Electrochemical Pseudo-Titration of Water-Soluble Antioxidants

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Abstract
An amperometric test for the antioxidant power (AOP) of biological and food samples is presented. The gist of the method is to measure by linear sweep voltammetry the anodic current produced during the oxidation of the labile species present in the sample and then rationalizing this signal with a mathematical treatment that allows the pseudo-titration of antioxidants (AOs) around a given threshold potential. As a result, the AOP of the sample is calculated. This method allows the discrimination of the most biologically relevant AOs that react rapidly and at low oxidation potentials, from less reactive AOs that are oxidised slowly and at much higher oxidation potentials. This methodology was applied for measuring the AOP of blood, saliva, and natural drinks like orange juice.

Keywords: Antioxidants, Electrochemical pseudo-titration, Antioxidant power

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Dedicated to Professor Erkang Wang on the Occasion of His 80th Birthday

1 Introduction

The reaction of oxidation consisting of a transfer of electron(s) from one species to another, often corresponds to a structural and functional damage of the oxidised species. In biological systems, reactive oxygen species (ROS; hydroxyl radical $^{\bullet}OH$, hydrogen peroxide H_2O_2, superoxide $O_2^{\bullet-}$, etc.) and reactive nitrogen species (RNS; nitrogen dioxide $^{\bullet}NO_2$, peroxynitrite $ONOO^-$, etc.) responsible for the oxidation of several target molecules, including proteins, cellular membranes, cells, DNA or RNA play a relevant role in the development of several diseases such as Alzheimer, Parkinson and cancer [1–7]. For instance, it has been shown recently that the oxidation of copper (I) ions complexed with β-amyloid peptides leads to the formation of β-amyloid agglomerates which precipitate as plaques in the brain and contribute to the generation of Alzheimer diseases [8]. Likewise, in other fields such as in alimentation, an oxidised product is often equal to a damaged product. In order to overcome oxidation and oxidative stress conditions, a series of species known as antioxidants (AOs) have evolved to prevent oxidation and/or to protect living cells, human tissues or food sources from oxidative species by inactivating them within redox reactions.

The AO species and their regulatory mechanisms, including catalytic cellular enzymes, scavengers, metal chelators responsible for controlling oxidation represent the AO defence system. AOs are a complex family of compounds, including hydrophilic and lipophilic molecules that act as neutralisers of oxygen or nitrogen radicals, as repairing machines of damaged membranes and preventive antioxidants [9]. AOs are present at different concentrations and locations in the human body. They include endogenous species that are produced by the cells and exogenous ones, mostly from dietary origins. The most common water-soluble AOs present in blood and saliva are uric acid (UA; endogenous), ascorbic acid (AA, vitamin C; exogenous) and glutathione (GSH; endogenous), while their hydrophobic counterpart is tocopherol (vitamin E; exogenous) [10–12].

The cellular and extra-cellular (i.e. in biological fluids such as blood and saliva) AOs concentration is tightly regulated and ideally maintains within an optimum physiological range. Thus, the ability of measuring the AOP in a simple, rapid and economic manner is important and

advantageous. However, the measurement of the concentration of AOs is not sufficient to establish the level of the AO defence system, since the differentiation among the different AO species has to be taken into account depending on their liability to react with oxidative species. In addition, the analysis of AOs in very complex matrices such as blood and saliva often requires a sample pretreatment. This increases the costs and time needed for the analysis and can also negatively affect the result. An additional constraint when quantifying AOs levels is the fact that AOs can undergo very rapid reactions within themselves or with other electrochemically active species. As a result, recycling or auto-oxidation of AOs can take place during analysis biasing the obtained results [13]. Finally, AOs include both hydro-soluble and lipo-soluble species and often AOs activity assays do not account equally for both of them [14–16]. Therefore, the quantification and rationalisation of AOs is a truly analytical challenge.

Despite the fact that AOs in several cases take part in homogeneous redox reactions with oxidising species (otherwise it can lead to critical damages in biological systems), the determination of antioxidant activity is not restricted to quantification methods based on homogeneous reactions. Indeed, both homogeneous and heterogeneous based methods for antioxidant activity quantification show advantages as well as limitations and not necessarily identical thermodynamics and kinetics from the ones taken place at different biological systems. Therefore, relative antioxidant activity scales can be defined by using either a reference redox species or a defined $E_{threshold}$ potential (vide infra). In fact, one of the aims of the present manuscript is to take advantage of the $E_{threshold}$ potential to distinguish among all the possible electrochemically detected antioxidants, the most relevant ones (i.e. the ones with faster kinetics and lower overpotentials) (vide infra). To assess the total antioxidant capacity (TAC) of not only one antioxidant species, but of a whole complex sample (e.g. blood and saliva), several methodologies have been employed such as, the trolox equivalent antioxidant capacity (TEAC), the ferric reducing/antioxidant power assay (FRAP), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical test, the reducing antioxidant capacity evaluated (RACE) by electrolysis, among others [12,17–21]. The main principle of such strategies is based on quantifying the concentration of AOs that can be consumed by a given amount of oxidative species and in some cases finding the equivalence between the signal obtained from the measured sample and a known AO concentration, for instance trolox. With this aim, spectrophotometric, chemiluminescence and electrochemical techniques have been applied [22–28].

