4. **Fluorescence quenching by photoinduced electron transfer between 9-anthracene carboxylic acid and substituted uracils:**

Substituted and nonsubstituted uracils are biological ribonucleic acid (RNA) pyrimidine bases and found to undergo interaction with 9-anthracene carboxylic acid (9-ANCA) in aqueous solution. The bare uracil, 5-fluoro uracil and 5-methyl uracil were tested for understanding interaction with fluorescent probe 9-ANCA in excited state. The extent of quenching of fluorescence of 9-ANCA was correlated with the concentration of uracils and analytical methods have been developed for the analysis of uracils.

4.1. **PET between 9-ANCA and uracil: Molecular Interaction Studies**

The ground-state complex formation, between 9-ANCA and uracil was checked by recording the absorption spectra of a mixture of 9-ANCA and uracil using solution of concentrations similar to those used in quenching studies [1]. The absorption spectra of 9-ANCA with and without uracil is shown in Fig.4.1. The banded absorption spectra of 9-ANCA remained unaltered in presence of uracil that eliminated the possibility of ground state charge transfer (CT) complex formation.

The excitation spectra of 9-ANCA without quencher (spectrum A) and with quencher (spectrum B) in aqueous solution are shown in Fig.4.2. The excitation spectra are identical spectrally with banded absorption spectra. The structured spectrum indicates that the 9-ANCA exists as an isolated molecule in aqueous solution [2]. The comparison of the spectra with those reported without uracil shows that the presence of uracil decreases the fluorescence intensity without any spectral modification.
Fig. 4.1: Absorption spectra of 9-ANCA in the (A) absence and (B) presence of uracil in aqueous solution

Fig. 4.2: Excitation spectra of 9-ANCA (A) without uracil and (B) with uracil in aqueous solution

This observation indicates the electronic interaction of 9-ANCA with uracil in the excited state. From Fig. 4.2, it is also observed that the characteristic aromaticity band of 9-ANCA seen at 260 nm is disappeared due to presence of uracil. The decrease in intensity of excitation band and
disappearance of aromaticity band led to consider interaction in the excited state of 9-ANCA.

4.1.1 Fluorescence quenching of 9-ANCA by uracil:

The quenching experiment was set by keeping concentration of 9-ANCA constant to a value of $2.0 \times 10^{-5}$ mol dm$^{-3}$ and that of uracil was varied from $3.0 \times 10^{-3}$ to $27.0 \times 10^{-3}$ mol dm$^{-3}$. The details of the experimental set up are given in the Table 4.1. The solution mixtures were degassed by passing nitrogen gas for about 10 min. The fluorescence spectra of the mixtures were recorded and results are presented in Fig.4.3.

Table 4.1: Experimental set for fluorescence quenching studies of 9-ANCA by varying concentrations of Uracil

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Vol. of 9-ANCA $2.0 \times 10^{-4}$ mol dm$^{-3}$ in ml</th>
<th>Vol. of uracil $3.0 \times 10^{-2}$ mol dm$^{-3}$ in ml</th>
<th>Vol. of water in ml</th>
<th>Total volume in ml</th>
<th>$10^5 [9$-ANCA$]$ mol dm$^{-3}$ in mixture</th>
<th>$10^3$ [Uracil] mol dm$^{-3}$ in mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.0</td>
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<td>4.5</td>
<td>0.0</td>
<td>5.0</td>
<td>2.7</td>
<td>27</td>
</tr>
</tbody>
</table>

From Fig.4.3 it is seen that the intensity of emission bands of 9-ANCA decreases regularly with increasing amounts of uracil without any spectral modification. The expected broad red shifted emission band characteristic of an
exciplex is not seen even at higher concentration of uracil. The absorption band of uracil (268 nm) is widely separated from the emission region of 9-ANCA (370 nm – 500 nm). The zero degree of overlap of emission spectrum of 9-ANCA with the absorption spectrum of uracil, rules out the possibility of excitation energy transfer from excited 9-ANCA to ground state uracil. Therefore the fluorescence quenching of 9-ANCA by uracil should occur through proton or electron transfer.

![Steady-state fluorescence spectra of 9-ANCA in the absence (A) and presence of varying concentrations of uracil (B to J) 3, 6, 9, 12, 15, 18, 21, 24 and 27.0 x 10^-3 mol dm^-3 respectively in aqueous solution](image)

Fig.4.3: Steady-state fluorescence spectra of 9-ANCA in the absence (A) and presence of varying concentrations of uracil (B to J) 3, 6, 9, 12, 15, 18, 21, 24 and 27.0 x 10^-3 mol dm^-3 respectively in aqueous solution

In the present fluorescence quenching studies 9-ANCA is selectively excited to its first excited singlet state. It is known that the aromatic carboxylic acids exhibit a decreasing acidity in first excited singlet relative to the ground state. The excited state pKa of 9-ANCA due to π→π* transition is above 7.0 and thus indicates that the 9-ANCA in excited state becomes more basic [3]. Therefore its ability to loose the electron or take up the proton occurs at a faster rate in the aqueous medium as given below,
The uracil is a RNA base. Hence it has tendency to donate electron rather than hydrogen atom to 9-ANCA. Hence the probable mechanism should be transfer of electron from ground state uracil to excited 9-ANCA. The results obtained on electrochemical studies of 9-ANCA-uracil system in aqueous medium helped us to confirm the transfer of electron from uracil to excited 9-ANCA prior to complex formation. Figure 4.4 is the cyclic voltammogram of 9-ANCA-uracil mixture. The cyclic voltammogram shows reversible oxidation-reduction peak potential. The redox process was quasi-reversible in nature, which is evident from obtained peak separation ($\Delta E_p$) value 600 mV. The $\Delta E_p$ value was greater than 59 mV at 100 mV/s scan rates. The ratio of cathodic to anodic peak height was one, establishing a quasi-reversible one-electron transfer process [4].

