A selective and sensitive electrochemical sensor for detecting uric acid (UA) has been developed. The UA electrochemical sensor was made from single-walled carbon nanotubes (SWCNTs), which were coated on the surface of a gold wire electrode using the chemical vapor deposition (CVD) method. The performance of UA electrochemical sensor was characterized using cyclic voltammetry. The obtained calibration curve followed a linear equation, $y_{pa} = 132.753 + 1.512c$, with $R^2 = 0.99597$. The LOD and sensitivity of the SWCNTs-modified gold electrode for UA detection obtained were 5.05 nM and 5.240 μA mm$^{-2}$ nM$^{-1}$, respectively. The reaction between the electrode and UA demonstrated the diffusion-controlled behavior. No interference signals from ascorbic acid, glucose and urea were observed during the UA measurements. However, the signal for dopamine appeared in the voltammogram, although it was located at a different potential. Moreover, the dopamine signal disappeared when the measurement was conducted at concentrations of less than 1 mM. These results confirm that SWCNTs-modified gold electrodes are favorable for detecting uric acid.

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Uric acid (UA) is an oxidation product of purine metabolism. Normal levels of UA in human serum can be in the range of 231 – 475 μmol/L and 154 – 350 μmol/L for men and women, respectively. High levels of uric acid in the human body can cause diseases such as cardiovascular, hypertension, coronary artery disease, etc. On the other hand, low levels of uric acid can cause multiple sclerosis. Therefore, it is important to monitor the levels of UA in human serum and urine. Various methods have been reported for the determination of UA, e.g., capillary electrophoresis, analyte pulse perturbation, HPLC and gas chromatography. These methods need complicated preparation, their analysis is time-consuming, and some of them require reagents that are relatively expensive. Yet, we have found that the electrochemical method for the determination of UA is relatively faster, simpler and low-cost.

UA is commonly found in biological fluids with ascorbic acid (AA) and dopamine (DA), glucose and urea. All of these substances have similar electrochemical behaviors at bare electrodes and thus interfere with each other. The voltammogram shows that the oxidation potentials of AA, DA, glucose and urea are very close to each other, and can sometimes even yield in overlapping.

The development of electrochemical sensors has increased in several years in order to improve the performances in terms of selectivity and sensitivity. Gold electrodes are commonly used in electrochemical sensors. The use of modified gold electrodes in electrochemical sensors has been previously reported, for example gold electrodes coated with gold nanoparticles for sugar detection, gold nanoparticle-modified gold electrodes for dopamine detection, polyaniline-invertase-gold nanoparticle-modified gold electrodes for sucrose detection, and multi-walled carbon nanotubes (MWCNTs)-chitosan/poly(amidoamine)/DNA nanocomposite-modified gold electrode for uric acid and dopamine detection.

Another modified sensor has been fabricated and reported for the determination of uric acid and dopamine. These modified sensors are glassy carbon/multi-walled carbon nanotubes-holmium fluoride nanoparticles (GC/MWCNTs-HoFNPs), in doped CeO$_2$ nanoparticles modified glassy carbon paste electrode (nano-In-CeO$_2$/GCPE) and single-walled carbon nanotubes (SWCNTs). The limit of detection (LOD) of the GC/MWCNTs-HoFNPs electrode is 0.06 μM, while that of the nano-In-CeO$_2$/GCPE electrode is 0.0074 μM for uric acid determination. Moreover, in our previous study, SWCNTs have been developed for dopamine detection at 0.2 M acetate buffer solution (pH 4). According to our study, SWCNTs have an advantage as a sensor compared to other materials. Due to its unique properties, such as high electrical conductivity, high surface area, chemical stability and significant mechanical strength, it could facilitate electron transfer reactions between the materials and other molecules. In this work, we used CVD to fabricate single-walled carbon nanotubes (SWCNTs)-modified gold electrode for the determination of UA in 0.1 M phosphate buffer (pH 7.0). The performance of the SWCNTs-modified gold electrodes is demonstrated by their improved LOD compared to GC/MWCNTs-HoFNPs and nano-In-CeO$_2$/GCPE electrodes.

