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RESEARCH ARTICLE

SPECTROSCOPIC ANALYSIS OF EGG ALBUMIN WITH AMANTADINE IN SPAN 40

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ABSTRACT

Spectroscopic analysis was used to study the effect of the interaction of egg albumin with amantadine in SPAN-40 solution. Protein binding properties of Egg albumin-amantadine complex were investigated by fluorescence spectroscopic technique. From the fluorescence spectral data stern-volmer quenching constant and the quenching rate constant have been calculated and reported. Other constants calculated for the surfactant SPAN 40 micelle were aggregation number, radius, surface area per head group and packing parameter.

INTRODUCTION

Antioxidant biomolecules are used as therapeutic agents in many disease like cancer (Salim, 1996), diabetes (Desco et al., 2002), cardiovascular diseases (Mimic-Oka et al., 1999), autoimmune diseases (Nanazi, 2009) and neuron degenerative disorders (Singh et al., 2004). This is because over productions of reactive oxygen species encourage progress of clinical disease process. The balance of reactive oxygen species and antioxidant molecules is major mechanism in preventing damage by oxidative stress. Therefore, the dietary supplement of antioxidant such as vitamins and flavonoids has been used to prevent the occurrences of many chronic disease e.g., intestinal anti-inflammatory effect (Camuesco et al., 2004; Peluso, 2006). However herbal medicines with antioxidant properties are used for various purposes to control disease and epidemiological data authenticate the wide spread acceptance of these agents.

Among all, amantadine is one of the most abundantly and widely distributed antioxidant biomolecules in the edible and medicinal plants. Here, fluorescence quenching study has been carried out to understand the interaction of amantadine with egg albumin in SPAN 40 solution.

MATERIALS AND METHODS

Egg albumin, amantadine and SPAN 40 were purchased from Sigma Aldrich company, Bangalore and were used without further purification.

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The concentration of Egg albumin for the fluorescence measurements was 1×10^{-4} mol L⁻¹. The stock solution of the EA was added to different amantadine concentration (0.2×10^{-5} mol L⁻¹ to 14×10^{-5} mol L⁻¹). The concentration of the SPAN 40 was 0.1 M. Fluorescence measurements were made by SHIMADZU RF 5301 PC spectrofluoro photometer.

RESULTS AND DISCUSSION

The fluorescence spectra of Egg albumin in 0.1M micellar concentration of SPAN 40 both in presence and absence of the quencher, amantadine, show no observable change in spectral shape and maxima. Further, observation of similar absorption spectra of a solution containing any concentration of the quencher after carrying out the fluorescence indicates that no detectable photoproduct is formed under the experimental condition. No new fluorescence peak is also observed at longer wavelength. The excitation spectra monitored at different emission wavelengths also remain the same. These observations indicate that there is no ground state complexation of egg albumin and amantadine. Fig.1 show the fluorescence quenching spectra of egg albumin without and with different concentrations of amantadine in 0.1 M concentration of SPAN 40.

According to the Stern-volmer equation

$$\frac{I_0}{I} = 1 + K_{sv}[Q] = 1 + K_q \tau_0 [Q] \quad (1)$$

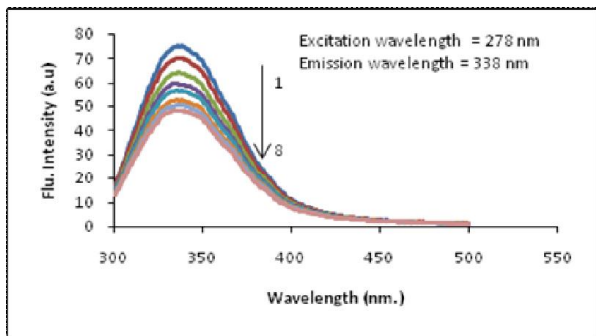


Fig. 1. Steady – state fluorescence spectra of EA with different concentrations of Amantadine (mol L⁻¹) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.08, (6) 1.0, (7) 1.2, (8) 1.4 in 0.10 M concentration of SPAN 40

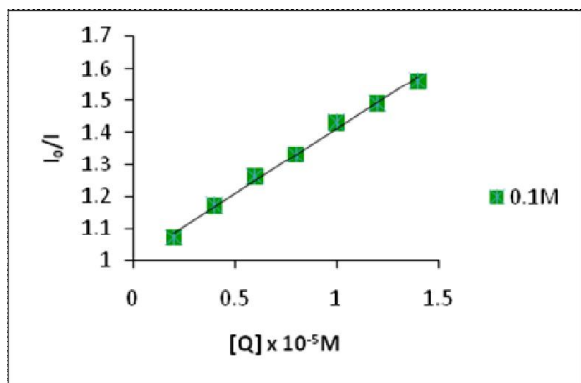


Fig. 2. Stern-Volmer plots of Egg albumin with Amantadine in 0.01 M concentration of SPAN 40

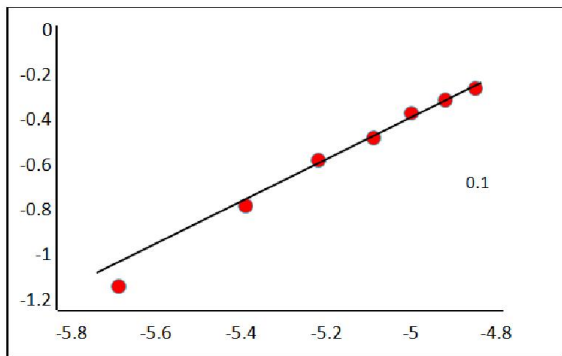


Fig. 3. Double log plot of Amantadine quenching effect on EA fluorescence at 0.01 M concentrations of SPAN 40

where, I₀, and I are the fluorescence intensities before and after the addition of the quencher, K_q is the quenching rate rate constant, K_{SV} is the stern-volmer quenching constant, [Q] is the

quencher concentration and τ₀ is the average lifetime without quencher a graph was drawn for (I₀/I) against amantadine concentration [Q] in SPAN 40 solution (Fig.2).

From this Stern-Volmer Constnat K_{SV} and bimolecular quenching rate constant K_{SV} and bimolecular quenching rate constant K_q of Egg albumin with amantadine in 0.1M concentration of SPAN 40 have been calculated as K_{SV} = 0.7 and K_q = 1.45. The value of binding constant K was determined from the intercept of (log (I₀-I)/I versus (log[Q]) as shown in Fig.3. The value of K is 3.42 x 10⁴ L mol⁻¹ and values of binding site n is found to be 0.97. The linear correlation coefficient of the curve is higher than 0.92, indicating that the interactions between amantadine and EA agreed well with the site-binding model according to eqn,

$$\log \left[\frac{I_0 - I}{I} \right] = \log k + n \log [Q] \tag{2}$$

aggregation number, radius, surface area per head group and packing parameter of SPAN 40 micelle of 0.1 M concentration of SPAN 40 were calculated. These values are given below. Aggregation number is 4499.28, radius of micelle is 83.49, area of the micelle 19.46, and critical aggregation parameter is 1.085.

Conclusion

The interaction of the anti-oxidant flavonoid amantadine, with egg albumin in SPAN 40 solution was studied by fluorescence spectroscopic technique. Using fluorescence technique, the binding constant K for the binary complex of anti oxidant flavonoid, amantadine and egg albumin were calculated. The number of binding sites were also calculated. Micelle parameters were calculated and reported.

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