A Project Report of Summer Internship on:

"How CuO nanostructures regulate the fibrillation Of Human Serum Albumin?"

Submitted by

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My acknowledgement would be incomplete without thanking the biggest source of my strength, my parents for the constant support given to me all the times. Last but not the least, I would like to praise and thank God, the almighty, who has granted countless blessings, knowledge and opportunity.

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1) INTRODUCTION:-

Development of amyloid fibrils from unfolded and partially folded proteins results in numerous neurodegenerative disorders[1-3]. Such types of disorders are mainly associated with the accumulation of intracellular toxic amyloid fibrils with cross β-sheet rich content[4]. inhibition or disturbance of this fibrillation process is regarded as one of the emergent propositions in the prevention of all amyloid-related diseases. Due to the ultra-small size, nanoparticals(NPs) have distinct properties compared with the bulk form of the same materials. In recent times, nanomaterial-modulated fibrillations of various amyloidogenic proteins have gained attention as they are found to play a pivotal role in promoting, hindering, or disintegrating the fibrillation process[5-7]. Owing to the dimensional similarity of the nanomaterials with the protein molecules, they are also known to offer significant therapeutic potential by regulating the fibrillation process. The physicochemical properties of the nanomaterials including their chemical composition[8], surface charge[9] and surface chirality[10] have been recognized as key factors for controlling the propensity of various proteins to form fibrils. Few researchers have also studied the effect of size and shape of the engineered nanoparticles on the modulation of amyloid protein aggregation[11-13] however, the mechanistic details of the influence of the various morphologies of the nanostructures on the fibrillogenesis are still elusive.

1)a) WHAT ARE AMYLOIDS:-

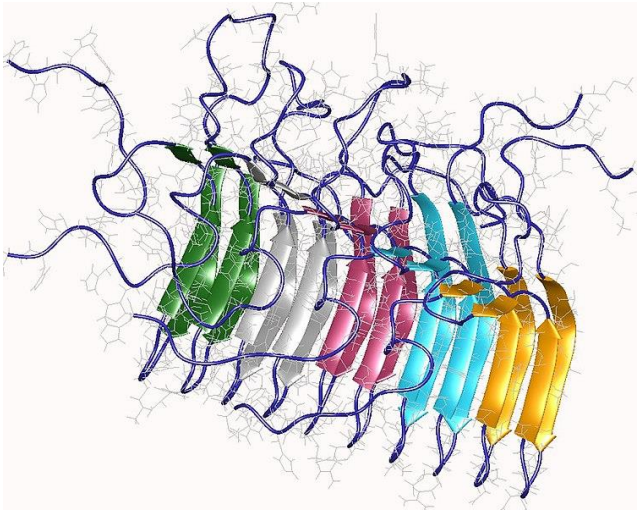

Figure 1 : Amyloid of HET-s(218-289) prion pentamer, podospora anserina.

Amyloids are aggregates of proteins that become folded into a shape that allows many copies of that protein to stick together, forming fibrils. In the human body, amyloids have been linked to the development of various diseases. Pathogenic amyloids form when previously healthy proteins lose their normal physiological functions and form fibrous deposits in plaques around cells which can disrupt the healthy function of tissues and organs.

Such amyloids have been associated with more than 50 human diseases, known as amyloidoses, and may play a role in some neurodegenerative disorders. Some amyloid proteins are infectious; these are called prions in which the infectious form can act as a template to convert other non-infectious proteins into infectious form. Amyloids may also have normal biological functions; for example, in the formation of fimbriae in some genera of bacteria, transmission of epigenetic traits in fungi, as well as pigment deposition and hormone release in humans.

Amyloids have been known to arise from many different proteins and polypeptides and formed of long unbranched fibers that are characterized by a **cross-beta sheet quaternary structure** in which antiparallel chains of β-stranded peptides are arranged in an orientation perpendicular to the axis of the fiber. Each individual fiber may

be 5–15 nanometres in width and a few micrometres in length. Amyloid is usually identified using fluorescent dyes, stain polarimetry, circular dichroism, or FTIR.

