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Flow Injection Analysis of Kojic Acid in Cosmetics by a Carbon-Nanotube Modified Screen-Printed Electrode

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ABSTRACT

A novel carbon-nanotube modified screen-printed electrode (CNT/SPE) was successfully prepared and validated to be a useful detector in the flow injection system. Compared to a bare screen-printed electrode (SPE), CNT/SPE improved the detection limit for kojic acid (KA). The detection limit on $S/N = 3$ was as low as 0.01 ppm. Furthermore, several factors, including the pH, applied potential, flow rate and coating quantity of CNT, were assessed. The attractive response of CNT/SPE has allowed it to be used for constant-potential FIA (Flow-Injection Analysis) of KA and indicates great potential for monitoring chromatographic effluents. The linear relationship between KA concentration (0.1-120 ppm) and the current response was established. Meanwhile five different CNT/SPEs for the detection KA of 20 ppm yielded a RSD value of 4.43%, indicating acceptable reproducibility of the present analysis. Finally, two real samples on the market were analyzed using the proposed system with appreciable results.

Key words: carbon nanotubes, screen-printed electrode, kojic acid, flow injection analysis, cosmetics

INTRODUCTION

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one) (KA) is a fungal metabolic product which inhibits tyrosinase activity. The inhibition mechanism is the formation of a complex of KA with copper ion, a coenzyme partner of the tyrosinase. The ketone group at position 4 and hydroxyl group at position 5 in KA are specific donor groups. Due to the repression of tyrosinase activation in the skin, melanocytes become nondendritic, thus decreasing the formation of melanin. Although effective as a skin-lightening agent, KA was reported to have a high sensitizing potential and thus causing irritant contact dermatitis⁽¹⁾. Upon exposure to air or sunlight KA induces a strange brown shade and loses its bleaching efficacy⁽²⁾. The use of KA as ingredient in cosmetic formulations has been regulated because it is not stable. Therefore, it is necessary to develop a sensitive assay for the determination of KA for cosmetics and cellular mechanistic studies.

Few analytical methods were reported for the determination of KA. UV-visible approach to detect KA is popular but has suffered from poor sensitivity⁽³⁻⁵⁾. The potential of voltammetry with chemically modified screen-printed carbon electrode for the determination of KA has been demonstrated in our previous study⁽⁶⁾.

The carbon nanotube (CNT) represents a new kind of carbon-based material and is superior to other carbon materials mainly in, for example, closed topology and tubular structural feature and unique electronic and

mechanical properties⁽⁷⁾. CNTs have been claimed to be extremely useful as chemical sensors, in particular via electrochemical detection. The ability of CNT-modified electrodes to promote electron-transfer reactions has been widely demonstrated in several compounds of clinical, biological and environmental interest⁽⁸⁻¹¹⁾.

Here a novel carbon-nanotube modified screen-printed electrode (CNT/SPE) was prepared, and the amplification characteristics of KA obtained in the presence of CNT were described. A number of hydrodynamic parameters of CNT/SPE/FIA, such as applied potential, flow rate and components of eluent, were also experimentally investigated. Furthermore the CNT/SPE has been proved to be an adequate tool for the flow injection analysis (FIA), where a stable, highly sensitive and reproducible response was needed. The proposed system was then applied to analyze two real cosmetic samples.

MATERIALS AND METHODS

Kojic acid was obtained from Sigma (St. Louis, MO, USA). Sodium hydroxide, disodium hydrogen phosphate, phosphoric acid and all remaining chemicals of the highest purity were obtained from Showa (Tokyo, Japan). Aqueous solutions were prepared using doubly distilled deionized water by the reverse osmosis technique. Carbon nanotube was supplied by Powertip Co. (Hsinchu, Taiwan).

