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Voltammetric Peak Separation of Dopamine from Uric Acid in the Presence of Ascorbic Acid at Greater Than Ambient Solution Temperatures

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Peak overlap in voltammetry poses challenges for the quantitative analysis of electroactive species. Dopamine and uric acid are typically challenging to determine voltammetrically because of their very similar oxidation peak potentials. We report preliminary results of the use of a screen-printed carbon electrode for the determination of dopamine and uric acid in an electrolyte solution maintained above ambient temperatures. Higher temperatures resulted in dramatic shifting of the dopamine oxidation peak toward lower potentials, while the uric acid peak was essentially stationary. Ascorbic acid, an interference in voltammetric uric acid determinations, is effectively suppressed at higher temperatures. This resulted in a greater peak separation of dopamine from uric acid at higher temperatures, which is desirable for better peak integration. In addition, greater current responses for both species were recorded at higher temperatures. The cause for such an increase in peak current is unraveled using ac impedance measurements. Presented are preliminary results for determining dopamine and uric acid at temperatures higher than ambient. Much improved voltammetric peak separation and sensitivity is obtained at these higher temperatures compared to ambient.

Liner sweep voltammetry may be applied for quantifying more than one electroactive species in a mixture, provided peak potential overlap does not occur. When peak overlap does occur, various schemes including masking[1,2] and the use of chemical modified electrodes (CMEs)[3−5] may be attempted to suppress the electroactivity of interfering species) at the potential of interest.

Increasing the temperature of the working electrode, termed hot-wire voltammetry,[6−8] has been used for voltammetric applications, with marked increases in analyte current signals and thus resulting in lower detection limits. Wang et al has reported the use of a heated carbon paste electrode for the determination of nucleic acids.[9] As much as a 34-fold signal increase compared to an ambient temperature signal was obtained depending upon the applied temperature. The use of hot disk microelectrodes has also been reported with resulting increases in signal currents of tested species.[10] Fundamental electrochemical studies that revealed rate and equilibrium constants have been reported for hydropyridazines,[11] hydrazines,[12] dialkylamino maleates,[13] and other electroactive species[14−16] through experiments at nonambient temperatures, mostly low temperatures. Additional voltammetric peaks and peak-shifting behavior were observed in these studies when temperatures were adjusted from ambient.

We report preliminary results for the voltammetric determination of dopamine and uric acid in the presence of ascorbic acid using a screen-printed carbon electrode at temperatures higher than ambient. Changes in dopamine and uric acid concentrations in biological samples are an indication of possible body abnormalities or diseases.[17] Reports in the literature focus on the use of CMEs to address the difficulty of determining these two species because of their similar oxidation peak potentials.[18−20] In the presented approach, the electrolyte solution containing dopamine and uric acid is directly heated, thus avoiding the more complex electronics needed for direct electrode heating. In addition to increased current signals, we have observed significant shifting of the dopamine peak potential. This offers a tremendous advantage for the simple separation of these overlapping electroactive species for peak integration and thus quantitative analysis. Furthermore, the response of ascorbic acid, a major interferring

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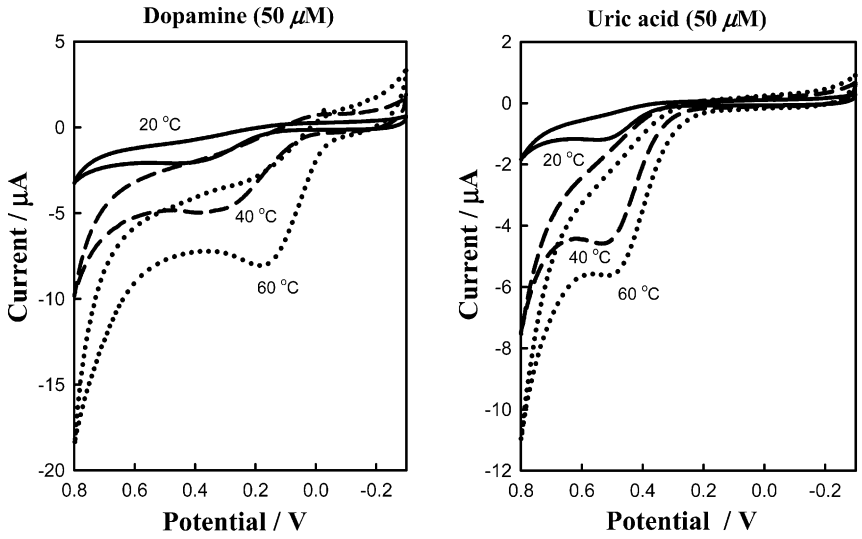

