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PII: S1572-6657(20)30027-8

DOI: https://doi.org/10.1016/j.jelechem.2020.113844

Reference: JEAC 113844

To appear in: *Journal of Electroanalytical Chemistry*

Received date: 30 September 2019

Revised date: 15 December 2019

Accepted date: 9 January 2020

Please cite this article as: A.C. Gonzalez-Baro, D. Izquierdo, A. Heras, et al., UV/Vis spectroelectrochemistry of o-vanillin: Study of the antioxidant properties, *Journal of Electroanalytical Chemistry*(2020), https://doi.org/10.1016/j.jelechem.2020.113844

UV/Vis Spectroelectrochemistry of *o*-vanillin: study of the antioxidant properties.

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Abstract

UV/Vis absorption spectroelectrochemistry is a very useful technique to study complex electrochemical mechanisms. In this work, we have studied the electrochemistry of *o*-vanillin in presence and absence of oxygen. This compound exhibits good antioxidant properties. The products of the reaction of o-vanillin with the superoxide anion, electrogenerated when oxygen is reduced in an aprotic medium, have been detected using UV/Vis absorption spectroelectrochemistry. This technique allows us to shed more light on the antioxidant properties of *o*-vanillin, providing valuable information not only on the antioxidant properties of this compound but also about other compounds that follow a similar mechanism. In this work we have deconvolved the electrochemical signal in the different components that are related to the processes taking place at the electrode and in the solution adjacent to it.

Keywords

Electrochemistry, spectroscopy, spectroelectrochemistry, o-vanillin, antioxidant

Introduction

Spectroelectrochemistry (SEC) can be defined as the instrumental technique that combines *in-situ* and simultaneous spectroscopic and electrochemical measurements during a chemical process [1–6]. Most of the times, the chemical reaction is electrochemically controlled, being the optical signal used to obtain valuable information about the chemical process. Among the different possible experimental set-ups, UV-Vis absorption SEC (UV/Vis-SEC) has been widely used for a better understanding of complex mechanisms of reaction [7,8]. In this work, UV/Vis-SEC has been used to detect the products generated when *o*-vanillin (2-hydroxy-3-methoxybenzaldehyde), *o*-HVa, acts as antioxidant. *o*-Vanillin is a positional isomer of vanillin (4-hydroxy-3-methoxybenzaldehyde) a plant natural product used as food flavoring agent with known therapeutic activities [9]. Both species have antioxidant properties at milimolar concentrations [10], the assay of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging shows that o-vanillin is a more effective quencher of DPPH radical than vanillin [10] .

Antioxidants are molecules that decrease or prevent the oxidation of other substances [11]. Nowadays, these compounds are receiving an important

interest because they can play an important role in the prevention of different diseases [12]. Thus, the development of new methods to evaluate the power of antioxidants to scavenge free radicals or to understand how these molecules protect against reactive oxygen species (ROS) is very important [13]. Antioxidant properties have been studied using different analytical methods, being spectroscopy and electrochemistry very useful techniques for this purpose [14–23]. However, to the best of our knowledge, the *in-situ* combination of these two analytical methods in one single experiment to follow the antioxidant properties of a molecule has not ever been attempted. From its own nature, SEC should be a very useful technique to follow this type of processes [1,2,5]. On one hand, the redox state of the molecules in solution can be easily controlled by the potential, and on the other hand the optical signal can interrogate the solution adjacent to the electrode about the changes in composition that takes place during the generation of radicals. Moreover, the combination of the information obtained from the electrochemical and the optical signals can be used to deconvolve the current in the different components that are related to the processes taking place at the electrode and in the solution adjacent to it. In this work, we demonstrate that the spectroscopic signal can be used to find out if all the superoxide ion has reacted with the antioxidant, obtaining an estimation of the fraction of charge involved in the reaction between antioxidant and radical. Particularly, UV/Vis-SEC has been used to study the antioxidant properties of *o*-HVa against superoxide, an interesting radical anion, because it is one of the ROS present at *in-vivo* systems. The study demonstrates the valuable information that can be obtained about this complex process.

