Design and development of novel biocompatible nanomaterials for multifunctional applications.

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by

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6. Conclusions and Future Scope

6.1 Conclusions

This thesis presents a simple and facile technique for fabricating ultra-small multicolor fluorescent BSA capped $CaCO_3$ prenucleation clusters. Various sophisticated characterizations like TEM, HR-XRD, XPS, Fluorometer, Fluorescence microscope (Confocal Microscopy) Fluorescence lifetime spectrometer etc. were used to show their successful synthesis. The synthesized fluorescent $CaCO_3$ prenucleation clusters emerged as a successful tool for bio-imaging due to existence of excellent biocompatibility, eminent photostability, pH stability and their physiological stability. Such class of nanomaterial is envisioned to have multifarious application in the area of health sector.

Next, these ultra-small $CaCO_3$ clusters were utilized to compute their nucleation rate and interfacial energy pattern at high temperature and respective conversions. For this purpose thermogravimetric (TGA) analysis was first utilized to study the nucleation of $CaCO_3$ ionic pre-nucleation clusters at high temperature determine their nucleation rate (J) and pre-exponential kinetic factor (A_α) and thermodynamic parameters ($\Delta H, \Delta G \; and \; \Delta S$), with related conversions. An iterative technique calculated activation energy to be as 128.041kJ/mol with little or no systematic error in the narow range of 0.005.The differential function $f(\alpha) = (1-\alpha)^3$ of random nucleation mechanism was detected by $z(\alpha)$ master plots and A_α was computed by utilizing this differential function by keeping integral limits in the range from $T_{\alpha-\Delta\alpha} \; to \; T_\alpha$. The value of frequency factoe $A_\alpha(nuclei\; \mu m^{-2} min^{-1})$ lied in the range from $10^{20} \; to \; 10^{30} \; min^{-1}$, at all conversion values from 0.1 to 0.85. Mathematically, nucleation rates and interfacial energy models were proposed and nucleation rate varied exponentially with temperature and conversion values. The mean value of nucleation rate was 22337 (10 ^{0}C/min), 33636 (15 ^{0}C/min),

and 88140 min^{-1} (20 ^{0}C/min). While, Interfacial energy varied linearly with temperature. Interfacial energy was found to increase with conversion with polynomial of order 2. The mean value of interfacial energy was 66.77, 67.39, and 68.05 mJ/m^2 at 10, 15 and 20 ^{0}C/min, respectively. The existence of nucleation in $CaCO_3$ pre-nucleation at 500 °C were validated experimentally by XRD and z(α) master plots. For experimental validation, these clusters were allowed to stay in nitrogen atmosphere inside an oven and heated subsequently at 500 °C at heating rate of 10 ^{0}C/min. Post heating of such ultra-small clusters produces crystals of CaS and $CaCO_3$, as evinced by XRD and z(α) master plots. Curent research opens a new avenue in nanoscience for determination of nucleation rates and interfacial energy of ultra-small clusters at high temperature and respective conversions.

Next, for oncological treatments new class of material is developed which is activated by NIR light to produce heat, abbreviated as alpha tin nanoparticles co-existing with beta tin. The present study reports the successful synthesis of alpha tin nanoparticles with beta tin at room temperature by using reduction approach. This reduction approach produces tiny tin nanoparticles at room temperature which are basically utilized for the first time at the interface of biology and engineering by performing mathematical FFT modeling, *in-vivo* biocompatibility assessments by using Wistar RATS, and *in-vitro* malignant cells treatment through NIR absorption. This substrate free tin nanoparticles are anticipated not only use in biological domains, but also in other areas such as superconductivity, one dimensional layer similar properties to graphene materials, lithium and sodium ion batteries and so on. These nanocrystals tiny size allows them for their first time utilization for biological applications. This one pot facile synthesis were anticipated to unlock their ample utilization in coming future.

Novelty as well as major modification involved in our synthesis was concluded as below:

Chapter 3:

Novelty: Ultra-small fluorescent clusters of $CaCO_3$ (~1.3 nm) were synthesized using BSA as a capping agent and ascorbic acid as reducing agent for the first time in contrast to large sized non-fluorescent $CaCO_3$ nanoparticles in calcite form (>10 nm).[509]

Modification: After adding BSA protein with Moringa oleifera as a slow reductant and maintaining a basic pH (10.7) with NaOH addition, which facilitated environment CO_2 absorption and drove the reaction slow, ensuring the formation of $CaCO_3$ nanoclusters, which differs from conventional approaches, which directly utilized Na_2CO_3 precursor, which produces fast crystallization, resulting in the creation of large size $CaCO_3$ nanoparticles in the form of calcite.[510]

Chapter 4:

Novelty: The significant contribution of this research lies in its quantitative analysis of nucleation rate and interfacial energy at various high temperature levels (>100°C) for extremely small nanoclusters (1-2 nm). This detailed investigation marks the study of nucleation phenomena for nanoclusters of such diminutive size for the first time.

Modification: The utilization of TGA for the first time for predicting and understanding nucleation rates and interfacial energy for various nano systems by computing activation energy (E_α) and pre-exponential kinetic factor A_α accurately at high temperature in contrast to experimental studies that typically use two different sophisticated techniques like in situ grazing incidence small-angle X-ray scattering (GISAXS) and ex situ AFM for determination of E_α and A_α separately, for accurate estimation of J at room temperature only[511, 512].

