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Ion- and pH-Dependent Fluorescence Quenching of Serotonin in Aqueous Solution: Evidence for Mixed Dynamic and Static Mechanisms

Abdourahmane Khonté [1,2]*, Abdou Dieng [1], Coura Dione [1], Jean Pierre Bakhoum [2], Diégane Sarr [1], Pape Abdoulaye Diaw [1], Atanasse Coly [2]

[1] *Département de chimie, UFR Sciences Appliquées et Techniques d'Information et de Communication (SATIC), Université Alioune Diop (UAD), BP 30, Bambey, Sénégal.*

[2] *Département de chimie, Faculté des Sciences et Techniques, Université Cheikh Anta Diop (UCAD), BP 5005, Dakar, Sénégal*

**Corresponding author, Email address: abdourahmane.khonte@uadb.edu.sn*

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Abstract: Serotonin (5-hydroxytryptamine, 5-HT) is an indole-based neurotransmitter exhibiting intrinsic fluorescence that is highly sensitive to its aqueous microenvironment. This work investigates the effects of inorganic ions, ionic strength, and pH on serotonin fluorescence using steady-state spectrofluorimetry (λ_{ex} = 276 nm, λ_{em} ≈ 335–340 nm). In dilute solutions (10^{-8}–10^{-5} mol L^{-1}), fluorescence intensity increases linearly with concentration, indicating monomeric behavior without aggregation. The addition of various inorganic salts (NaCl, KNO_3, $MgSO_4$, $CaCl_2$, Na_2CO_3, Na_2HPO_4, K_2SO_4) results in significant fluorescence quenching without any spectral shift, confirming that the electronic structure of the indole chromophore remains unchanged. Stern–Volmer and Perrin analyses reveal that fluorescence quenching proceeds via a mixed mechanism involving both dynamic (collisional) and static (ground-state complex formation) processes. Multivalent ions induce stronger quenching effects due to higher charge density and enhanced electrostatic interactions. Deviations from linear Stern–Volmer behavior further support the coexistence of multiple quenching pathways. In addition, fluorescence intensity shows a strong pH dependence, reaching a maximum near neutral conditions, consistent with protonation–deprotonation equilibria of the indole system. Overall, these results demonstrate that serotonin fluorescence is highly sensitive to ionic and acid–base environments, making it a promising optical probe for studying ion–solute interactions in aqueous and biological systems.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an indole-based biogenic amine that plays a central role in regulating physiological and neurological processes such as mood, sleep, and appetite (Lakowicz *et al.*, 2000), (Callis and Vivian *et al.*, 2007). Beyond its biological importance, serotonin exhibits intrinsic fluorescence due to its indole chromophore, making it a useful model compound for studying excited-state processes in aqueous environments (Callis *et al.*, 2011). Indole derivatives, including serotonin and tryptophan, are well known for their sensitivity to their microenvironment. Their fluorescence properties are strongly influenced by solvent polarity, hydrogen bonding, pH, and ionic strength, which modulate radiative and non-radiative relaxation pathways (Petrich *et al.*, 1983), (Bakker *et al.*, 2010), (Ball *et al.*, 2008). In particular, these environmental factors can

significantly affect fluorescence intensity through changes in excited-state dynamics. Fluorescence quenching is a key process governing the emission behavior of fluorophores in solution. It results from interactions between excited fluorophores and surrounding species and can proceed through dynamic (collisional) or static (ground-state complex formation) mechanisms (Eftink *et al.*, 1981), (Ware *et al.*, 1962). While the Stern–Volmer model is widely used to describe dynamic quenching, deviations from linearity often indicate the coexistence of multiple mechanisms, including static contributions (Kalyanasundaram *et al.*, 1977). In aqueous ionic systems, fluorescence quenching is further influenced by inorganic ions, which modify solvent structure, ionic strength, and electrostatic interactions. Multivalent ions, in particular, may induce stronger effects due to their higher charge density and stronger interaction with polar functional groups of fluorophores (Chen *et al.*, 2020), (Xu *et al.*, 2021), (Zhang *et al.*, 2022). Despite extensive studies on indole fluorescence, the combined effects of different inorganic ions, ionic strength, and pH on serotonin fluorescence remain poorly understood (Li *et al.*, 2024), (Kumar *et al.*, 2024). In particular, the relative contributions of dynamic and static quenching mechanisms under controlled ionic conditions have not been systematically clarified. In this context, the present study aims to systematically investigate the fluorescence behavior of serotonin in aqueous solution under varying ionic and pH conditions. The objectives are to (i) characterize its photophysical properties, (ii) analyze fluorescence quenching using Stern–Volmer and Perrin models, and (iii) elucidate the coexistence of dynamic and static quenching mechanisms in ionic media (Wang *et al.*, 2023), (Zhang *et al.*, 2021).

