J. Mountain Res. P-ISSN: 0974-3030, E-ISSN: 2582-5011 DOI: https://doi.org/10.51220/jmr.v21-S2.24
Vol. 21(S2), (2026) 193-199

Optical Interaction Between The Dye Pair Fluorescein-Safranine T Doped In Polyvinyl Alcohol Polymeric Matrix

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Received: 30.09.2025; Revised: 31.10.2025; Accepted: 22.11.2025

Abstract: Energy transfer and migration in molecular and ionic systems have wide applications in laser, optical sensors, biological probes, luminescent solar collectors etc. The excitation energy transfer between the dye pair fluorescein (donor) and safranine T (acceptor), doped in a polyvinyl alcohol (PVA) matrix, was investigated at room temperature under steady state emission conditions. Thin PVA films doped with varying concentrations of dyes were prepared and fluorescence-absorption spectra were subsequently recorded. The overlap integral between donor fluorescence and acceptor absorption has been calculated using absorption and fluorescence spectra, which indicates the energy transfer probability from donor to acceptor. The corresponding critical transfer distance between donor-acceptor (R_{D-A}), reduced concentration has also been calculated. Increase in emission intensity of safranine T and simultaneous decrease in emission intensity of fluorescein was observed with the increasing concentration of safranine T and at constant concentration of fluorescein. The large values of overlap integral and critical transfer distance suggest long range dipole-dipole energy transfer mechanism for the dye pair fluorescein-safranine T.

Keywords: Fluorescence • Excitation energy transfer • Dipole-dipole interaction • Optical excitation • Dye pair.

Introduction

Excitation energy transfer is a photophysical process, where the physical state of a molecule may change without any change in the chemical structure. It involves transfer of excitation energy absorbed by donor fluorescent molecule to other acceptor fluorescent molecule through non-radiative process (Misra et al. 2002). This process is experimentally demonstrated by simultaneous quenching of the donor fluorescence and electronic excitation of the acceptor. Fluorescence resonance energy transfer (FRET) between luminescent systems has been of a great interest due to its versatility in investigating the structure, dynamics and micro-environmental properties of the polymers and biopolymers (Joshi et al. 2006; Kaur et al. 2020). FRET studies in dye doped polymers are fascinating as they are used for fiber lasers and fiber amplifiers, fluorescence chemosensors, optoelectronics, organic light emitting diodes, molecular imaging, drug delivery carriers, luminescent solar collectors etc. (Joshi et al. 2008). FRET studies have also been conducted using rare earth ion pairs doped in various glass matrices to investigate energy transfer for applications in the field of lasers, optical devices etc. (Joshi et al. 2008; Dhondiyal et al. 2011, 2021, 2022).

One commonly known polymeric matrix for holographic recording is PVA, a polar water-soluble polymer, which undergoes crosslinking with hydrogen bonds. It is humidity-sensing thermoplastic material having high affinity for water due to hydrophilic functional group and is biologically decomposable in nature. It is biocompatible, stable with variation in temperature and nontoxic to living tissues and cells, so used for different applications (Finch 1992).

Fluorescein dye has high molar absorptivity in the visible region, good fluorescence quantum

yield and is highly photostable, which makes it a highly useful and sensitive fluorescent marker. Fluorescein remains in different ionic forms in aqueous solutions, so pH strongly influences its fluorescence and absorption properties (Sjöback et al. 1995). Safranine T is a phenazinium dye having its absorption spectrum (λ_m ~ 520 nm) in aqueous solutions and has attracted significant attention as a sensitizer in photoinitiation systems and for various other applications, including solar energy conversion (Savenko & Kostjukov 2022). Chatterjee et al. (2005) reported the quantum yield (ϕ_D) of fluorescein as 0.91. For the FRET analysis, the orientation factor is assumed to be 2/3, based on the random orientation of the donor and acceptor transition dipoles in PVA matrix (King et al. 2012). The efficiency of FRET depends on several factors, including the distance between the donor and acceptor molecules (typically within 1–10 nm) and the degree of spectral overlap between the donor's fluorescence spectrum and the acceptor's absorption spectrum and relative orientation of donor fluorescence dipole moment and acceptor absorption dipole moment (Hoefling et al. 2011). The present study is therefore conducted to investigate the energy transfer between the dye pair fluorescein-safranine T doped in PVA.

