Characterization of the Interaction between Quercetin and Egg Albumin in SPAN 40 Solution

S. Bakkialakshmi[*], B. Bhavani[a] and P. Selvarani[b]
*Department of Physics, Annamalai University, Annamalainagar, Tamilnadu, India-608 002.
[a]Christ Institute of Technology, Pondicherry
[b]Auxilium College of Arts and Science for women, Pudukottai

Abstract

Dietary polyphenols are very essential and are wieldy studied compounds due to their various biological activities. The anti-bacterial protein, lysozyme is beneficial drug carrying ability. Hence their binding at molecular level is essential perspective in the field of pharmaceutics. The bindings of quercetin with Egg albumin in a surfactant, SPAN 40 have been investigated using UV-vis, fluorescence and molecular docking studies. Quercetin is found to be the quencher of Egg albumin fluorescence and the quenching mode involved here is found dynamic in nature. The energy transfer parameters are calculated and a possibility of energy transfer from Egg albumin to quercetin is observed. Molecular docking study gives the distance of quercetin to egg albumin.

Keywords: *Quercetin, Egg albumin, Fluorescence, Binding constant, Docking.*

I. INTRODUCTION

Enzymes are essential for various biologically important pathways and they are also acting as the targets in therapies, and pharmaceutics. The anti-bacterial protein, lysozyme is an enzyme and it is abundant in various types of secretions like tears, nasal mucus, human milk, saliva, etc. It is available in large amount in Egg albumin. It has various pharmaceuticals and pharmacological properties like anti-tumor, anti-viral, anti-histaminic, anti-inflammatory, immune modulatory properties [1-5]. Lysozyme has the capacity to cure various diseases by binding with various drugs and realizing them in their target site. The protein is important in medicinal point of view due to its drug binding ability. Hence the study on lysozyme and ligand interaction at molecular level is very important.

Lysozyme is a globular protein and contains 129 amino acid residues, is immensely important due to its biological properties (6-8), in accordance with the capability of destruction of bacterial cell membrane. In the ligand binding site two tryptophan (Trp) residues, trp 62 and 108 are present which are found to be the most effective fluorophores among available six tryptophans in the overall structure.

Flavonoids are the largest category of resources. The intake of flavonoids decreases the chance of various diseases like cancer, cardiovascular disease, stroke (9-11), because these compounds have radical scavenging, anti-inflammatory, anti-cancer, anti-tumer and anti-oxidative properties (11,12).

Quercetin (3, 3′, 4′, 5, 7 – pentahydroxy flavones) is a member of naturally occurring, highly important compounds, the flavonoids, which are ubiquitous phenolic compounds, found in plants, fruits, flowers and plant derived foods (13). Flavonoids consist of two aromatic rings (Fig.1) linked by an oxygen-containing hetrocyle. Quercetin has anti-oxidant and metal ion chelating capacity. It possess various biological and biochemical effects including anti-inflammatory and cardio protective activities (14,15) to understand the actions of bioflavonoids require studying of their interaction with all possible biological targets, including nucleic acids (16), enzymes (17, 18) and proteins (19).

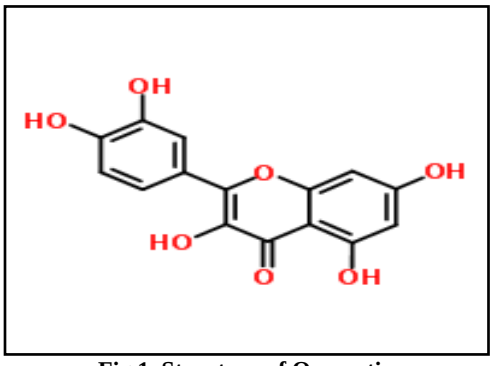

Fig.1. Structure of Quercetin

The binding of quercetin (Q) with egg albumin (EA) in SPAN 40 solution using UV/Vis, fluorescence, and molecular docking studies has been investigated in this paper. The binding affinities, number of binding sites and binding distances are determined for Egg albumin- quercetin complexes. Molecular docking study has been executed to substantiate the experimental findings. The interaction of small drug like molecules with the biological

targets under physiological conditions finds significant applications in the field of pharmaceutical and biomedical research for drug delivery or drug targeting host systems.

