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Antioxidant properties of phenols

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Abstract

The current understanding of the antioxidant properties of phenols (in homogeneous solutions) is reviewed, with particular emphasis on the role of the solvent. Phenols (ArOH) are known to reduce the rates of oxidation of organic matter by transferring a H atom (from their OH groups) to the chain-carrying $ROO^{\bullet}$ radicals, a mechanism that most likely involves a concerted transfer of the hydrogen as a proton and of one electron between the two oxygen atoms, O–H•••O• (proton-coupled electron transfer mechanism). The antioxidant capabilities of phenols are strongly reduced by hydrogen-bond accepting solvents since the hydrogen-bonded molecules ArOH•••S are virtually unreactive toward $ROO^{\bullet}$ radicals. The magnitude of these kinetic solvent effects is determined by the solute acidity α_2^H of ArOH (range 0 to 1) and solvent basicity β_2^H (range 0 to 1). Hydroxyl solvents (alcohols) have a double effect on ArOH. On the one hand, they act as hydrogen-bond accepting solvents and reduce the conventional rates of the $ArOH + ROO^{\bullet}$ reaction. On the other hand, these solvents favour the ionization of ArOH into their phenoxide anions ArO^{-}, which may react with $ROO^{\bullet}$ very rapidly by electron transfer (sequential proton loss electron transfer mechanism). The overall effect is therefore determined by the ionization degree of ArOH. Other aspects of the kinetics and thermodynamics of $ArOH + ROO^{\bullet}$ are also discussed.

Introduction

Organic matter, both synthetic and biological, when exposed to air undergoes a multistep oxidation without combustion known as autoxidation or peroxidation, which in the early stages converts the C-H bonds of organic molecules into C–OOH bonds by O_2 addition to the C backbone. The initial hydroperoxide products (C–OOH) may decompose into various stable oxygen compounds and produce, with the aid of light or transition metals (e.g. Fe or Cu), radicals that further accelerate oxidation (autocatalytic effect). The subject has been thoroughly reviewed by many authors (e.g. Frank 1950; Ingold 1961; Mayo 1968; Porter 1986) and therefore will only be touched on in brief here. In this context, I want to recall the reader's attention to the fact that this process is generally viewed as deleterious, particularly in humans where it seems to be implicated in the onset of many degenerative diseases such as atherosclerosis, arthritis, inflammation, heart disease, cancer and aging (Halliwell & Gutteridge 2006). However, it must be remembered that this kind of oxidation is fundamental for the natural degradation of enormous quantities of anthropogenic pollutants released into the environment. Volatile organic compounds (VOC), for instance, are degraded in the troposphere via radical reactions triggered predominantly by the hydroxyl radical $HO^{\bullet}$, which in turn results from the photolysis of ozone (O_3) at wavelengths of 350 nm or less, Reactions 1–3 (Reeves & Penkett 2003):

$$O_3 \xrightarrow{h\nu} O(^1D) + O_2 \quad (1)$$

$$O(^1D) + H_2O \longrightarrow 2HO^{\bullet} \quad (2)$$

$$VOC + HO^{\bullet} \longrightarrow \xrightarrow{O_2} \text{oxygenated compounds} \quad (3)$$

Phenols (ArOH) are known to have the outstanding property of suppressing or delaying spontaneous autoxidation of organic molecules (Ingold 1961) through mechanisms that will be discussed in some detail in the following paragraphs. These compounds might therefore

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prevent the consequences of autoxidation of biomolecules. Indeed, the consumption of fruits and vegetables, which contain plenty of phenolic antioxidants, has been demonstrated to significantly reduce the risk of cancer (Block et al 1992). These properties have sparked an exponential interest in phenolic antioxidants, particularly after the discovery that the main lipid-soluble antioxidant present in human blood is a phenol, namely α-tocopherol (Burton et al 1983). In 2006, for instance, the number of publications that can be traced back to studies on antioxidants exceeds 10 000, of which about 14% expressly concern 'phenols as antioxidants'. Excellent reviews and books on this topic are also available (Denisov & Khudyakov 1987; Denisov & Denisova 2000; Barclay & Vinqvist 2003). Despite all this, there seems to be no common language yet among the various specialists of this complex topic. Many works lack kinetic detail and little attention is usually paid to the influence of the medium on the antioxidant properties of phenols as a result of which comparison among the published data is somewhat difficult. In this article, the current understanding of phenolic antioxidants is reviewed in terms of kinetics, energetics and mechanisms of action in homogeneous solutions, which may be useful in the interpretation of available data and selection of novel antioxidants.

Uninhibited autoxidation: a brief survey

The oxidation of a hydrocarbon (R-H) in the liquid phase by atmospheric oxygen can adequately be described at low temperatures by the following radical chain process, Reactions 4–7, which includes the three fundamental steps of chain reactions, that is initiation, propagation and termination (Mayo 1968; Mahoney 1969):

$$\text{Initiation:}\quad \text{Radical source} \longrightarrow R^{\bullet} \tag{4}$$

$$\text{Propagation:}\quad R^{\bullet} + O_2 \xrightarrow{fast} ROO^{\bullet} \tag{5}$$

$$R-H + ROO^{\bullet} \xrightarrow{slow} R^{\bullet} + ROOH \tag{6}$$

$$\text{Termination:}\quad 2\,ROO^{\bullet} \xrightarrow{fast} ROH + R'{=}O + O_2 \quad \text{or } ROOR + O_2 \tag{7}$$

In order for this sequence to be a chain reaction, it is necessary that the rate of propagation be faster than the rate of termination (Denisov & Khudyakov 1987).

Autoxidation requires a source of radicals in order to be initiated and sustained since the H-atom abstraction from hydrocarbons by ground-state dioxygen ($^3O_2$) is usually very slow. In kinetic works, peroxy compounds, transition metals/ROOH or H_2O_2 and azo-compounds have been extensively employed, while in living organisms ionizing and non-ionizing radiation, exogenous molecules (e.g. medicines, cosmetics), endogenous (intracellular) sensitizers, enzymes such as xanthine oxidase, NADPH oxidase, NADH cytochrome reductase and lipoxygenases, are all potential radical initiators (Girotti 2001).

Molecular oxygen is, for instance, converted by a few enzymes to superoxide radical anion $O_2^{\bullet-}$ (the less reactive oxygen radical) or hydroxyl radical $HO^{\bullet}$ (the most reactive oxygen radical) with or without Fe(III)/Fe(II) involvement (Halliwell & Gutteridge 2006). In aqueous solutions, the $O_2^{\bullet-}$ radical is in equilibrium with its protonated radical form, $O_2^{\bullet-} + H^+ \leftrightarrows HOO^{\bullet}$. At pH 7.4 the $[O_2^{\bullet-}]$ is about 500-times greater (pK_a of $HOO^{\bullet} \approx 4.7$; Bielski 1978) than that of $HOO^{\bullet}$ but the latter species is much more reactive. In healthy subjects, it has been estimated that approximately 1.7–17 kg per year of dioxygen is converted to $O_2^{\bullet-}$ (Halliwell & Gutteridge 2006) and hence this is likely the most abundant oxygen radical in the human body. This radical is believed to be responsible for O_2 toxicity in living organisms. Mice lacking superoxide dismutates, a family of $O_2^{\bullet-}$-removal enzymes (see below), survive only a few days after birth (Halliwell 2006). Superoxide radical anions may combine under physiological conditions with $NO^{\bullet}$ (nitric oxide) to yield $ONOO^-$ (peroxynitrite anion), which in the protonated form, ONOOH (peroxynitrous acid), undergoes O–O homolysis to produce the reactive radicals $HO^{\bullet}$ and $NO_2^{\bullet}$ (nitrogen dioxide) (Richeson et al 1998; Halliwell 2006). Most $HO^{\bullet}$ radicals are generated in-vivo by the reaction of metal ions (Fe^{2+} and Cu^+) with H_2O_2 (Fenton reaction). Metal ions can also decompose lipid hydroperoxides (ROOH) to alkoxyl and peroxyl radicals according to the following reactions:

$$ROOH + Fe^{2+} \longrightarrow RO^{\bullet} + Fe^{3+} + HO^- \tag{8}$$

$$ROOH + Fe^{3+} \longrightarrow ROO^{\bullet} + Fe^{2+} + H^+ \tag{9}$$

These reactions are largely used to trigger autoxidation in studies conducted in model systems. Given that during autoxidation the amount of ROOH increases, the rate of initiation governed by Reactions 8 and 9 tends to increase as well, and, in kinetic studies, this may lead to erratic results. By contrast, azo-compounds, such as azo-bis(isobutyronitrile) (AIBN), slowly decompose on thermolysis in solution and generate carbon-centred radicals at a constant rate (see Figure 1), which are then converted to peroxyl radicals by the dissolved oxygen at close to the diffusion-controlled limit (i.e. $k_5 \geq 10^9\ M^{-1}\,s^{-1}$). In aqueous solutions 2,2′-azobis(amidinopropane) (ABAP) is extensively employed but this azo-initiator produces positively charged peroxyl radicals (Paul et al 2000).

Oxidative chains are carried by peroxyl radicals that react with RH by abstracting a H atom from C-H bonds. New C-centred radicals are therefore formed, Reaction 6, and the process continues cyclically until two $ROO^{\bullet}$ radicals encounter and quench. Peroxyl radicals therefore act as a catalyst of the formal reaction $R{-}H + O_2 \rightarrow ROOH$. When the concentration of dissolved oxygen in solution exceeds 1 mM, the chain-terminating process consists of the sole bimolecular self-reaction of peroxyl radicals, Reaction 7, otherwise the reactions $R^{\bullet} + ROO^{\bullet}$ and $R^{\bullet} + R^{\bullet}$ are also possible (Foti et al 2005). Typical values of $2k_7$ for alkylperoxyl radicals are about 10^3–10^4 for tertiary, about 10^7 for secondary and about $10^8\ M^{-1}\,s^{-1}$ for primary alkylperoxyls (Foti & Ingold 2003). The mechanism

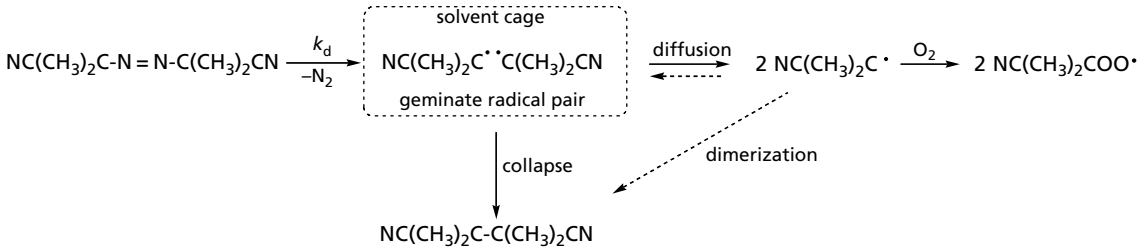


Figure 1 Decomposition of azo-bis(isobutyronitrile) in the presence of dioxygen. Reaction paths indicated by dotted arrows are highly improbable because of the presence of O_2.

of combination and quenching of $ROO^{\bullet}$ involves the reversible formation of an unstable tetroxide (ROOOOR), which rearranges to various products according to the structure of R (Foti & Ingold 2003).

It is possible to demonstrate (Mahoney 1969) that the rate of uninhibited autoxidation under steady rate of initiation (R_i) and low [ROOH] is given by:

$$R_{O_2} = \frac{k_6}{(2k_7)^{1/2}} \; [RH] R_i^{1/2} \qquad (10)$$

Kinetic experiments have widely demonstrated the validity of this equation in the early stage of oxidation. The ratio $k_6/(2k_7)^{1/2}$ (oxidizability) is a measure of the susceptibility of RH to undergo autoxidation. Linoleic acid, for instance, undergoes autoxidation with a rate at 50°C that is 9-fold that of cumene, $k_6/(2k_7)^{1/2}$ being at this temperature $0.027\ M^{-1/2}\ s^{-1/2}$ (Foti & Ruberto 2001) and $0.003\ M^{-1/2}\ s^{-1/2}$ (extrapolated from data reported by Zawadiak et al 2004), respectively (all else being equal).

Oxidation of unsaturated hydrocarbons commences at the allylic position, $R_1–CH=CH–CH_2–R_2$ via H-atom abstraction (though radical addition to the π bond is also possible). The C–H bond elongation and cleavage by radical attack is facilitated by the presence of double bonds that stabilize (by resonance) the intermediate C-centred radical, $R_1–CH=CH–{}^{\bullet}CH–R_2 \leftrightarrow R_1–{}^{\bullet}CH–CH=CH–R_2$. The propagation rates for stearic, oleic and linoleic acids (ratio 1:1000:10 000) demonstrate the increasing activation of methylene groups for H-atom abstraction. Polyunsaturated fatty acids are therefore subjected to fast autoxidation, the process being orders of magnitude faster than with the saturated counterparts. Linoleic acid is initially attacked at the bis-allylic position 11 ($k_6 \approx 163\ M^{-1}\ s^{-1}$ at 50°C with secondary $ROO^{\bullet}$; Foti & Ingold 2003) generating a resonance-stabilized pentadienyl radical that undergoes O_2 trapping at either terminus (see Figure 2) (Tallman et al 2004). Over 97% of the oxygen absorbed in the autoxidation process is fixed into hydroperoxides (Porter & Wujek 1984) whose distribution (see Figure 2) depends on oxidation conditions (Tallman et al 2004). Autoxidation thus changes dramatically the structure and polarity of polyunsaturated fatty acids in cell membranes. This can cause damage to the cell membrane such as a decrease of fluidity, easiness of phospholipid exchange between the two halves of the bilayer, permeability to substances that do not normally cross it other than through specific channels (e.g. K^+ and Ca^{2+}), and damage to membrane proteins; continued oxidation may eventually lead to loss of membrane integrity (Halliwell 2006).

In the above paragraph, it is implicitly assumed that ground-state molecular oxygen (i.e. the oxygen we breathe), $^3O_2$, was the reagent involved in the oxidation reactions. Appropriate photoexcitable compounds (sensitizers) can convert $^3O_2$ into singlet molecular oxygen, $^1O_2$ ($^1\Delta_g$), by energy transfer (approx. 22 kcal mol^{-1}) (Greer 2006). This latter excited species readily oxidizes polyunsaturated fatty acids to hydroperoxides with no radical intermediates (type II photosensitized oxidation) (Girotti 2001). Indeed, the atoms of the –OOH group derive exclusively from $^1O_2$ and the target lipid and the binding sites are more numerous. For instance, thermal autoxidation of linoleoyl chains leads to conjugated 9-OOH and 13-OOH (see Figure 2); in contrast, the $^1O_2$-mediated peroxidation of linoleic esters leads to 9-OOH, 10-OOH, 12-OOH and 13-OOH (Greer 2006). Generally, photosensitized oxidation of lipids sets off both the radical-mediated (type I) and the $^1O_2$-mediated (type II) pathways (Girotti 2001).

