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# Effect of polar solvents on $\beta$-carotene radical precursor

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## Abstract

$\beta$-Carotene forms radicals in chloroform upon photo-excitation (i) in the femtosecond time-scale by direct electron ejection into chloroform and (ii) in the microsecond time-scale by secondary reactions with chloroform radicals formed in the faster reactions. The precursor for $\beta$-carotene radical cation decays in a second-order reaction in the mixed solvents, with a rate decreasing for increasing dielectric constant of cosolvent (acetic acid $<$ ethanol $<$ acetonitrile $\sim$ methanol). The precursor is assigned as an ion pair from which the $\beta$-carotene radical cation is formed in neat chloroform, but in more polar solvents it reacts at least partly through disproportionation in a bimolecular reaction promoted by the presence of ions. The stabilization of the radical precursor by increased solvent polarity, allowing for deactivation of the precursor by an alternative reaction channel, is discussed in relation to the balance of pro- and antioxidative properties of $\beta$-carotene at lipid/water interfaces.

**Keywords:** β-*Carotene, radical, solvent effect, antioxidant*

## Introduction

Antioxidative effects of carotenoids in food can be assigned to three mechanisms: (i) scavenging of reactive radicals; (ii) quenching of reactive excited states of photosensitizers; and (iii) quenching of singlet oxygen [1,2]. Quenching of excited states by carotenoids yielding protection against light induced oxidation is well-established and is important for many food products [3]. A clear antioxidative effect of carotenoids against oxidation of lipids induced by radicals has also been clearly demonstrated in food model systems [4]. Such effects on thermal oxidation have been difficult to confirm for food with carotenoids added. There is, however, some indication that carotenoids act as antioxidants in conjugation with phenolic compounds like the tocopherols and other plant phenols [5–7]. Such antioxidant synergism as seen in liposomes seems to depend on a regeneration of the carotenoid from one-electron oxidized carotenoid radicals by the phenolic compounds [8,9].

Carotenoid radicals and their formation and decay have mainly been studied in lipophilic solvents in which carotenoids are soluble. The interaction between one-electron oxidized carotenoids and plant phenols other than the tocopherols are, however, expected to occur at the water/lipid interface of membranes where the lifetime of the carotenoid radicals also may be important for the balance between pro- and antioxidative effects of carotenoids [6,7]. Solvents effect was recently shown to be important for electron transfer processes including carotenoids [10]. In order to study the reactivity of

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carotenoid radicals and their precursors under conditions of increasing solvent polarity (as at the water/lipid interface), we have designed experiments in which decay of one-electron oxidized carotenoid could be followed in solvent mixtures using laser flash photolysis.

## Materials and methods

### *Chemicals*

$\beta$-Carotene was from the same sources and purified as previously described [11]. Methanol and acetonitrile (HPLC grade, Caledon Laboratories, Georgetown, Ontario, Canada) for spectroscopy were used as received. Chloroform ($>99.0\%$, Beijing Chemical Plant, Beijing, China) was purified by passing through an alumina column (Wusi Chemical Reagent Ltd., Shanghai, China) before use. Acetic acid ($>99.5\%$, Wendaxigui Chemical Plant, Tianjin, China), ethanol ($>99.0\%$, Beijing Chemical Plant) and tetramethylammonium hydroxide (97%, Sigma, St. Louis, MO) were used as received. Tetramethylammonium acetate was made *in situ* from acetic acid and tetramethylammonium hydroxide by neutralization.

