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How Do Phenolic Compounds React toward Superoxide Ion? A Simple Electrochemical Method for Evaluating Antioxidant Capacity

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The reactivities of different phenols and polyphenols versus superoxide ion ($O_2^{\bullet -}$) were investigated as an easy-to-handle electrochemical method for evaluating antioxidant capacities. In view of this application, the $O_2/O_2^{\bullet -}$ couple and associated reactions between $O_2^{\bullet -}$ and polyphenols (or phenols) were examined in an aprotic solvent [dimethylformamide (DMF)] by cyclic voltammetry. Comparisons based on simple criteria (reversibility of the O_2 reduction in the presence of the phenolic compound, electron stoichiometry, or apparent kinetic constants) allow discriminations between the possible mechanistic pathways (acid−base or radical reaction type). The results highlight that the proton-transfer and radical-transfer pathways are both present for monophenols and polyphenols, with the relative contributions of the two pathways depending on the phenol structure. In agreement with the literature, polyphenols containing an *o*-diphenol ring (as in flavonoids) were found to present the highest reactivities.

Polyphenols are natural compounds that are ubiquitous in plants[1] and have a chemical structure containing phenolic units.[1,2] They are often described as antioxidant molecules,[3] that is, substances that, when present at low concentration, decrease or prevent the oxidation of other molecules.[4] They are found in a number of food products[1−5] (fruits, vegetables, beverages, cereals), nutraceuticals,[6] and medications[7] and are commonly used as additives for preventing oxidation processes in food.[8] These molecules are receiving great interest from consumer, food, and pharmaceutical manufacturers because epidemiologic studies have suggested beneficial influences in preventing various pathologies such as cancer, cardiovascular, and neurodegenerative diseases.[9] Many protective effects have been ascribed to the antioxidative activities of polyphenols,[10] notably in relation with their power of scavenging free radicals (radical chain breaking) and protection against ROSs (reactive oxygen species).[4,11]

In this connection, numerous investigations have been devoted to the chemical and physicochemical properties of polyphenols (especially flavonoids) and of their radicals in relation to their activity. Studies have reported on the determinations of oxidation potentials,[12] pK_a values, and spectral properties,[13] as well as the reactivities of polyphenol radicals and the formation of secondary oxidized products.[14] However, there are still controversies about the meaning of these data, for example, concerning

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apparent oxidation potentials[13] or even concerning the structure of the produced phenoxyl radicals.[14,15] Moreover, in addition to the fact that phenolic compounds are acids, able to rapidly exchange H^+, they are often described as efficient H-atom donors[16] or as being involved in coupled proton−electron transfer (CPET),[17] resulting in a manifold of possible mechanistic pathways.

However, even if the mechanisms responsible for the observed properties are still the subject of active debates, many methods have been proposed for estimating antioxidant capacity, especially in terms of radical-scavenging capacity.[3] The most popular methods are certainly those based on the reaction of an antioxidant with a defined radical, generally a colored radical allowing spectrometric determinations of its concentration, typically, 1,1-diphenyl-2-picrylhydrazyl (DPPH) or 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$).[18,19] As expected, absolute characterizations differ according to the considered radical.[20] Following this strategy, other radicals could be envisaged as standard for estimating the antioxidant power of polyphenols. Superoxide radical anion, $O_2^{\bullet -}$, appears to be a particularly interesting species because of its existence in vivo as a ROS (reactive oxygen species) and because of various possible reaction pathways that make $O_2^{\bullet -}$ a good candidate for probing mechanisms.[4] For example, $O_2^{\bullet -}$ is known to react as a nucleophile with alkyl halides (nucleophilic substitution), as a moderate one-electron reducing agent with organic substrates and several transition-metal complexes (electron transfer), or as a weak base with acidic compounds (proton transfer).[21] $O_2^{\bullet -}$ is also described as a H-abstractor, notably against phenolic compounds (H-atom transfer, radical transfer).[22] A few spectrophotometric methods[22,23] or electrochemical biosensors based on the dosage of $O_2^{\bullet -}$ after reaction with a polyphenol have been proposed for evaluating antioxidant capacities. Typically, $O_2^{\bullet -}$ is first generated by an enzymatic system and reacts with the polyphenol. The residual $O_2^{\bullet -}$ concentration is then determined by spectroscopy or superoxide dismutase (SOD) based sensors in the case of electrochemical detection. Above all, these approaches attempt to measure IC_{50} values (i.e., the substrate concentration necessary to consume 50% of the superoxide radical) and provide very limited information about the involved mechanisms. In this context, there is a clear need for understanding the dominating mechanism involved between phenolic compounds and radicals.

