analytical chemistry

Anal. Chem., 1997, 69(16), 3385-3390, DOI:10.1021/ac9611733

Figure S1. First derivative EPR spectrum of radicals generated in potassium peroxodisulfate powder by a 4 hour UV-irradiation exhibiting near unaxial symmetry g_3 = 2.006 ≈ g_2 < g_1 = 2.031, and no hyperfine splitting larger than the linewidth. Present EPR spectrum was recorded at 298 K.

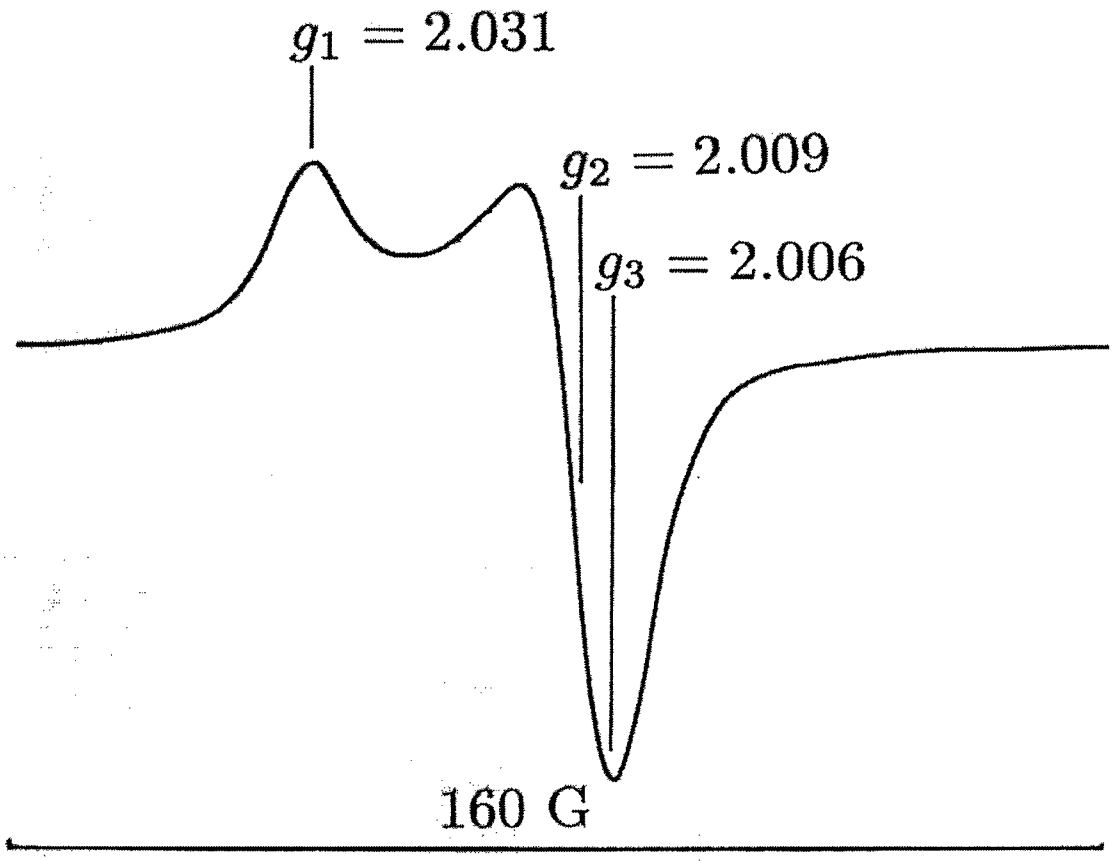


Figure S2. Effect of some phenols on luminol-specific ELL of UV-irradiated $K_2S_2O_8$: caffeic acid (■), protocatehuic acid (●), p-coumaric acid (✳), p-iodophenol (▲), p-cresol (◆), and additionally NO_2^- (○). Conditions: pH = 12, 1.00×10^{-7} M luminol, 10.0 mg samples of UV-irradiated $K_2S_2O_8$, injection volume 1.00 mL.

