Talanta 105 (2013) 267–271

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Component analysis of fluorescence spectra of thiol DAB dendrimer/ZnSe-PEA nanoparticles

M. Algarra [a,*], K. Radotić [b], A. Kalauzi [b], B. Alonso [c], C.M. Casado [c], J.C.G. Esteves da Silva [d]

[a] Centro de Geologia, Departamento de Geociências, Ambiente e Ordenamento do Território do Porto, Faculdade de Ciências da Universidade do Porto, Porto, Portugal
[b] Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11000 Beograd, Serbia
[c] Departamento de Química Inorgánica, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain
[d] Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Porto, Portugal

ARTICLE INFO

Article history:
Received 20 March 2012
Received in revised form
30 August 2012
Accepted 10 September 2012
Available online 15 September 2012

Keywords:
ZnSe Quantum dots
Dendrimers
O-phosphorylethanolamine
Log-normal model
Non-linear fitting

ABSTRACT

The fluorescence spectroscopy technique is an accurate method and has great utility in the interpretation of complex systems based on several emission bands. An interpretation of the system requires determination of the number, positions and intensities of the spectral components. In this work, the emission spectra of the synthesized ZnSe complex coated with *O*-phosphorylethanolamine (ZnSe-PEA), both with and without thiol DAB dendrimer generation 5 (S-DAB G_5), were analyzed using a combination of asymmetric (log-normal) and symmetric (Gaussian) models. The method applied for the deconvolution of fluorescence spectra has proven to be very sensitive for observing the stability of the ZnSe-PEA complex after binding with S-DAB. The ZnSe-PEA emission spectrum contains two components. The positions of the emission maxima of these two components are not significantly affected by the presence of S-DAB G_5 in the complex, which revealed the presence of a stable complex at a pH of 7. By applying the spectral deconvolution method, strong evidence was obtained that suggested that the ZnSe-PEA complex is stable after complexation with S-DAB G_5.

1. Introduction

Rapid advancements in nanotechnology have motivated research interests in developing a variety of nanostructured materials. In this context, semiconductor quantum dots (QDs), which are nanometer-sized crystals, have been demonstrated to be powerful probes for fluorescence analysis; their distinct properties provide them with unique capabilities and make them the most promising nanostructured material due to their high photochemical stability and size-tunable photoluminescence [1,2]. QDs can be attached to biomaterials such as monoclonal antibodies, peptides and oligonucleotides by self-assembly through noncovalent interactions. However, this attachment method has inherent instability drawbacks under environmental conditions such as low pH, high temperature, etc.

Stabilization with organic molecules in aqueous media, such as thiol groups, has been performed to improve the spectral properties of QDs. Glutathione, which is a thiol-containing tripeptide [3,4], mercaptoethanol [5,6], succinic [7,8], mercaptopropionic [9–12] or thioglycolic acids [13] and cysteine, [14,15] etc. are capable of providing improved biocompatible capping for QDs compared to many other water-soluble ligands. These stabilizing ligands are effective in controlling particle agglomeration and ripening. Macromolecules, such as dendrimers, are synthetic spheres with a well-defined surface, including a core, branches and a considerable number of terminal groups [16,17]. The excellent biocompatibility and low toxicity of macromolecules lead to many potential applications, such as DAB and PAMAM dendrimers, which are used as capping agents for QDs and have resulted in their application in a variety of research areas [18–26]; they enhance the biocompatibility and cellular uptake of these nanoparticles [27–29]. Other strategies to obtain stabilized systems in aqueous media include the use of gold nanoparticles (AuNPs); in this case, dendrimers are used as templates and the AuNPs are generally located within the dendritic arms [30–36].

In this work, ZnSe QDs with *O*-phosphorylethanolamine capped by the thiolated DAB dendrimer were synthesized with the objective to obtain less toxic systems for further applications. *O*-phosphorylethanolamine (PEA) is a specific synthetic ligand for the C-reactive protein, whose determination in serum samples can provide a rapid diagnosis of infections and inflammation in patients and has been the focus of considerable interest as a marker of inflammation associated with cardiovascular diseases [26,37–41].

In this paper, the luminescence spectra of the synthesized ZnSe-PEA complex, both in the presence and absence of the thiol DAB dendrimer (S-DAB), were analyzed using a combination of asymmetric (log-normal) and symmetric (Gaussian) models [42,43].