2. Materials and methods

2.1. Chemicals and Reagents

Serotonin (5-hydroxytryptamine, 5-HT) hydrochloride of analytical grade was used as the fluorescent probe in this study. All inorganic salts employed as quenchers, including NaCl, KNO_3, $MgSO_4$, Na_2CO_3, NaH_2PO_4, Na_2HPO_4, K_2SO_4, and $CaCl_2$, were of analytical purity and used without further purification. All solutions were prepared using ultrapure water (Millipore system, resistivity > 18.2 MΩ·cm) in order to eliminate any possible contamination from trace ions that could interfere with fluorescence measurements. Serotonin stock and working solutions were freshly prepared prior to each experiment to minimize potential degradation or photochemical alteration, and concentrations were adjusted in the range of 10^{-8} to 10^{-5} mol L^{-1} depending on the experimental requirements. The quencher solutions were prepared over a concentration range from 0 to 0.6 mol L^{-1} to ensure sufficient coverage for quenching analysis and model fitting.

2.2. Instrumentation and Spectroscopic Measurements

Fluorescence measurements were carried out using a spectrofluorometer equipped with a xenon arc lamp and double monochromators for both excitation and emission, ensuring high spectral resolution and selectivity. Quartz cuvettes with a 1 cm optical path length were used for all measurements. Excitation spectra were recorded in the range of 220–320 nm, while emission spectra were collected between 300 and 450 nm. All measurements were performed at a controlled temperature of 25 ± 0.5 °C to avoid temperature-induced variations in diffusion processes and fluorescence lifetimes. The excitation wavelength was fixed at 276 nm, corresponding to the maximum absorption of serotonin in the near-UV region, and the emission maximum was monitored around 340 nm.

2.3. Fluorescence Quenching Experiments

For each experimental condition, fluorescence spectra were recorded after incremental addition of inorganic salts to serotonin solutions, and steady-state fluorescence intensities were measured under identical instrumental settings. All spectra were corrected for instrumental response to ensure accurate comparison of fluorescence intensities. Each measurement was repeated at least three times, and the reported values represent the average of independent replicates to ensure reproducibility and statistical reliability.

2.4. Data Analysis and Quenching Models

Fluorescence quenching experiments were analyzed by monitoring the variation of fluorescence intensity in the absence (I_0) and presence (I) of quencher. The resulting data were interpreted using classical Stern–Volmer and Perrin models to distinguish between dynamic and static quenching mechanisms. In systems showing deviation from linearity, polynomial fitting was additionally applied to account for mixed quenching contributions and possible higher-order interactions between serotonin and ionic species.

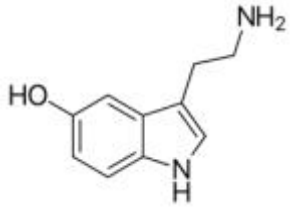
2.5. pH-Dependent Fluorescence Studies

The effect of pH on serotonin fluorescence was investigated by adjusting the solution pH over a range from 1 to 13 using dilute hydrochloric acid (HCl) and sodium hydroxide (NaOH). The pH of each solution was measured using a calibrated pH meter before fluorescence analysis. This allowed the evaluation of protonation-dependent changes in fluorescence intensity and the assessment of acid–base effects on the electronic structure of serotonin.

2.6. Experimental Conditions and Reproducibility

All experimental procedures were performed under ambient atmospheric conditions and protected from direct light exposure to minimize photodegradation of the fluorophore. The combination of controlled chemical preparation, precise spectroscopic measurements, and systematic variation of ionic and pH conditions ensured reliable characterization of serotonin fluorescence behavior in aqueous media.

Table 1. Chemical Properties of Serotonin

	Chemical Properties		Formula
Serotonin	Formula	$C_{10}H_{12}N_2O$	
	Molecular	176.22 g mol^{-1}	
	Water solubility (25°C)	25.5 $g.L^{-1}$	
	Melting point	167-168 °C	
	pKa (23.5°C)	10,16	
	Log Kow (25°C)	1.34	

