Effect of Carboxyl group surface functionalized-Carbon quantum dots on HEWL amyloidogenesis

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Protein misfolding occur due to the failure in protein quality control mechanism present within the cells. These misfolded proteins undergo self-assemble and turn into fibrillar structures known as amyloid fibrils. Amyloid forming proteins possess high ordered beta sheet structures in back bone and hydrogen bonds help out in making stable interaction with other similar structures. Amyloid formation is considered as main pathological event in the onset of neurodegenerative diseases which include Alzheimer's disease, Parkinson's disease, Huntington disease, Type-II diabetes, and others. Amyloid fibrils are formed via intermediate states and process followed by proteins is reported to be nucleated condensation polymerization mechanism and associated with many pathophysiological conditions. Voluminous research is being carried out to find effective therapeutics for amyloidosis. In recent times, carbon quantum dots (CQDs) gained attention of active researchers due to their unique semi-conducting, physio-chemical properties, large surface area to volume ratio, optical properties and bio-compatibility. In the study we have synthesized CQDs using kitchen spice mix and these particles are surface functionalized with Carboxylic group. We have demonstrated the characterization of modified CQDs and their action against *in vitro* amyloid forming model protein Hen Egg White Lysozyme (HEWL). The results shed some light towards the potential candidature of surface functionalized CQDs as therapeutics against amyloidosis.

Keywords: *Protein Quality Control, Amyloidogenesis, Neurodegenerative diseases, CQD, Surface functionalization, HEWL*

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Introduction

Amyloid fibrils formation is the predominant factor in many of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease and others. In recent times, the carbon quantum dots (CQD) are evolving as potential candidates targeting amyloid.[1] CQD possess excellent semi-conductive property, high surface area to volume ratio, optical stability and bio-compatible. In the current study we have implemented kitchen spices *Syzygium aromaticum, Cinnamomum verum, Elettaria cardamomum* and *Larus nobilis towards* CQD synthesis and surface functionalized with carboxylic group of Citric acid (CQD-CA). To monitor effect of CQD-CA against amyloidogenesis, we exploited model protein Hen Egg White Lysozyme (HEWL) forming amyloid at *in-vitro* conditions. Results showed will shed some light on the potential of surface modified CQD as therapeutics against amyloidosis.

