Feature Article

Anodic Voltammetry of Xanthine, Theophylline, Theobromine and Caffeine at Conductive Diamond Electrodes and Its Analytical Application

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Received: August 6, 2001
Final version: November 20, 2001

Abstract

Boron-doped diamond (BDD) electrodes were used to examine the electrochemical oxidation of xanthine and its naturally occurring *N*-methyl derivatives, theophylline, theobromine and caffeine. Voltammetric studies showed that the mechanism of the overall reaction is similar to that of the oxidation of purine derivatives at the pyrolytic graphite electrode. The effects of pH, concentration and potential sweep rate on the voltammetric response were thoroughly investigated, and it was found that BDD exhibits excellent behavior, in terms of very well-defined, reproducible oxidation peaks, for xanthine, theophylline, theobromine and caffeine determination. The results enabled the measurement of the oxidation peak current to be used as the basis for a simple, accurate and rapid method for determining the investigated compounds, within a concentration range of 1 to 400 μM for theophylline, theobromine and caffeine, and of 1 to 100 μM for xanthine. Promising results were obtained for caffeine determination in real samples of commercially available products, without separation from the matrix.

Keywords: Boron-doped diamond, Xanthine, Voltammetry, Detection, Sensitivity

1. Introduction

Xanthine (3,7-dihydro-1*H*-purine-2,6-dione) belongs to the purine group, and it is thought to have a critical biological importance, because it plays a prominent role as an intermediate in adenine and guanine degradation to uric acid [1]. Furthermore, xanthine is sparingly soluble in water, and metabolic abnormalities can occasionally lead to its precipitation as aggregates, although xanthine "stones" are quite rare [2].

The *N*-methyl derivatives of xanthine (see Table 1), including theophylline (3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione), theobromine (3,7-dihydro-3,7-dimethyl-1*H*-purine-2,6-dione), and caffeine (3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione), are alkaloids that are widely distributed in plant products and beverages and are known to have many physiological effects, such as gastric acid secretion, diuresis, and stimulation of the central nervous system [3]. Besides, these compounds are considered to be risk factors for asthma, kidney malfunction and cardiovascular diseases [4].

Because of the apparent parallelism between electrochemical and enzymatic oxidations [5–7], it is also likely that the study of the electrochemical oxidation of naturally occurring purines could help, at least in part, in understanding the complex mechanism of enzymatic and perhaps in vivo reactions.

This is why there have been numerous studies aiming to develop reliable methods for the evaluation and quantitation of xanthine and its *N*-methyl derivatives, and several methods have been proposed, including UV-spectrometry [8], thin-layer chromatography [9], electrophoresis [10], and HPLC [11]. Glassy carbon (GC) electrodes have been used for the amperometric detection of caffeine, theophylline and theobromine in beverages, either directly or after solid-phase extraction [12], and for the voltammetric determination of xanthine within the concentration range from 50 to 200 μM [13]. Differential-pulse voltammetry using GC electrodes has also been employed for the accurate determination of caffeine in drug formulations, and a linear calibration range of 0 to 250 μM was reported [14]. Compared to bare GC electrodes, GC electrodes modified with a lead-ruthenium pyrochlore oxide in a Nafion matrix exhibited a marked enhancement of the current response for caffeine determination by square-wave voltammetry, over the 5–200 μM range [15]. The use of carbon (or graphite) and platinum electrodes in the anodic determination of xanthines is rendered difficult by interference from oxidation background currents that are not precisely reproducible. This is because relatively large positive potentials (>1.4 V vs. SCE) are required for reasonable sensitivity, both in amperometry for GC [12] and voltammetry for GC [14, 15] and pyrolytic graphite (PG) [16]. The optimization of the peak current is important for analysis, but this relies on the availability of electrode materials that

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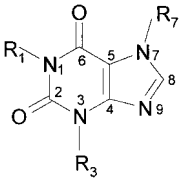
Table 1. Structure of xanthine and its naturally occurring *N*-methyl derivatives.

Structure	R_1	R_3	R_7	*Compound*
	H(11.5)[a]	H(7.5)[a]	H(11.0)[a]	Xanthine
	CH_3	CH_3	H(8.6)[a]	Theophylline
	H(11.0)[a]	CH_3	CH_3	Theobromine
	CH_3	CH_3	CH_3	Caffeine

[a] pK_a values for xanthine and methylated xanthines were taken from [22].

have the ability to detect compounds that are difficult to oxidize, as in the work reported here.

