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HIPOCHROMIC EFFECT IN RIBOFLAVIN SOLUTIONS

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The process of self-assembly of riboflavin molecules in aqueous and binary mixtures of solvents was investigated by a spectroscopic method. It was shown that the self-assembly of vitamin B2 molecules by the dipole-dipole interaction of van der Waals forces, as a result of which resonant splitting of excited electronic levels of riboflavin occurs. In concentrated solutions and in binary mixtures of solvents, the observed hypochromic effects are due to a decrease in the intensity of the absorption capacity of self-aggregated riboflavin molecules relative to their monomers. The absorption band of self-assembled riboflavin molecules is determined by the obtained linear dichroism spectra in a laminar hydrodynamic flow.

Keywords: Riboflavin, self-aggregation, luminescence, absorption, resonant splitting, electronic levels, hypochromic effect, linear dichroism, dipole-dipole interactions.

Introduction

Recently, interest in silicon carbide films as a promising material for nanoelectronics and Riboflavin (vitamin B2) is an important and necessary drug for the development and viability of the human body. Vitamin preparation is used in the form of powders, and in the form of aqueous solutions [1, 2]. Self-assembly is a process in which only the components of the final structure are involved [3, 4]. The main condition for the self-assembly of nanoparticles is the formation of a high-molecular local volume [5]. One of the methods for the formation of a local volume with high concentrations is carried out by thermal evaporation of the solvent from a drop of a solution of the dissolved test compound. The authors of [6, 7] obtained ring structures of nanoparticles on the surface of a glass substrate. The assembly of molecules itself can also be carried out in concentrated solutions and in binary mixtures of solvents. The choice of methods for obtaining self-assembled molecules is one of the most pressing issues in this area.

In the process of self-aggregation, depending on the nature of the solvents used, the concentrations of the compounds under study, there is a significant deformation of the electronic spectra of vitamin B_2 and food dyes, in the form of a hypochromic effect [8, 10]. Identifying the nature of the hypochromic effect is one of the urgent problems of condensed matter spectroscopy. The solution of this question may lead to the development of the thermo and photo stability methods of riboflavin depending on the degree of self-aggregation of molecules.

1. The equipment and technique of the experiment.

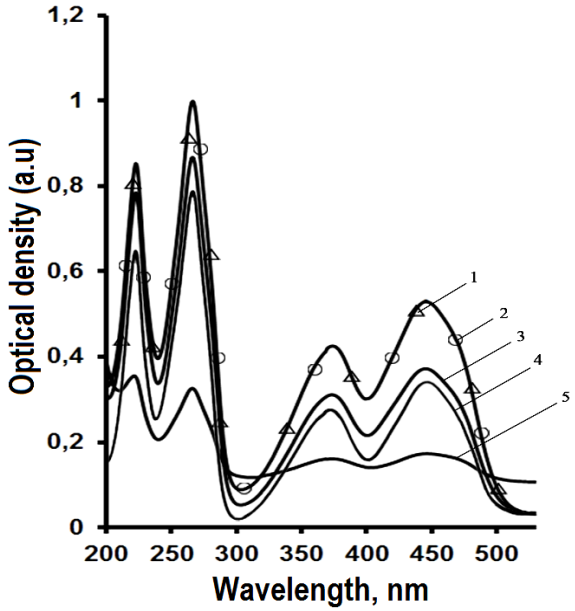
In the work used powder riboflavin brand "HCH". Electronic absorption spectra were measured on a Specord 50 SA spectrophotometer (Analytik Jena, Germany) allowing measurements in the range of 190-1100 nm. The measurement of fluorescence spectra was carried out on an installation assembled on the basis of two monochromators of the type MDR-76 with photoelectron registration. A FEU-38 (Russia) was used as a photodetector. For the convenience of comparing the absorption and fluorescence spectra, they are normalized to unity. The dispersion of the optical rotation and the linear dichroism spectra were taken on a Jasko-20 dichrograph from an optical set-top box of the double Fresnel parallelepiped used in the visible and UV portions of the spectral region. The following solvents were used: distilled water, ethanol, acetone, chloroform purified according to the procedures, as well as their binary mixtures: ethanol + acetone, ethanol +

chloroform. Binary mixtures were prepared in such a way that the concentration of the test compound remained constant, the ratio of the binary mixture of solvent changed. In the second case, the composition of the binary solvent mixture remained constant, the concentration of riboflavin varied.