Inhibited autoxidation

Many substances can reduce the rate of autoxidation, thereby acting as antioxidants or retarders (see below). Several mechanisms behind such effects have been unveiled and these allow one to classify antioxidants essentially in two general classes: preventive and chain-breaking. To the first class belong those compounds that reduce the initiation rate by eliminating the 'source of initiating radicals', for instance compounds that destroy hydroperoxides or H_2O_2, metal ion chelators and $^1O_2$ quenchers.

In living organisms, a few enzymes are prominent examples of preventive antioxidants. Superoxide dismutases (SOD) remove $O_2^{\bullet-}$ by catalysing the reaction $2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$. The large quantities of H_2O_2 produced (especially in the brain) by SOD and other enzymes (Halliwell 2006) are then disposed of by catalases, which convert H_2O_2 to $H_2O + O_2$, glutathione peroxidases, which catalyse the redox reaction of H_2O_2 with glutathione GSH, $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$, and peroxiredoxins, which are thought to be the most important H_2O_2 removal enzymes in animals (Halliwell 2006).

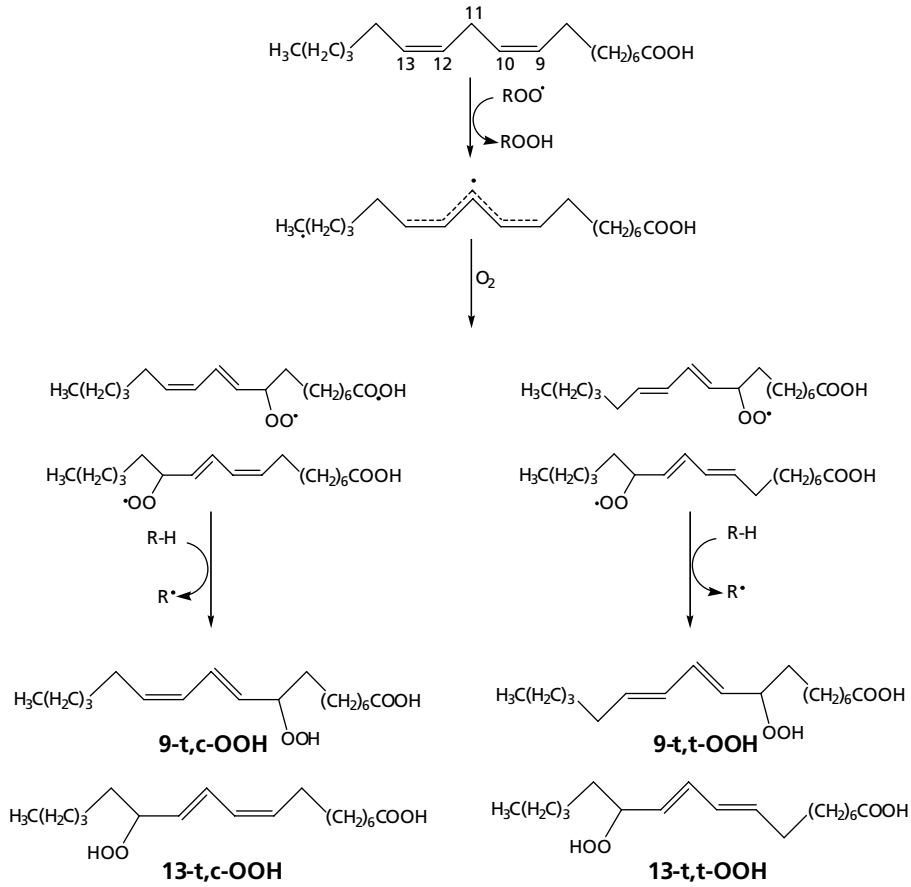


Figure 2 Peroxidation process of linoleic acid. Distribution of linoleoyl hydroperoxides depends on oxidation conditions.

Alkyl- and arylphosphites are widely used by the polymer industries as inhibitors of autoxidation as these compounds reduce hydroperoxides stoichiometrically (Denisov & Denisova 2000):

$$\mathrm{ROOH + P(OPh)_3 \longrightarrow ROH + O{=}P(OPh)_3} \qquad (11)$$

Chain-breaking antioxidants function instead by 'neutralizing' peroxyl radicals responsible for chain propagation. The chain length, that is the number of oxidized molecules of substrate per initiating radical, decreases accordingly.

The most prominent examples of chain-breaking antioxidants are represented by aromatic H atom donors such as phenols, aromatic non-tertiary amines and thiophenols. These compounds inhibit oxidation by transferring a H atom to the chain-carrying $ROO^{\bullet}$ radicals (H-atom transfer or HAT mechanism) as exemplified for phenols in Reaction 12. This elementary process was invoked for the first time by Bolland and Have to explain the inhibitory effects of representative phenols on the thermal autoxidation of ethyl linoleate (Bolland & Have 1947). Very recently, the hypothesis has been advanced that this formal HAT mechanism may in reality hide a more complex mechanism called proton-coupled electron transfer (PCET). This mechanism involves a prior formation of a hydrogen-bonded complex between ArO–H and a lone pair on the $O^{\bullet}$ of $ROO^{\bullet}$. The hydrogen is then transferred as a proton between the two O atoms and one electron moves simultaneously from the 2p lone pair of the O on phenol to the orbital containing the unpaired electron on $O^{\bullet}$ of $ROO^{\bullet}$ (Mayer et al 2002; Mayer & Rhile 2004; see also Litwinienko & Ingold 2007).

$$\mathrm{ArOH + ROO^{\bullet} \underset{(\leftarrow)}{\longrightarrow} ArO^{\bullet} + ROOH} \qquad (12)$$

The resonance-stabilized aryloxyl radical ($ArO^{\bullet}$) of efficient phenolic antioxidants (e.g. sterically protected phenols), generated in Reaction 12, is usually unreactive toward dioxygen and the substrate (Reactions 13 and 14, respectively), and therefore it is generally unable to continue oxidation (Ingold 1961) (see below for a few exceptions). It decays via the bimolecular self-reaction, Reaction 15, or

(especially for aryloxyl radicals of hindered phenols) by reacting with another $ROO^{\bullet}$ radical, Reaction 16 (see below).

$$ArO^{\bullet} + O_2 \xrightarrow{very\ slow} Ar(=O)OO^{\bullet} \quad (13)$$

$$ArO^{\bullet} + RH \xrightarrow{very\ slow} ArOH + R^{\bullet} \quad (14)$$

$$ArO^{\bullet} + ArO^{\bullet} \xrightarrow{fast} products \quad (15)$$

$$ArO^{\bullet} + ROO^{\bullet} \xrightarrow{fast} non\text{-}radical\ products \quad (16)$$

Chain terminations of inhibited autoxidation do not therefore occur via Reaction 7 but rather via the two consecutive reactions, Reactions 12 and 16 (Reaction 15 is also chain-terminating if Reaction 14 does occur). By assuming quasi-stationary conditions and with the simplifications stated above on the behaviour of $ArO^{\bullet}$, it is possible to demonstrate that the rate of inhibited autoxidation under steady rate of initiation (R_i) and low [ROOH] is given by:

$$R_{O_2}^{inh} = \frac{k_6[RH]R_i}{n k_{12}[ArOH]} \quad (17)$$

where k represents the rate constant of the pertinent reactions and n is the stoichiometric factor of ArOH. Kinetic studies show that the rate law of inhibited processes in many cases conforms to Equation 17, at least in the early stage of oxidation. However, many other equations for the rate of inhibited oxidation have been derived by Denisov and Khudyakov by extending the number of possible reactions (Denisov & Khudyakov 1987).