II. MATERIALS AND METHODS:-

Egg albumin, quercetin and SPAN 40 were obtained from sigma Aldrich Company Bangalore. They were used for taking readings without further purification. UV – Vis studies were conducted on a SHIMADZU 1650 PC UV-VISIBLE SPECTROPHOTOMETER in the region of 250 – 350 nm, at room temperature. The fluorescence measurements were carried out at room temperature on SHIMADZU RF 5301 PC SPECTROFLUOROPHOTOMETER using a 1 cm quartz cell.

The 3D crystal structure of Egg albumin (PDB ID 4XEN) is obtained from the Protein Data Bank.

III. RESULTS AND DISCUSSION

A. Absorption Characteristics Of Egg Albumin With Quercetin

UV/Vis absorption measurement is a simple but effective method of detecting complex formation[20,21]. Complex formation between quercetin and EA is evident from the data UV/Vis absorption spectra.

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 SPAN 40 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. UV/Vis absorption spectra of EA and those of EA with the addition of different concentrations of quercetin were recorded. It is obvious that the intensity of UV absorption of EA decrease with the addition of quercetin in SPAN 40 solution.

Fig.2 shows the absorption spectra of EA from 250 nm to 350 nm in the presence of different concentrations of quercetin in 0.02 M concentration of SPAN 40. It is evident that absorbance of EA decreased regularly with the variation of quercetin concentrations and the maximum peak position of quercetin-EA shifted from 280 nm 278 to nm. Moreover, a slight blue shift of the maximum peak of albumin at 278 nm, probably due to complex formation between quencher and albumin was also noticed [22].

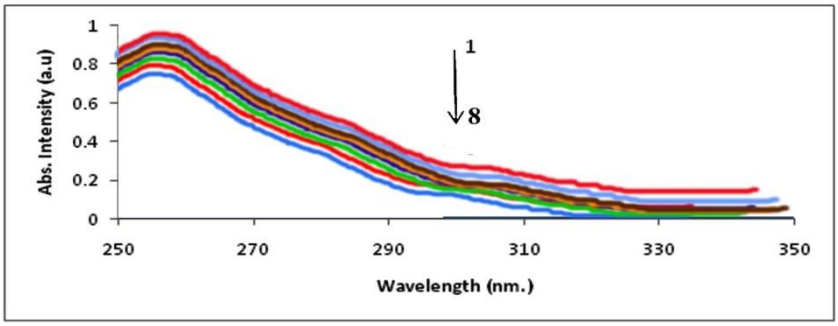

Fig. 2: UV/Vis Absorption Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.02 M Concentration of SPAN 40

The absorption of spectrum of Egg albumin in the absence and presence of quercetin in 0.04, 0.06 and concentrations of SPAN 40 are shown in Figs.3,4 and 5 and respectively. It may be noted that all the other concentrations of SPAN 40 (0.04, 0.06, and 0.08M and) also exhibited a similar behavior as like as 0.02 M concentration of SPAN 40.

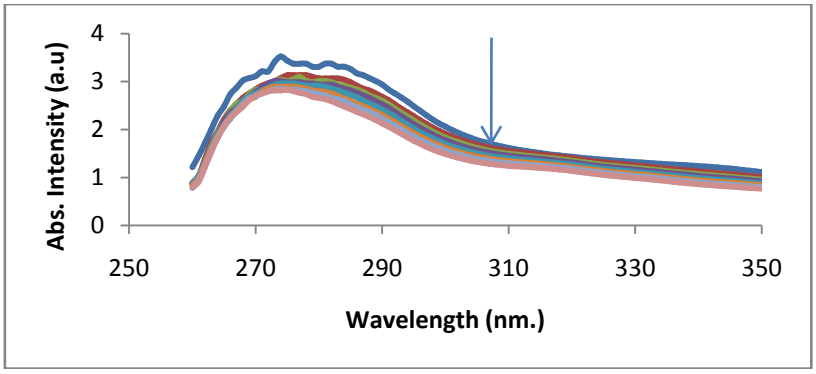

Fig. 3: UV/Vis Absorption Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.04 M Concentration of SPAN 40