Figure 1. Cyclic voltammograms of dopamine and uric acid (50 μM) at various temperatures using a screen-printed carbon electrode in a 0.1 M PBS. Scan rate, 100 mV/s.

agent in voltammetric uric acid determinations, was greatly suppressed at higher temperatures. We report preliminary results of this approach for the change in uric acid and dopamine peak potentials in a pH 8 buffer solution. To the best of our knowledge, there is no reported analytical application of using higher temperature solutions in voltammetry with a resulting shift in analyte peak potentials. The detection of uric acid in a human urine sample was finally demonstrated as a real sample application.

EXPERIMENTAL SECTION

Cyclic voltammetric and linear sweep voltammetric experiments were carried out with a CHI 1030 electrochemical workstation (CH Instruments, Austin, TX). The three-electrode system consists of a screen-printed carbon electrode (SPE) (Zensor R&D, Taichung, Taiwan) as the working electrode (geometric area, 0.2 cm^2), an Ag/AgCl reference electrode, and a platinum (geometric area, 0.07 cm^2) auxiliary electrode. The impedance measurements were done on the Autolab frequency response analyzer with FRA2 module that was controlled by an IBM-compatible PC. The electrochemical impedance was measured at 10 discrete frequencies per decade from 0.01 Hz to 100 kHz at an amplitude of 5 mV (rms). The acquired data were analyzed on the basis of equivalent electrical circuits by a nonlinear least-squares method.[21] Fitting constraints were imposed in such a way that further iterations will be stopped when the χ^2 change is less than 0.001% as compared to previous iteration. Less than 5% fluctuations between the experimental and fit data were assumed satisfactory in confirming validity of the selected fitting circuit.

Dopamine and uric acid were purchased from ICN Biomedicals Inc. (Aurora, OH). All the other compounds used in this work were of ACS-certified reagent grade. A pH 8 phosphate buffer solution (PBS) of $I = 0.1$ M was used as the base electrolyte.

The SPE was washed thoroughly with deionized water before subsequent experiments in pH 8 PBS. Scan rates from 5 to 300 mV/s were used over the potential range of −0.4 to 0.7 V. The SPE was equilibrated in pH 8 PBS over this same potential range at 100 mV/s until the current response was consistent. Buffer solutions containing uric acid and dopamine contained in a 20-mL beaker were immersed in a small hot water bath in order to adjust and maintain the desired elevated temperatures. Solution temperature was carefully monitored to maintain the desired experimental temperature. Temperature changes we accomplished by simply adding aliquots of hot water to the water bath until the desired experimental temperature was achieved. Because of the rapid completion of each experiment, the temperature easily remained constant.

RESULTS AND DISCUSSION

Cyclic voltammetric potential scans from −0.2 to 0.7 V at 100 mV/s using solution temperatures of 20, 40, and 60 °C for dopamine and uric acid at 50 μM are depicted in Figure 1. At increasing solution temperatures, both species show an increase in current signals mostly due to the increase in mass-transfer effect. The peak potential for uric acid is essentially constant at the investigated temperatures, while dopamine peak potentials undergo a significant shift toward lower oxidation potentials with increasing temperature. This is a significant observation for the separation these two species for quantitative voltammetric analysis. In Table 1, temperature versus current response and peak potential are provided for both dopamine and uric acid. From 20 to 60 °C, current response for both species increases ∼4-fold. However, a more interesting result can be seen for peak potentials versus temperature. An approximate 280 mV potential shift occurs with dopamine, while a negligible shift occurs for uric acid.

