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New Fluorescent Nanomaterial Based on Silver Atoms and Organic Dye for Biosensing and Bioimaging Applications

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We report on synthesis and studies of strongly fluorescent nanostructures formed of several Ag atoms with the aid of organic dye Thioflavin T that serves as sensitizer and molecular support. The 1:1 stoichiometry between silver atoms and dye molecules is observed in this complex formation. These novel structures are formed in a simple one-step way by photoreduction on illumination by UV light and are characterized by excitation and emission maxima at 340 nm and 450 nm correspondingly. These materials offer good prospects for different applications in biosensing and bioimaging technologies.

Keywords: Fluorescence, Nanostructures, Silver Atoms, Thioflavin T, Bioimaging.

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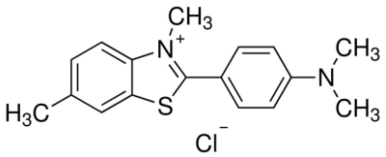
1. INTRODUCTION

Different fluorescent nanocomposites are being developed for biosensing and bioimaging. They address strong demands regarding high brightness and photostability and also desired wavelength range of excitation and emission, possibility of functionalization, the absence of toxicity, etc. We are focused on the development of nanostructures that involve fluorescent clusters of several atoms of silver. Previously such clusters were obtained by different methods of reductions of silver salt solutions (by application of UV light, chemical reducers, microwaves, etc.) with the necessary presence of different scaffolds (polymers, DNA, SH-reagents) that suppress continuous growth of nanoparticles [1-5]. By now all these systems do not allow precise determination of cluster composition and achieving sufficient stability. With this aim, we decided to use electron-rich organic dyes as both photoreducing agents and also supports of reduced silver atoms that form silver clusters composed of several (2-4) atoms.

2. MATERIALS AND METHODS

Thioflavin T is a well-known organic dye that is frequently used in different biotechnologies, in particular, for determination of cellular deposits of pathological protein aggregates. Its formula is presented below.



Thioflavin T.

In our work we applied Thioflavin T from Sigma-Aldrich, cat. No T3516-5G with 75% purity. After solubilization it was mixed with $AgNO_3$ dissolved in series of polar organic solvents and water and then illuminated this mixed solution with near UV light. The conditions for illuminations were the following. The lamp DeLux EBT – 01, 26 Wt , 780 Lm, maximal wavelength 360 ± 15 nm, illuminations time 3 hrs at a distance of 10 cm in common small test tubes.

The time dependence of the appearance of fluorescence intensity and the excitation and emission spectra were studied on spectrofluorimeter PTI Quan-taMaster 40 in common instrument conditions 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.

The dependences of excitation spectra on emission wavelength and of emission spectra on excitation wavelength were obtained with intervals of 10 nm over the correspondent bands. Excitation and emission monochromator slits were set at 5 nm.

3. RESULTS

The nanocomposite structures obtained by photoreduction as indicated above based on the dye and silver salts were highly fluorescent. Their fluorescence parameters were similar for the emitters synthesized in water, ethanol (96 %), *2*-propanol. The spectra in *2*-propanol are presented below (Fig. 1).

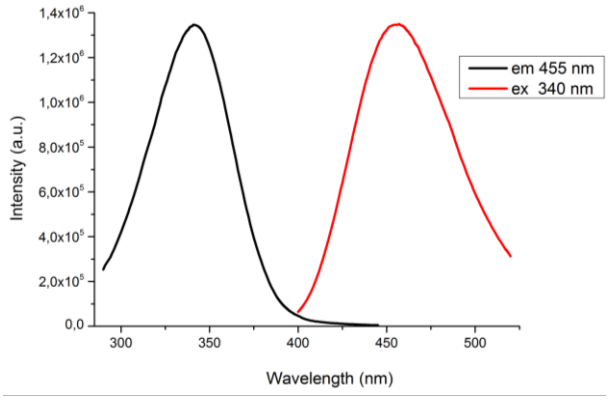


Fig. 1 – Fluorescence excitation (left) and emission (right) spectra of silver clusters - Thioflavin T nanocomposites in *2*-propanol. The silver clusters were formed in solution of $AgNO_3$ in the presence of Thioflavin T (the molar ratio 1:1) obtained by UV photoreduction

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In the case of *2*-propanol the fluorescence emission of silver clusters showed the highest stability with observation time, whereas in water the system demonstrated the loss of stability (the appearance of silver deposits) during several hours. The clusters formed in *2*-propanol were characterized in most detail regarding stichiometry of cluster formation, exposure time, etc. The fluorescence increase over that of parent Thioflavin T was 15-20-fold, and at the conditions of illumination, in which the dye bleaches rapidly, the cluster retains its strong emission.

As we can see from Fig. 1, the spectra are characterized by excitation and emission maxima at 340 nm and 450 nm correspondingly. Small variations of these spectra are observed as a function of solvent, and they will be analyzed in our future studies. It is essential that Thioflavin T without silver salt addition in the same conditions of illumination behaves differently. Its emission is characterized by a shifted fluorescence spectrum at 465-470 nm and much lower (~15-20-fold) fluorescence intensity. Upon illumination it is photobleached with the loss of intensity, so that the fluorescence spectrum of oxidized form is observed on a very low level at 415-420 nm.

It could be also noticed that fluorescence of studied materials is characterized by a very strong Stokes shift that indicates some strong relaxation process in the excited state. Its origin is not understood and requires special studies for its elucidation. In practical terms, this fact is beneficial, since it allows providing excitation and collecting emission at strongly different wavelengths, thus avoiding re-absorption and light-scattering effects.

