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SPECTROSCOPIC ANALYSIS OF FLUORESCENCE QUENCHING AND ENERGY TRANSFER IN THE HUMAN GAMMA GLOBULIN–TANNIC ACID INTERACTION

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Abstract: This study investigates the interaction between Human Gamma Globulin (HGG) and Tannic acid using UV–Visible and fluorescence spectroscopic techniques. UV–Vis absorption spectra indicated complex formation between the protein and ligand. Fluorescence emission spectra showed a gradual decrease in fluorescence intensity of HGG with increasing concentration of Tannic acid. Stern–Volmer analysis suggested that the quenching mechanism follows static quenching. Binding constants and number of binding sites were calculated using double logarithmic plots. Furthermore, Fluorescence Resonance Energy Transfer (FRET) analysis was performed to estimate the binding distance between donor and acceptor molecules. The results reveal strong binding interaction between Human gamma globulin and Tannic acid, forming a stable complex.

Keywords: Human gamma globulin, Tannic acid, UV–Vis spectroscopy, Fluorescence quenching, FRET.

1. INTRODUCTION

Proteins are essential biomolecules that perform a wide range of biological functions including immune response, molecular transport, enzymatic catalysis, and cellular regulation (Lakowicz, J. R. (Ed.). (2006)). Understanding the interaction between proteins and small bioactive molecules is important for studying biochemical mechanisms and drug–protein binding. Human Gamma Globulin (HGG), an important plasma protein belonging to the immunoglobulin family, plays a significant role in immune defence mechanisms. Investigation of the interaction between HGG and biologically active compounds can provide valuable information about protein structure, stability, and binding behaviour (Feng, R., et al. (2014)).

Polyphenols are naturally occurring compounds widely present in fruits, vegetables, cereals, and beverages such as coffee (Belay, A., et al. (2016)). These compounds exhibit several biological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties Tannic acid (TA), a commercial form of tannin, is a kind of polyphenol with abundant hydroxyl groups, showing strong interactions with macromolecules including polysaccharides, proteins, and other synthetic polymers (Li, S., et al. (2010).) The interaction of polyphenols with proteins may influence both protein structure and biological activity, making such studies important in biochemical and pharmaceutical research.

Spectroscopic techniques such as UV–Visible absorption spectroscopy and fluorescence spectroscopy are widely used to investigate protein–ligand interactions. UV–Visible spectroscopy provides information about structural changes (Chen, Z., et al. (2013)) and complex formation between proteins and ligands, while fluorescence spectroscopy is highly sensitive for studying conformational changes and quenching mechanisms. Intrinsic fluorescence of proteins mainly arises from aromatic amino acids such as tryptophan and tyrosine, which serve as useful probes for monitoring molecular interactions.

In addition, Fluorescence Resonance Energy Transfer (FRET) is an effective method for studying molecular distances and energy transfer between donor and acceptor molecules (Eftink, M. R., & Ghiron, C. A. (1976)). In the present study, the interaction between Human Gamma Globulin and Tannic acid has been investigated using UV–Visible absorption spectroscopy and fluorescence spectroscopy. The fluorescence quenching mechanism, binding parameters, and energy transfer efficiency were analyzed to understand the molecular interaction between the protein and ligand.

2. MATERIALS AND METHODS

2.1. Experimental Materials

Human Gamma Globulin (HGG, IgG), a Y-shaped immune protein (~160 kDa), was used as the primary macromolecule. Tannic acid (TA), is a kind of polyphenol with abundant hydroxyl groups, served as the ligand. Both HGG (90% purity) and TA were obtained from Sigma-Aldrich, Bangalore.

2.2. Sample Preparation

1 M Phosphate buffer (pH 7.4) was used to maintain physiological conditions. HGG stock (1x 10^{-5} M) and TA stock (10^{-4} M) were freshly prepared using triply distilled water. Verified via melting point analysis and UV-Vis spectral consistency across various excitation wavelengths.

