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Energy Transfer Calculations Based on Fluorescence Spectra of Acriflavine and Rhodamine B Laser Dyes

In this work, the fluorescence spectra of a mixture of two laser dyes; acriflavine and rhodamine B in different solvents; ethanol and DMSO, were recorded and studied. The energy transfer efficiency of the prepared samples was determined and studied by comparing some parameters such as the critical transfer distance, the emission lifetime, and the rate constant for energy transfer at different concentrations of these solutions. The emission lifetime of the dye mixture in DMSO was shorter than that in ethanol by 35.6%.

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1. Introduction

Fluorescence energy transfer is the transfer of the excited state energy from a donor (D) to an acceptor (A) [1]. This transfer occurs without the appearance of photon and is primarily a result of dipole-dipole interaction between the donor and the acceptor. The rate of energy transfer depends upon the extent of overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the relative orientation of the donor and acceptor transition dipoles and the distance between these molecules [2]. The nonradiative energy transfer occurs as a result of dipole-dipole coupling between the donor and the acceptor, and does not involve the emission and reabsorption of photons. The other process is radiative process, which depends upon other properties of the sample, such as size of the sample, container, optical densities of the sample at the excitation and emission wavelengths and the precise geometric arrangements of the excitation and emission axes [3].

In contrast to these trivial factors nonradiative energy transfer depends upon the molecular details of donor-acceptor pairs. It is important to note here that the phenomenon of energy transfer also contains molecular information, which is different from revealed by other phenomena such as solvent relaxation, excited state reactions, and fluorescence quenching or fluorescence polarization. These other spectral properties of fluorescence reveal primarily the interactions with the other molecules in the surrounding solvent shell. The important parameter for energy transfer that nonradiative energy transfer is effective over distance ranging of 50Å. The intervening of solvent or other macromolecules has little effect on the efficiency of the energy transfer, which depends primarily on the D-A distances [4].

So, briefly the transfer of electronic energy from one molecule to another generally occurs in one of the following two ways.

- 1- Radiative energy transfer mechanism [5].
- 2- Non-Radiative energy transfer mechanism:
which includes
 - a- Resonance energy transfer [6].
 - b- collision energy transfer [7].

Energy transfer in a mixture of dyes have been established as an effective mechanism for extending the wavelength of lasing, tenability, enhancement of power of the dye laser and low pump power requirement; dye lasers have some limitations as the dye solution used as an active medium absorbs energy from the excitation source in a very limited range and so the emission band also has these limitations. If a dye laser has to be used as an ideal source its spectral region needs to be extended. In order to extend the spectral region of operation mixtures of different dye solutions/dye molecules embedded in solid matrices are being used. The work on energy transfer between different dye molecules in such mixtures in various solvents and solid matrices is, therefore, of great importance. The use of such energy transfer in dye lasers is also helpful in minimizing the photo-quenching effects and thereby, increasing the laser efficiency [8,9].

The energy transfer is manifested by a quenching of donor emission, an increased emission of acceptor excited via the donor, a decrease in donor photobleaching rate, and a decrease in donor lifetime [10]. Figure (1) shows the energy level scheme of the resonant transitions of a donor- acceptor pair [11].

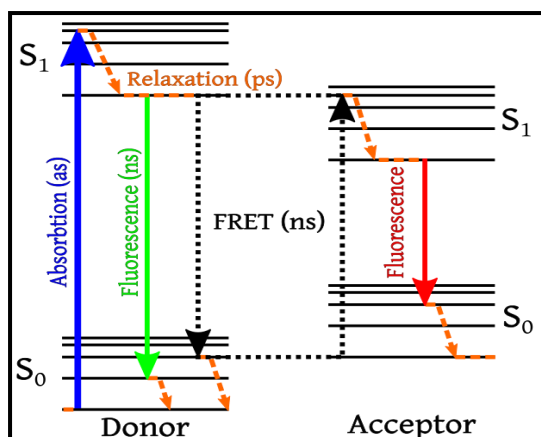


Fig. (1) Energy level scheme of the resonant transitions [11]

