Sorbic Acid and Its Degradation Products: Electrochemical Characterization

Ilanna C. Lopes a b, Paulina V. F. Santos a, Victor C. Diculescu a, Mário César U. de Araújo b & Ana Maria Oliveira-Brett a

a Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal
b Departamento de Química, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Paraíba, Brasil

Available online: 06 Jan 2012

To cite this article: Ilanna C. Lopes, Paulina V. F. Santos, Victor C. Diculescu, Mário César U. de Araújo & Ana Maria Oliveira-Brett (2012): Sorbic Acid and Its Degradation Products: Electrochemical Characterization, Analytical Letters, 45:4, 408-417

To link to this article: http://dx.doi.org/10.1080/00032719.2011.644738

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.
Electrochemistry

SORBIC ACID AND ITS DEGRADATION PRODUCTS: ELECTROCHEMICAL CHARACTERIZATION

Ilanna C. Lopes,1,2 Paulina V. F. Santos,1 Victor C. Diculescu,1 Mário César U. de Araújo,2 and Ana Maria Oliveira-Brett1

1Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal
2Departamento de Química, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Paraíba, Brasil

The electrochemical redox behavior of sorbic acid (SA), an important food preservative, was investigated at a glassy carbon electrode using cyclic, differential pulse, and squarewave voltammetry over a wide pH range. The oxidation of SA is an irreversible, diffusion-controlled, and pH-independent process that occurs with the transfer of only one electron and does not involve the formation of any electroactive oxidation product. Adsorption of SA at GCE electrodes was also observed. Following incubation in different pH electrolytes, the degradation of SA was electrochemically detected by the appearance of a new oxidation peak at lower potential value. The degradation products, formed homogenously in solution, undergo irreversible oxidation and lead to the formation of two oxidation products that strongly adsorb on the electrode surface and are reversibly oxidized. SA degradation was also confirmed using HPLC and UV-Vis spectrophotometry. A mechanism for oxidation of SA and its degradation products in aqueous solutions was proposed.

Keywords: Degradation products; Oxidation; Sorbic acid; Voltammetry

INTRODUCTION

Every year large quantities of food are discarded due to contamination and spoilage by fungi. Fungal spoilage is manifested by visible growth of hyphae, sporulation on the food surface, alteration in taste or texture due to formation of fungal metabolites, and, in some cases, by formation of mycotoxins. In order to prevent...
spoilage and aging, various compounds have been approved and widely used for addition to foods, drugs, and cosmetic products as preservatives, acting as antimicrobial agents and/or antioxidants.

Sorbic acid (trans, trans-2,4-hexadienoic acid) (SA) is an antimycotic agent against molds and yeasts, also being effective against a wide range of bacteria, and is used as preservative in pharmaceutical, cosmetic, and food products. During recent decades SA and its sodium, potassium, and calcium salts have been accepted as “generally recognized as safe” substances and have become the leading preservatives for food as well as for pharmaceutical and cosmetic preparations (Tang and Wu 2005). Despite its advantages as a preservative and considerable stability in the pure dry, crystalline state, SA has been shown to be unstable in aqueous solutions, leading to several degradation products, which may be involved in chemical reactions and result in a change of the organoleptic and nutritional properties of food preparations. Yarramraju et al. (2007) simultaneously investigated the SA in aqueous solutions and its degradation products and found 12 SA degradation products, 8 of which could be characterized (acetone; 2-methylfuran; crotonaldehyde; α-angelicalactone; 2-acetyl, 5-methylfuran; toluene and 2,5-dimethylfuran), and some of them are known to be toxic. The SA degradation has been found to be influenced by a number of factors such as temperature, pH, presence of salts, trace metal ions, sugar, glycerol, and amino acids (Arya 1980; Arya and Thakur 1988; Hildegard and Sabalitschka 1965).

Several assay methods have been described for the analysis of SA and its degradation products, including static headspace gas chromatography (HS-GC) (Yarramraj et al. 2007), solid-phase extraction (SPE), and high-performance liquid chromatography (HPLC) with electrochemical and UV detection (Techakriengkrai and Surakarnkul 2007), capillary zone electrophoresis with UV detection (Tang and Wu 2005), UV spectrophotometry (Chen and Ni 2009), and potentiometry (Santini et al. 2009).

However, less is known about the electrochemical behavior of SA and its degradation products. From this point of view, the objective of this paper was to investigate the electrochemical behavior of SA and its degradation products in aqueous solutions for different incubation periods and pHs, using cyclic, differential pulse and square-wave voltammetry at a glassy carbon electrode, and complemented by HPLC and UV-Vis spectrophotometry.