* Corresponding author. Tel.: +351 220 402 568; fax: +351 220 402 659.
E-mail address: magonzal@fc.up.pt (M. Algarra).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.09.008

Symmetric models for fluorescence emission components, such as Gaussian, Lorentz and a combination of the models, are often used when Raman bands are present in the system, which exhibit symmetric shapes. When varying excitation wavelengths are applied, these Raman bands are easily identified as migrating emission peaks, whose positions linearly increase with the excitation wavelengths [42]. However, because the majority of experimentally recorded emission spectra have an asymmetric shape, the asymmetric models are more suitable for their analysis. Asymmetry arises because the vibration force constants of the ground and excited states are not the same. However, when a new type of emitting mechanism is involved (such as in case of dendrimers), it is also necessary to apply these more general mathematical descriptions because they may be applied to components with a wide range of degrees of asymmetry, including the symmetric ones.

Our objective was to investigate the stability of the ZnSe-PEA complex after its complexation with the S-DAB dendrimer using a spectral deconvolution treatment, which has not been previously reported.

2. Experimental

2.1. Reagents

Zinc nitrate (99.9%, ($Zn(NO_3)_2 \cdot 4H_2O$)), selenium powder (Se, 99.99%), sodium borohydride ($NaBH_4$, $\geq 96\%$), 3-mercaptopropionic acid (MPA, 99%), triethylamine (TEA) and *O*-phosphorylethanolamine (PEA) were purchased from Sigma-Aldrich Química S.A. (Spain) and used without further purification. All solutions were prepared using deionized water that had a resistivity greater than 18 MΩ cm.

The thiolated polypropylenimine dendrimer DAB-AM-(64), generation 5 (S-DAB-G_5), was synthesized at the Autonomic University of Madrid and it was synthesized as follows.

2.2. Synthesis of the thiolated S-DAB-G_5 dendrimer

A solution with a 5% excess of 3-mercaptopropanyl-N-hydroxysuccinimide ester was added dropwise to a stirred solution of the dendritic polyamine DAB-AM-(64, G_5) and triethylamine in CH_2Cl_2 under a flow of Ar at room temperature [44]. After stirring overnight, the resulting reaction mixture was washed three times with H_2O and the organic layer was subsequently dried over $MgSO_{4,anh}$ and evaporated. S-DAB-G_5 was obtained as colorless, waxy solids and it was insoluble in organic solvents but soluble in aqueous NaOH (0.1 M). The yields were 60%. NMR spectra (*data not shown*) exhibited the low-field shift of the dendrimer resonances, which confirmed complete functionalization with the thiol groups.

2.3. Synthesis of the S-DAB-ZnSe-PEA QDs nanoparticles

Synthesis of the S-DAB-ZnSe-PEA QDs was performed as follows. 10 mg of the S-DAB dendrimer was dissolved and stirred in deionized H_2O (50 mL) for 72 h. Then, 7 mg of $Zn(NO_3)_2$ was added. The mixture was stirred after total dissolution (24 h) to ensure the complexation of Zn^{2+} with the S-DAB dendrimer. MPA (17.6 μL) was then added to this solution and left for 24 h. Subsequently, TEA was used to adjust the pH to 12 and then 200 μL of a Se solution as NaHSe was added, as previously reported [26]. Finally, PEA (14.3 mg) was added to obtain S-DAB-ZnSe-PEA QDs after 24 h. All the synthesis procedures were conducted at room temperature under a flow of N_2 to avoid the oxidation of Se.

2.4. UV absorption and steady state fluorescence spectroscopies. Data analysis

A UV–vis spectrophotometer (Hewlett-Packard HP8452A diode array) and a photoluminescence spectrophotometer (Horiba JovinYvon Fluoromax 4 TCSPC) were used to characterize the S-DAB ZnSe-PEA QDs. The UV-vis absorption spectrum of the QDs was obtained in the wavelength range of 180–400 nm. The fluorescence of the nanoparticles was measured in the wavelength range of 300–740 nm. The spectra were obtained with an integration time of 0.1 s and the widths of the slits were fixed at 5 nm for both excitation and emission. All the measurements were performed at room temperature. For each compound, a series of emission spectra were collected by exciting at different wavelengths in the range 240–300 nm with a step size of 5 nm.

