

Research Article

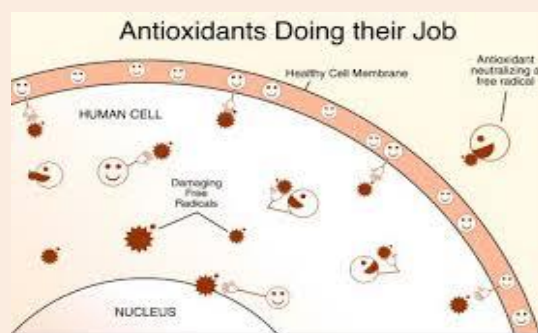
Kinetics and Mechanism of Protection of Thymine from Sulphate Radical anion by Vitamin E under Anoxic Conditions

M. Sudha Swaraga*¹ and M. Adinarayana²¹St. Pious X PG College for Women, HMT Nagar, Nacharam, Hyderabad, 500076, India¹Department of Chemistry, Osmania University, Hyderabad, 500 007, India²Post-Graduate College of Science, Saifabad, Osmania University, Hyderabad, 500 004, India**Abstract**

The oxidation of thymine by sulphate radical anion ($SO_4^{\bullet-}$) have been followed by measuring the absorbance of thymine at 264 nm spectrophotometrically. The rates and the quantum yields (ϕ) of oxidation of thymine by sulphate radical anion have been determined in the presence of different concentrations of vitamin E (α -tocopherol). Increase in [vitamin E] is found to decrease the rate of oxidation of thymine suggesting that vitamin E acts as an efficient scavenger of sulphate radical anions and protects thymine from it. Sulphate radical anions compete for thymine as well as for vitamin E. The rate constant of sulphate radical anion with vitamin E has been calculated to be $1.34 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. The quantum yields of photooxidation of thymine have been calculated from the initial rates of oxidation of thymine and the light intensity absorbed by peroxydisulphate (PDS) at 254 nm, the wavelength at which peroxydisulphate is activated to sulphate radical anions. From the results of experimentally determined quantum yields (ϕ_{exptl}) and the quantum yields calculated (ϕ_{cal}) assuming vitamin E acting

Keywords: Oxidation of vitamin E, Repair of thymine by vitamin E, Oxidation by sulphate radical anion, Protection of thymine

only as a scavenger of sulphate radical anions show that ϕ_{exptl} values are lower than ϕ_{cal} values. The experimentally found quantum yield values at each vitamin E concentration and corrected for $SO_4^{\bullet-}$ scavenging by vitamin E (ϕ^1) are also found to be greater than ϕ_{exptl} values. These observations suggest that the thymine radicals are repaired by vitamin E in addition to scavenging of sulphate radical anions.

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Introduction

The lethal effects of ionizing radiation on cellular systems involve radical induced chemical changes in essential biomolecules, particularly in deoxyribonucleic acid (DNA) [1]. Ionizing radiation causes damage to DNA by direct effect and indirect effect. The former is caused by the absorption of energy of ionizing radiation by the DNA molecule itself, the later by water radicals generated upon absorption of energy of ionizing radiation by water. On the absorption of energy of ionizing radiation DNA molecule undergoes a chemical change giving radical cation, which on spontaneous deprotonation gives DNA radical, the chemistry of which is similar to DNA radicals produced by water ($\bullet\text{OH}$) radicals. When DNA is subjected to ionizing radiation many different changes can occur in DNA [2], ranging from various kinds of base modifications to single and double strand breaks. Even though sugar radicals are actually responsible for strand break formation in DNA, experimental results clearly indicate that base radicals can contribute significantly via transfer of radical sites from base moiety to sugar moiety [3,4].

