UDC (535.34+535.37):577.164.12

THE NATURE OF ELECTRONIC SPECTRA OF SELF-COMBINED RIBOFLAVIN MOLECULES

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The self-assembly of riboflavin molecules in aqueous and binary mixtures of solvents was studied by a spectroscopic method. It is shown that the self-assembly of vitamin B2 molecules occurs as a dipole-dipole and strong inductive-inductive interaction, as a result of which a resonant splitting occurs by excitation of electronic levels of riboflavin molecules. For self-aggregated molecules in the spectra of linear dichroism, hidden adsorption bands are identified, on the basis of which schemes of electronic transitions to absorption are constructed.

Keywords: riboflavin, self-aggregation, luminescence, fluorescence, spectrum, absorption, structure, hydrogen bonds, electronic transitions.

Introduction

Self-assembly is a process in which only components of the final structure participate [1, 2]. The main condition for the self-assembly of nano particles is the formation of a high-molecular local volume [3]. One of the methods for the formation of a local volume with high concentrations is by thermal evaporation of the solvent from a drop of a solution of the dissolved test compound. The authors [4, 5] obtained ring structures of nano particles on the surface of the glass substrate. Self-assembly of molecules can also be carried out in concentrated solutions and binary mixtures of solvents. The choice of the technique for obtaining self-assembled molecules is one of the topical issues of this direction. The process of the formation of aggregates is accompanied by a significant deformation of the electronic spectra in the form of a hypochromic effect [1-4].

1. Objects and methods of research.

Riboflavin powders of the brand "ChDA" are used in the work. Electronic absorption spectra were measured on an EMC-30PC-UV spectrophotometer, which allows measurements of optical density values with an accuracy of 0.3% and a high resolution in the range of 190-1100 nm. Measurement of the fluorescence and luminescence excitation spectra was carried out using an apparatus assembled on the basis of two monochromators of the MDR-76 type with photoelectron registration. For the convenience of comparing the absorption and fluorescence spectra, they are normalized to unity. The optical rotation dispersion and the linear dichroism spectra were recorded on a Jasko circular dichrograph using the Fresnel double parallelepiped optical attachment calculated for the visible and UV portions of the spectral region.

As solvents, we used: bidistilled water, ethyl alcohol, acetone, chloroform purified according to the procedure [6]. Binary solvents were also used: alcohol + acetone, alcohol + chloroform. Self-assembly of riboflavin molecules was carried out in binary mixtures of solvents: alcohol + acetone, alcohol + chloroform. Quantum-chemical calculation of the electronic structure of the charge distribution and dipole moment, mainly of the excited state of the electrons, was carried out using the MORAS 2009[7], software package, by the semiempirical AM1 method for standard elections parameters [8]. Preliminary optimization of the geometry of molecules using the limited Hartree-Fock method and the Polak-Ribier algorithm with an accuracy of 0.001kcal / (A^0mol) and taking into account various versions of the initial confirmations was preliminarily carried out.

2. Experimental results and their discussion of the influence of the concentration and composition of solvent binary.

In our early works, the concentration dependence of the electronic spectra of riboflavin in water was studied. It is shown that in the highly concentrated aqueous solutions (10^{-5} m ÷ 2 · 10^{-3} m) there is a decrease in the absorbing and luminescent capacity of the solution, i.e., a hypochromic effect is observed. These phenomena are explained that at a concentration of 10^{-5} ÷ 10^{-6} m, riboflavin molecules are in the monomeric state. The observed hypochromic effect in the high-concentration range refers to the self-aggregates of the riboflavin molecules. We observed significant deformations of the electronic spectra in binary mixtures of solvents [7-9].

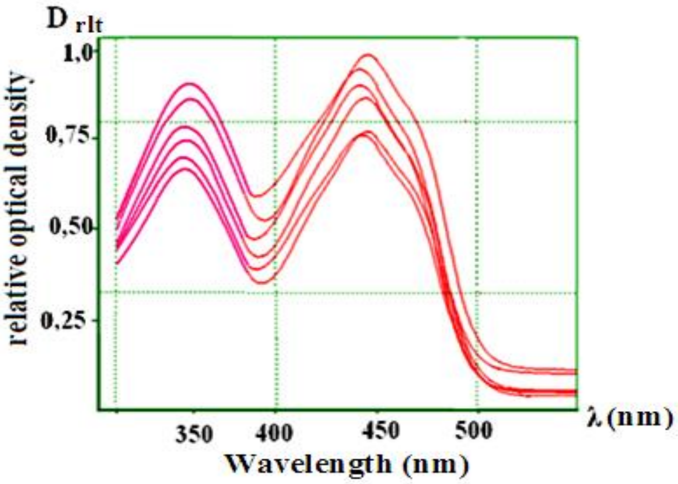

Fig.1a. Absorption spectrum of riboflavin (C 10^{-4} M) in a binary mixture of alcohol + acetone solvents depending on the fraction of acetone added (0-1, 20-2, 30-3, 50-4, 70- 5) in% by volume ratio.

