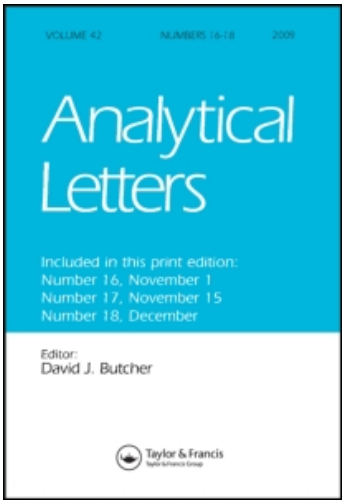

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Electrochemical Study of the Antioxidant Activity and the Synergic Effect of Selenium with Natural and Synthetic Antioxidants

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Electrochemistry

ELECTROCHEMICAL STUDY OF THE ANTIOXIDANT ACTIVITY AND THE SYNERGIC EFFECT OF SELENIUM WITH NATURAL AND SYNTHETIC ANTIOXIDANTS

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In this paper we propose two different electrochemical methods such as Cyclic Voltammetry (CV) and Osteryoung Square Wave Voltammetry (OSWV) to study the free radical scavenging ability of Selenium (Se) and natural and synthetic antioxidants. The originality of this paper is based on the study of the synergic effect of Se, not only with α-Tocopherol, but with a variety of antioxidants. As a result, we find an important synergism, in vitro, between Se and some other natural and synthetic antioxidants in the aqueous medium.

Keywords: Antioxidants; Aqueous medium; Electrochemical; Scavenging ability; Selenium; Synergism

INTRODUCTION

It has been well documented that the superoxide anion ($O_2^{\bullet -}$) and its related products such as hydroxyl radicals ($OH^{\bullet -}$), singlet oxygen ($^1O_2$), and non-free radical species, hydrogen peroxide (H_2O_2), are potent reactive oxygen species (ROS), which cause harmful effects in biological systems (Hall and Braughler 1989; Greenstock 1981; Bump and Brown 1990; Biaglow, Mitchell, and Held 1992). Furthermore, $O_2^{\bullet -}$ could reacts with nitric oxide (NO) producing an extremely reactive oxidants, peroxynitrite (ONO_2), and the hydroxyl radical. Moreover, they could be generated through exogenous sources such as exposure to pollutants, ionizing irradiation, and other external factors (Halliwell and Gutteridge 1989). High level of generation of ROS and other radical species leads to oxidative stress (OS). The ROS may be defined as a disruption of the balance between the levels of oxidants to reductants in the living organism. Thus, oxidative stress can result

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from increased exposure to oxidants or from decreased protection against them, or even from both problems happening simultaneously (Davies 2000).

Selenium (Se) is an important element from an environmental and biological point of view as it is essential in a very narrow concentration range; whereas, outside this range, deficiency or toxicity occurs (Sager 2006). The Se is a natural antioxidant that delays the oxidation of polyunsaturated fatty acids and preserves the elasticity of tissue (Skřivanová et al. 2007). This element is a cofactor of a large number of selenium-dependent enzymes such as an antioxidant enzyme glutathione peroxidase (GSH-Px) and thioredoxin reductase, which are involved in a cell's protection from severe oxidation by free radicals (Birringer, Pilawa, and Flohé 2002). Otherwise, high concentrations of selenium can cause the loss of hair and nails, and also, the irritation of skin and eyes.

Edible vegetable oils, and elaborate meal as some cosmetic products, tend to encounter problems of autoxidation, which would affect their quality and may endanger human health. The antioxidants to be used are determined by various factors including legislation, cost, stability, and effectiveness. Among natural antioxidants, caffeic acid (CA) and gallic acid (GA) are used in processed food, cosmetics, and food packing materials to prevent rancidity induced by lipid peroxidation and spoilage. They are phenolic acids found in various agricultural products (Milić, Djilas, and Čanadanović-Brunet 1998). The CA scavenged alkoxyl radicals as a result of metal-ion breakdown of hydroperoxide-enriched methyl linoleate of sunflower oil (at concentrations of 0.5, 1.0 and 2.0 mM) (Marinova, Toneva, and Yanishlieva 2009). The GA was found to be a strong antioxidant in emulsion or lipid systems (Cheng et al. 2007) and exhibits antimutagenicity.

On the other hand, synthetic phenolic antioxidants (SPAs), such as tertiary butylhydroquinone (TBHQ), are commonly used because of their chemical stability, low cost, and availability. But, the safety of these SPAs was questioned due to their potential risk (Madsen and Bertelsen 1995). The TBHQ, although it has not been approved as a food antioxidant in some countries, is permitted to be used in foods up to a maximum limit of 200 mg/kg in some other countries (Okubo et al. 2003; Pinho et al. 2000).