2. Results and discussion

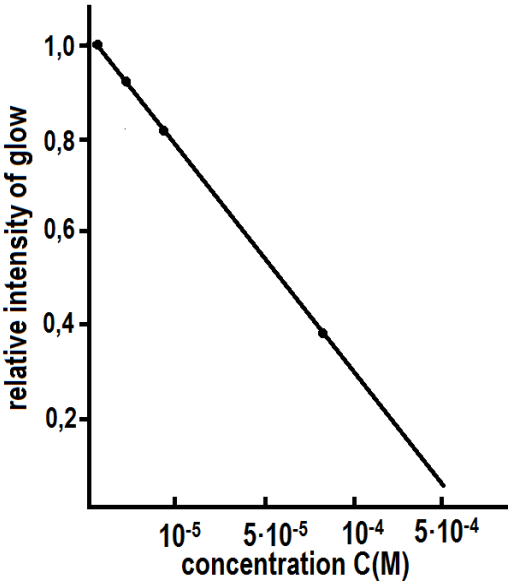
2.1. Riboflavin aggregates

In this case, self-assembling of vitamin B2 was carried out by two methods. The first method was that the concentration of the test compound remained constant, the ratios of the binary mixtures changed. In the second case, the compositions of binary mixtures remained constant, but the concentration of vitamin B2 changed.



a)

Fig.1a. Absorption spectrum of riboflavin in water (1.2) ($C=2\cdot10^{-5}$M) and binary mixtures of alcohol + chloroform (3-5) ($C=4\cdot10^{-5}$M) from the share of added chloroform (0-2; 20-3; 40-4; 60-5) in % volume ratio.



b)

Fig.1b. The dependence of the relative yield of light on the concentration of riboflavin molecules in a binary mixture of solvents alcohol + chloroform (0.35 + 0.65).

Another condition for the use of binary solvents was that they were infinitely dissolved among themselves. As an example, Fig. 1a shows the absorption spectra of riboflavin at a constant concentration ($C = 4 \cdot 10^{-5}$ M), the ratios of alcohol and chloroform changed. From figure 1a it can be seen that the absorption spectrum of riboflavin in pure alcohol coincides with the band of vitamin B2 obtained in dilute aqueous solutions. However, as the proportion of chloroform in binary mixtures increases, the integral absorption capacity of riboflavin decreases (curves 3–5, Fig. 1a). In contrast to the absorption spectra, the shape of the fluorescence spectra of the studied molecules with a constant ratio of the binary solvent does not depend on the concentration of the solution and only a decrease in the relative emission yield is observed (Fig. 1b). These phenomena were explained by us the concentration quenching of luminescence. We observed similar concentration quenching of luminescence in binary solvent mixtures for natural dyes. This process is associated with the aggregation of the studied compounds [8-10].

From temperature experiments, the binding energy of self-aggregates of riboflavin molecules was determined. This energy corresponds to the value of 16-20 KJ / mol, which refers to the energy

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of the hydrogen bond. On the basis of the experimental results obtained, it can be assumed that a certain binding energy belongs to the alcohol + chloroform system, and riboflavin molecules unite with each other under the action of van der Waals forces. To determine which of the van der Waals forces will lead to the self-aggregation of riboflavin molecules, the distribution of charges on the atoms of vitamin B2 was determined.

2.2. Electronic bands of riboflavin monomers and self-aggregates

On the basis of the results obtained, the dipole moments of the ground (μ) and excited (μ*), electronic states of the compound under study were calculated [9]. The calculated values of the dipole moments are μ=7.222 Db and μ* = 23.538 Db, respectively. These values show that riboflavin molecules self-aggregating as results dipole-dipole interaction.

In order to determine the nature of the hypochromic effect in riboflavin solutions, the frequencies ν_{00} were determined at the corresponding intersection point of the normalized absorption and luminescence spectra in the frequency scale for dilute solutions. These values for diluted riboflavin solutions are ν_{00} = 20.200 cm^{-1} (Fig. 2). The most probable transitions to absorption and luminescence for monomeric and self-aggregates of riboflavin molecules were also determined.