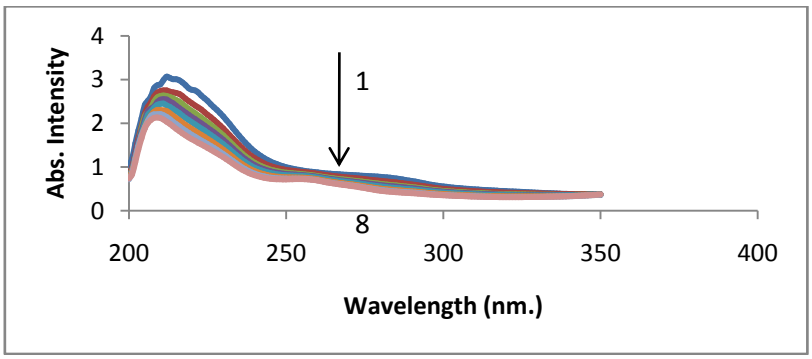

Fig. 4: UV/Vis Absorption Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.06 M Concentration of SPAN 40

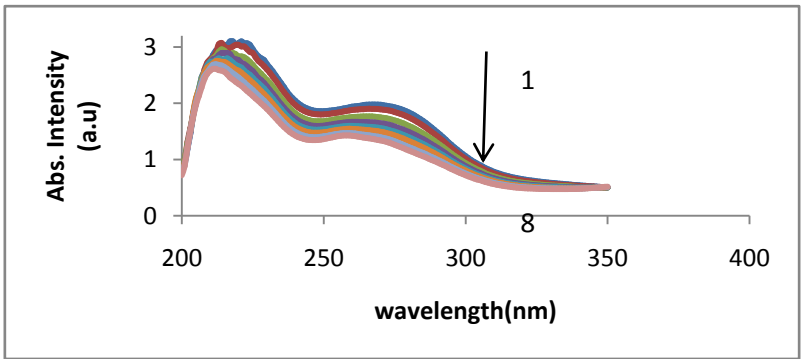

Fig. 5: UV/Vis Absorption Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.08 M Concentration of SPAN 40

B. Fluorescence Quenching of Egg Albumin by Quercetin in SPAN 40

The fluorescence spectra of Egg albumin in different micellar concentrations of SPAN 40 both in presence and absence of the quencher, show no observable change in spectral shape and maxima. Fig.6 shows the fluorescence quenching spectrum of Egg albumin without and with different concentrations of quercetin in the solution of 0.02M SPAN 40 concentration. There is appreciable quenching even at low concentration of quercetin (0.2 x 10^{-5}M), the shape of the fluorescence spectra remains the same with no change in the position of the 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 is all the media. These observations indicate that there is no ground state complexation of Egg albumin and quercetin.

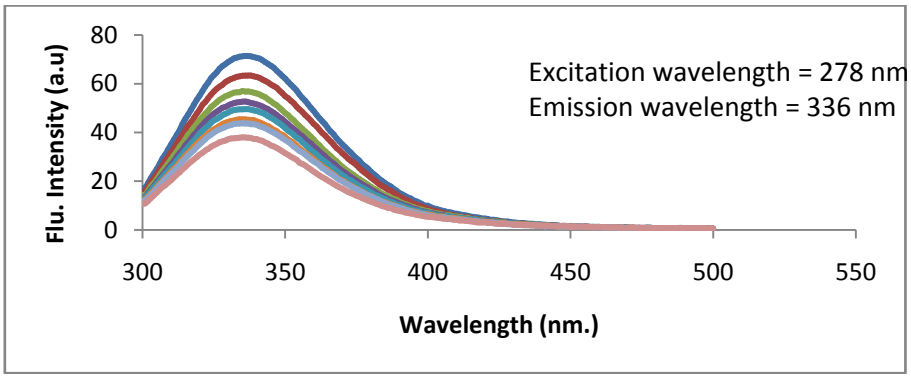

Fig. 6: Steady – state fluorescence Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.08, (6) 1.0, (7) 1.2, (8) 1.4 in 0.02 M Concentration of SPAN 40