In recent years, a group of vitamins has aroused special interest for their ability to protect against cardiovascular diseases, cancer, cataracts, deficiencies in immune response, age-related problems, etc. One of these antioxidant vitamins, Vitamin E (α-tocopherol) (Perrin and Meyer 2002), is a lipophilic compound which is incorporated into biological membranes and is especially effective as a lipid–peroxyl radical scavenger (Arnao, Cano, and Acosta 1999; Rimm et al. 1993). The antioxidants properties of α-tocopherol (α-T) are based on the ability of the chromanoxyl ring to interact with different free-radicals (Leibovitz, Hu, and Tappel 1990).

Antioxidant capacity may be considered in nutritional analysis of fruits and vegetables. Moreover, the correct pharmaceutical election of the antioxidant mixture may help to remove ROS in patient with certain diseases and thus improve their clinical outcome. Dietary antioxidants can enhance the cellular defense and help to prevent oxidation damage to cellular components. Since antioxidant capacity of different samples depends on synergistic interactions between different antioxidant compounds, as well as on the way of action of each one of them, it is

necessary to use adequate procedures in the determination of their antioxidant capacity. Analytical methods have been developed to evaluate different aspects of the antioxidant action (Murase et al. 1997). These methods provide an uncertain measurement; therefore, an improved method should be developed for assessing antioxidant activity (Aruoma 2003; Frankel and Meyer 2000). In recent years, analyses with electrochemical detection constitute a methodology used extensively (Brand-Williams, Cuvelier, and Berset 1995; Jiao, Sun, and Wang 2002). Some advantages of this method include speed, accuracy, and precision. Electrochemical methods are developed for the investigation of antioxidant properties of biological fluids particularly of blood, estimation of its total antioxidant capacity (TAC), and clarification of the role in an antioxidant defense system (Gyurcsányi et al. 2002; Garay and Lovrić 2002). Square-wave voltammetry (SWV) is one of the electrochemical techniques more widely applied in quantitative analysis, especially due to its high sensitivity, which is a consequence of the rejection of most of the capacitive currents (O'Dea, Osteryoung, and Osteryoung 1981; Vogel 1961).

The aim of the present study was to investigate the synergic effects of Se with different natural and synthetic antioxidants which could be found in food and medicine. Thus, we firstly study the free radical scavenging individual ability of selenium (Se), α-tocoferol (α-T), gallic acid (GA), caffeic acid (CA), tert-butylhydroquinone (TBHQ), and Trolox (Trx), using its effect over $O_2/O_2^{\bullet -}$ couple in ACN (acetonitrile, aprotic media) by cyclic voltammetry. The same experiment was carried out using square wave voltammetry and was used for the $DPPH/DPPH^{\bullet -}$ couple in phosphate buffer (protic media). After that, we were able to determinate the synergism between Se and the other studied antioxidants.

MATERIALS AND METHODS

Reagents and Solutions

The free radical form (90% purity) of 1,1-Diphenyl-2-picrylhydrazyl ($DPPH^{\bullet -}$) and 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), α-tocopherol (α-T), gallic acid (GA), tert-butylhydroquinone (TBHQ), tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) were purchased from Sigma-Aldrich (Argentina). Tetramethylammonium $(CH_3)_4NOH$, tetramethylammoniumchlorid $(CH_3)_4NCl$, tetraethylamonium acetato $(CH_3\text{-}CH_3)_4NC_2H_3O_2$, were purchased from Fluka AG. The SeO_2 was purchased from Aldrich. Acetonitrile solvent was of analytical grade. The stock standard solution was prepared by dissolving 0.1405 g of SeO_2 (Se IV) in phosphate buffer:ethanol 40% and in acetonitrile (ACN) diluting to the mark in a 100 mL volumetric flask. The remaining antioxidant standard solutions were prepared by diluting a precise amount of drug in the different solutions described previously. Then, these standards were ultra-sonicated and were kept in darkness until they were used.

All other reagents employed were of analytical grade and were used without further purification. All solutions were prepared with ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultra pure water system, and the samples were diluted to the desired concentrations using a 10 mL Metrohm E 485 burette.

Apparatus and Electrodes

Electrochemical experiments were performed using a BAS 100B/W electrochemical analyzer (Bioanalytical System, West Lafayette IN). Cyclic and square-wave voltammograms were obtained using a three-electrode system consisting of a glassy carbon (GC) working electrode (BAS MF-2012; 3.0 mm diameter, 0.071 cm^2 geometrical area), a Pt wire counter electrode, a 3 M NaCl Ag/AgCl reference electrode (BAS MF-2052) in protic media, and a Ag/AgNO3 (0.01 M in ACN) reference electrode in aprotic media.

