Synthesis and Characterization of Amino acid Functionalized Silver Nanoparticles and their Interaction with Lipid Membrane

M.Sc. Thesis

by

PUJA KUMARI

1903131019

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Synthesis and Characterization of Amino acid Functionalized Silver Nanoparticles and their Interaction with Lipid Membrane

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PUJA KUMARI

DISCIPLINE OF CHEMISTRY

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Synthesis and characterization of amino acid functionalized silver nanoparticles and their interaction with lipid membrane** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DISCIPLINE 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. Anjan Chakraborty, Associate Professor, IIT Indore.

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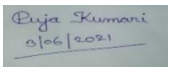

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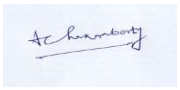

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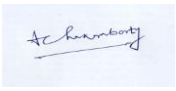

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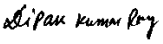

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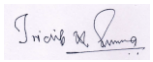

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Puja Kumari

M.Sc. 2nd year

Dedicated to My Family

ABSTRACT

The effect of the nature of the bare NPs surface in presence of the same ligands in the lipid bilayer and nanoparticles interaction (lipid corona formation) is not well studied in the literature. Therefore, we are interested to study the intermixing of the amino acid functionalized gold and silver nanoparticles with the lipid bilayer. In the present contribution, we have synthesized aromatic amino acid functionlized silver nanoparticles to look into the intermixing of these with the lipid membrane and compare this with our previous results with amino acid functionalized gold nanoparticles. Firstly, both the concentration of sodium hydroxide and amino acids play a crucial part in the formation of colloidal stable silver nanoparticles, These silver nanoparticles have well fluorescence properties because of clusterization of the amino acids on the NPs plane. The Ag-Tyr NPs at low lipid measurement of zwitterionic lipid vesicles are stable which is opposite to our previous results for Au-Tyr NPs. This specifies the part of bare NPs plane important part in the NPs and lipid bilayer interaction.

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NOMENCLEATURE

nm	Nanometer
mL	Milliliter
µL	Microliter
mM	Millimolar
°C	Degree Celsius

ACRONYMS

Ag NPs	Silver Nanoparticles
Ag-AA NPs	Amino acid functionalized silver nanoparticles
Phe	Phenylalanine
Tyr	Tyrosine
Trp	Tryptophan
MLV	Multi-Lamellar Vesicle
LUV	Large Unilamellar Vesicle
GUV	Giant Unilamellar Vesicle
SUV	Small Unilamellar Vesicle
DMPC	1,2-dimyristoyl-sn-glycero-3-

	Phosphocholine
$AgNO_3$	Silver nitrate
NaOH	Sodium Hydroxide
Ag-Tyr NPs	Tyrosine functionalized silver Nanoparticles
Ag-Trp NPs	Tryptophan functionalized silver nanoparticles
SPR	Surface Plasmon Resonance

Chapter 1

Introduction

(1.1) Silver Nanoparticles: A nanoparticle is a small particle in the range between 1-100 nm. Silver nanoparticles (Ag NPs) have wide range of advancement in different fields of biomedical, catalysis, sensors, biology, antimicrobial activities [1-4]. The formation of the silver nanoparticles is silver nitrate, reducing agent, and stabilizing agent [5-8]. Natural resources and their components are also used to synthesize the Ag NPs from plants and their extracts, bacteria, fungi, and biopolymers [9].

