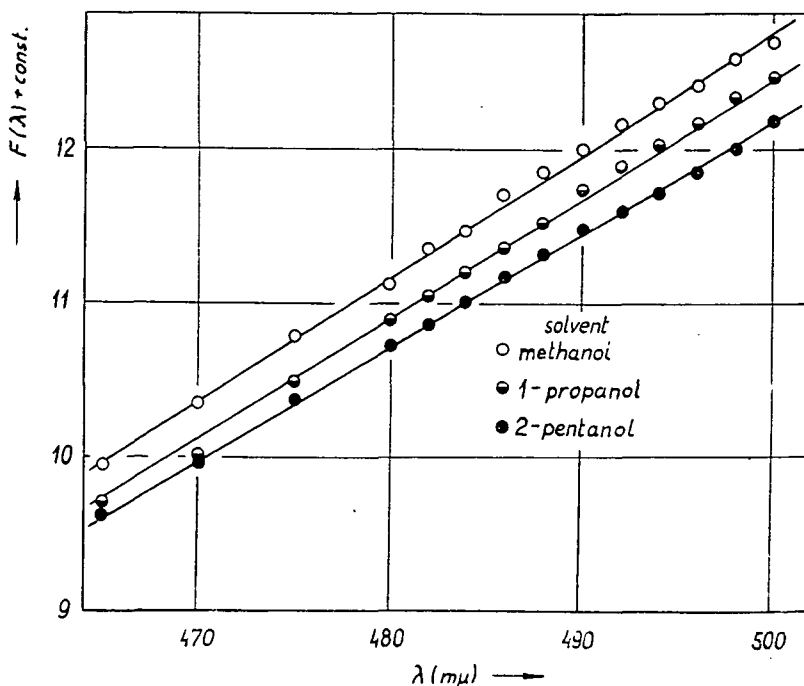


Fig. 2

$\rho c \approx 0,5$ (ρ and c are the density in g/cm³ and the specific heat in cal/g grad of the alcohols, respectively). On assuming a value of $r' = 3 \text{ \AA}$, $T_0^* - T \approx 250^\circ$. Making use of the data in Tab. 1 eq. (3) yields a series of values of the order of 5 \AA for r ($5,8 \text{ \AA}$ in methanol and $4,3 \text{ \AA}$ in 2-pentanol). This seems to be a reliable value because the average distance of the neighbouring excited solute particles is obviously much greater.

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Authors are indebted to I. BECK and S. CSÁKI for their valuable help in carrying out the experiments and calculations.

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ВЛИЯНИЕ РАСТВОРИТЕЛЯ НА СПЕКТРАХ ПОГЛОЩЕНИЯ И ЛЮМИНЕСЦЕНЦИИ СПИРОВЫХ РАСТВОРОВ ТРИПАФЛАВИНА

И. Кечкемети, Л. Салаи, З. Варконц

В случае разных спиртовых растворов трипафлавина исследовалось выполнение соотношения Степанова, описывающие связь между спектрами поглощения и люминесценции.