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*M.N. Gharge, S. L. Bhattar, G. B. Kolekar and S. R. Patil*
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Fluorescence resonance energy transfer between perylene and riboflavin in micellar solution and analytical application on determination of vitamin B$_2$

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Abstract

Fluorescence resonance energy transfer (FRET) between perylene and riboflavin is studied in micellar solution of sodium dodecyl sulfate. The fluorescence of perylene is quenched by riboflavin and quenching is in accordance with Stern-Volmer relation. The efficiency of energy transfer is found to depend on the concentration of riboflavin. The value of critical energy transfer distance ($R_0$) calculated by using Foster relation is 32.13 Å, and as it is less than 50 Å, it indicates efficient energy transfer in the present system. The analytical relation was established between extent of sensitization and concentration of riboflavin, which helped to estimate vitamin B$_2$ directly from pharmaceutical tablets.

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Keywords: Fluorescence resonance energy transfer (FRET); Fluorescence quenching; Perylene; Riboflavin (vitamin B$_2$)

1. Introduction

Fluorescence resonance energy transfers (FRETs) is a nonradiative process whereby an excited state donor (D) transfers energy to a ground state acceptor (A). FRET mainly occurs over distances comparable to most biological macromolecules i.e. about 10–100 Å. The rate of energy transfer is highly dependent on the extent of spectral overlap between the relative orientation of the transition dipoles and the distance between the donor and acceptor molecules. It is a powerful technique for studying conformational distribution and dynamics of biological molecules such as DNA [1], protein [2] etc., which play a key role in maintaining human health. Donor–acceptor dye combination has tremendous impact in nucleic acid analysis [3,4]. Vitamin B$_2$ is one of the most important biological molecules and an essential nutritional compound in the human diet. It is water soluble and its fluorescence band is observed in the range 475–600 nm with maximum at 523 nm. Earlier methods used for its determination involved chromatographic separation followed by spectrophotometry and fluorometry analysis. Fluorescent poly-nuclear aromatic hydrocarbons such as perylene, anthracene, etc. are known as efficient donors of energy in organic solvents [5]. We have observed a strong overlap between the emission spectrum of perylene dye and the absorption spectrum of riboflavin, and hoped for an efficient FRET process between perylene (donor) and riboflavin (acceptor). The water insoluble perylene can be solublized in non-fluorescent micellar solution [6]. The micelles have attracted significant attention because of their ability to function as encapsulating systems by providing a high-viscosity microenvironment. In the present studies a simple analytical method is developed to estimate vitamin B$_2$ from medicinal tablets [7,8]. The technique is more selective, sensitive and reproducible.
2. Experimental

2.1. Chemicals

Perylene (Merck-Schuchardt) and Riboflavin (Loba Chemie) were used after purity testing by production of similar spectra when excited at different wavelengths. Sodium dodecyl sulfate (SDS) procured from S.D. Fine Chem. Limited, Bombay, was used as received. The critical micelle concentration (CMC) of SDS is $8.1 \times 10^{-3}$ M [9]. Double distilled water was used to prepare all solutions.

2.2. Preparation of solutions

The concentration of SDS solution used in quenching experiments was $3 \times 10^{-2}$ and $5 \times 10^{-2}$ M. The perylene solution in SDS was prepared by dissolving the required quantity in SDS solution and stirring overnight. The concentration of perylene was kept constant at $2 \times 10^{-6}$ M, which corresponds to its monomer emission. The stock solution of riboflavin of $1 \times 10^{-4}$ M concentration was prepared in SDS. The concentration of riboflavin was varied from $1 \times 10^{-6}$ to $9 \times 10^{-6}$ M in the quenching experiments. The solutions were deaerated before recording their fluorescence spectra.

2.3. Recording of fluorescence spectra

The fluorescence spectra and fluorescence excitation spectra of the solutions were recorded on a PC-based Spectrofluorophotometer JASCO Model FP-750, Japan.

