Mechanism of mercury electrooxidation in the presence of hydrogen peroxide and antioxidants

by

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Abstract

The oxidation process occurring on mercury electrodes in the presence of hydrogen peroxide has been analized in basic solutions by polarography and voltammetry in their linear scan and differential pulse modes. The process involves Hg(I) ion – in addition to Hg(II) ion – and hydroperoxide radicals, these last formed at trace levels. To demonstrate this radical generation radical scavengers (antioxidants), characterized with the DPPH$^{\bullet}$ radical scavenging assay, were employed. The interaction of the radicals with the antioxidant originate the decrease in signal, but the antioxidant itself does not react with the hydrogen peroxide in the absence of Hg. In the absence of antioxidant, the process is two-electron, being the rate-determining step the second electron transfer; at high concentrations of antioxidant, the oxidation corresponds to a reversible one-electron transfer followed by a chemical reaction between the hydroperoxide radicals and the antioxidant. A reaction scheme is proposed for intermediate antioxidant concentrations.

1. Introduction

More than forty years ago, the oxidation and reduction processes originated by the H_2O_2 on mercury electrodes were investigated in acidic media using polarographic and galvanostatic methods [1]. In the study, it was concluded that the anodic reaction corresponds not the H_2O_2 oxidation, but to the oxidation of Hg to Hg^{++} ions and the subsequent formation of a complex with sulfate ions.

Kikuchi and Murayama [2] proposed that the anodic wave observed on mercury electrodes in the presence of H_2O_2 is due to the oxidation of Hg to Hg^{++} followed by the formation of a mixed complex with HO_2^- and OH^- ions, as is shown in scheme I. This assumption was confirmed by Suznkievic and coworkers by using the titration of H_2O_2 with $HgCl_2$ in basic solutions [3]. A mechanism involving ROS (reactive oxygen species) generation has been recently proposed [4].

$$Hg \rightleftharpoons Hg^{2+} + 2e^-$$

$$Hg^{2+} + HO_2^- + HO^- \rightleftharpoons Hg(HO_2)(OH)$$

Scheme I

Reactive oxygen species (ROS) are radicals generated by organic and inorganic peroxides, including those originated by H_2O_2 or water itself, i.e., $HO^\bullet$, $HO_2^\bullet$, $O_2^{\bullet-}$… Free radicals are produced in moderate quantities in the organism, and apart from their role as secondary redox messengers [5], they participate also in important biological functions as the cellular proliferation stimulation, the physiological regulation, etc. [6]. The excessive production of ROS of exogenous or endogenous origin imply the occurrence of oxidative stress [7]. Such species play a significant role in the antioxidant stress and damage to DNA, proteins and lipids

[8] and in chronic diseases such as Alzheimer's, Parkinson's, Crohn's, cancer, etc. [9, 10]. The reactivity of radicals is associated to the chain reactions they initiate. Secondary antioxidants (that is, antioxidants with radical scavenging activity) interrupt the propagation of free radicals and prevent the metabolic activation of carcinogens [11, 12].

Spices and condiments have been recognized long time ago for their physiological and medicinal properties [13, 14]. The antioxidant activity of these compounds, specially phenols towards free radicals or ROS produced either by cell metabolism or as a response to external factors, is due to the scavenge of radicals and ROS [15]. This results in an inactivation of radical species [16], thus avoiding or preventing the abovementioned degenerative disorders [17-20].

To evaluate the antioxidant activity of natural products, different assays have been used, but a comparison of the results is difficult due to the different experimental methodologies adopted. Antioxidant activities of plant extracts (or their pure components) have been determined by an accelerated test [19, 20], by using stable radicals such as $ABTS^{+\bullet}$ [21] or $DPPH^{\bullet}$ [22], by ESR spin trapping technique and by measuring the oxygen consumption in lipid oxidation [23]. All these procedures present drawbacks as their requirements of using specific reagents and the time-consuming sample preparation.

Plant phenolics deserve special mention due to wide-ranging benefits they offer to plants and other living organisms. These advantages are, in essence, the result of their inherent physicochemical properties associated with the phenol functional group. The antioxidant character of phenolic compounds has been recently determined from the decrease in the polarographic current of hydrogen peroxide solutions originated by the addition of increasing amounts of the antioxidants [3, 24-27]. A variant of the method using

the differential pulse voltammogram was applied to other alimentary compounds [4, 28]. In all cases, the antioxidant action was related to the scavenging character of the antioxidants.

The aim of this work was to report the experimental evidences supporting a reaction mechanism of mercury electrooxidation in the presence of hydrogen peroxide and antioxidants, able to prove the ROS generation in this anodic process of hydrogen peroxide on mercury electrodes.

2. Experimental

2,5-dihydroxybenzaldeyde, eugenol, geraniol, sesamol and DPPH$^{\bullet}$ (2,2-Diphenyl-1-picrylhydrazyl) free radical, 95%, were purchased from Aldrich (or Sigma-Aldrich) and the rest of chemicals were Merck analytical grade reagents. All the reactants were used without further purification.