### *Laser flash photolysis*

Sub-microsecond time-resolved absorption spectra were recorded at room temperature as described previously [11,12]. The excitation laser pulses at 532 nm with a repetition of 1 Hz were generated from a $Nd^{3+}$:YAG laser (Tempest 300, New Wave Research). The pump energy was $\sim 5$ mJ/pulse at the sample cell. The optical path length of the flowing-type cuvette was 5 mm. A volume of sample (25 mL) was kept in an ice-cooled reservoir and was circulated between the reservoir and the sample cuvette. A set-up based on a gated photodiode array detector (1420, EG&G, Princeton, NJ) was used for measuring the multicolour difference absorption spectra in the visible region. NIR kinetics at individual wavelengths was recorded with a xenon lamp probe (CW, 500 W) and an APD detector (C5460, Hamamatsu Photonics, Hamamatsu, Japan) and the transient was digitized by an oscilloscope (TDS620B, Tektronix, Beaverton, OR). Analyses of the time-resolved spectral data was done based on Matlab 5.2 (Mathworks Inc., Natick, MA), Mathcad 2001 (Parametric Technology Corp., Needham, MA) and Origin 7.0 (OriginLab Corp., Northampton, MA).

## Results

Upon excitation of $\beta$-carotene in chloroform by laser flash photolysis, an instantaneous bleaching was seen on the nanosecond time-scale parallelled by the rise of structured absorption bands in the visible and near infrared spectral region (Figure 1A). The time evolutions for the maximum absorption at 550 nm and 720 nm in Figure 1B could not be described by a single exponential, but the observation is in agreement with previous findings [13], i.e. a faster component in the microsecond time-scale and a slower component in the millisecond time-scale. The absorption with maximum at 550 nm and 720 nm decayed in the fast reaction with similar time constants around 10 µs, which moreover was similar to the time constant for the rise of the absorption at 1000 nm. Based on these spectral information and previous assignments [12,13], we suggest the reaction sequence as shown in Scheme 1.

The initial $\beta$-carotene radical cation $\beta\text{-Car}^{\bullet+}$ and a chloroform radical $CHCl_3^{\bullet-}$ formed in the femtosecond time-scale by direct electron ejection from singlet excited state to chloroform, as evidenced by an instantaneous absorption on the nanosecond time-scale, and is followed by formation of a $\beta$-carotene intermediate species absorbing at $\sim 720$ nm assigned as an ion pair between the $\beta$-carotene radical cation and $CHCl_2^-$ formed by reaction of $CHCl_2^{\bullet}$ with $\beta$-carotene. The absorption around 550 nm has been assigned to an addition radical in which electron transfer from $\beta$-carotene to the solvent radical

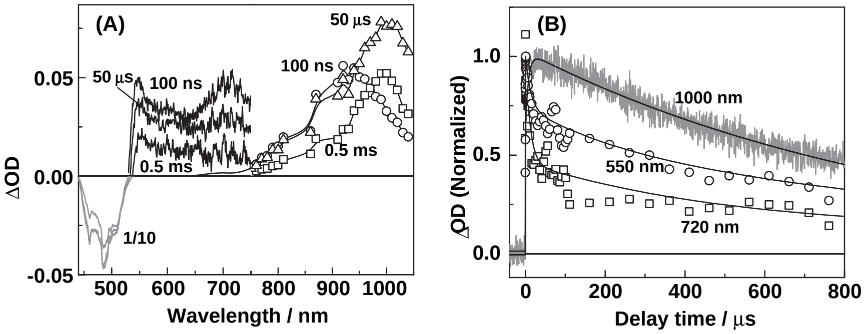

Figure 1. Transient absorption spectra at indicated delay times (A) and time evolution of absorbance at indicated wavelengths (B) for $\beta$-carotene ($5 \times 10^{-5}$ M) in chloroform following 532 nm excitation. The spectra in visible region were obtained by multi-channel detection and in near infrared region constructed from time dependence of absorption at single-wavelengths. The solid lines in (B) are bi-exponential fits to the experimental points serving as guides to the eyes.

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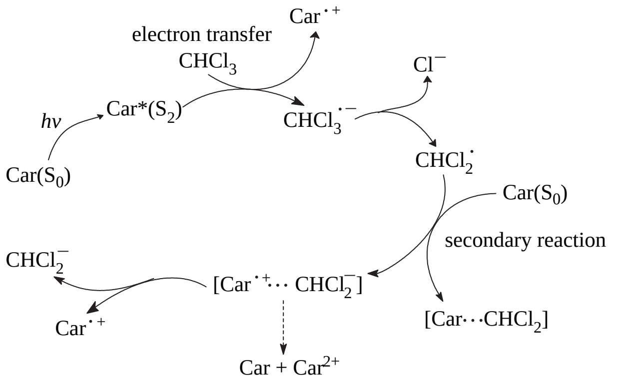

Scheme 1. Reaction sequence following excitation of $\beta$-carotene in chloroform or chloroform rich solvents with direct formation of $\beta$-carotene radical cation and a chloroform radical initiating further $\beta$-carotene radical cation formation.

