



Preparation and Evaluation of Tunable Emission Zinc Oxide Quantum Dots for Gene Delivery

Jomon N. Baby^a, K. Pramod^{a,f,*}, Syril John^b, M. R. Aji Alex^c, C. V. Suneesh^d, Sumeet Kapoor^c, M. A. Tahir^e

^aCollege of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram, Kerala, India.

^bDept of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, S. A. S Nagar, Mohali, Punjab, India.

^cCentre for Biomedical Engineering, Indian Institute of Technology (IIT), Hauz Khas, New Delhi, India.

^dDepartment of Chemistry, University of Kerala, Kariavattom Campus, Trivandrum, Kerala, India.

^eBiogenero Labs Pvt Ltd., Unit No.1, Sy. No 179, C.K Palya, Hullahalli, Sakalvara Post, Bangalore, India.

^fCollege of Pharmaceutical Sciences, Govt. Medical College, Kozhikode – 673008, Kerala, India.

*Corresponding author's E-mail: pramodkphd@yahoo.com

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ABSTRACT

Quantum dots (QDs) possess unique photochemical and photophysical properties and can be excited for multicolor emission with a single light source. Gene therapy is an emerging technique in treatment of cancers. In the present study zinc oxide QDs were prepared by precipitation technique. The quantum dots synthesized at precipitation pH 6, 8 and 10 emitted orange (590 nm, under 365 nm excitation), yellow (560 nm, under 328 nm excitation) and green (494 nm, under 325 nm excitation) fluorescence respectively. The particle size and PDI were found to decrease on decreasing the pH during preparation of QD. Zeta potential was found to become more positive on decreasing the pH during preparation of QD. From the gel retardation study it was noticed that plasmid DNA encoding TNF- α gene cannot be efficiently conjugated directly on to the quantum dot. Further studies are warranted by employing an efficient capping agent to facilitate DNA binding.

Keywords: Tunable emission, Zinc oxide, Quantum dots, Gene delivery.

INTRODUCTION

Quantum dots (QDs) possess unique photochemical and photophysical properties that are not available from either isolated molecules or bulk solids. In comparison with organic dyes and fluorescent proteins, these quantum-confined nanoparticles are brighter, more stable against photobleaching, and can be excited for multicolor emission with a single light source.¹

QDs can be utilized in real-time fluorescent imaging of cancer cells both *in vitro* and *in vivo*.² Gene therapy is an emerging technique in treatment of cancers.^{3,4} Conjugating QDs with gene can be utilized for long-term intracellular and intranuclear tracking studies.⁵ ZnO is considered as a "GRAS" (generally recognized as safe) substance by the FDA.

Gene therapy has emerged as a promising approach for curing of inherited conditions and cancer.^{6,7} Gene therapy involves the expression of an exogenous gene in order to cure a disease that is being caused by an altered gene.

The objective of the present work was to prepare and evaluate tunable emission zinc oxide quantum dots for gene delivery.

MATERIALS AND METHODS

Zinc acetate dihydrate ($\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$) and lithium hydroxide monohydrate ($\text{LiOH} \cdot \text{H}_2\text{O}$) were purchased from Sigma-Aldrich Co., (MO, USA). Ethanol (99.9%) was purchased from Jiangsu Huax Co., Ltd., Jiangsu, China.

Preparation of tunable emission zinc oxide quantum dots by precipitation method

Zinc oxide quantum dots (ZnO QDs) were prepared through a precipitation method by using ethanolic solution of lithium hydroxide monohydrate (LHM) as the precipitation agent. In general, 44 mg of zinc acetate dihydrate (ZAD) (0.2 mmol) was added in 20 mL of absolute ethanol. The mixture was dissolved completely by being stirred at room temperature for 30 min. LHM (14 mg) was dissolved in 20 mL of absolute ethanol. The ZAD solution was then added to the LHM solution. The pH value of the solution was measured to be 10. After reaction for 2 h, the solution became turbid, indicating ZnO QDs were formed. The pH values of the solutions were tuned to 8 and 6 when 10 and 5.5 mg of lithium hydroxide were added respectively. The obtained ZnO QDs that precipitated at different pH values were first washed thrice using absolute ethanol to remove unreacted precursors. The purified quantum dots were then dispersed in 20 mL absolute ethanol for storage till use.⁸

Evaluation of tunable emission zinc oxide quantum dots

Photoluminescence spectroscopy

Photoluminescence (PL) spectra were recorded in open-sized 1 cm path-length quartz cuvettes using a Perkin-Elmer LS 55 spectrofluorometer.⁸

Zeta potential



Zeta potential was determined by the electrophoretic mobility of quantum dots in U-type tube at 25 °C, using Zetasizer Nano ZS Particle Sizer (Malvern Instruments Ltd, Worcestershire, United Kingdom).

