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Kinetics and mechanism of regeneration of β-carotene from *tert*-butoxyl radical induced β-carotene radical cation by α-tocopherol: A synergistic interaction

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ABSTRACT

The rates of oxidation of α-tocopherol and β-carotene by tert-butoxyl radicals (t-BuO•) were studied spectrophotometrically. Radicals (t-BuO•) were generated by the photolysis of tert-butyl hydroperoxide (t-BuOOH) in presence of tert-butyl alcohol to scavenge •OH radicals. The rates and the quantum yields (ϕ) of oxidation of α-tocopherol by t-BuO• radicals were determined in the absence and presence of varying concentrations of β-carotene. An increase in the concentration of β-carotene was found to decrease the rate of oxidation of α-tocopherol, suggesting that β-carotene and α-tocopherol competed for t-BuO• radicals. From competition kinetics, the rate constant of β-carotene reaction with t-BuO• was calculated to be 5.54 × 10^8 dm^3 mol^{-1} s^{-1}. The quantum yields ϕ_{expt} and ϕ_{cal} values suggested that α-tocopherol not only protected β-carotene from t-BuO• radicals, but also repaired β-carotene radicals, formed by the reaction of β-carotene with t-BuO• radicals.

Keywords: α-tocopherol, β-carotene, regeneration, synergism, *t*-BuO• radicals

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INTRODUCTION

Oxygen is vital for aerobic life processes. About 5% or more of the inhaled oxygen is converted to Reactive Oxygen Species (ROS) such as $O_2^{-\bullet}$, •OH and H_2O_2 by univalent reduction of oxygen which is highly toxic to cells [1,2]. Cellular antioxidant systems and the free-radical scavengers normally protect a cell from toxic effects of the ROS. However, when generation of the ROS overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins and nucleic acids) [3-6] occurs, leading finally to various pathological conditions including cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of the vital organs, cancer and premature aging [1-7]. These radicals are found to react rapidly in the presence of oxygen with biological targets such as lipids, nucleic acids, carbohydrates, proteins, etc. to form endogenous alkyl hydroperoxides and these reactions have been shown to affect the biological structure, function and subsequent cellular processing of these molecules. Alkyl hydroperoxides on homolysis produce alkoxyl and hydroxyl radicals. The alkoxyl radicals were shown to react with the nucleobases and nucleosides as well as antioxidants, histone proteins and amino acids [8]. The generation of these radicals may play a role in the formation of DNA double strand breaks and inter-strand crosslinks [9,10]. The free radical mediated oxidative stress results also in oxidation of membrane lipoproteins, glycoxidation and oxidation of DNA subsequently leads to cell death.

In a biological system, an antioxidant can be any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate. The oxidizable substrate may be any molecule that is found in foods or biological materials, including carbohydrates, DNA, lipids and proteins. During the past two decades, intensive research has been carried out on naturally occurring antioxidants from different sources [11-13]. The main drive behind this search was to reduce the use of synthetic compounds as food additives because of their potential negative health effects and as a result of consumer demand.

Synergism is the cooperative effect of antioxidants or an antioxidant with other compounds to produce enhanced activity than the sum of the activities of the individual component when used separately [14]. Antioxidant synergism in food was first reported by Olcott and Mattill [15]. Two types of synergism are observed: Involving primary antioxidants only and involving a combination of primary antioxidants with metal chelators or peroxy scavengers. Several mechanisms are involved in synergism among antioxidants: one among them involves combination of two or more different free radical scavengers in which one antioxidant is regenerated by others. Regeneration of a more effective free radical scavenger (primary antioxidant) by a less effective free radical scavenger (coantioxidant, synergist) occurs mostly when one free radical scavenger has a higher reduction potential than the other.

Antioxidants may act synergistically due to differences in reactivity towards different oxidants thereby yielding a better overall protection in combination than either could individually or may be due to direct interaction between them. Strong synergistic activity has been observed in the mixtures of natural tocopherols and citric acid. The synergistic effect of this mixture is caused by the chain breaking ability of tocopherols and metal chelation of citric acid [16].