Forster has developed a theory for non-radiative energy transfer in terms of the resonance dipole-dipole interaction mechanism, and has shown that rate constant for dipole energy transfer K_{ET} between donor D and acceptor A, is given by

$$K_{ET} = 1.25 \times 10^{17} \frac{\phi_D}{n^4 \tau_D R^6} \int_0^\infty \frac{F_D(v) \epsilon_A(v) dv}{v^4} \quad (1)$$

where ϕ_D is the quantum yield of donor emission, τ_D is the lifetime of the emission, n is the solvent refractive index and R is the distance in nm between D^* and A, $F_D(v)$ is the emission spectrum of the donor, expressed in wave number and normalized to unity and $\epsilon_A(v)$ is the molar excitation coefficient of A at the wave number v [1].

The critical transfer distance (R_0) is generally used to indicate the strength of the interaction between donor and acceptor molecules and by definition, is the distance where the rate constant for energy transfer K_{ET} is equal to the rate constant for fluorescence by donor in the absence of acceptors $1/\tau$. Equation (1) can re-written as:

$$K_{ET} = \frac{1}{\tau_D} \left(\frac{R_0}{R} \right)^6 \quad (2)$$

substituting Eq. (2) into Eq. (1) gives:

$$R_0^6 = 1.25 \times 10^{17} \frac{\phi_D}{n^4} \int_0^\infty \frac{F_D(v) \epsilon_A(v) dv}{v^4} \quad (3)$$

The conditions favoring energy transfer are a large overlap between fluorescence spectrum of the donor and the absorption spectrum of acceptor, a large value of ϵ_A , and a large value of fluorescence quantum yield of donor.

$$\frac{\tau_D}{\tau} = 1 + \tau_D K_{AD} [A] \quad (4)$$

when $\tau = 1/2\tau_M$, $[A]_h = [A]_{1/2}$: half-value concentration, which is given by

$$[A]_{1/2} = 1/K_{SV} \quad (5)$$

where

$$K_{SV} = \tau_D K_{AD} \quad (6)$$

then the critical transfer distance between donor and acceptor is

$$R_0 = \frac{7.35}{\sqrt[3]{[A]_{1/2}}} = 7.35(K_{SV})^{1/3} \quad (7)$$

Resonance transfer is often the dominant mechanism of energy transfer in concentrated

solutions and can occur over intermolecular distance of 10-100 Å [12].

2. Experimental Part

The solutions of acriflavine and rhodamine B dyes of primary concentration of 10^{-3} M were prepared by dissolving the appropriate amounts of these dyes (weighted by Mattler balance of 0.1mg sensitivity) in the solvents. The weight, W , of the dye (in gm) was calculated using the following equation: $W = M_w V[M]/1000$ (8) where M_w is the molecular weight of dye (gm/mole), V is the volume of the solvent (ml), and $[M]$ is dye concentration ($M = \text{mole/l}$). The concentration of each dye was then diluted to get concentrations in the range of 10^{-3} M to 1×10^{-5} M according to:

$$[M_1]V_1 = [M_2]V_2 \quad (9)$$

where, $[M_1]$ is the high concentration, V_1 is the volume before dilution, $[M_2]$ is the low concentration, and V_2 is the total volume after dilution.

The absorption spectra of the prepared samples were recorded using UV-VIS-NIR spectrophotometer (model UV-3600) in UM, covering the wavelengths range (150-2500nm), supplied by SHIMATZU (Japan). Liquid and solid (disk, and thin film) samples can be used with this instrument by changing the sample chamber for each case.

The fluorescence spectra were obtained using two instruments. The first one was constructed with wavelength range of 185-630 nm. Laser-induced fluorescence spectra of the dyes were measured using 405nm, 50mW, CW laser diode as excitation sources (other light sources could also be used). The second instrument was Quanta Master™ 40 Steady State Spectrofluorometer. The emission wavelength range of this instrument is 180-900 nm using CW xenon arc lamp as the excitation source with adjustable slits.