Particle size and polydispersity index

Mean particle size and polydispersity index were determined by using dynamic light scattering technique. The nanoparticles dispersed in ultrapure water were taken in a cuvette and its size and size distribution were determined using Zetasizer Nano ZS Particle Sizer (Malvern Instruments Ltd, Worcestershire, United Kingdom) at 25 °C.

Gel retardation assay

Agarose gel electrophoresis is a method for separating and visualizing DNA fragments. The fragments are separated by charge and size and forming then to move through an agarose gel matrix, which is subjected to an electric field. The electric field is generated by applying potential across an electrolytic solution (buffer). When boiled in aqueous buffer, agar dissolved and upon cooling solidified to a gel.

1.4 % agarose gel was prepared in tris-EDTA buffer (TE buffer). This was melted in hot water bath at 90°C and then melted agarose was cooled down to 45°C, and poured into gel casting apparatus with gel comb. After setting, the comb was removed from gel. The platform with gel was placed into an electrophoresis tank with sufficient electrophoresis buffer (Tris-acetate-EDTA buffer) to immerse the gel. Samples [ZnO QDs complexed with plasmid DNA encoding TNF- α gene (pDNA-TNF- α)] were prepared with an appropriate amount (3 μ L) of fluorescent dye ethidium bromide and were added to gel. The gel was allowed to run. The stained gel was then observed using Gel documentation system (E-gel imager, Invitrogen, Carlsbad, CA, USA).

RESULTS AND DISCUSSION

Preparation of tunable emission zinc oxide quantum dots by precipitation method

Fig.1 shows the photographs of the prepared zinc oxide QDs dispersed in ethanol. Upon illuminating with UV 360 nm wavelength, various visible color emissions, such as green, yellow, and orange, were observed for the ZnO quantum dots that precipitated at pH 10, 8, and 6 respectively. It appeared that, on decreasing the precipitation pH from 10 to 6 a red shift (bathochromic shift was observed).

Evaluation of tunable emission zinc oxide quantum dots

Photoluminescence spectroscopy

The zinc oxide quantum dots, which were precipitated at varying pH values, depicted different visible emissions, under their respective peak UV excitations (Fig. 2). The quantum dots synthesized at precipitation pH 6 emitted an orange fluorescence at around 590 nm under 365 nm

excitation. Likewise the emission color was tuned to yellow (560 nm, under 328 nm excitation) and green (494 nm, under 325 nm excitation) on adjusting the precipitation pH to 8 and 10 respectively. The results were in agreement with reported data.⁸

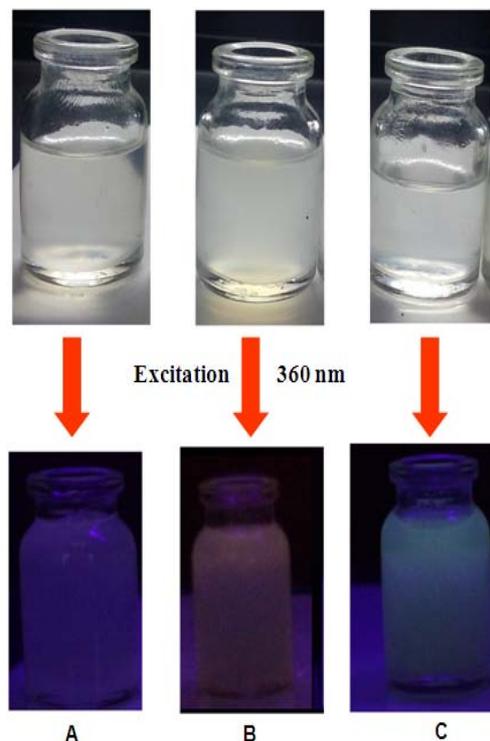


Figure 1: Photographs of ZnO quantum dots prepared at (A) pH 6-emitting orange color, (B) pH 8-emitting yellow color, (C) pH 10-emitting green color under 360 nm excitation.

