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Studies on binding affinities of phenylalanine based Schiff base metal complexes on bovine serum albumin

R. Biju Bennie[1], *S. Theodore David[1], C. Joel[1], S. Daniel Abraham[1], M. Seethalakshmi[1] and S. Iyyam Pillai[2]

[1]P.G. Department of Chemistry, St. John's College, Tirunelveli, India
[2]P.G.and Research Department of Chemistry, Pachaiyappa's College, Chennai, Tamilnadu, India

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ABSTRACT

A novel Schiff base ligand (PQ-phe) derived from 9,10-Phenanthrenequinone and L-phenylalanine have been synthesized and complexed with Mn(III) and Fe(III) metal ions. They were characterized. The interaction of the synthesized Schiff base complexes with Bovine Serum Albumin (BSA) was investigated by fluorescence and UV-VIS spectroscopic techniques. It was found that the fluorescence quenching of BSA by Schiff base metal complexes occurs as a result of complex formation between the BSA and the metal complexes ie., static quenching. The number of binding sites and binding energy were calculated.

Keywords: 9,10-Phenanthrenequinone, L-phenylalanine, Schiff base, BSA, Static quenching.

INTRODUCTION

Schiff bases have been playing an important role in the development of co-ordination chemistry. Many Schiff base ligands have been designed to mimic the function of natural carriers recognizing and transporting specific metal ions, anions or neutral molecules and in understanding and reproducing the catalytic activity of metallo-enzymes and proteins.[1] Almost all transition metal complexes of Schiff bases show good antibacterial, antiviral, antimalarial and antitubercular activity.[2-4] Amino acid based Schiff base complexes have recently received considerable attention due to their biological importance.[5]

Proteins are the most abundant macromolecules in cells and are crucial to maintaining normal cell functions. Bovine serum albumin (BSA), one of the major components in plasma protein, plays an significant role in transporting and metabolizing of many endogenous and exogenous compounds in metabolism. Since the serum albumin has been considered to be non-antigenic and biodegradable, and also readily available, the albumin has been used as a bio-material, such as drug delivery and novel hydrophilic carriers. Recently, the Schiff bases conjugation has been proved to be an effective method to improve their bioactivity.[6] Binding of Schiff base metal complexes with the most abundant carrier proteins (serum albumins) have also been a subject of interest as such drug-protein binding greatly influences absorption, drug transport, storage, metabolism and excretion properties of typical drugs in vertebrates. Due to the scarcity of the information regarding the interaction of amino acid based Schiff base complexes with BSA, we have decided to synthesize Schiff base metal complexes derived from 9,10-Phenanthrenequinone and L-phenylalanine and to study their interaction with BSA using fluorescence and UV-VIS spectroscopic techniques. The quenching mechanism, binding sites and binding energy were also calculated.

MATERIALS AND METHODS

1.1 Materials and methods

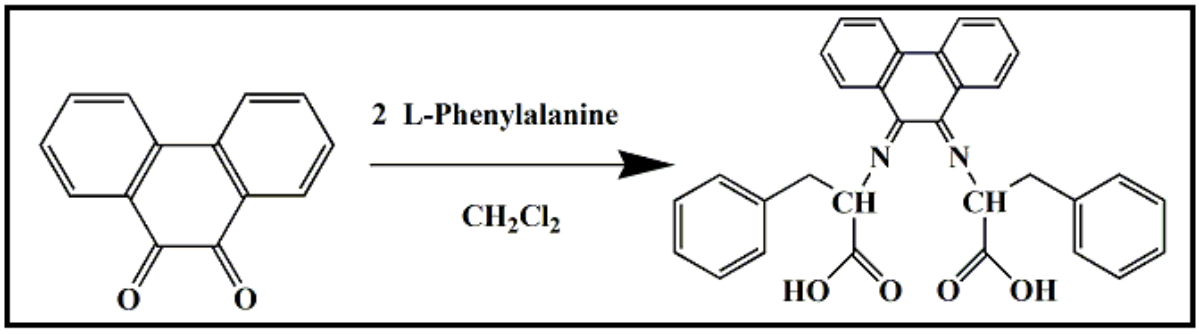
All chemicals and solvents used were of analytical reagent grade. 9,10-Phenanthrenequinone, BSA and Mn(III) acetate were purchased from Sigma and L-phenylalanine, Ferric chloride and tris-buffer from HIMEDIA. Sodium chloride and hydrochloric acid were purchased from LOBA and were used to prepare Tris-HCl buffer of pH 7.4.