Electrochemical-based sensors have been used for measuring the AO activity due to their simple, fast and reliable analytical performance on biological and food samples [29–36]. Moreover, electrochemical strategies can also take into account the complete range of chemical and biochemical interactions between all present species, including recycling or auto-oxidation processes. Unlike other methods based on the measurement of a single endpoint, they also represent the advantage of measuring AO activity from a dynamic point of view, since kinetic aspects of the reactions between AOs, other reactive species and the surface of the sensor can be measured. Despite of this, small efforts have been made in order to discriminate at once between fast and slow reacting species, allowing the quantification of a global AO activity.

Herein, we report a simple electrochemical procedure, based on a pseudo-titration of a linear sweep voltammogram for rapidly measuring the hydro-soluble AOP in a variety of samples. This pseudo-titration voltammetry (PTV) of the original electrochemical current–potential signal is carried out by multiplying a dimension-less mathematical function representing a virtual and ideal oxidant and allows to discriminate between the fast and most biologically relevant AOs, able to stop oxidative cascade reactions, from slow and low reactive ones, unable to counter balance an oxidative stress condition. The PTV was used to quantify in a single measurement the activity of water-soluble AOs, such as ascorbic and uric acid, in simple aqueous solutions and in complex mixtures like blood, saliva and orange juice, respectively.

2 Experimental

2.1 Reagents, Chemicals and Samples

Phosphate buffered saline (PBS) was purchased from Sigma-Aldrich (Germany). Uric acid (UA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Fluka (Switzerland). Ascorbic acid (AA; vitamin C) was purchased from Riedel-de-Haën (Switzerland). All reagents were of analytical grade and solutions were freshly prepared with deionised water produced by a Milli-Q plus 185 model from Millipore (Zug, Switzerland).

All solutions of AA, Trolox and UA were prepared in PBS (pH 7.4). Natural samples were either purchased from local supermarkets (orange juices) or provided by voluntary persons (saliva and blood). Only fresh orange juice, saliva and blood samples were used for electrochemical measurements without any pre-treatment to assure the reliability of the obtained results.

2.2 Electrodes

Electrode strips were prepared by screen-printing using a simple two parallel track geometry. One electrode was made with carbon ink (Electra Polymer & Chemicals Ltd., Roughway Mill, Dunk Green, UK) and the other track was made from a Ag/AgCl ink (Ercon, Wareham, MA, USA). The electrode area was defined by printing an insulating layer.

2.3 Apparatus and Electrochemical Measurements

Electrochemical measurements were performed using a commercial EDEL potentiostat electrochemical analyser (Edel therapeutics, Switzerland) in a two-electrode arrangement. The working electrode (WE) was a screen-printed carbon electrode operated in conjunction with a screen-printed counter/reference electrode (Ag/AgCl, CE/RE). All potentials are reported with respect to the Ag/AgCl CE/RE. For the electrochemical measurements, the sample is deposited over the two-electrode setup in the case of blood and saliva samples, otherwise the electrodes were immersed in the sample solution. Linear sweep voltammograms (LSVs) were recorded from 0 V to 1.2 V with a scan rate of 100 $mV\,s^{-1}$ under ambient conditions. For each LSV a fresh WE was employed.

3 Results and Discussion

3.1 Principle of Electrochemical Pseudo-Titration

Common potentiometric titrations are based on the measurement of the potential change when a so-called analyte solution is titrated by the controlled addition of a reagent that can convert quantitatively the analyte from one oxidation state to another. The titrant consumes the analyte and, accordingly to the Nernst-Equation, the potential noticeably changes when the analyte concentration is significantly decreased. In this way, the equivalence point of the titration is determined and employed to calculate the analyte concentration. In conventional amperometric or coulometric methods a constant potential is applied at which either the analyte or the titrant are electrolysed. The measured current increases or decreases and a significant change in the slope defines the endpoint of the titration.