Solutions of 0.1 M in both sodium carbonate and phosphoric acid were used as supporting electrolytes. The aqueous solutions were prepared with ultrapure water type I (resistivity 18.2 MΩ.cm at 25º C) obtained from a Millipore Simplicity® system. The ionic strength was adjusted to 0.5 M with solid KNO_3 and the *pH* was adjusted with solid NaOH. Antioxidants were dissolved in ethanol and the stock solution concentrations were $5x10^{-3}$ M. These solutions were stored in darkness at 277 K to avoid decomposition.

The concentration of hydrogen peroxide in the cell was $5x10^{-4}$ M. In the experiments made at variable antioxidants amounts, the percentage of ethanol in the cell was 30% because the ethanol content modifies the DPV peak area [28]. For this reason, the solutions were prepared with a fixed amount of supporting electrolyte, 100 µL of $5x10^{-2}$ M H_2O_2, variable volumes of the stock solution of antioxidant in ethanol and completing the total volume with

pure ethanol. This was made by preparing a separate solution for each concentration of antioxidant. The H_2O_2 was added after the solutions were purged with purified nitrogen.

Measurements were made on a CHI650A electrochemical workstation from IJCambria coupled to an EF-1400 controlled growth mercury electrode from BAS instruments. In both the DME and in the HMDE mode, the drop area was 6.70×10^{-3} cm^2. The temperature was kept at 298±0.1 K. All potentials were measured against an Ag|AgCl|KCl, 3M electrode (BAS MF-2052). A platinum counter electrode BAS MW-1034 was used. The parameters selected in dc polarography and differential pulse voltammetry (DPV) were: pulse amplitude 0.05 V, pulse width 0.05 s and pulse period 0.2 s. In both dc and DP polarography the drop-time was fixed at 2 s.

The reproducibility of the measurements was ensured by repeating the experiments and the standard deviations of the data were less than 5%.

Spectrophotometric measurements. DPPH• radical scavenging assay

The maximum wavelength of the UV–visible absorption band of the DPPH• is 515 nm and the action of an AO causes the decrease of this band or its eventual disappearing through the reactions to give DPPH and AO•. The amount of antioxidant required to decrease the initial concentration of DPPH• to 50% (efficient concentration or EC_{50}) is a measurement of the antioxidant activity. The reverse value, namely anti-radical power, ARP = $1/EC_{50}$, should be higher as the antioxidant is more efficient.

UV measurements were made on a Genesys 10 UV spectrophotometer from Thermo Electron Corporation with quartz cuvettes of path-length 1.0 cm.

Different concentrations of antioxidants were added to DPPH$^{\bullet}$ methanolic solution. The initial DPPH$^{\bullet}$ concentration was $6x10^{-5}$ M. The DPPH$^{\bullet}$ concentration in the reaction medium was calculated from a calibration curve with the equation:

$Abs_{515\ nm} = 12.195 \times c_{DPPH} - 0.0137$

as determined by linear regression.

3. Results and discussion

Solutions containing hydrogen peroxide give single signals in direct current, dc, and differential pulse, DP, polarography, as well as in linear-sweep and differential pulse voltammetry. In dc polarography, the curve generally shows a polarographic maximum that prevents the use of this technique for mechanistic purposes [3, 24-28]. In some situations, a well-defined polarogram can be obtained, as is the case shown in figure 1: a maximum suppressor as Triton-X100 can eliminate the maximum but the resulting wave is deformed, being wider than the original polarogram. In fact, in these conditions, the logarithmic analyses made in the form of E vs. $\log[i/(i_L-i)]$ plots, in the range of potentials close to the half-wave potential, have slopes much higher than those expected [2]. Moreover, the analyses were not entirely linear. In the same way, DP polarograms are also distorted.

– – Figure 1 – –

On the other hand, both the limiting currents, in dc polarography, and the peak currents, in DP polarography, are nearly pH-independent at pH values higher than 8.5, decreasing slightly as the pH was decreased. Below pH 7 the oxidation of mercury overlaps strongly with the oxidation signal.

In linear-sweep cyclic voltammetry, the oxidation-reduction process was investigated at pH>7.5. Figure 2 shows a typical cyclic voltammogram. It can be appreciated that the reverse scan shows a reduction peak at potentials close to the oxidation peak, this indicating that the cathodic process corresponds to the reduction of the species that is oxidized in the oxidation scan. Moreover, the form of the voltammogram show evidences of superficial processes, this confirming the polarographic results.

– – Figure 2 – –

In all cases, the characteristic potentials (i.e. half-wave potentials in dc polarography and peak potentials in the rest) remained almost unchanged in very basic buffered solutions (pH>11.5). In the rest of the pH-range studied (8-11.5) these potentials shifted 45-50 mV per pH unit towards negative values as the pH was increased. This indicates that the H^+ ion is involved in the process at pH<11.5. It is evident that the acid-base dissociation of the hydrogen peroxide preceding the oxidation process is the cause of this pH dependence, because the dissociation pK_a of H_2O_2 is 11.7.