Materials and Methods

PVA was procured from Central Drug House (P) Ltd., Mumbai; fluorescein and safranine T were obtained from Aldrich Chemical Company Inc., USA and BDH Chemicals Ltd., Poole, England respectively. Thin PVA films were prepared using cold water, both with and without dye incorporation. PVA and the required concentrations of dyes were dissolved in a known volume of distilled water and stirred for 30 minutes at room temperature to prepare thin films. The resulting dye–PVA mixtures were gently heated to around 40°C with occasional stirring until a homogeneous viscous solution was obtained. This mixture was then poured into a polypropylene dish and dried in an incubator to form transparent films of the desired shape and size. After dye incorporation, the films were immersed in methanol to remove any unbound dye molecules. The absorption spectra were recorded using a JASCO(V-550) spectrophotometer, and the fluorescence spectra were measured using a JASCO(FP-777) spectrofluorometer to study the luminescence characteristics of the dye-doped polymer films.

The absorption and emission spectra of dye molecules exhibit broad bands in the ultraviolet–visible region. According to Forster (1959), the rate of energy transfer between excited donor (D*) and unexcited acceptor (A) is distance dependent and corresponds to dipole-dipole interaction between the two species. Considering the overlap between donor fluorescence and acceptor absorption, the various spectroscopic and energy transfer parameters for dye pair fluorescein (donor) and safranine T (acceptor) doped in polymer (PVA) are calculated and presented in Table 1 and 2.

Results

The emission and absorption spectra of pure fluorescein (donor) (concentration 1 x 10^{-4} mol L^{-1}) in PVA were calculated. Fluorescein shows absorption at 505 nm and emission at 524 nm, on excitation by 470 nm of visible radiation. The absorption spectra of safranine T at different concentrations 0.2 x 10^{-4}, 0.4 x 10^{-4} and 0.6 x 10^{-4} mol L^{-1} in PVA were obtained by plotting optical density versus wavelength (nm). The overlapped spectra of fluorescein emission at concentration (1x10^{-4} mol L^{-1}) and safranine T absorption at concentrations (0.2x10^{-4}, 0.4x10^{-4}, and 0.6x10^{-4} mol L^{-1}) in PVA shown in Fig. 1, indicate significant overlap between donor's fluorescence spectrum and acceptor's absorption spectrum.

