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Molecular Spectroscopic Study On Binding Of Quercetin To Egg Albumin In CTAB

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Abstract- Steady – state and time – resolved fluorescence spectroscopy techniques were used to study the interaction of the flavonoid, quercetin [QCT] with egg albumin (EA) in different concentrations of cationic (Cetyl trimethyl ammonium bromide (CTAB) micelle. The binding constant and the binding site have been calculated. Micelle parameters such as aggregation number (Nagg), Radius of micelle (Ro), Head group area (ao) and critical aggregation parameter (ρ) have been calculated. To conform the complex formation, UV absorption spectra, FTIR spectra and SEM analysis have also been carried out.

Key words- Cetyl trimethyl ammonium bromide, Egg albumin, Quercetin, Fluorescence, FTIR**.**

1. INTRODUCTION

Flavonoids are a large class of naturally occurring polyphenols widely distributed in plants. Being both dietary and biologically active compounds, flavonoids have attracted much attention as investigators as potent species capable of affecting various biological process in living organism. It has been recognized that flavonoids display anticancer, antiviral, anti-inflammatory, and heart disease protective activities [1,2]. They are able to modulate various enzymes [3,4]. These highly potent biological activities of flavonoids are thought to result from their antioxidant and free radical scavenging properties. Flavonoids are also capable of chelating transition and noble metal ions what may result in enhancing their anti-inflammtory and anti – oxidant properties [5].

Quercetin (QCT) is a member of flavonoids, which are ubiquitous phenolic secondary metabolites found in plants, flowers, and plant derived foods [6]. The basic structure of flavonoids (Fig. 1) is usually characterized by three rings. They are two aromatic rings (A and B) which are joined by a three – carbon linked pyrone ring (C), forming a C6 – C3 – C6 skeleton unit where side group is usually hydroxyl, methoxyl or glycosyl. Abundant in the human diet, QCT posses various biological and biochemical effects including anti-inflammatory, antineoplastic and cardioprotective activities [7,1]. Additionally, QCT is among the group of phytoestrogens (Plant derived molecular with estrogenic or anti-estrogenic effects) suggested to reduce risks of certain cancers [8]. A full understanding of the modes of action of Bioflavonoids, however requires the study of their interaction with all possible biological target, including nucleic acids [9], enzymes [10,11], and other proteins [12].

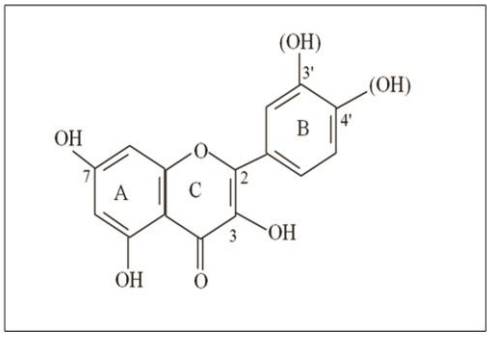

Fig. 1. Structure of Quercetin

In this work, the interaction of Egg albumin with QCT has been performed in CTAB solutions under physiological conditions utilizing fluorescence method in combination with time – resolved fluorescence and UV absorption spectrum. Furthermore FTIR spectra and SEM analysis also support to confirm the complex formation of egg albumin and quercetin in CTAB solution.

2. EXPERIMENTAL DETAILS

2.1 *Materials*

Egg albumin, Quercetin and Cetyl trimethyl ammonium bromide (CTAB) was purchased from Sigma Aldrich Company, Bangalore. Triply distilled water was used throughout the study.

2.2. *Sample Preparation*

The concentration of Egg albumin was maintained at 1.0 x 10-4 M and the concentration of the quencher (QCT) was varied in the range 0.2-0.14mM. The concentration of CTAB was varied in the range 0.02 – 0.10 M which is above the critical micellar concentration (CMC) of CTAB (0.92mM) [13].

2.3 Methods

2.3.1. UV/Vis Absorption Experiments

The absorption spectra of Egg albumin in water and in different micellar concentrations of CTAB both in presence and absence of the quencher, quercetin, have been recorded using Shimadzu 1650 PC UV-Visible Spectrophotometer. .