Nonlinear fitting of all the fluorescence spectra was performed using the Nelder-Mead simplex algorithm as implemented in the MATLAB 6.5 software program. Deconvolution into two asymmetric (log-normal):

$$F_\lambda = \begin{cases} F_m \exp\left\{-\left(\ln 2/\ln^2 \rho\right)\ln^2[(1/a-1/\lambda)/(1/a-1/\lambda_m)]\right\}, \lambda > a \\ 0, \lambda \leq a \end{cases} \quad (1)$$

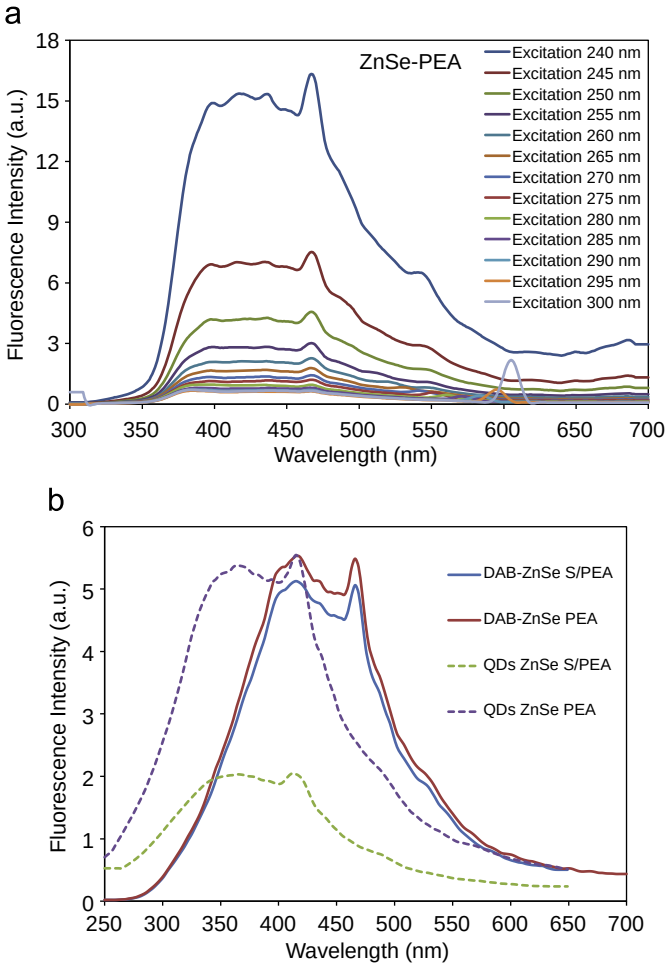

Fig. 1. A: Series of emission spectra of the ZnSe-PEA complex, which were obtained after excitation at various wavelengths. B: Overlaid emission spectra of the ZnSe-PEA and S-DAB-ZnSe-PEA complexes, both of which were excited at 240 nm.

and one (or eventually two in the case of Raman bands) symmetric (Gaussian) components

$$F_\lambda = \frac{A}{\sqrt{2\pi\sigma}}\exp\left(\frac{-(\lambda-\lambda_0)^2}{2\sigma^2}\right) \quad (2)$$

were applied as the most suitable deconvolution methods because both fluorophores (log-normal) and an artifact or Raman (Gaussian) emission band were present in the spectra.

Specifically, the final deconvolution formula took the following form:

$$F_\lambda = \sum_{i=1}^{N_L} F_{m,i}\exp\left\{-\left(\ln 2/\ln^2\rho_i\right)\ln^2[(1/a_i-1/\lambda)/(1/a_i-1/\lambda_m)]\right\} + \sum_{j=1}^{N_G}\frac{A}{\sqrt{2\pi\sigma_j}}\exp\left(\frac{-(\lambda-\lambda_{0,j})^2}{2\sigma_j^2}\right)+C_o \quad (3)$$

where N_L denotes the number of log-normal components, N_G represents the number of Gaussian components and C_o is an additive constant.

3. Results and discussion

A set of fluorescence spectra recorded during the excitation of ZnSe-PEA at different wavelengths (λ_{ex}), starting from the excitation maximum at 240 nm with a 5 nm step, is shown in Fig. 1A. The initial excitation wavelength of 240 nm was selected based on our previous measurements of excitation spectra. The presence of S-DAB in the complex induced a red-shift of the emission maximum and increased the fluorescence intensity, as shown in Fig. 1B. Fluorescence spectra of the different raw components of the system, i.e., PEA and ZnSe, were recorded under the same experimental conditions, in order to analyze their contribution in the formed complex system.