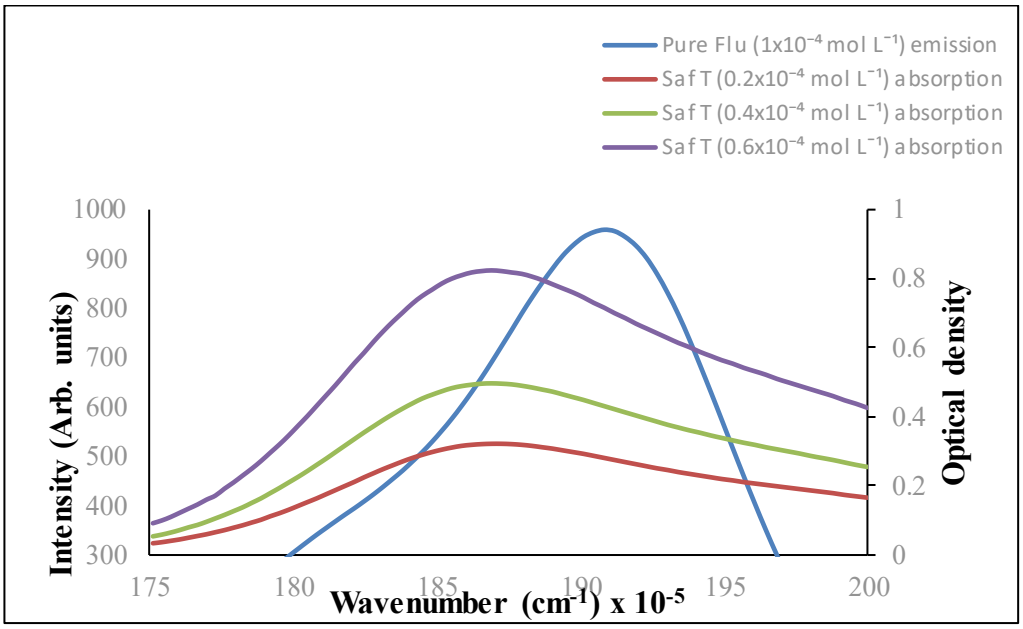

Fig. 1: Overlapped spectra of fluorescein emission and safranine T absorption

Small overlapping observed between absorption and fluorescence spectra of donor (fluorescein) itself was also observed with the help of a graph between intensity and wavenumber(cm^{-1}) x 10^{-5}.

Only two absorption peaks were observed in the absorption spectrum of dye mixture fluorescein and safranine T corresponding to dyes doped in PVA as shown in Fig. 2 at different concentrations of safranine T with constant concentration of fluorescein.

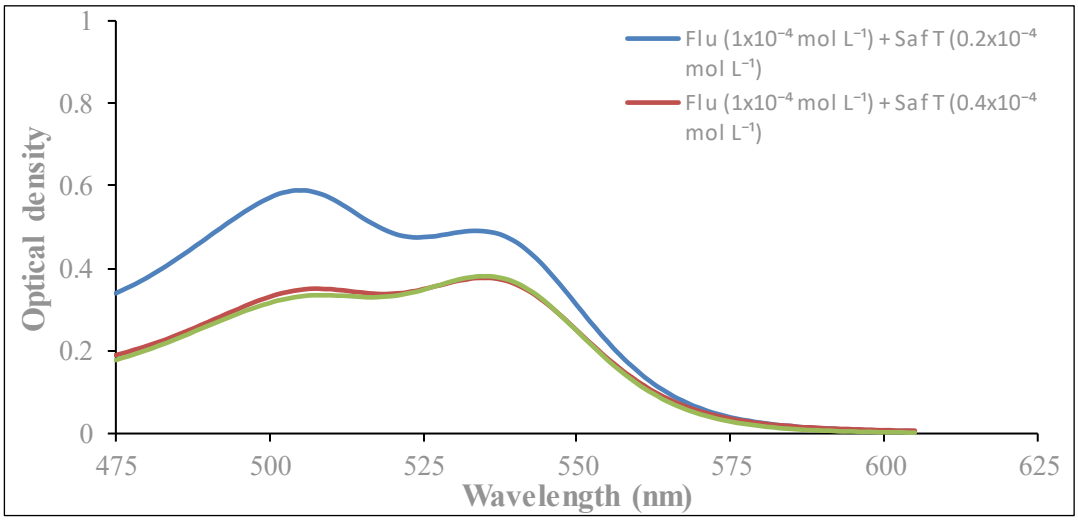

Fig. 2 Absorption spectrum of mixture of fluorescein and safranine T

Table 1: Relative emission intensity of fluorescein and safranine T in fluorescein-safranine T system doped in PVA, excited by visible radiation of 470 nm.

Donor Conc. (mol L^{-1}) (Fluorescein)	Acceptor Conc. (mol L^{-1}) (Safranine T)	Emission Peaks (nm)	Emission Intensity (arb. units)
$1x10^{-4}$	----------	524, ----	1236, -----
$1x10^{-4}$	$0.2x10^{-4}$	520, 554	578, 411
$1x10^{-4}$	$0.4x10^{-4}$	518, 561	525, 670
$1x10^{-4}$	$0.6x10^{-4}$	514, 570	400, 1161

Fluorescence spectra of fluorescein, safranine T and their mixtures shown in Fig. 3 indicate that the fluorescence intensity of pure fluorescein (curve 1) was prominent than that of pure safranine T (curve 3) at excitation wavelength of 470 nm (Table 1). The overall decrease in fluorescein emission with doping of increasing concentration of safranine T (curve 2i, ii, iii of Fig. 3) shows energy transfer from excited fluorescein molecules to unexcited safranine T molecules.

