

<http://dx.doi.org/10.5935/0103-5053.20140046>

*J. Braz. Chem. Soc.*, Vol. 25, No. 3, 602-609, 2014.  
Printed in Brazil - ©2014 Sociedade Brasileira de Química  
0103 - 5053 \$6.00+0.00

# Article

## Electrochemical Study of Simple Coumarin and its Determination in Aqueous Infusion of *Mikania glomerata*

Daniela M. Miyano,<sup>a</sup> Thays Lima,<sup>a</sup> Fábio R. Simões,<sup>a</sup> Mauro A. La-Scalea,<sup>a</sup>  
Hueder P. M. Oliveira<sup>b</sup> and Lucia Codognoto<sup>\*a</sup>

<sup>a</sup>Departamento de Ciências Exatas e da Terra, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Rua Prof. Artur Riedel, 275, Bairro Eldorado, 09972-270 Diadema-SP, Brazil

<sup>b</sup>Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Rua Santa Adélia, 166, Bairro Bangu, 09210-170 Santo André-SP, Brazil

O presente estudo teve por objetivos o desenvolvimento e a aplicação de um método eletroquímico para a determinação da cumarina simples em meio aquoso utilizando o eletrodo de diamante dopado com boro (BDDE). Os estudos foram realizados em pH 8,0 utilizando a voltametria cíclica (CV) e observou-se um processo de redução irreversível controlado por difusão com um pico de redução em torno de  $-1,6$  V. Entretanto, foi possível observar por voltametria de onda quadrada (SWV) que no mesmo pH a redução da cumarina possui um caráter reversível. Além disso, esta reversibilidade se tornou mais evidente com o aumento do pH da solução. Um estudo cronoamperométrico mostrou que o processo de redução da cumarina envolve dois elétrons. A partir dos parâmetros otimizados da SWV uma curva analítica foi construída no intervalo linear de  $0,5 \times 10^{-5}$  a  $10,0 \times 10^{-5}$  mol L<sup>-1</sup>. Os limites de detecção e de quantificação foram  $1,5 \times 10^{-6}$  mol L<sup>-1</sup> e  $4,5 \times 10^{-6}$  mol L<sup>-1</sup>, respectivamente. A cumarina foi determinada em amostras de *Mikania glomerata* (infusão aquosa) com valores de recuperação entre 92 e 104%.

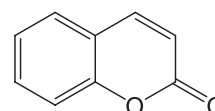
The present study aims the development and application of an electrochemical method for simple coumarin determination in aqueous media by using a boron-doped diamond electrode (BDDE). The studies were carried out at pH 8.0 by cyclic voltammetry (CV) and registered an irreversible reduction process controlled by diffusion with the peak potential recorded around  $-1.6$  V. The square wave voltammetry analysis (SWV) showed the reversible behavior of the electrochemical reduction of coumarin at the same pH. Additionally, the reversibility of the process was improved by increasing the solution pH. The chronoamperometry study showed that the coumarin reduction process involves two electrons. From the optimized SWV parameters, the analytical curve was constructed in a linear range between  $0.5 \times 10^{-5}$  and  $10.0 \times 10^{-5}$  mol L<sup>-1</sup>. The limits of detection and quantification were  $1.5 \times 10^{-6}$  mol L<sup>-1</sup> and  $4.5 \times 10^{-6}$  mol L<sup>-1</sup>, respectively. The coumarin was determined in an aqueous infusion of *Mikania glomerata*, showing recovery values between 92 and 104%.

**Keywords:** coumarin, *Mikania glomerata*, boron-doped diamond electrode

### Introduction

The medicinal plants market has been increasing in the last two decades.<sup>1,2</sup> *Mikania glomerata*, commonly known as guaco, was formalized in the Brazilian Pharmacopeia in 1929 and has been widely applied to the treatment of diseases as an anti-allergic, bronchodilator, anti-asthmatic and anti-inflammatory drug. Its properties are attributed to the simple coumarin, or 1,2-benzopyrone (Figure 1), which is found

highly concentrated in the upper leaves of *Mikania glomerata*. Its pharmaceutical formulations, syrup and oral solution, were included as reference for medicine and complementary inputs in primary healthcare.<sup>1</sup> Simple coumarin is also used for other applications such as perfume fixative, paint, spray additive, food flavoring and cleaning supplies.<sup>2</sup>



**Figure 1.** Chemical structure of simple coumarin.

\*e-mail: [lucia.codognoto@unifesp.br](mailto:lucia.codognoto@unifesp.br)