The reactivities of superoxide versus different substituted monophenols, particularly the unsubstituted phenol itself, have been extensively studied in aprotic solvents using transient electrochemistry.[21,24] Electrogeneration of $O_2^{\bullet -}$ by reduction of dissolved oxygen in aprotic solvents is a convenient process because no byproduct is generated and $O_2^{\bullet -}$ is a long-lived species in aprotic media.[21,25] In organic solvents [dimethylformamide (DMF), dimethyl sulfoxide (DMSO), etc.], in the presence of a monophenol, the reduction mechanism of oxygen to form hydrogen peroxide was described as a series of proton transfers associated with homogeneous or heterogeneous electron-transfer steps [electrochemical−chemical−electrochemical/disproportionation (ECE/DISP) mechanism].[21,24,26] In the presence of weak proton donors (H_2O), $O_2^{\bullet -}$ reduction could also involve coupled proton−electron transfer (CPET).[27]

By comparison, electrochemical mechanistic studies of superoxide with polyphenols are scarce, and most of them were dedicated to the oxidation processes of typical polyphenols.[28] Some investigations used pulsed radiolysis in water,[29] but reactions of antioxidants with $O_2^{\bullet -}$ are often slower than "natural" superoxide decay in aqueous solution, which limits the available experimental time range. Radiolysis was realized in basic aqueous solution to avoid disproportionation (pH 10), allowing a classification of their reactivity.[29b] However, at this pH, phenolic compounds are generally in anionic forms that could lead to different reaction pathways.

We recently presented some preliminary results showing that the reaction of $O_2^{\bullet -}$ toward flavonoid molecules can be observed by cyclic voltammetry.[30] In the present work, we have explored and developed this possibility to gain more insight into the mechanisms and kinetics involved in the reaction of $O_2^{\bullet -}$ with different polyphenols that could be correlated with the antioxidant capacity. Combining experimental data and numerical simulations of the voltammograms, we propose a classification based on simple criteria (reversibility, stoichiometry) and/or the determination of apparent kinetic constants. This analysis was treated with a simplified mechanistic hypothesis involving proton-transfer and/or radical-transfer reaction pathways. We

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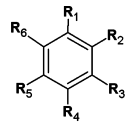
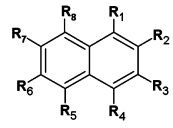
Table 1. Investigated Phenolic Compounds

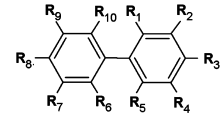
		substitution pattern									
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
		Benzenic Compounds									
1	phenol	OH	H	H	H	H	H				
2	4-chlorophenol	OH	H	H	Cl	H	H				
3	*p*-cresol	OH	H	H	CH_3	H	H				
4	2,4-dimethylphenol	OH	CH_3	H	CH_3	H	H				
5	2,6-di-*tert*-butyl-*p*-cresol	OH	$C(CH_3)_3$	H	CH_3	H	$C(CH_3)_3$				
6	catechol	OH	OH	H	H	H	H				
7	resorcinol	OH	H	OH	H	H	H				
8	hydroquinone	OH	H	H	OH	H	H				
9	3,5-di-*tert*-butylcatechol	OH	OH	$C(CH_3)_3$	H	$C(CH_3)_3$	H				
10	*tert*-butylhydroquinone	OH	$C(CH_3)_3$	H	OH	H	H				
11	phloroglucinol	OH	H	OH	H	OH	H				
12	*n*-propylgallate	$C(O)-O-C_3H_7$	H	OH	OH	OH	H				
		Naphthalenic Compounds									
13	2,3-dihydroxynaphthalene	H	OH	OH	H	H	H	H	H		
14	2,7-dihydroxynaphthalene	H	OH	H	H	H	H	OH	H		
		Biphenolic Compounds									
15	2,2′-biphenol	H	H	H	H	OH	H	H	H	H	OH
16	4,4′-biphenol	H	H	OH	H	H	H	H	OH	H	H



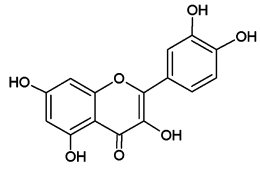
Benzenic compounds



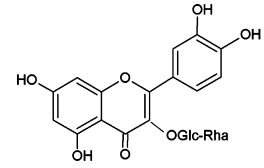
Naphthalenic compounds



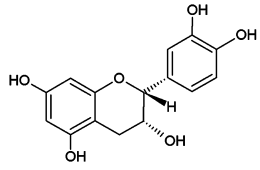
Biphenolic compounds



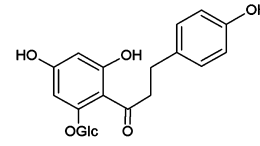
Quercetin (**17**)



Rutin (**18**)



Epicatechin (**19**)



Phloridzin (**20**)

have selected various types of phenolic compounds that could show different reactivities toward superoxide and used them as the first standard (Table 1). The considered phenolic compounds differ in terms of the number of phenol nuclei, the number and position of the hydroxyl functions, and chemical nature of the substitutions.

RESULTS AND DISCUSSION

Electrochemical Behavior of O_2 in the Presence of Phenols or Polyphenols. Our reactivity estimations are based on modifications of the voltammogram of O_2 reduction in DMF recorded at low scan rate (0.1 V s^{-1}) when a phenolic compound is added. Use of an aprotic medium, such as DMF, avoids disproportionation of the electrogenerated anion $O_2^{\bullet-}$,[25] making this radical stable even at the time scale of low-scan-rate voltammetry.[24,25] As other interests, many polyphenols are soluble in DMF (at least in the 10^{-3} mol L^{-1} range) and are only oxidizable at positive potentials, and thus, their electrochemical signal do not interfere with the measurements.

