PHOTOLUMINESCENCE FROM SELF-ASSEMBLED NANOCOMPOSITES

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PHOTOLUMNISCENCE FROM SELF ASSEMBLED NANOCOMPOSITES

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **PHOTOLUMNISCENCE FROM SELF-ASSEMBLED NANOCOMPOSITES** in the partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DEPARTMENT OF CHEMISTRY Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the time period from July 2020 to June 2021 under the supervision of Dr.Tusharkanti Mukherjee, Associate Professor, Department of Chemistry, Indian Institute of Technology, Indore.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

Annapurna Saxena

Signature of the student
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This is to certify that the above statement made by the candidate is correct to the best of my/our knowledge.

Tushar kanti Mukherjee

3/06/2021

Signature of the Supervisor

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Abstract

Various neurogenerative disorders such as Parkinson's disease, Alzheimer and other amyloidosis have not found a cure yet and thus need to be studied. These neurogenerative disorders involve protein misfolding, thus resulting in the formation of amyloid. Our main objective is to study the photoluminescence of protein and the fabrication of protein assemblies. Photoluminescence involves spontaneous emission of radiation from electronically or vibrationally excited species upon upon absorption of radiation. Fluorescence is photoluminescence. We have also tried to explore the physiochemical properties of these assemblies. In this thesis, we have attempted to inspect human serum albumin's time and temperature-dependent fluorescence, one of the most abundant protein in the human body, with a polyethylene glycol(PEG crowder).

LIST OF FIGURES:

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Chapter 1: INTRODUCTION

The fundamental understanding of protein misfolding and their fluorescence properties are of current interest.Misfolded proteins structure totals which are identified with different neurogenerative issues, for example, “Alzheimer's sickness, Parkinson's illness, and various kinds of amyloidosis.”[1-2]. A more profound comprehension of amyloid isn't just fundamental for the treatment of different sickness yet in addition valuable for the sound planning of amyloid fibrils as novel “biomaterial and nanodevices” [3] Fluorescent polymer nanoparticles have drawn considerable attention in recent years for theireasyfictionalization, biocompatibility, and metal-free property.

Protein investigation has colossal significance in a wide scope of examination fields, for example, organic chemistry and immunodiagnostics. Protein remains in highly crowded environment in cells structure, and activity gets affected. Protein stability can be comprehended by the net balance of forces which determines whether a protein will remain in native or a denatured state. Protein stability can be explained either thermodynamic or kinetic. Thermodynamic stability of protein is related to denatured state in equilibrium with native protein. Kinetic stability is regarding to significant energy barrier separating native and denatured or partially denatured state. Thermodynamic stability of protein is defined as the differences in free energy between native and denatured states under physiological conditions[4].Under certain conditions, such as an exposure to heat or chemical agents proteins can denature. That is the three dimensional structure breaks down and loses its biological functionality. Once the denaturingof agent is removed, the primary structure of the protein dictates the refolding process and the polypeptide forms its native conformation. Under denaturing condition, a segment of a protein becomes unstable and begins to break down. As that segment denatures, it causes disruptions in other segments of the polypeptide. In this sense, many segments of protein cooperate together to break down the overall structure of protein.To study its properties and how it behaves in a different climate, various spectroscopic techniques have been employed. Out of the multiple proteins

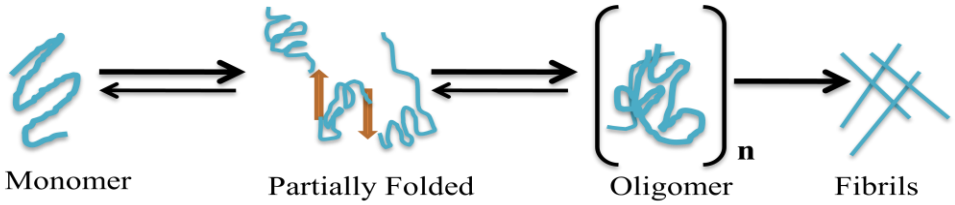
present,HSA(Human Serum Albumin) protein is of incredible premium as it comprises over half of the blood plasma proteins and actas a vehicle protein in light of its ability of official, conveying anextraordinarily different scope of endogenous and exogenous accumulates through the circulatory system to their objective organs.[5-6] It is a single polypeptide chain of 585 residues which includes 16 histidine residues which help in accepting and donating protons and act as a buffer. Secondary structure of HSA is made up of α-helix which make the globular monomeric protein. Albumin is composed of three domain which is further composed of two sub-domains a) which has six α-helix b) four α-helix.Although a few fluorescent probes for serum albumin detectionhave been accounted for, the vast majority of them demonstrated low selectivity for HSA, ortheir recognition limits werelow enough not to make them relevant inreal viable fields. In this way, it is vital todevelop a delicate fluorescent test for HSA assurance in practical examples. HSA shows fluorescence in the UV visible range(~340 nm) upon excitation at 295 nm due to the presence of intrinsic protein tryptophan. Concentration-dependent and subsequent structural alternations of serum albumin need to be explored.