In order to mimic and understand the mechanism of direct effect of ionizing radiation on DNA, Bansal and Fessenden [5] have used sulphate radical anion a strong electrophilic radical to create radical cation in uracil and substituted uracils. Ravi et al. [6] reported that 5-yl radicals obtained from oxidation of thymine by phosphate radical anion ($\text{PO}_4^{\bullet-}$) have been repaired to a greater extent at about $50\mu\text{M}$ of dithiothreitol. Sudha et al. [7,8] reported that 5-yl radicals obtained from oxidation of thymine and uracil by $\text{SO}_4^{\bullet-}$ have been repaired to a greater extent at about $50\mu\text{M}$ of caffeic acid. It has been reported that a number of biochemical reactions in mammalian systems generate reactive oxygen species that are capable of damaging crucial biomolecules such as DNA, proteins and membrane lipids [9,10]. The major reactive oxygen species generated due to oxidative stress and / or by ionizing radiation are the hydroxyl radical (OH^\bullet), the superoxide anion ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and peroxy radical (ROO^\bullet). If these radicals are not effectively scavenged by the antioxidant defense mechanism in the tissues, oxidative stress results [11]. Tocopherols are present in cellular membranes and edible oils and act as biological antioxidants due to their ability to inhibit the free radical chain reaction of lipid peroxidation [12,13]. The antioxidant activity of the tocopherols (TocH) is mainly due to their ability to donate their phenolic hydrogens to peroxy radicals (LOO^\bullet). In this paper we report the results on the protection of thymine from sulphate radical anion by vitamin E. From the competition kinetic studies of $\text{SO}_4^{\bullet-}$ with thymine and vitamin E the rate constant of $\text{SO}_4^{\bullet-}$ reaction with vitamin E have been evaluated. Further an attempt has also been made to evaluate the percentage of scavenging of the $\text{SO}_4^{\bullet-}$ and the extent of repair of thymine radicals by vitamin E.

Experimental

Thymine and Peroxydisulphate were purchased from E.Merck, while Vitamin E (α - tocopherol) was from Sigma chemicals and used as received. The solutions of vitamin E were always prepared afresh-using HPLC grade acetonitrile and that of thymine and peroxydisulphate with double distilled water. Stock solutions of thymine and vitamin E were always freshly prepared and were deaerated by bubbling nitrogen. The solutions of potassium salt of peroxydisulphate were standardized using cerimetry using ferroin indicator. Peroxydisulphate solution was added to a measured excess of ferrous ammonium sulphate and back titrated with a standard ceric ammonium sulphate solution as reported by Kapoor et al. [14]. At room temperature this reaction is rapid enough for analytical purposes and equivalency of ferrous ion to peroxydisulphate is 2 to 1. Required amounts of vitamin E was then injected as aqueous – acetonitrile (1:3) solution into the mixture of thymine and peroxydisulphate solutions present in a specially designed 1-cm path length quartz cuvette which is suitable for both irradiations in the quantum yield reactor as well as for absorbance measurements. The absorbance measurements were made at 264 nm, which is the λ_{max} of thymine, on a HITACHI UV-visible spectrophotometer (model 3410). Irradiations, were performed at room temperature (25°C) with high-pressure mercury lamp using Quantum yield reactor, model QYR-20. The irradiations were interrupted at definite intervals of time and the absorbance was noted from which the rate of reaction and the quantum yields of oxidation are calculated. The light intensity at 254 nm was measured by peroxydisulphate chemical actinometry [15].

Results and Discussion

N_2 saturated aqueous solutions of the reaction mixture containing thymine ($0.5 \times 10^{-4} \text{ mol dm}^{-3}$), peroxydisulphate ($2.50 \times 10^{-4} \text{ mol dm}^{-3}$) and with varying concentrations of vitamin E were irradiated and the absorbance at 264 nm (λ_{max} of thymine) with time were noted. (Table 1 and Figure 1)

The absorbance of thymine in the reaction mixture at different intervals of irradiation time have been obtained by subtracting the contribution of absorbance of vitamin E by carrying out a parallel experiment with vitamin E alone at the same time intervals of time measured under similar experimental conditions of the oxidation of thymine by sulphate radical anion in the presence of vitamin E (Table 1). From these the rates of oxidation of thymine were calculated from the plots of absorbance versus time using microcal origin computer program on personal computer Table 2. The initial rates of oxidation of thymine by sulphate radical anion have been found to decrease with increase

in [vitamin E] (**Table.2**). The quantum yields of oxidation of thymine were calculated from the rates of oxidation of thymine by sulphate radical anion and the light intensity absorbed by peroxydisulphate at 254 nm, the wavelength at which peroxydisulphate is activated to sulphate radical anions. The quantum yields of oxidation of thymine (ϕ_{exptl}) at different [vitamin E] are presented in **Table.2**.