1)b) AMYLOID FIBRILATION AND NEURODEGENERATIVE DISEASES:-

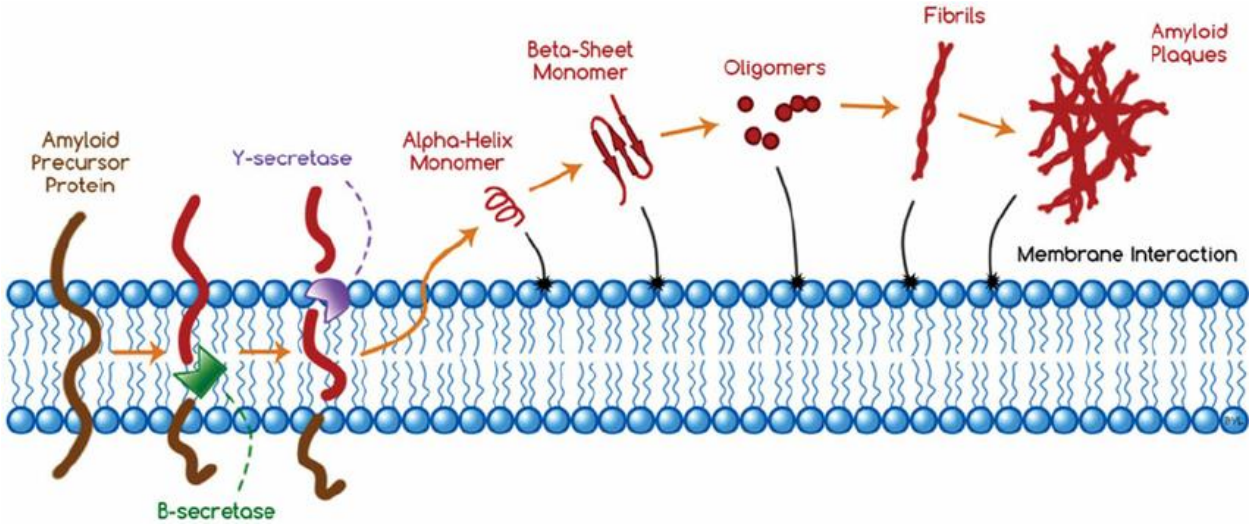

Figure 2 : Schematic showing amyloid origin and the mechanism of the amyloid fibril formation and toxicity.[14]

The fibrillation process of amyloids involves the self-assembly of protein monomers into the short-lived oligomers, which in turn transforms into long-lived oligomers that produces the fibrils eventually. Neurodegeneration is the progressive loss of structure or function of neurons, including death of neurons. Many neurodegenerative diseases – including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and Huntington's disease – occur as a result of

neurodegenerative processes. Several neurodegenerative diseases are classified as proteopathies as they are associated with the aggregation of misfolded proteins.

- **alpha-synuclein:** can aggregate to form insoluble fibrils in pathological conditions characterized by Lewy bodies, such as Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Alpha-synuclein is the primary structural component of Lewy body fibrils. In addition, an alpha-synuclein fragment, known as the non-Abeta component (NAC), is found in amyloid plaques in Alzheimer's disease. Alpha-synuclein can damage membranes by inducing membrane curvature, and cause extensive tubulation and vesiculation when incubated with artificial phospholipid vesicles.The tubes formed from these lipid vesicles consist of both micellar as well as bilayer tubes. Extensive induction of membrane curvature is deleterious to the cell and would eventually lead to cell death[15].Apart from tubular structures, alpha-synuclein can also form lipoprotein nanoparticles similar to apolipoproteins[16].
- **tau:** hyperphosphorylated tau protein is the main component of neurofibrillary tanglesin Alzheimer's disease.
- **beta amyloid:** the major component of senile plaques in Alzheimer's disease.
- **prion:** main component of prion diseases and transmissible spongiform encephalopathies.

1)c) HUMAN SERUM ALBUMIN AS A MODEL PROTEIN FOR FIBRILLOGENESIS:

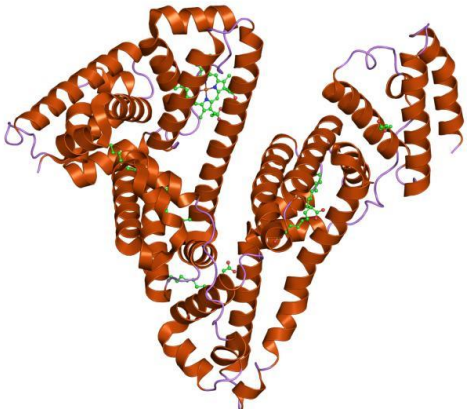

Figure 3: Human serum albumin

Human serum albumin (HSA) is the serum albumin found in human blood. It is the most abundant protein in human blood plasma; it constitutes about half of serum protein. It is produced in the

liver. It is soluble in water and monomeric. Albumin transports hormones, fatty acids, and other compounds, buffers pH, and maintains oncotic pressure, and various other functions. Albumin is synthesized in the liver as preproalbumin, which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin. It has a serum half-life of approximately 20 days. It has a molecular mass of 66.5 kDa.

Albumin is considered as a model protein for studying the fibrillogenesis due to its propensity to aggregate in vitro under suitable condition[17-18]. Their fibrillation can be easily accomplished by disturbing the ambient physiological conditions of pH, temperature and ionic strength[18-20]. Besides, the effects of various additives such as – alcohol, sugar, surfactants and metal ions on HSA aggregation have been well studied[21-24]. On the contrary, only a few reports are available on nanomaterials modulated fibrillation of HSA[25-26] while, the morphological effects of the nanomaterials on fibrillation of HSA is yet to be elucidated.