$CHCl_2^{\bullet}$ has not occurred [14]. The [$\beta$-$Car^{\bullet +}$ … $CHCl_2^-$] ion pair is suggested to be the precursor of $\beta$-$Car^{\bullet +}$ absorbing at 1000 nm and longer wavelengths.

For excitation under otherwise identical conditions in chloroform/methanol (3:1), the transient absorption spectra at similar delay times, 100 ns, 50 µs and 0.5 ms, are shown in Figure 2A. Less bleaching and more positive absorption at longer wavelength were instantaneously observed. As compared to the evolutions in chloroform in Figure 1B, it is evident the time evolutions in the mixed solvent shown in Figure 2B were different. The formation of the species absorbing around 720 nm continued for a longer time than in neat chloroform and the decay was slower, as was the formation of the species absorbing at 1000 nm and around 800 nm as a spectral feature not seen for chloroform. Notably, the decay of the absorption at 720 nm and 550 nm in the mixed solvent had qualitatively different time profiles and the 720 nm decay did not correspond to the rise at 1000 nm.

Other polar cosolvents (acetonitrile, ethanol and acetic acid) than methanol had a similar effect on the lifetimes of the radical cation precursor as seen in Figure 3, in which the evolutions at 720 nm are seen to be more sensitive to the solvent polarity than the evolutions at 550 nm. This difference in decay rate is important since it indicates that two different species are present despite the similar rate of decay in neat chloroform. The decay of the absorption at 720 nm in the mixed solvents, but not in neat chloroform, was found to follow second-order kinetics, as seen for the chloroform/methanol 3:1 mixture in Figure 4A. A calculation of the second-order rate constant would require conversion of absorbance to a concentration that depends on the unavailable numerical value of the extinction coefficient $\varepsilon$ of reacting species. Since ΔOD are normalized in Figure 3, the relative rate constants at 720 nm obtained for the chloroform/methanol 3:1 mixture as seen in Figure 4B and for the three other mixed solvents are directly comparable. The solvent effects on the second-order rate constant were analysed in terms of the dielectric constant of the mixed solvents, as calculated following Jouyban et al. [15], and the value $\log(k_2)$ is seen to depend linearly on $(\varepsilon - 1)/(2\varepsilon + 1)$, as shown in Figure 4B, which is in agreement with theoretical

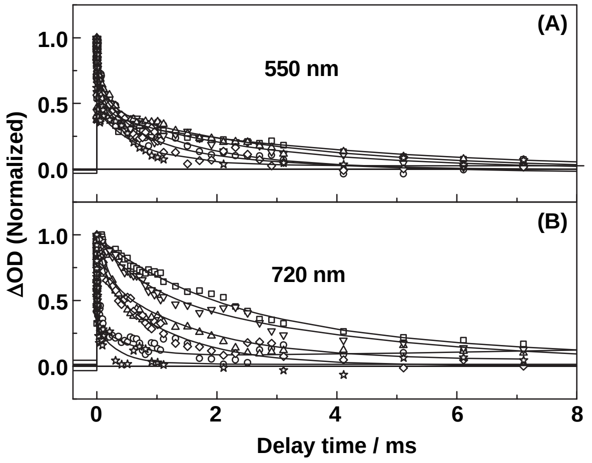

Figure 3. Time evolutions of absorbance of $\beta$-carotene ($5 \times 10^{-5}$ M) in the binary chloroform 3:1 solvents with acetonitrile (□), methanol (▽), ethanol (△), acetic acid (◇) or n-hexane (☆) as cosolvents and in neat chloroform (○) at 550 nm (A) and 720 nm (B) following 532 nm excitation.