In a typical measurement, cyclic voltammograms (CVs) were first recorded in the absence of substrate in order to determine the initial cathodic and anodic peak currents (I_{pc}^{o} and I_{pa}^{o}, respectively) of the quasireversible reduction of O_2 to $O_2^{\bullet-}$.[21,24] The stability of $O_2^{\bullet-}$ in DMF is attested by the anodic oxidation current observed during the reverse scan. The large difference between the cathodic and anodic peak potentials, E_{pc} and E_{pa}, is indicative of slow heterogeneous electron-transfer kinetics ($k_s \approx 5 \times 10^{-3}$ cm.s^{-1}). Then, series of CVs were recorded in the presence of increasing concentrations of the studied phenolic compound (Figure 1A–D). Additions of phenolic substrate lead to a decrease of the $O_2^{\bullet-}$

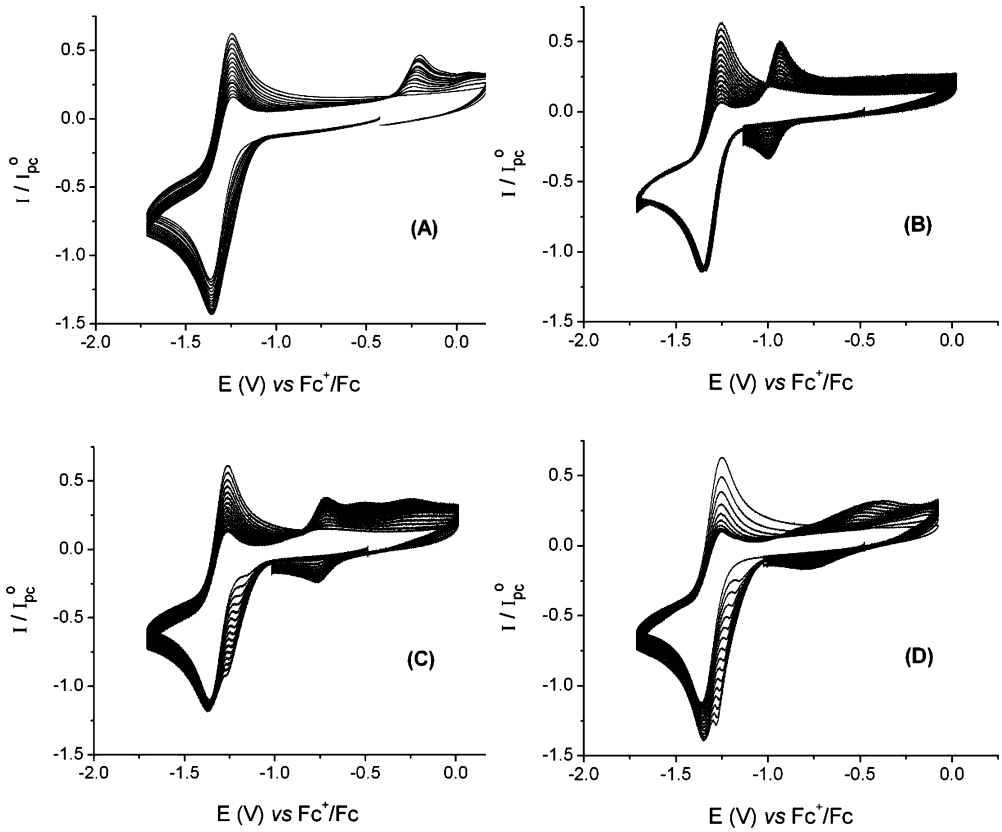


Figure 1. Cyclic voltammograms of the $O_2/O_2^{\bullet-}$ system in the presence of increasing concentrations of phenolic compounds. 10^{-4} mol L^{-1} successive additions of (A) 4-chlorophenol, (B) *tert*-butylhydroquinone, (C) catechol, and (D) quercetin. Glassy carbon disk electrode (2-mm diameter), 0.1 mol L^{-1} DMF + Bu_4NPF_6, scan rate = 0.1 V s^{-1}.

anodic peak current, I^S_{pa}, regardless of the phenolic structure. Depending on the nature of the phenolic compounds, this decrease could be concomitant with an increase of the cathodic peak current (as in Figure 1A) or with the appearance of a prepeak (Figure 1C,D).

Simultaneously, anodic peaks appear on the reverse scans that correspond to the products formed after reaction with $O_2^{\bullet-}$. When the added compound is a monophenol (Figure 1A), the irreversible anodic peak that is visible at potentials between about −0.4 and −0.3 V can be ascribed to the oxidation of generated phenolate anion by comparison with an authentic sample.[31] In the case of *tert*-butylhydroquinone, the reversible process (observed on the reverse scan around −0.8 V) is indicative of the formation of the corresponding quinone (see Figure 1B). Concerning other polyphenols like flavonoids, one or more broad oxidation peaks appear (see, for example, Figure 3D, below). A partial reversibility of these processes is sometimes detected, suggesting the formation of quinonic compounds (or semiquinones) and of polymeric materials such as those seen when authentic polyphenol materials are directly oxidized in an aprotic solvent.[12,32]

