

Spectroscopic Studies of New Fluorescent Nanomaterial Composed of Silver Atoms and Organic Dye

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The novel fluorescent nanostructures are synthesized in a simple one-step process by UV light illumination of silver salt in a mixture with organic dye Thioflavin T. The latter serves both as a sensitizer in photoreaction and as molecular support. The most stable composite structures are obtained in 2-propanol. They are characterized by absorption spectra that are quite different from that of the dye and by strong excitation and emission bands with the maxima at 340 nm and 450 nm correspondingly. We suggest that this photoreaction product consists of two silver atoms and two dye molecules. We believe that this new fluorescent nanoscale material will find many applications in biosensing and bioimaging technologies.

Keywords: Fluorescence, Nanostructures, Silver Atoms, Thioflavin T, Organic-Inorganic complexes.

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1. INTRODUCTION

Many new phenomena were discovered in the research on noble metal atoms forming nanoscale structures. This story started with the discovery by Michael Faraday of stable suspensions formed by colloid gold. Next the experiments came on plasmonic effects observed with gold and silver nanoparticles that lead to dramatic enhancement in intensities of Raman and fluorescence bands. The trend of new discoveries continued with the important findings of intense fluorescence of clusters formed from only several atoms of these metals [1-6]. These results stimulated many activities in basic research with an attempt to understand, predict and optimize the observed effects. Simultaneously there started a broad range of applications in sensing and imaging technologies, in information recording and other areas. Being photostable and environmentally friendly, these new nanomaterials promise a lot of attractive features in biotechnological applications.

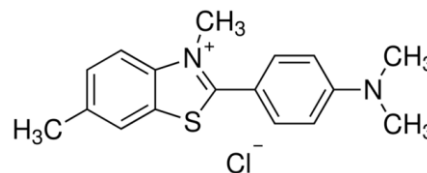
Recognizing the advantages of these new fluorescent materials, the researchers realized immediately that such noble metal clusters formed in different conditions are unstable and need efficient molecular support in order to avoid their growing into colloids or sediments. A variety of ligands were applied to stabilize them, starting from low-molecular thiols to DNA fragments [3] and synthetic polymers [2,4-6]. Some of these molecular constructs feature insufficient stability in time and with the change of external conditions. But their most important negative aspect comes from inability to achieve strongly stoichiometric cluster-to-ligand correspondence in a small-size composite unit.

Such possibility appears as a result of our research. We show that when the clusters are formed by photo-reduction of silver salt in the presence of organic dye, the stable dye-metal composites are formed with stoichiometry 2:2. In previous work [7] we demonstrated the fluorescence spectra of obtained clusters together with the description of optimal conditions for their synthe-

sis. The present report is focused on transformation of light absorption and fluorescence spectra in the course of formation of these clusters in alcohols.

2. MATERIALS AND METHODS

In this research we used Thioflavin T, which is a well-known organic dye that is frequently applied in different biotechnologies, in particular, for determination of cellular deposits of pathological protein aggregates. It is a cationic dye that is solubilized in polar solvents together with an anion:



Thioflavin T.

In our work we used Thioflavin T from Sigma-Aldrich, cat. No T3516-5G of 75% purity. After solubilization in a series of polar organic solvents it was mixed with AgNO₃ solution. Then this mixed system was illuminated with near-UV light using a home-built photoreactor based on the lamp DeLux EBT-01, 26 Wt, 780 Lm, maximal wavelength 360 ± 15 nm. Illumination was performed in common small test tubes at a distance of 10 cm and its duration was variable on the scale of hours.

The excitation and emission spectra and their evolution with time were studied on spectrofluorimeter PTI QuantaMaster 40 in a standard cuvette at room temperature. The obtained material is characterized by strong excitation and emission bands located at ~340 nm and ~450 nm correspondingly. Excitation and emission monochromator slits were set at 5 nm.

Absorption spectra were studied on spectrophotometer Perkin-Elmer Lambda Bio+ in standard conditions at room temperature.

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3. RESULTS

Fig. 1 demonstrates the changes that occur in absorption spectra as a result of UV illumination. High transparency and the absence of visual turbidity of both reactant solutions and of formed nanoclusters witness against the presence of any micellar or other colloid formations. This fact is clearly derived from the absorption spectra.