3. Results and Discussion

3.1 Photophysical properties of serotonin in aqueous solution

Serotonin (5-hydroxytryptamine, 5-HT) contains an indole chromophore whose photophysical behavior is governed by a conjugated aromatic π-electron system, structurally similar to indole and tryptophan. The excitation and emission spectra recorded in aqueous solution at room

temperature reveal a maximum excitation band centered at 276–280 nm, while the emission maximum is located at approximately 335–340 nm (**Figure 1**). These spectral features are characteristic of $\pi \rightarrow \pi^*$ electronic transitions localized on the indole ring system, consistent with previous reports on indole-based fluorophores in polar environments (Callis *et al*., 2007), (Petrich *et al*., 1983). Following photon absorption, serotonin undergoes ultrafast vibrational relaxation and internal conversion within the excited singlet manifold before reaching the lowest vibrational level of the first excited singlet state (S_1). Fluorescence emission then occurs via radiative relaxation to the ground state (S_0). This sequence of events is typical of indole derivatives in polar protic solvents, where solvent relaxation plays a critical role in stabilizing the excited state and modulating emission efficiency (Bakker *et al.*, 2010), (Ball *et al.*, 2008). The spectral profile, characterized by a single well-defined emission band without secondary shoulders or additional peaks, indicates that fluorescence originates predominantly from a single emissive species. This strongly suggests that serotonin remains in a monomeric state under the investigated conditions. The absence of spectral broadening or secondary bands further excludes the formation of excimers or aggregated species in dilute aqueous solution.

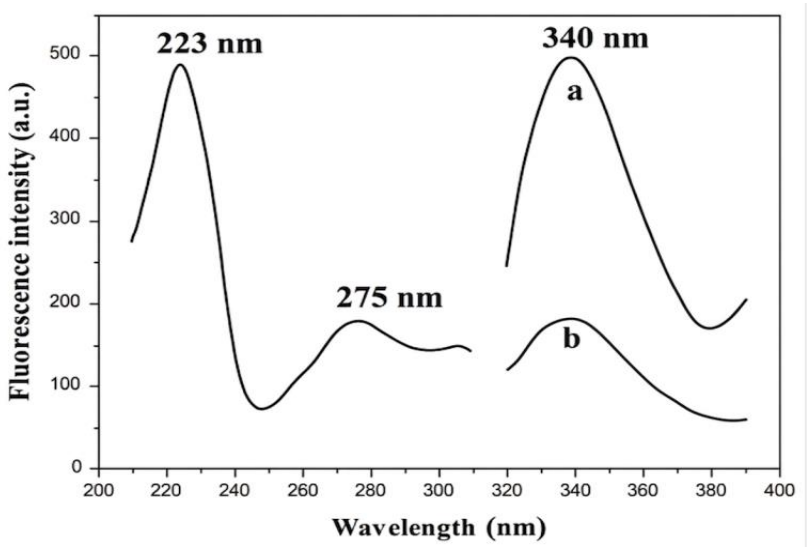


Figure 1. Excitation and emission spectra of 5-HT in aqueous solution ($\lambda_{ex} \approx 276$ nm ; $\lambda_{em} \approx 340$ nm)

The fluorescence intensity increases linearly with serotonin concentration in the range 10^{-8}–10^{-5} mol L^{-1}, confirming that the system follows the Beer–Lambert regime in fluorescence response. This linearity demonstrates that, within this concentration domain, the population of excited states scales proportionally with the number of absorbing molecules, without significant loss due to intermolecular interactions. However, at higher concentrations, slight deviations from linearity may arise, which are commonly attributed to self-quenching processes. These processes result from diffusion-controlled collisions between excited-state and ground-state molecules, leading to non-radiative deactivation pathways (Eftink *et al.*, 1981), (Ware *et al.*, 1962). Such behavior is widely reported for aromatic fluorophores and reflects the increasing probability of intermolecular encounters in solution as concentration increases.

From a mechanistic perspective, these observations confirm that serotonin behaves as a typical indole-based fluorophore whose photophysical properties are highly sensitive to its microenvironment. In particular, solvent polarity, hydrogen-bonding interactions, and ionic strength are expected to strongly influence both radiative and non-radiative decay pathways, as will

be demonstrated in the following sections (Lakowicz *et al.*, 2000), (Callis *et al.*, 2011). Although absolute quantum yield measurements were not performed in this study, the fluorescence response of serotonin can be discussed in terms of relative quantum yield variations under different environmental conditions.

3.2 Influence of inorganic ions on serotonin fluorescence

The addition of inorganic salts to serotonin solutions induces a progressive decrease in fluorescence intensity, as illustrated in **Figure 2**. In contrast, the emission maximum remains essentially constant at approximately 340 nm, indicating that the spectral position is not affected by ionic strength. This invariance of the emission wavelength is a key observation, as it demonstrates that the electronic structure of the indole chromophore is not significantly altered by the presence of dissolved ions. Consequently, the observed decrease in fluorescence intensity cannot be attributed to chemical degradation, irreversible photochemical processes, or structural modification of serotonin. Instead, the observed behavior is characteristic of fluorescence quenching processes, in which the excited state of the fluorophore is deactivated through interactions with surrounding species without modification of its ground-state electronic structure (Eftink *et al.*, 1981), (Ware *et al.*, 1962), (Lakowicz *et al.*, 2000).