In general, analytical methods for coumarin and other organic compounds in medicinal plants are based on spectroscopic and chromatographic techniques.<sup>3-10</sup> However, the applicability of these methods to plant extract samples could be difficult due to the matrix complexity. Moreover, these methodologies are usually time-consuming, costly and laborious. In this sense, electroanalytical methods appear to be alternatives as they are faster, low cost and more easily applied for samples with simple treatment, although few works have described electroanalytical methods for coumarin determination in natural samples.<sup>11-13</sup> It is worth mentioning that the classic work by Harle and Lyons<sup>11</sup> described coumarin's polarographic behavior, indicating that in the range  $6.8 < \text{pH} < 11.2$  two forms of coumarin, lactone and coumaric acid, are present. Furthermore, Wang *et al.*<sup>13</sup> presented the electroanalytical reduction of coumarin using a glassy carbon electrode (GCE), a lead-modified GCE (Pb/GCE) and a mercury-modified GCE (Hg/GCE). The authors investigated coumarin levels using direct current as well as the cyclic (CV) and the differential pulse (DPV) voltammetries; eventually, a reduction wave was observed around  $-1.4 \text{ V vs. Ag/AgCl}$ , reaching the more sensitive conditions in the pH range of 8.07 to 8.96.

The boron-doped diamond electrode (BDDE) presents some advantages compared to other carbon allotropes, such as glassy carbon and pyrolytic. This material presents many attractive properties, which include a wide electrochemical window and high chemical stability.<sup>14,15</sup> Thus, the BDDE applications have been increasingly used for the development of electroanalytical methods to determine inorganic and organic compounds, such as pesticides,<sup>16,17</sup> polyamines,<sup>18</sup> serotonin and histamine,<sup>19</sup> and uric acid,<sup>20</sup> as well as medicines such as paracetamol, ascorbic acid and caffeine,<sup>21</sup> captopril,<sup>22</sup> dopamine,<sup>23</sup> fosamprenavir (an anti retroviral drug),<sup>24</sup> and lornoxicam in serum.<sup>25</sup> In general, the results of these studies agree at the 95% confidence level with those found in chromatographic methods.

This work seeks to address the development of a methodology for coumarin determination in medicinal plants by using square wave voltammetry (SWV) combined with BDDE. This methodology was applied for simple coumarin analysis in aqueous infusions of guaco leaves.

## Experimental

### Reagents

1,2-benzopyrone (simple coumarin) was purchased from Acros Organics. The *Mikania glomerata* infusions were prepared with guaco leaves purchased at a local commercial market. All of the solutions were made from

analytical grade reagents and ultra-pure water (MilliQ, Millipore Corporation). The support electrolyte was a solution containing Britton-Robinson (BR) buffer.

### Apparatus

A conventional three-electrode cell with the Ag/AgCl system and a Pt wire were used as the reference and auxiliary electrodes, respectively. The working electrode was a boron-doped diamond (BDD) film (8000 ppm boron and area =  $0.25 \text{ cm}^2$ ) on a silicon wafer. Prior to the experiments, BDDE was submitted to anodic treatment once ( $+3.0 \text{ V vs. Ag/AgCl}$  for 10 min) to remove hydrophobic film. After that, a cathodic treatment was realized ( $-3.0 \text{ V vs. Ag/AgCl}$  for 10 min) for surface conditioning. When electrodic surface recovery was necessary, cathodic treatment was used for 30 seconds.<sup>26</sup> An electrochemical analyzer Autolab<sup>®</sup> PGSTAT128N (Eco Chemie, Netherlands) was used for all voltammetric measurements. Data acquisition and conducting potentiostat were achieved using a computer and GPES software.

### Study of the electrochemical behavior of simple coumarin

The electrochemical behavior of coumarin ( $5.0 \times 10^{-5} \text{ mol L}^{-1}$ ) in BR buffer (pH 8.0) was analyzed using the cyclic voltammetry (CV) technique in the scan range from 0.5 to  $-1.6 \text{ V vs. Ag/AgCl}$  reference electrode. The scan rate was evaluated from 10 to  $100 \text{ mV s}^{-1}$ .

The square wave voltammetry (SWV) technique was used for the analytical determination of simple coumarin. Parameters such as pH, frequency, amplitude and the step potential were optimized, aiming to improve the sensibility and selectivity. The voltammograms were registered from  $-1.30$  to  $-1.75 \text{ V vs. Ag/AgCl}$ .

For the chronoamperometry study, 10 mL of the supporting electrolyte were added in an electrochemical cell ( $0.1 \text{ mol L}^{-1}$  BR buffer, pH 8.0) with 500  $\mu\text{L}$  of a stock solution of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  coumarin. A potential of  $-1.8 \text{ V}$  was applied to the system in time intervals of 0.4, 0.6, 0.8 and 5 seconds.

