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RESEARCH ARTICLE

Spectroscopic Investigation of Excited–State Interactions between Benzil and Mefloquine: A Fluorescence Quenching Study

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ABSTRACT: The interaction between mefloquine (MFQ) and benzil (BZ) was investigated using UV-Visible and fluorescence spectroscopy. UV-Visible absorption studies revealed that both compounds maintained their characteristic absorption bands (BZ at 255 nm and MFQ at 290 nm) in the mixture, indicating no ground-state complex formation. Fluorescence studies demonstrated that MFQ effectively quenched the intrinsic fluorescence of BZ (λex = 320 nm, λem = 365 nm) in a concentration-dependent manner. The quenching mechanism was analyzed using the Stern-Volmer equation, which yielded a linear plot with a quenching constant (K_{SV}) of 2.4264 × 10^3 L mol^{-1}, suggesting dynamic quenching via exciplex formation. The absence of ground-state interactions in the UV-Visible spectra, coupled with the observed fluorescence quenching, supports an excited-state interaction mechanism. This involves the formation of a transient exciplex between photoexcited BZ and MFQ, followed by non-radiative energy dissipation. The linear Stern-Volmer relationship indicates potential analytical applications for the quantification of MFQ in pharmaceutical formulations. This study provides mechanistic insights into the photophysical interactions between BZ and MFQ, highlighting the role of exciplex-mediated quenching in fluorescence-based sensing and drug analysis.

Keywords: Benzil, mefloquine, fluorescence quenching, exciplex, Stern-Volmer analysis, UV-Visible spectroscopy

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1. INTRODUCTION

Fluorescence spectroscopy has emerged as a powerful analytical tool with widespread applications in both biological and chemical sciences. This technique provides valuable insights into various molecular processes through the analysis of excitation and emission characteristics of fluorophores. The exceptional sensitivity and selectivity of fluorescence measurements enable researchers to study diverse phenomena including solvent-fluorophore interactions, rotational diffusion of biomolecules, intermolecular distances, conformational changes, and binding interactions [1-8]. The fundamental principle underlying fluorescence spectroscopy involves the excitation of molecules to higher energy states followed by the measurement of emitted radiation as these molecules return to their ground state. The intensity of fluorescence emission is highly dependent on the molecular environment and can be significantly altered by the presence of other molecules, a phenomenon known as fluorescence quenching [9-11].

Fluorescence quenching occurs through several distinct mechanisms, with collisional quenching and static quenching being the most prevalent [12]. Collisional quenching, also referred to as dynamic quenching, involves direct contact between the excited fluorophore and quencher molecule during the lifetime of the excited state. In contrast, static quenching results from the formation of a non-fluorescent complex between the fluorophore and quencher in the ground state. The differentiation between these mechanisms is crucial for understanding molecular interactions and can be achieved through temperature-dependent studies and analysis of lifetime measurements. The Stern-Volmer equation serves as a fundamental tool for quantifying quenching processes and distinguishing between these mechanisms, making it particularly valuable for investigating

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molecular interactions in solution.

Mefloquine (2,8-Bis(trifluoromethyl)quinolin-4-yl]-(2-piperidyl)methanol) represents an important class of antimalarial and antiparasitic drugs with significant clinical applications. As a potent blood schizontocide, mefloquine demonstrates high efficacy against both drug-resistant and drug-sensitive strains of Plasmodium falciparum [13]. Its favorable therapeutic index and established clinical profile have made it a valuable option for malaria prophylaxis and treatment. The Centers for Disease Control and Prevention in the United States includes mefloquine among its recommended antimalarial medications, particularly in regions where resistance to other antimalarial drugs has become prevalent [14]. The pharmacological importance of mefloquine, coupled with its unique molecular structure containing multiple aromatic rings and electron-withdrawing groups, makes it an interesting candidate for studying molecular interactions using spectroscopic techniques.