The stoichiometric factor of ArOH is calculated as follows (Mahoney 1969):

$$n = \frac{1}{[ArOH]_0}\int_0^T R_i dt = \frac{R_i \times T}{[ArOH]_0} \quad (18)$$

where T and $[ArOH]_0$ are the induction period (see below) and the initial concentration of inhibitor. The product $R_i \times T$ gives the moles per litre of peroxyl radicals released by the initiator in the time T. The value of n, therefore, represents the number of oxidative chains terminated by 1 molecule of inhibitor. These numbers are generally found to be close to 1.0 or 2.0 for various phenols, and this suggests that phenols react with $ROO^{\bullet}$ via simple stoichiometric processes (Mahoney 1969). Kinetically controlled experiments are needed for an accurate determination of n and k_{12}.

Equation 17 shows that in order for a phenol to be an effective antioxidant, that is $R_{O_2}^{inh} << R_{O_2}$, its inhibition rate constant k_{12} must be several orders of magnitude greater than the propagation rate constant k_6, that is $k_{12} >> k_6$ (Barclay & Vinqvist 2003). Indeed, the antioxidant ability is not an absolute property of ArOH but actually depends on the substrate to be protected. Inhibitors capable of delaying peroxidation of highly oxidizable (high k_6 values and/or low $2k_7$ values) substrates are characterized by high values of the rate constant k_{12}. On the other hand, substrates relatively insensitive to dioxygen (or better $ROO^{\bullet}$) may be protected by weak antioxidants. Cumene, for instance, oxidizes slowly, with a k_6 of 0.18 $M^{-1}\,s^{-1}$ at 30°C, and thus its autoxidation can effectively be inhibited by small quantities of the simple phenol C_6H_5OH even though this inhibitor is characterized by a small k_{12} of approximately 10^3 $M^{-1}\,s^{-1}$. By contrast, the same phenol functions as a simple retarder of linoleic acid autoxidation since in this case $k_6 \approx 60$ $M^{-1}\,s^{-1}$ at 30°C.

Effective antioxidants are characterized by a definite lifetime (induction period T) that is measurable from the profile of oxidation (oxygen uptake) (Mahoney 1969). Since the induction period is dependent on n and R_i (see Equation 18), its determination under controlled kinetic conditions allows one to calculate either the stoichiometric factor, n, or the rate of initiation, R_i. In a few cases, the induction period has also been used to evaluate the quantity of antioxidants present in used engine lubricating oils (Mahoney et al 1978) and human plasma (Burton et al 1983).

Inhibitors that do not possess large values of k_{12} with respect to k_6 (retarders) exert a weak inhibition of autoxidation and do not show a clear induction period since they are being slowly consumed. Termination of radical chains occurs via Reaction 12 and Reaction 7, but in this case the process is not as efficient as with chain-breaking antioxidants. Equation 17 is no longer valid and more complicated equations have been derived (Denisov & Khudyakov 1987; Foti & Ruberto 2001). Typical profiles of oxygen uptake for autoxidation of a hydrocarbon substrate in the presence of a real antioxidant and a retarder are shown in Figure 3. Thus, according to the pattern of oxygen uptake profiles in kinetically controlled experiments, antioxidants can easily be recognized as simple retarders or chain-breaking antioxidants. This has recently been done with melatonin (Antunes et al 1999; Fowler et al 2003), which has often been described as a potent antioxidant (even more active than α-tocopherol) (Figure 4). As a matter of fact, it was shown that at a concentration of 60.4 μM, melatonin caused a minimal retardation of the AIBN-initiated peroxidation of styrene at 30°C. By contrast, α-tocopherol at a concentration

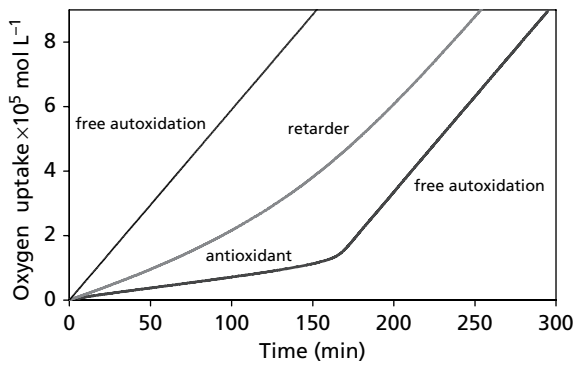


Figure 3 Typical profiles of oxygen-uptake during peroxidation of a substrate without inhibitors and with a retarder or an antioxidant.

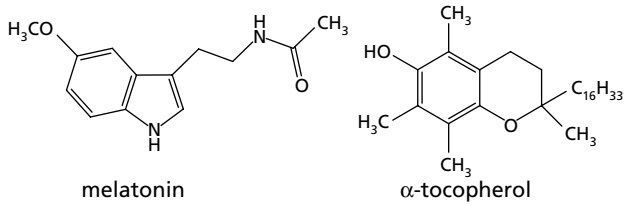
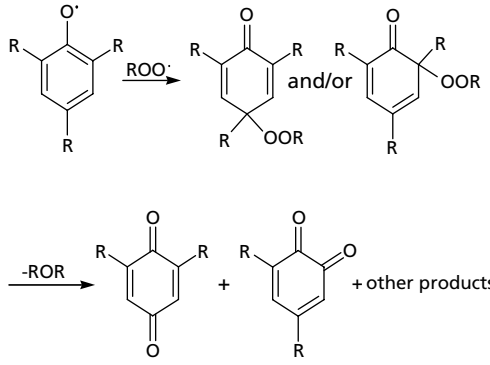


Figure 4 The structures of melatonin and α-tocopherol.

10-times lower (5.80 μM, other things being equal) determined a sharp inhibition of peroxidation for about 78 min (induction period). Further investigations showed that melatonin is a rather weak preventive antioxidant of the metal-ion deactivating subclass (Antunes et al 1999).

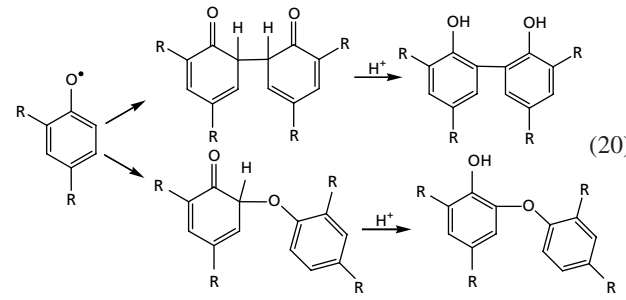
The fate of ArO• radicals

The aryloxyl radical ArO• may give rise to various reactions that will be described very briefly here. These reactions are of interest since the antioxidant ability of ArOH is itself influenced by the fate of ArO•. The value of the stoichiometric factor, n, is, for instance, determined by the rates of the concurrent reactions, Reactions 15 and 16. If Reaction 16 largely prevails then n → 2, with a consequent beneficial effect on the length of the induction period (see above) and on the oxidation rate (see Equation 17). The ArO• radical is able to quench another ROO• radical via (in many cases) ring addition, Reaction 19; the *ortho*- or *para*-peroxide may decompose to *ortho*- or *para*-quinones (Coppinger 1964).