Differential pulse voltammetry was revealed as the most reliable technique for the study of the oxidation process, because the well definition and low distortion of the signal. The area of the DPV peak of the H_2O_2 oxidation on mercury was proportional to the peroxide concentration [29], and decreased as the contents of ethanol in the medium was increased, this indicating that the oxidation involves superficial processes, as commented above.

The equation 1, corresponding to first-order processes, was used for the analysis of the DP voltammograms [29]:

$$I = 4I_P[L/(1+L)^2] \qquad (1)$$

where $L=\exp[-(E-E_P)/b]$, I_P and E_P being the peak intensity and the peak potential, respectively, and b is a parameter which has the same value and meaning as the slope of the dc logarithmic analysis [29, 30].

This equation corresponds to a symmetrical peak, which area is proportional to both the number of electrons involved in the process and the reactant concentration. As can be seen in Fig. 3, the DPV peaks of H_2O_2 agree with equation 1. The fitting between theoretical and experimental data is good in the first part of the curve, but the end part is distorted by the oxidation of the mercury of the electrode surface, being the distortion more evident for the peaks recorded at lower concentrations of H_2O_2. So, the b values were obtained from the fitting of the experimental results to equation 1 in the first part of the DPV peak. Such b values were of 38-42 mV, corresponding to a two-electron process in which the rate-determining step, r.d.s., is the second electron transfer [29, 30].

– – Figure 3 – –

The addition of an antioxidant to the reaction mixture causes a decrease in the intensity of the DPV peak with respect to that obtained in the absence of this compound. This decrease is greater as the antioxidant concentration is increased, as is shown in figure 4.

– – Figure 4 – –

The scavenging properties of the compounds here used have been proved by using the DPPH$^\bullet$ radical scavenging assay. 2,5-dihydroxybenzaldeyde, eugenol, geraniol and sesamol are found in natural or synthetic spices and condiments [31]. The main experimental problem of this method arises from the determination of the steady state concentration, since even for antioxidant species having a fast kinetics, after 24 h the decrease of absorbance continues, though in low extension, and so it is difficult to obtain reproducible measurements. In this

work, it was considered that the steady state was reached when the absorbance remained constant during at least 10 min in the uncertainty limits of the spectrophotometer, i.e. ±0.001 absorbance units. The results obtained are gathered in Figure 5 for sesamol and 2,5-dihydroxybenzaldehyde as an example.

– – Figure 5 – –

As can be seen, both compounds are active in the test, the steady state concentration depending on the start concentration of antioxidant. The parameter EC_{50}, that is, the concentration of antioxidant that causes a decrease of 50% in the $DPPH^{\bullet}$ radical concentration, is obtained from these values. This is illustrated in figure 6 for the abovementioned compounds and also for eugenol.

– – Figure 6 – –

The antioxidant character increases as the EC_{50} decreases, because a lower concentration of compound is needed to scavenge the radical used as probe. In short, the four compounds here used are radical scavengers.

From the fittings to equation 1 shown in figure 7 for sesamol, the values of the b and I_P parameters were obtained, and the peak areas were calculated. At high antioxidant concentrations, such areas reached limiting values corresponding to one-half of the original areas. In addition, the b value in these conditions is close to 60 mV, corresponding to an EC process, that is, a process in which the r.d.s. is a chemical reaction placed after one reversible electron transfer [29, 30].

– – Figure 7 – –

In a first approach, it could be assumed that the decrease of the peak intensity is due to the reaction of the antioxidant with the species HO_2^- (or H_2O_2 itself). If this reaction takes place, a parallel decrease the concentration of the complex occurs. However, there are three reasons that support that this is not the cause of the decrease of intensity: First, the reaction rate of the interaction of the hydrogen peroxide and sesamol must increase as the antioxidant concentration is increased. This implies that the decrease of intensity must progress beyond the 50%, and at high antioxidant concentrations no signal would be detected. Second, if the antioxidant reacts with the H_2O_2, it must also react with this molecule in the solution and in the absence of the electrochemical process. Third, if scheme I were true, the shape of the DPV peak (the b value) must be the same both in the presence and in the absence of antioxidant, because the electrochemical process must be of the same type irrespective the antioxidant concentration, conversely to the experimental findings.

The justification for the first reason has been given above, that is, there is a limiting value of the peak area, namely one half of the area in the absence of antioxidant.

Two experimental evidences support the second reason. First, the reaction between hydrogen peroxide and the antioxidant in the solution must take place continuously. If this reaction occurs, successive voltammograms recorded with the same solution must decrease in intensity, conversely to that experimentally found: voltammograms recorded along 3h give the same values of intensity and half-width.