Approximate Reactivity Ranking Based on $C_{60\%}$ and $C_{10\%}$ Values. We proposed this approach as the electrochemical equivalent to spectrometric procedures based on DPPH or ABTS radical that are commonly used for estimating antioxidant capacities.[18−20] By analogy with these methods, we define for each substrate two characteristic concentrations, $C_{60\%}$ and $C_{10\%}$, as the phenolic concentrations needed to decrease the initial anodic peak current, I^o_{pa}, to 60% and 10%, respectively, of its initial value in pure DMF at $v = 0.1$ V s^{-1} (see the Supporting Information). In a first attempt, $C_{60\%}$ was considered for drawing the comparative reactivity scale. As seen in Figure 2, $C_{60\%}$ values are often lower than the O_2 concentration in DMF (around 10^{-3} mol L^{-1} under air),[24] and thus, the $C_{10\%}$ criterion seems more adapted for kinetics comparisons. For an easy reading, phenolic

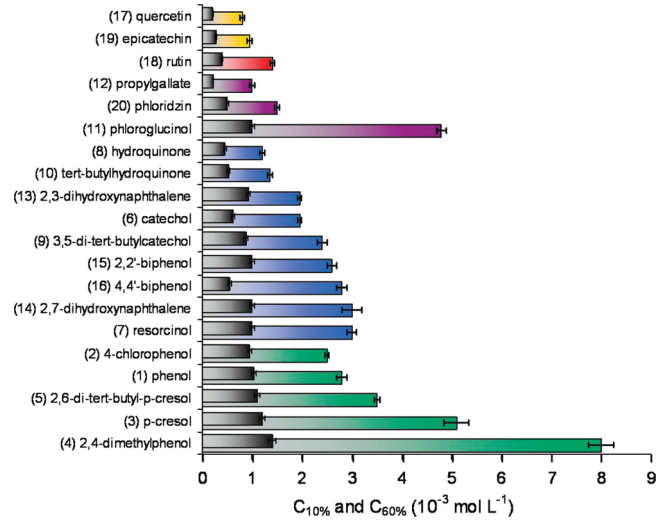


Figure 2. $C_{10\%}$ (colored) and $C_{60\%}$ (gray) values for various phenolic compounds. OH-phenolic function number: 1 (green), 2 (blue), 3 (purple), 4 (red), 5 (yellow).

(31) We checked that voltammograms obtained after addition of Me_4NOH base in a N_2-saturated solution of the monophenol showed similar peaks.

(32) Cotelle, N.; Hapiot, P.; Pinson, J.; Rolando, C.; Vezin, H. *J. Phys. Chem. B* **2005**, *109*, 23720.

compounds are classified in Figure 2 by their OH-phenolic function number (increasing from the bottom to the top of the histogram) and inside the same number series, by the decreasing $C_{10\%}$ values corresponding to an increasing reactivity.

As expected, reactivity depends on the number and chemical nature of the substituents present on the phenolic compound. Electron-donating groups decrease the reactivity of the phenolic compound, for example, in alkyl substitutions. Indeed, the reactivity of *tert*-butylhydroquinone (**10**) is lower than that of hydroquinone (**8**). For similar reasons, the three alkyl-substituted monophenols (**3**−**5**) are less reactive than phenol (**1**). Concerning the flavonoids, interpretation is somewhat more complicated, as reduction prepeaks appear upon addition of the polyphenols (Figure 1C,D). However, based on their $C_{10\%}$ values, it appears that flavonoids, such as epicatechin and quercetin, are the most reactive molecules, in agreement with antioxidant capacity estimations based on spectroscopic techniques.[20]

Possible Reaction Mechanisms. If $C_{10\%}$ values are interesting to draw rapid reactivity rankings, simple determinations of this concentration provide little information about the involved mechanisms. Obviously, reactions with the various antioxidants imply different chemical pathways as evidenced by the diverse resulting compounds (phenolate, quinone, condensation products, etc.; see voltammograms in the Supporting Information). Two main classes of reaction corresponding to different possibilities of associations between simple electron- and proton-transfer steps are generally considered for the reactivity of phenols or polyphenols: proton-transfer and/or H-transfer pathways (see, for example, refs 4 and 11).

Proton-Transfer Pathway. This is the major mechanism for the reaction between monophenols and $O_2^{\bullet -}$ in aprotic solvents (e.g., DMF, DMSO) and is the simplest situation as only one OH is concerned.[24] As demonstrated before, the mechanism involves a first proton transfer between the electrogenerated $O_2^{\bullet -}$, acting as a weak base, and the phenolic compound (AH), acting as a Brönsted acid (reaction 2). The produced radical $HO_2^{\bullet}$ is easier to reduce than the starting superoxide.[24,25,33] It could exchange a second electron at the electrode surface, reactions 1, 2, and 5, leading to an ECE-type mechanism or in solution by the superoxide, reactions 1−3, leading to a DISP mechanism[26]

$$O_2 + e^- \leftrightarrows O_2^{\bullet -} \qquad E^o, \alpha, k_s \tag{1}$$

$$O_2^{\bullet -} + AH \leftrightarrows HO_2^{\bullet} + A^- \qquad k_{f_{H^+}}, k_{b_{H^+}};\quad K_{H^+} = k_{f_{H^+}}/k_{b_{H^+}} \tag{2}$$

