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Spectroscopic studies for determining the interaction between Sinapic acid with Ovalbumin

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Abstract

Proteins may be classified as aerated dietary products based on their stability and foaming capability. When gas is disseminated in aqueous solutions to create foams, surface-active chemicals keep the bubbles from bursting. In our everyday activities, proteins and polyphenols are often combined. Sinapic acid (SA) is one of the most widely distributed polyphenols. It has been examined if the foam would remain stable when coupled with ovalbumin (OVA) and Sinapic acid (SA). The results of this study showed that polyphenols may be used in the food industry as a stabilizing agent to improve the functional characteristics of foam and provided some fundamental understanding of how polyphenols alter the foaming properties of OVA. UV-visible spectroscopy was used to study and characterize the relationship between ovalbumin and Sinapic acid .The experimental results indicated that the fluorescence quenching mechanism between Sinapic acid (SA) and ovalbumin (OVA) was static quenching which was proved again by the analysis of fluorescence lifetime by time resolved fluorescence Spectro fluorimeter .Utilizing FT-IR spectra, the structural and functional groups of the tyrosine (Try) and tryptophan (Trp) residues were also examined. The binding constant and the total number of SA binding sites on OVA were also determined in this research.

Key words: Ovalbumin (OVA), Fluorescence, Sinapic acid (SA), Synchronous fluorescence, FT-IR.

1. Introduction

One essential protein source is ovalbumin found in egg white protein (EWP). Due to its remarkable gelling and foaming properties, EWP is well known in the culinary industry and often used in foods like meringues, cakes, cookies, and chocolate mousses. (1)

Ovalbumin (OVA), the main component of EWP (2), is a typical spherical phosphor-glycoprotein with a molecular weight of 45 kDa and 385 single amino acid chains (P-gp). (3)

A key functional component and attractant in many foods are foaming. It enhances the amazing feeling of dishes like meringues, soufflés, whipped toppings, chiffon desserts, and leavened bread products that are related to it (4-7). Bubbles in foams, which are gases dispersed in aqueous solutions, are stabilized by surface-active chemicals (8–9).

Protein produces a sticky protein film around the gas or air droplet when it is dissolved in water and vigorously stirred (10). When deciding whether proteins are acceptable for aerated food products, foaming capability and stability are key factors. Ostwald's maturity, draining, and coalescence are the last phases that Bubbles go through before finally becoming unstable, bursting, and reducing free energy (11). Research has been done on protein modifications to improve foaming properties, including high-intensity ultrasound (12), phosphorylation under wet-heating conditions (13), and oxidative modification (14).

Polyphenols, which include flavonoids, stilbenes, phenolic acids, lignans, and other subclasses, are a biologically active class of plant compounds (15). Protein-polyphenol combinations are common in our daily lives. Nearly all dietary plants, including fruits, vegetables, cereals, etc., contain phenolic acids (PAs), the majority of which are ester-bound to carbohydrates in the form of O-glycosides (16-18). The hydroxybenzoic acids and hydroxycinnamic acids, which are produced synthetically from cinnamic acid or p-coumaric acid by hydroxylation and methylation, are two of the most significant components of the PAs. Gallic acid, syringic acid, caffeic acid, ferulic acid and Sinapic acid (SA) are few of the most prevalent PAs (19). Different reaction products of PAs that are created during enzymatic browning in food might result in unfavourable alterations in flavour and appearance (20). However, PAs function as antioxidants, scavenging free radicals and preventing lipid oxidation; as a result, they are a significant food and beverage additive (21).

Sinapic acid (SA) is a natural antioxidant. While there have been few studies on how polyphenols affect the functional properties of proteins, such as solubility, foaming, and emulsification, which are visible to the naked eye and are of the greatest concern to consumers and processors, the majority of current research on SA and protein has focused on improving the antioxidant capacity of protein (22). Analyzing the structural alterations of OVA and quantifying its intrinsic tryptophan fluorescence helped to further clarify the mechanism behind the interaction between SA and OVA. The research demonstrated that polyphenols might be utilized to enhance the functional qualities of food foam and gives some basic information on how polyphenols affected the foaming properties of OVA.

Through the study of native and polyphenols-bound proteins in an aqueous phase, the binding of Sinapic acid and ovalbumin was identified. Time-resolved fluorescence, synchronous fluorescence, and UV/Visible analyses supported the verification of OVA alterations. In addition to the experimental tests, FT-IR research was used to examine the effects of polyphenols on the secondary structure of proteins.

