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Application of Functionalized ZnS Quantum Dots as a Fluorescence Probes for Determination of Amino Acids

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Abstract. In this work, L-cysteine capped-ZnS quantum dots were synthesized in aqueous medium, and their interaction with some of the amino acids was studied with fluorescence spectra. The results demonstrated that histidine could effectively quench the QDs emission more than other amino acids. Electron transfer process between the capping ligands and histidine was mainly responsible for the remarkable quenching effect of histidine, because according to the structure of histidine, it is the strongest acceptor among essential amino acids. Under optimum conditions, the quenched fluorescence intensity increased linearly with the concentration of histidine ranging from 1.33×10^{-6} *to* 1.49×10^{-4} $molL^{-1}$. The limit of detection for histidine was 0.05×10^{-7} mol L−1.

Keywords: Amino acid; Nanoparticles; L-Cysteine-capped nano-ZnS; Fluorescence.

1. Introduction

Quantum dots (QDs) are nano-sized semiconductor crystals which exhibit size-dependent physicochemical properties such as a tunable, narrow emission spectrum, excellent photo stability and a broad excitation spectrum [1-5]. Thus, luminescent semiconductor quantum dots (QDs), have gained increasing attention in the past decade [6-8] .In 1998, Alivisatos and coworkers [9] and Chan and Nie [10] demonstrated the first applications of QDs for biology. Studies of luminescent quantum dots first appeared in the early 1980s [11-14]. The surfaces of quantum dots can be coated with sulfhydryl groups using molecules like mercaptoacetic acid or dihydrolipoic acid. Biomolecules can also be bound to the surface by hydrophobic or electrostatic interactions [15]. Thus we modified quantum dots with l-cysteine because of its thiol group therefore it was used as a sensor for detection of histidine.

Amino acids in their native form are generally weak chromophores (do not absorb UV light) and do not possess electrochemical activity. Therefore, the need for monitoring amino acids is a challengeable problem. Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. The 20 amino acids that are found within proteins convey a vast array of chemical versatility. Humans can produce 10 of the 20 amino acids. The others must be supplied in the food. The essential amino acids are arginine (required for the young, but not for adults), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In this work we suggest a novel way for detection of histidine with a good detection limite.

2. Experimental

2.1. Reagent

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l-Cysteine, zinc acetate, sodium sulphide nine hydrate, methanol, and other routine chemicals were purchased from Merck. All aqueous solutions were prepared with double distilled deionized water. The stock solutions of amino acids were prepared by dissolving suitable amount of amino acids in 50mL doubly deionized water, and was further diluted whenever necessary.

2.2. Apparatus

The fluorescence studies were performed using a Perkin-Elmer LS50 spectrofluorimeter. UV–Vis spectra and absorbance measurements were recorded on a PerkinElmer Lambda2 UV/Vis spectrophotometer. An EcoMet p25 pH-meter with a Metrohm double junction glass electrode was used for pH adjustment. FT-IR spectra were recorded with AVATAR-360-FT-IR infrared spectrometer.

2.3. Synthesis of L-cysteine capped ZnS quantum dots

L-cysteine-capped ZnS QDs were synthesized based on the method described elsewhere [16]. Briefly 0.2mmol of Zn $(CH_3COO)_2$ and 0.2mmol of l-cysteine were added into a 250 ml round bottom flask. 200 ml of double distilled deionised water was added into this flask and purged with pure nitrogen gas for at least 60 min under magnetic stirring. The pH value of solution was adjusted about 7 using 0.5M Tris solution, as you know the Isoelectric Point (pH) of L-cysteine is around 5, so the pH was set upper than 5 to deprotonation of L-cysteine.0.2mmol of Na_2S was dissolved in a 10ml of double distilled deionised water and added slowly to the stirred solution using a syringe. Then the mixture was refluxed under nitrogen atmosphere for 10 h. The cysteine-capped nanoparticles were obtained in a powder form through ethanol precipitation. These nanoparticles were treated with ethanol in three or four repeated cycles to remove the contaminants. The resulting l-cysteine-capped nanoparticles were dried under vacuum for further use.

2.4. Measurement procedures

2.5mgL^{-1} of l-cysteine-capped-ZnS nanoparticle solution was suspended into phosphate buffer solution (PBS) (pH 7.8). Then, a series of different concentration amino acids standard solution were transferred into a 10mL volumetric flask and finally diluted to 10.0mL with water and mixed thoroughly. The relative fluorescence intensity was measured at λem/λex = 333/290 nm.