2.3. Spectroscopic Techniques

UV-Vis Absorption: Measured using a Shimadzu 1800 PC (range 200–500 nm) to study ground-state complex formation. Fluorescence Quenching: Performed on a Shimadzu RF 5301 PC. Protein was excited at 280 nm; emission was recorded at 300–450 nm (Harding, S. E., & Chowdhry, B. Z. (2001)).

Quenching mechanism was calculated using the following Stern-Volmer Equation:

$$\frac{F_0}{F} = 1+K_q\tau_0\,[Q] = 1+K_{SV}\,[Q] \qquad (1)$$

Binding Constant (K_a) and Binding Sites (n) was calculated using the following Double-Log Equation:

$$\text{Log}\left(\frac{F_0-F}{F}\right) = \log K_a + n\log\,[Q] \qquad (2)$$

2.4. Energy Transfer (FRET)

Förster Resonance Energy Transfer (FRET) was used as a "spectroscopic ruler" to calculate the distance (r) between HGG (donor) and TA (acceptor) based on the spectral overlap of protein emission and ligand absorption.

3. RESULT AND DISCUSSION

3.1 UV–Visible Spectroscopic Analysis of HGG–TA Interaction:

This study examines the interaction between Human Gamma Globulin (HGG) and Tannic acid (TA) using UV–Visible spectroscopy. The absorption spectra were recorded in the range of 200–800 nm under physiological conditions. Pure HGG showed a characteristic peak at 278 nm due to aromatic amino acids.

As the concentration of TA increased, the absorption peak at 278 nm became more intense. There was no significant shift in the position of the HGG absorption peak at 278 nm. The increase in the absorption band at 278 nm in response to TA indicates that the HGG conformation is changed due to TA binding and the changes are centered near tryptophan residues. The hyperchromic effect observed in biomolecules upon exposure to TA provides evidence for the formation of HGG-TA complexes.

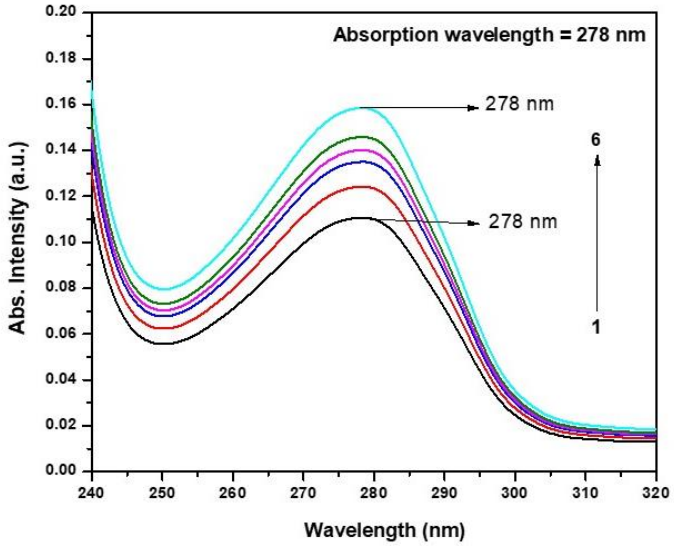

Fig. 3.1 UV-Vis absorption spectra of Human Gamma Globulin with different concentrations of Tannic acid (mol L-1) (1) 0.0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8 & (6) 1.0.

3.2 Determination of Binding Constant of HGG–TA Complex:

The binding interaction between Human Gamma Globulin (HGG) and Tannic acid (TA) was analyzed using UV–Vis absorption spectroscopy. The Benesi–Hildebrand method was applied to calculate the binding constant based on absorbance changes at 280 nm, which reflect alterations in the environment of aromatic amino acids. The interaction was assumed to follow a 1:1 stoichiometry between HGG and TA. A double reciprocal plot of $1/(A - A_0)$ versus $1/C_{TA}$ showed good linearity ($R^2 = 0.99$), confirming the formation of a stable complex.

