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Potentiality of chemometric approaches for the determination of (+)-catechin in green tea leaves at the surface of multiwalled carbon nanotube paste electrode

Saeed Masoum*, Mohsen Behpour, Fatemeh Azimi, Mohammad Hassan Motaghedifard

Faculty of Chemistry, Department of Analytical Chemistry, University of Kashan, Kashan, Islamic Republic of Iran

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ABSTRACT

Differential pulse voltammetry technique assisted by chemometric methods such as multivariate curve resolution-alternating least squares (MCR-ALS) has been proposed as a valuable approach for (+)-catechin determination in the presence of gallic acid at the surface of multiwalled carbon nanotube paste electrode. Central composite design and response surface methodology were used to optimize the influencing parameters. To determine (+)-catechin in the presence of unexpected electroactive interference with a very high degree of overlapping, second-order electrochemical data were generated by changing the pulse height as an instrumental parameter. After potential shift correction, MCR-ALS results show that second-order calibration could be applied with great success for electroanalytical determination of highly overlapped electroactive species. The linear least-squares calibration curve based on the area under concentration profile was provided over the range of 0.10–2.69 μM for (+)-catechin, whereas detection limit was found to be 0.017 μM. Also in this study, the effect of rotational ambiguity associated with a particular MCR solution under a set of constraints was investigated.

1. Introduction

The subject of quantitative analysis in complex and natural samples that consist of additional components, which are not expected and known as unexpected interferences, that is, those not included in calibration set, has been an interesting and important field in analytical chemistry [1]. In these cases, to cope with the problems related to high overlapping components signal, especially when non-modeled component present in sample, different multivariate data analysis techniques have been developed to quantify the analyte of interest accurately [2,3]. One of the interesting intrinsic property of second-order calibration methods is the so-called "second-order advantage," which permits analyte quantification even in the presence of unexpected interference and even it is implemented in well-known algorithms for the adequate processing of second-order data. Application of soft-modeling multivariate curve resolution (MCR) coupled with alternating least squares (ALS) in achieving the second-order advantage is proved to be a powerful technique for resolution and quantification of complex mixture [4]. MCR-ALS is a complex second-order multivariate chemometric method. One of the oldest chemometric procedures was introduced for two-component systems by Lawton and Silvestre in 1971 [5]. The necessity of using this method is justified when there are serious matrix interferences, due to the second-order advantage. The proposed method has been used in combination with voltammetric [6] and hyphenated systems [7] under the condition of extreme spectral overlapping among sample components and can be applied mathematically without using pre-defined chemical models and sample pre-treatment [8,9].

Phenolic compounds have been shown to play important roles in human health with attention to their potent antioxidant activity to scavenge free radicals [10]. High intake of green tea as a rich natural source of polyphenols, particularly flavonoids, has been reported to prevent or delay a number of many chronic diseases including cancer, cardiovascular, inflammation, and many degenerative diseases [11,12]. The main flavonoids present in green tea include catechins (Flavan-3-ols). Green tea also contains phenolic acid (such as gallic acid, caffeicacid, etc.) and flavonols (such as quercetin, myricetin, etc.). The beneficial effects associated with green tea have been attributed to its polyphenols, particularly catechins such as epigallocatechin-3-gallate (EGCG) and epigallocatechin (EGC or (+)-catechin), the first and second major catechins that are in vivo strong antioxidants [13,14]. It has been shown that green tea catechins enhance liver function [15,16], reduce body fat [13], and decrease body weight after long-term consumption (usually 12 weeks) [17]. Due to the specific chemical finding by green tea catechins, they consist of high-level antioxidant activity, even more than vitamin C and D [18]. Therefore, it is important to establish a simple, reliable, and free interference analytical

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* Corresponding author. Tel.: +98 3615912338.
E-mail address: masoum@kashanu.ac.ir (S. Masoum).

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method for the determination of (+)-catechin as the second main substance in green tea sample in developing high quality green tea product. Determination of polyphenolic compounds has been widely investigated in the food technology and human health fields. Traditional techniques such as spectrophotometery and HPLC [19] are expensive and time-consuming with low efficiencies. Thus, they are being replaced by other innovative techniques such as electrochemical approaches based on modified electrodes. Electrochemical reaction and adsorption of polyphenol species on multiwalled carbon nanotube (MWCNT) paste electrode surface have been studied for a long time and have been a matter of concern. Due to the similarity in the structures of (+)-catechin (epigallocatechin) flavonoid and gallic acid (phenolic acid), oxidation peak potential of gallic acid has strongly overlapped with (+)-catechin. Therefore, gallic acid is one of the usual interferences in the determination of (+)-catechin. The oxidation peak current of the catechin in a 0.10-M phosphate buffer solution (PBS) was optimized by experimental design for its determination in the presence of gallic acid. Response surface methodology (RSM) was applied to assess individual and interactive effects of the three main independent parameters on the obtained data efficiency. RSM uses an experimental design such as the central composite design (CCD) to fit a model using least-squares technique [20]. Satisfactoriness of the proposed model is then revealed using the diagnostic checking tests provided by analysis of variance (ANOVA).

