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ARTICLE

On the Photophysics of Electrochemically Generated Silver Nanoclusters: Spectroscopic and Theoretical Characterization

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Ligand-free atomic silver nanoclusters (AgNCs) were successfully synthesized following the electrochemical procedure developed by Lopez-Quintela and col. (D. Buceta, N. Busto, G. Barone, J. M. Leal, F. Domínguez, L. J. Giovanetti, F. G. Requejo, B. García and M. A. López-Quintela, *Angew. Chemie - Int. Ed.*, 2015, **54**, 7612–7616) who have identified the presence of Ag₂ and Ag₃ AgNCs. The goal of this work was to get information on the photophysics of these AgNCs, which was achieved by combining information from excitation/emission matrix (EEM) and time resolved emission spectroscopy (TRES) along with DFT/TD-DFT calculations. This procedure allowed deconvolving the emission and excitation spectra of the AgNCs mixture, with further assignment of each transition and lifetimes associated to Ag₂, Ag₃⁺ and Ag₄²⁺ clusters. This deconvolution together with the theoretical calculations allowed suggesting for the first time the radiative and non-radiative excited state deactivation mechanism for these clusters.

Introduction

With the advent of nanotechnology, metal nanocluster (MNCs) have been an important subject of study due to their potential applications as biosensors,¹ low-consuming LEDs,² biological markers,^{3,4} in medicine due to their biological activity,^{5,6} etc. It is well known that, when the size of the metal system is sufficiently reduced, quantum confinement effects appear in the material and make new optical and chemical properties to arise.⁷ The main effect of quantum confinement in metal nanoclusters is the appearance of a HOMO-LUMO energy-gap due to a discretization of the energy levels within the nanocluster that behaves as a molecule-like system.^{8,9} This energy discretization is responsible for the high fluorescence quantum yield and great thermodynamic stability, which are highly sensitive to chemical environment, cluster size, total charge, etc.⁷ In this sense, many experimental techniques (chemical reduction,¹⁰ electrochemical synthesis,¹¹ photoreduction,¹² etc.) and templates (DNA,^{13–16} polymers,^{17,18}

proteins,¹⁹ etc) have been used to generate these MNCs in order to achieve a better control of their optical and dynamical properties.

The templates confer stability against coalescence as well as particular optical properties, but in turn preclude obtaining information on the intrinsic optical properties of the bare MNCs.

Very recently, in a promising bottom-up approach, Lopez-Quintela and co-workers developed a simple electrochemical synthesis of highly fluorescent, thermodynamically stable and ligand-free AgNCs in water as small as two and three silver atoms.²⁰ By using the spherical jellium model approximation for MNCs in combination with high resolution mass spectrometry (HR-MS) they assigned the optical properties of the solution containing AgNCs to Ag₂ and Ag₃. Although the charge states of these clusters is not discussed by the authors, the analysis of the reported HR-MS allows assigning Ag₂⁰ and Ag₃⁺.²⁰ They also studied the interaction of these clusters with DNA by various experimental techniques and particularly by quenching of the AgNCs fluorescence by the addition of DNA and reported that Ag₃⁺ selectively intercalates in DNA but Ag₂ does not.²⁰ Anticancer activity was also analysed in mice with lung tumours, observing that the co-administration of cisplatin with Ag₃ NCs strongly potentiates the anticancer efficiency by reducing tumor size due to the DNA intercalation property of Ag₃⁺.⁵

Despite the broad applicability and the promising results on the specific intercalation with DNA, a detailed understanding of the photophysical properties of the AgNCs is still missing. In this aspect, the optical and structural characterization is necessary for a rational and addressed-synthesis of new AgNCs containing nanomaterials. In this regard, here we report a

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spectroscopic and theoretical characterization of the photophysical properties of ligand-free AgNCs in water, synthesized by following the electrochemical method developed by Lopez-Quintela and col.²⁰