Calibration plots for dopamine and uric acid from 5 μM to 1 mM at individual temperatures of 20, 40, and 50 °C are presented in Figure 2. For both species, an increase in response sensitivity with increasing temperature can be observed. A marked jump in sensitivity is observed for dopamine from 20 to 40 °C. This may have something to do with the fact that higher temperatures resulted in dramatic shifting of the dopamine oxidation peak toward lower potentials. When cyclic voltammetry was conducted

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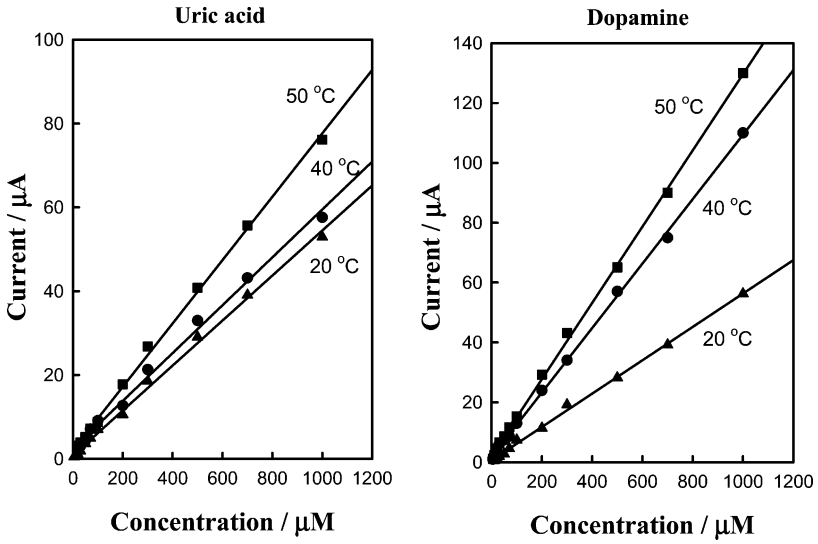

Figure 2. Calibration curves for uric acid and dopamine at various temperatures for concentrations from 5 μM to 1 mM.

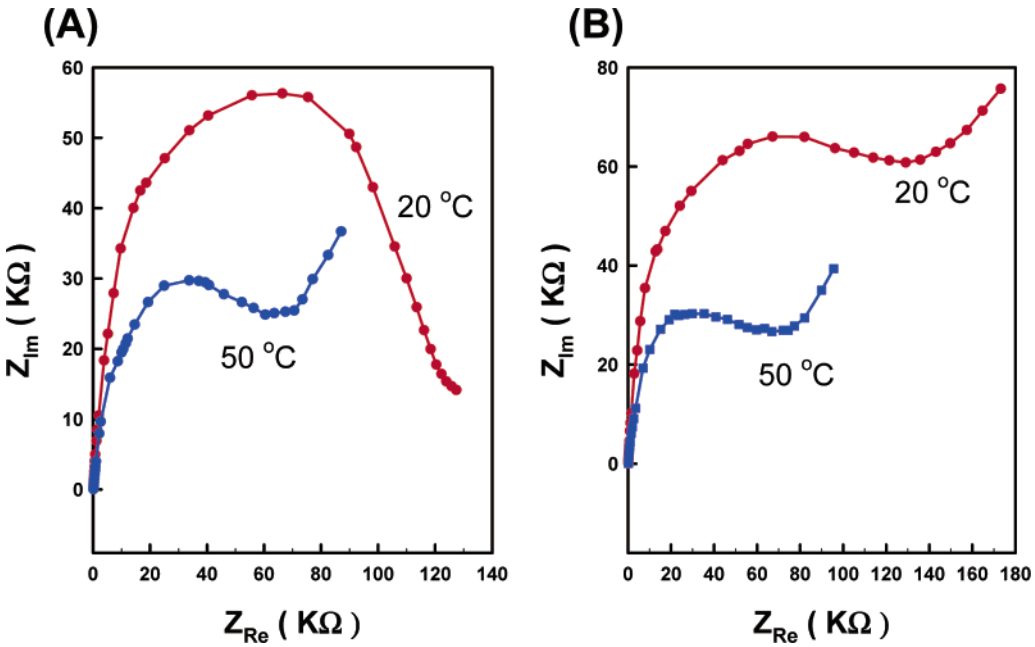

Figure 3. Resulting impedance spectrum of (A) 50 μM dopamine at 0.15 V and (B) 50 μM uric acid at 0.5 V at conditions of 50 and 20 °C using a screen-printed carbon electrode in a 0.1 M PBS.

