

# Spectroscopic Study of Interaction Between Extracellular Fungal Proteins and Biosynthesized Silver Nanoparticles

Swarup Roy\* and Tapan Kumar Das

**Abstract**— *Aspergillus foetidus* have been used to synthesize biological silver nanoparticles and extracellular proteins were also isolated from the same strain. The interaction between fungal isolated protein and silver nanoparticles was investigated by UV- Vis spectroscopy, fluorescence spectroscopy and circular dichroism technique. UV- Vis spectroscopic data demonstrates that formation of the complex between fungal proteins and silver nanoparticles occur. Fluorescence spectrum results clearly suggest that silver nanoparticles have a strong ability to quench the intrinsic fluorescence of protein by quenching mechanisms. The number of binding sites 'n' and binding constants 'K' were calculated according to the relevant fluorescence data. Thermodynamic parameters  $\Delta H$ ,  $\Delta G$  and  $\Delta S$  were calculated at different temperatures and the results showed that Van der Waals force and hydrogen bonding played a major role. Negative  $\Delta G$  values imply that the binding process is spontaneous. Circular dichroism results also revealed a conformational change.

**Index Terms**— *Aspergillus foetidus*, Extracellular Proteins, Silver nanoparticles, Spectroscopy

## 1 INTRODUCTION

Noble metallic nanoparticles are found to be increasing research attention in all fields of science for the past few decades due to having their attractive physicochemical properties. Among the metallic nanoparticles, silver nanoparticles (SNP) have been showing considerable attention because of their wide range of applications [1], [2], [3]. Even at low concentration of SNP it could show antibacterial and antifungal activities [4]. Hence, the demand of silver nanoparticles as antimicrobial agents is gradually increasing. To understand the mode of action of SNP against fungi an attempt should be taken to study of mechanism of interaction of *Aspergillus foetidus* mediated biosynthesized SNP with the extracellular proteins of the same fungi. Hence, present investigation emphasizes on understanding the biophysical study of interactions between SNP and extracellular fungal proteins with the study of UV-Visible, fluorescence, and circular dichroism (CD) spectroscopic analysis.

## 2 Experimental

### 2.1 Materials

Silver nitrate (Merck) was used in this experiment. The stock solution of isolated protein ( $1\text{mgml}^{-1}$ ) was prepared dissolving it in a 0.1(M) phosphate buffer (pH- 7.4). All of the other chemicals were of analytical reagent grade and double distilled water was used throughout.

### 2.2 Apparatus

Fluorescence spectra were recorded on a Agilent Technologies

Cary Eclipse Fluorescence Spectrophotometer. The absorption spectra were obtained from a Cary 100 UV-VIS Spectrophotometer Agilent Technologies and circular dichroisms were recorded in Jasco J-815 CD spectrometer.

### 2.3 Methods

#### 2.3.1 Synthesis and Characterization of SNPs

Silver nanoparticles were synthesized using cell filtrate of a fungus, *Aspergillus foetidus* and 1mM final concentration of silver nitrate. The biosynthesized SNP were characterized by different techniques as reported in our earlier report [5], [6].

#### 2.3.2 Isolation of fungal extracellular protein

Extracellular proteins were precipitated by using acetone precipitation of protein and the pellets formed were air dried & finally dissolved in 0.1(M) phosphate buffer (pH- 7.4). Protein content was determined by following the method of Lowry et al. [7].

#### 2.3.3 UV-Visible spectroscopy

Spectral changes of fungal protein were monitored after adding different concentrations of silver nanoparticle (0, 10, 20, 30, 40, 50, 60, 70 and 80  $\mu\text{M}$ ) in the range of UV- Visible absorption (250–450 nm).

#### 2.3.4 Intrinsic fluorescence

The intrinsic emission of protein was seen at the excitation wavelength of 270 nm. The experiments were repeated in the presence of different concentrations of SNP (0, 20, 40, 60, 80, 100, and 120  $\mu\text{M}$ ). The experiments were also carried out at different temperatures as 20, 30 & 40 °C.

