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SYNERGISTIC INTERACTION OF CHLOROGENIC ACID WITH PHOTOCHEMICALLY GENERATED tert-BUTOXYL RADICAL INDUCED ASCORBIC ACID RADICALS – A KINETIC APPROACH TO REGENERATION OF ASCORBIC ACID

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ABSTRACT

The rates of oxidation of ascorbic acid and chlorogenic acid (CGA) by *tert*-butoxyl radicals (*t*-BuO$^{\bullet}$) were studied by measuring the absorbance of ascorbic acid at 265 nm and CGA at 328 nm spectrophotometrically. Radicals (*t*-BuO$^{\bullet}$) were generated by the photolysis of *tert*-butyl hydroperoxide (*t*-BuOOH) in presence of *tert*-butyl alcohol to scavenge OH$^{\bullet}$ radicals. The rates and the quantum yields (ϕ) of oxidation of CGA by *t*-BuO$^{\bullet}$ radicals were determined in the absence and presence of varying concentrations of ascorbic acid. An increase in the concentration of ascorbic acid was found to decrease the rate of oxidation of CGA, suggesting that ascorbic acid and CGA competed for *t*-BuO$^{\bullet}$ radicals. From the competition kinetics, the rate constant for the ascorbic acid reaction with *t*-BuO$^{\bullet}$ radicals was calculated to be 6.65×10^{9} dm^{3} mol^{-1} s^{-1}. The quantum yields (ϕ_{expt}) were calculated from the experimentally determined rates of oxidation of CGA under different experimental conditions. Assuming that CGA acts as a scavenger of *t*-BuO$^{\bullet}$ radicals only, the quantum yields (ϕ_{cal}) were theoretically calculated. ϕ_{expt} and ϕ_{cal} values suggested that ascorbic acid not only protected CGA from *t*-BuO$^{\bullet}$ radicals, but also regenerated CGA from CGA radical, formed by the reaction of CGA with *t*-BuO$^{\bullet}$ radicals. Results indicated a possible synergistic interaction between CGA and ascorbic acid in which 59.9 % of CGA was regenerated by ascorbic acid. Keywords: Chlorogenic acid, ascorbic acid, regeneration, synergism, *tert*-butoxyl radicals, oxidation.

INTRODUCTION

Reactive oxygen species (ROS) and their likely involvement in some human physiopathologies have attracted growing interest from the health sector over few decades. Oxidative stress, caused by an imbalance between antioxidant systems and the production of oxidants, including ROS, seems to be associated with many multifactorial diseases, especially cancers, cardiovascular diseases and inflammatory disorders.[1-8] The mechanisms by which these pathologies develop generally involve oxidative alteration of physiologically critical molecules, including proteins, lipids, carbohydrates and nucleic acids, along with modulation of gene expression and the inflammatory response.[9-12] Oxidative DNA damage has been thought to be an important source of mutation leading to aging[7] and a wide range of degenerative diseases such as immune-system decline, brain dysfunction and cataracts.[8] Organic peroxides form an important part of various chemical, pharmaceutical and cosmetic products. Upon reduction or oxidation by the cytochrome P450 enzyme family, by other heme proteins and by low molecular weight metal ion complexes, these hydroperoxides produce alkoxyl and hydroxyl radicals.

Although lethal effects of the hydroxyl radicals on DNA and its constituents have been studied[4] extensively, relatively little is known about the biological effects of alkoxyl radicals and the key cellular targets for these species. Organic oxygen radicals, particularly alkoxyl radicals may participate in metabolic and pathological processes.[9] *tert*-Butyl hydroperoxide (*t*-BuOOH) has been chosen as a model peroxide which on homolysis gives $^{\bullet}$OH and *t*-BuO$^{\bullet}$ radicals. Previous studies on the reactivity of *tert*-butoxyl radicals suggest that these species might be expected to attack both the sugar and the base moieties of DNA.[10] The experimental evidence indicates that base radicals also contribute to strand breaks by transfer of their radical sites from base moiety to sugar moiety.

