

**Measurement of total antioxidant capacity by electrogenerated iodine at disposable  
screen printed electrodes**

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## **ABSTRACT**

Total antioxidant capacity is an important parameter for the evaluation of the oxidative status in different kinds of biological samples such as plant extracts, or in food industry. We report a fast, easy, portable, cost-effective electroanalytical method to measure total antioxidant capacity, based on the reaction of natural antioxidants with electrogenerated iodine using disposable platinum screen-printed electrodes. This reaction can be measured by the increment of the electrochemical current signal of iodide oxidation to iodine during a voltammetric cycle. Iodine reacts with reducing compounds such as glutathione, ascorbate, gallic acid and NADH without interference of the corresponding oxidized counter-parts. The addition of ascorbate oxidase also allows the concentration of ascorbate to be determined. The method was tested with real samples of plant extracts and the results correlated well with those obtained with a standard spectrophotometric method.

## **KEYWORDS**

Disposable screen-printed electrodes, cyclic voltammetry, total antioxidant capacity, ascorbate, iodide/iodine redox couple, plant extracts.

## INTRODUCTION

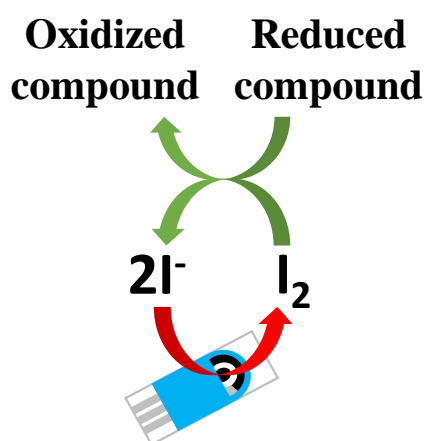
The total antioxidant capacity (TAC) is defined as the ability of a compound (or a mixture of compounds) to inhibit the oxidative degradation in living systems, such as preventing lipid peroxidation [1]. Therefore, it is an important parameter to take into account in all biological media such as plants, food, plasma, saliva, etc. Specifically, reducing compounds from natural sources (e.g. ascorbate, glutathione or polyphenols) scavenge free radical and reactive oxygen species (ROS) such as hydrogen peroxide and superoxide, preventing oxidative stress [2-4]. Under normal physiological conditions, these compounds are mainly in their reduced forms. An increase in ROS can produce the oxidation of these crucial compounds, which may lead to oxidative stress and programmed cell death [5]. Therefore, the monitoring of the “antioxidant status” is very useful in the study of metabolism as well as in the effect against oxidative stress of antioxidant compounds [6]. In studying the potential health benefits of antioxidants against oxidative stress, it is important to highlight that these compounds, in a sample, can exert some additive and synergistic effects [7], so it is difficult to define the antioxidant effect of each single compound. For this reason, it seems more helpful to quantify the effect of antioxidant compounds in terms of TAC.

A variety of different analytical methods are used to determine the antioxidants content in plants, food and other biological fluids including colorimetry [8], fluorescence [9] and chemiluminescence [10]. Colorimetric methods are the most extensively used. However, these methods have some disadvantages such as high costs, long analysis times and often require laborious technical handling and chemical derivatisation [11]. In addition, they are prone to interference from color and turbidity of samples. In spite of these disadvantages, they are commonly accepted assays for the evaluation of the antioxidant degree in different samples [12, 13].

As an alternative, electroanalytical methods have major advantages, being cheap, technically straightforward and having good sensitivity. Electrochemical techniques are being increasingly used as versatile tools to measure biologically relevant compounds related to oxidative stress [14-17] and the antioxidant capacity of different samples [18-22]. Some authors have used bare electrodes in the measurement of TAC with the drawback that the different natural antioxidants are oxidized to different potentials [23, 24]. To solve this fact, some authors have determined the TAC following the reduction of some radicals or the damage caused to biological molecules. For example, the amperometric detection of the DPPH radical on a glassy carbon electrode [25], a carbon nanotube modified-glassy carbon electrode [6] and a graphene/poly (3,4-ethylenedioxythiophene):poly (styrene sulfonate) nanocomposite-modified screen-printed carbon electrode [26] was applied to measure TAC in vegetable extracts and herbs, respectively. On the other hand, DNA based electrochemical sensors were also developed to measure the antioxidant power, by measuring the protective effect of different antioxidants against the damage of DNA in the presence of NO radicals [27] and superoxide [28]. However, the use of radicals as mediators presents the drawback of their instability or non-desired reactions.

The development of new instrumentation with the possibility of miniaturization is one the main goals of electrochemical techniques in the analysis of bioactive compounds [15, 29]. Recent important advances in the design and fabrication of disposable screen printed sensors for the electrochemical detection have been achieved. These devices are highly accurate, portable and rapid. They have opened a promising way of sensing, since they not only address the issue of cost effectiveness and miniaturization but also satisfy other requirements of quality like good sensitivity and limit of detection.

The reaction between iodine and the antioxidant ascorbate is widely known and has led to the determination of this vitamin by redox titration [30]. In a similar way, an analytical method to measure antioxidant levels in food and medical samples using polyvinylpyrrolidone as an iodophor was patented some years ago [31]. The *in situ* oxidation of aqueous iodide at a platinum electrode produces molecular iodine near the electrode surface [32, 33]. The iodine electrogenerated under aqueous conditions is able to oxidize reducing compounds. Then the iodine turns back to iodide, which is again electrochemically oxidized, involving the increase in the measurable current peak and allowing the analytical determination of some important antioxidants by cyclic voltammetry [33-36]. This process is represented in Fig. 1. As far as we know, this approach has not been used to determine TAC in biological samples. Therefore, the general aim of this paper was to develop a feasible electroanalytical method using the system iodide/iodine as mediator to measure TAC using disposable platinum screen-printed electrodes. The validity of the assay was tested with real samples of extracts from plants growth under non-stress and stressful conditions.