#### 2.3.5 Circular dichroism spectroscopy

Protein solution was prepared in 0.1(M) phosphate buffer (pH- 7.4). 100  $\mu\text{gml}^{-1}$  of Protein solution was used to obtain the spectra. All spectra were taken in triplicate in the scan range, 190 to 230 nm and a back ground-corrected against buffer blank.

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### 3. Results and Discussion

#### 3.1 Absorption Characteristics of BSA-SNP interaction

Figure 1a show the absorption spectra of fungal protein and fungal protein in presence of increasing concentration of bio-synthesized SNP.

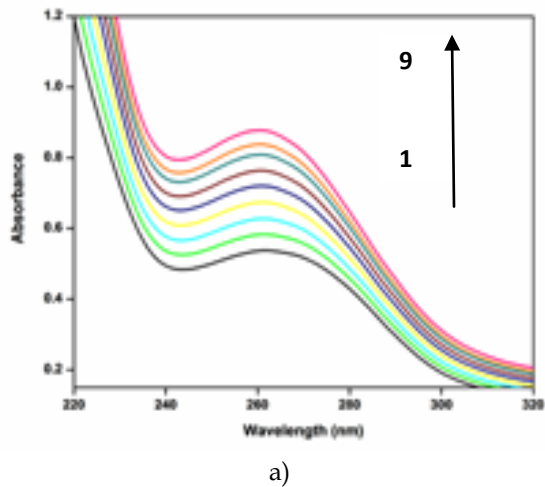


Fig. 1 UV- visible absorption spectra (bottom to top) of fungal protein in presence of SNP× 10<sup>-5</sup>; 0, 1, 2, 3, 4, 5, 6, 7 & 8 respectively;

It was found in Figure 1 as the nanoparticles concentration increases, the intensity at the wavelength of 270 nm increases significantly with a blue shift of 4 nm. This increase in intensity can reflect the formation of the ground state complex between fungal protein and SNP.

The values of the binding constant,  $K_{app}$ , were obtained from the protein absorption at 270 nm according to the methods published in the literature [8], the data were treated using linear reciprocal plots based on the Eq. (1) [9].

$$\frac{1}{A_{obs} - A_0} = \frac{1}{A_C - A_0} + \frac{1}{K_{app}(A_C - A_0)[SNP]} \quad \text{Eq. 1}$$

Where  $A_0$  is the absorbance of protein at 270 nm in the absence of SNP and  $A_C$  is the recorded absorbance at 270 nm for protein at different SNP concentrations. The double reciprocal plot of  $1/(A_{obs}-A_0)$  versus  $1/[Q]$  is linear and the bonding constant ( $K_{app}$ ) can be estimated to be  $5.6 \times 10^2 \text{ Lmol}^{-1}$  from the ratio of the intercept to the slope [8].

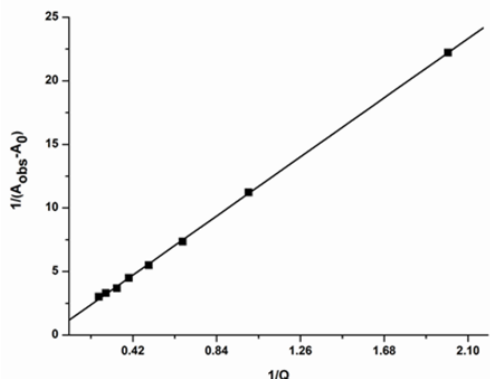


Fig. 2 Determination of  $K_{app}$  of fungal protein-SNP complex;  $1/(A_{obs}-A_0)$  versus  $1/[Q]$  plot.