In addition, experiments were made by recording the linear-sweep cyclic voltammogram of antioxidants in the presence of increasing amounts of H_2O_2. No effects on the oxidation wave were observed even at hydrogen peroxide concentrations 10 times higher than the antioxidants [4], as shown in figure 8 for sesamol.

In conclusion, no reaction occurs involving these two reactants.

– – Figure 8 – –

Finally, the shape of the DPV peak, and the value of the area at high antioxidant concentrations corresponds to a one-electron process, in which the r.d.s. must be a chemical reaction involving antioxidant, placed after the first electron transfer. Therefore, the overall process changes from a from a two-electron oxidation to a one-electron reaction.

For all these reasons it can be concluded that scheme I is not complete and an intermediate must react with the antioxidant.

The oxidation of mercury in the absence of hydrogen peroxide takes place at potentials 0.1 to 0.3 V more positive than the peak here analyzed, and involves the formation of Hg (I) ions after one electron transfer [32]. It is reasonable to think that this species must be the abovementioned intermediate. Moreover, the interaction with the antioxidant (radical scavenger) indicates that, in the presence of H_2O_2, there are radicals involved in the reactions, and the only reasonable possibility is the formation of such radicals along the electrochemical process.

The scheme II shows a reaction pathway that takes into account all the above results and conclusions.

$$Hg \rightleftharpoons \frac{1}{2} Hg_2^{2+} + e^- \quad (2)$$

$$\frac{1}{2} Hg_2^{2+} \rightarrow Hg^{2+} + e^- \quad (3)$$

$$\frac{1}{2} Hg_2^{2+} + HO_2^- \rightarrow Hg + HO_2^{\bullet} \quad (4)$$

$$Hg^{2+} + HO_2^- + HO^- \rightleftharpoons Hg(HO_2)(OH) \quad (5)$$

Scheme II

In the absence of antioxidant, the reaction 3 to give Hg (II) ions is the predominant, the reaction 4 takes place at trace levels, and the reaction pathway is reduced to that represented in Scheme I. In this case, the DPV peak area corresponds to a two-electron transfer, and the shape of the peak can be described by equation 1 with b=2.303RT/1.5F=39.6 mV at 298 K [29, 30], assumed that the r.d.s is the second one electron transfer (reaction 3).

The antioxidants act by reacting with the hydroperoxide radical produced in reaction 4, thus decreasing the Hg (II) ion concentration and, consequently, decreasing also the oxidation current. At high antioxidant concentrations, reactions 3 and 5 do not take place at measurable rates and the process can be described with scheme III.

$$Hg \rightleftharpoons \frac{1}{2} Hg_2^{2+} + e^- \qquad (2)$$

$$\frac{1}{2} Hg_2^{2+} + HO_2^- \longrightarrow Hg + HO_2^{\bullet} \qquad (4)$$

$$HO_2^{\bullet} + \text{Antioxidant} \longrightarrow \text{products} \qquad (6)$$

Scheme III

Now, the DPV peak area corresponds to a reversible (or quasireversible) one-electron transfer followed by a chemical reaction (4+6), and the shape of the peak can be described by equation 1 with b=2.303RT/F=59.7 mV at 298 K [29, 30].

At intermediate antioxidant concentration values, the scheme consists of reactions (2)+(3)+(4)+(5)+(6) and the values of the peak area and b parameter are intermediate between the values obtained in the absence and in the presence of high concentrations of sesamol.

Figure 9 shows the values of the b parameter, together the percentage of decrease of the peak area, with the addition of variable amounts of sesamol.

– – Figure 9 – –

4. Conclusions

Solutions containing hydrogen peroxide give single signals in dc and DP polarography and voltammetry, with evidences of superficial processes coupled with the oxidation reactions.

The antioxidants 2,5-dihydroxybenzaldeyde, eugenol, geraniol and sesamol are radical scavengers as is deduced from the $DPPH^{\bullet}$ radical scavenging assay.

From the analysis of the interaction with the radical scavengers it was concluded that the electro-oxidation of mercury in the presence of hydrogen peroxide is not a simple sequence of two electron transfers followed by a complex formation, but hydroperoxide radicals are formed at trace levels.

This interaction allows the use of voltammetric techniques to evaluate the antioxidant character of substances which react with these radicals, as was proposed in reference 4.

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Figure captions

Figure 1. dc polarogram of 5×10^{-4} M H_2O_2 at pH=11.49 and 0.03% triton X-100.

Figure 2. Linear-sweep cyclic voltammogram of 5×10^{-4} M H_2O_2 at pH 10.50

Figure 3. Fitting of equation 1 (lines) to experimental DP voltammograms (symbols) of H_2O_2 at pH=10.50 and 30% ethanol and different concentration values. Squares: 0.1 mM; triangles: 0.4 mM; circles: 1 mM.