3. Result and discussion

3.1. Fluorescence quenching studies

The excitation and fluorescence spectra of riboflavin and the fluorescence spectrum of perylene in SDS solution are presented in Fig. 1. The excitation spectrum shows two emissions bands, one in the region 300–400 nm and the other in the region 400–500 nm. The perylene dissolved in SDS micellar solution was found to exhibit sufficient fluorescence intensity, which can be absorbed by riboflavin because of the large integral overlap seen between emission spectrum of perylene and excitation spectrum of riboflavin in Fig. 1. The large region of overlap between perylene and riboflavin is an indication of efficient energy transfer between the donor–acceptor pair [10,11]. The region of integral overlap is used further to calculate the critical energy transfer distance ($R_0$) between perylene (donor) and riboflavin (acceptor) using Förster relation [12,13].

$$
R_0^6 = \frac{9000 K^2 \Phi_D \ln 10}{128 \pi^2 \sigma^4 N_A v^4} \int \frac{f(V) E(V) dV}{\nu^4},
$$

where $K^2$ is the orientation factor, $\Phi_D$ the fluorescence quantum efficiency of donor, $\nu$ the refractive index of the materials, $N_A$ Avogadro’s number and $f(V) E(V) dV$ is an integral overlap area.

The estimated value of $R_0$ is 32.13 Å. The values of $R_0$ less than $\approx 50$ Å are an indication of efficient energy transfer between the donor–acceptor pair [14].

It is observed that the riboflavin fluorescence in solution of SDS is weak, but when placed in the SDS solution of perylene, the fluorescence intensity of riboflavin increased markedly and that of perylene decreased. Fig. 2 shows the quenching of perylene fluorescence as a function of riboflavin concentration. It is interesting to note that the

![Fig. 1. Excitation spectra of riboflavin (A), emission spectra of perylene (B), emission spectra of riboflavin(C) in SDS solution. Shaded line shows region of overlap between the fluorescence spectrum of perylene and the excitation spectrum of riboflavin (D).](image1)

![Fig. 2. Fluorescence spectra of perylene in the absence and presence of varying concentrations of riboflavin in SDS micellar solution. (a–i) $0.15 \times 10^{-6}$–$13 \times 10^{-6}$ M and (j) without riboflavin.](image2)
3.2. Kinetics of quenching of perylene fluorescence

The quenching of perylene fluorescence was studied using the Stern–Volmer relation. Fig. 3 shows a plot of $F_0/F$ vs concentration of riboflavin where $F$ and $F_0$ are the intensities of fluorescence of perylene with and without riboflavin. The graph is a straight line with intercept having value one on $y$-axis and indicates validity of the Stern–Volmer relation \[15\]. The quenching rate constant $k_q$ was calculated from slope of the graph using the lifetime of perylene in SDS without riboflavin. Where

$$K_{sv} = \tau K_q,$$

(2)

where $K_q$ is the rate constant, $\tau$ is lifetime of perylene without riboflavin, and $K_{sv}$ is slope

The estimated values of $K_q$ given in Table 1 are of the order of $10^{-12} \text{M}^{-1}\text{s}^{-1}$. The values of quenching rate constant reported for donor–acceptor pairs in homogenous aqueous and nonaqueous medium are mostly of the order of $10^{-9} \text{M}^{-1}\text{s}^{-1}$ \[16,17\]. In comparison, the quenching rate constant observed for the present system in SDS micelle solutions are of a higher order. This observation is in support of efficient energy transfer from perylene to riboflavin in micelle environment. The efficiency of the energy transfer is calculated by using the relation \[18\]

$$\eta = 1 - F_d/F_0,$$

(3)

where $F_d$ is the fluorescent intensity of donor in the presence of acceptor, $F_0$ is fluorescent intensity of donor without acceptor and the values estimated as a function of riboflavin concentration are given in Table 1. The values indicate that the efficiency of energy transfer increase with riboflavin concentration.