2. Materials and Methods

Ovalbumin (from chicken egg white, lyophilized powder, ≥ 98%), and Sinapic acid (D7927) were purchased from Sigma-Aldrich Chemical Co. A stock solution of OVA (1×10^{-5}M) was prepared by phosphate buffer. The stock solution of SA (1×10^{-4}M) was prepared by using ethanol. The solutions were prepared just before taking absorption and fluorescence measurements.

2.1. Spectroscopic Instruments Details

Absorption spectra were recorded between 200 and 400 nm with a SHIMADZU model (SHIMADZU 1800 PC UV/Vis Spectrophotometer). Fluorescence spectra of each solution were recorded between 280 and 800 nm using an RF-5301 PC (Shimadzu Corporation, Kyoto, Japan) fluorescence spectrophotometer. Fluorescence lifetimes were measured by using a time-resolved HARIIBA – JOBIN YVON [SPEX-SF B-III] spectrofluorometer. FT-IR spectra for the liquid sample of SA and OVA is measured from 4000 to 400 cm^{-1} on an Agilent resolutions pro330 spectrometer.

3. Results and Discussion.

3.1. Absorption spectral characteristics of SA with OVA

The interactions between drugs were investigated using UV-Vis spectroscopy (23). The structural changes and an understanding of the formation of a complex between different molecules and proteins were investigated with a suitable and effortless spectroscopic technique such as UV –Vis spectroscopy.

ISSN 2515-8260 Volume 10, Issue 1 2023

As a consequence, UV-Vis spectral findings were shown in Fig.1. The excitation wavelength is observed at 281 nm at pH 7.4. The intensity of the recorded UV – Vis Spectra of OVA increased when increasing concentration of SA up to (1.0 [mol L^{-1}]). In Table.1, the absorption spectrum data of OVA at various SA concentrations were summarised. From Fig.1.UV-visible spectra of OVA with an increasing addition of SA, the strength of UV-absorption system increased gradually, and no obvious change of absorption maximum wavelength was detected. Since the amide group of OVA absorbs UV light, an increase in UV absorption of SA suggests that hydrogen bonding between the phenolic hydroxyl of SA and the amide group of OVA may take place. Hydrogen bonding increased the intensity of the π electron clouds on the aromatic ring of SA and lowered the transition energy, resulting in a hyperchromic effect (24).

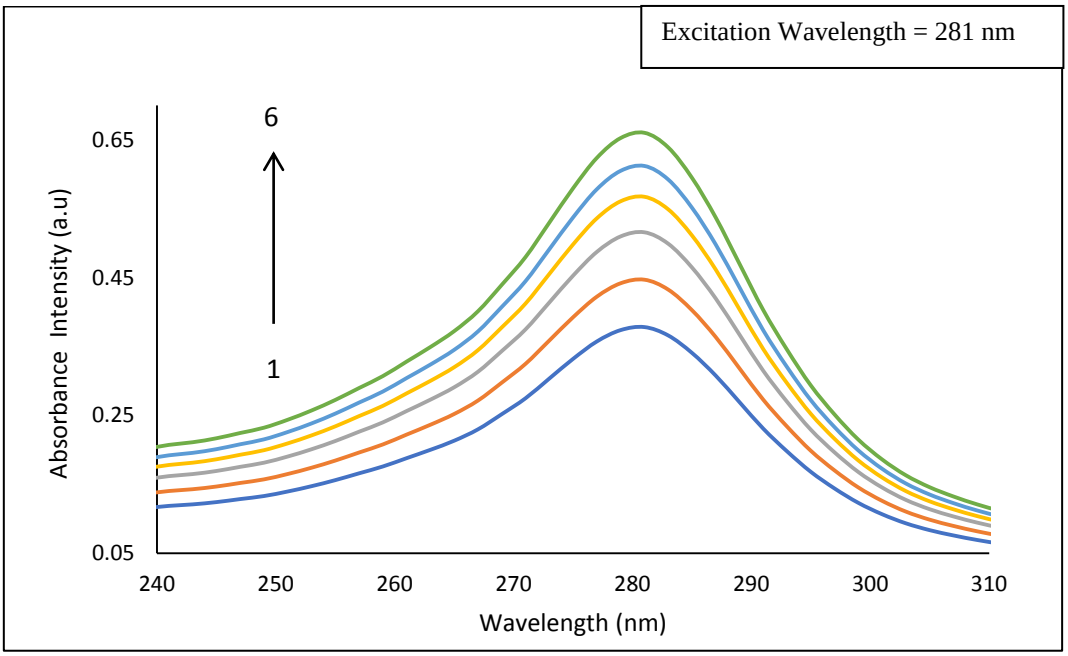

Figure.1. UV/Vis absorption spectra of Ovalbumin with different concentrations of Sinapic acid (mol L^{-1}) (1) 0.0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8 and (6) 1.0