Before each analysis the working electrode was carefully polished using PK-4 polishing Kits BAS MF-2060 and rinsed following the general guideline for polishing electrodes recommended for BAS Electrode Polishing and Care, BAS A-1302. The polished electrode was activated electrochemically in 1 M KOH (Merck) aqueous solution by a potential step of 1.2 V over 5.0 min. Its electrochemical area ($A = 0.070\,cm^2$) was calculated from Cottrell plots (Bard and Faulkner 2001), studying the oxidation of 1.99×10^{-3} M ferrocyanide in 0.5 M KCl. The ferrocyanide diffusion coefficient in this reaction medium has already been reported as $D = 7 \times 10^{-6}\,cm^2\,s^{-1}$(Mackay et al. 1990).

Electrochemical Studies $O_2/O_2^{\bullet -}$ Couple

In order to choose the most suitable supporting electrolyte for the CV, solutions of tetrabutylammonium hexafluorophosphate (Bu_4NPF_6), tetramethylammonium $(CH_3)_4NOH$, tetramethylammoniumchlorid $(CH_3)_4NCl$, tetraethylamonium acetate $(CH_3\text{-}CH_3)_4NC_2H_3O_2$ were tested. The CV voltammograms showed well-formed voltammetric peaks using Bu_4NPF_6 between 0.03–0.07 M. A lower background current with high sensitivity was achieved using Bu_4NPF_6 0.065 M, so we consider this concentration as an appropriate medium for the experiments.

The redox potential for $O_2/O_2^{\bullet -}$ couple was determined by measuring current–potential responses in oxygen saturated ACN solution at scan rates $50\,mVs^{-1}$ using Bu_4NPF_6 0.066 M as a supporting electrolyte. After each measurement, the solution was purged with O_2 for 2 min to obtain a saturated media (99.98%). With a glassy carbon electrode, a defined reversible redox couple was observed at $50\,mVs^{-1}$ for each solution. An excellent reproducibility of Cyclic Voltammograms for $O_2/O_2^{\bullet}$ was obtained due to the stability of O_2 in anhydrous aprotic solvents (Nagano, Takizawa, and Hirobe 1992), and the ratio of anodic (Ipa) to cathodic (Ipc) current peak at all scan rates was almost the unit. The peak potentials were independent from the scan rates. The linearity of current peak vs. $v^{1/2}$ suggests that the rate of electron transfer was controlled by diffusion. The thermostatic cell was maintained at $25.0 \pm 0.5°C$.

OSWV Studies DPPH/$DPPH^{\bullet -}$ Couple

For the study of antioxidant activity in protic medium, an electrochemical open cell consisting of a glassy carbon (GC) working electrode (model BAS MF-2012, 3.0 mm diameter), an Ag|AgCl|3MNaCl reference electrode (BAS MF-2052), a Pt wire counter electrode, and a nitrogen supply tube was used.

The electrochemical thermostatic cell was maintained at $25.0 \pm 0.5°C$. The solution was previously de-oxygenated by bubbling on nitrogen for 10 min prior to measurements. The measurements of the $DPPH/DPPH^{\bullet -}$ couple were carried out using OSWV under the following conditions: step E: 4 mV, S.W. amplitude: 25 mV, S.W. frequency: 15 Hz, samples per point: 256, potential range: 0−500 mV, sensitivity $= 1 \times 10^{-5}\,AV^{-1}$. The current peak was determined using the BAS 100 W software.

Ten milliliters of 5 mM stock solution of DPPH were prepared in ethanol. The solution was ultra-sonicated (Transsonic 460/H, Elma, Germany) during 2 hrs and kept in the dark to minimize its light-decomposition. All electrochemical measurements were perform in a phosphate buffer solution (0.033 M, pH = 7.4) containing 0.033 M KCl and 40% (v/v) of ethanol. Student's t-test was used for statistic analyze with $p \leq 0.05$.

Electrochemical Determination of the Antioxidant Activity and Synergic Effect

Increasing concentrations of each compound were added to the electrochemical cell in protic and aprotic media for the determination of the antioxidant activity. The inhibition current was measured at different concentrations until the reaction reached equilibrium. The studies of the synergic effect were carried out with successive and increasing concentrations of a mixture of Se with other studied antioxidants in protic and aprotic media. The current was determined after the reaction reached equilibrium. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Feasibility Study on Superoxide Anion Scavenging by CV