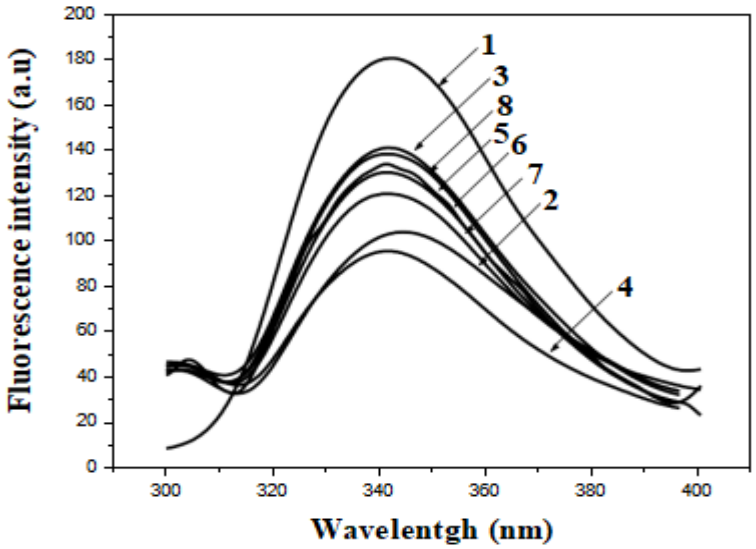


Figure 2. Emission spectra of 5-HT in the presence of increasing concentrations of inorganic salts (2×10^{-4} mol L^{-1}) (1: water; 2: Na_2SO_4; 3: Na_2HPO_4; 4: Na_2CO_3; 5: NaCl; 6: K_2SO_4; 7: $CaCl_2$; 8: KNO_3) at 25 °C in aqueous solution (λ_{ex} = 276 nm; λ_{em} = 340 nm).

In aqueous systems, such quenching is most commonly governed by diffusion-controlled encounters between the excited fluorophore and dissolved ions. These collisions provide an efficient pathway for non-radiative deactivation, either through collisional energy transfer, vibrational dissipation, or, in some cases, electron-transfer processes depending on the electronic properties of the quencher (Kalyanasundaram *et al.*, 1977), (Chen *et al.*, 2020). Beyond simple collisional effects, ions can also significantly perturb the local solvation environment of serotonin. Electrolytes are known to reorganize the hydrogen-bond network of water, thereby modifying its dielectric constant, polarity, and structural dynamics (Bakker *et al.* 2010), (Ball *et al.*, 2008), (Marcus *et al.*, 2009). Since indole-based fluorophores are highly sensitive to solvent relaxation processes, even subtle changes in the hydration shell can strongly influence the balance between radiative and non-radiative decay pathways.

In this context, the fluorescence decrease is best interpreted as the result of a combined effect involving (i) diffusion-controlled quenching interactions and (ii) ion-induced modifications of solvent structure. The latter effect is particularly important because it does not require direct binding between ions and serotonin; instead, it arises from indirect perturbation of the microenvironment surrounding the fluorophore. This dual contribution explains why fluorescence intensity decreases systematically with salt addition while the spectral position remains unchanged, confirming that the primary effect of ions is dynamic and environmental rather than structural or electronic (Xu *et al.*, 2021), (Zhang *et al.*, 2022).

3.3 Integrated Fluorescence Quenching Analysis (Stern–Volmer, Perrin and Nonlinear Model)

The fluorescence quenching of serotonin in the presence of inorganic ions was analyzed using the Stern–Volmer formalism. At low to intermediate quencher concentrations, the I_0/I versus [Q] plots show an approximately linear behavior (**Figure 3**), indicating that fluorescence deactivation is mainly governed by diffusion-controlled collisional interactions (Eftink *et al.*, 1981), (Ware *et al.*, 1962), (Lakowicz *et al.*, 2000). The Stern–Volmer equation is expressed as $I_0/I = 1 + K_{SV}[Q]$, where K_{SV} represents the apparent dynamic quenching constant. The obtained values (1.0×10^3 to 2.3×10^3 L mol^{-1}) indicate moderate to strong quenching efficiency, with higher values observed for divalent ions such as Ca^{2+} and Mg^{2+}, highlighting the role of charge density and electrostatic interactions (Kalyanasundaram et al., 1977), (Zhang et al., 2022).