The electrochemical pseudo-titration presented here is based on a similar concept, where the mathematical modulation of a current–potential curve is performed, which represents the electrochemical oxidation of water-soluble AOs in titrant-free solutions. When investigating multicomponent systems, it has to be taken into consideration that all electrochemical reactions taking place at the solution-electrode interface are controlled by the applied potential, the kinetics of the electrochemical reaction and by the mass transport. According to Butler-Volmer equation, the kinetic influence on the reaction of a quasi-reversible redox system can be expressed by Equation 1 [37]

$$\frac{I}{I_{da}} = \frac{nFAc^*_R k^0 \exp\left(\frac{\alpha nF(E-E')}{RT}\right)}{I_{da} + nFAc^*_R k^0 \exp\left(\frac{\alpha nF(E-E')}{RT}\right) + \frac{\delta_O}{D_O} I_{da} k^0 \exp\left(\frac{-(1-\alpha)nF(E-E')}{RT}\right)} \quad (1)$$

where I is the measured current, n is the number of transferred electrons, F is the Faraday constant, A is the area of the electrode, c^*_R is the concentration of the reduced form of the redox couple in bulk solution, α is the transfer coefficient, E is the applied potential, E' is the formal redox potential, R is the universal gas constant, T is the temperature, k^0 is the standard reaction rate constant, $\delta_{O/R}$ is the diffusion layer thickness for the oxidised and reduced redox species, respectively, and $D_{O/R}$ is the diffusion coefficient of the oxidised and reduced redox species, respectively. In Equation 1, I_{da} is the steady-state limiting anodic diffusion current of the oxidation and can be expressed as

$$I_{da} = \frac{nFADRc^*_R}{\delta_R} \quad (2)$$

Therefore, Equation 1 describes a normalised current.

Figure 1 a shows the calculated normalised current–potential curves of reactions taking place at an equal formal redox potential of $E' = 0.6$ V for different standard reaction rates (solid lines). The diffusion coefficients and diffusion layer thickness were assumed to be equal for the oxidised and reduced species; hence $\delta_{O/R} = \delta_{O/R} = \delta$ and $D_O = D_R = D$, respectively.

As can be seen in Equation 1 and Figure 1 a, fast reactions are indicated by steeper slopes. In biological systems, the oxidation of the most relevant AOs, such as AA, follows fast kinetics and takes place at lower E's compared to the oxidation of e.g. water. Therefore, they are rapidly consumed in case of an increasing anodic potential sweep (or during the increase of the oxidative species concentration) whereas other compounds are oxidised at higher potentials. Therefore, AOs with low E' and fast kinetics are more important for the antioxidant defence system than the ones having a high E' with slow kinetics. With the aim to amplify the signal coming from the biologically relevant group of AOs (and to minimise the signal of biologically non-relevant AOs), the current–potential curve obtained for each sample can be treated by a dimensionless modulation curve (Equation 3) that is correlated to the mathematical expression of the Fermi-Dirac distribution.

$$f(E) = \frac{1}{1 + \exp\left(\frac{\lambda nF(E-E_{threshold})}{RT}\right)} \quad (3)$$

Indeed, it is worth remembering that the Nernst Equation stems from a Fermi–Dirac distribution between an oxidised and reduced state.

In Equation 3, λ is a factor modulating the shape of the curve and $E_{threshold}$ is the adjustable threshold potential which shifts the curve towards smaller or higher potentials. The pseudo-titrated voltammograms (PTVs) are obtained by the multiplication of the current by the Fermi-Dirac function according to Equation 4. Please note that the current for the theoretical treatment is normalised for easier comparison of the generated data.