Due to its excellent electrochemical features, such as wide potential window in aqueous solutions [17], low background current [18, 19], and long-term stability of response [20, 21], conductive diamond is an electrode material that has attracted great interest, especially in electroanalysis. The aim of the present work is to study the electrochemical oxidation of xanthine and its naturally occurring *N*-methyl derivatives (theophylline, theobromine and caffeine) at conductive diamond electrodes, with an eye to possible analytical applications.

2. Experimental

Microwave plasma-assisted chemical vapor deposition was used in order to obtain boron-doped polycrystalline diamond coatings on Si(100) wafers. The procedure has been described previously in detail [19]. Voltammetric measurements were performed in a glass cell, at room temperature, with a Hokuto Denko HA-502 potentiostat, a Hokuto Denko HB-111 function generator and a Riken Denshi x – y recorder. Boron-doped diamond (BDD) films were used as working electrodes (0.07 cm^2 exposed area), while a platinum foil was used as the counter electrode. As a reference, a saturated calomel electrode (SCE) was used.

Xanthine, theophylline, theobromine and caffeine were obtained from Wako Chemicals and were used as received. All of the chemicals were of analytical-reagent grade, and all solutions were prepared from Milli-Q water (Millipore). Air-saturated Britton-Robinson buffer solution (0.04 M) containing 0.1 M $NaClO_4$ was used as the supporting electrolyte, and the pH was measured with a conventional glass electrode.

3. Results and Discussion

3.1. Cyclic Voltammetry

Figure 1 shows cyclic voltammograms recorded for theophylline (50 μM) at a sweep rate of 20 mV s^{-1} at BDD and GC electrodes in Britton-Robinson buffer (pH 1.8). The oxidation potentials were 1.3 V and 1.25 V (vs. SCE) for BDD and GC respectively. At BDD, the voltammogram was

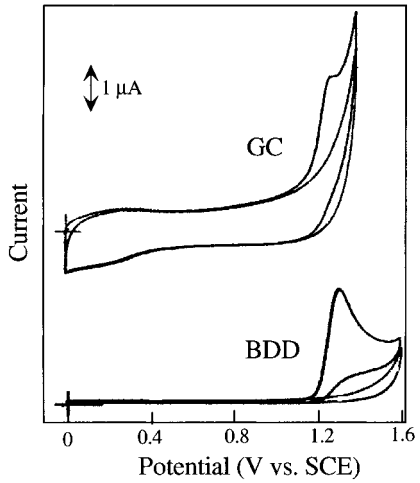


Fig. 1. Cyclic voltammograms for theophylline at a) BDD electrode and b) GC electrode at pH 1.8. Concentration, 50 μM; sweep rate, 20 mV s^{-1}.

well-defined with a signal-to-background (*S*/*B*) ratio of 92, whereas at GC, oxygen evolution reaction interfered with the oxidation curve, making it ill-defined. The *S*/*B* ratio at GC was 0.85. This value is ca. 100 times lower than that obtained at BDD. The slightly higher peak current observed at BDD may be attributed to surface roughness of the electrode. Another important observation is the difference in the background current. At the oxidation potential, the background current was 30 nA for BDD and 1.6 μA for GC. The low background current and the wide potential window for BDD are due to the near absence of intrinsic electrochemical processes, as well as the near absence of porosity [22]. Similar cyclic voltammetric features were observed for the oxidation of xanthine, theobromine and caffeine. The large difference in the background currents and S/B ratios indicate the superiority of BDD for the detection of xanthines.

3.1.1. Effect of pH

Xanthine, theophylline, theobromine and caffeine each exhibited an oxidation peak at the BDD electrode in a specific pH range, which depended on the particular compound (results not shown). It was observed that increasing pH results in a negative shift of the peak potential

for theophylline. It is clear from the pH dependence results that the most analytically desirable response is obtained at low pH. At higher pH, the background current (presumably due to oxygen evolution) begins to interfere, and also the peak height for the oxidation of the compound itself decreases, as discussed later in more detail.

These trends were also found for the other compounds examined. Nevertheless, xanthine and theophylline exhibited well-defined and reproducible peaks over a wide pH range (1.8 to 12.0), whereas in the case of theobromine and caffeine, the oxidation peak tended to become rather ill-defined at pH values above ca. 8, because it occurred very close to background discharge.