Table.1. Absorption spectral data of OVA with different concentrations of SA at pH 7.4.

S.No	Concentration of SA (mol L^{-1})	Absorbance (a.u)
1.	0.0	0.27
2.	0.2	0.29
3.	0.4	0.31
4.	0.6	0.33
5.	0.8	0.35
6.	1.0	0.38

ISSN 2515-8260 Volume 10, Issue 1 2023

3.2. Fluorescence quenching of OVA by SA

Fluorescence quenching may be caused by a number of different mechanisms, including excited state events, molecular rearrangements, energy transfer, the creation of ground-state complexes (static quenching), or collisional interactions (dynamic quenching) (25).

Fluorescence quenching displays the variations in fluorescence emission spectra at various Sinapic acid concentrations with OVA as seen in Fig.2. It was evident from the spectrum that the fluorescence intensity of OVA decreases at around 340 nm with an increase in Sinapic acid content, as indicated in Table.2. Fig.2. Shows Fluoresces emission spectra OVA quenched by SA no spectral shift was observed and indicating that interaction with SA have no influence on the environment of fluorophores in protein (26).

Most proteins could emit intrinsic fluorescence upon UV light absorption because of the amino acids Trp, Tyr, Phe, and other individual residues present in the molecular structure (27). Fluorescence quenching is the process of weakening the protein's fluorescence intensity through various molecular interactions. Protein-polyphenol binding data have been studied using this phenomenon. It demonstrates that the addition of SA significantly reduced the OVA fluorescence intensity.

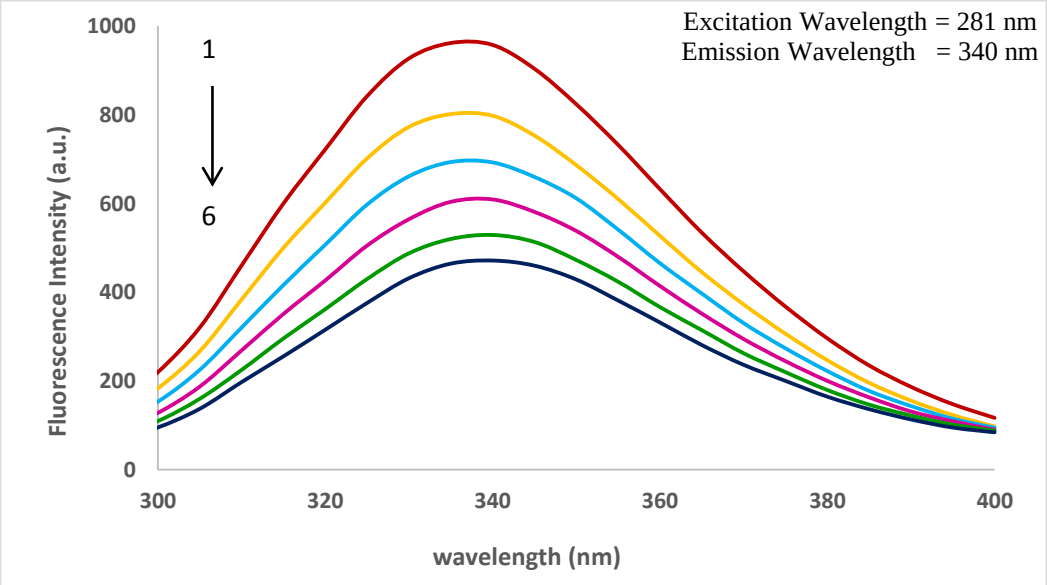

Figure.2. Steady-state fluorescence spectra of OVA with SA (1) 0.0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8 and (6) 1.0 (mol L^{-1}) with emission wavelength = 340 nm in PBS medium (pH 7.4).