(1.2) Amino acid functionalized silver nanoparticles: Amino acids possess both -NH_2 and -COOH functional group and side chain (-R) group. It is well known that in most of the chemical transformation reaction –NH_2 and –COOH group play an important role while R group remain intact. Aromatic amino acids like phenylalanine, tryptophan, and tyrosine are essential aromatic amino acids that can be used to prepare the silver nanoparticles by on site conversation method. These aromatic amino acids behaving as reducing and capping agents and play important role in the preparation of different sized amino acid functionalized Ag NPs. It is well established that tryptophan and tyrosine can be used in electron/hydrogen transport via radical intermediates in biological systems [10-16]. So, different ligand functionalized silver nanoparticles are prepared using aromatic amino acids, $AgNO_3$, NaOH. First, we optimized the reaction condition to form colloidal stable ag NPs by varying the concentration of aromatic amino acids, and NaOH. Although peptide functionalized (in end group tryptophan or tyrosine) fluorescence silver nanoparticles have been previously synthesized, however, their fluorescence properties are unstable [17-18].

(1.3) Lipid Bilayers(Liposomes): Phospholipids and protein membrane are main constituents of the cell membrane [19]. The lipid bilayer is the barrier between the cell and extracellular components [20]. The lipid bilayer is composed of lipid. Lipid has two parts: the first one is a polar head group (phosphate and choline group) and another one is the hydrophobic tail part

which consists of a long hydrocarbon chain. When phospholipids are immersed in polar solvent like water, a second lipid layer is attached and lipid bilayer is formed so to seize the hydrophobic tail from water.

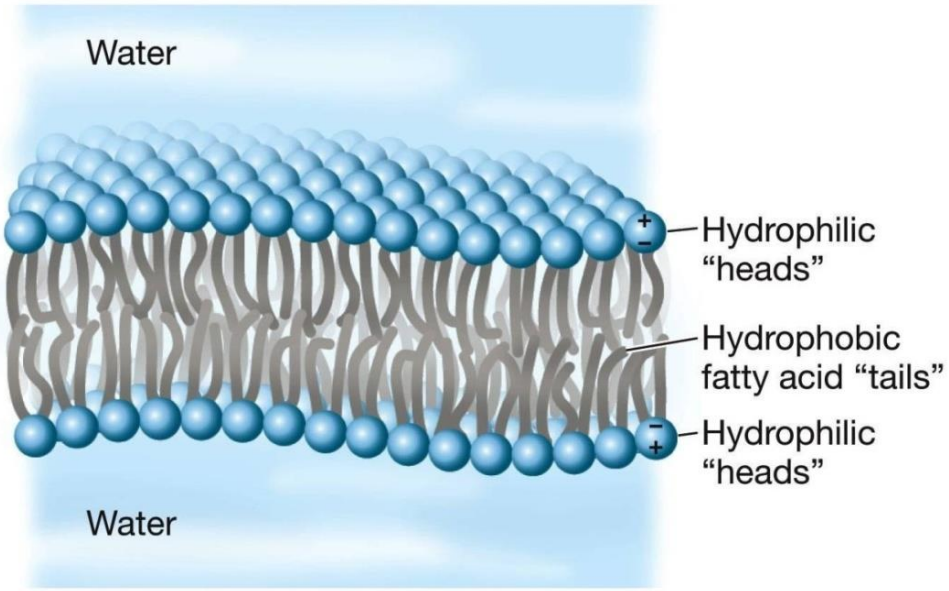

Figure: Phospholipid bilayer [21]

Long hydrocarbon chains group each other to form the lipid bilayer. Phospholipid formed liposomes are called PC liposomes. Recently, lipid membranes draw significant interest due to their interaction with metal ions, amino acids, polymer, nanoparticles [22-29]. Several methods are used to prepare the lipid bilayers from lipid molecules (i) reverse-phase evaporation [30] (ii) thin-film hydration [31] (iii) solvent injection [32]. lipid bilayers can be classified in terms of their size as), GUV- Giant Unilamellar Vesicle (>1µm), LUV- Large Unilamellar Vesicle (> 100 nm), MLV- Multi-Lamellar Vesicle (>0.5µm), and SUV- Small Unilamellar Vesicle (20-100 nm).

