

Simultaneous voltammetric determination of flavouring substances in Christmas spices

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A new electroanalytical method for the simultaneous determination of flavouring substances has been developed when utilising anodic oxidation of eugenol, myristicin, and anethole in an acidic medium in combination with square-wave voltammetry and the glassy carbon electrode. Electroanalytical performance of the respective method was verified on analysis of model solutions, as well as real samples of Christmas spices. In overall, the method proposed has shown some potential for implementation into analysis of medicaments, where one can find the above-specified substances as active pharmaceutical ingredients or flavouring agents, and where only minimal matrix effect can be expected.

Keywords: Square-wave voltammetry, Glassy carbon electrode; Simultaneous determination; Flavouring substances; Christmas spices.

Introduction

So-called Christmas spices is a blend of dried parts from medicinal plants used to flavour cosmetics, perfumes, sweet dishes, hot drinks, alcoholic beverages, and some pharmaceutical products. Typical ingredients in Christmas spices are: (common) anise, star anise, cloves, cinnamon, nutmeg, or vanilla beans. They usually contain one or more flavouring substances (aka essential oils) responsible for their typical smell, namely eugenol (EG) in cloves, myristicin (Myr) and elemicin (Ele) in nutmeg, *trans*-anethole (TAN) in star anise, *trans*-cinnam-aldehyde (TCA) in cinnamon, and vanillin (VA) in vanilla beans; see Figure 1. Extracts from these spices as

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tinctures or flavouring agents in the pure form are applied in traditional medicine. Regarding EG, it has local anaesthetic and antiseptic effects, thus being often used in dentistry. In addition, EG exhibits antioxidant, antimicrobial, inflammatory, anti-mutagenic and anti-cancerous properties [1].

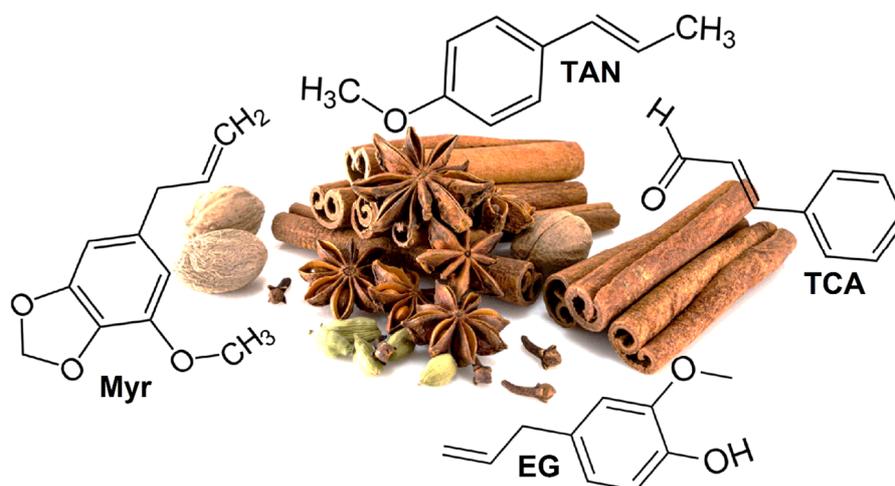


Fig. 1 Christmas spices (nutmeg, cinnamon, cloves, and star anise) with molecular structures of the respective aromatic substances

Myr and Ele are then known for their psychoactive effects due to similar chemical structure to 3,4-methylene-dioxymethamphetamine (MDMA) [2]. The third substance, TAN, is included in many cold and cough remedies because it promotes expectoration. As the main ingredient of syrups for newborns, TAN also supports the secretion of digestive enzymes, which has a beneficial effect on bloating and abdominal cramps [3]. Moreover, this compound helps with intestinal catarrh and speeds up the digestion process. In mid 2000s, it has been proved that both TCA and VA are dietary antimutagens effectively inhibiting spontaneous and induced mutations [4].

Since flavouring agents are volatile substances, various analytical methods, mainly based on gas chromatography coupled with mass spectrometry (GC-MS) in combination with the previous enrichment (microextraction) technique have been reported for their determination in food and pharmaceuticals [5]. Besides, several scientific reports admit a possibility to apply also electroanalytical methods because these substances are electrochemically active and capable of undergoing anodic oxidation at carbonaceous electrodes [6–11]. However, it is necessary to mention that hitherto reported voltammetric methods have been developed for the determination of flavouring substances as the single analytes. This has motivated us to focus our attention on a new approach applicable also to the simultaneous determination of three important aromatic substances, namely: EG, Myr and TAN.