The PTI (Photon Technology international) lifetime instrument was used to measure the fluorescence lifetime for the prepared samples. This instrument includes nanosecond pulsed LED excitation sources in different ranges from 300 to 700 nm, and fast photomultiplier (PMT) in the spectral range 185-900 nm as a detector.

3. Results and Discussion

The energy transfer measurements of ACF dye (as donor) and RhB dye (as acceptor) in liquid and solid phases are presented using 405 nm as excitation wavelength. Two different type of solvent used, ethanol and DMSO as a proton transfer and non-proton transfer solvent, respectively. The study includes the absorption spectra, fluorescence spectra, fluorescence lifetime decay curves in each solvents.

The absorption spectra of mixture of ACF and RhB dyes dissolved in ethanol and DMSO are illustrated figures (2) and (3), respectively.

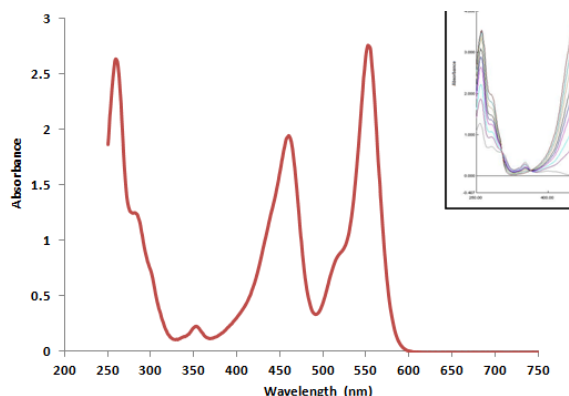


Fig. (2) Absorption spectrum for the mixture of 5×10^{-4} M ACF and 1×10^{-4} M RhB dyes in ethanol. Inset for 5×10^{-4} M ACF with different concentrations of RhB dye

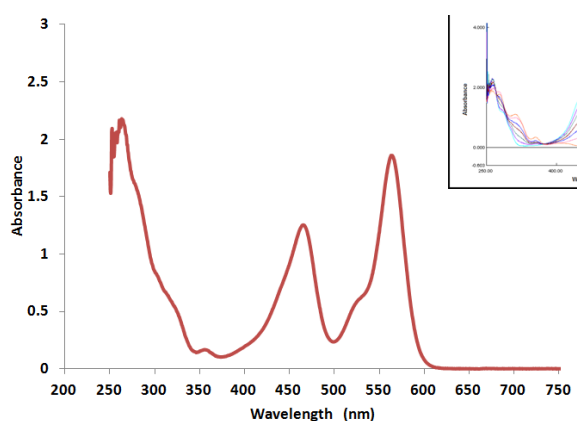


Fig. (3) Absorption spectrum for the mixture of 5×10^{-5} M ACF and 1×10^{-4} M RhB dyes in ethanol. Inset for 5×10^{-5} M ACF with different concentrations of RhB dye

The fluorescence spectra for the ACF+RhB mixture dissolved in ethanol and DMSO were recorded using PTI QM40 instrument taking 405 nm as the excitation wavelength. This wavelength was chosen because the absorption of RhB is negligible at this wavelength. For the energy transfer mechanism, the donor (ACF dye) concentration was fixed at 5×10^{-4} M as it was one of the most efficient concentration in ethanol mixed with different concentrations of the acceptor (RhB dye) in the range of 1×10^{-3} to 1×10^{-6} M. While the ACF concentration was fixed at 5×10^{-5} M in DMSO with different concentrations of RhB dye in the range of 1×10^{-3} to 1×10^{-5} M. Figures (4) and (5) show the fluorescence spectra for the mixtures in ethanol and in DMSO, respectively.

As one can see from figures (4) and (5) that the fluorescence intensity of ACF decreases as the concentration of RhB increases, while that of RhB increases. This is because of the energy transfer from ACF to RhB.