**EXPERIMENTAL**

**Materials and Reagents**

Sorbic acid (SA, ≥99.0%), acetonitrile (HPLC gradient grade) and formic acid were purchased from Sigma. Fresh stock solutions of SA $10^{-4}$ M (for Square Wave Voltammetry), $10^{-4}$ M and $10^{-3}$ M (for Differential Pulse Voltammetry) and $5 \times 10^{-3}$ M (for Cyclic Voltammetry), were prepared. All SA solutions were prepared in purified water and kept at 4°C before performing the experiments. Each day a new fresh solution of SA was prepared. After analysis of the SA solutions during the day, the solutions were transferred from the electrochemical cell to 0.65 mL microcentrifuge tube (Costar) and stored and used after different time periods.
All supporting electrolyte buffer solutions, with 0.1 M ionic strength (Oliveira et al. 2007), were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity $\leq 0.1 \mu$S cm$^{-1}$). The pH measurements were performed with a Crison micropH 2001 pH-meter (Barcelona, Spain) with an Ingold combined glass electrode. All experiments were carried out at room temperature ($25 \pm 1^\circ$C).

Voltammetric Parameters and Electrochemical Cells

Voltammetric experiments were carried out using a µAutolab Type II potentiostat in combination with GPES 4.9 Software (Metrohm-Autolab, Utrecht, The Netherlands). Measurements were carried out using a glassy carbon (GCE, $d=1.5$ mm) (Cypress Systems Inc., USA) working electrode, a Pt wire counter electrode, and an Ag/AgCl (3 M KCl) (Cypress Systems Inc., USA) as reference, in a 0.2 mL one-compartment electrochemical cell of capacity 2 mL. The experimental conditions for differential pulse voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s$^{-1}$. For square wave voltammetry (SWV) a frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 mV s$^{-1}$ were used. For cyclic voltammetry (CV), the experimental parameters used were scan rate 100 mV s$^{-1}$.

The GCE was polished using diamond spray (particle size 1 µm, Kemet International Ltd, UK) before each electrochemical assay. After polishing, the electrode was rinsed thoroughly with Milli-Q water. Following this mechanical treatment various voltammograms were recorded in supporting electrolyte buffer solution, in order to obtain a stable baseline voltammogram.

Spectrophotometric and Chromatographic Parameters

The UV-VIS measurements were performed using a spectrophotometer SPECORD S100, running with Aspect Plus Version 1.5 (Analytik Jena GmbH, Jena, Germany). The experimental conditions for absorption spectra were: integration time 41 ms and accumulation 10 points. All UV-Vis spectra were measured from 200 nm to 400 nm, in a quartz glass cuvette with an optic path of 1 cm.

The SA HPLC experiments were carried out on an Alliance Waters 2690 Separations Module HPLC using a C18 column of reverse-phase ODS-3 V from Inertsil, at room temperature. The detector used was a Waters 996 Photodiode Array Detector (PDA 996) from Waters S.A., USA, set at the wavelength of 260 nm and the volume of sample injected was 50 µL. 5 × 10$^{-6}$ M SA solutions were prepared in pH 3.4 0.1 M acetate buffer, since at this pH the degradation of SA was faster. The mobile phase was acetonitrile/water (35:65, v/v), with 0.05% formic acid, pH $\approx 2.8$, isocratic method.

RESULTS AND DISCUSSION

Electrochemical Oxidation of SA

Cyclic voltammetry. Initial experiments on the voltammetric behavior of SA were performed in a N$_2$ saturated solution of 7.5 × 10$^{-4}$ M SA in pH 7.0 0.1 M
phosphate buffer using a GCE. The CVs were recorded starting from 0.00 V and cycling between a positive potential limit of +1.50 V and a negative potential limit of −1.00 V. Independently of the initial scan direction (toward positive or negative values) only one anodic peak occurred in all cases showing that SA only undergoes oxidation. Therefore, all experiments were carried out between 0.00 V and +1.50 V.

On the positive-going scan of the first CV in a fresh solution of $7.5 \times 10^{-4}$ M SA in pH 7.0 0.1 M phosphate buffer, an anodic peak 1a occurred at $E_{pa}^1 = +1.38$ V. Changing the scan direction, no cathodic peak was observed showing that oxidation of SA is irreversible. Subsequent CVs in the same solution without cleaning the GCE surface did not show any new oxidation peak demonstrating that the oxidation process of SA does not involve the formation of any electroactive oxidation product. The decrease of peak 1a current, on the second and third scans, was due to the adsorption of SA and/or its non-electroactive oxidation product.

