Design and development of novel biocompatible nanomaterials for multifunctional applications.

Thesis submitted in partial fulfillment

for the Award of Degree

Doctor of Philosophy

by

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Dedicated

to

My Beloved Grandmother,

Dr. Savitri Sabbarwal,

and

My Father,

Satish Kumar Sabbarwal

CERTIFICATE

It is certified that the work contained in the thesis titled **'Design and development of novel biocompatible nanomaterials for multifunctional applications'** by **Shivesh Sabbarwal** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

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List of Abbreviations and Symbols

Abbreviation	Nomenclature
CTI	Computed tomography imaging
MRI	Magnetic resonance Imaging
SPECT	Single-Photon Emission Computed Tomography
BI	Bioluminescence imaging
FI	Fluorescence imaging
PTT	Photothermal therapy
NIR	Near-infrared light
FCPN	Fluorescent calcium carbonate prenucleation clusters
Qd’s	Quantum dots
BSA	Bovine Serum Albumin
MTT Assay	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide
CTCF	Corrected total cell fluorescence (CTCF) analysis
TGA	Thermogravimetric analysis
CCPN	Calcium carbonate prenucleation clusters
m_0	Initial weight of CCPN before TGA
m_f	Final weight of CCPN after TGA
α	Conversion
J_0	Kinetic barrier of nucleation
A_α	Pre-exponential kinetic factor
E_α	Activation energy of nucleation
R	Gas constant
ΔG	Change in Gibbs free energy
ΔH	Change in enthalpy
ΔS	Change in entropy
$exp(\Delta G/RT)$	Thermodynamic barrier
J	Nucleation Rate
$f(\alpha)$	Differential function
$g(\alpha)$	Integral function
β	Heating Rate
vyazovkin AIC	Vyazovkin Advanced isoconversional method
T_p	Peak temperature in DTG curve
K_B	Boltzmann constant
h	Plank constant
σ	Interfacial energy of nanomaterial
ρ_{nm} ,M, v_0, N_A	Density, molar mass, specific volume, Avogadro Number
$z(\alpha)$	Product of differential and integral function
FFT	Fast fourier transform
IFFT	Inverse Fast fourier transform