Experimental Section

The synthesis of AgNCs was carried out according to the procedure reported by López-Quintela and coworkers.²⁰ Briefly, nanoclusters were synthesized in a two-electrode cell with two plate electrodes (2.0 cm² surface area) separated by 1 cm. Previous to the experiment, a silver plate (working electrode) was polished in 0.05 μm Al₂O₃ (Buehler) followed by an electropolished for 600 s at 2 V and a flamed platinum plate (counter electrode), and then thoroughly washed with ultrapure water (Milli-Q, Millipore, resistivity of 18 MΩ cm). The synthesis of AgNCs was carried out by applying a potential step at 2.0 V for 600 s in N₂ deaerated ultrapure water at room temperature with a freshly electropolished silver electrode. The potentiostatic chronoamperometry procedure was carried out with an Autolab PGSTAT100 electrochemical interface using the NOVA 1.10 software package. After the synthesis, Na₂S (JT Baker) 1 M was added to the solution to precipitate remaining Ag⁺ cations. In some experiments KCl (Sigma-Aldrich) 1 M was added instead of Na₂S to explore the effect of the identity of the anion on the optical properties of the clusters. Finally, the solution was filtered by a PTFE-membrane filter (Nalgene) with 0.2 μm pore size, concentrated with a rotary evaporator at 40 °C and stored in the dark.

UV-Vis spectra were obtained with a Shimadzu UV-1700 PharmaSpec spectrophotometer. Emission and excitation spectra were recorded on a Quanta Master QM2 spectrofluorometer from Photon Technology International equipped with a Hamamatsu R928 PMT and a photon counting detector. The excitation-emission matrices (EEM) were obtained by measuring excitation and emission spectra of the nanocluster solution every 10 nm. Time-resolved emission spectroscopy (TRES) experiments were carried out in a Horiba Delta-Flex Fluorimeter, exciting the sample with a 267 nm diode laser source. A collagen suspension was used to obtain the instrumental time response (prompt). Fluorescence decay curves were measured at emission wavelength between 300 nm and 500 nm every 10 nm. DAS-6 fluorescence analysis software was used for data analysis. The fitting was performed by deconvolution of the time-resolved decay using the instrumental time response of the prompt.

High-resolution mass spectra of the samples were recorded in a Bruker Daltonics ESI-microTOF-QII mass spectrometer operating in positive mode. For full MS scan analysis, the spectra were recorded in the 100 to 1000 m/z range. Internal calibration was performed in high-precision calibration mode (HPC) by employing sodium formate. For the analysis, 10 μL of formic acid 0.1 M (LCMS grade, Sigma-Aldrich) was added to 1.0 mL of the solution containing AgNCs which was injected in the electrospray (ESI) source at 0.1 mL/min flow rate.

Computational Details

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Calculations of the ground and excited state geometries and energies of different naked silver nanocluster were performed with the Gaussian 09 software package,²¹ at the DFT/CAM-B3LYP level. The Stuttgart/Dresden pseudopotential (SDD) was used to represent silver atom cores in all cases. The polarizable continuum model (PCM)²² was used as an implicit solvent scheme in all calculation to take into account solvent effects. Excitation spectra were obtained by single point calculation of the excited state of interest at the ground state equilibrium geometry of each AgNCs. A SIMPLEX algorithm²³ was used to fit a linear combination of the calculated excitation spectra to the experimental excitation spectra.

Results and Discussion

The UV-vis and fluorescence emission spectra of the synthesized AgNC exhibit the characteristic optical properties previously reported by López-Quintela and col.^{5,20,24} Two bands are observed in the UV-vis spectra at 220 nm and 290 nm (Figure 1.a), while the emission spectra with excitation at 230 nm exhibits three distinct bands at 305 nm, 350 nm and 410 nm (Figure 1.b). The emission bands at 305 nm and 410

Figure 1: Absorption (a) and emission (b) spectrum upon excitation at $\lambda_{ex} = 230$ nm, for the AgNCs synthesized in this work. The inset in (a) shows the shoulder between 250 and 350 nm.

nm were already observed by Buceta et al.,²⁰ with possible assignment to Ag₂ and Ag₃ clusters respectively, assuming the spherical jellium model approximation, while the band at 350 nm remains still elusive. A detailed analysis of the HR-MS

reported by these authors²⁰ shows that the charge states of the above mentioned clusters are 0 and +1, respectively and then the identified clusters are Ag₂ and Ag₃⁺.