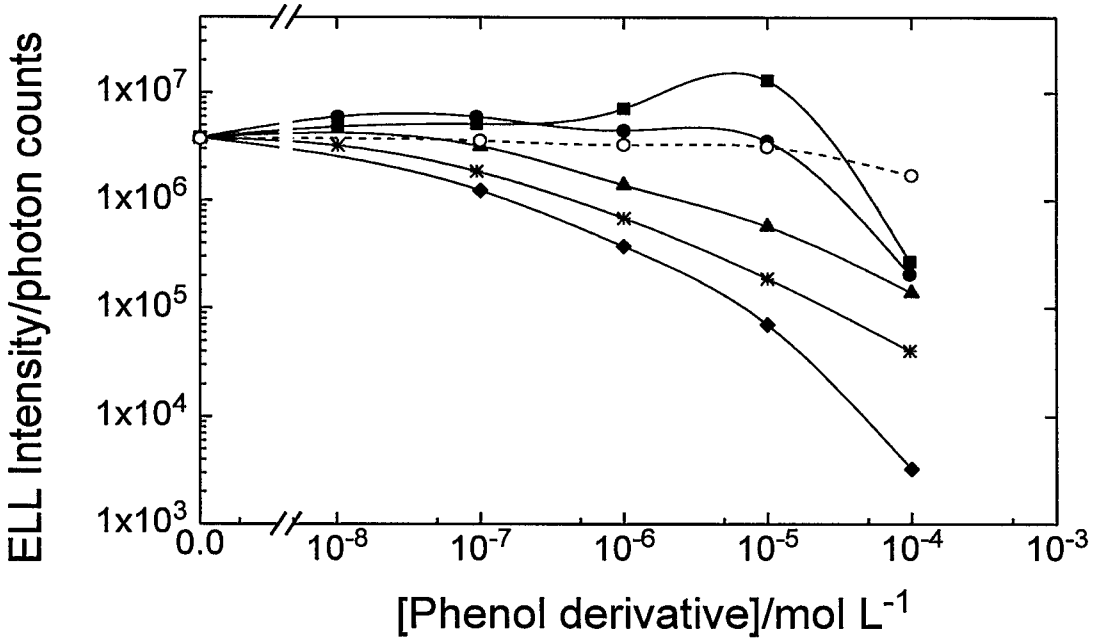
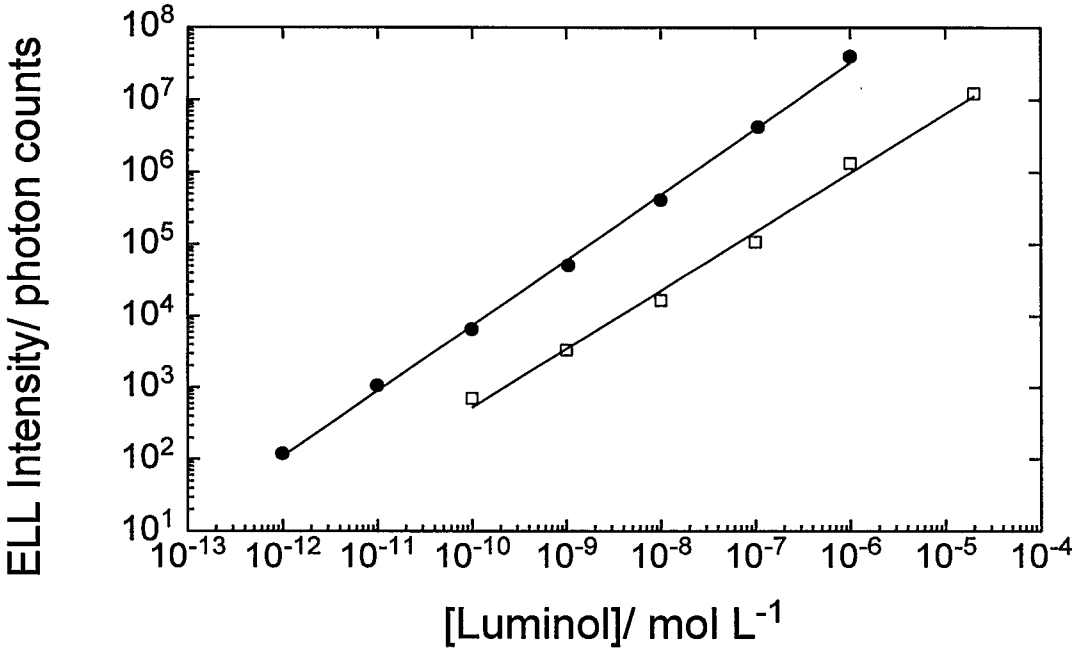


Figure S3. ELL and CL intensity as a function of luminol concentration. ELL (●), conditions: pH = 12, 10.0 mg samples of UV-irradiated $K_2S_2O_8$, injection volume 1.00 mL. CL (□), conditions: pH = 12, 2.00×10^{-3} M H_2O_2, 1.00×10^{-6} M $K_3Fe(CN)_6$; 300 μL sample of H_2O_2 solution was injected in the LL-cell containing 100 μL luminol mixed with 300 μL catalyst solution.