The IR spectra were recorded on Perkin Elmer FT-IR spectrometer with samples prepared as KBr pellets. ^{13}C-NMR spectra were recorded in $CDCl_3$ by using TMS as an internal standard on a BRUKER 400 instrument. EI mass spectra were recorded on a JEOL DX-303 EI mass spectrometer. UV-visible spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer. The emission spectra were recorded on Perkin-Elmer LS-45 Fluorescence spectrometer.

1.2 *Synthesis of the Schiff base ligand*

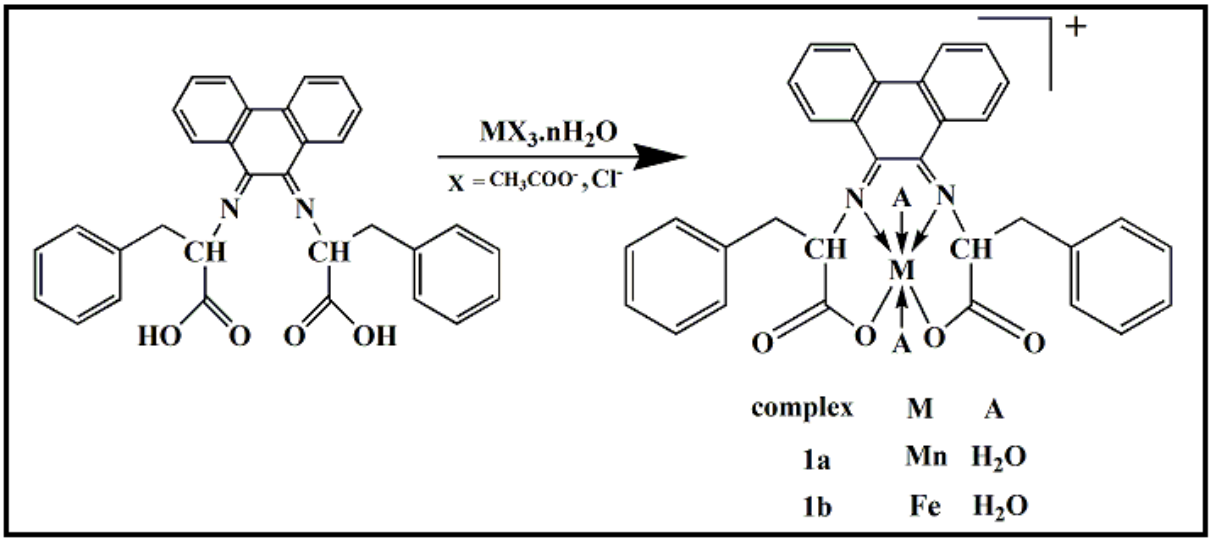
To 1mmol of 9,10-Phenanthrenequinone in dichloromethane (40ml), 2mmol of L-phenylalanine (0.16g) was added.. The solution was stirred for 8h, reduced in volume and the precipitate filtered, washed with water, diethyl ether and dried in *vacuo*. (Scheme.1)

Scheme. 1 Preparation and structure of the Schiff base ligand



1.3 *Synthesis of the Schiff base ligand*

To 1 mmol of the ligand in 25 ml of methanol, a solution of metal(III) (1 mmol) in 25 ml of methanol was added drop wise with constant stirring. The pH was maintained around 9. The reaction mixture was stirred under reflux for 8 h and the volume was reduced to half of the initial volume under reduced pressure. The precipitated metal complex was filtered, washed several times with diethyl ether and then dried in *vacuo*. (Scheme.2)



Scheme. 2 Preparation and structure of the Schiff base complexes

1.4 Preparation of stock solutions

The concentrations of the stock solutions of BSA (C_{BSA} ~ 2 x 10^{-6} $molL^{-1}$) in Tris buffer (0.05 $molL^{-1}$ Tris, 0.15 $molL^{-1}$ NaCl and the pH is maintained at 7.4 using conc. HCl) are determined from absorption spectroscopy. The concentration is calculated by dividing absorbance at 280 nm by the molar extinction coefficients of BSA (ε_{280} = 44,300 $M^{-1}cm^{-1}$).[7] The stock solutions of metal complexes were prepared by dissolving them in DMSO and diluting them to a final concentration of 10 x 10^{-6} $molL^{-1}$.

1.5 BSA binding studies

1.5.1 Absorption studies

The absorption spectra of BSA and BSA-metal complex system were recorded in the range of 250-310 nm. The concentration of BSA and metal complex was 2 x 10^{-6} $molL^{-1}$.