Two antioxidants whose bond dissociation energy difference is high exert a synergistic antioxidant effect [17]. Regeneration of the antioxidant is fast when a synergist has a higher BDE than the primary antioxidant. Also, the primary antioxidant can be regenerated [18] when the rate constant for regeneration of the primary antioxidant is atleast $1.0 \times 10^3\ M^{-1}\ s^{-1}$. Regeneration of the antioxidant can be accomplished by electron transfer from a synergist to a primary antioxidant [19]. Quercetin and α-tocopherol show a synergism in decreasing the oxidation of lard by the mechanism in which α-tocopherol acts as a free radical scavenger while quercetin acts as a metal chelator [20].

A number of studies have shown that β-carotene and other carotenoids have lipid-soluble antioxidant activity [21]. In homogenous lipid solutions, in membrane models and also in intact cells, β-carotene has been studied mostly and it is a less effective antioxidant than α-tocopherol [22]. Mixtures of carotenoids have been found to be more effective than any one single carotenoid in protecting liposomes against lipid peroxidation and this synergistic effect is most pronounced if lycopene or lutein is present in the mixture. It is possible that β-carotene and α-tocopherol act cooperatively as antioxidants in membranes and lipoproteins.

A synergistic or antagonistic effects occurring between pairs of antioxidants can be explained by regeneration mechanisms, depending on the Bond Dissociation Energies (BDE), redox potentials, chemical structure of molecules and on the possible formation of stable intermolecular complexes [17]. It is generally accepted that β-carotene is more lipophilic than α-tocopherol and will be more likely to be in the interior of a membrane. However, the β-carotene radical is a charged species while the α-tocopherol radical is uncharged. Thus the β-carotene radical may reorient so that the charge is near the polar interface of the cell membrane and hence be more accessible to aqueous phase antioxidants such as ubiquitous ascorbic acid. Palozza and Krinsky [23] showed that β-carotene and α-tocopherol can act synergistically in the membrane system and in rat liver microsomes. However Bohm *et al.* [24] showed that α-tocopherol protects β-carotene and not vice versa. Willson [25] proposed that they have similar electron donor abilities while Tappel [26] suggested that β-carotene is the weaker antioxidant. In the view of the various mechanistic studies of antioxidant interactions, we have undertaken the present study to investigate the nature of molecular interactions (synergistic/antagonistic) occurring between the antioxidants β-carotene and α-tocopherol in the presence of *t*-$BuO^{\bullet}$ radicals using competitive kinetic method.

The *t*-$BuO^{\bullet}$ radicals have been generated by steady-state photolysis of *tert*-butyl hydroperoxide in the presence of *t*-BuOH to scavenge the hydroxyl radicals in aqueous solution [27]. In the present paper, the reactions of *t*-$BuO^{\bullet}$ radicals with β-carotene have been studied in the presence of α-tocopherol to assess the protection by α-tocopherol towards oxidation of β-carotene by *t*-$BuO^{\bullet}$ radicals and also regeneration, if any offered by α-tocopherol towards β-carotene radicals.

EXPERIMENTAL SECTION

α-Tocopherol and β-carotene were purchased from Sigma Chemical Co., St. Louis, USA and used as received. All solutions were prepared afresh using double-distilled water. *tert*-Butyl hydroperoxide (*t*-BuOOH) was used as received from Merck-Schuchardt of Germany. There is no contamination of other peroxides in the assay of the sample. *t*-BuOOH was estimated by iodometric method [28].

The irradiations were carried out at room temperature in a quantum yield reactor model QYR-20 supplied by Photophysics, England, attached with 400 W medium pressure mercury lamps. The quartz cuvette containing the sample was irradiated and the irradiations were interrupted at definite intervals of time and the absorbance was noted. The light intensity corresponding to the irradiating wavelength (254 nm) was measured using peroxydisulphate chemical actinometry [29]. On photolysis, *t*-BuOOH was activated at 254 nm to generate $^{\bullet}$OH and *t*-BuO$^{\bullet}$ radicals by homolytic cleavage of –O-O-bond [30]. The $^{\bullet}$OH radicals produced were scavenged using sufficient concentration of *t*-BuOH [27]. In a typical kinetic run, the aqueous reaction mixture of α-tocopherol and *t*-BuOOH was taken in a specially designed 1 cm path length quartz cuvette, suitable for both irradiations and absorbance measurements. The absorbance measurements were made at the λ_{max} of α-tocopherol (294nm) on a Chemito UV-Visible spectrophotometer (model 2100).

