

Fluorescent Few-Atom Clusters of Silver Formed in Organic Solvents on Polymeric Supports

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Few-atom silver clusters are fluorophores with a set of attractive properties including sub-nanometer size, high quantum yield and large Stokes shift. Sharing high photostability with semiconductor quantum dots but being of much smaller size, lacking blinking and with expected lack of toxicity, they are especially attractive for biological imaging, down to single molecules. No less promising are their applications in chemical sensing and biosensing as well as for molecular optic and electronic devices on a single molecular level. We demonstrate that it is not a unique property of water that can provide the formation and stability of silver clusters. They can be produced on photoreduction in different organic solvents using the same polymeric template. Unique photophysical properties of these clusters share both similarities and differences to that of organic dyes.

Keywords: Fluorescence, Probes, Sensing, Imaging, Silver few-atom clusters, Polymeric scaffolds.

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1. INTRODUCTION

The ability of clusters of silver composed of only several atoms to emit fluorescence in the visible range of spectra has attracted many researchers interested in biosensing and bioimaging applications [1-3]. These new fluorescence emitters can also be the basis of optical recording of information with extremely high density and for other technological areas. They are usually obtained by reduction of silver salts in aqueous solutions. We developed a technology for obtaining these clusters using photoreduction on polymeric scaffolds in different organic solvents.

2. SYNTHESIS OF FLUORESCENT SILVER CLUSTERS

Synthesis of silver clusters was performed in a series of organic solvents using an amphiphilic polystyrene-*block*-poly(methacrylic acid) block copolymer (PS-*b*-PMAA). The PMAA homopolymer was already reported to be an excellent template for silver clusters in aqueous medium,[4] and in the current work the PS block was selected to extend the solubility to polar and apolar organic solvents. We achieved photoreduction of silver ions in different organic solvents, including those of high and low polarity. This study demonstrates that it is the matrix that matters much stronger than the major solvent [5].

3. FLUORESCENCE MEASUREMENTS

Recording of fluorescence spectra and analysis of excitation-emission functions was performed on Cary Eclipse spectrofluorimeter in a standard cuvette at room temperature. 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 5 nm. For anisotropy measurements excitation and emission polarizers were used.

Two samples (illumination time of 9 h and 6 h) were used in these studies. With these samples we provided three series of experiments. 1). Dependence of excitation spectra on emission wavelength (λ_{em}) with the steps of 10 nm in λ_{em} variation. 2). Dependence of emission spectra on excitation wavelength (λ_{ex}) within the long-wavelength band of absorption with the steps of 10 nm. 3). Excitation spectra at different λ_{em} and emission spectra at different λ_{ex} at excitations in the near-UV range of absorption. Probing the structural and dynamic heterogeneity by stepwise shifts of excitation or emission wavelengths with recording of correspondent spectra is a frequently used tool with common application in the studies of polymers and biopolymers. In addition, fluorescence anisotropy was measured over fluorescence excitation and emission spectra.

4. STABILITY AND FLUORESCENCE PROPERTIES

The strong variations in stability and fluorescence properties (positions of spectra, quantum yields ϕ and lifetimes) for clusters synthesized in different solvents are observed (see. Table 1).

In this study quantum yields in toluene and tetrahydrofuran could not be measured because of high turbidity of the samples, which is due to the formation of polymer micelles. According to the images of these clusters obtained by transmission electron microscopy, silver clusters are formed at the periphery of these micelles. The most stable and optically transparent samples were obtained in dimethylformamide. In this solvent the samples could be stored for many months without any changes of their fluorescence properties.

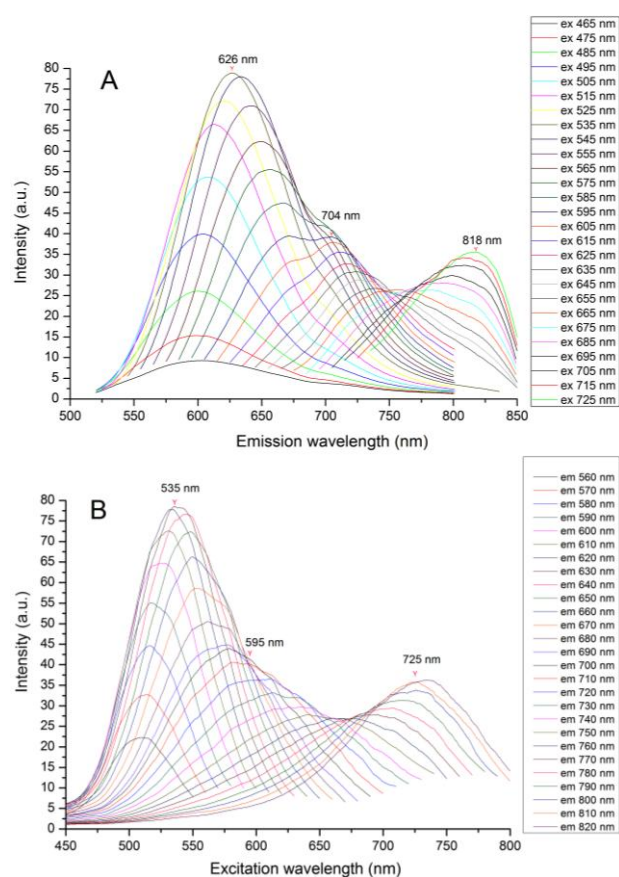
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Table 1 – Fluorescence properties of silver clusters formed in different solvents.