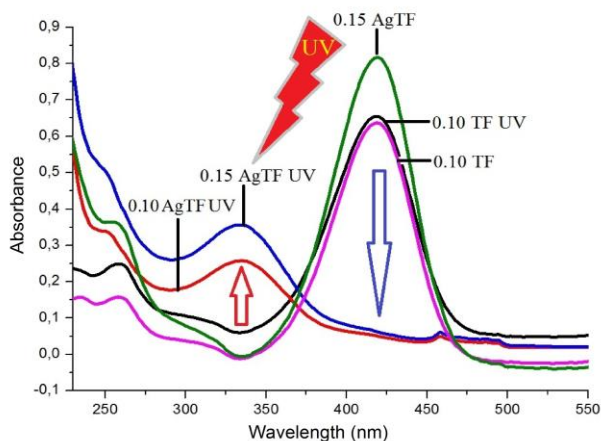


Fig. 1 – Absorption spectra of intact Thioflavin T (TF) and after its treatment with UV light (TF UV), its mixture with AgNO_3 , intact (AgTF) and after UV treatment (AgTF UV). Concentrations of components (millimolar in stoichiometry 1:1) are indicated.

Thioflavin T is characterized by strong and relatively narrow absorption spectrum with the maximum at 440 nm and very weak fluorescence intensity in solutions excited at this wavelength. Illumination of this solution does not bring essential changes of these spectra. Since the silver salt is transparent in this range of wavelengths, its addition does not change the absorption spectrum. Meantime, the most dramatic changes are observed when this mixture is illuminated with the UV light. The 440 nm band disappears completely and, instead, there appears the band at 330-340 nm that demonstrates clearly the synthesis of new species.

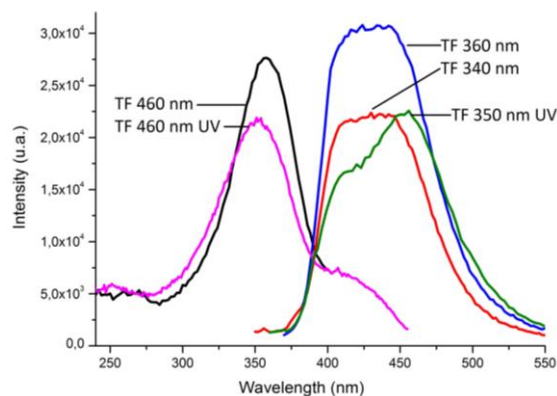


Fig. 2 – Fluorescence excitation (left) and emission (right) spectra of Thioflavin T in 2-propanol. Illumination for 1 hr results in decrease of fluorescence intensity and deformation of spectra. Excitation and emission wavelengths and UV treatment are indicated.

It is interesting to follow the changes of fluorescence spectra with illumination time, first of Thioflavin, and then on the addition of silver salt solution. Thioflavin T is characterized by a very weak emission when it is excited at 340-360 nm. This emission is attributed to a contaminant, which is believed to be an oxidized form of the dye. Illumination of Thioflavin solution in 2-propanol results in gradual photobleaching. Fluorescence intensity is decreased and some deformation of spectra is observed, probably due to fluorescence of photooxydation products (Fig. 2).

Addition of silver ions to thioflavin solution without illumination does not change the fluorescence spectra. Only a small decrease of intensity is observed, which may be due to collisional quenching by heavy atom (the results are not shown).

Dramatic changes in this system are observed on illumination. The strong fluorescence band appears with the maximum at 466 nm already after 1 hour of illumination (Fig. 3). It shifts to shorter wavelengths (454-457 nm) when this treatment continues for 3-4 hours. At 5 hours some decrease of intensity starts to be observed. So we consider 3-4 hours of light treatment to be optimal for the reaction to occur.

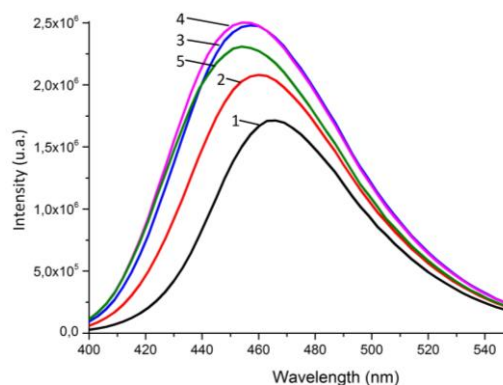


Fig. 3 – Fluorescence emission spectra of the system containing Thioflavin T and silver ions in 1:1 molar ratio in 2-propanol at different illumination times (hrs).