1.5.2 Fluorescence studies

In a typical fluorescence measurement, 2 ml BSA was added to quartz cell (1.0 x 1.0 cm). Fluorescence quenching spectra were recorded in the range of 320-400 nm. The width of the excitation and emission slit was set to 15 and 4 nm respectively. An excitation wavelength of 285 nm was chosen. The BSA was titrated by successive additions of 2 x 10^{-6} $molL^{-1}$ metal complex solutions. Titrations were done manually by using micro-injector.

1.5.3 Fluorescence quenching mechanism

The decrease in fluorescence intensity of BSA by different molecular interactions is called quenching. Fluorescence quenching could proceed via different mechanisms usually classified as dynamic quenching and static quenching. Dynamic quenching is caused by diffusion and static quenching is caused by formation of non-fluorescent ground-state complex.[8] The fluorescence quenching is described by the Stern-Volmer relation.[9]

$$F_0/F = 1+ K_{sv}[Q] = 1+ K_q\tau_0[Q]$$

Where, F_0 and F represent the fluorescence intensity in the absence and presence of quencher respectively. K_{sv} is a linear Stern-Volmer quenching constant and [Q] is the concentration of quencher, K_q is the quenching rate constant and τ_0 is the fluorescence lifetime of the protein in the absence of quencher. In the case of fluorescence quenching of BSA, a linear plot of F_0/F against [Q] was obtained and from the slope K_{sv} was calculated. The value of K_{sv} represent a single quenching mechanism, either static or dynamic.[10]

2.5.4 Analysis of binding sites

Number of binding sites can be calculated from fluorescence titration data using the following equation,[11]

$$\log [(F_0-F)/F] = \log K_b + n \log [Q]$$

K_b and n are the binding constants and binding sites of metal complexes. The corresponding K_b and n values were evaluated from the slopes and intercepts of the linear fitting plots of log $[(F_0-F)/F]$ vs. log [Q].

2.5.5 Analysis of binding energy

The Gibbs free energy changes (ΔG) can be calculated by Van't Hoff equation. [12]

$$\Delta G = -RT \ln K$$

Where K is analogous to the effective binding constants K_b at the corresponding temperature and R is the gas constant. The negative ΔG value confirms the spontaneity of binding.

RESULTS AND DISCUSSION

1.6 Structural characterization of ligand

The structure of the ligand has been confirmed by the following spectral data.

3.1.1 FT IR spectra

The IR spectrum (Fig 1) of the Schiff base ligand shows an important intense peak at 1673 cm^{-1} due to the imine ν(-C=N) vibration. The intense peak at 1589 cm^{-1} is due to (COO^-) asymmetric stretching and the peak at 1409 cm^{-1} is due to (COO^-) symmetric stretching vibration of carboxylic acid group.

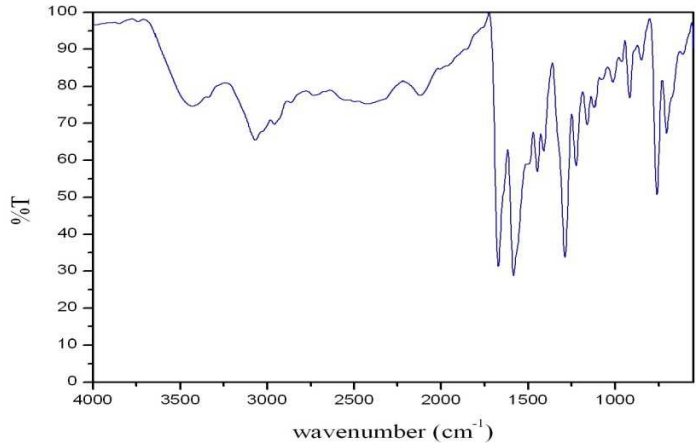


Fig 1: FT-IR spectrum of the Schiff base ligand(PQ-phe)

