

# Selective Monitoring of Rutin and Quercetin based on a Novel Multi-wall Carbon Nanotube-coated Glassy Carbon Electrode Modified with Microbial Carbohydrates $\alpha$ -Cyclosophorohexadecaose and Succinoglycan Monomer M3

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Multi-wall carbon nanotube (MWNT)-modified glassy carbon electrodes (GCE) were prepared for simultaneous determination of rutin and quercetin. Microbial carbohydrates,  $\alpha$ -cyclosophorohexadecaose ( $\alpha$ -C16) and succinoglycan monomer M3 (M3) were doped into MWNTs to prepare a  $\alpha$ -C16-doped MWNT-modified GCE (( $\alpha$ -C16 + MWNTs)/GCE) and a M3-doped MWNT-modified GCE ((M3 + MWNTs)/GCE), respectively. The sensitivities of the ( $\alpha$ -C16 + MWNTs)/GCE to rutin and quercetin were  $34.7 \mu\text{A}\cdot\mu\text{M}^{-1}\cdot\text{cm}^{-2}$  and  $18.3 \mu\text{A}\cdot\mu\text{M}^{-1}\cdot\text{cm}^{-2}$ , respectively, in a linear range of  $2 \sim 8 \mu\text{M}$  at pH 7.2. The sensitivities of the (M3 + MWNTs)/GCE was  $2.44 \mu\text{A}\cdot\mu\text{M}^{-1}\cdot\text{cm}^{-2}$  for rutin and  $7.19 \mu\text{A}\cdot\mu\text{M}^{-1}\cdot\text{cm}^{-2}$  for quercetin without interference.

**Key Words:**  $\alpha$ -Cyclosophorohexadecaose, Quercetin, Rutin, Square wave voltammetry, Succinoglycan monomer

## Introduction

Flavonoids have various pharmacological and therapeutic activities including the potential to make difficult-to-detect biomolecules more easily detectable.<sup>1-5</sup> Conventional labor-intensive detection methods for flavonoids based on chromatography and spectroscopy have been recently replaced with more comprehensive electrochemical methods.<sup>6,7</sup> However, flavonoids abundant in a variety of fruits and vegetables are chemically much less soluble in water than in organic solvents. Direct electrochemical detection of flavonoids, hence, requires modification of the sensing electrode. A strong adsorption interaction between carbon nanotubes (CNTs) and a glassy carbon electrode (GCE) provides enhanced sensitivity and excellent chemical and electrical properties to CNT-modified GCE. The CNTs-adsorbed GCE surface also blocks additional adsorption of electrical-inactive byproducts of flavonoids.<sup>8</sup> Recent works have reported electrochemical selective monitoring of some flavonoids *via* the separation of the peak potentials ( $E_p$ ) of individual flavonoid constituents in a mixture.<sup>9</sup>

In this work, two different carbohydrate-modified GCEs are introduced for simultaneous determination of rutin and quercetin which cannot be accomplished with the use of a conventional  $\beta$ -cyclodextrin ( $\beta$ -CD)-doped multi-wall carbon nanotube (MWNT) film. The microbial carbohydrates  $\alpha$ -cyclosophorohexadecaose ( $\alpha$ -C16) from *X. oryzae* and succinoglycan monomer M3 (M3) from *R. meliloti* were used for modifying GCEs through a medium of MWNTs and resulted in a  $\alpha$ -C16-doped MWNT-modified GCE (( $\alpha$ -C16 + MWNTs)/GCE) and a M3-doped MWNT-modified GCE ((M3 + MWNTs)/GCE). Complexation capability due to the large cavity of  $\alpha$ -C16 can provide  $\alpha$ -C16-doped MWNTs with a better response to the relatively large molecules of rutin. A MWNT-modified GCE (MWNTs/GCE) was also prepared in order to clarify the electrochemical activities of  $\alpha$ -C16 and M3. The sensing performance of the