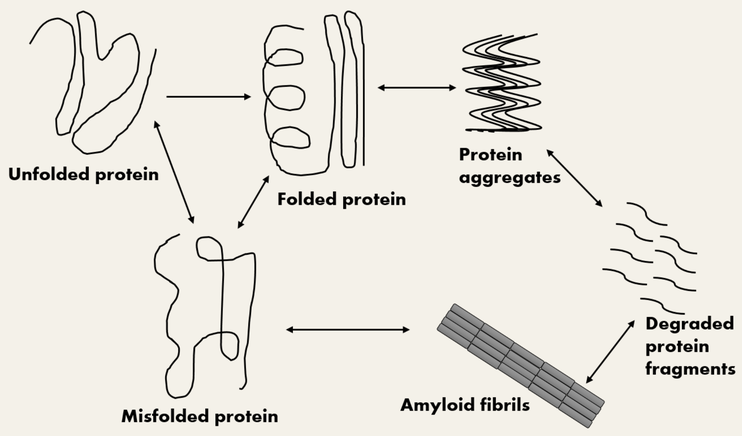

Figure 1: Schematic representation of amyloid fibrils formation

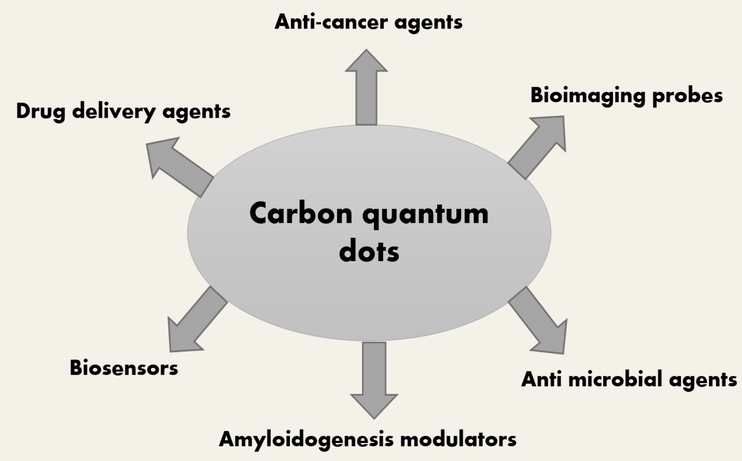

Figure 2: Applications of CQDs

Methods

Synthesis of surface functionalized CQD:

To synthesize CQD-CA we have followed hydrothermal treatment method at 190°C for 4 h using a Teflon-lined stainless-steel autoclave.

HEWL Amyloid preparation:

We followed the Gazova group method reported in 2018 with some modifications. Briefly, 35 µM HEWL was dissolved in freshly prepared 70 mM Glycine-HCl buffer with pH 2.7, and allowed to form amyloid *in vitro* at 65°C, 840 rpm agitation for 5h on the hot plate magnetic stirrer.[2]

Fluorescence quenching and thermodynamic equilibrium studies:

Intrinsic fluorescence quenching of protein and Guanine hydrochloride mediated protein denaturation was studied in presence and absence of CQD-CA.[3,4]

Cell viability and ROS elimination:

MG-63 cell-line used for performing standard MTT assay to evaluate cell viability expressed by the CQD-CA. DCFH-DA dye-based study was made to verify action of CQD-CA on the ROS generation induced by amyloid fibrils.[5,6]

Results

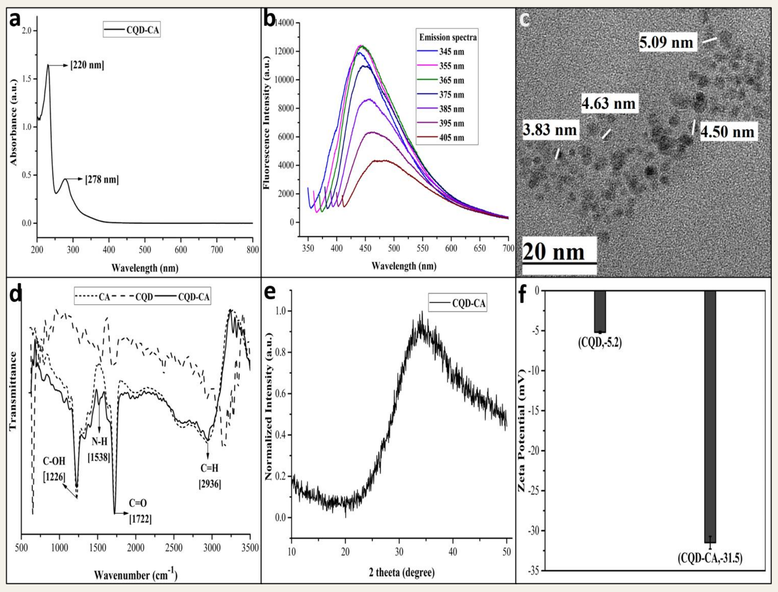

Figure 3: Characterization of CQD-CA (a) Absorbance spectra, (b) Fluorescence emission behavior, (c) TEM image, (d) FTIR spectra, (e) X-ray diffraction pattern, and (f) Zeta potential value.

Results contin..

- CQD-CA possess absorbance in UV region, the characteristic peak position at 278 nm retrieved from unmodified CQDs correspond to C=C bond, and 220 nm represent the carboxylic group absorbance peak correspond to C=O bond.
- Exhibited excitation dependent fluorescence emission behavior due to presence of in different sizes of quantum dots.
- FTIR spectra of CQD-CA confirm the surface functionalization with presence of C=O band at 1722 cm-1, and C-OH band at 1226 cm-1 wavenumber, similar to FTIR bands in only Citric acid spectra.
- XRD pattern reveals about the amorphous structure of CQD-CA due to the close packing of carbon atoms.
- Zeta potential value indicate that higher value exhibited by the CQD-CA are stable in solvent and prevent agglomeration of particles.

