Abstract

CUPRAC is a method used to determine antioxidants quantifying the chromophore \([\text{Cu(Nc)}_2]^+\), generated upon increases of added antioxidants to a \(\text{Cu(II)}\) containing solution. In this work, an electrochemical alternative to quantify this complex is presented using cyclic voltammetry and chronoamperometry, compared with the classical spectroscopic determinations by UV-Vis. The final results show that the analysis performed by electrochemical methodologies is statistically similar, affording an efficient determination the total antioxidant capacity.

Keywords: CUPRAC; Antioxidants; Electrochemical; Cyclic Voltammetry; Chronoamperometry.

1. Introduction

There is a great interest in studying the antioxidant capacity in foods, due to their properties to neutralize free radicals like Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which are involve in aging and degenerative diseases as cancer, Alzheimer and Parkinson.\(^1\) For this reason there is a particular interest in polyphenolic compounds, because they are the most commonly found in the nature.\(^2\) For example, Gallic acid (3,4,5-trihydroxybenzoic acid) and its derivatives can be present in foods and wines, and it is also used as food additive like...
natural antioxidant. In the other hand, flavonoids are other group of antioxidants broadly distributed in vegetables and fruits; many of these compounds present activity as: antioxidant, anti-inflammatory, anti-allergic, anti-cancer and anti-hemorrhagic. Since all these benefits to the health have been described, there is an increasing interest in studying and quantifying these compounds in wines, vegetables and fruits.

Antioxidant properties of phenols and flavonoids are important due to their use in biological and industrial processes. One method to quantify Total Antioxidant Capacity (CAT), introduced by Apak and co-workers, is Cupric Ion Antioxidant Capacity (CUPRAC) involve cupric ions –Cu(II)–, Neocuproine (Nc) and antioxidant molecules (AO) and can be represented as:

\[ nCu^{2+} + 2nNc + mAO_{red} \leftrightarrow n[Cu(Nc)_2]^+ + mAO_{ox} \]  

Quantification by this method is performed by measuring the variation of the amount of the complex \([Cu(Nc)_2]^+\) upon gradual increases of the amount of antioxidant added to the solution, which in turn is oxidized by Cu(II), this quantification can be developed by electrochemical methods as cyclic voltammetry and chronoamperometry. In this work, an alternative of the electrochemical quantification of the complex \([Cu(Nc)_2]^+\) - an electroactive chromophore – is presented.

### Nomenclature

- **Nc**: Neocuproine
- **WE**: Working electrode
- **GC**: Glassy carbon
- **CUPRAC**: Cupric Reducing Antioxidant Capacity
- **Ia**: Anodic Current
- **Ic**: Catodic current

### 2. Materials and Methods

#### 2.1. Reagents

Ammonium acetate (NH₄Ac, 98.0%), Neocuproine (Nc), CuCl₂ 2H₂O (99%), Trolox (TX, 97%); Methanol (99.99%) and Ethanol (99.99%), some of them from Sigma Aldrich, were employed.

#### 2.2. Solutions

CUPRAC solutions were prepared following the procedure to quantify the complex \([Cu(Nc)_2]^+\) using cyclic voltammetry, chronoamperometry and spectrophotometry.

### 3. Results and Discussion

#### 3.1. Spectroscopic and Electrochemical CUPRAC

CUPRAC solutions were analyzed by UV-Vis spectroscopy, upon addition of different volumes from a solution containing the antioxidant Trolox (Figure 1); the experimental response shows an increasing absorbance at 454 nm of CUPRAC solution as this signal is proportional to the concentration of the complex \([Cu(Nc)_2]^+\) in solution.

Solutions in the same conditions were studied by cyclic voltammetry and the voltammograms obtained are shown in Figure 2. In this case a reversible process, associated to the one electron reduction (peak Ic) of Cu(II) in solution. Upon addition of the antioxidant, the formation of the complex \([Cu(Nc)_2]^+\) leads to progressive increases of the oxidation peak Ia, without performing a previous electrochemical reduction. The current from this peak now becomes proportional to the amount of antioxidant added and was further used to perform the required quantification.
Fig. 1. Absorption spectra of CUPRAC solutions of increasing concentrations of Trolox, the arrow indicates the changes in the spectra with increasing concentration of added Trolox (0, 1.29x10^4, 2.5 x 10^5 and 4.18 x 10^4 mol dm^-3)

Cyclic voltamograms were used to establish the conditions to perform the potential pulses for chronoamperometric experiments as is previously reported.5

3.2. Analytical performance of CUPRAC techniques

The analytical performance of the three methodologies to quantify the CUPRAC reagent were evaluated, leading to linear correlations between the signal vs concentration comparison with correlation factors higher than 0.97;5 The Linear range of these functions were 0.11 x 10^-5 - 5.5 x 10^-5 mol dm^-3 for UV-Vis spectroscopy; 8.69 x 10^-5 - 80 x 10^-5 mol dm^-3 for cyclic voltammetry and 4.8 x 10^-5 – 80 x 10^-5 mol dm^-3 using chronoamperometric measurements; these values show that a wider range of work is achieved using electrochemical techniques. In terms of precision, the sequence obtained reveal that the highest correspond to UV-Vis spectroscopy, chronoamperometry and finally cyclic voltammetry with values of: 2.57, 2.78 and 9.42 mol dm^-3 x 10^-4 respectively. In terms of the trueness using
the “Student’s t” statistical test, the values obtained were 0.16 in cyclic voltammetry, 0.95 in chronoamperometry and 1.98 in spectroscopic, showing that there are no significant statistical differences between any technique employed.\(^5\)

### 3.3. Conclusions

All these studies and analysis were employed to find an alternative to quantify the chromophore CUPRAC, which is traditionally determined by spectrophotometric techniques. The obtained results showed it is possible to quantify the complex \([\text{Cu(Nc)}_2]\) employing electrochemical techniques as cyclic voltammetry, by following as the analytical signal the oxidation current. The analytical performance was studied to verify the difference between all the techniques, and one of the most important analyses was the Student’s t that does not show difference between them; besides the precision of electrochemical techniques is better. Also, electrochemical techniques have the advantage of being of use avoiding interferences like the presence of dyes in samples or analysis of samples with turbidity.

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### References