3. Results and discussion

3.1. Characterization of ZnS quantum dots

The morphology of the functionalised ZnS nanoparticles was studied by the SEM. The SEM image of ZnS QDs (Fig. 1) shows that the shapes' of the l-cysteine-capped particles are dispersed spherical with the size of around 20 nm in diameter.

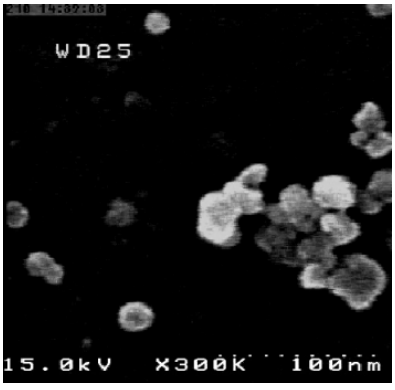

Fig. 1: Scaning Electron Micrographs (SEM) images of functionalized ZnS nanocrystals.

The optical properties of l-cysteine- capped ZnS QDs were characterized by UV–visible absorption spectrometry. The characteristic absorption peak of the l-cysteine-capped ZnS nanoparticles is located at 300nm as shown in Fig. 2.

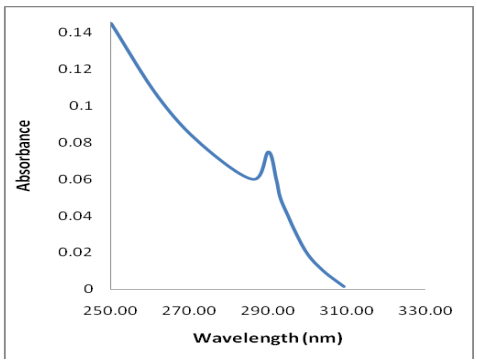

Fig. 2: Absorption spectrum of the l-cysteine-capped ZnS QDs.

Fig. 3 shows the FT-IR spectra of free l-cysteine and lcysteine-capped ZnS nanoparticles. The IR absorption band around 1600 cm^{-1} (COO^{-}), 1350cm^{-1} (COO^{-}), 3500–3000cm^{-1} (OH, COOH), indicate the –COO^{-} group. The peak at 2900–3420cm^{-1} (N–H) indicates –NH2 group and 600–800 cm^{-1} (C–S) indicate the C–S group, while the peak at 2550–2750cm^{-1} (S–H) represents –S–H group. There are coexisting IR absorption bands of –COO^{-}, observed on both L-cysteine and L-cysteine-capped ZnS QDs. Therefore carboxylic acid and amino group are present on the surface of the ZnS QDs, while the S–H group vibration (2550–2670cm−1 S–H) is absent on the surface of the l-cysteine-capped ZnS QDs. The reason for disappearance of S–H group vibration on the surface of ZnS nano particles is due to the formation of covalent bonds between thiols and the surface of ZnS.

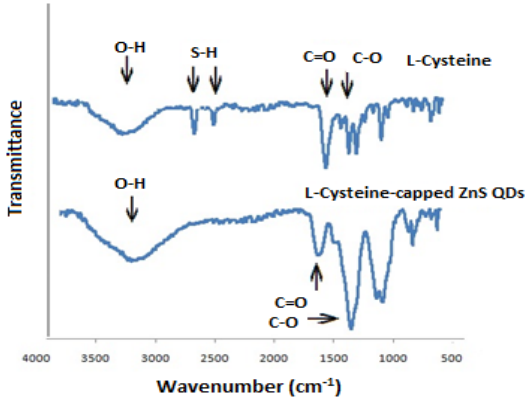

Fig. 3: FT-IR spectra of free l-cysteine and l-cysteine-capped ZnS QDs.

3.2. The effect of the pH on fluorescence intensity

We have studied the effect of pH on fluorescence of QDs to obtain an optimum pH to develop a sensitive fluorescence sensor for histidine. The fluorescence intensity was studied against pH in the range 4.0 and 9.0 in order to obtain the optimum conditions for the determination of histidine,it is shown in Fig.4. The optimum net fluorescence intensity was obtained in the pH range between 7.30 and 8. Therefore a phosphate buffer solution (PBS) (pH 7.8) was used for the determination of histidine studies. The reason for the low fluorescence intensity in acidic medium is the result of dissociation of the Zn^{2+}–l-cysteine nanoparticles due to the protonation of the surface-binding thiolates [17].When pH increases, not only the deprotonation of the thiol group in the lcysteine molecule ,but also deprotonation of carboxylate group in the histidine may occur. This deprotonation may strengthen the covalent bond between Zn and l-cysteine molecule,also between histidine and capped l-cysteine .This leads to increase the fluorescence intensity with increase of pH. At higher pH the fluorescence intensity decreases due to precipitation of Cu $(OH)_2$.