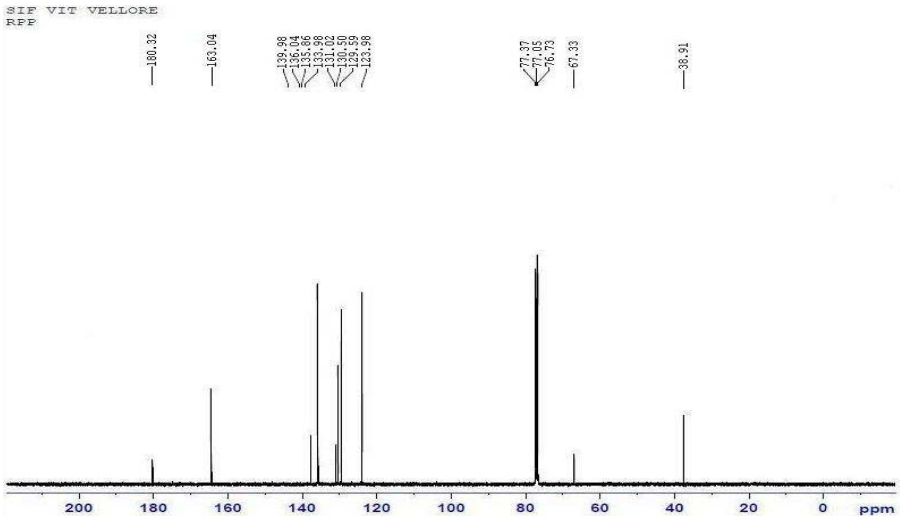


Fig 2: ^{13}C NMR spectrum of the Schiff base ligand (PQ-phe)

3.1.2 ^{13}C-NMR spectra

^{13}C-NMR Spectrum (Fig. 2) of the ligand shows signals in the range δ 123.98-139.98 which are due to aromatic carbons. The signal at δ 163.04 corresponds to the two azomethine carbons. Signal at δ 180.32 is due to the two carboxylate carbons.

3.1.3 EI mass spectra

The EI mass spectrum (Fig 3) of the ligand shows the molecular ion(M^+) peak at m/z =502 corresponding to the molecular weight of the ligand. The peaks at m/z = 472, 293, 250, 234, 207, 179, 151, 104 and 75 corresponds to various fragments $C_{32}H_{26}N_2O_4$, $C_{18}H_{16}N_2O_2$, $C_{16}H_{13}NO_2$, $C_{16}H_{13}NO$, $C_{14}H_{18}N_2$, $C_{10}H_9NO_2$, $C_9H_9O_2$, C_8H_8 and C_6H_5 respectively.

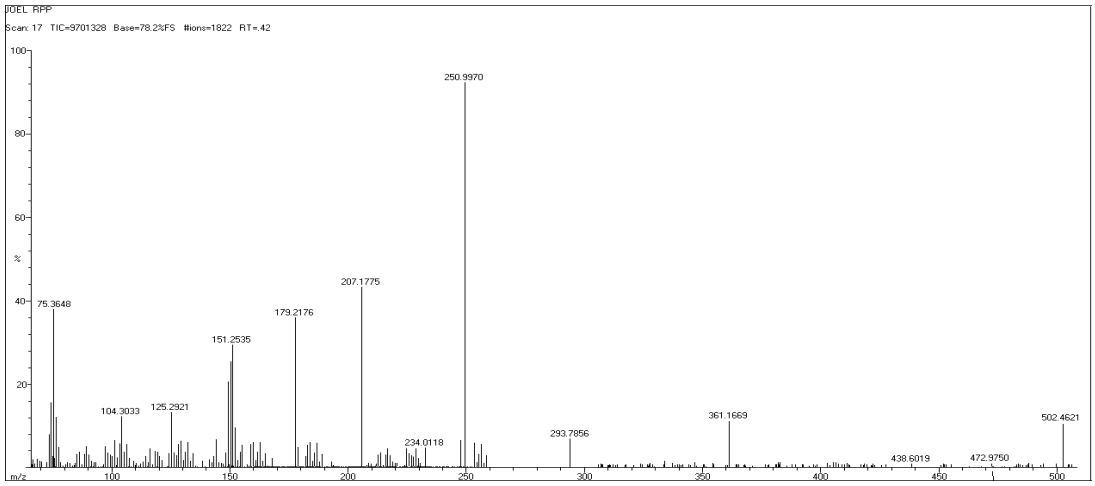


Fig 3: EI Mass spectrum of the Schiff base ligand(PQ-phe)

3.2 Structural characterization of metal complexes

3.2.1 FT-IR spectra

The FT-IR spectra of both the Mn(III) and Fe(III) complexes of PQ-phe showed a shifting in C=N stretching to a lower frequencies (1642 cm^{-1} and 1648 cm^{-1}) when compared to that of the free ligand (1673 cm^{-1}). This indicates the coordination of imino nitrogen to the metal ions.[13] The bands at 532 cm^{-1}, 530 cm^{-1} and 438 cm^{-1}, 430 cm^{-1} corresponds to M-O and M-N bands of Mn(III) and Fe(III) complexes respectively.