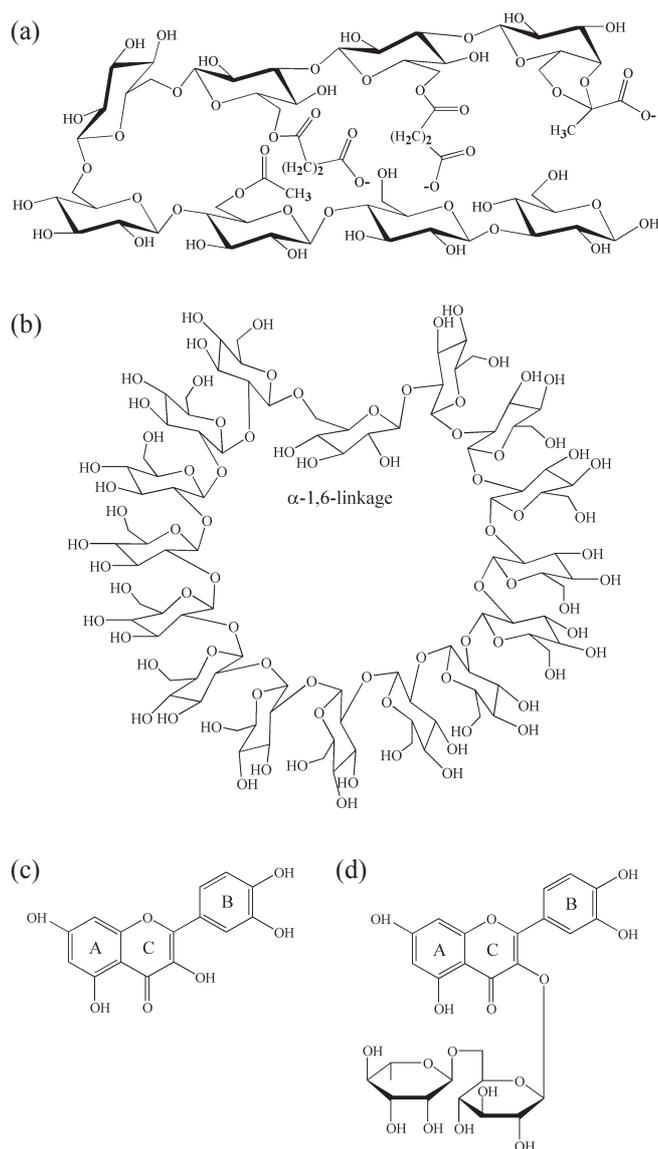
( $\alpha$ -C16+MWNTs)/GCE and the (M3+MWNTs)/GCE were assessed by cyclic voltammetry (CV) and square wave voltammetry (SWV) in terms of sensitivity and selectivity in a sodium phosphate buffer (SPB) solution at pH 7.2 by comparing the peak current density ( $J_p$ ) and the  $E_p$  arising from the redox reactions of rutin and quercetin in a potential range of  $0 \sim 0.5$  V vs. Ag/AgCl.

## Materials and Methods

**Reagents and materials.** M3 is an octasaccharide, having one acetyl group, one pyruvyl group, and two succinyl groups as substituents, derived from *R. meliloti* 1021. The oligosaccharide  $\alpha$ -C16 is a ring-shaped hexadecasaccharide isolated from *Xanthomonas oryzae*. The molecular structures of M3 and  $\alpha$ -C16 are shown in Figs. 1 (a) and (b), respectively. Figs. 1 (c) and (d) show the molecular structures of quercetin and rutin, respectively. MWNTs dissolved in ethanol (SolCNT #3011, Cluster Instruments Co., Ltd., 1 wt %) were used as an entrapping matrix for  $\alpha$ -C16 and M3. Rutin trihydrate (> 90%) quercetin (> 98%), SPB (pH 7.2), and ethanol (> 99.5) were used without further purification.

**Equipment.** Electrochemical measurements were performed in an electrochemical workstation composed of a potentiostat/galvanostat (VersaSTAT3), an IBM-compatible PC, a lab-made electrochemical cell, and a Faraday cage. Pt wire (> 99.99%) was used as the counter electrode. The working electrode potential was always recorded versus the Ag/AgCl (Sodium saturated) reference electrode.