**Figure 1.** Schematic diagram of an electrocatalytic cycle with the iodide/iodine pair redox couple as a mediator in the oxidation of reducing compounds on the surface of a screen printed electrode.

## EXPERIMENTAL

### Reagents

L-Ascorbic acid (disodium salt, ASC), Ascorbic Acid Assay Kit II, L-dehydroascorbic acid (DHA), ellagic acid, gallic acid, glutathione (oxidized and reduced, GSSG and GSH, respectively), nicotinamide adenine dinucleotide (oxidized and reduced,  $\beta$ -NAD<sup>+</sup> and  $\beta$ -NADH, respectively), potassium carbonate, sodium iodide and sodium perchlorate were purchased at their highest available purity from Sigma-Aldrich (Spain) and were used as received. All solutions were prepared with deionized water (resistivity of no less than 18.2 M $\Omega$ ·cm at 25°C) (Millipore, Watford, UK). Standard antioxidants solutions were freshly prepared every day and bubbled with N<sub>2</sub> gas prior to calibration experiments to avoid spontaneous oxidation.

### Plant extracts

Plant extracts from *Pisum sativum* (pea) leaves were supplied by the Fruit Technology Group ([http://www.cebas.csic.es/dep\\_spain/mejora/biotecnologia/biotec\\_lineas.html](http://www.cebas.csic.es/dep_spain/mejora/biotecnologia/biotec_lineas.html), CEBAS, CSIC, Murcia, Spain). Pea seeds (*Pisum sativum*) were imbibed in distilled water for 24 h. Then, seeds were washed twice with distilled water and germinated and grown in vermiculite. The grown chamber was set at 25/18°C, 80% relative humidity and 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light, with a 16-h photoperiod. First, plants were cultivated in aerated distilled water for 7 days [37]. Plants were then transferred to aerated half-strength Hoagland solution for another 7 days. After this period, 100 mM

NaCl was added to the nutrient solution in some plants (stressed plants) that were grown under these conditions. The Hoagland solution was replaced once a week. After 15 days of growth in the presence of NaCl, the plants were harvested. Controls plants (non-stressed plants) were grown in the same conditions but in the absence of NaCl.

The production of extracts was performed as follows: Pea leaves (1 g) were frozen in liquid nitrogen, then ground in a mortar and pestle in 3 mL of 1 M HClO<sub>4</sub> solution. After that, the homogenate was centrifuged at 12,000 rpm for 15 minutes at 4 °C, and the supernatant removed and stored at –80°C. Under these conditions, the chemical composition to be expected in the extracts is the following: low molecular weight carbohydrates (e.g. glucose), small peptides (e.g. glutathione), aminoacids (e.g. cysteine), different phenolic compounds (e.g. salicylic acid), vitamins (e.g. ascorbic acid), low molecular weight organic acids (e.g. oxalic acid) and mineral substances. This protocol has previously been optimized to measure the antioxidants content in plant extracts [37]. The use of liquid nitrogen and acidic conditions is necessary to avoid spontaneous oxidation of antioxidants in the samples and to precipitate proteins.

The samples were neutralized with K<sub>2</sub>CO<sub>3</sub> 5 M to pH 6.5 just prior to the analytical measurements. The plant extracts have been named as follows: 1) Controls: C1 and C2 and 2) Plants subjected to salt stress: S1 and S2.

### **Electrochemical measurements**

Electrochemical experiments were performed using a computer-controlled potentiostat, AUTOLAB PGSTAT128N (Eco Chemie B.V., The Netherlands). All solutions were previously incubated in a Thermomixer Comfort Eppendorf (Hamburg, Germany) at 25°C before each measurement. The electrochemical measurements were carried out on disposable platinum screen-printed electrodes (Dropsens, DRP-550),

which consist of a platinum working electrode (diameter 4 mm), a platinum counter electrode and a quasi-silver reference electrode. Approximately 50  $\mu\text{L}$  of the sample solution were deposited onto the chip covering the three electrodes prior to running the electrochemical experiment.

The method measures the antioxidant capacity by the reduction of the electrochemically generated iodine from iodide at the platinum electrode surface. This redox process does not involve the transfer of protons and hence buffered conditions are not necessary. The electrochemical detection was performed by diluting the samples with the appropriated quantity of 0.2 M  $\text{NaClO}_4$  and mixing them with a solution of 1 mM NaI [34]. Cyclic voltammetries were performed from 0.2 V to 0.8 V and vice versa with a scan rate of  $100 \text{ mV s}^{-1}$ .

To discriminate the ascorbate concentration from TAC in the sample, electrochemical measurements were performed both in the absence and in the presence of the enzyme ascorbate oxidase (AOX). AOX was left to act 15 minutes before the measurement so that all the ascorbate present in the sample was oxidized. The maximum peak current obtained for each sample in the presence of AOX was subtracted from its corresponding peak signal in the absence of the enzyme to set the zero of ascorbate concentration. All solutions were prepared in 0.2 M  $\text{NaClO}_4$ . Previously standards of ascorbate were prepared and measured in the interest range of concentration to establish the calibration straight line. Temperature was controlled at  $25^\circ\text{C}$ .