Figure 2: Excitation (a) and emission (b) spectra of the AgNCs recorded at different emission and excitation wavelength. (c) and (d) are the emission-excitation matrix obtained from (a) and (b), respectively. Arrows in (c) indicate the position of the four islands analysed here.

To obtain more information about the optical properties and identity of the AgNCs, excitation and emission spectra were recorded at different emission and excitation wavelengths every 10 nm. Figure 2 shows both emission and excitation spectra along with the generated fluorescence emission-excitation matrix (EEM). Ramsay et al. showed the power of the fluorescence EEM spectroscopy technique towards the identification and characterization of complex mixtures of nanoclusters.²⁵ The EEM generated from both excitation and emission scans (Figure 2.c and Figure 2.d) exhibits four distinct islands with very large energy difference between the excitation and emission maxima ($\Delta E_{\text{ex-em}}$) as reported in Table 1. It is important to note that the islands observed in the EEM could correspond either to different types of silver nanocluster or to different electronic transitions of the same cluster. Time-resolved emission spectroscopy is a suitable technique to deconvolve and characterize emission from complex mixtures.

Fluorescence emission decay curves upon excitation at 267 nm, were obtained between 280 and 500 nm with an emission wavelength-spacing of 10 nm (Figure S1). All curves were globally fitted by using deconvolution of the time resolved signal considering a 4-exponential decay function leading to the following set of excited state lifetimes for all the emission wavelengths explored: $\tau_1 = 1.10$ ns, $\tau_2 = 4.50$ ns, $\tau_3 = 17.1$ ns and $\tau_4 = 0.031$ ns. Excited state lifetimes in the nanosecond range have already been reported for silver and gold nanoclusters.^{26,27} Decay associated spectra (DAS) were obtained by plotting the amplitude factors (A_1 , A_2 and A_3) corresponding to the exponential decays associated to τ_1 , τ_2 and τ_3 , respectively, against the emission wavelength as shown in Figure 3. The shortest lifetime $\tau_4 = 0.031$ ns was required to fit the lower emission wavelengths decays. The plot of the amplitude factor A_4 associated to τ_4 shows a continue decrease from 280 nm up to 325 nm (Figure S2). Then, it is assigned to

Figure 3: Decay associated spectra (DAS) obtained by plotting the amplitudes of exponential decay function (associated to three different excited state lifetime) as a function of the emission wavelength as obtained from the simultaneous fitting of all the decays of a TRES recorded upon excitation at 267 nm. The emission decays were recorded every 10 nm.

the scattered light from the excitation laser source whose temporal width is ≈ 0.025 ns. Although a distorted Gaussian-shape is observed in the DAS, this could correspond to emissions from different electronic states. Moreover, Table S1 and Figure S3 show that the quality of the fitting of the TRES profiles above 430 nm is poorer than at shorter wavelengths due to the low signal intensity. Then, the fitting of the TRES to four exponential decays above 430 nm becomes unreliable and the DAS in this spectral region is meaningless (See Supporting Information).

The wavelengths at the maxima of A_1 , A_2 and A_3 in Figure 3 match the emission maxima for islands 1-4 in the EEM (Figure 2.c and Figure 2.d). Hence, the lifetime values obtained in the TRES experiment are assigned to each island (Table 1). It is important to note that A_2 has two emission maxima at 305 nm and 410 nm and this may be related to two decay processes with similar lifetimes.

It is acknowledged that at 267 nm mainly emitters associated to islands B and C are excited. A more accurate information from emitters associated to islands A and D would be obtained by exciting at 240 nm and 310 nm, respectively. However, these wavelengths are not available at the present in our fluorimeter and therefore, we rely on the current results.

As mentioned previously, from the combination of EEM and TRES results it was shown that the emission spectrum of the solution is deconvolved in three emission spectra, each one associated to a different excited state lifetime. Therefore, the three spectra and excited state lifetimes can be assigned to three different AgNCs; two of them tentatively assigned to Ag_2 and Ag_3^+ by comparison with previous results of Buceta et al.²⁰ It is worth noting that these two silver nanoclusters (Ag_2 and Ag_3^+) are species with close-shell electronic configuration, which are thermodynamically more stable and their formation is favored over those with open-shell electronic structures.²⁸

To assign the third AgNC we consider the following close-shell specie (Ag_4 or Ag_4^{2+}). DOI: 10.1039/D0CP02136A