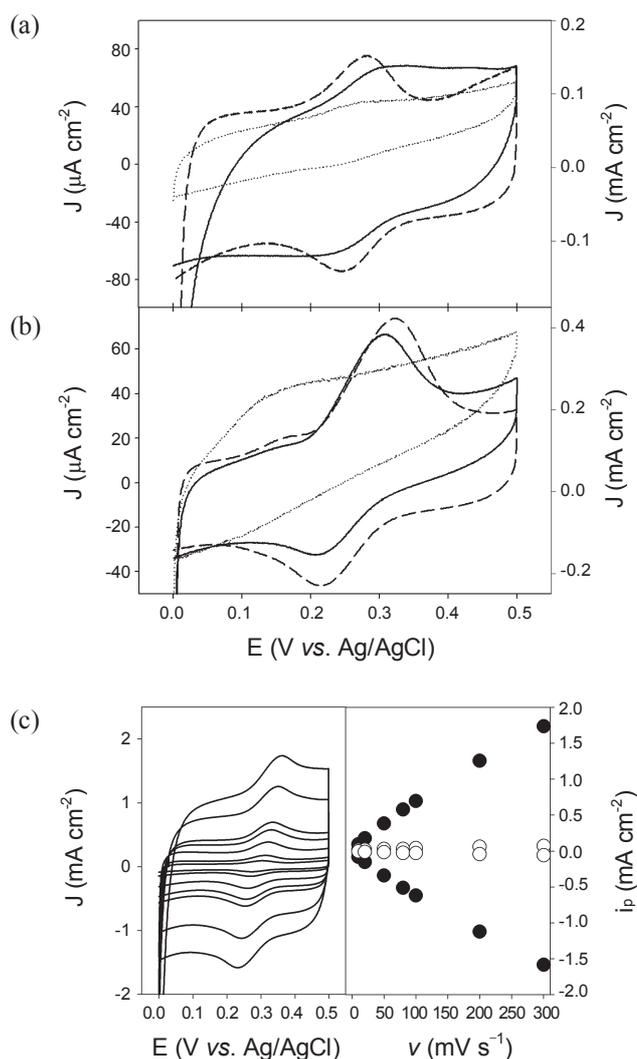
**Preparation and identification of  $\alpha$ -C16 and M3.** *X. oryzae* from the Korean Agricultural Culture Collection (KACC) was grown in a triptone glucose yeast (TGY) medium. After extraction with 5% trichloroacetic acid and chromatographic purification,  $\alpha$ -C16 was identified using Fourier transform nuclear magnetic resonance spectroscopy (FT-NMR) and matrix assisted laser desorption/ionization time of flight mass spectroscopy



**Figure 1.** Molecular structure of succinoglycan monomer M3 (a),  $\alpha$ -cyclophosphorohexadecase (b), quercetin (c), and rutin (d). Succinoglycan monomer M3 is an open-structured octasaccharide featuring two succinyl substituents, while  $\alpha$ -cyclophosphorohexadecase has a closed ring structure composed of fifteen 1, 2 linkages and one  $\alpha$ -1, 6 glucosidic linkage.

(MALDI-TOF).<sup>10,11</sup> *R. meliloti* 1021 was cultured in a glutamate mannitol salts (GMS) medium at 30 °C for 5 days. After multiple concentrations and centrifugations of the culture supernatants, M3 was separated from other succinoglycan monomers and analyzed by 500 MHz NMR spectroscopy.<sup>12,13</sup>