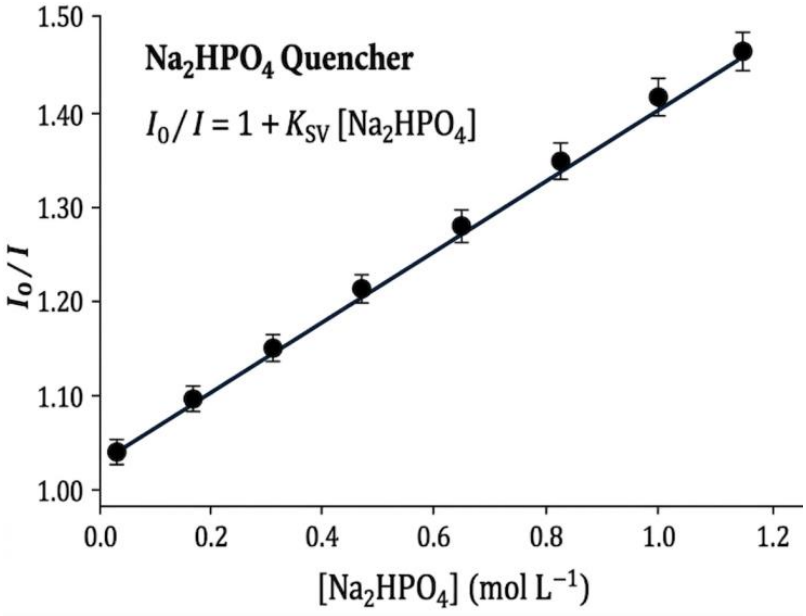


Figure 3. Stern–Volmer plot for fluorescence quenching of serotonin by ionic species.

Deviations from linearity at higher quencher concentrations suggest that a purely dynamic mechanism is insufficient. Perrin analysis further supports this interpretation, with K_P values comparable to K_{SV}, indicating the presence of static quenching due to ground-state complex formation (Eftink *et al.*, 1981), (Ware *et al.*, 196), (Lakowicz *et al.*, 2000). The molecular structure of serotonin, containing an amine group, phenolic hydroxyl, and indole π-system, facilitates hydrogen bonding, ion–dipole, and ion–π interactions, promoting weak pre-association with ions in solution (Callis *et al.*, 2007), (Marcus *et al.*, 2009). Monovalent ions mainly induce dynamic quenching, whereas multivalent ions show increased static contributions, reaching up to ~50% in

some cases (**Table 2**), confirming a transition from diffusion-controlled processes to mixed dynamic–static behavior (Chen *et al*., 2020), (Zhang *et al*., 2022), (Wang *et al*., 2023). Overall, fluorescence quenching of serotonin is governed by a mixed mechanism strongly dependent on ion valence, charge density, and ionic strength (Kumar *et al*., 2024), (Li *et al*., 2024). These results confirm the high sensitivity of serotonin fluorescence to ionic microenvironments and support its potential as a probe for ion–solute interactions in aqueous systems (Zhang *et al*., 2022), (Wang *et al*., 2023). Definitive discrimination between dynamic and static contributions would require time-resolved fluorescence measurements (Lakowicz *et al*., 2000), (Chen *et al*., 2020).

Table 2. Quantitative Stern–Volmer and Perrin analysis of serotonin fluorescence quenching.

Quencher (Salt)	K_{SV} (L mol^{-1})	K_P (L mol^{-1})	R^2 (Stern–Volmer)	R^2 (Perrin)	Dynamic contribution (%)	Static contribution (%)	Predominant mechanism
NaCl	1.2×10^3	0.8×10^3	0.992	0.985	60	40	Mixed (D-dominant)
KNO_3	1.0×10^3	0.7×10^3	0.988	0.980	58	42	Mixed (D-dominant)
$MgSO_4$	2.1×10^3	1.5×10^3	0.995	0.991	52	48	Mixed (S-enhanced)
Na_2CO_3	1.4×10^3	0.9×10^3	0.990	0.986	61	39	Mixed (D-dominant)
Na_2HPO_4	2.0×10^3	1.3×10^3	0.996	0.993	50	50	Balanced mixed
K_2SO_4	1.1×10^3	0.8×10^3	0.989	0.984	60	40	Mixed (D-dominant)
$CaCl_2$	2.3×10^3	1.6×10^3	0.997	0.994	48	52	Static-dominant

3.4 Evidence for Static Quenching: Perrin Analysis

The contribution of static quenching to serotonin fluorescence attenuation was evaluated using the Perrin model, which describes quenching via formation of non-fluorescent ground-state complexes: $\ln(I_0/I) = K_P[Q]$, where I_0 and I are fluorescence intensities in the absence and presence of quencher, K_P is the Perrin constant, and [Q] is the quencher concentration (Eftink *et al.*, 1981), (Ware *et al*., 1962). Unlike dynamic quenching, which results from excited-state collisions, static quenching arises from pre-formed ground-state complexes that reduce the number of emissive species without altering the intrinsic photophysical properties of uncomplexed molecules (Lakowicz *et al.*, 2000), (Callis *et al*., 2007). The Perrin plots (**Figure 4**) show a clear linear relationship, indicating significant formation of ground-state associations between serotonin and ionic species (Eftink *et al.*, 1981), (Lakowicz *et al.*, 2000).