### **Spectrophotometric measurements**

Spectrophotometric measurements were carried out using a UV/Vis Perkin-Elmer Lambda 35 (Perkin Elmer Instruments, Waltham, USA) spectrophotometer



coupled to a PCB 150 Water Peltier System. The commercial Ascorbic Acid Assay Kit II from Sigma-Aldrich was used. The basis of this spectrophotometric method (Ferric Reducing/Antioxidant and Ascorbic Acid (FRASC) assay) consists of the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by the antioxidants present in the sample, which results in a change of color at 593 nm. This change in absorbance is related on the quantity of antioxidants in solution. The addition of AOX to parallel samples oxidizes any ascorbate present and allows also the quantification of ASC. The colorimetric detection was performed by diluting the samples with the appropriated quantity of water (5  $\mu\text{L}$  of sample in 200  $\mu\text{L}$  of total volume) and mixing them with the Master Reaction Mix (FRASC buffer, Ascorbic acid probe and iron chloride solution in the proportion 80:10:10). The absorbance at 593 nm was measured both in the absence and the presence of AOX. This enzyme was incubated in the blanks for 15 min to set the zero of ascorbate. Previously ascorbic acid standards were prepared and measured in the interest range of concentration to establish the calibration straight line. Temperature was controlled at 25 °C.

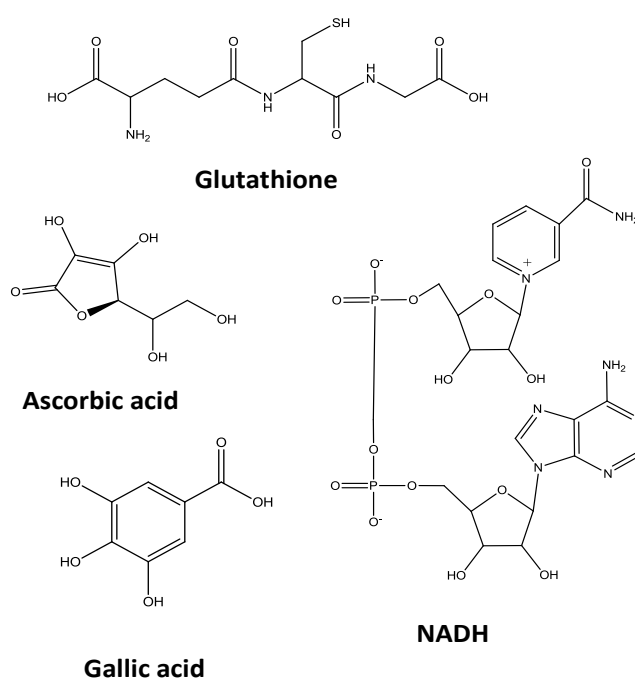
### **Fitting the data**

Data were represented and fitted by least squares linear regression using the SigmaPlot Scientific Graphing Software 13.0 for Windows.

## RESULTS AND DISCUSSION

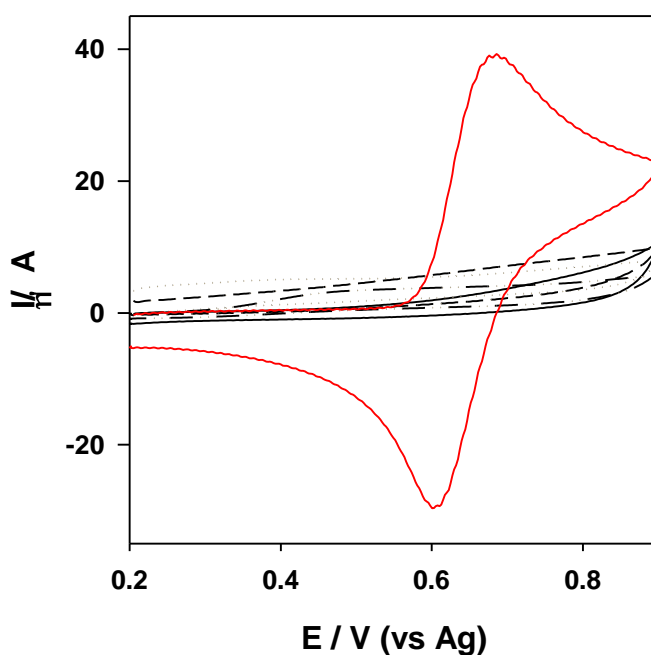
### Iodide/iodine redox assay

The chemical structures of some representative antioxidant compounds which are widely present in plant organisms (GSH, ASC, NADH and gallic acid) are shown in Fig. 2. These model compounds have been chosen as they show strong antioxidant capacity and they are important markers of the antioxidant protection of organisms [1]. GSH and ASC are critical factors in protecting organisms against toxicity and disease both in animals and plants and are involved in the glutathione-ascorbate pathway in plants [2-4, 38]. NADH plays a central role in mitochondrial respiratory metabolism and is one of the most used coenzymes in cellular metabolism. Hundreds of enzymes use NADH (or NADPH) to catalyze reduction-oxidation reactions reversibly [39, 40]. Gallic acid is a natural phenolic compound, strong antioxidant and widely used as a standard antioxidant to determine the phenolic content in plant extracts [41]. All these compounds, although have different chemical structure, have the same function: to protect the cell from oxidative stress.