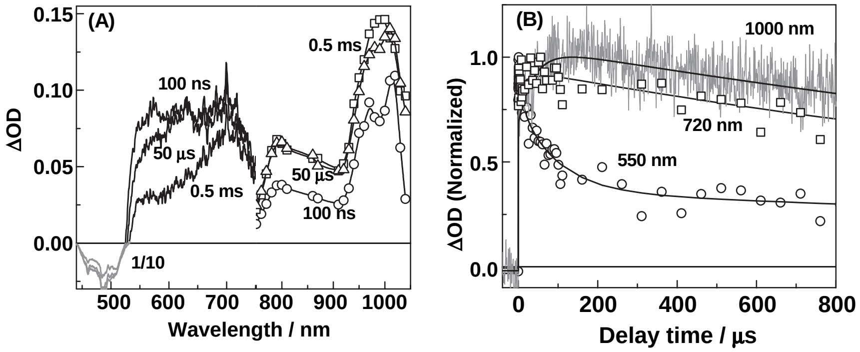

Figure 2. Transient absorption spectra at indicated delay times (A) and time evolution of absorption at indicated wavelengths (B) for $\beta$-carotene ($5 \times 10^{-5}$ M) in chloroform/methanol (3:1) following 532 nm excitation. The spectra in visible region were obtained by multichannel detection and in near infrared region constructed from time dependence of absorption at single-wavelengths. The solid lines in (B) are bi-exponential fits to the experimental points serving as guides to the eyes.

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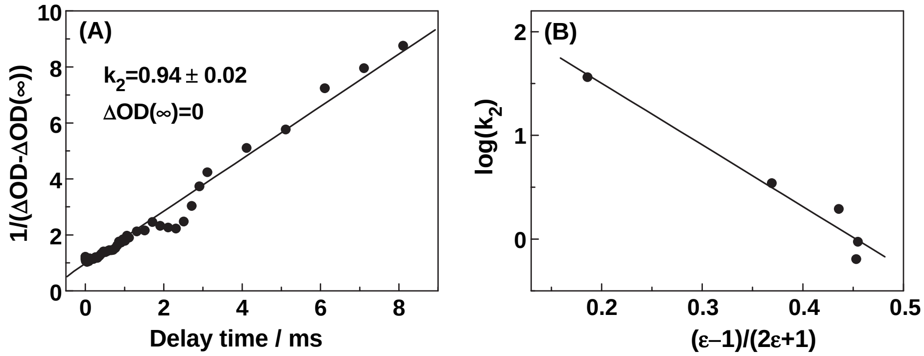

Figure 4. (A) Second-order plot for decay of absorbance at 720 nm for $\beta$-carotene ($5 \times 10^{-5}$ M) in binary chloroform/methanol 3:1 solvent with ΔOD = 0 at infinity time, cf. Figure 3B. The slope provides the relative second-order rate constant. Similar second-order plots were obtained for the other mixed solvent but not neat chloroform. (B) Logarithmic plot of dependence of relative second-order rate constant on solvent dielectric constant for the solvent mixtures as calculated accordingly to Jouyban et al. [15] from the dielectric constant for the solvent components.

predictions for a reaction between polar molecules [16].

Regarding the possible influence from the protic and aprotic properties of solvents, we have examined the kinetics for $\beta$-carotene in the chloroform/$n$-hexane 3:1 mixture (Figure 3). It is seen from Figure 3B that the 720-nm trace for the case of $n$-hexane decays significantly faster than other cases. $n$-Hexane is both non-polar and aprotic. Therefore, this observation favours a dielectric constant dependence of the kinetic behaviour of the $\beta$-Car$^{\bullet +}$ precursor, as further proven by the $\log(k_2) \sim (\varepsilon - 1)/(2\varepsilon + 1)$ correlation plotted in Figure 4B.