**Differential pulse voltammetry.** The effect of pH on the electrochemical oxidation of $1.5 \times 10^{-4}$ M SA fresh solutions in $3.4 < \text{pH} < 11.9$ using DPV was investigated.

In $3.4 < \text{pH} < 7.0$, the oxidation of SA presented a pH independent peak 1a, at $E_{pa}^1 \approx +1.36$ V, with the width at half height $W_{1/2} \approx 87$ mV, corresponding to the transfer of one electron (Brett and Brett 1993).

**Electrochemical Behavior of Chemically Degraded SA**

The same solution of SA used after 5 h showed new oxidation peaks at lower potential, Fig. 1A, corresponding to the oxidation of the homogeneously chemically degraded SA (cdSA), and the cdSA in different buffers electrochemical behavior was investigated.

**Figure 1.** DP voltammograms background-corrected in $3 \times 10^{-5}$ M SA in pH 3.4 0.1 M acetate buffer: (A) after (●●●) 0 h, (●●●) 5 h, (●●●) 48 h, and (●) 14 days incubation in buffer; and (B) after 14 days incubation: (●●●) first, (●●●) second, and (●●●) third scans in solution and (●) first scan after transferring the electrode to buffer.
**Differential pulse voltammetry of cdSA and pH effect.** The degradation behavior of $3 \times 10^{-5}$ M SA in pH 3.4 0.1 M acetate buffer, was followed by DPV after different incubation times and between experiments the GCE surface was always cleaned. The DP voltammograms obtained after 5 h incubation showed peak $2_a$ at $E_{pa}^2 = +0.83$ V, due to cdSA, Fig. 1A. The DP voltammograms recorded in the same solution after longer incubation times, 24 h, 48 h, 96 h, 7d, and 14d showed a progressive increase of peak $2_a$ current with increasing incubation time. At the same time, the disappearance of SA oxidation peak $1_a$ was observed, Fig. 1A. After 14 d incubation, peak $2_a$ reached a constant current showing that the degradation of SA was completed after this period of time.

These experiments showed that upon incubation in buffer, structural modifications in the SA occurred with time. The decrease of peak $1_a$ current with incubation time corresponds to the decrease of SA concentration whereas the appearance of peak $2_a$ corresponds to the oxidation of the cdSA formed in buffer electrolyte solution.

Successive DP voltammograms were also recorded in a solution of SA in pH 3.4 0.1 M acetate buffer after 14 d incubation, Fig. 1B. On the first scan, peak $2_a$ occurred at $E_{pa}^2 = +0.82$ V. On the second scan two new oxidation peaks $3_a$, at $E_{pa}^3 = +0.48$ V, and $4_a$, at $E_{pa}^4 = +0.57$ V, were observed. These two peaks correspond to the oxidation of the adsorbed cdSA oxidation product formed on the GCE surface during the first potential scan. Increasing the number of scans, both peaks $3_a$ and $4_a$ increased due to the formation of more oxidation products at the GCE surface. The adsorption of cdSA oxidation products, peaks $3_a$ and $4_a$, at the GCE surface was also observed, Fig. 1B.

The electrochemical oxidation behavior in $3.4 < \text{pH} < 11.9$ of cdSA and of its oxidation products was studied in a $3 \times 10^{-5}$ M SA 14-d old solution in different buffer electrolytes.

The cdSA oxidation peak $2_a$ was observed for pH $< 11.9$. For $3.4 < \text{pH} < 9.2$, peak $2_a$ potential was displaced to less positive values with increasing pH, Fig. 2A.

![Figure 2](image-url) **Figure 2.** (A) 3D-plot of DP voltammograms background-corrected after 14 days incubation of $3 \times 10^{-5}$ M SA in different buffer electrolytes as a function of pH. (B) second SW voltammograms after 20 days incubation of $3 \times 10^{-5}$ M SA in pH 3.4 0.1 M acetate buffer; $f = 50$ Hz, $\Delta E_s = 2$ mV, pulse amplitude $50$ mV, $v_{eff} = 100$ mV s$^{-1}$; $I_t$ – total, $I_f$ – forward, $I_b$ – backward currents.
following the equation $E_{pa}^{2a}(V) = 1.03 - 0.059\, \text{pH}$. The slope of the line, $59\, \text{mV}$ per pH unit, showed that the oxidation mechanism involves the same number of electrons and protons (Smith 2006). The width at half height of peak $2a$, $W_{1/2} \approx 73\, \text{mV}$, means that the oxidation process involves the transfer of one electron and one proton. For pH $> 9.2$, peak $2a$ potential is pH independent, a mechanism involving the transfer of one electron, as the cdSA oxidation product undergoes chemical deprotonation in more alkaline electrolytes (Diculescu, Kumbhat, and Oliveira-Brett 2006), and pH $\approx 9.2$ for cdSA was determined.