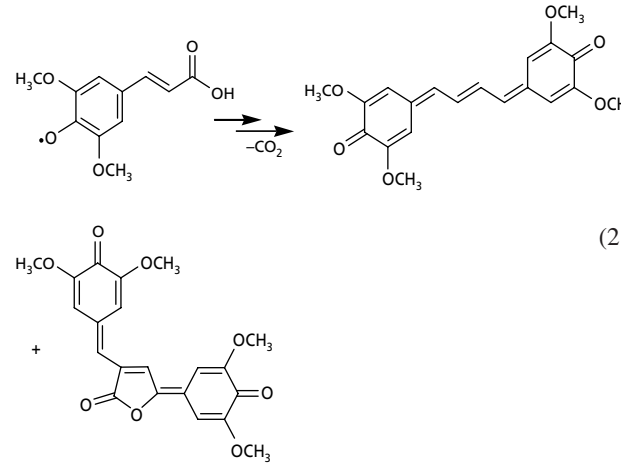
(19)

In the case of a predominant decay of ArO• via the self-reaction, Reaction 15, the stoichiometric factor will be close to 1.0, unless the parent phenol is regenerated via disproportionation, in which case n → 2. Self-quenching of ArO• usually gives rise to C–C or C–O products (Weiner & Mahoney 1972), which may themselves exert a weak antioxidant activity on the evidence of the oxidation rate which occasionally does not return to the uninhibited rate after the consumption of ArOH. The mechanisms of action of retarders may be complex but in a few cases this residual antioxidant activity may be due to in-situ formation of phenols from the slow (in apolar media) tautomeric relaxation of the initial keto-form of C–C or C–O dimers of ArO• as shown in Reaction 20 (Mahoney & Weiner 1972 a, b).



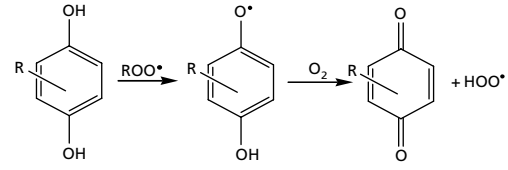
(20)

In a few cases, the ArO• self-reactions may have valuable synthetic uses. One interesting case is represented by the formation of stable and highly conjugated *p*-quinomethanes from the coupling of ArO• radicals derived from *p*-hydroxycinnamic acids, Reaction 21 (Daquino & Foti 2006).



(21)

We have assumed above that the ArO• radicals do not contribute to the oxidative chains. There are a few cases, however, in which they do. The aryloxyl radicals of hydroquinones, catechols, aminophenols and aromatic diamines, for instance, may react with dioxygen and generate HOO• radicals (Reaction 22), which keep the chain reaction going (Denisov & Denisova 2000). Antioxidant abilities and stoichiometric factors of these compounds therefore decrease according to the rate of the second step of Reaction 22.



(22)

Another surprising case of pro-oxidation caused by $ArO^{\bullet}$ radicals has been reported by Bowry, Stocker and Ingold. It regards the effect of α-tocopheroxyl radical (α-$TO^{\bullet}$) on the human low density lipoprotein (LDL) (Bowry & Stocker 1993; Bowry & Ingold 1999). The LDL particle contains on average 6–12 molecules of α-tocopherol (α-TOH) and approximately 1450 bis-allylic CH_2 groups in lipids (LH). In two studies on LDL peroxidation, it had been observed that 'peroxidation occurred at much the same rate in the formally α-TOH inhibited period of peroxidation as in the subsequent uninhibited (α-TOH consumed) period. Both studies also noted that the minor quinolic antioxidant, ubiquinol-10 (CoQH2) was consumed before α-TOH' (Bowry & Ingold 1999). Under the kinetic conditions adopted in these studies it was very unlikely that an LDL particle could contain more than one radical at a time. Because of the ratio $[\alpha\text{-TOH}]_{LDL}/[CH_2]_{LDL}$ and the involved rate constants, the $ROO^{\bullet}$ radical reacted preferentially with α-TOH. The resulting α-$TO^{\bullet}$ radical, surviving for a long time in the particle (no other $ROO^{\bullet}$ radical was readily available to quench it; moreover, α-$TO^{\bullet}$ being highly lipophilic was unable to diffuse into the aqueous phase) therefore became the chain carrier of a peroxidation process involving the lipid (LH) inside the LDL particle, Reactions 23–25. This process is now termed 'tocopherol-mediated peroxidation' (TMP). Termination reactions of TMP most likely involve the antioxidants ascorbate (water/LDL interphase) or ubiquinol-10 (LDL), which react with α-$TO^{\bullet}$ and quench it, Reaction 26. The semiquinone radical $Q_{10}H^{\bullet}$ produced in Reaction 26 is readily oxidized by O_2 to ubiquinone-10, and the resulting superoxide ion ($O_2^{\bullet-}$/$HOO^{\bullet}$) produced in this reaction migrates to the aqueous phase outside the LDL particle, Reaction 27.

$$ROO^{\bullet}/LOO^{\bullet} + \alpha\text{-TOH} \longrightarrow ROOH/LOOH + \alpha\text{-}TO^{\bullet} \quad (23)$$

$$\alpha\text{-}TO^{\bullet} + LH \xrightarrow{ca.0.1 M^{-1}s^{-1}} \alpha\text{-TOH} + L^{\bullet} \quad (24)$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \quad (25)$$

$$Q_{10}H_2 + \alpha\text{-}TO^{\bullet} \longrightarrow Q_{10}H^{\bullet} + \alpha\text{-TOH} \quad (26)$$

$$Q_{10}H^{\bullet} + O_2 \longrightarrow Q_{10} + HOO^{\bullet} (\leftrightarrows O_2^{\bullet-} + H^{+}) \quad (27)$$

Finally, it is occasionally observed that the addition of hydroperoxides ROOH during the lag phase of inhibited autoxidations determines an acceleration of the rate of oxidation. Thermal homolysis of the RO–OH bond, that is $ROOH \rightarrow RO^{\bullet} + HO^{\bullet}$, can be the cause of such an increase at high temperatures. At low temperatures, however, this increase of rate has been accounted for by the suggestion that the $ArO^{\bullet}$ radicals can be involved in chain-restarting reactions with ROOH, that is Reaction 12 (Mahoney 1969; Denisov & Khudyakov 1987). The effects of this reaction are marked (depending on [ROOH]), particularly with non-hindered phenols being generally negligible for hindered phenols (Mahoney 1969). The rate constant k_{-12} for the aryloxyl radical of 4-methoxyphenol is, for instance, large, being approximately 650 M^{-1} s^{-1} at 60°C; on the contrary, the aryloxyl radical of 2,4,6-tri-*tert*-butylphenol reacts slowly with ROOH, with k_{-12} being only 0.87 M^{-1} s^{-1} at the same temperature (Denisov & Khudyakov 1987). As a result, the former antioxidant shows a particularly complex kinetic behaviour (Mahoney 1969).

The rate constant of inhibition, k_{12}

The values of the inhibition rate constant k_{12} are largely determined (in apolar solvents) by the thermochemistry of the formal H-atom transfer, Reaction 12, that is by the bond dissociation enthalpy (BDE) of the phenolic OH (the BDE of ROO-H is relatively constant, 86–88 kcal mol^{-1}). Generally, the lower the BDE of ArO–H, the higher the k_{12}, though solvent and steric effects play an important role. The OH BDE is influenced by the electronic and steric effects of the substituents present in the aromatic ring (Wright et al 2001; Brigati et al 2002). Generally, electron-withdrawing groups increase the OH BDE (up to +10 kcal mol^{-1} for *o*-NO_2 with respect to C_6H_5OH), while electron-realizing groups weaken the OH bond by several kcal mol^{-1} (−2.0 and −2.5 kcal mol^{-1} for *ortho*- and *para*-CH_3 up to −11.5 kcal mol^{-1} for *ortho*-NH_2) (Wright et al 2001). These substituent effects are interpreted in terms of stabilization or destabilization of the aryloxyl radical or the parent phenol (Franchi et al 1999). In the absence of steric hindrance the k_{12} determined in apolar solvents correlates with the BDE of ArO–H (Foti et al 2002):

