DETERMINATION OF ANTI-CANCER DRUG EMODIN USING A SILICA-GEL-MODIFIED CARBON PASTE ELECTRODE

Artigo

Dai Long Vu[a], Bensu Ertek[b], Yusuf Dilgin[b] and Libor Cervenka[c,*]

[a]Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
[b]Department of Chemistry, Science & Art Faculty, Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey
[c]Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 532 10 Pardubice, Czech Republic

In this paper, a silica-gel-modified carbon paste electrode (Si-gel/CPE) was used to determine the anti-cancer drug emodin by anodic stripping differential pulse voltammetry (ASDPV). The effects of the silica-gel content, the pH of the supporting electrolyte, and the scan rate on the oxidation current of emodin were investigated. The oxidation currents of emodin obtained from ASDPV measurements were linearly correlated with the concentration in the range of 5.0×10^{-9} to 300.0×10^{-9} mol L^{-1}. The limit of detection was determined to be 1.5×10^{-9} mol L^{-1}. The current method was successfully applied to determine emodin in a knotweed root sample, with recovery rate of 92.5% to 98.3%.

Keywords: emodin; carbon paste electrode; voltammetry.

INTRODUCTION

Emodin [1, 3, 8-trihydroxy-6-methylanthraquinone] is an anthraquinone naturally occurred in many herbal medicines such as *Rheum palmatum*, *Polygonum multiflorum*, *Cassia acutifolia* and *Aloe vera* with anti-microbial, immunosuppressive and anti-inflammatory activities.[1,2] Recently, the effectiveness of emodin and its derivatives in the treatment of cancer have been illustrated.[3-5] Herbal medicines containing anthraquinones are being increasingly used for cosmetics, food and pharmaceuticals due to their wide therapeutic and pharmacological properties. For these reasons, the determination of emodin has been of great importance. Several methods have been developed for this purpose including HPLC,[6] LC-MS,[7] fluorescence[8] as well as electrochemical methods.[9-12] Compared with those methods above, electrochemical techniques have some advantages such as high sensitivity, accuracy, simplicity, low expense and the possibility of miniaturization. Among all the carbon electrodes, carbon paste electrode (CPE) is an appealing and widely used electrode material in the fields of electrochemistry, electroanalysis, etc. due to its attractive advantages, such as simple preparation, low-cost implementation, renewability, low background current, and wide potential window.[13] Silica gel (Si-gel), a granular and porous form of silicon dioxide, possesses some excellent electrochemical properties such as high surface area, strong sorption ability and easy surface modification. For these reasons, it has been used for modification of carbon paste electrodes in order to enhance the electrochemical behavior of various organic molecules.[14-18] Si-gel CPE was used for the determination of emodin by anodic stripping differential pulse voltammetry (ASDPV) in our report for the first time, to our best knowledge.

EXPERIMENTAL

Reagents and equipments

All the reagents were purchased in Sigma-Aldrich (Czech Republic). Deionized water was used in this study (G ≤ 0.055 μS).

Dissolved oxygen was removed from all the solutions by purging with argon for 15 min (purity 99.99%, Linde Technoplyn, Czech Republic).

A stock solution of emodin (10^{-2} mol L^{-1}) was freshly prepared in methanol and was kept in a dark bottle during the experiments. Briton Robinson (B-R) buffer solution was used as supporting electrolyte.

A three electrode system consisting of CPEs (working), Ag/AgCl/3.0 mol L^{-1} KCl (reference) and platinum wire (counter electrode) connected to PalmSens (Ivium Technologies, Netherland) was used for electrochemical measurement. The surfaces of modified CPE and unmodified CPE were regenerated by renewing and polishing them on wet filter paper before each measurement.