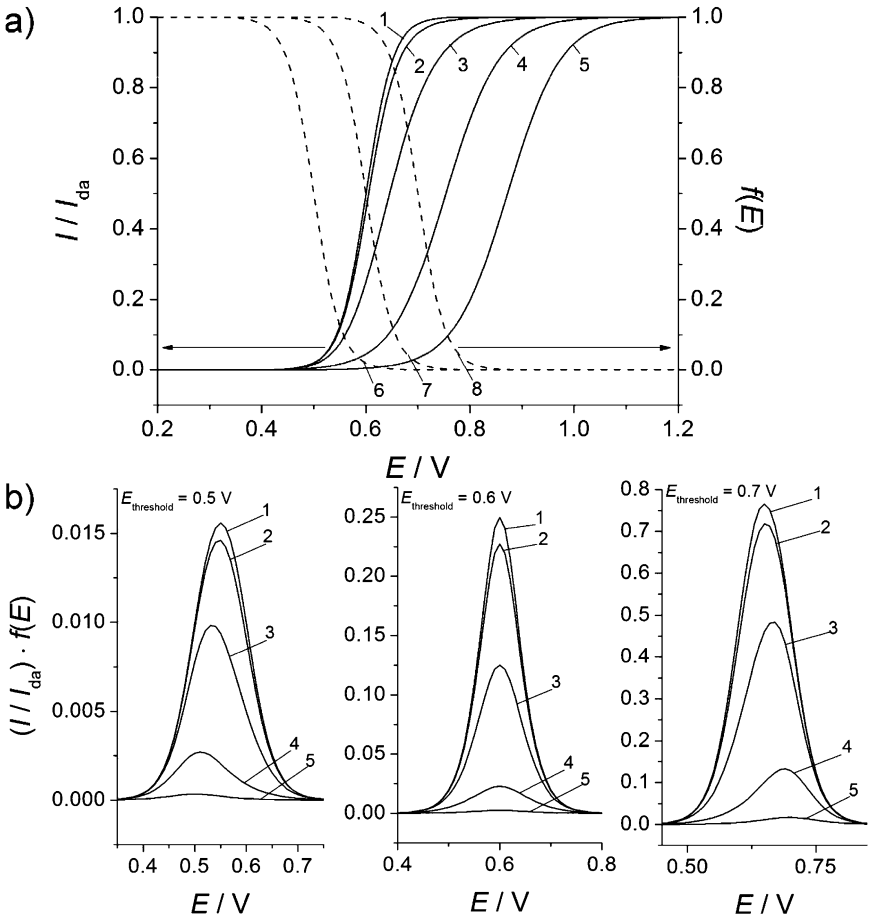

Fig. 1. Principle of pseudo-titration voltammetry (PTV). a) Current–potential curves for diffusion-limited reactions (solid lines, Equation 1) using different standard reactions rates: $k^0 = 10\ \mathrm{cm\,s^{-1}}$ (curve 1), $0.1\ \mathrm{cm\,s^{-1}}$ (2), $0.01\ \mathrm{cm\,s^{-1}}$ (3), $0.001\ \mathrm{cm\,s^{-1}}$ (4) and $0.0001\ \mathrm{cm\,s^{-1}}$ (5). $D = 10^{-5}\ \mathrm{cm^2\,s^{-1}}$, $\delta = 5 \cdot 10^{-4}$ cm, $\alpha = 0.5$, $E' = 0.6$ V. Fermi-Dirac plots of Equation 3 (dashed lines) using different $E_{\mathrm{threshold}}$ and $\lambda = 1$. $E_{\mathrm{threshold}} = 0.5$ V (6), 0.6 V (7) and 0.7 V (8). b) Pseudo-titration voltammograms (PTVs).

$$\frac{I}{I_{\mathrm{da}}} \cdot f(E) = \frac{I}{I_{\mathrm{da}}\left[1 + \exp\left(\frac{\lambda n F (E - E_{\mathrm{threshold}})}{RT}\right)\right]} \tag{4}$$

Figure 1b shows three exemplary PTVs applying three different $E_{\mathrm{threshold}}$ values ($E_{\mathrm{threshold}} = 0.5$ V, 0.6 V and 0.7 V) for $\lambda = 1$ and a formal potential of the reaction of $E' =$ 0.6 V. Peaked shapes are achieved where peak heights are related to the heterogeneous reaction rates and selected $E_{\mathrm{threshold}}$. Thus, the obtained modified current (I_{mod}) represents at its highest value the equivalence point of the investigated AO contained in the analysed sample. This procedure does not take into account the species whose oxidation processes are induced at higher potentials and with slow kinetics. Indeed, it can be seen clearly that slow kinetics are almost neglected within the final titrated signal (curve 5). To convert the LSV signal into more biologically relevant information, two main parameters can be tuned in Equations 3 and 4, namely $E_{\mathrm{threshold}}$ and λ. The main aim of the $E_{\mathrm{threshold}}$ parameter is to help the discrimination process between relevant and non-relevant antioxidants by defining a boundary. The value of such limit is an intrinsic parameter of each matrix sample, for instance, the most common water-soluble AO species present in blood, saliva and natural juices are oxidised at potentials lower than 0.6 V (vide infra). Therefore, in order to amplify their electrochemical signal an optimum $E_{\mathrm{threshold}}$ value for such type of samples is equal to 0.6 V. Notice, that this value might not be the optimum one for other samples such as cosmetic creams, where not only the media, but also the present AOs differ drastically from water-based samples. In this theoretical example, $E_{\mathrm{threshold}} = 0.6$ V makes a good compromise between peak height and suppression of slow kinetics. A further improvement of the signal treatment can be achieved by adjusting λ (Figure 2). For the PTV method it is important to specify the selected $E_{\mathrm{threshold}}$ and λ since it influences basically the result.

Compared to $\lambda = 1$ (panel in the middle of Figure 1b), a value of $\lambda = 0.5$ (Figure 2b, left panel) gives higher currents of the fast reactions whereas slow kinetics are still negligible. However, a further decrease of λ to 0.25 (Figure 2b, right panel) does not amplify the fast kinetic signals significantly, but the signal for slow kinetics. From