The photochemical reaction of α-tocopherol in the presence of *t*-BuOOH was followed by measuring the absorbance of α-tocopherol at 294 nm at which β-carotene was totally transparent.

It is known that *t*-BuOOH is activated to radical reaction by the absorption of light at 254 nm [31]. However, the substrates used in the present work, viz., α-tocopherol and β-carotene have strong absorption in this region. But, in the absence of *t*-BuOOH in the reaction mixture, α-tocopherol, β-carotene or α-tocopherol- β-carotene mixture did not undergo any observable chemical change on shining the light. Even though a small fraction of the total light intensity was absorbed by *t*-BuOOH directly in the presence of β-carotene and/or α-tocopherol, a considerable chemical change was observed with β-carotene as well as α-tocopherol. If α-tocopherol and β-carotene acted as only inner filters, the rates of the reaction of α-tocopherol or β-carotene with *t*-BuO$^{\bullet}$ radicals would have been decreased with increase in concentration of α-tocopherol or β-carotene. But, the results in Tables 1 and 2 were contrary to this. One another fact against the inner filter concept was that the rate of oxidation of α-tocopherol in the presence of β-carotene would have been much less than the experimentally observed values (Table 4). Hence, we proposed that the excited states of α-tocopherol and β-carotene acted as sensitizers to transfer energy to *t*-BuOOH to produce radical species. This type of sensitizing effect was proposed in similar systems earlier [32]. Therefore, the light intensity at 254 nm was used to calculate the quantum yields of oxidation of α-tocopherol as well as β-carotene under different experimental conditions.

RESULTS AND DISCUSSION

The oxidation of β-carotene by *t*-BuO$^{\bullet}$ radicals was carried out by irradiating the reaction mixture containing known concentrations of β-carotene and *t*-BuOOH in the presence of sufficient amount of *t*-BuOH to scavenge the $^{\bullet}$OH radicals completely [27].The reaction was followed by measuring the absorbance of β-carotene at 491 nm (λ_{max} of β-carotene) with time. The initial rates and quantum yields of oxidation of β-carotene by *t*-BuO$^{\bullet}$ are presented in Table 1. The initial rates of photooxidation of α-tocopherol by *t*-BuOOH in presence of *t*-BuOH were calculated from the plots of absorbance of α-tocopherol at 294 nm *vs* time using microcal origin computer program on a personal computer (Table 2). UV-visible absorption spectra of α-tocopherol in presence of *t*-BuOOH and *t*-BuOH at different irradiation times were recorded (Fig. 1). In order to find the protection offered to β-carotene by α-tocopherol towards oxidation by *t*-BuO$^{\bullet}$, the reaction mixture containing known concentrations of α-tocopherol and *t*-BuOOH was irradiated in presence of varying concentrations of β-carotene. The reactions were followed by measuring the absorbance of α-tocopherol at 294 nm (Fig. 2) at which β-carotene was transparent and the rate data are presented in Table 3. The photooxidation of α-tocopherol by *t*-BuO$^{\bullet}$ at different concentrations of β-carotene was also studied (Fig. 3) and the data are presented in Table 4.