Preface and Thesis Organization

Nanomaterials are gaining compelling interest among various scientific communities owing to their vast applications in the area of energy, health and environment. Over the decades, ample amount of work has been done on nanomaterials due to their unique opto-electronic properties when compared to the bulk materials. Among various nanomaterials investigated, nobel metals such as Au, Ag and Cu are most popular fluorescent material owing to their several unique properties like fluorescence, near infrared absorption, catalytic capability, and enzymatic settings. In recent years, the research is turning out to create more biocompatible materials owing to existence of long-term toxicity during *in-vivo* study on nobel metals. Herein, an attempt to synthesize fluorescent biocompatible nanomaterials based on alkaline earth metal has been taken up, to harness their multicolor emission. To synthesize such type of ultra-small clusters, a low cost protein Bovine Serum Albumin (BSA) is utilized and leaf extract is used as a reducing agent. Such type of synthesis uses BSA protein and leaf extract can be abbreviated as bio-mineralization based synthesis, and these clusters in the current study are basically used as a bio imaging agent. The synthesized fluorescent nano clusters ($CaCO_3$) demonstrate an average size of around 1.3 nm, exhibiting fluorescence lifetimes that range from 1.05 ns to 30.60 ns. These clusters contain varying numbers of atoms, ranging from 16 to 936 molecules. Additionally, their penetration depths, ranging from 4 μm to 6 μm, are attributed to their diverse sizes, highlighting their adaptability for a wide range of applications. Additionally, the research highlights the biocompatibility of these clusters, as indicated by an observed increase in cell viability over a 7-day incubation period. On the contrary, understanding nucleation kinetics of ultra-small clusters at high temperatures provides critical insights into the thermodynamic behavior of the system, including the activation energy required for nucleation. This knowledge is instrumental in designing efficient synthesis routes and predicting the stability of resulting clusters across varying conditions. Moreover, this understanding allows researchers to tailor the synthesis process to optimize cluster properties for specific applications, enhancing their performance and functionality in catalysis, optics, and biomedical fields. Hence, the next research was focused towards the calculation of nucleation rate of ultra-small clusters in high temperature range (100 °C- 600 °C). But there was no engineered technology to calculate the same mathematically. To address this issue, I employed thermogravimetric (TGA) analyses to assess the nucleation rate of ultra-small clusters across a high-temperature range (100 °C- 600 °C) and varying conversion levels (0.1-0.9). Through this method, numerical parameters such as the activation energy (128.041 kJ mol^{-1}) with a systematic error of $\leq$0.005, and the pre-exponential kinetic factor (1020–1030 min^{-1}) were determined. Additionally, nucleation rates corresponding to different temperature increments were elucidated: at 10 °C min^{-1}: 22,337, 15 °C min^{-1}: 33,636 min^{-1}, and 20 °C min^{-1}: 88,140. The interfacial energy with temperature was also analyzed, revealing values of 66.77 mJm^{-2} at 10 °C min^{-1}, 67.39 mJm^{-2} at 15 °C min^{-1}, and 68.05 mJm^{-2} at 20 °C min^{-1}. The temperature required for crystallization (500 °C) was highlighted, providing crucial insights into the synthesis process and the thermodynamic behavior of the clusters. Moreover, the development of a bioimaging agent for diagnosing diseases such as cancer is a crucial step in early detection and treatment planning. The clusters being within the 10 nm barrier of kidney filtration limit and the constituent elements present in the cluster are among one of the elements already present in the body, its metabolism will only lead to formation of harmless by products such as CO_2 and calcium, which underscores their biocompatibility and safety for medical use. However, for the treatment of cancerous tissues, a different approach is required. Thus, tin (Sn) nanoparticles have been selected for their biocompatibility, small size (less than 10 nm), and high photothermal efficiency (42.4%). Hence these nanoparticles hold promise for targeted cancer therapy, delivering localized heat to cancer cells while minimizing damage to healthy tissues. This dual approach, employing bioimaging agents for diagnosis and therapeutic nanoparticles for treatment, highlights the multifaceted nature of nanotechnology in healthcare, offering innovative solutions for combating diseases like cancer. But synthesis of alpha tin nanoparticles at room temperature were understood challenging owing to their instability under ambient conditions. Many scientific groups synthesized alpha tin nanoparticles with the help of large sized substrates (>70 nm) like CdTe, InSb and silicon with matching lattice constant and used high temperature for the synthesis. To address this issue, we successfully synthesized alpha tin nanocrystals in solvent water at ambient temperature without utilizing any bulky substrates by tuning their direct band gaps. Hence, the current synthesis includes the synthesis of substrate free tin nanocrystals and their biomedical applications. With a photothermal efficiency of 42.4%, it efficiently converts light into heat for therapeutic use. Stable at room temperature, it ensures practicality in various settings. Its strong absorption in the NIR region (800-1100 nm) indicates its suitability for targeted therapies. *In-vivo* studies on Wister rats report no adverse effects on vital organs, affirming its safety. The absence of poisonous substances in synthesis underscores its biocompatibility. With a promising potential for cancer treatment, it demonstrates efficacy under low-power NIR light ($0.83 W/cm^2$). Cellular response prediction is facilitated by FFT-weighted images on a scale from 0 to 100, allowing precise evaluation. With ultra-small, free-standing tin nanocrystals arranged in a diamond-cubic structure, measuring just 4.9 nm, this material exhibits highly desirable physical properties. Synthesized in aqueous phase using deionized water, it offers versatility and compatibility with biological systems. Hence, this thesis is mainly consisting of six chapters followed by references and publications list. A brief introduction about separate chapters is demonstrated below;

Chapter 1 includes the detailed investigation about the requirement of biocompatible nanoclusters, and a brief description about pros and cons of various cancer diagnostic and treatment tools. The various new methods for metallic cluster synthesis such as chemical, hydrothermal, biomineralization, microwave, sonochemical, photochemical, and chemical etching routes are discussed in current chapter. The reason of selection of biomineralization synthesis method was also discussed in the current chapter. In spite of above, reason of selection of optical fluorescence imaging and photothermal therapy is also discussed in detail. Ultimately a brief paragraph on problem statement is defined.