### Prediction of diffusion coefficients

The diffusion coefficient predictions of the studied compounds in the aqueous phase with infinite dilutions were carried out based on a previously published method,<sup>27</sup> in which the Wilke-Chang equation was applied:

$$D = \frac{7.4 \times 10^{-8} (xM)^{0.5} T}{\eta V^{0.6}} \quad (1)$$

where  $D$  is the diffusion coefficient of the solute in water ( $\text{cm}^2 \text{s}^{-1}$ ),  $\eta$  is the viscosity of water (centipoise) at the temperature of interest ( $\eta = 0.8937 \text{ cp}$  at  $25 \text{ }^\circ\text{C}$ ),  $M$  is the molar mass of water ( $\text{g mol}^{-1}$ ),  $T$  is the temperature (K),  $x$  is the association parameter of water (2.53), and  $V$  is the molar volume of the solute ( $\text{cm}^3 \text{mol}^{-1}$ ). The solute molar volume was calculated starting from the ratio between the Van der Waals molecular volume and the Le Bas molar volume.<sup>27</sup> From quantum chemical calculations, the molar volume of simple coumarin was estimated. The calculations were based on the density functional theory (B3LYP) with 6-31 g basis set to optimize the coumarin molecule, and for the calculations involving solvents, the IEFPCM model was used.

### Studies with *Mikania glomerata* infusions

The infusions were prepared by the dissolution of 3.0 g of dry guaco leaves in 150.0 mL of boiling water. A volume of 250  $\mu\text{L}$  of the infusion sample was transferred to the voltammetric cell containing 10 mL of the supporting electrolyte (0.10 mol  $\text{L}^{-1}$  BR buffer, pH 8.0). After dissolved air had been removed from the solution by bubbling with nitrogen for 10 min, coumarin was determined by SWV through the standard addition method, using the BDD working electrode. The voltammetric parameters comprise the frequency ( $f$ ): 100  $\text{s}^{-1}$ , the pulse amplitude ( $a$ ): 100 mV, and the scan increment ( $\Delta E_s$ ): 4 mV.

The recovery experiments were carried out by the addition of 250  $\mu\text{L}$  of the coumarin stock solution ( $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ) in 1 mL of infusion sample. In sequence, 250  $\mu\text{L}$  of this solution was added to 10 mL of supporting electrolyte, followed by standard additions from the coumarin stock solution which resulted in an analytical plot. All measurements were performed in triplicate. The recovery efficiencies (% $R$ ) for the system under investigation were calculated using equation 2, where the value [coumarin] found refers to the concentration obtained by extrapolation of the analytical curve in the corresponding spiked infusion samples:

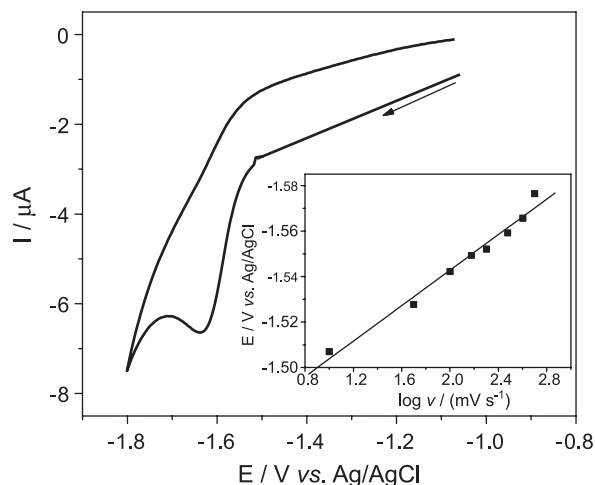
$$\%R = 100 \frac{[\text{coumarin}]_{\text{found}}}{[\text{coumarin}]_{\text{added}}} \quad (2)$$

## Results and Discussion

### Electrochemical behavior of coumarin

The cyclic voltammogram of coumarin on the BDDE in the aqueous solution exhibited an irreversible reduction peak at  $-1.65 \text{ V vs. Ag/AgCl}$  at pH 8.0 (Figure 2). The

scan rate variation analysis demonstrated that the reduction process of coumarin at BDDE is controlled by diffusion, since the peak current increases linearly with the square root of the scan rate (results not shown). Additionally, the peak potential ( $E_p$ ) vs.  $\log v$  plot (Figure 2 inset) showed a linear relationship ( $r = 0.993$ ), in which the peak potential was shifted to a negative direction when the scan rate was increased, with a slope value of 39 mV per ten fold of  $v$ , which confirms the irreversibility of the process in this experimental condition.<sup>28</sup>