2.3.2. Fluorescence Steady – State Measurements

The steady – state fluorescence quenching measurements were carried out in a Shimadzu RF5301PC Spectrofluorophotometer. The excitation wavelength was 280 nm. The emission was monited at 339nm. The excitation and emission slit width (5nm) and scan rate (200 nm/s) were constantly maintained for all the experiments.

2.3.3 Fluorescence Quenching Experiments

For the quenching experiments, various concentrations of quencher were chosen. Fluorescence intensities were obtained for different quencher concentration and plotted according to the equation,

$$\frac{I_o}{I} = 1 + K_q \tau_0 [Q] \qquad (1)$$

The slopes afforded the Ksv values. The lifetime τo of EA without any added quencher and with different concentrations of the quencher were recorded. To extract Kq values, the experimental lifetimes were plotted against quencher concentration according to the Stern – Volmer equation,

$$\tau_0 / \tau = 1 + K_q \tau_0 [Q] \qquad (2)$$

where τ0 and τ are lifetime of EA in the absence and presence of quencher.

2.3.4. Fluorescence Lifetime Measurement

Fluorescence lifetime measurements were carried out in a Hariba – Jobin Yvon [spex-sf 13-11] spectrofluorimeter. The interchangeable nano LED (280 nm) was used as excitation source. The fluorescence decay of EA was measured with a monochromator – Photo multiplier setup. The data points were fitted by mono exponential decay functions. The data analysis was carried out by the software.

2.3.5 FTIR Measurements

FTIR spectra of Egg albumin without and with quercetin in different concentrations of CTAB were recorded using Thermonicolet is5 FTIR spectrophotometer.

2.3.6 SEM Analysis

Joel Sem Model, Jsm – 5610 Lv Scanning Electron Microscope was used to record the SEM photographs of Egg albumin with different concentrations of CTAB in the presence and absence of quercetin.

3. RESULTS AND DISCUSSION

3.1 UV/Vis Absorption Studies

The ground state complex formation if any between Egg albumin and quercetin was checked by recording the absorption spectra of a mixture of EA and quercetin in different concentrations of CTAB using concentration similar to those used in quenching studies. The absence of any new peak and the fact that absorption spectrum of EA was unaltered in the presence of the quencher eliminate the possibility of ground state charge transfer complex formation. For example, as a typical case, the absorption spectrum of Egg albumin in the absence and presence of quercetin in 0.02 m concentration of CTAB is shown in fig. 2. It may be noted that other concentrations, (0.04, 0.06, 0.08 and 0.10M) of CTAB also exhibited a similar behavior.

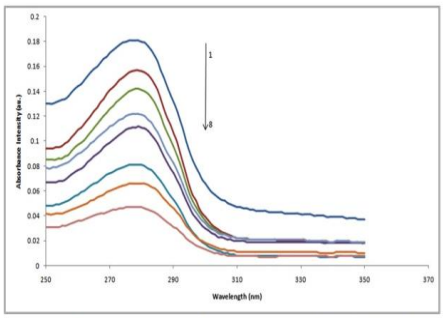

Fig. 2 Absorption spectra of Egg albumin withtout and with different concentrations of quercetin mol L^{-1} (1) 0.0 (2) 0.2, (3)0.4, (4) 0.6, (5) 0.8, (6)1.0, (7)1.2, (8) 1.4 in 0.02 M concentration of CTAB

3.2 Steady – State Fluorescence Study

The fluorescence spectra of Egg albumin in water and different micellar concentrations of CTAB both in presence and absence of the quencher [Fig. 3) (0.02M concentration of CTAB)], show no observable change in spectral shape and maxima. Although there is appreciable quenching even at low concentration of quercetin (0.2 x 10-5M), the shape of the fluorescence spectra remains the same with no change in the position of the maxima. Furthermore, 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 in all the media. These observations indicate that there is no ground state complexation of Egg albumin and quercetin. Decrease in the

fluorescence intensity of Egg albumin in all concentrations of CTAB (0.02, 0.04, 0.06, 0.08, 0.10M) 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.