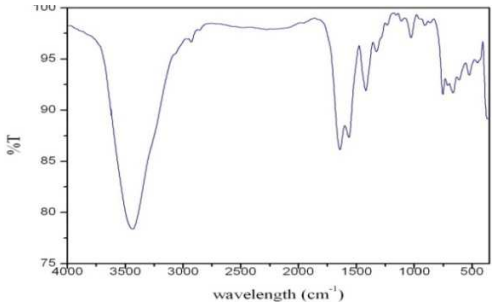


Fig 4a: FT-IR spectra of Mn(III)-PQ-phe

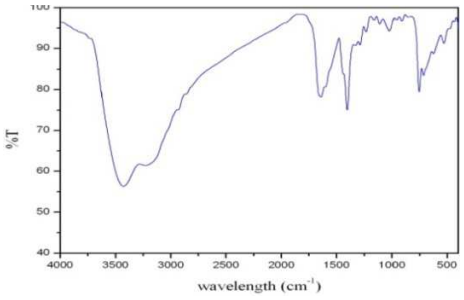


Fig 4b: FT-IR spectra of Fe(III)-PQ-phe

3.2.2 Electronic spectra and molar conductance

The molar conductance values of 134[14] and 195[15] $\Omega^{-1}mol^{-1}cm^2$ for Mn(III)-PQ-phe and Fe(III)-PQ-phe respectively suggests the 1:3 electrolytic nature of the metal complexes. The electronic spectra of Mn(III)-PQ-phe shows three transition bands at 750 nm, 575 nm and 350 nm corresponding to $^5B_1g \rightarrow {}^5A_1g$, $^5B_1g \rightarrow {}^5B_2g$ and $^5B_1g \rightarrow {}^5Eg$ transitions respectively, confirming the octahedral geometry for the complex.[16] The electronic spectra of Fe(III)-PQ-phe exhibits three bands at 750 nm, 600 nm and 325 nm, due to $^6A_1g(S) \rightarrow {}^4T_1g(G)$, $^6A_1g(S) \rightarrow {}^4T_2g(G)$ and $^6A_1g(S) \rightarrow {}^6A_1g(S) \rightarrow {}^4Eg$ transitions respectively, which demonstrates octahedral geometry for the complex.[17]

3.3 BSA binding studies

3.3.1 Fluorescence spectroscopy

The fluorescence intensity of BSA was quenched around 350 nm on addition of increasing concentration of the metal complexes but the emission band did not move to shorter or longer wavelength. BSA contains three fluorophores namely, tryptophan, tyrosine and phenylalanine. The intrinsic fluorescence of BSA is mainly due to tryptophan, because phenylalanine has a very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is ionized, or is near an amino group, a carbonyl group, or a tryptophan residue.[18]

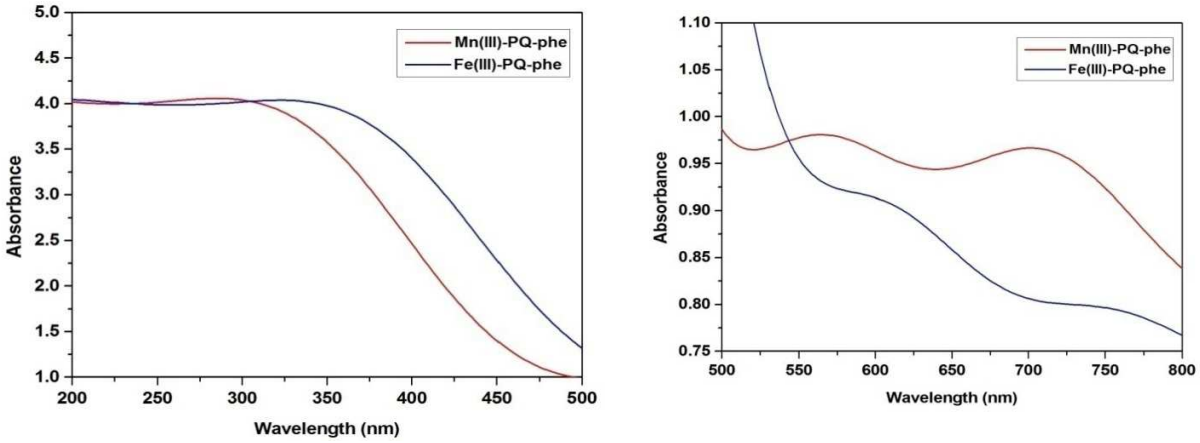


Fig 5: Electronic spectra of Mn(III)-PQ-phe and Fe(III)-PQ-phe

The decrease in fluorescence intensity of BSA on addition of metal complexes is due to the interaction of the metal complexes with BSA. The effect of the metal complexes on BSA is shown in figures 6a and 6b. The quenching of BSA may be either dynamic or static. Dynamic quenching is caused due to collision. Static quenching occurs as a result of complex formation between BSA and the metal complexes. The quenching mechanism of metal complexes with BSA was probed by Stern-Volmer equation. The K_{sv} values obtained from the linear plot of F_0/F vs. [Q] is furnished in Table 1. The fluorescence lifetime of BSA in the absence of quencher (τ_0) is 10^{-8} s. The quenching rate constant K_q (= Ksv/ τ_0) is calculated (Table 1).

