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## SPECTROSCOPIC RESEARCHES OF THERMODESTRUCTION OF RIBOFLAVIN IN AGGREGATIVE STATE

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*By spectroscopic researches have been established that water solution of riboflavin undergoes destruction at temperature influence. And at that depending on concentration of researched compound in solutions the share of molecules which undergo destruction is in an interval of (4-28) %. It is shown that powders of riboflavin are steady enough against temperature influences. We made a conclusion that sterilization of water solution of riboflavin used in medical and biologic practice is not to share temperature influence at (373-393) K.*

**Keywords:** riboflavin, solution, destruction, concentration, powder, temperature, sterilization.

### Introduction

Riboflavin (vitamin B<sub>2</sub>) is one of the most important water-soluble vitamins, participating in many biochemical processes [1, 2]. However in practice are often used solutions of organic compounds where their molecules are in various aggregative state, and each of which possesses the electronic spectra [3]. Thus in multicomponent mixesthere can be various intermolecular interactions (IMI) which influence to spectral-luminescent characteristics of organic compounds. Along with the spectral-luminescent characteristics the essential parameter defining working resource of solutions and medical preparations is their photo stability and thermal stability. Photo physical and photochemical properties as well as the processes occurring in solutions of riboflavin are well enough studied in [4-7]. However in the course of manufacture of the various medicines based on riboflavin for their sterilization thermal processing is used, which in result can considerably change the property of these medicines [8]. Therefore studying of thermo stability and development of distinct methods of stabilization of riboflavin in solutions has the important practical value.

### 8. Objects and technique of research

Powders of riboflavin of DA marks of industrial production [9] are used in this scientific work. As solvent the distilled water was used. Electronic spectra of absorption were measured on spectrophotometer Specord 50 SA (Analytikjena, Germany) allowing to carry out measurements with accuracy (+/-0.003 D) and the permission (0.3 nanometers) in a range 190-1100 nm.

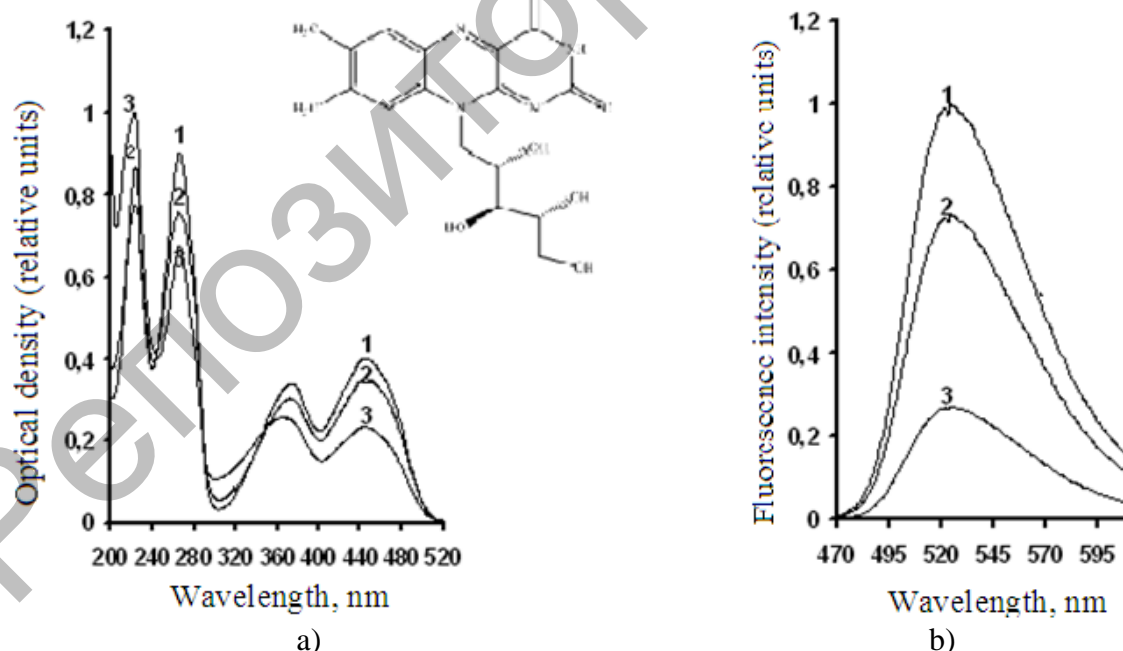
Measurement of spectra of fluorescence and excitation was carried out on the luminescent installation assembled on the basis of two monochromators of MDR-76 type with photo electronic registration (PER-38). Spectra captured by means of quartz cuvette allowing varying a thickness of a measured layer from 0.1 to 50 mm. Also the phenomenon of reabsorption was considered.