Effect of added phenolic compounds on the ELL

Previous studies[10] show that p-iodophenol enhances the chemiluminescence associated with the horseradish peroxidase-catalysed oxidation of luminol. p-Iodophenol reacts rapidly with the peroxidase reactive intermediates (rate constant value is ca 10^7 M^{-1} s^{-1})[10] supporting the hypothesis that the enhancement is due to the acceleration of the enzyme turnover. In addition, it has been shown that some phenoxyl radicals (Ph-O·) can oxidize luminol (S1). The reaction is reversible and the rate constants for forward and reverse reaction are often almost equal:

$$Ph\text{-}O\cdot + LH^- \leftrightarrow L\cdot^- + Ph\text{-}OH \quad (S1)$$

For coumaric acid (4-hydroxycinnamic acid) the rate constants for forward and reverse reaction are 4.8×10^7 M^{-1} s^{-1} and 3.4×10^6 M^{-1} s^{-1}, respectively[10]. Therefore, when the concentration of this phenol is too high it can act as a luminol radical scavenger.

In our system, p-iodophenol, coumaric acid and p-cresol quenched luminol-specific ELL as is shown in Fig. S2. The quenching effect of nitrite is reproduced in Fig. S2 to provide easier comparison with the effects of inorganic sulfate radical scavengers. The redox potentials for phenoxyl radicals are: 0.87 V and 0.77 V for p-iodophenol and p-cresol, respectively[S1]. The phenoxide ions ($Ph\text{-}O^-$) in our system can react with sulfate and hydroxyl radicals yielding phenoxyl radicals:

$$Ph\text{-}O^- + SO_4\cdot^- \rightarrow Ph\text{-}O\cdot + SO_4^{2-} \quad (S2)$$

$$Ph\text{-}O^- + \cdot OH \rightarrow Ph\text{-}O\cdot + OH^- \quad (S3)$$

The rate constant k_{34} for coumaric acid and sulfate radicals is 1.6×10^9 M^{-1} s^{-1}, a decarboxylation also taking place in this case[8]. Reaction (S3) is characterized by the following rate constants[12]: k(p-cresol + ·OH) = 1.2×10^{10} M^{-1} s^{-1}, k(p-coumaric acid + ·OH) = 8.1×10^9 M^{-1} s^{-1} and around 10^9 M^{-1} s^{-1} for p-iodophenol. However, the p-iodophenol solution was prepared in DMSO, known as hydroxyl radical scavenger [k(DMSO +·OH) = 7×10^9 M^{-1} s^{-1}][12]. An ELL enhancing effect due to the added phenoxides could only be expected, if the oxidation step of LH^- is fast enough. The longer lifetime of phenoxide radicals in comparison to sulfate and hydroxyl radicals allows phenoxides to mediate oxidation of LH^-. However, it seems not possible to make luminol detection by the present ELL method more sensitive with simple phenoxide additives.

The anions of caffeic (3,4-dihydroxycinnamic acid) and protocatehuic (3,4-dihydroxybenzoic acid) acids denoted as QH_2 (the charge of carboxyl group omitted for clarity) are known to enhance luminol-O_2-hexacyanoferrate chemiluminescence in strongly alkaline conditions and quench the luminol-H_2O_2-horseradish peroxidase

chemiluminescence[S2]. Dias et al. have proposed that the most strongly reducing anions of phenolic acids increases the conversion of luminol into endoperoxide (LO_2^{2-}) via the following reactions:[S2]

$$Q^{2-} + Fe^{3+} \rightarrow Q^{\cdot -} + Fe^{2+} \quad \text{(S4)}$$

$$Q^{\cdot -} + O_2 \rightarrow Q + O_2^{\cdot -} \quad \text{(S5)}$$

$$Q^{\cdot -} + O_2^{\cdot -} \rightarrow QO_2^{2-} \quad \text{(S6)}$$

$$QO_2^{2-} + L^{\cdot -} \rightarrow LO_2^{2-} + Q^{\cdot -} \quad \text{(S7)}$$

where Q^{2-} is the corresponding phenoxide ion of deprotonated phenolic acid, $Q^{\cdot -}$ the enhancing species (phenoxyl radical), Q benzoquinone form and QO_2^{2-} the phenol endoperoxide. From the quenching effect of these phenolic acids on the luminol-H_2O_2-horseradish peroxidase chemiluminescence, Dias et al. concluded these compounds to be too weak oxidants to oxidize luminol to luminol radical.