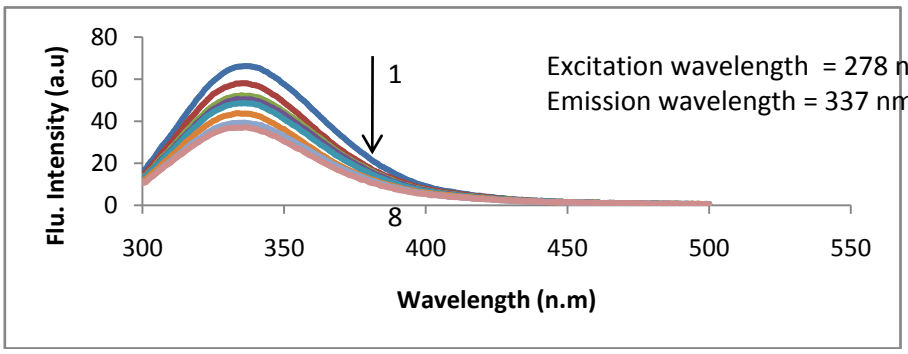

Fig. 7: Steady – State Fluorescence Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.04 M Concentration of SPAN 40

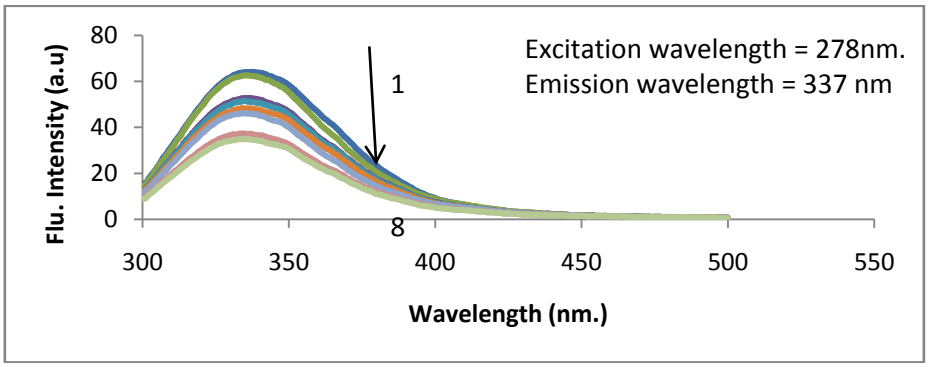

Fig. 8: Steady – State Fluorescence Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.06 M Concentration of SPAN 40

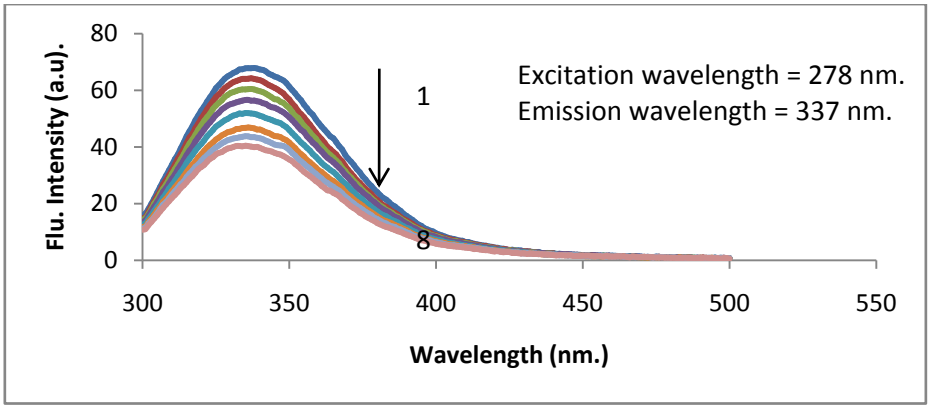

Fig. 9: Steady – state Fluorescence Spectra of EA with Different Concentrations of Quercetin (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0, (7) 1.2, (8) 1.4 in 0.08 M Concentration of SPAN 40