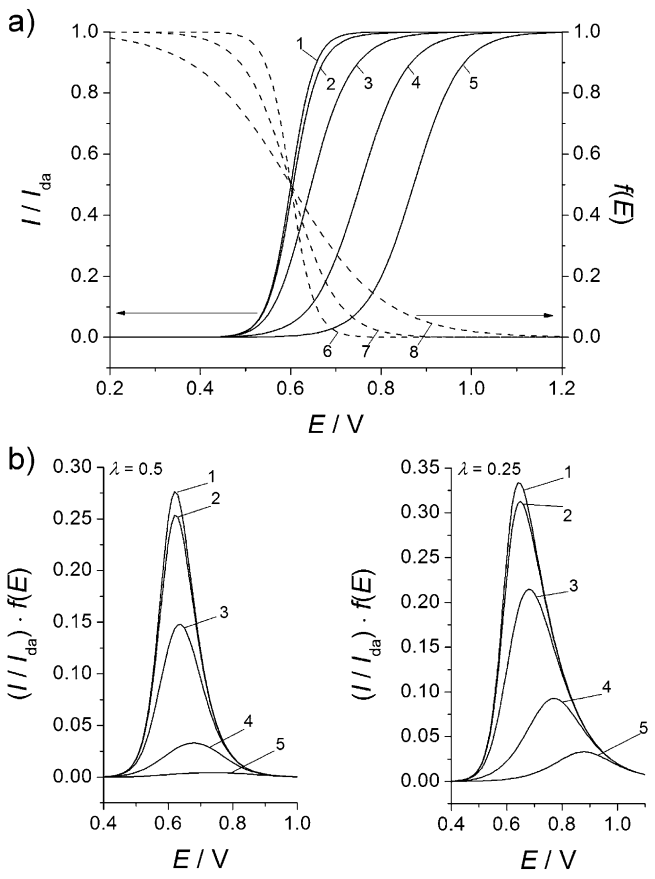

Fig. 2. Optimisation of λ. a) Current–potential curves for diffusion-limited reactions (solid lines, Equation 1) using the same different standard reactions rates as in Figure 1. Fermi–Dirac plots of Equation 3 (dashed lines) using different λ and $E_{threshold}$ = 0.6 V. λ = 1 (curve 6), 0.5 (7) and 0.25 (8). b) PTVs with λ = 0.5 and 0.25.

this theoretical point of view $\lambda = 0.5$ is demonstrated to be the most appropriate value. Note, that λ is not related to the transfer coefficient α. It is important to highlight that cascade-like oxidations take place in natural multi-component systems (see Supporting Information SI-1), which cause a step-wise or overlaid increase of the measured current. Additionally, recycling of AOs can take place when the oxidised AO species are reduced by other AO species. Despite the latter two aspects that make the signal interpretation of complex samples more difficult, the herein proposed pseudo-titration strategy allows the integration of such phenomena to adapt tangible values from several types of electrochemical measurements. The concept of electrochemical pseudo-titration (i.e. the exclusion of slow reaction kinetics) is not only applicable to steady-state conditions (based on the Nernst-Equation) as described above, but it can also be transferred in the same way to non steady-state conditions, such as the ones achieved experimentally in the present manuscript (vide infra).

3.2 Analysis of Ascorbic Acid Solution–Pseudo-Titration Voltammetric Reference System

The direct electrooxidation of AA at carbon electrodes is well documented [38]. AA is widely present in several biological fluids such as saliva and blood, food sources and industrial products such as manufactured food and cosmetics [11]. It is one of the most important hydrosoluble antioxidants and therefore used as a reference system for the presented pseudo-titration to corroborate the applicability of the optimal values chosen in the previous section. LSVs were performed in solutions of different concentrations of AA (c_{AA}) spanning the physiological range as shown in Figure 3a. Due to the fact, that LSVs are based on a potential ramp, the potential scan rate (herein 100 mV/s) is an additional parameter besides $E_{threshold}$ and λ, which has to be specified in order to interpret and compare the measurements. For each c_{AA} three LSVs were carried out in three freshly prepared solutions demonstrating the reproducibility of the measurement. The current increases until a first peak current is reached at around 0.5 V related to the oxidation of AA. At higher potentials the measured currents stay almost constant until a second weak oxidation peak appears, mainly due to the oxidation of the PBS compounds. At even higher potentials the oxidation of water may set in as indicated by a significant increase in current at $E > 1.1$ V. The currents are multiplied by Equation 3 to give the modulated currents I_{mod}

$$I_{mod} = \frac{I}{1 + \exp\left(\frac{0.5 \cdot nF(E-0.6\mathrm{V})}{RT}\right)} \quad (5)$$

where λ was set to 0.5 as demonstrated from the theoretical treatment in Section 3.1 to be the most suitable one. $E_{threshold}$ was chosen as 0.6 V. It is slightly larger than the formal redox potential in order to increase the impact of the oxidation of AA on the PTV (detailed description in SI-2 and SI-3). Figure 3b shows the PTVs obtained in AA solutions.

By applying the optimised values to the analysis of different AO containing samples, a rational antioxidant power (AOP) of the sample can be calculated as the integral of the PTVs:

$$\mathrm{AOP} = \int I_{mod}\, f(E)\, \mathrm{d}E \quad (6)$$

The AOP value, measured in Watt, could provide reliable information about the state of the antioxidant defence system of a person, for instance.

Figure 4 shows the modulated peak currents $I_{mod,p}$ from Figure 3b and the calculated AOP_{AA} for ascorbic acid as a function of c_{AA}. The values for AOP_{AA} are also listed in Table 1.