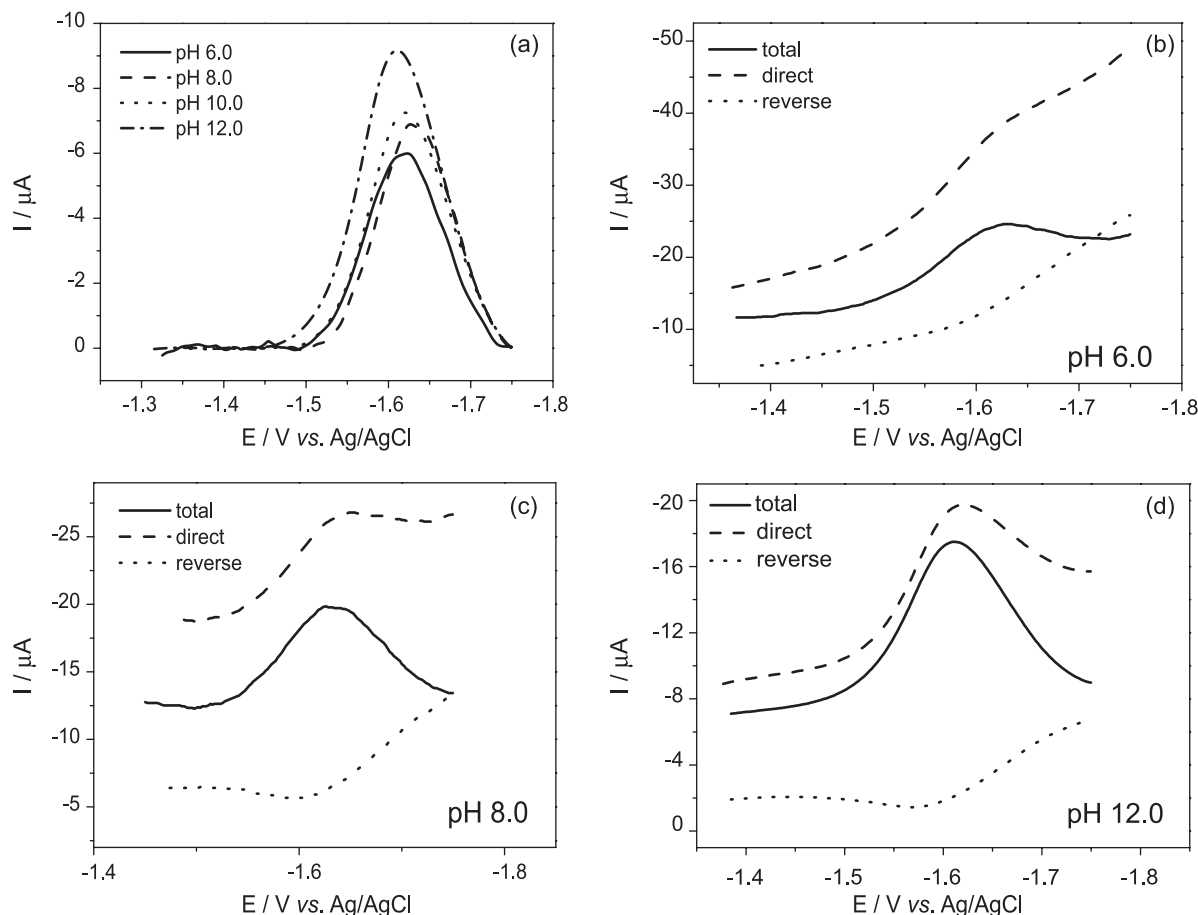


**Figure 2.** Cyclic voltammogram of  $5.0 \times 10^{-5} \text{ mol L}^{-1}$  coumarin in 0.1 mol  $\text{L}^{-1}$  BR buffer at pH 8.0,  $v = 100 \text{ mV s}^{-1}$ . Inset: linear dependence on the peak potential with the logarithm of the scan rate.

The influence of the acidic and basic media was evaluated using the coumarin voltammetric behavior using the BDDE in a 0.1 mol  $\text{L}^{-1}$  BR buffer in the range  $2.0 < \text{pH} < 12.0$  by SWV (Figure 3). At pH values lower than 6.0, no peak was observed for coumarin reduction, since the water reduction reaction overlaps the coumarin signal in this pH region, due to the high current generated by the supporting electrolyte decomposition. On the other hand, while the  $E_p$  value was displaced non-linearly at  $\text{pH} > 6.0$ , the peak current ( $I_p$ ) increased (Figure 3a).

