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A versatile electrochemical sensor for quality control and antioxidant evaluation of cosmetic ingredients and formulations.

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1. Introduction

Oxidative stress plays a pivotal role in a wide range of biological processes and diseases, resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses. ROS, including superoxide anion ($O_2•^-$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), are reactive molecules that, when present in excess, can damage cellular components such as lipids, proteins, and DNA. This damage is associated with aging, inflammatory diseases, and skin disorders, underscoring the importance of antioxidant systems in maintaining cellular health [1,2].

To mitigate oxidative stress, the body relies on a network of antioxidant defenses, including enzymes like Superoxide Dismutase (SOD) and Catalase (CAT). SOD catalyzes the dismutation of superoxide radicals into less reactive molecules, while CAT converts H_2O_2 into water and oxygen, thus preventing further oxidative damage (Figure 1). Despite the efficiency of these enzymatic systems, the measurement of antioxidant activity, especially in complex matrices such as skin, remains a significant challenge for researchers and formulators in cosmetic and pharmaceutical industries [3,4].

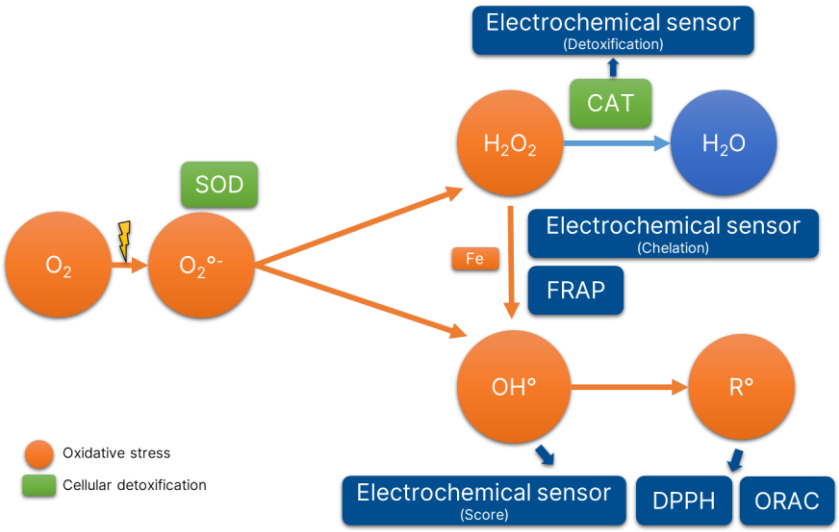

Figure 1: Oxidative Stress and Antioxidant Mechanisms.

Conventional methods for evaluating antioxidant capacity, such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ORAC (Oxygen Radical Absorbance Capacity), and FRAP (Ferric Reducing Antioxidant Power), are widely used but each has limitations. DPPH measures radical scavenging ability, ORAC quantifies peroxyl radical scavenging, and FRAP assesses the reducing power related to metal ion chelation (Figure 2). However, these assays often fail to detect active compounds with complex properties, such as amphiphilic antioxidants or those that work through mechanisms other than radical scavenging [5,6].