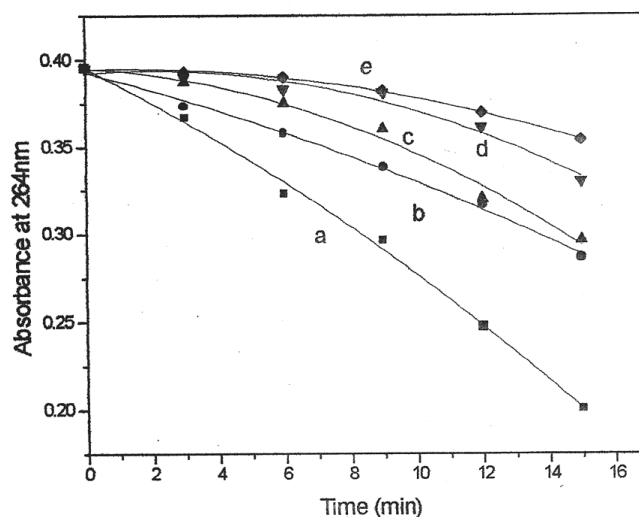


Figure 1 Effect of vitamin E on the photooxidation of thymine by PDS

- (a) [Thymine] = 5.00×10^{-5} mol dm⁻³, vitamin E = 0.00
 (b) [Thymine] = 5.00×10^{-5} mol dm⁻³, vitamin E = 1.00×10^{-6} mol dm⁻³
 (c) [Thymine] = 5.00×10^{-5} mol dm⁻³, vitamin E = 1.00×10^{-5} mol dm⁻³
 (d) [Thymine] = 5.00×10^{-5} mol dm⁻³, vitamin E = 2.00×10^{-5} mol dm⁻³
 (e) [Thymine] = 5.00×10^{-5} mol dm⁻³, vitamin E = 5.00×10^{-5} mol dm⁻³

Table 1 Photooxidation of thymine in presence of peroxydisulphate at various [vitamin E] in aqueous acetonitrile anoxic solutions

Irradiation time (min)	Absorbance at 264nm in presence of different [vitamin E]				
	0.00μM	1.00μM	10.00μM	20.00μM	50.00μM
0	0.395	0.395	0.395	0.395	0.395
3	0.367	0.373	0.387	0.390	0.393
6	0.323	0.366	0.375	0.383	0.390
9	0.296	0.338	0.360	0.381	0.382
12	0.247	0.317	0.320	0.361	0.370
15	0.200	0.286	0.296	0.330	0.354

[Thymine] – 5.0×10^{-5} mol dm⁻³, [PDS] = 2.50×10^{-4} mol dm⁻³, Temp – 298K
 pH = 7.5, Light intensity – 2.16×10^{15} quanta s⁻¹

Table 2 Effect of [vitamin E] on the quantum yields of photooxidation of thymine in presence of peroxydisulphate (PDS) under anoxic conditions

S.No	$10^5 \times [\text{vitamin E}]$	$10^8 \times \text{rate}$ ($\text{mol dm}^{-3} \text{s}^{-1}$)	ϕ_{exptl}	P	ϕ_{cal}	ϕ'	% Scavenging	% Repair
1	0.0	3.00	1.326	1.00	1.326	1.326	0.00	0.00
2	0.1	2.06	0.911	0.788	1.044	1.157	21.2	12.74
3	1.0	0.711	0.314	0.272	0.360	1.154	72.8	12.97
4	2.0	0.334	0.148	0.157	0.208	0.943	84.2	28.88
5	5.0	0.100	0.044	0.070	0.093	0.628	93.0	52.64

Light intensity = 2.16×10^{15} quanta s^{-1} ; [PDS] = 2.5×10^{-4} mol dm^{-3} ;
 [Thymine] = 5.00×10^{-5} mol dm^{-3} ; pH~7.5, Temp = 298 K