4.1.2 Mechanism of electron transfer process:

The uracil is weak photosensitive hence the mechanism of excitation energy transfer from 9-ANCA to uracil quencher is improbable. The uracil is RNA pyrimidine base hence the mechanism of charge transfer from excited 9-ANCA to uracil was considered and investigated. It is believed that the reactants are mobile and free to approach to close distances within the lifetime of the excited partner and structural factors keep the reactants separated at a fixed distance during the lifetime of the excited partner. In a fluid medium, donor and acceptor molecules, which are mobile, and unrestrained by structure or environment may form intermolecular encounter complex prior to quenching. The complex formation then follows molecular diffusion in solution by a series of one-dimensional random steps. Eventually molecules colloid,
separate and undergo further collisions. A typical collision time is of the order as $10^{-9}$ to $10^{-10}$ s. The transfer of charge takes place during the lifetime of the collision complex and the charge transfer (CT) species immediately forms either contact ion pair or an exciplex depending upon the polarity of the solvents used [5].

![Cyclic voltammogram of 9-ANCA-uracil system in aqueous solution, [9-ANCA] $2.0 \times 10^{-5}$ mol dm$^{-3}$ and [Uracil] $9.0 \times 10^{-3}$ mol dm$^{-3}$](image)

The increased polarity may lead to form contact radical ion pair (CRIP) by electron transfer, which results to decrease the intensity of exciplex emission wavelength [6]. The more polar solvents separate the ionic species to form solvent separated radical ion pair (SSRIP). Thus in polar solvents, exciplex can dissociate and solvent separated ions can be stabilized by interaction with solvents molecules. It is known that in the formation of solvent separated ions via the exciplex pathway, the free energy gained from solution and coulombic interaction of the solvent separated ions must exceed the free energy required to dissociate the exciplex. It is believed that the spectral characteristics of 9-ANCA in presence of uracil do not show the structureless broad emission band, characteristics of exciplex formation by electron transfer.
Hence it is suggested that the non-emissive complex between uracil and excited 9-ANCA must be found by electron transfer in excited state, which will be referred as CT complex. Based on experimental results and available information following mechanism involving electron transfer is proposed for interaction of 9-ANCA with uracil (Scheme-I).

\[
\begin{align*}
[9\text{-ANCA}] + h\nu & \rightarrow [9\text{-ANCA}]^* S_1 & \text{Excitation} \\
[9\text{-ANCA}]^* S_1 + [\text{Uracil}] S_0 & \rightarrow [9\text{-ANCA} \text{----Uracil}]^* \rightarrow [(9\text{-ANCA}) (\text{Uracil})]^* \\
& \text{Collisional complex} & \text{excioplex} \\
& \text{Excitation} & \text{Polar solvents} \\
& (\text{Encounter complex}) & (9\text{-ANCA})^- (\text{Uracil})^+ \\
& \text{Contact radical ion pair} & \text{Solvent separated radical ion pair} \\
[\text{CRIP}] & \text{[SSRIP]} & [9\text{-ANCA}]^- \cdots \cdots [\text{Uracil}]^+ \\
& & \text{Free ions}
\end{align*}
\]

**Scheme I: Mechanism of electron transfer process**
4.1.3 Kinetics of fluorescence quenching of 9-ANCA by uracil in aqueous solution:

The considered fluorescence quenching process is bimolecular reaction, which competes with radiative process, and all other molecular processes [7-9]. The singlet excited 9-ANCA molecules are short lived species and before returning to the ground state, they undergo intersystem crossing (ISC), internal conversion (IC), fluorescence (F) and enter in bimolecular fluorescence quenching with ground state uracil after PET. Following mechanism is proposed and kinetics of quenching process is discussed by applying steady state approximation.
where $I_a$ intensity of radiation absorbed, $k_{IC}$ and $k_{ISC}$ are rate constant for internal conversion and intersystem crossing processes.

Steady state approximation to process without Uracil:

$$I_a = k_{ISC} [9-ANCA]^{*}_{s_{1}} + k_{IC} [9-ANCA]^{*}_{s_{1}} + k_{f} [9-ANCA]^{*}_{s_{1}}$$

$$\frac{[9-ANCA]^{*}_{s_{1}}}{I_a} = \frac{I_a}{k_{ISC} + k_{IC} + k_{f}}$$

The quantum efficiency of 9-ANCA fluorescence without quencher ($F_{f0}^0$) is given by

$$F_{f0}^0 = \frac{k_{f} [9-ANCA]^{*}_{s_{1}}}{I_a}$$

$$= \frac{k_{f} I_a}{k_{ISC} + k_{IC} + k_{f}} \times \frac{1}{I_a}$$

$$F_{f0}^0 = \frac{k_{f}}{k_{ISC} + k_{IC} + k_{f}} \quad (1)$$
Steady state approximation to the processes with Uracil:

\[ I_a = k_{ISC} [9-\text{ANCA}]^*_S S_1 + k_{IC} [9-\text{ANCA}]^*_S S_1 + k_f [9-\text{ANCA}]^*_S S_1 + k_a [9-\text{ANCA}]^*_S [\text{Uracil}] \]

\[ [9-\text{ANCA}]^*_S S_1 = \frac{I_a}{k_{ISC} + k_{IC} + k_f + k_q [\text{Uracil}]} \]

The quantum efficiency of perylene fluorescence in presence of Uracil \( \Phi_f \) is given by

\[ \Phi_f = \frac{k_f [9-\text{ANCA}]^*_S S_1}{I_a k_f} \]

\[ \Phi_f = \frac{k_f [9-\text{ANCA}]^*_S S_1}{k_{ISC} + k_{IC} + k_f + k_q [\text{Uracil}]} \]  

\[ \Phi_f^0 \]

\[ \Phi_f = 1 + K_{sv} [\text{Uracil}] \]  

where

\[ K_{sv} = \frac{k_q}{k_{ISC} + k_{IC} + k_f} \]