The preparation of Si-gel/CPE

Si-gel (pore size 60 A[0], 5-25 μm particles size, Sigma-Aldrich) and graphite powder 5.5-7.0 μm (CR-5, Maziva Týn n. L., s.r.o., Czech Republic) were weighed and the total mass was controlled at 0.5 g. The mass content of silica gel varied from 3 to 21%. After that, 130 μL of mineral oil (M5904, Sigma-Aldrich, Germany) was added to the powder and exhaustively hand mixed together in ceramic mortar by ceramic pestle. The resulting paste was packed into the teflon piston holder (3.0 mm inner diameter).[19] The unmodified CPE was also prepared without the addition of silica gel.

Electrochemical procedure

The effect of the amount of silica gel in carbon paste composite material upon the oxidation current of emodin was investigated using ASDPV in B-R buffer solution at pH 3.0 at a scan rate of 50 mV/s (potential step 0.025V; potential pulse 0.025 V; potential time 0.05 s) after deposition of emodin (-0.2V, 120 s). The influence of pH between 2.0 and 8.0, deposition potential from -0.4 to 0.0 V and deposition time ranged from 30 to 240 s was also investigated at a scan rate of 50 mV/s after deposition of emodin (-0.2V, 120 s). In order to study the effects of the scan rate on the peak potentials and peak currents, the cyclic voltammograms of the Sil-gel/CPE were recorded at various scan rates in the potential range between -0.4 and +0.4 V.

*e-mail: libor.cervenka@upce.cz

Preparation of sample

Knotweed root (*Reynoutria bohemica*) sample was obtained from Institute of Botany of the Academy of Sciences of the Czech Republic. The sample was milled to powder (particle size < 0.12 mm) and a portion was extracted in 10 mL methanol using ultrasonic bath for 30 min. The pellet was discarded by centrifugation (2000 rpm, 5 min) and 100 µL of appropriate dilution of the extract was added to B-R buffer solution (pH 3.0) and the content of emodin was determined using voltammetry technique described below. The same procedure was applied with the samples spiked with standard emodin solution for recovery study.

Student's t-test was used for determination of statistical differences between results at the probability level (p) 0.05 (Origin Pro v. 9, OriginLab Corp., USA)

RESULTS AND DISCUSSION

Enhancement effect of Si-gel on the oxidation of emodin

The oxidation currents of emodin significantly increased (p < 0.01) with the increase of silica gel content and reached the highest value at 15% of silica gel (1.77 ± 0.01 µA). Further increase in silica gel content (up to 25%) did not enhance the oxidation current of emodin (p < 0.05). These results were in good agreement with a study on activated silica-gel modified CPE for the determination of quercetin in term of the trend.[19] The decrease of oxidation current with the increase of the mass content of silica gel may be caused by the reduction of conductivity resulted from a larger amount of this modifier. In this study, 15% of silica gel was chosen as optimum for the bulk modification of CPE. The effect of silica gel modification towards emodin oxidation is depicted in Figure 1.
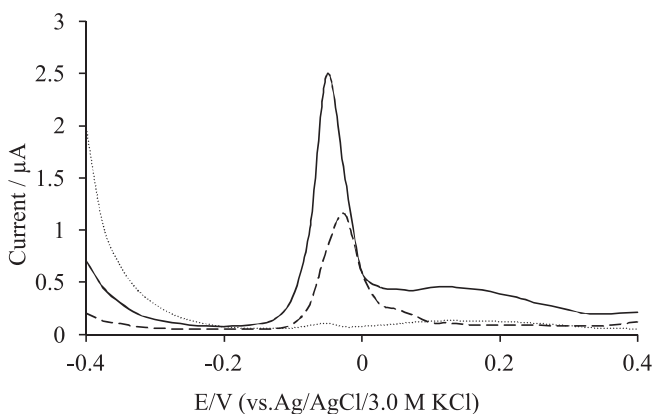

Figure 1. *Anodic stripping differential pulse voltammetry curves of 0.25 µM emodin in B-R buffer solution at the pH 3.0 using bare CPE (— —) and Sil-gel/CPE (——) after deposition at -0.2 V for 90 s. Bare CPE (·····) in B-R buffer solution served as control. Potential range -0.4 to +0.4 V, potential step 0.025 V, potential pulse 0.025 V, pulse time 0.05 s, scan rate 50 mv/s*