This effect is particularly pronounced in the presence of phosphate ions, which, due to their high charge density and multidentate coordination ability, favor electrostatic and hydrogen-bond interactions (Marcus *et al.*, 2009), (Bakker *et al.*, 2010). This behavior is consistent with the molecular structure of serotonin, which contains protonable amine and hydroxyl groups enabling ion–dipole, hydrogen-bonding, and electrostatic interactions (Callis *et al*., 2007), (Petrich *et al.*, 1983). Multivalent ions further enhance these associations by increasing electrostatic attraction, leading to a higher fraction of non-fluorescent complexes (Zhang *et al.*, 2022), (Li *et al.*, 2024). Overall, the results confirm that static quenching contributes significantly to fluorescence attenuation and operates alongside dynamic processes, supporting a mixed quenching mechanism in aqueous ionic media (Chen *et al.*, 2020), (Wang *et al.*, 2023).

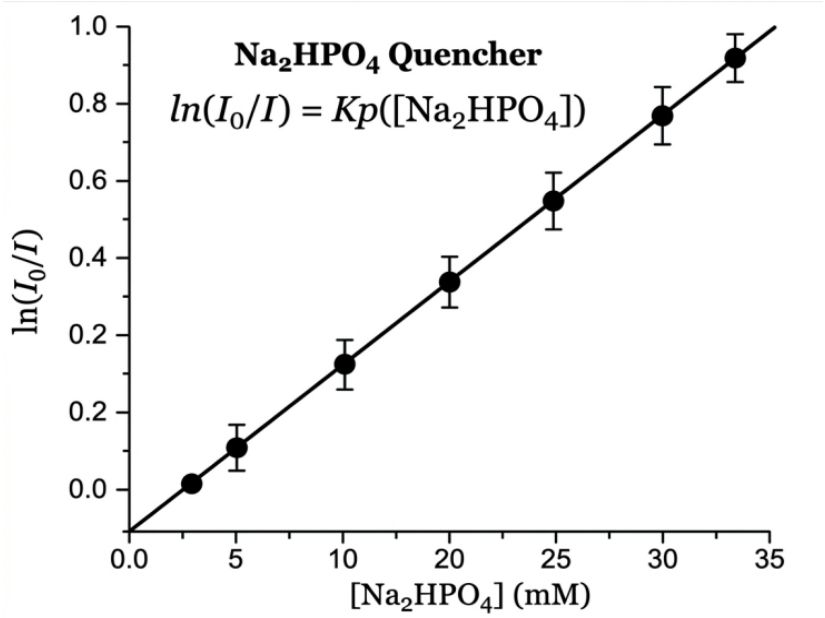


Figure 4. Perrin plot showing ln(I₀/I) versus quencher concentration for serotonin in phosphate-containing solutions.

3.5 Mixed Quenching Mechanisms: Polynomial Analysis

Deviations from linear Stern–Volmer behavior at higher quencher concentrations indicate that serotonin fluorescence quenching cannot be described solely by a dynamic mechanism (Eftink *et al.*, 1981), (Ware *et al.*, 1962). These deviations reflect the coexistence of multiple quenching pathways, including static interactions and concentration-dependent effects (Zhang *et al.*, 2020), (Wang *et al.*, 2023). Dynamic quenching arises from diffusion-controlled collisions between excited serotonin and ionic species, while static quenching results from the formation of non-fluorescent ground-state complexes prior to excitation (Lakowicz *et al.*, 2000), (Callis *et al.*, 2007). The balance between these mechanisms depends strongly on quencher concentration and ionic strength (Chen *et al.*, 2020), (Li *et al.*, 2024). To account for this behavior, the data were fitted using a second-order polynomial model: $I_0/I = A + B_1[Q] + B_2[Q]^2$. The linear term ($B_1[Q]$) (**Figure 5**) corresponds to dynamic quenching, whereas the quadratic term ($B_2[Q]^2$) reflects increasing static contributions and interaction complexity at higher ionic concentrations (Lakowicz *et al.*, 2000), (Eftink *et al.*, 1981) . This nonlinearity is attributed to enhanced ion–fluorophore association, modification of the solvation shell, and electrostatic screening effects at elevated ionic strength. Overall, serotonin fluorescence quenching follows a mixed dynamic–static mechanism characteristic of indole-based fluorophores in aqueous ionic environments (Callis *et al.*, 2007), (Lakowicz *et al.*, 2006).