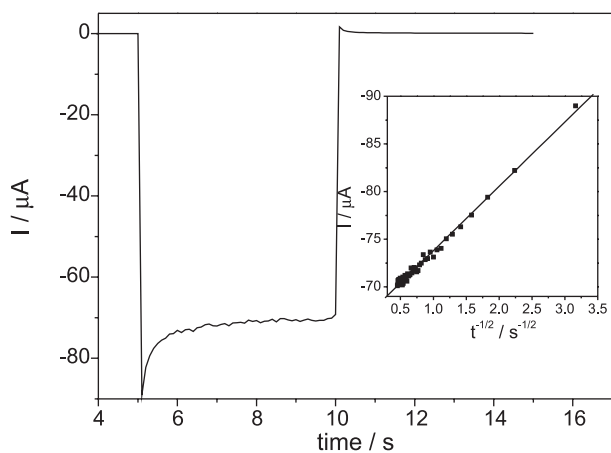
Unexpectedly, from what was observed by CV, the voltammetric reduction of coumarin presented a quasi-reversible character that is clearly influenced by solution pH. Using SWV, it was possible to observe the recording of a reverse current (anodic component), which improved its definition with the increase in pH. Figures 3b, 3c and 3d show the current decomposition as a function of the pH. It is possible that the reverse current could only be seen because SWV is more sensitive than CV.

To better understand the electrochemical behavior of simple coumarin on BDDE, chronoamperometric experiments were aimed at determining the number



**Figure 3.** (a) Square wave voltammograms of  $5.0 \times 10^{-5} \text{ mol L}^{-1}$  coumarin at different pH values in  $0.1 \text{ mol L}^{-1}$  BR buffer ( $a = 50 \text{ mV}$ ,  $f = 100 \text{ s}^{-1}$  and  $\Delta E_s = 2 \text{ mV}$ ). Current components for the simple coumarin reduction by SWV at pH values (b) 6.0; (c) 8.0; and (d) 12.0.

of electrons involved in the process. Figure 4 shows a chronoamperogram registered for simple coumarin. The relationship between the registered current and the square root of time was linear and the estimate of the number of electrons was made by applying Cottrell's equation,<sup>3</sup> as shown in Table 1.



**Figure 4.** Chronoamperogram of  $5.0 \times 10^{-5} \text{ mol L}^{-1}$  coumarin at  $-1.8 \text{ V}$  in a time interval of 5 s. Inset: Cottrell's linear relationship.

From the data shown in Table 1, it can be stated that 2 electrons are involved in the reduction process of simple coumarin. The molar volume was calculated considering coumarin in vacuum and water; for both results, similar values of diffusion coefficients were predicted and the same number of electrons calculated. It is important to highlight that the Wilke-Chang<sup>27</sup> equation considers the aqueous medium involving an association parameter of water. Therefore, based on these results and corroborated with the literature data,<sup>29</sup> we assume that coumarin reduction occurs in the carbonyl group with the involvement of two electrons and two protons to form the hydroxyl group as a reduced species. Consequently, it is possible to infer that an intermediate species in the radical form can be more stable in alkaline media. This possibility, to a smaller extent, would explain the reversibility observed by SWV, since it was not observed by CV in the same experimental conditions.

#### Optimization of SWV conditions

Optimization of the analytical procedure involved a systematic study of the experimental parameters affecting

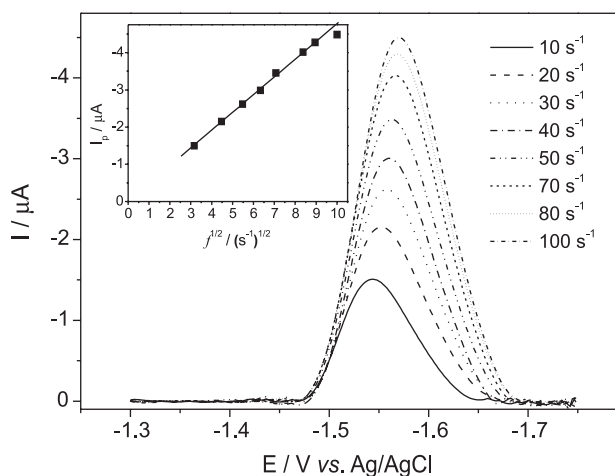
**Table 1.** Number of electrons involved in the process calculated with the chronoamperometric data