(1.4) Studying the nano-bio interaction of nanoparticles with lipid bilayer: Lipid bilayer and the metal nanoparticles interaction is an

emerging field as they usually produce biocompatible systems [33-38]. The interaction of inorganic nanoparticles and the different lipid bilayers is well studied in the literature [39-42]. In lipid the phosphate group head served as the most important role in metal oxide nanoparticles with lipid bilayer [43-45]. Our group recently studied the interaction so to functionalized the gold nanoparticles with the aromatic amino acid(Au-AA NPs) with different concentrations of different surface charged lipid bilayers [46]. The results showed that at high value of the concentration of lipid, the Au-AA NPs form lipid corona whereas lower value of l, the Au-AA NPs undergo lipid-induced aggregation with not only positive charged vesicles but also with the neutral charged vesicles. In this present contribution, we are interested to investigate the consequence of the nature of the nanoparticles (hydrophilic Ag and hydrophobic Au) in the lipid corona formation and lipid-induced aggregation where capping and stabilizing ligands (amino acids) are the same.

Chapter2

EXPERIMENTAL SECTION

(2.1) Chemicals and Reagents:. Silver nitrate ($AgNO_3$), Phenylalanine (Phe), tryptophan (Trp), tyrosine (Tyr), and 4-(2-hydroxyetyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. The neutral phospholipid DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids. Sodium Hydroxide(NaOH) and Milli-Q water were purchased from Merck. all these chemicals were used as received without further purification. Milli-Q water is used to prepared all the solutions. We kept all the glassware in 3:1 HCl/HNO3 (aqua regia) overnight and cleaned it properly before doing any experiments.

(2.2) Synthesis of amino acid functionalized Silver nanoparticles: We have taken a green synthesis in situ technique to prepare the silver nanoparticles (Ag NPs) by using aromatic amino acids with slight modification. We have varied the concentration of the amino acids (0.2×10^{-3} to 2.0×10^{-3} M) and sodium hydroxide (5×10^{-3} to 50×10^{-3} M)to optimize the reaction condition to synthesize the colloidal and stable AgNPs. Briefly, 0.125 mL of 7.5×10^{-4} M aqueous metal ion solution was mixed in 3.875 mL of Milli-Q the resulting solutrion with water was heated to 80 °C for 30 min. At last the required value of amino acid added with concentration of NaOH and the final solution was kept under stirring for 2 hours. The formation of colloidal Ag NPs was initially studied by the color changes (from colorless to yellow) of the solution.

(2.3) Preparation of three Silver Nanoparticles with the optimized condition: We have optimized the condition of the formation of stable and colloidal Ag NPs by varying the concentration of amino acids and NaOH. $AgNO_3$ solution (0.75 mM) was mixed in 3.875 mL of Milli-Q water and finally the solution was heated at 80 °C for 30 min. Then, 1 mL of 5

mM amino acid solution (solution concentration 1mM) (for all the three amino acids) was added followed by 5 mM NaOH (for Trp and Tyr) and 10 mM NaOH (for Phe) under vigorous stirring for 2 hours. The formation of colloidal and stable Ag NPs was primarily observed by changing the colour of solution(from colorless to yellow). All the solutions were first put at room temperature then 4 °C for the further experimental work.

(2.4) Fluorescence spectrum of Nanoparticles: We have taken the fluorescence emission and excitation spectrum of both the stock and diluted (diluted 10 times of the stock solution) solution of tryptophan and tyrosine functionalized Ag NPs. We excited all these samples in the range of 280-400 nm and the emission spectrum recorded. For the UV-Visible spectrum, we also diluted 10 times of all the stock solutions.

(2.5) Preparation of Lipid Vesicles: We prepared the zwitterionic DMPC lipid vesicles in HEPES buffer solution (pH=7.0) following the ethanol injection method. At first, the buffer solution was heated at 70 °C at large value of trasition temperature of the DMPC lipid for 1 hour. Then the specific amount of lipid was dissolved in ethanol (0.01% of the hydrating solution) and injected into already heated buffer solution. For doing any experimental work, the solution was cooled for 4-5 hours after one hour of heating. The concentration of the final lipid solution was 0.8 mM.