It was found that the peak potential decreases linearly with increasing pH for all of the investigated xanthines, in agreement with previously reported results for the oxidation of several biologically important xanthines at the pyrolytic graphite electrode [23]. Nevertheless, unlike theobromine and caffeine, in which cases E_p vs. pH relationships exhibited a single slope, for xanthine and theophylline, linear regression statistical analysis suggested a slope change at pH values above 8.0 and 9.0, respectively (Table 2). The shift of the peak potential with pH of ca. 60 mV per pH unit for xanthine and theophylline indicates the involvement of identical numbers of protons and electrons in the potential-controlling process. At higher pH values, it is difficult to evaluate the slopes precisely, due in part to the proximity to the background discharge. Nevertheless, the lower slopes of the E_p vs. pH relationships (Fig. 2), 43 and 40 mV per pH unit, are consistent with the fact that in this pH range, the overall oxidation reactions of xanthine and theophylline involve more electrons than protons, e.g., three protons and four electrons. The reaction mechanism will be discussed later in detail.

It should be noted that the pH values corresponding to the changes in slope (Fig. 2) agree quite well with the pK_a values for the first proton dissociations for xanthine and theophylline, which involves the NH protons from positions 3 ($pK_a = 7.5$) and 7 ($pK_a = 8.6$), respectively [24]. In the case of theobromine and caffeine, the variation of the peak potential as a function of pH is not affected by acid dissociation. No protons are available for dissociation in the case of caffeine, whereas the single NH proton (position 1) in theobromine dissociates only at high pH ($pK_a = 11.0$) [24].

Linear sweep voltammograms recorded at several pH values for all of the investigated xanthines also showed a

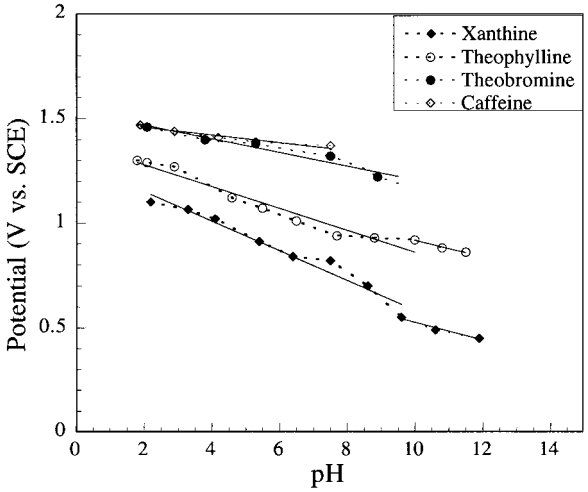


Fig. 2. Variation of the peak potential as a function of pH for: 1) theophylline oxidation; 2) xanthine, 3) theobromine and 4) caffeine oxidation. Concentration, 50 μM; sweep rate, 20 mV s^{-1}.

variation of the peak current as a function of pH. Although many studies have been devoted to the electrochemical oxidation of xanthines, it seems that the effect of pH on the peak current is quite variable. Thus, at GC and PG electrodes, Goyal found the peak current for xanthine oxidation to be virtually constant over a wide pH range (1.8 to 10.7) [25]. Nevertheless, for the same process, Dryhurst found a more or less continuous decrease of the peak current with increasing pH at a PG electrode [26]. At GC, Yao et al. found that the peak current for xanthine and hypoxanthine oxidation was much larger at both low and high pH compared to neutral pH [13].

Figure 3 shows the effect of pH on the peak current for the oxidation of all four compounds at the BDD. For theophylline, the peak current reaches lower values around neutral pH, as previously reported for xanthine and hypoxanthine oxidation at GC [13]. A similar relationship was also obtained for the oxidation of xanthine, theobromine and caffeine at the BDD electrode. However, in the case of theobromine and caffeine, at pH values higher than ca. 8 and 9, the oxidation peaks occurred very close to background discharge. Although the reasons for this behavior remain ambiguous, it is likely that it is related to a strongly pH-dependent adsorption of these compounds [13, 26]. This could explain, at least in part, the apparently contradictory

Table 2. Linear relationships between E_p and pH for the oxidation of xanthine, theophylline, theobromine and caffeine at the BDD electrode. Concentration: 50 μM, sweep rate: 20 mV s^{-1}.