Cottrell's slope <sup>a</sup>	time / s	Vacuum		Water	
		V / (cm <sup>3</sup> mol <sup>-1</sup> ) 93.117	D / (cm <sup>2</sup> s <sup>-1</sup> ) 1.10 × 10 <sup>-5</sup>	V / (cm <sup>3</sup> mol <sup>-1</sup> ) 103.16	D / (cm <sup>2</sup> s <sup>-1</sup> ) 1.03 × 10 <sup>-5</sup>
		n <sup>a</sup>		n <sup>a</sup>	
5 ± 2 × 10 <sup>-6</sup>	0.4	2.0 ± 0.8		2.2 ± 0.8	
5 ± 1 × 10 <sup>-6</sup>	0.6	2.2 ± 0.6		2.3 ± 0.6	
5.4 ± 0.7 × 10 <sup>-6</sup>	0.8	2.4 ± 0.3		2.5 ± 0.3	
6.0 ± 0.5 × 10 <sup>-6</sup>	5.0	2.7 ± 0.2		2.7 ± 0.2	

<sup>a</sup>Average values from 3 measurements.

the SWV response; namely, the frequency ( $f$ ), the pulse amplitude ( $a$ ) and the scan increment ( $\Delta E_s$ ). The results of these studies will be presented separately, as follows.

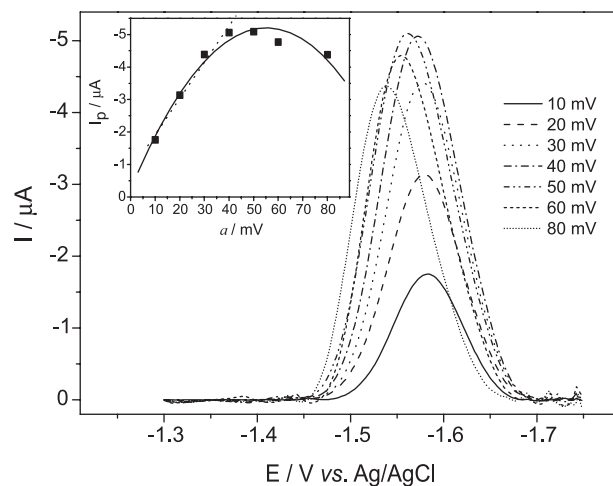
In SWV, the frequency is the most important parameter, since it determines the intensity of the signal and, in turn, the sensitivity of the technique. The voltammograms obtained at frequencies ranging from 10 to 100 Hz with  $5.0 \times 10^{-5}$  mol L<sup>-1</sup> coumarin are presented in Figure 5 and clearly show the influence of frequency on the SWV profiles for coumarin reduction on BDDE. The relationship between peak current and square root of the frequency was found to be linear over the entire range tested (Figure 5 inset). The  $I_p$  vs.  $f^{1/2}$  linear relationship is normally associated with an electrode process controlled by mass transport; in this case, by semi-infinite linear diffusion.<sup>30</sup>



**Figure 5.** Effect of frequency on the square-wave voltammograms for  $5.0 \times 10^{-5}$  mol L<sup>-1</sup> coumarin in 0.1 mol L<sup>-1</sup> BR buffer, pH = 8.0, with  $a = 50$  mV and  $\Delta E_s = 2$  mV. Inset: linear dependence of the peak current with the square wave frequency.

In order to analyze the relationship between peak current and pulse amplitude for  $5.0 \times 10^{-5}$  mol L<sup>-1</sup> of coumarin, SWV was performed with various amplitudes in the range of 10 to 80 mV, as shown in Figure 6. Here, it was found that peak currents increase linearly with amplitude, but the

half-peak width also increases, impairing the sensitivity of the method.<sup>31</sup> As an optimal amplitude for coumarin on the BDDE, 50 mV was chosen, as demonstrated in Figure 6. Finally,  $\Delta E_s$  with a value of 4 mV was used in this investigation, since it had no influence on the peak current in the range from 1 to 10 mV.



**Figure 6.** Effect of amplitude of the square wave voltammograms for  $5.0 \times 10^{-5}$  mol L<sup>-1</sup> coumarin in 0.1 mol L<sup>-1</sup> BR buffer, pH = 8.0, with  $f = 100$  s<sup>-1</sup> and  $\Delta E_s = 2$  mV. Inset: linear dependence of the peak current of the square-wave amplitude.

#### Analytical performance

To establish the analytical procedure, a series of experiments was carried out using standard solutions of coumarin and the conditions described above concerning the relation to both the maximum peak current (sensitivity) and the minimum half-peak width (sensitivity).