(2.6) Lipid Vesicle-Nanopartcle mixture preparation: To investigate the interaction of tryptophan and tyrosine functionalized silver nanoparticles (Ag-Trp NPs and Ag-Tyr NPs) with the zwitterionic DMPC lipid vesicles, we have chosen two different lipid concentration. One is high lipid concentration (0.8 mM) and another is low lipid concentration (0.0125 mM). Then, in a fixed Ag NPs solution, two different concentrations (high and low) lipid vesicles were added and the mixture was kept overnight.

(2.7) Instrumentation: The absorption spectra of the aromatic amino acid functionalized silver nanoparticles (Ag-AA NPs) was recorded by using Varian UV-vis spectrophotometer (Cary 100 Bio) in a quartz cuvette (10 × 10 mm^2). FluoroMax-4p spectrofluorometer from Horiba Jobin Yvon (model:

FM100) was used to record the fluorescence spectra of the Ag-AA NPs. OriginPro 8.1 software was used to analyze all the absorption and fluorescence spectra. We maintained room temperature (25 °C) throughout. The DLS and ζ potential of the synthesized Ag-Trp NPs and Ag-Tyr NPs was measured using NanoPlus ζ/particle size analyzer (NanoPlus-3 model).

Chapter 3

RESULTS AND DISCUSSION

(3.1) Optimization and Characterization of differently amino acid functionalized Ag NPs:

Herein, different amino which are aromatic acids namely a) Tryptophan b) Tyrosine c) Phenylalanine functionalized silver nanoparticles (Ag-AA NPs) was synthesized by an on site conservation in which aromatic amino acids act as not only stabilizing but also reducing agent. The structure of the three aromatic amino acids is shown below **(figure 2)**.

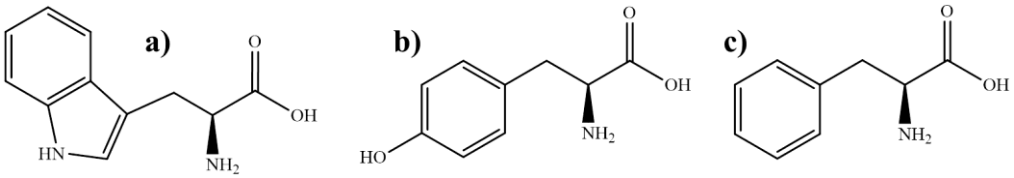

Figure 2: Molecular structure of a) Tryptophan b) Tyrosine c) Phenylalanine.

As mentioned earlier in the material section, we optimized the condition of the formation of colloidal and stable Ag NPs by varying the concentration of the amino acids and NaOH. Firstly, for synthesizing tryptophan functionalized silver nanoparticles (Ag-Trp NPs), the concentration of NaOH was varied at a constant concentration of $AgNO_3$ (0.75 mM) and Trp (1 mM). With large concentrated of NaOH from 5 to 20 mM, we have observed a broad SPR peak of AgNPs, indicating the formation of larger Ag-Trp NPs **(figure 3a)**. So, the optimized NaOH concentration is 5 mM. Then, see the changes in amino acid concentration in the formation of Au-Trp NPs, we have varied the concentration of tryptophan from 0.2 mM to 2 mM at given value of $AgNO_3$ (0.75 mM) and NaOH (5 mM). The UV-Visible spectra show that at a low concentration of tryptophan (0.2 mM and 0.5 mM) the SPR peak was broadened and in high concentration (2 mM), the SPR peak was shifted to a longer wavelength **(figure 3b)**. So, at optimized concentration [$AgNO_3$ (0.75 mM), Trp (1 mM), and NaOH (5 mM)], the SPR peak of the Ag-Trp NPs seen at ~ 409 nm.