Self-oligomerisation is a reversible phenomenon. The protein collaborates with other protein through different communications, for example, “hydrogen bonding, Vander Waals or covalent interactions”.[7-8] These interactions destabilise the native protein and facilitate the self-association.[9-10] Unfolding and denaturation of various native serum albumins at physiological condition have been studied and explored. But the protein unfolding and self-aggregation at high protein concentration need to be further explored. The intrinsic fluorescence of protein has drawn much larger attention in recent years. The capacity to imagine protein amyloid structures without extraneous marking gives occasions to the advancement of in vitro dynamic total teststhat are not susceptible to perturbation of the aggregating protein by “dyes or labels”[11].The fluorescent proteins can act as“fluorescent probe” towardvarious cations, hence can be employed as a biosensor.

Intrinsic fluorescence of proteins can be utilised for sensing and biosensing of different metal ions. “Fluorophores that are both selective and sensitive are prerequisite for luminescent sensor development”[12].A predetermined number of fluorophores have both these characteristics for estimating change metal ions.Transitionmetals are the basic building blocks of all enzymes, and contributing as electron transport, maintenance of basic integrity,catalysis,and neuronal functions. [13-14] Out of various transition metal, copper is of great importance as it is an integral part of various biological functions such as electron transport oxygen metabolism and transport.Dietary admission of copper at fitting sum is required to keep up the typical development of mind and sensory systems, to continue the flexibility of veins, to intercede the arrangement of collagen and elastin, and to control “haemoglobin” level.[15]Proteins containing copper have a noticeable part in the sensory system of humans. BothMenkes' and Wilson's neurological problems, are associated with the body's failure to use copper viably. Current methods to determine the copper level in the biological system are ineffective as these methods destroy the sample. Organic fluorophores are very sensitive, less photostable and undergo photobleaching very soon.Thus,a new system which can replace organic fluorophore and gives the effective measurement of metal ions in the biological system needs to be employed and explored.As of late, the fluorescent proteins from different creatures become suitable candidate for sensor improvement. This incorporates the green fluorescent protein (GFP) andits variations from the Pacific Northwest jellyfish specifically “*Aequorea Victoria”*, the redfluorescent protein (DsRed) from the tropical coral in particular “*Discosomasp”*., and thefar-red fluorescent (HcRed) protein from the reef coral in particular “*Heteractis crispa”*. [16]

Liquid–liquid phase separation (LLPS) of biological polymer is a critical phenomenon in the formation of intracellular ‘membraneless’ organelles.[17]Proteins can assemble into something called “liquid phase” and can form droplets in liquid phase which is well separated from solvent. These droplets are well distinguished in solvent and can been seen as with the evolution of time and addition of crowder. In this report, we have analyzed the concentration-

dependent time evolution of intrinsic fluorescence of human serum albumin with PEG-8000(polyethylene glycol)crowder.



Scheme 1: Schematic Representation of Fibril Formation via Intermediate Oligomers

Chapter 2: EXPERIMENTAL SECTION

Materials and Method:

Human Serum Albumin (HSA), PolyethyleneGlycol(PEG)-8000, Disodium Phosphate (Na_2HPO_4) and monosodium phosphate(NaH_2PO_4) were purchased from Sigma Aldrich(India).

Preparation of pH 7.4 Phosphate Buffer: pH 7.4 phosphate buffers were prepared by dissolving 103.75mg disodium phosphate and 15.6mg of monosodium phosphate in 100 mL milliQ water.