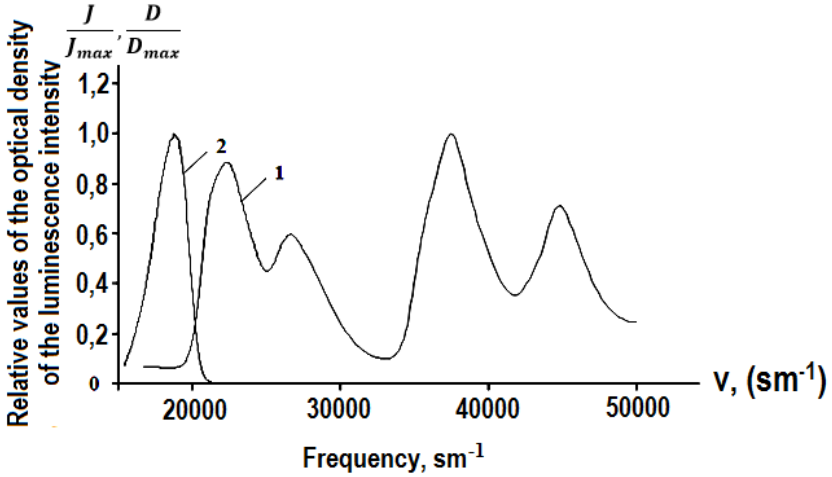

Fig.2. Normalized absorption spectra (1) and fluorescence (2) of dilute solutions of riboflavin ($c = 2 \cdot 10^{-5}$) in water and in alcohol.

The most probable frequency transitions in absorption (ν_p^a)are determined from Fig. 2, for dilute aqueous solutions and binary mixtures of solvents correspond:

$\nu_p^{a_1}$=22300 sm^{-1} and $\sigma_p^{a_1}$=4530 sm^{-1}; $\nu_p^{a_2}$=26800 sm^{-1} and $\sigma_p^{a_2}$=8580 sm^{-1}

$\nu_p^{a_3}$=37450 sm^{-1} and $\sigma_p^{a_3}$=4660 sm^{-1}; $\nu_p^{a_4}$=44840 sm^{-1} and $\sigma_p^{a_4}$=5780 sm^{-1},

where, σ_p^a is the half-width of the corresponding absorption bands. For a dilute aqueous solution of riboflavin, the maximum of the emission intensity corresponds to the frequency, ν_p^f=19050 sm^{-1} and the half-width of this band has the value σ_p^f=2500sm^{-1}.

From fig. 1a, it follows that on the background of the hypochromic effect of absorption and emission bands, the corresponding bands of self-assembled riboflavin molecules do not appear. To obtain the relevant information, we investigated the linear dichroism spectra of the molecules under study. One of these possibilities was realized using the Jasko-20 circular dichrograph with the optical prefix of the double Fresnel parallelepiped. The optical set-top box is designed for studies in the visible and UV part of the spectrum [8, 9].

It was established experimentally that when pumping a solution of self-assembled molecules through a flow cell, they become optically active. Such a flow-through cuvette was developed and

applied to remove the linear dichroism of food dyes and vitamins [11]. Only in this case, the dichrograph registers a different from the zero line of the circular spectrum of linear dixroism. For a clear removal of the linear dichroism spectrum, the rate of solution passage through a flow cell (2mm / hour) was experimentally determined. Such a value of speed was chosen in order to ensure the laminarity of hydrodynamic molasses. In this case, linearly polarized light falls at an angle of 45° on the measuring cell. It has been established experimentally that when pumping solutions of self-assembled molecules through a flow cell, they become optically active.

In fig.3 shows the linear dichroism spectrum of self-collected riboflavin molecules in the frequency scale obtained in a laminar hydrodynamic flow.