The presence of –NH groups in the inner structure of the dendrimer also affects the non-radiative decay rate by decreasing it and increasing the transitions to the ground state of the ZnSe-PEA QDs [45]. One of the most intriguing aspects is that the functional units involving ligands can be placed in specific sites in the dendrimers, interior and/or surface, which is ideal for developing functional materials required for vectorial interactions [46].

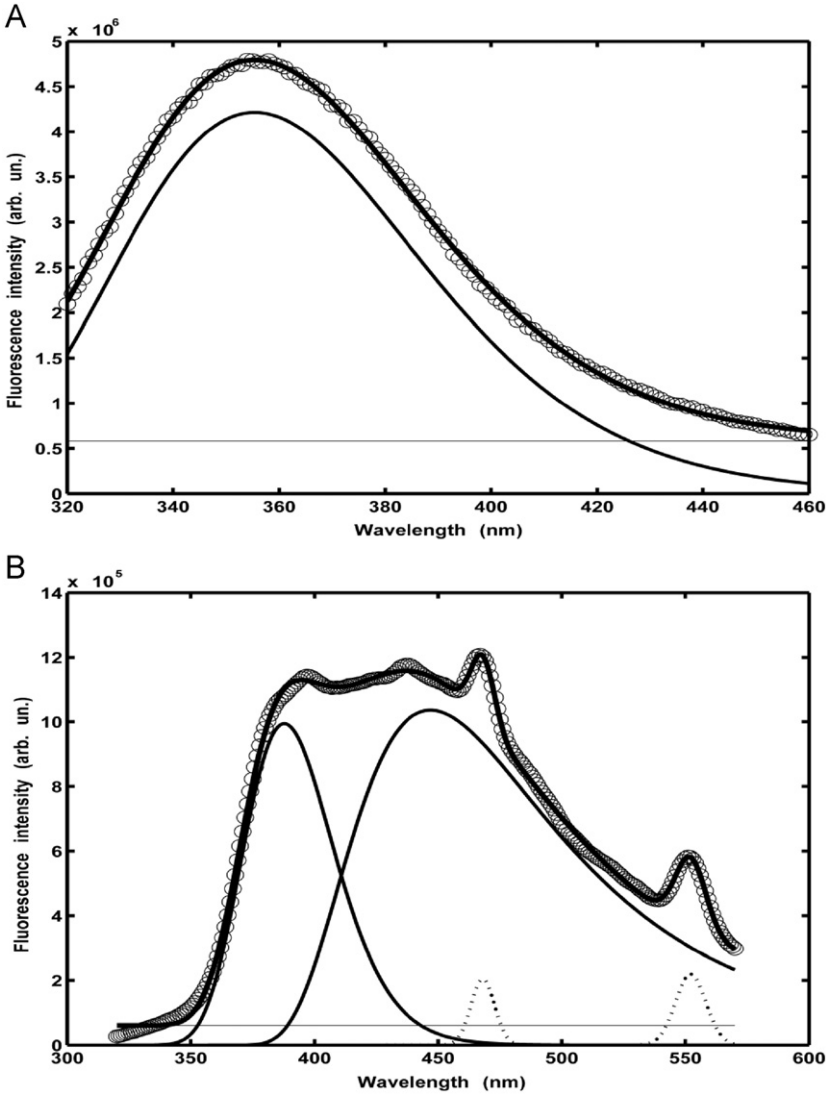

Fig. 2. A: An example of one log-normal component deconvolution of a S-DAB emission spectrum, which was obtained by excitation at 275 nm. Log-normal component: $F_m=4.21\times10^6$; $\rho=1.05$; $a=114.7$; $\lambda_{em}=355.4$; additive constant: co$=5.81\times10^5$, square error per point: err$^2/N=1.02\times10^9$, R square adjusted: $R^2_{adj}=0.99949$; B: deconvolution into two log-normal and two Gaussian components of an emission spectrum of ZnSe-PEA, which was excited at 275 nm. Fitted parameter values according to formulas (1) and (2); log-normal components: $F_{m1}=9.94\times10^5$, $F_{m2}=1.04\times10^6$; $\rho_1=1.12$, $\rho_2=1.50$; $a_1=262.1$, $a_2=353.3$; $\lambda_{m1}=387.8$, $\lambda_{m2}=446.9$, Gaussian components: $A_1=2.42\times10^6$, $A_2=3.40\times10^6$; $\sigma_1=4.7$, $\sigma_2=6.2$; $\lambda_{01}=468.1$, $\lambda_{02}=552.2$, additive constant: co$=6.04\times10^4$, square error per point: err$^2/N=1.29\times10^8$, R square adjusted: $R^2_{adj}=0.99911$.