Preparation of Stock solution of HSA: 1mM stock solution of HSA was prepared by dissolving 16.60mg of HSA in 0.5 mL buffer solution at room temperature.

Preparation of PEG-8000crowder: 40% PEG-8000 stock solution was prepared by dissolving 2 g of PEG-8000 in 5 mL phosphate buffer. Then, 750µL of this 40 % solution was used for 10 % PEG-8000 solution.

Preparation of HSA at different temperature: 1µM, 3µM, 5µM, of HSA was prepared by dissolving 3, 9, and 15 µLofphosphate buffer in a glass vial and was kept at room temperature. Likewise another set was prepared and it was kept at 37°C in an oven.

Preparation ofHSA+ PEG solution: 1 µM HSA +PEG (10%) solution, was prepared by dissolving 3µL HSA with 750 µL PEG which was further dissolved in 2247µLof phosphate buffer. Likewise, for 3µMHSA+ 10 % PEG solution, 9µL, HSA was dissolved in 750µL PEGwhich was further dissolved in 2241µL of phosphate buffer. Similarly, for 5µMHSA+ 10 % PEG solution, 15µLHSA dissolved in 750µL PEG which was further dissolved in 2235µL of phosphate buffer. These solutions were prepared in two sets; one set was placed at room temperature and the another at 37°C in an oven.

Turbidity Measurements: The turbidity of HSA and (HSA +PEG) solutions can be calculated according to the following equation:

$$T = 100-(100\times10^{-A})$$

Where, T is the turbidity and A is the absorbance at 400 nm.

Instrumentation: Absorption spectra were recorded by using a Varian UV–vis spectrophotometer (Cary 100 Bio) in a quartz cuvette (1 cm × 1 cm). Photoluminescence (PL) spectra were recorded by using a Fluoromax-4 spectrofluorometer (Horiba JobinYvon, model FM-100) with excitation and emission slit widths at 5 nm.Field-emission scanning electron microscopy (FESEM) measurements were performed by using a Supra 55 Zeiss field-emission scanning electron microscope.

Chapter 3: RESULTS AND DISCUSSION

Biological polymers are very famous for liquid-liquid phase transition (LLPS) present inside the cellular body in different forms such as granules and nucleoli to perform various biological activities. In order to investigate the LCD (low concentration domain)-mediated LLPS under suitable conditions, we have utilised intrinsically ordered HSA protein and a crowding agent PEG-8000. For the LLPS regime, we have examined two results one is only with protein and another is protein in presence of crowder PEG.