Antioxidants are substances, when present in small quantities prevent or delay the oxidation of cellular organelles by minimizing the damaging effects of ROS/RNS or oxidative stress. Many studies have now confirmed that exogenic antioxidants, especially supplied by foods, are essential for counteracting oxidative stress.[11-13] These antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids. They are required in the management of pathophysiological conditions, most of which involve free radical damage.

The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last 20 years.

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] In a combination of two or more free radical scavengers, rapid reaction with free radicals occurs because of the differences in bond dissociation energies (BDE) or steric hindrance of free radical scavenger.[15] These differences result in one scavenger being used faster than the other and regenerate the primary scavenger by transferring its radical to another scavenger.

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. Interaction among antioxidants can be synergistic, antagonistic or merely additive. Peyrat-Maillard *et al.* have studied the synergistic and antagonistic effects occurring between pairs of phenolic antioxidants in a mixture.[17] A synergistic or antagonistic effects occurring between pairs of antioxidants can be partly explained by regeneration mechanisms, depending on the chemical structure of molecules and on the possible formation of stable intermolecular complexes.[18,19] 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. The antioxidant system of ascorbic acid and tocopherols is an example in which tocopherols (E = 500 mV) act as primary antioxidant and ascorbic acid (E = 330 mV) act as a synergist.[20] Ascorbic acid donates a hydrogen atom to α-tocopheryl radical and thus regenerate α-tocopherol. In addition to that, it can shift the redox potential of food systems to the reducing range and can act synergistically with chelators and regenerate primary antioxidants other than tocopherols.

Ascorbic acid (AA) or vitamin-C is widespread in nature but sparingly associated with fats of oils because of its hydrophilic nature.[21] Ascorbic acid in the free form is the commonly used antioxidant in foods. In vivo, ascorbic acid acts as a primary antioxidant and in tissues it is essential for the prevention of oxidative cellular damage by hydrogen peroxide. In foods, water soluble ascorbic acid acts as a secondary antioxidant and participates in various antioxidative and related functions. Ascorbic acid is capable of quenching various forms of oxygen derived radicals (singlet oxygen, hydroxyl radicals and superoxide). Recently, it was

demonstrated that ascorbic acid is indeed capable of reducing the α-tocopheroxyl radicals in miscellar and membrane systems.[22,23]

Chlorogenic acid (CGA), an ester of caffeic acid with quinic acid, is found in a wide range of fruits and vegetables. Coffee, one of the most widely consumed beverages in the world, contains high amounts of CGA. Phenolcarboxylic acids such as CGA exert beneficial effects on human health through prevention of degenerative pathologies such as cardiovascular diseases and cancer.[24,25] It was found that CGA inhibited NO production in lipopolysaccharide (LPS) stimulated mouse macrophage like cells (RAW 264.7 cells) and scavenged various radicals such as superoxide anions and hydroxyl radicals. It scavenges radicals generated in the aqueous phase[26], increases the resistance of LDL to lipid peroxidation[27] and inhibits DNA damage.[28] In vivo, when added to the diet, it inhibits chemically induced carcinogenesis of the large intestine, liver and tongue in rats and hamsters.[29,30]

Antioxidants such as CGA do not act in isolation, but rather constitute an intricate network in the presence of coantioxidants such as glutathione, ascorbate or other phenolic compounds such as α-tocopherol.[25] There remains a great interest in the possible health promoting effects of antioxidants, but the synergistic mechanism by which these compounds coexist in foods is in need of further study. The regeneration effect of one antioxidant by another antioxidant is a potentially beneficial reaction that needs to be further studied in human and animal models. It is in this context that a systematic kinetic study of interaction of CGA with ascorbic acid in the presence of *t*-BuO$^{\bullet}$ radicals was carried out to get an insight into the possible synergistic/antagonistic molecular mechanisms which help in selection of co-antioxidants while adding to the food preservatives.