Table 1 – Effect of [β-carotene] and [*t*-BuOOH] on the rates and quantum yields of photooxidation of β-carotene by *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium

10^5 × [β-carotene] (mol dm^{-3})	10^3 × [*t*-BuOOH] (mol dm^{-3})	10^9 × Initial rate (mol $dm^{-3} s^{-1}$)	Quantum yield (φ)
0.5	5.0	0.3163	0.00210
0.8	5.0	0.4784	0.00318
1.0	5.0	0.5324	0.00354
2.0	5.0	1.0570	0.00703
5.0	5.0	2.5001	0.01662
8.0	5.0	4.8375	0.03216
10.0	5.0	6.7900	0.04516
5.0	10.0	5.8250	0.03874
5.0	15.0	8.0240	0.05340

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 294 nm, pH ~ 7.5, Temperature = 298 K

Table 2 – Effect of [α-tocopherol] and [*t*-BuOOH] on the rates and quantum yields of photooxidation of α-tocopherol by *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium

10^5 × [α-tocopherol] (mol dm^{-3})	10^3 × [*t*-BuOOH] (mol dm^{-3})	10^9 × Initial rate (mol $dm^{-3} s^{-1}$)	Quantum yield (φ)
0.5	5.0	0.6868	0.00046
0.8	5.0	1.6569	0.00110
1.0	5.0	3.673	0.00244
2.0	5.0	11.627	0.00773
5.0	5.0	33.691	0.02240
8.0	5.0	63.357	0.04213
10.0	5.0	69.840	0.04645
5.0	1.0	19.000	0.01236
5.0	10.0	39.132	0.02602
5.0	15.0	48.756	0.03242

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 294 nm, pH ~ 7.5, Temperature = 298 K

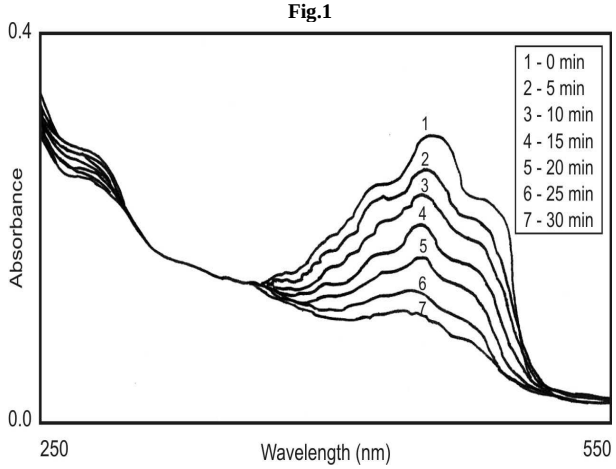
The oxidation rate of β-carotene in the presence of *t*-BuOH refers exclusively to the reaction of *t*-$BuO^{\bullet}$ with β-carotene. These rates were found to increase with increase in concentration of β-carotene as well as *t*-BuOOH. The quantum yield values were also increased with increase in [β-carotene] as well as [*t*-BuOOH] (Table 1).

The rate of oxidation of α-tocopherol increased with increase in concentration of α-tocopherol (Table 2). The quantum yields of oxidation of α-tocopherol were calculated from the initial rates and the light intensity at 254 nm. These values were also increased with increase in concentration of α-tocopherol (Table 2). Having known the rates of *t*-$BuO^{\bullet}$ radical reactions with β-carotene as well as α-tocopherol under varying experimental conditions, both β-carotene and α-tocopherol were introduced for the competitive studies with *t*-$BuO^{\bullet}$ radical. Aqueous solutions of reaction mixture containing α-tocopherol and *t*-BuOOH were irradiated in presence of varying concentrations of β-carotene (Fig. 3). The initial rates and quantum yields of oxidation of α-tocopherol by *t*-$BuO^{\bullet}$ radicals were found to decrease with increase in concentration of β-carotene (Table 4). Comparison of the initial rates and quantum yields of oxidation of α-tocopherol in presence and absence of β-carotene clearly indicated that the initial rates and quantum yields of oxidation of α-tocopherol were substantially decreased in presence of β-carotene (Table 4). These observations clearly demonstrated that β-carotene and α-tocopherol were in competition for *t*-$BuO^{\bullet}$ radicals.