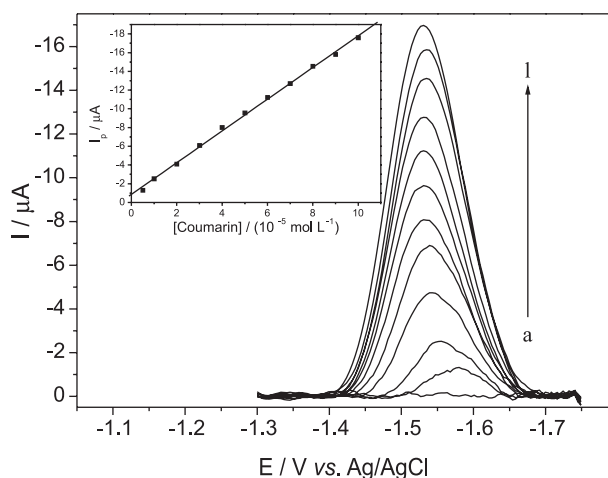
The linearity, linear range and sensitivity were obtained from calibration plots using an external standard at eleven concentration levels, in triplicate, between  $0.5 \times 10^{-5}$  to  $10.0 \times 10^{-5}$  mol L<sup>-1</sup> coumarin in 0.1 mol L<sup>-1</sup> BR buffer pH 8.0 (Figure 7). The linearity was tested using a pure-error lack-of-fit test with simple regression, which was not significant at the 5% level. The sensitivity (slope of the

**Table 2.** Comparison of the analytical parameters obtained using different electrode materials and/or techniques for coumarin determination

Technique	Linear range	LOD	LOQ	Ref.
DIP-APCI-MS	0.1-10 mg L <sup>-1</sup>	7.2 mg kg <sup>-1</sup>	25.3 mg kg <sup>-1</sup>	33
HPLC-UV/VIS	0.024-6.250 mg L <sup>-1</sup>	0.72 mg kg <sup>-1</sup>	2.42 mg kg <sup>-1</sup>	34
HPLC-MS	2.0-3000.0 mg L <sup>-1</sup>	0.17 mg L <sup>-1</sup>	0.56 mg L <sup>-1</sup>	35
HPLC-UV/VIS	0.25-25.0 ng μL <sup>-1</sup>	30 mg kg <sup>-1</sup>	80 mg kg <sup>-1</sup>	36
Fluorescence spectroscopy	0.37-14.61 mg L <sup>-1</sup>	0.15 mg L <sup>-1</sup>	–	37
DPV-mercury film electrode	2-60 mg L <sup>-1</sup>	–	–	13
SWV-BDDE	0.73-14.61 mg L <sup>-1</sup>	0.22 mg L <sup>-1</sup>	0.66 mg L <sup>-1</sup>	Present work

DIP-APCI-MS: direct inlet probe-atmospheric-pressure chemical ionization-mass spectrometry; HPLC-MS: high performance liquid chromatography-mass spectrometry; DPV: differential pulse voltammetry; LOD: limit of detection; LOQ: limit of quantification.

calibration plot) and linearity (linear regression coefficient) were calculated as 0.17 A mol<sup>-1</sup> L and 0.999, respectively. The corresponding linear equation was determined as  $I_p (\mu\text{A}) = -0.86 \mu\text{A} + 0.17 \text{ A} / \text{mol C}$ , where C is coumarin concentration in mol L<sup>-1</sup>. The inter-assay precision, expressed as the estimate relative standard deviation and established through the analysis of a  $1.0 \times 10^{-5}$  mol L<sup>-1</sup> coumarin solution (n = 10) was 1.1%.



**Figure 7.** SWV responses for the reduction of different coumarin concentrations: blank (a); 0.5 (b); 1.0 (c); 2.0 (d); 3.0 (e); 4.0 (f); 5.0 (g); 6.0 (h); 7.0 (i); 8.0 (j); 9.0 (k); 10.0 (l)  $\times 10^{-5}$  mol L<sup>-1</sup> in 0.1 mol L<sup>-1</sup> BR buffer pH 8.0. Inset: linear dependence of  $I_p$  with coumarin concentration.

The limits of detection (LOD) and quantification (LOQ) were calculated as  $3\sigma_B / b$  and  $10\sigma_B / b$ , respectively, where  $\sigma_B$  is the standard deviation of the blank current at the potential value corresponding to the peak current and  $b$  the slope of the calibration plot.<sup>32</sup> The calculated value of LOD for coumarin on the BDDE was  $1.5 \times 10^{-6}$  mol L<sup>-1</sup> (0.22 mg L<sup>-1</sup>) while the LOQ was  $4.5 \times 10^{-6}$  mol L<sup>-1</sup> (0.66 mg L<sup>-1</sup>).

Table 2 shows a comparison among the analytical parameters obtained by the present work for coumarin determination by using BDDE with those registered in the

literature obtained by different methods. It is possible to observe that our results are compatible with the literature.