1)d) PYSIOLOGICAL IMPORTANCE OF COPPER AND EFFECT OF CuO NANOPARTICALS ON PROTEIN FIBRILATION:-

Copper, a necessary trace element i.e. micronutrient, play vital roles in our body. Copper is an important component of many enzymes in the body and play an important role in cell energy production. Activity of these enzymes is highest in the heart, brain, liver and kidney. In addition, enzymes that are responsible for connective tissue proteins formation (collagen and elastin) require copper. Copper is necessary for the development and maintenance of blood vessels, skin, bones and joints.It helps in preserving cells of the nervous system. Copper is involved in the release of iron from their depot, the creation of bone marrow and maturation of red blood cells.Copper is essential for the synthesis of phospholipids of cell membranes and thus maintain myelin that separates the nerve cell from the environment and regulates the levels of the neurotransmitters.Copper is a component of the enzyme copper-zinc superoxide dismutase (Cu / Zn SOD), which serves as an antioxidant essential to preserve the body from damage caused by free radicals. Matching the copper and zinc is important for the normal functioning of the body. Copper is very important in creating the immune response to infection. During the inflammatory process or infection, the mobilization of the two compounds in their structure include ion copper superoxide dismutase and ceruloplasmin. Copper is also necessary for the maturation and

function of T cells. And also it subsists in the body bound to amyloidogenic proteins[27-28]. Cu nanoparticles have been reported to exhibit the ability to inhibit proteins aggregation[30-31]. In addition, Cu (II) ions have been found to significantly affect fibrillation of peptides and proteins[32-33] and their interaction as well as their effect on HSA fibrillation has also been investigated in earlier reports[23, 34]. It may also be noted that besides Cu (II) ion and Cu nanoparticles, CuO nanoparticles have also been studied in various biological studies e.g. magnetic resonance imaging, interaction study with HSA etc[35-36]. Thus, to gain better insight on the morphological effect of nanostructures on the amyloid fibrillation process CuO has been chosen as a platform for the present study.

1)e) OBJECTIVE:-

Herein, the rod, sphere, flower and star shaped morphologies of CuO nanostructures have been synthesized using aqueous based chemical precipitation technique and characterized by Fourier transformed infrared (FTIR) spectroscopy, powder X-ray diffraction (XRD) studies, scanning electron microscope (SEM). Their potency to inhibit the fibrillation of HSA have been investigated. The influence of synthesized nanostructures on the HSA fibrillation process has been monitored through the Thioflavin T (ThT) fluorescence spectroscopy and circular dichroism (CD) spectroscopy study.

2) MATERIALS AND METHODS :-

2)1) MATERIALS:-

All chemicals were of analytical grade and were used without further purification. Copper acetate monohydrate (Cu(CH3COO)2.H2O, Merck Ltd, Mumbai, 98%), Acetic acid (Merck Ltd, Mumbai, 99%), Tartaric acid (Merck Ltd, Mumbai, 98%), Citric acid (Merck Ltd, Mumbai, 98%), HPLC grade ethanol (Merck Ltd, Mumbai) and Sodium hydroxide (NaOH, Merck Ltd, Mumbai, $\geq$ 97%) were used in this experiment. Human serum albumin (HSA),and Thioflavin T (ThT) were purchased from Sigma Chemical Co. (St. Louis) and were used as received.

2)2) METHODS AND TECHNIQUES:-

2)2)a) SYNTHESIS OF CuO NANOSTRUCTURES:-

The CuO nanoparticles were prepared by co-precipitation method using copper acetate and NaOH as well as various acids(acetic acid, tartaric acid and citric acid) as precursors. 0.1 M Aqueous solution of copper acetate was prepared and was mixed with excess of NaOH at 100 °C by maintaining the solution pH at 10. Then, the solution was cooled to room temperature and allowed to settle overnight. Supernatant was discarded carefully and the remaining black precipitate of CuO was washed with water several times and separated by centrifugation. The residue was dried in a vacuum oven at 120 °C for 6 h to obtain the dry powder of CuO[38] (referred as CuOR in the text). The above procedure for obtaining CuO was repeated with the addition of acetic acid, tartaric acid and citric acid in the initial aqueous solution of copper acetate and the thus-obtained black powder have been referred to as CuOP, CuOF and CuOS respectively in the following text.

2)2)b) CHARACTERIZATION OF CuO NANOSTRUCTURES:-

(i) X-RAY DIFFRACTION STUDIES:-

Powder XRD was a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The crystalline phase of CuO nanostructures in the synthesized samples were ascertained through powder X-ray diffraction (XRD) studies, carried out on a Bruker AXS Diffractometer D8 powder XRD, Germany, using Cu-Kα radiation ($\lambda = 1.5418$ Å) at the applied voltage of 40 kV and at a scan rate of 3° min^{-1} between the 2θ range of 30 - 80°.

(ii) FOURIER TRANSFORMED INFRARED (FTIR) SPECTROSCOPY STUDIES:-

FT-IR spectroscopy is useful in measuring the absorption of IR radiations by a sample, and the results were shown by means of a wavelength. The evaluation of the IR spectrum includes the correlation of the absorption bands (vibrational bands) and the chemical compounds in the sample. It is used to determine functional groups in molecules. IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups. Generally, stronger bonds and light atoms will vibrate at a high stretching frequency (wavenumber). Hence the presence of surface functional groups in the synthesized CuO samples

were established through Fourier transformed infrared (FTIR) spectroscopy, using Perkin-Elmer Spectrum RX-II, Model no. 73713, USA, within a spectral range of 4000–400 cm^{-1}.

