Development of Water Soluble, White Light Generating Highly Biocompatible Lanthanide Oxide Nanoparticles for Applications in Health Care.

Thesis submitted in partial fulfillment

For the Award of Degree

Doctor of Philosophy

BY

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2023

Dedication

I am dedicating this Ph.D. thesis to my family and the people of my country, India, who paid for the cost of this study.

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It is certified that the work contained in the thesis titled "**Development of Water Soluble, White Light Generating Highly Biocompatible Lanthanide Oxide Nanoparticles for Applications in Health Care**" by **Vivek Kumar Verma** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

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I, Vivek Kumar Verma, certify that the work embodied in this Ph.D. thesis is my own bonafide work and was carried out by me under the supervision of **Dr. Manoj Kumar** for a period from **July 2018 to September 2023** at the School of Biomedical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credit to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not wilfully copied any other's work, paragraph, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, thesis, etc., or available on websites and have not included in this thesis and have not cited as my own work.

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It is certified that the above statement made by the student is correct to the best of my knowledge.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Nomenclature
MRI	Magnetic resonance imaging
CT	Computed tomography
PET	Positron emission tomography
SPECT	Single-photon emission computed tomography
US	Ultrasound
UV	Ultraviolet
NMR	Nuclear magnetic resonance
SPIONs	Superparamagnetic iron oxide nanoparticles
SPR	Surface plasmon resonance
Gd_2O_3	Gadolinium oxide
NPs	Nanoparticles
NCs	Nanoclusters
IVIS	In vivo imaging system
MALDI-TOF	Matrix-assisted laser desorption/ionization
CTCF	Corrected total cell fluorescence
QDs	Quantum dots
FRET	Förster resonance energy transfer
BSA	Bovine albumin serum
WLE	White light emission
CIE	Commission Internationale de I'Eclairage
KAS	Kissinger-Akahira-Sunose
FWO	Flynn-Wall-Ozawa
Vyazovkin AIC	Vyazovkin Advanced isoconversional
TGA	Thermogravimetric analysis
β	Heating rate
α	Conversion point
J0	Kinetic barrier of nucleation
J	Nucleation rate
T_P	Peak temperature
K_B	Boltzmann constant
$E\alpha$	Activation energy of nucleation
$A\alpha$	Pre-exponential kinetic factor
ΔH	Change in enthalpy
ΔG	Change in Gibbs free energy
ΔS	Change in entropy
$f(\alpha)$	Differential function
$g(\alpha)$	Integral function
Exp ($\Delta G/RT$)	Thermodynamic barrier
M0	Initial weight of Gd_2O_3 before TGA
Mf	Final weight of Gd_2O_3 after TGA
R	Universal gas constant
$Z(\alpha)$	Product of differential and integral function
h	Plank constant

FWHM	Full-width half maxima
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
FTIR	Fourier-transform infrared spectroscopy
HRTEM	High-resolution transmission electron microscopy

PREFACE AND THESIS ORGANIZATION

Recently, biomedical imaging technologies have been widely used for early disease identification and diagnosis. Several imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), photoacoustic imaging (PAI), positron emission tomography (PET), single-photon-emission computed tomography (SPECT), and optical imaging (OI), play important roles in the observation of biological system structures and functions and provide important information about the pathogenesis, progression, and treatment of diseases such as cancer. Because of their relative drawbacks, monomodal imaging techniques typically cannot achieve the requirements of high sensitivity and spatial resolution. As a result, combining two or more imaging modalities, known as dual or multi-modal imaging, is a common technique to circumvent these constraints. Nowadays, merging diverse components into a single platform is the most frequent technique for utilizing their unique functions. The complicated components impede this strategy and synthetic procedure, causing inevitable interference, poor reproducibility, and uncertain pharmacokinetics, and hence less accessible for clinical use. Alternatively, one component with multiple contrasting capacities remains more desirable due to the lower interference, simpler fabrication, defined structures, and far better reproducibility than the composite agents. In this thesis work, we exclusively developed a novel platform for the synthesis of nanoparticles for multimodal imaging (MRI and fluorescent imaging) by a one-pot facile synthetic route. Surface functionalization with commercially available polymer bovine serum albumin and chitosan enables precise control over size and makes nanomaterial biocompatible. Developed nanoprobes were characterized by several microscopic and spectroscopic techniques to confirm the synthesis of ultrasmall Gd_2O_3 nanoclusters. Furthermore, post-characterization studies such as *in-vitro* and *in-vivo* biocompatibility,

hemocompatibility, real-time imaging in mice, *in-vitro* imaging in HaCaT cells (Human keratinocytes), and U-87 MG (Human glioblastoma cells), and in-vitro relaxivity measurement suggested it as a potential contrast agent and confirmed its candidature toward multimodal imaging.

The current thesis work is divided into six chapters.

Chapter 1 of the thesis covered a detailed description of multimodal imaging techniques and their advantages and disadvantages. It also provides insight into their multidimensional applications in disease prognosis. This chapter provides the details of contrast agents and discusses various synthetic routes to synthesize Gd_2O_3 nanomaterials for imaging purposes. This chapter also provides a detailed discussion of capping agents used in the synthesis of Gd_2O_3 nanomaterials.

Chapter 2 consists of an extensive literature survey on the synthetic approach and multifunctional aspects of Gd_2O_3-based nanomaterials. This chapter also gives insight into the effect of texture, shape, and size effect of Gd_2O_3 nanomaterials on its properties and prospective application. The literature survey also addresses the possible applications of the Gd_2O_3 nanomaterials in the biomedical field. In addition, this study also addresses the importance and associated challenges with determining the nucleation rate and interfacial energy at elevated temperatures, as well as the corresponding conversions.

Chapter 3 explains a facile one-pot novel technique to synthesize water-soluble multifluorescent Gd_2O_3 nanoclusters by utilizing BSA protein and ascorbic acid as capping and reducing agents, respectively. The prepared clusters exhibited tunable fluorescence, high quantum yield, excellent photostability, wide-range pH, and ionic strength tolerability. These multifunctional properties of Gd_2O_3 nanoclusters are availed in labelling HaCaT cell lines with negligible toxicity.

Chapter 4 of the thesis reports a novel strategy to compute various parameters such as activation energy, preexponential factor, nucleation rate, interfacial energy, and thermodynamic parameters of Gd_2O_3 nanoclusters using non-isoconversional thermogravimetric models at high temperatures. The Vyazovkin AIC method was utilized to estimate activation energy, which was found to be 143.98 kJ/mol. In this work, we proposed four different models for calculating nucleation rate and interfacial energy.

Chapter 5 provides the one-pot facile synthesis of Gd_2O_3 nanoclusters using a chitosan polymer template. The prepared Gd_2O_3 clusters show a wide range of emissions range without mixing any dopants. The prepared clusters exhibit negligible cytotoxicity, excellent water dispersibility, prolonged photostability, and high absolute quantum yield. The prepared clusters exhibit high longitudinal relaxation along with luminous properties and hence could be applied in both MR imaging and fluorescence imaging. Besides the in vitro study, this chapter includes real-time rodent imaging using an IVIS (*in-vivo* imaging system), hemocompatibility assay, and *in-vivo* toxicological study.

Chapter 6 of the thesis summarizes the whole study in a nutshell with the future prospects of prepared nanoclusters in various fields and highlights the major upshot of every chapter for achieving the objective of the whole study in a collaborative manner. The composition reported in this study primarily focused on developing novel nanoplatforms for multimodal imaging applications by functionalizing them with different capping agents. The current work is envisioned to contribute significantly in the areas of science, engineering, and nanomedicine with targeted drug delivery.