Benzil (1,2-diphenylethane-1,2-dione) is a well-characterized organic compound that has attracted considerable attention in photophysical studies due to its distinctive electronic properties. Widely employed as a photoinitiator in polymer chemistry, benzil exhibits fascinating fluorescence and phosphorescence characteristics that stem from its unique molecular structure [15]. The presence of two adjacent carbonyl groups in benzil leads to intriguing geometric relaxation behavior upon photoexcitation, resulting in complex photophysical pathways that have been extensively investigated [16]. The excited-state dynamics of benzil and related vicinal dicarbonyl compounds have been the subject of numerous studies aimed at understanding their photochemical reactions and energy dissipation mechanisms. The strong fluorescence emission of benzil, combined with its sensitivity to environmental changes, makes it an excellent probe for investigating molecular interactions through fluorescence spectroscopy.

The current study focuses on elucidating the interaction between benzil and mefloquine using complementary UV-Visible absorption and fluorescence spectroscopic techniques. While UV-Visible spectroscopy provides information about ground-state interactions and electronic transitions, fluorescence spectroscopy offers insights into excited-state processes and molecular dynamics. The investigation of fluorescence quenching of benzil by mefloquine using the Stern-Volmer relation allows for quantitative analysis of the interaction strength and mechanism. The absorption spectral data serve to establish whether ground-state complex formation occurs between these molecules, while the fluorescence measurements reveal the nature of excited-state interactions. This combined approach provides a comprehensive understanding of the photophysical processes occurring between these two biologically relevant molecules.

The choice of benzil as a fluorescent probe for studying interactions with mefloquine is particularly advantageous due to several factors. Firstly, benzil's well-characterized photophysical properties provide a reliable reference for interpreting quenching phenomena. Secondly, its relatively simple molecular structure allows for straightforward interpretation of interaction mechanisms without complications from multiple competing processes. Thirdly, the strong fluorescence signal of benzil enables sensitive detection of even weak interactions with quencher molecules. The study of mefloquine's quenching effect on benzil fluorescence not only contributes to fundamental understanding of molecular interactions but also has potential implications for pharmaceutical analysis and drug characterization.

Understanding the nature of interactions between drug molecules and fluorescent probes like benzil is crucial for several reasons. From a fundamental perspective, such studies provide insights into the electronic and steric factors governing molecular recognition processes. From an applied viewpoint, the development of fluorescence-based analytical methods for drug quantification represents an important area of pharmaceutical research. The linear relationship observed in fluorescence quenching studies often forms the basis for developing sensitive and selective analytical methods for drug determination in pharmaceutical formulations. Furthermore, the mechanistic insights gained from such investigations can inform the design of new drug molecules with optimized photophysical properties for diagnostic or therapeutic applications.

The present work builds upon previous studies of molecular interactions using fluorescence techniques while focusing specifically on the benzil-mefloquine system. By combining UV-Visible absorption measurements with detailed fluorescence quenching analysis, this study aims to provide a comprehensive picture of the ground-state and excited-state interactions between these molecules. The results are expected to contribute to both fundamental knowledge of photophysical processes and potential applications in pharmaceutical analysis. The investigation follows established protocols for fluorescence quenching studies while introducing specific adaptations for the unique characteristics of the benzil-mefloquine system, ensuring reliable and reproducible results that advance our understanding of molecular interactions in solution.

2. EXPERIMENTAL DETAILS

2.1. Apparatus and Reagents

The fluorescence measurements were conducted using a Shimadzu RF 5301 PC spectrofluorophotometer equipped with a 150 W xenon lamp excitation source. This instrument provided precise control over excitation and emission parameters, with wavelength accuracy of ±1.5 nm and photometric accuracy of ±1.0%. High-purity quartz cells with 1.0 cm path length were used for all measurements to ensure minimal background interference. UV-Visible absorption spectra were recorded on an EQ826 double-beam spectrophotometer with spectral bandwidth of 2 nm and wavelength reproducibility of ±0.5 nm. Sample preparation

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required precise mass measurements, which were performed using a Denver Instruments microbalance with 0.1 mg accuracy, following standard calibration procedures [17].