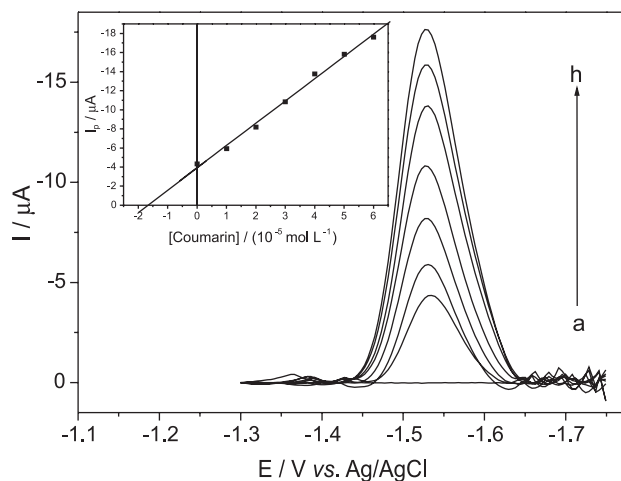
#### Determination of coumarin in aqueous infusion *Mikania glomerata*

After the extraction process, 0.25 mL of the infusion were added to 10 mL of the 0.1 mol L<sup>-1</sup> BR buffer at pH 8.0 in the electrochemical cell. The square wave voltammogram registered for the infusion is presented in Figure 8. The diluted infusion sample in the electrochemical cell presented an amount of  $1.6 \times 10^{-5}$  mol L<sup>-1</sup>, corresponding to a concentration of  $6.3 \times 10^{-4}$  mol L<sup>-1</sup> of simple coumarin in the original aqueous infusion. Therefore, around 14 mg of coumarin was found in 3 g of guaco dry leaves. The coumarin concentration was determined by using the standard addition method; the results are shown in Table 3.

In sequence, for the recovery studies, the infusion sample was spiked with coumarin ( $0.6 \times 10^{-5}$  mol L<sup>-1</sup> in the electrochemical cell) and several standard solutions were added up to a concentration of  $6.0 \times 10^{-5}$  mol L<sup>-1</sup>. All of the square wave voltammetric responses were collected and the initial concentration was recovered by linear regression (Figure 8). The percentage found for each recovery experiment is also included in Table 2. They clearly demonstrate that the BDDE allowed an excellent percentage of recovery, even in highly complex samples, thus offering the possibility of analytical determinations in medicinal plants.

## Conclusions

The voltammetric behavior registered for simple coumarin by using CV showed that the compound presents an irreversible reduction peak around -1.6 V and that this reduction process is controlled by diffusion using the BDDE. On the other hand, the pH study showed that



**Figure 8.** Square wave voltammograms for coumarin in the infusion sample: blank (a); infusion sample (b); coumarin 1.0 (c); 2.0 (d); 3.0 (e); 4.0 (f); 5.0 (g); and 6.0 (h)  $\times 10^{-5}$  mol L $^{-1}$ , in 0.1 mol L $^{-1}$  BR buffer, pH 8.0,  $f = 100$  s $^{-1}$ ,  $a = 50$  mV, and  $\Delta E_s = 4$  mV. Inset: current peak vs. coumarin concentration.

**Table 3.** Results for coumarin in the infusion samples

Sample	Coumarin in the infusion / (mol L $^{-1}$ )	Recovery / %
1	$6.4 \times 10^{-4}$	98
2	$6.3 \times 10^{-4}$	100
3	$6.0 \times 10^{-4}$	92
4	$6.7 \times 10^{-4}$	104
5	$6.2 \times 10^{-4}$	98

the voltammetric behavior of coumarin is different when registered using SWV. In this case, it was observed that the reversibility was better defined with an increased pH. From the chronoamperometric study, it was estimated that two electrons are involved in the reduction process of simple coumarin. The determination in the aqueous infusion of guaco showed LOD and LOQ of  $1.5 \times 10^{-6}$  mol L $^{-1}$  and  $4.5 \times 10^{-6}$  mol L $^{-1}$ , respectively, and the recovery values were around 92 to 104%. The results indicate that the use of BDDE combined with square wave voltammetry can be an efficient alternative for the quantification of coumarin in aqueous infusions of *Mikania glomerata*; the method was easy to apply and required almost no sample treatment.

## Acknowledgments

The authors thank FAPESP (Proc. No. 2008/50588-6) and CNPq, Brazil, for scholarships and financial support for this work.

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*Submitted: October 15, 2013*

*Published online: February 28, 2014*

**FAPESP has sponsored the publication of this article.**