Figs. 6, 7, 8, and 9 show the fluorescence quenching spectra of egg albumin without and with different concentrations quercetin in the solutions of 0.02 M, 0.04M, 0.06M, and 0.08M concentrations of SPAN 40. Decrease in the fluorescence intensity of egg albumin in all concentrations of SPAN 40 (0.02M, 0.04M, 0.06M, and 0.08M) 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. Fluorescence quenching of EA results from a decrease of quantum yield of fluorescence. The Stren-Volmer equation is often applied to describe fluorescence quenching and analyze the quenching mechanism [23].

$$\frac{I_o}{I} = 1 + K_{sv}[Q] = 1 + K_q \tau_o [Q] \quad \text{…….. (1)}$$

Where I_o and I are the fluorescence intensities before and after 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_o is the average lifetime without quencher.

According to eqn (1) a graph was drawn for (I_o /I) against quencetin concentration [Q] in SPAN 40 solution. A linear plot was observed. Fig. show the linear plot of I_o /I against [Q] in all the concentrations (0.02M, 0.04M, 0.06M, and 0.08M) of SPAN 40. From the slope the Stern-Volmer quenching constants (K_{sv}) were calculated. The bimolecular quenching rate constants (K_q) were obtained for the different concentrations of SPAN 40 and the corresponding electrochemical data were compiled in Table 1.

Table-1 Stern – Volmer (K_{SV}) and Bimolecular Quenching Rate Constant (k_q) of Egg Albumin with Quercetin in Different Concentrations of SPAN 40

Concentration of CTAB (M)	K_S V (L Mol^{-1})	K_q L $mol^{-1}S^{-1}$	R^2	SD
0.02	0.40	1.04	0.97	0.25
0.04	0.55	2.41	0.98	0.23
0.06	0.60	3.62	0.96	0.27
0.08	0.65	6.46	0.99	0.23
0.10	0.70	1.45	0.99	0.21

The obtained K_q values differ among the different concentrations of SPAN 40 studied. The observed minimum K_q value may be to a weak quenching.

C. Binding Constants and Number of Binding Sites

Large K_q beyond the diffusion controlled limit indicates that some type of bindings interaction exists between fluorophore and quencher [24]. For

static quenching, the relationship between the intensity and the concentrations of quencher can be described by the binding constant formula [25].

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

$$nQ + B \rightarrow Qn \ldots B \quad \ldots\ldots\ldots\ldots\ldots (2)$$

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

$$K = [Q_n \ldots B] / [Q_n][B] \quad \ldots\ldots\ldots\ldots\ldots (3)$$

If the overall amount of biomolecular (bound or runbound with the quencher) is B_o then,

$$[B_o] = [Q_n \ldots B] + [B] \quad \ldots\ldots\ldots\ldots\ldots (4)$$

Here, [B] is the concentration of unbound biomolecules, then the relationship between fluorescence intensity and the unbound biomolcules as $[B] / [B_o] = I / I_o$

That is

$$\log\left[\frac{I_o - I}{I}\right] = \log K + n \log [Q] \quad \ldots\ldots\ldots\ldots\ldots (5)$$

Where K is the binding constant, and n is the number of binding sites. Thus, a plot of log (I_0 - I) versus log [Q] can be used to determine K as well as n.

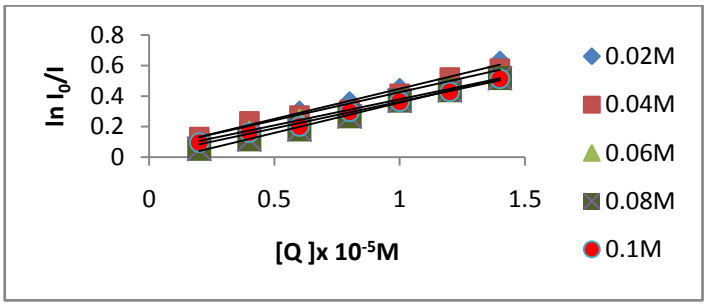

Fig. 10: Plot of log (I_0-I) Vs. [Q] x 10^{-5}M of Egg Albumin with Quercetin in Different Concentration of SPAN 40

The value of K was determined from the intercept of log (I_0 - I) versus log [Q] as shown in Fig. 10. The value of K are 1.76 x 10^6, 5.75 x 10^5 , 3.14 x 10^5 and 5.96 x 10^4 L mol^{-1} for EA in 0.02, 0.04, 0.06 and 0.08M concentrations of SPAN 40 respectively and the values of n were found to be nearly 1.3 (above 0.90) for all concentration of SPAN 40. The linear correlation interaction between quercetin and EA agreed well with the site-binding model according to equation (5).