Quantum efficiency is proportional to area under the fluorescence band which in turn proportional to the intensity of band corresponding to maximum emission wavelength.
\[ \frac{I_0}{I} = 1 + K_{sv} [Uracil] \]  \hspace{1cm} (4)

The above equation is well known Stern-Volmer relation [10-13] and validity of this equation is tested by the plots of \( I_0/I \) vs. \([Uracil]\) for the results obtained in aqueous solution. The Stern-Volmer quenching plot of \( I_0/I \) vs. \([Uracil]\) was linear and gave consistent results, indicating that the quenching process is purely dynamic in nature as shown in Fig.4.5. The values of Stern-Volmer constant \( K_{sv} \) is obtained from the slope of this graph and then used further to calculate the quenching rate constant.

\[ K_{sv} = \frac{k_q}{k_{isc} + k_{ic} + k_f} \]  \hspace{1cm} (5)

where,

\[ \therefore K_{sv} = \frac{k_q}{\sum k_i + k_f} \]
\[ \sum k_i = k_{isc} + k_{ic} \]

\[ K_{SV} = \tau \times k_q \] \hspace{1cm} (6)

‘\(\tau\)’ is the actual lifetime of the 9-ANCA in absence of uracil. The value of ‘\(\tau\)’ of 9-ANCA is 2.3 nano seconds [14]. The estimated values of \(k_q\) and \(K_{sv}\) are given in the Table 4.2.

**Table 4.2: Kinetic parameters obtained for 9-ANCA- uracil system in aqueous solution**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>(K_{SV}) (\text{mol}^{-1}\text{dm}^3)</th>
<th>(k_d) (\text{mol}^{-1}\text{dm}^3\text{s}^{-1})</th>
<th>(k_q) (\text{mol}^{-1}\text{dm}^3\text{s}^{-1})</th>
<th>(k_{et}) (\text{mol}^{-1}\text{dm}^3\text{s}^{-1})</th>
<th>(k_q / k_d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water</td>
<td>11.10</td>
<td>8.38 (\times) 10(^9)</td>
<td>4.82 (\times) 10(^9)</td>
<td>1.97 (\times) 10(^{10})</td>
<td>0.5751</td>
</tr>
</tbody>
</table>

The estimated values of quenching rate constant \((k_q)\) in aqueous solution are in agreement with values reported earlier for PET studies of a normal diffusion controlled quenching process [15]. The present result also indicates a strong interaction between aromatic molecule 9-ANCA and uracil which exclude the possibility of formation of emissive charge transfer complex (CT) as an intermediate. The diffusion controlled rate constant \((k_d)\) is of same order as the quenching rate constant i.e.\(10^9\) \(\text{mol}^{-1}\text{dm}^3\text{s}^{-1}\). The calculated value of ratio \(k_q / k_d\) is less than one. This data indicates that the polarity of the solvent might be keeping the charged species separated and diffusion is less favored. Hence an exciplex emission is seen to be diminished in the highly polar water solution. The observed results and behavior of the donor-acceptor molecules explained on the basis of quenching model are in close agreement with theory. The bimolecular quenching by diffusion-controlled rates coincides nicely with
dynamic Stern-Volmer correlation. The photo-induced intermolecular electron transfer from uracil to the singlet excited 9-ANCA has been proposed as the quenching mechanism.

4.1.4 Estimation of electron transfer rates:

In present studies it was observed that electron transfer (ET) occurs with much faster rate and prior to ET donor and acceptor have to diffuse together [16, 17]. Under diffusive conditions, the reorganization energy appears to play the major role in governing rate constants higher than that of diffusion controlled rate constants (kd). It is known that when quenching rate constants leveled off by diffusion rate constant, the contribution of intramolecular reorganization must have very negligible role in determining ET dynamics under diffusive conditions [7].

It is believed that the donor (uracil) and the acceptor (excited 9-ANCA*) molecules diffuse together to form an encounter complex, which then undergoes reorganization to reach the transient state, where ET takes place from the donor to the acceptor to give an ion-pair. The parameters kd and k_d are the diffusion controlled rate constants for the formation and dissociation of (ANCA*-uracil) complex, k_et and k_e are the forward and backward ET rate constants and k_p is the sum of all rate constants causing the disappearance of the (ANCA*-uracil+) state.

The diffusional rate constant is higher (kd > k_d) than that of quenching rate constant. The diffusional rate constant kd (mol^-1 dm^3 s^-1) calculated by using the relation [18],

\[ k_d = \frac{RT}{3000 \eta} \]

where, \( \eta \) is the dynamic viscosity of the medium at the room temperature. The diffusional rate constant kd and k_d are believed to be nearly same and are greater than the quenching rate constants. The electron transfer rates were estimated by using the following relation,
\[ k_{et} = \frac{k_d x k_q}{K (k_d - k_q)} \]  \hspace{1cm} (8)

Assuming \( K = \frac{k_d}{k_q} = 1 \text{ dm}^3\text{ mol}^{-1} \), \( K \) is diffusional equilibrium constant, the values of \( k_{et} \) under diffusive condition \( k_{et-d} \) were estimated for the system, where \( k_d > k_q \) and are listed in Table 4.2.

The \( k_{et} \) values are seen greater than the quenching rate constants. These results are in accordance with the studies of electron transfer from aromatic amines to the excited coumarin dye in acetonitrile where electron transfer rate constants are greater than quenching rate constants [16] and also for other systems in homogenous medium [17].

