NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

Journal Name

RSCPublishing

ARTICLE

Evidence of oriented attachment in the growth of functionalized ZnTe nanoparticles for potential applications in bio-imaging

Rekha Dunpall,[a] Sixberth Mlowe[a] and Neerish Revaprasadu[a*]

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

This study reports a solution based, low temperature route to the synthesis of water soluble cysteine capped zinc telluride (ZnTe) nanoparticles under the influence of variations in pH and reaction time. The optical properties of the ZnTe nanoparticles display broad emission peaks ranging between 365-415nm, which make them useful for imaging and biological labelling applications. Transmission electron microscope (TEM) and high resolution TEM studies indicated that the particles are uniform in size and shape, however at the 2 hour reaction time interval the nanoparticle growth enters a transition phase where the morphology changes from nanospheres to nanorods. The formation of the ZnTe nanoparticles relative to reaction temperature, pH and time is typical of the oriented attachment growth mechanism. X-ray diffraction patterns confirmed the crystalline cubic phase. The surface charge of the functionalised ZnTe was also determined. The fluorescence properties and optical stability of the ZnTe nanoparticles in DNA plasmid pGEMT Easy were studied using fluorescence (365-395 nm filter) and phase-contrast (UV 2A 330-380 nm filter) microscopy techniques.

Introduction

The synthesis of functionalized semiconductor nanoparticles has attracted interest in the fields of nanoscience and medicine.[1-3] Scientists are constantly searching for alternative materials that may be utilized in bio-imaging and labelling applications. A key component in designing such nanomaterials incorporates water solubility, functionalization, relatively low toxicity and materials that are not susceptible to photobleaching. Semiconducting materials may be engineered at sizes that are smaller than or comparable to those of a cell, protein or gene. In addition their surface features may be altered to facilitate its interaction and entry into the biological entity of choice resulting in a variety of applications across plant biology and the biomedical sciences.[4-7] A challenge in synthesizing semiconducting nanomaterials for bio-applications is the toxicity of the component ions. ZnTe is an interesting II-VI semiconducting nanomaterial that is less toxic when compared to cadmium based chalcogenides.[7]

ZnTe has been synthesized via physical routes such as mechanical alloying[8-9] and pulsed laser ablation.[10] It is a challenge to prepare ZnTe nanostructures with controllable size and shape because of the lack of suitable precursors for controlling the thermodynamics and kinetics of the nanocrystal nucleation stage. ZnTe in the form of nanodots,[11-14] nanoflowers,[14] nanowires and nanorods[14-17] have been reported using various colloidal and high temperature routes. In many cases the growth of the anisotropic structures is attributed to the oriented attachment growth mechanism.[18-22]

An aqueous route for the synthesis of fluorescent ZnTe/dendrimer nanocomposites was reported by Ghosh *et al*., their study investigated antimicrobial properties of the ZnTe nanocomposites and revealed their potential in therapeutic applications.[23] Water-dispersible 1D Te@ZnTe core–shell nanoparticles have also been reported whereby by the ZnTe nanoparticles were used as precursors. The 1D nanoparticles have a crystalline Te core and an amorphous ZnTe shell.[24]

This study reports a solution based, significantly lower thermal route for the synthesis of functionalized ZnTe nanoparticles. The synthesis method used in this work was adapted from the route previously reported for the synthesis of cysteine capped CdSe nanoparticles.[25]

Experimental details

Materials

Zinc chloride, L-cysteine ethyl ester hydrochloride, tellurium powder, sodium borohydride, deionised water (HPLC grade) and acetone were obtained from Sigma Aldrich. All the chemicals were of analytical grade and used as purchased.

Synthesis of cysteine capped ZnTe nanoparticles

Tellurium powder (0.32 mmol) was mixed with 20 ml of deionized water in a three necked round bottom flask at room temperature. Sodium borohydride (0.81 mmol) was added to the reaction mixture under inert conditions (Equation 1). After 4 hours 20 ml of 3.2 x 10^{-4} M $ZnCl_2$ (zinc salt) and L-cysteine ethyl ester hydrochloride (capping agent) was added to the reaction mixture in molar ratios of 1:10 (Equation 2). The reaction mixture was then heated at 60 °C for 3 hours under nitrogen gas. The pH of the reaction was adjusted to 4, 7 and 11 within the first 30 minutes of adding the zinc salt. An aliquot of the reaction mixture was removed and purified for characterisation at 1, 2 and 3 hours to determine the effect of reaction time on nanoparticle growth and morphology. The ZnTe nanoparticles were precipitated using acetone. This product was centrifuged to give the pellet that was then dried under vacuum and weighed out to give a dark brown material that was readily dispersed in water.