In order to investigate, electrochemically, the antioxidant effect of Se over $O_2^{\bullet -}$, the redox potentials of Se were measured in acetonitrile by cyclic voltammetry and then compared with those obtain for α-T, GA, CA, TBHQ and Trx. The redox potential of $O_2/O_2^{\bullet -}$ couple was found to be −775 mV vs. Ag/AgNO3 (0.01 M in ACN) reference electrode. Cyclic voltammograms for the $O_2/O_2^{\bullet -}$ couple, at different concentrations of Se were measured. The Figure 1 shows the CV for different concentrations of Se in an O_2 saturated solution of ACN. With the addition of Se, the oxidation current peak of $O_2^{\bullet -}$ decreased until 20 μM was obtained when the signal disappeared. The reduction current of $O_2/O_2^{\bullet -}$ couple (−800 mV) increased because an irreversible reduction of Se took place near this potential. The current peaks are attributed to the oxidation/reduction of dissolved O_2, which had not reacted with Se. The insert in Fig. 1 reveals a lineal decrease of $O_2^{\bullet -}$ scavenging by Se within 0–20 μM ($r = 0.998$).

The same experiment was realized for consecutive and increasing additions of CA, GA, TBHQ, α-T, and Trx antioxidants (see as described in the following section).

Quantitative comparison/IC_{50}. Superoxide anion scavenging ability of the antioxidants was measured using a plot of normalized current vs. concentration.

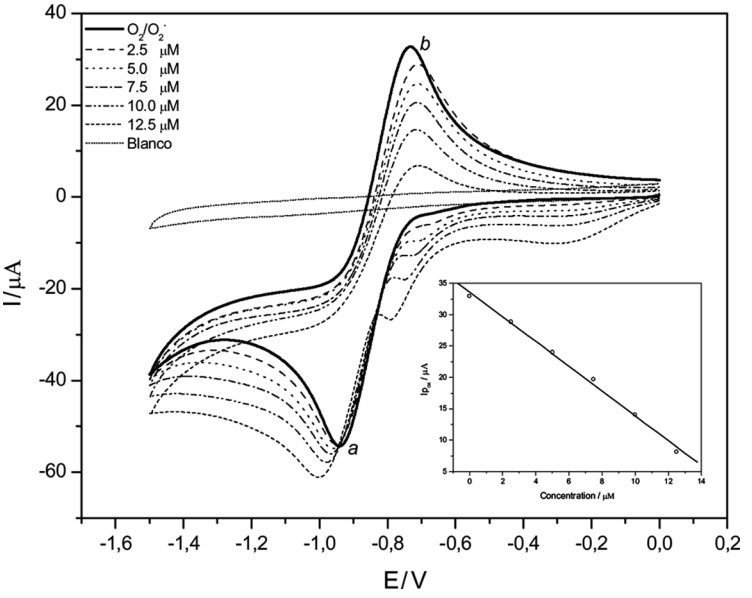

Figure 1. Cyclic voltammograms of the $O_2/O_2^{\bullet -}$ couple. Behavior of glassy carbon electrode at different concentrations of Selenium, in O_2-saturated ACN. Scan rate: $50\,mVs^{-1}$, supporting electrolyte 0.065 M Bu_4NPF_6. The insert in Fig. 1 reveals a lineal quenching of $O_2^{\bullet}$ by Se within the range of the studied concentration ($r = 0.998$).

Figure 2 shows the percentage of the inhibition current for the superoxide anion formation (scavenging effects) determined at different concentrations of each studied compounds. This behavior was inversely related to the antioxidant capacity of a compound.

The scavenging effects were calculated as follow:

$$\text{Scavenging effects} : I\,\% = \frac{I_0 - I_x}{I_0}\,100; \tag{1}$$

where I_x and I_0 are the current intensities of the samples, with and without antioxidants, respectively.

The antioxidant capacity of a compound was evaluated according to their IC_{50} index (expressed as the amount of antioxidant needed to decrease the current intensity to 50%). Moreover, we also calculated, graphically, the time needed to reach the steady state at IC_{50} concentration (TIC_{50}). The TIC_{50} is an index which depends on the reactivity of the antioxidants and its concentration. The time at steady state was used in order to guarantee that the reaction did not continue. Both factors (IC_{50} and TIC_{50}) can be combined as an anti-radical efficiency parameter ($AE = 1/IC_{50}$ TIC_{50}), in order to easily characterize the behavior of an antioxidant compound. While the lower number is IC_{50}, the higher number is the antioxidant activity of the compound.