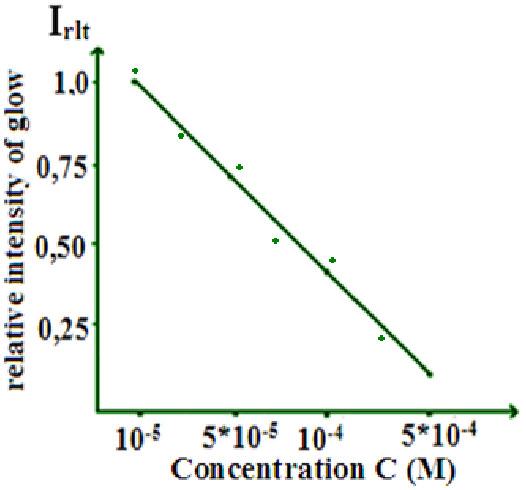

Fig.1b. Dependence of the relative yield of luminescence on the concentration of riboflavin molecules in a binary solvent mixture of alcohol + acetone (0.35 + 0.65)

Binary solvents were chosen in such a way that the concentration of the test compound remained constant, the binary ratios of the solvent mixtures varied. In the second case, the composition of binary mixtures of solvents remained constant, the concentration of vitamin B_2 changed. Other conditions for the use of binary solvents were that solvents were infinitely dissolved among themselves. As an example, Fig. 1 shows the absorption spectra of riboflavin at a constant concentration (C 10^{-4} M) in binary mixtures of alcohol + acetone solvents as a function of the fraction of acetone added.

As can be seen from Fig. 1, the absorption spectrum of riboflavin in pure alcohol practically coincides with the band of vitamin obtained in dilute aqueous solutions. However, with an increase in the fraction of acetone in binary mixtures, the fall of the integral absorption bands of riboflavin is observed. In these cases, the relative intensity of the luminescence of the vitamin molecules is also observed. Similar changes in the electronic spectra, as well as in the relative yield of luminescence at a constant ratio of binary mixtures of solvents, are observed with an increase in riboflavin concentration. Fig. 1, b shows the dependence of the luminescence yield on the concentration of riboflavin molecules with constant mixtures of binary solvents alcohol + acetone (0.35-0.65). As can be seen from Fig. 1b, at a constant ratio of binary mixtures of solvents, concentration quenching of the luminescence is observed. Such concentration quenching of luminescence was observed by us in binary mixtures of solvents for arylethylene molecules and food dyes. These phenomena are explained by the manifestation of aggregation of the molecules under study in binary mixtures of solvents [12-14]. The observed phenomena in binary mixtures of alcohol $+CHCl_3$ alcohol solvents and alcohol + acetone are also possibly associated with the process of self-aggregation of vitamin molecules.

The process of aggregation in binary mixtures of solvents for riboflavin molecules can be explained on the basis of the following reasoning. When dissolving riboflavin in alcohol, each molecule of riboflavin will appear in the solvate of an alcoholic solvent. In this case, the MMVs are eliminated directly between the molecules of the dissolved compound, and they will be in the monomeric state.

The addition of an insoluble component of solvents, whereby these components are combined by a hydrogen bond, $ROH \ldots OR,\ \ R$ or $OH \ldots ClR_2,$ where R, R_1 и R_2 respectively, the lagging part of the molecules of alcohol, acetone and chloroform, respectively. As a result, the MMW solvate around the dissolved substance weakens. An increase in the fraction of the second component of the binary solvent leads to a further weakening of the solvate around each molecule of riboflavin. In these cases, the molecules of the test compound come out of the solvate and there is the possibility of interaction between the molecules of the dissolved molecules. These processes lead to the formation of a local volume with a high concentration, which lead to self-aggregates of the vitamin. Self-assembly of riboflavin molecules is carried out. At the first stage of aggregation, dimers are formed. As the fraction of insoluble component $CHCl_3$, acetone increases, the process of aggregation of the medication molecules intensifies and complex or more complex aggregates of the vitamin B_2 are formed. To confirm this assumption, we conducted a temperature study of the electronic spectra of solutions of riboflavin, where a hypochromic effect is observed in the absorption and emission bands.