(iii) SCANNING ELECTRON MICROSCOPE ANALYSIS:-

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. Hence the morphology of the synthesized CuO nanostructures were analyzed by Carl Zeiss MERLIN scanning electron microscope (SEM), Germany, outfitted with a field emission gun at an accelerating voltage of 5 kV. Diluted CuO samples were drop casted on aluminum foil and coated with gold prior to microscopic study.

2)3) HUMAN SERUM ALBUMIN (HSA) FIBRILATION STUDIES:-

(A) FIBRIL FORMATION:-

(i) The HSA stock solution was freshly prepared by dissolving HSA in Milli-Q water.

(ii) It was then stored at 4^0 C.

(iii) The accurate HSA concentration was calculated by UV-Vis spectrophotometer (Shimadzu UV-2450) using molar extinction coefficient of 35,219 M−1 cm−1 at 280 nm[38].

(iv) The stock solutions of each of the CuO nanostructures were prepared by dispersing 0.25 mg of the respective CuO samples in 1 ml of phosphate buffer [of pH 7.0 (20 mM)].

(v) It was then subjected to ultrasonication for 2 min prior to use.

(vi) To study the influence of the various CuO nanostructures on the inhibition of HSA fibrillation, 200 µL of the each of the CuO stock solutions were separately added into 50 µM HSA at pH 7.0 (20 mM phosphate buffer) in presence of 60% (v/v) ethanol.

(vii) Samples were incubated at 37 ºC for 24 h**.**

(viii) Samples were centrifuged to separate the CuO by precipitation from HSA solutions and supernatant was collected prior to further studies.

(ix) As a control, the HSA solution (without the CuO) was incubated in phosphate buffer of pH 7.0 (20 mM) under the same experimental condition[24].

The experimental techniques used for studying the inhibition of fibrillation of HSA in the presence of CuO nanostructures [such as – ThT fluorescence (fluorescence spectroscopy),and CD spectroscopy] have been carried out using only the supernatant, after separation of CuO from the protein solution through centrifugation.

(B) <u>ThT FLUORESCENCE</u>:-

The fibrillation of HSA solutions in presence and absence of CuO nanostructures was monitored using Thioflavin-T (ThT) fluorescence study.

(i) The stock solution of ThT dye was prepared in phosphate buffer.

(ii) the concentration was determined spectrophotometrically using the molar extinction coefficient at 416 nm of 26600 $M^{-1}cm^{-1}$. [39]

(iii) To monitor the ThT binding, the aliquots withdrawn from various sets of solutions at definite time intervals and incubated with ThT for 5 min at room temperature.

(iv) The final concentrations of protein and ThT dye were kept 2 µM 10 µM respectively**.**

(v) The fluorescence of the aliquots were measured at 25 °C using a Horiba Jobin Yvon Fluoromax 4 spectrofluorimeter with an excitation wavelength of 450 nm.

(vi) the emission spectra were observed within the wavelength range of 470 nm to 600 nm.

(vii) The slit width and integration time were kept at 5 nm and 0.3 s respectively.

(viii) All spectra were corrected with respect to the corresponding blank.

(ix) Each measurement was done at least three times and error bars represent the standard deviation from the mean value.

(x) The possibility of reduction of fluorescence due to the absorption of incident and emitted light, the inner filter effect has been taken into account and all the fluorescence spectra were corrected by the following relation[40].

$$F_{corrected} = F_{observed} \times 10^{\frac{(Aex+Aem)}{2}} \quad \text{……..……..(1)}$$

Where $F_{corrected}$ and $F_{observed}$ are the corrected and observed fluorescence intensities of the samples respectively, A_{ex} and A_{em} are the absorbance of the samples at the excitation and emission wavelength respectively.

(C) <u>CIRCULAR DICHROISM (CD) SPECTROSCOPY</u>:-

(i) Far-UV CD spectra of the samples were observed on a JASCO-810 automatic recording spectrophotometer under a constant nitrogen flow at 25 °C.

(ii) The CD spectra were acquired in a strain free quartz cuvette of 0.1 cm path length, in the wavelength range of 190 to 240 nm at a scan rate of 50 nm per min.

(iii) The aliquots of native HSA, HSA fibrils, HSA fibrillar solutions containing different CuO nanostructures were diluted with 20 mM phosphate buffer (pH 7.0) to achieve final HSA concentration of 2 µM for each solution.

(iv) The changes in secondary structural contents of HSA was determined by using the online DICHROWEB server[41].

(v) Each spectrum was corrected with respect to the appropriate blank.

(vi) Each experiment has been performed in triplicate and error bars represent the standard deviation from the mean value.