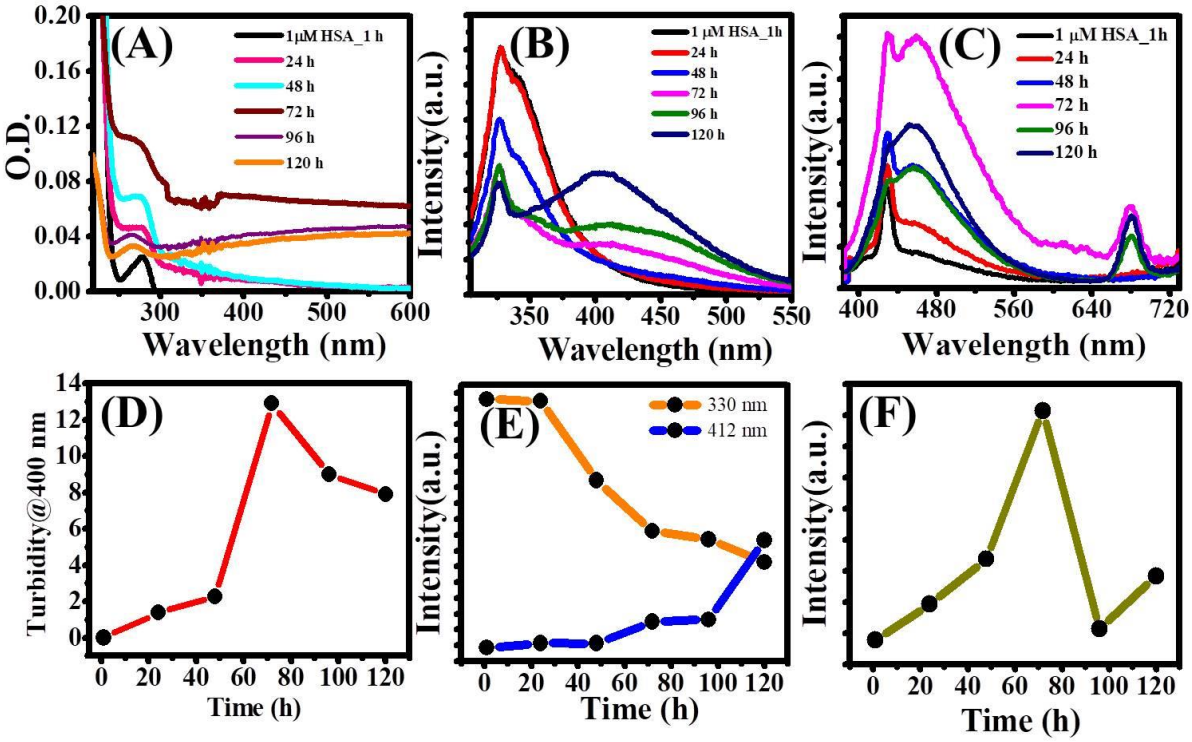


Figure 1: (A) UV-vis spectra of 1μM HSA atdifferent time interval.Fluorescence spectra of 1μM HSA atdifferent time interval with excitation wavelength of (B) 295 nm, and (C) 375 nm.(D) Changes in turbidity values as a function of time by taking absorbance values at 400 nm. (E) Changes in fluorescence intensity at 330 and 412 nm upon excitation at 295 nm. (F) Changes in fluorescence intensity at 430 nm upon excitation at 375 nm. All the measurements were performed at RT.

In the first experiment, figure 1(A) shows the UV-vis spectra, and figure 1(B), (C) shows the fluorescence spectra of 1 μM HSA protein at different time interval with excitation wavelength of 295 and 375 nm at room temperature. In the initial time period, solution mixture was transparent shows the highly soluble dispersed phase of HSA monomers. But upon increasing the incubation time, the solution becomes turbid over the period of 3 days which highlights the formation of aggregates between the HSA monomers. For clear understanding, we have plotted turbidity profile of 1 μM HSA protein as a function of time as shown in figure 2(D). After 48 h from the initial time zone, turbidity value was sudden increase and uplifted from 1 to 13. Figure 1(E) shows the decrease and increase in fluorescence intensity at 330 nm and at 412 nm, respectively at excitation wavelength of 295nm of 1 μM HSA protein incubated at different time period and at room temperature. While figure 1(F) shows the increase in fluorescence signal at 430 nm over the period upon excitation at 375 nm at room temperature.