Figure 4. DP voltammograms of 5×10^{-4} M H_2O_2 and different amounts of 1 mM antioxidant solutions at pH 10.50 and 30% ethanol in the medium. Final volume: 10 mL.

Figure 5. $DPPH^{\bullet}$ radical scavenging assay. Determination of the steady state concentration for different antioxidant/ $DPPH^{\bullet}$ ratios.

Figure 6. $DPPH^{\bullet}$ radical scavenging assay. Dependence of the $DPPH^{\bullet}$ steady state concentration with the antioxidant/$DPPH^{\bullet}$ ratio for three antioxidants. Vertical lines place at the 50% value indicate the EC_{50} parameter for each antioxidant.

Figure 7. Fitting of equation 1 (lines) to experimental DP voltammograms (symbols) of 5×10^{-4} M H_2O_2 at pH=10.50 and 30% ethanol. Circles: in the absence of sesamol. Squares: in the presence of 0.1 mM sesamol.

Figure 8. Peak currents (○) and peak potentials (□) of the linear-sweep voltammograms of sesamol recorded on a glassy carbon electrode in the presence of variable amounts of H_2O_2.

Figure 9. Decrease of the DPV peak area (solid squares, continuous line) and variation of b parameter value (hollow circles, dotted line) with the added amounts of $5x10^{-3}$ M sesamol solution.

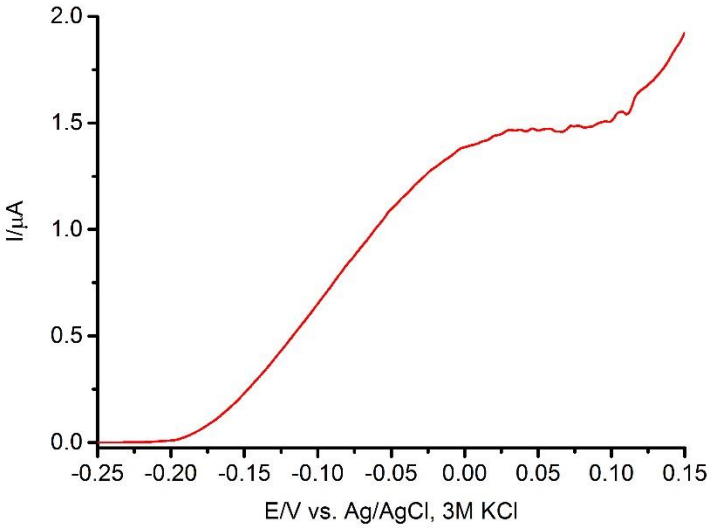


Figure 1

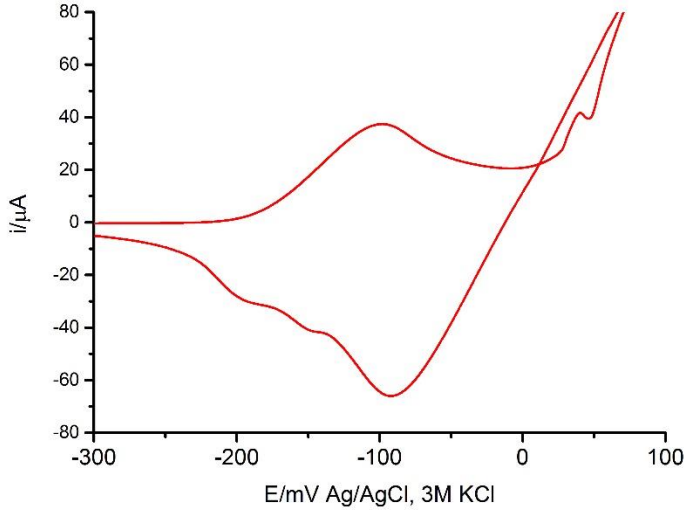
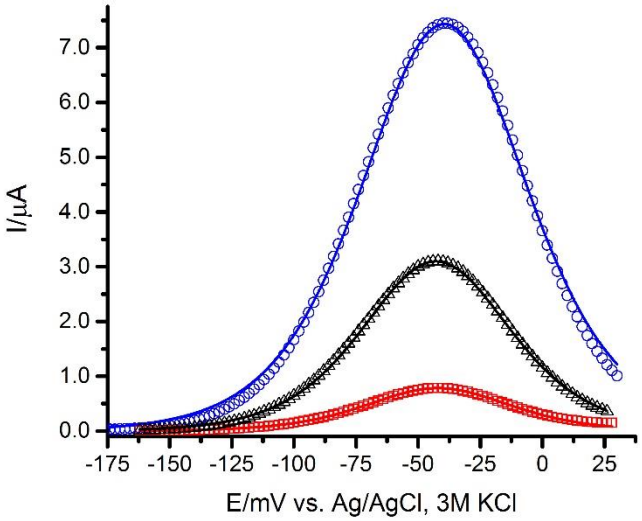