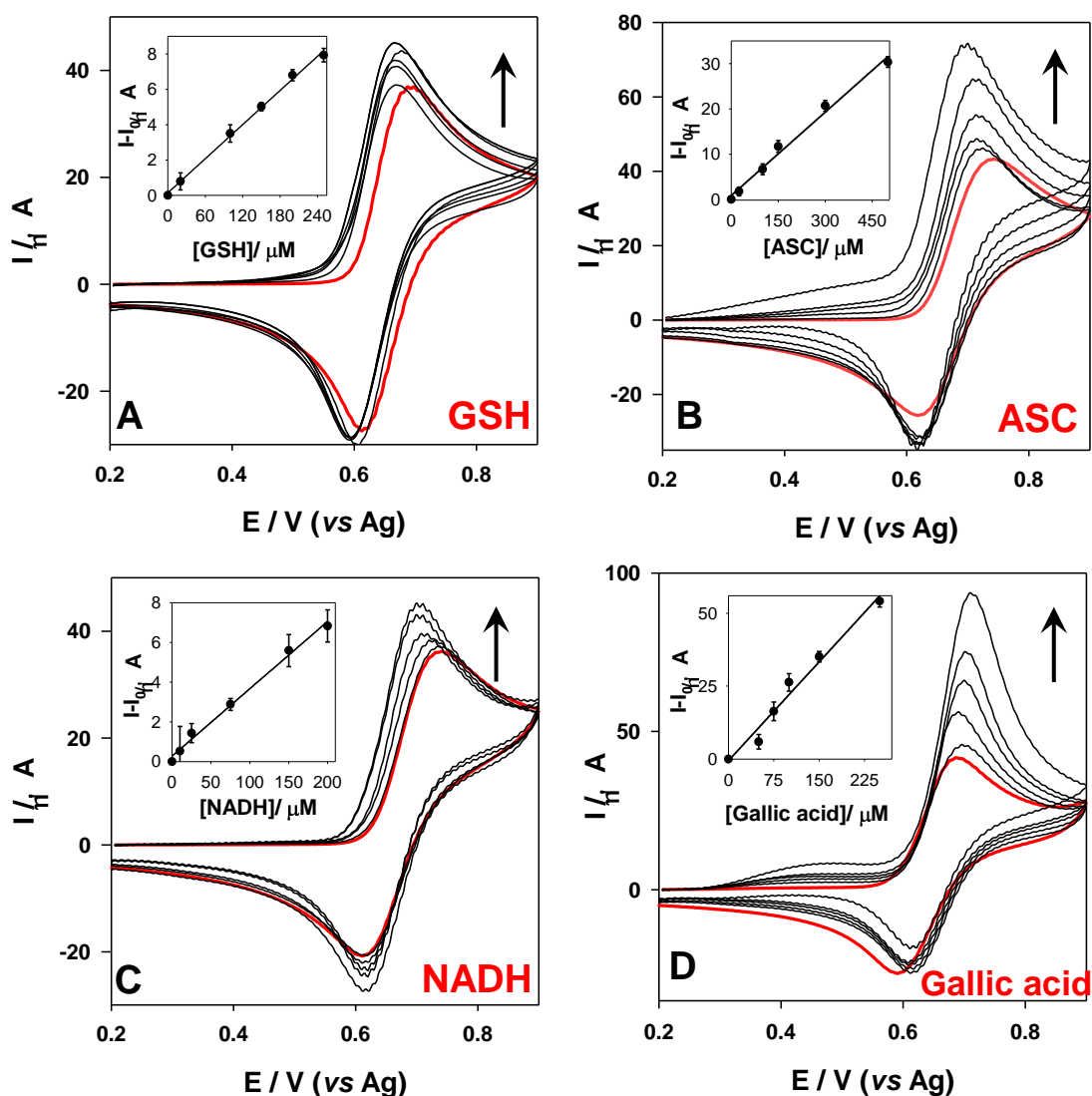
**Figure 2.** Chemical structures of GSH, ASC, gallic acid and NADH involved in defense mechanisms against oxidative stress.

The voltammetric response of 1 mM iodide was recorded at a platinum screen printed electrode (Fig. 3, red line). A quasi-reversible diffusional redox peak at about 0.65 V (mid-point potential vs. Ag) was observed. The anodic (positive) voltammetric peak corresponds to the one-electron oxidation of the iodide ion to iodine and the cathodic (negative) voltammetric peak is the iodine reduction to iodide. Despite the fact that only a single process was observed in the voltammetry, the presence of small quantities of triiodide ion cannot be discarded [34, 42]. The comparison of this voltammogram (red line) with those obtained for GSH, ASC, NADH and gallic acid (solid, dotted, dashed dotted and dashed black lines, respectively), showed that the direct electrochemical determination of these antioxidants is not possible since no measurable signal was detected on platinum electrodes.



**Figure 3.** Voltammetric response of 1 mM NaI (red solid line), 100  $\mu$ M GSH (black solid line), 100  $\mu$ M ASC (black dotted line), 100  $\mu$ M NADH (black dashed-dotted line) and 100  $\mu$ M gallic acid (black dashed line) at 100  $\text{mV s}^{-1}$ . All solutions were prepared in 0.2 M  $\text{NaClO}_4$ .

Fig. 4 shows some voltammograms of the iodide/iodine solution with increasing concentrations of the reducing compounds GSH, ASC, NADH and gallic acid. We could see a clear increase in the positive peak of the iodide oxidation dependent on concentration when these antioxidants were added to the iodide solution and cyclic voltammetries were performed. This correlation showed good linearity in the range of micromolar for all the compounds (see the corresponding insets in Fig. 4). The antioxidants were able to be oxidized by iodine (electrochemically generated), which is again reduced to iodide as depicted in Fig. 1.



**Figure 4.** Voltammetric response of 1 mM NaI in the absence (red line in each plot) and in the presence of different increasing antioxidants concentrations: (A) GSH: 20, 100, 150, 200 and 250  $\mu M$ , (B) ASC: 0, 25, 100, 150, 300 and 500  $\mu M$ , (C) NADH: 0, 10, 25, 75, 150 and 200  $\mu M$ , (D) Gallic acid: 50, 75, 100, 150 and 250  $\mu M$  at 100  $mV s^{-1}$ . All solutions were prepared in 0.2 M  $NaClO_4$ . The inset of each figure shows the current variation of the anodic peak vs. the antioxidant concentration. Error bars were calculated as the standard deviation of three different measurements.