#### 3.2 Characteristics of Fluorescence Spectra

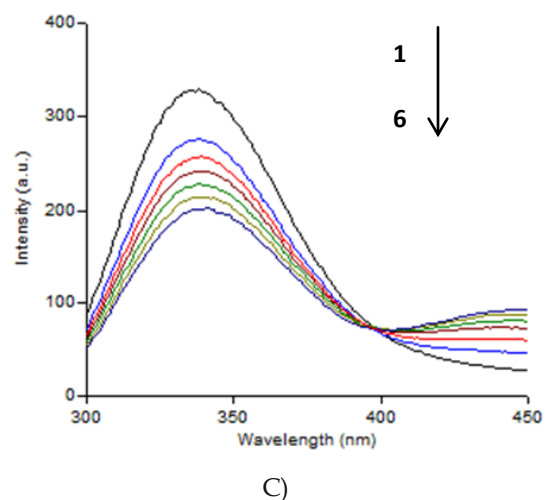
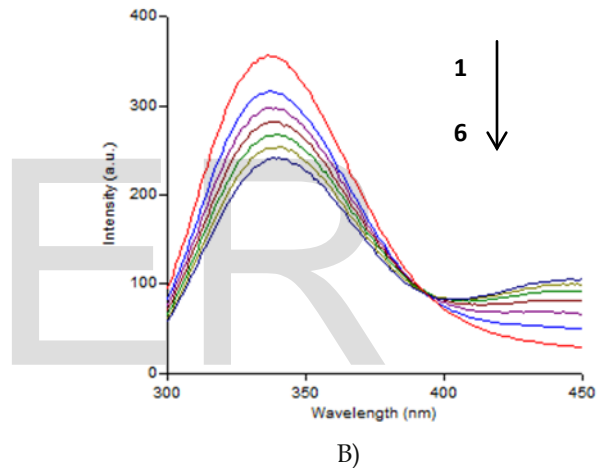
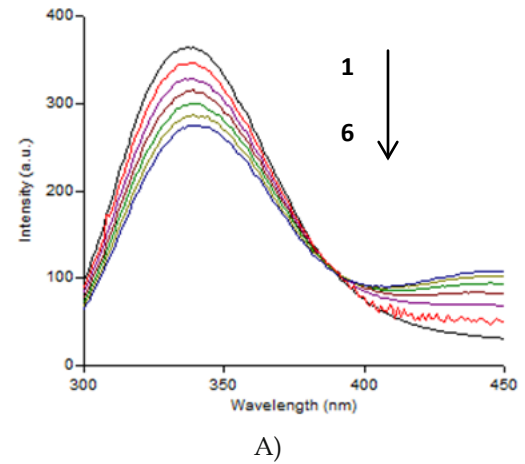


Fig. 3 The fluorescence quenching spectra of fungal protein by SNP at 20 °C, 30 °C & 40 °C (A), (B) & (C) respectively,  $\lambda_{ex} = 270 \text{ nm}$ ;  $C_{(protein)} = 1 \text{ mgL}^{-1}$ ;  $C_{(SNP)}/(10^{-5} \text{ mol L}^{-1})$  (a-k): 0, 2, 4, 6, 8, 10 & 12.

Figure 3 shows the fluorescence emission spectra of fungal

protein and fungal protein incubated with SNP upon excitation at 270 nm. It can be observed that with increase of SNP concentration the emission intensity of fungal protein decreases gradually with a blue shift. Fluorescence quenching means any process that decreases the fluorescence intensity of a sample solution. The quenching constant increases with increasing temperature for dynamic quenching, however, it decreases with increasing temperature for static quenching [10]. The quenching data can be described by the Stern-Volmer equation, Eq. (2) [10]

$$F_0 / F = 1 + kq\tau_0[Q] = 1 + K_{SV}[Q] \quad \text{Eq. 2}$$

Where  $F_0$  and  $F$  represent the fluorescence intensities in absence and presence of quencher,  $kq$  is the bimolecular quenching rate constant,  $K_{SV}$  is the Stern Volmer constant,  $\tau_0$  is the average lifetime of the molecule without quencher and  $[Q]$  is the concentration of the quencher. Quenching data are presented as plots of  $F_0/F$  versus  $[Q]$ .