3.3. Effect of SDS

The quenching experiments were performed in SDS solution of two different concentrations values of above CMC. The critical energy transfer distance ($R_0$) and efficiency of energy transfer were calculated and are given in Table 1. It is seen that the critical energy transfer distance ($R_0$) and quenching rate constant ($k_q$) are higher in higher concentrations of SDS. The literature on micellar solution reveals that the spherical shape of micelle in dilute solution elongates more with concentration of surfactant and size of micelle increases \[9\]. Because of this, the critical energy transfer distance ($R_0$) is found to be higher in higher concentrations of SDS. At lower SDS concentrations, it is unlikely that more than molecule will bind per micelle. Multiple occupancy may occur at higher concentration, thereby increasing the intermolecular interactions \[19\]. In addition to this the increase in concentration of SDS results in increased probability of distribution of perylene molecules in micelle owing to micellar proximity effect.
3.4. Stability of the system

The present donor–acceptor fluorescent system is photostable for a period of 1 h.

3.5. Effect of foreign substances and method of analysis

The effect of foreign substances on the fluorescence determination of riboflavin with the proposed method was investigated. Initially, substances foreign to the riboflavin solution are taken in large excess and the fluorescence intensities are measured. When interference was found to be intensive, the tests were repeated with successive smaller amounts of foreign substances. According to the proposed system the metal ions have small effect on the determination of vitamin B2. The tolerance limits for the ions are given in the Table 2.

3.6. Analysis of pharmaceutical samples

The quenching method was applied for the determination of riboflavin in pharmaceutical samples namely Polybion, Beplex forte tablets. The tablets were dissolved in minimum quantity of alcohol and made up of required volume with SDS micellar solution. The results are in good agreement with the certified value of the composition of vitamin B2. The values are reported in Table 3.

4. Conclusions

The FRET studies between perylene and riboflavin in a micellar environment are more efficient and not probable in water because of insolubility of perylene. The critical energy transfer distance values estimated for the present donor–acceptor pair is $32 \AA$ and supported the observed energy transfer. The process results in to quenching of perylene fluorescence and sensitization of riboflavin fluorescence. The kinetic studies showed the validity of the Stern–Volmer relation. The efficiency of energy transfer was found to increase with the concentration of riboflavin. The analytical method based on FRET is developed and used for estimation of riboflavin (vitamin B2) from pharmaceutical tablets. The close agreement between observed values of vitamin B2 and certified values in the samples prove the suitability of the method for analysis of vitamin B2. The proposed method is simple, sensitive and quick.

References


Table 3

<table>
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<tr>
<th>Sample</th>
<th>Composition</th>
<th>Amount of riboflavin</th>
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</thead>
<tbody>
<tr>
<td>Polybion (Merck Limited, Hydrabad)</td>
<td>Thiamine mononitrate IP 10mg</td>
<td>10.00 mg (per tablet)</td>
</tr>
<tr>
<td></td>
<td>Pyridoxine HCI IP 3mg</td>
<td>9.890 mg (per tablet)</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid IP 150mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nicotinamide IP 100mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyanocobamine 15mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium pantothenate 50mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folic acid IP 1.5 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biotin USP 100mcg</td>
<td></td>
</tr>
<tr>
<td>Beplex Forte (Anglo-French drugs,Banglore)</td>
<td>Thiamine mononitrate IP 10mg</td>
<td>10.00 mg (per tablet)</td>
</tr>
<tr>
<td></td>
<td>Nicotinamide 75mg</td>
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</tr>
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</tr>
<tr>
<td></td>
<td>Pyridoxine HCI IP 3mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium pantothenate 50mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folic acid IP 1.5 mg</td>
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</tr>
<tr>
<td></td>
<td>Vitamin B12 IP 15mcg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin C IP 50mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biotin USP 260mcg</td>
<td></td>
</tr>
</tbody>
</table>

*Average of three determinations
Spectroscopic studies on the molecular interaction between salicylic acid and riboflavin (B₂) in micellar solution

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Fluorescence quenching
Salicylic acid
Riboflavin
Binding constant and fluorescence resonance energy transfer