Table 1 shows that α-T, with an IC_{50} of 0.46 μM, exhibits the highest AE value (98.1×10^{-3}), so it could be considered the compound with the highest antioxidant

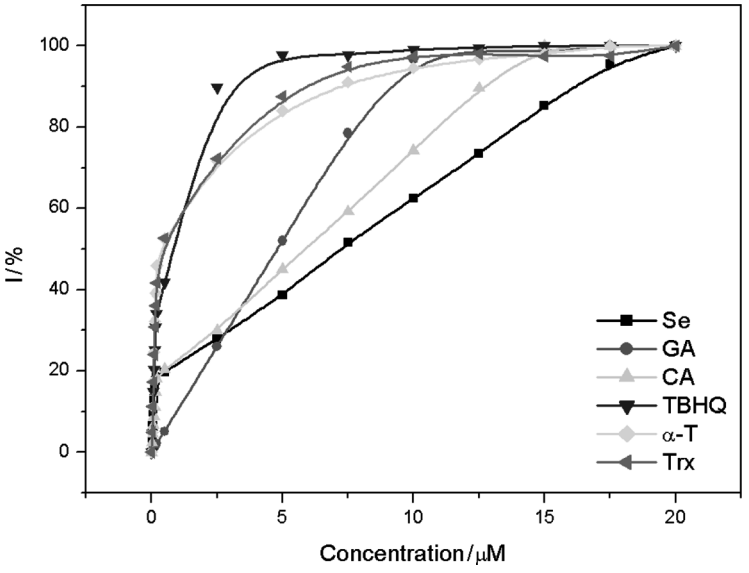

Figure 2. Superoxide radical scavenging ability of antioxidants. Inhibition capability (I %) of the current generated by superoxide after successive additions of antioxidant. The normalized data are shown as inhibition percentage of the peaks current mean, obtain by CV in ACN, supporting electrolyte 0.065 M Bu_4NPF_6, Scan rate: 50 mVs^{-1} (n = 5).

activity, and this is caused by a fast rate of reaction. This characteristic has great importance in biological systems as free radicals have a very short half-life.

Among quinone derivates, GA, CA, and TBHQ, TBHQ presents the lower IC_{50} and the higher AE. The GA has lower IC_{50} than CA, although their TIC_{50} were inverted; CA has higher AE than GA. The IC_{50} values of α-T and Trx were the lowest of all, with the α-T being smaller than Trx (standard antioxidant). In the case of Selenium, 7.29 μM and 57.7 min was necessary to arrive at a stable status; therefore, it had the higher IC_{50} and TIC $_{50}$, and the lower AE.

In addition, we performed an assay to determine a synergic effect between Se and α-T, GA, CA, TBHQ, and Trx. As a result, we did not find a synergetic effect in ACN solutions in comparison to their own antioxidant effect (see Tables 1 and 2).

Table 1. Superoxide radical scavenging parameters. IC_{50}, TIC_{50}, and antioxidant efficacy AE values of tested compounds in ACN, supporting electrolyte 0.1 M Bu_4NPF_6

Antioxidant	IC_{50} (μM)	TIC_{50} (min)	AE ($\times 10^{-3}$)*
Se	7.29 ± 0.3	57.7 ± 0.7	2.4 ± 0.1
GA	4.76 ± 0.2	49.1 ± 0.8	4.3 ± 0.2
CA	5.87 ± 0.09	36.1 ± 0.6	4.7 ± 0.2
TBHQ	0.78 ± 0.01	32.5 ± 1.0	39.3 ± 2.0
α-T	0.46 ± 0.01	22.3 ± 0.9	98.1 ± 2.2
Trx	0.50 ± 0.02	21.4 ± 0.8	93.9 ± 2.1

*AE = 1/IC50 × TIC50.

Table 2. Superoxide radical scavenging parameters. IC_{50}, TIC_{50}, and antioxidant efficacy AE values of mixtures (Se + antioxidants) in ACN, supporting electrolyte 0.1 M Bu_4NPF_6

Mix of Antioxidant	IC_{50} (μM)	TIC_{50} (min)	AE ($\times 10^{-3}$)*
Se:GA	2.88 ± 0.07	51.1 ± 0.7	6.8 ± 0.2
Se:CA	$5.12 + 0.2$	$50.2 + 0.8$	$3.9 + 0.1$
Se:TBHQ	$4.32 + 0.1$	$35.5 + 1.0$	$6.5 + 0.2$
Se:α-T	$0.71 + 0.02$	$25.2 + 0.6$	$55.9 + 1.1$
Se:Trx	0.98 ± 0.01	20.3 ± 0.5	50.1 ± 1.0

*AE = 1/IC50 × TIC50.

Figure 3 shows the inhibition capability of the current generated for the ion superoxide after successive additions of the antioxidants mixtures; the Se:α-T present the higher antioxidant activity.

Feasibility Study on $DPPH^{\bullet -}$ Scavenging by OSWV

The DPPH method allows the evaluation of not only the electron or hydrogen atom-donating properties of the antioxidants, but also the rate of their reaction to the free radicals. The $DPPH^{\bullet -}$ scavenging ability of Se, α-T, GA, CA, TBHQ and Trx were studied with consecutive and increasing concentrations, using OSWV technique in aqueous medium.