Table.2. Fluorescence spectra of OVA with different concentrations of SA at pH 7.4

S.No	Concentration of SA (mol L^{-1})	Fluorescence intensity (a.u)
1.	0.0	957.5
2.	0.2	797.9
3.	0.4	692.6

4.	0.6	609.2
5.	0.8	529.0
6.	1.0	471.4

The fluorescence quenching for interacted molecule quencher [Q] and fluorescer [F] can be analyzed by the Stern-Volmer equation (28)

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

where F_0 and F denote the steady-state fluorescence intensities in the absence and the presence of the quencher [Q] respectively. K_q is the biomolecular quenching rate constant, τ_0 is the average lifetime of the protein, [Q] is the concentration of the quencher, K_{sv} is the Stern -Volmer quenching constant (29). The above equation is applied to determine K_{sv} by linear regression of a plot of (F_0/F) against [Q].

The classical Stern-Volmer plots OVA in the presence of Sinapic acid. The Stern-Volmer quenching constant and biomolecular quenching rate constant (K_q) are shown in Fig.3.

Table.3. Stern - Volmer (K_{sv})and bimolecular quenching rate constant (K_q) of Ovalbumin with sinapic acid.

Quenchers	K_{sv}x 10^5(L mol^{-1})	K_q(L $mol^{-1}S^-$	R[a]	S.D[b]
Sinapic acid	1.12	2.59	0.99	0.61

a→is the correlation coefficient, b→is the standard deviation.

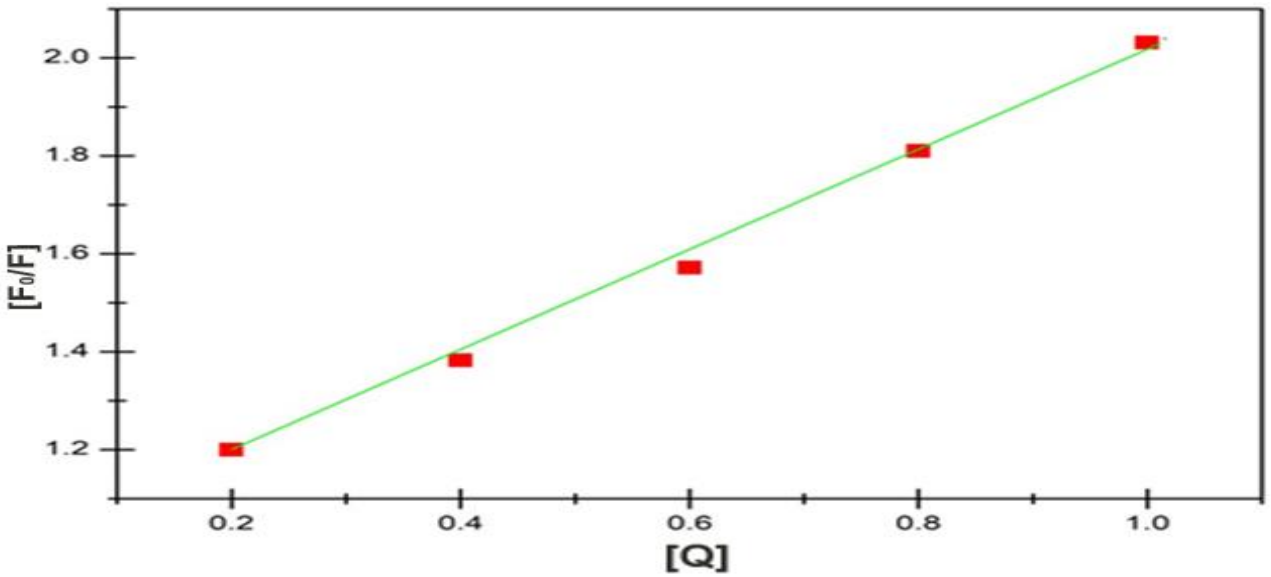

Fig.3.Stern-Volmer plot for the quenching of OVA by Sinapic acid concentration

ISSN 2515-8260 Volume 10, Issue 1 2023

3.3 Synchronous fluorescence Spectroscopy

The alterations in the fluorophore functional groups' molecular microenvironment were found using synchronous fluorescence spectra (SFS). To learn more about the conformational changes seen in Fig.4, synchronous fluorescence spectra were performed on OVA coupled with SA. The effects of SA on the SFS of OVA at the two Δλ are shown in Fig.4 (a) **Δλ = 15 nm** and (b) **Δλ =60 nm**.