The decay of the species absorbing at 720 nm was further studied in the presence of increasing concentration of tetramethylammonium hydroxide as a base, acetic acid as an acid and tetramethylammonium acetate as a salt in the chloroform/methanol 3:1 mixture. The base and the salt but not the acid increased the rate of decay of the species absorbing at 720 nm, as seen in Figure 5. The presence of tetramethylammonium hydroxide was not found to affect the reaction order of the decay, as seen for the second-order analysis of the reaction in Figure 6A. The relative second-order rate constants showed a linear dependence on the concentration of tetramethylammonium hydroxide (Figure 6B) and a similar effect is seen for tetramethylammonium acetate.

## Discussion

The nature of the solvent plays an important role for the balance between pro- and antioxidative effects of carotenoids [17]. The change in solvent from neat chloroform to a 3:1 mixture of chloroform and methanol was found to decouple the formation of the species absorbing around 1000 nm from the decay of the species absorbing with a maximum at 720 nm, as is clearly seen from comparison between Figures 1 and 2. The decay of the species absorbing at 720 nm has been found to show a biexponential decay in a previous study [13], while this decay for the mixed solvents including the chloroform/methanol mixture

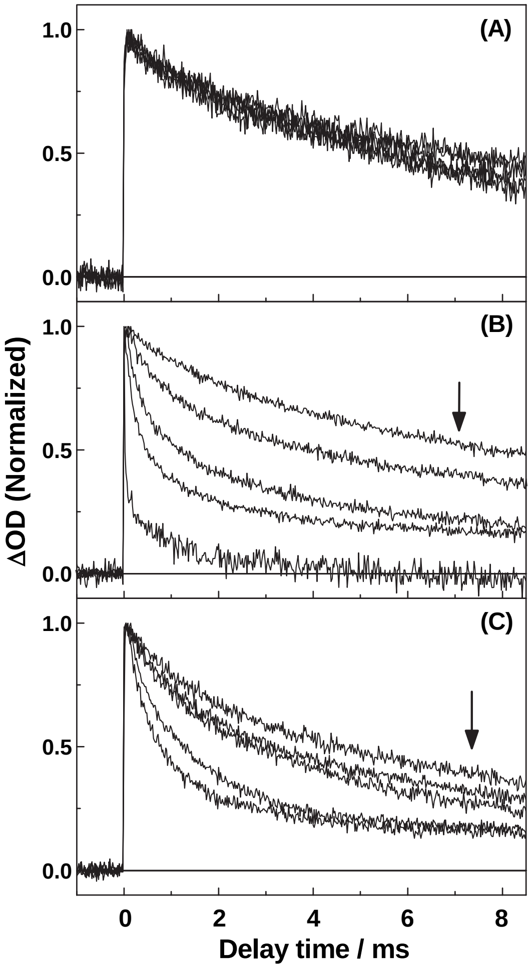

Figure 5. Time evolutions of $\beta$-carotene ($5 \times 10^{-5}$ M) in the chloroform/methanol mixture (3:1) at 720 nm following 532 nm excitation for increasing concentration (0 M; $8 \times 10^{-5}$ M; $4 \times 10^{-4}$ M; $8 \times 10^{-4}$ M; $1.6 \times 10^{-3}$ M) of acetic acid (A), of $(CH_3)_4N^+OH^-$ (B) and of $CH_3COON(CH_3)_4$ (C).

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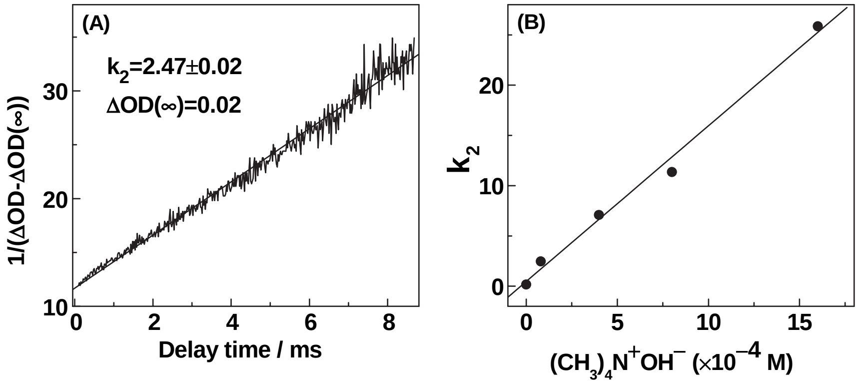

