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Studying on Synthesis of Highly Luminescent Quantum Dots Based on Zinc and Their Application for *Escherichia coli* O157: H7 and Methicillin-Resistant *Staphylococcus aureus* Detection

Diem Thi Bui[1], Duy Khanh Pham[2], Xuan Truong Mai[2], Van Khiem Nguyen[2], Thanh Thao Bui[3], Ngoc Quyen Tran[2,4], Nguyen Thi Ngoc Nhi[5], Tai The Diep[5*] and Bich Thi Luong[2*]

[1]Industrial University of Ho Chi Minh City, Vietnam.
[2]Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Vietnam.
[3]Faculty of Chemical Engineering, Ho Chi Minh City University of Technology, Vietnamese National University in Ho Chi Minh City, Vietnam.
[4]Institute of Research and Development, Duy Tan University, Da Nang City 550000, Vietnam.
[5]Pasteur Institute of Ho Chi Minh City, Vietnam.

Authors' contributions

This work was carried out in collaboration between all authors. Authors BTL, DTB and TTD designed the study. Authors XTM, NTNN and DTB performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BTL, DTB and TTD managed the analyses of the study. Authors NQT, DKP, VKN and TTB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The synthesis of highly luminescent quantum dots ZnSe/ZnS:Mn(5%)/ZnS và ZnSe:Ag(1%) in aqueous phase was studied. This synthetic method is for green chemistry. Silver and manganese are used as a dopant to increase photoluminescence quantum yields of obtained products. Mercaptopropionic acid (MPA) is used as a capping agent which is a sufficient bridge to combine the quantum dots and antibody, bacteria via protein or EDC (1-ethyl-3-(3-dimethylaminopropyl)

**Corresponding author: E-mail: marialuongbich@gmail.com, marialuongbichcnd@gmail.com;*

carbodiimide hydrochloride). *Escherichia coli* O157: H7 and *methicillin-resistant Staphylococcus aureus* (*E. coli* O157: H7 and MRSA) are leading causes of food poisoning and they are also detected via luminescent signals. The results showed that the quantum dot complex was capable of detecting bacteria at 101 CFU / ml for 15 – 30 minutes. The specificity of the reaction is 100%. Optimal antibody concentration for detection of bacteria from 10 mg to 30 mg. Rapid detection of MRSA and *E. coli* O157: H7 on food and clinical samples are going on further test.

Keywords: Luminescent quantum dots; synthesis of quantum dots in aqueous phase; biosensor.

1. INTRODUCTION

Luminescence nanoparticle is a particle of size from 1 to 30 nm, emitted under the excitation source such as the light energy of ultraviolet or infrared. Quantum dots are semiconductor nanoparticles that have a crystal structure of 1-10 nm in size. When semiconductor nanoparticles absorb photons from light-emitting sources, negative electrons (e-electrons) are excited on the conduction and leave positive holes (h-holes) carrying positive charge in the region. Chemistry forms the exciton pair, which then jumps to lower energies and releases energy corresponding to the appropriate wavelength and gives the corresponding fluorescent colour [1]. The luminescent nanoparticles have been studied and synthesized by various methods. There are three main methods: synthesis in organic environment, phase transformation synthesis from organic to water phase and in aqueous phase. Based on the fluorescence properties of quantum dots, they become one of the sources of materials for many different applications. Jun-Jie Zhu and his colleagues have studied the synthesis of ZnSe/ZnS photoluminescence by phase transformation, and have initially applied in a variety of fields, such as biomedical [2], photoconductive [3], and a carrier for drug delivery [4].

Snee et al. [5] synthesized ZnSe/ZnS:Mn in organic media, luminescent quantum dots were attached to dye-dyes to increase luminescence sensitivity, study. Luminescent quantum dots are well dispersed in the water environment and are easily dispersed in the bioreactor through surface stabilizers with two functional groups -SH and -COOH, so they easily interact with biological agents such as antibodies and biological cells. The nanoparticles, after interacting with the biological agent, will produce different luminescence signals compared to their specific luminescence [6-9]. This will serve as a basis for the detection of bacteria and the production of biological sensors based on the sensitivity of the fluorescence system after binding of antibodies to quantum dots through the effect Förster resonance energy transfer (FRET) from quantum dots to spheres and either bacteria, cell or DNA) [7-12].