### 4.1.5 Energetics of electron transfer process:

Electron transfer (ET) reaction involves the crossing of the surface free energy of the reactant to the product at transition state [19-20]. Feasibility of PET from ground state donor (uracil) to an excited state acceptor (9-ANCA) mainly depends upon free energy change (\( \Delta G^0 \)) for the ET reaction. For the present system, the (\( \Delta G^0 \)) values were calculated using the following Rehm-Weller relation. [21],

\[
\Delta G^0 = E_{(D/D^+)} - E_{(A/A^-)} - E_s - \frac{e^2}{\varepsilon_s r_0} \]  \hspace{1cm} (9)

where \( E_{(D/D^+)} \) and \( E_{(A/A^-)} \) are the oxidation and reduction potentials of uracil and 9-ANCA respectively, \( E_s \) is the singlet excited energy of the 9-ANCA in the \( S_1 \) state, \( e \) is the charge on electron, \( \varepsilon_s \) is the static dielectric constant of reaction medium and \( r_0 \) is the chemical separation between interacting 9-ANCA and uracil molecules and obtained by using Edwards volume addition method. The value of free energy change for 9-ANCA-uracil system in aqueous solution was listed in Table 4.3.
Table 4.3: Thermodynamic and binding parameters for 9-ANCA-uracil system in aqueous solution

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Quencher</th>
<th>$\Delta G^\circ$ (eV)</th>
<th>Double logarithm Equation</th>
<th>$K$ in mol$^{-1}$dm$^3$ Binding const.</th>
<th>$n$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Uracil</td>
<td>-1.49</td>
<td>$\log (I_0-I)/I = \log 0.8897 + 0.8555 \log [Q]$</td>
<td>7.7571</td>
<td>0.8555</td>
<td>0.9917</td>
</tr>
</tbody>
</table>

The more negative $\Delta G^\circ$ values observed for 9-ANCA-uracil system indicate the faster rate of electron transfer in aqueous medium [22]. Thus the present result indicates a strong interaction between 9-ANCA and uracil in aqueous solution.

4.1.6 Binding Parameters:

The binding constant of 9-ANCA with uracil was calculated from the data of steady state fluorescence quenching of 9-ANCA by uracil in aqueous solution. The binding constant ‘$K$’ and the binding number ‘$n$’ are calculated by using the following equation [23].

$$\log_{10} \left( \frac{I_0 - I}{I} \right) = \log_{10} K + n \log_{10} [Q]$$  \hspace{1cm} (10)

where, $K$ is the binding constant and $n$ is the number of binding sites, and $I_0$, $I$ are the fluorescence intensities of 9-ANCA in the absence and presence of quencher respectively. Figure 4.6 displays the plot of $\log_{10} ([I_0 - I]/ I)$ against $\log_{10} [Q]$ and the corresponding binding data with correlation coefficient ($R^2$) is given in Table 4.3. In present system the number of binding sites found to be 0.8555. It can be seen that the interaction between 9-ANCA and uracil is strong. Fluorescence quenching of 9-ANCA resulting from binding with uracil indicates that electron transfer between 9-ANCA and uracil has occurred. The
values of binding constant given in Table 4.3 are smaller when compared with the binding constants reported for the systems forming a stable complex. The lower values support the mechanism of solvent separated radical ion pairs rather than a strong stable complex [24-25].

Fig.4.6: Plot of $\log_{10} \left( \frac{(I_0 - I)}{I} \right)$ vs. $\log$ [Uracil]
4.2 PET between 9-ANCA and 5-Fluoro uracil: Molecular Interaction Studies

The normalized excitation ($\lambda_{em} = 413$ nm) spectrum A and fluorescence ($\lambda_{ex} = 363$ nm) spectrum B of 9-ANCA in aqueous solution are shown in Fig.4.7. The excitation and emission spectra are structured mirror images of each other. The excitation spectrum of 9-ANCA shows bands appeared at 333nm, 345nm, 363nm and 383 nm, it is seen that the fluorescence spectrum matches well with excitation spectra. This indicates that the behavior of 9-ANCA in ground and excited state is similar.

![Normalized excitation and emission spectra of 9-ANCA](image)

Fig.4.7: Normalized (A) excitation and (B) emission spectra of 9-ANCA in aqueous solution

The excitation spectra of 9-anthracene carboxylic acid without quencher (spectrum A) and with quencher (spectrum B) in aqueous solution are shown in Figure 4.8. The excitation spectra are identical spectrally with banded absorption spectra. The presence of 5-fluoro uracil (5-FU) decreases the fluorescence intensity of 9-ANCA without any spectral change. It can be seen that by the presence of 5-FU aromaticity of 9-ANCA becomes reduced and
these observations led to consider molecular interaction in the excited state of 9-ANCA.

**Fig.4.8: Excitation spectra of 9-ANCA (A) without 5-fluoro uracil and (B) with 5- fluoro uracil in aqueous solution**

From the electronic absorption spectra shown in Fig.4.9 of 9-ANCA in the presence and absence of 5-FU, it seen that the presence of 5-FU does not alter the spectral characteristics in aqueous solution. It indicates the absence of ground state charge transfer complex formation between 9-ANCA and 5-FU [1].
Fig. 4.9: Absorption spectra of 9-ANCA in the (A) absence and (B) presence of 5-fluoro uracil in aqueous solution

4.2.1 Fluorescence quenching of 9-ANCA by 5-FU:

The details of the experiments set to study the systematic quenching of 9-ANCA by 5-FU in aqueous solution is given in the Table 4.4. For the quenching experiments, the concentration of 9-ANCA was kept constant at a value $2.0 \times 10^{-5}$ mol dm$^{-3}$ while the concentration of quencher (5-FU) was varied from $1.0 \times 10^{-3}$ mol dm$^{-3}$ to $18.0 \times 10^{-3}$ mol dm$^{-3}$. The samples were carefully degassed using nitrogen gas for about 10 min. and freshly prepared solutions were used for all experiments. The quenching of 9-ANCA by 5-FU is given in Fig 4.10.
Table 4.4: Experimental set for fluorescence quenching studies of 9-ANCA by varying concentrations of 5-FU in aqueous solution

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Vol. of 9-ANCA 2.0 x 10^{-4} mol dm^{-3} in ml</th>
<th>Vol. of 5-FU 2.0 x 10^{-2} mol dm^{-3} in ml</th>
<th>Vol. of water in ml</th>
<th>Total volume in ml</th>
<th>$10^5$ [9-ANCA] mol dm^{-3} in mixture</th>
<th>$10^5$ [5-FU] mol dm^{-3} in mixture</th>
</tr>
</thead>
<tbody>
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<td>4.5</td>
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</tr>
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</tbody>
</table>