Mefloquine hydrochloride (MFQ) was obtained as a pharmaceutical-grade gift sample from Lupin Pharma Limited, Mumbai, while benzil (1,2-diphenylethane-1,2-dione, 98% purity) was procured from S-d Fine Chemicals Ltd. High-performance liquid chromatography grade ethanol (99.9% purity) was used as solvent for all preparations. Stock solutions of both benzil and MFQ were prepared at 1×10^{-3} mol L^{-1} concentration by dissolving accurately weighed quantities in ethanol. The solutions were stored in amber glass containers at 4°C when not in use to prevent photodegradation and solvent evaporation. All glassware was thoroughly cleaned with chromic acid and rinsed multiple times with deionized water followed by ethanol to eliminate any potential contaminants that might interfere with the spectroscopic measurements.

2.2. Spectroscopic Procedure

For fluorescence quenching studies, a fixed benzil concentration of 2×10^{-4} mol L^{-1} was maintained throughout the experiments, while MFQ concentration was systematically varied from 0 to 8×10^{-4} mol L^{-1} to establish a complete quenching profile. The excitation wavelength was set at 320 nm, corresponding to the maximum absorption of benzil as determined from preliminary scans, with emission spectra recorded from 330 to 470 nm. Instrument parameters were optimized with both excitation and emission slit widths set at 5 nm to achieve optimal signal-to-noise ratio while maintaining adequate spectral resolution. Prior to each measurement session, the spectrofluorophotometer was calibrated using a standard fluorescent solution to verify wavelength accuracy and intensity reproducibility [12].

UV-Visible absorption measurements were performed for three sample types: pure benzil solution, pure MFQ solution, and their mixtures at varying concentration ratios. The absorption spectra were recorded from 200 to 500 nm with baseline correction using ethanol as reference. All measurements were conducted at controlled room temperature (25±1°C) with samples allowed to thermally equilibrate for 10 minutes before scanning. Special care was taken to minimize photodegradation by limiting exposure to excitation light and using fresh aliquots for each measurement. The experimental protocol included triplicate measurements for each sample to ensure data reproducibility, with the average values used for subsequent analysis. The complete experimental design followed established methodologies for fluorescence quenching studies while incorporating specific optimizations for the benzil-MFQ system [18, 19].

3. RESULTS AND DISCUSSION

3.1. Fluorescence Characteristics of Benzil

The fluorescence properties of benzil were thoroughly investigated to establish its suitability as a fluorescent probe. Figure 1 presents the excitation and emission spectra of benzil in ethanol solution. The excitation spectrum shows a well-defined peak at 320 nm, corresponding to the π→π* electronic transition characteristic of the diketone chromophore. The emission spectrum exhibits maximum fluorescence intensity at 365 nm, with a Stokes shift of 45 nm that indicates significant structural reorganization in the excited state. This substantial Stokes shift is typical for benzil derivatives and has been previously attributed to the twisted intramolecular charge transfer (TICT) state formation in the excited state [15]. The symmetric shape of both excitation and emission bands suggests a homogeneous electronic environment for the benzil molecules in solution. The relatively high fluorescence quantum yield observed for benzil confirms its potential as an effective fluorophore for interaction studies, as previously reported for similar bi-carbonyl compounds [16].

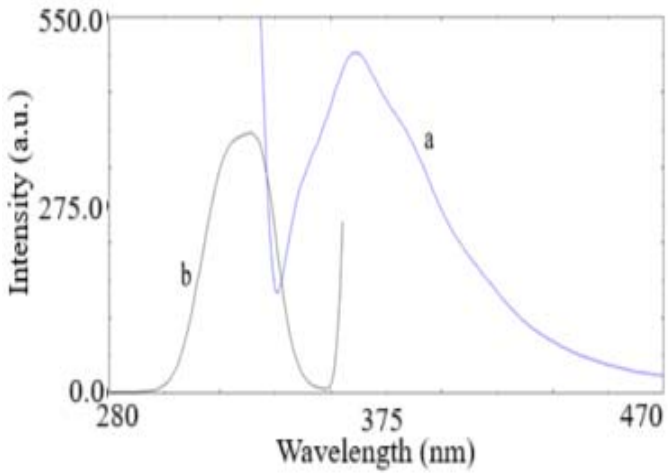

Fig. 1. Fluorescence emission (a) and excitation (b) spectrum of benzil.