The OSWV experiments were performed in excess of the $DPPH^{\bullet -}$ radical in order to saturate the H-donating capacity of the antioxidants. The final concentration

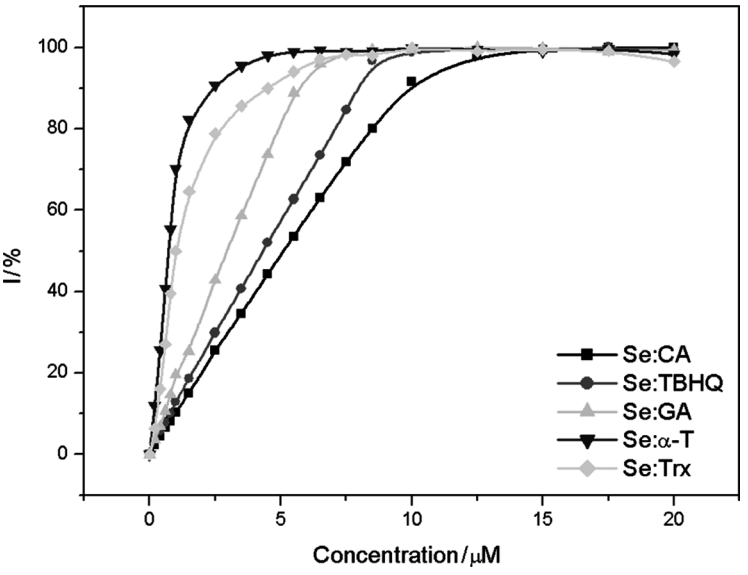

Figure 3. Superoxide radical scavenging ability of Mixtures (Se + antioxidants). Inhibition capability (I %) of the current generated by superoxide right after successive additions of mixtures. The normalized data are shown as inhibition percentage of the peaks current mean (n = 5) obtain by CV in ACN, supporting electrolyte 0.065 M Bu_4NPF_6, Scan rate: 50 mVs^{-1}.

of $DPPH^{\bullet -}$ in the cell was 0.3 mM, while the antioxidant concentrations were added in the range 0.00–5.00 μM. The study was carried out with a phosphate buffer of 0.033 M containing 0.033 M KCl and 40% (v/v) of ethanol. The electrochemical behavior of antioxidants was investigated within a pH range of 3.0–8.4. The voltammograms exhibited single well-defined waves in this range of pH. This wave shifted toward less positive potentials measurements, while the pH increased, but at pH 7.4 its intensity was almost constant. So, this pH was selected for the next experiments. The current peak of $DPPH^{\bullet -}$ radical decreased with the sequential addition of Se (Figure 4). The inset in this Figure 5 shows a lineal quenching of $DPPH^{\bullet -}$ by Se in a range 0.05–2.5 μM.

Figure 5 shows the normalized data of the inhibition current of $DPPH^{\bullet -}$ formation, which was achieved at different concentrations for each studied compounds. The reaction shows a biphasic behavior with most of the compounds, with a fast decay in current intensity during the first minute, followed by a slow step until equilibrium was reached. In an aqueous medium, α-T and Trx have the highest AE values. Table 3 shows IC_{50}, TIC_{50}, and AE in buffer phosphate. These results demonstrated that in protic solvents, the oxidized quinones suffered a nucleophilic attack by the solvent, leading to a regeneration of its catechol structures, which allowed them to scavenge two additional radicals. This mechanism is better in aqueous solution than in aprotic solvents because of the higher $DPPH^{\bullet -}$ radical scavenging activity of the antioxidants (Saito and Kawabata 2004).

Quantitative comparison/IC_{50}. For the purpose of comparison of the antioxidant activity of the studied compounds, the TIC_{50} parameter has been defined as