In addition, Ag_2 , Ag_3^+ and Ag_4^{2+} have been previously identified in solution as intermediates species in the formation of silver nanoparticles, among which the Ag_4^{2+} cluster was reported to be the most stable one.^{29,30} Moreover, Kononov and co-workers have worked intensively during the last few years in the spectroscopic characterization of small AgNCs interacting with different biomolecules. By chemical reduction of $AgNO_3$, in presence of DNA as a template, they were able to generate luminescent Ag_3^+ and Ag_4^{2+} clusters.¹⁶

Therefore, we assumed that the three AgNCs responsible for the optical properties reported in this work are: Ag_2 , Ag_3^+ and Ag_4^{2+} . The solution was finally analyzed by HR-MS that allowed confirming the presence of Ag_2 and Ag_3^+ clusters, as previously reported.²⁰ In addition we determined the presence of species containing Ag_4^{2+} (see Supporting Information). It must be noted that the signal to noise ratio was very low as a consequence of the low concentration of clusters in the solution. Therefore, this result is not conclusive, but another evidence of the presence of the above mentioned specie.

Island	λ_{ex} (nm)	λ_{em} (nm)	ΔE_{ex-em} (cm ⁻¹)	Lifetime (ns)
A	240	350	13095	17.1 ± 0.5
B	275	305	3576	4.50 ± 0.09
C	280	340	6303	1.10 ± 0.03
D	310	410	7868	4.50 ± 0.09

Table 1: Wavelength at the maxima of the excitation (λ_{ex}) and emission (λ_{em}) spectra for the islands observed in the EEM for the AgNCs mixture, energy difference between the excitation and emission maxima (ΔE_{ex-em}) and lifetime assignment.

To add evidence on the assignment, ground and excited states optimization of the three clusters were performed as detailed in the computational section. The absorption spectra of the lowest energy structures of each AgNC were also calculated. While the appropriate comparison of the calculated spectra should be made with the experimental absorption spectrum of the AgNCs mixture, the strong absorbance of the sample below 225 nm precludes a good comparison. Therefore, the comparison was made with the total fluorescence excitation spectrum obtained as the sum of the experimental excitation spectra recorded over the emission spectral range (225 – 600) nm. Figure 4 shows the calculated excitation spectra for the most stable structures of Ag_2 , Ag_3^+ and Ag_4^{2+} in comparison with the total fluorescence excitation spectra obtained experimentally. It is worthy to note that the two characteristic bands observed in the absorbance spectrum, namely the bands at 220 nm and at 290 nm are also observed in the total fluorescence excitation spectra. The oscillator strengths of each transition of the calculated spectra are plotted as blue vertical lines. The main electronic transitions along with the corresponding molecular orbitals are presented in Tables S2, S3 and S4.

It is noticed that only the calculated spectrum of Ag_4^{2+} could fit the experimental total fluorescence excitation spectrum. Nevertheless, the three AgNCs share similar features, namely a

Figure 4: Comparison between calculated excitation spectra for the most stable structures of Ag_2 , Ag_3^+ and Ag_4^{2+} (black solid line) and the experimental total fluorescence excitation spectrum (red line).

band around 200 nm, another band around 300 nm and the lowest energy band ($\lambda > 300$ nm) corresponding to the HOMO-LUMO transition. While it is generally accepted that the larger the cluster size the smaller the HOMO-LUMO energy gap,³¹ this is only true for clusters with the same charge state. In this case, the HOMO-LUMO transition energy increases with the AgNC size ($\text{Ag}_2 < \text{Ag}_3^+ < \text{Ag}_4^{2+}$) but so do the positive charge on the nanocluster, thus becoming the HOMO-LUMO transition energies incomparable on the basis of the cluster size only. The energy gap law (EGL)³² establishes a correlation between the excited state lifetime (including radiative and non-radiative processes) and the energy gap between the states involved in the non-radiative processes. This law is commonly used to

correlate excited state lifetimes with HOMO-LUMO energy gaps. This is correct if one compares the excited state lifetimes of the first excited states of different systems that involve the HOMO $\rightarrow$ LUMO transition. Otherwise, if the excited state lifetimes correspond to other states, it is not correct to correlate them with the HOMO-LUMO energy gap, but with the ΔE of the state responsible for the non-radiative deactivation. In consequence, the lower the energy gap between the two states involved in internal conversion or intersystem crossing processes the shorter the excited state lifetime of the emitting state.