In this study, the "green" synthesis of ZnSe: Ag and ZnSe / ZnS: Zn nanoparticle ZnSe / ZnS quantum dots in water is studied and initial application of obtained quantum dots in the detection of *Escherichia coli* O157: H7 and *methicillin-resistant Staphylococcus aureus* (*E. coli* O157: H7 and MRSA) is mentioned.

2. EXPERIMENTAL

2.1 Synthetic Quantum Dots

2.1.1 Synthesized ZnSe quantum dot aggregation: Ag

NaHSe solution was prepared from Se, NaBH4 powder, and water in N2 gas. For the synthesis of ZnSe:Ag (1%), the reaction system was prepared in a 3-necked flask containing the following solution mixture. It was made by mixing of 10 ml of 0.1M zinc acetate and 0.01 M AgNO3 with 90 ml of water, and 40 ml of mercaptopropionic acid (MPA) 0.1 M was added. Then, using a 2M NaOH solution to adjust the pH of the reaction mixture at pH 3.8, the reaction was agitated under air evaporated with N2 gas for 30 min. Applying NaHSe solution to the reaction mixture, the reaction system was kept stirring at 90-100°C for 3 hours.

2.1.2 Synthesized ZnSe/ZnS:Mn(5%)/ZnS quantum dots [13]

ZnSe/ZnS:Mn(5%)/ZnS is synthesized by the following steps: at first the synthesis of ZnSe was conducted, reaction system was prepared: in the 3-neck flask containing the following solution mixture of 10 ml zinc acatate 0.1 M with 90 ml of water, and 40 ml of mercaptopropionic acid solution (MPA) of 0.1M. Then, using 2M NaOH solution to adjust pH = 8, the reaction system was stirred and air evaporated with N2 30 minutes, raise the system temperature to 90-95°C [7].

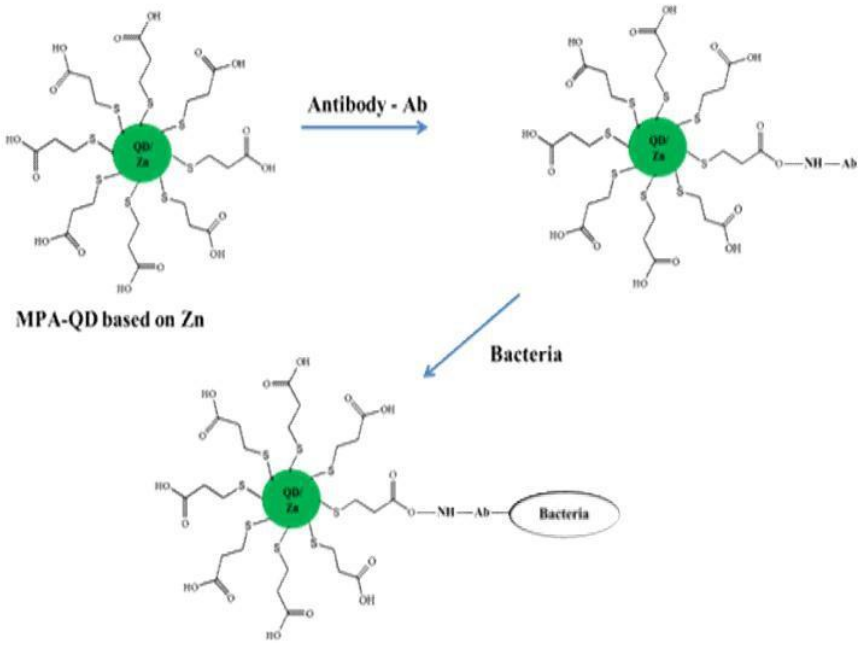

Fig. 1. Schematic mapping of antibodies (Ab) and bacteria on luminescent quantum dots via MPA bridge

