

Voltammetric Determination of *Arbutin* on Carbon Paste Electrode

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Abstract: *Arbutin* (hydroquinone- β -D-glukopyranoside) belongs among the most frequent active substances in skin-whitening cosmetics. In this matrix, its content does not require sensitive, but rather simple, quick and cheap methods of determination, which can be supplied by electrochemical methods. Method for determination of *Arbutin* was developed using differential pulse voltammetry at a carbon paste electrode in Britton-Robinson buffer (pH 2). The concentration dependence proved a linear response in the concentration range of 4–100 $\mu\text{mol L}^{-1}$ with the limit of quantification of about 2.4 $\mu\text{mol L}^{-1}$. The practical applicability of the method was tested on the determination of *Arbutin* in cosmetic cream "*Unitone4 α -Arbutin*". The content found (20.4 ± 1.1) mg g^{-1} *Arbutin* is in very good agreement with the declared value of 20 mg g^{-1} .

Keywords: Voltammetry; Carbon paste electrode; *Arbutin*; Determination; Skin-whitening cosmetics.

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Introduction

Skin-whitening cosmetics are a branch of “beauty industry” dealing with the corrections of pigmentation spots such as freckles, vitiligo, lentigo and liver spots, but also with the general lightening of the skin. The desired effect is most easily achieved by the suppressing of production of pigment melanin through the inhibition of tyrosinase in the melanin-producing cells, melanocytes [1,2].

There are several compounds with the desired effect; however, many of them are suspected of adverse effects to the human health, including the most effective one, suspected carcinogen hydroquinone [3].

Arbutin (hydroquinone- β -D-glucopyranoside) is glycosylated hydroquinone, occurring naturally at relatively high concentrations in many kinds of plants, such as bearberry, cranberry, pear, thyme, marjoram and also several kinds of cereals [4]. In the glycosidic form, the cosmetic effect of hydroquinone is weaker [5], but its toxicity is strongly decreased, though not completely removed [6]. These properties make *Arbutin* frequently used active substance in the whitening cosmetics.

Arbutin is determined mainly either in the matrices of the producing plants, body fluids of exposed organisms or in the cosmetics. In the first two cases, combination of a separation method and a sensitive detection technique is usually used, such as HPLC with coulometric detection [7], HPLC-MS [8], gas chromatography with flame-ionization detection [9], or capillary zone electrophoresis with spectrometric detection [10]. In cosmetics, simpler methods are preferred, such as HPLC with spectrophotometric detection [11-13] or electrochemical methods. Two such methods are described, using screen printed electrode; one of them utilizes modification of the electrode surface by clay materials [14], the other one employs flow injection analysis with online oxidation by manganese oxide [15]. Sensitivity in these cases reaches down to the submicromolar level, but the determination still requires powerful apparatus. For many samples, however, such a high sensitivity is not necessary as the content of *Arbutin* in the cosmetics is rather high. Then, also simple and undemanding methods might be preferable and of interest.

The aim of the presented work is the development of a simple method for the determination of *Arbutin* in cosmetics using a carbon paste electrode (CPE), utilizing its favorable properties such as wide potential window, low background current, adsorptive properties, and easy handling and surface renewal [16,17].

Experimental

Chemicals

The stock solution of *Arbutin* (98 %, Sigma-Aldrich) ($c = 1 \text{ mmol L}^{-1}$) was prepared by dissolving the exact amount of the substance in deionized water and it was kept at a laboratory temperature. It was proved spectrophotometrically, that the solution is stable for at

least six months. Britton-Robinson (BR) buffers served as supporting electrolytes. All chemicals used for buffer preparation were of analytical grade purity and obtained from Lachema Brno, Czech Republic. Other used chemicals were methanol (p.a., Merck, Germany) and deionized water (Millipore, USA).

Electrodes and Apparatus

A carbon paste electrode was prepared by thorough mixing of 0.25 g graphite CR-2 (Grafit Tyn, Czech Republic) and 0.1 mL mineral oil (Fluka, Switzerland). Voltammetric measurements were carried out using Eco-Tribo-Polarograph, controlled by software Polar Pro 5.1 (both PolaroSensors, Prague, Czech Republic). Carbon paste was packed in the Teflon piston-driven holder with 2 mm inner diameter [18]. Differential pulse voltammetry (DPV) was employed with carbon paste electrode (CPE) as the working electrode, a platinum wire in the role of auxiliary electrode, and an Ag/AgCl (3 M KCl) electrode as the reference (model "RAE 113" Monokrystal Turnov, Czech Republic), to which all the potential values are referred. The pH of the solutions was measured with a pH meter Jenway 3510 (Jenway, UK) with a combined glass electrode.