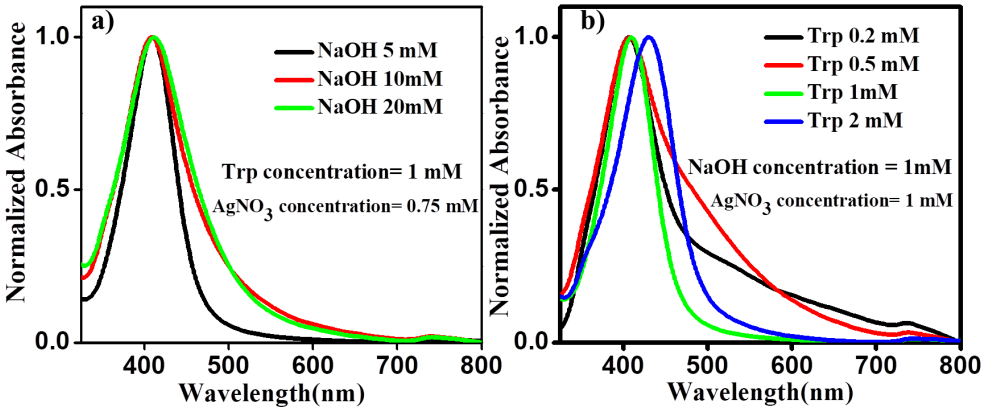

Figure 3: UV-Visible absorption spectrum of Ag-Trp NPs at various concentrations of NaOH (a) and Trp (b) at a fixed concentration of $AgNO_3$ (0.75 mM).

Similarly, for tyrosine functionalized silver nanoparticles (Ag-Tyr NPs), we have studied the effect of tyrosine and NaOH concentration. With the increase of the NaOH concentration, the SPR peak of the Ag-Tyr NPs is broadened **(figure 4a)**. Also, at low concentration (0.2 mM and 0.5 mM) and high concentration (2 mM) of Tyr, the SPR peak of the Ag-Tyr NPs is broadened and shifted in longer wavelengths respectively **(figure 4b)**. So, at optimized concentration [$AgNO_3$ (0.75 mM), Tyr (1 mM), and NaOH (5 mM)], the SPR peak of the Ag-Tyr NPs was observed at ~ 416 nm.

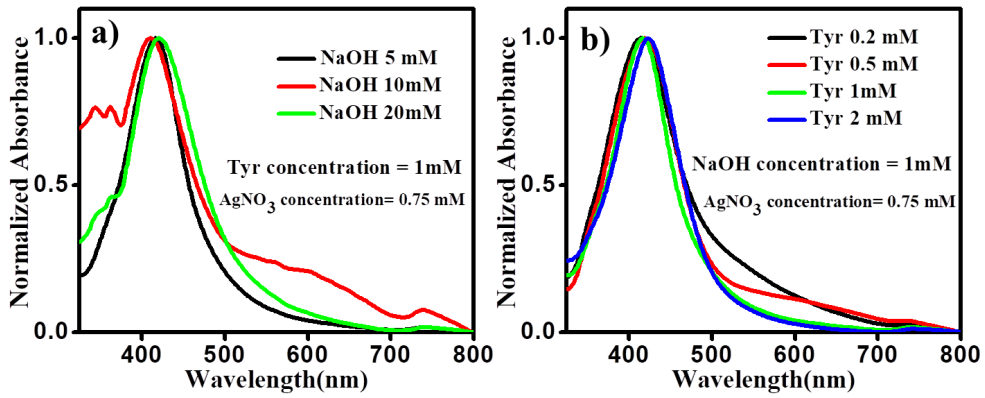

Figure 4: UV-Visible absorption spectrum of Ag-Tyr NPs at various concentration of NaOH (a) and Tyr (b) at a fixed concentration of $AgNO_3$ (0.75 mM).