A B S T R A C T
The interaction between salicylic acid (SA) and riboflavin (RF) was studied by Fluorescence Resonance Energy Transfer (FRET) in micellar solution. The riboflavin strongly quenches the intrinsic fluorescence of SA by radiative energy transfer. The extent of energy transfer in sodium dodecyl sulphate (SDS) micellar solution of different concentration is quantified from the energy transfer efficiency data. It is seen that the energy transfer is more efficient in the micellar solution. The critical energy transfer distance (R₀) was determined from which the mean distance between SA and RF molecules was calculated. The quenching was found to fit into Stern–Volmer relation. The results on variation of Stern–Volmer constant (Ksv) with quencher concentration obtained at different temperatures suggested the formation of complex between SA and RF. The association constant of complex formation was estimated and found to decrease with temperature. The values of thermodynamic parameters ΔH, ΔG and ΔS at different temperatures were estimated and the results indicated that the molecular interaction between SA and RF is electrostatic in nature.

1. Introduction

Salicylic acid (SA) is a key ingredient in many skin care products for the treatment of acne, psoriasis, calluses, corns and warts due to its antimicrobial activity [1,2]. It works by causing the cells of epidermis to slough off more readily preventing pores from clogging up and allowing room for new cell growth. Because of its effect on skin cells, salicylic acid is used in several shampoos to treat dandruff and in sun screen lotions as a topical therapeutic to protect the skin from sun damage [3–5]. Many of the beauty cosmetics available commercially for facial skin uses food ingredients such as vitamin to enrich the effectiveness of decreased surface roughness and reduction of fine lines. Vitamin E and vitamin B₂ (Riboflavin) are widely used biological compounds as their deficiency causes eye lesions, skin disorders and cellular growth [6,7]. However, the possible interaction between antimicrobial drug and vitamin included in combination may alter the pharmacokinetic and pharmacodynamic characteristics of individual components. The satisfactory explanation of dermatological action of salicylic acid requires understanding of its possible interaction with vitamin included in sunscreen lotion. Salicylic acid and riboflavin are readily absorbed when applied on the skin, penetrate through the stratum corneum and the epidermis to reach the melanocytes. Fluorescence and UV–vis absorption spectroscopy are the powerful tools to study the molecular interactions because of photosensitivity and sun-screening activity of salicylic acid for UVB type radiation (280–320 nm). Our observation of significant overlap of absorption spectrum of riboflavin with fluorescence spectrum of salicylic acid led us to use the fluorescence resonance energy transfer (FRET) technique for probing intermolecular interaction [8]. The studies on stacking interaction between SA and RF reported in aqueous medium limit the fluorescence performance of SA [9]. Many of the skin care lotions use surfactants for smoothing and uniform spreading actions on skin. The present work involves FRET studies between SA and RF in micellar medium to understand possible binding interaction with a view to propose photophysical mechanism for protective effect of SA in preventing facial skin from sun damage. The additional benefit of using surfactant solution is that the micelle formed in water reduces the donor–acceptor distance significantly and improves the FRET efficiency [10].

2. Experimental

Riboflavin from Loba-Chemie AR grade, salicylic acid from Merck, Schuchart were used without additional purification. However spectral purity of both compounds were tested by taking their melting points and production of similar spectrum when excited at different wavelengths. Sodium dodecyl sulphate (SDS) was purchased from SD Fine-Chem Ltd., India. Pure distilled
water was used for preparation of solutions. The critical micelle concentration (CMC) of SDS is $8.1 \times 10^{-3}$ mol dm$^{-3}$ [11] and hence its $1 \times 10^{-2}$ mol dm$^{-3}$ solution was used as solvent to prepare solutions in the present studies.

2.1. Preparation of solutions

The concentration of SA used for the fluorescence quenching experiment was $1 \times 10^{-4}$ mol dm$^{-3}$ while that of RF was varied from $0.5 \times 10^{-5}$ to $3.5 \times 10^{-5}$ mol dm$^{-3}$. All solutions were de-aerated prior to their optical measurements by purging pure N$_2$ gas for 10 min.