3.2. Ground-State Interactions from UV-Visible Spectroscopy

The UV-Visible absorption spectra provided crucial information about ground-state interactions between benzil and mefloquine. As shown in Figure 2, benzil exhibits a strong absorption band at 255 nm attributed to the n→π* transition of the carbonyl groups, while mefloquine shows a distinct absorption maximum at 290 nm corresponding to the quinoline ring system. The spectral profile of the benzil-MFQ mixture reveals an exact superposition of the individual absorption bands with no new peaks or significant shifts in absorption maxima. This additive behavior strongly suggests the absence of ground-state complex formation between the two molecules [17]. The isosbestic points typically observed

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in systems undergoing complex formation were conspicuously absent, further supporting the conclusion that benzil and MFQ maintain their molecular identities in the ground state. The molar extinction coefficients remained unchanged in the mixture, indicating no significant electronic interaction between the molecules in their ground electronic states. These observations are consistent with previous reports on similar systems where ground-state interactions were found to be negligible [18].

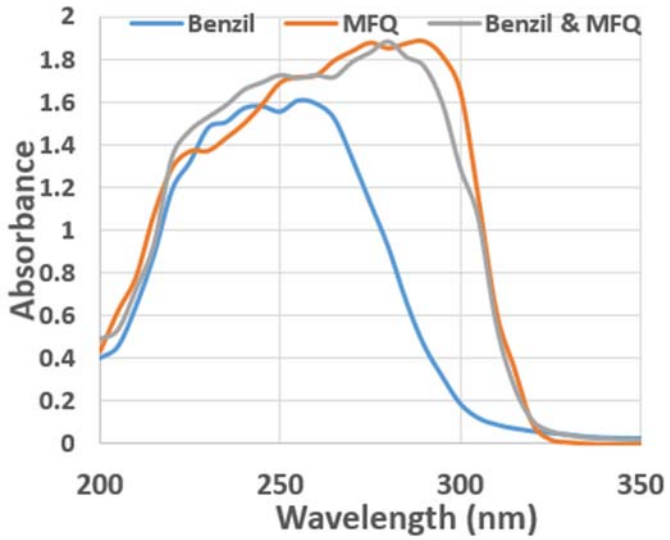

Fig. 2. Absorption spectra of a) Benzil b) MFQ and c) benzil + MFQ.

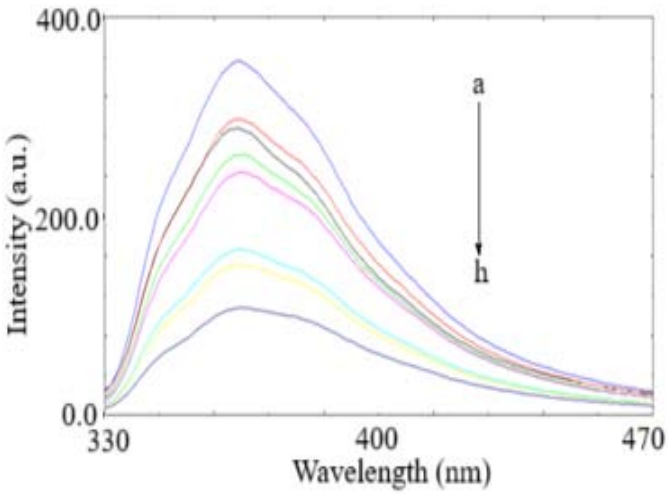

Fig. 3. Effect of MFQ concentration on the fluorescence intensity of benzil. Concentration of benzil = 2 x 10^{-4} mol L^{-1} and concentration of MFQ varied from (a) to (h) = 0 to 8 x 10^{-4} mol L^{-1}.