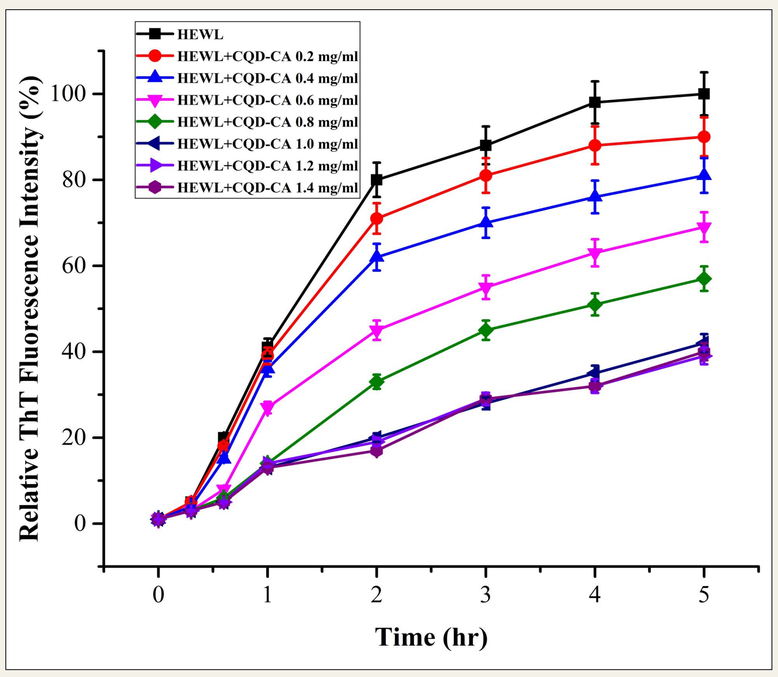

Figure 4: HEWL amyloid formation evaluated by implementing amyloid binding dye Thioflavin T in presence of different concentrations of CQD-CA. Excitation wavelength was 440 nm, and emission wavelength was 485 nm. ($p<0.005$)

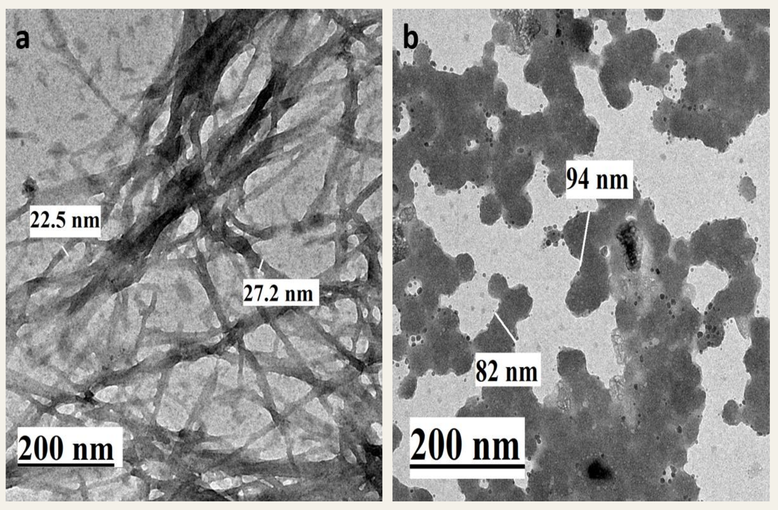

Figure 5: TEM images (a) mature fibrils, (b) amyloid formation restricted to spherical oligomers presence of CQD-CA