The present study results were in agreement with the study by Miller (30-31). The steady state Δλ excitation and emission wavelengths at 15nm and 60nm of synchronous fluorescence spectra represent characteristic information about the Tyr or Trp residues, respectively. As the concentration of SA increased, the fluorescence intensity of OVA decreased. The emission intensities of OVA at Δλ = 15 nm decreased with no shift occurred. The considerable fluorescence quenching showed that the phenolic acids came closer to tyrosine residue during the binding process but no effect on the microenvironment of the Tyr residues (32). When Δλ= 60 nm, the emission intensity of OVA decreased (Fig. 4a and 4b), which indicated that phenolic acids were bound in the vicinity of the Tyr residues. When comparing the binding distance between phenolic acids and chromophore molecules (Tyr and Trp residues), the fluorescence intensity of Tyr and Trp residues in the presence of phenolic acids were obtained when Δλ were stabilized at 15 or 60 nm. As shown in Fig. 4a and 4b, when Δλ = 15 nm, the slope of the SA system was higher than that of Δλ= 60 nm (33)

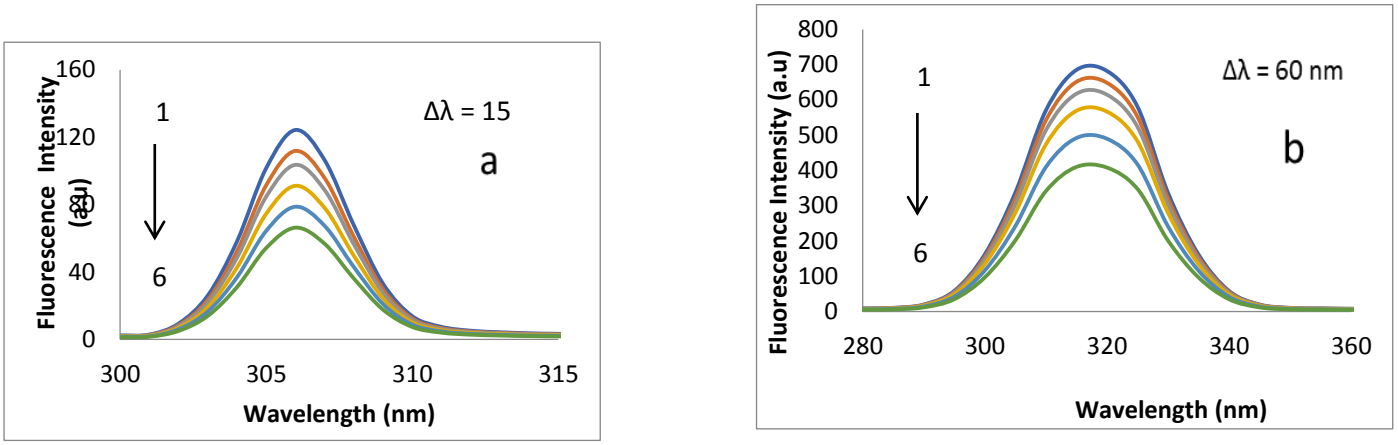

Figure.4.Synchronous Fluorescence Spectra for OVA with SA a) Δλ = 15 nm and b) Δλ =60 nm

3.4. Binding constant (K_a) and Binding site (n) of OVA with Sinapic acid (SA)

Fluorescence quenching techniques can be used to determine the binding constant (K_a) and the number of binding sites (n) dependent on the interaction of SA with OVA. Under the presumption that proteins have independent binding sites, the values of K_a and n can be determined by using the formula.(34)