Experimental

Chemicals and Materials.

o-Vanillin (*o*-HVa, Merk), tetrabuthylammonium hexafluorophosphate ($TBAPF_6$, Merck), dimethylsulfoxide (DMSO, Merk) were analytical grade and used as received without further purification. For safety considerations, all handling and processing were performed carefully during all experiments. Argon was used to purge the solutions to work in absence of oxygen.

Instrumentation

In-situ operando UV/Vis SEC measurements were performed using a UV-VIS SPELEC instrument (DropSens) combined with a home-made reflection cell that consists of two polytetrafluoroethylene (PTFE) bodies that contains a three electrode system and a bifurcated reflection probe (DRP-RPROBE, DropSens). This spectroelectrochemical cell works in a near normal reflection configuration and in a semi-infinite diffusion regime. The bottom body is used to hold a glassy carbon working electrode (WE, CHI Instruments). The upper body contains the reflection probe that is placed in front of the WE, a Pt wire as counter electrode (CE), and a Ag wire as pseudo-reference electrode (RE). Four magnets in each body and an O-ring help to fix the bodies, avoiding solution leakages. A small recess is made in the bottom body to place the O-ring while the magnets allow us to assemble/disassemble the SEC cell in a simple way. The SPELEC instrument was controlled by DropView SPELEC software (DropSens),

performing simultaneous and time-resolved SEC experiments, with a totally synchronized data acquisition. In all the experiments, the spectrum of the sample before starting the experiment is taken as reference. Thus, absorbance is presented as increment of absorbance respect to the reference spectrum.

Assessment of the charge involved in an electrochemical process using the absorbance.

Charge (Q) and absorbance (A_λ) are correlated by eq. 1 [32]:

$$A_\lambda = \varepsilon \frac{10^3}{nFS} Q \quad (1)$$

where ε is the molar absorption coefficient, n is the number of electrons, F is the Faraday's constant and S is the electrode area. Therefore, if the slope of the linear correlation between A_λ and Q (eq. 1) is known, the faradic charge related to one of the reactants, products or compounds that suffer a quantitative reaction with the products can be calculated from the absorbance in any electrochemical process.

For example, for an electrode process where the product of the electrochemical reaction (eq. 2) is quantitatively consumed in a subsequent chemical reaction (eq. 3):

$$O + ne^- \rightarrow R \quad (2)$$

$$R + B \rightarrow C + D \quad (3)$$

assuming that O, R, B and D do not absorb at the same wavelength than the product of the chemical reaction C, if the slope of eq. 1 is known for C, obtained from an electrochemical reaction of this compound, the inverse of eq. 1 can be

used to calculate the charge involved in the generation of C (Q_c.). In all cases, the stoichiometry of the reactions must be considered. It should be noted that the derivative of the absorbance respect to time and the electrical current are related by the same slope that eq. 1.

The total current (i_t) involved in the electrode process governed by eq. 2 and eq. 3 can be deconvolved in two different components:

$$i_t = i_c + i_r \quad (4)$$

where i_c is the current related to the generation of C from the reaction of R with B and i_r is the current related to the generation of R that does not react with B. Thus, i_c can be obtained from the derivative of Qc respect to time, dQ_c/dt.

For a voltabsorptometric experiment, i_t is the current measured in the voltammetry, i_c can be obtained from the derivative voltabsorptogram respect to time at a characteristic wavelength of C, and therefore, i_r can be easily obtained using eq. 4.

Results and discussion

The reactivity of different phenols and polyphenols versus superoxide ion ($O_2^{\bullet-}$) using cyclic voltammetry has been used to evaluate electrochemically the antioxidant capacity of different molecules [22,23]. Superoxide ion can be generated electrochemically in aprotic organic solvents as dimethylsufoxide (DMSO), dimethylformamide (DMF), acetonitrile or ionic liquids [22–28]. The use of an aprotic medium avoids the disproportionation of the electrogenerated anion $O_2^{\bullet-}$, making this radical stable even performing voltammetric experiments at low scan rates [22].