MATERIALS AND METHODS

CGA and ascorbic acid 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.[31] 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.[32] 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.[33] The $^{\bullet}$OH radicals produced were scavenged using sufficient concentration of *t*-BuOH.[34] In a typical kinetic run, the aqueous reaction mixture of CGA 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 CGA (328 nm) on a Chemito UV-Visible spectrophotometer (model 2100). The photochemical reaction of CGA in the presence of *t*-BuOOH was followed by measuring the absorbance of chlorogenic acid at 328 nm at which ascorbic acid was totally transparent. It is known that *t*-BuOOH is activated to radical reaction by the absorption of light at 254 nm.[32] However, the substrates used in the present work, viz., CGA and ascorbic acid have strong absorption in this region. But, in the absence of *t*-BuOOH in the reaction mixture, CGA, ascorbic acid or CGA - ascorbic acid 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 ascorbic acid and/or CGA, a considerable chemical change was observed with ascorbic acid as well as CGA. If ascorbic acid and CGA acted as only inner filters, the rates of the reaction of ascorbic acid or CGA with *t*-BuO$^{\bullet}$ radicals would have been decreased with increase in concentration of ascorbic acid or CGA. 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 CGA in the presence of ascorbic acid would have been much less than the experimentally observed values (Table 4). Hence, we proposed that the excited states of CGA and ascorbic acid 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 ascorbic acid as well as CGA under different experimental conditions.

RESULTS AND DISCUSSIONS

The oxidation of ascorbic acid by *t*-BuO$^{\bullet}$ radicals was carried out by irradiating the reaction mixture containing known concentrations of ascorbic acid and *t*-BuOOH in the presence of sufficient amount of *t*-BuOH to scavenge the $^{\bullet}$OH radicals completely.[32] The reaction was followed by measuring the absorbance of ascorbic acid at 265 nm (λ_{max} of ascorbic acid) with

time. The initial rates and quantum yields of oxidation of ascorbic acid by *t*-BuO$^{\bullet}$ are presented in Table 1. The initial rates of photooxidation of CGA by *t*-BuOOH in presence of *t*-BuOH were calculated from the plots of absorbance of CGA at 328 nm *vs* time using microcal origin computer program on a personal computer (Table 2). UV-visible absorption spectra of CGA in presence of *t*-BuOOH and *t*-BuOH at different irradiation times were recorded (Fig. 1). In order to find the protection offered to ascorbic acid by CGA towards oxidation by *t*-BuO$^{\bullet}$, the reaction mixture containing known concentrations of ascorbic acid and *t*-BuOOH was irradiated in presence of varying concentrations of CGA. The photooxidation of CGA by *t*-BuO$^{\bullet}$ at different concentrations of ascorbic acid was also studied (Fig. 2) and the data are presented in Table 3. The reactions were followed by measuring the absorbance of CGA at 328 nm (Fig. 3) at which ascorbic acid was transparent and the rate data are presented in Table 4. The oxidation rate of ascorbic acid in the presence of *t*-BuOH refers exclusively to the reaction of *t*-BuO$^{\bullet}$ with ascorbic acid. These rates were found to increase with increase in concentration of ascorbic acid as well as *t*-BuOOH. The quantum yield values were also increased with increase in [ascorbic acid] as well as [*t*-BuOOH] (Table 1). The rate of oxidation of CGA increased with increase in concentration of CGA (Table 2). The quantum yields of oxidation of CGA were calculated from the initial rates and the light intensity at 254 nm. These values were also increased with increase in concentration of CGA (Table 2). Having known the rates of *t*-BuO$^{\bullet}$ radical reactions with ascorbic acid as well as CGA (Table 2) under various experimental conditions, both ascorbic acid and CGA were introduced for the competitive studies with *t*-BuO$^{\bullet}$ radical.