$$4NaBH_4 + 2Te + 7H_2O \xrightarrow[2\ h]{\text{Room temperature}} 2NaHTe + Na_2B_4O_7 + 14H_2 \quad (1)$$

$$NaHTe + ZnCl_2 \xrightarrow[60\ °C,\ 3\ h]{C_5H_{11}NO_2S\cdot HCl} \text{Cysteine-capped ZnTe} + NaCl + HCl \quad (2)$$

Fluorescence of ZnTe nanoparticles in the presence of DNA plasmid pGEMT easy

An aliquot of 2 µl of DNA plasmid was treated with 50 ug/ml of cysteine capped ZnTe nanoparticles and placed on a heating block at 37˚C. The sample was placed on a slide and viewed under a fluorescence and phase-contrast microscope

Optical studies and characterisation

A Perkin-Elmer Lamda 20 UV-vis spectrophotometer was used to carry out optical measurements in the 200-1100 nm wavelength range at room temperature. Samples were placed in quartz cuvettes (1cm path length) and the absorbance was recorded. At Room temperature photoluminescence (PL) spectra was recorded on a Perkin-Elmer LS 55 luminescence spectrometer with xenon lamp over range of 200-800 nm. The samples were placed in quartz cuvettes (1cm path length) and the excitation peaks were analysed and recorded. Samples for TEM analysis) were prepared by putting an aliquot solution of ZnTe nanoparticle material onto an amorphous carbon substrate supported on a copper grid and the grid was allowed to air dry. A Philips CM120 BIOTWIW transmission electroscope sample viewed at 80K was used for TEM analysis. A JOEL JEM 3010 URP high resolution transmission electron microscope (HRTEM) operated at 300 kV was used for high resolution analysis of a small aliquot of the ZnTe samples. Images were recorded using a 1024 x 1024 CCD camera. SEM measurements were done using a Zeiss Ultra Plus FEG SEM at 10 kV and EDX at 20 kV. XRD measurements of the Au-ZnTe samples were performed using a Bruker aXS D8 advanced diffractometer with Cu-Kα radiation (λ= 1.5406 Å) operated at 40 kV and 40 mA. The Zeta potential of cysteine capped ZnTe was determined by a Zetasizer, Nanoseries, Malvern Instrument. Samples were filtered several times through a 0.22 mm Millipore membrane filter prior to recording surface charge measurements. Fluorescence images were recorded using a Nikon Eclipse 80i fluorescence and phase-contrast microscope. The aqueous sample of ZnTe nanoparticles was placed on a slide and viewed under a 365-395 nm filter set.

Results and discussion

Cysteine capped ZnTe nanoparticles were synthesized using a one pot route method under inert experimental conditions. During the reduction step a dark purple colour was observed after 4 hours. This solution turned dark brownish to black in colour after the addition of zinc chloride and L-cysteine ethyl ester hydrochloride. This method was carried out at acidic, neutral and alkaline pH. The growth of the nanoparticles was investigated at 1, 2 and 3 hour time intervals. The ZnTe nanoparticles synthesized at pH 4, 1-3 hour intervals showed interesting features in their growth patterns, size and shape and therefore was subjected to in-depth analysis in this study.

The cysteine capped ZnTe nanoparticles synthesized at pH 4 showed an absorption peak at 269 nm (Fig. 1.A). This absorption peak is attributed to the Zn-ligand complex as reported previously for cysteine capped CdTe.[13,26] The optical band gap of the ZnTe nanoparticles can be estimated from the UV-spectra by extrapolating the linear portion of the curve into X-axis. The blue shift observed from both the absorption edges and peaks can be related to the quantum confinement effect (QCE). For the ZnTe samples synthesized at acidic reaction conditions, there is evidence of quantum confinement. The absorption band edge of the ZnTe synthesized at pH 4, 2hrs is observed at 295 nm (4.2 eV), which is higher than the bulk value of 548 nm (2.26 eV) [29] as shown in Fig. 1A and C.