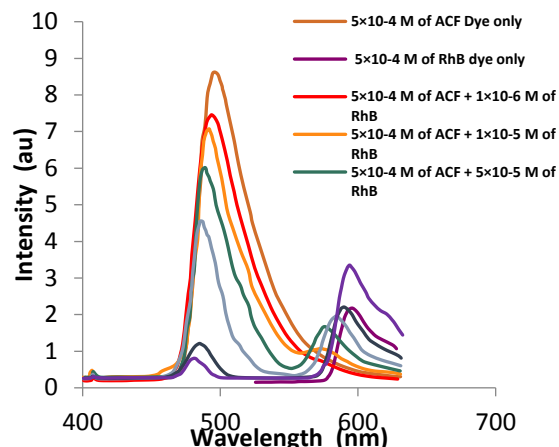


Fig. (4) Fluorescence spectra of ACF+RhB in ethanol for 5×10^{-4} M of ACF with different concentrations of RhB

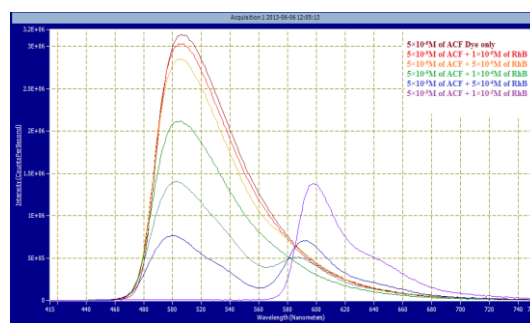


Fig. (5) Fluorescence spectrum of ACF+RhB in DMSO for 5×10^{-5} M of ACF with different concentrations of RhB

The parameters of fluorescence energy transfer that can be calculated from the fluorescence spectrum of the mixture of two dyes includes Stern-Volmer constant K_{SV} , half-value concentration $[A]_h$, efficiency of energy transfer F_{AD} , critical transfer distance R_0 , and energy transfer rate parameter K_{AD} .

The parameters of above energy transfer between donor and acceptor for the mixture of ACF and RhB dyes were determined by measuring the quenching of donor fluorescence, or reduction of fluorescence lifetime. The former was applied in the present work. Since the fluorescence quantum yield of donor ϕ_{AD} is proportional to the peak of fluorescence intensity I , from the Stern-Volmer relation for energy transfer, we have

$$\frac{I_0}{I} = 1 + K_{SV}[A] \quad (10)$$

where I_0 and I are the peak fluorescence intensities of donor without and with acceptor, respectively

Figure (6) shows the Stern-Volmer plot of I_0/I versus the acceptor (RhB) concentration $[A]$, for ACF-RhB mixtures in ethanol and DMSO.

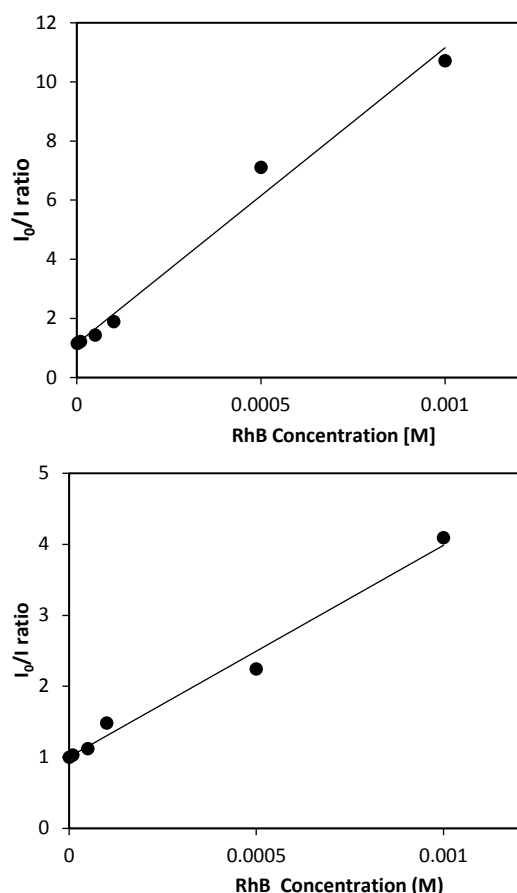


Fig. (6) The I_0/I ratio of ACF+RhB dye mixture as a function of the concentration of RhB dye in 5×10^{-4} M ethanol (upper), and in 5×10^{-4} M DMSO (lower)