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

ISSN 2515-8260 Volume 10, Issue 1 2023

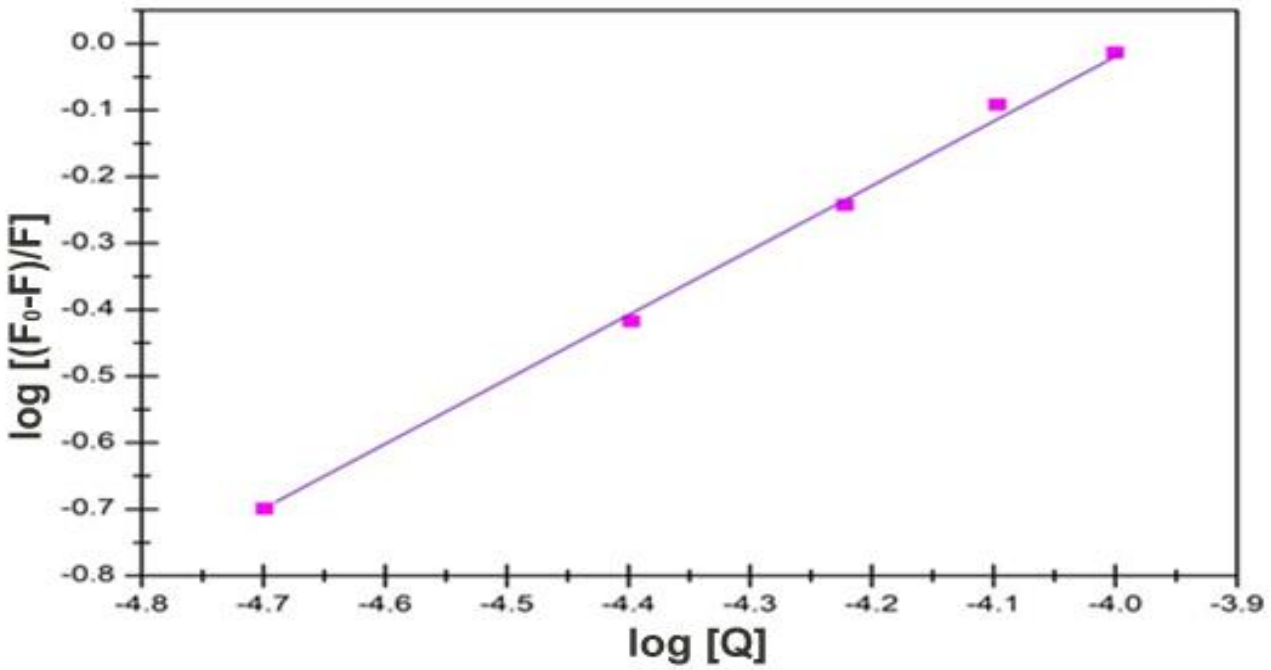

Fig.5.The plot of log (F_0-F)/F versus log [Q]

Table.4. The binding constant (Ka), binding site (n) correlation coefficient(R), change in free energy ΔG_g (for ground state) and ΔG_e(for excited state) of ovalbumin with Sinapic acid

Quenchers	Ka x 10^5(L mol^{-1})	n	R	ΔG_g (kJ mol^{-1}) x 10^3	ΔG_e (kJ mol^{-1}) x 10^3
Sinapic acid	1.10	1.08	0.99	-5.75	16.72

The fluorescence intensities of the protein with and without quencher are F and F_0, respectively, and [Q] is the initial quencher concentration. Fig.5. depicts the results of fluorescence quenching studies, while Table.4.mentions the computed results. These results suggested that OVA had only one equivalent binding site with SA, implying that a complex formed between SA and OVA. In general, if K_a is greater than 10,000, the binding is strong (35). Ka value of 1.10x10^5 from Table.4 demonstrates that SA and OVA had a strong binding interaction. As a consequence, the OVA and the SA might immediately interact, changing the biological function of OVA. Free energy of ground state (ΔGg) and excited state (ΔGe) were calculated and tabulated.

3.5. Time-resolved fluorescence spectra of OVA with Sinapic acid

Time-resolved fluorescence spectral studies were performed to understand how encapsulation within the SA cavity influences the stability and statics in the excited states. SA is carried out to substantiate time-resolved fluorescence decay curves and are shown in Table.5. The lifetime and decay curves of OVA and OVA with SA are well displayed in Fig.6. The time-resolved fluorescence decay of OVA combines with SA showed that the tri-exponential decay indicated three lifetimes' values (τ_1 =2.8 × 10^{-9}s/2.4 × 10^{-9} s, τ_2 = 6.5× 10^{-9} s/5.3 × 10^{-9} S and τ_3= 7.48× 10^{-9} S/5.4× 10^{-9}s) for the presence and absence of SA respectively.

