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Fluorescence Study on the Interaction of Egg Albumin with Quercetin in SPAN-40

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S. Bakkialakshmi[*], B. Bhavani[a] and P. Selvarani[b]

**Department of Physics, Annamalai University, Annamalainagar, Tamilnadu, India-608 002.*

[a]Dr.S.J.S. Paul Memorial College of Engineering and Technology, Pondicherry

[b]Auxilium College of Arts and Science for women, Pudukottai

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ABSTRACT

A fluorescence investigation on the interaction of Egg albumin (EA) with quercetin [Q] in SPAN 40 (Surfactant) solution was reported in this paper. Decrease in the fluorescence intensity of egg albumin in 0.1M concentration of SPAN 40 without the appearance of any new band in the presence of quercetin indicates that no emissive exciplex is formed between the Egg albumin and quercetin. Addition of quercetin to the solution of Egg albumin resulted in the quenching of its fluorescence emission. Stern-volmer quenching constant and the quenching rate constant were calculated and tabulated. Aggregation number, radius, surface area per head group and packing parameter of SPAN 40 micelle were also calculated for the surfactant SPAN 40.

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1. INTRODUCTION

The interactions of small molecules with biomolecules such as proteins and nucleic acid have aroused great interest among chemists and biologists. The study of supramolecular interaction between them is useful for understanding the structures and functions of biomolecules. The quantitative determination of proteins is very important in clinical tests and biological techniques because it is often used as a reference for the measurement of other components in biological systems.

The traditionally used methods include the coomassie brilliant blue G-250 (1), Lowery method (2), and bromophenol blue method (3). In recent years, many dye-binding methods have been widely proposed for protein determination by spectrophotometry (4,5), fluorometry (6,7), light scattering technique (8,9), and electrochemical method (10,11). Compared with spectroscopic methods, fluorescence method is simple, radiable and practical with low detection limit and wide dynamic range.

2. MATERIALS AND METHODS

Egg albumin, quercetin and SPAN 40 were purchased from Sigma-Aldrich Company, Bangalore and were used without further purification.

The concentration of Egg albumin for the fluorescence measurements was 1 x 10^{-4} mol L^{-1}. The stock solution of the EA was added to different quercetin concentration (0.2 x 10^{-5} mol L^{-1} to 14 x 10^{-5} mol L^{-1}). The concentration of the SPAN 40 was 0.1 M.

Fluorescence measurements were made by SHIMADZU RF 5301 PC spectrofluorophotometer.

3. 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, quercetin, 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 quercetin.

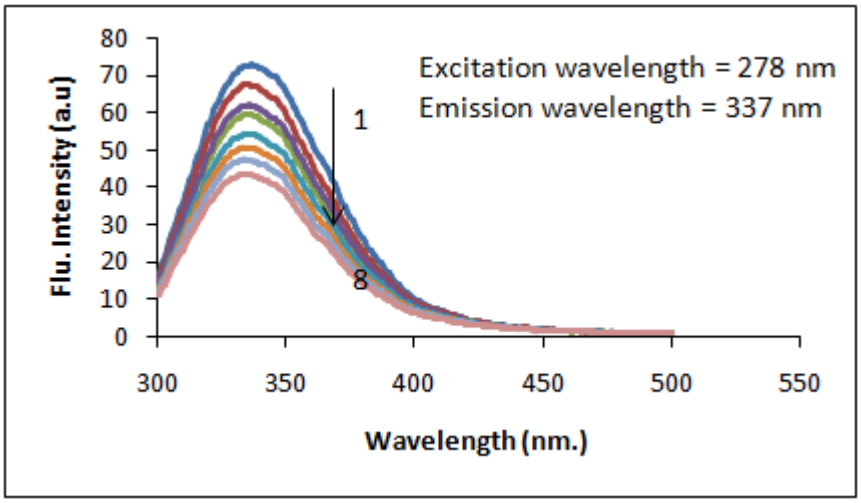

Fig. 1: Steady–state fluorescence spectra of EA with different concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.08, (6) 1.0, (7) 1.2, (8) 1.4 in 0.10 M concentration of SPAN 40

Fig.1 shows the fluorescence quenching spectra of egg albumin without and with different concentrations of quercetin in 0.1 M concentration of SPAN 40.