Solvent	$\lambda_{ex}(nm)$	$\lambda_{em}(nm)$	$\phi, \%$
DCE	370	460	33
Toluene	495	575	-
THF	537	610	-
DMF	532	625	24

Second electronic transition was found in the near-UV (320-330 nm). At this excitation, in full correspondence with Kasha rule, all emission is collected in the red-NIR wavelength range corresponding to that excited in the visible wavelength range. As we will demonstrate below, this emission is strongly depolarized.

In all studied solvents the strong Stokes shift is observed, which may characterize the whole family of Ag^0 clusters as fluorescence emitters. Detailed studies in dimethylformamide (DMF) allowed revealing three types of clusters that were distinguished by distinct fluorescence excitation and emission spectra, which is depicted in Figs. 1, A and B.

**Fig. 1** – Fluorescence excitation (A) and emission (B) spectra of studied few-atom silver clusters in DMF at different emission and excitation wavelengths correspondingly.

The series of fluorescence spectra presented in Fig. 1 strongly suggest that the observed three types of clusters must be clearly identified. The difference in positions of their excitation and emission bands could be attributed to difference in the cluster composition. Based on the known properties of similar clusters in noble gas matrices, we attribute these clusters to Ag_2^0 , Ag_3^0 and Ag_4^0 types. This suggestion should be confirmed in further

measurements.

The fact that the three observed bands in excitation and emission spectra really belong to three different emissive species is witnessed by the correspondence of their excitation and emission spectra, so that selective excitation of one band results in correspondent band in emission. The spectroscopic parameters characterizing these bands and correspondent Stokes shifts are presented in Table 2.

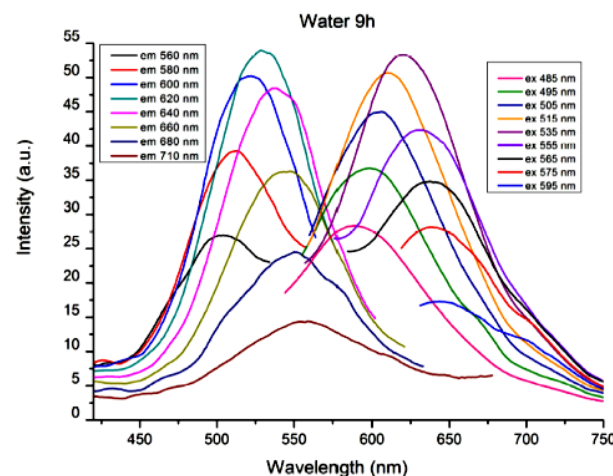
Table 2 – Positions in excitation and emission maxima in cm^{-1} and in nanometers and the values of Stokes shifts observed for three maxima in fluorescence spectra of silver clusters in DMF.

Excitation maximum	Emission maximum	Stokes shift
18690 cm^{-1}	15950 cm^{-1}	2740 cm^{-1}
534 nm	626 nm	
16810 cm^{-1}	14200 cm^{-1}	2610 cm^{-1}
594 nm	704 nm	
13790 cm^{-1}	12220 cm^{-1}	1570 cm^{-1}
25 nm	818 nm	

We observe also that each of these bands exhibits characteristic features of inhomogeneous broadening - strong dependences of excitation spectra on emission wavelength and of emission spectra on excitation wavelength.

The latter dependence does not appear on excitation at the 2-nd band in the near-UV. A qualitative model based on inhomogeneous broadening of spectra is suggested to explain these effects. Involvement of this mechanism has to be accounted in the analysis of spectroscopic heterogeneity of different metal clusters in solutions.

Strong inhomogeneous broadening effects are also observed when the clusters are formed and studied in water, which is required for many biological applications (Fig. 2).