Fig. 1 shows a cyclic voltammetry experiment where the potential was scanned at 0.010 V s^{-1} between +0.00 V to -1.10 V, starting at 0.00 V in DMSO containing 0.1 M $TBAPF_6$ as supporting electrolyte, in an air-saturated solution. The corresponding cyclic voltammogram (CV) is related to the generation of superoxide ion during the cathodic scan and the corresponding reversible oxidation to oxygen in the anodic scan, confirming the stability of the electrogenerated anion $O_2^{\bullet-}$. A difference of 0.137 V between the cathodic and anodic peak potentials is obtained, which indicates a slow heterogeneous electron-transfer kinetics [28].

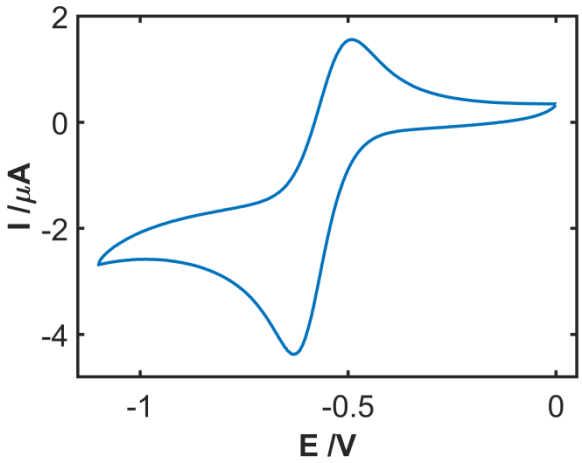

Fig. 1. CV obtained during the potential scan in 0.1 M $TBAPF_6$ in DMSO. Potential was scanned at 0.010 V s^{-1} between +0.00 V and -1.10 V. Initial potential: 0.00V. The CV was collected in an air-saturated solution.

The electrochemistry of *o*-HVa depends strongly on the presence or absence of oxygen in solution because of the antioxidant character of this compound. Fig. 2 shows two cyclic voltammetry experiments where the potential was scanned at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +1.30 V and finishing at the initial potential, 0.00 V, in a 10^{-3} M *o*-HVa solution with 0.1 M $TBAPF_6$ as

supporting electrolyte in DMSO. Red curve corresponds to the experiment in absence of oxygen and blue curve corresponds to the experiment in presence of oxygen. As can be observed the two CVs are strongly different in both the cathodic and the anodic scan.

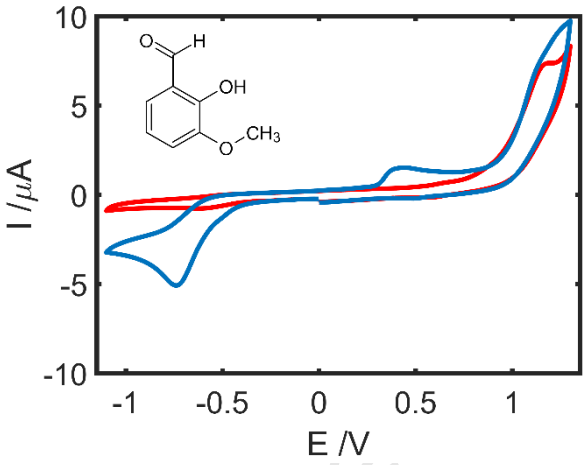

Fig. 2. CVs obtained during the potential scan in a 10^{-3} M o-HVa solution with 0.1 M $TBAPF_6$ in DMSO. Potential was scanned at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +1.30 V and finishing at the initial potential, 0.00 V. Experiments performed in absence (red line) and in presence (blue line) of oxygen. Inset: chemical structure of o-HVa