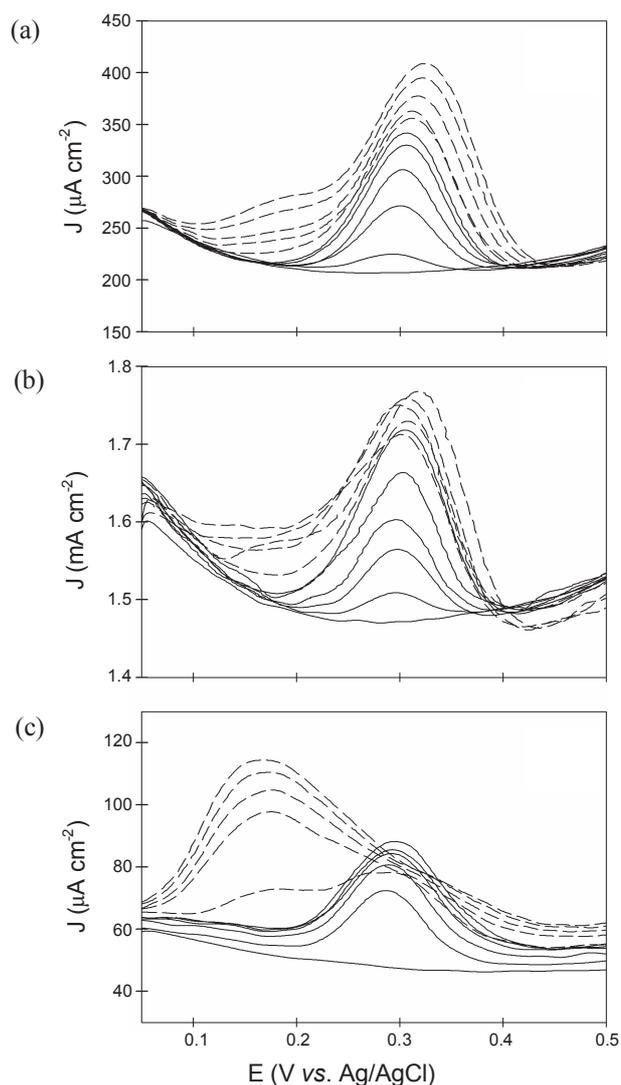
**Preparation of modified GCEs.** The GCE (3.0 mm in diameter, Tokai Carbon, Japan) was polished with 0.3 micron alumina powder on a polishing cloth after each use to remove adsorbed impurities. After rinsing the surface thoroughly with deionized water (D.I. water), the polished GCE was sonicated for 5 minutes to remove trace amounts of alumina powder from the surface and rinsed again with D.I. water. The electrode tip was then dipped into a 30% nitric acid solution for 20 minutes, followed by the electrode surface being scanned twenty times in a 0.5 M



**Figure 2.** Cyclic voltammograms of the multi-wall carbon nanotube-modified glassy carbon electrode (solid line), the  $\alpha$ -C16 + MWNTs/GCE (dashed line), and the succinoglycan monomer M3-doped multi-wall carbon nanotube-modified glassy carbon electrode ((M3 + MWNTs)/GCE), shown as a dotted line, either in the presence of 7.5  $\mu\text{M}$  rutin (a) or 7.5  $\mu\text{M}$  quercetin (b). Electrolyte = 0.15 M sodium phosphate buffer (SPB) solution (pH 7.2);  $\nu = 0.02 \text{ V}\cdot\text{s}^{-1}$ . Note that the right axes in (a) and (b) are only for the  $\alpha$ -C16 + MWNTs/GCE. (c) shows CV diagrams of the  $\alpha$ -C16 + MWNTs/GCE in a SPB solution (pH 7.2) containing 7.5  $\mu\text{M}$  rutin at 0.01  $\text{V}\cdot\text{s}^{-1}$ , 0.02  $\text{V}\cdot\text{s}^{-1}$ , 0.05  $\text{V}\cdot\text{s}^{-1}$ , 0.08  $\text{V}\cdot\text{s}^{-1}$ , 0.1  $\text{V}\cdot\text{s}^{-1}$ , 0.2  $\text{V}\cdot\text{s}^{-1}$  and 0.3  $\text{V}\cdot\text{s}^{-1}$ , and the plots of  $i_p$  vs.  $\nu$  for the  $\alpha$ -C16 + MWNTs/GCE (closed circle) and for the (M3 + MWNTs)/GCE (open circle) are also shown.

sulfuric acid solution in a range of  $-0.2 \sim 1.2 \text{ V}$  at  $0.05 \text{ V}\cdot\text{s}^{-1}$ .

