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Fast and Accurate Electrochemical Measurement of Total Antioxidant Capacity as an Alternative to Spectrophotometrical Methods

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Abstract

Total Antioxidant Capacity or TAC is an indicator of the sample ability to scavenge free radicals despite its complex composition. It has been measured in biological fluids as an inverse biomarker of oxidative stress, which has been related to disease. Classical spectrophotometric methods present some limitations including sample pretreatment required leading to long assay procedures, native pH alteration, low stability of some reagents, high detection limits, low sensitivity and sample's colour interference. Various electrochemical techniques have raised as more precise alternatives that overcome most of the limitations in classical methodologies and have been gaining popularity specially for food and beverage analysis. In this mini review, an electrochemical measurement of antioxidant capacity with applications *in vitro* and *in vivo* is presented.

Keywords: Total Antioxidant Capacity; Electrochemical; Voltammetry; Biological samples; Oxidative stress

Abbreviations: TAC: Total Antioxidant Capacity; TEAC: Trolox Equivalent Antioxidant Capacity, GAE: Gallic Acid Equivalents; CEAC: Vitamin C Equivalents Antioxidant Capacity

Introduction

Antioxidants are compounds that neutralize free radicals, very toxic by-products of cell metabolism, and so preventing the damage that they cause. This ability, which is usually referred to as total antioxidant capacity or TAC, depends on different parameters, for example, antioxidant concentration, molecular weight and the synergies among them [1]. The antioxidant capacity is an increasingly interesting biomarker since it is inversely proportional to oxidative stress.

Classic Methods

With the aim of determining the TAC of a sample, more than 25 assays have been developed until date [2]. They can be classified into direct (when a free radical is used) or indirect (if the reaction does not involve a free radical) [3]. In general, the basis of these assays is to put the sample in contact with a compound, which absorbs at a specific wavelength either in its oxidized or reduced form. Then, a measure of the absorbance gives the amount of the compound reduced by the sample. This antioxidant capacity is typically referred to the concentration of a model antioxidant like Trolox, gallic acid or ascorbic acid giving the following units: TEAC (Trolox Equivalent Antioxidant Capacity), GAE (Gallic Acid Equivalents) or CEAC (Vitamin C Equivalents Antioxidant Capacity). However, while many methods have been described, they still present some limitations. Firstly, the pH, solvents and temperatures set for the assay are usually different from the native conditions of the sample, and so antioxidant capacity may be affected [4]. Secondly, the size and complexity of the indicator affect the binding ability of the antioxidants, and so the larger and complex the indicator compound is, the higher the probability of underestimating the sample's TAC [5]. In addition, some key antioxidants cannot be measured with some of the classic techniques, like glutathione [6]. Finally, the combination of all the factors mentioned above, make antioxidant capacity obtained with the different methodologies not comparable among them, even if they were measured with the same units [7].

Electrochemical Methods

Electrochemistry is a very sensitive and reproducible technique that has been already described as a powerful alternative to

classical spectrophotometric methodologies [8]. It allows a quick measurement of TAC without modifying sample native conditions and could potentially be established as a standardized measurement of antioxidant capacity that overcomes the drawbacks of intercomparisons and uses an international system derivative unit for antioxidant capacity. From all the electrochemical methods, the most used are voltammetry, bioamperometry, amperometry, potentiometry and coulometry. Since those methods present lower detection limits, higher sensitivity and quickness, they have been gaining popularity in recent years, especially in the beverage's analysis field [9] (mainly wine [10-15], tea [16,17] and juice [10,18-20]) rather than in biological samples (urine [21,22], plasma [23-25] and blood [26,27]). The present portable device and voltammetric method comprises the application of increasing potentials and the measurement of the intensity of the current at each one. In this way, a complete oxidation of the sample is carried out. Individual peaks are considered the response of a specific antioxidant and through a mathematic algorithm a measure of the total antioxidant capacity of the sample obtained and expressed in micro-coulombs. The total charge of antioxidants is divided in two sections, from which the ones at a lower potential of oxidation are considered the fast and the others the slow antioxidants.

Conclusion

The measurement of TAC with electrochemical methods have already been described. However, they are not widely used within the scientific community. This new promising voltammetric method can be used at physiological pH, leading to a total determination of antioxidant capacity, with potential applications both *in vitro* and *in vivo*. Moreover, it is able to distinguish between slow and fast antioxidants, an interesting feature not present in other TAC assays.

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