Table.5. Fluorescence lifetime and relative amplitudes of OVA with different concentrations of Sinapic acid

Concentration of Sinapic acid (M)	Lifetime (ns)			Average Lifetime $\tau \times 10^{-9}$ sec	Relative amplitude			χ^2	S.D 10^{-11} sec		
	τ1	**τ2**	**τ3**		**B_1**	**B_2**	**B_3**		**τ1**	**τ2**	**τ3**
OVA	2.8	6.5	7.4	4.8	30.7	72.3	21.1	1.2	2.1	5.3	4.1
OVA+SA (0.4)	2.7	6.4	7.2	4.7	29.2	64.7	12.5	1.1	2.0	4.8	4.0
OVA+SA (0.8)	2.6	5.5	5.6	4.6	28.2	59.3	11.5	1.0	1.6	3.9	3.2
OVA+SA (1.2)	2.4	5.3	5.4	4.3	26.9	58.2	8.33	1.0	1.5	3.3	2.4

The consolidated data and the decay curve confirmed the formation of the complex based on the hike in a lifetime and relative amplitude values by the addition of SA concentration in an aqueous solution. The enhanced lifetimes of OVA during the addition of SA lead to the restriction of rotational degrees of freedom with a consequent impact on the depletion of non-radiative decay channels. The observed enhancement in lifetime indicates that the OVA molecule experiences less polar hydrophobic environments within the SA cavity resulting in the reduction of non-radiative decay processes. Further, the increase in fluorescence lifetime is a result of the significant interactions of the OVA with hydrophobic SA. The relative amplitude of free OVA and bound OVA with SA is also continuously enhanced which suggests the formation.

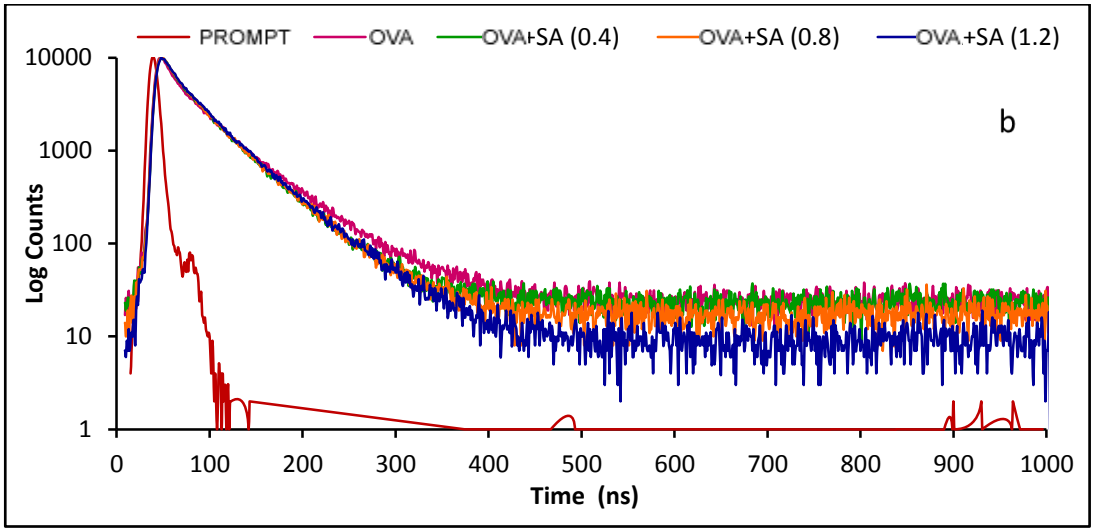

Figure.6. Time-resolved fluorescence spectra of OVA with different concentrations of Sinapic acid (mol L^{-1}) 1) 0.0 2) 0.4, 3) 0.8 and 4) 1.2

3.6. FT-IR spectroscopic study of OVA and SA

The conformational changes and the stability of OVA combined at different concentrations with Sinapic acid were determined by its functional and structural compounds which are shown in Table.6. and Fig.7.

Proteins have produced numerous insights into the folding mechanism as well as the identification of the folded protein can be analyzed through FT-IR.

The key elements controlling the conformational sensitivity are the amide bands with hydrogen bonding and coupling between transition dipoles. The amide band was primarily of amide-I detected in the peak range (1600–1700 cm–1) and amide-II in the range (1500-1600 cm-1).

The amide-I band is frequently utilized for visible analysis of protein secondary structure elements and the amide-II band, on the other hand, has a relatively complex association. The C=O stretching vibration of the peptide backbone, which is influenced by the interactions between the amide groups and the strength of the hydrogen bond, creates the amide-I band. Fig.7. (a) show the FT-IR spectra of OVA and Fig.7. (b), 7. (c) OVA with different concentrations of SA.