Figure 6. (A) Second-order plot for decay of absorbance at 720 nm for β-carotene in binary chloroform/methanol mixture (3:1) with $\Delta OD = 0.02$ at infinity time (in presence of $8 \times 10^{-5}$ M $(CH_3)_4N^+OH^-$), cf. Figure 5. The slope provides the relative second-order rate constant. (B) Dependence of the relative second-order rate constants on the concentration of tetramethylammonium hydroxide.

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could be described as a second-order reaction (Figure 4A). The notable difference can be explained by the formation of another β-carotene derivative than β-Car$^{\bullet+}$ also absorbing around 1000 nm. The two-electron oxidized form of β-carotene, which is known to have absorption with a maximum around 800 nm [18], could be formed by a disproportionation reaction also incorporated in Scheme 1:

$$[\beta\text{-Car}\cdots\text{CHC12}]^{\bullet} \rightarrow \beta\text{-Car}^{\bullet} + +\text{CHC12}^{-} \qquad (1)$$

The two-electron oxidized form of β-carotene is expected to be longer lived than β-Car$^{\bullet+}$, which explains the increasing absorption at 1000 nm for a delay time up to 0.5 ms in chloroform/methanol, the decrease in absorption in chloroform for the same time span, as seen from a comparison between the transient spectra of 50 μs and 0.5 ms obtained for photolysis of β-carotene in the two solvents. Notably, in the mixed solvent an absorption maximum around 800 nm appears which is not seen in chloroform in support of this assignment. While formation of the β-carotene radical cation from the precursor previously assigned as an adduct:

$$\beta\text{-Car}\cdots\text{CHCl}_2{}^{\bullet+} + \text{CHCl}_2^{-} \qquad (2)$$

seems to dominate in neat chloroform [13], the increase in dielectric constant of the polar solvents seems to stabilize an adduct in which electron transfer already occurred, $[\beta\text{-Car}^{\bullet+}\ldots\text{CHCl}_2^{-}]$. This ion pair may then be the actual reactant in the disproportionation reaction

$$2[\beta\text{-Car}^{\bullet+}\cdots\text{CHCl}_2^{-}] \rightarrow \beta\text{-Car} + \beta\text{-Car}^{2+} + 2\text{CHCl}_2^{-} \qquad (3)$$

We base this conclusion on the observed second-order kinetics for the decay of the species absorbing at 720 nm in the solvent mixtures and further on the observed decrease in rate for the reaction for increasing dielectric constant of the solvent as seen in Figure 4B in agreement with a stabilization of the ion pair relative to the precursor originally proposed [13]. The species absorbing around 550 nm is less sensitive to the solvent polarity, in agreement with a less polar character.

The effect of electrolytes on the decay reaction for the species absorbing at 720 nm provides further information of the transition state of the reaction in the chloroform/methanol solvent. Acetic acid had no effect, while both tetramethylammonium hydroxide and acetate increased the reaction rate without changing the reaction order, as seen in Figure 6A. Rather than being an effect of the presence of base, we suggest this effect to be due to the presence of ions in the solvent. For low concentrations of ions, as for the present experiments, solvent polarity is decreased as compared to the solvent without ions present [19]. The effect of the ions could accordingly be due to a change in solvent polarity caused by the ions or the effect could be accounted for by an electrostatic shielding of the ion pair, facilitating formation of the transition state in the bimolecular disproportionation reaction. Other effects such as molecular mobility should, however, also be considered [20].

## Conclusion

Carotenoid radical cations, as formed by electron transfer to protein or lipid radicals, may deplete other antioxidants like the tocopherols in lipophilic environments or even propagate oxidation, in effect being pro-oxidative. The present study shows that a precursor of the carotenoid radical cation on moving towards a more polar environment may become stabilized and that another reaction channel could open up, preventing formation of the otherwise aggressive radical through disproportionation.