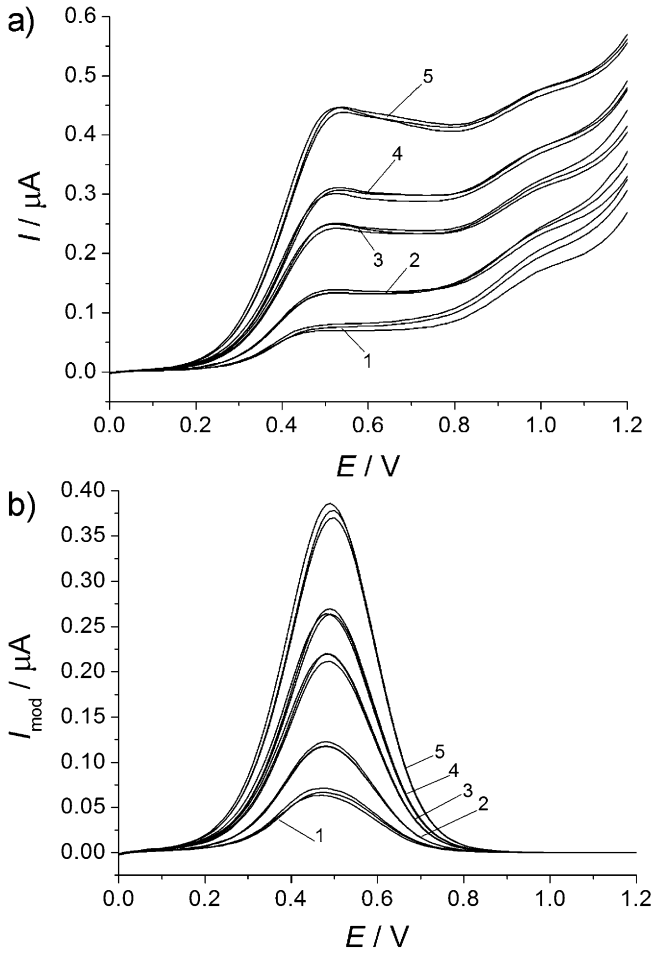

Fig. 3. a) LSVs in PBS solutions (pH 7.4) containing various concentrations c_{AA} of water-soluble AA. For each c_{AA} three different samples were measured. Start potential 0.0 V vs. Ag/AgCl, potential scan rate 100 mV s^{-1}. b) Pseudo-titrated voltammograms (PTVs), $E_{threshold}=0.6$ V, $\lambda=0.5$. For a) and b) c_{AA} was 20 µM (curves 1), 50 µM (2), 100 µM (3),133 µM (4) and 200 µM (5).

The calibration plots (Figure 4) demonstrate the linear relationship of the modulated PTV peak current and AOP_{AA} with respect to c_{AA}. The error bars represent the standard deviation of a triplicate (Table 1). These results prove the applicability of the presented technique and the possibility to determine the concentration of the AO with comparable accuracy as in conventional potentiometric and amperometric electrochemical titrations. Thus a $E_{threshold}$ of 0.6 V was applied to all investigated systems presented herein.

3.3 Comparison of the PTV for Ascorbic Acid, Trolox and Uric Acid Solutions

In order to illustrate the application of PTV to various water-soluble AO containing systems, the electrochemical oxidation of UA, a metabolite present in both blood and saliva which harbours a relatively high antioxidant activity [39–41], and Trolox, a water-soluble vitamin E equivalent [41], were investigated and compared with the result obtained with the reference system AA. Figure 5 shows the original currents (a) and the PTVs (b) after applying Equation 5.

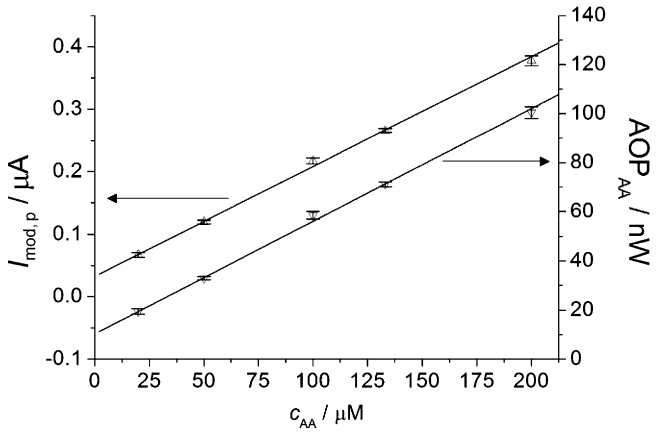

Fig. 4. Calibration curves for $I_{mod,p}$ from Figure 3 and calculated AOP_{AA}.

Table 1. Antioxidant power of ascorbic acid (AOP_{AA}) for different concentrations c_{AA}. *AV* = Average, *SD* = Standard deviation.