During the experiment, in each buffer electrolyte, several DP voltammograms were recorded without cleaning the GCE surface. On the second DP voltammogram, for $3.4 < \text{pH} < 11.9$ peaks $3a$ and $4a$ occurred, corresponding to the oxidation of cdSA oxidation products. Their electrochemical behavior was studied as a function of pH. Increasing the pH, the potential of both peaks $3a$ and $4a$ was shifted to less positive potential values. The dependence was linear and the slope $59\, \text{mV}$ per pH unit, the width at half height for both peaks $54\, \text{mV}$, so peaks $3a$ and $4a$ oxidation processes involve the transfer of two electrons and two protons. For pH $> 9.2$, the cdSA peaks $3a$ and $4a$ are pH independent, in a mechanism involving the transfer of two electrons.

The peak $2a$ current varies with the incubation time and the degradation of SA is faster in acid electrolytes, Fig. 3A, in agreement with the literature (Arya 1980) which showed that the degradation of SA in aqueous solutions follows first-order or pseudo-first-order reaction kinetics and the rate of reaction is very much dependent on the protons concentration. The rate of reaction decreases with increasing pH and becomes negligible for pH $> 5.0$. As SA pH $\approx 4.75$, only non-dissociated SA molecules are susceptible to oxidative degradation in aqueous solutions and ionized molecules are degraded to a negligible extent.

**Square wave voltammetry.** To characterize the cdSA, SW voltammograms were recorded in different supporting electrolytes; a similar behavior was observed in
all electrolytes, and for pH 3.4 0.1 M acetate buffer is shown in Fig. 2B. The reversible character of the electron transfer reaction can be checked in one scan using SW voltammetry, since the current is sampled in both the positive- and the negative-going pulses.

The SW voltammograms were recorded in $3 \times 10^{-5}$ M SA solution after 20-days in pH 3.4 0.1 M acetate buffer. The first SW voltammogram showed the cdSA irreversible peak $2_\alpha$, at $E_{pa} = +0.87$ V. In the second SW voltammogram recorded without cleaning the GCE, Fig. 2B, the reversible cdSA oxidation products, peak $3_\alpha$, at $E_{pa} = +0.51$ V, and peak $4_\alpha$, at $E_{pa} = +0.62$ V, appeared, Fig. 2B, and peak $2_\alpha$ current decreased due to the adsorption of cdSA oxidation products on the GCE surface.

**Spectrophotometric experiments.** Spectrophotometric measurements were carried out in $2.5 \times 10^{-5}$ M SA in $2.1 < \text{pH} < 11.9$ to complement the voltammetric studies.

For pH $< 4.0$, SA showed a one absorption band at $\lambda \approx 263$ nm, due to the conjugated double bond carbonyl system (Arya 1980; Lozano et al. 2007). For pH $> 4.0$, the SA absorption band shifted to $\lambda \approx 254$ nm with increasing pH, Fig 3B. These variations involve the carboxylic acid groups: the degree of ionization of weak acid changes with pH and SA is in a neutral form in strong acid medium (Chen and Ni 2009).

The absorption spectra recorded for pH 6.1, 7.0, and 8.1 SA solutions, at 0 h, were all similar. After 15 d incubation, the absorption band of the parent molecule at $\lambda \approx 254$ nm decreased for pH 8.1 and 7.0, and completely disappeared at pH 6.1, Fig. 3B. This indicates that the chemical degradation of SA in aqueous solution decreased with increasing pH. A decrease in absorbance accompanied by a simultaneous increase in total carbonyls and malonaldehyde content and a slight decrease in pH has already been observed (Arya 1980).

**HPLC study of SA.** The HPLC with diode array detection measurements were carried out in order to complement the voltammetric studies. Aliquots from $5 \times 10^{-6}$ M SA solutions were injected in the mobile phase immediately after the preparation and also after 24 d incubation.

The chromatogram obtained for the fresh SA solution showed only one SA peak, with a retention time of 5.5 min, Fig. 4A, with maximum absorbance at $\lambda \approx 262$ nm.