The experiment carried out in absence of oxygen (Fig. 2, red line) only shows an ill-defined irreversible anodic peak around +1.17 V that can be related to the oxidation of *o*-HVa but any reduction peak evolves. Compton *et al.* [29] studied electrochemically the oxidation of vanillin and proposed that during the vanillin oxidation not only the *o*-quinoid form is generated but also other dimerization products are formed. On the contrary, in presence of oxygen (Fig. 2, blue line), an irreversible reduction peak is observed at -0.75 V and two irreversible oxidation peaks evolve at +0.42 and +1.17 V. Clearly, this last anodic peak, observed in presence and in absence of oxygen, has to be ascribed to the

oxidation of *o*-HVa. However, the first oxidation peak at +0.42 V cannot be directly ascribed to *o*-HVa because it was not observed either in absence of oxygen or when the potential scan starts in the anodic direction, even in presence of oxygen (data not shown). From the results shown in Fig.1, the reduction peak at -0.75 V has to be related to the reduction of oxygen to generate superoxide ion, but in this case, no-reversible anodic peak is observed. In order to shed more light to the reactions taking place during the electrochemical experiments, a near normal reflection setup was used to obtain simultaneous spectral information about the compounds generated during this electrode reaction.

Fig. 3 displays the SEC responses obtained for the two experiments shown in Fig. 2. As can be seen, the evolution of the spectra obtained during the experiment in absence of oxygen (Fig. 3.A) is completely different from the one in presence of oxygen (Fig. 3.B). In agreement with the CV (red line, Fig. 2) in absence of oxygen, only a change of absorbance around +1.17 V is observed.

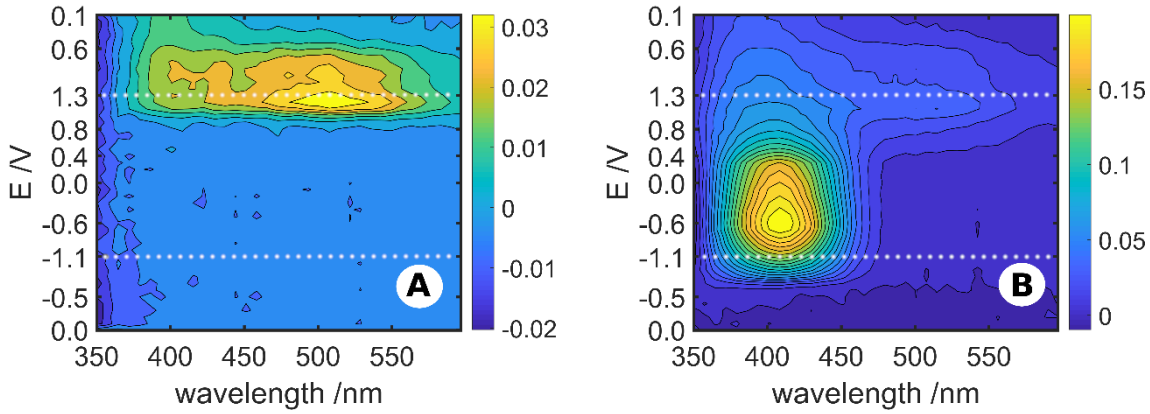

Fig. 3. Evolution of the absorption spectra (as contour plot) obtained during the CV in a 10^{-3} M o-HVa solution with 0.1 M $TBAPF_6$ in DMSO. Potential was scanned at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +1.30 V and finishing at the initial potential, 0.00 V. Experiments performed (A) in absence and (B) in

presence of oxygen. White dotted horizontal lines at the vertex potential (-1.10 V and +1.30 V) have been added for a better understanding of the experiment.

A small absorption band peaking at 507 nm evolves concomitantly with the oxidation peak observed in the voltammogram. Therefore, the oxidation of *o*-HVa yields a compound absorbing at this wavelength. In the case of the experiment in presence of oxygen (Fig 3.B), an absorption band centered at 410 nm evolves during the reduction of the oxygen. This absorption band increases during the cathodic scan, disappearing during the anodic scan. Clearly, this band cannot be assigned to the reduction of oxygen, because superoxide ion absorbs in the UV region [30,31].