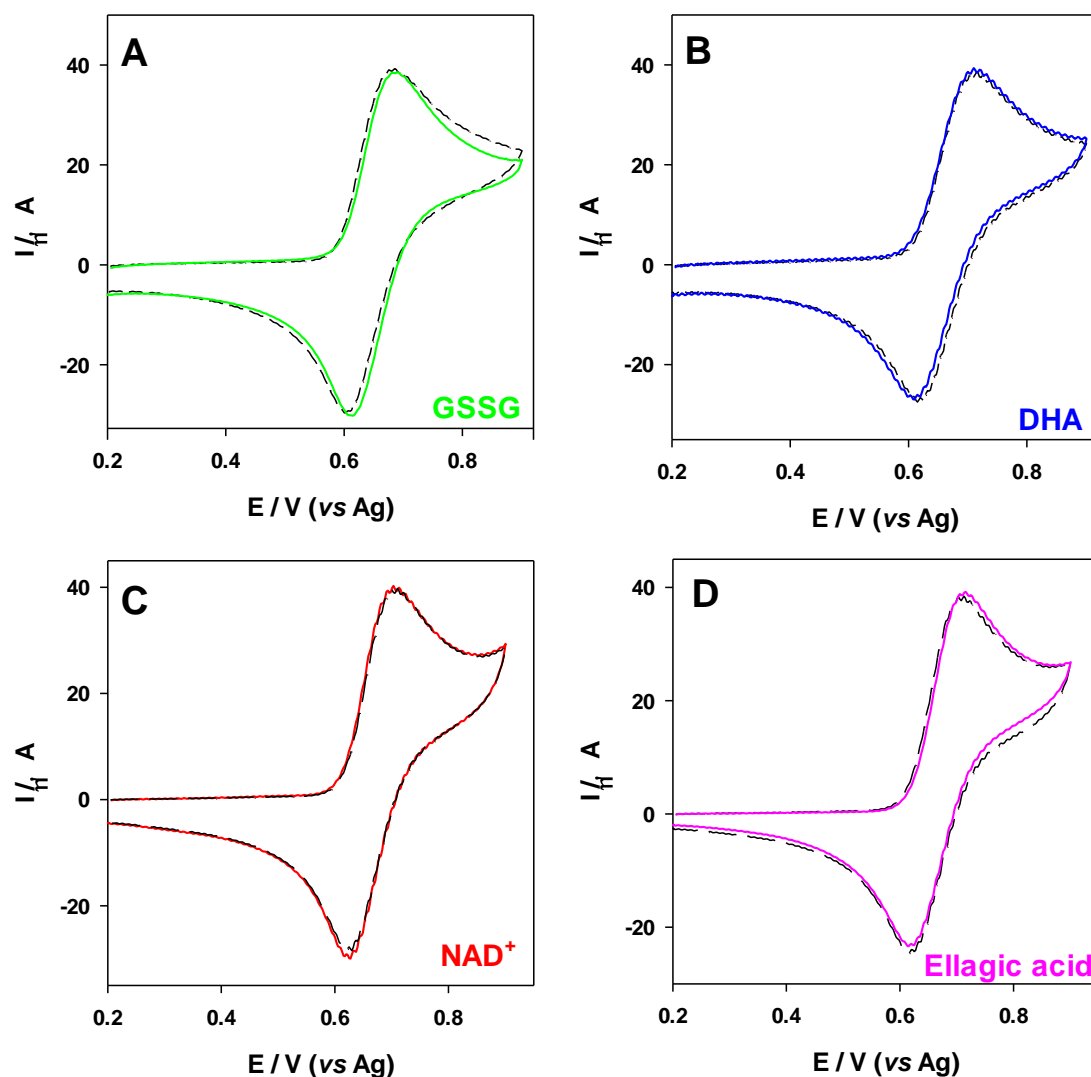
Table 1 summarizes the analytical performance (limits of detection and sensitivity) for the antioxidants GSH, ASC, NADH and gallic acid. Micromolar limits of detection were obtained for all the compounds (7-22  $\mu\text{M}$ ), which was considered to be enough to their measurement in plant extracts. The sensitivity evaluated by this electrochemical method was as follows (in decreasing order): gallic acid > ASC > NADH > GSH.

**Table 1.** Limits of detection ( $3\sigma/\text{S}$ ) and sensitivity (S) for the studied biological antioxidant compounds.

Compound	LOD ( $\mu\text{M}$ )	Sensitivity ( $\mu\text{A } \mu\text{M}^{-1}$ )
Gallic acid	22.2	0.23 $\pm$ 0.01
Ascorbate	16.9	0.069 $\pm$ 0.002
NADH	7.2	0.037 $\pm$ 0.001
Glutathione	10.5	0.032 $\pm$ 0.001

In a real sample, there is a mixture of reducing and oxidizing compounds. A method which measures TAC necessarily has to be able to detect only the reduced compounds but insensitive to the oxidized forms. Fig. 5 shows the cyclic voltammetry response of 1 mM iodide in presence of the oxidized counter parts of the antioxidants herein investigated. The addition of the corresponding oxidized (GSSG, DHA,  $\text{NAD}^+$  and ellagic acid) did not have any effect on the voltammetry. Therefore, the analytical method is able to detect only the antioxidants, not the oxidized compounds, which is very important to analyze the oxidative status of the samples. This is particularly

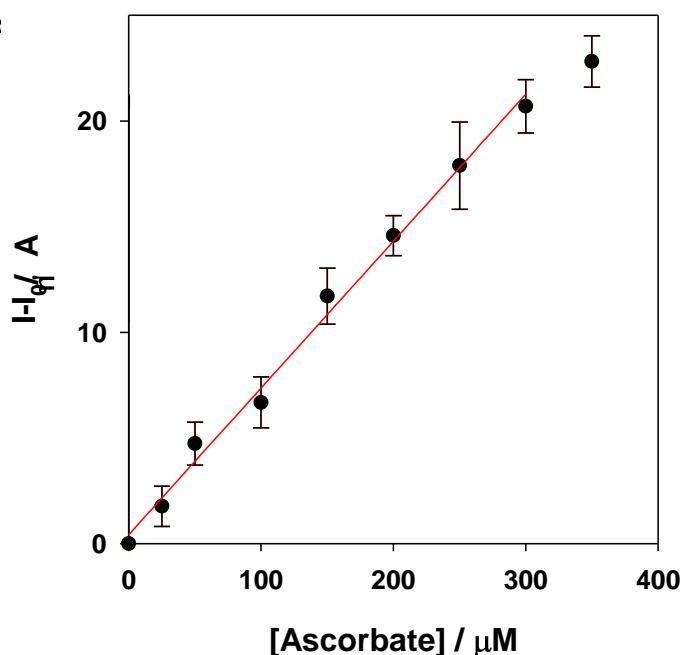
important for DHA, the oxidation product of ASC, bearing in mind its relatively high levels found in leaves [43, 44].