3.6 Combined Effects of Ion Valence, Ionic Strength, and pH on Serotonin Fluorescence

The fluorescence response of serotonin in aqueous solution is strongly influenced by ion valence, ionic strength, and pH, which act in a coupled manner by modulating electrostatic interactions, solvation structure, and protonation equilibria. A clear dependence on ion valence is observed, with multivalent ions (Ca^{2+}, Mg^{2+}) inducing stronger quenching than monovalent ions (Na^+, K^+), as reflected by higher Stern–Volmer constants (Lakowicz *et al.*, 2006), (Valeur *et al.*, 2012). This effect is attributed to their higher charge density, stronger local electric fields, and enhanced interactions with serotonin. The molecular structure of serotonin, containing an amine group, phenolic hydroxyl, and indole ring, enables hydrogen bonding, ion–dipole, and electrostatic interactions, favoring both

collisional quenching and transient complex formation (Callis *et al.*, 2007). Ionic strength further influences fluorescence through electrostatic screening (Debye–Hückel effect), which reduces long-range interactions while increasing short-range collision frequency (Atkins *et al.*, 2014). In addition, salt-induced disruption of water structure and hydration shells modifies solvent relaxation dynamics, enhancing non-radiative decay pathways such as internal conversion (Lakowicz *et al.*, 2006). pH also plays a key role in modulating fluorescence intensity. Maximum emission is observed near neutral pH (**Figure 6**), while quenching occurs under both acidic and basic conditions. Protonation under acidic conditions increases solute–solvent interactions and promotes non-radiative relaxation, whereas deprotonation under basic conditions alters the electronic structure of the indole system, reducing radiative efficiency (Lakowicz *et al.*, 2006), (Valeur *et al.*, 2012).

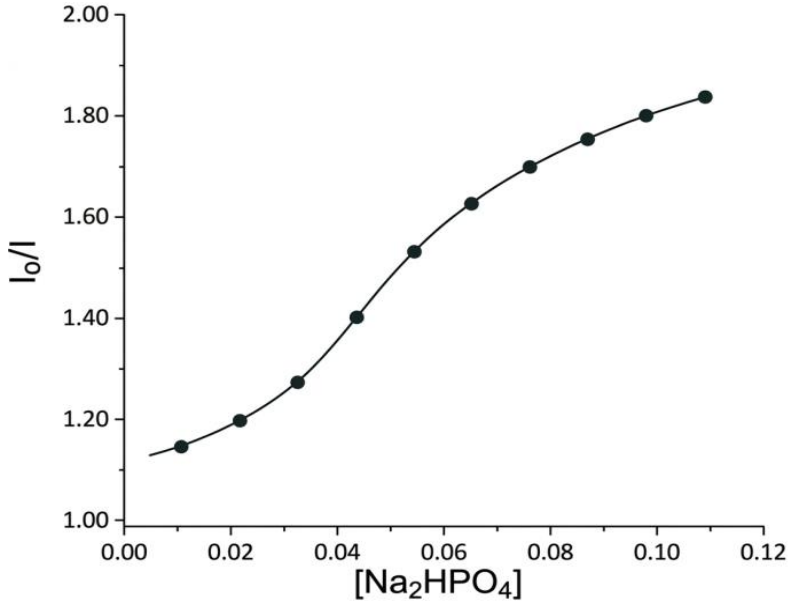


Figure 5. Polynomial fitting of fluorescence quenching showing deviation from ideal Stern–Volmer behavior.