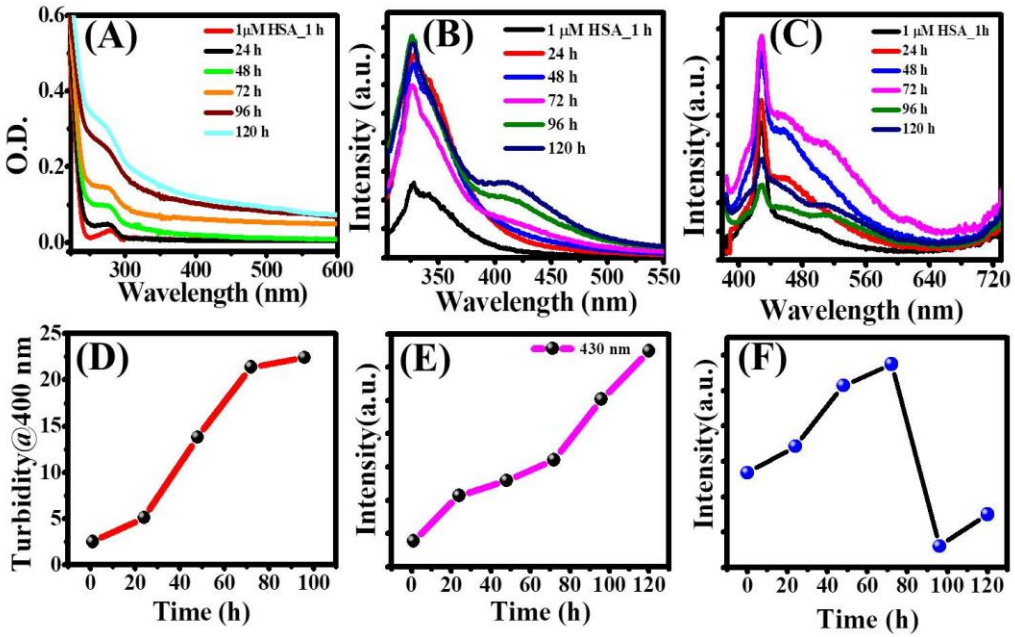
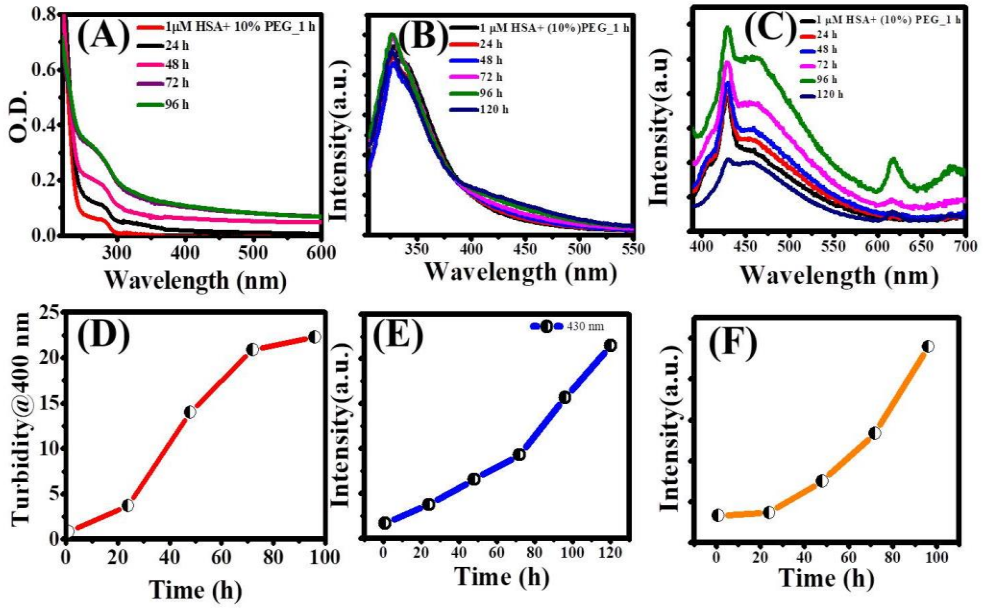


Figure2:(A) UV-vis spectra of 1 μM HSA at different time interval. Fluorescence spectra of 1μM HSA at different time interval with excitation wavelength of (B) 295 nm, and (C) 375 nm. (D) Changes in turbidity values as a function of time by taking absorbance values at 400 nm. (E) Changes in fluorescence intensity at 430

nm upon excitation at 295 nm. (F) Changes in fluorescence intensity at 430 nm upon excitation at 375 nm.

All the measurements were performed at 37ºC.As the phase separation of protein strongly depends on temperature, therefore we have also studied this LLPS of HSA protein at 37 ℃. Figure 2 shows the UV-vis and fluorescence spectra of HSA protein at 37 ℃ with their corresponding turbidity and intensity plots. We have observed the similar trend but with higher turbidity values (maximum value = 24) and more fluorescence intensities at both excitation wavelengthsin comparison to room temperature.

Figure 3:(A) UV-vis spectra of 1 μMHSA in presence of 10 % PEG-8000 at



different time interval. Fluorescence spectra of 1μM HSA in presence of 10 % PEG-8000 at different time interval with excitation wavelength of (B) 295 nm, and (C) 375 nm. (D) Changes in the turbidity values as a function of time by taking absorbance values at 400 nm. (E) Changes in fluorescence intensity at 430 nm upon excitation at 295 nm. (F) Changes in fluorescence intensity at 460 nm upon excitation at 375 nm. All the measurements were performed at RT.