The binding constant (K_g) was determined to be **1.57×10^4 M^{-1}**, indicating a strong interaction between HGG and TA within the ideal range for protein–ligand binding. The negative Gibbs free energy ($\Delta G = -18.42$ kJ/mol) suggests that the binding process is spontaneous. Overall, the results confirm that TA binds strongly to HGG through non-covalent interactions, likely dominated by hydrophobic forces, (Li, S., et al. (2010)) forming a stable HGG–TA complex.

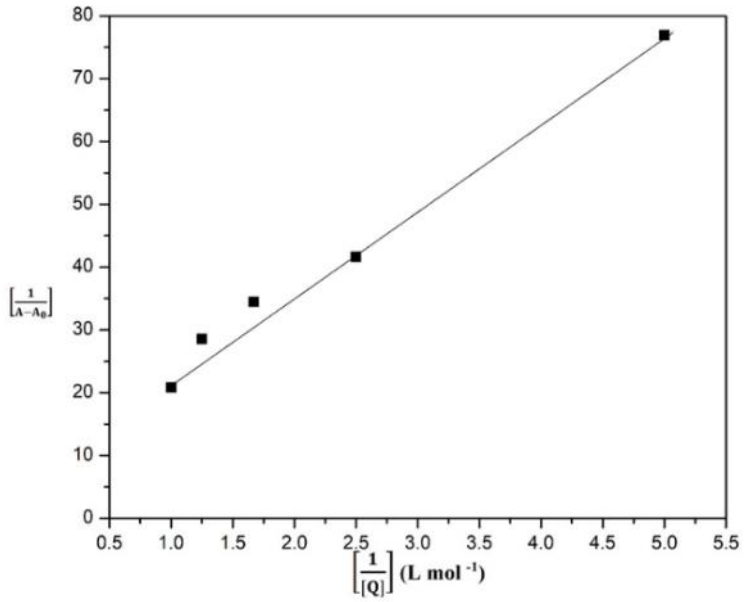

Fig. 3.2 Double reciprocal plot of $\frac{1}{A-A_0}$ versus $\frac{1}{C_{TA}}$ for HGG with Tannic acid (TA)

Table 3.1 Binding constant of Human Gamma Globulin with Tannic acid (TA)

Quencher	Binding constant K_g x10^4 ($Lmol^{-1}$)	R^a	ΔG_g
Tannic acid (TA)	1.57	0.99	-18.42

a→ is the correlation coefficient.

3.3 Fluorescence Spectroscopic Study of HGG–TA Interaction:

The interaction between Human Gamma Globulin (HGG) and Tannic acid (TA) was investigated using fluorescence spectroscopy, focusing on intrinsic fluorophores such as tryptophan, tyrosine, and phenylalanine residues. Among these, tryptophan plays a dominant role due to its high sensitivity to changes in the local environment (Lakowicz, J. R. (Ed.). (2006)). HGG exhibited a strong fluorescence emission peak at 337 nm when excited at 278 nm, primarily due to tryptophan residues. Upon the gradual addition of TA, a significant decrease in fluorescence intensity (quenching) was observed, indicating interaction between TA and HGG. This quenching suggests changes in the microenvironment surrounding tryptophan residues, likely due to conformational alterations in the protein.

Additionally, a slight red shift (3 nm) in the emission maximum was observed, indicating that TA binds within a hydrophobic region of the protein. This results in increased hydrophobicity and displacement of water molecules from the binding site. Overall, the results confirm that TA interacts strongly with HGG, causing structural changes and modifying the local environment of fluorescent amino acid residues.

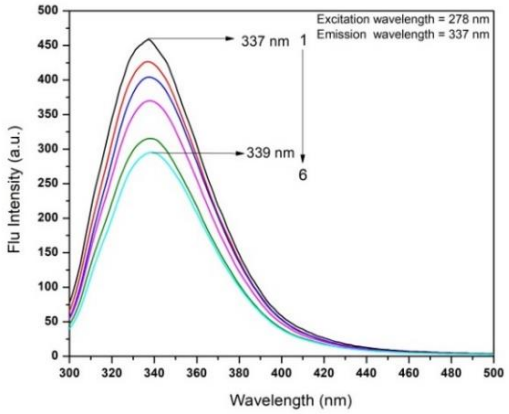

Fig. 3.3 Fluorescence spectra of Human Gamma Globulin with different concentrations of TA (mol L^{-1}) (1) 0.0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8 & (6) 1.0.