The rate constant of the reaction of *t*-$BuO^{\bullet}$ with α-tocopherol has been reported [32] to be 7.29×10^8 dm^3 mol^{-1} s^{-1} under similar experimental conditions of the present work. The rate constant for the reaction of *t*-$BuO^{\bullet}$ with β-carotene was calculated by the adenosine competition method, which was very similar to the method [21] used to determine the rate constant for the reaction of $^{\bullet}OH$ radicals with polyhydric alcohols in competition with KSCN. In the present study, solutions containing α-tocopherol and varying amounts of β-carotene in presence of *t*-BuOOH was irradiated for 2 min and the decrease in absorbance of α-tocopherol was measured. The decrease in absorbance of α-tocopherol reflected the amount of *t*-$BuO^{\bullet}$ radicals that had reacted with α-tocopherol. From the known rate constant of the reaction of α-tocopherol with *t*-$BuO^{\bullet}$ radical under similar experimental conditions of the present work ($k_{\alpha\text{-tocopherol}}$ = 7.29×10^8 dm^3 mol^{-1} s^{-1}), the rate constant of *t*-$BuO^{\bullet}$ radical reaction with β-carotene ($k_{\beta\text{-carotene}}$) can be calculated using the following equation:

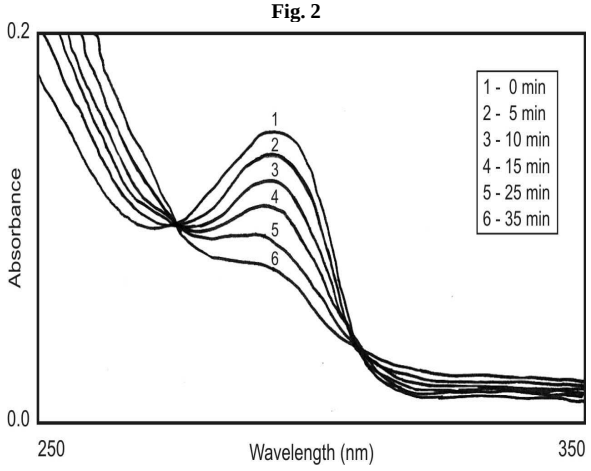
$$\frac{[\text{Absorbance of chlorogenic acid}]_o}{[\text{Absorbance of chlorogenic acid}]_{\alpha\text{-tocopherol}}} = 1 + \frac{k_{\alpha\text{-tocopherol}}\,[\alpha\text{-tocopherol}]}{k_{\text{chlorogenic acid}}\,[\text{chlorogenic acid}]} \quad (1)$$

Fig.1



Absorption spectra of photooxidation of β-carotene in the presence of tert-butyl hydroperoxide at different irradiation times; [β-carotene] = 5 × 10^{-5} mol dm^{-3}, [t-BuOOH] = 5 ×10^{-3} mol dm^{-3}, Light intensity = 2.7168 ×10^{15} quanta s^{-1}, λ_{max} = 451 nm, pH ~ 7.5, temperature = 298 K, [t-BuOH] = 1.0 M

Fig. 2



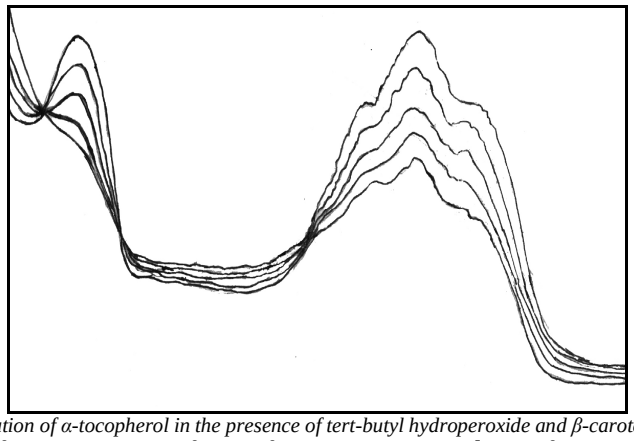
Absorption spectra of photooxidation of α-tocopherol in the presence of tert-butyl hydroperoxide at different irradiation times; [α-tocopherol] = 5 ×10^{-5} mol dm^{-3}, [t-BuOOH] = 5 ×10^{-3} mol dm^{-3}, Light intensity = 2.7168 ×10^{15} quanta s^{-1}, λ_{max} = 294 nm, pH ~ 7.5, temperature = 298 K, [t-BuOH] = 1.0 M