In addition, the rate for the non-radiative processes could depend strongly on the state's density of the systems since it favors the coupling between different electronic states. The density of states increases with cluster size and, as a consequence, the excited state lifetime decreases. Consequently, the longest excited state lifetime determined in this work ($\tau_3 = 17.1$ ns) is tentatively assigned to Ag_2 cluster, while $\tau_2 = 4.50$ ns and $\tau_1 = 1.10$ ns are assigned to Ag_3^+ and Ag_4^{2+} , respectively. The assignment of all the bands observed in the experiments was performed by taking into account the above discussion and the comparison with the theoretical spectra, considering the calculated transition energies and their corresponding oscillator strength.

The band observed at $\lambda_{\text{ex}}/\lambda_{\text{em}}$ 280/340 nm (island C), associated to $\tau_1 = 1.10$ ns was assigned to Ag_4^{2+} . The calculated excitation spectrum for this cluster shows a band located at 300 nm, corresponding to the excitation to the first, second and third excited states (Table S4), which is the closest band among the other calculated spectra to the maximum of the experiment total fluorescence excitation spectrum (280 nm). In accordance, an absorption band at ≈ 275 nm has already been assigned to a stable Ag_4^{2+} cluster, in pulsed radiolysis experiments.^{29,30,33–35} Also, this band is associated to the shortest excited state lifetime (1.10 ns), which correlates with the largest density of state as compare to the smaller clusters, allowing for a faster internal conversion process. On the other hand, island C correlates with the emission band at 340 nm, which is the most prominent band in our results (Figure 2.c.) and the only one that is absent in the emission spectrum reported by Buceta et al.²⁰ who have only observed Ag_2 and Ag_3^+ clusters. Therefore, it makes sense that the absent band in their results corresponds to Ag_4^{2+} cluster.

The lifetime $\tau_2 = 4.50$ ns was assigned to Ag_3^+ . The bands associated to this lifetime, located at $\lambda_{\text{ex}}/\lambda_{\text{em}}$ 275/305 nm (island B) and 310/410 nm (island D), may be assigned to the calculated excitation bands at 263 and 346 nm for the Ag_3^+ cluster, corresponding to transitions from the ground to the fifth and first/second excited state, respectively; as shown in Table S3. In accordance, a long lived transient absorption band located at ≈ 310 nm has been previously assigned to Ag_3^+ cluster during pulsed radiolysis formation of Ag nanoparticles.³⁵ The larger $\Delta E_{\text{ex-em}} = 7868$ cm^{-1} of the $\lambda_{\text{ex}}/\lambda_{\text{em}}$ 310/410 nm band as compare to the $\Delta E_{\text{ex-em}} = 3576$ cm^{-1} of the $\lambda_{\text{ex}}/\lambda_{\text{em}}$ 275/305 nm band suggests a larger equilibrium geometry change of the cluster between the ground and first/second excited state than between the ground and fifth

Figure 5: (a) Ground state (GS), first (S_1) and fifth (S_5) excited state scan of Ag_3^+ varying Ag – Ag – Ag angle and (b) Ground state (GS), first (S_1), second (S_2), third/fourth (S_3/S_4) and fifth/sixth (S_5/S_6) excited states scan of Ag_2 varying Ag – Ag distance. Vertical axis indicates energy relative to ground state PEC minimum. Excitation and emission processes are represented by colour arrows. S_5 scan in (a) is shown up to 180° due to a strong mixture of the excited states, that cannot be resolved by this computational methodology.

excited state. Indeed, the calculated equilibrium geometries for the ground and fifth excited states shows a triangular (D_{3h} symmetry) structure, while the corresponding geometry of the first excited state is linear ($C_{\infty v}$ symmetry).