Compound	*pH range*	*E_p [V vs. SCE]*	*R^2*
Xanthine	1.8 – 8.0	1.18 – 0.071 pH	0.9811
	9.0 – 12.0	0.65 – 0.043 pH	0.9819
Theophylline	1.8 – 9.0	1.3 – 0.052 pH	0.9763
	9.0 –12.0	0.93 – 0.040 pH	0.9765
Theobromine	1.8 – 8.9	1.47 – 0.032 pH	0.9791
Caffeine	1.8 – 7.5	1.47 – 0.19 pH	0.9788

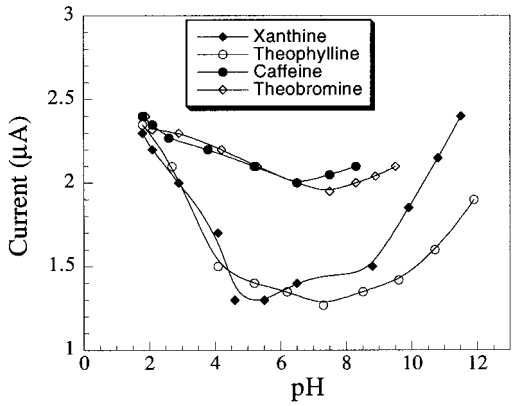


Fig. 3. Effect of pH on the peak current for the oxidation of theophylline (1), xanthine (2), theobromine (3), and caffeine (4) at the BDD electrode, concentration; 50 μM; sweep rate, 20 mV s^{-1}.

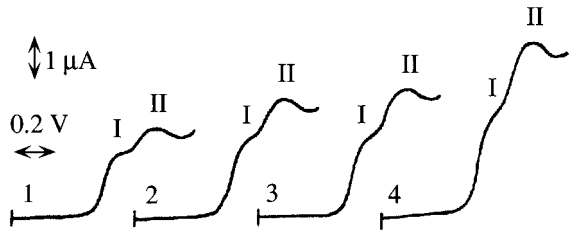


Fig. 4. Influence of the sweep rate on the anodic linear-sweep voltammograms for theophylline oxidation. Sweep rate: 1) 50, 2) 83, 3) 100, 4) 167 mV s^{-1}; concentration, 50 μM; pH, 4.6; initial potential, 0.6 V.

data reported in the literature concerning the variation of the peak current as a function of the pH for the oxidation of xanthines.

The study of the effect of pH on the voltammetric oxidation of xanthine and theophylline at conductive diamond showed that in some pH ranges an additional peak appears, at more positive potentials than the main peak, as in the case of hypoxanthine oxidation at GC [13]. At BDD electrodes, post-peaks were observed within pH ranges of ca. 3.0 to 8.0 and ca. 4.0 to 8.5, for xanthine and theophylline, respectively and are attributed to strong reactant adsorption (see also below). However, it was found that complications due to adsorption were easily avoided by operating at low pH.

Taking into account all of the results thus far, it is clear that in the case of xanthine, theophylline, theobromine and caffeine oxidation at BDD electrodes, acidic media (pH <3.0) are the most suitable for analytical purposes. At low pH, the three main advantages are 1) relatively high peak current, 2) relatively little interference from background current (probably oxygen evolution) and 3) freedom from extraneous peaks due to possible adsorption.

3.1.2. *Effect of the Sweep Rate*

The study of the effect of the sweep rate (within a range of 5 to 200 mV s^{-1}) on the anodic peak current at the BDD electrode was also carried out for all of the investigated xanthines. It was found that in the case of theobromine and caffeine, the peak current increased linearly with the square root of potential sweep rate, with zero intercept, indicating diffusional control within the investigated pH range, in good agreement with previously reported results for caffeine oxidation at chemically modified electrodes [15]. A rather similar dependence of the peak current on the sweep rate was found for xanthine and theophylline within the pH ranges where no post-peaks appeared (1.8 to 3.0 and 8.0 to 12.0 for xanthine, and 1.8 to 4.0 and 8.5 to 12.0 for theophylline), with the only difference being a small non-zero intercept. The intercept is an indication of a change in slope from a higher value (for reversible behavior) at low sweep rates to a lower value (for irreversible behavior) at higher sweep rates [27].

Concerning the additional peaks observed with xanthine and theophylline at the BDD electrode in the medium pH range (see above), the effect of the sweep rate on the voltammetric behavior was examined. Figure 4 shows linear-sweep voltammograms obtained for a 50 μM theophylline solution (pH 4.6) at several sweep rates. It can be seen that, in addition to the peak that appears over the whole investigated pH range (peak I in Figure 4), a post-peak (peak II) appears at a more positive potential particularly at higher sweep rates. This type of behavior has been reported by other workers, e.g., Hansen and Dryhurst [23]. A variety of similar results has been reported including both post-peaks [26, 28, 29] and pre-peaks [23] and have ascribed to adsorption of reactants [30] or products, respectively. Our results concerning the oxidation of both xanthine and theophylline indicate possible adsorption of reactants at the conductive diamond surface at intermediate pH, although the linear dependence of the current vs. square root of sweep rate plot tends to indicate otherwise.