As an example, a typical result from the one component log-normal deconvolution of S-DAB and a two log-normal plus two Gaussian components deconvolution of the ZnSe-PEA QDs emission spectra, which were obtained by excitation at 275 nm, are shown in Fig. 2A and B, respectively. Each component obtained from the deconvolution of the integral spectrum corresponds to a fluorophore in the molecule. By varying the excitation wavelengths, we were able to observe various fluorophores emitting at different specific wavelengths (component positions). It is known that fluorophores do not change their emission maxima with a variable excitation wavelength. Therefore, in a series of multi-component deconvolutions performed at different excitation wavelengths, the component positions tend to group at certain fixed points, which correspond to the emission maxima of the present fluorophores. These components are statistically detectable as local peaks of the approximate probability density (APD) curve, which was applied to all the calculated component positions.

Fig. 3 presents the positions of the components in the ZnSe QDs emission spectra in both the presence and absence of S-DAB bound in the complex. By comparing the component positions in these three cases, it is clear that S-DAB (Fig. 3A) can be deconvoluted with only one log-normal component, whereas the complexes ZnSe-PEA (Fig. 3B) and S-DAB-ZnSe-PEA (Fig. 3C) required two log-*n* components in addition to the Gaussian component. It is clear that the binding of S-DAB to the complex did not significantly affect the positions of the two components or the number of components in the complex. The S-DAB component was not present in the spectrum of the S-DAB-ZnSe-PEA complex.

In a previous study for PAMAM, which is a dendrimer that is structurally similar to DAB and S-DAB, strong fluorescence was observed although it does not contain fluorophores [47]. It was hypothesized that functional groups in the PAMAM dendrimer could be densely packed under suitable conditions because of its structural stability resulting from covalent bonds. Therefore, it is possible for the overcrowded functional groups to construct a somewhat new type of 'fluorophores'. A similar structure may be expected for the S-DAB dendrimer; therefore, one can expect that its emission originates from the packed functional groups. There was a remarkable difference in the fluorescence properties between the different generation G_2 and G_4-NH_2-terminated PAMAM dendrimers. It can be assumed that the backbone of the dendrimer played the key role in forming the novel fluorescent center [48]. Here, the presence of the dense thiol terminal groups (–SH) of the dendrimer generation (G_5) does not change this effect, which may be explained for the different natures, in terms of electronegativity, of the shell involved.

The fact that S-DAB did not change the position of the two spectral components after binding to the ZnSe-PEA QDs complex indicates that this complex is stable at a pH of 7. This result indicates that the applied method for the deconvolution of fluorescence spectra is very sensitive for observing the fine structural changes in the investigated molecular complex after the binding of S-DAB. The same approach may also be applicable in other binding studies, such as the binding of dendrimers to