In this research for the analysis of electrochemical data, we applied a simple method based on the changes in pulse height as an instrumental variable to generate electrochemical second-order data. Due to the non-bilinear behavior of the electrochemical data, the potential shift correction procedure is utilized for the correction of the observed shifts in the data. Then, MCR-ALS is applied on the corrected data to quantitative analysis of the analyte of interest using a series of constraints that ensure some physiochemical meaning to solution. The major limitation of all MCR methods is the rotational ambiguity due to not applying enough constraint to restrict the feasible band boundaries makes the estimated concentration of analyte of interest deviates noticeably from the accurate solution [21]. Calculation of maximum and minimum band boundaries of feasible solutions permits the evaluation of the effects of constraints on decreasing the rotational ambiguities associated with MCR solutions.

2. Experimental Procedures

2.1. Materials

The entire chemicals used in this investigation were of analytical grade: (+)-catechin (purity> 98%) and gallic acid (purity> 98%) from Sigma-Aldrich; phosphoric acid and sodium hydroxide from Merck. All standard solutions were prepared daily by dissolving in a small volume of buffer solution. Ultrapure water was prepared from a water purification system (smart 2 pure, TKA, Germany). The buffer solution (pH = 2) used as supporting electrolyte was prepared from 0.1 mol L^{-1} of phosphoric acid solution. The pH of buffer was adjusted by adding 0.1 mol L^{-1} of sodium hydroxide solution. Pure paraffin (>99%) from Merck was used as pasting liquid for the preparation of carbon paste electrode. The graphite powder (>99%) from Merck and MWCNTs from Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences (purity = 90%, 10–20 nm in diameter) were utilized as modifiers for the preparation of modified carbon paste as a working electrode.

2.2. Instrumentation and software

Differential pulse voltammograms were recorded by a potentiostat/galvanostat (SAMA 500, electroanalyzer system, Islamic Republic of Iran), which is connected to a three-electrode cell that had been linked to a computer running the SAMA software for the transformation of initial signals. The electrochemical cell was equipped with a modified paste electrode as a working electrode, an Ag/AgCl electrode (Metrohm) and a wire platinum electrode (Metrohm) as reference and counter electrode, respectively. The pH was measured with a 691 pH meter (Metrohm).

MCR-ALS was implemented using the graphical user interface provided by Tauler in Matlab environment [22] and MCRC software that presented by Jalali-Heravi group [23].

2.3. Fabrication of MWCNT-modified carbon paste electrode

The carbon paste was made by hand mixing of 0.5 g graphite powder with an appropriate amount (seven to eight drops, ∼ 0.2 mL) of paraffin oil in a mortar. For preparing modified carbon paste electrode, 0.002 g MWCNT was added to carbon paste and was mixed well for 30 min until a uniformly and homogeneously wetted paste was obtained. The modified carbon paste was packed into the end of a Teflon syringe. A new surface was obtained by pushing an excess of the paste out of the syringe and polishing with a weighing paper. Electrical contact was prepared by pushing a copper wire down into the syringe.

2.4. Experimental design and data analysis

A robustness study was achieved by means of an experimental design and multivariate analysis. A CCD was applied, which is composed of a two-level full or fractional factorial design, star points, and at least one point at the center of the experimental region (replicated center points) [24,25]. The star points allow the model to estimate the curvature response and are located at the center and both extreme levels of the experimental domain. In a full uniformly CCD, the number of runs is equal to $N = 2^f + 2f + r$, where f is the number of parameters and r is the replicate number of the central point. In this study, three parameters (amount of modifier, scan rate, and step potential) were investigated. All factors are studied in five levels. With this number of parameters and three center points, a complete CCD would result in a number of runs equal to 17. The experiments were fully randomized to avoid operator intervention and generate protection against the factors that may affect the obtained results, but are not of primary interest [26]. The structure of CCD allows the development of the second-order polynomial model that can be represented by the following equation [27,28]:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_{11} x_{11} + \beta_{22} x_{22} + \beta_{33} x_{33} + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

The predicted response (Y) is assessed as a function of three factors and was correlated with the set of effects, three first-order effects (using linear coefficient (β_i) related to x_1, x_2, and x_3 terms), three second-order effects (using quadratic coefficient (β_{ii}) related to x_{11}, x_{22}, and x_{33} terms), and three interaction effects (using interaction coefficient (β_{ij}) related to x_1x_2, x_1x_3 and x_2x_3 terms). Design and analysis of experiments were performed by the statistical software package, MINITAB® (Minitab Inc. Release 16.0).

2.5. Preparation of real sample

Using the hot water extraction method, only 2% w/w of the catechin is extracted, therefore, the following extraction procedure in the presence of 80% ethanol/water was implemented. In this case, the amount of catechin extracted from green tea leaves increases to 20% w/w. Therefore, to provide the green tea extract, 1.0 g green