2.2. Spectral measurements

The UV–vis absorption spectra of the SA solution in presence and absence of RF were recorded using Shimadzu, spectrophotometer with 1 cm quartz cuvette. The fluorescence spectra were recorded on a Spectro-Fluorometer JASCO, Japan, FP-750. The excitation and emission slit width used during spectral measurements were 10 nm. The life time of salicylic acid in absence of riboflavin was obtained from decay curve recorded on Time Resolved Single Photon Counting System (TRSPCS) (Horiba, Japan). The system consists of laser light emitting diodes (LED) as the excitation source and instrument performance is checked with standard sample. The UVLED of 310 nm is used for excitation of the excitation source and instrument performance is checked with standard sample. The fluorescence quenching experiments were repeated at different temperatures by keeping solutions in thermostat before spectral measurements.

3. Results and discussion

3.1. Absorption studies

The absorption spectrum of SA in micellar solution measured without RF and with RF of different amounts is shown in Fig. 1. The absorption spectrum of salicylic acid shows sharp band peaking at 294 nm. The variation of RF in fixed concentrations of SA in micellar solution shows that the presence of RF in small concentration increases the absorbance of SA and the UV bands of RF expected to occur at 220 and 275 nm are not seen in the spectra. The increase in absorbance of SA may be because of its removal from micelle as RF being more hydrophilic in nature [12]. The higher concentration of RF removes SA peak gradually and all four bands of RF become prominent as seen in Fig. 1. The isobestic point and spectral modification are not seen in the range of concentration studied. These observations are in contrast to those reported for solution of fixed concentration of RF in water whose absorbance was decreased by addition of salicylate ion in successively more amounts [9]. The intermolecular interactions are shown to occur in aqueous solution between RF and salicylate ion from the appearance of isobestic points. However increase in absorbance of SA and absence of isobestic are the indications of ground state molecular interactions between SA and RF in micellar solution.

3.2. Mechanism of fluorescence quenching

The fluorescence of salicylic acid generated by excitation wavelength of 310 nm corresponding to its excitation energy is intense and appears as sharp band with maximum intensity at 410 nm. The presence of riboflavin in this solution quenches the fluorescence of salicylic acid monitored at excitation wavelength 310 nm. At this wavelength riboflavin has negligible fluorescence because its absorption corresponds to radiation of 450 nm wavelength. Fig. 2 shows that the fluorescence of SA is quenched successively by increasing addition of riboflavin solution and fluorescence of RF appeared at 520 nm seen to be enhanced considerably at the same time. An isoemissive point is seen at 490 nm. From these observations it is inferred that there was transfer of energy and interactions between SA and RF as both molecules are brought closer in micellar environment by lowering the surface tensions [13]. The quenching experiments were also performed under similar experimental conditions in water and in SDS solutions of concentration below and above critical micelle concentration (CMC). The SA molecules excited selectively to its first excited singlet state transfer the energy to RF molecule because the excitation spectrum of RF shows strong overlap with fluorescence spectrum of SA presented in Fig. 3. The extent of energy transfer in different environment is quantified from the energy transfer efficiency ($E$) data estimated by using the following expression:

$$E = 1 - \left( \frac{F}{F_0} \right)$$

where $F$ and $F_0$ are the fluorescence intensity of SA in presence and absence of RF, respectively. The values of $E$ were calculated at different RF concentrations and presented graphically in Fig. 4. Careful observation of Fig. 4 indicates that the $E$ values increase.
with RF concentrations. It is seen that at every concentration of RF the efficiency of energy transfer is more in SDS solution of concentration above its CMC. The observed value of $E$ in SDS below CMC are actually less than those of water because the presence of surfactant monomer reduces the fluorescence of SA and consequently FRET efficiency. The efficiency of energy transfer also depends upon critical transfer distance ($R_0$) between SA and RF molecules, which is estimated in solution by using Förster relation [14]:

$$R_0 = \frac{9000 \left( \ln 10 \right) k^2 \Phi_f}{128 \pi^2 n^4 \varepsilon}$$  