Hence, the values of Stern-Volmer constant K_{SV} were calculated from the slope of best fit straight lines in Fig. (6). These values are listed in table (1). The values of $[A]_h$, R_0 , F_{AD} , and K_{AD} [calculated from Eqs. (4)–(7)] are listed in table (1) too.

The energy transfer rate parameter K_{AD} was calculated from Eq. (6), using the measured values τ_D , which is the lifetime of the donor (ACF) alone without the acceptor (RhB). The lifetime values for 5×10^{-4} M of ACF were measured using PTI life instrument to obtain $\tau_D = 4.279$ ns, giving $K_{AD} = 2.115 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$ in ethanol, and $\tau_D = 2.753$ ns giving $K_{AD} = 1.0825 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$ in DMSO.

The total energy transfer efficiencies (F_{AD}) for the two solutions of ACF+RhB system were calculated at different concentrations. The plots are as shown in Fig. (7). The shape of these plots is exponential in nature and the values in the former case (ACF+RhB in ethanol) are at little higher side as compared to the latter one in DMSO. Theoretical calculations and the results from these considerations indicate that:

(i) At very low concentration range, $[A] < 2 \times 10^{-4}$ M, the radiative transfer is the dominant mechanism. At these concentrations, simultaneous photon emissions at two separate spectral regions are expected.

(ii) In the range of concentration in which photon emissions is possible only in acceptor band spectrum, $[A] > 2 \times 10^{-4}$ M, these relation show that both radiative and non-radiative energy transfer mechanism play important role in both cases.

Table (1) Parameters of fluorescence energy transfer for ACF-RhB mixtures in ethanol and DMSO

Acriflavine dye concentration					
5×10^{-4} M in ethanol			5×10^{-5} M in DMSO		
K_{SV} (M^{-1})	$[A]_h$ (M)	R_0 (Å)	K_{SV} (M^{-1})	$[A]_h$ (M)	R_0 (Å)
10.006×10^3	9.99×10^{-5}	158.405	2.980×10^3	3.3×10^{-4}	106.361
$[RhB]$ (M)	F_{AD} in ethanol		$[RhB]$ (M)	F_{AD} in DMSO	
1×10^{-6}	0.0099		1×10^{-5}	0.02894	
5×10^{-6}	0.0476		5×10^{-5}	0.1297	
1×10^{-5}	0.0909		1×10^{-4}	0.2296	
5×10^{-5}	0.333		5×10^{-4}	0.5984	
1×10^{-4}	0.5		1×10^{-3}	0.7487	
5×10^{-4}	0.8334		----	----	
1×10^{-3}	0.909		----	----	
τ_D of ACF only ns	K_{AD} in ethanol $\text{M}^{-1}\text{s}^{-1}$		τ_D of ACF only ns	K_{AD} in DMSO $\text{M}^{-1}\text{s}^{-1}$	
4.279	2.115×10^{12}		2.753	1.0825×10^{12}	

The critical transfer distance R_0 corresponds to an average distance of the acceptor molecule in a sphere with radius R_0 having the excited donor molecule at the center. The values of R_0 are larger as compared to that of collisional transfer (the R_0 values for collisional transfer are reported to be in the range of 1–10 Å [13]), especially in ethanol solution is larger as compared to that of DMSO solution. So to avoid the Dexter-type transfer, the distance between donor and acceptor should be $> 10 \text{ Å}$.