Experimental

Chemicals and materials.—Uric acid [C$_5$H$_4$N$_4$O$_3$, 99.0%] and dopamine [C$_7$H$_7$N$_2$O$_2$, 98%] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and used without any purification. L(+)-Ascorbic acid [C$_6$H$_8$O$_6$], D(+)-Glucose [C$_6$H$_12$O$_6$], Urea [CO(NH)$_2$], 99.5%, ethanol [C$_2$H$_5$OH], di-sodium hydrogen phosphate [Na$_2$HPO$_4$], and sodium dihydrogen phosphate monohydrate [NaH$_2$PO$_4$.H$_2$O] were purchased from Merck KGaA (Darmstadt, Germany) and used without any purification. Gold wire (99.999%, Tanaka Kikinzoku Kogyo, Japan) with diameter 0.8 mm was used for preparation of SWCNTs-modified gold electrode. Demineralized water was used for cleaning and chemical preparation.

Instrumentation.—Electrochemical measurements were carried out using a potentiostat from an electrochemical analyzer (model 700B, equipped with ALS/CH700B software) and eDAQ (potentiostat E161 and e-corder 410, equipped with e-chem software version 2.0.1).

Synthesis of single-walled carbon nanotubes (SWCNTs) on gold electrodes.—SWCNTs were synthesized on the surface of an Au wire using chemical vapor deposition, as described in our previous reports. The Au wire surface was completely covered with SWCNTs as shown in Figure 1. Field-emission scanning electron microscopy (FE-SEM, Hitachi SU-8000) measurements indicated that the thickness of the SWCNTs layer was ca. 20 μm. FE-SEM and transmission electron microscopy (TEM, JEOL 2000FX)
observations showed that the pieces of SWCNTs were bundled with each other, resulting in tubes with diameters of 5–20 nm (Figures 2a and 2b, respectively). In TEM images, the surface edges of the SWCNTs layers, and individual tube structures with an estimated wall thickness of 0.30–0.35 nm were observed, indicating that the structures were SWCNTs. Raman spectroscopic measurements (HORIBA (Jobin Yvon) LabRAM HR-800) revealed that the intensity ratio of the G-band to the D-band ($I_G/I_D$) was ca. 20, using a 514.5-nm (2.41-eV) excitation laser (Figure 3). This high $I_G/I_D$ value indicated that the synthesized SWCNTs were highly crystalline. The radial breathing mode of the SWCNTs in Raman spectra indicated that the diameter distribution of the SWCNTs was 0.9–1.6 nm which was in good agreement with the TEM result (Figure 2b). XPS measurement (Thermo VG Scientific, Sigma Probe HA6000II) using Al K$_{α}$X-ray (1486.68 eV) source for excitation was carried out to analyze functional groups at the SWCNTs surface. The result indicated that there were some carbon-oxidized functional groups such as C-O, C=O and O-C=O (Figure 4).

**Electrochemical measurements.**—Electrochemical experiments were performed by cyclic voltammetry. A three-electrode cell system was used in all measurements unless otherwise described, with platinum as the counter electrode (CE), Ag/AgCl (sat’d KCl) as the reference electrode (RE), and SWCNTs-modified gold electrode as the working electrode (WE). The potential was swept from $-0.2$ to $0.5$ V vs. Ag/AgCl (sat’d KCl), with 20 seconds of rest time before measurement. The scan rates were 0.01, 0.05, 0.1, 0.2 and 0.3 V/s.