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

10^5 × [ascorbic acid] (mol dm^{-3})	10^3 × [*t*-BuOOH] (mol dm^{-3})	10^8 × Initial rate (mol dm^{-3} s^{-1})	Quantum yield (φ)
0.5	5.0	5.4483	0.03623
0.8	5.0	6.9531	0.04624
1.0	5.0	8.5443	0.05682
2.0	5.0	9.9211	0.06598
2.0	10.0	11.213	0.07457
2.0	15.0	12.682	0.08434

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 265 nm, pH ~ 7.5, Temperature = 298 K

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

10^6 × [CGA] (mol dm^{-3})	10^3 × [*t*-BuOOH] (mol dm^{-3})	10^9 × Initial rate (mol dm^{-3} s^{-1})	Quantum yield (φ)
20.0	5.0	9.6908	0.00644
10.0	5.0	7.0008	0.00465
8.0	5.0	5.2798	0.00351
5.0	5.0	2.7845	0.00185
2.0	5.0	2.2974	0.00152
20.0	10.0	11.203	0.00745
20.0	15.0	13.157	0.00875

Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

Aqueous solutions of reaction mixture containing ascorbic acid and *t*-BuOOH were irradiated in presence of varying concentrations of CGA (Fig. 2). The initial rates and quantum yields of oxidation of CGA by *t*-BuO$^{\bullet}$ radicals were found to decrease with increase in concentration of ascorbic acid (Table 4). Comparison of the initial rates and quantum yields of oxidation of ascorbic acid in presence and absence of CGA clearly indicated that the initial rates and quantum yields of oxidation of ascorbic acid were substantially decreased in presence of CGA (Table 4). These observations clearly demonstrated that ascorbic acid and CGA was in competition for *t*-BuO$^{\bullet}$ radicals. The rate constant of the reaction of *t*-BuO$^{\bullet}$ with CGA has been reported[35] to be 3.20×10^9 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 ascorbic acid was calculated by the adenosine competition method, which was very similar to the method[36] 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 ascorbic acid and varying amounts of CGA in presence of *t*-BuOOH was irradiated for 2 min and the decrease in absorbance of CGA was measured. The decrease in absorbance of CGA reflected the amount of *t*-BuO$^{\bullet}$ radicals that had reacted with CGA. From the known rate constant of the reaction of CGA with *t*-BuO$^{\bullet}$ radical under similar experimental conditions of the present work (k_{CGA} = 3.20×10^9 dm^3 mol^{-1} s^{-1}), the rate constant of *t*-BuO$^{\bullet}$ radical reaction with ascorbic acid ($k_{\text{ascorbic acid}}$) can be calculated using the following equation:

$$\frac{[\text{Absorbance of ascorbic acid}]_o}{[\text{Absorbance of ascorbic acid}]_{\text{chlorogenic acid}}} = 1 + \frac{k_{\text{chlorogenic acid}}\,[\text{chlorogenic acid}]}{k_{\text{ascorbic acid}}\,[\text{ascorbic acid}]}$$

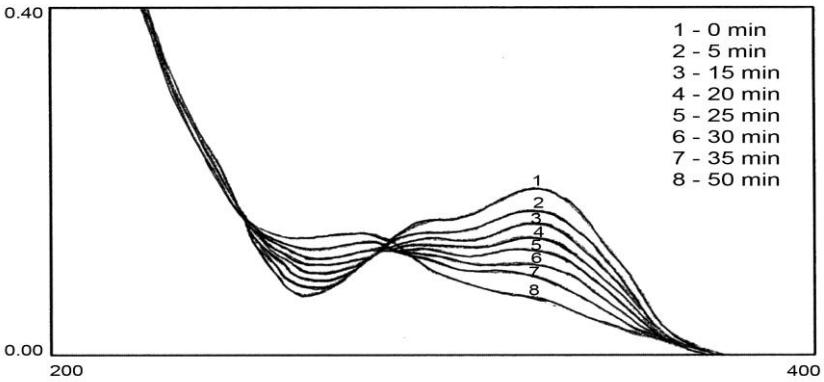


Fig. 1

Absorption spectra of photooxidation of CGA in the presence of *tert*-butyl hydroperoxide at different irradiation times in *t*-BuOH-water (1:4 v/v) medium, [CGA] = 10.0×10^{-6} mol dm^{-3}, [*t*-BuOOH] = 5×10^{-3} mol dm^{-3}, Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, temperature = 298 K