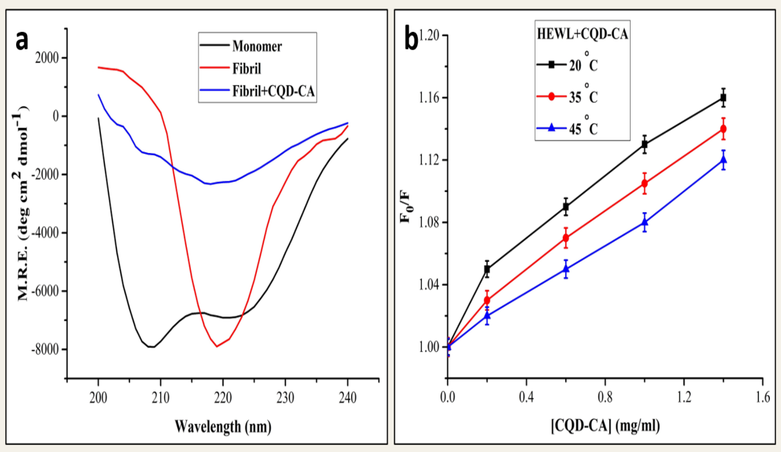

Figure 6: (a) Circular dichroism spectra of monomer, mature fibrils formation in presence and absence of CQD-CA, (b) Stern-Volmer plot of HEWL tryptophan residue fluorescence quenching exhibited by CQD-CA . The quenching was said to be static in nature due to decrease in fluorescence with increase in temperature, and quenching was due to the formation of low fluorescence complex of HEWL+CQD-CA in ground state.

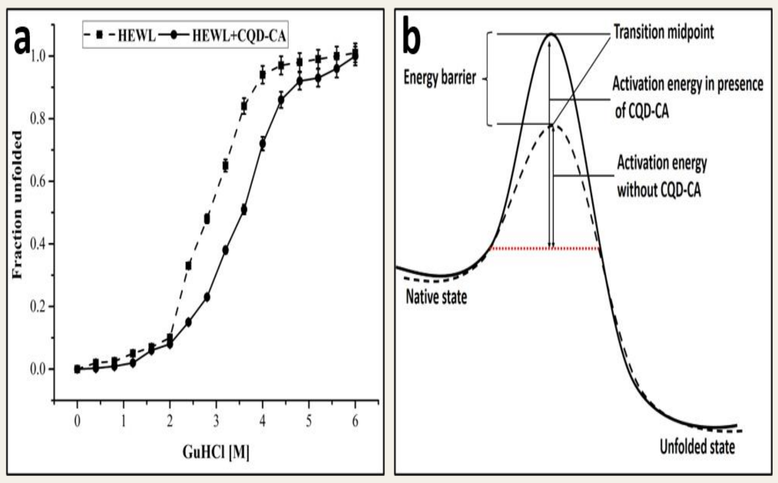

Figure 7: (a) HEWL denaturation mediated by the Guanidine hydrochloride in presence and absence of CQD-CA, (b) Increased energy barrier required for HEWL protein to cross the transition midpoint and undergo denaturation to unfold in the presence of CQD-CA.

Results contin..