We could think that this band is due to the reduction of *o*-HVa, but, as can be deduced from the CV in absence of oxygen (red line, Fig. 2), *o*-HVa is not reduced at this potential. Therefore, this band has to be related to a chemical reaction between *o*-HVa and superoxide ion that has been generated during the reduction of oxygen. Finally, the mentioned band at 507 nm due to the oxidation of *o*-HVa is observed around +1.17 V.

Voltabsorptograms at 507 and 410 nm shows the specific evolution of the absorbance with potential at these two wavelengths, allowing us to obtain a better understanding of the processes taking place on the electrode and at the solution adjacent to it (Fig. 4).

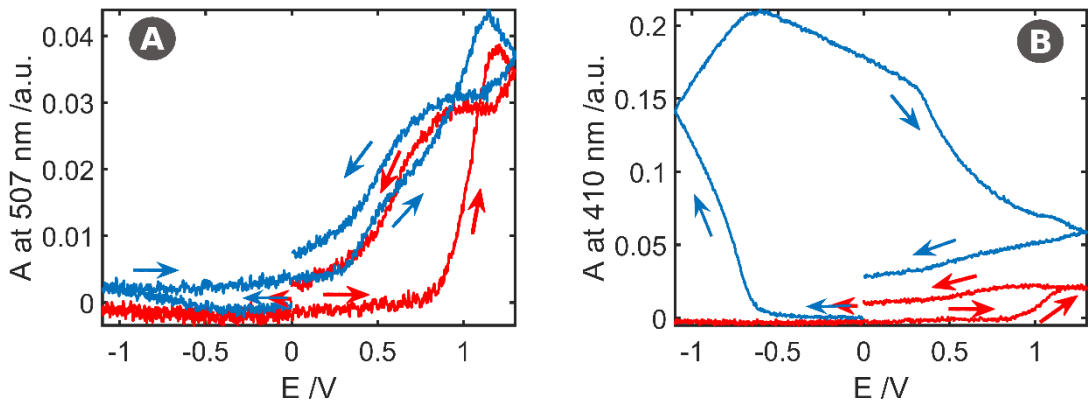

Fig. 4. Voltabsorptograms at (A) 507 nm and (B) 410 nm, obtained during the CV in a 10^{-3} M o-HVa solution with 0.1 M $TBAPF_6$ in DMSO. Potential was scanned at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +1.10 V and finishing at the initial potential, 0.00 V. Experiments performed in absence (red line) and in presence (blue line) of oxygen.

From the evolution of the absorbance band around 507 nm with the applied potential (Fig. 4.A) we can conclude that this band is related to the oxidation of *o*-HVa, in good agreement with the CV. Apparently, in the case of the oxygenated solution, absorbance increases at a lower potential than the observed in the deaerated solution, but this increment can be ascribed to the tail of the absorption band around 410 nm (Fig. 3.B). Visible absorption bands are usually wide, with the overlapping of close spectral bands being very common. More interesting is the voltabsorptogram of the band peaking at 410 nm (Fig. 4.B). As can be observed, the deaerated solution does not show any change of absorbance during the cathodic scan and only a small increase of absorbance is appreciated during the oxidation of *o*-HVa around +1.00 V (Fig. 4.B, red line). This change in the anodic region has to be ascribed to the tail of the band at 507 nm. On the contrary, the experiment carried out in presence of air (Fig. 4.B, blue line) shows a deep increase of absorbance at 410 nm around

-0.75 V during the cathodic scan. Absorbance increases continuously from potentials lower than -0.55 V in the forward scan, and it starts to decrease from +0.42 V onwards, in the backward scan. At these cathodic potentials, *o*-HVa is not reduced (Fig. 4.A) but oxygen is reduced to yield superoxide ion (Fig. 4.B). Therefore, a compound with antioxidant properties, as *o*-HVa is, chemically reacts with the anion $O_2^{\bullet-}$ yielding a compound that absorbs at 410 nm. Once this compound is generated in the cathodic scan, it is oxidized at +0.42 V during the anodic scan. From the SEC information we can deduce that the compound generated by a chemical reaction of *o*-HVa and $O_2^{\bullet-}$ is *o*-vanillinate anion, *o*-Va^-.