## Acknowledgements

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## References

[1] Burton GW, Ingold KU. β-carotene—an unusual type of lipid antioxidant. Science 1984;224:569–573.

[2] Edge R, McGarvey DJ, Truscott TG. The carotenoids as antioxidants—a review. J Photochem Photobiol 1997;41:189–200.

[3] Bradley DG, Min DB. Singlet oxygen oxidation in food. Crit Rev Food Sci Nutr 1992;31:211–236.

[4] Jørgensen K, Skibsted LH. Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. Z Lebensm Unters Forsch 1993;196: 423–429.

[5] Schroeder MT, Becker EM, Skibsted LH. Molecular mechanism of antioxidant synergism of tocotrenols and carotenoids in polm oil. J Agric Food Chem 2006;54:3445–3453.

[6] Palozza P, Krinsky NI. β-carotene and α-tocopherol are synergistic antioxidants. Arch Biochem Biophys 1992;297: 184–187.

[7] Han RM, Tian YX, Becker EM, Andersen ML, Zhang JP, Skibsted LH. Puerarin and conjugated bases as radical scavengers and antioxidants: molecular mechanism and synergism with β-carotene. J Agric Food Chem 2007;55: 2384–2391.

[8] Becker EM, Ntouma G, Skibsted LH. Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organization. Food Chem 2007;103:1288–1296.

[9] Mortensen A, Skibsted LH. Real time detection of reactions between radicals of lycopene and tocopherol homologues. Free Radic Res 1997;27:229–234.

[10] Sliwka HR, Melø TB, Foss BJ, Abdel-Hafez SH, Partali V, Nadolski G, Jackson H, Lockwood SF. Electron- and energy-transfer properties of hydrophilic carotenoids. Chem Eur J 2007;13:4458–4466.

[11] Han RM, Wu YS, Feng J, Ai XC, Zhang JP, Skibsted LH. Radical cation generation from singlet and triplet excited states of all-trans-lycopene in chloroform. Photochem Photobiol 2004;80:326–333.

[12] Han RM, Tian YX, Wu YS, Wang P, Ai XC, Zhang JP, Skibsted LH. Mechanism of radical formation from the excited state of zeaxanthin and astaxanthin in chloroform. Photochem Photobiol 2006;82:538–546.

[13] Mortensen A, Skibsted LH. Kinetics of photobleaching of β-carotene in chloroform and formation of transient carotenoid species absorbing in the near infrared. Free Radic Res 1996;25:355–368.

[14] El-Agamey A, McGarvey DJ. Evidence for a lack of reactivity of carotenoid addition radicals towards oxygen: a laser flash photolysis study of the reactions of carotenoids with acylperoxyl radicals in polar and non-polar solvents. J Am Chem Soc 2003;125:3330–3340.

[15] Jouyban A, Soltanpour S, Chan HK. A simple relationship between dielectric constant of mixed solvents with solvent composition and temperature. Int J Pharm 2004;269:353–360.

[16] Espenson JH. Chemical kinetics and reaction mechanisms. New York: McGraw-Hill; 1981. p 170.

[17] El-Agamey A, Cantrell A, Land EJ, McGarvey DJ, Truscott TG. Are dietary carotenoids beneficial? Reactions of carotenoids with oxy-radicals and singlet oxygen. Photochem Photobiol Sci 2004;3:802–811.

[18] Jeevarajan JA, Wei CC, Jeevarajan AS, Kispert LD. Optical absorption spectra of dications of carotenoids. J Phys Chem 1996;100:5637–5641.

[19] Gore RC, Briscoe HT. The dielectric constants of solutions of some organic acids in ethyl alcohol and benzene. J Phys Chem 1936;40:619–625.

[20] Wisniewska A, Widomaska J, Subczynski WK. Carotenoid-membrane interactions in liposomes: effect of dipolar, monopolar, and nonpolar carotenoids. Acta Biochim Pol 2006;53:475–484.

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