3. Temperature studies.

It was experimentally established that an increase in the temperature of the solution, where a hypochromic effect occurs, an increase in the absorbing and luminescent capacity of the solution was observed. At a temperature of 75-80^{0}C, the absorption and emission spectra of the monomeric molecules of the test compound are restored. From the temperature experiments, the binding energy of self-aggregates of riboflavin molecules was determined. This energy corresponds to a value of 16-20 KJ / mol, which corresponds to the energy of the hydrogen bond. On the basis of the obtained experimental results, it can be assumed that a certain binding energy refers to the alcohol + acetone, alcohol + chloroform system, and the riboflavin molecules combine under the action of van der Waals forces. To find out which of the Van der Waals forces lead to the very aggregation of riboflavin molecules, it is definitely the distribution of charges on the atoms of vitamin B2. Also, dipole moments in the main μ and μ, * electronic states of the test compound were determined [9]. The calculated values of the dipole moment are $\mu = 7.222$ Db $\mu = 23.538$ Db. These values show, when self-aggregating riboflavin molecules, a strong dipole-dipole interaction occurs. For the nature of electronic states, we determined the frequencies of the 0-0 transition and the most probable transitions to absorption and luminescence were calculated for monomeric and self-aggregates of riboflavin molecules. For this purpose, the electron spectra were translated in the frequency scale and normalized to unity by the intensity (Fig. 2).

As can be seen from Fig. 2, the frequencies corresponding to the intersection point normalized absorption and emission spectra, i.e. for dilute solutions correspond to the values of the frequencies of the purely electronic transition $\nu_{00} = 20.200$ sm^{-1}. The most probable frequency transitions in the absorption (ν_p^a) (from left to right on the frequency scale) for dilute solutions of aqueous and binary solvent mixtures correspond to:

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

$$\nu_p^{a_3} = 37450 sm^{-1} \quad \sigma_p^{a_3} = 4660 sm^{-1}; \quad \nu_p^{a_4} = 44840 sm^{-1} \quad \sigma_p^{a_1} = 5730 sm^{-1};$$

Where $\sigma_p^{a_1}$ is the values of the half-width of the corresponding absorption bands. For a dilute aqueous solution of riboflavin, the maximum intensity of the glow corresponds to the frequency $\nu_p^f = 19050\ sm^{-1}$ and the half-width of this band is significant $\sigma_p^f = 2500\ sm^{-1}$.

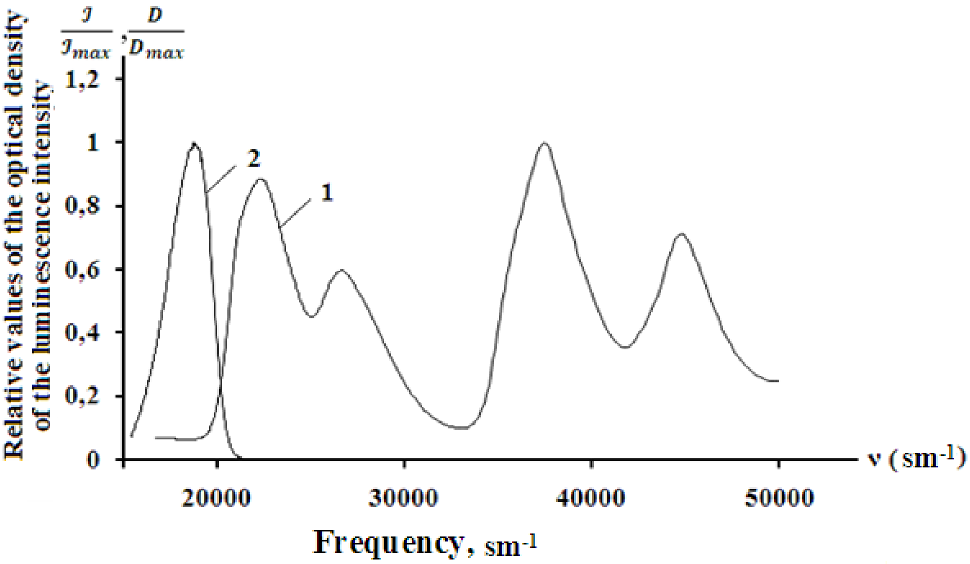

Fig.2. Normalized absorption spectra (1) and fluorescence (2) diluted solutions of riboflavin.