Solution mixtures composed of carbohydrates and MWNTs for coating the electrode substrates were prepared as follows. An ethanolic MWNT solution at 1% (w/w) concentration was diluted in D.I. water to a concentration of  $2 \text{ mg}\cdot\text{mL}^{-1}$ . Ten milligrams each of  $\alpha$ -C16 and M3 were separately dissolved in 0.5 mL portions of the dilutions. After vortexing during mixing, 10  $\mu\text{L}$  portions of each solution mixture were dropped on the cleaned GCE surface and dried in a vacuum desiccator for 24 hours to

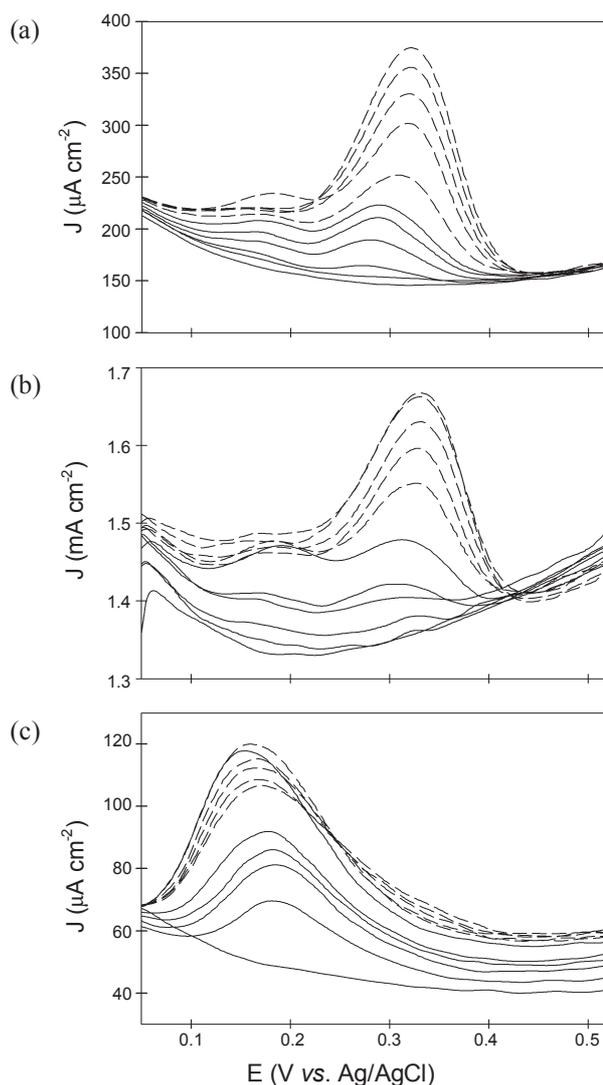


**Figure 3.** Square wave voltammograms of the multi-wall carbon nanotube-modified glassy carbon electrode (a), the  $\alpha$ -cyclophosphorohexadecase-doped multi-wall carbon nanotube-modified glassy carbon electrode (b), and the succinoglycan monomer M3-doped multi-wall carbon nanotube-modified glassy carbon electrode (c) for the oxidation of rutin and quercetin in a 0.15 M sodium phosphate buffer solution at pH 7.2. Amplitude = 0.025 V; step potential = 0.002 V; frequency = 15 Hz. A 10  $\mu$ L portion of rutin solution (0.1% in ethanol) was added to 10 mL of electrolyte successively five times to obtain solid lines. 10  $\mu$ L of quercetin solution (0.1% in ethanol) was then injected to the rutin-dispersed electrolyte solution five times to obtain dashed lines.

prepare the ( $\alpha$ -C16 + MWNTs)/GCE, the (M3 + MWNTs)/GCE, and the MWNTs/GCE.