**Figure 5.** Voltammetric response of 1 mM NaI (black dashed line in each plot) in the presence of some oxidized compounds at  $100 \text{ mV s}^{-1}$ : A) 100  $\mu$ M GSSG (green), B) 100  $\mu$ M DHA (blue), C) 100  $\mu$ M  $NAD^+$  (red), D) 100  $\mu$ M ellagic acid (pink). All solutions were prepared in 0.2 M  $\text{NaClO}_4$  except ellagic acid. The stock solution of ellagic acid was prepared in a solution containing 0.2 M  $\text{NaClO}_4$  and 98% ethanol (at

the proportion 40:60, respectively). 250  $\mu\text{L}$  of this last solution were added to the solution containing NaI to give a final volume of 1 mL.

To express the TAC of plant extracts in a quantifiable and easily understood manner, different antioxidant standards equivalents can be used [45-47]. Under our experimental conditions, the best sensitivity was obtained for gallic acid. However, this antioxidant compound has poor water solubility which makes it difficult for practical use. Therefore, we consider more appropriate the analysis of antioxidant properties in terms of ascorbate equivalents. Ascorbate equivalents or vitamin C equivalent antioxidant capacity (VCEAC) has been used by other authors to express the TAC [45]. Furthermore, ASC is extremely important in plants, not only because of its direct antioxidant effects, but also because this compound is a substrate for the redox enzyme ascorbate peroxidase. ASC is present at high levels in all parts of plants and can even reach concentrations of 20 mM in chloroplasts [48]. Fig. 6 shows the linear calibration of ASC using this electroanalytical method ( $I-I_0$  ( $\mu\text{A}$ ) =  $(0.069 \pm 0.002)$  [ASC] ( $\mu\text{M}$ ),  $n = 3$ ). The limit of detection was determined to be 16.9  $\mu\text{M}$  (see Table 1) using  $3\sigma/S$ , where  $\sigma$  is the standard deviation of solution in the absence of ASC and  $S$  is the sensitivity of the a:





**Figure 6.** Plot of current increment of the anodic peak *vs* ASC concentration, where  $I_0$  is the oxidative current of the iodide/iodine in the absence of ASC. Error bars were calculated as the standard deviation of three different measurements.

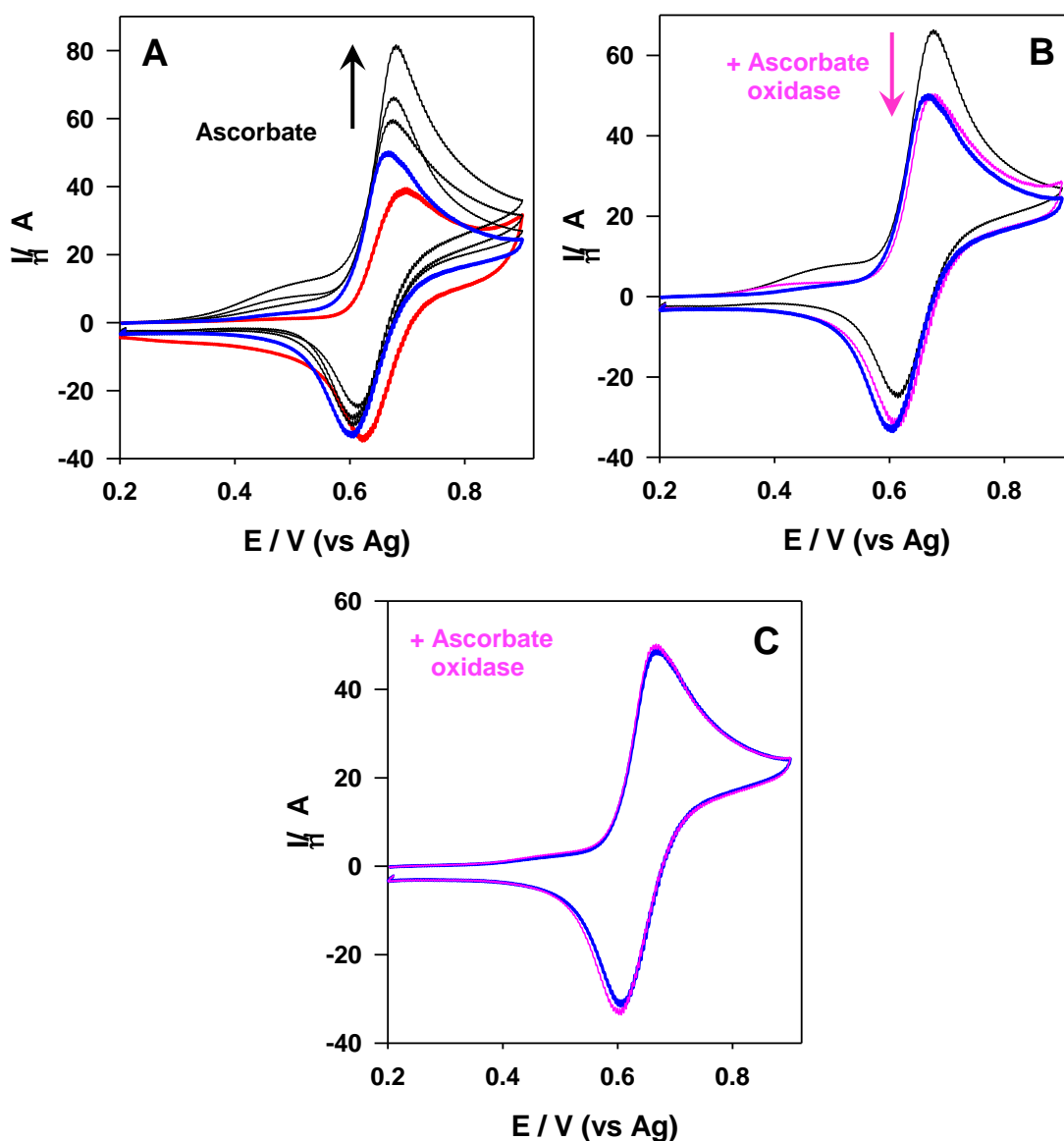
Although other authors have obtained better limits of detection with the use of DPPH radical [6,25,26], the preparation of this compound shows some technical disadvantages, such as the need of an ultrasounds bath for two hours and high protection from light to minimize its decomposition during the preparation process. On the other hand, the use of a FIA system by a continuous flow injection of DPPH solution [6] makes it more difficult the portability of the assay and shows the handicap of the generation of large quantities of waste solution.