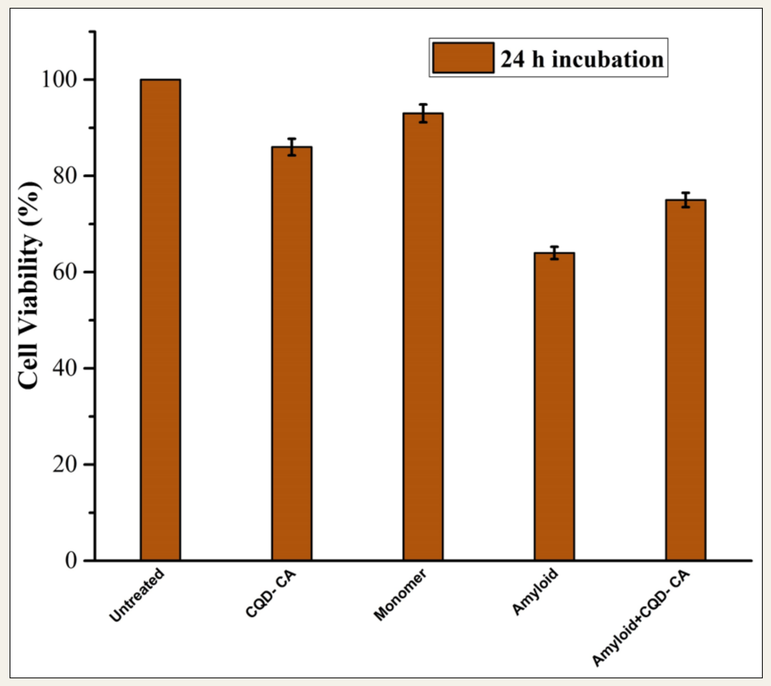

Figure 8: MTT assay of CQD-CA, amyloid fibrils formed in presence and absence of CQD-CA. Only CQD-CA displayed more than 80% cell viability. Amyloid fibrils showed 60%, where as amyloid formed in presence of CQD-CA displayed almost 75% cell viability.

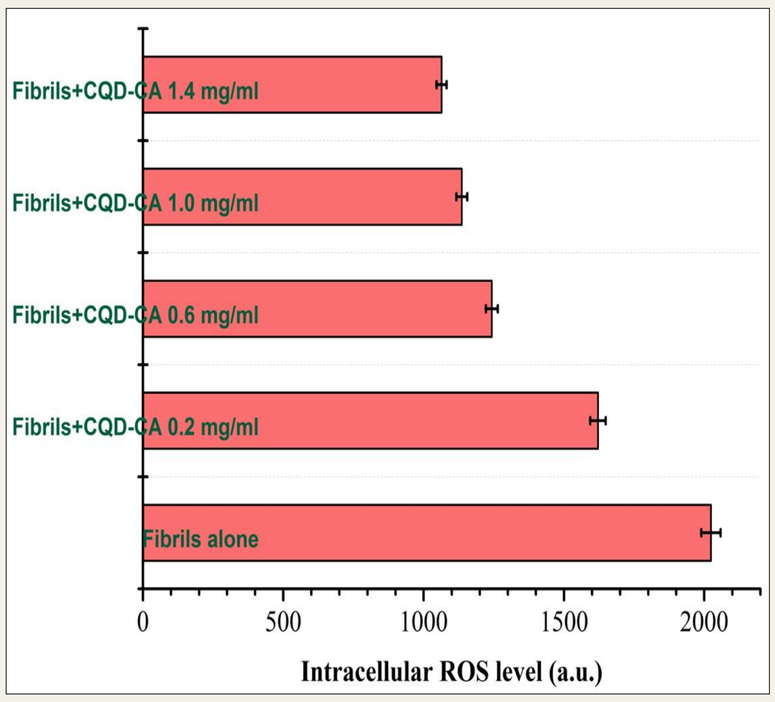

Figure 9: DCFH-DA dye-based assay for identifying the ROS levels induced by amyloid in the presence of different concentrations of CQD-CA

Conclusion

- The high amorphous structure CQD-CA synthesized with an average size of 4.54 nm in diameter, and were highly stable in nature.
- 1.2 mg/ml concentration of CQD-CA exhibited maximum amyloid inhibition capacity of 61%. TEM images confirm the amyloid inhibition with the formation of spherical oligomers having more than 80 nm in diameter.
- Circular dichroism spectra reveal the formation of amyloid fibrils (by change in secondary structure peak position from 208 nm presence of a-helix structure in monomer to B-sheet (peak at 217 nm). Reduction in mean residue ellipticity value of HEWL fibrils formed in presence of CQD-CA revealing the reduction in beta sheet content of amyloid.
- Formation of stable complex between HEWL and CQD-CA revealed from Stern-Volmer plot is further indication for the potential of CQD-CA in amyloid inhibition. Showed increase in energy barrier, and more denaturant is required for protein unfolding.
- CQD-CA exhibited more than 80 % cell viability, and formation of relatively less toxic amyloids compared to toxic mature fibrils.
- DCFH-DA assay revels that intracellular ROS levels induced by amyloid fibrils were reduced with increase in concentration of CQD-CA.

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