Figure 2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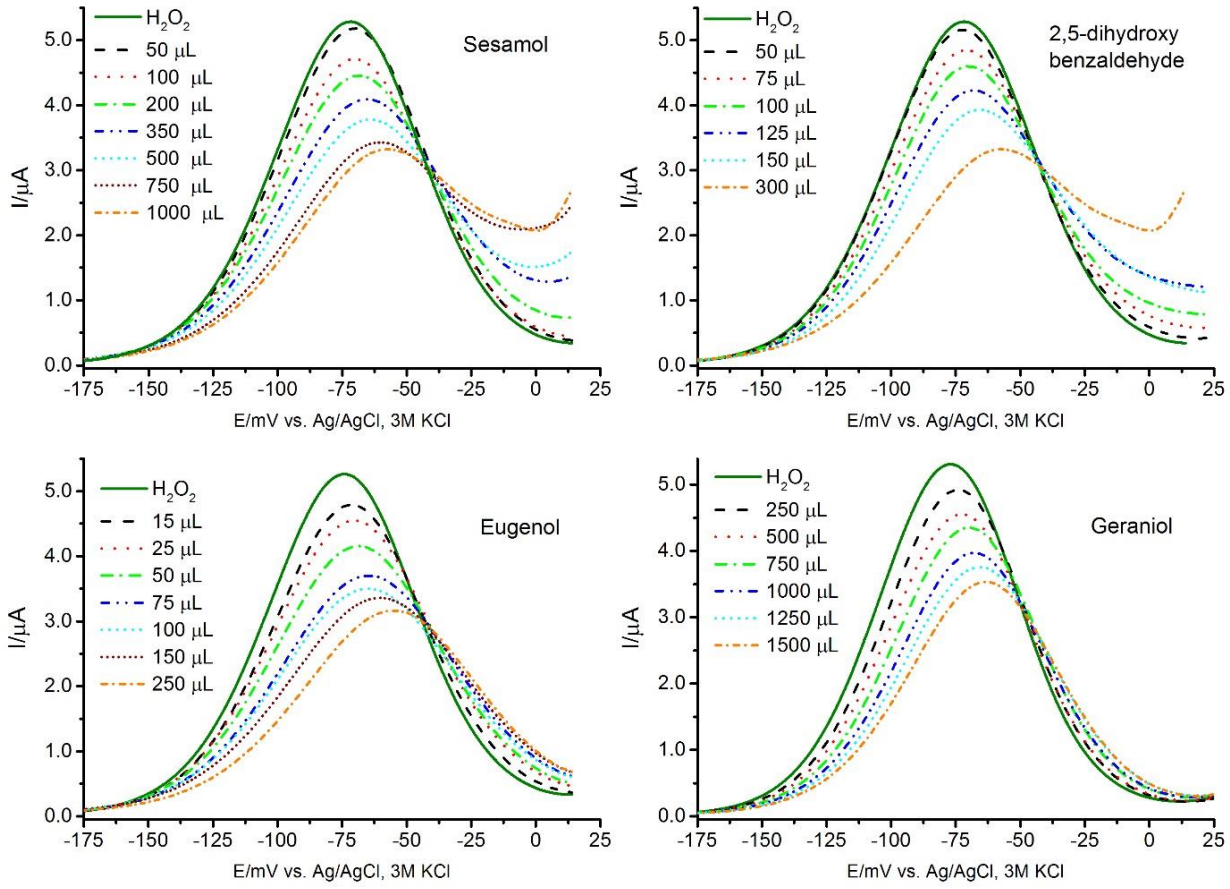
25
E/mV vs. Ag/AgCl, 3M KCl