In order to confirm that the product generated during the chemical reaction between *o*-HVa and $O_2^{\bullet-}$ is *o*-Va^-, experiments in a 5.10^{-4} M *o*-HVa solution, in presence of 0.05 M LiOH, with 0.1 M $TBAPF_6$ in DMSO were performed. A lower concentration of *o*-HVa was selected because its deprotonated form exhibits an absorption band around 410 nm and, therefore, the light beam is strongly absorbed and a noisy optical signal is obtained at higher concentrations. In this electrolytic medium, *o*-HVa can be found in its deprotonated form as *o*-Va^-, and its spectroelectrochemical behavior can be specifically studied.

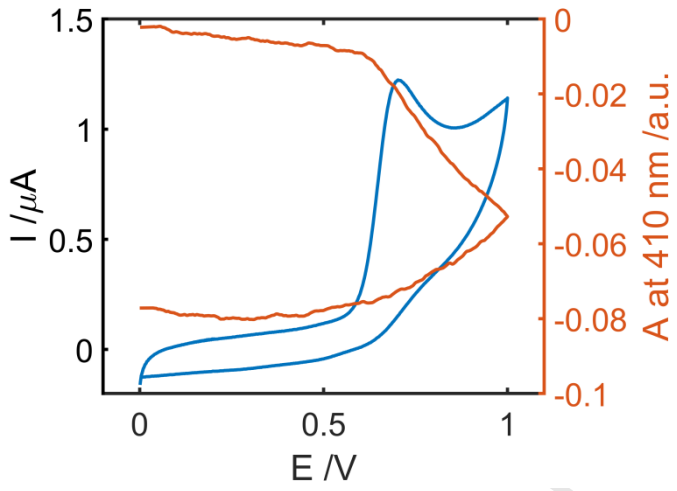

Fig. 5. CV (blue line) and voltabsorptogram at 410 nm (orange line) obtained during the potential scan in a 5.10^{-4} M o-vanillin solution with 0.05 M LiOH and 0.1 M $TBAPF_6$ in DMSO solution, in presence of oxygen. Potential was scanned at 0.010 V s^{-1} from 0.00 V to +1.00 V, finishing at the starting potential, 0.00 V.

Using this electrolytic solution, an anodic peak around +0.70 V evolves due to the oxidation of the anion *o*-Va$^-$ (Fig. 5, blue line). Concomitantly, the absorption band peaking around 410 nm that is directly related to this oxidation decreases during the anodic scan (Fig. 5), when the initial solution is taken as reference spectra. The position of the anodic peak changes respect to the data shown in Fig. 2 (blue line) because of the different composition of the solution. In presence of LiOH the reduction of oxygen is observed around -0.52 V (data not shown). The spectra corresponding to the oxidation of the anion is completely similar to the one observed in the experiments in absence of LiOH (Fig. 3.B), indicating that the same compound is being oxidized.

Two possible mechanisms of reaction are generally considered for the reactivity of phenols and polyphenols that can be considered for the reaction of o-HVa: proton-transfer and/or H-transfer pathways [22]. In the two cases the phenolate

ion is generated [22] and therefore, more investigations should be done to clarify the mechanism followed in the case of o-HVa.

Nevertheless, once detected the products of the reaction taking place between *o*-HVa and $O_2^{\bullet-}$, SEC measurements in normal configuration allows us to deconvolve the electrochemical process. From the spectroscopic data is deduced that the compound generated during the reduction of oxygen is the same that is consumed in the anodic scan, at +0.42 V, the o-vanillinate anion (*o*-Va^-). Therefore, if the charge transferred during this oxidation peak is plotted respect to the absorbance at 410 nm, a linear regression model can be constructed in the potential range at which *o*-Va^- is oxidized, obtaining the following relationship ($Q = 655.41*A_{410nm}$, $R^2 = 0.999$) in the potential range from +0.40 V to +0.50 V.