The chromatogram obtained after 24 d incubation of SA solution presented three peaks, Fig. 4B. The peak of SA, at $\lambda \approx 262$ nm, appeared $\sim 0.002$ a.u. smaller but at the same retention time, and the other two new peaks with retention times of 3.8 min, with maximum absorbance at $\lambda \approx 246$ nm, and 9.5 min, with maximum absorbance at $\lambda \approx 256$ nm. Therefore, the SA chemical degradation involves breakdown of the molecule into two parts yielding the cdSA.

**Oxidation and chemical degradation mechanisms of SA.** The electrochemical experiments in fresh solutions of SA showed its oxidation at carbon electrodes. The oxidation takes place at the double bonds between C2–C3 or C4–C5. The double bond between C2 and C3 is close to the carboxyl moiety and its oxidation may be shielded, consequently more difficult to oxidize. Therefore, the SA oxidation
mechanism proposed, Scheme 1, involves the breaking the double bond between C4 and C5 with addition of OH$^{-}$ at position C5, peak 1$ _{a} $, followed by chemical deprotonation of the oxidation product.

Studies on the stability of SA showed that upon storage in acid electrolytes, the absorption band due to the conjugated double bond of the carbonyl system in the SA moiety decreased in a time dependent manner indicating the chemical degradation of SA. Malonaldehyde, crotonaldehyde and acrolein have been referred to as the major SA degradation products (Arya 1980; Hildegard and Sabalitschka 1965).

Figure 4. HPLC-UV chromatogram obtained from 5 x 10$^{-6}$ M SA solution in pH 3.4 0.1 M acetate buffer: (A) (●●●) 0 h and (■■■) after 24 days incubation time in buffer; and (B) enlarged between 3 and 10 min.

Scheme 1. Proposed oxidation mechanism of (a) sorbic acid and (b) sorbic acid degradation products: crotonaldehyde (R: = CH$_2$), malonaldehyde (R: = O).
The electrochemical results showed that upon chemical degradation of SA electrochemically active compounds, cdSA, were formed, Figs. 1 and 2. The rate of cdSA formation decreased with increasing pH and became negligible above pH 5.0, and an increase in total malonaldehyde and carbonyls content was observed, the compounds considered the main degradation products of SA. In agreement with the electrochemical results which showed a very small peak 2a in neutral and alkaline electrolytes, only non-dissociated SA molecules are susceptible to degradation and ionized molecules are degraded only to a negligible extent, Figs. 2A and 3A.

Malonaldehyde, crotonaldehyde and acrolein are the main products of chemical degradation of SA (Arya 1980). However, acrolein is a planar molecule with the $\pi$ systems of the carbon-carbon and carbon-oxygen double bonds overlapped, which increases the stability of the conjugated system. Therefore, the oxidation of acrolein requires a much greater energy than the potential applied to the GCE. For this reason, peak 2a is considered to be due exclusively from malonaldehyde and crotonaldehyde electrochemical oxidation. In the proposed mechanistic pathway for malonaldehyde and crotonaldehyde oxidation, Scheme 1, peak 2a, leads to the formation of 2-hydroxymalonaldehyde (MacDonald and Dunford 1989; Marder and Schuerch 1959) and 2-hydroxcrotonaldehyde, which also undergo reversible oxidation, peaks 4a–4c and 3a–3c, respectively.

CONCLUSIONS

The electrochemical behavior of SA was studied by cyclic and differential pulse voltammetry using a glassy carbon electrode. It was shown that SA undergoes irreversible, pH-independent electrochemical oxidation at high potentials.

Upon storage in acid electrolytes, the SA oxidation peak disappeared which gave rise to new anodic peaks of malonaldehyde and crotonaldehyde from chemically degraded SA (cdSA). The SA chemical degradation was confirmed by spectrophotometric measurements and HPLC analysis. A systematic electrochemical study of cdSA was carried out. Malonaldehyde and crotonaldehyde formed in the chemical degradation of SA are both irreversibly oxidized at a glassy carbon electrode involving the transfer of one electron and one proton. This process leads to the formation of hydroxymalonaldehyde and hydroxcrotonaldehyde as oxidation products, which undergo a reversible redox reaction. The irreversibility of cdSA oxidation and the reversibility of hydroxymalonaldehyde and hydroxcrotonaldehyde oxidations were confirmed by SW voltammetry. Based on the results, a mechanism for the oxidation of both SA and of cdSA was proposed. The electroanalytical determination of SA and cdSA is foreseen, which would provide very important and useful data for toxicity evaluation.

REFERENCES