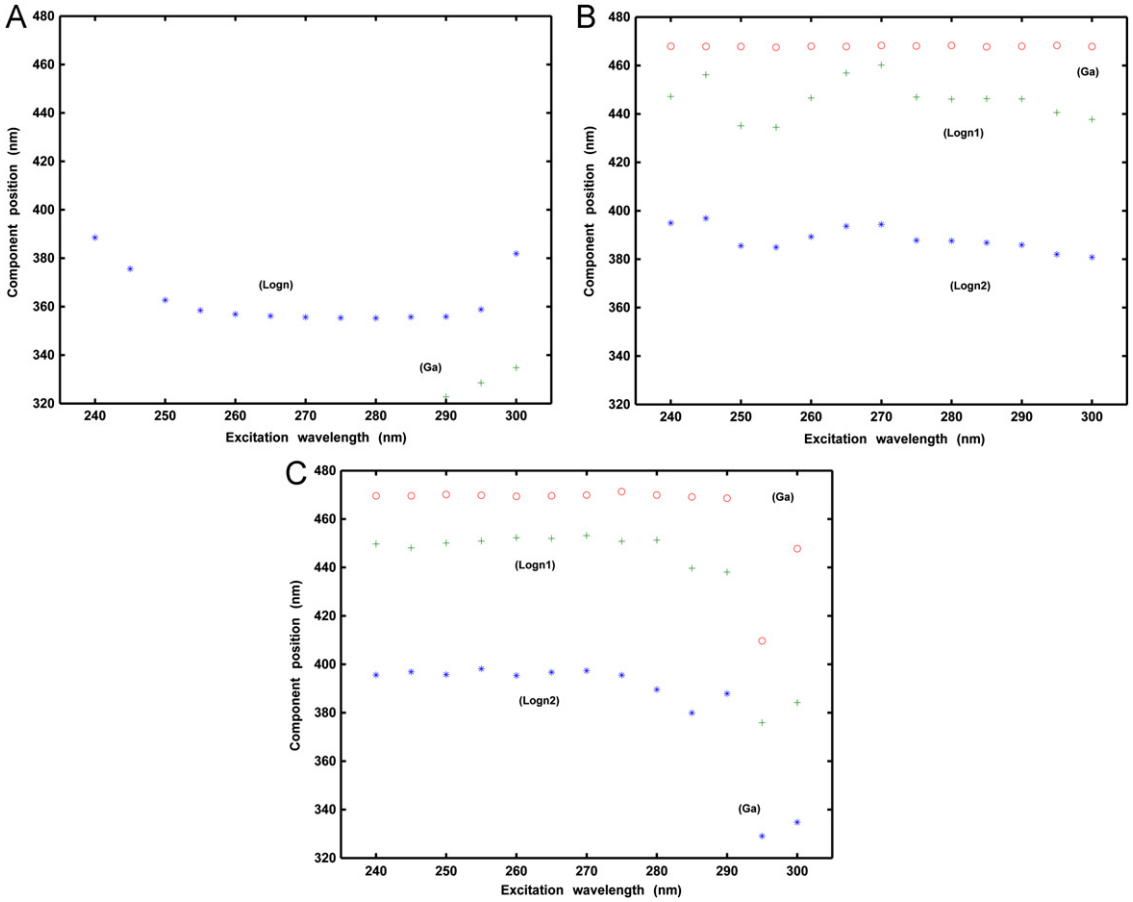

Fig. 3. Dependence of the position of the component maxima, obtained from the deconvolution of emission spectra on the excitation wavelength. A: Pure S-DAB; B: ZnSe-PEA in the absence of S-DAB and C: in the presence of S-DAB in the complex. Each point (or pair of points) is the result of the deconvolution of one spectrum. Position of the components obtained by the deconvolution of emission spectra is shown in the Fig. 4.

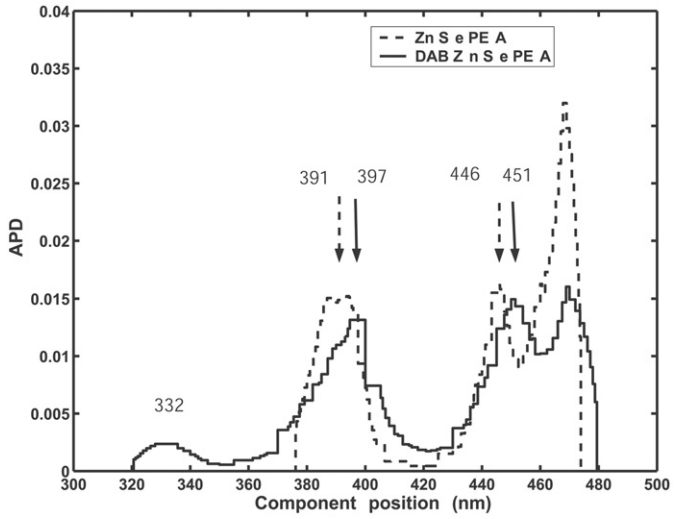

Fig. 4. Approximate probability density (APD) for component positions for the ZnSe-PEA complex in the presence and absence of S-DAB. The most probable positions (numerical values in nm) of the log-normal components are indicated by arrows. A smeared peak at 332 nm originated from a Raman Gaussian component.

proteins, in which the stability of both molecules has to be determined after complexation. Such complexes have potential for biomedical applications, such as the C-reactive protein [49]; therefore, their stability is an important property.