Figure 4.10 shows that the intensities of emission bands of 9-ANCA decreases with increasing concentration of 5-FU without formation of an exciplex. The 5-FU absorption and 9-ANCA absorption wavelengths are very widely separated. Hence selective excitation of one in presence of other is possible. It also confirms that excitation energy transfer from excited 9-ANCA to ground state 5-FU is forbidden. Hence the probable mechanism of quenching should be proton or electron transfer.
Fig. 4.10: Steady state fluorescence spectra of 9-ANCA in the absence (A) and presence of varying concentrations of 5-FU (B to J) 2, 4, 6, 8, 10, 12, 14, 16 and $18.0 \times 10^{-3}$ mol dm$^{-3}$ respectively in aqueous solution.

As the 9-ANCA in excited state is more basic in nature it has ability to lose the electron or take up the proton with faster rate in the aqueous medium. The 5-FU is the fluoro derivative of RNA base of uracil. Hence it has tendency to donate electron rather than to donate the proton, which is not expected from the electron withdrawing nature of fluorine atom. The PET occurring in the present system is confirmed by electrochemical studies by cyclic voltammetry. Figure 4.11 is the cyclic voltammogram of 9ANCA-5FU mixtures. The cyclic voltammogram shows reversible oxidation-reduction peak potential.

Fluorescence intensities were obtained at different quencher concentrations and plotted according to the Stern Volmer equation,

$$\frac{I_o}{I} = 1 + k_q \tau_0 [Q] \quad (1)$$

where, $I_o$ and $I$ are the fluorescence intensities for the 9-ANCA in absence and in presence of the quencher (5-FU) respectively, $K_{sv}$ is the Stern-Volmer
constant, \( \tau_0 \) is the fluorescence lifetime in the absence of a quencher and \( [Q] \) is the concentration of quencher [10-13].

\[
\frac{I_0}{I} \text{ vs. } [5\text{-FU}]
\]

**Fig.4.11: Cyclic voltammogram for 9-ANCA-5FU system in aqueous solution, [9-ANCA] 2.0 \times 10^{-5} \text{ mol dm}^{-3} \text{ and } [5\text{-FU}] 8.0 \times 10^{-3} \text{ mol dm}^{-3}**

The Stern-Volmer quenching plot of \( \frac{I_0}{I} \) vs. [5-FU] was linear and gave consistent results shown in Fig.4.12. The steady state bimolecular quenching rate constants and the electrochemical data measurements are compiled in Table 4.5. The quenching rate constant depends on the nature of the substituents at 5-position of uracil. The presence fluorine atom increases the electron donating efficiency of uracil molecules and hence observed \( k_q \) values of (9-ANCA-5-FU) are greater than that of (9-ANCA-uracil) system.
Fluorescence Quenching of 9-ANCA by Uracils

4.2.2 Kinetics of fluorescence quenching:

The bimolecular fluorescence quenching of 9-ANCA by 5-FU may occur along four pathways i) the formation of (excited) charge transfer complex ii) energy transfer iii) proton transfer or iv) electron transfer. To test the formation of exciplex, we have measured the $k_q$ values in comparatively less polar solvents i.e. ethanol. The $k_q$ values decreased from more polar solvent water to less polar solvent ethanol and no significant quenching process is observed. The inverted solvent effect has not been observed in this case. From this observation, the formation of an exciplex can be ruled out by the absence of the inverted solvent effect. The mechanism of energy transfer from singlet excited 9-ANCA to 5-FU is easily ruled out because, the excitation energy of singlet state ($E_s$) of 9-ANCA (3.122 eV) is lower than that of 5-FU. A mechanism involving hydrogen atom transfer from 5-FU to 9-ANCA can be excluded because of thermodynamic parameters calculated from the Rehm-Weller equation. Hence the probable mechanism should be electron transfer (ET). To understand the nature of interaction, the $k_q$ values are correlated with the oxidation potential of 5-FU and reduction potentials of 9-ANCA. The

Fig. 4.12: Stern-Volmer (SV) plot for the fluorescence quenching of 9-ANCA by 5-FU in aqueous solution

![Stern-Volmer plot](image-url)
probable mechanism proposed for 9-ANCA- 5-FU system consisting ET from 5-FU to 9-ANCA is given as scheme II.

Scheme II: Fluorescence quenching mechanism of 9-ANCA by 5-FU in aqueous solution by ET

It is believed that the donor (5-FU) and the acceptor (excited 9-ANCA*) molecules diffuse together to form an encounter complex, which then undergoes reorganization to reach the transient state, where ET takes place from the donor to the acceptor to give an ion-pair. The parameters $k_d$ and $k_d$ are the diffusion controlled rate constants for the formation and dissociation of (ANCA*- 5-FU), $k_{et}$ and $k_{et}$ are the forward and backward ET rate constants and $k_p$ is the sum of all rate constants causing the disappearance of the (ANCA - uracil*) state.

The diffusional rate constant is higher ($k_d > k_q$) than that of quenching rate constant. The diffusional rate constant $k_d$ (mol$^1$dm$^3$s$^{-1}$) is calculated by using the relation [18],

$$k_d = \frac{RT}{3000 \eta} \quad \text{------------------- (2)}$$

where, $\eta$ is the dynamic viscosity of the medium at the room temperature. The diffusional rate constant $k_d$ and $k_d$ are believed to be nearly same and are greater than the quenching rate constants. The electron transfer rates were estimated by using the following relation,

$$k_{et} = \frac{k_d \times k_q}{K (k_d - k_q)} \quad \text{------------------ (3)}$$
assuming $K = k_d / k_q = 1$ dm$^3$ mol$^{-1}$, $K$ is diffusional equilibrium constant, the values of $k_{et}$ under diffusive condition $k_{et,d}$ were estimated for the system where $k_d > k_q$ and are listed in Table 4.5.