As mentioned above, the principle of the corresponding procedure(s) would be the anodic oxidation at the glassy carbon electrode (GCE) in an acidic medium, when the performance of such electroanalytical method could be verified on analysis of Christmas spice extracts.

Experimental

Chemicals and reagents

Eugenol (99%), myristicin (analytical standard), and *trans*-anethole (99%) were purchased from Merck KGaA (Darmstadt, Germany). Perchloric acid (75%), hydrochloric acid (35%), sulfuric acid (96%), phosphoric acid (85%), glacial acetic acid (99.8%), boric acid, sodium hydroxide, and ethanol (96%) were obtained from Lach-Ner, s.r.o. (Neratovice, Czech Republic). An ultrapure water with resistivity lower than 18.3 M Ω cm was prepared in a Milli-Q[®] deionization unit from Merck Millipore (Burlington, MA, USA).

Instrumentation

Each voltammetric measurement was carried out in standard electrochemical cell containing 10 mL of 0.05 mol L⁻¹ HClO₄ as supporting electrolyte and incorporating the GCE (type "6.09395.014", Metrohm, Herisau, Switzerland) with a surface diameter of 3 mm, the reference Ag/AgCl/3 mol L⁻¹ KCl (the same supplier) and a Pt-sheet fixed in a glass rod (Elektrochemické detektory Turnov, Czech Republic) acting as auxiliary electrode. All the electrodes were connected to a potentiostat/galvanostat (Autolab/PGSTAT101) operated with Nova 1.11 software from already mentioned Metrohm company.

Sample preparation

Stock ethanolic solutions of 0.01 mol L⁻¹ standards (96% v/v) were prepared by dissolving their respective amounts in 10 mL volumetric flasks. Commercially available anise, clove, and nutmeg spices selected for demonstrative analysis were from Vitana, a.s. (Byšice, Czech Republic).

Regarding preparation of the samples, 2 g of each spice was ground for 10 min using a pestle and mortar from ceramics. The spice grits obtained were transferred into 50 mL volumetric flasks and filled up to the mark with 96% ethanol. The flavouring substances were extracted in an ultrasonic bath (model "K-18LM", Kraintek Czech, s.r.o.; Hradec Králové, Czech Republic) when the sonication

took two subsequent sequences – at a high intensity for 20 min and at a low intensity for another 60 min. Afterwards, the extracts were filtered through folding filter paper and stored in a refrigerator at 5 °C.

Voltammetric measurements

From each spice extract, a dose of 0.5 mL was mixed with 9.5 mL of 0.05 mol L⁻¹ HClO₄ and subjected to voltammetric analysis, whereas the calibration in the range of 20–200 μmol L⁻¹ was used as the method of choice for quantification of the flavouring substances determined.

Prior to measurements, the surface of GCE was renewed by thorough polishing with alumina slurry (Al₂O₃ particle size of 1.0 μm) for 30 s and subsequent rinsing by stream of distilled water. If needed, this renewal was made even more often. Simultaneous detection of flavouring substances was performed in 0.05 mol L⁻¹ HClO₄ using square-wave voltammetry (SWV) within a potential range from 0 to +1.6 V, at potential step (E_{step}) of 5 mV, with potential amplitude (E_{amp}) of 25 mV, and a frequency (f) of 20 Hz.

Results and discussion

Electrochemical behaviour of flavouring substances

Generally, the position of the anodic peak (E_p^a) is usually determined by the willingness of a substance to release the electrons at the electrode. From the molecular structure point of view, the compounds investigated (EG, Myr and TAN) can be considered as differently substituted methoxybenzene, known also under trivial name anisole [12]. Electrochemical properties of the individual derivatives are then given by mesomeric effects of the respective substituents together with pH of the working electrolyte that enables the protonation or deprotonation of the whole molecule.

In accordance with the literature, anodic oxidation of EG at +0.69 V vs. Ag/AgCl in strong acidic media leads to the formation of the corresponding *o*-quinone with the participation of two electrons and subsequent nucleophilic addition of water with the release of one proton and molecule of methanol [8]. Under the same working conditions, Myr provides one broad anodic peak at +1.14 V. Due to the presence of electron-donating etheric oxygen on the benzene ring, it can be predicted that its electron-rich aromatic structure will most likely be oxidised to form the corresponding catechol (analogously like MDMA) with the participation of two electrons and protons, followed by spontaneous nucleophilic addition of water and subsequent release of molecular formaldehyde, HCH=O (see e.g. [13]). In the first step, TAN is anodically oxidised to a radical cation

TAN⁺⁺ at +0.92 V which readily reacts with the present nucleophilic agent, which is often molecule of water, and a TAN⁺ species are formed. In second step, these unstable intermediates are further transformed at +1.25 V, followed again by a nucleophilic addition giving rise to electrochemically inactive product [11].