Table-2 Binding Constant (K_a), Binding site (n), Correlation Coefficient (R), Change in Free Energy ΔGg (for Ground State) and ΔGe (for Excited State).

Concentration of SPAN 40 (M)	K_a $Lmol^{-1}$	N	R	ΔGg $kJmol^{-1}$	ΔGe KJ mol^{-1}
0.02	$1.76X10^6$	1.30	0.99	-42.29	71.29
0.04	$5.75X10^5$	1.21	0.97	-23.28	98.55
0.06	$3.14\ X10^5$	1.15	0.92	-22.16	56.54
0.08	$5.98\ X10^4$	1.02	0.99	-18.21	70.05
0.10	$3.42\ X10^4$	0.97	0.99	-16.82	74.48

The result illustrates that there is a strong binding force between quercetin and EA, and that the binding site formed would be one. Table 2 presents the calculated binding constant and binding site values.

D. Mechanism of Quenching

The quenching of Egg albumin can be explained by a number of possible mechanisms such as electron transfer, energy transfer, proton transfer, or hydrogen atom transfer. It can be seen from a scrutiny of the above said figures, fluorescence intensity of EA decreases steadily and with the addition of quencher there is almost no shift in the emission wavelength (λ_{emi} = 336 nm). The quenching constant K_q are much higher than the maximum scatter collision quenching constant of the various quenchers [2.0 x 10^{10} L mol^{-1} S^1] which indicates that the quenching mechanism of quercetin – EA interaction is not initiated by dynamic collision but by compound formation [26]. 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 exists four types of non-covalent interactions in the binding of the ligands to proteins. These are hydrogen bonds, van dar walls forces, hydrophobic and electrostatic interactions

[27]. 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 K, thermodynamic parameter is evaluated using the following equation,

$\Delta G = -RT\ 1n\ K$ …….. (6)

R is the gas constant and this 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 [28].

E. Fluorescence Resonance Energy Transfer between Quercetin and Egg Albumin in SPAN 40

A lot of information about molecular details of the donor – acceptor pair can be obtained from non-radiation energy transfer [29], fluorescence resonance energy transfer occurs when the emission spectrum of the donor overlaps the absorption spectrum of the acceptor. The dependence of the energy transfer rate on the interaction distance has been widely used to measure the distance between the donor and the acceptor.

Generally, the maximum distance is in the range of 7-10 nm [30]. According to the Forster non-radiation energy transfer theory [31,32], energy transfer is related not only to the distance between the acceptor and donor, but also to the critical energy transfer distance (R_0),

$$E = \frac{R_0^{\ 6}}{R_0^{\ 6} + r^6} \quad \text{…… (7)}$$

Where R_0 is the critical transfer distance when the transfer efficiency is 50% and r the mean distance between the centers of the donor and acceptor dipoles. The donor and acceptor here are EA and quercetin respectively. E is the energy transfer efficiency calculated with equation (7),

$$E = 1 - \frac{I}{I_0} \quad \text{……(8)}$$

Where I and I_0 are the fluorescence intensity of EA with and without quercetin respectively. And R_0 can be given by,

$R_0^{\ 6} = 8.8 \times 10^{-25}\ K^2\ N^{-4}\ \phi\ J$ ……(9)

Where K^2 is the spatial orientation factor of the dipole, N, the refractive index of the medium, ϕ the fluorescence quantum yield of the donor in the absence of the acceptor, J expresses the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor,

J is given by,

$$J = \frac{\Sigma F(\lambda)\,\varepsilon(\lambda)\lambda^4\Delta\lambda}{\Sigma F(\lambda)\Delta\lambda} \quad \text{……..(10)}$$

where F(λ) is the fluorescence intensity of donor at wavelength range λ and ε (λ), the molar absorption coefficient of the acceptor at wavelength λ with unit of $Cm^{-1}mol^{-1}$ integrating the overlap of the UV absorption spectrum of quercetin and the fluorescence emission spectrum of EA.