Now, to synthesize phenylalanine functionalized silver nanoparticles (Ag-Phe NPs), we again varied the concentration of the phenylalanine and NaOH. The UV-Visible spectra show that with the increase of the concentration of the phenylalanine from 0.5 to 2 mM, a secondary peak is observed along with the presence of the primary SPR peak which indicates the less colloidal stability of Ag-Phe NPs **(figure 5a)**. At optimized concentration [$AgNO_3$ (0.75 mM), Phe (1 mM), and NaOH (10 mM)], the SPR peak of the Ag-Phe NPs was observed at ~ 414 nm **(figure 5b)**.

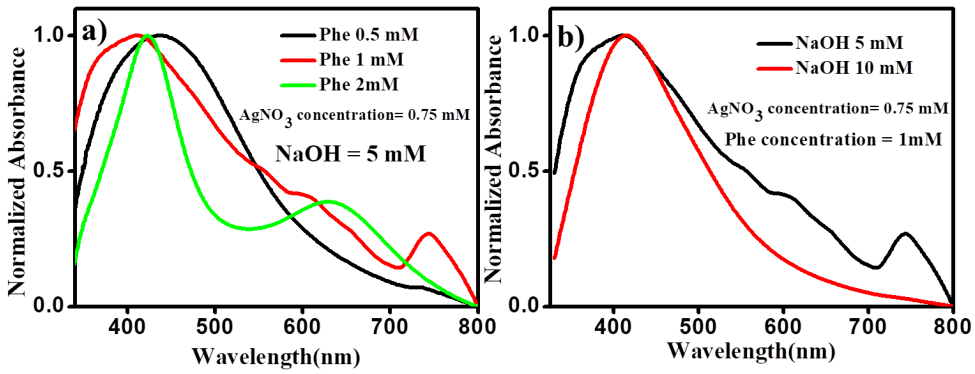

Figure 5: UV-Visible absorption spectrum of Ag-Phe NPs at various concentrations of Phe (a)and NaOH (b) at a fixed concentration of $AgNO_3$ (0.75 mM).

The UV-Visible spectra of all the Ag-AA NPs in optimized condition are shown in **figure 6**. The less colloidal stability of the Ag-Phe NPs than Ag-Trp NPs and Ag-Tyr NPs proves that the secondary amine group of the Trp and the –OH group of Tyr important in nucleation and stabilization of the Ag-Trp NPs and Ag-Tyr NPs respectively.

At last, we have successfully synthesized the Ag-AA NPs. The formation of the stable colloidal Ag-AA NPs was primarily established by UV-Visible spectroscopy.