Furthermore, the values of K_{AD} in the present work [$\sim 10^{12} \text{ M}^{-1}\text{s}^{-1}$] are much larger as compared to that of radiative energy transfer [$\sim 10^9 \text{ M}^{-1}\text{s}^{-1}$] or to collisional transfer K_{diff} [$\sim 10^9$ – $10^{10} \text{ M}^{-1}\text{s}^{-1}$], where for example K_{diff} is given by

$$K_{diff} = 8 RT/3000 \eta \quad (11)$$

where R is the gas constant, T is the temperature of the solution, and η is its viscosity. For example $K_{diff} = 5.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ for ethanol solution at room temperature [14]

This indicates that the resonance energy transfer is the dominant mechanism for energy transfer.

The achieved results from figures (6) and (7), and table (1) indicate that the energy transfer efficiency increases with increase of the acceptor (RhB) concentration at a fixed donor (ACF) concentration, and approaches unity at high concentration, leading to leveling off the energy transfer mechanism or in other words it becomes the dominant mechanism. This is in agreement with the obtained results of other donor acceptor pairs [13,15].

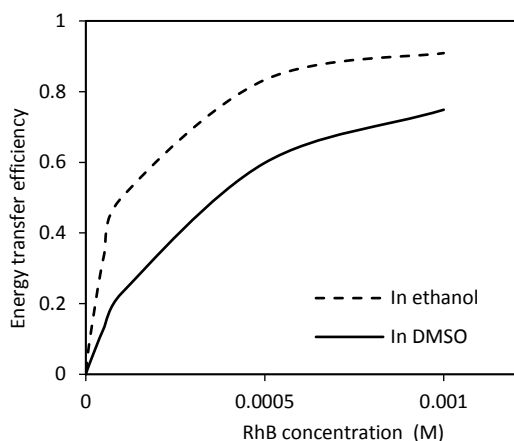


Fig. (7) Energy transfer efficiency of ACF+RhB mixture as a function of RhB concentration in ethanol and DMSO

The energy transfer represent one of the parameters used to reduce the photobleaching of acceptor dye, as it is known by increasing the bleaching time, the magnitude of absorption features in the visible region of the spectrum decreases and the rate of decreases depend on the wavelength of bleaching, shorter wavelengths leading to increase the bleaching rate [16,17]. So according to this, one of the important parameters that used to decrease the influence of photobleaching via energy transfer by use the mixture of two dyes as a donor and acceptor.

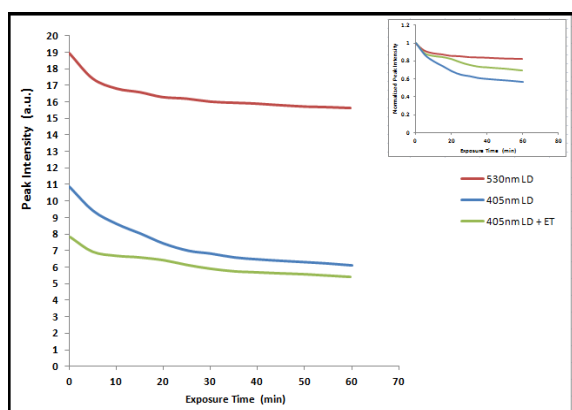


Fig. (8) Peak fluorescence intensity vs. time of exposure for disks of RhB and ACF+RhB mixture using 405nm and 530nm LDs. Inset is the normalized decay

The energy emitted by ACF dye only is absorbed by the RhB dye to get their characteristic emission. In this mechanism the RhB dye excited by longer wavelength than that 405 nm LD leading to decrease of bleaching rate. The continuous exposure for the disk sample of RhB dye doped PMMA to each of 405 nm and 530 nm LDs of 1mW output power for one hour and record the decay curve for each case and by record the decay curve for a disk sample combine of ACF and RhB dyes for 405nm LD, as shown in Fig. (8). The achieved result gives an indication of the energy transfer reduce the bleaching rate of RhB dye from 43.8 to 30.6%. The acceptor photobleaching

technique can be employed to measuring FRET in different fields for example in biomedical field with cell surface protein label; it depends on the increase in donor fluorescence after complete acceptor photobleaching represent a measured of the FRET efficiency.