Optimisation of simultaneous electrochemical detection

The aim of these experiments was to find a suitable composition of the working electrolyte and optimal constellation of instrumental parameters for the square-wave voltametric mode chosen for measurements as it had allowed us to achieve the highest possible current yields of the corresponding electrode reactions.

Based on some previous findings in the literature [6–11], different types of working media with 5% (v/v) ethanol were tested, namely: 0.1 mol L⁻¹ acetate buffer (pH 4.5), 0.1 mol L⁻¹ phosphate buffer (pH 6), 0.1 mol L⁻¹ Britton-Robinson buffer (pH 2), and diluted solutions of mineral acids at concentrations 0.05 and 0.1 mol L⁻¹. The maximal current yields have been attained in acidic solutions, when a solution of 0.05 mol L⁻¹ HClO₄ with 5% (v/v) ethanol content was the working medium of choice, due to well-defined peaks of all the flavouring substances examined, as well as favourable signal-to-noise characteristics.

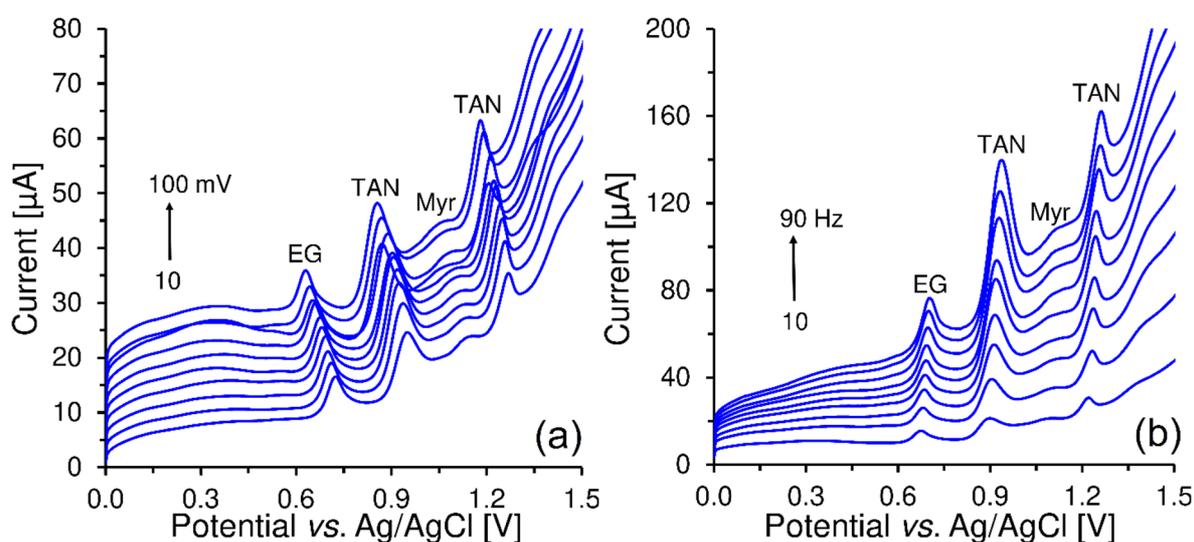


Fig. 2 Square-wave voltammograms of model mixture (100 $\mu\text{mol L}^{-1}$ EG, TAN, and Myr) recorded at GCE at $E_{\text{step}} = 5 \text{ mV}$, $f = 20 \text{ Hz}$, and varying E_{amp} values (a) or at $E_{\text{step}} = 5 \text{ mV}$, $E_{\text{amp}} = 25 \text{ mV}$, and varying f values (b)

Note: Both experiments were performed in 0.05 mol L⁻¹ HClO₄ with 5% (v/v) ethanol content

As evident from comparison of Figure 2a and Figure 2b, the potential amplitude (E_{amp}) did not have any significant effect on the increase of the anodic peaks of the substances of interest in a model mixture containing each compound

at the same concentration of $100 \mu\text{mol L}^{-1}$. Next, a study with observation of the effect of the frequency (f) followed, inevitable for definition of the rate of polarization (v) at a constant potential step (E_{step}). By considering the relatively low current response of Myr compared to those for EG and TAN in acidic solution (with optimum at pH 4), a potential amplitude of 25 mV and frequency of 20 Hz were selected as the proper values.