Then, NaHSe solution was added into the reaction mixture and kept stirring at 90-100°C for 3 hours. The reaction system would be cooled to room temperature. The shell layer (ZnS: Mn) covered by the core layer (ZnSe) is formed by adding to the reaction system in step 1 a mixture of 7.9 ml of Zn(CH3COO)2 0.1 M and 5 ml of Mn(CH3COO)2 0.01 M at a rate of 1 drop per second. The outer coating of ZnS shell is covered with the following two layers: the reaction system is stirred at 80°C, 8.3 ml of Zn(CH3COO)2 0.1 M solution were added, after 20 minutes of stirring, 8.3 ml of Na2S solution 0.1 M is added to the reaction system and agitated for 1 hour. The obtained product is crystallized in isopropyl alcohol and washed several times with centrifuge, dried in vacuum at room temperature. The obtained product was prepared for structural and optical properties analysis.

UV–vis absorption spectra were obtained using an Optizen 2120UV spectrophotometer (Science and Technology Development) in order to clarify the excitation wavelength of obtained samples. Fluorescence measurements were performed using WGY-10 fluorescence spectrophotometer. All optical measurements were carried out at room temperature at the excitation wavelength (315 nm).

2.2 Application of Luminescent Nanoparticles to Detect Bacteria

Quantum dots were diluted in sterile distilled water into concentrations of 10-1, 10-2, 10-3. The test bacteria are diluted to a solution of McF 0.5 equivalent to 108 CFU / ml. Research and evaluation of optimal conditions for protein A binding on luminescent photon nanoparticles (protein A).Gel column was used with built-in protein A (commercial column) and washed with sticky pad to stabilize the column. Applying 2 ml of nanoparticles to the column with 1 ml / 10 minutes, then 25 µl antibody was given against E.coli, and Staphylococcus through the column at 1 µl / 1 minute 2 ml of column buffer solution wa also applied to stabilize the column and wash the unsustainable bonds from the column. 1 ml of bacterial solution was added at 108 CFU / ml via column at 1 ml / 20 min. The results were prepared for the control 25 µl of *E.coli* antibody and Staphylococcus interacts with 2 ml of a luminescent nanoparticle solution for 20 minutes. Addition of 1 ml of bacterial solution was taken at 108 CFU / ml via column at 1 ml / 20 min. Examinations of the solutions under UV light were taken and their results were also recorded [9-11].

3. RESULTS AND DISCUSSION

3.1 Analysis of Optical Structure and Properties of Quantum Dots

Luminescence properties of ZnSe:Ag nanoparticles were determined. The luminescence of the ZnSe:Ag agglomerates at different silver salt doping concentrations and the same pH value of 8 was shown by fluorescence spectra (Fig. 2a). PL spectrum show that the amount of doped Ag was increased (1, 3, 5, 7%), the light intensity decreases. Maximum light intensity when doped Ag content is 1%. The Ag+ emits at the energy band relating to 460-490 nm of wavelength. When amount of doped Ag was increased, the position of the peak of the emission also varies with the longer wavelength, which proves that the doped amount was raised, the radius of the nanoparticles. At the same time, due to the competition of Ag+ions in the crystals, the increase in the doping concentration of Ag is slightly lower.

The IR spectra show the functional groups oiscillations of the MPA and the ZnSe: Ag sample doped with concentrations of 1, 3, 5, 7 (%), the SH group of the MPA no longer demonstrates that it has formed a bond on the surface of the crystal ZnSe. At the same time, there is still the tip -OH and C = O of the COOH group of the MPA, so the COOH tail is still present (Fig. 3a). This helps to increase the dispersion of water and helps it to have better applications in biology, more compatible with biological cells.