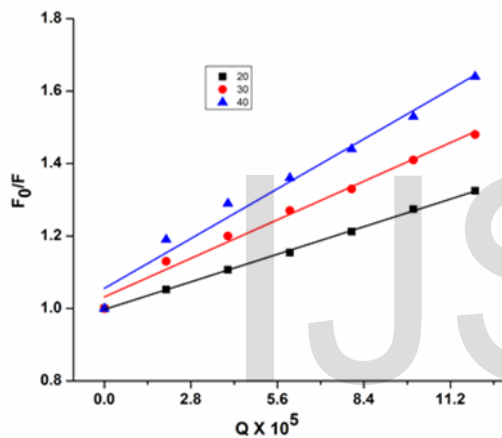


Fig. 4 Stern Volmer plot for SNP & fungal protein; a) at 20°C, b) at 30°C & c) at 40°C respectively.

This is because  $F_0/F$  is expected to be linearly dependent upon the concentration of the quencher. A plot of  $F_0/F$  versus  $[Q]$  yields a slope equal to stern Volmer quenching constant. The values of  $K_{SV}$  at different temperatures are shown in Table 1. The linearity of the  $F_0/F$  vs.  $[Q]$  plots is shown in Fig. 4. As shown in Table 1, the quenching constant  $K_{SV}$  increases with increasing temperature which indicates that the probable quenching mechanism of fungal protein is a dynamic quenching procedure and complex between silver nanoparticle and fungal protein may be formed.

### 3.3 Binding Constant (K) and Number of Binding Sites (n)

The number of binding sites ( $n$ ) and the binding constant ( $K$ ) between silver nanoparticle and fungal protein have been calculated using the Eq.3 for the quenching process [11].

$$\log \frac{F_0 - F}{F} = \log K + n \log [Q] \quad \text{Eq. 3}$$

A plot of  $\log [(F_0 - F)/F]$  vs.  $\log [Q]$  gives a straight line, whose slope equals to  $n$  (the number of binding sites of SNP on fun-

gal protein) and the intercept on Y-axis equals to  $\log K$ . Figure 5 denotes the double logarithm plots and Table 1 gives the corresponding results. The values of ' $n$ ' (Table 1) at the experimental temperatures were approximately equal to one which indicates that there is a single binding site in fungal protein for SNP which is dependent of temperature very little in the range 293 K to 313 k.

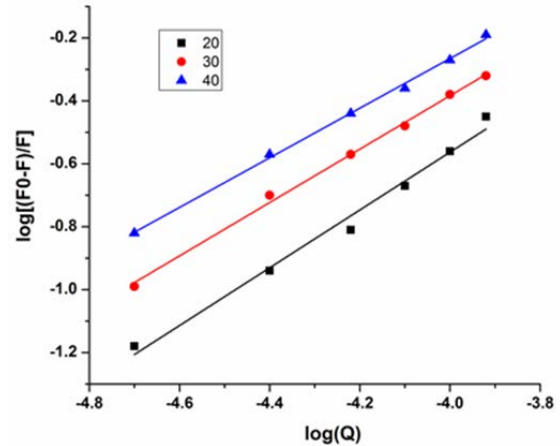


Fig. 5 Plots of the SNP quenching effect on fungal protein fluorescence at different temperature (20, 30 & 40°C respectively).

### 3.4 Thermodynamic Parameters and Nature of Binding Forces

Determination of parameters such as  $\Delta H$  and  $\Delta S$  of binding interactions provide us to determine the type of binding force & Gibbs free energy ( $\Delta G$ ) provides us to determine the spontaneity of the binding process.

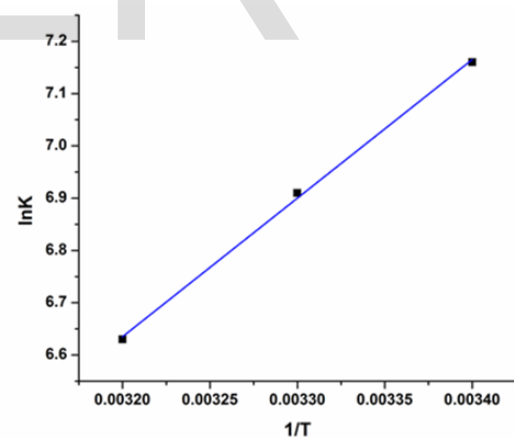


Fig. 6 The Van't Hoff plot for the interaction of fungal protein and SNP.