Analytical performance of the developed voltammetric method

As documented by the data in Table 1, the electrochemical detection of the individual aromatic substances at the electrodes from carbonaceous materials is most effective in acidic or mild acidic solutions.

Table 1 Comparison of hitherto reported voltammetric methods for determination of flavouring substances

Analyte	Sensor	Working medium	Linear range [$\mu\text{mol L}^{-1}$]	LOD [$\mu\text{mol L}^{-1}$]	Ref.
EG	CPE/GO/SnO ₂	0.1 mol L ⁻¹ PB (pH 3)	0.05 to 440	0.02	[6]
	BDDE	0.1 mol L ⁻¹ AcB (pH 6)	3 to 99	0.1	[7]
	CPE/GO	0.1 mol L ⁻¹ BRB (pH 2)	0.1 to 17	0.007	[8]
	PGE	0.1 mol L ⁻¹ BRB with 0.1 mol L ⁻¹ KCl (pH 2)	0.3 to 50	0.09	[9]
	GCE	0.05 mol L ⁻¹ HClO ₄	14 to 163	5.6	This work
Myr	GCE	0.05 mol L ⁻¹ HClO ₄	15.8 to 182	4.7	This work
TAN	BDDE	0.1 mol L ⁻¹ AcB (pH 6)	4.7 to 118	0.16	[10]
	La ₂ O ₃ /CPE	0.1 mol L ⁻¹ AcB (pH 6)	4.7 to 118	0.03	[10]
	CPE/SDS	0.1 mol L ⁻¹ LiClO ₄ in MeCN	2.4 to 196	0.7	[11]
	GCE	0.05 mol L ⁻¹ HClO ₄	8.9 to 189	2.1	This work

Abbreviations used: AcB, acetate buffer; BDDE, boron-doped diamond electrode; CPE, carbon paste electrode; GO, graphene oxide; MeCN, acetonitrile; PGE, pencil graphite electrode; SDS, sodium dodecyl sulphate.

As the simultaneous determination of more analytes in a mixture required certain compromise in the terms of optimizing the conditions for each substance extra, it was not possible to achieve detection limits comparable to the previously reported voltammetric methods that had been developed for the determination of the single analyte(s). Despite this, the respective calibrations I_p vs. c_A resulted in fine linear dependences (over two orders of magnitude) with coefficients of determinations $R^2 > 0.9930$, which was found sufficient for practical analysis of real sample of Christmas spices, which is confirmed also by the data in Table 2.

The repeatability calculated for five replicates during model measurements in the mixture (with each compound at $100 \mu\text{mol L}^{-1}$ in concentration) could be expressed for the individual substances as the relative standard deviation, RSD, $\pm 0.9\%$ for EG, $\pm 5.5\%$ for Myr, and $\pm 7.7\%$ for TAN, respectively. Among others, these values had also reflected a different solubility of the individual flavouring substances in 5% (v/v) ethanol. More specifically, the least soluble TAN evidently passivated the electrode surface, thus indicating a significant contribution of adsorption. This was in contrast with highly water-soluble EG that had provided the most precise detection.

Table 2 Analytical parameters of the developed voltammetric method

Analyte	Sensor diameter [mm]	Sensitivity [$\mu\text{A L } \mu\text{mol}^{-1}$]	y-intercept [μA]	R^2
EG	3	0.167	2.179	0.9957
Myr	3	0.308	6.264	0.9933
TAN	3	0.129	6.078	0.9957

Note: Presented data were obtained from separate electrochemical detection using square-wave voltammetry at glassy carbon electrode

Figure 3a offers typical voltammograms obtained during simultaneous calibration measurements, from which the subtracted values were used to calculate the content of individual flavouring substances in both model and real samples (ethanolic extracts of Christmas spices). However, as can be seen in Figure 3b, any presence of TAN significantly has decreased the Myr signal (red curve). Fortunately, this phenomenon was not observed in the case of EG; see again red curve and almost unchanged signal at *ca.* $+0.65 \text{ V}$. Moreover, the TAN content in nutmeg has been found negligible, and therefore it does not interfere with the Myr determination.

The accuracy of the voltammetric method developed was verified using analysis of three model samples and three extracts of Christmas spices. As seen via data in Table 3, excellent recovery-rate values around 100% confirms the desired precision and correctness of the presented method.

Also, a very close agreement between the determined and declared contents of flavouring substances in Christmas spices have proved the practical usefulness of this new electroanalytical approach.