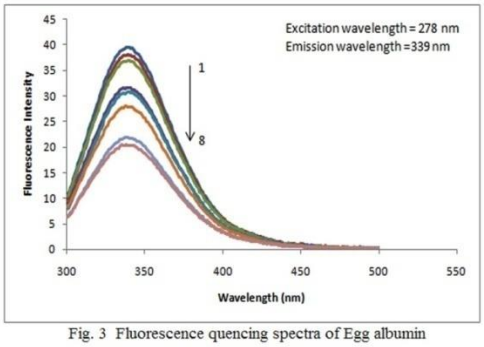

Fig. 3 Fluorescence quencing spectra of Egg albumin withtout and with different concentrations of quercetin mol L^{-1} (1) 0.0 (2) 0.2, (3)0.4, (4) 0.6, (5) 0.8, (6)1.0, (7)1.2, (8) 1.4 in 0.02 M concentration of CTAB

Fig. 3 shows the effect of increasing concentration of quercetin on the fluorescence emission of Egg albumin in 0.02 M concentration of CTAB. Addition of quercetin to the solution of Egg albumin resulted in the quenching of its fluorescence emission. According to eqn (1) we got linear plot [shown in Fig. 4] of the I_0/I against quercetin concentration in the CTAB solution.Stern volmer quenching constants (K_{sv}) have been calculated from the slope of the plot. The bimolecular quenching rate constant (K_q) was obtained and the corresponding electrochemical data were compiled in Table 1.

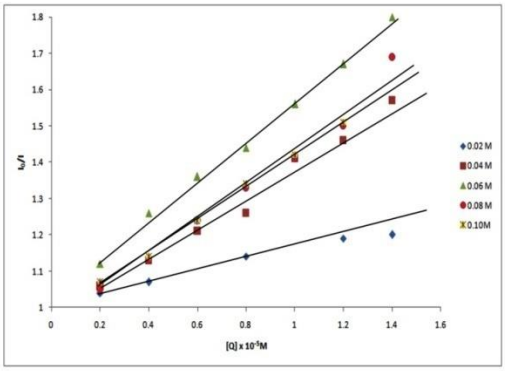

Fig. 4. Stern – Volmer plots of Egg albumin with quercetin in different concentration of CTAB

Table 1 Stern – volmer (K_{SV}) and bimolecular quenching rate constant (K_q) of Egg Albumin with Quercetin in different concentrations of CTAB

Concentration of CTAB (M)	K_{SV} x 10^5 (L Mol^{-1})	K_q x 10^{13}L $mol^{-1}s^{-1}$	R^2	SD
0.02	0.45	1.21	0.92	0.34
0.04	0.55	1.55	0.99	0.19
0.06	0.70	1.97	0.99	0.24
0.08	0.95	2.72	0.98	0.22
0.10	1.15	3.25	0.91	0.27

The obtained K_q values differ among the different concentrations of CTAB studied. The observed minimum K_q value may be due to a weak quenching.

3.3 Binding Constant and Number of Binding Sites

Large Kq beyond the diffusion – controlled limit indicates that some type of binding interaction exists between fluorophore and quencher [14].For static quenching, the relationship between the intensity and the concentration of the quencher can be described by the binding constant formula [15].

The relationship between the fluorescence intensity and the quencher medium can be deduced from the following equation.

$$nQ + B \rightarrow Qn\ldots B \qquad (3)$$

where B is the fluorophore, Q is the quencher and Qn….B is the postulated complex between a fluorophore and n molecules of the quencher. The constant K is given by,

$$K = [Qn\ldots B] / [Q]\,[B] \qquad (4)$$

if the overall amount of biomolecules (bound or unbound with the quencher) is B0, then [B0] = [Qn….B] + [B], here [B] is the concentration of unbound biomolecules, then the relationship between fluorescence intensity and the unbound biomolecular as [B] / [B0] = I/Io that is,

$$\text{Log}\left[\frac{F_0 - F}{F}\right] = \log k + n \log[Q] \qquad (5)$$

where K is the binding constant and n is the number of binding sites.