Table.6. shows Pure OVA has α - helix rich in confirmation by the extreme band in the amide-I province that centres at 1653.284 cm-^{1}. For the OVA with SA complex, the typical peak moves by 1653.063cm-1 and 1653.587. In all three spectra, the fundamental characteristics of the OVA secondary structure were preserved in the presence of SA. According to the FT-IR analysis, it could be conformed that the SA was involved in the complexation and that the furan ring is present outside of the OVA cavity.

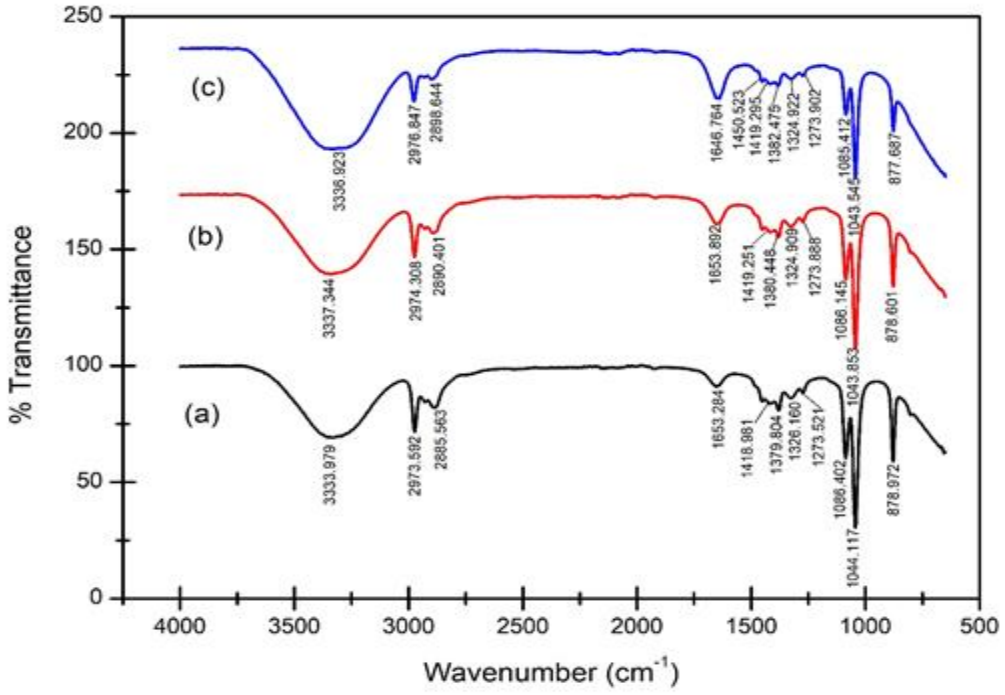

Fig.7. FT-IR spectra of (a) OVA (b) OVA+0.4mol L^{-1}SA (c) OVA+1.0mol L^{-1}SA

Table.6. FT-IR peak assignments of OVA without and with different concentration of Sinapic acid

Wavenumber cm^{-1}			Peak assignments
OVA	**(OVA+ 0.4 mol L^{-1}SA)**	**(OVA+1.0 mol L^{-1}SA)**	
3333.979	3336.359	3338.781	O-H Stretching
2973.592	2974.177	2975.490	C-H Stretching

2885.563	2886.482	2895.561	C-H Stretching
1653.284	1653.063	1653.587	C=N Stretching
1418.981	1419.221	1419.441	C-C Stretching
1379.804	1380.102	1381.309	C-N Stretching
1326.160	1325.944	1324.455	CH_3 Implement Bending
1273.521	1273.922	1273.988	CH_2 Implement Bending
1086.402	1085.144	1085.768	C-O Stretching
1044.117	1043.922	1043.650	C-O Stretching
878.972	878.697	878.149	C-H Aromatic

4. Conclusion

In the present work, the interactions between SA and OVA have been analyzed using fluorescence and UV–Vis spectroscopy and binding parameters have been obtained. A static quenching mechanism was identified for the intrinsic quenching of the OVA fluorescence by SA. The obtained number of binding sites showed the presence of a single class of binding site for the SA molecule on OVA confirmed. According to the results of UV and FT-IR spectroscopic studies, it can be noted that SA induced conformational changes in the albumin structure. The results show that SA bind to OVA with high affinity and strongly quench its intrinsic fluorescence.

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