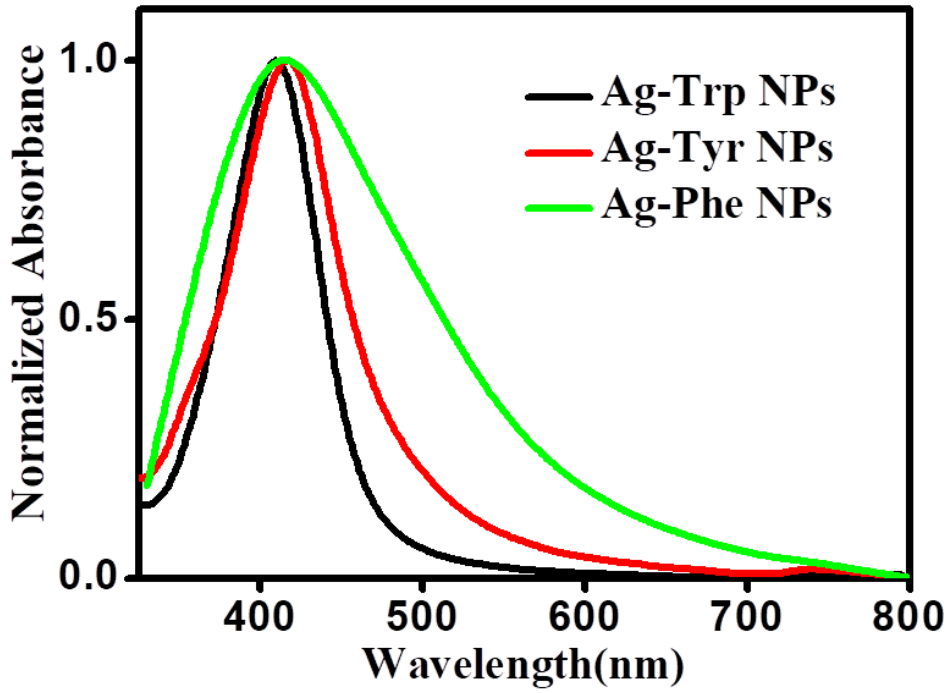

Figure 6: UV-Visible absorption spectrum of Ag-AA NPs at an optimized concentration of the $AgNO_3$, amino acids, and NaOH.

(3.2) Fluorescence Emission and Excitation spectra of Ag NPs:

The excitation wavelength-dependent fluorescence properties of the Ag-Trp and Ag-Tyr NPs were observed **(figure 7a and 7c)**. Maximum emission for the Ag-Trp NPs was detected at 390 nm with a humped peak at 436 nm while for Ag-Tyr NPs, maximum emission peak seen at 405 nm with humped peak at 454 nm **(figure 7b and 7d)**. The fluorescence of these blank amino acids is well reported in the literature. Tryptophan shows emission at 350 nm while tyrosine shows at 300 nm in a water medium. The distinct fluorescence properties indicate that the fluorescence property of the Ag-Trp and Ag-Tyr NPs is not coming from the blank amino acids. Our UV-Visible data suggest that size of the Ag NPs is in between 40-45 nm. It is well known that NPs in this size range is not fluorescent. So, the emission property of the Ag NPs is coming from the ligand (amino acids). The different fluorescence properties may be due to the different microenvironments of the aromatic amino acids on the silver nanoparticles surface [47].