Effect of pH and accumulation parameters

It was found that the oxidation peak currents of emodin on Si-gel/CPE gradually increased with the decrease of pH values indicating the higher enhancement effect of silica-gel in more acidic conditions (Figure 2A) and reached the highest current at pH 3.0 (p < 0.05). As shown in Figure 2B, the oxidation peak currents of emodin increased when the deposition potential changed from -0.4 to -0.2, followed by steep decreasing.

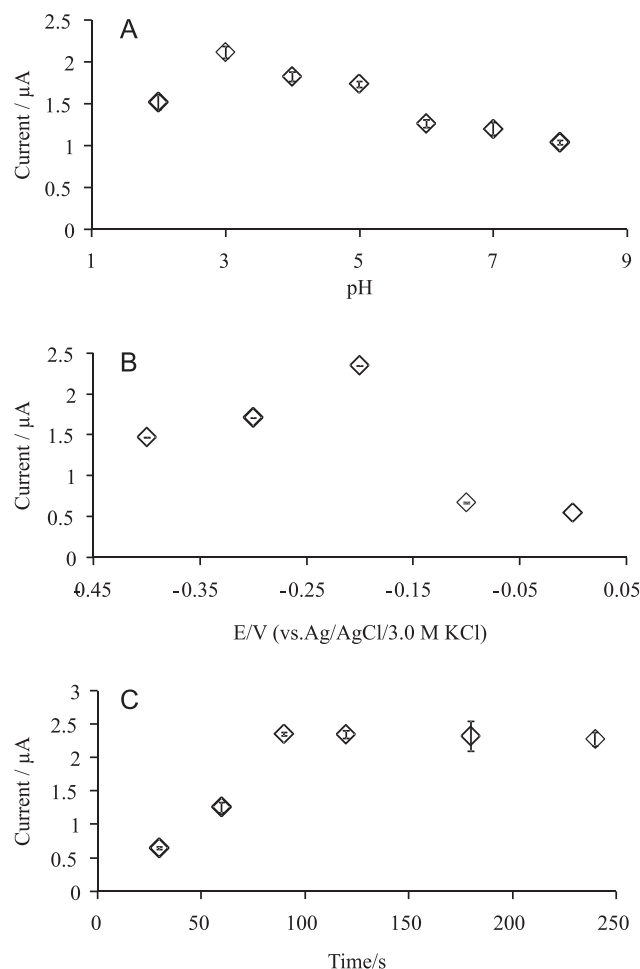The influence of accumulation time in the pre-concentration step

Figure 2. *The oxidation peak currents of 0.25 µM emodin as a function of A) pH; B) deposition potential and C) deposition time. Error bar represents the standard deviation of five measurements*

is shown in the Figure 2C. The oxidation current increased rapidly with the increase of accumulation time and became relatively stable at around 90 s (p > 0.05). Emodin belongs to the 9,10-anthraquinone derivates with three hydroxyl groups (Figure 3) which makes this molecule slightly acidic. In addition, silica gel also acts as a weak acid with estimated pKa value to 4.0 due to its high content of silanol group on the surface.[20]
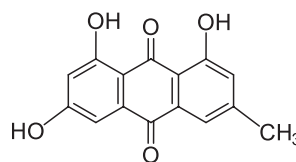

Figure 3. *The structure of emodin*

Putting these findings together, we may imply that the specific interactions (presumably hydrogen bonds) among silanol groups and hydroxyl groups of emodin gives raise under acidic condition. With increasing pH, deprotonization of both silanol and hydroxyl groups occurred leading to repulsion of negatively charged emodin from negatively charged silica gel surface.[21] A possible mechanism for the reaction of silica gel with emodin at the modified CPE is attributed to the following steps:

(1) pre-concentration step (buffer solution at pH 3.0, deposition potential -0.2 V, deposition time 90 s).

$$\equiv Si - OH_{(CPE)} + HO - Emodin_{(solution)} \leftrightarrow \equiv Si - OH \cdots H - O - Emodin_{(CPE)}$$
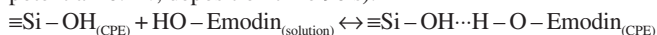
(2) stripping step (buffer solution at pH 3.0, positive scan from -0.3 to +0.1 V)

$$\equiv Si - OH \cdots H - O - Emodin_{CPE)} \leftrightarrow \equiv Si - OH + Emodin_{(ox)} + ne^-$$
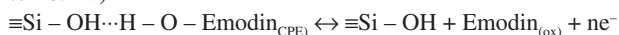
Effect of scan rate

Cyclic voltammograms of Si-gel/CPE in B-R buffer solution at pH 3.0 at various scan rates are shown in Figure 4. As indicated, the oxidation peak potentials slightly shifted to the more positive potentials with increasing of the scan rate between 20 and 150 mV/s. The oxidation currents is linearly proportional to the scan rate (Figure 4, inset) (I_a = 0.0096 V/s + 0.6366; R^2 = 0.9981). These findings indicate that the electrochemical process of emodin at Si-gel/CPE was surface controlled.
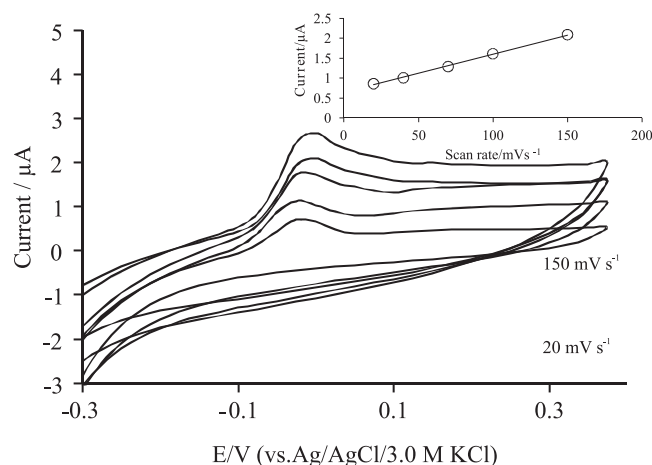

Figure 4. Cyclic voltammograms of Si-gel/CPE at various scan rates (from 20 to 150 mV s⁻¹) in the presence of 0.5 μM of emodin in B-R buffer at the pH 3.0. Inlet: the oxidation current of emodin at various scan rates, I(μA) = 0.0096 (V/s) + 0.637, (R² = 0.9981). Potential range from -0.4 to +0.4 V, potential step 0.025 V, potential pulse 0.025 V, pulse time 0.05 s

Analytical application

For further analysis of emodin, the optimum conditions for ASDPV were chosen as: supporting electrolyte (B-R buffer at pH 3.0), accumulation potential (-0.2 V) and accumulation time (90 s). The linear response of the oxidation current of emodin upon its concentration was determined in the range between 5.0 and 300.0×10^{-9} mol L⁻¹ (Figure 5). The limit of detection (LOD) using the equation LOD = $3s_b$/m, where s_b is the standard deviation of the blank response and m is the slope of the calibration plot was found to be 1.5×10^{-9} mol L⁻¹ and limit of quantification (LOQ) was 5.0×10^{-9} mol L⁻¹ (signal/noise = 10).