Concentration	20 µM	50 µM	100 µM	133 µM	200 µM
AV (nW)	19.4	33.0	58.5	71.3	100.4
SD	0.8	0.7	1.5	0.8	1.1

The calculated AOPs are 58.48 nW (AA, mean value of Table 1), 82.34 nW (Trolox) and 57.53 nW (UA) demonstrating that the AOPs of these three relevant AOs are similar. Although the original current–potential curves have different shapes the PTVs give comparable curves with respect to the AOPs. We want to stress that other values for the adjustable parameters $E_{threshold}$ and λ of Equation 3 would result in slightly different ratios of the AOPs. However, the model gives a good estimation of the AOP.

3.4 Analysis of Complex Antioxidants

We used the present PTV method for the determination of the antioxidant activities in industrially produced orange juices, in saliva and in capillary whole blood. The results of the electrochemical pseudo-titration and the corresponding original LSVs of these three real samples are shown in Figure 6 and in the insets in Figure 6, respectively. While showing slight differences in the original LSVs with respect to peak potentials, peak currents and curve shapes, the PTVs of the three sample types appear much more similar and comparable among each other. The calculated AOPs of orange juice are higher than the ones of saliva, followed by the one of blood as shown in Table 2. The tested orange juices demonstrate that depending on their quality and industrial fabrication their AOPs can vary considerably (Figure 6a). Despite Fig-

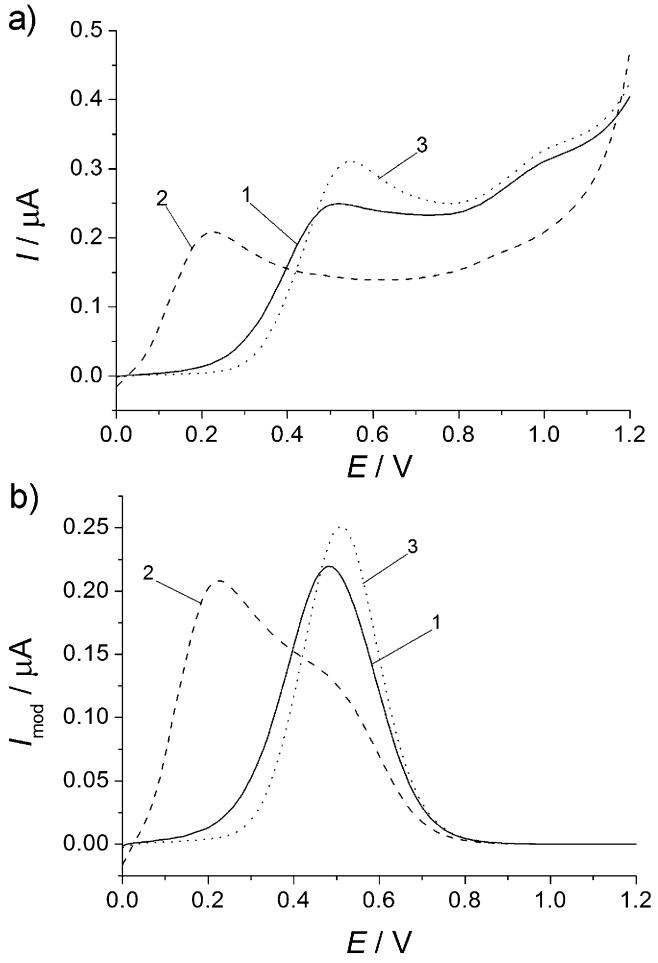

Fig. 5. LSVs (a) and PTVs (b) in different PBS solutions (pH 7.4) containing 100 µM water-soluble AA (curve 1), Trolox (2) or UA (3). Start potential 0.0 V vs. Ag/AgCl, potential scan rate 100 mV s^{-1}. $E_{threshold}$ = 0.6 V, λ = 0.5.

Table 2. AOP of measured samples of orange juice (OJ), saliva and blood.

Sample no.	AOP_{OJ}	AOP_{saliva}	AOP_{blood}
1	478 nW	282 nW	67 nW
2	343 nW	309 nW	88 nW
3	577 nW	250 nW	82 nW