Fig. 4 presents the final result from the deconvolution as an approximate probability density (APD) for the component positions. It is evident that in presence of S-DAB in the complex, there is a slight red-shift of the component positions. This result is in agreement with the red-shift of the emission spectrum maximum in the presence of S-DAB in the molecule (Fig. 1B). However, the S-DAB component was absent in the emission spectrum of the S-DAB-ZnSe-PEA complex (Fig. 3). This complete quenching of the S-DAB emission when it is present in the complex may be explained by the energy transfer from S-DAB to the complex and the relaxation of the system to low energies, which is reflected in the red-shift of the emission maximum of the complex (Fig. 1B).

4. Conclusions

Our results reveal that the emission spectrum of ZnSe-PEA contains two components. The positions of the emission maxima of these two components are not significantly affected by the presence of the dendrimer S-DAB (G_5) in the complex, which reveals that the complex is stable at a pH of 7. This result is important in its application in binding studies in bio-analysis systems.

Acknowledgments

The authors would like to thank the Ciência 2007 and FEDER Project PTDC/QUI/71001/2006 from FCT (Lisbon, Portugal). They also thank the Ministry of Science and Technology of the Republic of Serbia which supported this study with Grant 173017. Spanish Ministerio de Ciencia e Innovación (Project CTQ2009-12332-C02-01) is also acknowledged.