Powders and solutions of riboflavin subjected thermal processing in densely corked cuvettes and boiling under normal conditions, in drying box at 98<sup>0</sup>, 120<sup>0</sup>, 180<sup>0</sup>, 190<sup>0</sup> and 200<sup>0</sup> C. At temperature researches of powders of riboflavin thin layers of small dispersive powders were put on special "pure horizontal substrate" which has been placed in the special chamber. Hot air was entered into the chamber through branch pipes in the range of temperatures 20<sup>0</sup>-180<sup>0</sup>C. Powders of riboflavin ere dissolved in the distilled water after thermal processing. For temperature influence of water solutions of riboflavin was used the heat-resistant capsule. With a view of reduction of

pressure of water steams in a capsule the cooling block has been built in, with which help water steams were condensed. The formed condensate back came across in a cuvette, being in a heat-resistant capsule. Dispersion of optical rotation and spectra of linear dichroism captured on circular dichrograph Jasko-20 applying calculated and made on visible and UV spectral areas of optical prefix - a double parallelepiped of Frenel. Spectra of a nuclear magnetic resonance captured on spectrometer WM-360 (USA) in deuterated water ( $D_2O$ ) and dimethyl sulfoxide ( $CD_3SOCD_3$ ). IR absorption spectra captured on spectrophotometer UR-20 (Germany). The resulted spectra of absorption and fluorescence were normalizes to unit for convenience of comparison.

## 9. The received results and their discussion

Water solutions of powders of riboflavin which have been subjected to temperature influence were exposed to spectroscopic research. Such researches are caused that water solutions of vitamin  $B_2$  are its medicinal form. With a view of sterilization of water solutions of riboflavin thermal treatment is usually used. Solution thermal treatment has been carried out by water steam at temperature  $373^{\circ}K$  within 20 minutes. The spectrum of absorption and fluorescence of water solutions of riboflavin (fig. 1 a and b) has been received. Apparently from fig. 1a water solutions of riboflavin in ultra-violet and visible area of a spectrum are typical for molecules of vitamin  $B_2$ , and has four strips of absorption  $I_{max} = 223, 268, 375$  and  $445$  nanometers. The fluorescence strip is in area of  $525$  nanometers (fig. 1 a,b curve. 1). It shows that vitamin molecules are in monomeasured conditions. Water solutions of riboflavin were exposed to temperature influence  $100^{\circ}C$ ,  $120^{\circ}C$  and  $150^{\circ}C$  during 20 minutes (Fig. 1). Spectra of absorption of vitamin  $B_2$  are resulted at  $T = 293, 373$  and  $423^{\circ}K$  accordingly curve 1-3. Apparently from fig. 1a at temperature influence on water solutions of riboflavin essential deformation of electronic spectra of an investigated preparation is observed. Thus along with falling of absorptive abilities of a strip from  $I_{max}=445, 375$  and  $268$  nanometers is observed growth of value of optical density of a solution at  $I_{max}=220$  nanometer.



**Fig. 1.** Absorption spectra (a) and fluorescence (b) of the solution of riboflavin in water ( $s=2 \cdot 10^{-5}$ ) sustained at temperature  $\sim 423^{\circ}K$  during: (1) - 0, (2) - 20, (3) - 60 minutes.

In these case for a strip from  $I_{max}=375$  nanometer is observed hypsochromium mixture son 7 nanometers (fig. 1a, curve 2-3). Hypsochromicity of absorptive strips is accompanied also by reduction of intensity of fluorescent ability of water solutions of riboflavin. Thus, the form of a

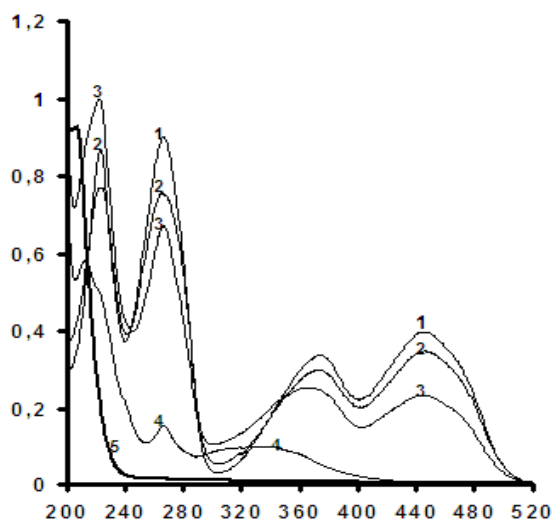
spectrum of a luminescence remains invariable (fig. 1, curve. 2-3).