Anodic linear-sweep voltammograms recorded for the oxidation of the investigated xanthines at the BDD electrode also showed a shift of the peak potential towards higher values with increasing sweep rate, for example, shifting from 1.240 V at 5 mV s^{-1} to 1.280 V at 300 mV s^{-1} for theophylline at pH 1.8. Under our experimental conditions (small ohmic drop, due to the low current and high conductivity of the solution), this result is consistent with a relatively small electron transfer rate constant.

3.1.3. *Effect of the Concentration*

The examination of the effect of concentration showed that, at pH 1.8, the relationships between the peak current and the concentration were linear, with zero intercept, for theophylline, theobromine and caffeine, over the concentration range of 1 to 400 μM. Linear dynamic range and the results of least-squares analysis, i.e., sensitivity along with its standard deviation are given in Table 3. The analytical performance will be discussed later in detail. A similar linear calibration plot, passing through the origin, was found for xanthine (pH 1.8) within the concentration range 1 to

Table 3. Parameters of the calibration curves (I = aC) and correlation coefficients for xanthine, theophylline, theobromine and caffeine determination (pH, 1.8; sweep rate, 20 mV s^{-1}). s_a: standard deviation in slope.

Compound	*Concentration range [μM]*	*Sensitivity (a) ± s_a [μA / μM]*	R^2
Xanthine	1 – 100	0.046 ± 0.00015	0.9997
Theophylline	1 – 400	0.043 ± 0.000072	0.9980
Theobromine	1 – 400	0.045 ± 0.000026	0.9989
Caffeine	1 – 400	0.042 ± 0.00031	0.9986

100 μM. Above ca. 150 μM, the curve tended towards a plateau, probably due to the limited solubility of xanthine in aqueous buffers [25].

At pH 1.8, the peak potential was only slightly dependent on the concentration, shifting slightly towards more positive values with increasing concentration. Within the concentration range from 1 to 100 μM, this shift was ca. 10 mV for caffeine and theobromine, and ca. 20 mV for xanthine and theophylline. The contribution of the ohmic drop to this shift should be less than 0.05 mV. Similar dependence of the peak potential on the concentration was previously ascribed to the adsorption of the reactant in the case of uric acid oxidation at PG [26, 29], as well as xanthine and hypoxanthine oxidation at GC [13].

For xanthine and theophylline, the effect of concentration was also investigated within the pH ranges where additional peaks appeared. Thus, it was observed that at very low concentration (below 2 μM), only the post-peak occurs (i.e., for oxidation of adsorbed reactant), while as the concentration increases, the relative magnitude of the diffusive peak increases. This behavior is evident for xanthine and theophylline adsorption at the BDD electrode within the pH ranges from 3.0 to 8.0 and from 4.0 to 8.5, respectively [31]. Over these pH ranges, a linear dependence was found between the total current (peak I + peak II) and the concentration, although with a small non-zero intercept. At the same time, increasing concentration resulted in larger shifts of the peak potentials for both of these compounds. However, the shapes of the voltammograms over these pH ranges render the detailed analysis difficult.

Summarizing the linear sweep voltammetry results, it is clear that quantitative analysis can only be reliably carried out at low pH (<3). At such pH values, excellent linear calibration was found for the range 1 – 400 μM for theophylline, theobromine and caffeine, and the range 1 – 100 μM for xanthine.

3.2. Mechanistic Studies

Cyclic voltammetric measurements were performed for xanthine, theophylline, theobromine and caffeine oxidation at the BDD electrode over the entire pH range, with sweep rates ranging from 5 to 300 mV s^{-1}. No cathodic peaks were observed on the reverse sweep for any of the investigated compounds, which indicates that the overall oxidation of xanthines is an irreversible process.