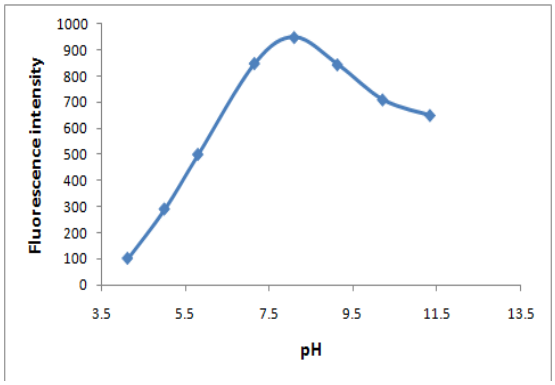

Fig.4: Effect of pH on the fluorescence intensity of l-cysteine-capped ZnS QDs.

3.3. Analytical performance of new senthesized QDs

The fluorescence spectra of l-cysteine-coated ZnS QDs and its fluorescence titration with histidine were recorded at optimum experimental conditions. The fluorescence of l-cysteien-coated ZnS QDs is significantly decreased with increasing the concentration of histidine Fig.6.

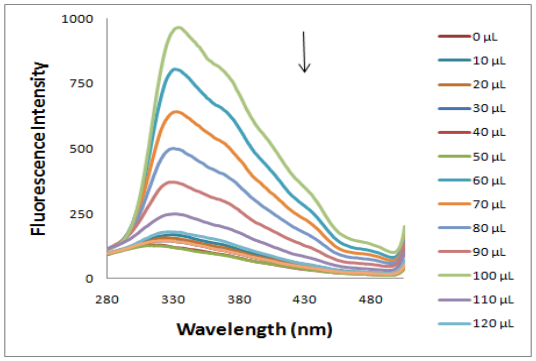

Fig.6: Effect of histidine concentration on the fluorescence intensity of l-cysteine-capped ZnS QDs.

A very good linear relationship (R = 0.997) was observed the range between 1.33×10^{-6} to 1.49×10^{-4} mol L^{-1} when using the well-known Stern-Volmer equation Fig.7. And the detection limit, calculated following the 3_ IUPAC criteria, was 0.05×10^{-7} mol L^{-1}.

$$Log\ (F_{0/}F) = 1 + K_{sv}C \quad (1)$$

where F_0 and F are the intensity in the absence and presence of the quencher, K_{SV} is the Stern-Volmer quenching constant, and C is the concentration of the quencher. The K_{SV} was found to be 16×10^{6} M^{-1}.

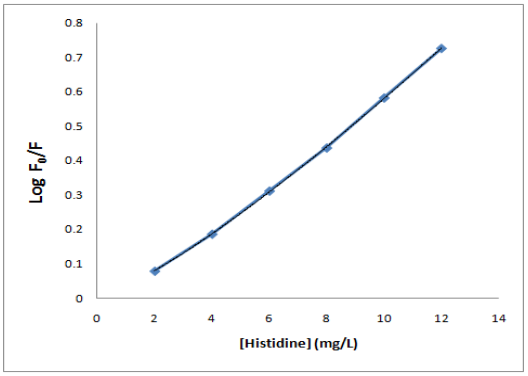

Fig. 7: Stern–Volmer plot for interaction between l-cysteine-capped ZnS QDs and histidine.

4. Effect of other lanthanide ions on l-cysteine-capped ZnS QDs

The fluorescence emission of l-cysteine QDs was significantly decreased without any emission band shift with the addition of histidine .Fig.6. As can be seen in Fig.8 the influence of other amino acids such as isoleucine, valine, threonine, alanine, glycine, proline etc,was very weak, i.e., l-cysteine -QDs were rather selective towards histidine which makes it very attractive for the selective recognition of histidine.Quantum dots appear to behave like any other fluorophore with regard to energy transfer [15].

According to the structure of histidine, it is the strongest acceptor among essential amino acids, thus l-cysteine capped ZnS QDs were rather selective towards histidine which makes it very attractive for the selective recognition of histidine.