The charge transferred, exclusively used to generate *o*-Va^- by the chemical reaction with the superoxide ion, can be calculated from the absorbance at 410 nm using the relationship that has been calculated between charge and absorbance. This charge that corresponds exclusively to the action of the antioxidant, *o*-HVa in this case, can be subtracted from the total charge consumed during the electrochemical experiment, and therefore, the charge related to the superoxide ion that has not reacted with the antioxidant can be assessed. Taking the derivative of the calculated charges respect to time, the CV can be deconvolved in two components. A lower concentration of *o*-HVa than in the previous experiments was selected to better illustrate this deconvolution of the CV. Thus, the amount of electrogenerated anion $O_2^{\bullet-}$ is higher than the amount of *o*-HVa in solution. The total consumption of superoxide is avoided by using this lower *o*-HVa concentration. In this way, a

fraction of the superoxide ion electrogenerated did not react with *o*-HVa, and the deconvolution of CV can be more easily observed. Under these experimental conditions, the fraction of charge corresponding to anion $O_2^{\bullet-}$ that reacts with the antioxidant and the fraction corresponding to the reduced O_2 that has not reacted with the antioxidant can be estimated. Fig. 6 shows the CV (blue line) registered by scanning the potential at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +0.60 V and finishing at the initial potential, 0.00 V, in a $5 \cdot 10^{-4}$ M *o*-HVa solution with 0.1 M $TBAPF_6$ in DMSO.

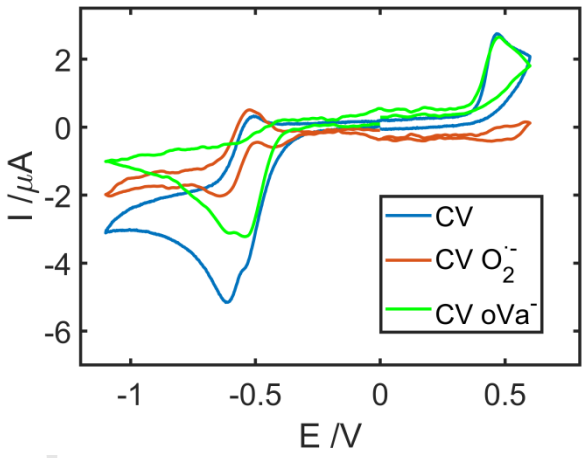

Fig. 6. CV (blue line) registered during the potential scan in a $5 \cdot 10^{-4}$ M o-HVa solution with 0.1 M $TBAPF_6$ in DMSO. Potential was scanned at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +0.60 V and finishing at the initial potential, 0.00 V. Recalculated CV from the absorbance at 410 nm (green line, CV o-Va$^-$) and difference between CV and CV o-Va$^-$ (red line, CV $O_2^{\bullet-}$).

As can be observed in Fig. 6, the CV (blue line) obtained under these experimental conditions shows a main reduction peak around -0.60 V with a

shoulder at -0.50 V. This shoulder is related to the reduction of O_2 to anion $O_2^{\bullet-}$ that reacts with *o*-HVa, yielding *o*-Va^- anion that absorbs around 410 nm. The relationship between charge and absorbance at 410 nm, shown above, can be used to recalculate the corresponding CV related to the amount of *o*-Va^- generated (green line, Fig. 6). The second overlapping peak that emerges at -0.60 V is related to the formation of superoxide ion that is not reacting with *o*-HVa because its concentration is so low that some superoxide anion remains in solution. This fraction of current can be calculated by using the difference between the experimental CV and the CV assessed from the generation of *o*-Va^-. The typical reversible CV of the reduction of oxygen in aprotic media, similar to that shown in Fig. 1, can be observed (red line, Fig. 6). In any case, other parasite or side reactions could be possible, and therefore, it is necessary to confirm that there is not any other reaction to be considered prior to recalculate the charge related to the superoxide that has not reacted with the antioxidant compound.