In Eq. (1), [Absorbance of ascorbic acid]$_o$ and [Absorbance of ascorbic acid]$_{chlorogenic\ acid}$ are the absorbance values of ascorbic acid in the absence and presence of CGA, 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 ascorbic acid ($k_{ascorbic\ acid}$) was calculated at different concentrations of CGA and ascorbic acid and the average of these was found to be 6.65×10^9 dm^3 mol^{-1} s^{-1}. As ascorbic acid had strong absorption at 294 nm, it is not possible for the direct determination of protection and repair offered to CGA by ascorbic acid. However, one could calculate indirectly the extent of protection offered to CGA by ascorbic acid from competition kinetic studies measured at 328 nm, λ_{max} of CGA. The method was as follows:

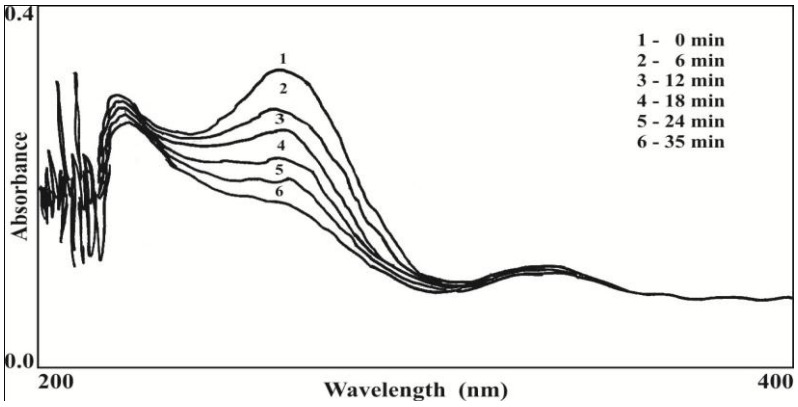


Fig. 2

Absorption spectra of photooxidation of CGA in the presence of *tert*-butyl hydroperoxide and ascorbic acid at different irradiation times; [CGA] = 2×10^{-5} mol dm^{-3}, [*t*-BuOOH] = 5×10^{-3} mol dm^{-3}, [ascorbic acid] = 2×10^{-5} mol dm^{-3}, Light Intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, temperature = 298 K

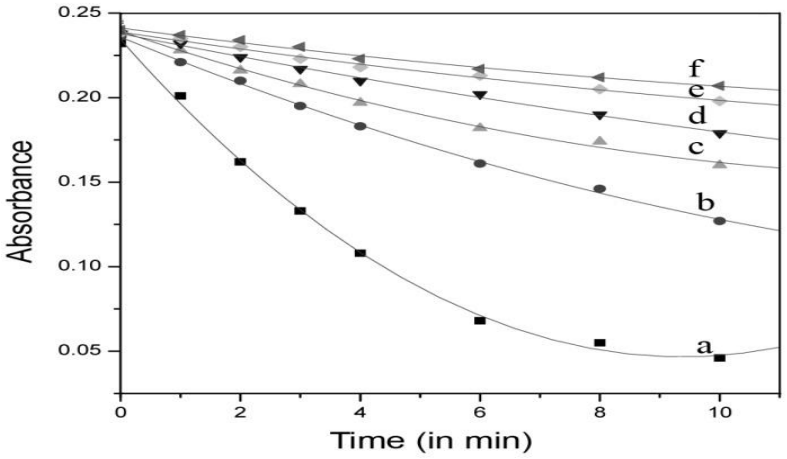


Fig. 3

Effect of [CGA] on the oxidation of ascorbic acid by *t*-BuO• in *t*-BuOH-water 1:4 (v/v) neutral medium.

When the system containing ascorbic acid, CGA and *t*-BuOOH was irradiated, the probability of *t*-BuO• radicals reacting with ascorbic acid $\{p_{(t\text{-}BuO^{\bullet} + \text{ ascorbic acid})}\}$was calculated using the following equation:

$$p_{(t\text{-}BuO^{\bullet} + \text{ascorbic acid})} = \frac{k_{\text{ascorbic acid}}\,[\text{ascorbic acid}]}{k_{\text{chlorogenic acid}}\,[\text{chlorogenic acid}] + k_{\text{ascorbic acid}}\,[\text{ascorbic acid}]}$$

If ascorbic acid scavenged only *t*-BuO• radicals and did not give rise to any other reaction (e.g. reaction with CGA radicals), the quantum yield of oxidation of CGA (ϕ_{cal}) at each concentration of ascorbic acid may be given by equation:

where ϕ^{o}_{expt} is the quantum yield of oxidation of CGA in the absence of ascorbic acid, and *p* is the probability given by Eq. (2).