In Eq. (1), [Absorbance of α-tocopherol]$_o$ and [Absorbance of α-tocopherol]$_{\beta\text{-carotene}}$ are the absorbance values of α-tocopherol in the absence and presence of β-carotene respectively at the same interval of time. Experiments of this kind can be carried out with great accuracy. Using Eq. (1), the rate constant for the reaction of *t*-BuO$^{\bullet}$ radical with β-carotene ($k_{\beta\text{-carotene}}$) was calculated at different concentrations of α-tocopherol and β-carotene and the average of these was found to be 5.54×10^8 dm^3 mol^{-1} s^{-1}. As β-carotene had strong absorption at 294 nm, it is not possible for the direct determination of protection and repair offered to β-carotene by α-tocopherol. However, one could calculate indirectly the extent of protection offered to β-carotene by α-tocopherol from competition kinetic studies measured at 294 nm, λ_{max} of α-tocopherol. The method was as follows:

When the system containing β-carotene, α-tocopherol and *t*-BuOOH was irradiated, the probability of *t*-BuO$^{\bullet}$ radicals reacting with α-tocopherol {$p_{(t\text{-BuO}^{\bullet} + \alpha\text{-tocopherol})}$}was calculated using the following equation:

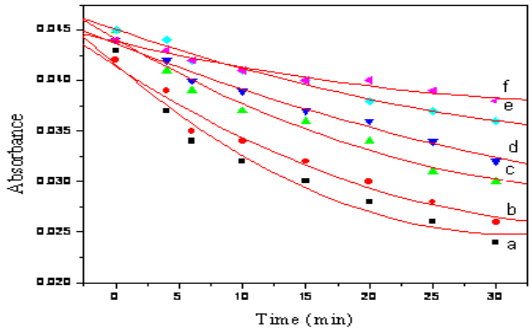
$$p_{(t\text{-}BuO^{\bullet} + \text{chlorogenic acid})} = \frac{k_{\text{chlorogenic acid}}[\text{chlorogenic acid}]}{k_{\alpha\text{-tocopherol}}[\alpha\text{-tocopherol}] + k_{\text{chlorogenic acid}}[\text{chlorogenic acid}]} \quad (2)$$

Fig. 3



Absorption spectra of photooxidation of α-tocopherol in the presence of tert-butyl hydroperoxide and β-carotene at different irradiation times; [α-tocopherol] = 2 ×10^{-5} mol dm^{-3}, [t-BuOOH] = 5 ×10^{-3} mol dm^{-3}, [β-carotene] = 5 ×10^{-5} mol dm^{-3}, Light Intensity = 2.7168 ×10^{15} quanta s^{-1}, λ_{max} = 294nm, pH ~ 7.5, temperature = 298 K

Fig. 4



Effect of [β-carotene] on the oxidation of α-tocopherol by t-BuO$^{\bullet}$ in the presence of β-carotene in t-BuOH-water (1:4 v/v) medium; [α-tocopherol] = 5 ×10^{-5} mol dm^{-3}, [t-BuOOH] = 5 ×10^{-3} mol dm^{-3} at 298 K. [β-carotene] = (a) 0.0, (b) 1 ×10^{-5} mol dm^{-3}, (c) 2 ×10^{-5} mol dm^{-3}, (d) 5 ×10^{-5} mol dm^{-3}, (e) 8 ×10^{-5} mol dm^{-3}, (f) 10 ×10^{-4} mol dm^{-3}, Light intensity = 2.7168 ×10^{15} quanta s^{-1}, λ_{max} = 294nm, pH ~7.5, Temperature = 298 K

If α-tocopherol scavenged only *t*-BuO$^{\bullet}$ radicals and did not give rise to any other reaction (e.g. reaction with β-carotene radicals), the quantum yield of oxidation of α-tocopherol (ϕ_{cal}) at each concentration of β-carotene may be given by equation:

$$\phi_{cal} = \phi^{o}_{expt} \text{ X } p \quad (3)$$

where ϕ^{o}_{expt} is the quantum yield of oxidation of α-tocopherol in the absence of β-carotene, and p is the probability given by Eq. (2).