Figure 3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50 µL
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Sesamol
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E/mV vs. Ag/AgCl, 3M KCl
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50 µL
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2,5-dihydroxy benzaldehyde
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1250 µL
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Geraniol
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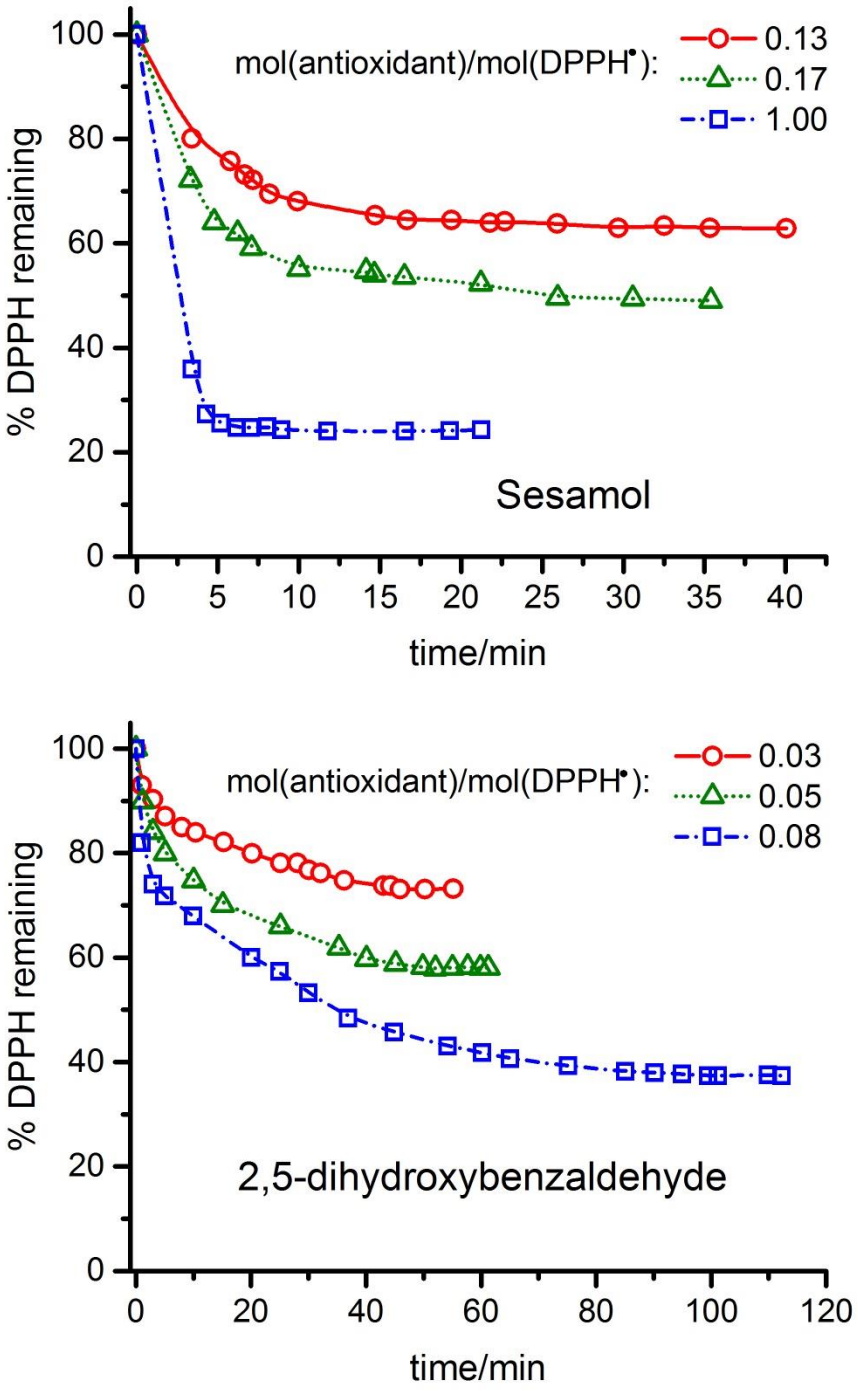
E/mV vs. Ag/AgCl, 3M KCl

Figure 4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0.17
1.00
Sesamol
% DPPH remaining
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0
20
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mol(antioxidant)/mol(DPPH•):
0.03
0.05
0.08
2,5-dihydroxybenzaldehyde
% DPPH remaining
time/min
0
20
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60
80
100
120