The repeatability of the sensor response was evaluated by performing 5 successive measurements of a sample containing 1 mM NaI and 100  $\mu$ M ASC using the same screen- printed electrode, yielding RSD = 4.7 %. The reproducibility of the analytical response obtained with five different screen-printed electrodes was also analyzed, with a result of RSD = 3.6%.

#### **Application of the method in synthetic mixtures of antioxidants**

Fig 7A shows the cyclic voltammograms of the system iodide/iodine (red line) in the presence of a synthetic mixture of the antioxidants GSH, NADH and gallic acid (blue line) and after the addition of increasing concentrations of ASC (black lines, from bottom to top). The maximum peak current of the iodide oxidation increased linearly as

the ASC concentration was increased (Fig. 7A) with a similar slope than in Fig. 6. When AOX was added to the solution containing GSH, NADH, gallic acid and ASC, the voltammetric signal decreased until the same intensity than the solution in the absence of ASC (pink and blue lines in Fig. 7B, respectively). The addition of the enzyme allows us to set the concentration zero of ascorbate since the current signal of iodide/iodine redox couple did not change when the enzyme was added to a solution without ASC (Figure 7C, pink and blue lines, respectively). Therefore, AOX did not interfere on TAC measurements. These results demonstrated that our method is able to determinate the TAC in ascorbate units in a complex mixture. Moreover, the AOX addition allows the ascorbate concentration to be independently measured in the sample. It is important to stand out that this strategy is extrapolable to the measurement of other different antioxidants using enzymes based on oxidases to establish their corresponding zero in concentration.

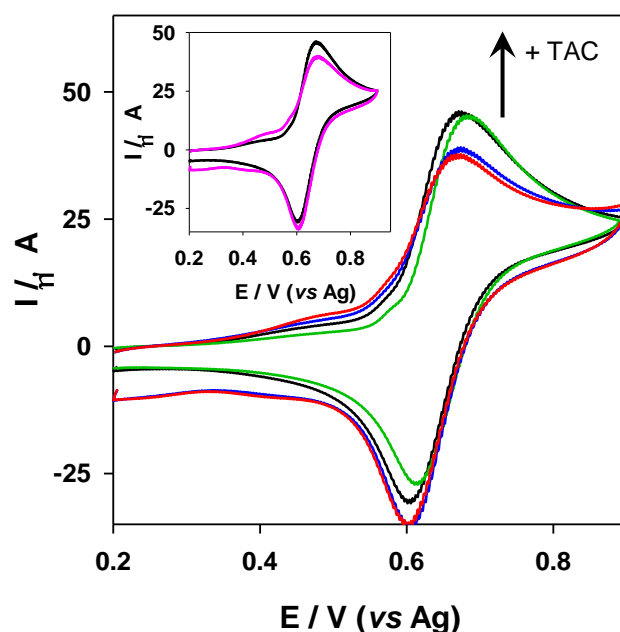


**Figure 7.** Voltammetric response of 1 mM NaI after the addition of ASC and AOX at 100  $\text{mV s}^{-1}$ . A) 1 mM NaI (red line), 1 mM NaI in the presence of a synthetic mixture of 50  $\mu\text{M}$  GSH, 50  $\mu\text{M}$  NADH and 50  $\mu\text{M}$  gallic acid (blue line), and 1 mM NaI in the presence of the previous synthetic mixture of antioxidants containing 200, 300 and 500  $\mu\text{M}$  ASC (black, from bottom to top, respectively). B) 1 mM NaI in the presence of a synthetic mixture of 50  $\mu\text{M}$  GSH, 50  $\mu\text{M}$  NADH and 50  $\mu\text{M}$  gallic acid (blue line), after the addition of 300  $\mu\text{M}$  ASC (black line) and after the addition of 300  $\mu\text{M}$  ASC and of

AOX (pink line). C) 1 mM NaI in the presence of a synthetic mixture of 50  $\mu$ M GSH, 50  $\mu$ M NADH and 50  $\mu$ M gallic acid (blue) and the same solution after the addition of AOX (pink). AOX (108 units of activity) was incubated during 15 min.