Since FRET efficiency largely depends on the molecular closeness of donor-acceptor in the given matrix, so the variation of FRET efficiency with varying concentration of safranine T in case of fluorescein - safranine T dye mixture was studied. Fluorescein concentration was kept constant at 1 x 10^{-4} mol L^{-1} whereas safranine T concentration varied from 0.2x10^{-4} mol L^{-1} to 0.6x10^{-4} mol L^{-1}. The FRET efficiency increases linearly with increase in safranine T concentration in the dye mixture.

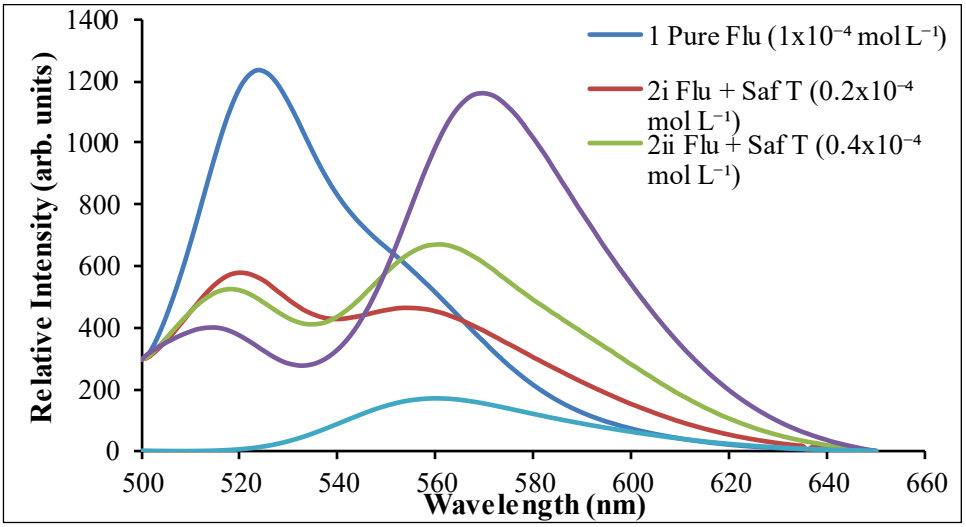

Fig. 3 Fluorescence spectra of pure fluorescein, pure safranine T and their mixture Flu + Saf T in PVA with acceptor (Saf T) concentration variation

The values of spectral overlap integral (Ω_{DA}), critical transfer distance (R_o), critical acceptor concentration (C_{OA}), reduced concentration for donor-acceptor overlapping (γ_{DA}), the donor acceptor distance (R_{DA}) and energy transfer efficiency (η) were determined from the fluorescence spectra of the fluorescein–safranine T dye mixture doped in PVA and are presented in Table 2.

Table 2: Calculated values of spectroscopic parameters for fluorescein-safranine T system in PVA.

Host Polymer	Acceptor Conc. (mol L^{-1})	Ω_{DA} (10^{-14} cm^6 mol^{-1})	R_O (nm)	C_{OA} (mol L^{-1})	γ_{DA} (10^{-3})	R_{DA} (nm)	η (%)
PVA	0.2 x 10^{-4}	59.75	6.30	1.79 x 10^{-3}	11.17	6.19	53.2
	0.4 x 10^{-4}	67.78	6.40	1.70 x 10^{-3}	23.43	6.10	57.5
	0.6 x 10^{-4}	86.41	6.70	1.48 x 10^{-3}	45.69	5.95	67.6

The Ω_{DA} varied from 59.75 x10^{-14} to 86.41x10^{-14} cm^6 mol^{-1} and the R_{DA} varied from 6.19 to 5.95 nm. Maximum energy transfer efficiency was 67.6 % for 0.6 x 10^{-4} mol L^{-1} acceptor concentration. The fluorescence maximum of fluorescein is shifted from 524 nm to 514 nm, while the fluorescence maximum of safranine T shifted from 554 nm to 570 nm,

corresponding to a blue shift in fluorescence maximum of fluorescein and a red shift in fluorescence maximum of safranine T with increasing concentrations of safranine T.

Discussion

Our results show fluorescein absorption at 505 nm and emission at 524 nm, comparable to emission wavelength at 510 nm as reported by Blagoi et al. (2005). Fluorescein emission and safranine T absorption spectra at different concentrations in PVA exhibit significant overlapping (Fig. 1), Saha et al. (2017) also observed similar overlap in dye pair fluorescein-rhodamine 6G. The appropriate overlapping of the fluorescence spectrum of fluorescein and absorption spectrum of safranine T rationalizes the selection of these two dyes suitable for the energy transfer study (Blagoi et al. 2005; Grauer et al. 1984; Sahare et al. 2008). Small overlapping between absorption and fluorescence spectra of fluorescein itself indicates the possibility of excitation energy transfer between similar molecules, known as energy migration (Kalinin & Johansson 2004). The presence of two absorption peaks observed in the absorption spectrum of dye mixture fluorescein and safranine T as shown in Fig. 2, rules out the possibility of any type of chemical interaction in these molecules when fluorescein and safranine T are mixed together in PVA at the concentration used.

Saha et al. (2017) also reported direct energy transfer from fluorescein to rhodamine 6G at excitation wavelength of 430 nm and prominent peak of fluorescein, similar results were observed in our analysis for fluorescein safranine T dye pair, where the prominent peak of fluorescein was observed. The energy transfer observed in Fig. 3 may be contributed to the non-radiative transfer from excited fluorescein molecules to unexcited safranine T molecules via FRET, which may also be confirmed by the average donor acceptor distance observed close to 6 nm lying in the range of 1–10 nm (Hoefling et al. 2011) and FRET depends on the concentration of safranine T as also reported by Chatterjee et al. (2005).

Similar distance dependent FRET study has been conducted by Hoefling et al. (2011) based on the molecular closeness of donor-acceptor in the given matrix. The linear increase in FRET efficiency with increase in safranine T concentration can be explained due to close proximity of fluorescein and safranine T with increase in acceptor concentration (Bhuin et al. 2021). It has been observed in Table 2, that the distance between fluorescein-safranine T decreases with increase in safranine T concentration, while the value of spectral overlap integral increases (Haugland et al. 1969), resulting an increase in FRET efficiency. Energy transfer efficiency increase with increasing acceptor concentration has been reported by Guang Chao et al. (2013).

The observed blue shift in fluorescence maximum of fluorescein with increasing concentration of safranine T appears to be due to radiative energy transfer to the acceptor and the red shift in the acceptor fluorescence can be attributed to radiative migration among acceptors as also reported by Misra and Misra (2007) and Mishra et al. (2006).

The energy transfer phenomenon from fluorescein to safranine T studied from the measurements of steady state emission shows a considerable overlap between the fluorescence spectrum of donor (fluorescein) and absorption spectrum of acceptor (safranine T), which is a prerequisite condition for FRET. Energy transfer between dye pair fluorescein - safranine T in PVA polymeric matrix was observed primarily by non-radiative mechanism. The energy transfer efficiency varies from 53.2 % to 67.6 % with variation in safranine T concentration from $0.2x10^{-4}$ to $0.6x10^{-4}$ mol L^{-1}.

Acknowledgment

We are deeply indebted to our respected teacher and supervisor, Late Prof. B. C. Joshi, Former Head, Department of Physics, S.S.J. Campus, Almora, for his invaluable guidance,

insightful discussions, constant encouragement, and unwavering patience throughout this study. One of the authors, Bhawna Sanwal, gratefully acknowledges the Council of Scientific and Industrial Research (CSIR), New Delhi, for providing financial support.

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