The calculated E = 0.4566; Ro = 38.94 A° and r = 40.09 A°. The overlap spectrum is shown in Fig.11. The compiled data are present in Table. 3.

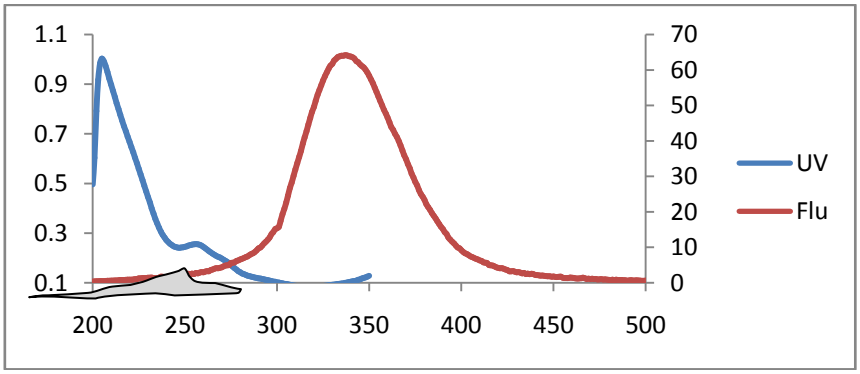

Fig.11: The Overlap of UV Absorption of Quercetin with the Fluorescence Emission Spectrum of EA .

Table-3 Efficiency Transfer Energy (E), Critical Energy Transfer Distance (R_o) of EA with Quercetin FRET

Quencher	Energy (E)	R_o Å	J (cm^3M^{-1})	rÅ
Quercetin	0.4566	38.94	8.32×10^{-40}	40.09

F. Micellar Size

From a structure point of view, the most relevant parameter of a micellar system is the mean micellar aggregation number. To analyze the effect of Egg albumin addition on the mean aggregation number of SPAN 40 micelles, the well established quenching method firstly proposed by [33] on the basis of previous analysis performed by Tachiya [34]. The procedure is based upon the quenching of a luminescent probe by a known concentration of a quencher. The quenching experiments were analysed by using the following equation,

$$\ln \frac{I_0}{I} = \frac{N_{agg}}{[S] - CMC}[Q] \qquad \text{.....(11)}$$

Where I_0 and I are the fluorescence intensities in the absence and presence of the quenchers respectively, N_{agg} 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. 12 shows the obtained quenching results according to equation (11). The mean aggregation numbers of SPAN 40 micelles, are listed in Table 4.

G. Molecular Docking Results

Recently molecular docking becomes an important tool to investigate the protein – ligand binding study. The docking poses of Egg albumin and quercetin in Egg albumin are represented in figs 13 and 14 respectively. For discussion we have chosen the first docked conformation in each cash because it posses the minimum energy.