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

$10^5 \times$ [ascorbic acid] (mol dm^{-3})	$10^5 \times$ [CGA] (mol dm^{-3})	$10^8 \times$ Rate (mol dm^{-3} s^{-1})	Quantum yields ϕ
2.0	0.0	9.9211	0.06598
1.0	0.0	8.5443	0.05682
0.8	0.0	6.9531	0.04624
0.5	0.0	5.4483	0.03623
2.0	2.0	5.1542	0.03428
1.0	2.0	3.8324	0.02548
0.8	2.0	2.2136	0.01472
0.5	2.0	1.8334	0.01219

[t-BuOOH] = 5.0×10^{-3} mol dm^{-3}, Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

The calculated quantum yield (ϕ_{cal}) values at different CGA 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 ascorbic acid molecules was consumed in the system than expected and the most likely route for this was H atom donation by ascorbic acid to CGA radicals. In Table 4, are presented the fraction of *t*-BuO• radicals scavenged by ascorbic acid at different CGA concentrations. These values referred to the measure of protection offered to CGA due to scavenging of *t*-BuO• radicals by ascorbic acid. 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 CGA radicals by ascorbic acid occurs. In the absence of any "repair" of CGA radicals by ascorbic acid, the ϕ' values should all be equal to ϕ^o_{expt}. The observed increase in ϕ' with increasing CGA concentration (Table 4) clearly indicated the repair of CGA radicals. The extent of repair may be quantified by the following equation:

$$\% \text{ Regeneration} = \frac{(\phi' - \phi^o_{expt})}{\phi^o_{expt}} \times 100$$

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 ascorbic acid acts only as a *t*-BuO$^{\bullet}$ radical scavenger. This showed that ascorbic acid acted not only as an efficient scavenger of *t*-BuO$^{\bullet}$ radicals, but also as an agent for the repair of CGA radicals.

The nature of interactions of CGA with other antioxidants is essential for understanding the effects of this compound in oxidative stress conditions in vivo. Whether a synergistic or antagonistic effect is observed for the mixtures derived from radical oxidation may depend on the chemical structure of the molecules, Bond Dissociation Energy (BDE), redox potentials, microenvironment of the reaction and the possible formation of stable intermolecular complexes.

Table 4 – Effect of varying [CGA] on the rate and quantum yield of photooxidation of ascorbic acid in the presence of *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium

$10^5 \times$ [CGA] (mol dm^{-3})	$10^8 \times$ Rate (mol dm^{-3} s^{-1})	ϕ_{expt}	ϕ_{cal}	p	ϕ'	% scavenging	% regeneration
0.0	9.9211	0.06598	0.06598	1.0000	0.06598	100.0	0.0
0.2	8.3714	0.05567	0.05462	0.8279	0.06725	82.79	1.9
0.5	7.0568	0.04693	0.04342	0.6581	0.07131	65.81	8.1
0.8	6.3477	0.04221	0.03603	0.5461	0.07730	54.61	17.2
1.0	6.0882	0.04049	0.03235	0.4904	0.08256	49.04	25.1
2.0	5.8343	0.03428	0.02143	0.3248	0.10554	32.48	59.9

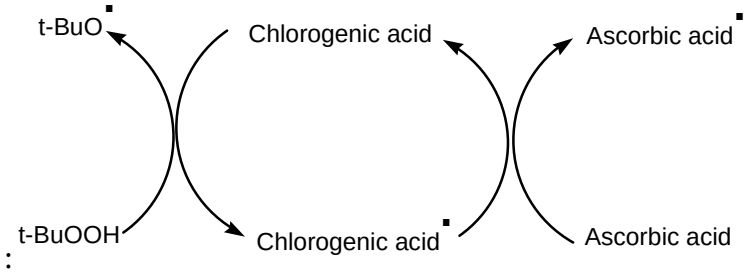
[ascorbic acid] = 2.0×10^{-5} mol dm^{-3}, [t-BuOOH] = 5.0×10^{-3} mol dm^{-3},
Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