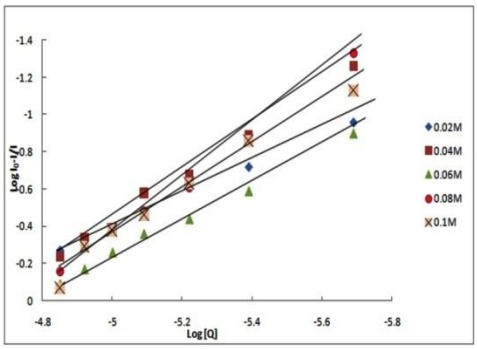

Fig. 5. Double log plot of quercetin quenching effect on Egg albumin fluoresence in different concentration of CTAB

The value of K was determined from the intercept of log [(Io – I)/I] versus log[Q] as shown in Fig. 5. The value of binding constant [K] and number of binding sites (n) for quercetin in all CTAB concentrations have been calculated and shown in Table. 2. The correlation coefficient for all the 0.97 curves were larger than indicating that the interaction between Egg albumin and quercetin in CTAB solution agrees well with the site binding model underlying eqn. (5).

Table 2 Binding constant (K_a), binding numbers (n), correlation coefficient (R), change in free energy ΔG_g (for ground state) and ΔG_e (for excited state).

Concentration of CTAB (M)	K_a $Lmol^{-1}$	n	r	ΔG_g $KJmol^{-1}$	ΔG_e KJ mol^{-1}
0.02	$1.13x10^5$	1.05	0.96	-44.54	50.82
0.04	$4.7x10^4$	1.0	0.99	-36.91	62.69
0.06	$3.31x10^4$	0.95	0.99	-65.53	71.73
0.08	$2.45x10^4$	0.90	0.99	-41.14	59.03
0.10	$1.97x10^4$	0.90	0.98	-39.04	53.62

3.4. Mechanism of Quenching

The quenching of Egg albumin can be explained by a number of possible mechanisms such as electron transfer, energy transfer, and proton transfer on hydrogen atom transfer. Fig. 3 shows the fluorescence emission spectra of EA with various quantities of quercetin 0.02M concentration of CTAB.. it can be seen from a scrutiny of the all the concentration of CTAB (0.02, 0.04, 0.06, 08, 0.1M) figures, fluorescence intensity of EA decreases steadily and with the addition of quencher there is almost no shift in the emission wavelength (λemi = 339nm). The quenching rate constant Kq are much higher than the maximum scatter collision quenching constant of the various quenchers [2.0 x 1010 L mol-1 s1] which indicates that the quenching mechanism of quercetin – EA interaction is not initiated by dynamic collision but by compound formation [16]. That is, drug is bound to EA and a drug – EA complex is formed, which resulted in the quenching of the fluorescence of the fluorophore.

Essentially, there exist four types of non-covalent interactions in the binding of the ligands to proteins. These are hydrogen bonds, van der waals forces, hydrophobic and electrostatic interactions [17]. Thermodynamic parameters, free energy (ΔG), standard enthalpy (ΔH) and standard entropy (ΔS) will provide an insight into the binding mode. Among these parameters, ΔG reflects the possibility of reaction; ΔH and ΔS are principal evidence for determining the active forces. Through, the binding constant ka, thermodynamic parameter is evaluated using the following equation,

$$\Delta G = - RT \ln Ka \qquad (6)$$

R is the gas constant; ΔG value is given in Table 2.

The negative sign for ΔG means that interaction is spontaneous and also indicates that the electron transfer processes studied are thermodynamically favourable. The hydrophobic force may play a major role in the reaction [18].

3.5 Fluorescence Lifetime Measurements Of Egg Albumin With Quercetin In Different Ctab Concentrations

Fluorescence lifetime measurement is a very useful technique for understanding the type of interaction between the donor and the acceptor systems. In general, the measurement of fluorescence lifetime is the most definite method to distinguish static quenching and dynamic quenching [19].

The decay curves of Egg albumin in the absence and presence of quercetin in different CTAB concentration were shown in Fig. 6. The lifetime of EA remains not same in both conditions; hence the merging of the kinetic traces was not observed (The plots not look like a single decay curve). This shows that the quenching of Egg albumin was dynamic in nature the same case was observed for all concentration of CTAB (Table.3).