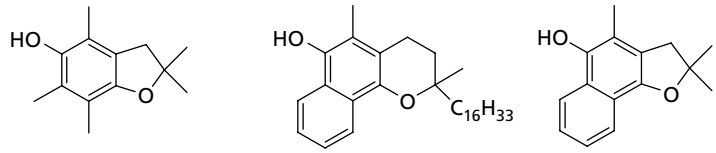
$$\log(k_{12}/M^{-1}\,s^{-1} \text{ at } 30°C) \approx 3.40 - 0.24 \times \Delta BDE(OH) \quad (28)$$

where ΔBDE(OH) is $BDE(ArO\text{–}H) - BDE(C_6H_5O\text{-}H)$ in kcal mol^{-1}. This equation allows the estimation of k_{12} for a large number of non-hindered antioxidants and retarders provided the values of OH BDE are known. Even for moderately hindered phenols, the experimental k_{12} values at 30°C are probably close to those calculated by Equation 28, which thereby represent a superior limit. The presence of bulky *ortho*-substituents (*tert*-butyl groups) reduces considerably the value of k_{12}. Based on the experimental rate constants and OH BDEs of 2,6-di-*tert*-butyl-4-methylphenol (BHT) (1.2×10^4 M^{-1} s^{-1} and approx. 80 kcal mol^{-1}) and 2,6-di-*tert*-butyl-4-methoxyphenol (BHA) (7.8×10^4 M^{-1} s^{-1} and approx. 77 kcal mol^{-1}), we can estimate that the steric hindrance of two *ortho*-*tert*-butyl groups reduces k_{12} by (at least) one order of magnitude with respect to non-hindered phenols of equal O–H strengths. Table 1 shows values of k_{12} and OH BDEs for various phenolic antioxidants.

Correlations similar to Equation 28 are also found for the reaction of ArOH with the $dpph^{\bullet}$ radical, Reaction 29 (Foti & Daquino 2006). The combination of these correlations leads to the equation $k_{12}/k_{29}^{2/3} \cong 4000$, which implies that the two rate constants k_{12} and k_{29} can be calculated one from the other. This fact outlines a possible 'shortcut' for

Table 1 Rate constants ($M^{-1}\,s^{-1}$) k_{12} for H-atom abstraction from a few selected phenols (ArOH) by $ROO^{\bullet}$ radicals and bond-dissociation enthalpies (BDE)[a] of ArO–H (kcal mol^{-1})

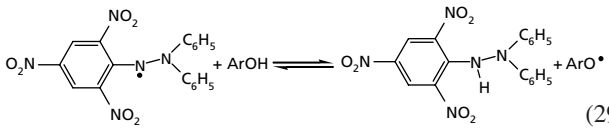
ArOH	OH BDE	Reference	k_{12}[b]	Temperature (°C)	Reference
4-CN-C_6H_4OH	89.7	Wright et al (2001)	6.5×10^2	65	Howard & Ingold (1963)
C_6H_5OH	86.7	Mulder et al (2005)	6.5×10^3	65	Howard & Ingold (1963)
3-MeO-C_6H_4OH	86.9	Wright et al (2001)	5.8×10^3	65	Howard & Ingold (1963)
4-Cl-C_6H_4OH	86.1	Wright et al (2001)	4.8×10^3	65	Howard & Ingold (1963)
4-Me-C_6H_4OH	85.0	Brigati et al (2002)	2.1×10^4	65	Howard & Ingold (1963)
4-OH-C_6H_4OH	82.2	Brigati et al (2002)	2.7×10^5	50	Foti & Ruberto (2001)
BHA	77.2	Brigati et al (2002)	7.8×10^4	30	Burton & Ingold (1981)
BHT	80.0	Brigati et al (2002)	1.2×10^4	30	Burton & Ingold (1981)
α-Tocopherol	77.1	Lucarini et al (1994)	3.2×10^6	30	Burton & Ingold (1981)
Catechol	80.7	Lucarini et al (2004)	5.5×10^5	30	Xi & Barclay (1998)
3,5-di-^{t}Butyl-catechol	78.2	Lucarini et al (2004)	1.5×10^6	30	Xi & Barclay (1998)
I	–		5.7×10^6	30	Burton et al (1985)
II	71.6	Foti et al (2002)	1.1×10^7	30	Barclay et al (1993)
III	70.7	Foti et al (2002)	2.9×10^7	30	Barclay et al (1993)



I II III

[a]The OH BDE values of a few phenols determined by electron paramagnetic resonance techniques have been adjusted downward by 1.1 kcal mol^{-1} as indicated in Mulder et al (2005); [b]the rate constants reported in Howard & Ingold (1963) have been corrected by a factor of 2.24 as reported in Burton & Ingold (1981). BHA, 2,6-di-*tert*-butyl-4-methoxyphenol; BHT, 2,6-di-*tert*-butyl-4-methylphenol.

estimating the antioxidant ability of ArOH by taking advantage of using a stable and commercially available radical ($dpph^{\bullet}$).



(29)

Finally, it must be observed that as the OH BDE of ArOH decreases the ionization potential tends to decrease as well. This can reduce the antioxidant ability of ArOH because of the direct reaction (especially in polar media) with O_2, which introduces into the system highly reactive $HOO^{\bullet}$ radicals:

$$ArOH + O_2 \longrightarrow ArOH^{\bullet+} + O_2^{\bullet-} \longrightarrow ArO^{\bullet} + HOO^{\bullet} \quad (30)$$

It has been calculated that for OH BDE lower than approximately 78 kcal mol^{-1} the electron transfer Reaction 30 is comparatively fast and may reduce the antioxidant activity of ArOH (Foti et al 2002).

The role of the hydrogen bonding

The intramolecular hydrogen bonding (HB) has important effects on the antioxidant properties of phenols. Various *ortho*-substituents may function as H-bond acceptors, however only the effects of methoxy and hydroxy groups will be described since these groups are largely diffused among natural phenols and have opposite effects on the antioxidant properties of phenols. The discussion can easily be generalized to other HB acceptors.

The OH group in 2-methoxyphenols is intramolecularly H-bonded to the adjacent methoxy group with a strength that has been evaluated to be approximately 4 kcal mol^{-1} by density functional theory calculations (Korth et al 2002). Consequently, the OH BDE of 2-methoxyphenols is greater than that of the corresponding 4-methoxyphenols (Brigati et al 2002). This causes the antioxidant efficiency (i.e. the rate constants k_{12}) of the former to decrease with respect to that of the latter. By contrast, 2-hydroxyphenols (catechols) are usually better antioxidants than the corresponding 4-hydroxyphenols (hydroquinones) and far better than 2-methoxyphenols. The value of k_{12} for catechol is, for instance, about 4-times greater than that of hydroquinone (per reactive OH) (Foti & Ruberto 2001) and 1–2 orders of magnitude greater than that of 2-methoxyphenol (Barclay & Vinqvist 2003). This apparent contrast between catechols and 2-methoxyphenols is readily explained by taking into account the structure of

catechols in which one OH group is not H-bonded and thus weaker than the other. Indeed, this HB-free OH is the one involved in Reaction 12. The striking fact is that the BDE of this HB-free O–H is also lower than either O–H bonds in the corresponding hydroquinones. The reason that has been suggested for this is that the intramolecular HB in the aryloxyl radical of catechols is stronger than that in the parent molecules (Foti et al 2002). This extra energy gain lowers the activation energy of Reaction 12 and as a result the antioxidant efficiency of catechols is boosted. The magnitude of this HB reinforcement in the aryloxyl radical of catechols has been a matter of controversy. By using the group additivity rule, Lucarini et al (2004) have recently reported that the difference in the strength of the intramolecular HB in the radical and parent catechol can be set to about 1.6 kcal mol^{-1}, a value much lower than predicted by theoretical calculations (4–6 kcal mol^{-1}).