The ϕ_{exptl} values were found to decrease with increasing concentration of vitamin E. The substances used in the present work viz, vitamin E and/or thymine did not undergo any chemical change on shining the light in the absence of peroxydisulphate. Vitamin E has molar absorption coefficient $348 \text{ dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ and thymine has $6250 \text{ dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ at 254 nm wavelength at which peroxydisulphate is activated to $\text{SO}_4^{\bullet-}$ radicals. Due to this more light is being absorbed by vitamin E and/or thymine and the concentration of $\text{SO}_4^{\bullet-}$ radicals produced from activation of peroxydisulphate should decrease with increase in concentration of vitamin E and/or thymine. However, during photo excitation alone in the presence of peroxydisulphate we have reported [16,17] that the quantum yields of oxidation of vitamin E and/or thymine were found to increase with increase in concentration of vitamin E and/or thymine. These results could be explained by assuming that vitamin E and/or thymine is excited by the absorption of light at 254 nm by acting as inner filter, which subsequently transfers energy to peroxydisulphate to give $\text{SO}_4^{\bullet-}$ radicals by acting as sensitizer. Thus the efficiency of production of $\text{SO}_4^{\bullet-}$ radicals increases, which increases the quantum yields of oxidation of vitamin E and/or thymine.

Therefore in the present work we propose that vitamin E as well as thymine act as sensitizers and transfers energy to peroxydisulphate to create $\text{SO}_4^{\bullet-}$ radicals. Since in this system there is competition between thymine and vitamin E for $\text{SO}_4^{\bullet-}$, the relative amounts of $\text{SO}_4^{\bullet-}$ reacting with thymine decreases with increasing [vitamin E]. The rate constant of the reaction of the sulphate radical anion with thymine has reported [18] to be $1 \times 10^9 \text{ dm}^3 \text{mol}^{-1} \text{s}^{-1}$. The rate constant for the reaction of sulphate radical anion with vitamin E has been calculated by the thymine competition method, which is very similar to the one chosen [19] to determine the rate constant for the reaction of OH^{\bullet} radicals with polyhydric alcohols in competition with KSCN. In the photolysis experiment, oxygen-free N_2 -saturated solutions containing thymine and varying amounts of vitamin E were irradiated for six minutes and the decrease of absorbance of thymine was measured. The decrease of absorbance of thymine reflects the number of sulphate radical anions that have reacted with thymine. From the rate constant of reaction of thymine with $\text{SO}_4^{\bullet-}$ ($k_{\text{thymine}} = 1 \times 10^9 \text{ dm}^3 \text{mol}^{-1} \text{s}^{-1}$), rate constant of $\text{SO}_4^{\bullet-}$ reaction with vitamin E ($k_{\text{vitamin E}}$) can be calculated using equation (1)

$$\frac{[\text{Absorbance of thymine}]_0}{[\text{Absorbance of thymine}]_{\text{vitamin E}}} = 1 + \frac{k_{\text{vitamin E}} [\text{vitamin E}]}{k_{\text{thymine}} [\text{thymine}]} \quad (1)$$

Where, $[\text{Absorbance of thymine}]_0$ and $[\text{Absorbance of thymine}]_{\text{vitamin E}}$ indicates the decrease in the absorbance of thymine in the absence and presence of vitamin E respectively, at the same interval of time. Experiments of this kind

can be carried out with great accuracy. The rate constant for the reaction of sulphate radical anion with vitamin E have been calculated with four different concentrations of vitamin E, and the average value obtained is. $1.34 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$

The probability of $\text{SO}_4^{\bullet-}$ radicals reacting with thymine $\{p (\text{SO}_4^{\bullet-} + \text{thymine})\}$ is calculated using the following equation.

$$p (\text{SO}_4^{\bullet-} + \text{thymine}) = \frac{[\text{Thymine}]k_{\text{thymine}}}{[\text{Thymine}]k_{\text{thymine}} + [\text{vitamin E}]k_{\text{vitamin E}}} \quad (2)$$

k_{thymine} and $k_{\text{vitamin E}}$ are the rate constants of $\text{SO}_4^{\bullet-}$ with thymine and vitamin e respectively. If vitamin E scavenges only $\text{SO}_4^{\bullet-}$ radicals and does not give rise to any other reaction (e.g. repair) the ϕ_{exptl} at each [vitamin E] should be given by equation (3).