Table 4.5: Kinetic parameters obtained for 9-ANCA-5-FU system in aqueous solution

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>$K_{SV}$ mol$^{-1}$ dm$^3$</th>
<th>$k_d$ mol$^{-1}$ dm$^3$ s$^{-1}$</th>
<th>$k_q$ mol$^{-1}$ dm$^3$ s$^{-1}$</th>
<th>$k_{et}$ mol$^{-1}$ dm$^3$ s$^{-1}$</th>
<th>$k_q / k_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>16.20</td>
<td>$8.40 \times 10^9$</td>
<td>$7.04 \times 10^9$</td>
<td>$5.2 \times 10^{10}$</td>
<td>0.8380</td>
</tr>
</tbody>
</table>

The estimated values of quenching rate constant ($k_q$) in aqueous solution are in agreement with values reported earlier for similar type PET studies for a normal diffusion controlled quenching process [15]. The present result also indicates a significant interaction between aromatic molecule 9-ANCA and 5-FU which also exclude the possibility of formation of emissive charge transfer complex (CT) as an intermediate. The diffusion controlled rate constant ($k_d$) is of same order as the quenching rate constant i.e. $10^9$ mol$^{-1}$ dm$^3$ s$^{-1}$. The calculated value of ratio $k_q / k_d$ is less than one. This data indicates that the polarity of the solvents might be keeping the charged species separated and diffusion is less favored. Hence an exciplex emission is seen to be diminished in the highly polar water solution. The observed results and behavior of the donor-acceptor molecules explained on the basis of quenching model are in close agreement with theory. The bimolecular quenching by diffusion-controlled rates coincides nicely with dynamic Stern-Volmer correlation. The photo-induced intermolecular electron transfer from 5-fluoro uracil to the singlet excited 9-ANCA has been proposed as the quenching mechanism.
4.2.3 Energetics of electron transfer process:

PET from ground state donor to an excited state acceptor mainly depends upon the change in surface free energy ($\Delta G^0$) for the electron transfer process [16, 20]. For the present system, the ($\Delta G^0$) values were calculated using the following Rehm-Weller relation. [26],

$$\Delta G^0 = E_{(D/D^+)} - E_{(A/A^-)} - E_s - \frac{e^2}{\varepsilon_s r_o} \tag{4}$$

where, $E_{(D/D^+)}$ and $E_{(A/A^-)}$ are the oxidation and reduction potentials of 5-FU and 9-ANCA respectively, $E_s$ is the singlet excited energy of the 9-ANCA in the $S_1$ state, $e$ is the charge on electron, $\varepsilon_s$ is the static dielectric constant of reaction medium and $r_o$ is the chemical separation between interacting 9-ANCA and 5-FU molecules and obtained by using Edwards volume addition method. The free energy change and binding parameters for 9-ANCA-5-FU systems in aqueous solution were listed in Table 4.6.

**Table 4.6: Binding parameters for 9-ANCA-5-FU systems in aqueous solution**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Quencher</th>
<th>$\Delta G^0$ (eV)</th>
<th>Double logarithm equation</th>
<th>K in mol$^{-1}$dm$^3$</th>
<th>$n$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5-Fluoro-uracil</td>
<td>-1.63</td>
<td>$\log (I_0/I) = \log 1.0987 + 0.9801 \log \left[Q\right]$</td>
<td>12.5516</td>
<td>0.9801</td>
<td>0.9989</td>
</tr>
</tbody>
</table>

The photo-induced electron transfer reaction between 5-FU and 9-ANCA was explored well by the calculation of the standard free energy changes with the Rehm-Weller equation. The more negative $\Delta G^0$ values observed for 9-ANCA and fluoro-substituted uracil indicate the faster rate of electron transfer in aqueous medium.
4.2.4 **Binding Parameters:**

The binding constant of 9-ANCA with 5-FU was calculated from the data of steady state fluorescence quenching of 9-ANCA by 5-FU in aqueous solution. The binding constant ‘K’ and the binding number ‘n’ are calculated by the following equation [23],

\[
\log_{10} \left( \frac{I_0 - I}{I} \right) = \log_{10} K + n \log_{10} [Q] \quad (5)
\]

where in the present case Io and I are the fluorescence intensities of 9-ANCA in the absence and presence of quencher respectively. Figure 4.13 displays the plot of \( \log_{10} \left( \frac{I_0 - I}{I} \right) \) against \( \log_{10} [Q] \) and the corresponding binding data is given in Table 4.6. The result shows that the interaction between 9-ANCA and 5-FU is very strong. Fluorescence quenching of 9-ANCA resulting from binding with 5-FU indicates the occurrence of electron transfer between 9-ANCA and 5-FU [24-25].

![Fig.4.13: Plot of log\(_{10}\) [(I\(_0\) – I) / I] to log\(_{10}\) [5-fluoro uracil]](image)
4.2.5 Applications:

Analysis of 5-FU from pharmaceutical samples:

To test the applicability of the proposed fluorescence quenching method, it was applied to the determination of 5-FU in pharmaceutical samples namely Fluorouracil cream USP, Florida 1% and Fluorouracil injection I.P. The drugs were dissolved in minimum quantity of ethylene glycol and diluted to required volume with water. The results are given in Table 4.5. Though 5-Fluorouracil formulation cream contains Methyl paraben and Propyl paraben, they do not interfere on fluorescence intensity measurements because of selective excitation of 5-FU. It could be seen from Table 4.7, the recovery and precision of this method is satisfactory.

