JCEC
THE JOURNAL OF ENGINEERING AND EXACT SCIENCES

OPEN ACCESS

The Journal of Engineering and Exact Sciences – jCEC, Vol. 10 N. 08 (2024)
journal homepage: https://periodicos.ufv.br/jcec
eISSN: 2527-1075
ISSN: 2446-9416

Electroanalytical Methods as a Tool for Determining the Antioxidant Capacity in Blood Samples

Métodos Eletroanalíticos como Ferramenta para Determinar a Capacidade Antioxidante em Amostras de Sangue

Article Info:
Article history: Received 2024-10-09 / Accepted 2024-12-12 / Available online 2024-12-12
doi: 10.18540/jcecvl10iss8pp20793

Fernanda Gonçalves de Oliveira
ORCID: https://orcid.org/0009-0000-9961-9740
Federal University of Rio de Janeiro, Brazil
E-mail: nandagoliveira@yahoo.com.br
Thiago da Silveira Alvares
ORCID: https://orcid.org/0000-0002-2261-8501
Food and Nutrition Institute, Federal University of Rio de Janeiro, Brazil
E-mail: alvares@macae.ufrj.br
Rodrigo de Siqueira Melo
ORCID: https://orcid.org/0000-0002-0816-462X
Multidisciplinary Institute of Chemistry, Federal University of Rio de Janeiro, Brazil
E-mail: rodrigosqm@macae.ufrj.br

Abstract
Aerobic metabolism is an essential process for energy production in cells, but it also generates reactive oxygen species (ROS), which, in excess, can cause cellular damage and contribute to the development of various diseases, such as cardiovascular diseases, cancer, and neurodegenerative disorders. The balance between ROS and antioxidants is crucial for health, as an imbalance can lead to oxidative stress, a significant risk factor for several clinical conditions. Electroanalytical techniques, such as cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV), have proven to be powerful tools for evaluating antioxidant capacity more efficiently and accurately than traditional spectrophotometric methods. These techniques offer notable advantages, such as high sensitivity, simplicity, and the ability to analyze complex biological samples rapidly. CV allows for the analysis of compounds with good sensitivity, DPV stands out for its high precision, while SWV offers excellent resolution and speed, making it ideal for clinical applications. The use of these methodologies has been expanded to analyze antioxidants in various biological matrices, enabling a more accurate assessment of oxidative stress and antioxidant status in specific clinical contexts. Additionally, electrochemical biosensors have become revolutionary tools in clinical diagnostics, allowing real-time monitoring of diseases related to oxidative stress. Innovations such as portable devices and integration with artificial intelligence promise to further enhance accessibility and treatment personalization, despite the challenges involved in device cost and standardization.
Keywords: Oxidative stress. Reactive oxygen species. Bioelectrochemistry.

Resumo
O metabolismo aeróbico é um processo essencial para a produção de energia nas células, mas também gera espécies reativas de oxigênio (EROs), que, em excesso, podem causar danos celulares e contribuir para o desenvolvimento de diversas doenças, como doenças cardiovasculares, câncer e distúrbios neurodegenerativos. O equilíbrio entre ROS e antioxidantes é crucial para a saúde, sendo

que um desequilíbrio pode levar ao estresse oxidativo, um fator de risco significativo para várias condições clínicas. As técnicas eletroanalíticas, como voltametria cíclica (VC), voltametria de onda quadrada (VOQ) e voltametria de pulso diferencial (VPD), têm se mostrado ferramentas poderosas para avaliar a capacidade antioxidante de maneira mais eficiente e precisa do que os métodos tradicionais espectrofotométricos. Essas técnicas oferecem vantagens notáveis, como alta sensibilidade, simplicidade e a capacidade de analisar amostras biológicas complexas de forma rápida. A VC permite a análise de compostos com boa sensibilidade, a VPD destaca-se pela sua alta precisão, enquanto a VOQ oferece excelente resolução e rapidez, sendo ideal para aplicações clínicas. O uso dessas metodologias tem sido ampliado na análise de antioxidantes em diversas matrizes biológicas, o que possibilita uma avaliação mais precisa do estresse oxidativo e do estado antioxidante em contextos clínicos específicos. Além disso, os biossensores eletroquímicos têm se tornado ferramentas revolucionárias no diagnóstico clínico, permitindo o monitoramento em tempo real de doenças relacionadas ao estresse oxidativo. Inovações como dispositivos portáteis e integração com inteligência artificial prometem aprimorar ainda mais a acessibilidade e a personalização dos tratamentos, apesar dos desafios que envolvem o custo e a padronização dos dispositivos.
Palavras-chave: Estresse oxidativo; Espécies reativas de oxigênio; Bioeletroquímica.

1. Introduction

The reactions of the aerobic metabolic pathway are associated with the generation of reactive oxygen species (ROS), such as the superoxide anion and hydroxyl radical, with their production balanced by antioxidant mechanisms (Fischer *et al*., 2005). Antioxidants are molecules that protect the body by reducing or repairing damage caused by ROS (Juan *et al*., 2021). Under certain conditions, the balance between ROS production and antioxidant concentration can be disrupted, resulting in ROS overproduction, a condition known as oxidative stress. While adequate levels of ROS are essential for maintaining cell proliferation and survival, dysregulation of the intracellular redox state can lead to dysfunction and cell death (Gęgotek *et al*., 2021). Elevated ROS levels are associated with the pathogenesis of various diseases, including cancer, metabolic syndrome, atherosclerosis, malaria, Alzheimer's disease, rheumatoid arthritis, neurodegenerative disorders, and preeclampsia (Allegra, 2021).

Antioxidant activity refers to the ability of a molecule to prevent or reduce the effects of free radicals, measured through assays that evaluate how antioxidants interact with reactive species in specific solutions (Pisoschi *et al*., 2015). The effectiveness of this activity depends on the reducing properties and chemical structure of antioxidants, which enable them to neutralize and scavenge reactive molecules. In contrast, antioxidant capacity measures the amount of free radicals a sample can eliminate, providing a quantitative assessment of the antioxidant's effectiveness in protecting cells (Lima *et al*., 2020). However, low antioxidant content does not necessarily indicate low antioxidant capacity, as this capacity is closely linked to the structure and behavior of the antioxidant when interacting with analogous compounds, such as in cases of synergy or antagonism (Morris, 2003).

Classical methods commonly used to determine antioxidant capacity are based on spectrophotometric techniques, which involve various reactions for scavenging free radicals (Hoyos-Arbeláez *et al*., 2017). While these methods are efficient and well-established, they present limitations, such as time-consuming sample preparation, prolonged and uncertain analysis durations, and the need for expensive reagents. Although UV-Vis methods are widely used, they have significant disadvantages, including the use of costly and environmentally harmful reagents, undefined reaction times, the need for sample pretreatment, and inaccuracies caused by interference from compounds that absorb within the same wavelength range. Furthermore, non-antioxidant substances can interfere with results, leading to an overestimation of antioxidant capacity (Suh *et al*., 2011).

In contrast, electroanalytical techniques offer a more efficient, rapid, and precise alternative for determining antioxidant capacity. These methods present several advantages, such as high sensitivity, simplicity, versatility, the use of relatively inexpensive equipment, the requirement for small sample volumes, and ease of handling. Another benefit is that many antioxidants are electroactive, enabling rapid analysis of complex or colored samples (Hoyos-Arbeláez *et al*., 2017). The most employed electroanalytical techniques for this purpose include cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry. These techniques assess parameters such as current intensity (I_p), peak potential (E_p), and charge (current × time), which are directly correlated to antioxidant capacity and activity, demonstrating greater selectivity and sensitivity compared to spectrophotometric methods (Gil & Couto, 2013).

The aim of this integrative review is to explore electroanalytical methods for determining antioxidant capacity, highlighting their advantages over classical methods, such as higher sensitivity, simplicity, and versatility, as well as the ability to perform rapid analyses of complex samples. The review will also address the main electroanalytical techniques used, including cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry, as well as their applications in quantifying antioxidant activity and evaluating protection mechanisms against reactive oxygen species.

2. Methodology

To explore electroanalytical methods for determining antioxidant capacity and to evaluate their advantages and limitations compared to classical methods, an integrative review of scientific literature was conducted. Articles were retrieved from recognized databases, including PubMed, Scopus, and Web of Science. The search employed the following descriptors and keywords combined with Boolean operators: *"antioxidant capacity," "electroanalytical methods," "cyclic voltammetry," "square-wave voltammetry," "differential pulse voltammetry,"* and *"antioxidant capacity."*

The inclusion criteria considered articles published between 2000 and 2024 that addressed the use of electroanalytical methods for assessing antioxidant activity or capacity, comparisons between classical (e.g., spectrophotometric) and electroanalytical techniques, and studies on low molecular weight antioxidant compounds in samples such as plant extracts, blood plasma, or tissue homogenates. Exclusion criteria included studies not available in full text, publications in languages other than English, and studies lacking empirical data or detailed methodological descriptions.

Information extracted from the selected articles included the type of electroanalytical method used (cyclic voltammetry, square-wave voltammetry, or differential pulse voltammetry), analyzed electrochemical parameters (peak potential, current intensity, and charge), sample types used in the studies, reported correlations between classical and electroanalytical methods, and the advantages and limitations of each method. Data were organized into thematic categories and critically analyzed to identify trends, gaps, and potential innovations in the application of electroanalytical techniques for determining antioxidant capacity.

The results were compared with classical methods to evaluate improvements in sensitivity, specificity, and practical applicability. The entire process was conducted in adherence to ethical standards and scientific integrity, prioritizing reliable sources and academically relevant articles to ensure the quality of the information included in the review.

This methodology aims to provide a solid foundation for analyzing the potential of electroanalytical methods in investigating antioxidant capacity, contributing to advancements in monitoring and controlling oxidative stress in various biological and clinical contexts.

3. Results and Discussion

3.1 Electroanalytical Behavior of Antioxidants

Phenolic compounds represent a group of non-enzymatic antioxidants characterized by their structure, which includes a benzene ring with hydrophilic substituents. These compounds can be classified into two main categories: phenolic acids (primarily hydroxybenzoic and hydroxycinnamic acids) and flavonoids. The initial phase of phenol oxidation can lead to the formation of a phenoxonium ion or a phenoxy radical (Figure 1). These products may undergo additional chemical reactions, such as coupling, proton loss, or nucleophilic attack (Ferreira *et al*., 2006).

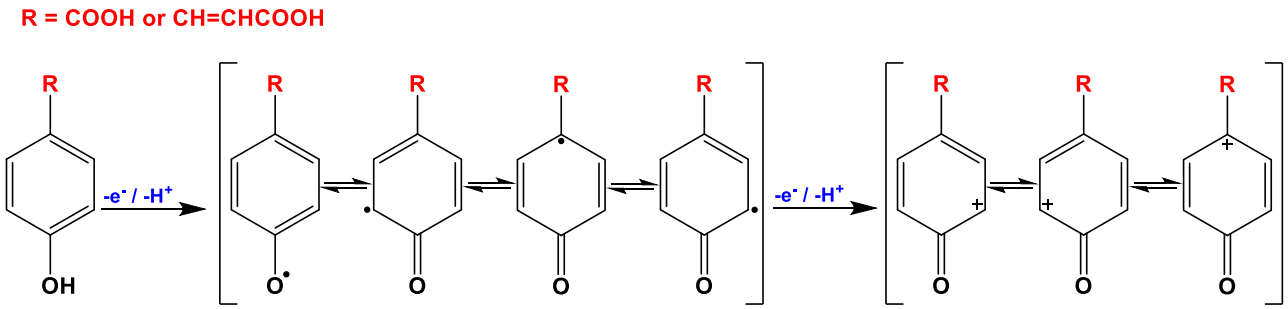

Figure 1 - Electrochemical oxidation of phenolic compounds.

Although phenoxonium ion intermediates may form, the likelihood of a positively charged structure on a carbon atom already experiencing electron deficiency due to the electron-attracting effect of a -COOH group is low. As a result, *p*- and o-hydroxybenzoic acids exhibit their peak potential shifted toward more positive potentials (anodic direction) compared to the meta isomer, which lacks a positively charged carbon atom in the -COOH group. Therefore, the meta isomer is the easiest to oxidize among the three derivatives. Additionally, steric effects in o-hydroxybenzoic acid may contribute to the potential shift.

3.2 Principles of Electroanalytical Methods

Electroanalytical techniques use a working electrode to detect analytes electrochemically. This electrode acts as a sensor, typically made of a fluid matrix (carbon paste) or a solid matrix (glassy carbon). Carbon paste electrodes offer the advantage of incorporating organic or inorganic compounds, thereby enhancing their selectivity and specificity. These electrodes are employed in voltammetric measurements, with cyclic voltammetry (CV), square-wave voltammetry (SWV), and differential pulse voltammetry (DPV) being the most effective techniques for studying antioxidant properties (Dorozhko & Korotkova, 2011). Electroanalytical techniques are fundamental tools for analyzing chemical compounds related to human health, enabling the detection and quantification of essential redox species such as drugs, biomarkers, and antioxidants. Among these methodologies, CV, DPV, and SWV stand out for their applicability in pharmacology, toxicology, and clinical analyses.

Cyclic voltammetry is a widely used electroanalytical technique to examine the redox properties of molecules in solution. This method measures the electric current during the variation of the applied potential, first in the anodic and then in the cathodic direction. This cycle allows the observation of current changes associated with the oxidation and reduction of the chemical species present. One of the main goals of CV is to identify relevant parameters, such as oxidation and reduction potentials and current intensity, which reflect the redox activity of the analytes. The resulting plots, known as cyclic voltammograms, display current peaks indicating redox reactions,

with their position and intensity directly related to the concentration and nature of the analyzed compounds.

CV has emerged as a powerful and versatile tool for evaluating antioxidants and antioxidant capacity in blood. Recent applications have demonstrated its utility in detecting and quantifying the redox activity of biologically relevant substances. Advances in this technique not only deepen our understanding of antioxidant mechanisms but also create opportunities for clinical and nutritional applications in health monitoring. For instance, Chevion reported one of the earliest procedures highlighting the utility of CV in analyzing low-molecular-weight antioxidants in plasma, biological samples, and plant extracts (Chevion *et al*., 2000). A basic CV experiment involves varying the voltage applied to the working electrode at a constant rate while recording the faradaic current generated during antioxidant oxidation. From the resulting voltammogram, key parameters used to characterize the antioxidant include peak potential (E_p), half-wave potential ($E_{1/2}$), and peak current (i_p) (Figure 2). Generally, the peak potential provides insights into the ease with which a molecule exchanges electrons (Sazhina, 2017), where peaks at low oxidation potentials are associated with compounds with high electron-donating capacity and vice versa. The peak current, in turn, provides combined information about the concentration of antioxidants dissolved in the solution or the average number of electrons exchanged (Milardovic *et al*., 2007).

The characteristics of CV include moderate sensitivity, allowing the detection of analytes at reasonable concentrations, though not as low as those achieved by more sensitive techniques. The analysis time is considered moderate, suggesting that obtaining results requires a manageable but not excessive amount of time. The complexity of the technique is medium, indicating the need for an appropriate level of skill and technical knowledge for its application. However, CV may present high interference, potentially compromising the precision of measurements in complex samples, necessitating rigorous control of experimental conditions. Background subtraction is a common practice to eliminate solvent or matrix signals. However, in samples containing multiple redox species or when a chemical species exhibits multiple peaks, the integrated area (Q) under the oxidation peak is considered a more comprehensive estimate of the total number of electrons exchanged (Sazhina, 2017).

Differential Pulse Voltammetry (DPV) stands out as a highly precise and sensitive electrochemical technique widely employed to investigate the redox behavior of antioxidants. In this method, the electric potential applied to the system is varied gradually in small increments (pulses), enabling a detailed analysis of oxidation and reduction reactions occurring at the electrode surface (Hoyos-Arbeláez *et al*., 2017). DPV differentiates itself from other voltammetric techniques by how the potential is applied. For each pulse, the potential increases by a fixed value, and the current is measured immediately before and after the pulse application. The difference between these two current measurements represents the recorded signal, which is plotted on a graph known as a voltammogram. This approach minimizes the contribution of capacitive current, resulting in voltammograms with significantly higher signal-to-noise ratios, thereby improving the detection of the electrochemical processes of interest.

DPV is recognized for its high resolution, which enables precise distinction of closely spaced electrochemical peaks—a feature that sets it apart from techniques like cyclic voltammetry (Nagao *et al*., 2020). This high resolution, combined with the possibility of optimizing experimental parameters, makes DPV ideal for analyzing complex mixtures, where the presence of different electroactive species may complicate individual compound identification. Furthermore, DPV is extremely sensitive, capable of detecting very low analyte concentrations, making it a valuable tool for analyzing samples with low antioxidant content.

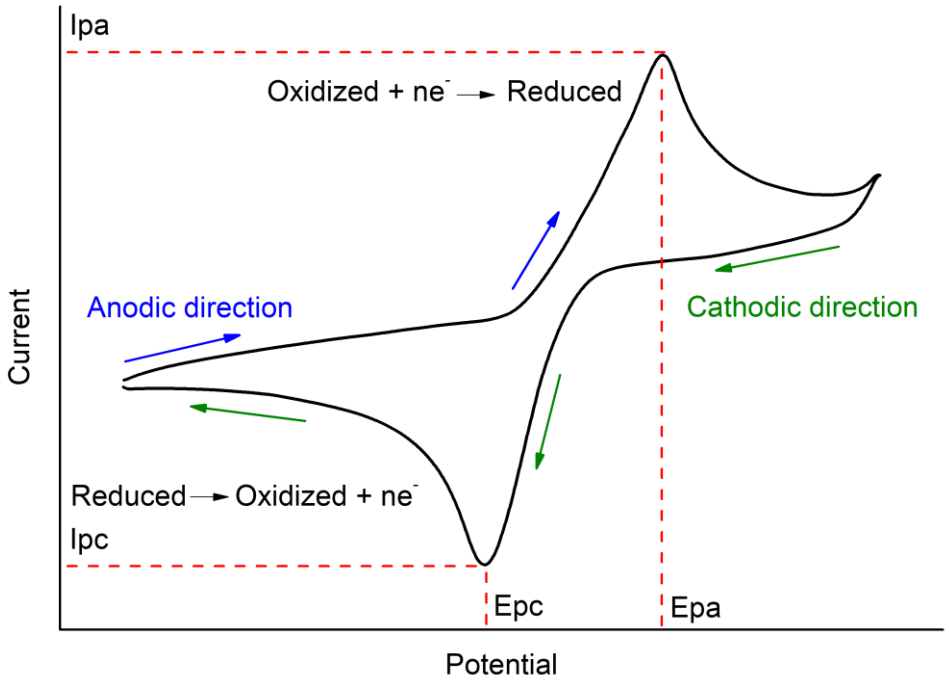

Figure 2 - Example of a cyclic voltammogram. The blue arrows indicate the anodic potential scan direction, while the green arrows represent the cathodic potential scan direction. E_{pa}: anodic peak potential; E_{pc}: cathodic peak potential; I_{pa}: anodic peak current; I_{pc}: cathodic peak current.

Square-Wave Voltammetry (SWV) is another highly sensitive and versatile electrochemical technique widely used for analyzing electroactive compounds, including antioxidants. It excels in providing detailed information about the redox processes occurring at the electrode-solution interface (Newair *et al.*, 2023). In SWV, the potential applied to the electrode varies as a series of square pulses superimposed on a staircase ramp. At each step of the ramp, two pulses of the same amplitude but opposite polarity are applied, and the current is measured at the end of each pulse pair. This pulse sequence produces voltammograms with sharper peaks and a significantly higher signal-to-noise ratio than other voltammetric techniques, such as cyclic voltammetry (Figure 3).

SWV is distinguished by its exceptional sensitivity, enabling the precise detection of minimal analyte concentrations (Maksimova, 2016). Its high resolution allows differentiation between very close electrochemical peaks, even in complex mixtures. Another advantage is its fast analysis time, making it suitable for applications requiring high throughput. Additionally, SWV minimizes interference from capacitive currents, resulting in clearer and more accurate signals. Its versatility allows for application across various electrochemical systems, in both aqueous and non-aqueous solutions.

The techniques of cyclic voltammetry, differential pulse voltammetry, and square-wave voltammetry play essential roles in the analysis of molecules relevant to human health. Each method has unique characteristics that make it suitable for specific applications, varying in sensitivity, complexity, and the type of sample analyzed. Selecting the most appropriate technique requires consideration of the analyte's specific properties, the sample matrix, and the study's objectives. Combining and appropriately using these electroanalytical techniques can provide a more comprehensive evaluation of the pharmacokinetics and efficacy of new drugs, monitor critical contaminants and biomarkers for human health, and contribute to advancements in therapies and diagnostics.

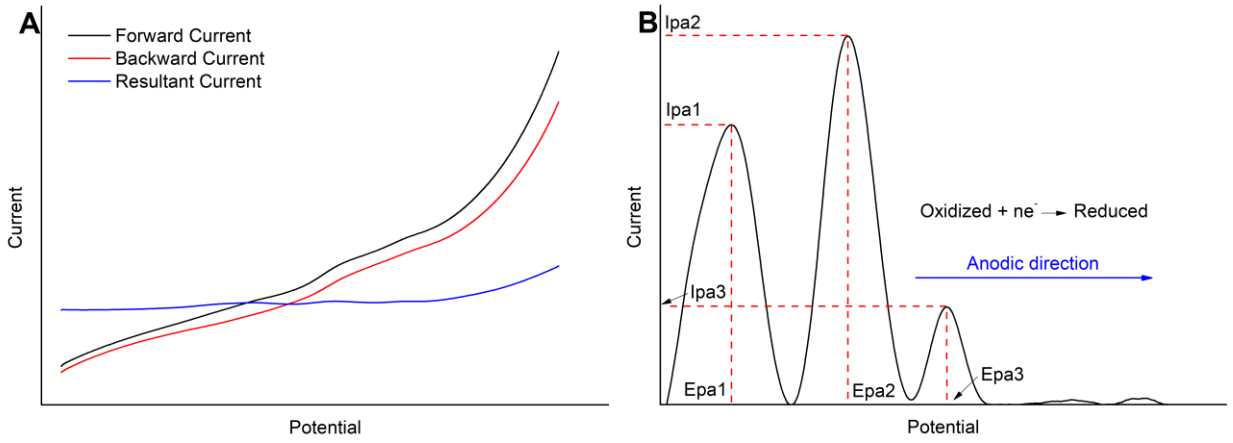

Figure 3 - Example of a square-wave voltammogram. A: Current in the positive direction (black), current in the negative direction (red), and resulting current (blue). B: After processing with baseline correction and smoothing tools, the resulting voltammogram with anodic peaks is obtained. In this example, three anodic peak currents and their respective anodic peak potentials are observed. The same processing and observation apply when the voltammogram is obtained in the cathodic direction.

3.3 Application of Electroanalytical Methods

Electrochemistry serves as a fundamental basis for various methods of evaluating antioxidant capacity. Traditionally, electron transfer in antioxidant capacity assays relied on redox reactions occurring in natural extracts. However, an innovative approach involves assessing antioxidant activity through electrochemical reactions on electrode surfaces (Blasco *et al*., 2007).

Redox Reaction (traditional): An oxidant gains an electron from the antioxidant, becoming reduced, while the antioxidant loses an electron and becomes oxidized. This reaction can be represented as follows:

$$\text{Oxidant} + e^- \text{ (from the antioxidant)} \rightarrow \text{Reduced Oxidant} + \text{Oxidized Antioxidant}$$

Electrochemical Reaction (new approach): The antioxidant loses an electron to the electrode, becoming oxidized. This reaction is represented as:

$$\text{Antioxidant} - e^- \text{ (to the electrode)} \rightarrow \text{Oxidant}$$

Voltammetry is widely used to characterize phenolic compounds and estimate the relative reducing capacity of flavonoids, phenolic acids, and other antioxidants in various matrices, including food, beverages, and biological samples. Zhang *et al*. (2011) investigated the electrochemical properties of 14 standard flavonoids, analyzing the oxidation potential and the area under the anodic wave. These data were compared to widely used spectrophotometric methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu (FC), FRAP (Ferric Reducing Antioxidant Power), and TEAC (Trolox Equivalent Antioxidant Capacity) to understand differences in antioxidant activity depending on the applied method. The results indicated that charge transfer efficiency during the first oxidation step was a key determinant for DPPH, FC, and FRAP assays, while TEAC was influenced by the total charge transfer in multiple oxidation steps. Furthermore, bond dissociation enthalpy values significantly correlated with the DPPH assay results ($r = 0.89$) but showed a weaker correlation with TEAC ($r = 0.57$), highlighting structural differences in antioxidant activity.

A study by Alcalde *et al*. (2019) examined the antioxidant capacity of polyphenols based on their chemical structures. This study employed various methods, including FC, FRAP, and TEAC, to identify differences related to the number and position of hydroxyl groups in polyphenols. Additionally, voltammetric methods using screen-printed carbon electrodes were recorded in the range of -0.2 to 0.9 V (relative to the Ag/AgCl reference electrode) to analyze the oxidation behavior of these substances. The results revealed weak correlations between the different tests, indicating that the compounds exhibited varied behaviors in response to the methodologies employed.

In the food sector, Yakovleva *et al*. (2007) used CV to characterize phenolics and flavonoids, while Artega *et al*. (2012) conducted a comprehensive study on low-molecular-weight antioxidants found in spices and pharmaceuticals. Among the compounds analyzed were gallic acid, sesamol, eugenol, thymol, carvacrol, vanillin, salicylaldehyde, limonene, geraniol, and 4-hexylresorcinol. Kilmartin *et al*. (2001) and Makhotkina *et al*. (2010) used voltammetric techniques with carbon electrodes to quantify antioxidants in wines. Anodic peak analysis was performed in wine model solutions with the addition of antioxidant standards at concentrations ranging from 0.1 to 0.2 mM. The results indicated that this approach effectively evaluates the antioxidant capacity of both red and white wines.

Kilmartin (2001) introduced CV in an acidic medium as an effective and rapid method to evaluate antioxidant capacity in complex mixtures, such as blood serum. This technique is based on the electrochemical oxidation of phenolic compounds present in the sample, where the most easily oxidized compounds, i.e., those with lower oxidation potentials, contribute more significantly to the total antioxidant capacity. This approach stands out for its speed, operational simplicity, and ability to provide relevant information about the antioxidant profile of biological matrices.

Rene *et al*. (2010) contributed to understanding phenolic oxidation mechanisms by investigating anodic peak potentials as a function of pH. The study proposed mechanisms involving proton and radical transfer depending on the polyphenol structure. Compounds containing o-diphenolic rings, such as flavonoids, exhibited the highest reactivity. These findings were corroborated by Simic *et al*. (2007), who analyzed phenolic compounds such as caffeic, salicylic, and coumaric acids, along with quercetin and rutin. The analysis revealed that compounds with multiple hydroxyl substitutions and conjugation exhibited greater antioxidant activity and low oxidation potentials (E_{pa} < 0.45 V). Compounds with higher potentials (E_{pa} > 0.45 V), however, displayed pro-oxidant behavior.

In biological samples, Photinon *et al*. (2010), highlighted caffeic acid as a model for determining phenolics and their antioxidant activity using a carbon electrode modified with iridium in thick-film sensors. The oxidation mechanism of this compound was studied in microfluidic systems coupled with mass spectrometry, enabling advances in antioxidant characterization. In subsequent studies by Beer *et al*. (2004), the total content of easily oxidizable phenols was calculated using the area under the voltammetric peak up to 500 mV (denoted Q_{500}). The results demonstrated a strong correlation with values measured by the Folin-Ciocalteu method, a widely recognized method for quantifying total phenols. Thus, voltammetry proved to be a reliable alternative for phenol analysis, with the potential to complement or replace traditional methods in certain applications, especially in studies involving antioxidants.

Voltammetric methods have shown promise for clinical studies, enabling the analysis of antioxidant capacity in various contexts and biological matrices. A pioneering study with human and equine plasma used a simple glassy carbon electrode to evaluate antioxidant activity (Martinez *et al*., 2006). For human plasma, responses revealed two broad voltammetric peaks in the potential ranges of 0.2–0.6 V and 0.6–0.9 V. In contrast, equine plasma showed no voltammetric signals when analyzed with an unactivated glassy carbon electrode. Key parameters such as peak potential (E_p), peak current density (i_p), and charge (Q) under the voltammetric waves were determined for each wave. These results highlighted marked differences in the concentration of low-molecular-weight antioxidants with low redox potentials between the two species.

Voltammetry has been extensively applied to investigate antioxidant status and oxidative stress in various clinical conditions, including healthy individuals, patients with chronic diseases

such as diabetes mellitus, and patients in acute situations, such as those undergoing total body irradiation before bone marrow transplantation. This technique has proven effective for rapid health status evaluation, enabling treatment monitoring, nutritional supplementation assessment, and even population screening. Additionally, voltammetry has been used to monitor the total oxidant scavenging capacity in plasma from patients with glycogen storage disease type Ia (Von Gierke's disease), focusing on uric acid quantification, recognized as one of the most effective antioxidants in the body (Koren *et al*., 2009). These examples illustrate the broad applicability of voltammetry in clinical contexts and its ability to provide detailed and relevant information on antioxidant activity in various conditions and biological matrices.

Square-wave voltammetry (SWV) has been widely used to investigate antioxidants, offering high sensitivity and specificity in various applications. Pohanka *et al*. (2009) employed SWV to analyze low-molecular-weight antioxidants (LMWAs) in the blood plasma of five black vultures (*Aegypius monachus Linnaeus*) accidentally intoxicated with lead. Blood samples were collected before and one month after treatment with Ca-EDTA. The voltammograms revealed two peaks: the first, at a potential of 466 ± 15 mV, was attributed to ascorbic and uric acids, while the second, at a potential of 743 ± 30 mV, indicated the presence of glutathione, tocopherol, ascorbic acid, and, to a lesser extent, uric acid. Separate assays with LMWA standards identified the individual contributions of each antioxidant.

An internal SWV analysis using printed graphite electrodes was conducted on blood samples from a 6-year-old patient diagnosed with neuroblastoma, a malignant disease of the head region (Cahová-Kuchaříková *et al*., 2005). The voltammogram revealed two peaks, labeled a1 and a2, located at 562 ± 20 mV and 839 ± 17 mV, respectively. The mean peak areas were 6.88×10^{-3} μA/V for peak a1 and 4.24×10^{-3} μA/V for peak a2. While peak a1 was present in all samples, peak a2 was almost absent in some, suggesting that the pathology may be associated with a specific group of endogenous antioxidants whose role requires further understanding.

4. Conclusions and Future Perspectives

Electroanalytical methods have emerged as essential tools for determining antioxidant capacity in biological samples, particularly in blood. Their ability to precisely measure antioxidant compounds and assess oxidative stress levels positions these techniques as foundational for advancements in clinical medicine and health research. Electrochemical biosensors have demonstrated transformative potential in clinical diagnostics due to their sensitivity, speed, and real-time analysis capabilities. These devices combine biological recognition elements, such as enzymes, antibodies, or DNA, with electrochemical platforms to detect specific compounds, such as low molecular weight antioxidants.

The use of nanomaterials, such as carbon nanotubes, graphene, and metallic nanoparticles, has enabled the construction of more selective and sensitive biosensors with detection limits that meet the demands of analysis in complex biological matrices like blood plasma. Furthermore, the use of modified surfaces, such as printed carbon electrodes or chitosan-based composites, enhances the ability to monitor a wide range of antioxidants, including ascorbic acid, glutathione, and tocopherol. These technological advancements make biosensors instrumental in rapid and effective diagnostics, particularly for conditions associated with oxidative stress, such as cardiovascular diseases, diabetes, and cancer. The ability to directly measure oxidative damage in biomolecules like DNA and lipids also highlights their relevance in early pathology identification and treatment monitoring.

The integration of electroanalytical methods with portable devices, also known as point-of-care (POC) devices, represents a significant evolution in the accessibility and efficiency of clinical diagnosis. These devices allow for analysis directly at the point of patient care, eliminating the need to transport samples to centralized laboratories. A promising example is the development of biosensors coupled with reading systems based on smartphones or portable devices, providing fast and easily interpretable results. Digital connectivity also enables real-time data transmission to medical platforms or health apps, allowing for remote and continuous monitoring of patients'

clinical status. This technology is particularly useful in emergency medical situations, rural communities, or regions with limited healthcare infrastructure, where the availability of rapid diagnostics can save lives. Additionally, POC devices offer advantages in screening at-risk populations, enabling early detection of oxidative stress-related conditions and facilitating preventive interventions.

Electroanalytical methods also play an increasing role in public health research and personalized medicine. In epidemiological studies, VC or VOQ-based biosensors can be used to measure antioxidant capacity on a large scale, providing valuable insights into the prevalence of risk factors in specific populations. For example, the relationship between oxidative stress levels and chronic diseases can be more effectively investigated with these tools, contributing to data-driven health policies.

In personalized medicine, these methods are even more promising. The ability to monitor individual antioxidant activity allows for adjustments in antioxidant treatments, dietary modifications, or specific supplementation, leading to more effective outcomes. Moreover, in therapies targeting oxidative stress, such as those for cancer or neurodegenerative diseases, biosensors can directly assess the body's response to treatment, enabling real-time adjustments. Analyzing antioxidants directly in patient blood can also be combined with other biomarkers, providing a comprehensive overview of health status. This is particularly important for conditions involving multiple physiological systems, such as diabetes or cardiovascular diseases, where oxidative stress plays a central role.

Despite the promising applications, some barriers still need to be overcome for these devices to be widely adopted. Challenges include the cost of manufacturing high-precision sensors, the need for standardization for clinical validation, and the integration of electrochemical results with other diagnostic tools. Future innovations include the development of fully automated systems for portable laboratories, with biosensors capable of simultaneously analyzing multiple biomarkers. Integration with artificial intelligence technologies may also enhance data interpretation, creating decision support systems for healthcare professionals. Another promising direction is the use of sustainable and biodegradable materials in sensor design, reducing environmental impact and promoting a more eco-friendly approach for disposable devices.

References

Alcalde, B., Granados, M., & Saurina, J. (2019). Exploring the Antioxidant Features of Polyphenols by Spectroscopic and Electrochemical Methods. *Antioxidants*, *8*(11), 523. https://doi.org/10.3390/antiox8110523

Allegra, M. (2021). Redox Systems, Oxidative Stress, and Antioxidant Defences in Health and Disease. *Antioxidants*, *10*(12), 1955. https://doi.org/10.3390/antiox10121955

Arteaga, J. F., Ruiz-Montoya, M., Palma, A., Alonso-Garrido, G., Pintado, S., & Rodríguez-Mellado, J. M. (2012). Comparison of the Simple Cyclic Voltammetry (CV) and DPPH Assays for the Determination of Antioxidant Capacity of Active Principles. *Molecules*, *17*(5), 5126–5138. https://doi.org/10.3390/molecules17055126

Beer, D. D., Harbertson, J. F., Kilmartin, P. A., Roginsky, V., Barsukova, T., Adams, D. O., Waterhouse, A. L. (2004). Phenolics: A Comparison of Diverse Analytical Methods. *American Journal of Enology and Viticulture*, *55*(4). https://doi.org/10.5344/ajev.2004.55.4.389

Blasco, A., González Crevillén, A., González, M., & Escarpa, A. (2007). Direct Electrochemical Sensing and Detection of Natural Antioxidants and Antioxidant Capacity in Vitro Systems. *Electroanalysis*, *19*(22), 2275–2286. https://doi.org/10.1002/elan.200704004

Cahová-Kuchaříková, K., Fojta, M., Mozga, A. A., & Paleček, E. (2005). Use of DNA Repair Enzymes in Electrochemical Detection of Damage to DNA Bases in Vitro and in Cells. *Analytical Chemistry*, *77*(9), 2920–2927. https://doi.org/10.1021/ac048423x

Chevion, S., Roberts, M. A., & Chevion, M. (2000). The use of cyclic voltammetry for the evaluation of antioxidant capacity. *Free Radical Biology and Medicine*, *28*(6), 860–870. https://doi.org/10.1016/s0891-5849(00)00178-7

Dorozhko, E. V., & Korotkova, E. I. (2011). Biologically active substances studied by voltammetric and spectrophotometric techniques. *Pharmaceutical Chemistry Journal*, *44*(10), 581–584. https://doi.org/10.1007/s11094-011-0521-2

Ferreira, M., Varela, H., Torresi, R. M., & Tremiliosi-Filho, G. (2006). Electrode passivation caused by polymerization of different phenolic compounds. *Electrochimica Acta*, *52*(2), 434–442. https://doi.org/10.1016/j.electacta.2006.05.025

Fischer, M. A. J. G., Gransier, T. J. M., Beckers, L. M. G., Bekers, O., Bast, A., & Haenen, G. R. M. M. (2005). Determination of the antioxidant capacity in blood. *Clinical Chemistry and Laboratory Medicine*, *43*(7), 735–740. https://doi.org/10.1515/CCLM.2005.125

Gęgotek, A., Jastrząb, A., Dobrzyńska, M., Biernacki, M., & Skrzydlewska, E. (2021). Exogenous Antioxidants Impact on UV-Induced Changes in Membrane Phospholipids and the Effectiveness of the Endocannabinoid System in Human Skin Cells. *Antioxidants*, *10*(8), 1260. https://doi.org/10.3390/antiox10081260

Gil, E. S., & Couto, R. O. (2013). Flavonoid electrochemistry: a review on the electroanalytical applications. *Revista Brasileira de Farmacognosia*, *23*(3), 542–558. https://doi.org/10.1590/s0102-695x2013005000031

Hoyos-Arbeláez, J., Vázquez, M., & Contreras-Calderón, J. (2017). Electrochemical methods as a tool for determining the antioxidant capacity of food and beverages: A review. *Food Chemistry*, *221*, 1371–1381. https://doi.org/10.1016/j.foodchem.2016.11.017

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & Pérez-Lebeña, E. (2021). The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *International Journal of Molecular Sciences*, *22*(9), 4642. https://doi.org/10.3390/ijms22094642

Kilmartin, P. A. (2001). Electrochemical Detection of Natural Antioxidants: Principles and Protocols. *Antioxidants & Redox Signaling*, *3*(6), 941–955. https://doi.org/10.1089/152308601317203495

Koren, E., Lipkin, J., Klar, A., Hershkovitz, E., Ginsburg, I., & Kohen, R. (2009). Total oxidant-scavenging capacities of plasma from glycogen storage disease type Ia patients as measured by cyclic voltammetry, FRAP and luminescence techniques. *Journal of Inherited Metabolic Disease*, *32*(5), 651–659. https://doi.org/10.1007/s10545-009-1242-5

Lima, A. P., Santos, Edson Nossol, Richter, E. M., & Rodrigo A.A. Munoz. (2020). Critical evaluation of voltammetric techniques for antioxidant capacity and activity: Presence of alumina on glassy-carbon electrodes alters the results. *Electrochimica Acta*, *358*, 136925–136925. https://doi.org/10.1016/j.electacta.2020.136925

Makhotkina, O., & Kilmartin, P. A. (2010). The use of cyclic voltammetry for wine analysis: Determination of polyphenols and free sulfur dioxide. *Analytica Chimica Acta*, *668*(2), 155–165. https://doi.org/10.1016/j.aca.2010.03.064

Maksimova, V. (2016). Electrochemical Evaluation of the Synergistic Effect of the Antioxidant Activity of Capsaicin and Other Bioactive Compounds in Capsicum sp. Extracts. *International Journal of Electrochemical Science*, 6673–6687. https://doi.org/10.20964/2016.08.34

Martinez, S., Valek, L., Rešetić, J., & Ružić, D. F. (2006). Cyclic voltammetry study of plasma antioxidant capacity – Comparison with the DPPH and TAS spectrophotometric methods. *Journal of Electroanalytical Chemistry*, *588*(1), 68–73. https://doi.org/10.1016/j.jelechem.2005.12.016

Milardovic, S., Kereković, I., & Rumenjak, V. (2007). A flow injection biamperometric method for determination of total antioxidant capacity of alcoholic beverages using bienzymatically produced ABTS+. *Food Chemistry*, *105*(4), 1688–1694. https://doi.org/10.1016/j.foodchem.2007.04.056

Morris, B. (2003). The components of the Wired Spanning Forest are recurrent. *Probability Theory and Related Fields*, *125*(2), 259–265. https://doi.org/10.1007/s00440-002-0236-0

Nagao, H., Carlos, Coldibeli, B., & Sartori, E. R. (2020). A differential pulse voltammetric method for submicromolar determination of antihistamine drug desloratadine using an unmodified boron-doped diamond electrode. *Analytical Methods*, *12*(8), 1115–1121. https://doi.org/10.1039/c9ay02785h

Newair, E. F., Al-Anazi, A., & Garcia, F. (2023). Oxidation of Wine Polyphenols by Electrochemical Means in the Presence of Glutathione. *Antioxidants*, *12*(10), 1891. https://doi.org/10.3390/antiox12101891

Photinon, K., Chalermchart, Y., Khanongnuch, C., Wang, S.-H., & Liu, C.-C. (2010). A Thick-film Sensor as a Novel Device for Determination of Polyphenols and Their Antioxidant Capacity in White Wine. *Sensors*, *10*(3), 1670–1678. https://doi.org/10.3390/s100301670

Pisoschi, A. M., Cimpeanu, C., & Predoi, G. (2015). Electrochemical Methods for Total Antioxidant Capacity and its Main Contributors Determination: A review. *Open Chemistry*, *13*(1). https://doi.org/10.1515/chem-2015-0099

Pohanka, M., Bandouchova, H., Sobotka, J., Sedlackova, J., Soukupova, I., & Pikula, J. (2009). Ferric Reducing Antioxidant Power and Square Wave Voltammetry for Assay of Low Molecular Weight Antioxidants in Blood Plasma: Performance and Comparison of Methods. *Sensors*, *9*(11), 9094–9103. https://doi.org/10.3390/s91109094

René, A., Marie-Laurence Abasq, Didier Hauchard, & Philippe Hapiot. (2010). How Do Phenolic Compounds React toward Superoxide Ion? A Simple Electrochemical Method for Evaluating Antioxidant Capacity. *Analytical Chemistry*, *82*(20), 8703–8710. https://doi.org/10.1021/ac101854w

Sazhina, N. N. (2017). Determination of Antioxidant Activity of Various Bioantioxidants and Their Mixtures by the Amperometric Method. *Russian Journal of Bioorganic Chemistry*, *43*(7), 771–775. https://doi.org/10.1134/s1068162017070147

Simić, A., Manojlović, D., Šegan, D., & Todorović, M. (2007). Electrochemical Behavior and Antioxidant and Prooxidant Activity of Natural Phenolics. *Molecules*, *12*(10), 2327–2340. https://doi.org/10.3390/12102327

Suh, H.-J., Kim, S.-R., Hwang, J.-S., Kim, M. J., & Kim, I. (2011). Antioxidant activity of aqueous methanol extracts from the lucanid beetle, Serrognathus platymelus castanicolor Motschulsky (Coleoptera: Lucanidae). *Journal of Asia-Pacific Entomology*, *14*(1), 95–98. https://doi.org/10.1016/j.aspen.2010.10.002

Yakovleva, K.E., Kurzeev, S.A., Stepanova, E.V. et al. (2007). Characterization of plant phenolic compounds by cyclic voltammetry. *Applied Biochemistry and Microbiology*, *43*(6), 661–668. https://doi.org/10.1134/s0003683807060166

Zhang, D., Le, C., Liu, Y., Wang, A., Ji, B., Wu, W., Zhou, F., Wei, Y., Cheng, Q., Cai, S., Xie, L., & Jia, G. (2011). Analysis of the Antioxidant Capacities of Flavonoids under Different Spectrophotometric Assays Using Cyclic Voltammetry and Density Functional Theory. *Journal of Agricultural and Food Chemistry*, *59*(18), 10277–10285. https://doi.org/10.1021/jf201773q