$$\phi_{\text{cal}} = \phi_{\text{exptl}}^0 \times p \quad (3)$$

Where ϕ_{exptl}^0 is the quantum yield of oxidation of thymine in the absence of vitamin E, and p is the probability given by equation (2). The ϕ_{cal} values at different vitamin E concentrations are presented in Table-2. It is clear from the data in Table.2 that the calculated quantum yield values (ϕ_{cal}) are larger than the experimentally measured quantum yield values (ϕ_{exptl}). The difference in ϕ_{cal} and ϕ_{exptl} values is proposed to be due to the prevention of chromophore loss by H atom donation to thymine radicals by vitamin E. From the rate constant of sulphate radical anion with vitamin E, the fraction of $\text{SO}_4^{\bullet-}$ radicals scavenged by vitamin E (Percentage scavenged = $(1 - p) \times 100$) at different [vitamin E] were calculated (**Table.2**). These values were a measure of protection of thymine due to scavenging of $\text{SO}_4^{\bullet-}$ radicals by vitamin E. **Table.2** also contains the ϕ^1 values, which are experimentally found quantum yield values at each vitamin E concentration corrected for sulphate radical anion scavenging by vitamin E

$$\phi' = \frac{\phi_{\text{exptl}}}{p} \quad (4)$$

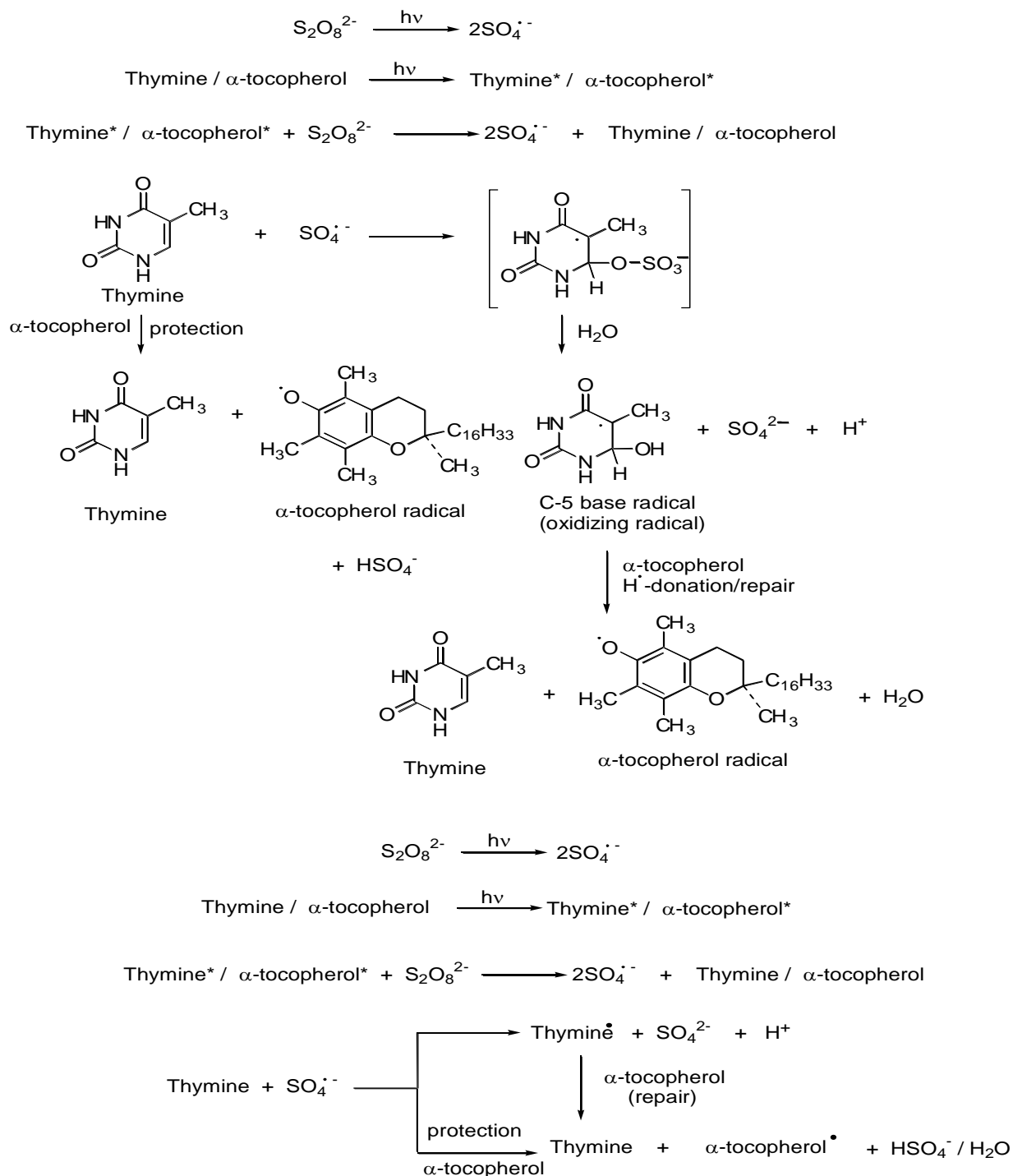
The ϕ^1 values represent the experimentally found quantum yield values if no scavenging of $\text{SO}_4^{\bullet-}$ radicals by vitamin E occurs and hence, in the absence of repair of thymine radicals by vitamin E, ϕ^1 values should all be equal to ϕ_{exptl}^0 . The observed decrease in the ϕ^1 values with increasing vitamin E concentration (**Table.2**) indicates the occurrence of repair of thymine radicals. The fraction of oxidation of thymine inhibited by repair of thymine radicals is given by equation (5).