Received results show that in the course of heat treatment of water solutions of riboflavin the preparation partially collapses. Thus, the quantity of the core substance – of riboflavin decreases in interval 4-20% (fig. 1). Besides, quality of this medicinal form will appear low because of presence at it of products of destruction of preparation. Temperature influences also were investigated with the help of carbon dioxide laser ILGN-701 with  $l_{\text{rad.}} = 10600$  nanometers and capacity of 60 V, which beams are scattered by germanium lens. The irradiation of the sample was carried out within 2-7 seconds. The IR, nuclear magnetic resonance and electronic spectra of vitamin B<sub>2</sub> temperature influence have been captured and compared.

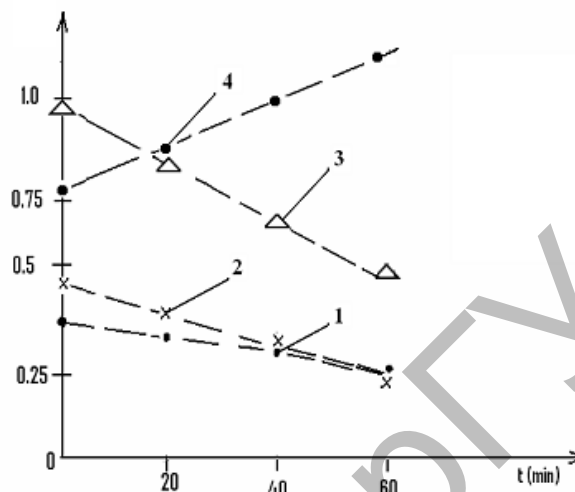
The received experimental results show that powder processing of riboflavin within the investigated temperatures during 20 minutes, and also processing of a dry powder by light carbon dioxide laser during 2-3 seconds does not lead to appreciable changes in IR and a nuclear magnetic resonance spectra, and intensity of absorption and a luminescence of solutions remains almost invariable. All these results show that in solutions where molecules of riboflavin are in monomer condition is observed thermal destruction of a preparation. In this time for a powder of figurative riboflavin process of destruction is not observed in the range of temperatures 290-423 K. Thus, investigated temperature influence on molecules being in a solution had various associations (aggregation).

For detailed research of processes of aggregation in solutions of food dyes binary mixes of solvents water-DMSO have been chosen. Thus, as well as in case of the concentrated water solutions deformation of electronic spectra was observed at transition from a water solution to dimethyl sulfoxide solution. In absorption spectra of riboflavin falling integrated absorptive abilities of a solution is observed. In this case the spectrum of absorption of the diluted solution in the chosen binary mix practically coincides with a spectrum of absorption of corresponding compound in water  $K = 2 \cdot 10^{-5}$ . In process of concentration growth the falling of integrated absorption abilities is observed. At the further increase in concentration of riboflavin are formed microcrystals of dissolved substance which drop out after a while in a deposit.

The spectroscopy of linear dichroism has appeared rather informative and exact quality monitoring of degree of aggregation of riboflavin in the water environment. In works of authors [15-17] it is shown that in a case when in a solution are formed associates of investigated compounds and pumping of such solution through one millimetric flowing to a cuvette with a velocity of 2 mm/sec, it is observed linear dichroism. Water solutions of riboflavin were exposed to temperature influence 373K, 393 K and 423K during 20 minutes. As an example on fig. 2 absorption spectra of riboflavin at 423 K are resulted. From fig. 2 at temperature influence on water solutions of riboflavin essential deformation of electronic spectra of an investigated preparation is observed. Thus, along with falling of absorptive abilities of a strip with  $l_{\text{max}}=445, 375$  and 268 nanometers, are observed growth of value of optical density of a solution at  $l_{\text{max}}=220$  nanometer. In these cases for a strip with  $l_{\text{max}}=375$  nanometer is observed hypochromic shift on 7 nanometers (fig. 2). Hypochromicity of absorption strips is accompanied also by reduction of intensity of fluorescent ability of water solutions of riboflavin. Thus, the form of a spectrum of a luminescence remains invariable. Hypochromicity of electronic spectra of absorption and a luminescence are defined by many authors to partial association of molecules in solutions. Absence of associates in solutions of riboflavin in case of influence on them of temperatures proves to be true following reasons. First, hanging of temperature of a solution leads to dissociation of molecules [18, 19]. Secondly, in case of temperature influence symmetric falling of all strips of absorption is not observed. Thus, it is experimentally established that temperature influence on water solutions of riboflavin leads to falling maxima of strips of absorption in the range from 24 to 42%.