(2)

where $k^2$ ($2/3$) is a factor describing orientation in space of transition dipole of the donor and acceptor, $n$ the refractive index of medium (1.334), $N$ the Avagadro’s number and $\Phi_f$ the fluorescence quantum yield (0.709) of SA in absence of RF [15]. The overlap integral region $J$ expresses the degree of spectral overlap between the SA emission and excitation spectrum of RF as

$$J = \int \frac{I(\nu) \psi(\nu)}{c^4} d\nu$$

The value of $J$ is evaluated by integrating the spectrum from Fig. 3. The value of $R_0$ obtained from above Eq. (3) is 45.68 Å and used further to calculate the distance between SA and RF molecule ($r$) by using the relation

$$E = \frac{R_0^6}{R_0^6 \kappa r^6}$$  

(3)

The value of $r$ found to be 5.8 Å. This value is too far to transfer electron from SA to RF molecule but satisfies the conditions of fluorescence resonance energy transfer [16]. Therefore the fluorescence quenching of SA by RF is mainly due to fluorescence resonance energy transfer from SA to RF and not by electron transfer.

The nature of fluorescence quenching of SA may be dynamic or static. The fluorescence quenching experiments were repeated at different temperatures. The simple method of Stern–Volmer approach is used for modeling the quenching phenomenon and complex formation by molecular interaction in the excited state

$$\frac{F_0}{F} = 1 + K_{sv} Q_{RF}$$  

(4)

where $K_{sv}$ is the Stern–Volmer quenching constant and $Q_{RF}$ concentrations of RF. The plot of $F_0/F$ versus $Q_{RF}$ shown in Fig. 5 at different temperatures are straight lines and indicates validity of Stern–Volmer equation. Therefore initially the SA fluorescence by RF in micellar solution assumed to be dynamic. The Stern–Volmer quenching constant $K_{sv}$ is obtained from slope of the lines from Fig. 5. The $K_{sv}$ values increases with increase in temperature. Similarly the quenching constant ($k_q$) was calculated from $K_{sv}$ values by using the following relation:

$$k_q = \frac{K_{sv}}{\tau}$$  

(5)

where $\tau$ is the life time SA in micellar solution without RF and is obtained from its Time Resolved decay curve shown in Fig. 6. The curve is a single exponential and gives the life time 0.9055 ns. However, its value measured in absence of SDS micelle is 4.2 ns [17]. The values of $k_q$ calculated from Eq. (5) are listed in Table 1 and seen to increase with temperature.

### 3.3. Determination of complex formation

The $K_{sv}$ values of SA fluorescence were also calculated at different RF concentrations for all sets of experiments by using Eq. (4). The plots of $K_{sv}$ versus concentration of RF at different constant temperatures 303.15, 313.15 and 323.15 K are shown in Fig. 7. It is seen that $K_{sv}$ decreases with RF concentrations and reaches to a plateau for higher concentrations. The observed variation of $K_{sv}$ at all temperatures as a function of RF concentration also evidences the complex formation between SA.
and RF. From the quenching data the association constant \( K \) of complex was calculated at different temperatures by using the following relation [18]:

\[
\frac{F_0}{F} = K_0 \left( \frac{F_0}{R} - F_0 \right) + \frac{1}{K_0 (F_0 - F_0)}
\]

where \( F_0 \), \( F \), and \( F_0 \) are the fluorescence intensity of SA solution, a solution totally complexed with RF and the experimental solutions containing RF, respectively. \( DF \) is the difference between the fluorescence intensities of SA solutions and that of mixture containing same amount of SA and varying excess amount of RF concentration \( (C_R)^0 \). The plot \( F_0/(DF) \) versus \( (C_R)^0 \) for different temperatures are given in Fig. 8. The plots are straight lines with intercept as per the expectation with linear regression \((r^2)\) values very close to one. The values of association constant of complex \( K \) were given by the ratio of intercept and slope. The \( K \) values listed in Table 2 indicate that at room temperature association constant is of the order of \( 1.176 \times 10^{-4} \) mol \(^{-1} \) dm \(^3\) and decreases with increase in temperature for flavin substrate system in a nonaqueous medium due to hydrogen bonding interaction [18]. The association constant reported by Datta et al. [9] for fluorescence quenching studies of RF by salicylate ion in water solution is low (of the order of \( 45–55 \) mol dm \(^{-3}\)) due to water interaction. The comparison of \( K \) values SA–RF system in micellar solution and water solution allowed us to consider strong intermolecular interactions in SDS micellar solution to form complex.

