Chapter 8:

Conclusions

The idea behind this work was to use the brilliant properties of carbon quantum dots (CQDs) for unconventional applications and to harness their untapped potential in recent research. CQDs are endowed with an innate fluorescence that combined with their biocompatibility paves a way for biomedical and nano-biotechnological applications. These CQDs have flexible synthesis protocol methods and carbon sources as well making their use more feasible. The composition of living matter perfectly matches with these nano-sized structures ameliorating the concern for any nano-toxicity arising from them. Most reports have used them as sensing tools and for bioimaging of various tissues. The purpose of this report was to further extend this work.

Through the first chapter, the sensing mechanism and potential of CQDs to detect food adulterants has been explored. The Tulsi-derived ~3 nm CQDs were brightly fluorescent (QY=11%) on excitation with 320 nm wavelength and were found to be rich in oxygen and nitrogen containing functional groups. The CQDs were found to be rich in antioxidant activity and were non-cytotoxic to bacterial and fungal cells alike. Malachite green (MG), a commonly found adulterant in green vegetables, was sensed by CQDs with a sensitivity of 18 nM LOD. The ringed structure is exists in most common dyes that are used to colour food consumables. This gives rise to the possibility of π-π interactions, hydrogen bonds, hydrophobic and even electrostatic interactions between them. These dynamic collisions and temporary interactions significantly affect the fluorescence emissions of CQDs. The sensitivity of such a change is big enough to be recorded for the easy detection of a food adulterant.

Through computational assessment it was found that the functional groups on the surface of the CQDs greatly enhance the interaction between the two. The detection of malachite green was possible in real water samples and in green leaves too. A small cubic prototype sensor was built for such detection. The difference in the fluorescence was highly obvious through optical examination by comparing the test cell with the control. This calorimetric sensor can be refined for market use and can fill the void for such a product to detect adulterants in daily consumables.

For the next part, the reducing nature of these CQDs was explored for the easy one-step synthesis of gold nanoparticles. CQDs acted as both the reducing agent as well as the capping agent for this reaction. The effect of different synthesis parameters during hydrothermal treatment of *Arjuna terminalia* was checked on the morphology of the gold nanoparticles. These parameters included temperature (160°C and 200°C) and solvent (water and ethanol). The blue-emitting CQDs thus produced were used in different concentrations (0.05, 0.1, 0.2 v/v) to spontaneously produce gold nanoparticles. Several observations were made on seeing the trend of gold nanoparticle formation. The CQD synthesized in ethanol at 160°C was most effective in forming well-dispersed gold nanoparticles that stayed stable for the longest duration as compared to other conditions. The ones synthesized using CQDs in water at 200°C were the least stable and less in concentration. The TEM results clearly showed the formation of solid quasihedral shapes and planar triangles and hexagons via water CQDs. On the other hand, lower temperature and ethanol as solvent for CQDs seemed to favour smaller spherical distributed gold nanoparticles that often came together to form nanoflowers of around 100 nm diameter. Collagen fibrils seemed to stabilise the unstable gold nanoparticles. This data can help in future biomedical applications. Moreover, β-mercaptoethanol through dative bonds self-assembled with the gold nanoparticles due to a very high affinity. This spontaneous and rapid reaction led to the 'snowing' of the gold nanoparticles to the base of the cuvette. This can definitely be used in future for solid state applications for assembling the gold nanoparticles out of solution. This study would surely shed light on the green CQD-assissted one-step synthesis of gold nanoparticles by making the possibility of tailoring gold nanoparticles into specific morphologies and size.

The possibility of using CQDs as a bioimaging probe to differentiate between healthy and damaged tissues was assessed. Blue-emitting CQDs synthesized from sandalwood with an exceptionally high lifetime of 8.6 ns were used as an imaging tool to discern the physiological effects of malachite green on exposure to plant and animal tissues. They were observed to easily penetrate into the nucleus where it causes mutagenic damage to DNA. Mung plants (*Vigna radiata*) incubated in various concentrations of the commonly accessible dye showed growth retardation, stunted roots, shoots and leaves. The point of great interest was that though the transverse section of the roots of control and dye-treated plants did not show a significant difference, their fluorescence images showed a remarkable difference. This technique can be used in future to detect the presence of an adulterant in various plants. The infiltration of the dye caused nuclear blebbing and cytological damage to MG-63 cells. The organs of golden hamster after administration of the dye for a week were examined. The stress parameters through intravenous examination showed that the LPO, SOD and ROS levels were imbalanced. Organ sections showed acute damage to the liver, spleen, adrenal gland and testes. CQDs used through intravenous route as well as applied topically, clearly distinguished the damages and morphological changes, more clearly than the usual HE staining. As such CQDs can be used for such histological inspections, organ damage, diagnosis and general bioimaging as a detection probe alternative to general HE staining.

Graphene oxide (GO) is a 2-D form of CQDs having a very similar composition but slight differences in bonds, surface area and morphology. For this reason, the interaction between proteins and GO with respect to electrostatic interactions was explored. Sequentially amino acids, peptides and proteins were interacted with GO suspension at different pH. It was seen that only with the positively charged amino acids, did GO form visible conjugates which were pH-reversible in nature. Similar observation was made with peptides where the overall positive charge on the peptides enabled a strong conjugate formation. In the case of proteins, initially no conjugate formation was seen unless the proteins were heated above their melting temperature. Through CD results it was clear that the slight unfolding of the proteins did not significantly alter its innate secondary structure. The slight changes may be because the new temporary electrostatic bond formation shifts the relative bond positions. These conjugates were found to be proteolysis-resistant through gel electrophoresis studies using pepsin. Such designs in future might pave a way for oral administration of proteins and other drug delivery applications.

Another dimension that was explored was the effect of modulating parameters (different species cultivars, pH, solvent and temperature) during the hydrothermal treatment to generate a plethora of CQDs synthesized using marigold (*Tagetes erecta*). The trend of fluorescence yield was observed and several points were noted. The lighter coloured petals yielded better fluorescence. Addition of hydroxyl ions greatly enhanced the fluorescence. Water, ethanol and PEG based samples yielded constant blue emitting CQDs. On the other hand, DMSO, isopropanol and acetone also steered towards green CQDs. Hydrothermal temperature of 200°C yielded the best emission in terms of quantum yield. Four brightest CQDs were chosen for further characterisation and employment as detectors for common food adulteration dyes. Malachite green was selected as the test dye and was strongly found to interact with DNA G-quadruplex secondary structure. The interaction caused the rigidification of the the movement of the rings causing a brilliant red fluorescence to arise. This mechanism can definitely be exploited for detection and understanding the conformation of biomolecules. A multi-detection device was constructed using the different fluorescence capabilities of all four CQDs that made it ultra-sensitive for a specific dye. This approach can be further be extended to construct simple sensors and devices for daily use.

The understanding developed through this study is just a start for developing multi-functional technologies that are competitive with the currently available technologies and may even supercede them. CQDs are beyond fascinating materials that have a potential to revolutionise the present areas of biomedical engineering, drug delivery, electronics, energy harvesting and even more areas that haven't utilised them yet. In future, taking cue from this study, many more characteristics, surface passivation technique, energy transfer and applications using CQDs will be carried on. Better use of recyclable waste for contributing towards waste management may be considered for making CQDs using it. The evaluation of CQD uptake by mammalian model systems will further be evaluated. New techniques and designs to fight metabolic and chronic diseases may be applied. The long-term positive impact of CQDs on general health will also be evaluated. A range of fresh ideas using these wonderful tools would be investigated in future.