The stock solutions were made by dissolving UA, dopamine, ascorbic acid, urea and glucose, each in 0.1 M phosphate buffer solutions. The various UA concentrations used to obtain the calibration curve were 0 nM, 2 nM, 4 nM, 6 nM, 8 nM, 14 nM, 16 nM and 20 nM. The LOD and sensitivity of UA were determined using the linear equation of the calibration curve. The calibration curve was plotted from the anodic peak current at the maximum potential. The selectivity of the SWCNTs-modified gold electrodes was analyzed by measuring 1 mM UA, dopamine, ascorbic acid, urea and glucose, and 4 nM dopamine solutions. Repeatability of the SWCNTs-modified gold electrodes was tested toward 1 mM uric acid and dopamine in 0.1 M phosphate buffer solutions at pH 7.0. This measurement was conducted at potential of $-0.2$ V to 0.5 V for 5 cycles with 5 duplications. The measurement was carried out at 2 days in a row. The data of repeatability measurement was analyzed using significance testing.

**Results and Discussion**

**Characterization of SWCNTs-modified gold electrode.**—The cyclic voltammogram of UA in phosphate buffer (PB) solution (pH = 7.0) using SWCNTs as electrodes are shown in Figure 5. In the absence of UA, there was no peak during measurements within the potential sweep from $-0.2$ to 0.5 mV. In the presence of UA, three oxidation peaks appeared at $-0.007$ V ($i_{pa}=107.547 \mu A$), $+0.286$ V ($i_{pa}=153.728 \mu A$) and $+0.385$ V ($i_{pa}=700.00 \mu A$). Two reduction peaks were also found at $-0.093$ V ($i_{pc}=-199.245 \mu A$) and $+0.290$ V ($i_{pc}=-334.339 \mu A$) during the measurement. Figure 5 shows that the process of oxidation reaction has higher peak current than that of reduction reaction. The SWCNTs-modified gold electrode has the ability to oxidize UA in three steps, making it different from other modified electrodes, where one of them has been reported by Ergün et.al. (2016). The GCE was modified by P(GBHA) and UA solutions were measured at potentials of $-0.2$ – $1.0$ V. The modified ele-

![Figure 1. Photograph of gold (Au) wire before (a) and after coated by SWCNTs (b).](image)

![Figure 2. SEM (a) and TEM (b) images of SWCNTs layer on the gold electrode.](image)

![Figure 3. Raman spectra of the G, D and 2D bands for the synthesized SWCNTs.](image)

![Figure 4. XPS results in the C (1s) region of the synthesized SWCNTs on the gold electrode.](image)
Figure 5. Cyclic voltammograms obtained for SWCNTs-modified gold electrode in 0.1 M phosphate buffer solution at pH 7.0 (A) and 1.0 mM UA in 0.1 M phosphate buffer solution, pH 7.0 (B), at scan rate of 100 mVs\(^{-1}\).

trode, however, only showed two step oxidation of UA, even though the measurements were carried out at similar potentials.

**Calibration curve for uric acid determination by SWCNTs-modified gold electrode.**—The detection performance of the SWCNTs-modified gold electrode was studied at different concentrations of UA. Differential Pulse Voltammogram of UA at various concentrations are shown in Figure 6A, where the peak of the anodic current increases with the increase in UA concentrations. Figure 6B shows the calibration curve obtained using an \(i_{pa}\) value of +0.305 V. The equation of linear regression and the correlation coefficient (R\(^2\)) are \(i_{pa} (\mu A) = 132.753 + 1.512c (\text{nmolL}^{-1})\) and 0.99597, respectively.

The LOD is defined as the smallest concentration that can be detected by the electrode or sensor\(^{26}\) and is determined using the following Equation 1:

\[
\text{LOD} = 3S_b/m,  \tag{1}
\]

where \(S_b\) is the standard deviation of the blank signal and \(m\) is the slope of the calibration curve. The LOD of the SWCNTs-modified gold electrode for UA has been found to be 5.05 nM. Calculation using the linear regression equation in Figure 6B shows that the sensitivity of the electrode is 5.240 \(\mu\text{Am m}^{-2}\text{nM}^{-1}\).

The performance of several electrodes for UA detection is shown in Table I. Comparison with the other working electrodes indicates that the SWCNTs-modified gold electrode has a lower LOD (Table I). Thus, the SWCNTs-modified gold electrode has demonstrated as an alternative sensor for UA determination.