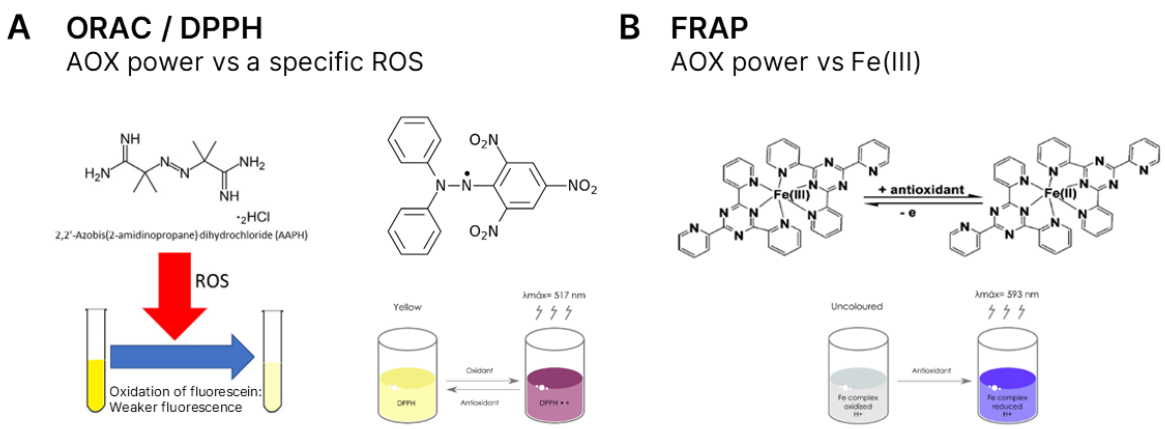

Figure 2: Principle of antioxidant capacity assessment by (A) ORAC (Oxygen Radical Absorbance Capacity), DPPH (2,2-diphenyl-1-picrylhydrazyl), and (B) FRAP (Ferric Reducing Antioxidant Power). DPPH measures radical scavenging ability, ORAC quantifies peroxyl radical scavenging, and FRAP assesses the reducing power related to metal ion chelation.

To address these limitations, an innovative electrochemical sensor offers a novel, electrochemical-based approach to antioxidant evaluation [7]. The electrochemical sensor integrates a dual-readout system, providing both a comprehensive electrochemical profile and a functional H_2O_2 detoxification assay. This platform not only detects antioxidant reactivity in a more nuanced manner but also simulates the detoxification of H_2O_2, mimicking the activity of CAT-like enzymes (Figure 1). This study explores the potential of the electrochemical sensor to complement existing antioxidant assays, providing deeper insights into the antioxidant capacity of cosmetic ingredients, particularly those that are difficult to assess with traditional methods.

2. Materials and Methods

Sample selection

A total of approximately 100 samples were selected for antioxidant evaluation, representing a diverse range of cosmetic ingredients. These included botanical extracts, marine extracts, and pure reference compounds. The selection encompassed both hydrophilic and lipophilic ingredients commonly utilized in cosmetic formulations, providing a comprehensive representation of the types of samples typically encountered in the industry.

Test Conditions and Standardization

The electrochemical sensor operates under a standardized protocol, ensuring consistent and reproducible results. This electrochemical sensor-based method uses two types of solvent systems:

- **Hydrophilic medium:** Phosphate-buffered saline (PBS)
- **Amphiphilic medium:** A PBS/Acetone mixture (75:25, v/v)

Each sample was tested at a concentration of 1 mg/mL in these solvent systems. If the solubility of the sample allowed, it was tested in both media to assess how solubility and environment influenced antioxidant performance.

Sensor Electrochemical Evaluation

1. **Sample Application**

   Each sample was prepared by diluting it in the appropriate electrolyte (PBS or PBS/Acetone), then applying it to the electrochemical sensor.

2. **Electrochemical Profile**

   During the analysis, a potential sweep was applied to the sensor, and the resulting current was recorded, producing a voltammogram, also called electrochemical profile (Figure 3A). This profile displays oxidation peaks, which correspond to the presence of electrochemical species in the sample such as antioxidants. The oxidation potential provides insight into the strength of the reducing species present in the sample [8].

3. **H_2O_2 Detoxification Model**

   For functional antioxidant evaluation, the electrochemical sensor mimics catalase-like behavior by testing the detoxification capacity of samples against hydrogen peroxide (H_2O_2), a relevant ROS (Figure 3B). The detoxification process is measured by assessing the decrease in the peak associated with H_2O_2 oxidation. A decrease in this signal indicates the sample's ability to reduce H_2O_2 before it reaches the sensor, simulating a biological detoxification mechanism.