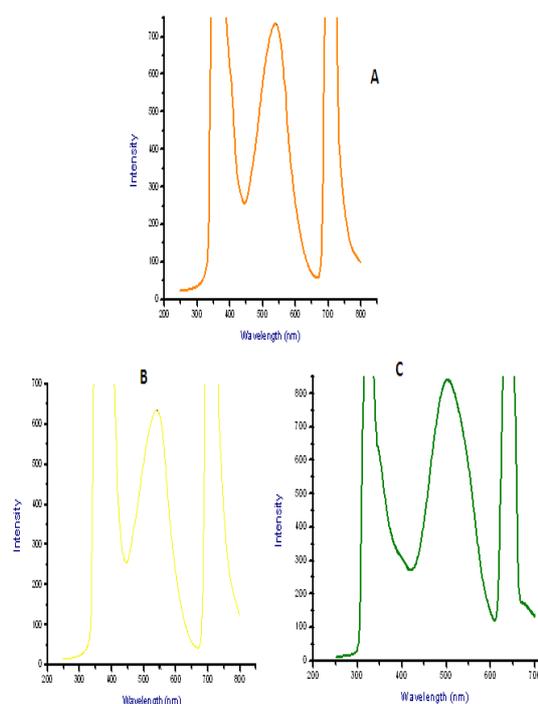


Figure 1: Photoluminescence spectra of ZnO QDs at varying pH (A) pH 6, (B) pH 8 and (C) pH 10

Zeta potential

The surface charges of the ZnO quantum dots were measured and the results are presented in Fig. 3. With a decrease in the pH value, the surface charge became more positive, i.e., -11.6 mV at pH 10, 8.56 mV at pH 8 and 11.6 mV at pH 6. Figure 4 shows zeta potential distribution plots of quantum dots precipitated at (A) pH 6 (B) pH 8 and (C) pH 10.

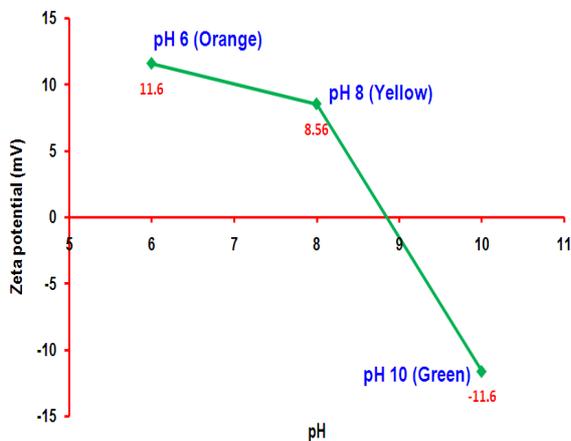


Figure 3: Plot of pH vs. zeta potential

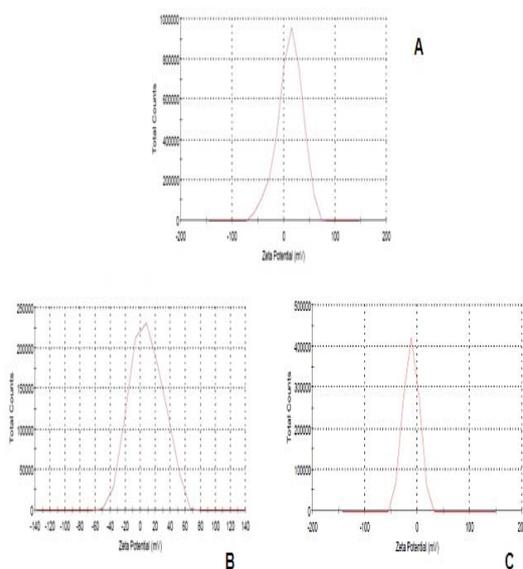


Figure 4: Zeta potential distribution plots of quantum dots precipitated at (A) pH 6 (B) pH 8 and (C) pH 10

The surface charge of oxides in solution is determined by the interaction between a surface attached OH⁻ group and OH⁻ or H⁺ groups in the surroundings, depending on the isoelectric point of the oxides. When the pH value is above the isoelectric point, the resultant oxides appear to have a negative charge because of the formation of O²⁻ on the surface, which is represented as follows:



The surface charge is positive due to the formation of Zn²⁺ below the isoelectric point, which is represented as follows:



Thus from the surface charge results obtained, it could be inferred that the isoelectric point of the zinc oxide quantum dots was nearby 9. This was in reasonable agreement with the reported value of 9.5 for zinc oxide.⁹

Particle size and polydispersity index

From the study, it was observed that, the particle size of the zinc oxide quantum dots tended to be larger, as the pH value ascends from 6 to 10 (Fig. 5). With regard to polydispersity index (PDI) any such correlation was not observed. Figure 6 shows size distribution data of QDs synthesized at (A) pH 6 (B) pH 8 and (C) pH 10.