3.4 Fluorescence Quenching Mechanism of HGG–TA Interaction:

The fluorescence quenching mechanism of Human Gamma Globulin (HGG) by Tannic acid (TA) was analyzed using the Stern–Volmer approach. Fluorescence quenching occurs due to interactions between the fluorophore and quencher, either through dynamic (collisional) or static (complex formation) mechanisms. The Stern–Volmer plot (F_0/F vs [Q]) showed a strong linear relationship (R^2 = 0.96), indicating the presence of a single quenching mechanism. The Stern–Volmer quenching constant (K_{sv}) was found to be **6.35×10^4 L mol^{-1}**, and the bimolecular quenching constant (K_q) was **2.29×10^{13} L mol^{-1} s^{-1}**.

Since the obtained K_q value is much higher than the typical maximum value for dynamic quenching (~10^{10} L mol^{-1} s^{-1}), the quenching mechanism is identified as **static quenching** (Eftink, M. R., & Ghiron, C. A. (1976)). This indicates the formation of a stable ground-state complex between HGG and TA rather than quenching through molecular collisions. Overall, the results confirm strong interaction between HGG and TA, with quenching occurring mainly via complex formation, supporting the existence of a stable HGG–TA complex.

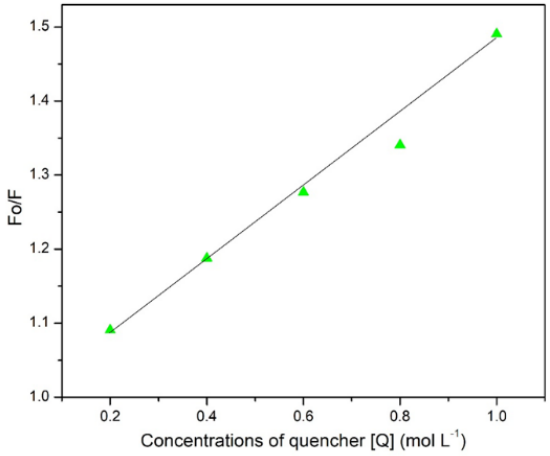

Fig. 3.4 The Stern Volmer plot of Human Gamma Globulin with TA.

Table 3.2 Stern – Volmer (K_{SV}) and bimolecular quenching rate constant (K_q) of Human Gamma Globulin with Tannic acid

Quencher	K_{SV} x 10^4 (L mol^{-1})	K_q x 10^{13} (L mol^1S^{-1})	R^a	$S.D^b$
Tannic Acid (TA)	6.35	2.29	0.96	0.44

[a]→ R is the correlation coefficient, [b]→ S.D is the Standard Deviation

3.5 Binding Parameters of HGG–TA Interaction:

The binding parameters between Human Gamma Globulin (HGG) and Tannic acid (TA) were determined using fluorescence quenching analysis. A double logarithmic plot was used to calculate the binding constant (K_a) and the number of binding sites (n) (Bolel, P., et al. (2012)). The plot showed good linearity ($R \approx 0.99$), confirming that the interaction follows a site-binding model. The binding constant was found to be **5.88×10^5 L mol^{-1}**, indicating strong binding affinity between HGG and TA. The number of binding sites ($n \approx 1.4$ -1.0) suggests the presence of a single primary binding site on the protein.

The negative free energy change ($\Delta G = -75.80$ kJ/mol) indicates that the binding process is spontaneous. Overall, the results confirm that TA strongly binds to HGG at a single site through a stable and spontaneous interaction.

