SPECTROSCOPIC RESEARCH OF THE INTERACTION BETWEEN 2,2'-BIPYRIDYL-BASED EUROPIUM COMPLEX AND HUMAN SERUM ALBUMIN

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Investigation of interactions of proteins with luminescent europium complex is interesting for the practical task of sensitive detection of proteins, as well as to study fundamental problems of peptide-lanthanide interactions (1). Presented work is devoted to research of chemical and spectroscopic properties of europium complex named OK-18 (europium trinitrate (2,2'-bipyridyl-6,6'-dikarbonilazandiil)tetrakis (methylene) phosphonate) (Figure 1) and to study its interaction with human serum albumin (HSA) using various spectroscopic techniques. This substance has a unique optical ability such as high values of the fluorescence intensity in aqueous solutions, which is more than the intensity of many other known coordination complexes of rare earth elements (REE).

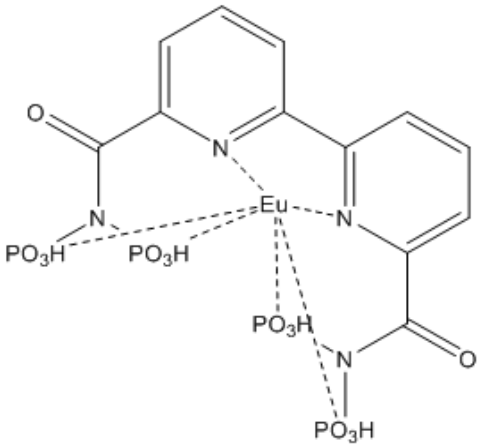

Figure 1: Structural formula of europium trinitrate (2,2'-bipyridyl-6,6'-dikarbonilazandiil)tetrakis (methylene) phosphonate (OK-18).

The investigation was carried out using the following methods: fluorimetry, spectrophotometry, IR spectroscopy, and dynamic light scattering method.

The first step of the investigation was the research of the influence of water on the fluorescent properties of OK-18. The experiment was conducted using the luminescence spectrometer «Hitachi F-7000» and the spectrophotometer Hitachi U-1900. The main maxima of europium in the luminescence spectrum (593 nm and 616 nm (2)) do not shift, thus, the chemical interaction between OK-18 and water doesn't occur and the complex is stable in aqueous solutions. In addition, the quantum yield was calculated and it consists about 14% in the DMSO solution and 16% in the water solution.

The second step was the examination of the stability of OK-18 in the alkaline solution. For this purpose were prepared solutions with different values of pH (7 – 10). Positions of main maxima in the luminescence spectrum do not change with pH alteration. Moreover, the quantum yield consists about 14-16% indicating the stability of OK-18 in the solutions with pH 7 - 10.

Further, the possibility of the chemical interaction between OK-18 and HSA was investigated. The experiment was conducted adding different volumes of the solution of OK-18 ($2 \cdot 10^{-5}$ M) to the solution of HSA ($1.5 \cdot 10^{-5}$ M) with a small amount of DMSO, which was offered to add to improve the interaction with the protein. The displacement of maxima 333 nm (the absorption of tyrosine) and 340 nm (the absorption or tryptophan) in the luminescence spectrum at the excitation wavelength 270 nm and 295 nm respectively was not observed. Therefore, preliminary conclusions about a lack of interaction between the complex and the protein were made.

Then, the interaction was investigated by IR spectroscopy. Characteristic peaks of protein (1650 cm^{-1} – C=O, C-H; 1550 cm^{-1} – C-N) do not shift by the addition of various volumes of the solution of OK-18 ($1.4 \cdot 10^{-3}$ M) to the solution of HSA ($1.5 \cdot 10^{-4}$ M). The experiment with addition of some quantity of DMSO cannot be carrying out due to higher intensity of peaks of DMSO.

Finally, the research was conducted using dynamic light scattering method (Photocor Complex), which allows to estimate hydrodynamic radii of particles in the solutions. Experimental results show that in the solution without DMSO there are two separated components – complex particles and HSA with radii 115±25 nm and 3.5±0.3 nm respectively which is in agreement with data of pure complex solution and HAS solution. In case of the adjunction small concentration of DMSO in the solution of OK-18 and HSA was detected a third component with radius 180±23 nm. We suppose this third component corresponds to particles which were formed as a result of interaction between DMSO and contaminants in the solution.

As a result of this work it has been shown that the coordination complex OK-18 is stable in the alkaline solution and does not manifest interaction with human serum albumin in the solution.

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