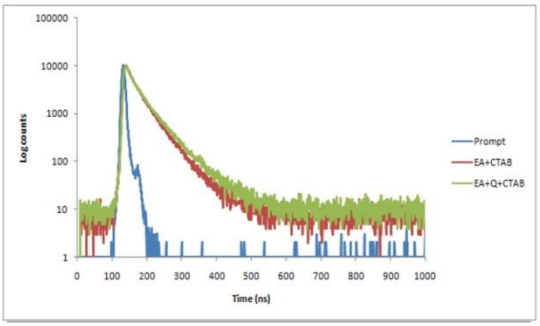

Fig. 6 Decay curves of Egg albumin

3.6. Micellar Size

From a structural point of view, the most relevant parameter of a micellar system is the mean micellar aggregation number. To analyse the effect of Egg albumin addition on the mean aggregation number of CTAB micelles, the well-established quenching method firstly proposed by Turro and Yekta [20] on the basis of previous analysis performed by Tachiya [21]. This procedure is based upon the quenching of a luminescent probe by a known concentration of quenchers. The quenching experiments were analysed by using the following equation,

$$\ln\frac{I_0}{I} = \frac{N_{agg}}{[S] - CMC}[Q] \qquad (7)$$

where Io and I are the fluorescence intensities in the absence and presence of the quenchers respectively. Nagg is the mean aggregation number, [S] is the total surfactant concentration and [Q] is the quencher concentration.

The results obtained in this quenching studies show how the Egg albumin fluorescence emission is quenched as the quencher concentration in the micellar system increase. Fig.7 shows the obtained quenching results according to equation (7). The mean aggregation number of CTAB micelles are listed in Table 4.

Table 3 Fluorescence life time and amplitudes of egg albumin without and with quercetin in different concentrations of ctab

Concentration of CTAB (M)	Concentration of Quercetin (M)	Lifetime (ns)			Average life time x 10^{-9} sec	Relative amplitude			χ^2	S.D x 10^{-11} sec		
		τ_1	τ_2 x 10^{-1}	τ_3		B_1	B_2	B_3		τ_1	τ_2	τ_3
0.02	0 1.4	1.96 1.96	4.73 x 10^{-9} 5.48 x 10^{-9}	4.30 x 10^{-10} 3.21 x 10^{-10}	3.73 4.12	26.13 27.51	67.55 64.87	6.32 7.62	1.22 1.05	1.57 1.11	4.12 3.87	5.39 3.93
0.04	0 1.4	1.59 2.01	2.42 x 10^{-10} 5.10 x 10^{-10}	4.44 x 10^{-9} 4.73 x 10^{-9}	3.56 3.62	23.93 29.32	4.84 7.50	71.24 63.17	1.31 1.38	1.08 1.98	6.78 6.73	2.77 4.08
0.06	0 1.4	1.74 1.81	4.48 x 10^{-9} 4.48x 10^{-9}	2.97 x 10^{-10} 3.68 x 10^{-10}	3.55 3.57	24.03 24.60	69.44 69.35	6.53 6.05	1.21 1.36	1.46 1.34	3.38 3.87	5.73 6.40
0.08	0 1.4	1.48 1.94	4.32 x 10^{-9} 5.16 x 10^{-10}	2.17 x 10^{-10} 4.62 x	3.49 3.57	22.47 28.52	72.86 6.87	4.69 64.61	1.16 1.23	9.43 1.95	2.62 7.19	5.93 3.70

				10^{-9}								
0.10	0 1.4	1.87 1.86	4.45 x 10^{-9} 4.49 x 10^{-9}	5.44 x 10^{-10} 3.41 x 10^{-10}	3.53 3.67	24.35 27.59	68.33 65.36	7.32 7.05	1.29 1.13	2.23 1.35	4.43 3.92	6.98 4.71

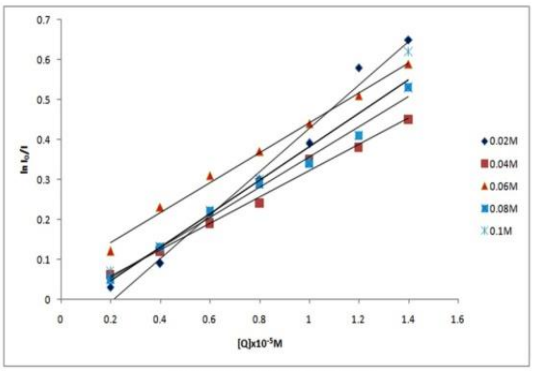

Fig. 7 Plot of ln (I_0/I) vs. [Q]x10^{-5} M

It has been proposed [22] that the surface area per head group, ao, is the most important controlling factor for micelle size. According to Tanford [23], the hydrophobic chain volume of the micelle v, and the critical chain length lc, can be obtained from,