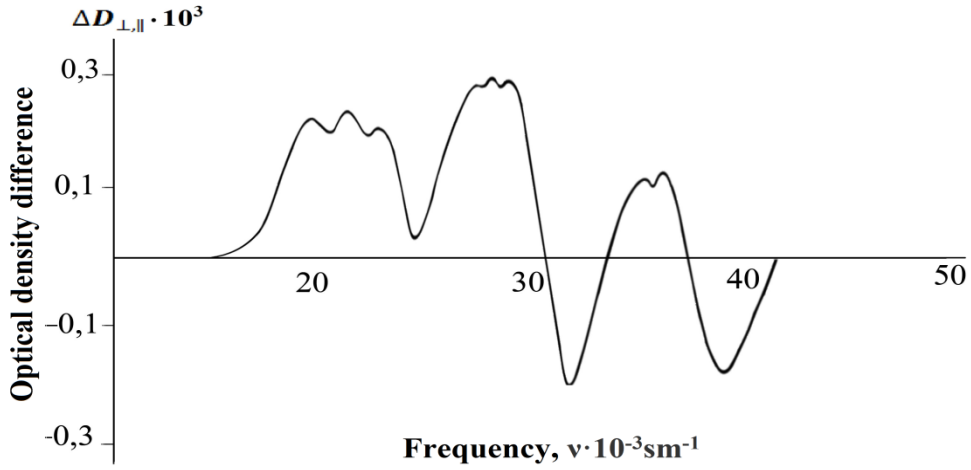

Fig.3. The linear dichroism spectrum of self-collected riboflavin molecules in a laminar hydrodynamic flow.

As can be seen from Fig. 3, the spectrum of linear dichroism is quite informative. The spectral characteristics of the observed bands differ significantly from the absorption spectra of riboflavin monomers obtained in dilute solutions.The parameters of the bands observed in the spectrum of linear dichroism are shown in Table 1.

Using the spectrum of linear dichroism (Fig. 3) and the data presented in Table 1, we can assume that in concentrated solutions, where there is a hypochromic effect, intermolecular interaction (IMI) appears in the form of exciton interaction, which is manifested in concentrated aqueous solutions and binary solvent mixtures of Riboflavin molecules.

Table 1. Energy parameters and optical density differences in linear dichroism of self-assembled riboflavin molecules.

Designations of bands in linear dichroism, ν_c	ν_{max} sm^{-1}	σ_c $\pm 100 sm^{-1}$	$\Delta D_{\perp\parallel} \cdot 10^3 \cdot D$	
			“+”	“-”
ν_{c_1}	21700	1700	0.26	
ν_{c_2}	27800	2000	0.32	
ν_{c_3}	31000	2000		0.22
ν_{c_4}	35000	3400	0.18	
ν_{c_5}	38500	3400		0.16
ν_{c_6}	47800	3800	0,03	

In the Table 1 are used following notations:

ν_c - numbering of electronic bands;

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ν_{max} -frequency corresponding to the maximum for concentrated solutions of riboflavin;
σ_c is the half-width of the band and
$\Delta D\perp\parallel$" +", $\Delta D\perp\parallel$" -" is the difference of the perpendicular and parallel components of optical density with positive and negative values of linear dichroism.

From the analysis of the literature data, it follows that the manifestation of an exciton interaction leads to resonant splitting of the excited electron state [12–13]. Figure 4 shows the scheme of electronic transitions between the normal and excited states of riboflavin in a dilute (m) and concentrated (a) aqueous solution.