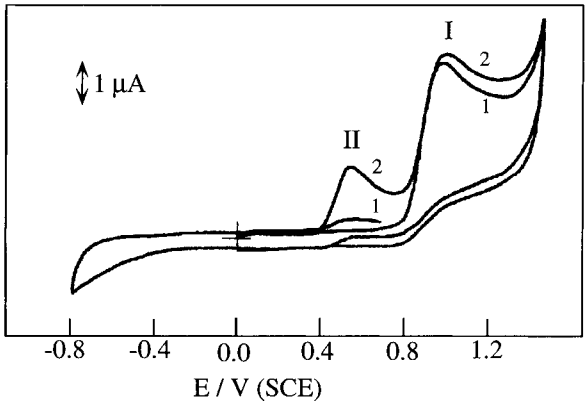
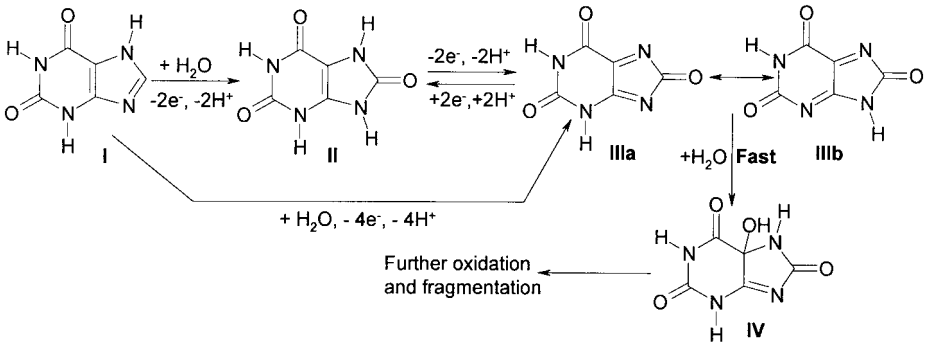


Fig. 5. Cyclic voltammograms at a BDD electrode for 50 μM xanthine (curve 1) and 50 μM xanthine + 40 μM uric acid (curve 2), sweep rate, 200 mV s^{-1}; pH, 5.3. The potential sweeps were initiated from 0.0 V. The small peak designated as curve 1 was obtained after one complete potential sweep cycle.

In the case of xanthine and theophylline, at sweep rates greater than 200 mV s^{-1}, a new anodic peak appeared during the second positive-going sweep of the voltammograms. Figure 5 shows the cyclic voltammogram with a second positive-going sweep, recorded for xanthine (curve 1 in Figure 5). It can be seen that a new, rather ill-defined peak (peak II in Figure 5) is observed at a less positive potential than that of the main peak for xanthine oxidation (peak I in Figure 5). The same behavior has been reported by Dryhurst [26], who ascribed the new peak to the oxidation of uric acid. This was also checked by the addition of uric acid to the solution. It is likely that the mechanism of the overall oxidation of xanthine at the conductive diamond electrode should be similar to that proposed for the PG electrode [23, 26, 29] shown in Scheme 1.

Thus, the electrochemical oxidation of xanthine (I) proceeds by an initial $2e^-$, $2H^+$ oxidation to uric acid (II). Since uric acid is more readily oxidized than xanthine (Figure 5), it is immediately oxidized to bis-imide (IIIa, IIIb) in a further $2e^-$, $2H^+$ reaction. Therefore, the single initial voltammetric peak for xanthine corresponds to the overall $4e^-$, $4H^+$ oxidation. It is likely that a small amount of the bis-imine (IIIa, IIIb) is reduced during the negative sweep back to uric acid, which is oxidized again, on the second positive-going sweep, in the range of peak II. Nevertheless, no cathodic peak for the bis-imine reduction to uric acid was



Scheme 1.

observed under our experimental conditions. This could be explained by the fast hydration of the bis-imine [26].

It is worth noting that an overall oxidation reaction involving the same number of electrons and protons ($4e^-$, $4H^+$) as suggested by the mechanism shown in Scheme 1, is in agreement with the slopes of ca. 60 mV per pH unit observed in the E_p vs. pH relationships for xanthine and theophylline, in the pH ranges 1.8–8.0 and 1.8–9.0, respectively (Table 2). According to the pK_a values for the first dissociation of a proton in xanthine ($pK_a = 7.5$) and theophylline ($pK_a = 8.6$), at pH values above 7.5 and 8.6, respectively, the predominant species should be the singly charged anion. In that case, the electroactive species should be the conjugate bases of xanthine and theophylline, formed by the loss of a proton from positions 3 and 7, respectively, and the overall oxidation reaction should then involve four electrons and only three protons. Indeed, as shown in Table 2, the variation of the peak potential for xanthine and theophylline oxidation as a function of pH exhibited slopes of 43 and 40 mV per pH unit, at pH values above 8.0 and 9.0, respectively. These values are close to that expected for the oxidation involving $4e^-$ and $3H^+$ (44 mV per pH unit), as already discussed.

Based upon voltammetric, coulometric and chromatographic measurements, the same mechanism was proposed for theobromine and caffeine oxidation, involving the corresponding *N*-methyl-derivative intermediates [16]. Nevertheless, the linear relationships between the peak potentials and pH for the oxidation of these compounds at the BDD electrode exhibited slopes lower than that expected for the overall $4e^-$, $4H^+$ process. This could be due either to the rather narrow range of pH, or to uncertainties introduced by the close proximity of the voltammetric peaks of theobromine and caffeine to the background discharge.