In our system with a fixed concentration $1x10^{-7}$ M of luminol, both phenolic acids (QH_2) behave as enhancers at low concentrations (Fig. S2). For caffeic acid the enhancing effect is observable even at 10^{-6} M and 10^{-5} M concentrations, but at higher concentrations it quenches the present ELL. As with the previous phenols, these compounds can also scavenge the sulfate and hydroxyl radicals. The oxidation potentials for protocatehuic acid and caffeic acid are: E^0= 0.084 V and E^0 = 0.119 V, respectively[S1], hence, it is clear that these phenoxide radicals cannot oxidize LH^-. So, if luminol chemiluminescence is observed, the initial key step, one-electron oxidation of luminol monoanions, must be provided by other oxidizing agents, and when the sulfate and hydroxyl radicals are too efficiently scavenged by phenolic carboxylates the quenching effect is indeed observed (Fig. S2). For both caffeic and protocatehuic acids the phenoxyl radicals are semiquinone radicals (denoted as QH·) which are rapidly deprotonated to anion radical form ($Q^{\cdot -}$) at pH = 12 [S3,S4]. The first reactions which occur in the presence of the phenoxides of these phenolic acids are:

$$QH^- + SO_4^{\cdot -} \rightarrow QH\cdot + SO_4^{2-} \quad \text{(S8)}$$

$$QH\cdot \rightarrow Q^{\cdot -} + H^+ \quad \text{(S9)}$$

The resulting semiquinone radicals react fast with the dissolved molecular oxygen (O_2 concentration ca. $2x10^{-4}$ M)[S4]

$$Q^{\cdot -} + O_2 \rightarrow Q + O_2^{\cdot -} \quad \text{(S10)}$$

where Q, is the benzoquinone form of the initial compound. The rate constant for reaction of luminol radical $L^{\cdot -}$ with oxygen[18,S6] is 550 M^{-1} s^{-1}, that is several orders of magnitude smaller than the rate constant for reaction (S10), which has been reported[S7-S9] for a series of quinones and hydroquinones to be around 10^8 M^{-1} s^{-1}. The phenoxyl radical ($Q^{\cdot -}$) reacts further with superoxide radical yielding phenol endoperoxide (QO_2^-), which reacts with luminol radical generating endoperoxide of luminol, as follows:

$$Q^{\cdot -} + O_2^{\cdot -} \rightarrow QO_2^{2-} \quad \text{(S11)}$$

$$QO_2^{2-} + L^{\cdot -} \rightarrow LO_2^{2-} + Q^{\cdot -} \quad \text{(S12)}$$

In conclusion, the enhancing effect of these phenolic acids is explained by the ability of their phenoxyl radicals ($Q^{\cdot -}$) to rapidly react with the dissolved O_2 present in the solution through the reaction (S10), in this way accelerating the conversion of luminol radical to luminol endoperoxide, (S11-S12) the key intermediate in the light emission pathway in these conditions.

Supporting Information References

S1 Wardman, P., *J. Phys. Chem. Ref. Data* **1989**, *18*, 1696-1755.

S2 Navas Dias, A.; Garcia Sanchez, F.; Gonzalez Garcia, J.A., *J. Biolumin. Chemilumin.* **1995**, *10*, 175-184.

S3 Kortum, G.; Vogel, W.; Andrussow, K., *Dissociation Constants of Organic Acids in Aqueous Solution*, Butterworths, London 1961.

S4 Neta, P., *Adv. Phys. Org. Chem.* **1976**, *12*, 223-297.

S5 Koppenol, W.; Butler, *J., Adv. Free Rad. Biol. Med.* **1985**,*1*, 91-95.

S6 Merenyi, G.; Lind, J.; Eriksen, T.E., *J. Phys. Chem.* **1984**, *88*, 2320-2323.

S7 Meisel, D., *Chem. Phys. Lett.* **1975**, *34*, 263-267.

S8 Meisel, D.; Fessenden, R., *J. Am. Chem. Soc.* **1976**, *98*, 7505-510

S9 Eberson, L., *Electron Transfer reactions in Organic Chemistry*, Springer-Verlag, New York 1987.