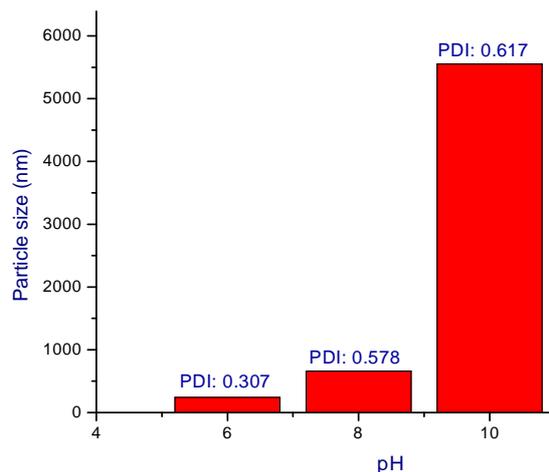


Figure 5: Particle size and polydispersity index data of QDs synthesized at varying pH

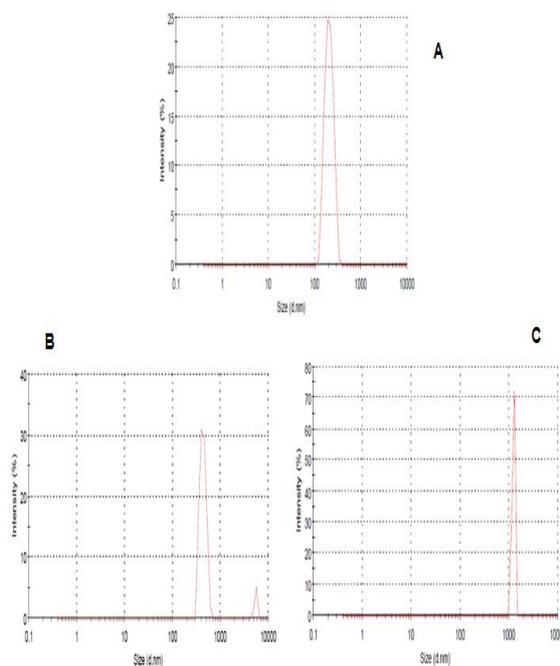


Figure 6: Size distribution data of QDs synthesized at (A) pH 6 (B) pH 8 and (C) pH 10

Gel retardation assay

The gel retardation assay was carried out to assess the possibility of binding DNA on to the surface of ZnO quantum dots. Lane 1 in the gel electrophoresis images

(Fig. 7) shows a band that is of naked plasmid DNA. Lane 2, 3, 4 and 5 shows the DNA complexed with the quantum dots of pH 6, 8 and 10 respectively. Since there was no retardation in any of these wells, it could be inferred that there is no electrostatic interaction between the DNA and quantum dots. From this study we came to a conclusion that DNA cannot be conjugated directly on to the quantum dot. Further studies are thus warranted by employing an efficient capping agent to facilitate DNA binding.

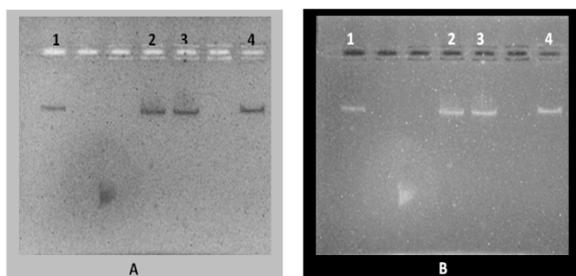


Figure 7: Gel electrophoresis images of ZnO QD with pDNA-TNF- α (A) in white background (B) in black background

CONCLUSION

In the present study zinc oxide QDs were prepared by precipitation technique. The photoluminescence spectra obtained for the QDs. The quantum dots synthesized at precipitation pH 6, 8 and 10 emitted orange, yellow and green fluorescence respectively. The particle size, polydispersity index and zeta potential of the prepared QDs were determined. The particle size and PDI were found to decrease on decreasing the pH during preparation of QD. Zeta potential was found to become more positive on decreasing the pH during preparation of QD. From the gel retardation study it was noticed that

DNA cannot be efficiently conjugated directly on to the quantum dot.

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