Figure 5

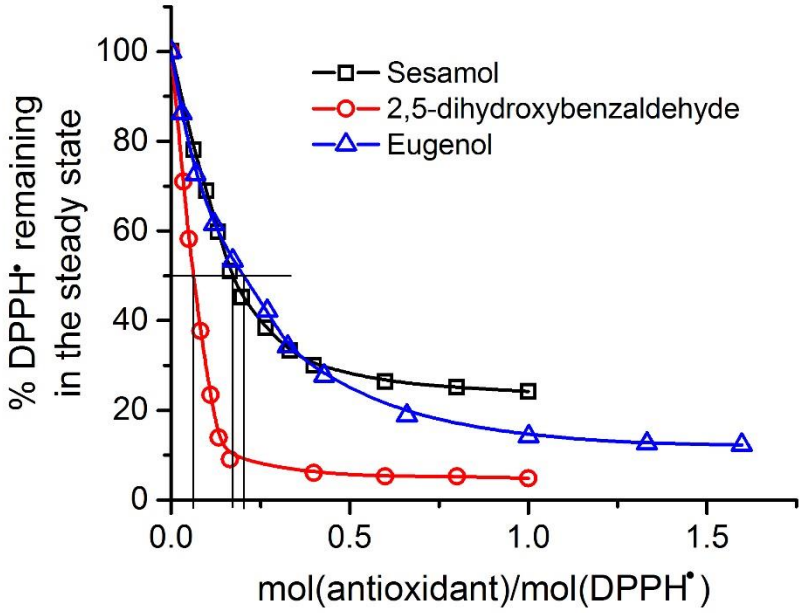

Figure 6

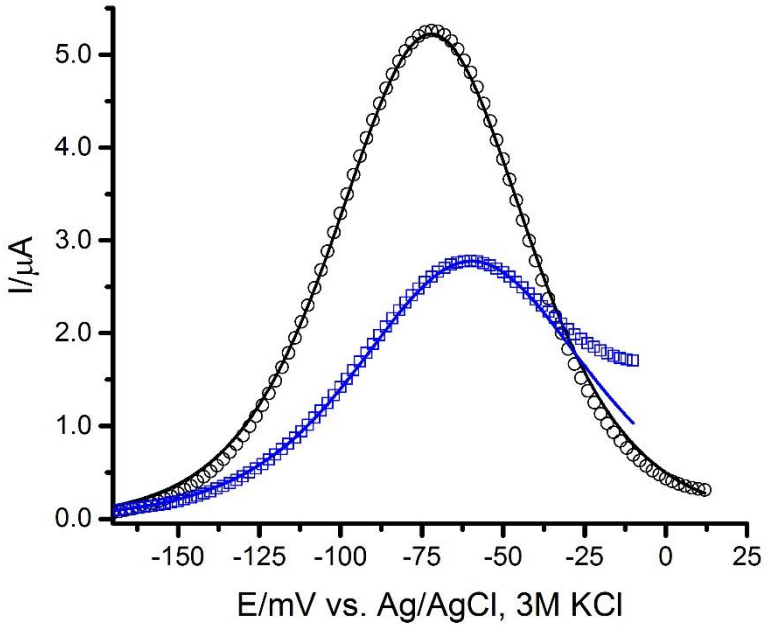

Figure 7

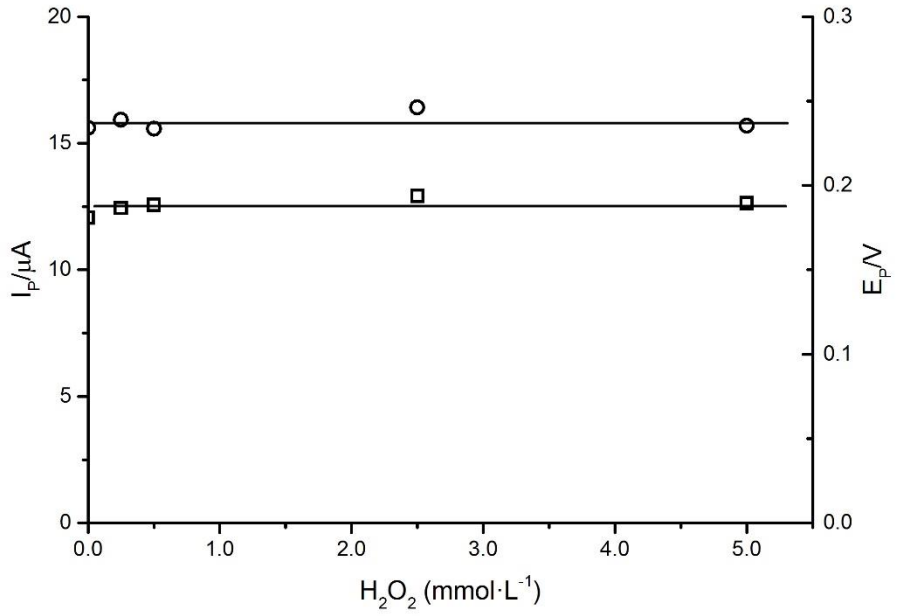

Figure 8

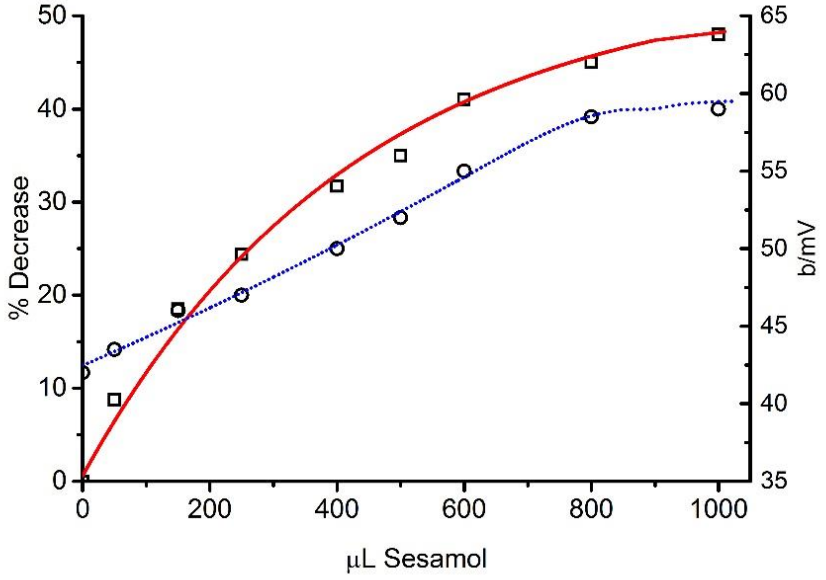

Figure 9