On the addition of the molecular crowder i.e. polyethylene glycol (PEG-8000), the human serum albumin showed the occurrence of turbidity which is due to

liquid like droplet formation. Figure 3(D) shows the increase in turbidity with the increase in time. Figure (B,C) further shows that the fluorescence intensity increases with incubation time of HSA and PEG. Figure (D,E) shows theintensity plot at 430nm, excited at 295 nm and at 375nm it thereby confirms that the fluorescence intensity increases with the addition of PEG crowder with time.

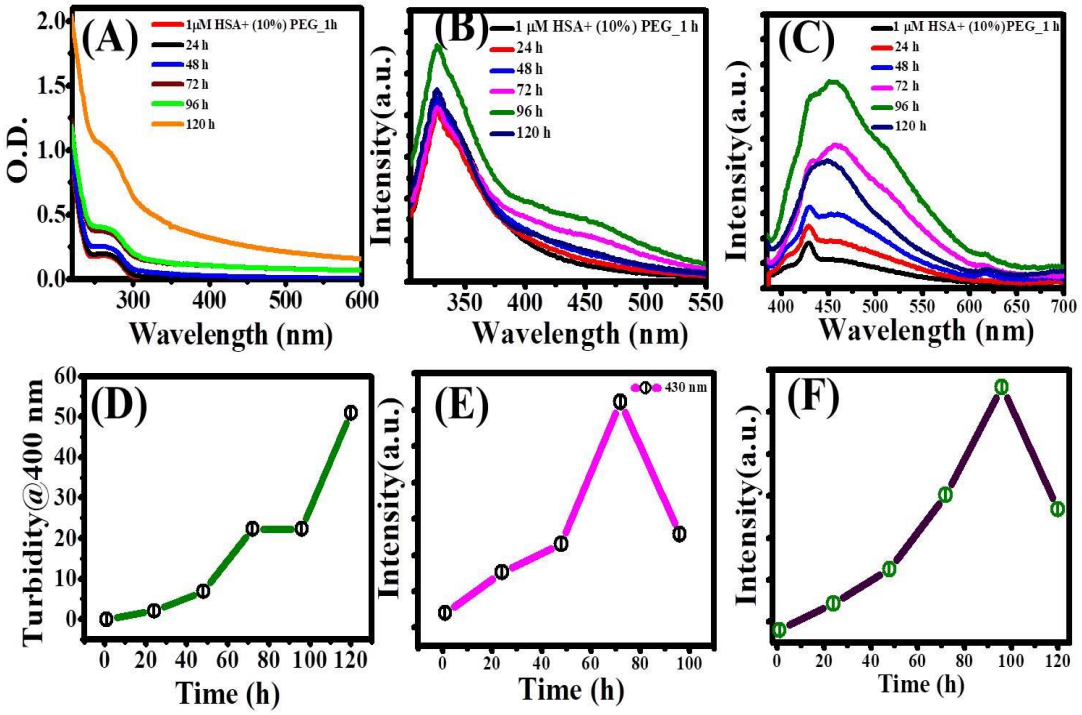


Figure 4:(A) UV-vis spectra of 1 μM HSA in presence of 10 % PEG-8000 at different time interval. Fluorescence spectra of 1μM HSA in presence of 10 % PEG-8000 at different time interval with excitation wavelength of (B) 295 nm, and (C) 375 nm. (D) Changes in the turbidity values as a function of time by taking absorbance values at 400 nm. (E) Changes in fluorescence intensity at 430 nm upon excitation at 295 nm. (F) Changes in fluorescence intensity at 460 nm upon excitation at 375 nm. All the measurements were performed at 37ºC.

As the phase separation of protein strongly depends on temperature, therefore we have also studied this LLPS of HSA protein at 37 ℃ in presence of crowder. Figure 4 shows the UV-vis and fluorescence spectra of HSA protein at 37 ℃ with

their corresponding turbidity and intensity plots. We have observed the similar trend but with higher turbidity values (maximum value = 55) and more fluorescence intensities at both excitation wavelengths in comparison to room temperature.