(3) RESULTS AND DISCUSSIONS :-

3)1) CHARACTERIZATION OF NANOPARTICLES:-

(i) X-RAY DIFFRACTION STUDIES:-

The indexed XRD pattern (Figure 4(A)) of various CuO nanostructures, are nearly equivalent to each other and match with the JCPDS file number 80-1916 indicating monoclinic structure with the space group of C2/c.

(ii) FOURIER TRANSFORMED INFRARED (FTIR) SPECTROSCOPY STUDIES:-

The FTIR spectra of CuO nanostructures are shown in Figure 4(b), where the characteristic peak positioned at ~430 cm^{-1} can be ascribed to Cu-O stretching band along (202) direction, peaks at ~520 and ~610 cm^{-1} can be assigned to Cu-O stretching along $(2\bar{0}2)$ direction[42]. The absorption peaks positioned at around 1540 and 1410 cm^{-1} in CuOP, 1615 and 1365 cm^{-1} in CuOF can be assigned to anti symmetric and symmetric C=O stretching modes of carboxylate groups respectively, probably due to the adsorbed acetate and tartarate anions on CuO surface[43]. Similarly, in the FTIR spectrum of CuOS, the peaks at ~1580 and ~1375 cm^{-1} may be attributed to stretching frequencies of C=O resulting from the citrate group adsorbed on CuOS surface[44]. In the FTIR spectrum of CuOS sample, the absence of peaks in between 1680 and 1750 cm^{-1} indicates the absence of unreacted acids, which has been used as chelating and capping agent. The XRD and FTIR spectra of all the samples confirm the purity of the CuO samples.

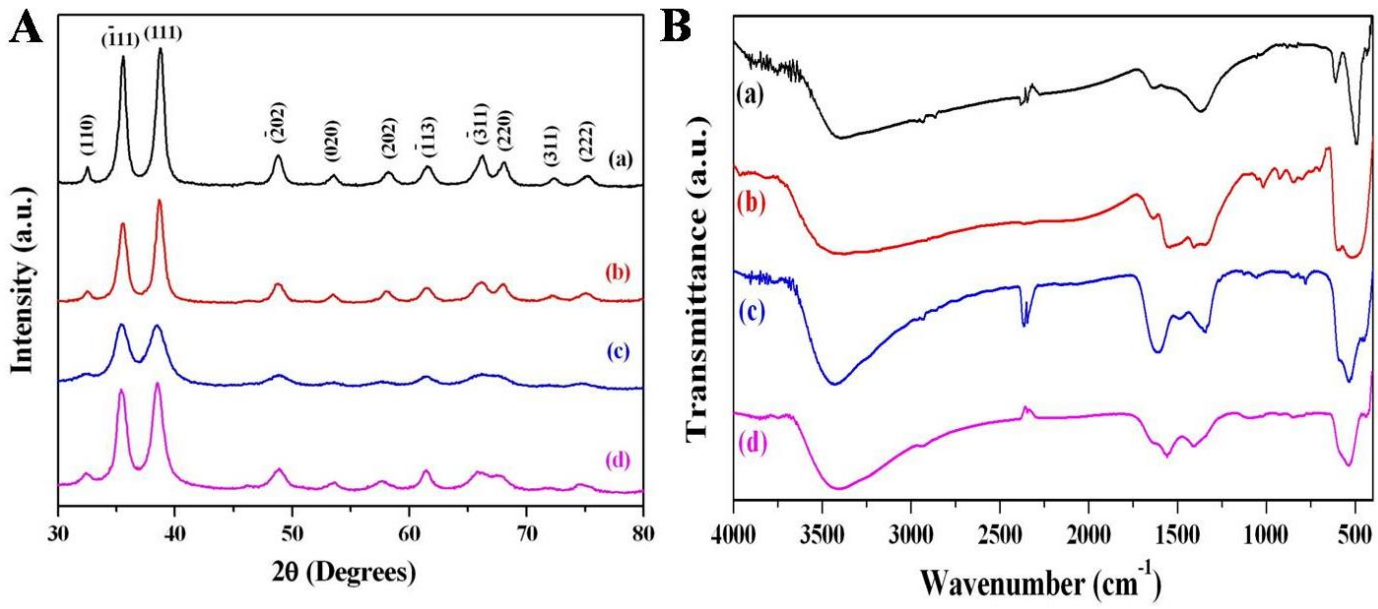

Figure 4: (A) Indexed XRD and **(B)** FTIR spectra of (a) CuOR, (b) CuOP, (c) CuOF and (d) CuOS nanostructures.

iii) SCANNING ELECTRON MICROSCOPE ANALYSIS:-

The surface morphology of the synthesized CuO nanostructures is visualized through the SEM, as shown in Figure 5. The SEM images of the CuO nanostructures demonstrate the homogeneous distribution of rod, sphere, flower and star shaped morphologies (Figure 5A, B, C and D respectively). The rod shaped particles of CuOR possess lengths in between 130-160 nm, the spherical particles of CuOP have diameters in between 15-25 nm and flower shaped CuOF consists of four to six branches with particle size in between 130-160 nm. The SEM image of CuOS sample shows the particles are star-shaped in morphology with the conical tip (quadpod,pentapod or hexapod) lengths varying between 80 – 100 nm (Figure 6D).