To get more insight into this matter a rigid scan calculation of the Ag-Ag-Ag angle was performed for the three electronic states involved and the corresponding potential energy curves (PECs) along this coordinate are shown in Figure 5.a. As observed in Figure 5.a. the minima on the PECs for the ground and fifth excited states, along the Ag-Ag-Ag angle coordinate, are located at 60° as expected for a triangular structure. Instead, the minimum on the PEC for the first/second excited state is located near 180° , which is consistent with a linear structure. Therefore, upon excitation to the fifth electronic

state (violet arrow) the emission will take place from the same region of the PEC leading to a moderate $\Delta E_{\text{ex-em}}$, as indicated by the blue arrows in Figure 5.a. On the contrary, the vertical excitation to the first/second excited state (green arrow) will take place at a geometry similar to that of the ground state, which lies above the small energy barrier for the opening of the triangle and it will relax within the time scale of a single vibration (few fs) to the most stable linear structure. Thus, the

emission will take place vertically from the excited linear structure to the ground state, in the region where the PEC reaches the maximum (red arrow in Figure 5.a), and as a consequence a large $\Delta E_{\text{ex-em}}$ is expected for this transition.

Finally, the lifetime $\tau_3 = 17.1$ ns was assigned to Ag_2 cluster. The band at $\lambda_{\text{ex}}/\lambda_{\text{em}}$ 240/350 nm (island A) associated to this lifetime may be assigned to the calculated excitation band located at 265 nm, which correspond to the transition from ground state to the fifth/sixth excited state (Table S2). This band has the largest $\Delta E_{\text{ex-em}} = 13095$ cm^{-1} among the AgNCs studied in this work, and this could be due to a large geometry difference between the electronic state involved in the transition, as in the case of the Ag_3^+ cluster, or to emission from a lower excited state populated by internal conversion from the optically excited state.

The calculated PECs (Figure 5.b) for the ground and fifth/sixth states show the minima at the same Ag-Ag bond distance (2.57 Å), suggesting that the large $\Delta E_{\text{ex-em}}$ is unlikely to be due to changes in geometry. On the other side, the energy gap (ΔE) between the optically excited state (4.69 eV) and the fourth/third excited states (4.4 eV) is very small ($\Delta E_{5-4/3} = 0.29$ eV), as well as the $\Delta E_{4/3-2} = 0.1$ eV between the fourth/third and the second excited states (4.30 eV). According to the EGL,³² the smaller the energy gap between two states the faster the internal conversion process from one to the other would be, making the fifth/sixth state almost a dark state that undergoes a very fast internal conversion to the fourth/third electronic states. The fourth/third states in turn deactivate very fast to the second excited state, but this state cannot be deactivated radiatively since its oscillator strength is zero. For that reason, it can only deactivate via internal conversion to the first excited state, which has considerably high oscillator strength (0.411). The non-radiative deactivation of this state to the ground state is very unlikely due to the large ΔE_{2-1} (3.03 eV) between them and a low density of states of Ag_2 . Therefore, this is the emitting bright state of the Ag_2 cluster. According to the proposed mechanism, the calculated $\Delta E_{\text{ex-em}}$ between the optically excitation at 264 nm (violet arrow) and the emission at 409 nm (red arrow) is ≈ 13363 cm^{-1} , in good agreement with the experimental value (13095 cm^{-1}). Considering the above assignment, the total fluorescence excitation spectrum was fitted by a SIMPLEX algorithm to a linear combination of the excitation spectra of the three (Ag_2 , Ag_3^+ and Ag_4^{2+}) clusters, weighted by a factor related to the relative concentration of each one. The result, shown in Figure 6, is in very good agreement with the experimental spectrum. The relative weights (W_i) obtained from the fitting were 0.04 for Ag_2 , 0.28 for Ag_3^+ and 0.68 for Ag_4^{2+} .

In the previous work by Buceta et al.²⁰, the authors reported that their solution was mainly composed by Ag_2 and Ag_3^+ clusters. Considering the charge and the chemical composition of the ions observed in the mass spectra reported by the authors, the silver clusters would correspond to Ag_2 and Ag_3^+ clusters. In their work, the maximum of the emission spectrum, upon excitation at 230 nm, was found at 305 nm with a smaller band at 425 nm. In this work, we assigned these bands to the Ag_3^+ cluster.

