Chapter-6

6 Conclusions and Future Scope

6.1 Conclusions

This thesis presents a new approach to synthesizing nanomaterials from biomolecules such as proteins and amino acids using facile synthesis techniques for biomedical applications. In favorable conditions, proteins undergo self-assembly to form nanostructures such as cages, fibers, and aggregates. Herein, we have used globular protein (BSA and lysozyme) to synthesize protein nanodots and hydrogels. The tunable temperature-dependent phases of BSA were synthesized using the hydrothermal method. Near melting temperature, protein starts to unfold, and a further rise in temperature leads to the sol-gel-sol transition. This is the first-kind-of report where hydrogels were synthesized using the hydrothermal method and without the use of any chemical crosslinker. Physical and optical characterization of the phases helps to understand the optical and mechanical properties of the phases for biomedical application. Hydrogel synthesized at 110^0C forms the most biocompatible hydrogel with excellent UVB absorbance properties, making it a good candidate for skin protection. To confirm the applicability of hydrogel as a UVB blocker or sunscreen material, *in vivo* and *in vitro* studies have been carried out. Results have verified that topical administration of H-110 is potentially effective in protecting skin from UVB-induced skin damage.

The sol phase synthesized at 200^0C showed the characteristic properties of carbon quantum dots and was named as protein nanodots. The synthesized protein nanodots showed advanced properties compared to other quantum dots synthesized from semiconducting or plant-based carbanous sources. The average diameter of PNDs ranges from 2-4 nm with homogenous particle size distribution. Photoluminescent studies confirm the high quantum

yield and longer decay lifetime of PNDs. The abundance of different amino acids in protein structure results in excellent surface functionality and possibilities for surface modification via covalent/non-covalent interactions. The synthesized PNDs were used to fabricate a bio-interface on a glassy carbon electrode for selective determination of melatonin in pharmaceutical and food samples. PNDs were conjugated with electrochemical deposited layers of gold nanoparticles and poly-l-lysine to increase the sensitivity and selectivity of the glassy carbon electrode. Fabricated electrode showed efficacy for the quantitative analysis of Mel in a linear range of 0.1 - 200μM with a limit of detection of 31.6nM, which outperformed the previously reported sensors. Appreciable stability and reproducibility of the sensor was also evaluated over a period of 28 days. Thus, the reported fabrication protocol and detailed investigation of melatonin on the developed bio-interface pave a path for tailoring protein nanomaterials, which though exhibit great potential to act as a recognition element but fail to find a way toward electrochemical applications due to compromised charge transfer.

To get more insight into the physicochemical properties of PNDs, globular protein lysozyme was used to synthesize nanodots at two different pH conditions. The optical and physical characterization of the pH-dependent PNDs showed similar properties. PNDs synthesized at physiological pH showed excellent biocompatibility with enhanced fluorescence and surface activity. These properties motivate us to use these materials as an image-guided drug carrier for melatonin. The conjugated system (MPNDs) shows advanced medical benefits compared to melatonin alone. MPNDs delivered higher cellular uptake with slow drug release in the breast cancer cell. The bioimaging and cell proliferation studies confirm the applicability of PNDs in image-guided drug delivery systems with increased efficacy of melatonin in breast cancer treatment.

We believe that the research work included in this thesis opens a window for exploring proteins as potential materials for functional nanomaterials synthesis with advanced functionality and applicability in biomedicine.

6.2 Future scope

Scientific reports published in the last decade show tremendous improvement in protein-based functional nanomaterial for biomedical applications. Although significant progress has been made in constructing, designing, and applying protein-based nanomaterial in the biomedical field, there is still scope for scientific understating to develop controlled nanostructures with high-sensitivity sensing/imaging and high-efficiency drug delivery. A lack of studies explains the deeper interaction or mechanism of protein self-assembly or force-driven nanostructures, which can readily help design and construct suitable structures for desired applications. Development in computational studies with advanced mathematical methods can help understand the formation mechanism of these nanostructures and lead to the synthesis of nanomaterial with more controlled parameters to get homogenous particle size shape and charge distribution with desirable surface chemistry. Protein-based nanostructures are becoming popular for bio-imaging and drug delivery due to the synergistic effects with other nanomaterials and biomolecules. However, like traditional nanocarrier systems, some protein-based nanostructures have substantial interactions with endothelial cells and destroy tight and adherent junctions, resulting in endothelial cell leakage. Therefore, it is necessary to conduct more studies to ensure the biological safety of PNNS after intravenous injection, especially in animal models and clinical trials.

To rapidly develop in biomedical research in the coming future, these areas require collaboration between interdisciplinary fields, including physics, chemistry, material

science, biomedical science, and electronics, to overcome the current hurdles. Through these efforts, the field of protein-based nanostructures will be continuously developed. It can provide more efficient and advanced probes for bio-imaging, biosensing, drug delivery, tissue engineering, and disease diagnosis.