**Fig.2.** Dependence of spectrum of absorption of water solution of riboflavin at temperature ICZK from time 0 (1), 40 (2), 60 (3), products of destruction (4) and carbamide ( $s=2,5 \cdot 10^{-4}M$ )



**Fig. 3.** Dependence of optical density of water solutions of riboflavin at temperature  $150^{\circ}C$  from time maxima of strips of absorption  $l_{max} = 370$  nanometers (1), 445 nanometers (2), 265 nanometers (3) and 220 nanometers (4)

On the basis of the received experimental results and the analysis of the literary data we have concluded that at thermal influence of solutions of riboflavin it is observed destruction of preparation of riboflavin. The cited data has been defined from analyses of spectra of absorption of a water solution of riboflavin. At the same time, the analysis of spectra of fluorescence which are more sensitive to environment changes show that at temperature 423 K quantity of molecules of a preparation undergoing to destruction can make up to 75 %. The same changes of quantity of riboflavin molecules which undergo of destruction is observed at constant sizes of temperature influence depending on change of time keeping of water solution of preparation at the given temperature. So, for example, at temperature 423 K for 20 minutes preservation of a solution during 20 minutes of ~15 % of molecules of riboflavin under goes destruction. In this time ~28% and ~42 % of molecules undergo destruction at keeping of a solution during 40-60 minutes accordingly. Essentially that in percentage share of molecules of riboflavin which undergo destruction depends both on temperature and time of thermal influence. At constant time of thermal influence of 40 minutes and temperatures 373K the share of molecules of riboflavin undergoing destruction makes 4 %, at 393 K - 7 % and in the same time at 423 K more than ~20 %.

The analysis of change of electronic spectra of water solution of molecules of riboflavin shows that in absorption spectra is observed two isobathic points at lengths of waves  $l=285$  nanometer and  $l = 335$  nanometers. Presence of these points specifies that in electronic spectra there are two centers of absorption. As these centers are defined absorption strips of riboflavin and strips concerning molecules formed as a result of thermal influence of initial compound. Apparently, at thermal influence on solutions of riboflavin the structure of the formed products differs from products of photo destruction. Such assumptions prove to be true by two reasons. The first is connected with absence of luminescent ability of products of destruction. In the second we assume that spectra of absorption of products of destruction are in ultra-violet part of a spectrum. For an estimation of a spectrum of absorption of products of destruction from a curve 3 have been subtracted a spectrum of adsorption of pure riboflavin. The received spectrum is resulted on fig. 2, curve 4. Apparently from fig. 2 maximum of a spectrum of absorption of products of thermal destruction is at length of a wave  $l_{max} = 210, 260$  nanometers and a wide strip in the range of length of a wave of 290-360 nanometers. In these cases all absorptive indicators are in ultra-violet area of a

spectrum. Thus, the long-wave strip of products of destruction has very low values of factor of absorption. From the analysis of spectra of absorption of products of destruction and the literary data [6, 14] we suppose that a principal cause of deformation of spectra of absorption and discoloration of a water solution of riboflavin is splitting of molecules of a preparation on carbamide and 1, 2 – dihydric – 6, 7 – dimethyl – 2 – keto– 1 – rybitil– 3 –quinoxylcarbon acid. Formation of such products at thermal influence is not compatible in medical-biological practice [14]. Therefore, we consider that for sterilization of a water solution of riboflavin used in medical and biologic practice it is not compatibly to use temperature influence ~ 373-393 K.

## Conclusion

Powders of riboflavin are steady enough against temperature influences. For riboflavin in water solutions a share of molecules undergone destruction are in the interval of 4-28 % in dependence of concentration of preparation and degree of temperature influence. It is established that the spectrum of absorption of products of thermal destruction of riboflavin is in ultra-violet part of a spectrum (carbamide and quinoxyl carbonacid), and it well coincides with an absorption strip of carbamide acids.

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