$$HO_2^{\bullet} + O_2^{\bullet -} \rightarrow HO_2^- + O_2 \qquad k_{f_3} \tag{3}$$

$$HO_2^{\bullet} + e^- \rightarrow HO_2^- \qquad k_{f_5} \tag{5}$$

$$HO_2^- + AH \rightarrow H_2O_2 + A^- \qquad k_{f_4} \tag{4}$$

$$O_2 + 2e^- + 2AH \rightarrow H_2O_2 + 2A^- \tag{6}$$

Because $O_2^{\bullet -}$ is a weak base, its protonation (reaction 2) by nonactivated phenols is thermodynamically uphill,[24] but remains efficient thanks to the subsequent homogeneous electron transfer (reaction 3) that displaces the H^+-transfer equilibrium.[24] In this situation, described as the DISP2 subcase, the reverse of reaction 2 is faster than reaction 3, reaction 2 acts as a pre-equilibrium compared with reaction 3, and the global kinetics depends on the parameter $K_{H^+}k_{f_3}C_{AH}$ (where K_{H^+} is the equilibrium constant for proton transfer, $K_{H^+} = k_{f_{H^+}}/k_{b_{H^+}}$, and C_{AH} is the concentration of phenol).[24]

Radical-Transfer Pathway. Concerning polyphenolic compounds, the most retained mechanism in the literature implies H-atom transfer (reaction 7).[19] As explained in the introduction, the H-donating capacities of polyphenols have essentially been examined toward radicals that do not exist in vivo.[19] Notably, flavonoids are described as good H-atom donors, and this feature is explained by their structure, which allows resonance in most cases through the phenolic nuclei and induces a stabilization of the generated radical $A^{\bullet}$ by reaction 7. The mechanistic and kinetics of the reactions between polyphenols and $O_2^{\bullet -}$ have been rapidly examined for only a few compounds.[30,34] Proposed mechanisms could be summarized by a simplified equation scheme (reactions 1−9)

$$O_2 + e^- \leftrightarrows O_2^{\bullet -} \qquad E^o, \alpha, k_s \tag{1}$$

$$O_2^{\bullet -} + AH \rightarrow HO_2^- + A^{\bullet} \qquad k_{f_{Rad}} \tag{7}$$

$$HO_2^- + AH \rightarrow H_2O_2 + A^- \tag{4}$$

$$A^{\bullet} \rightarrow \text{nonradical products} \tag{8}$$

$$O_2 + e^- + 2AH \rightarrow H_2O_2 + A^- + \text{nonradical products} \tag{9}$$

Key steps are both the H-atom transfer (reaction 7), which generates phenoxyl radical $A^{\bullet}$, and its rapid elimination to nonradical products (reaction 8).[12,32,35] In organic solvents, phenoxyl radicals rapidly dimerize or oligomerize to lead to nonradical products.[36] Formation of quinone or semiquinone is another possible evolution to nonradical products.[12]

Concerning the antioxidant capacity, it is thus important to discriminate not only the apparent reactivity but also the type of involved processes (proton transfer or radical transfer). Anodic current decrease upon polyphenol addition or apparition of new anodic peaks is not sufficient to discriminate the reaction pathway. More specific differences could be evidenced when simultaneously considering the reduction current intensity in addition to the reversibility analysis.[26] They are a function of the global number of exchanged electron: two for the proton-transfer pathway (reaction 6) and only one for the radical-transfer pathway (reaction

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(34) (a) Nanni, E. J.; Stallings, M. D.; Sawyer, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 4481. (b) Ziyatdinova, G. K.; Gil'metdinova, D. M.; Budnikov, G. K. *J. Anal. Chem.* **2005**, *60*, 49.
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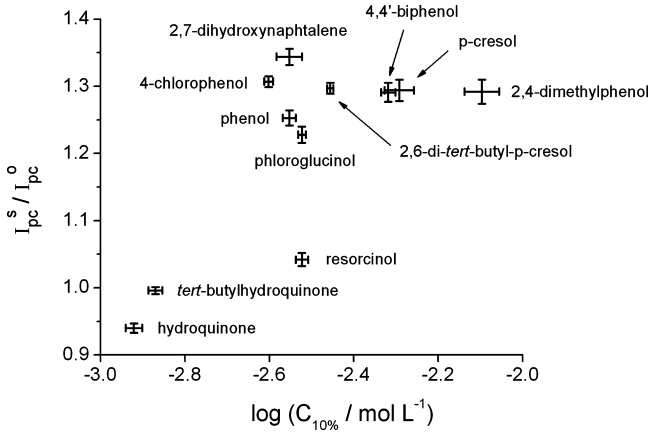

Figure 3. Reduction current ratio, I^S_{pc}/I^o_{pc}, measured at the concentration $C_{10\%}$.

9). However, both mechanisms might be concomitantly involved. If we examine our general results, the oxygen reduction current intensity always rises in the case of monophenols, whereas such an increase is observed for only some of the polyphenols.

Discrimination between Radical- and Proton-Transfer-Type Mechanisms. Analysis of the CVs was conducted in two steps: CVs without a prepeak and CVs presenting a prepeak.