$$\text{Percentage repair} = \frac{(\phi_{\text{exptl}}^0 - \phi')}{\phi_{\text{exptl}}^0} \times 100 \quad (5)$$

The data on percentage repair is presented in **Table 2**.

The experimentally determined quantum yield values (ϕ_{exptl}) are lower than the quantum yield values (ϕ_{cal}) calculated using equation (3) under the assumption that vitamin E acts only as a $\text{SO}_4^{\bullet-}$ radical scavenger. This shows that vitamin E is acting not only as an efficient scavenger of $\text{SO}_4^{\bullet-}$ but also acts as an agent for the repair of thymine radicals. It is therefore obvious that vitamin E is reacting not only with $\text{SO}_4^{\bullet-}$ radicals but also with thymine radicals. Behrens et al. [20] studied the oxidation of thymine by $\text{SO}_4^{\bullet-}$ using in situ photolysis of peroxydisulphate. It has been observed by them that majority of the radicals formed by the action of $\text{SO}_4^{\bullet-}$ on thymine are of oxidizing nature and

are the C-5 base radicals (5-yl radicals). This is attributed to the reason that C-5 position of thymine is not accessible for electrophilic addition of $SO_4^{\cdot-}$ due to steric hindrance of methyl group. From the results obtained in the present work (**Table 2**) indicated that the C-5 base radicals which are oxidizing in nature are efficiently repaired by vitamin E to the extent of 52% at about $50\mu\text{M}$ of [vitamin E]. The scheme of reactions of protection of thymine and repair of thymine radicals through H donation by vitamin E is given below.



Scheme 1 Reactions of Protection of Thymine and Repair of Thymine Radicals Through H Donation by Vitamin E

Conclusions

Photochemical oxidation studies of thymine in presence of various [vitamin E] have been carried out under different experimental conditions. From competition kinetic studies of thymine and vitamin E for $\text{SO}_4^{\bullet-}$ the rate constant of reaction of $\text{SO}_4^{\bullet-}$ with vitamin E has been calculated. From the experimental quantum yield values (ϕ_{exptl}) and the calculated quantum yield values assuming vitamin E acts as a scavenger (ϕ_{cal}), the percentage repair of thymine radicals have been calculated.

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