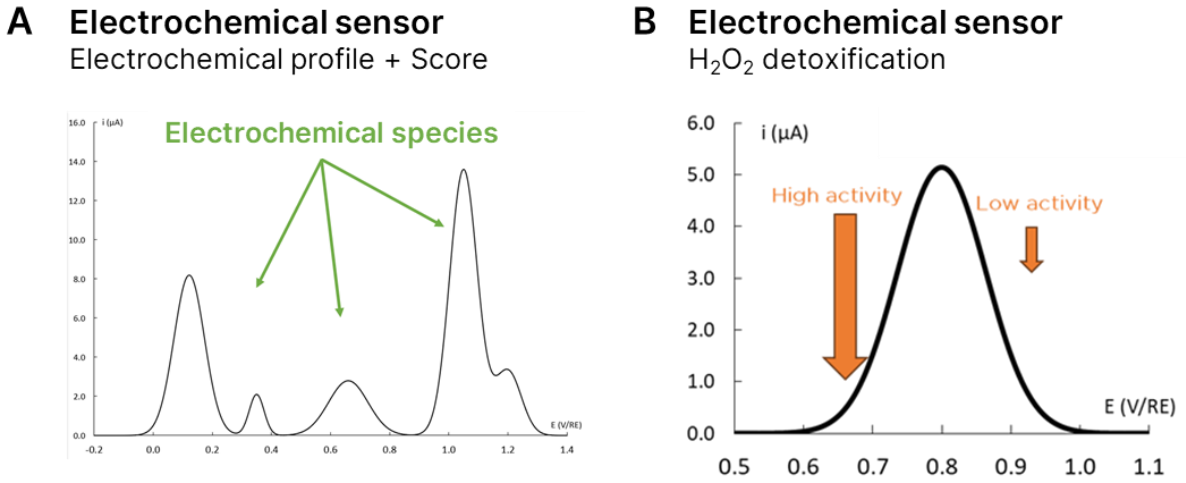

Figure 3: Principle of antioxidant capacity assessment by the electrochemical sensor. (A) Electrochemical profile + score. (B) H_2O_2 detoxification.

Conventional Antioxidant Assays

In addition to the electrochemical evaluation, traditional antioxidant assays were performed following widely accepted protocols to provide complementary data:

1. **DPPH Assay**

   The DPPH method quantifies the ability of antioxidants to neutralize the DPPH radical. The sample's antioxidant activity is measured by monitoring the decrease in absorbance at 517 nm after a 30-minute incubation at room temperature in the dark. The IC50 value is determined, representing the concentration of the sample required to inhibit 50% of the DPPH radical activity.

2. **ORAC Assay**

   The Oxygen Radical Absorbance Capacity (ORAC) assay measures the capacity of samples to neutralize peroxyl radicals generated by AAPH. A fluorescein probe is used, and fluorescence decay is monitored over time. The antioxidant capacity is then calculated relative to a Trolox standard curve, expressed in Trolox equivalents (µmol TE/g).

3. **FRAP Assay**

   The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the ability of samples to reduce Fe^{3+} to Fe^{2+}, resulting in the formation of a Fe^{2+}-TPTZ complex. This reaction is measured by absorbance at 593 nm, and antioxidant capacity is expressed in Trolox equivalents (µmol TE/g).

3. Results

3.1 Results Overview

The electrochemical sensor demonstrated promising results in assessing the electrochemical profile of a range of samples across different media. Electrochemical activity was detected in 33% of the samples in aqueous medium and 32% of the samples in amphiphilic medium. These findings suggest that adjusting sample concentration could enhance detection rates in both media.

Regarding detoxification activity, 38% of the samples exhibited measurable detoxification in aqueous medium, while 92% showed a detoxification activity in amphiphilic medium. This difference underscores the potential for lipophilic compounds to better express their antioxidant potential in amphiphilic environments. Optimizing test concentration in the standardized protocol could further improve the ability of the electrochemical sensor to reveal antioxidant potential without compromising sensor performance.

3.2 Correlations Observed

The study revealed positive correlations between the sensor's detoxification activity and traditional antioxidant assays:

- **Detoxification vs ORAC**: A positive correlation suggests that samples with stronger detoxification activity tend to also exhibit higher peroxyl radical neutralization.
- **Detoxification vs FRAP**: Similarly, detoxification activity was positively correlated with FRAP results, indicating that compounds with a higher redox potential are more effective at detoxifying reactive species.

However, no correlation was observed between the electrochemical profile and detoxification readouts from the electrochemical sensor, confirming that these two measurements are complementary. The electrochemical profile reflects intrinsic antioxidant activity, while the detoxification model focuses on the biological detoxification of H_2O_2, providing a broader view of a sample's antioxidant efficacy.