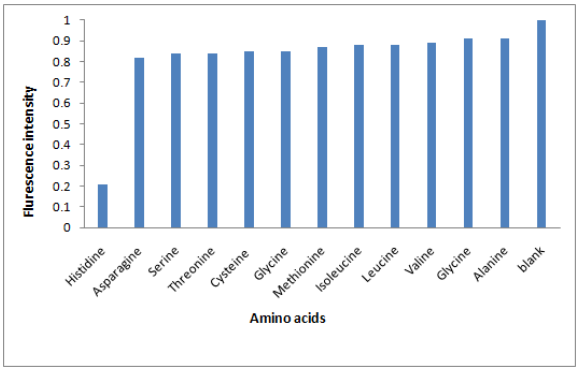

Fig. 8: Effect of amino acids on the luminescence l-cysteine-capped ZnS QDs

5. Conclusions

In conclusion, amino acids caused the fluorescence quenching of l-cysteine QDs. Here we demonstrated the potential application of luminescent QDs to develop new fluorescence sensors for the detection of amino acids .It is possible that changing the modifier of surface of QDs could make the method more effective. A good linear relationship was observed when using a modified Stern–Volmer plot. The detection limits I 0.05×10^{-7} mol.L^{-1}. The fact that QDs showed high fluorescence quenching sensitivity towards amino acids indicated that they are potential candidates' materials for detection of essential amino acids. This paper described for the first time the quantitative application of strong luminescence QDs for amino acids analysis.

6. References

[1] Aldana, J.; Wang, Y. A.; Peng, X. G., Photochemical instability of CdSe nanocrystals coated by hydrophilic thiols. *J Am Chem Soc* **2001,** *123* (36), 8844-8850.

[2] Guo, J.; Yang, W. L.; Wang, C. C., Systematic study of the photoluminescence dependence of thiol-capped CdTe nanocrystals on the reaction conditions. *J Phys Chem B* **2005,** *109* (37), 17467-17473.

[3] Rogach, A. L.; Katsikas, L.; Kornowski, A.; Su, D. S.; Eychmuller, A.; Weller, H., Synthesis and characterization of thiol-stabilized CdTe nanocrystals. *Ber Bunsen Phys Chem* **1996,** *100* (11), 1772-1778.

[4] Smith, A. M.; Nie, S., Chemical analysis and cellular imaging with quantum dots. *Analyst* **2004,** *129* (8), 672-7.

[5] Samia, A. C.; Dayal, S.; Burda, C., Quantum dot-based energy transfer: perspectives and potential for applications in photodynamic therapy. *Photochem Photobiol* **2006,** *82* (3), 617-25.

[6] Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S., Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* **2005,** *307* (5709), 538-544.

[7] Vastarella, W.; Nicastri, R., Enzyme/semiconductor nanoclusters combined systems for novel amperometric biosensors. *Talanta* **2005,** *66* (3), 627-633.

[8] Ye, Z.; Tan, M.; Wang, G.; Yuan, J., Development of functionalized terbium fluorescent nanoparticles for antibody labeling and time-resolved fluoroimmunoassay application. *Talanta* **2005,** *65* (1), 206-10.

[9] Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P., Semiconductor nanocrystals as fluorescent biological labels. *Science* **1998,** *281* (5385), 2013-2016.

[10] Chan, W. C.; Nie, S., Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* **1998,** *281* (5385), 2016-8.

[11] Kuczynskl, J. P.; MlIoravljevlc, B. H.; Thomas, J. K., Effect of the Synthetic Preparation on the Photochemical Behavior of Colloidal CdS. *J. PhyS. Chem.* **1983,** *87*, 3368-3370.

[12] Rossetti, R.; Nakahara, S.; Brus, L. E., Quantum size effects in the redox potentials, resonance Raman spectra, and electronic spectra of CdS crystallites in aqueous solution. *Journal of Chemical Physics* **1983,** *79* (2), 1086-1088.

[13] Weller, H.; Koch, U.; Gutiérrez, M.; Henglein, A., Photochemistry of Colloidal Metal Sulfides. 7. Absorption and Fluorescence of Extremely Small ZnS Particles (The World of the Neglected Dimensions). *Ber Bunsenges Phys Chem* **1984** *88*, 649-656.

[14] Ramsden , J.; Gratzel, M., Photoluminescence of small cadmium sulphide particles. *J Chem Soc Faraday Trans* **1984,** *80*, 919-933.

[15] Lakowicz, J. R., Principles of Fluorescence Spectroscopy. *springer Third Edition*.

[16] Gattas-Asfura, K. M.; Leblanc, R. M., Peptide-coated CdS quantum dots for the optical detection of copper(II) and silver(I). *Chem Commun (Camb)* **2003,** (21), 2684-5.

[17] Gao, M. Y.; Kirstein, S.; Mohwald, H.; Rogach, A. L.; Kornowski, A.; Eychmuller, A.; Weller, H., Strongly photoluminescent CdTe nanocrystals by proper surface modification. *J Phys Chem B* **1998,** *102* (43), 8360-8363.