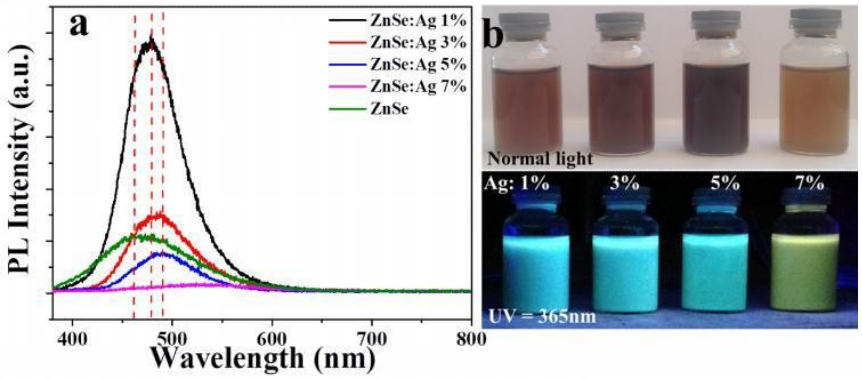

Fig. 2. (a) The PL spectrum and (b) real images under UV light (365nm) of ZnSe: Ag quantum dots solutions with doping of 1, 3, 5, 7%. (pH = 8)

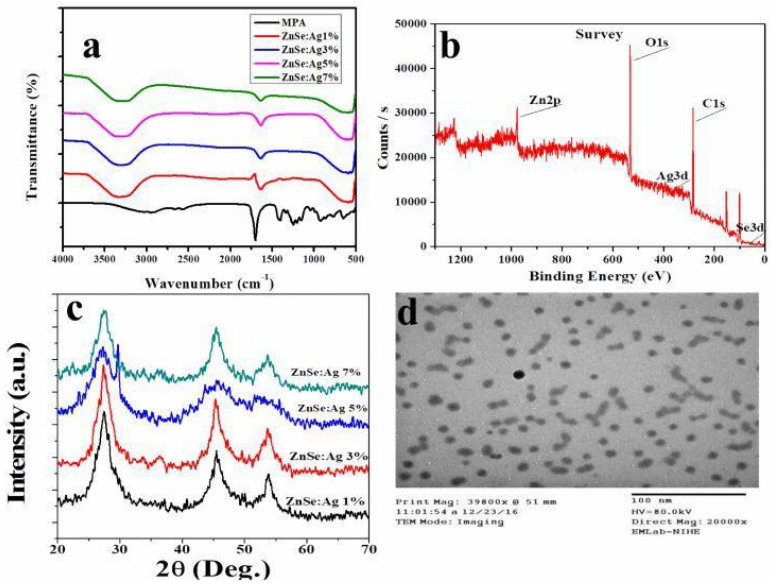

Fig. 3. a) IR spectrum, b) XPS spectra, c) X-ray diffraction patterns of ZnSe: Ag (pH = 8) Ag dopping at 1, 3, 5 and 7% concentrations; d) TEM image of ZnSe sample: Ag (1%)

The XPS spectrum (Fig. 3b) shows the presence of Zn, Se, Ag, C and O elements in the ZnSe granule: Ag is bonded to -H-CH2-CH2-COO-, suitable for the product obtained after synthesis.

From the X-ray diffraction pattern (Fig. 3c), the crystals are still forming crystalline cubic (Zinc Blende) crystals because of the diffraction peaks at 27, 370o, 45, 470o and 53, 850o relating to (111), (220) and (311), respectively. The agitation of small amounts of Ag in the ZnSe crystal does not alter the crystal structure of the original ZnSe. TEM images showed that the average size of the particles was 7.5 ± 0.3 (nm). Determination of luminescence properties of ZnSe/ZnS:Mn/ZnS quantum dots in the UV-Vis spectra (Fig. 4a), the absorption peak of the sputtered Mn quantum dots at different concentrations has an unequal shift, indicating that the size of the particle varies negligible when we change the content of Mn doped. Absorption band is about 325 - 330 nm.