$v, = (27.4 + 26.9\ n_c)\ (A^{\circ 3})$ (8)

and

$l_c = (1.5 + 1.265\ n_c)\ (A^{\circ})$ (9)

Table 4 Aggregation number,(N_{agg}), radius (R_o), surface area per head group (a_0), and packing parameter ($v/a_o l_c$) of CTAB micelle

Concentration	Aggregation number	Radius of the micelle (R_0)Å	Area of the micelle (a_0) Å^2	Critical aggregation parameters
0.02	955	47.81	30.06	0.703
0.04	2150.5	62.49	22.81	0.926
0.06	2807.25	68.24	20.84	1.013
0.08	3361.75	72.42	19.59	1.078
0.10	4459.5	79.49	17.79	1.187

where nc is the number of carbon atoms in the hydrophobic chain of the surfactant. In this way, assuming a spherical geometry, the micellar radius, Ro, and the surface area per head group were obtained. The corresponding values are listed in Table 5

Table : 5 Difference in FTIR absorption peak Intensities of Egg Albumin before and after complex formation in different concentrations of CTAB

Intensities (cm^{-1})				Difference in intensities prior to and after (%)		Tentative Assignment
EA SLS		EA Q SLS				
0.02	**0.10**	**0.02**	**0.10**	**0.02**	**0.10**	
2919	2917	2920	2918	0.08	0.45	C –H stretching
2850	2849	2851	2849	0.06	0.45	C-H stretching
1647	1654	1652	1653	0.07	0.05	C = Ostretching
1396	1395	1397	1396	0.03	0.10	C – H stretching

It is also included the critical packing parameter, v/aolc, which is a parameter controlling the micelle shape [22].

3.7. Fourier Transform Infrared Spectra

The changes of the FTIR spectra shown in Figs. 8 & 9 can reveal the formation of the Egg albumin and quercetin complexes in 0.02 M concentration of CTAB. The same can be observed for all other concentrations of CTAB (0.04, 0.06, 0.08 and 0.1 M) and the values are shown in Table 5.

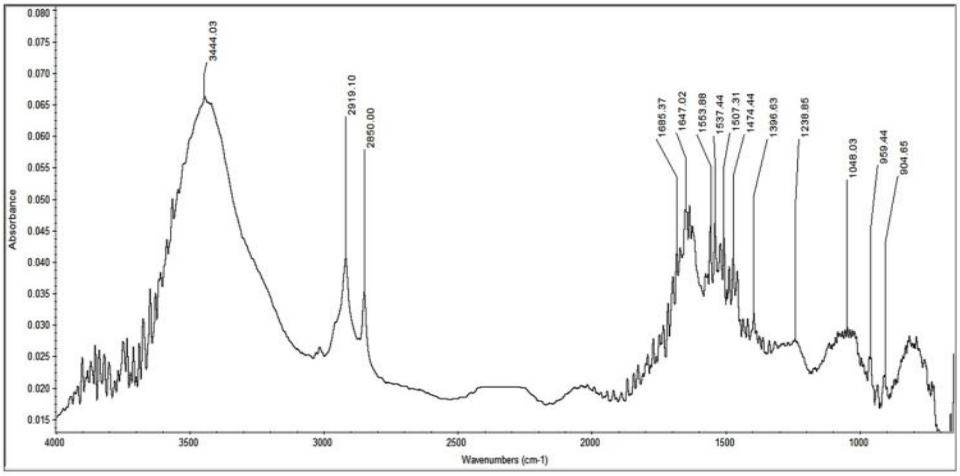

Fig. 8 FTIR spectra of Egg albumin in CTAB solution (0.02 M)