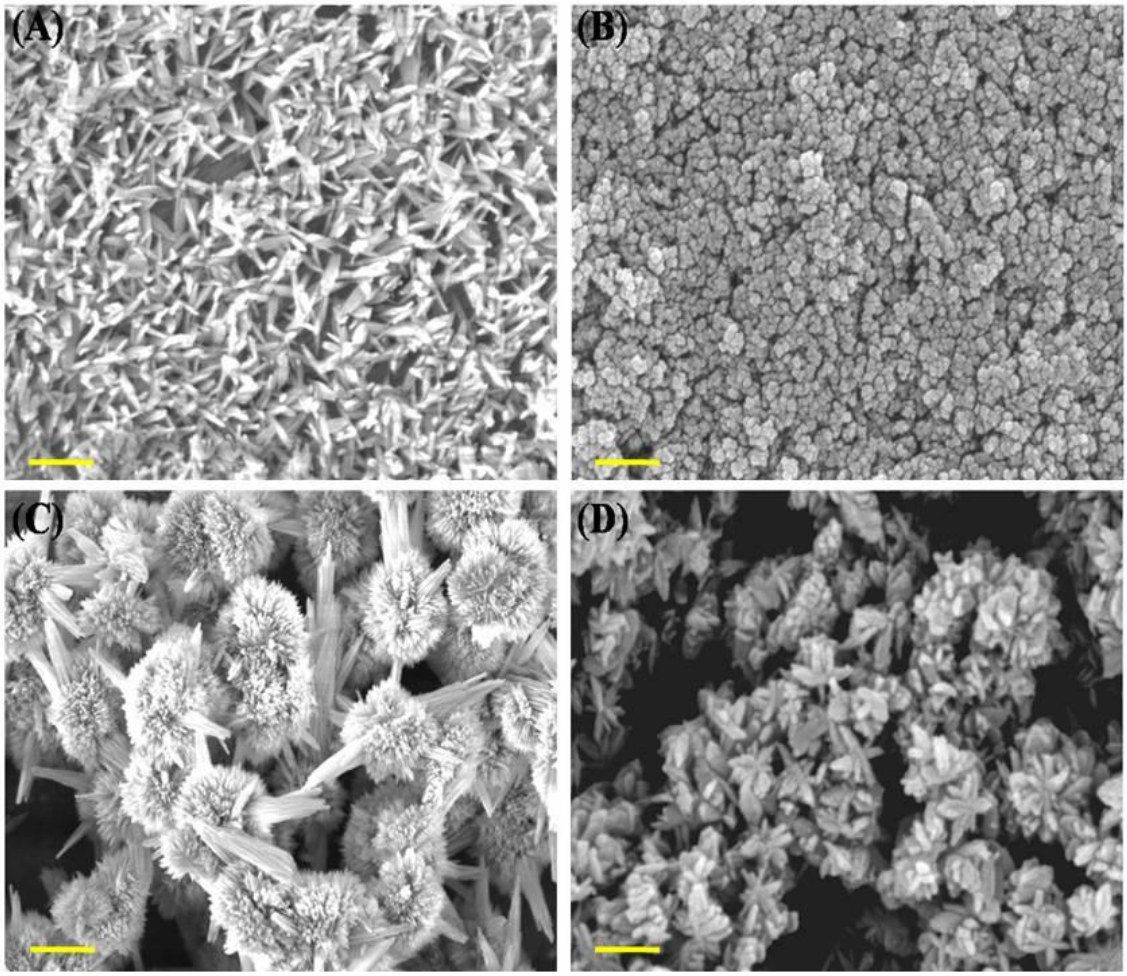

Figure 5: SEM images of (A) CuOR, (B) CuOP, (C) CuOF and (D) CuOS nanostructures. The scale bars represent 200 nm.

3)2) HUMAN SERUM ALBUMIN (HSA) FIBRILATION STUDIES:-

(i) ThT BINDING STUDY:-

Thioflavin T (ThT), a fluorescent dye, exclusively binds to cross β-sheet structures of amyloid fibrils and shows significant increase in fluorescence intensity at 485 nm when excited at 450 nm, allowing one to quantify the amount of amyloid fibrils[45]. The effect of the CuO nanostructures on the HSA fibrillation process has been investigated by the ThT binding fluorescence study. It is important to note that, ThT does not exhibit discernible change in fluorescence intensity in the control sets comprising native HSA and CuO nanostructures separately, when excited at 450 nm. The representative ThT fluorescence spectra of HSA

solutions in absence and presence of various CuO nanostructures after incubation at 37 ºC for 24h is presented in Figure 7. Co-incubation of CuOR, CuOP and CuOF with HSA causes ~21%, ~43% and ~53% reduction in the ThT fluorescence intensity respectively while CuOS results in the maximum reduction (~61%) in ThT fluorescence intensity, compared to the control HSA fibrillar solution. As the decrease in ThT fluorescence intensity is indicative of reduced formation of amyloid fibrils, it may be suggested that the star shaped CuOS is more efficient than other CuO nanostructures in inhibition of HSA fibrillation.