The PL spectrum given in Fig. 4(b), show that dopping into ZnSe crystals with Mn at different concentrations causes to various luminescent intensities. The brightness of ZnSe / ZnS: Mn / ZnS quantum dots solutions under UV light at 574 nm is optimal when the concentration of Mn doped is 5%, Mn2+. At 0.1% Mn2+ doping concentration, the Mn2+ luminance emission is weak, the orange intensity at the Mn2+ center is approximately equal to the green light intensity of the ZnSe crystal. The higher the Mn2+ concentration is applied, the higher the Mn emission is and the optimum is at 5%. However, increasing the doping concentration further, the Mn2+ luminescence center began to decrease. This is explained by the higher the Mn concentration is , the higher the magnetic field between Mn-Mn is, which reduces the fluorescence efficiency. At a concentration of 10% Mn, the fluorescence obtained is white. So the concentration of doping Mn greatly affects the luminous intensity and luminescent color of quantum dots.

The ZnSe core quantum dots have two emission peaks that is the emission of deep trap of ZnSe due to the suitable band gap (400-420 nm), [14,15] and another peak at 500-520 nm wavelength is surface trap emission of ZnSe

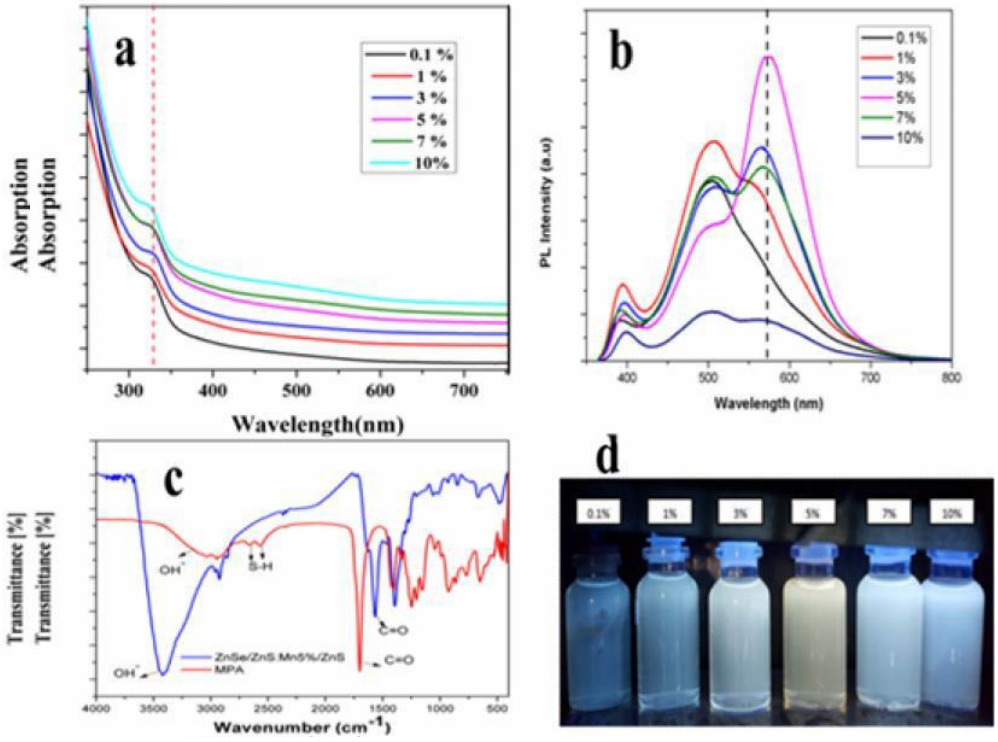

Fig. 4. a) UV-Vis spectrum, b) PL spectrum and c) IR of ZnSe / ZnS: Mn / ZnS at different concentrations; d) real images of ZnSe/ZnS:Mn/ZnS colloidal solutions under 365 nm wavelength