The calculated quantum yield (ϕ_{cal}) values at different β-carotene concentrations are presented in Table 4. The data showed that the ϕ_{cal} values were lower than the experimentally measured quantum yield (ϕ_{expt}) values. This indicated that more number of α-tocopherol molecules was consumed in the system than expected and the most likely route for this was H atom donation by α-tocopherol to β-carotene radicals. In Table 4, are presented the fraction of *t*-BuO$^{\bullet}$

radicals scavenged by α-tocopherol at different concentrations of β-carotene. These values referred to the measure of protection offered to β-carotene due to scavenging of *t*-BuO$^{\bullet}$ radicals by α-tocopherol. Using the ϕ_{exptl} values, a set of values, viz., ϕ' values were calculated from Eq. (4) and are presented in Table 4

$$\phi' = \frac{\phi_{expt}}{p} \qquad (4)$$

where ϕ's represent the experimentally found quantum yield values if no scavenging of β-carotene radicals by α-tocopherol occurs. In the absence of any "repair" of β-carotene radicals by α-tocopherol, the ϕ' values should all be equal to ϕ^{o}_{expt}. The observed increase in ϕ' with increasing α-tocopherol concentration (Table 4) clearly indicated the repair of β-carotene radicals. The extent of repair may be quantified by the following equation:

$$\% \text{ Repair} = \frac{(\phi' - \phi^{o}_{expt})}{\phi^{o}_{expt}} \times 100 \qquad \cdots(5)$$

The data on percentage repair is presented in Table 4. The experimentally determined quantum yield (ϕ_{expt}) values were higher than the quantum yield (ϕ_{cal}) values calculated using Eq. (3) under the assumption that α-tocopherol acts only as a *t*-BuO$^{\bullet}$ radical scavenger. This showed that α-tocopherol acted not only as an efficient scavenger of *t*-BuO$^{\bullet}$ radicals, but also as an agent for the repair of β-carotene radicals.

The nature of interactions of α-tocopherol with β-carotene is essential for understanding in oxidative stress conditions in vivo although it is more complicated. Carotenoids are regenerated by tocopherols [23,24,33] and tocopherols are regenerated by carotenoids [34]. But the carotenoid regeneration by tocopherols is more preferable partly because of the higher standard reduction potential of the carotenoid radical cation (780 mV) compared to α-tocopherol (500 mV) [35,24]. Regeneration of less effective free radical scavenger (β-carotene) by a more effective radical scavenger (α-tocopherol) contributes to a higher net interactive antioxidant effect with α-tocopherol regenerating β-carotene from β-carotene radical cation formed due to oxidation of β-carotene with *t*-BuO$^{\bullet}$ radicals to an extent of 43%.

The Bond Dissociation Energy (BDE) of the antioxidants along with the redox potentials control the regeneration of antioxidants. β-carotene with lower BDE of 74.0 kcal mol^{-1} shows more tendency to react with *t*-BuO$^{\bullet}$ radicals through hydrogen transfer than α-tocopherol [17,18] with BDE of 78.2-78.9 kcal mol^{-1}. Thus, in the initial step, β-carotene due to lower BDE reduces *t*-BuO$^{\bullet}$ radicals as given by the equation:

$$\beta\text{-Car} + \text{t-BuO}^{\bullet} \longrightarrow \beta\text{-Car}^{\bullet} + \text{t-BuOH}$$

The reduction potentials of β-carotene radical and α-tocopherol radical are 780 mV and 500 mV respectively. Due to the large difference in the redox potentials, the regeneration of β-carotene from its radical by α-tocopherol is possible with concomitant production of α-tocopheroxyl radicals as given in the equation:

$$\beta\text{-Car}^{\bullet} + \alpha\text{-TOH} \longrightarrow \beta\text{-Car} + \alpha\text{-TO}^{\bullet}$$