Similarly, it would be desirable to obtain normalized absorption and emission spectra for concentrated aqueous and binary mixtures of riboflavin solvents. However, in aqueous and binary mixtures of solvents of the medication, the absorption spectra will be structureless. Against the background of structurelessness in normalized spectra, electronic absorption and emission bands appear with the following frequencies:

$\nu^a = 22120sm^{-1},\ 37450sm^{-1},\ 4500\ sm^{-1},\ 19050\ sm^{-1}$.

These electronic bands, according to their maxima and in shape, correspond to dilute solutions of riboflavin. These results show that concentrated solutions of the vitamin B_2 have absorption bands of much lower intensity with respect to the electron bands of dilute solutions. Those sweep bands are hidden against the background of an unstructured adsorption solution.

4. Studies on the spectra of linear dichroism

It is noted in [10] that the spectra of linear dichroism are more informative with respect to absorption spectra. To obtain the relevant information on the position of the bands for concentrated solutions of the preparation, the possibility of measuring the spectra of linear dichroism was investigated. We realized one of these possibilities using the Jasco-20 circular dichrogram, using the optical prefix designed and manufactured for the visible and UV part of the spectrum, the Fresnel double parallelepiped. In practice, the measure of circular dichroism is the magnitude of ellipticity, determined by the ratio of the small and major axis of the ellipse [8-9].

It was experimentally established that when a solution of self-assembled molecules is pumped through a flow cell, they become optically active. Such a flow cell is developed and patented by the authors, and also used to remove the linear dichroism of food dyes and vitamins [11]. Only in this case dichrogrof registers a curve of the linear dichroism spectrum that is different from the zero line. To accurately remove the spectrum of linear dichroism, the rate of passage of the solution through a flow cell (2 mm / h) was determined experimentally. Such a speed value was chosen in order to ensure laminarity of the hydrodynamic molasses. In this case, linearly polarized light falls at an angle of 45° to the measuring cuvette. It has been experimentally established that when pumping solutions of self-assembled molecules through a flow cell, they become optically active. In Fig. 3 shows the spectrum of linear dichroism of self-assembled riboflavin molecules obtained in a laminar hydrodynamic flow in the frequency scale.

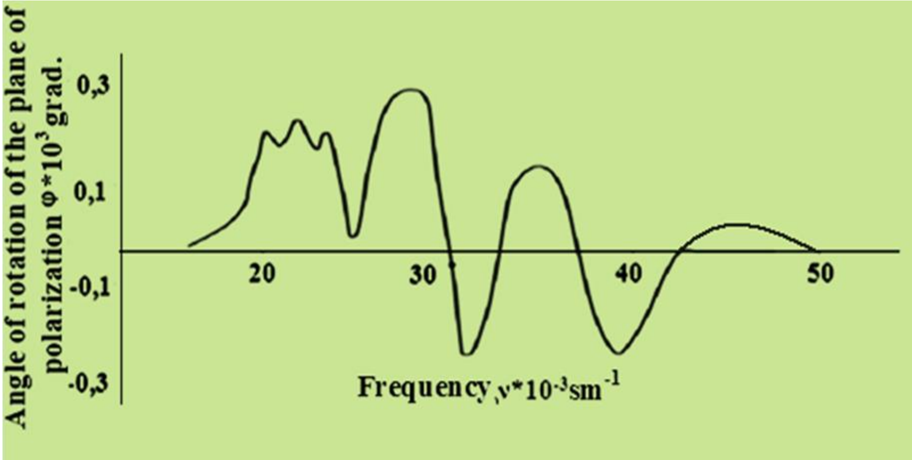

Fig.3. The spectrum of linear dichroism of self-assembled riboflavin molecules in a laminar hydrodynamic flow

As can be seen from Fig. 3, the spectrum of linear dichroism is quite informative. In this case, both long-wavelength and short-wave bands with positive and negative values of the rotation angle of the plane of polarization appear in the spectrum. The observed bands differ greatly in spectral characteristics from the parameters of the main bands of the absorption spectra of riboflavin monomers obtained in dilute solutions. Only in this case does the dichrograph register a curve of the linear dichroism spectrum that is different from the zero line. The parameters of the observed bands are given in Table 1.

Table 1. Energy parameters and angles of rotation of the plane of polarization of electronic bands, self-assembled molecules of complex riboflavin self-aggregates, obtained from the spectrum of linear dichroism.

Legend of new bands ν_c	ν_{Max}	σ_c	φ_+	φ_-
	sm^{-1}	$\pm 100 sm^{-1}$	$\varphi \cdot 10^2 grad$	$\varphi \cdot 10^2 grad$
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	

Here ν_c is the numbering of electronic strips; ν_{max} is frequency corresponding to the maximum for concentrated solutions of riboflavin; σ_c is the half-width of the band$\varphi_{(+)}, \varphi_{(-)}$ and the positive and negative rotation angle of the plane of polarization, respectively.