stabilized by MPA capping agent synthesized in aqueous phase. Therefore, the ZnSe/ZnS:Mn/ZnS core/shell/shell have three emission peaks that from 400-420, 500-520, and 580-600 nm due to the Mn dopant concentrations. Since the Mn2+ dopping concentration is high enough, the yellow or orange/red emission could be obtained*]* More interestingly, the low enough Mn2+ dopant concentrations were added, the white emission of the ZnSe/ZnS:Mn/ZnS core/shell/shell was obtained due to the well-control between blue, green and yellow/orange/red emissions so that the UV- white light was shown at the three emissions at different nm wavelength with the suitable intensity in PL spectra. Thus, the molar ratio between Zn and Mn is the most important parameter that affects on the colortunable emission [7,16]. The real images of obtained ZnSe / ZnS: Mn / ZnS with various manganese dopping concentrations under UV light (Fig. 4d). It shows that the 5% Mn doped sample gives the luminous intensity of the Mn2+ center corresponding to the PL measurement result (Fig. 5a).

Fig. 4c shows the IR spectral group of MPA and ZnSe / ZnS: Mn / ZnS doped Mn 5%, the SH group of MPA no longer demonstrates that it formed on the surface of the nanocrystal . At the same time, there is still -OH and C = O of the -COOH group of the MPA, indicating that the COOH group is still present. This helps to increase the dispersion of water and helps it to have better applications in biology, more compatible with biological cells.

From the X-ray diffraction pattern we can see that ZnNe/ZnS:Zn/ZnS quantum dots at other concentrations of Mn doped in Fig. 5a form crystalline cubic crystals Zinc Blende) because of the diffraction peaks at 27,370, 45,470 and 53,850 corresponding to the planes (111), (220), (311). Doping of Mn into ZnSe crystals did not alter the crystalline structure of the original ZnSe.

The TEM image of the ZnSe/ZnS:Mn/ZnS light emitting dots using MPA stabilizer with 5% Mn doped has a particle size of 19 nm. The size of the tang is much higher than that of the core (ZnSe: Ag 7.5 nm), which suggests that the ZnSe core was covered with a shell.

3.2 Results of Binding of Protein A and Antibody to Luminescence Nanoparticles (Quantum Dot-Protein A)

According to obtained quantum dots, the luminescence of different seeds may be due to the attachment of bacteria which would affect the luminescence of the grain. However, this does not affect the detection of strains. In addition, if the isolation can be detected through the luminescent nanoparticle suspension, antibodies should be followed by further parameters such as the difference between the bacterial solution and the bacteria-free one via electrophoresis (Fig. 6a and b). Initial results suggest that antibodies can be bound to quantum dots via the A. protein bridge. The reaction is incubated at 40°C for 5 hours. The *E. coli* O157: H7 antibody [5.5 mg / ml] and the MRSA antibody [1 mg / ml] were diluted in the above mixture into different concentrations of 1 µg, 5 µg, 10 µg, 20 µg, 30 µg. The entire quantum and antibiotic complexes were further incubated at 40°C overnight following the following scheme: Antibody-binding complexes were performed to determine the difference between antibody- and antibody-free nanoparticles (Fig. 6c and d). Quantum dots which have lower antibody would luminesce than non-antibody quantum dots. The complexes selected for subsequent experiments were