According to the Stern-volmer equation

$$\frac{I_0}{I} = 1 + K_{SV}[Q] = 1 + K_q \tau_0 [Q] \qquad [1]$$

where, I_0, and I are the fluorescence intensities before and after the addition of the quencher, K_q is the quenching rate constant, K_{SV} is the stern-volmer quenching constant, [Q] is the quencher concentration and τ_0 is the average lifetime without quencher a graph was drawn for (I_0/I) against quercetin connotation [Q] in SPAN 40 solution (Fig.2). From this Stern-Volmer Constant K_{Sv} and bimolecular quenching rate constant K_{SV} and bimolecular quenching rate constant K_q of Egg albumin with quercetin in 0.1M concentration of SPAN 40 have been calculated as K_{SV} = 0.7 and K_q = 1.45.

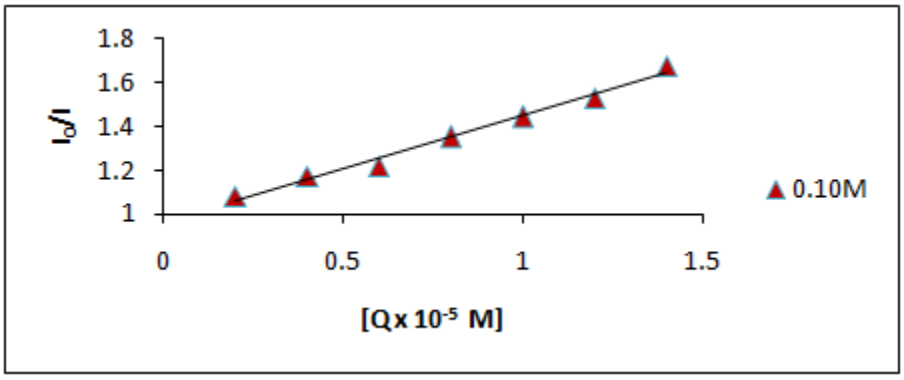

Fig. 2: Stern-Volmer plots of Egg albumin with Quercetin in 0.01 M concentration of SPAN 40

The value of binding constant K was determined from the intercept of (log (I_0-I)/I versus (log[Q]) as shown in Fig.3. The value of K is 3.42 x 10^4 L mol^{-1} and values of binding site n are found to be 0.97. The linear correlation coefficient of the curve is higher than 0.92, indicating that the interactions between quercetin and EA agreed well with the site-binding model according to eq.

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

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.

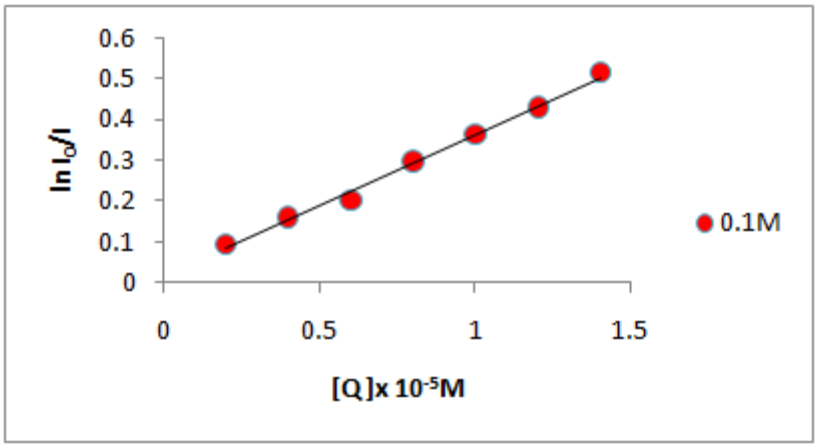

Fig. 3: Plot of ln (I_0-I) Vs. [Q] x 10^{-5}M of Egg albumin with Quercetin in 0.01 M concentration of SPAN 40

4. CONCLUSION

The results of fluorescence quenching experiments illustrate that there is a strong binding force between quercetin and egg albumin and that the binding site formed would be one. Drug, is bound to EA and a drug–EA complex is formed, which resulted in the quenching of the fluorescence of the Egg albumin.

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