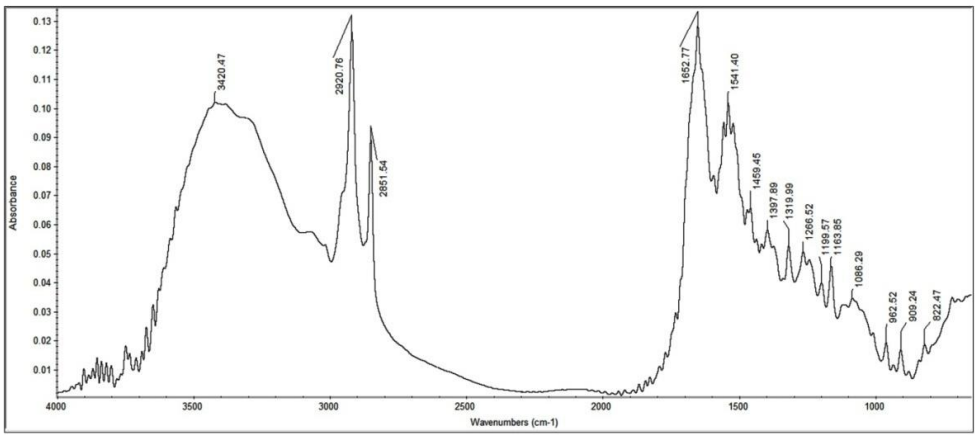

Fig. 9 FTIR spectra of Egg albumin with quercetin in CTAB solution (0.02 M)

The absorption intensity of the complexes is significantly weaker than that of Egg albumin. The complex infra-red peaks are observed in the range 1000 cm-1– 3000 cm-1 and are 0.03 to 0.45 % weaker than that of the Egg albumin molecule. As there is no changing in the wavenumber other than, change in the absorption intensities, it can be concluded that 0.03 to 0.45 % weaker complexes were formed of Egg albumin and quercetin in different CTAB concentration.

3.8. Scanning electron microscope (SEM) observation of Egg albumin with quercetin in CTAB

Egg albumin in CTAB was powdered separately and the structure of their particles in this powders was observed first, in the scanning electron microscope (Fig.10). Then the particles of the powdered form of the complexes (EA+QCT+CTAB) were also studied. These are shown in Figs.11-15.The SEM images of (EA+QCT) with 0.02,0.04,0.06,0.08,and 0.10M concentration of CTAB are shown in Figs 11, 12, 13, 14, 15 respectively. The structure of the particles of the complexes EA without Quercetin appears different from that of Egg albumin with quercetin in CTAB and it can be assumed as proof of the formation of new complex.