References

[1] A.P. Alivisatos, Science 271 (1996) 933.
[2] M. BruchezJr., M. Moronne, P. Gin, S. Weiss, A.P. Alivisatos, Science 281 (1998) 2013.
[3] Y. Zheng, S. Gao, J.Y. Ying, Adv. Mater. 19 (2007) 376.
[4] T. Jin, F. Fujii, Y. Komai, J. Seki, A. Seiyama, Y. Yoshioka, Int. J. Mol. Sci. 9 (2008) 2044.
[5] F. Dong, H. Han, J. Liang, D. Lu, Luminescence 23 (2008) 321.
[6] J.L. Nadeau, L. Carlini, D. Suffern, O. Ivanova, S.E. Bradforth, J. Phys. Chem. C 116 (2012) 2728.
[7] M. Thiry, K. Boldt, M.S. Nikolic, F. Schulz, M. Ijeh, A. Panicker, T. Vossmeyer, H. Weller, ACS Nano 28 (2011) 4965.
[8] H. Kuang, Y. Zhao, W. Ma, L. Xu, L. Wang, C. Xu, Trends Anal, Chem 30 (2011) 1620.
[9] W.R. Algar, U.J. Krull, J. Colloid Interface Sci. 359 (2011) 148.
[10] P.M. Ndangili, O.A. Arotiba, P.G.L. Baker, E.I. Iwuoha, J. Electroanal. Chem. 643 (2010) 77.
[11] S. Moeno, E. Antunes, T. Nyokong, J. Photochem. Photobiol. A Chem 218 (2011) 101.
[12] S. D'Souza, E. Antunes, C. Litwinski, T. Nyokong, J. Photochem. Photobiol. A Chem 220 (2011) 11.
[13] J. Tian, R. Liu, Y. Zhao, Q. Xu, S. Zhao, J. Colloid Interface Sci. 336 (2009) 504.
[14] C. Park, T.H. Yoon, Colloids Surf, B Biointerfaces 75 (2010) 472.
[15] D. Liu, P.T. Snee, ACS Nano 5 (2011) 546.
[16] J.M. Frechet, D.A. Tomalia, Dendrimers and Other Dendritic Polymers New York, John Wiley & Sons, NY, 2002.
[17] D. Astruc, E. Boisselier, C. Ornelas, Chem. Rev. 110 (2010) 1857.
[18] K.K. Ong, A.L. Jenkins, R. Cheng, D.A. Tomalia, H.D. Durst, J.L. Jensen, P.A. Emanuel, C.R. Swim, R. Yin, Anal. Chim. Acta 444 (2001) 143.
[19] B. Pan, F. Gao, H. Gu, J. Coll., Interface Sci. 284 (2005) 1.
[20] M.F. Ottaviani, S. Jockusch, N.J. Turro, D.A. Tomalia, A. Barbon, Langmuir 20 (2000) 10238.
[21] A.C. Wisher, I. Bronstein, V. Chechik, Chem. Commun. 15 (2006) 1637..
[22] B.B. Campos, M. Algarra, J.C.G. Esteves da Silva, J. Fluoresc. 20 (2010) 143.
[23] M. Algarra, B.B. Campos, M.S. Miranda, J.C.G. Esteves da Silva, Talanta 83 (2011) 1335.
[24] M. Algarra, B.B. Campos, B. Alonso, M.S. Miranda, Á.M. Martínez, C.M. Casado, J.C.G. Esteves da Silva, Talanta 88 (2012) 403.
[25] B.B. Campos, M. Algarra, B. Alonso, C.M. Casado, J.C.G. Esteves da Silva, Analyst 134 (2009) 2447.
[26] D. Gomes, M. Algarra, M.J. Diez de los Rios, M.M. Arrebola, M.E. Herrera-Gutiérrez, G. Seller-Pérez, J.C.G. Esteves da Silva, Talanta 93 (2012) 411.
[27] Z. Li, P. Huang, R. He, J. Lin, S. Yang, X. Zhang, Q. Ren, D. Cui, Mater. Lett. 64 (2010) 375.
[28] B. Pan, D. Cui, F. Gao, R. He, Nanotechnology 17 (2006) 2483.
[29] B. Pan, F. Gao, R. He, D. Cui, Y. Zhang, J. Colloid Interface Sci. 297 (2006) 151.
[30] M.E. Garcia, L.A. Baker, R.M. Crooks, Anal. Chem. 71 (1999) 256.
[31] V. Chechik, R.M. Crooks, Langmuir 15 (1999) 6364.
[32] R.M. Crooks, M. Zhao, L. Sun, V. Chechik, L.K. Yeung, Acc. Chem. Res. 34 (2001) 181.
[33] Y.G. Kim, S.K. Oh, R.M. Crooks, Chem. Mater. 16 (2004) 167.
[34] S. Srivastava, B.L. Frankamp, V.M. Rotello, Chem. Mater. 17 (2005) 487.
[35] D. Li, J. Li, Colloids Surf. A 257–258 (2005) 255.
[36] P.S. Kumar, S.K. Pal, S. Kumar, V. Lakshminarayanan, Langmuir 23 (2007) 3445.
[37] S.C. Smith, P. Greenland, S.M. Grundy, Circulation 101 (2000) 111.
[38] J. Cubedo, T. Padró, X.G. Moll, X. Pintó, J. Cinca, L. Badimon, J. Proteome Res. 10 (2011) 211.
[39] P. Libby, P.M. Ridker, A. Maseri, Circulation 105 (2002) 1135.
[40] P.M. Ridker, N.M. Rifai, L. Rose, J.E. Buring, N.R. Cook, N. Eng., Med 347 (2002) 1557.
[41] N. Vivona, G. Bivona, D. Noto, B.L. Sasso, A.B. Cefalù, G. Chiarello, A. Falletta, M. Ciaccio, M.R. Averna, Clin. Biochem. 42 (2009) 1713.
[42] K. Radotić, A. Kalauzi, D. Djikanović, M. Jeremić, R.M. Leblanc, Z. Cerović, J. Photochem. Photobiol. B Biol. 83 (2006) 1.
[43] D. Djikanović, A. Kalauzi, M. Jeremić, M. Mićić, K. Radotić, Colloids Surf, B Biointerfaces 54 (2007) 188.
[44] S. Connolly, S.N. Rao, D. Fitzmaurice, J. Phys. Chem. B 104 (2000) 4765.
[45] F.G. Sánchez, A.N. Díaz, M. Algarra, J. Lovillo, A. Aguilar, Spectrochim. Acta A 84 (2011) 88.
[46] K. Inoue, Prog. Polym. Sci. 25 (2000) 453.
[47] D. Wang, T. Imae, J. Am. Chem. Soc. 126 (2004) 13204.
[48] D. Wang, T. Imae, J. Am. Chem. Soc. 126 (2004) 8891.
[49] M. Algarra, B.B. Campos, D. Gomes, B. Alonso, C.M. Casado, M.M. Arrebola, M.J. Diez de los Rios, M.E. Herrera-Gutiérrez, G. Seller-Pérez, J.C.G. Esteves da Silva, Talanta 99 (2012) 574.