α-Tocopherol being more efficient antioxidant compared to β-carotene, regenerates β-carotene from β-carotene radical cation formed due to oxidation reaction of β-carotene with *t*-BuO$^{\bullet}$ radicals. This result is also reflected in Table 4 supporting our contention that α-tocopherol regenerates β-carotene from β-carotene radical cation. The proposed mechanism for the reaction between α-tocopherol and β-carotene may involve the reduction of β-carotene radical cation by α-tocopherol with concomitant production of α-tocopheroxyl radical is as in Scheme 1.

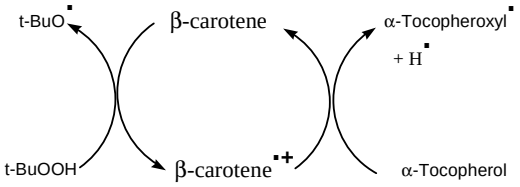
On the basis of the experimental results and the above discussion, the synergistic interaction of α-tocopherol with β-carotene and regeneration of β-carotene from β-carotene radical cation by α-tocopherol is suggested as in Scheme 2.

Table 3 - Effect of [α-tocopherol] on the rates and quantum yields of oxidation of α-tocopherol in absence and presence of β-carotene by *t*-BuO• in *t*-BuOH-water (1:4 v/v) medium

$10^5 \times$ [α-tocopherol] (mol dm^{-3})	$10^5 \times$ [β-carotene] (mol dm^{-3})	$10^9 \times$ Rate (mol dm^{-3} s^{-1})	Quantum yields ϕ
2.0	0.0	11.627	0.00773
1.0	0.0	3.6731	0.00244
0.8	0.0	1.6569	0.00110
0.5	0.0	0.6868	0.00046
2.0	2.0	7.5661	0.00503
1.0	2.0	2.4420	0.00162
0.8	2.0	0.5862	0.00039
0.5	2.0	0.0171	0.00011

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 294 nm, pH ~ 7.5, Temperature = 298 K

Scheme 1



Scheme 2

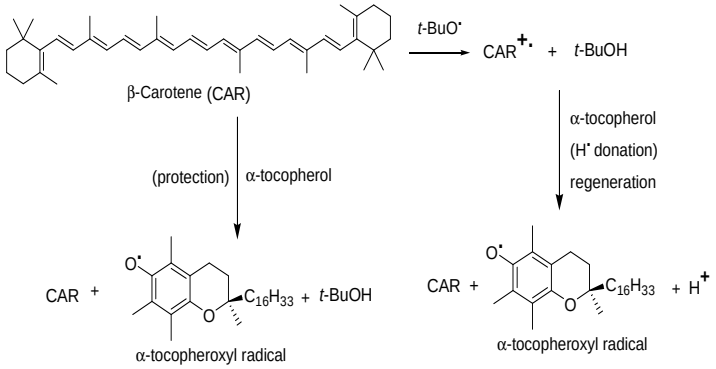


Table 4 - Effect of varying [β-carotene] on the rate and quantum yield of photooxidation of α-tocopherol in the presence of *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium

$10^5 \times$[β-carotene] (mol dm^{-3})	$10^9 \times$ Rate (mol dm^{-3} s^{-1})	ϕ_{expt}	ϕ_{cal}	p	ϕ'	% scavenging	% regeneration
0.0	11.627	0.00773	0.00773	1.0	0.00773	100.0	0.0
1.0	9.232	0.00614	0.00560	0.7246	0.00847	72.46	9.62
2.0	7.566	0.00503	0.00441	0.5699	0.00883	56.99	14.2
5.0	5.038	0.00335	0.00266	0.3448	0.00971	34.48	25.7
8.0	3.765	0.00250	0.00191	0.2475	0.01011	24.75	30.8
10.0	3.464	0.00230	0.00161	0.2083	0.01106	20.83	43.1

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 294 nm, pH ~ 7.5, Temperature = 298 K

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