4. Conclusions

In concluding remarks, the fluorescence spectra of a mixture of two laser dyes; acriflavine and rhodamine B in different solvents; ethanol and DMSO, were recorded and studied. The energy transfer efficiency of the prepared samples was determined and studied by comparing some parameters such as the critical transfer distance, the emission lifetime, and the rate constant for energy transfer at different concentrations of these solutions. The emission lifetime of the dye mixture in DMSO was shorter than that in ethanol by 35.6%.

References

- [1] H. Stegemeyer, "Book Review: Photoluminescence of Solutions. By C. A. Parker", *Angewandte Chemie International Edition in English*, 8(12),1969.
- [2] R. Ghazy et al., "Experimental Investigations on Energy-Transfer Characteristics and Performance of Some Laser Dye Mixtures", *Opt. Laser Technol.*, 34(2) (2002).
- [3] M. Isaksson et al., "On the Quantitative Molecular Analysis of Electronic Energy Transfer within Donor-Acceptor Pairs", *Phys. Chem. Chem. Phys.*, 9(16) (2007).
- [4] M.F.a.K. Mito, "Solid-State Dye Laser with Photo-Induced Distributed Feedback", *Jpn J. Appl. Phys.*, 39(10) Pt 1 ((2000) 5859.
- [5] J.B. Birks, "Photophysics of Aromatic Molecules", *Wiesly Interscience* (London, 1973).
- [6] J.A. Barltrop and J.D. Coyle, "Excited States in Organic Chemistry", *John-Wiley & Sons* (London, 1975).
- [7] T. Mitsugu and O. Yujiro, "Energy Transfer Mechanism and Amplified Spontaneous Emission Characteristics of Dye Mixture Solutions", *Jpn J. Appl. Phys.*, 22(9) (1983) 1392.
- [8] R.D. Singh et al., "Time-Resolved Spectra of Coumarin 30-Rhodamine 6g Dye Mixture", *Pramana – J. Phys.*, 34(1) (1990).
- [9] A.R.N. Sessa Bamini and V.S. Gowri, "Effect of Different Donors and a Polymer Environment on Photophysical and Energy Transfer Studies Using C540 as the Acceptor", *Pramana – J. Phys.*, 79(6) (2012) 1503.
- [10] E.M. Graham, "The Application of Fluorescence Lifetime Imaging Microscopy to Quantitatively Map Mixing and Temperature in Microfluidic Systems", PhD thesis, University of Edinburgh, 2007.
- [11] P.I.H. Bastiaens and T.M. Jovin, "Fluorescence Resonance Energy Transfer (FRET)

Microscopy in Cell Biology". 2nd ed., Academic Press (NY, 1998).

[12] S.R. Bobbara, "Energy Transfer between Molecules in the Vicinity of Metal Nanoparticle", MSc thesis, Queen's University, 2011.

[13] Z.F.M. Al-Bawi, MSc thesis, University of Technology, 1997.

[14] Z.S. Sadik, "Thin Film Energy Transfer Dye Laser", PhD thesis, Baghdad University, 2002.

[15] C. Vijila and A. Ramalingam, "Photophysical Characteristics of Coumarin 485 Dye

Doped Poly(Methyl Methacrylate) Modified with Various Additives", J. Mater. Chem., 11(3) (2001).

[16] Ed. McKenna et al., "Kinetic Model of Irreversible Photobleaching of Dye-Doped Polymer Waveguide Materials", J. Opt. Soc. Am. B, 21(7) (2004) 1294.

[17] D. Tomic and A.R. Mickelson, "Photobleaching for Waveguide Formation in a Guest-Host Polyimide", Appl. Opt., 38 (1999) 3893.