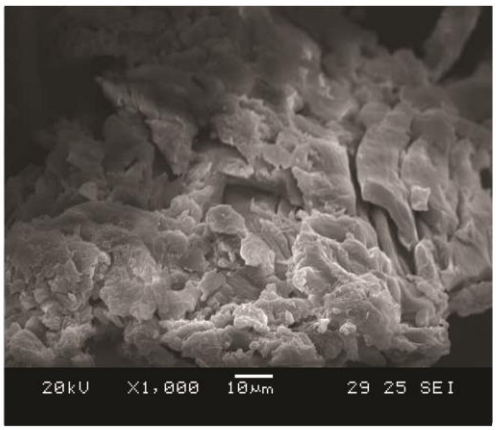

Fig. 10 SEM image of EA with Quercetin

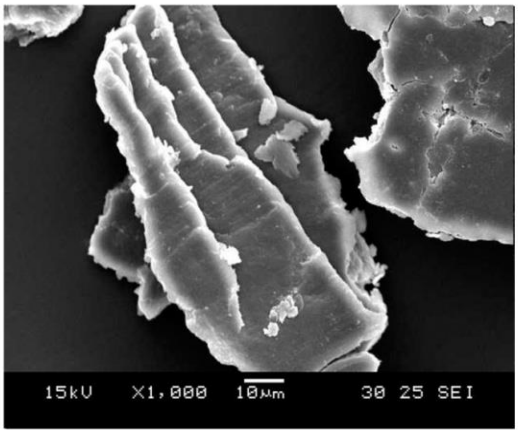

Fig.13 SEM Image of Egg albumin with quercetin in 0.06 M Concentration of CTAB

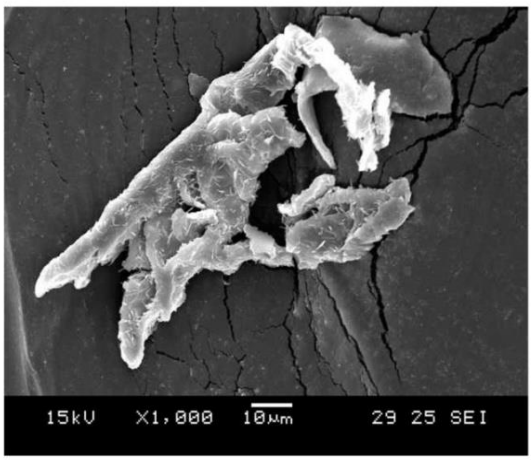

Fig.11 SEM Image of Egg albumin with quercetin in 0.02 M Concentration of CTAB

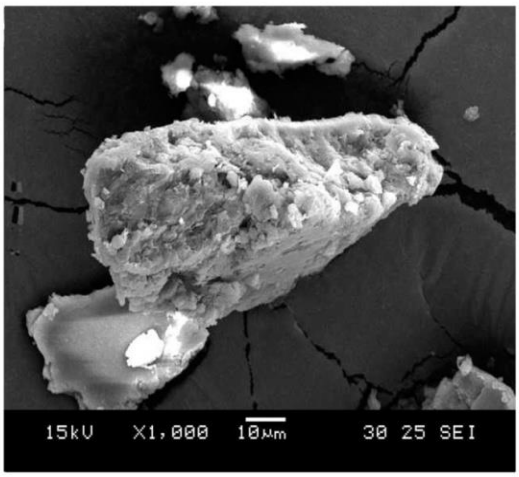

Fig.14 SEM Image of Egg albumin with quercetin in 0.08 M Concentration of CTAB

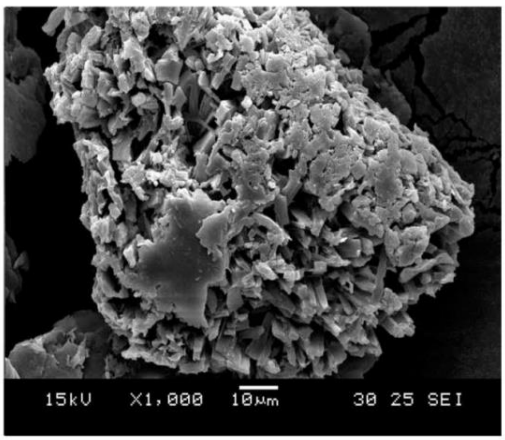

Fig.12 SEM Image of Egg albumin with quercetin in 0.04 M Concentration of CTAB

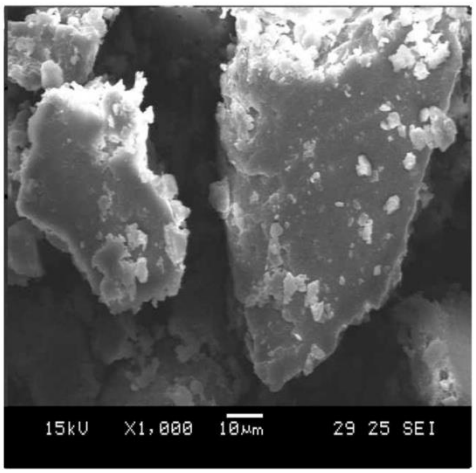

Fig.15 SEM Image of Egg albumin with quercetin in 0.10 M Concentration of CTAB

4. CONCLUSION

The application of plants as medicines by human dates to thousands years ago. Flavonoids of plants are natural products that

exhibit a various biological and pharmaceutical properties. In view of their broad incidence in nature and considerably low toxicities. Prospective development and use of these compounds as efficient pharmaceutical agents. Particularly as anticancer drugs is a matter of significant current interest. This paper has focused on the general features of the quercetin interaction with egg albumin in CTAB solution.

In this work, the interaction quercetin with EA in CTAB was studied by spectroscopic methods including fluorescence spectroscopy, UV-Visible absorption spectroscopy, time-resolved fluorescence spectroscopy, FTIR spectroscopy and SEM analysis. This experimental result indicates that the quenching mechanism of flourescne of EA by quercetin is a dynamic process; binding parameters calculated from Stern-Volmer method and scatcherd method showed that quercetin binds to EA with the binding affinities of the order 104L mol-1were discussed. The binding reaction is spontaneous and hydrophobic interaction play a major role in the reaction. Binding site was also determined.

The interaction study of quercetin with EA and structure – affinity relationships is of good importance in pharmacy, pharmacology and biochemistry.

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