**Effect of scan rates.**—The effect of scan rates, which depend on the potential sweep rate, as described in our previous study\(^{22}\), has been studied in order to assess whether the processes on the SWCNTs-modified gold electrode are controlled by diffusion or adsorption. Figure 7A shows the representative cyclic voltammogram of SWCNTs-modified electrodes in the presence of 1 mM UA at various scan rates. The increase in the scan rates shifted the \(E_{pa}\) slightly to more positive potentials, and increased the peak current. The peak current for the anodic oxidation is proportional to the square root of the scan rate (Figure 7B), and the linear regression equation obtained for this relation is \(i_{pa} (\mu A) = 66.0453 \times v^{1/2} (\text{mV}^{1/2}\text{s}^{-1/2}) - 138.1285\) (R\(^2\) = 0.9718). According to the Randles-Sevcik Equation\(^{27}\):  

\[
i_p = (2.69 \times 10^5) n^{3/2} ACD^{1/2}v^{1/2},  \tag{2}
\]

where \(i_p\) is the peak current, \(n\) is the number of electrons, \(A\) is the electrode area, \(C\) is the concentration, \(D\) is the diffusion coefficient,
Figure 7. Cyclic voltammograms obtained for SWCNTs-modified gold electrode in 0.1 M phosphate buffer solution at pH 7.0, containing 1.0 mM UA solution with different scan rates (10, 50, 100, 200 and 300 mVs$^{-1}$) (A). Plot of anodic current at 0.341 V vs. square root of the scan rate (B). Plot of anodic current at 0.341 V (a) and cathodic current at 0.324 V (b) vs. scan rate (C).
Scheme 1. Possible mechanism of the oxidation of uric acid.

\[
i_p = \frac{n F Q v}{4 R T}, \quad [3]
\]

where \(i_{pa}\) and \(i_{pc}\) are the peaks of anodic and cathodic current, respectively, \(n\) is the number of electrons, \(F\) is Faraday’s constant, \(Q\) is the electric quantity, \(v\) is the potential scan rate, and \(T\) is the thermodynamic temperature. The peaks of anodic and cathodic current are linearly proportional to the scan rate, based on the linear equation. This indicated that the electro-catalytic behavior of UA involved surface electron transfer.28,29

A possible mechanism for the oxidation of UA in solution using SWCNTs-modified gold electrodes is shown in Scheme 1. The oxidation reaction shows that the UA (I) releases two hydrogen ions and two electrons, and then forms a reactive di-imine species (II). The presence of nucleophilic species from water will attack di-imine species and produce an imine alcohol (III). The addition of water molecules can cause an intra-molecular degradation to allantoin (IV). The possibility of such reactions corresponds to the reaction reported by Dutt et al. (2003).31 According to Figure 5, SWCNTs responds to three oxidation peaks and two reduction peaks. The first peak is possibly due to the oxidation reaction of UA (I) becoming di-imine species (II). The second peak occurs because oxidation reaction of di-imine species (II) transformed into imine alcohol (III). Furthermore, the imine alcohol (III) will be oxidized to turn into allantoin (IV) and appear as the third oxidation peak in the voltammogram (Figure 5). The third peak is in accordance with the results that reported by Ghosh et al. (2015).4 Moreover, the first and third peaks are reversible and resulting in a reduction peak in the voltammogram (Figure 5). The second peak is not reversible because it is not observed as reduction peak in the voltammogram (Figure 5).