Procedures

Parameters of the DPV potential program were: pulse width of 100 ms, pulse height of 50 mV and scan rate of 20 mV s⁻¹. All measurements were made in triplicate. Concentration 100 μmol L⁻¹ was used during optimization measurements, unless stated otherwise. Carbon paste electrode was cleaned by wiping with the wet filtration paper before each measurement. Calibration dependences were evaluated by least squares linear regression method. The quantification limits were calculated as the concentration of the analyte which gave a signal ten times the standard deviation of the lowest evaluable concentration [19].

Real Samples Treatment

Cosmetic cream "Unitone 4 *α*-Arbutin" (Isis Pharma, USA) with 2 % declared content of active substance (*i.e.*, *Arbutin*) was used to confirm the applicability of the developed method. 0.1 g portions of sample were sonicated in 10 mL of solvent, e.g. water or methanol, for 10 min or until dissolved. A portion of 0.5 mL of this solution was diluted to 10 mL with BR buffer and subsequently used for the measurement.

Results and Discussion

In the first part of the work, the behavior of *Arbutin* in media of different pH was observed. *Arbutin* provides one voltammetric peak in anodic potential range, with potential decreasing linearly with increasing pH. The dependence can be described by equation $I [\text{nA}] = -49.1 \text{ pH} + 854$ ($R^2 = 0.9848$). The height of the peak gains maximum height in pH 2 and minimum in pH 7 and 8. Resulting voltammograms are shown in Fig. 1; medium of pH 2 was selected as optimum due to the highest and well developed peak.

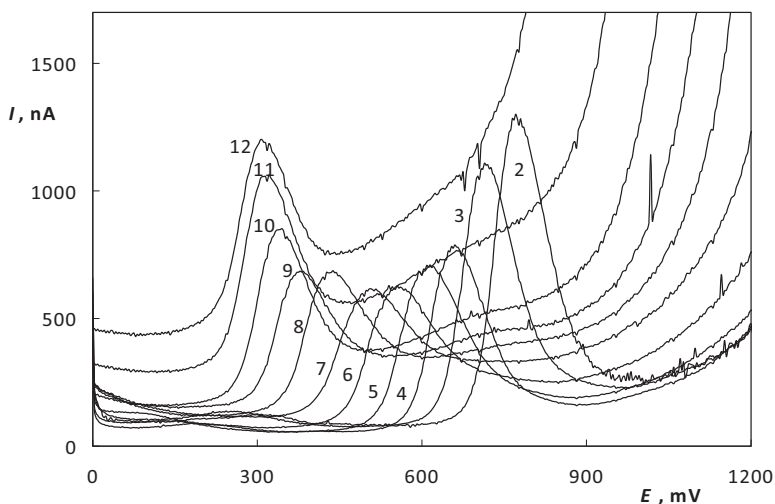


Fig. 1: DP voltammograms of *Arbutin* ($c = 100 \mu\text{mol L}^{-1}$) in BR buffer of the pH of choice.

Note: The numbers describing the curves correspond to the actual pH.

Attempt was made to increase the sensitivity of the determination by adsorption step, but no significant increase of the peak height was observed during the measurement of $5 \mu\text{mol L}^{-1}$ solution of *Arbutin* in BR buffer pH 2. The accumulation is thus not sufficient to improve the analysis sensitivity.

Concentration dependence was measured under the optimum conditions in the concentration range from 100 to $4 \mu\text{mol L}^{-1}$; resulting voltammograms of the lower concentrations are shown in Fig. 2. The dependence is linear within the whole indicated concentration range and follows the equation: $I [\text{nA}] = 9.7 c [\mu\text{mol L}^{-1}] - 19$ ($R^2 = 0.9952$). Limit of determination was found to be $2.4 \mu\text{mol L}^{-1}$.