Analysis of CVs without Prepeaks. We chose to follow the variation of the cathodic peak currents upon phenol addition, I^S_{pc}/I^o_{pc}, for the $C_{10\%}$ concentration. Figure 3 displays these I^S_{pc}/I^o_{pc} ratios as a function of log $C_{10\%}$, which allows a relative and comparative ranking of the phenolic compounds. Following the classification into radical- and proton-transfer-type mechanisms, the lowest log $C_{10\%}$ values correspond to higher reactivities of the phenol or polyphenol, and the lowest I^S_{pc}/I^o_{pc} ratio corresponds to the highest radical-transfer character.[37] As an example, 2,4-dimethyphenol was found to react with a dominating proton-transfer mechanism, and its kinetic constant is weaker than those of the other monophenols, in agreement with previous reports.[24] As expected, monophenols provide the highest acid−base character reaction with superoxide. In contrast, the lowest I^S_{pc}/I^o_{pc} ratios, corresponding to dominating radical-transfer mechanism, were observed for hydroquinones (I^S_{pc}/I^o_{pc} around 1 or slightly below). Hydroquinone is also the most reactive compound of the series. Resorcinol appears to be an intermediate case between the two types of mechanism, with an I^S_{pc}/I^o_{pc} ratio of around 1.05. Moreover, it is noticeable that the I^S_{pc}/I^o_{pc} values depend on the phenol but remain slightly below the theoretical values expected for a purely proton-transfer mechanism,[38] indicating the partial occurrence of a radical-transfer mechanism.

Analysis of CVs with Prepeaks (More Reactive Phenolic Substrates). Prepeaks appear on CVs when the most reactive polyphenols are added in substoichiometric concentrations; that is, when their concentrations are lower than that of dissolved oxygen in DMF (see Figure 1C,D and the Supporting Information). In such a case, the loss of reversibility of the O_2 reduction is mainly controlled by the amount of reactive phenolic functions. The described above $C_{10\%}$ or $C_{60\%}$ criterion does not permit a characterization of the kinetics except to say that these polyphenols display the highest reactivities. Information could be extracted from analysis of the prepeak potential; a higher potential shift ΔE_p (difference between the prepeak and initial O_2 reduction peak potential) could be correlated to a higher reactivity (see below). We first verified the prepeak current intensity to be proportional to the added concentration of polyphenols. Following this simplistic approach, we could provide a first approximate classification by considering the shift potentials ΔE under similar experimental conditions. ΔE_p for the flavonoid was measured when the prepeak current intensity was one-half that of O_2. ΔE_p was found to vary as follow: (**20**) phloridzin (93 mV) < (**19**) epicatechin (122 mV) < (**18**) rutin (130 mV) < (**9**) 3,5-di-*tert*-butylcatechol (144 mV) < (**17**) quercetin (148 mV).

Full Simulations of the CVs. To confirm previous treatments and hypotheses, we performed full simulations of the experimental CVs. These simulations provide additional information for mechanism classifications even if clearcut answers remain difficult to obtain because of the system complexity. More than one phenolic function is involved in the redox chemistry of polyphenol, and this number depends on the structure of the substrate. It results that manifold pathways are possible, as one could imagine many combinations of proton-transfer pathways or radical-transfer pathways. (See Scheme S1 in the Supporting Information). The overall mechanism was treated by the finite-difference method using the digital simulation program DigiElch.[39] (See the Supporting Information for details.)

For the less reactive phenolic compounds that display CVs without prepeaks, occurrence of a DISP2 kinetics situation was assumed to prevail for the acid−base part of the mechanism by analogy with previous investigations related to the electrochemical reduction of O_2 in the presence of monophenols.[24] The radical-transfer pathway was added to the mechanism as an irreversible competitive pathway ($k_{f_{Rad}}$) to the DISP2 pathway ($K_{H^+}k_{f_3}$). We considered as the main criteria values of I^S_{pc}/I^o_{pc} and I^S_{pa}/I^o_{pa} measured at the concentration $C_{10\%}$. The two constants $K_{H^+}k_{f_3}$ and $k_{f_{Rad}}$ were adjusted to reproduce the characteristics of the voltammograms. K_{H^+}, $k_{f_{H^+}}$, and $k_{f_{Rad}}$ at $C_{10\%}$ were chosen to be identical for each phenolic function. In a second step, values at $C_{60\%}$ were calculated using the same set of parameters and compared with experiments (see Table 2).

The same set of kinetics parameters permits the different experimental criteria to be reproduced with reasonable agreement, which supports the previous mechanistic propositions. It is also noticeable that in the monophenols series, derived values of K_{H^+} are in good agreement with data previously measured by double-potential-step chronoampererometry.[21,24] For hydroquinone, *tert*-butylhydroquinone, and 4,4′-biphenol, the number of involved OH-phenolic functions was found to be two, based on the small values of $C_{60\%}$ and $C_{10\%}$, as could be expected for this type of diphenol. Concerning the H-transfer character, hydroquinones **8** and **10** present the largest radical-transfer rate constants of the series, as derived from their lowest I^S_{pc}/I^o_{pc} and

(37) Considering that only the H^+-transfer mechanism leads to an increase of the oxygen reduction current and $A^\bullet$ is rapidly deactivated in a non-electrochemical way.