Table 4.7: Results of 5-fluoro uracil determination in pharmaceutical samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
<th>Amount of Fluorouracil</th>
<th>Recovery (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorouracil cream USP, Shalaks Phar. Pvt. Ltd New Delhi.</td>
<td>FU 1.00 % w/w Methyl paraben 0.2 % w/w Propyl paraben 0.05 %w/w</td>
<td>1.0 %</td>
<td>0.98%</td>
<td>98.00</td>
</tr>
<tr>
<td>FU injection I.P., Zuvius Life Sci. Pvt.Ltd. Mumbai</td>
<td>Fluorouracil per 10 ml</td>
<td>50.00 mg</td>
<td>50.00</td>
<td>48.62</td>
</tr>
</tbody>
</table>
4.2.6 Effect of foreign substances and method of analysis:

Under the optimal conditions, effects of interferences that usually used as the compatibility of coexisting substances on the fluorescence intensity of the system were studied [27-28]. Initially, foreign substances are taken in large excess in the 5-FU solution and the fluorescence intensities are measured. When interference was found to be intensive, the tests were repeated with successive smaller amounts of foreign substances. The most of metal ions have little effect on the determination of fluorouracil. The tolerance amount and a relative error of less than ±5 % are observed which are listed in Table 4.8

Table 4.8: Influence of coexisting substances on 9ANCA-5FU systems,

\[ [9-\text{ANCA}] \ 2.0 \times 10^{-5} \text{ mol dm}^{-3} \text{ and } [5-\text{FU}] \ 4.0 \times 10^{-3} \text{ mol dm}^{-3} \]

<table>
<thead>
<tr>
<th>Foreign substance</th>
<th>Concentration (mg/ml)</th>
<th>Relative error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn$^{2+}$</td>
<td>1</td>
<td>-2.09</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>1</td>
<td>-7.15</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1</td>
<td>-8.76</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>5</td>
<td>-3.31</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>1</td>
<td>-7.74</td>
</tr>
<tr>
<td>Ag$^{1+}$</td>
<td>1</td>
<td>-2.67</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>1</td>
<td>-9.07</td>
</tr>
<tr>
<td>Cr$^{3+}$</td>
<td>1</td>
<td>-2.43</td>
</tr>
<tr>
<td>Citrate</td>
<td>100</td>
<td>-0.93</td>
</tr>
<tr>
<td>SCN$^{-}$</td>
<td>100</td>
<td>-1.83</td>
</tr>
</tbody>
</table>
4.3 PET between 9-ANCA and 5-Methyl uracil: Molecular Interaction Studies

The excitation spectra of 9-ANCA without quencher (spectrum A) and with quencher (spectrum B) in aqueous solution are shown in Figure 4.14.

![Excitation Spectra](image)

**Fig.4.14: Excitation spectra of 9-ANCA (A) without and (B) with 5-methyl uracil in aqueous solution**

The structured spectra indicate that the 9-ANCA exists as an isolated molecule in aqueous solution [2]. The comparison of the spectra with those reported without 5-methyl uracil (5-MU) shows that the presence of 5-MU decreases the fluorescence intensity without any spectral modification. This suggests the significant electronic interaction of 9-ANCA with 5-MU in the excited state in aqueous solution. It can be seen that the aromaticity of the 9-ANCA reduced by addition of 5 MU.

4.3.1 Fluorescence quenching of 9-ANCA by 5-MU:

The details of the experiment set to study the systematic quenching of 9-ANCA by 5-MU concentration in aqueous solution is given in the Table 4.9. The experiment was set by keeping concentration of 9-ANCA constant to a
value of $2.0 \times 10^{-5}$ mol dm$^{-3}$ and that of 5-MU varied from $3.0 \times 10^{-3}$ mol.dm$^{-3}$ to $27.0 \times 10^{-3}$ mol dm$^{-3}$.

Table 4.9: Experimental set for fluorescence quenching of 9-ANCA by 5-methyl uracil

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Vol. of 9-ANCA 2.0 $\times 10^{-4}$ mol dm$^{-3}$ in ml</th>
<th>Vol. of 5-MU 3.0 $\times 10^{-2}$ mol dm$^{-3}$ in ml</th>
<th>Volume of water in ml</th>
<th>Total volume in ml</th>
<th>$10^5$ [9-ANCA] mol dm$^{-3}$ in mixture</th>
<th>$10^5$ [5-MU] mol dm$^{-3}$ in mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>00</td>
<td>4.5</td>
<td></td>
<td>0.0</td>
<td>00</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>4.0</td>
<td></td>
<td>0.3</td>
<td>03</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>1.0</td>
<td>3.5</td>
<td></td>
<td>0.6</td>
<td>06</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>1.5</td>
<td>3.0</td>
<td></td>
<td>0.9</td>
<td>09</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>2.0</td>
<td>2.5</td>
<td></td>
<td>1.2</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>2.5</td>
<td>2.0</td>
<td></td>
<td>1.5</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>3.0</td>
<td>1.5</td>
<td></td>
<td>1.8</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>3.5</td>
<td>1.0</td>
<td></td>
<td>2.1</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>4.0</td>
<td>0.5</td>
<td></td>
<td>2.4</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>4.5</td>
<td>00</td>
<td></td>
<td>2.7</td>
<td>27</td>
</tr>
</tbody>
</table>

Fluorescence quenching of 9-ANCA by 5-MU was studied in aqueous medium by using steady state fluorescence spectroscopy. The quenching of 9-ANCA by 5-MU is given in Fig 4.15. The dynamic quenching rate constants ($k_q$) were determined by the Stern-Volmer equation,

$$
\frac{I_o}{I} = 1 + k_q \tau_o [Q] \tag{1}
$$

where, $I_o$ and $I$ are the fluorescence intensities for the 9-ANCA in absence and in presence of the quencher respectively, $K_{sv}$ is the Stern-Volmer constant, $\tau_o$ is the fluorescence lifetime in the absence of a quencher and $[Q]$ is the concentration of quencher [10-13].
Fig.4.15: Steady-state fluorescence spectra of 9-ANCA in absence (A) and presence of varying concentrations of 5-MU (B to J) 3, 6, 9, 12, 15, 18, 21, 24 and 27 x 10^{-3} mol dm^{-3} respectively in aqueous solution