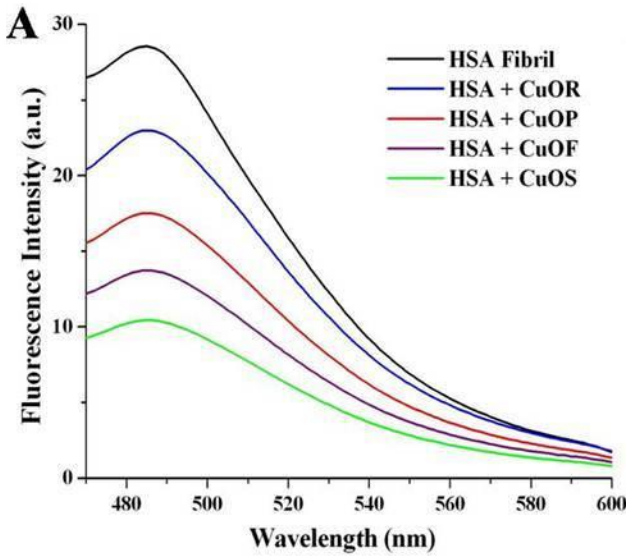

Figure 7: Representative ThT fluorescence spectra of various HSA solutions in the absence and presence of CuO nanostructures after incubation at 37 °C for 24 h.

Further, to validate the findings of ThT fluorescence assay, the inhibition of HSA fibrillation by CuO nanostructures is also investigated Quantitatively. Hence, Quantification of the amount of HSA fibrils in the different samples have therefore been carried out and reported through the analysis of the secondary structural contents of HSA protein probed through CD spectroscopy.

(ii)) CIRCULAR DICHROISM (CD) SPECTROSCOPY ANALYSIS:-

Circular dichroism (CD) spectroscopy is a sensitive technique to quantify the changes in various secondary structural contents of a protein during their fibrillation process. In present study, CD spectroscopy is used to probe the effect of CuO nanostructures on the conformational changes of HSA before and after inducing the fibrillation condition. Figure 8A represents the far–UV CD

spectra of native HSA and HSA fibrillar solutions in absence and presence of CuO nanostructures. Two negative minima (at 208 and 222 nm) observes in the CD spectrum of native HSA, indicate the α-helicity of the protein[46]. The negative bands at 208 nm and 222 nm respectively correspond to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the carbonyl groups in the polypeptide chains. Fibrillation of native HSA is marked by significantly decreased intensities (i.e., lower negative mdeg values) of these two bands in the CD spectrum, suggesting substantial loss of α-helicity along with enhancement of β- sheet structure. The HSA solution incubated in presence of the CuO nanostructures, show a noticeable increase in the mdeg values (i.e., more negative mdeg values) for both the bands (at 208 and 222 nm) compared to the control HSA fibrillar solution which suggests their ability to retain the α-helicity of HSA. The effect is however observed to be more pronounced in presence of CuOS compared to the other samples of CuO.

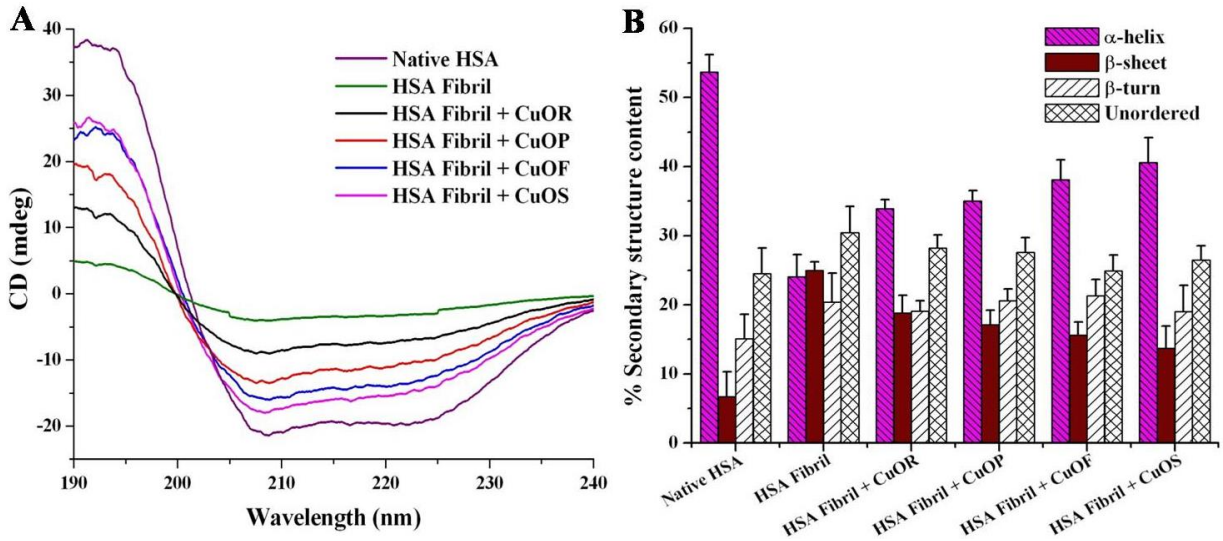

Figure 8: **(A)** Far-UV CD spectra and **(B)** corresponding histogram of % secondary structure content of native HSA and HSA fibrillar solution in absence and presence of CuOR, CuOP, CuOF and CuOS after incubation at 37 °C for 24 h in the presence of 60% (v/v) ethanol. Error bars represent standard deviation from the mean value estimated from at least three individual measurements.