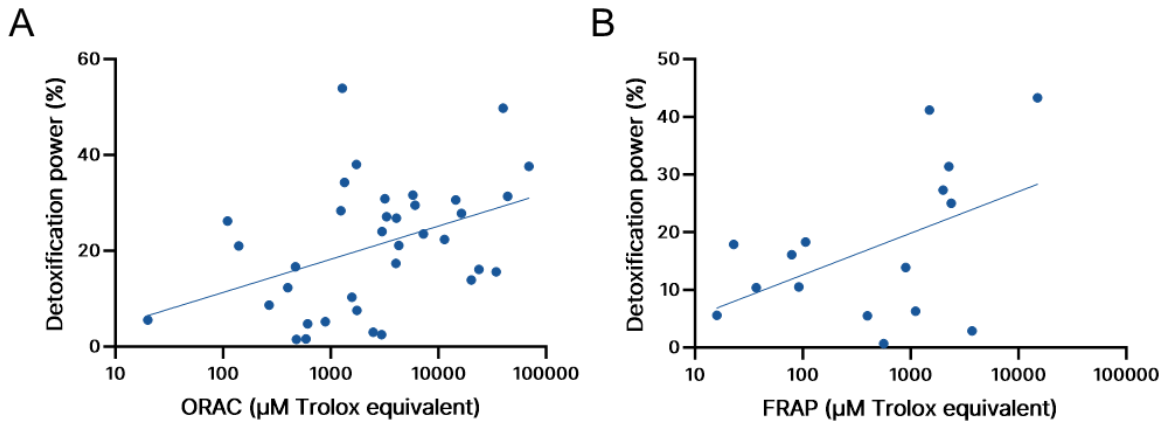

Figure 4: Correlation between the sensor's detoxification power and (A) ORAC or (B) FRAP. Nonlinear regression, semilog line.

4. Discussion

4.1 Methodological Considerations

The comparison between the electrochemical sensor and conventional antioxidant assays is limited by differences in the underlying mechanisms and sample preparation protocols. The electrochemical sensor measures electrochemical activity and biological detoxification, similar to catalase-like activity. On the other hand, methods like ORAC and DPPH focus on radical scavenging capacities (similar to SOD-like activity), while FRAP assesses redox potential and chelation, mimicking Fenton reaction inhibition. These methodologies, although valuable, should be seen as complementary rather than interchangeable due to their distinct mechanisms of action and targeted antioxidant pathways.

4.2 Advantages of the electrochemcial sensor

The sensor offers several distinct advantages over traditional antioxidant assays:

- **Simplicity and Speed**: The method is simple, rapid, and standardized, making it suitable for quality control screening and formulation guidance.
- **Versatility**: It is adaptable to various sample types, including both hydrophilic and lipophilic compounds, which are often challenging to assess with conventional methods.
- **Electrochemical Profile**: The electrochemical profile generated by the sensor provides valuable qualitative insights into a formulation's antioxidant activity, aiding in product development and QC.

- **Detoxification Model**: The detoxification model highlights the biological efficacy of samples in neutralizing H_2O_2, offering a functional measure of antioxidant potential that complements the results from other assays.

Together, these advantages make the electrochemical sensor a powerful tool for evaluating antioxidant activity in a more comprehensive and versatile manner.

5. Conclusion and Perspectives

The innovative electrochemical sensor offers unique and valuable insights into antioxidant activity, particularly for compounds that are underrepresented or missed by conventional methods such as ORAC, DPPH, and FRAP. It has the ability to:

- Identify active compounds that traditional assays may overlook
- Discriminate between compounds with similar antioxidant scores in classical assays

demonstrates its potential to provide a more comprehensive and nuanced understanding of antioxidant efficacy.

Future work could focus on the following areas to further enhance the capabilities of the electrochemical sensor:

- **Increasing test concentration**: By optimizing the test concentration, the detection rate of the electrochemical profile could be improved, which would also enhance detoxification performance and correlation with classical antioxidant assays.
- **Integration of the chelation model**: Incorporating the chelation model from the electrochemical sensor could provide valuable comparisons with FRAP results, broadening the scope of antioxidant evaluation and offering more insights into redox chemistry.

6. References

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