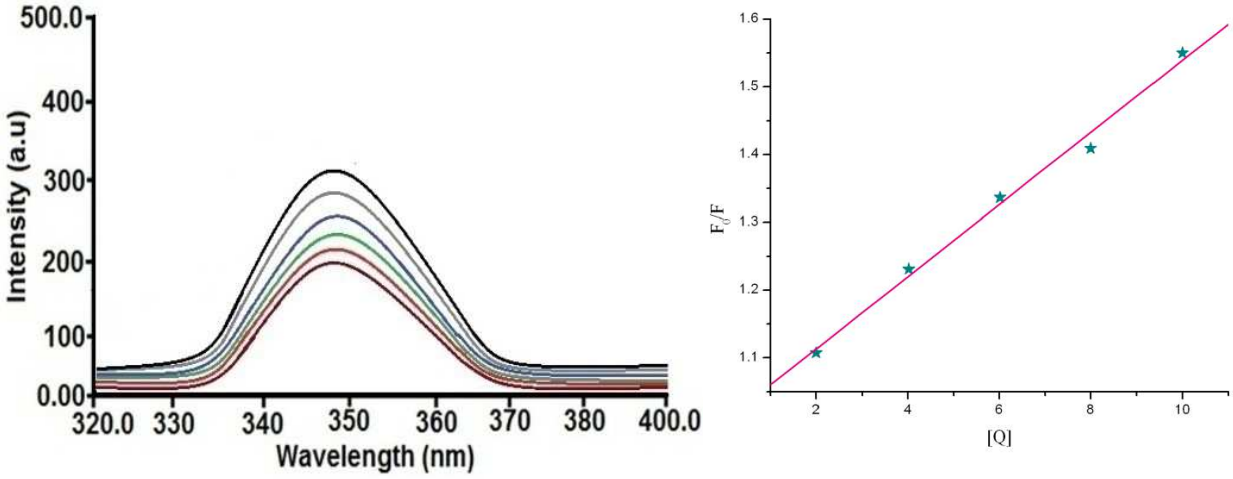


Fig 6a: Emission spectra and Stern-Volmer plot of BSA in the presence of various concentration of Mn(III)-PQ-phe (T=298 K and λ=280 nm), c(BSA)= 2.0 x 10^{-6} molL^{-1}, c(Mn(III)-PQ-phe) = 2, 4, 6, 8, 10 x 10^{-6} molL^{-1}

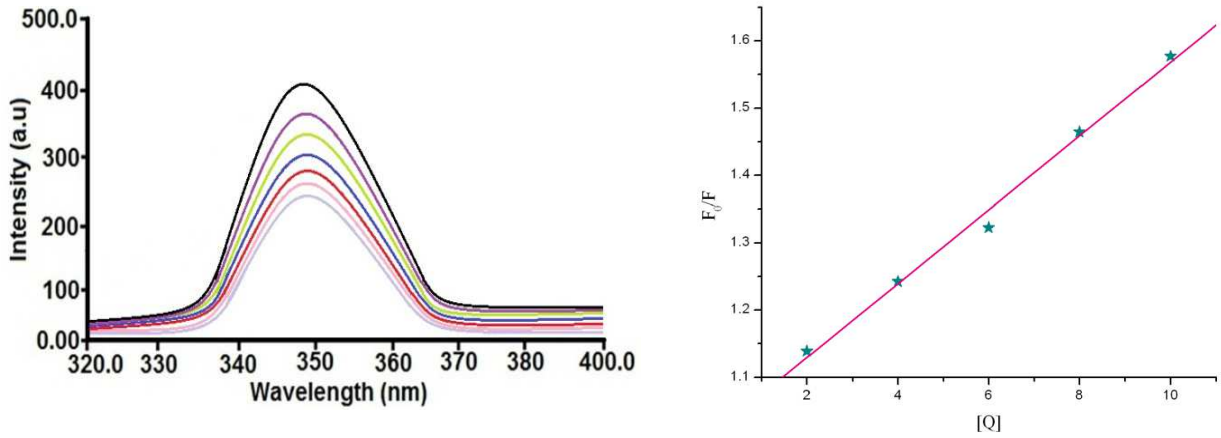


Fig 6b: Emission spectra and Stern-Volmer plot of BSA in the presence of various concentration of Fe(III)-PQ-phe (T=298 K and λ=280 nm), c(BSA)= 2.0 x 10^{-6} molL^{-1}, c(Fe(III)-PQ-phe) = 2, 4, 6, 8, 10 x 10^{-6} molL^{-1}