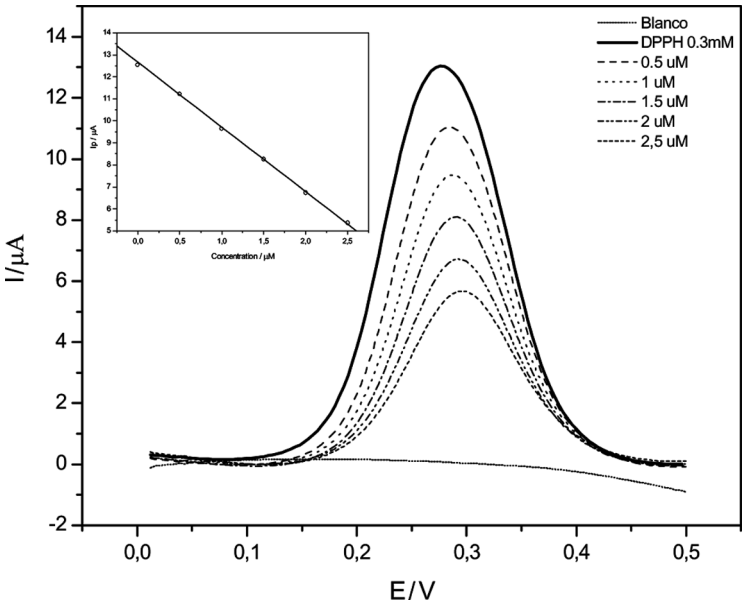

Figure 4. $DPPH^{\bullet -}$ scavenging effect of selenium. The OSWV of the $DPPH/DPPH^{\bullet -}$ 0.3 mM couple at different Selenium concentrations in phosphate buffer 0.033 M solutions containing 0.033 M KCl and 40% (v/v) of ethanol. Inset reveals lineal quenching of $DPPH^{\bullet -}$ by Se in the range of 0.01–2.5 μM.

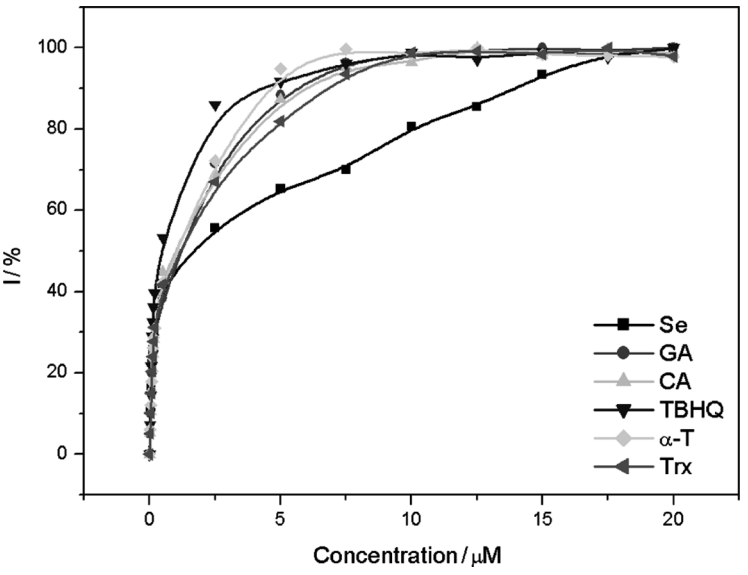

Figure 5. $DPPH^{\bullet-}$ scavenging effect of antioxidants. Inhibition capability (I %) of the current generated by $DPPH/DPPH^{\bullet-}$ 0.3 M couple right after successive additions of antioxidants. The normalized data are shown as inhibition percentage of the current peaks mean ($n = 5$) obtain by OSWV in phosphate buffer 0.033 M.

the time in which equilibrium is reached with a concentration of antioxidant equal to IC_{50}. The Se needed more time to react than the others antioxidants; its TIC_{50} was 40.1 min. (Table 3). On the contrary, Trx and α-T were the antioxidants that reacted more rapidly, with a TIC_{50} of 10.6 and 11.7 min, respectively. Furthermore, the time of reaction varied according to the origin of the different antioxidants. In this sense, the AE comprises these two aspects, in order to characterize easily the behavior of a substance as antioxidant. In Table 3, we can observe that compounds such as α-T, with an IC_{50} of 1.01 μM and a TIC_{50} of 11.7 min, show the highest AE value 84.5×10^{-3} of the tested compounds due to its fast rate of reaction. They are followed, in order of magnitude, by Trx, TBHQ, CA, GA, and Se, respectively.

Table 3. $DPPH^{\bullet-}$ radical scavenging parameters. IC_{50}, TIC_{50}, and antioxidant efficacy AE values of antioxidants in buffer phosphate (0.033 M, pH = 7.4) containing 0.033 M KCl and 40% (v/v) of ethanol

Antioxidant	IC_{50} (μM)	TIC_{50} (min)	AE ($\times 10^{-3}$)*
Se	1.70 ± 0.03	40.1 ± 0.7	14.6 ± 1.3
GA	1.15 ± 0.01	18.9 ± 0.5	46.3 ± 1.4
CA	0.97 ± 0.02	21.7 ± 0.6	47.4 ± 1.2
TBHQ	0.45 ± 0.01	29.6 ± 0.8	75.3 ± 1.1
α-T	1.01 ± 0.02	11.7 ± 0.3	84.5 ± 1.5
Trx	1.21 ± 0.03	10.6 ± 0.4	78.2 ± 1.7

*AE = 1/IC50 × TIC50.