Fig. 4 illustrates the fluorescence excitation and emission spectra of this photoreaction product. These spectra are quite different from that of parent Thioflavin T. One has to note that for the spectra obtained in the same conditions the intensity of photoproduct is higher by almost two orders of magnitude! This gives us a strong argument to consider that we synthesized a new product with characteristic photophysical behavior.

Interestingly, we observe a small peak in absorption and excitation spectra at 250-260 nm. Excitation at this band allows obtaining the fluorescence spectra in similar positions as on excitation at the main long-wavelength band (see Fig. 4). We suggest that this minor band belongs to the second electronic transition of the silver cluster so that the Kasha rule is observed.

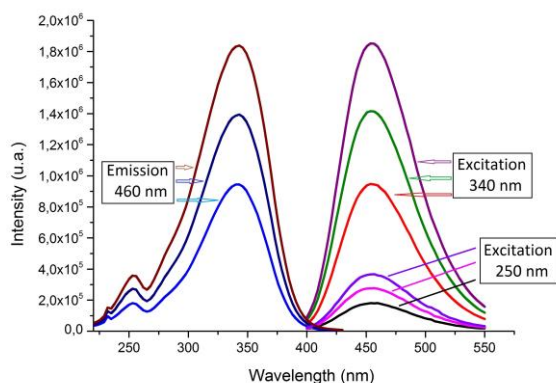


Fig. 4 – Fluorescence excitation (left) and emission (right) spectra in 2-propanol at indicated wavelengths. Illumination time 3 hrs.

The question arises, if the new fluorescent nanostructures can be synthesized by the action of UV light only in 2-propanol or this treatment can be provided in other solvents. The attempts to provide this synthesis in ethanol and water were quite successful. The correspondent spectra in ethanol are presented in Fig. 5.

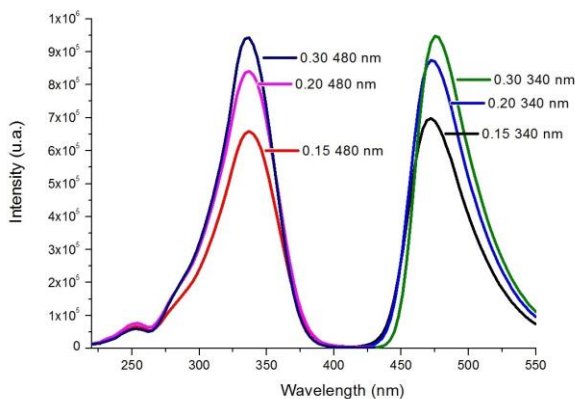


Fig. 5 – Fluorescence excitation spectra at emission 480 nm (left) and emission spectra excited at 340 nm (right) in 96% ethanol. The concentrations were 0.15, 0.20 and 0.30 mM. Illumination time was for 10 hrs and after that the samples were stored for 10 days.

The spectra in ethanol are narrower and shifted to longer wavelengths, probably due to the effect of sol-

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vent polarity on electronic transition. Meantime, these products demonstrate loss of stability on storage with the appearance of black sediments of silver with the loss of proportionality between fluorescence intensity and concentration (see Fig. 5). In water the system demonstrated the loss of stability (the appearance of silver deposits) during several hours. It should be noted that when some portion of formed product degrades, the remaining part retains fully its spectroscopic properties.

Al the samples studied by us demonstrate high level of spectroscopic homogeneity. In the broad excitation range (e.g. from 300 to 370 nm) the fluorescence spectra do not change their spectral position but only change the intensity in accordance with the profile of excitation spectrum. Similar observation was made regarding the excitation spectra as a function of emission wavelength.

Our experiments continue in the direction of determining the molecular structure of the complex. Preliminary data obtained in experiments on mass spectroscopy show the presence of particles with masses ~900 Da and the absence of larger particles. Experiments on size-exclusion chromatography are in progress to get an independent information on the particle size and to test their homogeneity. The NMR experiments are planned.

4. CONCLUSIONS AND PROSPECTS

According to our preliminary data, reported here metal-dye composites are the smallest brightly fluorescent metal-dye composites obtained so far. They possess an attractive set of properties including sub-nanometer size, high brightness and photostability. Their synthesis is clean and they are safe in production and applications. Moreover, similar to other products that contain silver they may possess bactericidal properties. However, the most prospective applications are expected in live cell imaging, since as the labels they can combine two types of imaging – by fluorescence and by contrast in electron microscopy.

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