### Results and Discussion

**Electrode reactions of rutin and quercetin on modified GCEs in a SPB solution at pH 7.2.** The CV diagrams of rutin (7.5  $\mu$ M) on modified-GCEs shown in Fig. 2 (a) indicate that the redox reaction of rutin is electrochemically reversible and generates only a single oxidative peak at about 0.28 V. Corresponding reduction peaks are observed at 0.25 V and the  $E_p$  separation is



**Figure 4.** Square wave voltammograms of the multi-wall carbon nanotube-modified glassy carbon electrode (a), the  $\alpha$ -cyclophosphorohexadecase-doped multi-wall carbon nanotube-modified glassy carbon electrode (b), and the succinoglycan monomer M3-doped multi-wall carbon nanotube-modified glassy carbon electrode (c) for quercetin and rutin oxidation in a 0.15 M sodium phosphate buffer solution at pH 7.2. Amplitude = 0.025 V; step potential = 0.002 V; frequency = 15 Hz. A 10  $\mu$ L portion of quercetin solution (0.1% in ethanol) was added to 10 mL of electrolyte successively five times to obtain solid lines. A 10  $\mu$ L portion of rutin solution (0.1% in ethanol) was then injected to the quercetin-dispersed electrolyte solution five times to obtain dashed lines.

30 mV. The redox peak of rutin below 0.5 V is due to the oxidation of 3',4'-dihydroxy groups on the ring-B of rutin and the corresponding reduction of 3',4'-diquinone.<sup>14</sup> Fig. 2 (b) shows CV diagrams of the redox reaction of quercetin (7.5  $\mu$ M). The MWNTs/GCE and the ( $\alpha$ -C16 + MWNTs)/GCE show two separate oxidative peaks at 0.15 V and 0.31 V, respectively. The (M3 + MWNTs)/GCE shows a single irreversible oxidation peak around 0.15 V. Fig. 2 (c) shows CV diagrams of the ( $\alpha$ -C16 + MWNTs)/GCE at various scan rates at 7.5  $\mu$ M of rutin. The plots of peak current ( $i_p$ ) vs. scan rate ( $\nu$ ) of both the ( $\alpha$ -C16 + MWNTs)/GCE and the (M3 + MWNTs)/GCE were linear in a

**Table 1.** Summary of sensitivities taken from the square wave voltammograms of carbohydrate-doped multi-wall carbon nanotube-modified glassy carbon electrodes

	Sensitivity (current density difference vs. flavonoid concentration) ( $\mu\text{A}\cdot\mu\text{M}^{-1}\cdot\text{cm}^{-2}$ )			
	Rutin	Rutin <sup>Q</sup>	Quercetin	Quercetin <sup>R</sup>
Multi-wall carbon nanotube-modified glassy carbon electrode	19.629 $\pm$ 1.5 <sup>b</sup>	20.137 $\pm$ 1.7 <sup>b</sup>	12.339 $\pm$ 1.5 <sup>b</sup>	9.277 $\pm$ 1.2 <sup>b</sup>
$\alpha$ -cyclodextrin-doped multi-wall carbon nanotube-modified glassy carbon electrode	34.719 $\pm$ 1.5 <sup>b</sup>	19.997 $\pm$ 0.9 <sup>b</sup>	18.314 $\pm$ 1.1 <sup>b</sup>	Not available
Succinoglycan monomer M3-doped multi-wall carbon nanotube-modified glassy carbon electrode	2.443 $\pm$ 0.4 <sup>b</sup>	Not available	7.193 $\pm$ 0.5 <sup>a</sup>	6.427 $\pm$ 0.5 <sup>a</sup>

<sup>a</sup>Peak currents shown at 0.17 V vs. Ag/AgCl was used to calculate the sensitivity slope. <sup>b</sup>Peak currents shown at 0.3 V vs. Ag/AgCl was used to calculate the sensitivity slope. <sup>Q</sup>sensitivity to rutin in the presence of 7.5  $\mu\text{M}$  quercetin dispersed in an electrolyte solution. <sup>R</sup>sensitivity to quercetin in the presence of 7.5  $\mu\text{M}$  rutin dispersed in an electrolyte solution.

range of 0.01  $\sim$  0.3 V  $\cdot$  s<sup>-1</sup>. The emerging linear relationship suggests that target flavonoids are successfully captured by the carbohydrate-modified MWNT film.