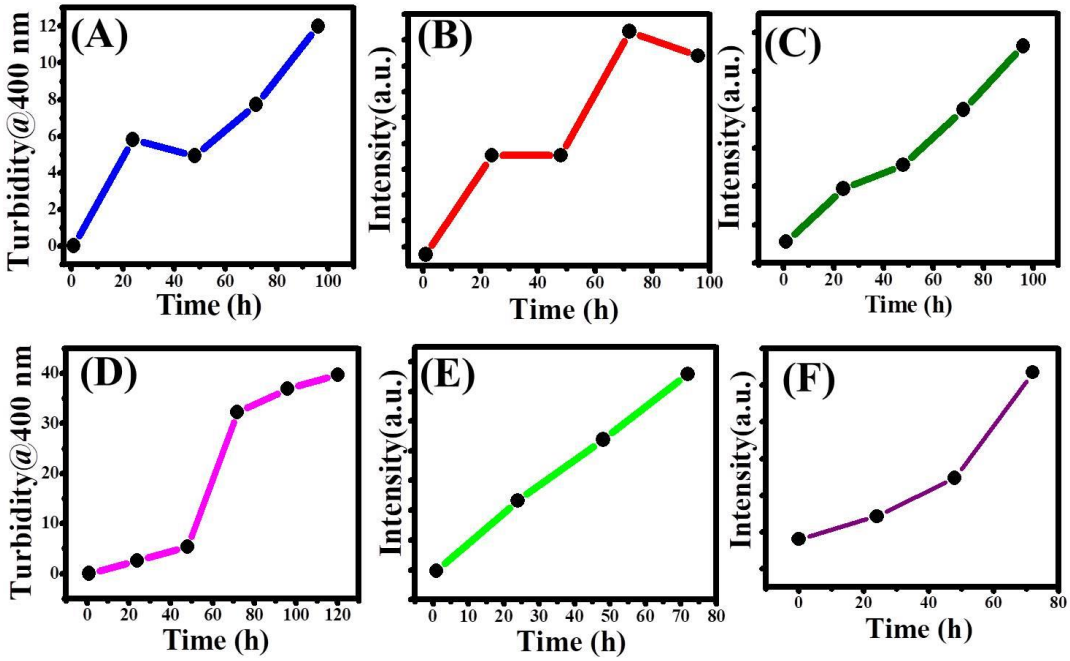


Figure 5: Changes in the turbidity values as a function of time by taking absorbance values at 400 nm of 3μM HSA (A) at RT, and (D) at 37ºC. Changes in fluorescence intensity at 430 nm upon excitation at 295 nmof 3μM HSA (B) at RT, and (E) at 37ºC.Changes in fluorescence intensity at 460 nm upon excitation at 375 nmof 3μM HSA (C) at RT, and (F) at 37ºC.

On increasing the concentration of HSA from 1 μM to 3μM at room temperature further showed increase in the turbidity value and intensity of fluorescence. Wehaveobserved that there was increase in turbidity at 37ºC as it can be seen from figure 6 (D). The turbidity gradually increased within 48 hours and then it increase drastically in next 24 hour and stabilized thereafter but the value of turbidity is more than the RT.