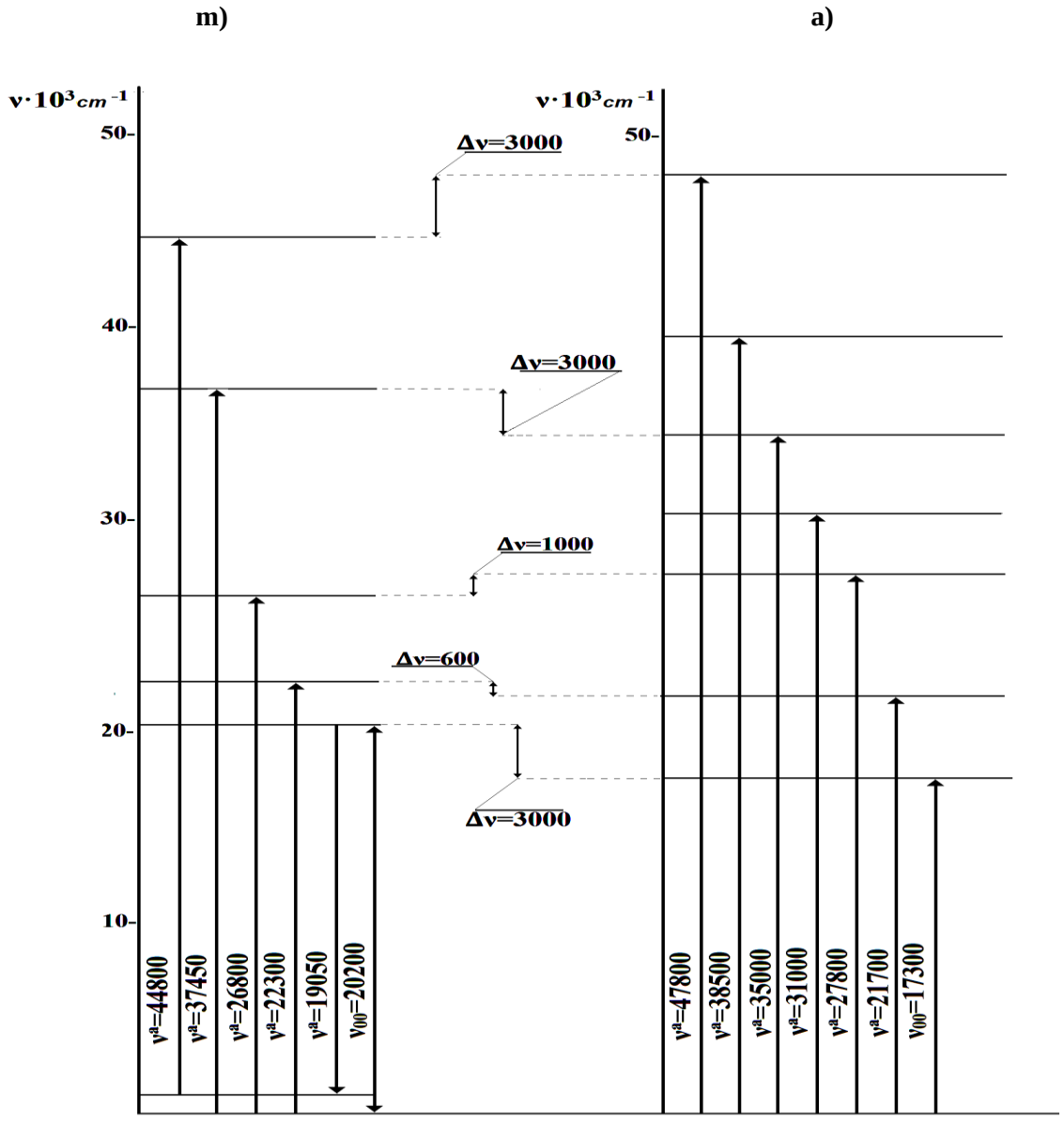

Fig. 4. Diagram of the frequency of electron transitions
of monomers (a) and self-aggregates (b) of riboflavin molecules

From fig. 4 it follows that indeed, in concentrated solutions a resonant splitting of the excited electronic state of the vitamin preparation is observed. As can be seen from rice 4, the magnitude of the resonance splitting is $\Delta\nu = 3000 \pm 200$ cm^{-1}. In electronic circuits, the thickness of the line indicates electronic transitions that are manifested in the absorption spectrum and linear dichroism.

At the same time, electronic transitions correlate with each other as ratios of optical density values.It also follows from Fig. 4 that, along with the splitting of electronic levels related to the frequency $\nu 00$ of the transition, splitting is also observed for other electronic levels, the excited states of self-assembled riboflavin molecules.

Conclusion

Thus, it was found that the self-assembly of vitamin B2 molecules is formed by the orientation of monomeric molecules, and they are combined by van der Waals forces. It is shown that as a result of a strong dipole-dipole interaction in a dimeric unit cell, resonant splitting of excited electronic levels occurs, and changes in the probability of electronic transitions in absorption. The first shows that the absorption bands of self-aggregates can be detected in the spectra of linear dichroism of the compounds under study. On the basis of which the nature of the hypochromic effect observed in concentrated solutions of vitamin B2 is explained.

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