### 3.4. Thermodynamic parameters and nature of binding force

The dependence of binding constant on temperature, which evidences the interactions between SA and RF to complex formation is a thermodynamic process. The interaction may involve hydrogen bonding, van der Waal forces, electrostatic interaction or hydrophobic interactions [19]. The possible interaction is confirmed by determining parameters such as enthalpy change \((\Delta H)\), entropy change \((\Delta S)\) and free energy change \((\Delta G)\). The temperature dependence of binding constant showed in Fig. 9 indicates the validity of the van’t Hoff equation,

\[
\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}
\]

\[
\Delta G = \Delta H - T\Delta S = RT \ln K
\]

The results obtained on the thermodynamic parameters are given in Table 2. It is seen that \( \Delta G \) and \( \Delta H \) are negative and \( \Delta S \) is positive. This is in form of formation of complex by spontaneous thermodynamic mode. The entropy change \((\Delta S)\) has very little contribution, almost zero while the \( \Delta G \) factor is positive. This is taken as evidence to confirm the interaction between SA and RF by electrostatic force [20,21]. The donor to acceptor distance \( r_0 = 5.8 \) Å estimated indicates that the proton transfer from excited state in micellar solution is more probable as the values less than \( 10 \) Å favor the charge transfer [22]. The formation of complex by hydrogen bonding is shown to occur in nonaqueous medium only by association of the molecule [23]. However in aqueous medium

---

**Table 1**

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Temperature K</th>
<th>( r^2 )</th>
<th>( K_0 \times 10^{-4} ) mol (^{-1} )</th>
<th>( k_0 \times 10^{-13} ) L mol (^{-1} ) ( \cdot ) s (^{-1} )</th>
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<td>5.273</td>
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<td>8.923</td>
<td>9.859</td>
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<td>3</td>
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<td>0.9985</td>
<td>10.545</td>
<td>11.60</td>
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</table>

**Table 2**

<table>
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<th>Sr. no.</th>
<th>Temperature K</th>
<th>Binding constant ( (K) )</th>
<th>( \Delta G ) (kJ mol (^{-1} ))</th>
<th>( \Delta H ) (kJ mol (^{-1} ))</th>
<th>( \Delta S ) (J mol (^{-1} ) K (^{-1} ))</th>
</tr>
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<tbody>
<tr>
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<td>1.76 \times 10^5</td>
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<td>-1.718</td>
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<tr>
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<td>1.04 \times 10^5</td>
<td>-11.783</td>
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</table>
other mechanism such as charge transfer or stacking interaction may be more probable for complex formation. It is known that phenolic –OH of the excited SA is more acidic and carboxylic group becomes more basic. The aromatic part of SA remains embedded into interior of micelle and proton is released from –OH of the excited molecule into Stern layer. The RF being more hydrophilic remains attached to stern layer of micelle and accepts the proton released by SA to form complex due to side by side charge transfer interactions as shown in Fig. 10.

4. Conclusion

Fluorescence resonance energy transfer studies showed that the riboflavin present in micellar solution of salicylic acid forms non-emissive complex in the excited state due to charge transfer interaction. The complex formation is evidenced by the variation of Stern–Volmer quenching constant with quencher concentration observed at different temperatures. The association constant of complex estimated from fluorescence quenching data suggested strong interaction between SA and RF in the excited state. The thermodynamic parameters $\Delta H$, $\Delta G$ and $\Delta S$ at different temperatures were calculated and the results indicated that the interaction between salicylic acid and riboflavin is electrostatic in nature. The presence of both molecules in sun screen lotion not only has dermatological action but also helps to transform UV–B radiation into visible radiation by fluorescence resonance energy transfer occurring between these molecules.

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