Table 1: Stern-Volmer constants(K_{sv}) and quenching rate constants(K_q)

complex	K_{sv} (x 10^4 Lmol^{-1})	K_q (x 10^{12} Lmol^{-1}s^{-1})
Mn(III)-PQ-phe	5.33	5.33
Fe(III)-PQ-phe	5.49	5.49

According to literature[19] for dynamic quenching, the maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0 x 10^{10} Lmol^{-1}s^{-1}.The obtained values of K_q are greater than 2.0 x 10^{10} Lmol^{-1}s^{-1} indicating that the quenching of BSA by the metal complexes is not dynamic. Therefore, the quenching mechanism arises due to static quenching ie., due to formation of complex between BSA and metal complexes.

3.3.2 Absorption spectroscopy

This was further confirmed by absorption spectra of BSA and BSA-metal complex solutions (Fig 7a and 7b). The absorption wavelength of BSA at 285 nm shifted slightly to the right on addition of solution of metal complexes indicating the complex formation between BSA and metal complexes.

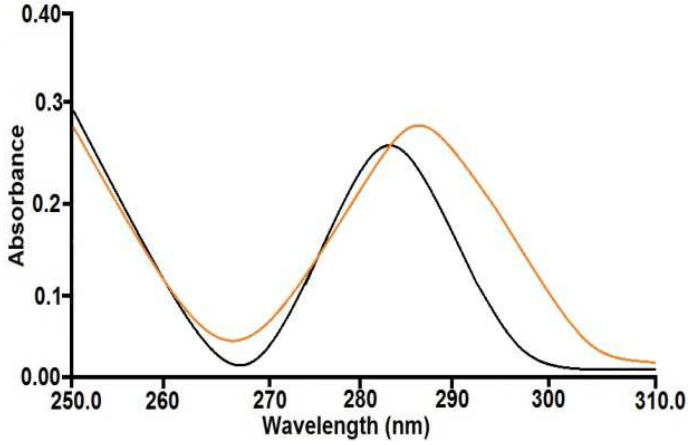


Fig 7a: UV-vis absorption spectra of BSA and BSA-Mn(III)-PQ-phe solutions: c(BSA) = c(Mn(III)-PQ-phe) = 2 x 10^{-6} molL^{-1}

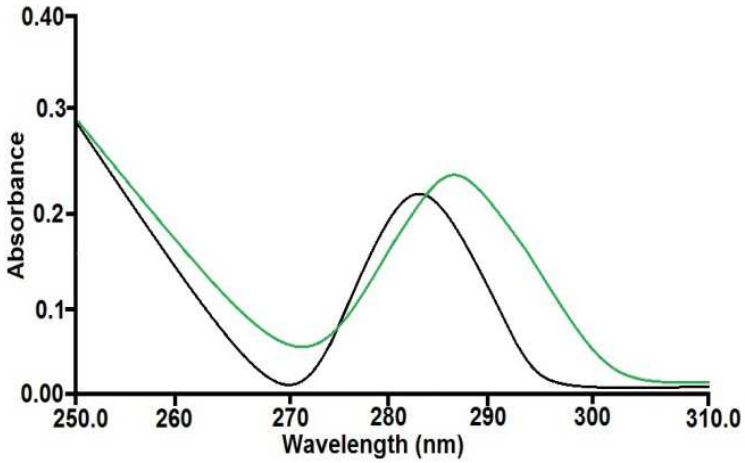


Fig 7b: UV-vis absorption spectra of BSA and BSA-Fe(III)-PQ-phe solutions: c(BSA) = c(Fe(III)-PQ-phe) = 2 x 10^{-6} $molL^{-1}$

3.3.3 Analysis of binding constants and binding sites

The linear fitting plots of log [(F_0-F)/F] vs. log [Q] (Fig 8a and 8b) gives the values of binding constants and binding sites which are furnished in table 2.