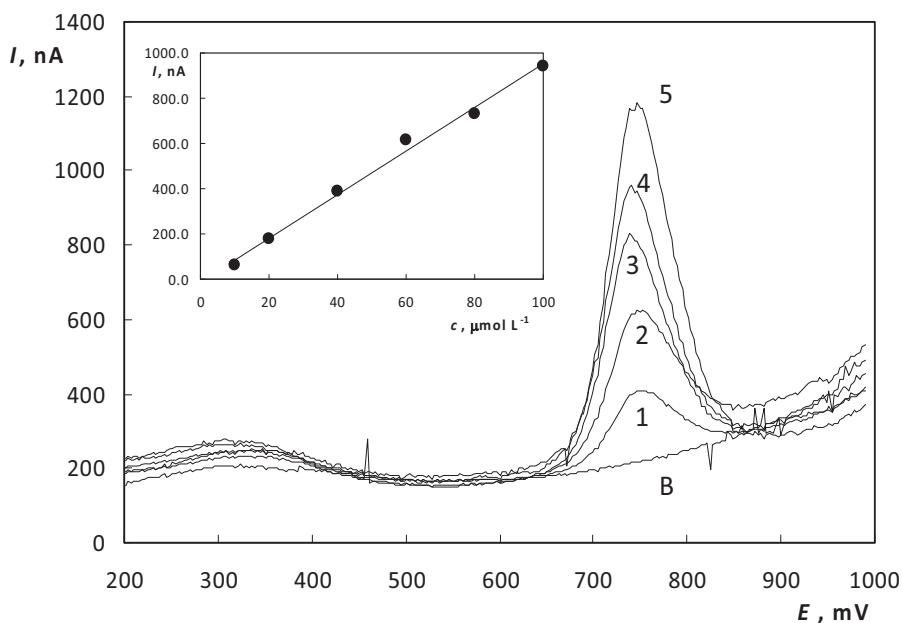


Fig. 2: DP voltammograms of Arbutin at concentrations of 0 (B); 10 (1); 20 (2); 40 (3); 60 (4); 80 (5), and 100 (6) $\mu\text{mol L}^{-1}$ in BR buffer (pH 2). Note: Inset shows the corresponding concentration dependence.

The applicability of the developed method was tested on the determination in whitening cream "Unitone 4 α -Arbutin" with declared content of 2 % Arbutin. The sample was treated as described in the section 2.4. Determination was performed with the standard addition method, with two consecutive additions of 0.2 mL of the stock solution of the analyte – Arbutin.

As a first attempt, water was used for the dissolution of Arbutin in the cream, but the found concentration was several times lower than the expected value. The cause might be in the insufficient transfer of the analyte from the hydrophobic components of the cream into the polar aqueous media, either due to the mechanical barriers of undissolved cream or due to the chemical affinity of Arbutin to the hydrophobic components. Therefore, further experiments were performed with methanol as the solvent of choice and, in this case, both declared and the real (found) content of Arbutin were in good agreement. Besides, the repeatability of the determination increased.

The results of the individual testing determinations are summarized in Table I, whereas a set of typical voltammograms shown in Fig. 3; the latter together with graphical representation of the standard addition method used.

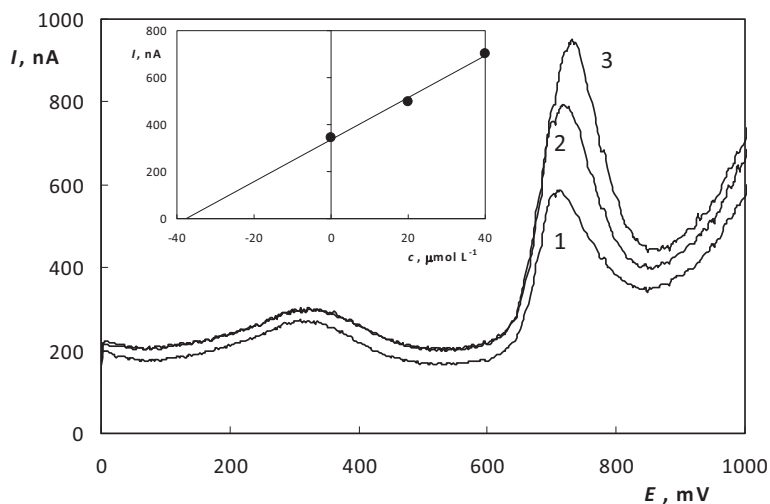


Fig. 3: DP voltammograms of the methanol extract of the cream sample without the addition (1), with the addition of 0.2 ml (2) and 0.4 ml (3) of the stock solution of Arbutin. Notes: Inset shows the corresponding standard addition method dependence to the concentration of Arbutin. As the supporting medium, BR-buffer (pH 2) was used containing methanol (95:5, v/v).

Table I: Summary of the declared and found content of Arbutin in the cosmetic cream. DP voltammetry, medium BR buffer pH 2.

Extraction solvent	Found content ^{a)} mg g ⁻¹	Confidence limit ^{a)} mg g ⁻¹	RSD %	Declared content mg g ⁻¹
Water	6.2	1.4	10.4	20
Methanol ^{b)}	20.4	1.1	2.4	20

Abbreviations & symbols used: RSD ... relative standard deviation; a) evaluated from three replicate measurements (n = 3); b) solution containing 5 % (v/v) of methanol.

Conclusions

Simple voltammetric method for the determination of Arbutin at a CPE was developed; BR buffer pH 2 being selected as optimal medium. The linearity of concentration dependences has been proved in the range of 4-100 $\mu\text{mol L}^{-1}$; limit of quantification being estimated to be 2.4 $\mu\text{mol L}^{-1}$. As found, the accumulation step did not increase the response of the electrode, indicating lesser affinity of the substance of interest to be accumulated [20].

The method developed has successfully been used for the determination of *Arbutin* in cosmetic product "*Unitone 4 α -Arbutin*" after the dissolution of the cream in methanol, as the extraction to water was not efficient enough. The found content of active substance in the cream was (20.4 ± 1.1) mg g⁻¹, which has been in very good agreement with the declared content of 20 mg g⁻¹ *Arbutin*.

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