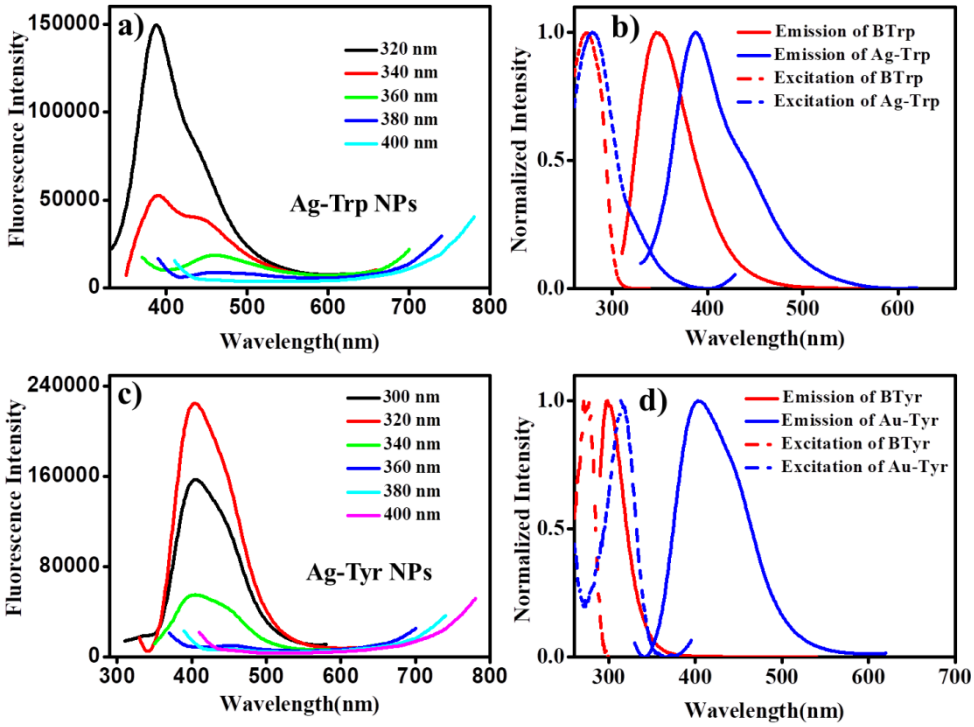

Figure 7: Excitation-wavelength-dependent fluorescence spectra of (a) Ag-Trp NPs and (c) Ag-Tyr NPs. The fluorescence excitation and emission spectra of the blank amino acids and amino acid functionalized Ag NPs at maximum emission wavelength for (b) Trp and (d) Tyr.

(3.3) Studying the interaction of Ag NPs with DMPC lipid vesicles: To check the interaction of the Ag-Trp NPs and Ag-Tyr NPs with zwitterionic lipid vesicles, the constant concentration of the Ag-AA NPs was incubated in various concentrations (0.4 mM and 12.5 μM) of the liposome overnight to reach the equilibrium. The UV-Visible absorption spectra of the Ag-Tyr NPs at high lipid (0.4 mM) of neutral (DMPC) lipid vesicles exhibited a red-shifted (~8 nm) SPR peak whereas, at low lipid concentration (0.0125 mM), ~5 nm red-shifted SPR peak was observed **(figure 8a)**. Our previous results for Au-Tyr NPs showed that low concentration of neutral DMPC lipid, visual aggregation of the NPs was observed [46]. This indicates that the nature of the NPs (Au or Ag) shows remarkable effect in the aggregation of the tyrosine functionalized nanoparticles. The UV-Visible studies states that the absorption spectra of the Au-Trp NPs in different lipid concentration has no spectral shift which is in accordance with our results for Au-Trp NPs **(figure**

8b).

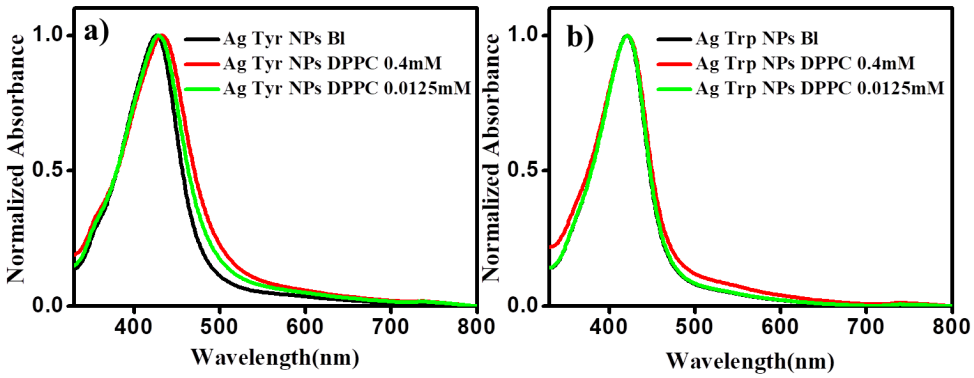

Figure 8: Normalized UV-Visible spectra of the Ag-Tyr NPs (a) and Ag-Trp NPs (b) in the variation of different DMPC lipid vesicles concentrations.

CONCLUSION

In summary, different aromatic amino acids functionalized silver nanoparticles were made in optimum conditions and varying the concentration of amino acids and NaOH. We also studied the interaction of these with neutral DMPC lipid vesicles. Our results show that both the concentration of the amino acids and NaOH have a significant part in the formation of the colloidal stable Ag NPs. These amino acids functionalized silver nanoparticles have excellent fluorescence properties which suggest that these Ag NPs can be used in the bioimaging of the cells. The interaction of the Ag-Tyr NPs with DMPC lipid vesicles is different than that of the Au-Tyr NPs. This indicates that the bare NPs surface also show the crucial part in the NPs with the lipid bilayer.

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