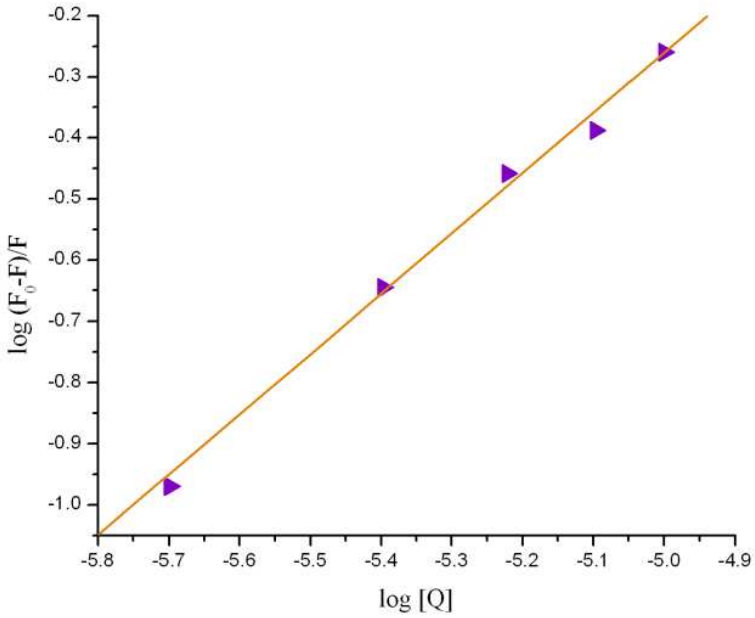


Fig 8a: Double-log plot of Mn(III)-PQ-phe quenching effect on BSA fluorescence at 298 K

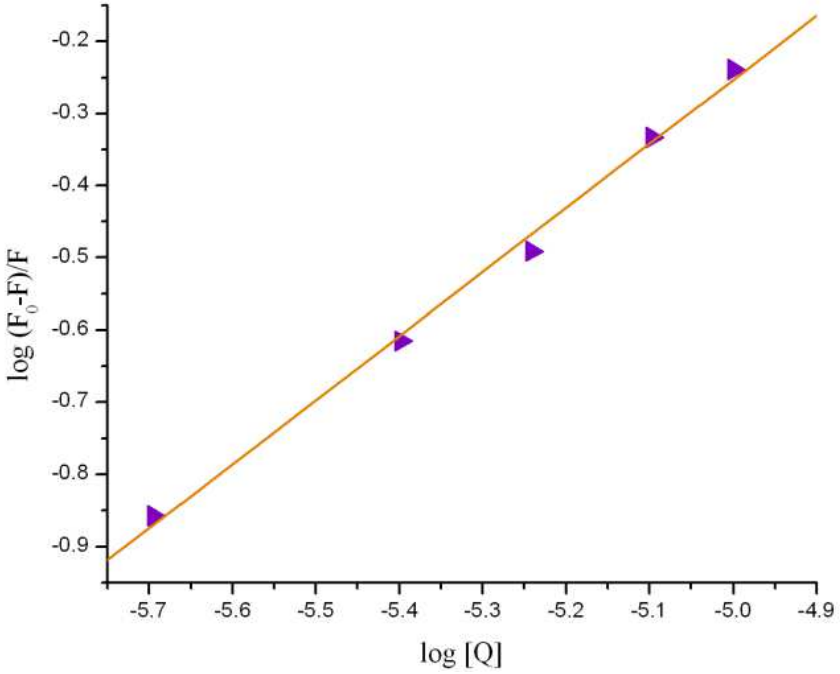


Fig 8b: Double-log plot of Fe(III)-PQ-phe quenching effect on BSA fluorescence at 298 K

Table 2: Binding constants, Binding sites and Binding energy values

Complex	Binding constant (x 10^4Lmol^{-1})	Binding site	Binding energy(ΔG)
Mn(III)-PQ-phe	4.67	0.98	-26.64
Fe(III)-PQ-phe	4.18	0.88	-26.36

These results suggest that there is a strong binding force between the BSA and the metal complexes. There is a hydrophobic cavity in BSA which is favorable for the hydrophobic metal complexes to enter into BSA and interacts with it. The number of binding sites for both the complexes is approximated to 1 which suggests that there is only one binding site on the protein. The negative ΔG values (Table 2) indicate the spontaneity of BSA-metal complexes binding process.

CONCLUSION

A new Schiff base ligand, PQ-phe, and its Mn(III) and Fe(III) complexes have been synthesized and characterized by IR, ^{13}C NMR, mass and electronic spectral analysis. The metal ions are six coordinate and the geometry can be described as octahedral. The molar conductance values reveal that all the complexes are 1:3 electrolytic in nature. BSA binding properties of the metal(III) complexes have been investigated by UV-Vis spectra, and fluorescence spectra. The mechanism of quenching of BSA by the metal(III) complexes was static quenching. The BSA-metal(III) complexes interactions were spontaneous based on the values of ΔG.

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