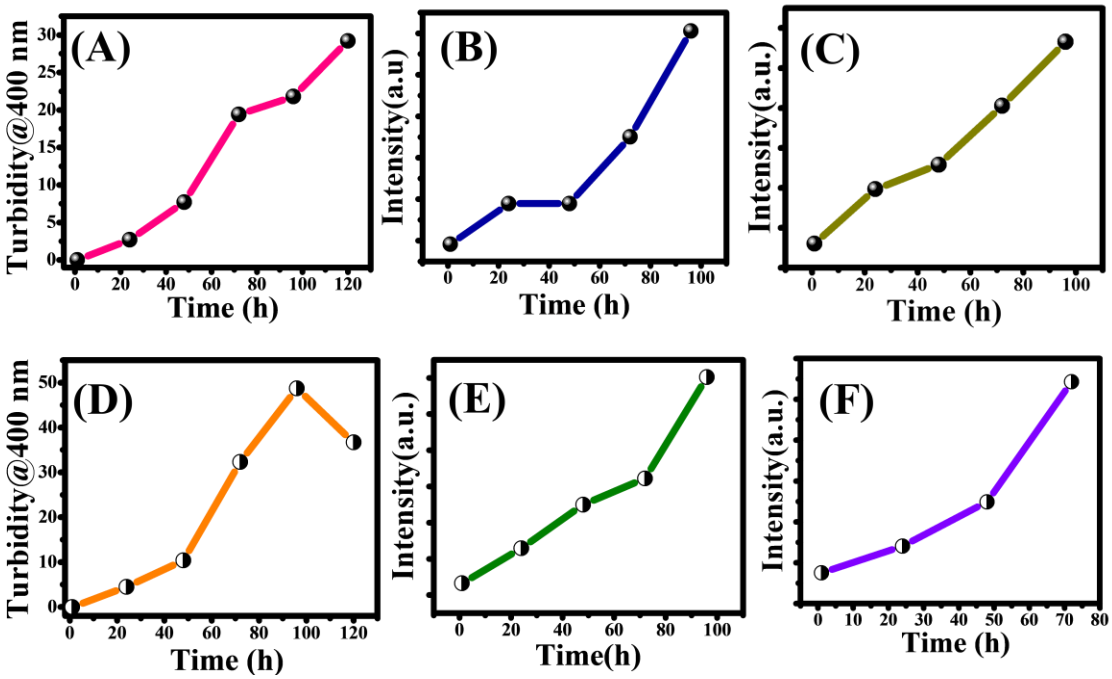


Figure 6: Changes in the turbidity values as a function of time by taking absorbance values at 400 nm of 3μMHSA in presence of PEG-8000 (A) at RT, and (D) at 37ºC. Changes in fluorescence intensity at 430 nm upon excitation at 295 nm of 3μMHSAin presence of PEG-8000 (B) at RT, and (E) at 37ºC.Changes in fluorescence intensity at 460 nm upon excitation at 375 nm of 3μMHSA in presence of PEG-8000(C) at RT, and (F) at 37ºC.

When only HSA was used the maximum turbidity obtained was 12, while on addition of the PEG-8000 crowder, the maximum turbidity obtained was nearly 30. Likewise on increasing the temperature the turbidity also increased as it can be seen from figure (D), at 37ºC the maximum turbidity obtained was near about 50. Intensity of fluorescence also increased with increase in temperature and addition of PEG crowder.

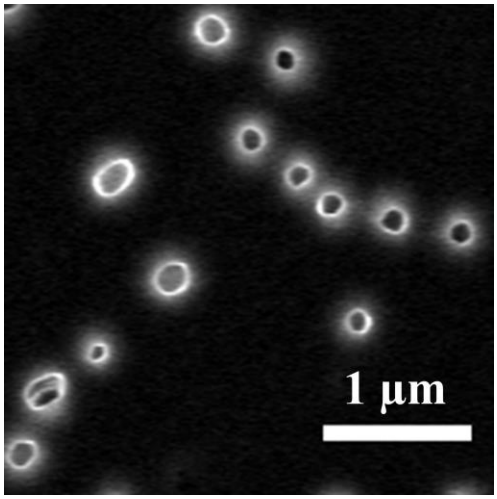


Figure 7: FE-SEM image of 3 µMHSA in presence of PEG-8000 incubated for 4 days at RT.

The FE-SEM image shows the formation of coacervates at 3 µM HSA concentrationincubated for 4 days at RT at room temperature. It is clear from the image that there was the formation of aggregate at this concentration of Human serum albumin. The FE-SEM image reveals the well-dispersed spherical droplets, indicates the formation of colloidally stable coacervates. However, we have not observed liquid-like droplets in the absence of PEG-8000.

Conclusions

In this thesis, we explored the photoluminescence of Human serum albumin at different temperatures and with PEG crowder. We have examined that HSA undergoes self-oligomerisation and shows turbidity. It can be seen that at room temperature, the maximum turbidity obtained was 12, while when the same Human serum albumin was kept at higher temperature, i.e. at 37ºC the maximum turbidity obtained was at 22. Further when crowding agent was added PEG-8000, there was increase in turbidity and the maximum value obtained was 25 at room temperature itself. To further explore the result SEM was done which confirmed the formation of liquid droplet which was well separated from the solution phase. Thus, it can be said that Human serum albumin shows temperature dependent oligomerisation and on addition of the crowder it undergoes liquid-liquid phase separation.

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