In general, regeneration between antioxidants occurs when BDE of an antioxidant is lower, or at least similar to that of other antioxidants.[37,38] The BDE of CGA (~80.0 kcal/mol) is higher than that of ascorbic acid (73.2 kcal/mol) for the removal of weaker hydrogen in the molecule. An antioxidant with higher antioxidant power (AOP) is regenerated by an antioxidant with lower AOP is termed as 'synergistic interaction'. CGA with higher AOP is regenerated by ascorbic acid having lower AOP to an extent of 59.9 %.

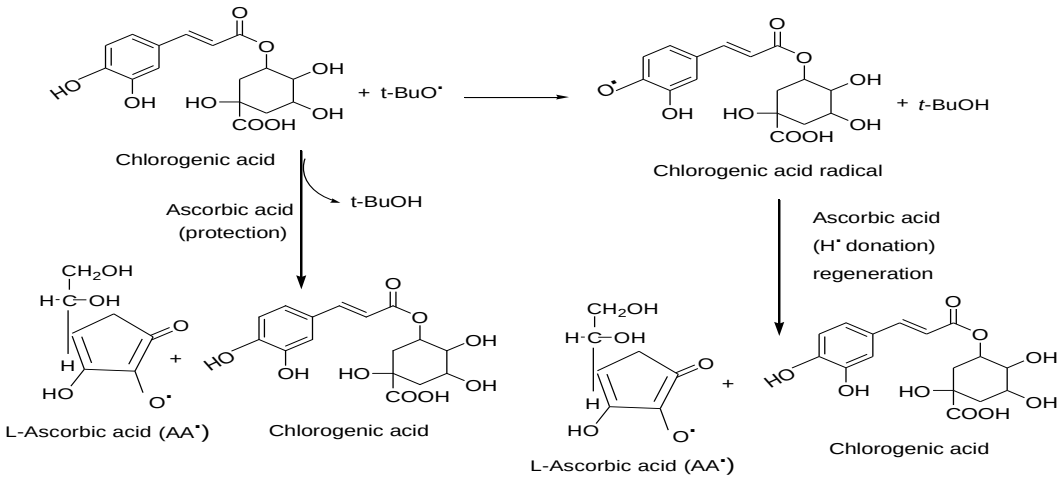
$$AA + CGA^{\bullet} \longrightarrow CGA + AA^{\bullet}$$

The redox potential of CGA (0.54 V) and ascorbic acid (0.28V) are also in conformity with the results in Table 4. Relatively higher redox potential of CGA makes it behave as primary

antioxidant and chlorogenic acid semiquinone radicals formed by the reaction of CGA with *t*-BuO• radicals are regenerated by ascorbic acid (AA) as shown below



The proposed mechanism for the reaction between CGA and ascorbic acid may involve the reduction of CGA radical by ascorbic acid at the LDL surface with concomitant production of CGA o-semiquinone (phenoxyl radical). On the basis of the experimental results and the above discussion, the scheme for the synergistic interaction of ascorbic acid with CGA and regeneration of CGA from CGA radical by ascorbic acid is given as follows:



Comparison of the results for the photooxidation of ascorbic acid in the absence and presence of CGA suggested that ascorbic acid acts as a synergist and CGA acts as a primary antioxidant. Stable intermolecular complexes could be formed between ascorbic acid and CGA as shown above which is similar to anthocyanin and CA or rutin in the co-pigmentation mechanism.[39,18] These interactions could be due to π-π stacking between the aromatic ring

of phenolic acid and ascorbic acid by hydrogen-bonding effects that would help in stabilizing the complex.[19] A higher stability of the complex formed between CGA with ascorbic acid due to better structural analogy and additional bonding between the two molecules, could explain 59.9% of CGA regeneration by ascorbic acid.

Possible interactions occurring in ascorbic acid/ CGA complex

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