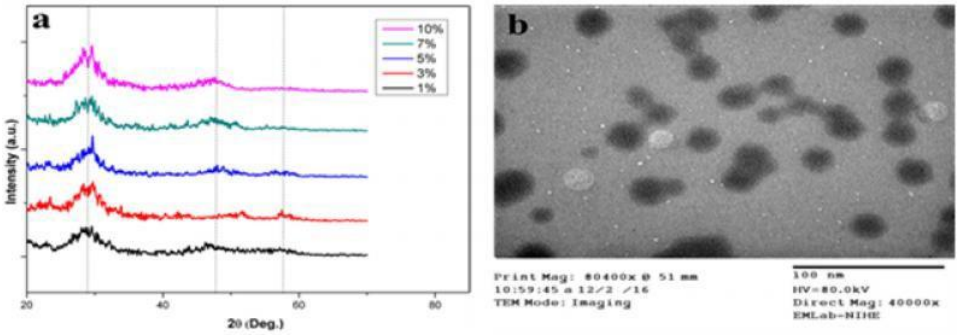

Fig. 5. a) XRD schema and b) TEM image of ZnSe / ZnS sample: Mn (5%) / ZnS

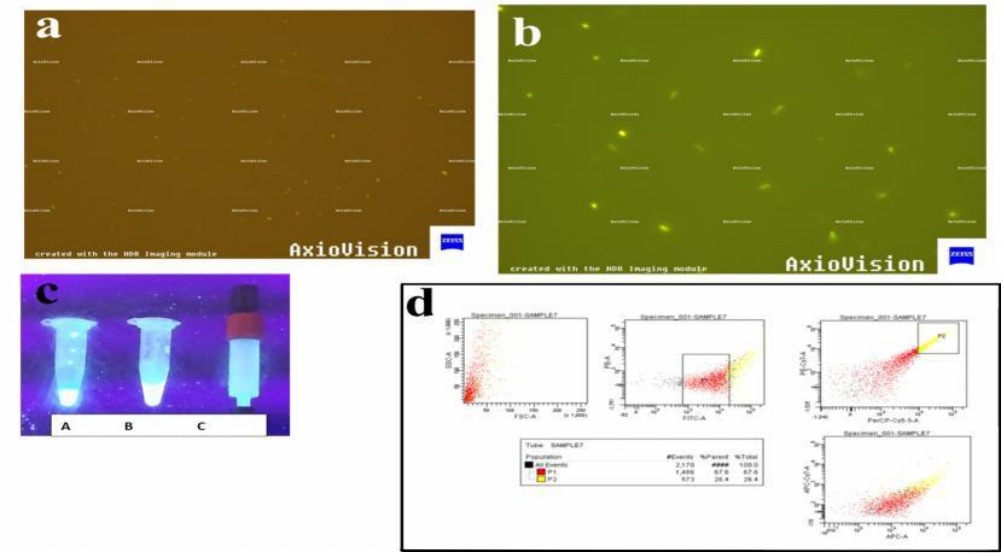

Fig. 6. Test results for binding protein A and antibody to luminescence nanoparticles. a) Screened for MRSA and b) E. coli O 157: H7 under fluorescence microscope; c) Results of testing protein A and antibody on seeds (A: luminescent nanoparticles, B: solution of luminescent nanoparticles and antibodies, bacteria, C: solution of luminescent nanoparticles, antibodies, proteins A and bacteria); d) The result of flowcytometry for good luminescence and antibody binding. Flow cytometry is performed on the FACSCalibur Calibrate BD

clear and antibody-binding luminescence complexes. Complexes for the signal are not known, although antibodies that can be attached to the particle remain unelected for further experiments. Mercaptopropionic acid (MPA) has become a bridge in which the thiol -SH group is bound to quantum dot linkage and the -COOH group of MPA binds directly to antibody resistance to detect and detect antibodies [12,13].

4. CONCLUSION

With ZnSe photons quantum dots: Ag (1%), ZnSe / ZnS: Mn (5%) / ZnS with size less than 20 nm, successfully synthesized in water phase, *E.coli* O 157: H7 and MRSA are quite good. Through protein A and EDC bridging, the proportions of the complexes A1, A2, A3 and B1, B2, B3 produce very good results. The ratio of complexes to antibody responses ranged from 5 µg to 30 µg. At a rate of 1 µg the reaction gives an unknown signal. Time to detect bacteria needs 30 minutes, however, bacteria can still be detected at 15 minutes, signal is not as good as 30 minutes. Direct detection of bacteria, for a high sensitivity of 101 CFU / ml, also uses bacterial detection from a specimen with a sensitivity of 102 CFU / ml. Specificity of 100% leaf reaction. The storage time is 40°C, for stable operation and does not affect the quality of the reaction. Based on the results of the study, compounds containing thiol and carboxylic acid (HS-R-COOH), especially 3-Mercaptopropionic acid, are used. MPA or HS- R-COOH is less toxic than EDC and with this design the cost is more acceptable due to the simplification of detection and analysis. Due to the close association between the COOH group and the amine of the bacterium, the photoluminescence of quantum dots after binding is altered, based on the difference in optical properties (intensity or wavelength) of the dot systems. Quantum-crosslinker- Ab-bacteria, bacteria will be detected.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sandra J, Rosenthal, Jerry C, Chang, Oleg K, James R, et al. Biocompatible quantum dots for biological applications. Chemistry & Biology. 2011;18(1):10-24.
2. Dong Z, Xiaoxing J, Cuie Z, Xiaolian S, Jianrong Z, Jun-Jie Z. Green synthesis and potential application of low-toxic Mn:

ZnSe/ZnS core/shell luminescent nanocrystals. Chem. Commun. 2010; 46(29):5226–5228
3. Vanessa W, Jonathan EH, Matthew JP, Moungi GB, Vladimir B. Alternating current driven electroluminescence from ZnSe/ ZnS:Mn/ZnS nanocrystals. Nano Lett. 2009;9(6):2367-2371.
4. William, Yu W, Emmanuel C, Rebekah D, Vicki L, Colvina. Water-soluble quantum dots for biomedical applications. Biochemical and Biophysical Research Communications. 2006;348(3):781-786.
5. Rahul T, Yingchuan C, Preston T, Snee. Efficient emission from Core/(Doped) Shell nanoparticles: Applications for chemical sensing. Nano Lett. 2007;7(11):3429-3432.
6. Wing CL, Ken TY, Indrajit R, Hong D, Rui H, Weiwei Z, et al, Aqueous phase synthesis of highly luminescent CdTe/ZnTe Core/Shell quantum dots optimized for targeted bioimaging. Small. 2009;5(11): 1302-1310
7. Bich TL, Eunsu H, Seokhwan J, Nakjoong K. Green synthesis of highly UV-orange emitting ZnSe/ZnS: Mn/ZnS core/shell/ shell nanocrystals by a three-step single flask method. RSC Advances. 2012;2(32): 12132-12135.
8. Sonal M, Jhimli S, Rajib D, Mitra MK. Biofunctionalised quantum dots for sensing and identification of waterborne bacterial pathogens. Journal of Experimental Nanoscience. 2010;5(5):438-446.
9. Luxin W, Chung SW, Xudong F, Azlin M. Detection of *Escherichia coli* O157:H7 and Salmonella in ground beef by a bead-free quantum dot-facilitated isolation method. Internationa l Journal of Food Microbiology. 2012;156(1):83–87.
10. Hu Y, Wang C, Bai B, Li M, Ronghui W, Yanbin L. Detection of *Staphylococcus aureus* using quantum dots as fluorescence labels. Int J Agric & Biol Eng. 2014;7(1):77-83.
11. Park IS, Kim DK, Nora A, Maria V, Namsoo K. Development of a direct-binding chloramphenicol sensor based on thiol or sulfide mediated self-assembled antibody monolayers. Biosensors and Bioelectronics. 2004;19(7):667–674.
12. Igor L, Medintz, Ellen R, Goldman, Michael E, Lassman, et al. A fluorescence resonance energy transfer sensor based on maltose binding protein. Bioconjugate Chem. 2003;14(5):909–918.
13. Bich Thi Luong, Eunsu Hyeong, Sujin Yoon, Jongwan Choi and Nakjoong Kim. Facile synthesis of UV-white light emission ZnSe/ZnS:Mn core/(doped) shell nanocrystals in aqueousPhase. RSC Adv. 2013;3:23395-23401.
14. Shavel A, Gaponik N, Eychmüller A. J. Phys. Chem. B. 2004;108:5905-5908.
15. Fang Z, Wu P, Zhong X, Yang YJ. Nanotechnology. 2010;21:305604(9pp).
16. Shao P, Wang HZ, Zhang QH, Li YG. J. Mater. Chem. 2011;22:17972-17977.

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