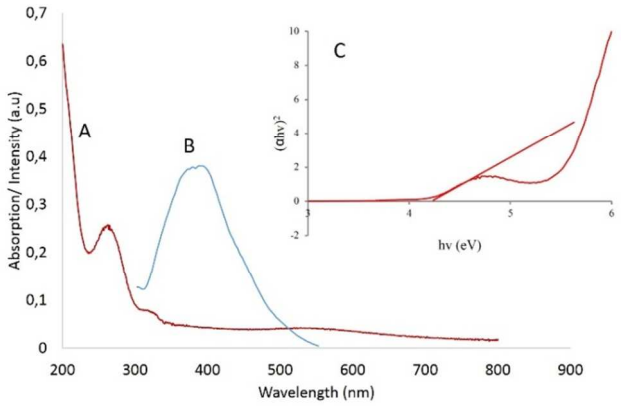


Fig. 1. Optical properties of cysteine passivated ZnTe nanoparticles synthesized at pH 4, 2 hours. Fig 1. A, shows an absorption peak at 269 nm. Fig 1. B, displays the emission spectrum in the 365-415nm range. Fig 1.C, displays the extrapolation of the band edge for the sample at pH 4, 2hours.

The corresponding photoluminescence spectrum shows narrow emission in the 365-420 nm range (Fig. 1B). The results are in accordance with previous reports which show emission in the 400-600 nm range.[27,28] The absorption properties of ZnTe

nanoparticles under varying pH conditions over different time intervals are shown in the supplementary data.

The ZnTe nanoparticles were characterized using TEM, HRTEM, XRD and SEM techniques. Fig.2A-I shows TEM micrographs of ZnTe nanoparticles at different pH and time intervals. The ZnTe nanoparticles synthesized at pH 7, 1-3 hour intervals appeared aggregated and undefined in shaped and size (Fig. 2. D-F). The ZnTe nanoparticles synthesized at pH 11, 1-3 hour intervals appeared close to spherical in shape and of irregular size (Fig. 2. G-I). The size and growth of ZnTe nanoparticles was controlled by time intervals, we restricted the growth of ZnTe nanomaterials to 3 hours because we observed relatively larger nanorods with an average size of 250 nm in length at 24 hours which was unfavourable for biological applications[2,3]. ZnTe nanoparticles from pH 4, 1 hour interval showed spherical nanoparticles that appeared closely agglomerated. ZnTe nanoparticles from pH 4, 2 hour interval showed a combination of nanospheres and nanorods. The ZnTe nanoparticles at pH 4, 1 hour time interval consisted of large spheres in the 40-50 nm size range.

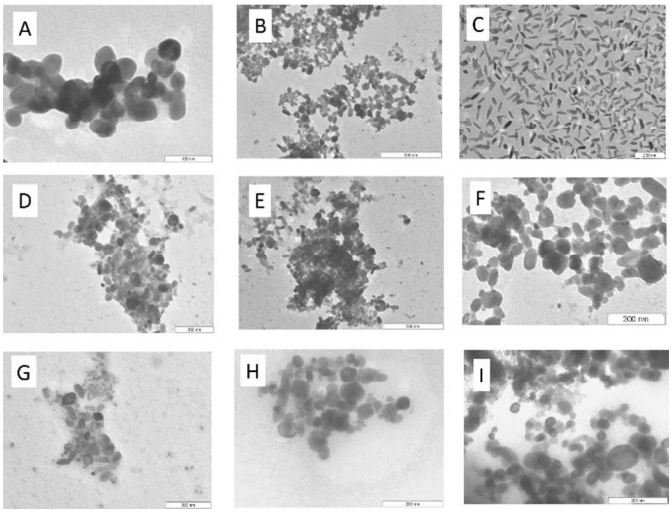


Fig. 2. A-I shows the morphological characterization of cysteine passivated ZnTe nanoparticles synthesized at varying pH and time intervals. Fig. 2. A, pH 4, 1 hour interval. Fig. 2. B, pH 4, 2 hours interval. Fig. 2. C, pH 4, 3 hours interval. Fig. 2. D, pH 7, 1 hour interval. Fig. 2. E, pH 7, 2 hours interval. Fig. 2. F, pH 7, 3 hours interval. Fig. 2. G, pH 11, 1 hour interval. Fig. 2. H, pH 11, 2 hours interval. Fig. 2. I, pH 11, 3 hours interval.

As the reaction proceeded to the 2 hour time interval the sample began to display a transition phase in which nanospheres develop into nanorods that are between 60-80 nm in length and 20-25 nm in diameter (Fig. 2. B and Fig. 3. A-C). This transition phase is similar to the morphology reported by Zhang et al, who described the growth of nanolumps or dots into nanowires.12 After 3 hours the transformation of the spheres into rods is completed as highlighted within the red ring in (Fig. 2 C and Fig. 4. A-C).

The formation of the ZnTe nanoparticles relative to reaction temperature, pH and time is typical of the oriented attachment (OA) growth mechanism which involves the controlled morphosynthesis of nanoparticles under the influence of crystal growth factors.[20] OA allows the nanocrystals to develop by the alignment and coalescence of neighbouring nanospheres by eliminating the capping agent boundaries.[21] It is common for OA to lead to the formation and growth of anisotropic nanostructures such as nanorods.[22] The growth patterns observed in the TEM images in (Fig 3.A-C and Fig. 4.A-C) clearly demonstrate oriented attached of nanospheres in the transition phase leading to the formation of well defined, uniform nanorods. Fig 5. A, shows spherical ZnTe nanoparticles (pH 4, 2 hours), that are in the process of alignment and coalescence. In Fig. 5.B-D the fusion of the particles is more evident. The clearly defined lattice fringes in varying directions are observed with the spacing of 0.36 and 0.39 nm in (Fig. 5. B, C.) is assigned to the (111) plane of cubic ZnTe, respectively. A higher surface energy of the zinc-blende can lead to the termination of the (111) faces resulting in strong dipolar interactions between the particles facilitating OA growth.[20-23]

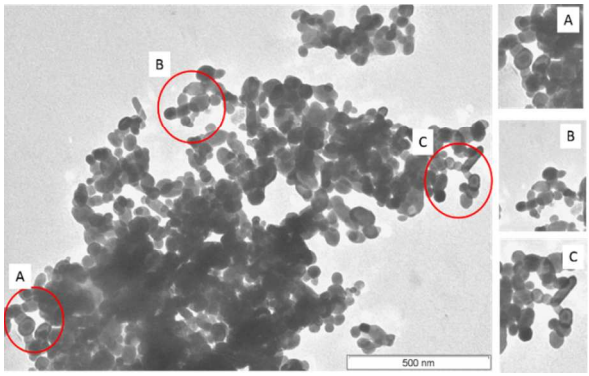


Fig. 3. A-C displays the oriented attachment observations of ZnTe nanoparticles using TEM analysis for pH 4, 2 hours interval

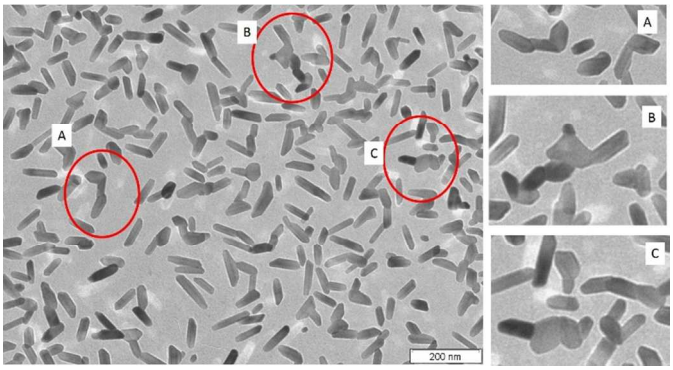


Fig. 4. A-C displays the oriented attachment observations of ZnTe nanoparticles using TEM analysis for pH 4, 3 hours interval

The SAED pattern observed for the ZnTe nanorods (Fig 5.F) corresponds to the cubic crystal phase. The use of surface passivating agents such as amines or thiols are commonly used to control and stabilize the particle growth at the order of nanometers to achieve quantum confinement effects.[29] In addition to the functionalization and stability that cysteine provides to ZnTe nanoparticles during the growth phase, it also functions as an agent for solubilisation in water, and finally for the possible conjugation with biomolecules to the free amine groups.[25] The correlation between the growth kinetics and properties of the nanomaterials is related to the

type of passivating agent employed during synthesis. In this case, functionalizing ZnTe with cysteine will allow for the attachment of various drugs or biological agents.

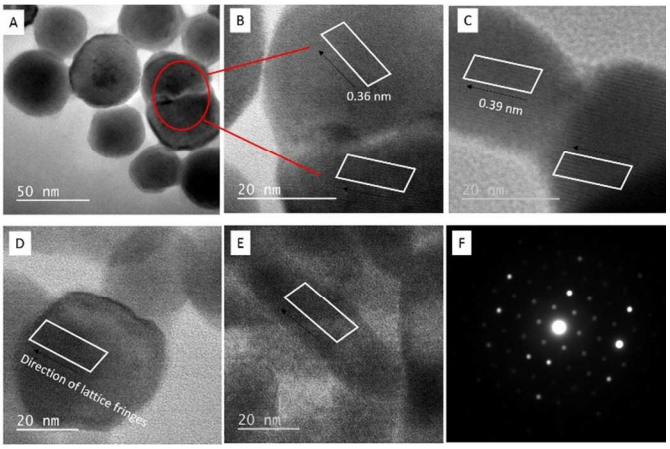


Fig. 5. Oriented attachment growth in cysteine capped ZnTe nanoparticles using HRTEM. A, B, C and D. demonstrates the oriented attachment mechanisms of growth. B and C indicates clear lattice fringes. E. Displays the orientation of lattice fringes observed in the nanorods. F. Shows the SAED pattern for the nanorods observed in E.

The SEM EDX and elemental mapping was utilized to provide more information on the surface morphology and elemental ratios of ZnTe nanoparticles. The SEM micrograph shown in Fig 6.A, confirmed the transition phase for the ZnTe nanoparticles synthesized at pH 4, 2hr, showing the presence of spherical and rod shaped particles. The EDX pattern shown in Fig 6.B, confirmed the presence of Te and Zn components. Fig 6.C, shows the elemental mapping of Zn and Te displaying their respective ratios.

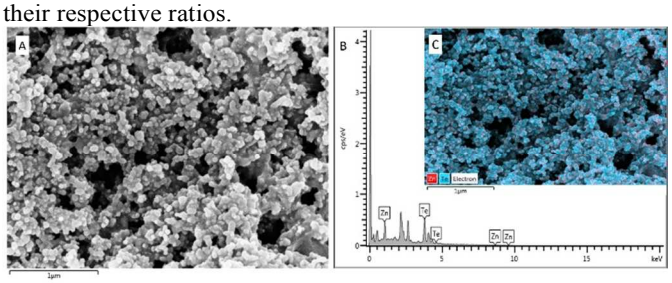


Fig. 6. Morphology characterisation of ZnTe nanoparticles (pH 4, 2 hour interval) using SEM, EDX and elemental mapping. Fig. 6. A, SEM micrograph displaying the surface morphology of ZnTe nanospheres and nanorods. Fig 6. B, EDX spectrum showing the presence of Te and Zn. Fig. 6. C, Elemental mapping of Te and Zn in the cysteine capped ZnTe nanomaterial

ZnTe nanoparticles can exist in both cubic zinc blende and wurtzite phases.11-17 The miller indices attributed to peaks (200), (220), (311) and (400) identified in Fig 7 correspond to the cubic phase of ZnTe (ICDD number: 015:0746). XRD diffraction peaks from unreacted Te, Zn, or other ZnTe phases are observed in the pattern.

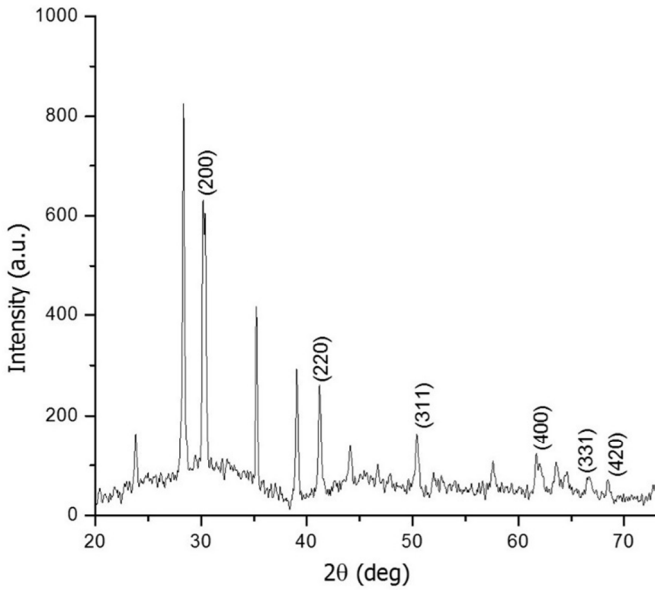


Fig. 7. XRD pattern for cysteine capped ZnTe nanoparticles

The Zeta potential is used to measure the surface charge of nanoparticles. In addition it may be used to determine how efficiently the capping agent has passivated the nanoparticle surface and confirm the size of the nanoparticles. The surface charge may be altered by the attachment of bioconjugates. Monitoring the surface charge allows control in electrostatic and hydrophobic interactions within biological systems resulting in intracellular targeting and drug carrier applications. The surface charge properties of cysteine capped ZnTe was evaluated and displayed a negative charge of -11.7 mV. Work reported by Ghost *et al.* showed that ZnTe nanoparticles capped with carboxyl, hydroxyl and succinamic acid displayed negative surface charges[23]. The negative charge displayed in cysteine capped ZnTe nanoparticles will accommodate the functionalization of this nanomaterial for further applications in cell targeting and bio-imaging.

The fluorescence properties and optical stability were studied using fluorescence and phase-contrast microscopy techniques. Fig 8A. shows the ZnTe nanoparticle emitting in the blue region under the influence of UV 2A 330-380 nm filter. The blue light corresponds to the absorption wavelength displayed in the UV spectroscopy results. Fig 8. B, shows the ZnTe nanoparticles fluorescing between the 365-395 nm filter. These findings primarily confirm the fluorescence properties of the nanospheres and nanorods present in the cysteine capped ZnTe nanomaterial. In addition it also provides a rationale for establishing more robust studies that can clearly demonstrate the use of ZnTe nanomaterials in bio-imaging applications across cell lines and whole animal studies. To date the literature has successfully demonstrated the use of various other quantum dots in bio-imaging of bacterial, plant and human cells.[4-6]

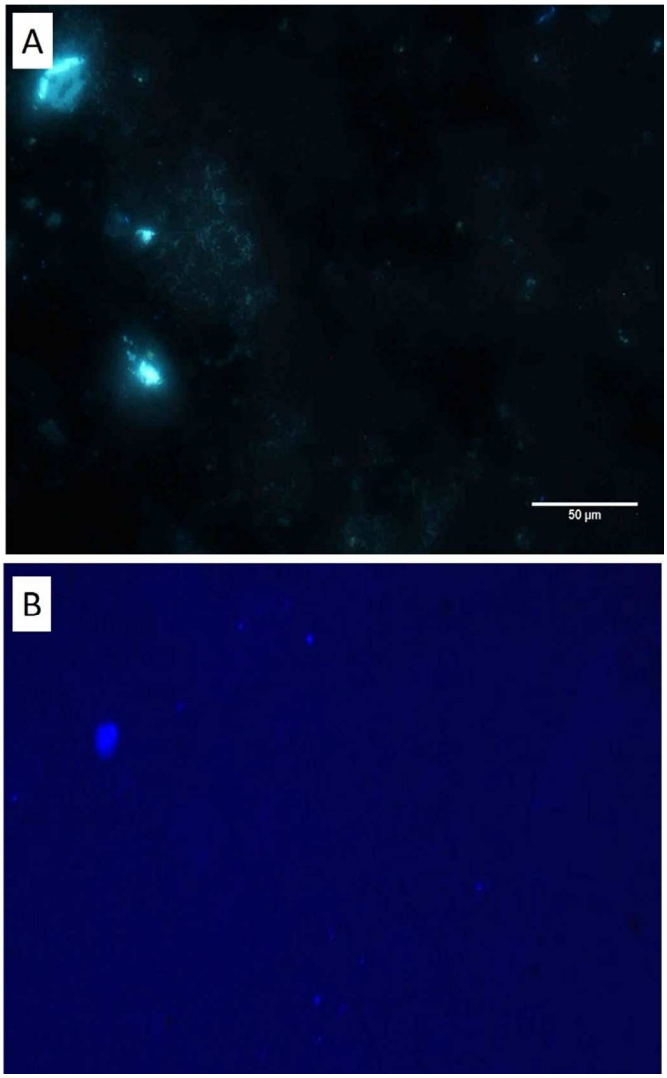


Fig. 8. Fluorescence of cysteine capped ZnTe nanoparticles. Fig. 8. A, UV 2A filter in the presence of DNA plasmid pGEMT Easy. Fig. 8. B, 365-395 filter set in the presence of DNA plasmid pGEMT Easy.

Conclusions

Cysteine capped ZnTe nanoparticles were synthesized at different pH using a simple solution based route. The ZnTe nanoparticles obtained at pH 4 showed interesting morphological features whereby a transition phase was identified in which nanospheres of 40-50 nm in size range developed into nanorods that were between 60-80 nm in length and 20-25 nm in diameter. This transformation was attributed to the oriented growth attachment mechanism. The particles synthesized at these optimum reaction conditions also showed quantum confinement and narrow band-edge emission. The cysteine capped ZnTe nanoparticles displayed a negative surface charge suggesting that the particles would be conducive to functionalization for further applications in cell targeting and bio-imaging. Initial fluorescence studies of the ZnTe nanoparticles within DNA plasmid shows emission in the blue region. Future research will involve the application of ZnTe nanoparticles using *in vitro* cell culture models to investigate their potential in bio-applications.

Acknowledgements

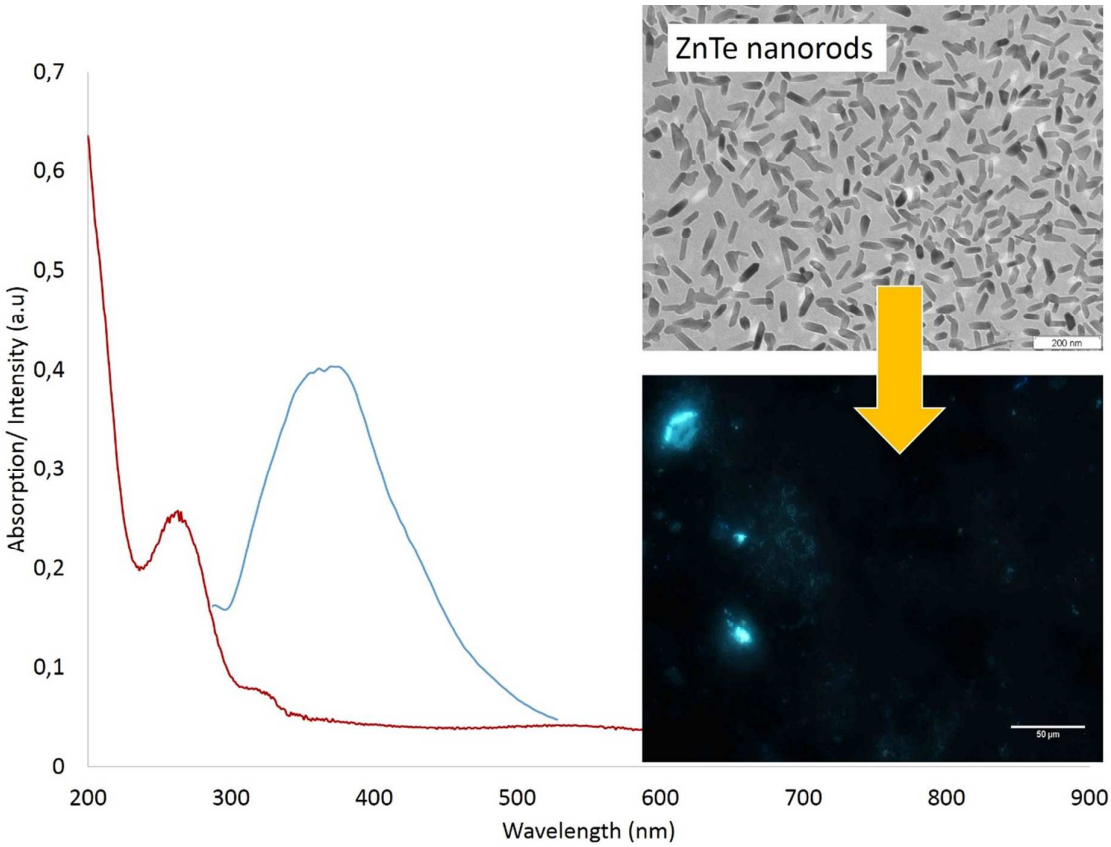
This work was supported by the Department of Science and Technology (DST) and National Research Foundation (NRF) of South Africa through the DST/NRF South African Research Chairs Initiative (SARCHi) program. The authors wish to acknowledge the Electron Microscopy unit of University of KwaZulu-Natal for training and analysis.

Notes and references

[a] Department of Chemistry, University of Zululand, Private Bag X1001, Kwa-Dlangezwa, 3886, South Africa.
E-mail: RevaprasaduN@unizulu.ac.za

1. L. Qi and X. Gao, Expert Opin. *Drug Deliv.* 2008, **5(3):** 263..
2. Y. Li, Y. Zhao, H. Cheng, Y. Hu, G. Shi, L. Dai and L. Qu. *J. Am.Chem. Soc.* 2012, **134,** 15.
3. D. Bera, L. Qian, T. Tseng and P. H. Holloway. *Materials.* 2010, **3,** 2260.
4. A. Valizadeh, H. Mikaeili, M. Samiei, S. M. Farkhani, N. Zarghami, M. kouhi, A. Akbarzadeh and S. Davaran. *Nanoscale Res Lett.* 2012, **7,** 480.
5. L. Shao, Y. Gao and F. Yan. *Sensors.* 2011, **11,** 11736.
6. L. A. Bentolila, X. Michalet, F. F. Pinaud, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir and S. Weiss. *Discovery medicine*. 2005, **5(26):** 213.
7. A. M. Smith, S. Dave, S. Nie, L. True and X. Gao. *Expert. Rev. Mol. Diagn.* 2006. 6(**2**), 231.
8. S. Patra, S.K. Pradhan. *Acta Materialia*. 2012, **60**,131.
9. S. Patra, S.K. Pradha. *J. Alloys Compd*. 2011, **509**, 5567.
10. Y. Lui, X. Zhang, R. Lui. R. Yang, C. Lui, Q. Cui. *J. Solid State Chem*. 2011, **184**, 684.
11. J. Zhang, K. Sun, A. Kumbhar, J. Fang. *J. Phys. Chem. C*. 2008, **112**, 5454.
12. B. Zhang, F. Guo, W. Wang. *J. of Nanomaterials*. 2012, doi:10.1155/2012/293041
13. N. Mntungwa, V. S. R. Pullabhotla, N. Revaprasadu. *Mater. Lett*. 2012, **81**, 108.
14. S. H. Lee, Y. J. Kim, J. Park. *Chem. Mater*. 2007, **19**, 4670.
15. J. Zhang, S. Jin, H. C. Fry, S. Peng, E. Shevchenko, G. P. Wiederrecht, T. Rajh. *J. Am. Chem. Soc*. 2011, **133**, 15324.
16. D. Kim, J. Kim, E. J. Kim, K. Koo. Korean *J. Chem. Eng.* 2011, 28(**4**), 1120
17. K.Yong, Y. Sahoo, H. Zeng, M. T. Swihart, J. R. Minter, P. N. Prasad. *Chem. Mater*. 2007, **19**, 4108.
18. F. Jiang, Y. Li, M. Ye, L. Fan, Y. Ding, Y. Li. *Chem. Mater*. 2010, **22**, 4632.
19. Q. Zhang, S. Lui, S. Yu. J. *Mater. Chem*. 2009, **19**, 191.
20. E.J.H, Lee, C. Ribero, E. Longo. E.R. Leite *J. Phys. Chem. B* 2005. **109**, 20842
21. J. Zhang, F. Huang, Z. Lin. *Nanoscale*, 2010, **2**, 18.
22. M. Gong, A. Kirkeminde, S. Ren. *Scientific Reports*. 2013. **3**:2092, DOI: 10.1038/srep02092
23. S. Ghosh, D. Ghosh, P. K. Bag, S. C. Bhattacharya, A. Saha. *Nanoscale*, 2011, **3**, 1139.

24. S. Xu, C. Wang, R. Wang, Z. Wang, Q. Xu, Y. Cui. *J. Mater. Chem*. 2011, **21**, 16427.
25. R. Dunpall, A. A. Nejo, V. S. R. Pullabhotla, A. R. Opoku, N. Revaprasadu, A. Shonhai. *IUBMB Life*, 2012, 64(**12**): 995.
26. N. Mntungwa, V. S. R. Pullabhotla, N. Revaprasadu. *Colloids Surf, B* 2013, **101**, 450.
27. Y. Jun, C.Choi, J. Cheon. *Chem. Commun*. 2001, 101.
28. D. K. Dwivedi, D. Dubeya, M. Dubeya. *J.Ovonic Res*. 2009, 5(**2**), 35.
29. D. P. Thompson and P. J. Boudjouk. *Org. Chem*. 1988, 53 (**9**), 2109.
30. S. Mlowe, A.A.Nejo, V.S.R. Pullabhotla, E. B.Mubofu, F.N. Ngassapa, P.O'Brien, N. Revaprasadu. *Mater. Sci. Semicond. Process*. 2013, **16**, 263



Cysteine capped ZnTe nanoparticles have been synthesized by a simple solution based route. The growth mechanism of the particles under certain reaction conditions suggests an oriented attachment mechanism. The particles show potential for bio imaging applications.

Table of Contents