(38) Theoretical values estimated by calculation when the constant for the radical-transfer pathway, $k_{f_{Rad}}$, is made equal to zero.

(39) (a) *DigiElch Version 4.0*; ElchSoft: Kleinromstedt, Germany, 2006; http://www.elchsoft.com/. (b) Rudolph, M. *J. Comput. Chem.* **2005**, *26*, 633.

Table 2. Kinetic and Thermodynamic Parameters Obtained from Digital Simulations for Proton-Transfer and Radical-Transfer Mechanisms of Phenolic Compounds toward Superoxide for Substrates not Leading to Voltammograms with a Prepeak

	substrate	number of reactive OH-phenolic functions	$K_{H^+}k_{f_3}$ [a]	$k_{f_{Rad}}$ [a]	$C_{10\%}$ [b]	I_{pc}^S/I_{pc}^o sim/exp [c]	I_{pa}^S/I_{pa}^o sim [d]	$C_{60\%}$ [b]	I_{pc}^S/I_{pc}^o sim/exp [e]	I_{pa}^S/I_{pa}^o sim [f]
1	phenol	1	4000	1200	2.8	1.25	0.10	1.03	1.18	0.61
						1.25			1.10	
2	4-chlorophenol	1	60000	3700	2.5	1.31	0.10	0.95	1.17	0.64
						1.31			1.16	
3	p-cresol	1	530	140	5.1	1.29	0.10	1.2	1.18	0.56
						1.29			1.10	
4	2,4-dimethylphenol	1	260	68	8	1.29	0.10	1.4	1.16	0.54
						1.29			1.08	
5	2,6-di-*tert*-butyl-p-cresol	1	800	450	3.5	1.30	0.10	1.1	1.16	0.59
						1.30			1.18	
7	resorcinol	1	240	1300	3	1.04	0.09	1	1.05	0.63
						1.04			1.02	
8	hydroquinone	2	250	75000	1.2	0.94	0.12	0.45	0.95	0.64
						0.94			0.98	
10	*tert*-butylhydroquinone	2	100	4000	1.35	1.00	0.10	0.53	1.01	0.59
						1.00			0.99	
11	phloroglucinol	1	360	190	4.8	1.23	0.10	1.0	1.14	0.63
						1.23			1.05	
14	2,7-dihydroxynaphthalene	1	3800	520	3	1.34	0.10	1.0	1.22	0.61
						1.34			1.17	
16	4,4′-biphenol	2	550	170	2.8	1.29	0.10	0.55	1.18	0.58
						1.29			1.08	

[a] In L mol^{-1} s^{-1}. [b] In 10^{-3} mol L^{-1}. [c] Comparison between simulated (sim) and experimental (exp) values measured at $C_{10\%}$. [d] Simulated $C_{10\%}$ (should be equal to 0.10). [e] Comparison between simulated (sim) and experimental (exp) values measured at $C_{60\%}$. [f] Simulated $C_{60\%}$ (should be equal to 0.60). All CVs were measured at a scan rate 0.1 V s^{-1}.

$C_{10\%}$ values. On the contrary, 2,7-dihydroxynaphtalene and monophenols, which display the largest I_{pc}^S/I_{pc}^o ratios, have the lowest radical-transfer contributions. More generally, we could notice that the most reactive compounds in the series also have the highest radical-transfer rate constants. Simulated CVs of O_2 reduction in the presence of 2,4-dimethylphenol and *tert*-butylhydroquinone are shown in Figure S-2 of the Supporting Information and exhibit the good agreement between experiments and simulations.

The last point to address concerns the polyphenols leading to CVs with prepeaks, which are also the most reactive compounds. As found in our study, but also reported in many previous reports, the $O_2/O_2^{\bullet -}$ system is a slow electrochemical couple.[21] Considering the value of the standard heterogeneous rate constant, k_s, of this system, the whole electrochemical kinetics is controlled by the first electron-transfer step. Thus, large potential shifts such as those observed with the most reactive flavonoids are not possible if we consider that the current at the electrode passes only through the $O_2/O_2^{\bullet -}$ couple (through reaction 1).[40] For this reason, we could reject the occurrence of DISP kinetic mechanisms and consider that ECE mechanisms are more likely to prevail for these very reactive polyphenols.

A first possible ECE-type mechanism is the H^+-transfer pathway where the second electron transfer goes by the heterogeneous reduction of $HO_2^{\bullet}$ radical (reaction 5). $HO_2^{\bullet}$ displays a reduction potential much more positive than that of oxygen ($E^o_{HO_2/HO_2^-} = -0.25$ V), and this radical is formed near the electrode surface, which also favors the occurrence of an ECE-type mechanism.[24,26] The H^+-transfer ECE mechanism was thus considered in the simulation in competition with the irreversible radical-transfer pathway. Simulations allowed us to reproduce a prepeak behavior in the CV but only if high proton-transfer rates (several 10^7 L mol s^{-1} constants) are introduced in the calculation (see Figures S-3 and S-4 of the Supporting Information). Such required values of k_{H^+} seem unrealistic in view of protonation kinetic constants that are obtained for other phenols,[24] and thus, this first mechanistic hypothesis does not explain the prepeak observation.