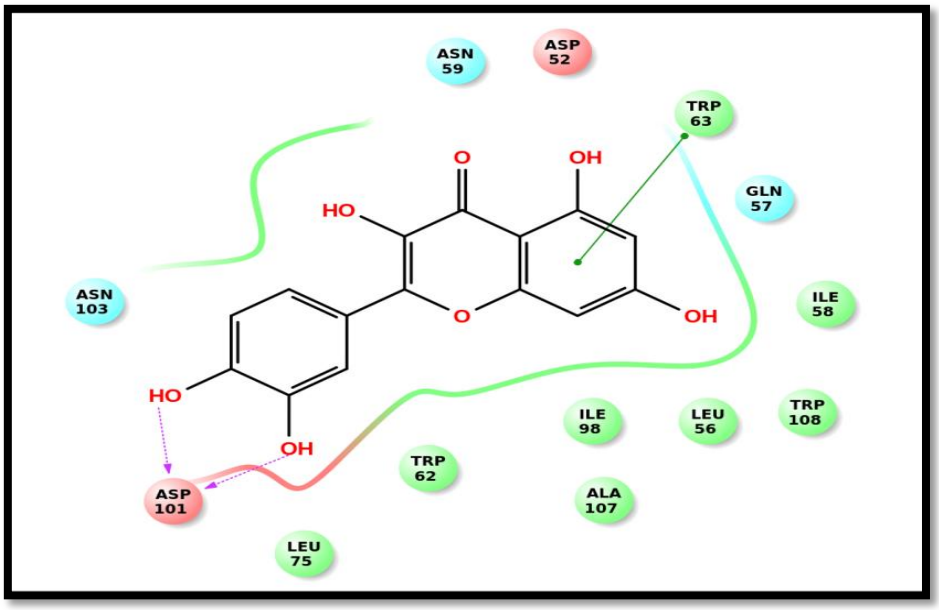

Fig 13: 2-Dimensional View of Molecular Docking of EA with Quercetin

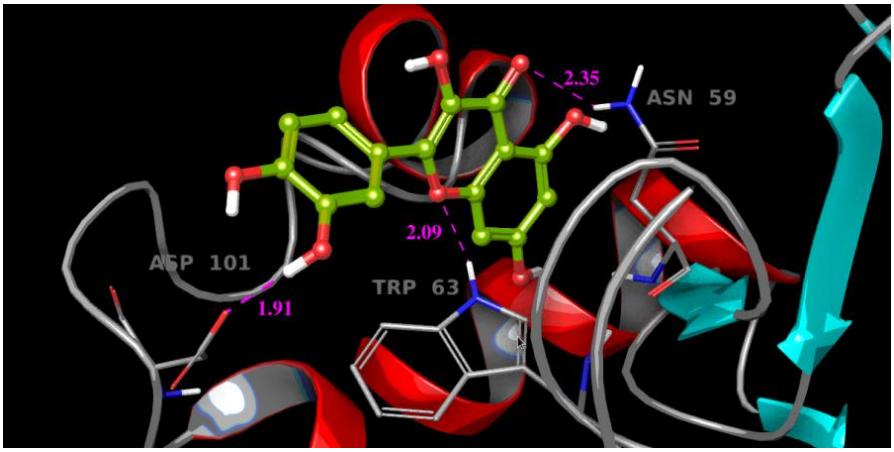

Fig 14: 3-Dimensional View of Molecular Docking of EA with Quercetin

It has been found that quercetin bind within H-bonding distance (Fig. 13) to the tryptophan residues. (Trp 63, ASP 101 & ASP 54) of Egg albumin. The expected interaction of the ligand with two tryplophan residues, Trp 63, ASP 101 & ASP 54 observed quenching of fluorescence intensity of egg albumin by quercetin. The distance of quercetin to Trp 63 BE 2.09 Å according to FRET results it has also been found that quercetin is present in the close vicinity to egg albumin. To recognize the residues involved in the binding, we have estimated me accessible surface area (ASA) of residues of native and complexed protein Table (5). High binding energy or total score represents a greater binding affinity of quercetin towards Egg albumin. The negative values of bindings energy or total score indicate the spontaneous binding of the polyphenol towards the protein.

Table 5: Docking Results

FLAVONOIDS	docking score	glide ligand efficiency	XP GScore	glide evdw	glide ecoul	glide energy	XP HBond	XP LipophilicEvdW	Potential Energy-OPLS-2005	Ionization Penalty
QUERCETIN	-6.84	-0.311	-6.84	-35.982	-9.165	-45.147	-2.281	-2.977	60.657	0.0113

IV. CONCLUSION

The interaction of dietary flavonoid quercetin, with Egg albumin have been executed using UV/Vis, fluorescence and molecular docking studies. The bindings affinities of the polyphenol is observed in the order of 10^4 M^{-1}. The compound is able to quench the fluorescence intensity of Egg albumin. The energy transfer parameters for the bindings are calculated and it is observed that there is a chance of energy transfer from donor (Egg albumin) to the acceptors (quercetin). Molecular docking study has been performed to substantiate the experimental facts. The bindings of dietary polyphenol with protein at the molecular level suggests a further insight into the success of the drugs in pharmaceutics.

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