Chapter 2 includes development of metallic fluorescent clusters and their properties, followed by the development of many synthesis techniques for fabricating nano systems based on Ca and Mg. The luminescent properties of various CaS phosphors were investigated and their flaws are also provided in the literature survey. More over development of fluorescent magnesium clusters were also mentioned in the literature review. On the other hand, hurdles to the calculation of nucleation rate and interfacial energy at high temperature and respective conversions are also addressed. Tin nanoparticles development are also discussed in the literature survey. Problem statement and objective of the thesis are also discussed.

Chapter 3 provides the synthesis of fluorescent $CaCO_3$ prenucleation clusters with high cellular uptake, low toxicity, eminent photostability, astonishing biocompatibility, excellent water dispersibility, and low cost of synthesis. Besides, such $CaCO_3$ material can easily get decomposed inside the body producing CO_2 and water as decomposition products. Besides high reactivity of magnesium and calcium, the synthesis of fluorescent BSA protected magnesium clusters (Mg-S) opens a new avenue for synthesis of BSA capped $CaCO_3$ prenucleation nanoclusters (FCPN). To perform such synthesis, M.oleifera leaf extract is used as reducing agent. To restrict the size of the $CaCO_3$, non-toxic, low cost and readily available, BSA protein was used as a capping and stabilizing agent.

Chapter 4 discusses computation of J_o (kinetic energy barrier) at high temperature and respective conversion for BSA capped $CaCO_3$ prenucleation clusters (CCPN) (1-2nm). A_α (pre-exponential kinetic factor) for $CaCO_3$ prenucleation clusters was determined through differential function $f(\alpha)$ linked to random nucleation process. This is the first time that TGA technology was used to calculate the nucleation rates and interfacial energy of such ultra-small nanoclusters at high temperatures and respective conversions followed by start of random nucleation within the matrix of CCPN through distortion of kinetic and thermodynamic boundary. The randomness of third-order nucleation was demonstrated using $z(\alpha)$ master plots. Moreover, thermodynamic parameters such as ΔG, ΔH, and ΔS were computed to estimate thermodynamic barrier required for nucleation to occur. Experimentally the existence of nucleation in $CaCO_3$ pre-nucleation clusters to Calcite/CaS crystal was performed at the high temperature (500 °C).

Chapter 5 discusses noteworthy global challenge that addresses the synthesis of alpha tin nanocrystals in their substrate-free form along with their beta counterparts, at ambient temperature. The reduction chemistry is utilized to create ultra-small free-standing tin nanocrystals, which are then employed at the interface of engineering and biology, utilizing sophisticated mathematical modeling through FFT, *in-vivo* toxicological assessments in Wistar rats, and *in-vitro* photothermal analyses, which have never been conducted before. The rapid disclosure of the substrate-free tin nanocrystals fabrication process is expected to have extensive implications not only in the realm of biological applications, but also in various other domains, such as superconductivity, the development of layered films similar to graphene materials, lithium-ion batteries and so on. Thereby, this innovation for synthesizing substrate-free alpha tin nanocrystals in solvent water at ambient temperature marks a significant achievement. These nanocrystals diminutive size, has allowed them to be used for the first time in biological applications. This straightforward, one-pot synthesis technique offers a promising avenue for unlocking their tremendous potential in the impending future.

Chapter 6 discusses the conclusions and future scope for all the developed materials for various biomedical applications and their nucleation rate and interfacial energy analysis at high temperature and respective conversion at high temperature by adopting thermogravimetric (TGA) analysis.