Table 1. Peak Potentials and Current Responses for Uric Acid (30 μM) and Dopamine (10 μM) at Various Temperatures

	dopamine		uric acid	
temp (°C)	E_p (V)	i_p (μA)	E_p (V)	i_p (μA)
20	0.455	2.105	0.547	1.209
40	0.355	4.976	0.531	4.564
60	0.175	8.045	0.522	5.631

Table 2. Parameters for the Circuit Elements Evaluated by Fitting the Impedance Data to the Equivalent Electrical Circuit for Bare SPE at Various Temperature

	20 °C/0.15 V (uric acid)	50 °C/0.15 V (uric acid)	20 °C/0.5 V (dopamine)	20 °C/0.5 V (dopamine)
R_{ct} (10^{-3} Ω)	150.2	82.8	124.1	75.6
R_s (Ω)	187.0	182.0	216.0	203.0

at different scan rates, good linearities between the peak currents and the square root of the scan rates for dopamine and uric acid indicate a diffusion-controlled process in solution. The behavior of shift in peak potential for dopamine at higher temperatures is thus not due to adsorption. To further understand the electrochemical behavior occurring, ac impedance experiments were performed using the SPE in a uric acid and dopamine (50 μM each) buffer solution at 20 and 50 °C (Figure 3). As shown in Table 2, the charge-transfer resistance (R_{ct}) increased approximately twice at peak potentials for each respective substance from 50 to 20 °C. In addition, the ac impedance dopamine 20 °C curve differed in shape from the other three experiments. The remaining three ac impedance experiments, dopamine at 50 °C, and uric acid at 20 and 50 °C, all resulted in similar profiles. The results of the

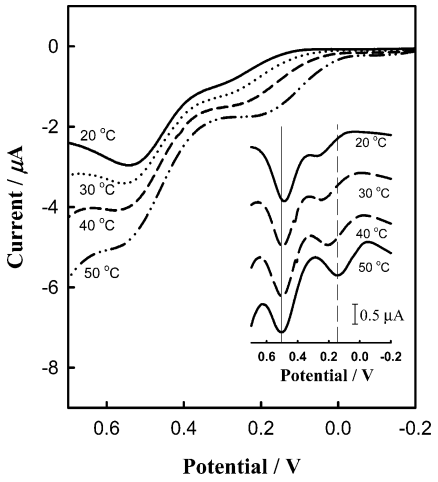

Figure 4. Linear sweep voltammetric scans with associated derivative voltammetric scans of a dopamine and uric acid mixture at various temperatures.

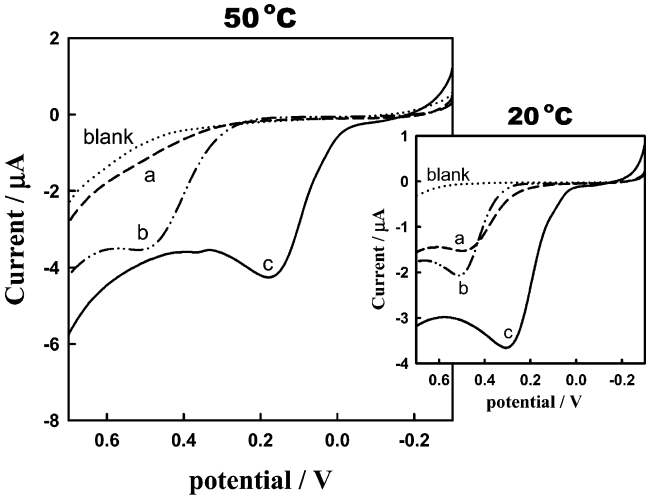

Figure 5. Linear sweep voltammagrams of uric acid, dopamine, and ascorbic acid (50 μM each) at conditions of 50 and 20 °C (inset).

dopamine experiment at 20 °C suggests to us adsorption is greatly decreased for dopamine at 50 versus 20 °C. This may explain the peak shifting that occurs for dopamine but not for uric acid. Our future investigations will focus on a more detailed investigation of the electron-transfer mechanism of both uric acid and dopamine.