Therefore, UV/Vis-SEC has demonstrated to be very useful to deconvolve the electrochemical signal in the different components that are related to the processes taking place at the electrode and in the adjacent solution.

The most interesting point of this methodology is that many of the antioxidant compounds can be followed by UV/Vis-SEC. Different types of antioxidants such us ascorbic acid, hydroquinone, inorganic complexes, can be followed using UV/Vis-SEC. Fig. 7 shows the CV and the voltabsorptogram of ascorbic acid at a characteristic wavelength, in this case 280 nm. CV was performed using a 10^{-3} M ascorbic acid solution with 0.1 M $TBAPF_6$ in DMSO, starting the experiment at 0.00 V in the cathodic direction to generate superoxide anion and

scanning the potential between -1.10 V and +1.00 V. As can be seen, absorbance increases during the reduction of oxygen and the compound generated during this reaction can be oxidized during the anodic scan. Therefore, the same methodology proposed to deconvolve the CV of *o*-HVa could be used in this experiment. Further studies have to be done, but UV/Vis-SEC seems a promising technique to quantify the antioxidant capacity of molecules.

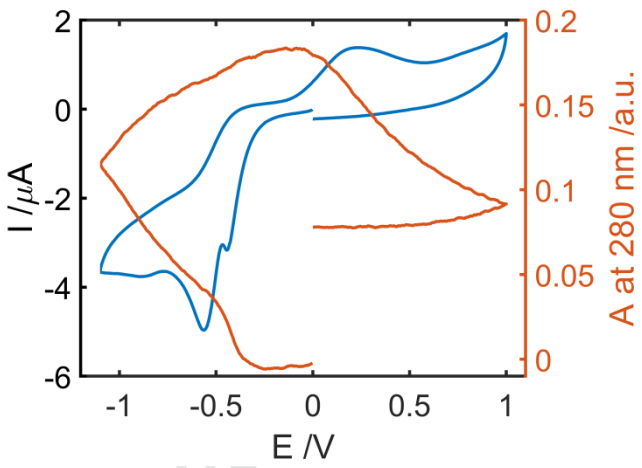

Fig. 7. CV (blue line) and voltabsorptogram at 280 nm (orange line) obtained during the potential scan in a 10^{-3} M ascorbic acid solution with 0.1 M $TBAPF_6$ in DMSO. Potential was scanned at 0.010 V s^{-1} from +0.00 V to -1.10 V, returning to +1.00 V and finishing at the initial potential, 0.00 V.

Conclusions

UV/Vis-SEC is a very useful technique to study the antioxidant properties of molecules. Particularly, we have studied the reaction of *o*-vanillin and superoxide ion in aprotic media, demonstrating the reaction between these compounds because of the antioxidant properties of *o*-vanillin. UV/Vis-SEC

allows us to deconvolve the electrochemical process by using the relationship between charge and the absorbance of the products generated in the chemical reaction between antioxidant and superoxide ion. From the results shown in this work, new analytical methods based on UV/Vis-SEC could be developed to study the antioxidant properties of molecules.

Acknowledgments

Authors acknowledge the financial support from Ministerio de Economía y Competitividad (CTQ2017-83935-R-AEI/FEDERUE), Junta de Castilla y León (BU297P18), Ministerio de Ciencia, Innovación y Universidades (RED2018-102412-T), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de La Plata, Argentina.

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Author Contribution

A.G-B and D.I. carried out the experiments. A.G-B., D.I., A.H and A.C. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- The products generated when o-vanillin acts as antioxidant has been detected.
- Spectroelectrochemistry (SEC) helps to deconvolve the electrochemical signal.
- SEC is a powerful technique to study the antioxidant properties of molecules.