The quantification of the amount of secondary structures in the native HSA as well as in the HSA fibrils (in presence and absence of the CuO nanostructures) has been carried out using the online DICHROWEB server and the corresponding histogram is represented in Figure 8B. Native HSA was found to contain ~54% α-helix and only ~7% β-sheet, while HSA fibrillar solution (in the absence of CuO nanostructures) showed ~24% α-helix and ~25% β-sheets. This indicates the conversion of the α-helix to β-sheet during fibrils formation, which is the hallmark of amyloid fibrillation. Conversely, a substantial variation in the secondary structural content is observed in the presence of CuO nanostructures where the amount of β-sheet is found to decrease along with an increase in the amount of α-helix with respect to the control HSA fibrils, as is evident from Fig. 5B. From the observations it can be inferred that the presence of CuO nanostructures hinders the α-helix to β-sheet conversion. The inhibition in the α-helix to β-sheet conversion is found to follow in the order: CuOR < CuOP < CuOF < CuOS where, CuOS showed maximum inhibition ability towards the conversion. This observation from the CD studies is well consistent with the result achieved from ThT fluorescence.

For studying the influence of surface directing agents, ThT fluorescence and CD spectroscopic studies for HSA fibrillation have also been carried out in presence of the various free acids (i.e., acetic/tartaric/citric), as a control. It may be mentioned that no noticeable changes in the ThT fluorescence spectra and CD spectra of HSA fibrillar solution were observed in presence of the acids at the concentration level used in this work. This reiterates that the observed changes in ThT fluorescence, CR binding study and CD spectroscopic studies could only be due to the presence of the various morphologies of CuO nanostructures in the HSA fibrillar solution.

(iii) <u>INHIBITING ABILITY OF CuO NANOSTRUCTURES</u>:-

The protein fibrillation process is associated with the irreversible structural transition of protein monomers from their native state to fibrils via the formation of partially destabilized self-assembled oligomeric intermediates[47]. Nanomaterials affect the fibrillation process either by promoting or inhibiting the nucleation step at the early stage of fibrillation. Herein, the HSA monomers get adsorbed on the surface of the nanostructures and the interaction of the HSA molecules with the nanostructures leads to the stabilization of their native structure, as revealed by the CD and ThT fluorescence studies. The adsorption of the HSA monomers onto the nanostructures results in the reduction of the concentration of free protein monomers, available

to proceed for fibrillation, in solution. This in turn affects the amyloid fibrillation process by causing hindrances in the nucleation phenomenon, the essential step of fibrillation process. The efficiency of protein-nanostructure interaction is found to be dependent on the physicochemical nature of the nanostructures i.e., size, shape and surface charge. Various types of binding forces such as van der Waals interactions, hydrogen bonding, hydrophobic interactions and electrostatic interactions are responsible for such type of interaction[48].

The star-shaped CuOS is endowed with high-index facet {211} whereas CuOF have {001} and {010} dual facets and CuOR possess only one facet {001}. The high index facets have elevated surface energies and higher surface roughness as compared to the low index crystal facets because of their high density of atomic steps, kinks and dangling bonds[49].

Sample	**Morphology**	**Growth directional facet**	**% Decrease in ThT fluorescence intensity**	**Relative % decrease in β-sheet content**
CuOR	Nanorods	{001}	21	25
CuOP	spherical NPs	-	43	32
CuOF	Nanoflowers	{001} {010}	53	38
CuOS	Nanostars	{211} {110}	61	45

Table 1: Characterization of CuO Nanostructures and Their Morphological Effects on HSA Fibrillation.

(4) CONCLUSIONS:-

The fibrillation process is established to be strongly dependent on the morphology of CuO nanostructures. The inhibiting efficiency of star shaped CuO nanostructures is found to be higher than the rod, spherical and flower shaped CuO nanostructures towards HSA fibrillation. The ThT binding study shows ~61% reduction of ThT fluorescence intensity and the CD binding study indicates a ~45% relative decrease in β-sheet content in presence of CuO nanostars as compared to control HSA fibrillar solution. I believe that this study provides fruitful information regarding the effect of morphological variation of nanostructures on fibrillation of proteins.

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15. Remodeling of Lipid Vesicles into Cylindrical Micelles by α-Synuclein in an Extended α-Helical Conformation[*]

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16. α-Synuclein Oligomers with Broken Helical Conformation Form Lipoprotein Nanoparticles[*]

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