Repeatability testing.—Repeatability of measurement for SWCNTs-modified gold electrode was carried out to determine the electrode stability when being tested repeatedly. Voltammogram of UA and DA for both the first and second days are shown in Figure 8. The repeatability of UA and DA measurements using the SCWNTs-modified gold electrode can be found at Tables II and III, consecutively. The oxidation peak from the measurements has been analyzed using F-test and t-test.
The F-test for the data of UA measurement at first day toward the second day indicates that the value of F calculated is higher than critical F value (Table IV). Hence, it can be concluded that H₀ is rejected, which also means that the measurement data has a precision with a significant difference of 95% confidence interval. The results from the t-test show that the value of t calculated is smaller than critical t value (Table V). This suggests that the UA measurement method used for the electrodes has an insignificant difference. According to the
Table V. t-test for UA measurement using SWCNTs-modified gold electrode.

|                      | First day | Second day |
|----------------------|-----------|------------|
| Mean                 | 0.000683  | 0.000705   |
| Variance             | 2.55E-10  | 7.9E-10    |
| Observations         | 5         | 5          |
| Hypothesized Mean Difference | 0         |            |
| t Stat               | -1.5267   |            |
| p(\(T < t\)) one-tail | 0.088844  |            |
| t Critical one-tail  | 1.94318   |            |
| p(\(F < f\)) two-tail | 0.177688  |            |
| t Critical two-tail  | 2.446912  |            |

Table VI. F-test for DA measurement using SWCNTs-modified gold electrode.

|                      | First day | Second day |
|----------------------|-----------|------------|
| Mean                 | 0.000874921 | 0.00112    |
| Variance             | 3.79311E-09 | 3.1E-09    |
| Observations         | 5         | 5          |
| F                    | 1.223582479 |            |
| p(\(F < f\)) one-tail | 0.42481227 |            |
| F Critical one-tail  | 6.388232909 |            |

Table VII. t-test for DA measurement using SWCNTs-modified gold electrode.

|                      | First day | Second day |
|----------------------|-----------|------------|
| Mean                 | 0.000875  | 0.00112    |
| Variance             | 3.79E-09  | 3.1E-09    |
| Observations         | 5         | 5          |
| Pooled Variance      | 5.0E-09   |            |
| Hypothesized Mean Difference | 0         |            |
| t Stat               | -6.6006   |            |
| p(\(T < t\)) one-tail | 8.46E-05  |            |
| t Critical one-tail  | 1.859548  |            |
| p(\(F < f\)) two-tail | 0.000169  |            |
| t Critical two-tail  | 2.306004  |            |

Results, it can be seen that the SWCNTs-modified gold electrode can still work well even if measured in UA for 2 days.

Different results have been observed at DA measurement. Table VI shows the F-test for data of DA measurement. The smaller value of F calculated that approaches the critical F value means that H₀ is accepted. This result confirms that the data has precision with no significant difference. Furthermore, t-test reveals that the value of t calculated is higher than critical t value (Table VII), which further concludes that H₀ is rejected. This also further hinted that the data have a significant difference. The significant testing has proved that the SWCNTs-modified gold electrode cannot work well for DA detection after the first day of measurement.

Interference study.—The response of the SWCNTs-modified gold electrode was studied against dopamine, glucose, urea and ascorbic acid. The voltammogram for the phosphate buffer (1), urea (2), glucose (3), ascorbic acid (4), dopamine (5) and UA (6) are shown in Figure 9A.

The voltammogram shows that no signals are found for urea, glucose and ascorbic acid. Unfortunately, dopamine gives a response with a peak current that resembles the uric acid response. Furthermore, the sensitivity of the SWCNTs-modified gold electrode for dopamine has been tested at lower concentrations similar to the LOD of the uric acid. No signal has been observed for 4 nM dopamine (Figure 9B (2)), which implied that dopamine did not interfere with the UA detection (Figure 9B (1)). Therefore, it can be concluded that SWCNTs-modified gold electrode has good selectivity for determination of UA at low concentrations.

Summary

The performance of the SWCNTs-modified gold electrode demonstrates that it is a good sensor for uric acid detection. The sensitivity and detection limit of the electrode are 5.240 µA mM⁻¹ nM⁻¹ and...
5.05 nM, respectively. It has been proved that SWCNTs can be used for selective and sensitive sensing of uric acid in the presence of urea, glucose, ascorbic acid and dopamine at low concentrations.

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