Batch-injection analysis with amperometric detection of the DPPH radical for evaluation of antioxidant capacity

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Introduction

The antioxidant capacity is the measure between an antioxidant solution, which can be composed by a mixture of antioxidant compounds, and free radicals. The DPPH scavenging radical method is one of the most employed methods to analyze the antioxidant capacity in several samples. Generally, spectrophotometry has been used to measure the consumption of the DPPH radical; however, the electrochemical techniques also have been used for this purpose. The association of batch injection analysis (BIA) with amperometric detection is a powerful tool for the analysis of food, environmental and pharmaceutical samples[1]. In this work, we propose the use of BIA with amperometric detection to determine the antioxidant capacity of plant and tea samples based on the selective and sensitive amperometric measurement of DPPH˙ consumption.

Results and Discussion

The initial investigation of the electrochemical process of DPPH˙ and antioxidants in ethanol–acetate buffer solution was carried out by cyclic voltammetry. Amperometric measurements were performed using a homemade electrochemical batch-injection cell[1]. A mixture of 0.2 mol L^{-1} acetate buffer (pH 5.5) and ethanol (40:60 v/v) was used as supporting electrolyte solution in all electrochemical measurements. A potential of 0.05 V was chosen based on a hydrodynamic voltammetry using the BIA system. BIA parameters such as injection speed of the programmable micropipette and injected volume were evaluated. A repeatability study (n =12) was conducted to evaluate the precision of BIA method for DPPH˙ determination. After the selection of the optimal conditions for proposed method the determination of the total antioxidant capacity of two standard antioxidants (GA and BHT), two plants (Moringa and Brazilian cherry) and two teas were performed. EC_{50}, which is defined as efficient concentration of antioxidant to scavenge 50% DPPH radicals after 1 h reaction, was determined using amperometric measurements for DPPH˙ consumption by different concentrations of antioxidants. The results for standard the antioxidants butylhydroxytoluene (BHT and gallic acid (not shown) and real samples (Figure 1) shows the agreement between the proposed BIA and spectrophotometric methods based on the measurement of DPPH˙ consumption.

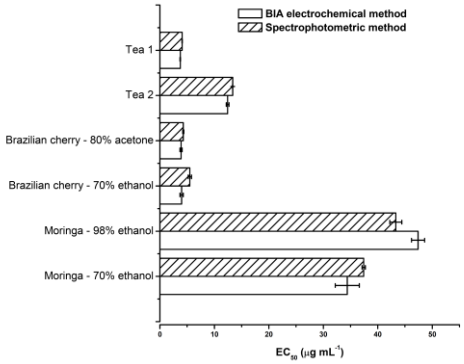

Figure 1. EC_{50} values for samples (Moringa, Brazilian cherry and teas) obtained by the proposed BIA electrochemical and spectrophotometric methods.

At the 95% confidence level, the calculated paired Student t-Test value (1.79) was smaller than the critical value (2.57, n=6), which indicates that there were no significant differences between the results. The lower EC_{50} (efficient concentration of sample to scavenge 50% DPPH radicals) indicates higher antioxidant capacity. Therefore, the Tea 1 sample presented higher antioxidant capacity than the Tea 2 sample, while the Moringa extracts presented lower antioxidant capacity than Brazilian cherry extracts.

Conclusions

This work demonstrated the first application of BIA with amperometric detection to determine the antioxidant capacity of real samples based on the measurement of DPPH˙ consumption. The BIA system with amperometric detection provided fast (180 h^{-1}), highly precise (RSD = 0.7%), sensitive and selective detection of DPPH˙, which was successfully applied to evaluate antioxidant capacity of tea and plant samples.

Acknowledgments

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