### **Application of the method in plant extracts**

In accordance with the above, the relative quantity of antioxidants in a plant sample can be evaluated by adding iodide solution and measuring its oxidative peak by cyclic voltammetry. To check the applicability of this methodology in real samples, it was used to compare the TAC of different plant extracts from pea leaves. In Fig. 8, cyclic voltammeteries of some plant extracts samples (C1, C2, S1, S2), in the presence of NaI, are shown. Among the plant extract samples, two of these came from plants grown under normal conditions (non-stressed biological replicates, C1 in black and C2 in green) and the other two from plants which were subjected to salt stress (stressed biological replicates, S1 in blue and S2 in red). As can be seen, the plant extracts from non-stressed plants have a higher positive current at 0.7 V than the extracts from stressed plants. A bigger current at this potential is related to the higher TAC in the samples, in agreement with the above results. Therefore, these measurements allow a good estimation of the difference in antioxidant power between plants subjected to optimal and stressful conditions. This methodology could be very useful in the study of how different oxidative stressful conditions can affect to the antioxidant properties of plants. The inset of Fig. 8 shows the decrease of the current signal when AOX was added to quantify ASC concentration.



**Figure 8.** Cyclic voltammetry at a disposable Pt screen printed electrode of different plant extracts from pea leaves (10 % plant extract in 0.2 M  $\text{NaClO}_4$ ) in the presence of 1 mM NaI. Non-stressed samples at  $100 \text{ mV s}^{-1}$ : C1 (black line) and C2 (green line); and stressed samples: S1 (blue line) and S2 (red line). Inset: Cyclic voltammetry of the sample C1 (10% plant extract in 0.2 M  $\text{NaClO}_4$ ) in the absence (black line) and the presence of AOX (pink line). AOX (108 units of activity) was incubated during 15 min.

Table 2 shows the data obtained for TAC and ascorbate concentration using this method in these plant extracts. In addition, the results obtained by the electrochemical method were compared to those obtained by a spectrophotometric method using a conventional commercial kit. A good correlation among the two methods was obtained showing a linear relationship between the antioxidant capacity measured by the electrochemical method (y) and that obtained by the spectrophotometric method (x) ( $y =$

$-0.004 + 1.255x$ ,  $R^2 = 0.999$ ) However, some differences can be seen in the ASC concentration, probably due to the interference of DHA in the colorimetric method [49]. This compound has been reported to be present in leaves extracts at relatively high concentrations [43, 44]. This comparison demonstrates the good performance in the estimation of TAC of the analytical method here proposed.

**Table 2.** Total antioxidant capacity (expressed in ascorbate equivalents) and ascorbate concentration in pea leaves extracts measured both by the electrochemical and the spectrophotometric (reference) method. The values are the mean of three replicates.

Sample	Electrochemical		Spectrophotometric		Proportional ratio (b/a)**
	Method*		method		
	Ascorbate	Ascorbate	Ascorbate	Ascorbate	
	equivalents	concentration	equivalents	concentration	
	(VCEAC) <sup>a</sup>	(mM)	(VCEAC) <sup>b</sup>	(mM)	
	(mM)		(mM)		
C1	1.53 ± 0.09	0.96 ± 0.02	1.9 ± 0.1	0.76 ± 0.09	1.2
C2	1.34 ± 0.08	0.91 ± 0.03	1.7 ± 0.1	0.59 ± 0.08	1.3
S1	0.5 ± 0.1	0.49 ± 0.05	0.6 ± 0.1	0.5 ± 0.1	1.2
S2	0.31 ± 0.09	0.29 ± 0.06	0.4 ± 0.1	0.18 ± 0.05	1.3

\*Fig. 8 shows the voltammograms from which VCEAC data have been calculated. Ascorbate concentration was calculated by the addition of AOX as illustrated in the inset for C1 sample.

\*\*Proportional ratio (b/a) corresponds to the ratio between the result obtained from the spectrophotometric method and that obtained from the electrochemical method.

## CONCLUSIONS

In conclusion, we have developed a simple, fast and easy method for determining the total antioxidant capacity in terms of ascorbate equivalents and the ascorbate concentration. The method is based on the reduction of the electrochemically generated iodine with different biological relevant compounds with antioxidant character, which provides information about the oxidative status of biological samples. Iodine reacts with reducing agents (antioxidants) but not with their corresponding oxidized species, which makes it possible to obtain a good estimation of the level of antioxidant capacity. This electrochemical method was applied in real plant extracts and compared with a commercial spectrophotometric method, obtaining a good correlation. Moreover, the insensitivity of the iodide/iodine mediator couple to pH, implies that the procedure may be well suited for application to a wide range of different analytical conditions. The analysis of the antioxidant properties is very important because it can be considered as a marker for the evaluation of oxidative stress in plants. This provides a better understanding of some processes occurring in plants and could also be used for the analysis of food quality in routinely assays (juices, fruits, vegetables and other commercial products).

## **FUNDING SOURCE**

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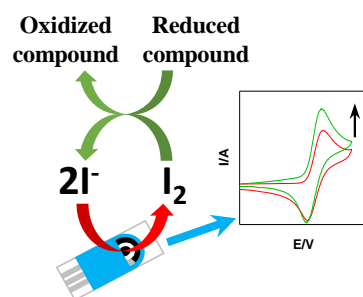


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## TABLE OF CONTENTS



## **ABBREVIATIONS USED**

<b>AOX</b>	Ascorbate oxidase
<b>ASC</b>	Ascorbic acid
<b>DHA</b>	Dehydroascorbic acid
<b>FRASC</b>	Ferric Reducing Ascorbate Assay Kit
<b>GSH</b>	Reduced glutathione
<b>GSSG</b>	Oxidized glutathione
<b>LOD</b>	Limit of detection
<b>NADH</b>	Reduced nicotinamide adenine nucleotide
<b>NAD<sup>+</sup></b>	Oxidized nicotinamide adenine nucleotide
<b>ROS</b>	Reactive oxygen species
<b>TAC</b>	Total antioxidant capacity
<b>VCEAC</b>	Vitamin C equivalent antioxidant capacity