In the Figure 4 inset, derivative voltammetric responses for uric acid (30 μM) and dopamine (10 μM) from corresponding linear sweep voltammagrams at various temperatures are presented. At 20 °C, the dopamine peak (~0.3 V) is on the shoulder of the uric acid peak (~0.5 V). The solid straight line represents the peak potential of uric acid, and the dashed line represents the peak potential of dopamine at 50 °C. As the temperature of the solution is increased from 20 to 50 °C the dopamine peak shifts and becomes well separated from the stationary uric acid peak. In addition to the observed dopamine peak shift, the area response for dopamine increases as the temperature increases. Easier peak integration results from these two phenomena occurring at higher temperatures.

Ascorbic acid is known to cause interference in the voltammetric determination of uric acid. The linear sweep voltammagrams for each of the three species of interest (uric acid, dopamine, ascorbic acid) at 20 and 50 °C is presented in Figure 5. The voltammagram at 20 °C reveals the high likelihood of peak overlap of ascorbic acid (trace a) and uric acid (trace b). However, notably evident is the much reduced response of the ascorbic peak at 50 °C compared to 20 °C. At this higher temperature, ascorbic acid is converted to an electroinactive species. This is a tremendous asset for the quantitation of uric acid with this presented procedure at elevated temperatures, because this major interference is eliminated.

A human urine sample was investigated with this proposed experimental design. In Figure 6, linear sweep voltammagrams from a 100-fold PBS diluted urine sample and subsequent spiked uric acid and dopamine levels at 50 °C is presented. Uric acid is present in the blank urine sample at a measured concentration of 2.05 μM according to a calibration curve fit (See Table 3 for recovery data.). We obtained statistically (CL = 95) quantitative percent recoveries at both uric acid and dopamine spiked levels indicating this procedure being free from interferences in a real sample.

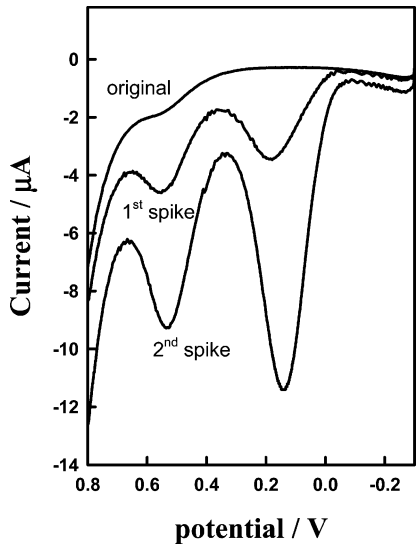

Figure 6. Derivative voltammetric scans of a human urine sample with spiking of uric acid and dopamine.

Table 3. Recovery of the Uric Acid and Dopamine in a Human Urine Sample

parameter	uric acid	dopamine
linear equation	$y = 4.84 \times 10^{-8}x + 1.34 \times 10^{-7}$	$y = 1.18 \times 10^{-7}x - 1.2 \times 10^{-6}$
r^2	0.9954	1.0000
original (μM)	2.05	0
spike (μM)	30	30
after spike (μM)	33.00	30.08
recovery (%)	102.96	100.26

CONCLUSION

The preliminary results of a unique voltammetric observation at higher temperatures have been presented. In summary, we have shown that two close peak potential species can be separated by increasing the analyte solution to temperatures higher than ambient. Oxidation peaks for uric acid and dopamine on a screen-printed carbon electrode were well overlapped at 20 °C but were well resolved at 50 °C. At higher temperatures, the dopamine peak

response shifted toward lower oxidation potentials, while the uric acid peak potential response remained the same. In addition, better selectivity was achieved for both uric acid and dopamine at higher temperatures. Also, at 50 °C, ascorbic acid is converted to an electroinactive species and thus will not interfere with the uric acid response. Our future investigations will involve a more detailed investigation of the mechanism of this peak increase and shifting phenomena and applications of biological samples that contain uric acid and dopamine.

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