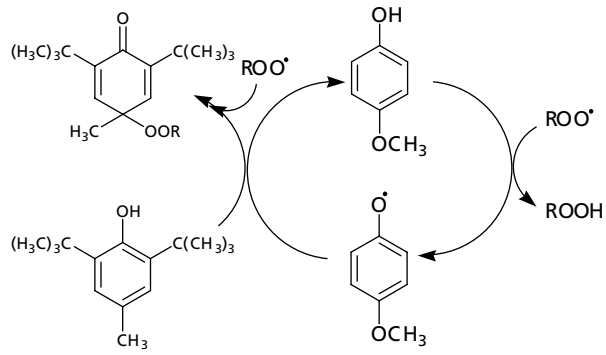
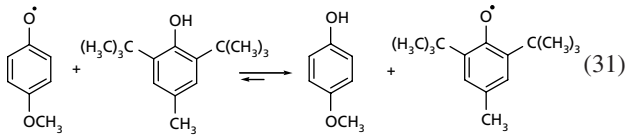


Figure 5 Synergistic effect between 2,6-di-*tert*-butyl-4-methylphenol and 4-methoxyphenol.

Synergism between antioxidants

Combinations of antioxidants frequently show a reinforcement of their individual action. This synergism is well documented for mixtures of hindered and non-hindered phenols. The rate of autoxidation of 9,10-dihydroanthracene at 60°C is almost unaffected by 0.068% (mol/mol) of BHT, however an identical quantity of 4-methoxyphenol (MP) exerts a considerable retardation. Interestingly, the retardation time is almost doubled when both phenols are present at a total concentration of 0.136% (Mahoney 1969) and BHT is the phenol to be consumed first. This synergistic effect was explained by Mahoney and DaRooge who in 1967 first introduced a mechanism of MP regeneration based on the following transfer equilibrium (Mahoney & DaRooge 1967):


(31)

As the OH BDE of MP is greater than that of BHT by 2.2 kcal mol^{-1}, this equilibrium is shifted to the right, that is towards the formation of MP at BHT expense. Indeed, BHT is not an efficient peroxyl radical scavenger since the value of k_{12} is approximately 10^4 M^{-1} s^{-1} while 4-methoxyphenol reacts faster with them, $k_{12} \sim 10^5$ M^{-1} s^{-1}. The resulting aryloxyl radical is then converted back to MP by Reaction 31, while the aryloxyl radical of BHT is readily quenched by one ROO$^\bullet$ radical (see Figure 5). In order for this mechanism to work appropriately it is important that the process of MP regeneration (Reaction 31) be faster than the chain-restarting reactions of the aryloxyl radical of MP with the substrate or ROOH, Reactions 14 and 12, respectively.

Many other cases of synergism are now known, among which the most prominent is represented by the regeneration of α-tocopherol from its radical by ascorbic acid (Barclay & Vinqvist 2003).

Kinetic solvent effects

Ingold and co-workers have observed and first quantitatively interpreted the kinetic effects that polar solvents (PS) have on reactions of H-atom abstraction by free radicals (Y$^\bullet$) from ArOH and other H-atom donors (Snelgrove et al 2001). They observed that in polar solvents these reactions are much slower than in saturated hydrocarbon solvents. The explanation that has been offered is that polar solvents block the phenolic OH into a hydrogen-bonded complex (PS•••HOAr) unreactive (for steric reasons) toward the Y$^\bullet$/ROO$^\bullet$ radicals. Yet, a small and quantifiable fraction of free phenol persists even in neat PS and the quenching of ROO$^\bullet$ radicals or other radicals is accomplished by this fraction clearly at a slower rate (see Figure 6).

This observation is of course of great importance in-vivo since cells contain plenty of water and polar molecules. The rate constants determined in saturated hydrocarbon solvents (hereafter indicated as k_{12}^0) may not be representative of the real antioxidant effect of ArOH in the cell compartments since water and polar groups exert a strong levelling effect on the rate differences, even though in these media electron transfer mechanisms can make Reaction 12 occur at a higher rate (see below).

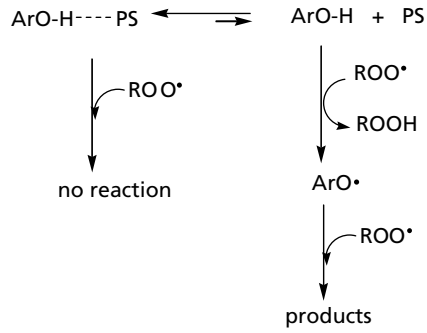


Figure 6 Kinetic solvent effects on the H-atom abstraction from ArOH by ROO$^\bullet$ radicals.

The magnitude of kinetic solvent effects, that is the reduction of rate, is independent of the nature of the radical $Y^{\bullet}$. In fact, it correlates pretty well with two parameters that describe the attitude of PS to act as hydrogen-bond acceptors (β_2^H scale from 0 to 1.0; Abraham et al 1990) and of ArOH to act as hydrogen-bond donors (α_2^H scale from 0 to 1.0; Abraham et al 1989) in the formation of the HB complex (PS•••HOAr):

$$\log k^{PS}_{ArOH/Y\bullet} \approx \log k^{0}_{ArOH/Y\bullet} - 8.3\alpha_2^H\beta_2^H \qquad (32)$$

Equation 32 allows the calculation of k_{12} in a range of solvents provided the value in one solvent is experimentally set. The calculated values are quite close to the experimental ones as long as Reaction 12 occurs via a HAT mechanism from ArOH to $ROO^{\bullet}$. Indeed, alcohols cause abnormal effects on Reactions 12 and 29. The experimental rate constants in these solvents are much larger than those predicted by Equation 32. In a few instances these rates are even greater than those in saturated hydrocarbon solvents (Litwinienko & Ingold 2003). These abnormal solvent effects demand a non-HAT mechanism. In these protic solvents, phenols dissociate into their corresponding phenoxide anions. It is this minute amount of ArO^{-} anions that quench efficiently the $dpph^{\bullet}$ and $ROO^{\bullet}$ radicals via an electron transfer mechanism, Reactions 33–35:

$$ArOH \rightleftharpoons ArO^{\ominus} + H^{\oplus} \qquad (33)$$

$$ArO^{\ominus} + dpph^{\bullet}/ROO^{\bullet} \longrightarrow ArO^{\bullet} + dpph^{\ominus}/ROO^{\ominus} \qquad (34)$$

$$dpph^{\ominus}/ROO^{\ominus} + H^{\oplus} \longrightarrow dpph\text{-}H\ /\ ROOH \qquad (35)$$

This ionic mechanism was discovered by Litwinienko and Ingold, and independently by Foti et al, and has been named sequential proton loss electron transfer (SPLET) (Litwinienko & Ingold 2003, 2004; Foti et al 2004). SPLET preferentially occurs in solvents of high dielectric constant where the HAT mechanism is almost entirely suppressed by kinetic solvent effects. Strong acids suppress the SPLET mechanism, while bases increase its contribution. This is because acids and bases shift equilibrium 33 in opposite directions. When the value of k_{12} or k_{29} in a given polar solvent is greater than predicted by Equation 32 or it can be reduced to the predicted limit by addition of an acid, then a contribution from SPLET is almost certain. All these effects on rates and mechanisms have very recently been reviewed by Litwinienko & Ingold (2007).

The importance of the medium: the case of curcumin

In the previous paragraph the role of the solvent in determining the mechanism and rate of the reactions of phenols with $ROO^{\bullet}$ radicals was emphasized. I want now to describe a peculiar case of solvent effects on the reaction mechanism of curcumin that has been a matter of controversy until recently (Litwinienko & Ingold 2004). Curcumin is the principal yellow pigment of the Indian curry spice turmeric (*Curcuma longa*), which is widely used in the Asian cuisine. Curcumin has been claimed to have many pharmacological properties generally ascribed to its radical scavenging properties such as antioxidant, anticancer and anti-inflammatory properties. In solution, curcumin exists in the tautomeric forms keto and enol (Figure 7), but the enolic form largely predominates (Kolev et al 2005).