Regarding the radical-transfer pathway, we could consider a second ECE-type mechanism. Indeed, when the radical-transfer reaction (reaction 7) becomes faster, radical A$^{\bullet}$ is formed near the electrode (as we discussed for $HO_2^{\bullet}$ in the proton-transfer pathway) and is more likely reduced before its deactivation. At this level of discussion, it is difficult to make a precise assessment about all of the possible deactivation ways; they are manifold, are not well-known, and would depend on the polyphenol structure. We have just tested this ECE scheme by considering a pure radical-transfer-type mechanism followed by the heterogeneous reduction of A$^{\bullet}$: A$^{\bullet}$ + e$^-$ → product (reaction 10). This scheme allows us to reproduce the prepeak phenomenon. We found that the potential shift ΔE_p increases with the rate constant $k_{f_{Rad}}$, but calculus requires high values of $k_{f_{Rad}}$ to fit the experimental ΔE_p values (around several 10^7 L mol s^{-1}). Are such values reasonable for H-atom transfer (reaction 7)? On one hand, pulse radiolysis studies report values on the order of 10^4 L mol^{-1} s^{-1} between quercetin and superoxide,[29b] but measurements where made at high pH (around 10) where the polyphenol is in anionic form. On the other hand, flash photolysis experiments performed with phenols in organic solvents, but reacting with other radicals, are commonly reported in the 10^7 L mol^{-1} s^{-1} range or higher.[16]

(40) Nadjo, L.; Savéant, J.-M. *J. Electroanal. Chem.* **1973**, *48*, 113.

Nevertheless, considering that high rate constants are required, we could conclude that the radical-transfer mechanism is more likely for flavonoids. However, the difficulty of reproducing the large potential shift of the prepeak might indicate some complications concerning the nature and modifications of the heterogeneous electron-transfer kinetics. This problematic issue remains open, and a clearcut answer would require detailed investigations and theoretical modeling that are presently beyond the scope of this work. Different hypotheses could be proposed such as the occurrence of coupled proton−electron transfer (CPET) or the formation of precomplex between O_2 and the substrate that renders the apparent heterogeneous electron-transfer kinetics faster.

All simulations confirm the expected tendency, namely, that a larger peak shift is indicative of a higher reactivity of the polyphenol, which supports our semiempirical analysis, and we could compare our ranking with literature. As expected, flavonoids present the highest reactivities, and more generally, our results confirm that catechol and, more generally, polyphenols containing a diorthophenol structure present the highest antioxidant activities. Indeed, the maximum effectiveness for the H-atom-transfer reaction requires a catechol-type structure in the B ring and the presence of the 3-OH group attached to the 2,3-double bond adjacent to the 4-carbonyl in the C ring.[18e,20] Quercetin fulfils all of these features. With this structure, the flavonoid phenoxyl radical shows the highest electron delocalization. Looking at our classification based on ΔE_p values, reactivities of flavonoids vary as follows: phloridzin < epicatechin < rutin < quercetin. By comparison with measurements based on DPPH or ABTS methods,[20] our results are globally in agreement with the literature, and the same general tendency is obtained.

CONCLUSIONS

Analysis of superoxide reactivity in the presence of polyphenolic compounds by transient electrochemical methods is a promising procedure for estimating the antioxidant capacities of polyphenols. At a first level, the procedure could be limited to the observation of both reversibility and stoichiometry of O_2 reduction upon polyphenol addition and permit rapid conclusions to be drawn about whether the studied compound is a potentially good radical scavenger. Lower reversibility means higher reactivity, as lower stoichiometry indicates a more likely radical-transfer-type mechanism. At a higher expertise level, these data could be associated with numerical simulations, allowing a better analysis of the mechanisms between phenolic compounds and superoxide radical that was correlated with their antioxidant activities. $C_{60\%}$ and $C_{10\%}$ are interesting indicators, as they permit a comparative classification of the reactivities of phenolic compounds toward superoxide. We could propose them as a possible electrochemical alternative to spectroscopic approaches such as those based on DPPH for estimating the antioxidant capacity. Different developments could be envisaged for practical analytical determinations. Notably, instead of using cyclic voltammetry, amperometric methods such as double-step chronoamperometry[24,26] could provide immediate numerical data. Such techniques could also permit operation at shorter experimental times with less influence of the heterogeneous electron-exchange kinetics and thus avoid the use of substoichiometric conditions. We could also emphasize that the procedure could be extended to the investigations of any compounds that present antioxidant properties and react either through proton-transfer or radical-transfer mechanisms, provided that the molecule is not electroreducible before the oxygen reduction.

Concerning the reactivity of polyphenols and phenols, flavonoids were found to be the best antioxidant candidates from among our examined series and the most reactive radical scavengers, in agreement with and as expected from literature reports. Interestingly, radical scavenging behavior was also detected for substituted monophenols, showing that a purely acid behavior versus superoxide is rarely the case.

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SUPPORTING INFORMATION AVAILABLE

Details on the electrochemical and simulation procedures, voltammograms of O_2 reduction in the presence of phenols or polyphenols, and comparisons with simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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