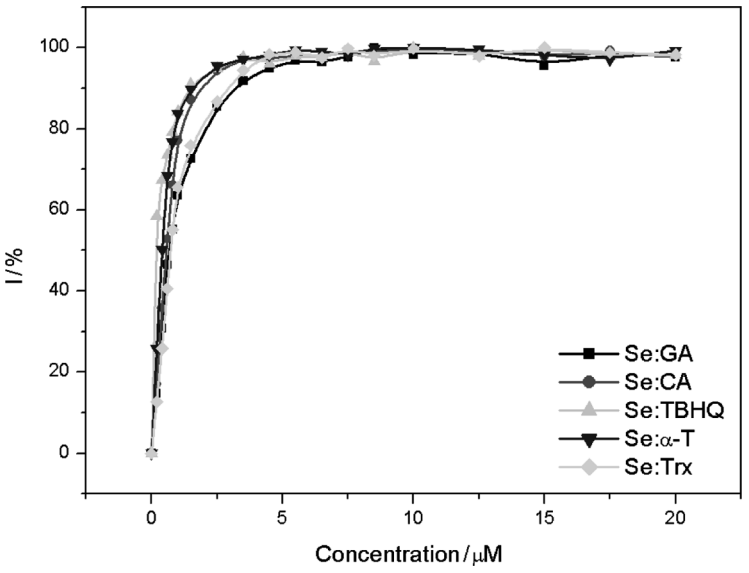

Figure 6. $DPPH^{\bullet -}$ scavenging effect of Mixtures (Se + antioxidants). Inhibition capability (I %) of the current generated by $DPPH/DPPH^{\bullet}$ 0.3 mM couple right after successive additions of mixtures. The normalized data are shown as inhibition percentage of the peaks current mean ($n = 5$) obtain by OSWV in phosphate buffer 0.033 M.

Figure 6 shows the normalized data of the current inhibition of DPPH formation (scavenging effects on couple $DPPH/DPPH^{\bullet -}$), when we attempted to establish a synergetic effect between Se and CA, GA, TBHQ, α-T, and Trx in aqueous medium. Table 4 shows a significant synergistic effect of Se with each antioxidant in the mixtures tested. The mixtures such as Se: α-T, with an IC_{50} of 0.38 μM and a TIC_{50} of 8.24 min, has the highest AE value 315.2×10^{-3} of the tested mixtures due to its fast rate of reaction. It is followed by Trx, TBHQ, CA, GA, and Se, respectively. The data in Tables 3 and 4 indicate that there was between 50 to 70% higher AE in the mixtures tested, compared with the effects that produce the antioxidants alone. Table 2 shows that there were significant differences ($p \leq 0.05$) among AE of the various mixtures in water solutions.

Table 4. $DPPH^{\bullet -}$ radical scavenging parameters. IC_{50}, TIC_{50}, and antioxidant efficacy AE values of mixtures (Se + antioxidants): in buffer phosphate (0.033 M, pH = 7.4) containing 0.033 M KCl and 40% (v/v) of ethanol

Mix of Antioxidant	IC_{50} (μM)	TIC_{50} (min)	AE ($\times 10^{-3}$)*
Se:GA	0.65 ± 0.01	14.86 ± 0.5	103.9 ± 3.1
Se:CA	0.50 ± 0.02	16.15 ± 0.1	123.1 ± 2.2
Se:TBHQ	0.15 ± 0.01	23.46 ± 0.4	286.8 ± 5.3
Se:α-T	0.38 ± 0.01	08.24 ± 0.3	315.2 ± 4.5
Se:Trx	0.68 ± 0.02	05.03 ± 0.2	291.8 ± 4.0

*AE = 1/IC50 × TIC50.

Probably, the biological role of selenium appears to lie in selenium-containing compounds that act as a carrier of antioxidants. These mixtures probably have an important synergetic effect to scavenging $DPPH^{\bullet -}$, which is of great relevance in biological systems.

CONCLUSIONS

Cyclic Voltammetry (CV) and Osteryoung Square Wave Voltammetry (OSWV) were used to evaluate the antioxidant activity of a wide variety of compounds as these methods, based on the applied glassy carbon electrode, showed good chemical stability, and nontransparent samples did not affect the response of the detector.

The protic and aprotic medium have shown a different behavior between $DPPH^{\bullet -}$ free radical and $O_2/O_2^{\bullet -}$ couple, both in terms of capacity and rate of scavenging. The α-T presented the greatest AE for both free radicals. When we analyzed the synergistic effect of Selenium with CA, GA, TBHQ, α-T, and Trx antioxidants, we demonstrated that AE increased considerably, which brought us to the conclusion that a strong synergistic effect exists between Selenium and the antioxidants tested in protic medium.

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