The Stern-Volmer quenching plots of I_o / I vs. [5-MU] were linear with correlation coefficient (R^2) is 0.9978 and gave consistent results, indicating that the quenching process is purely dynamic in nature as shown in Fig.4.16. The steady state bimolecular quenching rate constants and the electrochemical data measurements are compiled in Table 4.9. The quenching rate constant depends on the nature of the substituent at 5-position of uracil. The presence of electron donating methyl group in 5-MU, increases the electron donating efficiency and hence observed k_q values of (9-ANCA-5-MU) are greater than that of (9-ANCA-uracil) system and smaller than (9-ANCA-5-FU).
Fig. 4.16: Stern-Volmer (SV) plot for the fluorescence quenching of 9-ANCA by 5-MU in aqueous solution

4.3.2 Kinetics of fluorescence quenching:

The mechanisms of electron transfer from 5-MU to 9-ANCA remains same as presented in 9-ANCA-5-FU system. The diffusional rate constant is higher 

\(k_d > k_q\) than that of quenching rate constant. The diffusional rate constant \(k_d\) (mol\(^{-1}\) dm\(^3\) s\(^{-1}\)) is calculated by using the relation [18],

\[
k_d = \frac{RT}{3000 \eta}
\]

(2)

where, \(\eta\) is the dynamic viscosity of the medium at the room temperature. The diffusional rate constant \(k_d\) and \(k_d\) are believed to be nearly same and are greater than the quenching rate constants. The electron transfer rates were estimated by using the following relation,

\[
k_{et} = \frac{k_d \times k_q}{K (k_d - k_q)}
\]

(3)

assuming \(K = k_d / k_q = 1\) dm\(^3\) mol\(^{-1}\), \(K\) is diffusional equilibrium constant, the values of \(k_{et}\) under diffusive condition \(k_{et,d}\) were estimated for the system where \(k_d > k_q\) and are listed in Table 4.10.
Table 4.10: Kinetic parameters obtained for 9-ANCA-5-MU system in aqueous solution

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>$K_{SV}$ (mol$^{-1}$ dm$^3$)</th>
<th>$k_d$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</th>
<th>$k_d$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</th>
<th>$k_{et}$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</th>
<th>$k_q$ / $k_d$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>14.70</td>
<td>8.384 x 10$^9$</td>
<td>6.39 x 10$^9$</td>
<td>3.5 x 10$^{10}$</td>
<td>0.7621</td>
<td>0.9978</td>
</tr>
</tbody>
</table>

The photo-induced intermolecular electron transfer from 5-MU to the singlet-excited 9-ANCA has been proposed as the quenching mechanism. In present system, the rate of electron transfer is higher than uracil molecule while smaller than 5-fluorouracil.

4.3.3 Energetics of electron transfer process:

For the present system, the ($\Delta G^0$) values were calculated using the following Rehm-Weller relation. [26],

$$\Delta G^0 = E_{(D/D^+)} - E_{(A/A^-)} - E_s - \frac{e^2}{\varepsilon_s r_0} \quad \text{(4)}$$

where $E_{(D/D^+)}$ and $E_{(A/A^-)}$ are the oxidation and reduction potentials of 5 methyl uracil and 9-ANCA respectively, $E_s$ is the singlet excited energy of the 9-ANCA in the $S_1$ state, $e$ is the charge on electron, $\varepsilon_s$ is the static dielectric constant of reaction medium and $r_0$ is the chemical separation between interacting 9-ANCA and 5-MU molecule and obtained by using Edwards volume addition method. Cyclic voltammogram for the 9-ANCA and 5-MU system in aqueous solution is shown in Figure 4.17. The changes in free energy and binding parameters for 9-ANCA-5-MU system in aqueous solution were listed in Table 4.11.
Table 4.11: Binding parameters for 9ANCA-5MU system in aqueous solution

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Quencher</th>
<th>$\Delta G^0$ (eV)</th>
<th>Double logarithm Equation</th>
<th>K in mol$^{-1}$dm$^3$ Binding const.</th>
<th>n</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5-Methyl uracil</td>
<td>-1.54</td>
<td>$\log (I_0-I)/I = \log 0.9523 + 0.9389 \log [Q]$</td>
<td>8.9598</td>
<td>0.9389</td>
<td>0.9957</td>
</tr>
</tbody>
</table>

Fig.4.17: Cyclic voltammogram for 9ANCA-5MU system in aqueous solution, [9-ANCA] $2.0 \times 10^{-5}$ mol dm$^{-3}$ and [5-MU] $9.0 \times 10^{-3}$ mol dm$^{-3}$

Standard free energy change was calculated by using Rehm-Weller equation. The more negative $\Delta G^0$ values observed for 9-ANCA-5-MU system indicate the faster rate of electron transfer in aqueous medium.
4.3.4 Binding Parameters:

The binding constant of 9-ANCA with 5-MU was calculated from the data of steady state fluorescence quenching of 9-ANCA by 5-MU in aqueous solution. The binding constant ‘K’ and the binding number ‘n’ are calculated by the following double logarithm equation [25].

\[
\log_{10} \left( \frac{I_0 - I}{I} \right) = \log_{10} K + n \log_{10} [Q] \quad (5)
\]

where \( I_0 \) and \( I \) are the fluorescence intensities of 9-ANCA in the absence and presence of 5-MU respectively. Figure 4.18 displays the plot of \( \log_{10}(\frac{I_0 - I}{I}) \) against \( \log_{10}[Q] \) and the corresponding binding data is given in Table 4.10. It can be seen that the interaction between 9-ANCA and 5-MU is very strong and such system have high stability.

![Fig.4.18: Plot of log \(_{10}(\frac{I_0 - I}{I})\) vs. log \(_{10}\) [5-Methyl uracil]](image-url)
Chapter-IV  Fluorescence Quenching of 9-ANCA by Uracils

4.4 References: