



# ***An in vitro* assessment of the potential toxicity of Cadmium Selenide nanoparticles**

A thesis presented by

**Rekha Dunpall**

Submitted in fulfilment for the award of the degree of

**Master's (MSc) in Biochemistry**

Department of Biochemistry and Microbiology, Faculty of Science and Agriculture,

**University of Zululand**

**Supervisor: Dr A. Shonhai**

Department of Biochemistry and Microbiology,

University of Zululand

**Co-Supervisors: Prof A. R. Opoku**

Department of Biochemistry and Microbiology,

University of Zululand

**Prof N. Revaprasadu**

Department of Chemistry, University of Zululand

## **Abstract**

Nanotechnology is steadily finding its application in all aspects of the consumer industry, science and engineering. At a relative pace Cadmium Selenide (CdSe) nanoparticles are gaining increased attention for their potential use in biomedical applications such as bio-imaging of tissues, disease diagnosis and biological labelling due to its unique optical and electronic properties. Exposure of these particles to humans and other biological systems raise huge concerns with regards to their safety. In this study, water soluble cysteine capped CdSe nanocrystals, were prepared through a one pot green route method. The prepared CdSe nanocrystals were characterized using Transmission electron microscopy (TEM), High resolution transmission electron microscopy (HRTEM), Ultra violet spectroscopy (UV) and Photoluminescence (PL) analysis to establish the size, shape, dispersion, aggregation state, crystalline nature and optical properties of CdSe nanoparticles. The *in vitro* effects of CdSe nanoparticles on DNA stability, red blood cells (RBC's) and blood platelets were evaluated. DNA was exposed to CdSe nanoparticles and its assessment on DNA stability was confirmed by agarose gel electrophoresis and spectrophotometry. Damage to DNA structure was observed at 200  $\mu\text{g/ml}$  of CdSe. *In vitro* assays carried out on RBC damage included reducing power and chelating activity of iron. The results showed that the CdSe nanocrystals exhibited high reducing power and sufficient chelating activity, which would be able to impair the function of haemoglobin. CdSe nanoparticles promoted platelet aggregation in a dose dependent manner. Based on the findings of this study the biosafety of CdSe nanoparticles is not guaranteed and further studies need to be conducted to ascertain the safety of CdSe nanoparticles for possible use in biological systems.

## **Dedication**

This study is dedicated to my late Gurudev, Sri Saathiya Sai Baba. I am blessed everyday just knowing that you live forever in my heart. Thank you for always guiding me.

**Declaration**

I declare that this dissertation is my own, unaided work. It has been submitted for the degree of Master's in Science at the University of Zululand. It has not been submitted before for any degree or examination at any other University. I also state that all the sources that I have used have been duly acknowledged.

This \_\_\_\_\_ day of \_\_\_\_\_ 2012.

## **Acknowledgement**

I wish to thank my Supervisor Dr A. Shonhai, and co-supervisors Prof A. R. Opoku and Prof N. Revaprasadu for all the support, motivation, guidance and encouragement throughout the project. From all of you, I have gained many skills that will add endless value to my life and career. Thank you to Dr Shonhai for always pushing me to realize my potential and never giving up on me. Thank you to Prof N Revaprasadu (Chair of Nanotechnology, South Africa) and DST/NRF for the award of a scholarship that funded this project. It was a great honor for me to have been supervised by you all, I have learnt that hard work, determination and passion for research are the key to a promising academic career.

Thank you to my mentor, Dr A. Nejo, for all the support, motivation, guidance, grooming and constant encouragement throughout the project. From you, I have gained many skills that not only concern this project, but many aspects of scientific research. You have taught me a great deal on how to keep my head up and feet on the ground. Thank you all sincerely.

Thank you to all the staff at University of Zululand, all of whom have been a part of my daily life over the years.

I wish to thank you God and my parents, for always believing in me and standing by my side throughout the years. Without you I would never have made it this far your efforts to provide me with amazing opportunities in life have not gone unnoticed and is highly appreciated.

## **Table of contents**

Abstract	ii
Dedication	iii
Declaration	iv
Acknowledgements	v
Table of Contents	vi
Table of figures	ix
List of figures	x
List of Abbreviations	xi
List of Research outputs	xii

## **Chapter 1 –Introduction and literature review**

1.1 Introduction	1
1.2 The use of nanoparticles in biomedical applications	2
1.3 Nanotoxicology	4
1.4 Potential toxicity of CdSe nanoparticles	7
1.5 Chemical synthesis of CdSe nanoparticles	8
1.5.1 Colloidal route to CdSe nanoparticles	9
1.5.2 Organometallic route to CdSe nanoparticles	10
1.5.3 Single-molecular precursor route to CdSe nanoparticles	10
1.5.4 Synthesis of CdSe nanoparticles using a one-pot solution based route	11
1.6 Interaction of nanoparticles with biological material	11
1.6.1 Interaction of nanoparticles with DNA molecules	11
1.6.2 Interaction of nanoparticles with Red blood cells	12
1.6.3 Interaction of nanoparticles with blood platelets	14
1.7 Problem statement	16
1.8 Hypothesis	17
1.9 Specific objectives and methodological scope of this study	18

## **Chapter 2- Methodology**

2.1 Equipment	19
2.2 Reagents	19
2.3 Laboratory animals	19
2.4 Methodology	19

2.4.1 Synthesis of cysteine capped CdSe nanoparticles	19
2.4.2 Characterization of CdSe nanoparticles using UV-vis absorption spectroscopy	20
2.4.3 Characterization of CdSe nanoparticles using photoluminescence spectroscopy	20
2.4.4 Characterization of CdSe nanoparticles using TEM	21
2.4.5 Characterization of CdSe nanoparticles using HRTEM	21
2.4.6 Purification of pGEMT Easy plasmid DNA	21
2.4.7 Quantification of pGEMT Easy plasmid DNA	23
2.4.8 Confirmation of the integrity of plasmid DNA using restriction analysis	23
2.4.9 Agarose gel electrophoresis and spectrophotometric analysis of DNA exposed to nanoparticles	23
2.4.10 Assessment of the reducing power of CdSe nanoparticles on iron	23
2.4.11 Assessment of the chelation activity of CdSe nanoparticles on iron	24
2.4.12 Assessment of the interaction of CdSe nanoparticles with blood platelets	24
<b>Chapter 3- Results</b>	
3.1 The synthesis of cysteine capped Cadmium selenide nanoparticles	26
3.2 Characterization of CdSe nanoparticles	26
3.3 CdSe nanoparticles cause DNA damage	31
3.4 Possible interaction between CdSe nanoparticles and the iron in RBC's	34
3.5 CdSe nanoparticles promote platelet aggregation	37
<b>Chapter 4- Discussion</b>	
4.1 Discussion	39
<b>Chapter 5- Conclusion</b>	
5.1 Concluding remarks and suggestions for future studies	44
<b>Appendix A1- Special chemical reagents</b>	46
<b>A2- Preparation of reagents</b>	47
<b>Appendix B- Supplementary data</b>	50
<b>References</b>	53

## List of figures

<b>Figure 1.1:</b> Dose metrics involved in toxicological characterization of nano materials	5
<b>Figure 1.2:</b> Nanoparticles exposure routes	15
<b>Figure 3.1:</b> CdSe nanoparticles generated using various molar ratios of cadmium salt and coating agent.	26
<b>Figure 3.2:</b> TEM analysis of CdSe nanoparticles	29
<b>Figure 3.3:</b> HRTEM characterizations of CdSe nanoparticles	30
<b>Figure 3.4:</b> Restriction digest analysis of DNA plasmid pGEMT Easy	31
<b>Figure 3.5:</b> The <i>in vitro</i> effect of CdSe nanoparticles on plasmid DNA pGEMT Easy	32
<b>Figure 3.6:</b> Spectrophotometric analysis of exposed DNA	34
<b>Figure 3.7:</b> Reducing power activity of CdSe nanoparticles on iron	35
<b>Figure 3.8:</b> Chelation activity of CdSe nanoparticles on iron	36
<b>Figure 3.9:</b> Illustration of CdSe nanoparticles of various concentrations, interacting with blood platelets	37
<b>Figure 3.10:</b> CdSe nanoparticle effect on platelet function	38
<b>Figure B1:</b> UV Characterizations for each batch ratio of CdSe nanoparticles	49
<b>Figure B2:</b> PL characterizations for each batch ratio of CdSe nanoparticles	50



## **List of Tables**

<b>Table 3.1:</b> Optical characterization of CdSe nanoparticles	28
<b>Table B3:</b> Absorbance reading from the spectrophotometric analysis	51
<b>Table B4:</b> The effect of CdSe nanoparticles on blood platelets	51

## List of Abbreviations and symbols

<b>CdSe</b>	Cadmium selenide
<b>RBC's</b>	Red blood cells
<b>NaBH<sub>4</sub></b>	Sodium borohydride
<b>TEM</b>	Transmission electron microscope
<b>HRTEM</b>	High resolution transmission electron microscope
<b>UV</b>	Ultra- violet
<b>PL</b>	Photoluminescence
<b>DNA</b>	Deoxyribonucleic acid
<b>EDTA</b>	Ethylenediaminetetra-acetic acid
<b>TiO<sub>2</sub></b>	Titanium dioxide
<b>TAE</b>	Tris-acetic EDTA
<b>TCA</b>	Trichloro acetic acid
<b>ADA</b>	Acid dextrose anti-coagulant

## List of symbols

<b>°C</b>	Degrees Celsius
<b>µg</b>	microgram
<b>ng</b>	nano gram
<b>bp</b>	base pairs
<b>nm</b>	nanometers
<b>ml</b>	microliters
<b>A<sub>260</sub></b>	Absorbance at 260 nm
<b>A<sub>415</sub></b>	Absorbance at 415 nm
<b>A<sub>700</sub></b>	Absorbance at 700 nm

## List of research outputs

**Dunpall, R.,** Opoku, A. R., Nejo A. A., Revaprasadu, N. and Shonhai A.

An *in vitro* assessment of the potential toxicity of Cadmium selenide quantum dots. “Rejuvenating Science in Zimbabwe.” Harare, Zimbabwe, 26 October 2011. (Oral presentation)

**Dunpall, R.,** Opoku A. R., Nejo A. A., Revaprasadu, N. and Shonhai A.

The *in vitro* assessment of the interaction of CdSe nanoparticles on DNA, iron haemoglobin and blood platelets. (Manuscript currently in preparation)

**Dunpall, R.,** Opoku A. R., Nejo A. A., Revaprasadu, N. and Shonhai A.

The synthesis, characterization and *in vitro* assessment of the potential toxicity of cadmium selenide nanoparticles. NanoAfrica 2012. 4th International conference and nanotechnology, Bloemfontein 1-4 April 2012. (Oral presentation)

# **An *in vitro* assessment of the potential toxicity of Cadmium Selenide nanoparticles**

A thesis presented by

**Rekha Dulpall**

Submitted in fulfilment for the award of the degree of

**Master's (MSc) in Biochemistry**

Department of Biochemistry and Microbiology, Faculty of Science and Agriculture,

University of Zululand

**Supervisor: Dr A. Shonhai**

**Co-Supervisors: Prof A. R. Opoku**

**Prof N. Revaprasadu**

February 2012