If the enthalpy change ( $\Delta H$ ) does not vary significantly over the temperature range studied, then the thermodynamic parameters of  $\Delta H$  and  $\Delta S$  (entropy change) are determined using the Van't Hoff Eq. 4, where  $K$  is the binding constant at the corresponding temperature (Fig. 6).  $\Delta H$  and  $\Delta S$  can be determined from the slope and intercept of linear Van't Hoff plots.

The Gibbs free energy ( $\Delta G$ ) is estimated from the Eq 5.

$$\ln k = -\Delta H / RT + \Delta S / R \quad \text{Eq. 4}$$

$$\Delta G = \Delta H - T\Delta S = -RT \ln k \quad \text{Eq. 5}$$

The Eq. 5 yields the values of  $\Delta H$  and  $\Delta S$  to be  $-22.03 \text{ kJmol}^{-1}$  and  $-15.34 \text{ Jmol}^{-1} \text{ K}^{-1}$  respectively. The values of thermodynamic parameter  $\Delta G$  are listed in Table 1. The negative sign for  $\Delta G$  indicates the spontaneity of the binding of biosynthesized silver nanoparticle to extracellular fungal protein. Here, we found that negative values of  $\Delta H$  and  $\Delta S$  show that the binding process is mainly Van der Waals force and hydrogen bonding type between fungal protein and SNP [12]. Thus it can be concluded that weak forces play a major role in stabilizing the molecular complex between fungal protein-SNP.

Table 1 Binding parameters and thermodynamic parameters of fungal protein-SNP interaction.

T °C	K <sub>sv</sub> Lmol <sup>-1</sup> s <sup>-1</sup>	K Lmol <sup>-1</sup> (×10 <sup>3</sup> )	ΔG KJmol <sup>-1</sup>	ΔS Jmol <sup>-1</sup> K <sup>-1</sup>	ΔH KJm <sup>-1</sup>	n
20	0.027	1.28	-17.54	-15.3	-22	0.92
30	0.038	1	-17.38			0.85
40	0.049	0.75	-17.23			0.79

### 3.5 CD measurements

Figure 7 shows the helicity of fungal protein in the presence of increasing concentration of silver nanoparticle. In the wavelength region of 190–220 nm, the CD spectrum of a protein gives information about its conformation in relation to the secondary structure. The binding of silver nanoparticle to fungal protein changes the spectra and it is apparent that interaction of silver nanoparticle with fungal protein causes some conformational change of the protein.

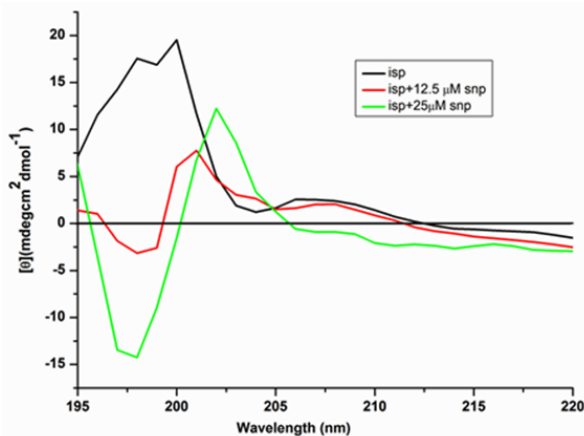


Fig. 7 UV-CD spectra, recorded for fungal protein in presence of SNP at a fungal protein concentration of 100 μg/ml and SNP concentrations of 1: 0, 2: 12.5 & 3: 25μM.

### 4. Conclusion

It can be concluded that binding of silver nanoparticle to fungal extracellular protein occurs; as there is a significant change in the quenching of protein fluorescence intensity, change in UV-Vis ab-

sorption spectra and also alteration of CD spectra.

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