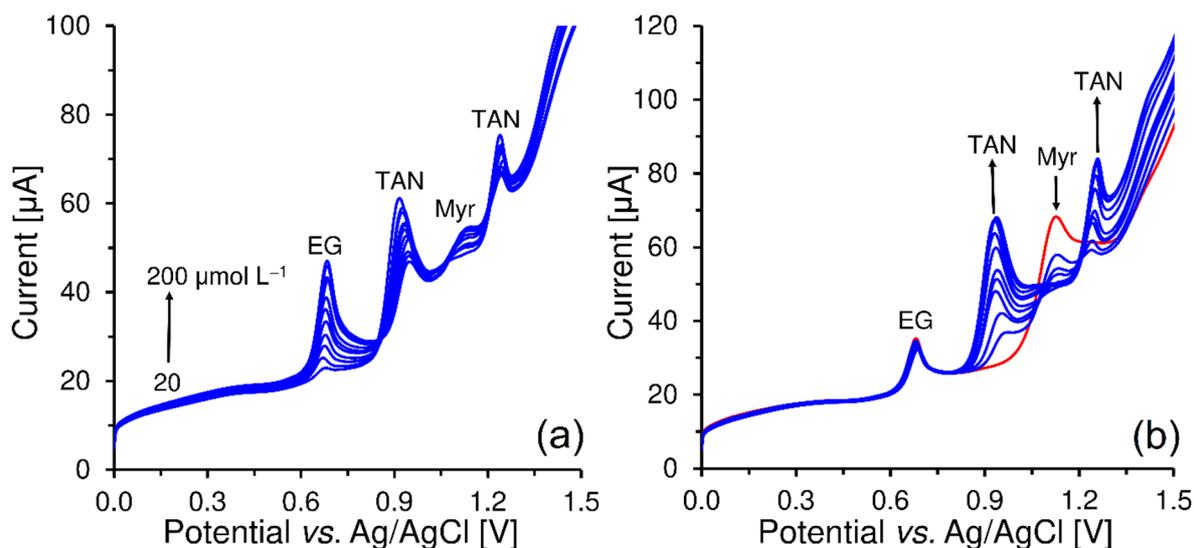


Fig. 3 Square-wave voltammograms obtained at GCE during calibration measurements of EG, TAN, and Myr (a) and TAN in the presence of $100 \mu\text{mol L}^{-1}$ EG and $200 \mu\text{mol L}^{-1}$ Myr (b). SWV-ramp: $E_{\text{step}} = 5 \text{ mV}$, $E_{\text{ampl}} = 25 \text{ mV}$, and $f = 20 \text{ Hz}$
Note: Both experiments performed in $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ with 5% ethanol (v/v)

Table 3 Accuracy of the developed voltammetric method

Sample	Analyte	Determined content [$\mu\text{mol L}^{-1}$]	Declared amount [$\mu\text{mol L}^{-1}$]	Recovery [%]
Model 1	EG	51.5 ± 4.7	51.8	99.4
Model 2	Myr	45.4 ± 6.2	46.3	98.0
Model 3	TAN	51.2 ± 2.8	48.3	106.0
		Determined content [mg per g]	Declared amount [mg per g]	References
Clove	EG	130.4 ± 15.5	93.8 to 146.5	[14]
Nutmeg	Myr	2.2 ± 0.1	0.6 to 2.9	[15]
Anise	TAN	15.5 ± 0.9	16.0 to 57.0	[16]

Note: Values of determined contents are presented as arithmetic means (\bar{x}) with the corresponding standard deviations (σ) for three to five replicate analyses.

Conclusions

In this article, it has been shown that a combination of anodic voltammetry in the square-wave mode with the glassy carbon electrode offers effective electroanalytical tool for the simultaneous determination of EG, Myr, and TAN in Christmas spices. However, due to rather pronounced passivation of the GCE surface by some reaction products after anodic oxidation of the analytes, the surface of the

working electrode had to be renewed quite often by time-consuming polishing, which naturally prolonged the overall time for analysis. Here, a possibility of using carbon paste-based electrodes with quick mechanical renewal of their surface [17] or, alternatively, their surface modification with surfactants [18,19] seems to be a way of how to suppress the surface passivation.

In prospect, it is assumed that new voltammetric methods like that reported herein could be applied in pharmaceutical and food analysis for samples with less complex matrix. Because this would be a principally different analytical approach than the hitherto standard GC-MS (see e.g. [20]), such voltammetric procedures could then serve for reference analyses during routine validations.

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