**Square wave voltammetric characterization of modified GCEs.** The first peak group shown around 0.17 V is due to the oxidation of quercetin and the other peak group observed at 0.3 V is due to the rutin oxidation. The  $i_p$  of rutin is pH dependent and commonly observed at 0.3 V under neutral conditions.<sup>15</sup> Fig. 3 (a) shows the SWV diagrams of the MWNTs/GCE for rutin oxidation (solid lines) and for quercetin oxidation in the presence of 7.5  $\mu\text{M}$  rutin (dashed lines). The rutin peaks continue growing not only with increasing rutin concentration but also as quercetin concentration increases in the presence of rutin. The rutin peak of the ( $\alpha$ -C16 + MWNTs)/GCE, which is 1.5 times higher than that of the MWNTs/GCE, as shown in Fig. 3 (b), is not affected by quercetin. The peak potentials of rutin and quercetin on the (M3 + MWNTs)/GCE were clearly observed at 0.28 V and 0.17 V, respectively (Fig. 3 (c)). Five minutes of preconcentration before potential scanning was allowed for normalization.<sup>9</sup> Because the redox reaction of rutin is electrochemically reversible (Fig. 2 (a)), the  $E_p$  of SWV diagrams taken from the rutin oxidation approximates the formal potential of rutin. The  $E_p$  of rutin varies linearly between 0.56 V  $\sim$  -0.02 V in a range of pH 2  $\sim$  pH 11.<sup>16</sup> Hence, the estimated  $E_p$  of rutin at pH 7.2 given by linear interpolation ((9  $\times$  0.56 V - 0.58 V  $\times$  5.2)/9) is 0.22 V. The roughly 60 mV difference from the experimental value may be due to M3 molecules doped into MWNTs.

Solid lines shown in Figs. 4 (a), (b), and (c) denote SWV curves of pure quercetin at various concentrations, while all dashed lines are curves of rutin in the presence of quercetin (7.5  $\mu\text{M}$ ). The MWNTs/GCE shows two oxidation peaks for quercetin and only one peak for rutin. The first small oxidation peak shown at 0.17 V is quercetin-specific and the second at 0.3 V is a response to both the quercetin and the rutin. The  $\alpha$ -C16-doped MWNT film enhanced SWV responses to quercetin and rutin. Indeed, Fig. 3 (b) and Fig. 4 (b) indicate that the binding strength between  $\alpha$ -C16 and rutin is stronger than that between  $\alpha$ -C16 and quercetin.  $\alpha$ -C16, having sixteen glucose rings, appears to fit the rutin molecule better than it fits the small quercetin molecule. The single broad oxidation peak of quercetin on the (M3 + MWNTs)/GCE at 0.17 V is strong, as shown in Fig. 4 (c), and overspreads the region where the potential rutin-

specific peak would appear. Greater sensitivity to quercetin for the M3-doped MWNT film arises from tight interaction between succinyl substituents in M3 and quercetin molecules.<sup>14</sup>

The peak current density ( $J_p$ ) of the rutin-specific peak shown at 0.3 V was used to draw calibration curves. In the case of the (M3 + MWNTs)/GCE, the quercetin-specific peak shown at 0.17 V was used instead. Table 1 summarizes the responses to flavonoids of all modified electrodes investigated in this work. The current density difference ( $\Delta J$ ) signifies that each  $J_p$  is normalized by means of subtracting the background current density at a potential from the  $J_p$  at the same potential.

## Conclusions

Novel microorganism-originated carbohydrates,  $\alpha$ -C16 and M3, were successfully extracted, purified and immobilized on a GCE surface by using MWNTs as a conductive matrix. Although, preaccumulated flavonoid showed a screening effect, the  $\alpha$ -C16 present in the MWNT film as a dopant appeared to render the ( $\alpha$ -C16 + MWNTs) composite more sensitive to both flavonoids. The structural advantage of  $\alpha$ -C16 for binding to rutin seems to enhance its sensitivity. The (M3 + MWNTs)/GCE clearly separates the oxidative peak potentials of rutin and quercetin. Some functional microbial carbohydrates, which could be utilized as key components of a sensing electrode, would enlarge the potential availability of microbial carbohydrates in food and medical biosensor applications.

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