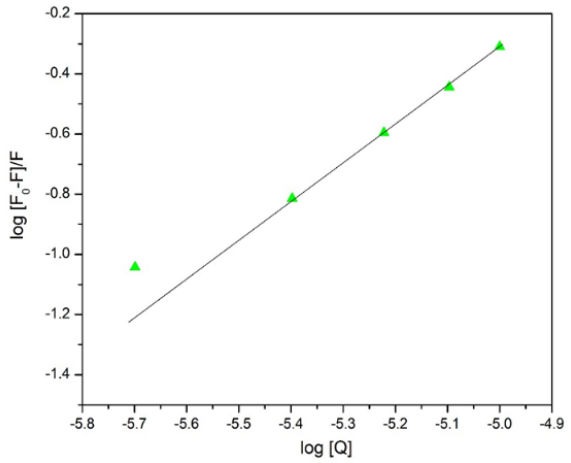

Fig. 3.5 Double log plot of Human Gamma Globulin with TA

Table 3.3 Binding constant (K_a), and binding sites (n) values of Human Gamma Globulin with TA

Quencher	K_a (L mol^{-1})	n	R[a]	S.D[b]	ΔG_e
Tannic acid (TA)	5.88×10^5	1.18	0.99	2.49	-75.80

[a]→ R is the correlation coefficient, [b]→ S.D is the Standard Deviation

3.6 FRET Analysis of HGG–TA Interaction:

Fluorescence Resonance Energy Transfer (FRET) was used to study the interaction between Human Gamma Globulin (HGG) as the donor and Tannic acid (TA) as the acceptor. FRET analysis helps determine the distance between interacting molecules and provides insight into binding mechanisms. The overlap between the fluorescence emission spectrum of HGG and the absorption spectrum of TA confirmed the possibility of energy transfer. The calculated FRET parameters showed an energy transfer efficiency (E) of **51.37**, Förster distance (R_0) of **2.33 nm**, and donor–acceptor distance (r) of **2.31 nm**.

Since the distance (r < 8 nm) falls within the effective FRET range, (Lakowicz, J. R. (Ed.). (2006)) efficient energy transfer occurs between HGG and TA. Additionally, the proximity between donor and acceptor supports the formation of a stable complex. The fact that r is close to R_0 further indicates a strong interaction and confirms a static quenching mechanism. Overall, FRET analysis demonstrates that TA binds near the tryptophan residues of HGG, enabling energy transfer and confirming close molecular interaction and complex formation.

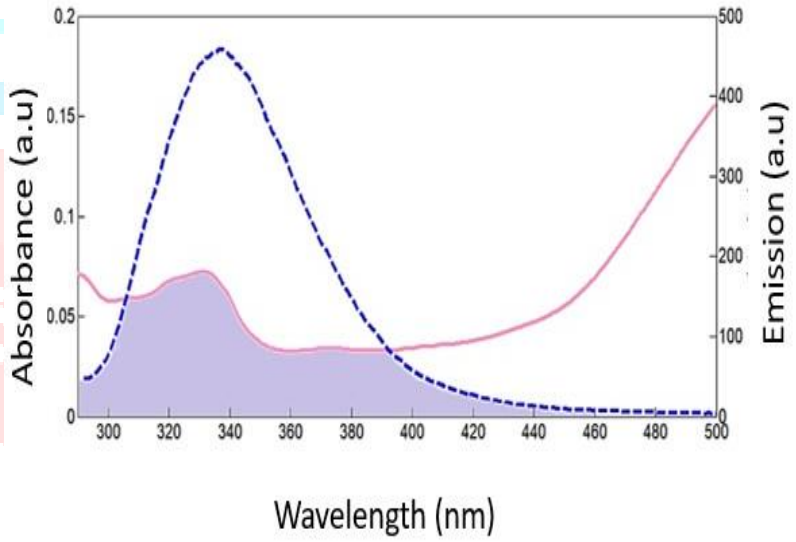

Fig. 3.6 The overlap of UV absorption spectra of TA (solid line) with the fluorescence emission spectra of Human Gamma Globulin (dashed line)

Table 3.4 Förster energy transfer parameters of Human Gamma Globulin with Tannic acid (TA)

Quencher	Energy(E)	R_o (nm)	J ($cm^{-3}LM^{-1}$) (10^{-15})	r (nm)
Tannic acid (TA)	51.37	2.33	7.44	2.31

3.7 Overall Summary of HGG–TA Interaction Study:

This research investigates the interaction between the phenolic compound Tannic acid (TA) and the protein Human Gamma Globulin (HGG), with potential applications in the food industry. The study combines UV–Vis absorption and fluorescence spectroscopy to understand the binding mechanism and structural changes in the protein.