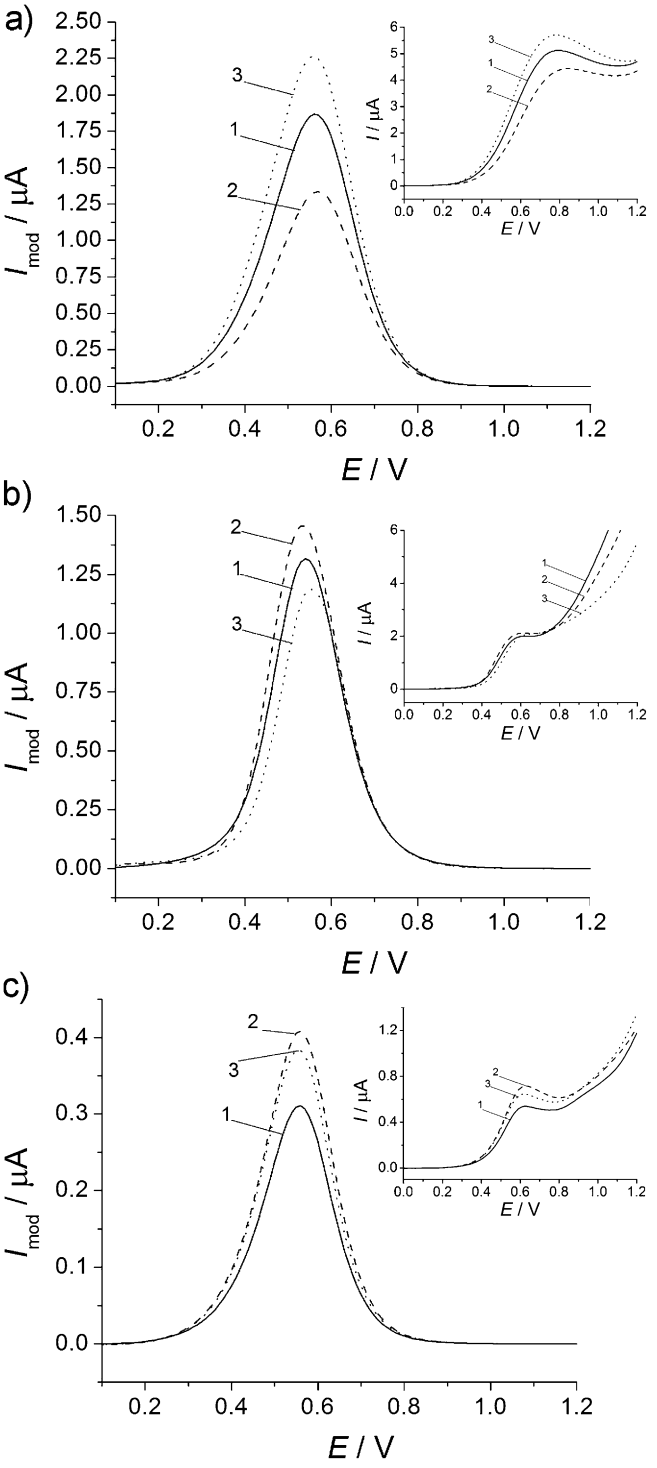

Fig. 6. PTVs (and LSVs as insets) of various representative native samples to detect their antioxidant activity; a) three different industrially produced orange juices b) three saliva samples of three different test persons; c) three blood samples of three different male test persons. Start potential 0.0 V vs. Ag/AgCl, potential scan rate 100 mV s^{-1}. $E_{threshold}$ = 0.6 V, λ = 0.5.

ure 6a only present the result for three orange juice samples, several experiments were performed confirming the observed trend. The results for saliva (Figure 6b) and blood (Figure 6c) were obtained from voluntaries without noticeable diseases related to oxidative stress. Since the AOP of blood of male and female humans differs, only results of male test persons are presented here. It has to be taken into consideration that some common AOs, such as GSH, are electrochemically difficult to detect due to a possible electrode fouling at, for instance, screen-printed carbon electrode caused by oxidised thiol groups on the surface of the electrode. Although most common AOs are preferentially measured in the PTVs of the real samples whereas compounds with slow kinetics are excluded, matrix effects might have to be considered depending on the investigated systems. As an example, Fig-

ure SI-4, in SI-4 shows three different measurements (i.e. LSVs and PTVs curves) of a blood sample from a single volunteer tested with the present method with a standard deviation of 8% for the calculated AOP. Therefore, we want to point out, that the calculated values for the AOP of saliva and blood samples do not represent an exact value for the health of a person, but it shows if the antioxidant level of a specific human is significantly lower or higher than the average of a healthy person. To fully demonstrate the potential of the PTV as a reliable indicative analytical tool for medical and clinical purposes in the investigative health sector, assays with a higher number of subjects have to be carried-out to unambiguously determine the relationship between AOP and oxidative stress related diseases.

4 Conclusions

The described pseudo-titration voltammetry of current–potential curves represents a new method of measuring the antioxidant power of biological systems. This method discriminates fast reactive species, representing the most biologically relevant antioxidants from the slow ones. It can be used for the characterisation of a variety of samples, including single antioxidants, such as vitamin C, uric acid and Trolox and complex mixtures, such as orange juice, blood and saliva. Further measurements and their detailed analysis shall provide more information about their use in the sector of health, where a reduced antioxidant activity is known to be associated with an increased risk of deficiency of the antioxidant defence system and a high value to its activation, in response to an increased oxidative stress. Likewise, the easy and rapid measurement of the antioxidant activity of industrially produced orange juice performed by the described method can be used for the monitoring of manufacturing process and quality control purposes in the agro-food and drink sectors. The used mathematical treatment for the electrochemical pseudo-titration relies on two manually adjustable parameters, namely the threshold potential $E_{\text{threshold}}$ and the curve shape modulation factor λ, and can be used for each kind of electrode material able to detect electrochemically AOs. Thus the presented concept is practically useful.

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