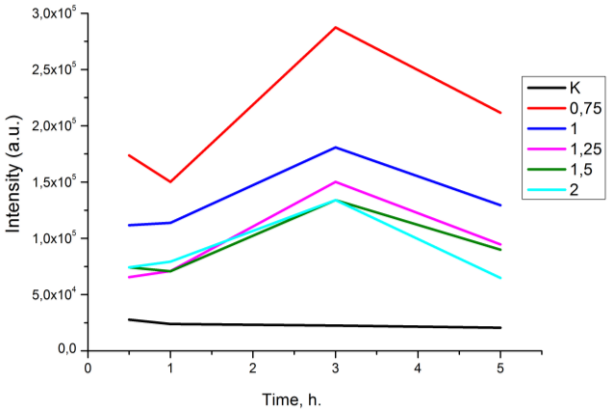

Fig. 2 – Dependence of fluorescence intensity at 450 nm (excitation 340 nm) on illumination time (hours) for different molar ratios (in the range 0.75-2.0) between Thioflavin T and silver ions in the illuminated solution in *2*-propanol. K indicates fluorescence of sole Thioflavin T

As we observe from Fig. 2, the fluorescence intensity of formed fluorescent nanoclusters increases with the illumination time and passes through a maximum at 3 hours. These conditions (the time 3 hours and the molar ratio 0.75) were used in our subsequent studies. It should be noted that since we used Thioflavin T preparation that was of 75% purity, we can derive that actual stoichiometry of dye molecules and Ag^0 atoms in the composite was 1:1.

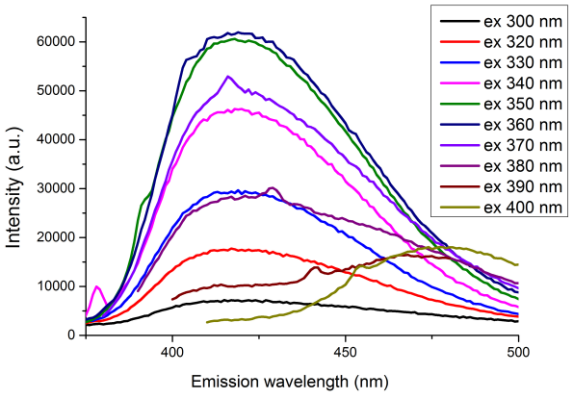

Fig. 2 – Dependence of fluorescence spectra on excitation wavelength for fluorescent Thioflavin T – Ag^0 nanocomposites in *2*-propanol

In order to verify whether fluorescence intensity comes from Thioflavin T (that could be enhanced) or from the formed nanocomposites we studied the excitation wavelength dependence of fluorescence emission spectra (Fig. 3). We observe that in the wavelength range from 300 to 370 nm the fluorescence spectrum does not change its position but only changes the intensity in accordance with the profile of excitation spectrum. However, with the increase of excitation wavelength from 380 nm and above a new spectral form starts to be observed that can be attributed to Thioflavin T that did not participate in the formation of nanocomposite.

High transparency of solutions of the formed nanoclusters and the absence of visual turbidity witness against the presence of any micellar or other colloid formations. Remarkably, after drying and subsequent re-solvation the cluster fully regains its fluorescent properties. According to our observations, these materials are strongly fluorescent in polymer media and when dried on chromatographic paper.

Our experiments continue in the direction of determining the molecular structure of the complex. Preliminary data using confocal fluorescence microscopy indicate that this complex when applied to cell incubation media can penetrate into the living cells and label their inner volume. It remains to establish the optimal concentrations and incubation times for this application. It can be stated however that under strong laser beam used in confocal microscope the formed nanocomposite exhibits much higher photostability than common fluorescent dyes used in histochemistry.

4. CONCLUSIONS AND PROSPECTS

Few-atom silver nanoclusters are new but already known fluorophores with an attractive set of properties including sub-nanometer size, high quantum yield and photostability [1, 6–9]. Being of much smaller size than semiconductor quantum dots and exhibiting the absence of toxicity and lack of blinking [6] they are attractive for biosensor applications, biological imaging [10, 11], opto-electronic devices [12, 13], chemical sensing [14–16] and optical recording media [17, 18]. In view of such broad range of applications many efforts are being made to optimize their properties.

Since the first report on stable fluorescent silver clusters in solution [19] much attention has been paid for achieving the necessary stability of these materials by varying the type of scaffold and synthesis procedure. Silver nanoclusters are usually encapsulated in water-soluble, polar, organic scaffolds that provide multiple binding sites for silver including polymers [20–25], dendrimers [19, 20], peptides [26, 27] and DNA oligonucleotides [6, 8, 28–31]. Meantime, there are applications that require small size of fluorescence reporter together with well defined molecular structure. The described herein technology of obtaining dye-Ag^0 atom composites can suggest such possibility.

According to the obtained data, the highest fluorescence emission was observed in the dye: Ag ion ratio 1:1, which , in view of the known fluorescent properties of Ag clusters in other systems, suggests the cluster composed of two silver atoms and two dye molecules. These only preliminary estimates, and exact molecular composition of these new materials has to be carefully verified. The suggestion that silver atoms are included into nanocomposite in the form of dimmer of the type Ag_2^0 comes from rather short wavelengths of their excitation and emission, whereas larger clusters formed on DNA scaffolds emit fluorescence in the red-near IR region [3, 8].

The area of possible applications of newly synthesized silver nanoclusters include fluorescence sensing and imaging within the living cells. Their functionalisation and inclusion into multifunctional nanocomposites have good prospects expanding the range of fluorescence reporters for biosensing technologies in microscopy, flow cytometry and microarray formats.

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