3.3. Analytical Performance Characteristics

The calibration data for xanthine, theophylline, theobromine and caffeine determination given in Table 3, are noteworthy because linear regression statistical analysis ($I = aC$) yielded excellent correlation coefficients and good precision. A zero intercept is an advantage, because it enables the use of the standard addition method. Furthermore, the single standard addition method is also possible. The zero intercept is not available with other techniques, for example, square-wave voltammetry at chemically modified electrodes [15].

Tests for reproducibility were also performed at several concentrations. The tests consisted of running linear-sweep voltammograms (within the potential range 0.8 to 1.6 V) at a sweep rate of 20 mV s^{-1}, in air-saturated Britton-Robinson buffer (0.1 M $NaClO_4$), at pH 1.8, for five aliquots of the same solution. Table 4 summarizes the results of the reproducibility tests for xanthine, theophylline, theobromine and caffeine.

It appears that the reproducibility is satisfactory at low concentration (below 5 μM) and very good at higher concentration (>10 μM), comparable with that reported for differential-pulse voltammetry at GC electrodes [14], square-wave voltammetry at chemically modified electrodes [32], and biosensor techniques [33]. In addition, the long-term stability of the response was found to be excellent. Negligible changes (<1%) in the voltammetric response were observed for a given electrode over a period of at least one month.

The accuracy of the procedure was assessed by analyzing two spiked samples of different concentrations, for each compound. The method of multiple standard additions was

Table 4. Reproducibility of voltammetric peak current for xanthine, theophylline, theobromine and caffeine, at various concentrations.

Compound	*Concentration [μM]*	*Peak current (average of 5 runs) [A]*	*Relative standard deviation of peak current [%]*
Xanthine	2	0.10	3.5
	14	0.65	0.7
Theophylline	3	0.15	1.7
	10	0.46	0.6
	200	8.48	0.3
Theobromine	3	0.16	1.2
	10	0.49	0.7
	100	4.62	0.6
Caffeine	5	0.21	2.2
	22	0.97	0.7
	150	6.29	0.3

Table 5. Parameters of the regression equations, $I = a\Delta C + b$, for multiple standard additions method applied to synthetic samples of xanthine, theophylline, theobromine and caffeine. s_a: standard deviation in slope; s_b: standard deviation in intercept.

Compound	*Expected concentration [μM]*	$a \pm s_a$ *[μA/μM]*	$b \pm s_b$ *[μA]*	R^2	*Calculated concentration [μM]*
Xanthine	2.0	0.044 ± 0.00083	$0.095 \pm 0.0.001$	0.9986	2.1
	10.0	0.037 ± 0.00031	0.358 ± 0.0002	0.9998	9.7
Theophylline	3.0	0.049 ± 0.00044	0.154 ± 0.001	0.9997	3.1
	90.0	0.036 ± 0.00096	3.217 ± 0.029	0.9972	89.4
Theobromine	5.0	0.050 ± 0.0023	0.269 ± 0.005	0.9933	5.4
	52.0	0.045 ± 0.0001	2.422 ± 0.003	0.9987	53.8
Caffeine	22.0	0.044 ± 0.00092	0.993 ± 0.011	0.9974	22.5
	180.0	0.038 ± 0.0008	6.764 ± 0.078	0.9987	178.0

Table 6. Parameters of the regression equations, $I = a\Delta C + b$, for multiple standard additions method applied for caffeine determination in real samples. For experimental details, see text.

Product	$a \pm s_a$ *[μA/μM]*	$b \pm s_b$ *[μA]*	*Caffeine content*	*Uncertainity in caffeine content*
Instant coffee	0.040 ± 0.0005	0.871 ± 0.0097	43.14 mg g^{-1}	1.09 mg g^{-1}
Tinned coffee	0.041 ± 0.0009	0.570 ± 0.0187	674.81 mg L^{-1}	36.4 mg L^{-1}
Cola	0.044 ± 0.0002	0.352 ± 0.0024	194.19 mg L^{-1}	22.14 mg L^{-1}

applied, and linear-sweep voltammograms were recorded under the same experimental conditions as above. The parameters of the regression equations, a and b along with their respective standard deviations for I (μA) vs. ΔC (μM), together with the expected and calculated concentrations, are shown in Table 5. The agreement between the expected and experimental concentrations was excellent at both low and high concentrations for all four compounds. Although we later show that it is practical to carry out analysis of real samples with minimal sample preparation, certain types of samples will no doubt require a separation step. We are also pursuing this approach in our laboratory and find excellent agreement between the present voltammetric results and flow injection analysis with amperometric detection. These results will be reported elsewhere.

