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Electrochemical Methods for Total Antioxidant Capacity and its Main Contributors Determination: A review

Abstract: *Backround:* The present review focuses on electrochemical methods for antioxidant capacity and its main contributors assessment. The main reactive oxygen species, responsible for low density lipoprotein oxidation, and their reactivity are reminded. The role of antioxidants in counteracting the factors leading to oxidative stress-related degenerative diseases occurence, is then discussed. Antioxidants can scavenge free radicals, can chelate pro-oxidative metal ions, or quench singlet oxygen. When endogenous factors (uric acid, bilirubin, albumin, metallothioneins, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase) cannot accomplish their protective role against reactive oxygen species, the intervention of exogenous antioxidants (vitamin C, tocopherols, flavonoids, carotenoids etc) is required, as intake from food, as nutritional supplements or as pharmaceutical products.

Literature study: The main advantages of electrochemical methods with respect to traditional, more laborious instrumental techniques are described: sensitivity, rapidity, simplicity of the applied analytical procedure which does not require complicated sample pre-treatment etc.

The paper reviews minutiously the voltammetric, amperometric, biamperometric, potentiometric and coulometric methods for total antioxidant capacity estimation. For each method presented, the electroactivity and the mechanism of electro-oxidation of antioxidant molecules at various electrodes, as well as the influences on the electroactive properties are discussed. The characteristics of the developed methods are viewed from the perspective of the antioxidant molecule structure influence, as well as from the importance of electrode material and/or surface groups standpoint.

The antioxidant molecule-electrode surface interaction, the detection system chosen, the use of modifiers, as well as the nature of the analysed matrix are the factors discussed, which influence the performances of the studied electrochemical techniques.

Conclusions: The electrochemical methods reviewed in this paper allow the successful determination of the total antioxidant capacity and of its main contributors in various media: foodstuffs and beverages, biological fluids, pharmaceuticals. The advantages and disadvantages of the electrochemical methods applied to antioxidant content and antioxidant activity assay are treated and interpreted, in the case of various analysed matrixes. Combining advanced materials with classical electrode construction, provides viable results and can constitute an alternative for the future.

Keywords: antioxidant activity, voltammetry, amperometry, potentiometry, foodstuffs, biological fluids.

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1 Introduction to oxidative stress and antioxidants

Oxidative stress was defined as the organism's status involving cell damage, by enhanced release of radical or non-radical oxygenated species [1]. Oxidative stress, representing a disproportion between oxidative and reductive factors in living organisms, has been more rigorously reconsidered as an interruption of the redox

circuits which are part of signaling transduction pathways, such as the cysteine moieties regulated by glutathione or thioredoxins. Taking over such a definition, led to methods for distinguishing disruptions of redox signaling and control. Hence, this is an answer to the requirement for uncomplicated and viable markers for the investigation and treatment of diseases, reactive oxygenated and nitrogenated species are responsible for [2,3].

During cellular respiration, the transfer of unpaired single electrons towards molecular oxygen may occur resulting in free radical generation [4]. Reactive oxygen species may also result in some cell-mediated immune functions [5]. These may be represented by either very unstable radicals containing a minumum of one unpaired electron, or by oxidizing non-radical species, that can promote the peroxidation of membrane lipids with accumulation of lipid peroxides [6,7]. Reactive oxygen species are present at physiological levels during normal cell functioning. If the respective amounts are exceeded, these oxidative species are capable of attacking vital biological molecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids or carbohydrates. It is known that namely, DNA damaging, is associated with mutations, thus being the cause of the genesis and pathophysiology of numerous diseases: atherosclerosis, type 2 diabetes, neurodegeneration (Alzheimer, Parkinson, amyotrophic lateral sclerosis), infections, cardiac impairment and cancer [8].

Different types of oxygen-centred free radicals, called reactive oxygen species (ROS), comprise superoxide anion radical ($O_2^{\cdot -}$), hydroxyl (HO·), peroxyl (ROO·) and alkoxyl (RO·) radicals, and nitric oxide (NO). Hydroxyl (half-life of nanoseconds) and alkoxyl (half-life of seconds) free radicals are endowed with high reactivity towards biomolecules, whereas, superoxide anion, lipid hydroperoxides and nitric oxide were reported as having lower reactivity [9]. Alongside the afore-mentioned radicalic oxygenated species, nonradicalic reactive oxygen species are also known, such as singlet oxygen, hydrogen peroxide, or hypochlorous acid [10].

During normal biological processes, reactive oxygen species are formed in small amounts, and the antioxidant systems of the organism can effectively deplete them. However, it has been confirmed that, under stress conditions such as the intake of drugs, the action of UV radiation or metabolic dysfunctions, these ROS can be generated in sufficient amounts, as to exceed the normal antioxidant defense capabilities of the organism, causing the oxidation of biomolecules and the initiation of radical chain oxidation in tissues [11,12]. Under these circumstances, antioxidants present in diet retard radical chain oxidation, and, to achieve this goal, they intervene in the initiation or propagation steps [7,13,14].

Antioxidants represent a wide class of chemical compounds that fight against the oxidative processes, including the degradation of nutrients found in diet, of materials such as rubber or plastic, of essential molecules found in biological media, etc [15,16].

Newly, with the continuously growing preoccupation for distinguishing secure food antioxidants, the choice is represented by natural antioxidants, especially plant-sourced. The reverse relationship between the consumption of fruits and vegetables and the development of oxidative stress-related disorders, confirmed by epidemiological studies, has been attributed to the presence of compounds endowed with high antioxidant activity [17-20]. The main biocompounds found in natural sources are phenolics (flavonoids or non-flavonoids), associated to health benefits resulted from the inhibition of low-density lipoprotein oxidation [19,21-25].

To accomplish their protective role, antioxidants act as free radical captors, metal chelating agents and singlet oxygen quenchers [26]. They can be classed as primary (named also long-term) antioxidants and as secondary (called also processing) antioxidants. Primary antioxidants are mainly represented by hindered phenols and secondary aryl amines, whereas the secondary antioxidants' class contains organophosphites and thioesters [27]. The primary antioxidants behave mainly as radical captors, hydrogen donors or chain breakers, while secondary antioxidants are mainly known as peroxide decomposers or singlet oxygen quenchers [25-27].

Endogenous antioxidants (part of the organism's complex defense system) are uric acid, bilirubin, albumin, metallothioneins, as well as enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase. Exogenous antioxidants are natural (vitamin C, vitamin E, carotenoids, phenolic compounds such as flavonoids, phenolic acids, anthocyanins, proteins such as transferrin, ceruloplasmin or albumin, some minerals like Se) or synthetic compounds, such as butylated hydroxyanisole, butylated hydroxytoluene, gallates, etc. It is recognized that vegetables, fruits, grains and beverages such as tea, juice and wine constitute significant sources of natural exogenous antioxidants [25,28,29]. Their intake has been confirmed to lower the oxidative stress [30-32]. Another adequate manner to fight against the deleterious action of free radicals is represented by the use antioxidants (of natural origin or synthetic) as dietary supplements [33].

The inhibition of the oxidation processes in foods, pharmaceuticals and cosmetics, as well as the reactive

oxygen species' intervention in degenerative disease occurrence, are topics subject to continuous investigation, requiring performant methods applied for the assessment of individual antioxidants, as well as antioxidant activity. Studies are directed towards assessing the effectiveness of antioxidants in ensuring protection for the product, to test compounds likely to be endowed with antioxidant activity or to estimate the role of endogenous defense systems in counteracting the pathological consequences of reactive oxygen species action. In view of the above-explained aspects, antioxidant measurement techniques can be diveded as: assessments relying on the inhibition of low-density lipoprotein oxidation evaluation, and those based on the measurement of the free radical scavenging capacity [34].

With respect to the assessment of the antioxidant ability in foodstuffs, the terminology of "antioxidant activity" or "antioxidant capacity" is employed, although, different meanings are attributed to these terms: the activity is correlated to the rate constant of the reaction of an individual antioxidant with a certain oxidant. The capacity is defined as the amount (expressed as number of moles) of a free radical that can be scavenged by a sample [35].

2 Electrochemical methods for total antioxidant capacity assessment

Recently, special interest has been bestowed on the application of electrochemical methods to antioxidant and antioxidant capacity determination, as they have the advantage of sensitivity, fastness, of requiring simple and relatively unexpensive instrumentation, small volumes of samples, with improvement of research resources use. The measured signal is independent on the distance that radiation travels in the analytical cell or on the turbidity, and the dynamic range is quite extended [36-40]. One aspect in favour of the electrochemical methods is the electroactivity of most antioxidants, the electron transfer being involved in antioxidant – free radical reactions, enabling a prompt screening of the antioxidant capacity of a series of organic biocompounds even in complex or coloured samples [37-41].

2.1 Voltammetry

Voltammetry as potentiodynamic assay is based on the recording of the current intensity at controlled potential variation and exploits the reducing ability of antioxidants or the reversibility of redox active substances which are either single or part of a sample [36-41]. The confirmed analytical advantages of the various voltammetric techniques are: enhanced sensitivity, a broad concentration range (10^{-12} to 10^{-1}M), numerous appliable solvents and electrolytes, wide working temperature ranges, fast analysis, simultaneous determination of analytes (organic and inorganic), the capacity to assess kinetic and mechanistic parameters (including reasonable estimation of unknown ones), the facility of different potential waveforms generation yielding low current intensity values, these features being sustained by a strongly developed theoretical backround [42].

Therefore, voltammetry has distinguished itself among the methods applied for both qualitative analysis and quantitation of biological molecules, such as L-ascorbic acid, even when present in trace amounts [43,44]. The method allows the study of the antioxidant molecules' electrochemical behavior, mutual influence and interaction with oxygenated species.

Nevertheless, an asserted drawback of voltammetry lies in the fact that the result of the quantitative determination of the sample does not always give account to its antioxidant ability. The parameter most oftenly referred to is the oxidation potential at the glassy carbon electrode, but its value was found to be strongly dependent on the reaction mechanism at the electrode's surface [41].

2.1.1 Cyclic voltammetry

This cyclic technique based on linearly sweeping the potential in time as a triangular waveform, was first used to assess the reducing capacity and reversibility of substances, pure or present in various matrixes. As the potential is scanned, the analytical peaks (signals) present on the intensity – potential dependence are the result of the antioxidant molecules' oxidative depletion at the electrode as the specific values of the formal potentials are attained [45]. Especially in recent decades, the oxidation potentials determined by cyclic voltammetry (CV), enable a comparative investigation of the antioxidant potency of phenolics like benzoic acids, hydroxycinnamic acids, and flavonoids, with distinction between substrate types [45-49]. Low oxidation potentials values reflect the propensity of a given molecule for electron donation and thus, for exhibiting significant antioxidant (antiradical) activity. Confirmed improvements of the voltammetric assay versus spectrophotometry (e.g. DPPH• method) were reported: fastness and cheapness, the fact that the oxidation potential value of each specific component can

be assessed with the same accurateness, regardless of the antioxidant power, in conditions of good peak separation. Voltammetric studies reported variations in the peak potential value smaller than ± 3 mV, imparting improved precision to the electrochemical assay. Whereas in DPPH• photometric experiments, the absorbance variations are subject to more inaccuracy, hence, notably at low antioxidant capacity values, the results may be prone to a great degree of uncertainty [41].

Nevertheless, in this technique, since the potential varies linearly with time, there is a significant charging current contribution that confines the detection limit value to approximately 10^{-5} M, thus affecting, to a certain extent, its use for quantitative measurements, as below this concentration, the Faradaic current is lower than the charging current. In addition, the charging current contribution increases with increasing scan rate; hence, charging current also restricts the appliable potential sweep rates [50]. Other limitation of cyclic voltammetry in complex media such as beverages or biological fluids, is its low resolution with respect to structural information, a shortcoming that often needs to be amended by coupling this electrochemical technique with spectroscopic ones [51]. Moreover, the analysis of complex natural samples, sugars or natural polymers may hamper either the procedure itself or the attribution of the potential values, given to interferences likely to occur [41].

Even though, cyclic voltammetry enabled with good results, the analysis of phenolic standards [46], of antioxidants found in wine [52], plant extracts [53-55] and biological fluids [48,56-60]. Cyclic voltammetry also proved reliable with respect to the determination of total antioxidant capacity of plasma, other body fluids and animal tissue homogenates, imparted mainly by the contribution of low molecular weight antioxidants [49,58-62].

It was evidenced that plasma (as biological information providing matrix) may be often used for free radical-initiated damage evaluation, as it contains the main targets of oxidative attack, particularly low-density lipoproteins and key antioxidants (like ascorbic acid and uric acid). Results of plasma analysis take into account the balance between antioxidant ability and the oxidative stress extent as an outcome of processes occuring in body tissues and nutritional behavior. Thus, a cyclic voltammogram of plasma illustrates the correlation between disease, diet, the action of free radicals and vitamin supplementation, and reflects the antioxidant capacity of subjects and the efficiency of the appliable treatment. The total antioxidant capacity may be illustrated by two key parameters: the oxidation potential with its half wave value which reflects the particular reducing power of a substance, and the anodic peak current intensity (i_a), which correlates to the concentration of the biocompound. It was found that the voltammograms of plasma characterized by oxidative stress showed significant diminutions of anodic currents and also proved decreases of ascorbic acid and uric acid levels, characteristic for oxidative stress [49].

The authors of the study have exploited the area under the anodic current wave, as they considered it a more suitable parameter than the peak current intensity, in taking acount on the sample's total antioxidant capacity. The reason for this choice was that the anodic current wave was viewed as representing more than the contribution of a single component [49].

Cyclic voltammetry was also proposed to determine the antioxidant capacity of skin, relying on the confirmed reducing abilities of low molecular weight antioxidants (LMWAs). The anodic current was recorded at 900 mV potential value, versus a saturated calomel electrode reference, using a Pt microelectrode placeable on the epidermis without the need of gel or water. This technique enabled a rapid, accurate and non-invasive determination of the total antioxidant capacity of the stratum corneum with results obtained in less than 1 minute. The analytical signal reflected regular alterations of the redox characteristics of the voltammetric microsensor [62].

In another voltammetric study, the total antioxidant capacity (TAC) of plasma was estimated, exploiting the one-electron reduction of oxygen at a glassy carbon electrode, in a 0.05 M $(C_2H_5)_4N^+ I^-$ solution in dimethylformamide, leading to generation of the superoxide anion-radical which reacts with antioxidants in a protonation reaction. Total antioxidant ability of plasma from subjects with purulent infections, expressed as alpha-tocopherol units, was found 55.6% lower than that corresponding to controls. The TAC diminution in inflammatory processes was ascribed to the action of phagocytic cells, mainly activated neutrophils as generators of oxidative species, such as $O_2^{\bullet -}$, H_2O_2 or HClO, that are directed towards pathogens, but these species can also impair the surrounding tissue [63]. It was further proved that a higher sensitivity in the analytical response of the voltammetric analyser based on molecular oxygen electro-reduction, could be obtained with a mercury film electrode in comparison with the glassy carbon electrode [64].

Several studies have been dedicated to the electrochemical behavior and quantitation of key phenolics contributing to the total antioxidant capacity of wines [45,65-69].

A thick-film sensor incorporating an iridium-carbon working electrode was employed for assessing

the polyphenols' content and the antioxidant capacity imparted by them in white wine. Caffeic acid was chosen as model (reference) antioxidant, given its ability to engender the greatest electro-oxidation current. Moreover, since it was identified as the major polyphenol in white wine, caffeic acid was presumed to be the major contributor to the white wine samples' antioxidant capacity. Furthermore, the use of iridium-containing carbon as the electrode material allowed lowering of the oxidation potential, hence minimizing noise, with measurements viable even in undiluted wine samples [65].

The total polyphenol content (TPC) and the assessed scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) were consistent with the determined caffeic acid amount. An increase of the oxidation current of caffeic acid was registered at wine addition and, at the same time, the oxidation peak at +0.35 V (*vs.* Ag/AgCl) on the voltammogram of white wines becomes more enhanced at caffeic acid addition, reconfirming the prevalence of this hydroxycinnamic acid. Fig. 1 A illustrates cyclic voltammograms of caffeic acid, wine, and caffeic acid-added wine and Fig. 1 B presents the voltammograms obtained at different dilutions of the white wine sample, as obtained by the authors [65].

Another cyclic voltammetric study aimed at assessing the total phenol content and the antioxidant capacity using a monitored chain reaction involving methyl linoleate. The kinetics of oxygen consumption which accompanied the oxidation of the model compound was followed at a glassy carbon working electrode using the Ag/AgCl reference. Voltammograms were recorded for samples diluted 400-fold in a model wine solution (120 ml L^{-1} ethanol, 0.033 M tartaric acid, pH = 3.60) at 100 mV s^{-1} scan rate. The current intensities were recorded having the blank signal as reference. The integrated surface below the voltammetric peak up to 500 mV potential value (Q_{500}), was chosen to express the total content of easily oxidizable polyphenols. All analysed wines showed a marked capacity to delay methyl linoleate oxidation in micellar solution [66].

The main peculiarity of the O_2 signals, noticed at wine, tea or coffee addition, was that at the beginning of the induction stage, the oxidation rate decreased with time and then it increased, after registering an inflection point. The advanced motivation for the noticed tendency was the greater reactivity of the polyphenols' oxidation products versus the peroxyl radicalrelative to initial polyphenols. The total phenol content and the shapes of the cyclic voltammograms were found to be influenced more by the grape variety and the viticultural and wine-making practices than by wine age [66].

Cyclic voltammetry at glassy carbon working electrode allowed the characterization of a range of compounds currently present in wine, such as phenolic acids and flavonoids, as well as ascorbic acid, and sodium metabisulfite; the process distinguishing among different antioxidant classes based on their comparative propensity to undergo oxidation. Antioxidants found at lower levels exhibit merely a shoulder figured on the voltammogram. In the case of red wines, the linear dependence is obtained at a 400 times sample dilution in comparison to the 10 times dilution applied to white wines. The observed results confirm the higher amount of phenolics characterizing red wines. Glassy carbon significantly reduced interferences

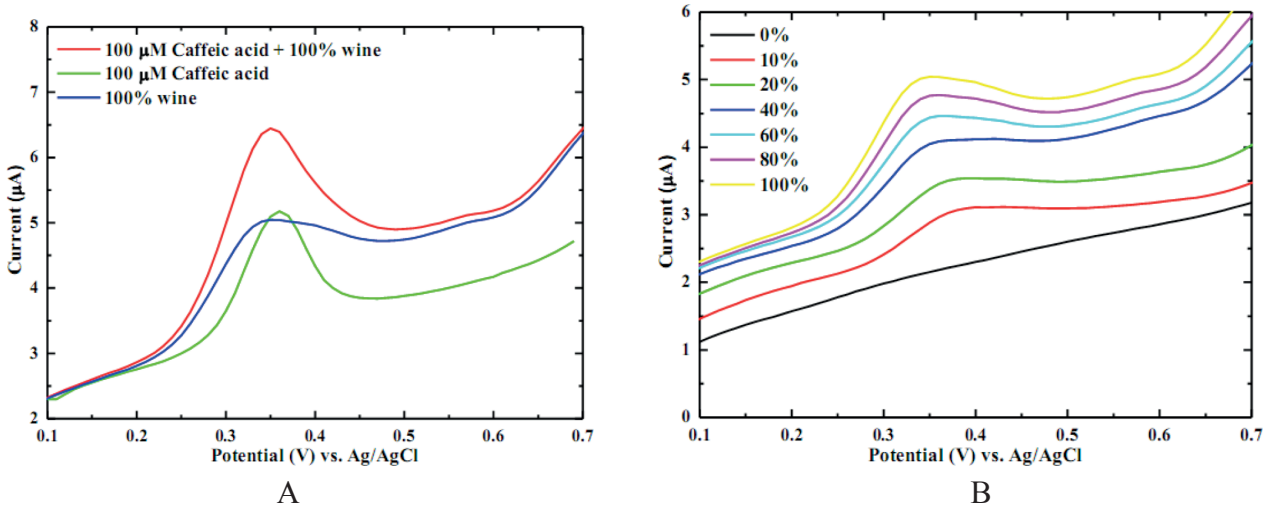

Figure 1: Cyclic voltammograms obtained at caffeic acid and white wine analysis; caffeic acid generated the most pronounced peak when added to the wine sample (A) and the analytical signal for the wine sample was recorded at different dilutions (B)[65].

given by ethanol (oxidizable at inert metal surfaces, like Pt and Au). Another mentioned advantage was the background current, low at the glassy carbon electrode and considerably higher at platinum or gold electrodes [45].

The cyclic voltammetric study [45] prove that the various phenolic classes are characterized by different reducing strengths and oxidation reversibilities. Phenolic acids and flavonoids with an ortho-diphenol group (caffeic, gallic and tannic acids, catechin and epicatechin) and morin, proved endowed with high antioxidative power, being the first oxidized at about 400 mV. Phenolics with significantly greater formal potentials (hence smaller antioxidant capacity and lowered reducing ability) which do not present an ortho-diphenol structure, such as ferulic acid, trans-resveratrol, malvin, as well as vanillic and p-coumaric acids, underwent slower oxidation, corresponding to their solitary phenol moiety, found in many cases close to a methoxy function. Anthocyanins, main antioxidant class in red wines, determine the occurence of a peak at 650 mV, and compounds with difficultly oxidizable groups generate peaks at higher potential values. It has been noticed that phenolics which yielded the first anodic peak in the 370 to 470 mV range can be subject to a second oxidation at around 800 mV, in the region of vanillic acid main peak: catechin and epicatechin due to the meta-diphenol groups on the A-ring, quercetin owing to the hydroxyl on the C-ring, and gallic acid due to a third -OH group adjacent to the orthodiphenol structure previously oxidized [45].

Sulfur containing antioxidants undergo slower oxidation at the carbon electrode and it is likely that the present method does not take account on their contribution, but this was not considered a problem for beverages and foodstuffs for which the amount of sulphur-antioxidants is small. Metabisulfite, reducing agent and preservative, was proved not to interfere in the electro-oxidation of phenolics. On the other hand, ascorbic acid oxidation took place at about 170 mV before the first wave of phenolics [45]. The charge passed to 500 mV or Q_{500} value, has been regarded as more sensitive to the concentration of phenols with low oxidation potential, than the peak intensity. Nevertheless, with respect to peaks attributed to phenolics with high oxidation potentials, it was asserted that they mainly allow qualitative assessment. The method had also the drawback of not providing also the antioxidant contribution of metal chelators [45].

The glassy carbon electrode was further employed to investigate the electro-oxidation of polyphenols presenting a catechol or galloyl group, which belong to the most significant polyphenol classes: catechin (a flavan-3-ol), rutin and quercetin (flavonols), caffeic, ferulic and coumaric acid (hydroxycinnamic acids), as well as gallic, syringic and vanillic acid (benzoic acids). Polyphenol oxidation to quinone was found to yield an anodic peak during the scan towards positive potentials, whereas the reduction of the quinone to the initial polyphenol generated a cathodic peak during the reversal potential sweep. The recorded cyclic voltammograms of the white wines showed two major peaks. The first peak at about 480 mV was assigned to catechol-containing hydroxycinnamic acids, the first polyphenol class in white wines. The second peak was obtained between 900 and 1000 mV potential values, and could be assigned to polyphenols with greater oxidation potentials, such as coumaric acid and its derivatives. Compounds resulting from the oxidation of original phenolics during wine storage and causing browning, are themselves oxidizable in the domain 800–1200 mV and also represent contributors to the second peak [67].

Red wines exhibited three voltammetric peaks. The first anodic peak is noticeable at about 440 mV, and results from the oxidation of catechin-type flavonoids, along with oligomeric and polymeric tannins. The second peak at about 680 mV can be associated with the malvidin anthocyanin [67] and was very close to potential values reported in previously discussed voltammetric studies [45], with a lower contribution provided by some stilbene derivatives such as trans-resveratrol. The third anodic peak noticed at 890 mV [67] is predominantly generated by the second oxidation of catechins (found in red wines in appreciable amount), previously reported at around 800 mV [45]. With respect to the study of the influence of S-containing wine preservatives [68], it was proved that the presence of sulfur dioxide alongside polyphenols altered the voltammetric signal, generating an anodic current increase, with a parallel decrease of the cathodic current. These observations can be attributed to a swift interaction of SO_2 with quinone generated by phenol oxidation, following two possible mechanisms: SO_2 can reduce quinone to original catechin with simultaneous oxidation of sulfite to sulfate or SO_2 can react with quinone to give a sulfonic acid derivative. Furthermore, on acetaldehyde addition, the cyclic voltammogram of catechin turned more reversible, until acetaldehyde concentration became greater than twice the sulfur dioxide one. At this stage, the cyclic voltammogram of catechin came to be fully reversible, owing to the interaction of the aldehyde with sulphur dioxide and corresponded completely to the aspect previous to any SO_2 additions [67].

A full voltammetric scan to 1200 mV was deemed necessary to assess total polyphenols in a wine sample.

With respect to the distinction between classes of phenolics, the best selectivity at determining catechol- and galloyl-containing polyphenols was provided by measuring the current intensity of the first peak, present between 430 and 500 mV potential values [67].

The electro-oxidation of caffeic, chlorogenic, sinapic, ferulic and p-coumaric acids was followed by cyclic voltammetry in acetate buffer pH = 5.60 at glassy carbon and modified glassy carbon electrodes. The antioxidant capacity of these phenolics was assessed, leading to a tendency of variation of the peak potential values as follows: caffeic acid (+0.31 V) > chlorogenic acid (+0.38 V) > sinapic acid (+0.45 V) > ferulic acid (+0.53 V) > p-coumaric acid (+0.73 V). The most enhanced antiradical power and lowest peak potential was characteristic for compounds with a catechol moiety (caffeic acid and chlorogenic acids). The developed method allowed phenolics analysis in orange juice [69].

Another cyclic technique at glassy carbon working electrode aimed at investigating the reducing ability and electro-oxidation of phenolics, as tightly related to the structures: namely, the molecules with more than two electron donating groups possess lower anodic peak potentials and more enhanced antioxidant capacities than monosubstituted phenols. Moreover, it was assessed that all compounds with an anodic potential smaller than 0.45V delay lipid peroxidation whereas compounds with an anodic potential greater than 0.45V behave as prooxidants [70].

The antioxidant capacity indexes IC_{30} and IC_{50} (the amount of antioxidant for which a 30% or 50% decrease in the peak current is obtained) have been used to rank the antioxidant capacities. The smaller IC value, the stronger the antioxidant capacity of the flavonoid towards DPPH·. The investigated flavonoids were classified on the basis of their relative scavenging ability: 5, 6, 7-trihydroxy flavone > 4,5,7 trihydroxyflavanone > 6-hydroxy flavanone > 6-methoxy flavanone > 6-methyl flavone > flavone. Hence, flavones containing three hydroxyl groups are endowed with the strongest antioxidant ability, and simple flavone possesses the weakest antioxidant power. The results obtained by the proposed electrochemical technique were validated by the classical UV-Vis spectroscopic method [71].

Cyclic and differential pulse voltammetry were also performed at a Pt electrode to assess kinetic parameters of flavonoids electro-oxidation. The electro-oxidation of these studied antioxidants was investigated along with its parameters, and it was considered adequate to provide an image of the manner they are metabolized by the organism. The assessed electro-oxidation heterogeneous rate constants ranged as follows: morin – 3.59×10^{-4}, rutin – 4.42×10^{-4}, dihydroxyflavone – 4.54×10^{-4}, trihydroxyflavone – 4.19×10^{-4}, hesperidin – 4.50×10^{-4} and quercetin – 4.63×10^{-4} cm s^{-1}. Xanthone and flavone were oxidized the quickest at the platinum electrode as indicate the small half-wave potential values and the high heterogeneous rate constants of 7.08×10^{-4} and 6.46×10^{-4} cm s^{-1}, respectively. The electrochemical oxidation reaction of flavonoids proved irreversible at a platinum electrode. The first oxidation peak was assigned to the oxidation of hydroxyl groups in ring B. This aspect was consistent with an electro-oxidation mechanism involving the transfer of one or two electrons, as dependent on the number of hydroxyl groups present in B ring. The hydroxyl groups in the rings A and C were oxidized at higher potentials, with further peaks generated [72].

The antioxidant activity of low molecular weight compounds recognized as main active principles in spices, condiments or drugs (gallic acid, sesamol, eugenol, thymol, carvacrol, vanillin, salicylaldehyde, limonene, geraniol, 4-hexylresorcinol, *etc.*) has been assessed by both voltammetry and photometry based on DPPH• radical scavenging. The investigation and interpretation of optimal conditions for the antioxidant activity assessment recommended the use of the lowest charging current achievable, as it was considered that in this case, the extrapolation of the charging current to the peak potential values gives more reliable results. So, it has been asserted that under these circumstances, the application of low scan rates would be recommended. Nevertheless, obtaining the voltammetric curves with a minimal distortion would require the application of not very lowered potential sweep rates but, to obtain a high sensitivity in intensity, the concentrations (and also the sweep rates) should be elevated [41].

As most antioxidant molecules have groups with acid-base properties (–OH, –COOH), the influence of pH on the analytical signal was followed. Generally, with increasing pH, the peak potential values tended to become less positive, and electro-oxidation took place with greater facility. Below (Fig. 2) several suggestive cyclic voltammograms of gallic acid (A) and eugenol (B) are presented, as obtained by Arteaga et al., reflecting pH influence on both peak position and analytical signal [41].

Moreover, the authors stipulated that elevated pH values may lead to dissociating mild acidic functions, and that these values do not comply with physiological media. Both acetic and phosphoric acid 0.1 M were employed as supporting electrolytes and solid NaOH helped to adjust neutral or basic pH values. Eventually, optimizing

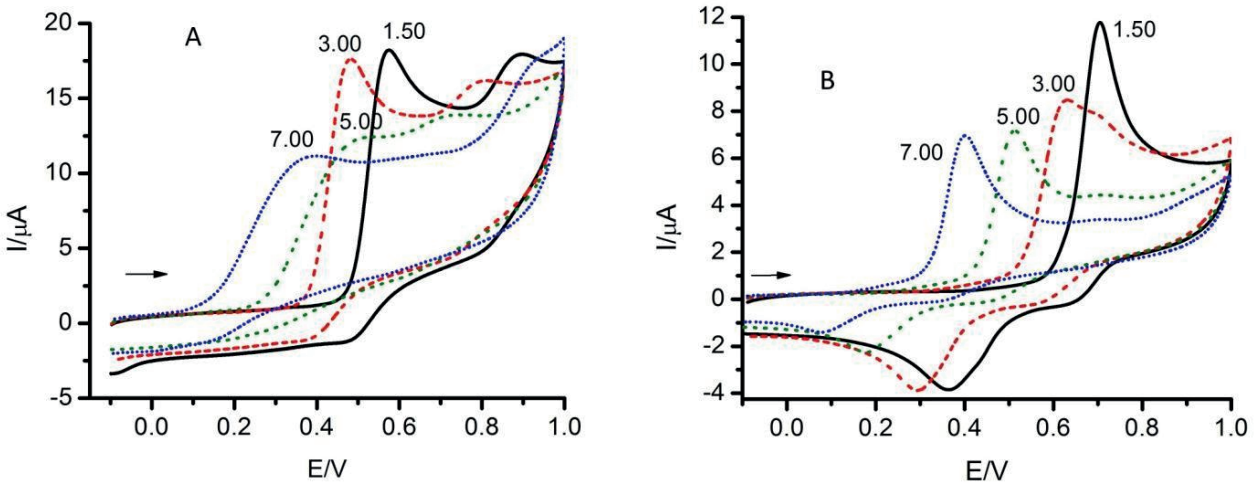

Figure 2: Cyclic voltammograms of gallic acid (A) and eugenol (B); arrows indicate the initial scan direction; experimental conditions: v = 0.1 V s^{-1}, c = 5 × 10^{-4} M and different pH values comprised between 1.50 and 7.0 [41].

working parameters led to the establishment of: pH = 7.0, scan rate 100 mV s^{-1}, for a 5 × 10^{-4} M antioxidant concentration value. Among the compounds tested, 2,5-dihydroxybenzaldehyde and ascorbic acid exhibited the lowest peak potential value, hence greatest antiradical power and best electro-activity [41].

Some vegetal matrixes often mentioned in literature in relationship to their high antioxidant activity, such as green tea, black tea, rosemary and coffee, were subject to voltammetric analysis of their corresponding dry extracts for total antioxidant capacity determination at a glassy carbon electrode. The authors also tested acerola and açaì, as less studied products from the Brazilian Amazon forest, which have only recently become the focus of interest in food and drug industries. Herb teas were also analysed. For the extracts obtained from each matrix, methanol, acetone or water was used, and the antioxidant capacity was provided by the anodic area on the voltammogram, and expressed as mg ascorbic acid equivalents. Among the products tested, the dry methanol extracts displayed the best total antioxidant capacity and the following classification was established: Green Tea > Black Tea > Rosemary > Arabica Coffee> Herb Tea > Acerola > Quality Tea > Acai. This implies that the solvents do not always have the same extractive power for all antioxidants present in the extract analysed [54].

Cyclic voltammograms recorded to 800 mV using a glassy carbon working electrode and a saturated calomel reference, at a 100 mV s^{-1} scan rate, in acetate buffer pH 3.0, led to the quantification of the electroactivity of compounds found in fruit tea infusions, and to the estimation of the antioxidant activity from the Q_{600} parameter (the charge passed to 600 mV). An enhanced anodic peak present at 440 mV in all teas, indicated that mainly ortho-dihydroxy-phenol and gallate moieties impart antioxidant capacity. The fruit tea with a significant rosehip amount showed the best antioxidant composite index and the most enhanced voltammetric peak at 440 mV.

On the cyclic voltammograms of analysed teas, up to three anodic peaks and one cathodic peak were noticed. The peak at the low potential value of 130 mV on the recorded cyclic voltammograms resulted from the facile oxidation of ene-diol present in the ascorbic acid structure to dehydroascorbic acid with its diketolactone structure, process involving the transfer of two protons and two electrons. The ascorbic acid content found in these fruit teas was attributed to their significant amount of rosehip or apple. Small intensity values of ascorbic peaks account for the diminished amount of this antioxidant in the above mentioned tea infusions, caused by boiling. Another stipulated reason for the decreased ascorbic acid level, was its depletion by O_2 present in the buffer or by other compounds [73]. The lack of the correspondent reduction peak illustrates the irreversibility of electrode reaction [73,74]. The anodic peak which appearred in the range 670–700 mV was attributed to the oxidation of the monophenol group or of the meta-diphenols on the flavonoid A ring [45,52]. This peak is particularly significant on the voltammogram of rosehip tea. Fruit teas rich in rosehip content also show this anodic peak [73].

The study of electro-oxidation and antioxidant activities of rutin and green tea extract solutions was also performed at a Pt working electrode. The cyclic and

pulse voltammograms showed the presence of glycoside rutin and other polyphenols in the extract of Gun Powder green tea; whereas, the extract of Sencha tea comprised other antioxidant compounds (essentially flavonoids) and was less oxidizable (oxidized at higher potential values). Hence, it has been inferred that the Gun Powder extract had, to some extent, more pronounced antioxidant activity than the Sencha extract. UV-VIS spectra also revealed the presence of chlorophyll, contributor to the antioxidant properties of green tea. It was concluded that the tested tea extracts may be regarded as potential stabilizing agents against oxidation [75].

A novel study allowed the facile and swift cyclic voltammetric estimation of the total antioxidant capacity of medicinal plants in ethanol/water mixture, relying on the antioxidant reaction with $ABTS^{\bullet+}$ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation. The $ABTS^{\bullet+}$ cation radical was obtained at a glassy carbon working electrode by in situ electrochemical oxidation of ABTS. The results proved consistent with those furnished by FRAP (ferric reducing antioxidant power) technique [76].

Cyclic voltammetry also provided the total antioxidant capacity of edible plants, vegetables, and fruits as well as reflected the product's quality over its shelf life [49,53]. Complete extraction of LMWAs could be achieved using water/acetic acid/acetonitrile. The authors [49,53] obtained the antioxidant activity from the peak areas, and the results were compared with those reported by the United States Department of Agriculture [61]. For each plant, the total antioxidant capacity (as vitamin C equivalents) was greater than the vitamin C content. This observation takes into account the presence of other LMWAs in each plant sample. Strawberry exhibited the highest antioxidant capacity, namely 169.6 ascorbic acid equivalents per 100 g sample.

In another study, several buckwheat extracts were subjected to cyclic voltammetric analysis [55]. The total antioxidant capacity provided by the total charge under the anodic current waveform gave results consistent with those furnished by spectrophotometry with $ABTS^{+\bullet}$ and DPPH•, and Folin-Ciocalteu reagent reducing capacity. Thus, the results of the antioxidant activity as well as the flavonoid profile of the analysed products proved that the variations in the antioxidant capacity of buckwheat and its derived products were consistent with the recorded alterations in flavonoid content [55].

Cyclic voltammetry at a glassy carbon working electrode was employed and compared to spectrophotometric methods [Trolox equivalent antioxidant capacity (TEAC), peroxyl radical trapping capacity (PRTC), DPPH radical scavenging activity, Folin-Ciocalteu reagent (FCR) reducing capacity], and photochemiluminescence (PCL), for antioxidant and reducing activity determination, of the prevalent onion flavonoids: quercetin (Q), quercetin-3-*O*-glucoside (Q3G), quercetin-4′-*O*-glucoside (Q4′G), and quercetin-3,4′-di-*O*-glucoside (Q3,4′G) were analysed by voltammetry and spectrophotometry (TEAC and PRTC). The cyclic voltammetric assessments showed that the best reducing activity can be asssigned to Q, while Q3G, Q4′G, and Q3,4′G proved only about 68.0, 51.0, and 30.0% of the reducing capacity of Q. This ranking was consistent with the free radical scavenging capacity determined by TEAC and PRTC assays [77].

The reducing ability of onion flavonoids assessed by cyclic voltammetry was 2 times superior to the antioxidant activity given by hydrosoluble components (ACW) evaluated by photochemiluminescence, and approximately 50% greater than PRTC, $DPPH^{\bullet}$ scavenging activity and the Folin-Ciocalteu reagent reducing capacity. On the other hand, the reducing power of onion flavonoids evaluated by cyclic voltammetry was 3 times lower than the one provided by the TEAC assay and around 4 times smaller than the antioxidant activity attributed to liposoluble components evaluated by photochemiluminescence (ACL). The best antioxidant activity of onion flavonoids was obtained cumulatively by photochemiluminescence (both ACW and ACL results) and Trolox equivalent antioxidant capacity results. The study of quercetin and its glucosides as part of the onions' antioxidant capacity proved a small contribution of Q, Q3G, and Q3,4′G, resulted from CV, TEAC, and PRTC; whereas, the most significant contributor was Q4′G. The importance of quercetin-4′-*O*-glucoside as a contributor to the total antioxidant capacity turned out to be the most significant in cyclic voltammetry, succeeded by peroxyl radical trapping capacity and Trolox equivalent antioxidant capacity assays. When assessing the comparative specificity of the methods employed, cyclic voltammetry yielded the best results, which ranked: CV > PRTC > TEAC, since it was confirmed that the lowest antioxidant gap is supplied by the voltammetric assay. These results recommend cyclic voltammetry as viable in the study of the antioxidative/reducing ability, relying on the electrochemical characteristics of the tested compounds [77].

Comparative cyclic voltammetric assays showed that the overpotential required in the electrochemical reaction of both vitamin E and CoQ(10) was higher when an oxidized boron-doped diamond electrode was employed than at the surface of a hydrogenated boron-doped diamond electrode. Thus, vitamin E molecules are more attracted to the hydrogenated boron-doped diamond electrode

than to the oxidized boron-doped diamond electrode. A stipulated reason could be the electrostatic interaction between the phenolic -OH group of the vitamin E molecule and the electrode surface functional groups. The signal-to-background ratio was greater at the hydrogenated boron-doped diamond electrode than at the glassy carbon electrode due to the lower background characteristics of the hydrogenated boron-doped diamond electrode [78].

A novel cyclic voltammetric technique for the determination of antioxidative activity of ferrocene derivatives relying on the interaction with 2,2-diphenyl-1-picrylhydrazyl radical was investigated. The anodic oxidation of ferrocene derivatives, presenting azomethine and 2,6-di-tert-butylphenol moities at a Pt electrode, takes place in three steps, as stipulated as an intramolecular proton-coupled electron transfer. As the oxidation process advances, a diminution of the current intensity corresponding to the peak of the DPPH radical anion is registered. It was concluded that conjugates of ferrocene and 2,6-di-tert-butylphenol act as effective antioxidant compounds [79].

Therefore, cyclic voltammetry allows rapid estimation of the antioxidant capacity of flavonoids and phenolic acids in different complex media rich in biocompounds like serum, beverages or various vegetal matrixes. The most efficient antioxidants being those easily oxidizable (having low oxidation potential). For some individual antioxidants, limits of detection below 10^{-5} M (Table 1) can be obtained: for curcumin, on a glassy carbon electrode using lithium perchlorate as electrolyte and ethanol as solvent [80], or for vanillic acid, when using carbonaceous electrodes from graphite, carbon microspheres or carbon nanotubes [81]. Nontheless, the total content of polyphenols present in complex samples such as wines, rich in compounds characterized by various oxidation potentials, and calculated using the area below the peak up to 500 mV [45,52,82], showed disparities with the results provided by Folin-Ciocalteu assay, due to the presence of phenolic compounds oxidized at potentials greater than 500 mV [82]. Phenolics with low antioxidant ability which generate a peak at around 800 mV cannot be included in the Q_{500} value, but contribute to the results of a Folin-Ciocalteu assay [83]. In further studies, it was found that the area under the voltammogram taken to 700 mV, at glassy carbon electrode, better correlated with a Folin Ciocalteu assay. However, hydroxycinnamic acid esters such as coutaric acid, oxidizable at potential values higher than 700 mV, cannot contribute to the results of both measures [84].

2.1.2 Differential pulse voltammetry

Differential pulse voltammetry involves two measurements of the current intensity for each potential pulse: one measurement before applying the potential pulse, and the second towards the end of the pulse period. By sampling the current just before the potential is changed, the effect of the charging current, which gave some limitations in cyclic techniques with respect to sensitivity, can be decreased [42]. Thus, in differential pulse techniques, the main advantage consists in actually measuring the $\Delta i/\Delta E$ value, where Δi represents the difference between the values of the current intensities, measured just before pulse application and at the end of the pulse period [42, 50]. In this way, the charging current decays exponentially during potential step application; and since the faradaic current decreases at a much slower rate, the first diminishes to a negligible level, when compared to the latter [50]. Hence, this double current sampling in DPV allows quantitative determinations at concentrations as low as 0.05 μM [51].

Another repercussion of double intensity measurement is that the differential pulse voltammograms are peak-shaped: the dependence of Δi on the applied potential reaches a maximum, so the intensity vs potential dependence has a peak shape, with a position correlated with the analyte type, and a height depending on the analyte concentration [85-87]. The sharper peaks, improved resolution and better sensitivity vs cyclic voltammetry are important in complex matrixes such as wines [88], fruits and their juices [89].

Enhancing of the faradaic current allows a more precise analysis of the electrode reaction from the mechanistic standpoint. Nevertheless, like cyclic voltammetry, differential pulse voltammetry necessitates the presence of a standard with structure related to the sample's compounds. The method requires rigorous control of parameters like pulse amplitude or pulse period, for optimizing resolution and minimizing noise [38].

Thus, the viability of DPV application has been investigated in quantifying polyphenols in complex media (e.g. beverages) comprising different phenolic classes such as phenolic acids and flavonoids, but also in antioxidant determination or oxidative impairment assessment in various clinical samples [90].

Differential pulse voltammograms aimed at exploring the analytical value of the electro-oxidation of polyphenolic compounds present in wines, in estimating the total phenol level [91], as main index of the total antioxidant capacity of these samples [45]. The total polyphenol amount was assessed at a glassy carbon electrode relying on the catechin voltammetric calibration

curve, considering the peak current density of its first anodic peak present between 0.440 and 0.475 V vs SCE, which shifts towards more positive values with the increase of catechin concentration. As reported in previous studies, this first peak of catechin occurs as outcome of the reversible oxidation of the 3',4'-dihydroxyl moiety on the B-ring [45,52,92]. It was found that this oxidation reaction depends on both pH and concentration value, and that its mechanism involved a two-electron-two-proton transfer [91]. The authors [91] stated that difficult to oxidize hydroxyl groups at C and A rings of the flavonoid molecule are likely to be oxidized at higher potentials, proving consistency with the results of CV studies [72,73], and this could be the cause of the second peak occurrence at 0.750 V on the voltammogram of catechin.

The exploiting of electrochemical parameters of the recorded pulse voltammograms of wines showed: all samples presented a first peak in the potential range 0.360–0.370 V versus SCE, which becomes more enhanced with increasing dilution, and, for at least a 400 times appliable dilution in acetate buffer solution pH 3.60, the peak intensity proved proportional to the dilution factor. The noticed oxidation peak in this potential range was attributed to phenolics with enhanced reducing ability (low oxidation potential), presenting ortho-diphenol (catechol) groups at B ring: flavonoids like catechin, epicatechin, quercetin, and several phenolic acids like gallic, caffeic, and tannic acids, confirming the results of cyclic voltammetry studies [45]. A second oxidation peak appears in the range 0.550–0.630 V, notable at lower dilutions (1/100 and 1/200); whereas at greater dilutions (1/400 or higher), this second peak is generally noticed as a shoulder. It was found that this second peak is most definite for wine samples rich in polyphenols (particularly red wines containing high anthocyanin amounts, like Dalmatia red wines). This observation is consistent with previous CV studies [45,52] that attribute the oxidation peak in this potential range to the oxidation of anthocyanins (e.g. malvidin). It was asserted that trans-resveratrol and several phenolic acids (e.g. ferulic acid) could also be contributors to the second peak [45]. A third oxidation peak was noticed in the range 0.760–0.780 V and was marked at low dilution factors. It has been previously determined that a peak in this potential range occurs from the electro-oxidation of phenolic acids characterized by high oxidation potentials [45,52]. In this particular case, several cinnamic acid derivatives (p-coumaric acid) and benzoic acids (vanillic acid) are mentioned, as well as some of the flavonoids that generated the first peak occurence (like catechin, epicatechin, quercetin, etc). It has been assessed that these compounds can be subject to a second oxidation, namely at the -OH group at position 3 on the C ring of the molecule, which is also in accordance with the results of cyclic voltammetric experiments [45]. This third peak was found larger in comparison to the second peak on the catechin voltammogram, as it represents the outcome of the oxidation of polyphenolics with more or less distinct oxidation potentials [91].

The total polyphenol content and polyphenol composition were characteristic for the geographical wine-producing region: red wines from Dalmatia, with a Mediterranean climate are the richest in polyphenols, and contain significant concentrations of relevant flavonoids and phenolic acids (catechin, epicatechin, quercetin, gallic acid, caffeic acid, p-coumaric acid) when compared to the red wines from the Slavonian continental region. Dalmatia red wines also exhibited the best antioxidant capacity. The results of antioxidant activity and total polyphenol content were well correlated [91].

Another differential pulse voltammetric method was developed, aiming at estimating the antioxidant properties of wines [93], using gallic acid as reference, whose electrochemical properties and antioxidant activity had been previously investigated [94]. The proposed method relied on gallic acid electro-oxidation at carbon nanotubes – modified carbon paste electrode, at 350 mV with respect to the Ag/AgCl reference electrode, in 0.1 M phosphate buffer solution with pH = 2.50. The modified carbon paste electrode enabled a reliable evaluation of the total phenolics in wines. Furthermore, the notable differences in the polyphenol content of red and white wines are consistent with the validated better antioxidant activity of red wines [93].

Gallic acid determination as standard phenolic antioxidant was also accomplished by differential pulse polarography in 0.04 M Britton-Robinson buffer, at pH = 10.0 which provided maximum sensitivity. Its peak was noticed at -160 mV with minimizing interferences from organic acids (ascorbic, oxalic, benzoic, tartaric) and inorganic salts by employing complexing agents such as ethylenediamine tetraacetic acid resulting in viable quantitation in fruit juices. The peak potential dependence on pH showed two linear portions broken at pH = 10.5, which corresponds to the pKa value of gallic acid which is related to its acid dissociation constant [95].

The electrochemical behavior of tocopherols at solid platinum electrodes was investigated, using differential pulse voltammetry, cyclic voltammetry and linear sweep voltammetry, in non-aqueous media. D-α-, D-γ-, and D-δ-tocopherol yielded well-shaped voltammograms, each compound showing a specific peak (at 0.550 V, 0.700 V and 0.750 V respectively, vs. a silver/ silver

chloride reference electrode). The 1:2 (v/v) mixture benzene/ethanol containing 0.12 M H_2SO_4 as supporting electrolyte, proved adequate as solvent/electrolyte system. A voltammetric assessment of the tocopherols' and cod liver oil's antioxidative activity towards the 2,2′-diphenyl-1-pycrilhydrazil radical was also performed. The peak intensity value on the tocopherols' voltammograms diminished with increasing DPPH concentration. On this basis, the differential pulse voltammograms were viable in the electroanalytical determination of α-, γ- and δ- tocopherol [96].

A pulse voltammetric method aimed at the investigation of the antioxidant activity of several sulfur containing biologically active substances, as part of the antioxidant defense of living cells by reducing the level of active oxygen and of the radical species or binding metal ions, thus inhibiting or delaying their pro-oxidative activity [97]. The investigation of the antioxidant activity in aqueous medium was based on three models: firstly, the interaction of antioxidants with ferrous ion to study the preventive activity of the tested compounds on the initiation stage of free radical oxidation, secondly and thirdly describing the interaction of an antioxidant with electrochemically generated hydroxyl radicals and hydrogen peroxide, to ascertain the inhibitory activity in the stages of propagation and branching of the free radical oxidation chain [98]. From the recorded pulse mode voltammograms at a platinum cathode, a dependence of the difference in the wave heights of Fe^{2+} reduction (in the absence and the presence of the biologically active substances) divided by the height of the initial wave of iron ions, on the concentration of the studied electroactive biocomponents was developed. The slopes of these dependences illustrated the antioxidant activity with these analytical data allowing the measure of the influence of the structure of the studied biologically active substances on their capacity to inhibit the radical oxidation chain [97].

In the presence of acetylcysteine, the limiting current of the wave of reduction of bivalent iron at -0.48 V in 0.1 M NaCl diminished proportionally as the concentration of the sulphurated compound. Similar changes in the iron ion reduction wave occur, under the influence of other sulphurated aminoacids or peptides, such as cysteine and glutathione. Furthermore, a new wave was generated at more positive potentials, which increased with the concentration of bioactive substances containing sulfhydryl groups that exert their antioxidant activity through the oxidation of -SH groups to disulfide bonds. Thus, it was considered that these obtained experimental results point to an interaction of S-containing biocompounds with ferrous ion, following a mechanism which was analogous to that occuring in biological systems. Ascorbic acid acts in the same way, with a similar influence on the wave of Fe^{2+} [97,98].

The developed electrochemical method for determining the efficiency of the tested antioxidants also exploited the changes in the pulse voltammograms of molecular oxygen reduction, to investigate the combined effect of ascorbic acid and S-containing biocompounds [97]. The relative values of the peak currents of the waves of hydroxyl radicals and hydrogen peroxide were plotted versus the concentration of ascorbic acid alone and in the presence of the analysed biologically active compounds. It was found that the presence of both ascorbic acid and sulfur-containing biologically active substances, results in an enhanced synergistic effect in their interaction with both hydroxyl radicals and hydrogen peroxide. This voltammetric study [97] confirms previously described aspects, proper to enhanced oxidative stress: in biological redox systems, when the source of antioxidant enzymes is consumed, an enhancement of the mutual influence (synergism) of ascorbic acid and endogenous low molecular antioxidants (particularly glutathione) occurs [99,100]. Under strong oxidative stress, ascorbic acid interacts with glutathione following both enzymatic and nonenzymatic pathways, and reduced glutathione can trap the active forms of oxygen thus increases the active concentration of ascorbic acid and promotes its antioxidant potency in a biological system [101].

An electrocatalytic DNA-modified carbon paste sensor with voltammetric detection was employed for assessing the total antioxidant capacity [25]. The electrode functioning relied on the partial damage of the DNA layer by Fenton OH• radicals, followed by electrochemical oxidation of the left undamaged adenine bases that can yield an oxidation product capable to catalyse NADH oxidation [25, 102]. The developed voltammetric biosensor was disposable and required simple, quick and reproducible preparation. The oxidative damage generated after the reaction of the DNA-modified carbon paste electrode with the Fenton reagent, was estimated from the electro-oxidation of the adenine molecules withstanding OH• initiated oxidation, at the carbon paste electrode, in a 0.05 mM NADH solution, containing 0.01 M $CaCl_2$ and 0.1 M tris–HCl, pH = 9.0. Antioxidants scavenged hydroxyl radicals, resulting in more adenine molecules unoxidized, and thus, increasing the current intensity emerged from the reduced coenzyme oxidation [25]. Ascorbic acid displayed the best scavenging role, so it was chosen as the model antioxidant, determining the highest electrocatalytic current increase, due to the presence

of a larger number of undamaged adenine molecules. The analytical performances of the biosensor allowed total antioxidant capacity evaluation in several beverages. As predictable, the highest level was found in the flavour, namely (480 ± 20) μM, as concentrated product [25].

The differential pulse determination of caffeic acid, gallic acid and Trolox was performed, after the study of their electrochemical behavior by cyclic voltammetry at carbon paste working electrode and optimization of scan rate, accumulation potential and accumulation time. High sensitivity was obtained for: 400 mV accumulation potential and 180 s accumulation time (for gallic acid) 1000 mV accumulation potential and 240 s accumulation time (for caffeic acid) and 1000 mV accumulation potential and 180 s accumulation time (for Trolox). Gallic acid proved best electroactivly resulting from the presence of three hydroxyl groups on the aromatic structure in comparison to caffeic acid (a dihydroxycinnamic acid) or Trolox (the water soluble homologue of vitamin E with one -OH group). Then, a biosensor with adsorptive transfer stripping voltammetric detection based on the oxidation of nucleobases by Fenton reagent, allowed for a rapid antioxidant screening of aqueous herb extracts with black tea proving the best protection from the deleterious action of OH• [103].

The performances of bare Pt and unmodified carbon paste electrodes in ascorbic acid determination as contributor to the total antioxidant capacity of soft drinks, were investigated in differential (Fig. 3) and cyclic voltammetry. Carbon paste electrodes provided lower detection limits (Table 1). The sensitivities given by the slopes of the calibration graph proved higher in cyclic voltammetry experiments: for a Pt working electrode, 65.42 μA mM^{-1} was the sensitivity obtained in CV studies, whereas 21.839 μA mM^{-1} was the sensitivity obtained in DPV. The ascorbic acid concentration ranged between 6.83 mg per 100 mL sample for soft drinks (Fanta Madness) and 54.74 mg per 100 mL for lemon juices freshly obtained from fruit [38].

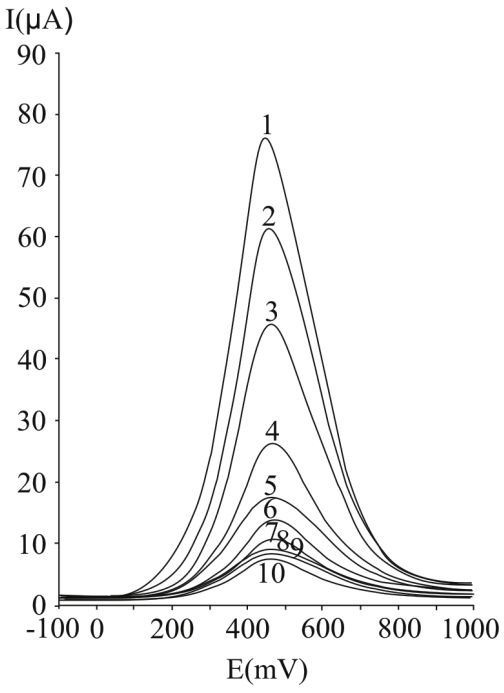

Figure 3: Differential pulse voltammograms obtained at a carbon paste working electrode for different ascorbic acid concentrations, expressed as mM: 20 (1), 15 (2), 10 (3), 5 (4), 2.5 (5), 1.25 (6), 0.625 (7), 0.31 (8) 0.15 (9) and 0.07 (10); 0.1 M KCl solution was used as supporting electrolyte [38].

2.1.3 Squarewave voltammetry

In this technique, a square-wave is superimposed on the potential staircase sweep with the current being recorded at the end of each potential change, hence the charging current current is minimized similar to DPV. Square-wave voltammetry (SWV) takes advantage of high sensitivity and lack of background signals. Its speed, resulted from the fast potential scan, allows for repetitive measurements with optimized signal-to-noise ratio. This analytical technique aimed first at studying electrode kinetics, but also at determining different electroactive species in trace levels. It has also been integrated in HPLC assays with electrochemical detection [42]. The complete scan can be done during a single mercury drop in about 10 seconds [85]. SWV turned out to be more sensitive even when compared to differential pulse voltammetry [83,104] and has a benefit a larger dynamic range and lower limit of detection vs linear sweep voltammetry [105] and cyclic voltammetry [106].

The main advantage of square wave voltammetry as a potential-step technique over linear sweep and cyclic voltammetry, is that charging current contribution is minimized by recording the current intensities when the faradaic current to charging current ratio is high. Other feature is its flexibility; as changes in applied potentials can be performed by tuning software as an alternative to hardware. Increasing the resolution of overlapped peaks is possible even when the half-wave potential values of the analytes are discriminated by less than 155 mV /n [107]. Due to square wave oscillations, both the results of forward and reverse electron transfer are quantified, so the current difference gives more information on reversible processes than in differential pulse techniques. Nevertheless,

when increasing square oscillation frequency, improved sensitivities can be obtained, even for irreversible electron transfer processes [50].

The speed, high analytical sensitivity and insensitivity to dissolved oxygen concentrations characterizing this technique, enable its applications to drug and biological sample analysis [42,108]. With respect to antioxidant assay in foodstuffs and beverages, flavonoids and nonflavonoids could be determined with detection limits of the order 10^{-9}–10^{-7} M in samples with high content in phenolics like teas and berries [109].

A simple and sensitive voltammetric method was developed, employing 4-hydroxybenzoic acid as a quencher for hydroxyl radicals produced by TiO_2-catalysed photogeneration aiming at estimating the antioxidant capacity. Square-wave voltammetric measurements performed in 50 mM phosphate buffer with pH = 7.40 quantified the amount of 3,4-dihydroxybenzoic acid resulting from the reaction between 4-hydroxybenzoic acid and OH·. The square-wave voltammogram enabled identification of the 3,4-dihydroxybenzoic acid peak at about 0.4 V, whereas that corresponding to 4-hydroxybenzoic acid was noticed at 0.8 V. Consecutively, 4-hydroxybenzoic acid can compete with the antioxidants present in the analysed sample for OH· scavenging, determining a diminution of the registered current intensity. The developed square voltammetric technique [110] was applied to comparatively assess the capacity as OH• scavengers of ascorbic acid, glutathione, caffeic acid, Trolox and α-lipoic acid (with a disulphide bond present in a dithiolane ring), that alongside its -SH containing reduced derivative (dihydrolipoic acid) proves antiradical properties [111]. It was noticed that Trolox and α-lipoic acid did not interfere on the analytical square voltammetric peak of 3,4-dihydroxybenzoic acid. Moreover, ascorbic acid, glutathione and caffeic acid did not hinder the quantification of 3,4-dihydroxybenzoic acid. The variation of the antioxidant capacity of the studied antioxidants followed a trend given by the IC_{50} values: DL-α-lipoic acid > caffeic acid > glutathione > Trolox » L-ascorbic acid [110].

The developed method's performances in quantifying the antioxidant capacities of various real samples were also tested: both mint and dandelion extracts, effective scavengers of OH· radicals, proved endowed with elevated antioxidant potential. The antioxidant capacity of plant extracts was greater than that exhibited by Trolox. Purified mint flavonoids displayed the greatest value of the antioxidant capacity as TEAC equivalents, and exhibited the smallest value of IC_{50}. The results were consistent with those supplied by fluorimetry [110].

SWV applying the standard addition method allowed best electrochemical discrimination (when compared to cyclic, linear sweep and differential voltammetric techniques) and quantitation of BHA, BHT and TBHQ in mayonnaise at glassy carbon and Pt electrodes in Britton–Robinson 0.1 M buffer (pH = 2.0) and HCl 0.1 M (pH = 2.0) both with 2 mL L^{-1} methanol, with minimizing matrix effects from citric, lactic acid, EDTA or sorbate. The peak intensity was found to decrease linearly with pH, and it has been proven that only pH = 2.0 enabled simultaneous detection of the three antioxidants as no oxidation of TBHQ and BHT could be observed at pH = 6.0 and pH = 8.0 [112].

2.2 Biamperometry

Biamperometric determinations exploit the reaction of the analyte with a redox pair such as Fe^{3+}/Fe^{2+}, I^2/I^-, $Fe(CN)_6^{3-}$ / $Fe(CN)_6^{4-}$, DPPH•/DPPH. The confirmed high selectivity of the biamperometric method allows the quantification of redox active analytes present in biological samples (urine, blood), and, also, of the total antioxidant capacity of foodstuffs and beverages [113-116]. Nevertheless, biamperometry requires very rigorous control of experimental conditions; the method's performances with respect to selectivity relying upon the specificity of the reaction between the oxidized or reduced form of the redox couple and the analyte [113-116].

The molar ratio DPPH•/DPPH should be carefully chosen: in studies using this redox couple, conditions were employed with a DPPH• concentration lower than the DPPH one, so the measured current intensity is limitated by the smaller concentration of the radical [23,114]. Antioxidant addition diminishes the concentration of DPPH• and increases the concentration of DPPH, thus giving rise to a current linearly dependent on antioxidant concentration [23, 114].

Another key parameter needing optimization in biamperometry is the potential applied: the investigation of the potential difference influence on the analytical signal, in a biamperometric antioxidant capacity assay in juices using the DPPH·/DPPH redox couple (Fig. 4) and Pt electrodes shows an increase of the analytical signal up to 200 mV. Higher working potentials should not be considered, because of possible reactions occuring at the electrode, concerning electroactive sample species, other than antioxidants [23].

DPPH•/DPPH biamperometry was employed in the analysis of fruit juices for their total antioxidant capacity, using two identical Pt electrodes [23] and tea, wine and

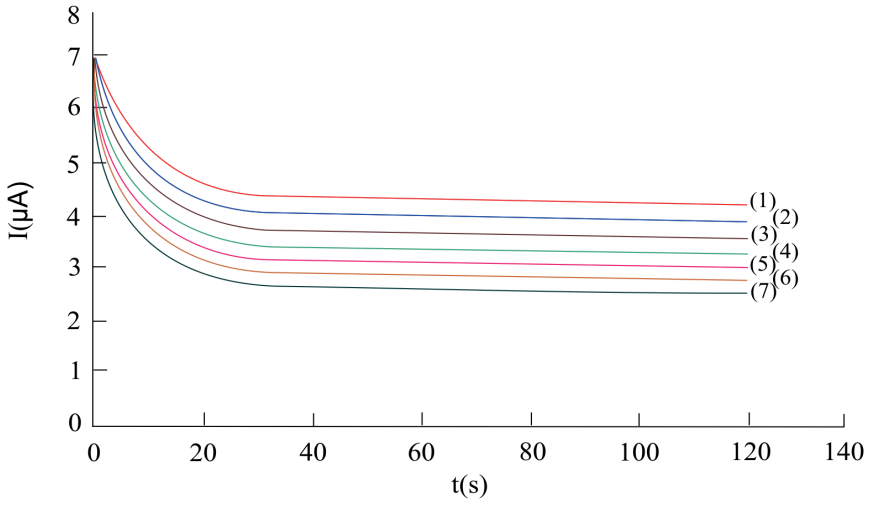

Figure 4: Chronobiamperograms obtained for different Trolox concentrations: (1) 0 μM, (2) 5 μM, (3) 10 μM, (4) 15 μM, (5) 20 μM, (6) 25 μM, (7) 30 μM; conditions:110 μM DPPH/100 μM DPPH·, potential difference, ΔE = 200 mV, as presented in [23].

coffee using glassy carbon electrodes [114]. With respect to fruit juice analysis, the greatest values of the total antioxidant capacity resulted for juices freshly obtained by fruit squeezing, namely 9.07 mM Trolox for orange juice and 6.25 mM Trolox for lemon juice [23].

A redox pair also currently applied in biamperometric antioxidant capacity determination is ABTS $^{+\cdot}$/ABTS. A peroxidase-containing flow system generated the cation radical, aiming at the assessment of antioxidant activity in juices, tea and wine at interdigitated microelectrodes [115], or the cation radical could be obtained by using glucose oxidase and peroxidase, for the analysis of wine and spirits with enhanced sensitivity, 0.165 nA/μM Trolox [116].

The antioxidant capacity of several samples of Brazilian woods, cabreúva (*Myrocarpus frondosus*), cabreúvavermelha (*Myroxylon balsamum*), imbuia (*Octea porosa*) and pequi (*Caryocar brasiliense*), and oak (*Quercus* sp.) extracts was assessed by ceric reducing antioxidant capacity (CRAC) analysis. The novel CRAC method applies chronoamperometric determinations to follow the decrease of Ce^{4+} concentration which are induced by its reduction by individual sample antioxidants. Owing to the elevated redox potential of the Ce^{4+}/Ce^{3+} couple, this system allows assessment of numerous antioxidants at a boron-doped diamond film cathode. The results given as Trolox Equivalents ranked as follows: oak (1.73) > cabreúva-vermelha (1.05) > cabreúva (0.90) > imbuia (0.71) > pequi (0.31). It was concluded that CRAC assay proved a viable technique in providing the antioxidant capacity of real samples [117].

The Ceric Reducing Antioxidant Capacity (CRAC) assay was also investigated with respect to the direct electron transfer facility, for determining the antioxidant capacity of eight organic compounds, relying on their ability to reduce the oxidized form of the redox couple [118]. The proposed methodology consisted in following the reduction of the Ce^{4+} concentration after its interaction, during 4 min, with the sample antioxidants. To this aim, the Cottrell equation expressing the diminution of the current intensity in time after imposing the due potential step, was exploited. This comparative study led to the following trend of variation of antioxidant capacities: tannic acid > quercetin > rutin > gallic acid ≈ catechin > ascorbic acid > butylated hydroxyanisole > Trolox. The results are in agreement with those provided by previous studies, and the consistency with the classical FRAP assay was confirmed as well [118].

2.3 Amperometry

In amperometry, the current generated at constant potential by the oxidation/reduction of the electroactive analysed compound, can be stoichiometrically correlated to the concentration of the latter [119-121]. It has been confirmed that the performances of the amperometric method with respect to sensitivity are subject to the chosen working electrode type, as well as to the potential value applied [85]. Moreover, it was found that a drawback of the electrochemical assay of antioxidant capacity in complex media, is represented by the difficulty to electro-

oxidize and determine at constant potential antioxidant macromolecules: thus, a study aiming at the comparative amperometric and spectrophotometric assay of the total antioxidant activity in bovine milk and whey demonstrated that flow injection amperometry and FRAP were suitable for the low molecular weight fractions, and less proper to the determination of protein antioxidant activity, while the ABTS method proved sensitive to high molecular mass components such as caseins and lipids [122].

In amperometric biosensors, the biorecognition elements can be enzymes, nucleic acids, lectins or whole cells. So in this case, amperometry benefits both the specificity imparted by the biocatalyst and the accuracy and rapidity of the electrochemical detection system, at the optimized potential value [119-122].

A flow system with amperometric detection for antioxidant capacity estimation, consisted of a bioreactor using Os-wired horseradish peroxidase. The H_2O_2 resulting from the enzymic xanthine was amperometrically monitored at -0.1 V vs. Ag/AgCl/KCl sat, and it was reported that this potential value applied avoided interferences. The antioxidant capacities of the analysed commercial beverages were consistent with those furnished by DPPH· and Folin–Ciocalteu assays [123].

A modified enzyme electrode was developed, by xanthine oxidase encapsulation in a sol-gel matrix fixed on a platinum screen-printed electrode. The hydrogen peroxide that emerged from the enzyme-catalysed hypoxanthine oxidation or by the unavoidable dismutation of superoxide radical anions, was detected amperometrically at +700 mV. The poly-*m*-phenylenediamine membrane imparted high permselectivity preventing the possible depletion of other electroactive sample species. The biosensor was used in the estimation of the antioxidant capacity of pharmaceuticals (e.g. acetylsalicylic acid) [124].

The amperometric enzymatic analysis of different samples of aromatic herbs, olives and fruits was done with a superoxide dismutase-based biosensor. The biosensor development implied superoxide dismutase immobilization in kappa-carrageenan. The enzyme gel was trapped between a cellulose acetate membrane and a dialysis membrane, and the amperometric transducer responded to H_2O_2. The enzyme sensor was sensitive to superoxide radical anion, the latter emerging from enzymic xanthine oxidation [125]:

$$\text{xanthine} + H_2O + O_2 \xrightarrow{\text{XOD}} \text{uric acid} + 2H^+ + O_2^{\bullet -}$$

In the next step, superoxide radical anion disproportionates, due to superoxide dismutase enzyme immobilized on the transducer yielding molecular oxygen and hydrogen peroxide:

$$2\,O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Consequently, H_2O_2 oxidation at 650 mV at the Pt anode of the amperometric sensor generates an analytical response proportional to the superoxide radical concentration value.

The presence of antioxidant compounds determined a diminution of the recorded amperometric analytical signal, as the electroactive superoxide anion radicals were consumed by antioxidant molecules in the sample. As far as fresh aromatic herbs samples were concerned, the highest antioxidant capacity was displayed by sage. Of all the fruit samples analysed, the highest antioxidant capacity was shown by medlar, followed by apricot and melon, while apple and pear did not turned out to be very active antioxidant fruits. On comparing fresh fruits and fresh aromatic herbs, the latter were found to be more powerful antioxidants [125].

Furthermore, it was asserted that the high potential value needed, namely 650 mV [126], can affect the selectivity and limit the applicability of superoxide dismutase biosensors based on hydrogen peroxide electro-oxidation at Pt electrodes [127]. To solve this drawback, another type of bioanalytical tool was developed, represented by the cytochrome-based biosensors, based on redox reactions involving this oxidase [128,129].

Immobilized cyt *c* proved reducible by superoxide anion, and it can be reformed afterwards at the electrode. The current intensity originating from the electron transfer superoxide anion radical - electrode, mediated by cytochrome *c* at the applied potential, is proportionally correlated to the radical concentration. Catalase addition helps avoid interference from H_2O_2, that may occur from $O_2^{\cdot -}$ disproportion. In this manner, the presence of antioxidants diminishes the radical amount and, proportionately, the measured intensity value resulting in viable quantitation of the antioxidant activity in different analysed matrixes [127-129].

The antioxidant capacity of flavonoids has been assessed comparatively [130] using a cytochrome *c*-modified electrode, and it was found to decrease in the order: flavanols > flavonols > flavones > flavonones > isoflavonones. Further studies proved an increase of the electron transfer rate proper to cyt *c* is achieved by employing mercaptoundecanoic acid/mercaptoundecanol self assembled-modified gold electrodes [131].

An amperometric biosensor employing two biorecognition elements, enabled the estimation of antioxidant activity of juices: the biosensor was developed by covalently co-immobilizing cyt c and xanthine oxidase on a mercaptoundecanol/mercaptoundecanoic acid self-assembled monolayer-modified screen-printed gold electrode. The viability of this technique was proved by estimating the antioxidant capacity of reference recognized antioxidants, such as ascorbic acid and Trolox as well as orange juices [132]. A further development of amperometric cytochrome *c* - based sensors involved cytochrome *c* and xanthine oxidase co-immobilization on the electrode by employing a composition-optimized self assembled monolayer of long-chain thiols. The biosensor allowed the estimation of the antioxidant capacity of alliin and two other substances found in garlic [133].

Peroxidase from Brassica napus hairy roots (PBHR) was employed in amperometric biosensor development for total polyphenolic level assessment in wine and tea [134]. The biosensor's analytical viability relied on the previously confirmed capacity of phenolics to act as electron-donors towards peroxidase, hence, promoting the catalysed H_2O_2 reduction [135,136]. Carbon paste electrodes were developed using peroxidase, ferrocene, and multi-walled carbon nanotubes mixed in mineral oil. The biosensor functioning relied on the decrease of H_2O_2 concentration in solution after the peroxidase-catalysed oxidation of phenolics. A semipermeable dialysis membrane proved able to minimize noises and electrode fouling which is likely to result from polymerization of oxidized phenolic compounds. The reaction environment consisted of: phosphate buffer solution pH = 7.0 (as optimum value with for peroxidase activity) and hydrogen peroxide at concentrations comprised between 0.005 and 1.5 mM. The highest total polyphenolic content (mg L^{-1}) was obtained for red wine, namely 1936 vs the t-resveratrol standard and 2988 vs the caffeic acid standard [134].

Coupling online microdialysis with amperometry at carbon microfiber electrodes, provided another analytical alternative for total antioxidant capacity and/or total wine phenolics estimation. Electrochemical cleaning was enough to avoid electrode inactivation owing to accomodating features of the artificially oxidized electrodes' surface. The improvement in sensitivity and response time was achieved through minimizing non-faradayic current in favor of the faradayic one owing to radial diffusion mass transfer of the analyte to the microelectrodes. In addition to that, it was proved that the microelectrodes' sensitivity is not prone to response alterations caused by stirring or flow irregularities as can happen with conventional millimeter-sized electrodes. The amperometric signals given by reference antioxidants such as caffeic acid and Trolox, at pH = 3.60 and 7.40 (obtained by adjusting artificial wine samples composed of 12 % ethanol and 0.05 M tartaric acid, with NaOH 1 M) were similar at 800 mV vs Ag/AgCl, showing that the recovery provided by the microdialysis probe was not pH dependent [137].

The results ranged from 98 ± 39.2 μmol Trolox Equivalents L^{-1} for Chardonnay dry Slovakia to 2743 ± 43.1 μmol Trolox Equivalents L^{-1} for Ruby Cabernet Dry California. The size of microelectrodes allowed for a facile automation of the antioxidant capacity/total phenolics measurement by 96-well microtitration plates. The results were in good agreement with those obtained by total reactive antioxidant potential (TRAP), oxygen radical absorbance capacity (ORAC), and chemiluminescence. It was found that, while the mean ratios between the antioxidant capacities of red and white wines were close for the amperometric, TRAP and ORAC methods (5.81, 4.36 and 5.76 Trolox Equivalents L^{-1}, respectively), the value determined by chemiluminescence was much greater (17.8 Trolox Equivalents L^{-1}). This apparent disparity was considered to be a result of the red wine interferents' contribution impairing enzyme activity and of the smaller antioxidant capacity of white wines [137].

The antioxidant capacity of caffeic, gallic, ferulic and p-coumaric acids was assessed by flow injection amperometry at a carbon/carbon nanotubes/polyethyleneimine (GC/CNT/PEI) electrode, in acetate buffer pH = 4.50 containing 0.1 M NaCl as carrier electrolyte. The GC/CNT/PEI electrode demonstrated enhanced analytical characteristics in the determination of the mentioned phenolics by amperometric flow injection: sensitivity, a linear range of three decades of concentration (Table 1) and a notably stable and robust electrochemical response, even when fouling effect compounds are present [138]. The glassy carbon electrode modified with carbon nanotubes dispersed in polyethyleneimine showed decreased overpotentials, improved sensitivity and better stability than the bare GCEs. The results were consistent with those furnished by spectrophotometry [138].

The reliability of the GC/CNT/PEI electrode in the analysis of wine samples was also tested. It was found that the signals recorded at both +0.30 and +0.70 V were proportionally correlable to the dilutions providing an extended range of appliable dilutions. The verified contribution of potential interferents such as ethanol, organic acids or sugars proved negligible at both +0.30 or +0.70 V in 10 times diluted model wine samples. The interference of sulphite was also investigated, at a concentration commonly encountered in real samples

which was considered 100 mg L^{-1}. An increase of just 4.4%, with respect to the polyphenols' analytical response, was noticed at +0.70 V while no signal fluctuations were recorded at +0.30 V. The oxidation products of polyphenols were swiftly removed by the flow of carrier electrolyte and could not react with sulphite close to the electrode, hence minimizing interferent effects, so the recorded signal could be ascribed to the samples' polyphenol content [138].

The greatest polyphenol content was obtained for red wine Izadi 1, namely 590 mg per liter gallic acid equivalents, measured at 0.70 V potential value. It was concluded that the enhanced electroanalytical features of the GC/CNT/PEI electrodes enable their use for polyphenol mapping in wines and the results of this assay can be well corroborated to HPLC or capillary electrophoresis. Another significant peculiarity of the method is its versatility in that it allows estimation of the low oxidation potential (highly electroactive) phenolics, as well as of the total polyphenol content, rapidly and directly, by merely tuning the working potential [138].

The total antioxidant capacity of different commercial products and biological samples was determined, relying on the electrochemical properties of the DPPH free radical and applying chronoamperometric measurements. The authors exploited the redox properties of DPPH in acetate: methanol buffer pH = 5.0–6.50 at a carbon glass electrode to develop amperometric calibration curves, employing the diffusion current intensity values [139]. The described growth of the diffusion layer with the time of potential pulse application [140] explains the current decrease in time and the low values obtained [139]. A sampling time of 3 seconds was found best to avoid the interference of the charging current. Both electrochemical and spectrometric methods with DPPH were employed in the determination of antioxidant capacity of fruit juices, creams and biological fluids. The total antioxidant capacity of juices determined by the electrochemical method ranged between $(1.38 \pm 0.12) \times 10^{-3}$ Trolox milliequivalents mL^{-1} (Boing Guava) and $(2.67 \pm 0.22) \times 10^{-3}$ Trolox milliequivalents mL^{-1} (Boing Mango) [139].

A recent amperometric method allowed for the assessment of the total antioxidant capacity of wines by the electrochemical reduction of DPPH• on a screen-printed gold electrode at -100 mV potential value, vs Ag/AgCl reference. It was confirmed that in the presence of red wine samples, DPPH•exhibited smaller amperometric signals at low pH than at high pH, indicating an enhanced scavenging ability of wine antioxidants in acid media [141]. The authors [141] compared the results obtained with previous observations, which stated that: both the flavilium ion form and the quinoidal base form of anthocyanins (present at acidic and neutral pH, respectively) proved powerful antioxidants [142]. On the other hand, for gallic acid, a diminution in the scavenging ability vs DPPH• was noticed beginning with pH values greater than 4.50, which has been assigned to the deprotonation of this reference antioxidant [143].

2.4 Potentiometry

These analytical tools correlate logarithmically the electrochemical cell's potential to the concentration/activity of the analysed compound [144]. Lowered potentials are correlated to enhanced electron-donating capacities and, consequently, to high antioxidant activities. Potentiometry was considered to present advantages compared with voltammetric or amperometric assay, in that it does not apply current or potential modulation. It uses simple apparatus, and the sample is viewed as the single independent variable [145]. Ion-selective potentiometry can suffer from drawbacks like possible deviation from Nernst's equation for potential that depends on changes of temperature or variations of ion activity [146].

A flow injection in vitro potentiometric method allowed for a rapid and reproducible evaluation of antioxidative ability proper to several aqueous plant extracts. This potentiometric technique relies on recording the potential shift which occurs in the potassium ferricyanide/potassium ferrocyanide mediator system (buffered in 0.2 M phosphate solution with pH = 2.50–10.50), as a result of its interaction with the sample antioxidants. The developed potentiometric method used as a detector a platinum electrode to estimate, comparatively, the antioxidant activity of some significant hydrosoluble antioxidants (ascorbic acid, pyrocatechol, pyrogallol, caffeic acid, chlorogenic acid, gallic acid, tannic acid, uric acid, l-cysteine, Trolox). A logarithmic dependence set between the measured analytical response and the antioxidant concentration allowed assessment of a wide antioxidant activity range by changing concentrations of ferricyanide from 5 to 0.01×10^{-4} M [94].

As the electroactivity of most of the above-mentioned compounds was explained by the presence of ene-diol structures, hydroxyl or sulfhydryl groups, it is important to add that the study of the electrochemical oxidation of uric acid in phosphate buffer (pH = 1.50–9.50), revealed that this reductive dihyropurinetrione is first oxidized in a two electron-two proton reaction to a highly unstable quinonoid diimine. At pH = 6.0, the quinonoid diimine anion undergoes hydrolysis yielding an anionic imine-

alcohol that is subject to ring contraction, resulting in 1-carbohydroxy-2,4,6,8-tetraaza-3,7-dioxo-4-ene-bicyclo-(3,3,0)-octane that eventually breaks down to allantoin. Mechanistic studies reported as end products: allantoin, 5-hydroxyhydantoin-5-carboxamide and, at pH = 3.0, alloxan [147].

The applied in vitro potentiometric method had as a benefit a high sampling rate (100 h^{-1}) and provided the total antioxidant activity of tea and herbal infusions as well as fruit extracts. The total antioxidant activity of aqueous fruit extracts ranged between 0.066 ± 0.002 ascorbic acid equivalents (for lemon) and 0.490 ± 0.001 (for orange). The tea infusions' values ranged between 3.60 ± 0.1 ascorbic acid equivalents for Dolche vita and 18.0 ± 0.2 for Sweet osman [94].

Another potentiometric method based on the change of the electrode potential corresponding to the ferricyanide/ferrocyanide mediator system aimed at estimating the oxidant/antioxidant status of seminal or follicular fluid samples. A platinum screen printed electrode was used as the indicator with the method relying on the simultaneous use of both forms of the ferricyanide/ferrocyanide mediator system to assess oxidant and antioxidant components of the seminal or follicular fluid samples from 99 male and 27 female subjects. The antioxidant activity of seminal fluids has been investigated in pathology conditions of reproductive system of male subjects and possibly related to male infertility symptoms [148].

A potentiometric assay for non-invasively providing the skin oxidant/antioxidant balance, relied, as measured analytical signal, on the potential change of the ferricyanide/ferrocyanide mediator system applied to the skin by means of a conductive gel. The change in the Pt indicator electrode potential was caused by the shift in the oxidized form to reduced form ratio of the mediator system. A decrease in the potential value indicates antioxidant activity of the analysed medium. Significant increases of antioxidant activities are obtained after ascorbic acid administration. In the case of samples displaying a diminution in antioxidant capacity and exhibiting a backward signal pointing to oxidant species occurence, this trend was ascribed to gastrointestinal impairment or to ascorbic acid irritating effect on gastric mucosa [149].

A recent potentiometric antioxidant capacity assessment in biological samples, food, drinks, combined the use of the free radical generator 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ as a mediator system. The sample underwent reaction with AAPH, so the antioxidants' concentration is lowered, as consequence of their interaction with peroxyl radicals generated from AAPH decay in phosphate buffer solution pH = 7.40 at 37°C. This method provides the possibility to analyse colored samples, such as whole blood and red cells [150].

Samples of human breast milk, commercial UHT milk and prebiotics supplemented infant formulas, have been analysed with respect to their total antioxidant activity, using iodine/iodide mediator redox couple, a Pt Fischer electrode as working electrode, a saturated calomel electrode reference and phosphate buffer 0.1 M, pH = 6.70 as electrolyte. The potentiometric assay revealed that human breast milk and infant formulas possess high redox potential, whereas commercial UHT milk has lowered potential [151].

2.5 Coulometry

In this technique, the electrochemical reaction generating the analytical signal can consist in the direct oxidation / reduction of the analyte at the electrode, or in the reaction of the analyte with a compound generated at the electrode surface by an electrolytical process. Hence, coulometric methods rely on the complete electrolysis of the analyte, the latter being quantitatively oxidized or reduced at the working electrode, or it can quantitatively react with a reagent formed at the surface of the electrode [152].

Coulometric titrations applied in total antioxidant capacity assessment require only a few minutes, and are easily tunable for automated analysis with generally unexpensive instrumentation. Interferent influence elimination is rendered possible by applying a potential at which the electrolysis of the interferents take place without the simultaneous electrolysis of analyte molecules. An advantage of coulometric titration is the electrochemical generation of the titrant allowing the use of unstable reagents [152], such as bromine, often employed in total antioxidant capacity assessment of blood or plasma [153,154]. It is possible to measure small quantities of charge, so coulometric titrations can be applied to determine concentrations which are too low for a conventional titration [152].

The amount of free liposoluble antioxidants in plasma was assessed (as tocopherol levels), by a simple coulometric method: antioxidants reacted with bromine electrogenerated from 0.2 M $(C_2H_5)_4NBr$ in 0.1M $HClO_4$/acetonitrile solution, and the measured redox potential was correlated with the total antioxidant capacity of plasma, by means of a logarithmic dependence. The analytical signals of plasma of patients with different ethiologies of chronic renal failure produced significant differences

between the levels of venous and arterial plasma: (15 ± 1) kilocoulombs L^{-1} vs (11.7 ± 0.7) kilocoulombs L^{-1}. The lowered antioxidant capacity of plasma was explained by the pro-oxidant transition metals (Fe, Cu, Mn, Ni and Cr) presence. Vitamin E and ximedon turned out to notably improve the total antioxidant capacity of plasma. This attests the correlation of processes involving antioxidants, free radicals and transition metals as reactive oxygen species generators in the radical chain processes which causes lipid peroxidation [153].

Another coulometric method used titration by electrogenerated bromine for the determination of total antioxidant capacity of blood and plasma. Electrochemical oxidation of bromide on a platinum electrode in acidic medium (0.1 M H_2SO_4) yields Br_2, Br_3^- and Br•, species which react with biologically active compounds with different structures, such as sulphur-containing amino acids and ascorbic and uric acids present in blood. Polyphenols participate in reactions following a radical mechanism, in electrophilic substitutions and redox reactions, and porphyrins can undergo addition to double bonds. The total antioxidant capacity of blood of patients suffering from chronic renal impairment and subject to longstanding hemodialysis proved 63% lower than that of control group due to oxidative stress (26 kilocoulombs L^{-1} versus 42 kilocoulombs L^{-1}, respectively) [154].

The electrochemical oxidation of antioxidants by an $ABTS^{+\cdot}$ solution prepared in phosphate buffer pH = 6.0 along with the spectrophotometric determination of the cation radical and the subsequent measurement of the amount of electricity, allowed the development of a novel method to measure antioxidant activity with a flat platinum electrode as cathode and a cylindrical platinum electrode as anode. Depending on the amount of antioxidants, the accumulation of cation-radical is retarded with subsequent utilization of a greater amount of coulombs. For a complete proof of the method, measurements were performed on a series of phenolics (quercetin, gallic acid, butylated hydroxyanisole, catechin, mirycetin, caffeic acid, ferulic acid), ascorbic acid and SO_2) and antioxidant-rich products derived from grapes. The greatest antioxidant capacity is shown by quercetin, 6.20 mmoles Trolox equivalents. With respect to the beverage samples, La Rioja red wines exhibit the best antioxidant capacity, 15.53 mM Trolox equivalents and 8.45 mM Trolox equivalents, respectively. For the complex grape-derived samples, the analytical signals of potentially electro-oxidizable substances (ethanol, acetic acid or SO_2) were found below the detection limit of the method [155].

3 Several analytical characteristics and applications of electrochemical methods to antioxidant capacity and its main contributors assay in different analysed media

The following Tables review the main performances (Table 1) and applications (Table 2) of the electrochemical methods presented and discussed, in various fields such as the analysis of foodstuffs and beverages, pharmaceutical and clinical analysis.

4 Critical and comparative considerations on the electrochemical methods applied to antioxidant assessment

Cyclic voltammetry, although applied with reliable results in several studies, can suffer from restricted values of detection limit (10^{-5} M) and poor sensitivity when compared to pulse techniques [42,50]. At the analysis of samples rich in various phenolics with different oxidation potentials such as wines, the analytical signal represented by the Q_{500} value is not sensitive to less oxidizable antioxidants, such as phenolics with high oxidation potentials [82]. Nevertheless, the problem could be addressed by considering the Q_{700} value [84]. Moreover, the assessed antioxidant capacity of tea infusions, estimated from the Q_{600} parameter, proved consistent with the results of classical spectrophotometric techniques (FRAP, ABTS and DPPH·) [73]. The cyclic voltammetric behaviour of phenolic acids found in orange juice at a glassy carbon electrode, provided their antiradical power with a ranking confirmed by the DPPH method [69].

Step voltammetric methods like DPV and SWV, result in lower detection limit (below 10^{-7} M, as shown by the results presented in Table 1) and better resolution, due to minimizing charging current. Differential pulse voltammograms proved able to better resolve the oxidation peaks than the linear sweep techniques for anthocyanin-rich samples like wines and grape extracts [88], and to allow a highly selective electro-oxidation of major flavonoids at pH = 7.50 enabling an easy and quick discrimination between phenolic acids and flavonoids in fruits and their juices at pH = 2.0 [89]. For the total polyphenol content assessment in wines, differential

Table 1: Some analytical characteristics of the electrochemical methods applied for antioxidant and total antioxidant capacity determination

Type of electrochemical detection	Electrode	Analysed medium	Anti oxidants	Detection limit	Relative standard deviation	Linear response	Ref.
Cyclic Voltammetry	Iridium-containing carbon (Ir-C)	Wine	Caffeic acid	-	-	0.0–25.0 mg L^{-1}	65
Cyclic Voltammetry	Glassy carbon	Roots from ready-to eat buckwheat sprouts	Total antioxidant capacity (vs Trolox)	-	0.51-2.74%	0.05-2.5 mM	156
Cyclic Voltammetry	Graphite Carbon microspheres Carbon nanotubes	Artificial wine solutions	Vanillic acid	2.85 µM 3.82 µM 4.13 µM	< 4%	10–400 µM	81
Square-wave Voltammetry	Glassy carbon	Fruits, nuts and juices	Ellagic acid	1.0 x 10^{-8} M	-	1.0 x 10^{-7}–1.5 x 10^{-6} M	157
Cyclic Voltammetry, Linear sweep voltammetry and Flow Injection Amperometry	Glassy carbon and spectrographic graphite	Bovine milk and derived products	Antioxidant capacity of milk, whey and its low-molecular-weight fractions	-	-	100–130 µM Trolox equivalents	158
Cyclic voltammetry	Glassy carbon	Spices	Curcumin	4.1 × 10^{-6} M	1.2-2.2 %	9.9 × 10^{-6}– 1.07 × 10^{-4} M	80
Cyclic voltammetry (bio-electronic tongue)	Biosensors based on redox enzymes: tyrosinase/ laccase and copper nanoparticles modified epoxy–graphite	Wine spiked samples	Polyphenols: catechol, caffeic acid and catechin	-	-	up to 200 µM	159
Differential Pulse Voltammetry	DNA-modified carbon paste	Flavoured beverages	Total antioxidant capacity (vs ascorbic acid)	50 nM	3.2% at 1 µM	0.05 - 1.0 µM	25
Differential Pulse Voltammetry	Carbon paste Platinum	Fruit juices and wines	Ascorbic acid	0.02 mM 0.087 mM	2.35% 2.09%	0.07-20 mM 0.31-20 mM	38
Differential Pulse Voltammetry	Carbon nanotubes modified carbon paste	Red and white wines	Antioxidant capacity (vs gallic acid)	3.0 x 10^{-7} M	5.3%	5.0 x10^{-7} - 5.0 x 10^{-5} M	93
Differential Pulse Voltammetry	Platinum		α-, tocopherol γ- tocopherol δ- tocopherol			2 ×10^{-8}-1×10^{-5} M 2.2×10^{-8}-1.4×10^{-6} M 2.2×10^{-8}-3.31×10^{-5} M	96
Differential Pulse Voltammetry	**Glassy carbon**	**Human serum blood**	Total antioxidant capacity	-	2.3-8.33%	-	160
Differential Pulse Voltammetry	**Glassy carbon**	Antitumor isoflavones isolated from *Maclura pomifera* and *Derris malaccensis*	**Pomiferin**	6.07x10^{-7} M	0.52%	0.5-20 µM	161

Continued **Table 1:** Some analytical characteristics of the electrochemical methods applied for antioxidant and total antioxidant capacity determination

Type of electrochemical detection	Electrode	Analysed medium	Anti oxidants	Detection limit	Relative standard deviation	Linear response	Ref.
Differential Pulse Voltammetry	Glassy carbon modified with a Zn-Al-NO_3 layered double hydroxide film		**Gallic acid** **Caffeic acid**	1.6 μM 2.6 μM	1.58%	4-600 μM 7.0-180 μM	162
Differential Pulse Voltammetry, Cyclic voltammetry	In situ activated bare glassy carbon	-	**Tertiary butyl hydroquinone**	67 nM	-	1.0 μM – 1.1 mM	163
Differential pulse polarography	**Dropping mercury**		**Gallic acid**	0.3 μM		1.0-50 μM	95
Square-wave voltammetry	Biosensor with purine base immobilized on glassy carbon	Flavour and flavoured waters, beverages	Total antioxidant capacity	-	0.02-35.62%	-	164
Square wave voltammetry	Nucleotide-based biosensors with glassy carbon transducer	Flavored water	Total antioxidant capacity (vs. ascorbic acid)	-guanine biosensor 0.10 mg L^{-1} -adenine biosensor 0.08 mg L^{-1}	-	-	165
Square wave voltammetry	Screen-printed sensors with carbon working electrodes	Standard antioxidants	Ascorbic acid N-acetylcysteine Melatonin	0.09 mM 0.04 mM 0.07 mM			166
Square wave voltammetry	4-[(4-decyloxyphenyl)-ethynyl]-1-methylpyridinium iodide modified glassy carbon	Total polyphenol content of mate herb extracts	Caffeic acid standard	$9.0x10^{-7}$ M $8.7x10^{-6}$ M	4.8%	$9.9x10^{-7}$ M-$3.8x10^{-5}$ M $4.7x10^{-5}$ M-$9.9x10^{-5}$ M	167
Amperometry	Biosensor based on peroxidase-modified carbon paste	Standard antioxidants and wine samples	t-resveratrol Caffeic acid	0.023 mg L^{-1} 0.020 mg L^{-1}	7.0% 8.4%	0.05 - 52 mg L^{-1} 0.06 - 69 mg L^{-1}	134
Amperometry	Glassy carbon		Trolox	$1.3×10^{-6}$M		$0.5-3.0×10^{-5}$ M	139
Amperometry (flow injection),	Carbon nanotube modified-glassy carbon electrode	Thai vegetables /herbs	Gallic acid Catechin Quercetin Caffeic acid Trolox	0.04 μM 0.02 μM 0.03 μM 0.08 μM 0.04 μM	1.3% 1.5% 1.5% 2.0% 1.2%	0.6-12 μM 0.3-6 μM 0.3-6 μM 0.6-12 μM 0.3-8 μM	168
Amperometry (flow injection)	Glassy carbon/ carbon nanotubes/ polyethyleneimine electrode	Standard antioxidants	Caffeic acid Gallic acid, Ferulic acid p-coumaric acid	< 0.1 μM	10.8% 11.0% 10.2% 13.5%	10^{-7}-10^{-4} M	138
Amperometry	Superoxide dismutase biosensor	Fruits, olives, fresh aromatic herbs	-	-	up to 8% for aromatic herbs; up to 13% for fruits	-	125

Continued **Table 1:** Some analytical characteristics of the electrochemical methods applied for antioxidant and total antioxidant capacity determination

Type of electrochemical detection	Electrode	Analysed medium	Anti oxidants	Detection limit	Relative standard deviation	Linear response	Ref.
Amperometry Cyclic Voltammetry	Superoxide dismutase biosensors with Pt anode for H_2O_2 amperometric detection Glassy carbon	Phytotherapeutic diet integrators	-	-	≤ 10%	-	169
Biamperometry (flow injection)	Interdigitated microelectrode (IME 1525.3 FD Au P)	Juices, tea and wine	Trolox		-	20-500 µM	115
Biamperometry (flow injection)	Bienzymatically produced ABTS+ and electrochemical detector using interdigitated gold electrode	Wine and spirits	Trolox		-	20 µM-2000 µM	116
Potentiometry, Linear sweep voltammetry Differential Pulse Voltammetry	Glassy carbon and iodide modified glasssy carbon	Model wine solution	Catechin as model polyphenol		-	1×10^{-5} - 6×10^{-4} M	170
Potentiometry	Platinum	Aqueous plant extracts	Ascorbic acid, Pyrocatechol, Pyrogallol, Caffeic acid, Chlorogenic acid, Gallic acid, Tannic acid, Uric acid, l-cysteine, Trolox		0.7–1.8%	1 µM - 10 mM	94

pulse voltammetry was regarded as an improvement vs cyclic voltammetry and Folin-Ciocalteu assay with respect to sensitivity and selectivity. The results of the polyphenol content DPV assessment showed a strong correlation with the antioxidant capacities determined by DPPH and particularly ABTS colorimetric measurements [91].

Mostly enhanced sensitivities, with detection limit values below 10^{-7} M, were obtained in square wave voltammetry assay [109]. SWV applying the standard addition method, proved more effective than cyclic and differential pulse voltammetry in discrimination of synthetic phenolic antioxidant present in mayonnaise at glassy carbon and Pt electrodes [112].

Amperometric biosensors benefit both the specificity imparted by the biocatalyst and the accuracy and rapidity of electrochemical detection at the optimized potential value. Though, even if characterized by a wide linear range, particularly in the case of nonenzymatic methods, the method suffers from the difficulty to electro-oxidize at constant potential high molecular mass antioxidants, and the measured signal does not always give account on their contribution [122]. Lowering the working potential is possible by changing the biocatalytical component or by using electrode modification with improved analytical characteristics in the case of high antioxidant content-samples, such as juices, wines or teas [132,134,138].

Biamperometric techniques are endowed with high selectivity, but require very rigorous control of experimental conditions, such as the molar ratio oxidized/reduced form of the redox couple and, also, the potential value applied: the increase of the latter although leading to an increase of the analytical signal is limited by the possible interference of electroactive compounds, other than antioxidants [23].

Ion-selective potentiometry does not need current or potential modulation, but can suffer from possible deviation of the analytical response from the Nernstian

Table 2: Some applications of electrochemical methods for the determination of antioxidant capacity and its main contributors in various analysed media

Detection mode	Electrode type	Antioxidants analysed	Analysed medium	Ref.
Cyclic voltammetry	Glassy carbon	Low molecular weight antioxidants (LMWAs)	Spices, condiments, drugs	41
Cyclic voltammetry	Carbon paste	Ascorbic acid, caffeic acid, quercetin, catechin, and hesperetin alone and in equimolar binary admixtures	-	47
Cyclic voltammetry	Glassy carbon	Low–molecular weight antioxidants (LMWAs)	Human plasma	49
Cyclic voltammetry	Glassy carbon	Total antioxidant capacity	Edible plant extracts	49, 53
Cyclic voltammetry	Glassy carbon	Total antioxidant capacity	Green tea, black tea, rosemary and coffee	54
Cyclic voltammetry	Glassy carbon	Total antioxidant capacity	Buckweat extracts	55
Cyclic voltammetry	Glassy carbon	Low–molecular weight antioxidants (LMWAs)	Plasma	57
Cyclic voltammetry	Pt microelectrode	Total antioxidant capacity imparted by low–molecular weight antioxidants	Skin	62
Cyclic voltammetry	Glassy carbon	Total antioxidant capacity	Plasma	63
Cyclic voltammetry	Iridium conaining carbon	Polyphenols	White wines	65
Cyclic voltammetry	Glassy carbon	Antioxidant potency composite index obtained from three recorded antioxidant analytical signals estimated to relative percentages	Fruit tea infusions	73
Cyclic voltammetry	Glassy carbon	Quercetin and its glucosides	Onions	77
Cyclic Voltammetry	**Glassy carbon**	Polyphenols	Sauvignon blanc grape juices	84
Cyclic Voltammetry	**Glassy carbon**	Flavonoids	Flavonoids	171
Cyclic Voltammetry	**Glassy carbon**	Polyphenols	Seaweeds	172
Cyclic Voltammetry	**Glassy carbon**	Flavonoids and phenolic acids	Tea infusions, Lipton brand	173
Cyclic Voltammetry	**Glassy carbon**	Methanolic extracts of spices	Clove, juniper, berries, nutmeg, cinnamon, rosemary, anise, oregano black pepper, ginger,basil, turmeric, red pepper, bay leaf, coriander, red sweet pepper, cumin, caraway	174
Differential pulse voltammetry Cyclic Voltammetry	DNA-modified carbon paste electrodes	Total antioxidant capacity	Lemon flavoured beverages	25
Differential pulse voltammetry	Carbon paste electrode modified with carbon nanotubes	Gallic acid, Total polyphenols	Wine analysis	93
Differential pulse voltammetry	Glassy carbon	Flavonoids and phenolic acids	Apples, pear and their juices	89

Continued **Table 2:** Some applications of electrochemical methods for the determination of antioxidant capacity and its main contributors in various analysed media

Detection mode	Electrode type	Antioxidants analysed	Analysed medium	Ref.
Differential pulse voltammetry, Linear sweep, Cyclic voltammetry	Glassy carbon	Anthocyanins	Chilean red wine, grape and raspberry	88
Cyclic voltammetry	Glassy carbon	Flavonoids	Synthetic solutions	175
Cyclic voltammetry	Glassy carbon	Antioxidant potential	Methanolic plant extracts	176
Differential pulse voltammetry	Mercury film and glassy carbon working electrodes	Antioxidant potential	Green tea extract, apple vinegar and pharmaceuticals	65
Cyclic voltammetry	Biosensor (superoxide dismutase); Glassy carbon	Total antioxidant capacity	Algae	177
Square wave voltammetry	Cobalt-phthalocyanine-modified carbon screen-printed electrodes	Total antioxidant capacity	Plant extracts	110
Square wave voltammetry	Carbon-polyurethane composite	Rutin	Green tea	178
Square wave voltammetry	Glassy carbon	Epigallocatechin gallate	Green tea	179
Square wave voltammetry	Glassy-carbon and spectral-grade paraffin-impregnated graphite rod	Ellagic acid	Strawberries, Raspberries and Blackberries	180
Square wave voltammetry	DNA modified carbon screen-printed electrodes	Trolox, uric acid, ascorbic acid, gallic acid, gluthatione, bovine serum albumin	Synthetic solutions	181
Square wave voltammetry	Carbon paste electrode	Flavonoids	Human urine	182
Square wave voltammetry	Screen-printed carbon electrodes	Antioxidant profile (phenolics)	Blackcurrant and strawberry juices	183
Square wave voltammetry	Carbon fiber disk ultramicroelectrode Pt band ultramicroelectrode	Tocopherols and Tert-butyl hydroxytoluene	Benzene/ethanol mixture (1:2) containing 0.1 mol L^{-1} H_2SO_4 Acetonitrile containing 0.1 mol L^{-1} $(C_4H_9)_4NPF_6$	184
Voltammetry and chronoamperometry (Ceric reducing antioxidant capacity assay)	Boron dopped diamond electrode	Antioxidant capacity	Free flavonoids and Fe^{2+}-flavonoid complexes	185
Amperometry	Xanthine oxidase/poly-*m*-phenylenediamine-modified electrode	Total antioxidant capacity	Pharmaceuticals - acetylsalicylic acid	124
Amperometry	Superoxide dismutase biosensor	Total antioxidant capacity	Fruits, olives, fresh aromatic herbs	125
Amperometry	Carbon microfiber electrode	Total antioxidant capacity	Wine analysis	137
Amperometry	Glassy carbon	Low–molecular weight antioxidant (LMWAs)	Bovine milk and whey	122
Amperometry	Tyrosinase-modified screen printed electrode	Total antioxidant capacity	Medicinal plants infusions	186

Continued **Table 2:** Some applications of electrochemical methods for the determination of antioxidant capacity and its main contributors in various analysed media

Detection mode	Electrode type	Antioxidants analysed	Analysed medium	Ref.
Amperometry (flow injection)	Glassy carbon/ carbon nanotubes/ polyethyleneimine electrode	Total polyphenols	Wine	138
Biamperometry	Platinum electrodes	Total antioxidant capacity	Fruit juices	23
Biamperometry	Interdigitated microelectrode (IME 1525.3 FD Au P)	Total antioxidant capacity	Juices, tea and wine	115
Biamperometry	Interdigitated gold electrode	Total antioxidant capacity	Alcoholic beverages (wines and spirits)	116
Potentiometry	Platinum electrode	Total antioxidant capacity	Tea infusions, herbal infusions and fresh fruit extracts	94
Potentiometry	Platinum screen printed electrode	Ascorbic acid, pyrogallol and catecholamines	Whole blood	149
Coulometry	Platinum electrode	Total antioxidant capacity	Blood and plasma	153
Coulometry	Platinum electrode	Total antioxidant capacity	Blood and plasma	154
Polarography	Dropping mercury electrode	Total antioxidant capacity	Propolis extracts	187
Polarography	Dropping mercury electrode	Flavonoids, phenolic acids, amino and organic acids and carbohydrates	Honey samples	188

dependence, and the measured signal can be subject to changes of temperature, shifts in ion activity or potential value of the redox couple on which the antioxidant determination relies [145, 146].

Even if less employed than voltammetry or amperometry, coulometric titrations allow electrochemical generation of the titrant, so reagents with low stability can be used such as bromine, mainly employed in total antioxidant capacity assessment of plasma [152-154].

5 Conclusions

The design of performant and accurate methods of antioxidant capacity assessment is important given the role of antioxidants in preventing the occurrence of oxidative stress-related diseases. The determination of antioxidant capacity in various media (foodstuffs, pharmaceuticals, biological fluids) is vital as it represents a quality indicator in the case of foods and beverages. The value of the antioxidant capacity of biological fluids (such as blood or plasma) is a relevant indicator of the level of oxidative stress and of the health status of the subjects.

The great majority of the antioxidant molecules are endowed with generally enhanced electroactivity, depending on their structure (the presence of active groups such as hydroxyl groups -OH, susceptible to undergo oxidation), as well as on the electrode material, as the incorporation of modifiers lowers the oxidation potential, enhancing the oxidation current peak.

Voltammetric investigation of phenolics electro-oxidation in complex sample proved a crucial influence of the structure, namely of oxidizable hydroxyl groups in different locations on the molecule: the most easily oxidizable compounds are flavonoids presenting an ortho-diphenol group at B ring, like catechin, epicatechin, quercetin and several phenolic acids – gallic, caffeic, and tannic acids. Further peaks were assigned to the oxidation of anthocyanins, stilbene derivatives (trans-resveratrol) and phenolic acids with higher oxidation potentials, such as benzoic and hydroxycinnamic acids that do not present an ortho-diphenol structure. Some of the flavonoids that generate the first peak in the domain of 370–470 mV can undergo second electro-oxidation of the less reactive hydroxyl groups on the rings A and C, contributing to peak occurence at about 800 mV. Sulphur-

containing antioxidants (e.g. metabisulfite) are oxidized after phenolic compounds at higher potential values. Ascorbic acid is oxidized before phenolic compounds, at lower potentials depending of the electrode's nature.

Although recent studies imply the use of various modified electrodes (it was proved that carbon nanotubes or nucleic acid modified carbon paste electrodes greatly enhance electro-oxidation and improve analytical characteristics, as can be seen in the results present in Table 1), reliable results in antioxidant assessment in wine and juices can also be achieved with unmodified electrodes, provided that adequate mechanical and/or electrochemical pretreating steps are performed.

Thus, the electrochemical methods developed for antioxidant capacity screening and its main contributors assessment, have proved rapid, sensitive, allowing the analysis of various media, with no need for complicated sample pre-treatment and with relatively low cost by exploiting either the peak current intensity or the value of the charge, obtained from the surface below the current wave.

Voltammetry can offer detection limits as low as 10^{-12} M, and presents advantages in comparison to more costly conventional instrumental methods: fastness, facility of working procedures including that of standard addition. Because of the relatively unexpensive instrumentation and minimum sample pretreatment, voltammetry offers a viable alternative in foodstuffs and beverages quality control as well as in biological fluids analysis. It enables assessment of antioxidant content, the study of antioxidant action mechanism and antioxidant mutual influence (synergism).

Potentiometric and amperometric biosensors exploit the specificity of the biocomponent and the accuracy and rapidity of the electrochemical transducer.

The viability of voltammetric, amperometric and potentiometric methods has been confirmed by their analytical characteristics obtained in numerous applications like food, clinical and pharmaceutical analysis.

The performances attained by the mentioned electrochemical techniques depend on the detection mechanism, on the modalities of sensor development (from bare electrodes to carbon nanotubes modified electrodes and various composites), on the interaction between the antioxidant molecule's and the electrode's functional groups, as well as on the characteristics of the analysed matrix (pH value, nature of the electrolyte, presence of interferent compounds). The applied electrochemical technique, the modality of electrode development and the type of reference antioxidant chosen, can be tuned to the nature of the analysed matrix and expected performances.

Combining advanced materials such as carbon nanotubes, screen-printing technique or ionic liquids with classical electrode construction (e.g. glassy carbon, carbon paste or metal electrodes) to obtain various composites provides viable results and can constitute an alternative for the future.

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