Chapter 5:

Novelty: Synthesis of ambient temperature stabilized α-Sn nanocrystals with their beta forms for the first time, which are free-standing, and their direct band gap increases from 0 to 2.38 eV due to the size of synthesized nanocrystals being less than that of Bohr exciton radius (12.5 nm), which imparts them stability at ambient temperature. These nanocrystals are used for the first time for *in-vivo* Toxicology analysis with photothermal therapy evicted by FFT Modeling.

Modification:

- ✓ Elimination of Hazardous Materials: This synthesis process never utilizes toxic substances like lithium[485] and lead-telluride[486], making it safer and more environmentally friendly.

- ✓ Removal of Bulky Substrates: Traditional approaches often rely on use of bulky Si/InSb substrates[489], but our approach is substrate free, which typically reduces synthesis complexity as well as high cost.

- ✓ Lower Synthesis Temperatures: Traditional approaches typically involve use of elevated temperatures (1400 °C) to stabilize α-Sn above 13.2 °C.[483] Hence, our room temperature synthesis approach is cheap, reduces energy consumption, and simplifies synthesis.

  We have shown in the thesis in Table 2 (Chapter 3), Table 1', and Table 1" how our synthesized materials are better than other examples in literature. The main innovation was to use environmental CO_2 which actually rely on automatic CO_2 absorption by using NaOH, which slowed down the carbonate synthesis and hence formation of ultra-small $CaCO_3$ clusters became possible. The calcium used in our clusters has a diminutive size that facilitates its efficient elimination from the body. In the event that a portion of the calcium is not expelled, it will be naturally

absorbed by the bones, while carbonate will be expelled via the lungs in the form of carbon dioxide (CO_2), hence serving as the primary factor contributing to biocompatibility. The logic and creativity used in the sample preparation of $CaCO_3$ clusters resulted in improved performance than other examples in literature.

- ✓ Tin nanocrystals were synthesized using $SnCl_2$ as a precursor in deionized water, along with using a capping agent first and therafter using a reductant. Despite attempts to prevent oxidation with nitrogen, black material reappeared. In a revised approach, after nitrogen infusion into the deionized water, $SnCl_2$ precursor was gradually introduced, followed by the dropwise addition of a reductant. This led to a shift in the solution's color from black to gray, with an average size of 4.9 nm. Analysis confirmed the presence of α-Sn and beta forms. The nanocrystals exhibited stability at room temperature, with a band gap increase to 2.38 eV due to quantum confinement, surpassing the formation of α-Sn nanocrystals. Therefore, the introduction of nitrogen and the sequential dropwise addition of the reductant into the precursor solution in deionized water enabled the synthesis of α-Sn nanocrystals. This logic and creativity used in the sample preparation of tin crystals resulted in improved performance than other examples in literature.

6.2 Future Scope

Based on the idea generated during the synthesis of ultra-small BSA capped $CaCO_3$ pre-nucleation clusters and tin nanoparticles, as well as their potential uses in bioimaging and cancer treatments, the research offered here lays a strong groundwork for numerous interesting directions for future research. These results provide a foundation upon which to build the following research priorities:

- **Refinement of Synthesis Techniques:** Our novel methodology for synthesizing ultra-small $CaCO_3$ pre-nucleation clusters could be refined to optimize quantum yield while maintaining environmentally friendly protocols without toxic solvents or substrates. This may entail trying different biomolecules for capping or changing reaction conditions to optimize cluster size and characteristics
- **Multifaceted Applications of $CaCO_3$ Pre-nucleation Clusters:** $CaCO_3$ pre-nucleation clusters' distinctive properties may enable research in fields outside bioimaging. These include drug delivery systems that use clusters' controlled release, sensitive metal ion detection platforms that use their binding, and catalytic applications that use their surface reactivity.
- **Mechanistic Insights through Thermogravimetric Analysis (TGA):** TGA might be used to study the synthesis methods of additional ultra-small clusters at other temperatures and conversions, building on our analysis of $CaCO_3$ pre-nucleation cluster nucleation rates and interfacial energies. Such mechanistic insight is necessary for rationally designing materials with customized crystallinity and characteristics.
- **Diversification of Tin Nanoparticle Applications:** Although our analysis mainly focused on the biological uses of tin nanoparticles, future research might investigate their usefulness in many fields. This may include research in the field of superconductivity, the development of innovative materials such one-dimensional layered diamond structured thin films similar to graphene, the exploration of energy storage systems such as lithium and sodium ion batteries, and the investigation of catalytic applications that leverage their distinctive surface features.

- By embarking on these avenues of inquiry, informed by the foundational knowledge established in our present research, we can further expand the frontiers of materials science and biomedical engineering, unlocking innovative solutions with far-reaching implications.
- It appears that herein none of the synthesis processes are scalable because they are confined within laboratory scale only. For instance, in a batch process spanning 15 hours, only 73 ml of $CaCO_3$ prenucleation clusters are produced. Similarly, within a 2-hour period, only 68 ml of tin nanoparticles are generated. These figures highlight the low amount of materials formed which typically creates, current constraints on scalability.