**Fig. 2** – Fluorescence excitation (left) and emission (right) spectra of studied few-atom silver clusters in water at different emission and excitation wavelengths correspondingly.

5. SPECTRAL DEPENDENCE OF ANISOTROPY

We discovered that in addition to excitation and emission bands in the visible, the studied clusters exhibit excitation bands also in the near-UV with normal

emission in the visible. We attributed them to 2-nd electronic transition, in analogy to correspondent transitions in organic dyes. It was interesting to study for these clusters the effects of variations of anisotropy of fluorescence over excitation and emission bands, including the excitation in the UV.

The strong variations in anisotropy are observed over excitation and emission bands (Fig. 3). High anisotropy of fluorescence emission for the band in the visible reaching the values 0.35-0.37 witnesses for the absence of rotational mobility of the cluster during fluorescence lifetime. This also suggests the presence of strong excited-state dipole moment generated in the excited state that can be due to strong interaction of the cluster with electron donor group of the ligand (presumably, of the carbonyl group of the polymeric support) forming the ligand-to-metal charge transfer (LMCT) states.

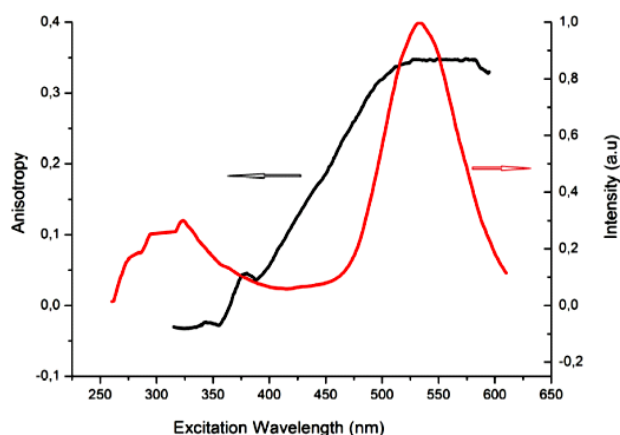


Fig. 3 – Fluorescence anisotropy (black) over fluorescence excitation spectrum (red) in the visible and near-UV ranges of spectra. The presented results were obtained in DMF.

An essential observation is that high anisotropy of fluorescence emission that is characteristic for the visible band is not observed for the UV band. This result extends the analogy of silver clusters and organic dyes, for which the first ($S_0 \rightarrow S_1$) and second ($S_0 \rightarrow S_2$) electronic transitions are commonly polarized in different directions. Therefore such observation is the witness for the fact that the UV band belongs to different high level electronic transition of the same fluorophore.

Strong intra-band heterogeneity is observed for the visible but not for the UV band (the results are not shown). When the fluorescence is excited at the red edge of the spectrum the emission anisotropy drops dramatically at the red-edge slope of emission. This result can be also explained within the concept of inhomogeneous environment of fluorescence emitters.

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6. CONCLUSIONS AND PROSPECTS

The silver nanoclusters exhibit strong similarity to organic dyes in a sense that they feature light absorption and luminescent properties originating from electronic transitions between discrete electronic energy levels [1-4]. The wide emission range that can be tunable from UV to the near-IR region and other properties, such as the outstanding photostability, the high fluorescence efficiency, the large Stokes shift, electroluminescence, chemiluminescence and electrogenerated chemiluminescence, the stimuli-responsive fluorescence – all that is characteristic for silver clusters consisting of several atoms that are very attractive for various applications [1,6,7]. Their good biocompatibility and the particularly small size make these fluorophores to become prospective versatile tools promising for applications in single-molecule studies, fluorescence imaging, fluorescence sensing, data storage devices and labeling.

A suitable scaffold is essential for the nanoclusters to remain stable. For several decades, solid inorganic scaffolds were used, such as cryogenic noble gases, inorganic glasses and zeolites, and they had greatly expanded our knowledge on the metal nanoclusters. They lead to a variety of applications including catalysis and 3D micropatterning [6]. The solution-based synthesis of fluorescent silver nanoclusters on dissolved polymeric matrices can be considered as a major breakthrough that has opened a new avenue for silver nanocluster research to fluorescence application areas where traditionally organic dyes and semiconductor quantum dots are the dominant fluorophores. This allows producing silver nanoclusters in scaffolds of molecular dimensions, so that they become suitable as fluorescent markers, e.g. to label proteins, organelles and the whole living cells.

These new materials are electro-luminescent [4] and suitable for electron microscopy [5]. They also exhibit anti-microbial properties [8]. Because the silver nanoclusters have a set of properties complementary to those of organic dyes and semiconductor quantum dots, we believe that their future looks bright

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