UV–Vis analysis confirmed the formation of the HGG–TA complex and revealed conformational changes in the protein. Fluorescence studies showed a significant decrease in the intrinsic fluorescence of HGG upon addition of TA, indicating strong interaction. The Stern–Volmer analysis demonstrated a linear relationship, confirming a single quenching mechanism. The high quenching constant (K_q) suggested that the process follows a **static quenching mechanism**, involving ground-state complex formation rather than dynamic collisions. Binding studies revealed a high binding constant ($K_a \approx 5.88 \times 10^5$ L mol^{-1}) and approximately one binding site, indicating strong affinity and a 1:1 interaction between HGG and TA. FRET analysis further confirmed close proximity between TA and tryptophan residues of HGG, supporting energy transfer and complex formation. Overall, TA exhibits strong quenching ability, high binding affinity, and stable complex formation with HGG through non-covalent interactions. These findings highlight the potential application of protein–phenolic interactions in food and biochemical systems.

Table 3.5 Quenching Ability of the Quencher for HGG

Quenchers	Maximum Intensity	Minimum Intensity	Quenching Ability	K_a x10^5 (L mol^{-1})
Tannic acid	459	296	162	5.38

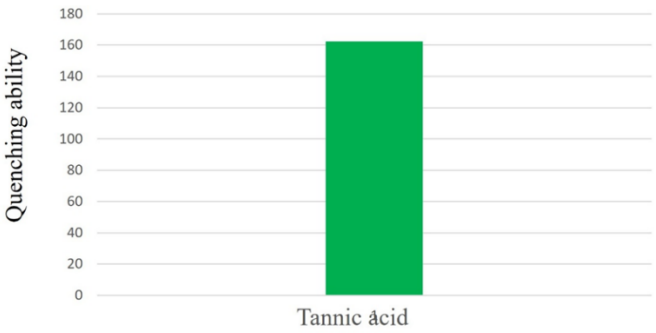

Fig.3.7 Quenching Ability of the Quencher for HGG

Table 3.6 Comparison table for all experimental results

Globulin	Phenolic compound	Quenching Constant K_q x 10^{13} (L mol^{1} s^{-1})	Binding Constant K_a X10^5 (L mol^{-1})	Quenching Ability	Energy (FRET)E
HGG	TA	2.29	5.88	162	34.37

From the above all results, it can be concluded that TA shows a high quenching effect for the globulin and exhibits higher binding affinity.

4. CONCLUSION

This study investigated the influence of Phenolic compound, Tannic acid (TA) on globulin through a combination of multi-spectroscopic studies. Fluorescence spectrum analysis reveals that TA quenched the fluorescence of HGG efficiently by static quenching mechanism, resulting from the formation of a ground-state complex with strong binding affinity. The comprehension of chemico-biological interactions is crucial for drug design, pharmacy, pharmacology, and biochemistry (Liu, C., et al. (2022)). This is achieved through the study of ligand binding to Globulins (Basu, A., & Suresh Kumar, G. (2016)). While derivatives of phenolic chemicals exhibit antiviral and anticarcinogenic properties, Protein evaluate their potential in the prevention of degenerative diseases and actively function as anticancer agents. These findings provide light on the relationship and process of phenolic chemicals' binding to amino acids and could serve as a helpful manual for future research in the food sector (Khan, M. S., et al. (2019) & Ying. L., Chao. W. and Guanghua. L., 2010).

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