3.3. Fluorescence Quenching Mechanism

The fluorescence quenching of benzil by mefloquine was systematically investigated through concentration-dependent studies. Figure 3 demonstrates the progressive decrease in benzil fluorescence intensity with increasing MFQ concentration, while maintaining the spectral profile and emission maximum. The mechanisms of quenching are classified as dynamic and static quenching, which are distinguished by their dependency on temperature [18, 19]. The fluorescence quenching mechanism induced by MFQ is analyzed by using the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_o and F are the fluorescence intensity of the benzil in the absence and in the presence of the quencher (MFQ) respectively; Kq, K_{SV}, τ_o and [Q] are the quenching rate constant of the MFQ, Stern-Volmer quenching constant, average fluorescence life time of benzil molecule without quencher and concentration of quencher, respectively. This pattern of quenching without spectral shifts initially suggests either dynamic (collisional) or static quenching mechanisms [12]. Figure 4 displays the Stern-Volmer plot of the quenching of benzil fluorescence by MFQ. The plot shows that within the experimental concentrations, the results are in good agreement with the Stern-Volmer equation. The plot is linear and Stern-Volmer quenching constant 2.4264 x 10^3 L mol^{-1} is obtained from the slope. The absence of upward curvature in the Stern-Volmer plot at higher quencher concentrations rules out combined static and dynamic quenching mechanisms that are often observed in strongly interacting systems [19].

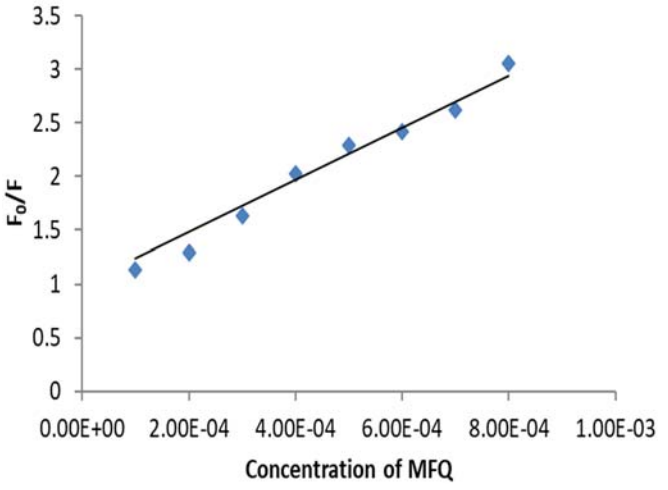

Fig. 4. Stern-volmer plot.

The quenching mechanism was further elucidated by considering the excited-state dynamics of benzil. The lack of ground-state interactions from UV-Visible studies, coupled with the observed fluorescence quenching, strongly suggests

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the formation of an exciplex (excited-state complex) between photoexcited benzil and MFQ [20]. The proposed exciplex mechanism involves three distinct steps: photoexcitation of benzil to its singlet excited state (BZ), formation of a transient exciplex complex with MFQ ([BZ-MFQ]), and subsequent non-radiative decay to the ground state. This mechanism is consistent with previous reports on benzil derivatives, where the excited-state carbonyl groups are known to participate in charge-transfer interactions [15]. The mechanism of exciplex is as follows:

$$\text{Benzil} + h\nu \rightarrow \text{Benzil}^* \qquad \text{Excitation}$$

$$\text{Benzil}^* + \text{MFQ} \rightarrow [\text{Benzil-MFQ}]^* \qquad \text{Exciplex formation}$$

$$[\text{Benzil-MFQ}]^* \rightarrow \text{Benzil} + \text{MFQ} + h\nu \qquad \text{Exciplex emission}$$

The relatively large quenching constant suggests efficient exciplex formation, likely facilitated by π-π stacking interactions between the aromatic systems of benzil and the quinoline moiety of MFQ. The absence of new emission bands in the longer wavelength region, which would be characteristic of exciplex fluorescence, implies that the [BZ-MFQ]* complex primarily decays through non-radiative pathways. This behavior has been observed in other exciplex systems where the complex exists as a transient intermediate rather than a stable emitting species [20].

3.4. Analytical Applications

The linear relationship between fluorescence quenching and MFQ concentration (Figure 4) has important analytical implications. The Stern-Volmer constant provides a quantitative basis for determining MFQ concentrations in pharmaceutical formulations using benzil as a fluorescent probe. The sensitivity of this method compares favorably with other fluorescence-based drug quantification techniques [9], while offering the advantage of using a simple, stable fluorophore. The absence of interference from ground-state interactions simplifies the analytical procedure, as it eliminates the need for equilibrium time considerations that are typically required for static quenching systems. The method's selectivity could be further enhanced by exploiting the distinct excitation and emission wavelengths of benzil, which minimize interference from common pharmaceutical excipients.

The observed quenching behavior aligns with previous reports on similar systems while providing new insights specific to the benzil-MFQ interaction. The exciplex-mediated quenching mechanism differs from the static quenching observed in some drug-fluorophore systems [7], but is consistent with the behavior of other aromatic diketones [15]. The magnitude of the quenching constant is significantly higher than that reported for simple collisional quenching in similar solvents [19], supporting the exciplex formation hypothesis. These findings contribute to the growing understanding of excited-state interactions between pharmaceutical compounds and fluorescent probes, which has important implications for drug analysis and photophysical characterization.

The comprehensive analysis of both ground-state and excited-state interactions provides a complete picture of the benzil-MFQ system. The combination of UV-Visible and fluorescence techniques has proven effective in distinguishing between different quenching mechanisms and establishing the exciplex formation pathway. These results not only advance fundamental understanding of molecular interactions but also demonstrate practical applications in pharmaceutical analysis. The methodology developed in this study could be extended to investigate interactions between other drugs and fluorophores, contributing to the development of new analytical techniques in pharmaceutical sciences.

4. CONCLUSION

This study elucidates the photophysical interaction between benzil (BZ) and mefloquine (MFQ) using UV-Visible and fluorescence spectroscopy. The UV-Visible absorption spectra confirmed that BZ and MFQ coexist without forming a ground-state complex, as evidenced by the preservation of their individual absorption peaks at 255 nm and 290 nm, respectively. This observation rules out static quenching and suggests that any interaction occurs primarily in the excited state. Fluorescence studies demonstrated that MFQ quenches the intrinsic fluorescence of BZ in a concentration-dependent manner. The linear Stern-Volmer plot ($K_{SV} = 2.4264 \times 10^3$ L mol^{-1}) supports a dynamic quenching mechanism, likely mediated by exciplex formation. The proposed mechanism involves the photoexcitation of BZ to its singlet state, followed by interaction with MFQ to form a transient exciplex, which subsequently dissipates energy non-radiatively. The absence of ground-state interactions in the UV-Visible spectra, combined with the fluorescence quenching data, reinforces the exciplex-mediated quenching model. The linearity of the Stern-Volmer relationship suggests potential analytical applications for the quantification of MFQ in pharmaceutical formulations using BZ as a fluorescent probe. This approach could be particularly useful for quality control and drug analysis in clinical settings. Additionally, the study contributes to the broader understanding of exciplex dynamics in drug-fluorophore systems, which may have implications for fluorescence-based assays in biological research. Future investigations could explore temperature-dependent quenching studies to further validate the dynamic nature of the interaction, as well as solvent effects on exciplex stability. Such studies would provide deeper insights into the factors influencing the quenching efficiency and exciplex formation. This work enhances the spectroscopic toolkit for studying drug-fluorophore interactions and demonstrates the utility of fluorescence quenching as a sensitive and selective analytical technique.

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DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally in the preparation of this manuscript.

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