Anodic voltammetry at the BDD electrode was further applied for the direct determination of caffeine, i.e., with only very simple sample pretreatment in instant coffee and two beverages, canned coffee and cola, all commercially available products. In order to reach the linear range of the calibration plot for caffeine determination, 408 mg instant coffee were dissolved in water, diluted to 10 mL, and then 25 mL of the supporting electrolyte (pH 1.8) was spiked with 60 μL of the above solution. For caffeine determination in tinned coffee and cola, volumes of 0.1 and 0.2 mL, respectively, of beverage were diluted to 25 mL with the supporting electrolyte (pH 1.8). It is likely that the dilution process helps to reduce the matrix effect of the real samples. The multiple standard addition method was applied to these samples by spiking (four spikes for each sample) with standard caffeine solution (8 μM $spike^{-1}$ for instant coffee and tinned coffee and 4 μM $spike^{-1}$ for cola). Before the addition of the standard solution, and after each spike, linear-sweep voltammograms were recorded, under the same experimental conditions as described above. The voltammetric responses thus obtained are shown in Figure 6, and the results for caffeine determination are summarized in Table 6.

The results obtained for caffeine determination by anodic voltammetry at BDD electrodes in commercially available products are in fair agreement with previously reported data [12, 15], illustrating the practical analytical utility of the method.

It is worth noting that under the experimental conditions for caffeine determination in real samples, theophylline and theobromine could also interfere. Nevertheless, for coffee this is not a major source of error, because the concentration of these two compounds in the investigated products is usually ca. 300-fold lower than the caffeine content [12]. For

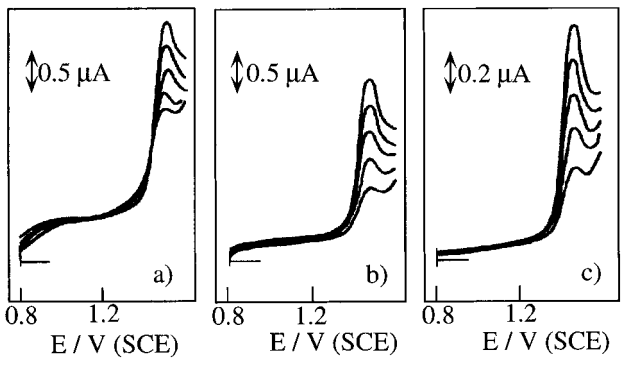


Fig. 6. Voltammetric responses for the determination of caffeine at a BDD electrode in commercial samples of a) instant coffee, b) canned coffee and c) cola with and without the successive addition of aliquots of standard caffeine solution. For other conditions, see text.

cola, typically only caffeine is present. For tea, theophylline and theobromine are present at higher levels, and therefore separation is necessary.

4. Conclusions

The study of the electrochemical oxidation of xanthine and its naturally occurring *N*-methyl derivatives (theophylline, theobromine and caffeine) at the conductive diamond electrode shows that the overall reaction takes place by a mechanism similar to that for the oxidation of purine derivatives at the pyrolytic graphite electrode. Cyclic votammograms obtained at BDD electrodes exhibited well-defined voltammograms with high signal to background ratios for all xanthine derivatives. Linear-sweep voltammetry measurements, carried out over a wide pH range, suggest that the electrochemical behavior of xanthine and theophylline in alkaline media is influenced to some extent by the dissociation of protons from positions 3 and 7, respectively, which occurs before the electrochemical oxidation.

The influence of experimental conditions on the voltammetric response of the BDD electrode was thoroughly investigated for the oxidation of xanthine, theophylline, theobromine and caffeine. Based upon these results, a simple, rapid, reproducible and accurate voltammetric method is proposed for the determination of the investigated compounds. The analytical performance characteristics of the method are comparable to those reported for the determination of xanthines by the use of chemically modified electrodes, biosensing techniques and differential pulse voltammetry. Additional advantages of electrochemical determination with BDD electrodes are the excellent stability of the response and the extreme robustness of the material, with essentially no need for electrode pretreatment or maintenance. The excellent results obtained for caffeine determination in three commercially available coffee and cola products, with very simple sample preparation, involving only dilution in electrolyte, demonstrates the practical analytical utility of the method.

5. Acknowledgements

This research was supported by the Japan Society for the Promotion of Science (JSPS), Research for the Future Program, "Exploratory research on Novel Artificial materials and Substances for Next Generation Industries".

6. References

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