Therefore, the differences observed in the emission spectra reported by Buceta et al.²⁰ and in the present work are reconciled considering the different relative concentration of the clusters. The different composition might be due to

Figure 6: Comparison between the theoretical spectrum as the sum of the calculated excitation spectra of Ag_2 , Ag_3^+ , and Ag_4^{2+} weighted by the factors obtained with the SIMPLEX fitting (black line) and the experimental total fluorescence excitation spectrum (red line).

different experimental conditions in the synthesis that up to now we are unable to determine.

It is interesting to compare the optical properties of the AgNCs studied in this work with those of similar size AgNCs supported in zeolite hosts.^{36–43} Although the spectroscopic features of LTA(Li)-Ag1 zeolite, after heat treatment at temperatures above 300 K, resembles the present results,⁴³ the rest of zeolites exhibit strong spectral red-shifts as compared to free AgNCs. This may be related to the very different surrounding that can affect the electronic structure of the system, and as a consequence the position of the excitation and emission bands.

On one side, this is supported by the fact that the nature of the co-cation (Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+}) strongly affect the optical properties and particularly the wavelength of the maximum of the emission spectra of the AgSNC-zeolites³⁷ and AgNC-zeolites clusters.³⁶

On the other side, the emission bands of Ag_2S and Ag_4S_2 clusters within zeolite structures may be assigned to phosphorescence from triple excited states that are populated by intersystem-crossing (ISC) from the singlet optically excited states⁴⁰ at variance with the free AgNCs studied in this work for which the emission takes place from singlet states. Similarly, the $\text{Ag}_4(\text{H}_2\text{O})_x^{2+}$ clusters (with $x = 2$ or 4) supported on zeolite have shown an efficient ISC and the concomitant red-shifted phosphorescence from triple states.⁴¹ The phosphorescence from triplet states is always red-shifted as compare to the fluorescence from the higher-lying singlet state and very likely, the interaction of the AgNCs or AgSNCs with the zeolite's networks facilitates the ISC as compared to the free AgNCs.

Finally, in addition to the phosphorescence from the triplet state, the $\text{Ag}_4(\text{H}_2\text{O})_x^{2+}$ clusters confined in LTA-zeolites also show red-

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shifted fluorescence, as compared to the AgNCs, and particularly Ag_4^{2+} cluster. In this case the authors found that the H_2O molecules act as ligands stabilizing the singlet and triple excited states as compare to the free Ag_4^{2+} . In the case of the Ag_4^{2+} synthesized in solution in this work, water molecules act just as a solvent since they move freely without the constrain imposed by the zeolite network. As a consequence, the interaction of water with the Ag_4^{2+} may be weak and have little or no participation on its optical properties.

Moreover, a close inspection to the Natural Bond Orbitals analysis reported by the authors (Tables S5 and S6 in Ref. 41) shows that the actual charge on the Ag_4 moiety is $< +1$ due to a partial charge transfer from the zeolite to the AgNC. The charge state of the AgNC have major influence on the electronic structure of the system, resulting in a variation of their excitation and emission response when compared to AgNC with different formal charges as in the case of Ag_4^{2+} in water solution.

Therefore, to understand the optical properties in host-guest systems, a detailed study of the composition and structure of the silver cluster with an increasing complexity of the crystalline network of the zeolite is desirable, using as a starting point the excited state deactivation mechanism proposed in this work.

Conclusions

The synthesis of few atoms AgNCs proposed by López-Quintela and col.²⁰ was successfully applied, although different relative concentrations of the clusters was found. By using EEM spectroscopy in combination with TRES and DFT/TD-DFT calculations we were able to deconvolve the emission and excitation spectra and to assign the different transitions and lifetimes associated to Ag_2 , Ag_3^+ and Ag_4^{2+} , whose presence in the solution was confirmed by HR-MS.

On the bases of the experimental and theoretical results, the radiative/non-radiative deactivation mechanism for these AgNCs was proposed for the first time.

These results are expected to be useful for studying, by optical techniques, specific interactions of each AgNC with different polymeric scaffolds (mainly oligonucleotides of variable length and bases sequence) or inorganic materials such zeolites commonly used to synthesize fluorescent hybrid AgNCs.

Conflicts of interest

There are no conflicts to declare

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We analyzed the photophysics of a mixture of electrochemically synthesized AgNCs by deconvolution of the emission and excitation spectra, together with theoretical calculations.