The fact of the presence of "positive" and "negative" directions of the angle of rotation of the plane of polarization shows that the observed spectrum is the result of MMW in the form of exciton interaction, which manifests itself in the self-assembly of riboflavin molecules in concentrated aqueous solutions. In favor of this assumption, one can attribute the fact of observation of both short-wave and long-wave bands in the spectrum of linear dichroism [11-13].

From the analysis of the literature data it follows that the manifestation of the exciton interaction leads to a resonant splitting of the excited electronic state. Figure 4 shows the scheme of electronic transitions between the ground and excited states of riboflavin in a dilute (a) and concentrated (b) aqueous solution.

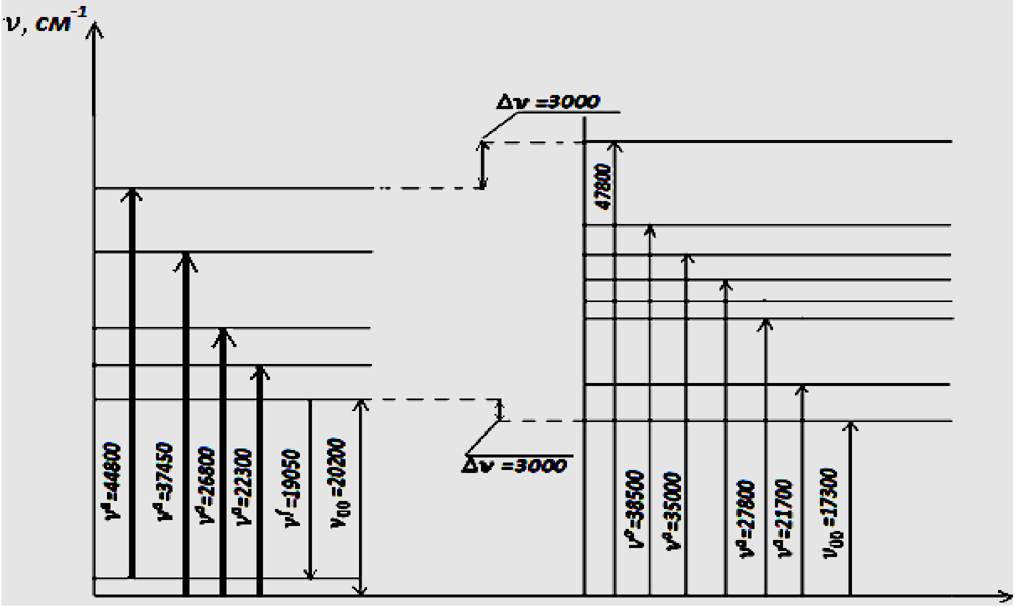

Fig.4. Scheme of frequencies of electron transitions of monomers (M) and self-aggregates ("a") of riboflavin molecules

From Fig. 4 it follows that, in fact, a resonant splitting of the excited electronic state of the vitamin preparation is observed in concentrated solutions. As can be seen from Fig. 4, the resonance expansion tips are Δν = 3000 cm^{-1} in electronic circuits, the line thickness indicates electronic transitions that appear in the absorption spectrum. In this case, the electronic transitions are correlated as a ratio of the optical densities. For the above scheme, we can write the following relations:

ΔE_c= ΔE +ΔD+$V_{1,2}$,

where ΔE_c and ΔE are the energy of the electronic transition for molecules in concentrated and dilute solutions, respectively.

ΔD is the difference between the energies of the interaction of excited and normal molecules.

$V_{1,2}$ is energy of excitenic interaction between molecules in a concentrated local volume of solvents.

The calculated values$V_{1,2}$ correspond to ≅ 1700cm^{-1}, which is characteristic for inductive coupling. Apparently, under the action of this force, self-assembly of molecules is formed.

Conclusion

It is thus established that the molecules of riboflavin self-aggregates are formed under the action of Van der Waals forces and dipole-dipole interactions are most probable. It is determined that a strong inductive-inductive interaction also occurs in solutions of vitamin B_2 as a result of which the splitting of the excited electronic states of riboflavin occurs during their self-aggregation. It is shown that self-assembled molecules of the flavone derivative have a sufficiently high value of optical activity and possess linear dichroism.

Acknowledgment

This work was supported in part by a grant OT-A4-07, Uzbekistan

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Article accepted for publication 11.06.2018