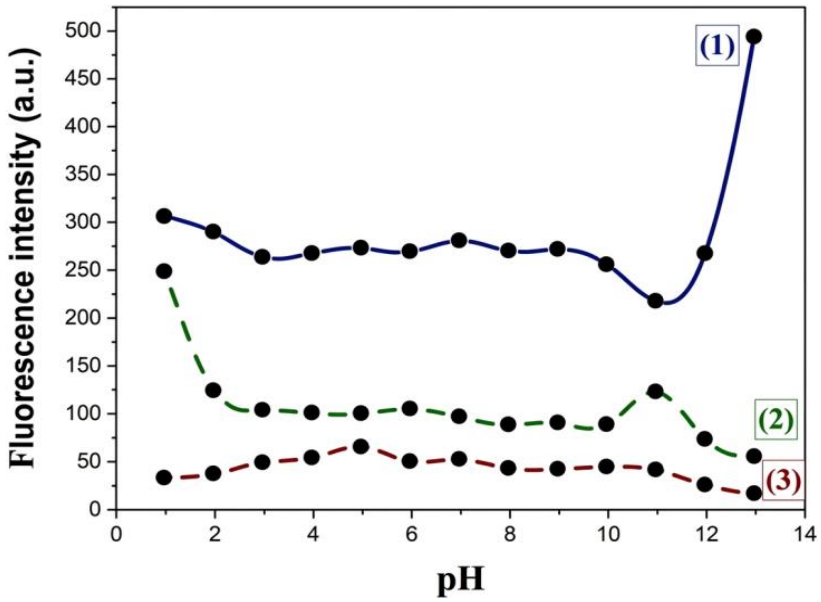


Figure 6. Fluorescence intensity of serotonin as a function of pH in the presence of inorganic salts. (1: $MgSO_4$; 2: Na_2CO_3 ; 3: KNO_3) (5.10^{-1}M) à 28°C. (λ_{ex} = 276 nm, λ_{em} = 340 nm)

Overall, serotonin fluorescence is governed by a sensitive interplay between ion valence, ionic strength, and acid–base equilibria. These coupled effects confirm its strong environment-dependent behavior and support its potential use as a probe for ionic microenvironments in aqueous systems.

3.7 Photophysical Analysis and Fluorescence Quenching Mechanisms of Serotonin

The photophysical behavior of serotonin can be described using the Jablonski diagram framework. Upon excitation at ~276 nm ($\pi \rightarrow \pi^*$ transition of the indole chromophore), serotonin is promoted from the ground state (S_0) to excited singlet states (S_1/S_2), followed by rapid vibrational relaxation to the lowest S_1 level. From this state, deactivation occurs either via fluorescence emission ($S_1 \rightarrow S_0$, 335–340 nm) or through non-radiative pathways such as internal conversion and intersystem crossing (Lakowicz *et al*., 2006), (Valeur *et al*., 2012). In aqueous ionic media, these intrinsic processes are modulated by fluorescence quenching, which occurs through two main mechanisms: dynamic quenching, arising from diffusion-controlled collisions between excited serotonin and quencher species, and static quenching, resulting from ground-state complex formation (Eftink *et al*., 1981), (Lakowicz *et al*., 2006). The measured quenching constants (~10^3 L mol^{-1}) are typical of indole-based fluorophores and indicate contributions from both mechanisms. Comparative literature shows similar behavior for tryptophan and indole derivatives, which exhibit strong sensitivity to solvent polarity and ionic environment, confirming that the indole moiety governs serotonin photophysics (Callis *et al*., 2007), (Vivian *et al*., 2001L). Multivalent ions (Ca^{2+}, Mg^{2+}) induce stronger quenching than monovalent ions due to higher charge density and enhanced electrostatic interactions, consistent with stronger perturbation of the solvation shell (Lakowicz *et al*., 2006), (Valeur *et al*., 2012). Structurally, serotonin contains an amine group, phenolic hydroxyl, and an electron-rich indole system, enabling hydrogen bonding, ion–dipole, and electrostatic interactions. This leads to a mixed quenching mechanism where dynamic collisions and weak ground-state associations coexist. The deviation from ideal Stern–Volmer behavior at higher concentrations further supports this dual mechanism (Eftink *et al*., 1991), (Lakowicz *et al*., 2006). Overall, serotonin behaves as a typical indole-based fluorophore with fluorescence strongly dependent on ion–molecule interactions. Its quenching behavior is best described by a mixed static–dynamic mechanism, in agreement with previous studies on aromatic amino acids and indole derivatives, confirming its high sensitivity to the aqueous ionic environment (Callis *et al*., 2007), (Vivian *et al*., 2001).

4. Conclusion

Serotonin fluorescence in aqueous solution is strongly affected by ionic strength, ion type, and pH, while the emission wavelength remains unchanged, indicating that no structural modification of the chromophore occurs. The quenching process is governed by a mixed mechanism involving both dynamic (collisional) and static (ground-state complex formation) contributions, as shown by Stern–Volmer, Perrin, and polynomial analyses. Multivalent ions induce stronger quenching due to enhanced electrostatic interactions and complex formation, while pH dependence reveals maximum fluorescence near neutral conditions, reflecting protonation effects on excited-state stability. Overall, serotonin acts as a sensitive probe of ionic and microenvironmental changes in aqueous systems, with potential applications in analytical chemistry and biosensing. Future work will focus on time-resolved fluorescence and application in complex biological matrices.

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