Controversial opinions about the reaction site of curcumin with various radicals have been expressed by different groups. The fast reaction of curcumin with $CH_3^{\bullet}$ and *tert*-$BuO^{\bullet}$ radicals in polar solvents was attributed by Jovanovic et al in 1999 to a HAT process from the central methylene of the eptadienone linkage. The C–H bonds of this methylene group were also considered responsible for the antioxidant properties of curcumin. By contrast, Barclay et al (2000) reported that 'curcumin is a classical phenolic chain-breaking antioxidant, donating H atoms from the phenolic groups not the CH_2 group as has been suggested'. This conclusion was based on the results of the inhibition of the AIBN-initiated autoxidation of styrene in chlorobenzene by curcumin and various synthetic derivatives.

Litwinienko and Ingold reconciled these contrasting opinions in 2004 by observing that polar and apolar solvents may activate different mechanisms (SPLET or HAT) and reaction sites (enolic or phenolic OHs) on curcumin (Litwinienko & Ingold 2004). There is no doubt that the preferential route for the reaction of curcumin with radicals in apolar solvents remains the H-atom abstraction from the phenolic OHs. On the other hand, polar solvents (especially alcohol solvents and water) activate the ionization of the enolic hydroxyl (the most acidic site in curcumin, $pK_a = 8.55$), with the consequent electron transfer reaction from this site (SPLET). Litwinienko and Ingold hold that the fast quenching of $dpph^{\bullet}$ caused by curcumin in polar solvents occurs via a SPLET process from the enol moiety. The elementary processes shown in Figure 8 have been formulated for this reaction (Litwinienko & Ingold 2004). Two steps appear to be the keystone of this mechanism, that is: (i) the fast ionization of one phenolic hydroxyl of the intermediate radical B because of the effects on the OH acidity of the strong electron-withdrawing enolic radical moiety; and (ii) the migration of the negative charge from the phenoxide portion of the radical anion C to the enolic

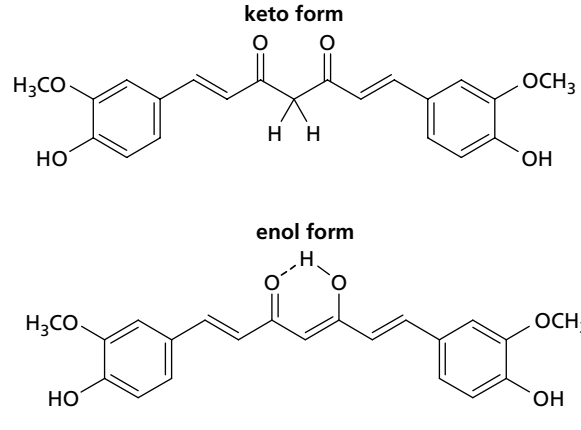


Figure 7 The keto and enol forms of curcumin.

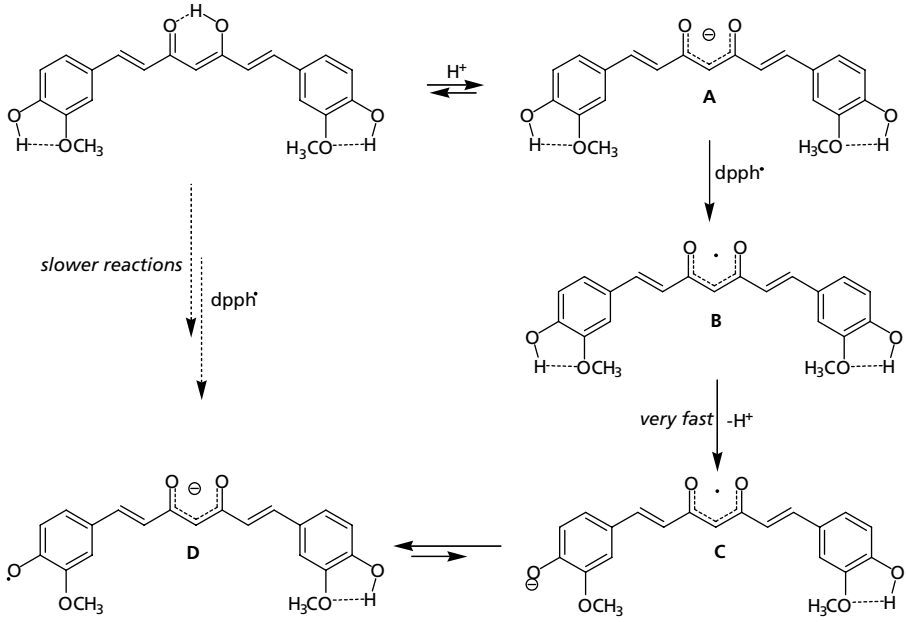


Figure 8 Reaction mechanism of curcumin with the dpph• radical in polar solvents.

radical group to form the more stable radical anion D. Other reaction paths from phenolic OHs or anions are considered to be slower than the previous one because of kinetic solvent effects or low acidity of the phenol moiety.

Conclusions

The analysis of kinetic and thermodynamic data gathered from half a century of studies indicates that the efficiency of phenols as inhibitors of the autoxidation of organic matter depends on a variety of factors. This is particularly true in complex systems such as living organisms. The design of new phenolic antioxidants must therefore take into consideration most of these physical/chemical constraints. There is no doubt that the BDE of the O–H bond to be broken is the first and possibly the most comprehensible factor determining the antioxidant activity of ArOH. Chain-breaking antioxidants have OH BDEs confined approximately over the range of 85–77 kcal mol^{-1}. The lower BDEs of this range characterize the most effective phenolic antioxidants (e.g. α-tocopherol and its analogues, catechols, flavonoids, ubiquinols). However, as the OH BDE decreases, the antioxidant may turn into a pro-oxidant since O_2 oxidizes it rapidly with formation of $HOO^{\bullet}$, $ArOH + O_2 \rightarrow ArO^{\bullet} + HOO^{\bullet}$. The decay kinetics of $ArO^{\bullet}$ are also important for the inhibition effects of ArOH, since this radical may contribute to chain-restarting reactions or may capture another $ROO^{\bullet}$ radical. The aryloxyl radicals of hindered phenols are less prone to react with the substrate (RH) or ROOH. However, special cases of isolation of $ArO^{\bullet}$ may determine an abnormal behaviour of ArOH (as with TMP in the human lipoprotein). The arguments put forward up to here are only a part of the picture. In fact, solvent effects strongly influence the rates and may modify the mechanisms of reaction of phenols with $ROO^{\bullet}$. Synergistic effects with various compounds may increase the antioxidant effect of a phenol in the system. Adventitious metal ions can be reduced by phenols to a lower oxidation state and as such decompose $ROOH/H_2O_2$ to strongly reactive radicals ($RO^{\bullet}$ and $HO^{\bullet}$). Phenols may also inactivate free metal ions by chelating them. Heterogeneous systems are obviously characterized by a plurality of physical phenomena that may blur the potential antioxidant activity of a phenol. The viewpoint expressed by Halliwell in 2000, 'The antioxidant paradox' can well be understood (Halliwell 2000). Although vitamin E, ascorbate and β-carotene are demonstrated to be excellent antioxidants in model systems, supplements of these compounds in clinical trials do not seem to have the expected beneficial effects on patients. The challenge for the future is therefore 'to be able to understand these relationships and how to manipulate them to our advantage to prevent and treat disease' (Gutteridge & Halliwell 2000).

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