The linearity ranges and calculated LOD obtained from the Si-gel/CPE were compared with other methods. The LOD of proposed method was lower in comparison with almost all the studies
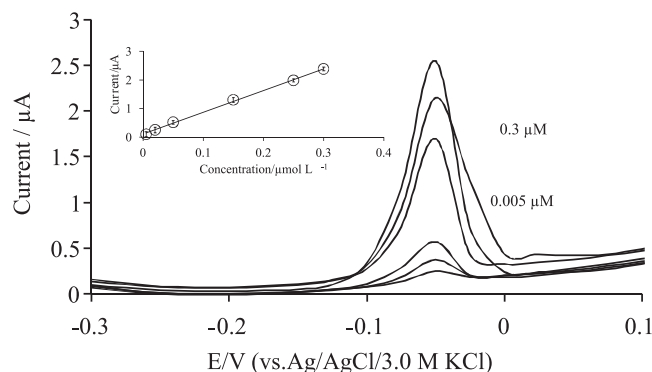

Figure 5. Anodic stripping differential pulse voltammetry curves of various concentration of emodin on Si-gel/CPE in Britton-Robinson buffer at the pH 3.0 after deposition (-0.2 V, 90 s). Potential range from -0.3 to +0.1 V, potential step 0.025 V, potential pulse 0.025 V, pulse time 0.05 s, scan rate 50 mV/s. Inlet: The plot of oxidation current vs. concentration of emodin

mentioned in the Table 1.[9-11] Moreover, there is also possibility to apply this Si-gel/CPE for the flow injection analysis system. The better LOD for emodin determination has been reported using pencil graphite electrode but the linear response of oxidation current of emodin to its concentration was limited.[12] The Si-gel/CPE electrode was proved to be extremely sensitive, simple renewable and easy in preparation and storage.

After 45 days of storage in room temperature, the oxidation current of emodin at the concentration of 10×10^{-9} mol L⁻¹ was not significantly different in comparison with that using freshly prepared electrode (p > 0.05). The accuracy of the method was investigated by the repeated measurements of 10 ×10^{-9} mol L⁻¹ and 100 ×10^{-9} mol L⁻¹ emodin (n = 15) and the relative standard deviation (RSD) was found to be 4.7% and 5.4%, respectively.

Interferences studies

Si-gel/CPE showed a remarkable selectivity towards emodin oxidation by ASDPV in the study. In the presence of emodin, the results show that the tolerable concentration ratios with respect to 0.1×10^{-6} mol L⁻¹ emodin were 30 folds of ascorbic acid, 80 folds of tartaric acid, 70 folds of rutin, 60 folds of oxalic acid, 50 folds of citric acid, 100 folds of glucose, dextrin and starch. The presence of interfering species influenced the oxidation current of emodin by less than 5% in all cases.

Sample determination

The proposed method was applied to emodin determination in knotweed root with results shown in Table 2. It can be seen the current method was sensitive for emodin determination with a good recovery in the range of 92.5-98.3%.

Table 1. Comparison of the efficiency of some modified electrodes used in the electrocatalysis of emodin

Methods	Linear dynamic range (μM)	Limit of detection (μM)	Samples	Reference
PGE[a]	0.0398-0.243	0.000517	Biological fluids	12
MIPs[b]	5.0-200	0.74	Tablets	11
GCE[c]	0.089-7.8	0.0078	Herbal drugs	9
MWCNTs/GCE[d]	1-100	0.3	-	10
Si-gel/CPE[e]	0.005-0.3	0.0015	Knotweed root	This work

a) pencil graphite electrode b) molecular imprinted polymer c) glassy carbon electrode d) multi-walled carbon nanotubes modified glassy carbon electrode e) silica gel modified carbon paste electrode.

Table 2. Determination of emodin in knotweed root sample and the recovery data (n = 6)

Spiked (mg/g)	Found (mg/g)	Recovery (%)
0.00	0.76	-
0.40	1.13	92.5
1.00	1.74	98.3

CONCLUSIONS

The electrochemical determination of emodin on the surface of Si-gel/CPE by ASDPV was firstly investigated in this study. The electrode is proved to be of high sensitivity, excellent selectivity, reproducibility and stability. It was shown that Si-gel/CPE remarkably enhanced the oxidation peak current of emodin comparing with that using unmodified CPE. The proposed method was successfully applied to the real sample.

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