The FIA system consisted of a pump (model Beckman 126), an auto-sampler (model SIC 09) with a 20- μ L injection loop (Tokyo, Japan) and a thin layer electrochemical detector (model BAS CC-5). V oltammetric

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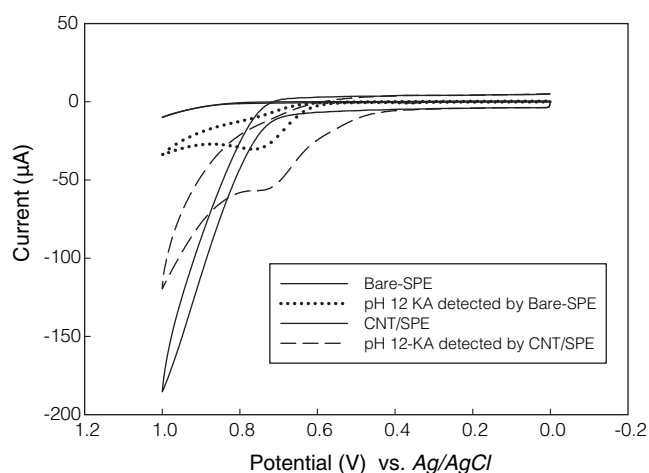


Figure 1. Cyclic voltammetric response at a bare SPE and the CNT/SPE with or without 20 ppm of kojic acid in 0.12 M phosphate solution (pH 12) at a scan rate of 100 mV/s.

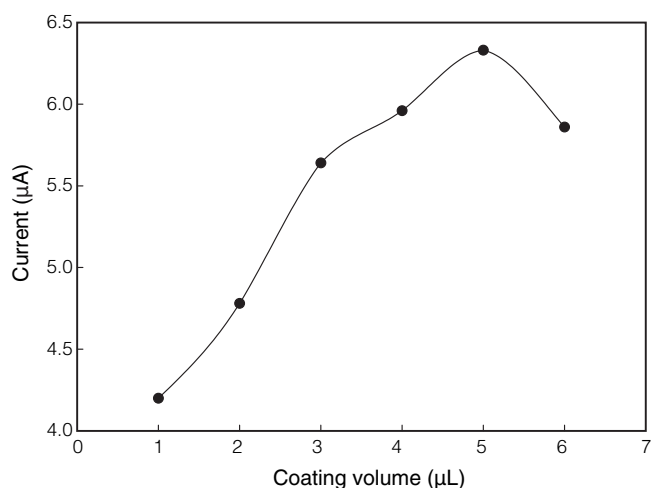
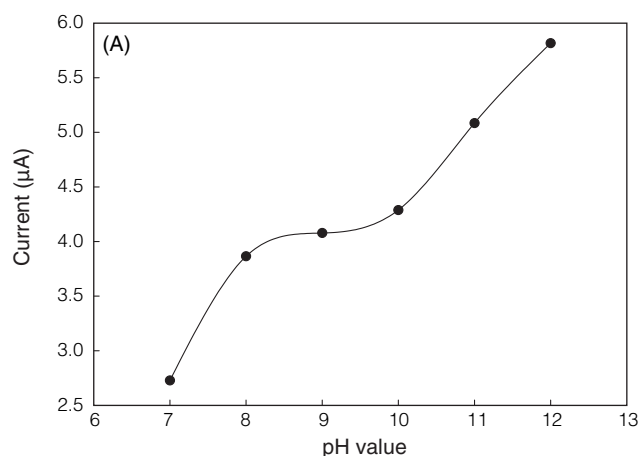


Figure 2. The effect of the CNT/SPE coating volume in the preparation of the CNT/SPE on the detection of 20 ppm kojic acid at an applied potential of 0.75 V vs. Ag/AgCl in 0.12 M phosphate solution (pH 12); flow rate = 0.5 mL/min.



measurements and FIA experiments were carried out on an electrochemical workstation (model CHI 621, TX, USA). A three-electrode cell assembled with a bare SPE or the CNT/SPE, Ag/AgCl reference (model BAS RE-5) and platinum disc auxiliary electrodes was used throughout the study. The SPE with a working area of 0.196 cm² and a conductive track radius of 2.5 mm was purchased from Zensor R&D (Taichung, Taiwan). The measured average resistance was $85.64 \pm 2.10 \Omega/\text{cm}$. Since dissolved oxygen did not interfere with the analysis of the detection potentials, no deaeration was needed in this study.

The CNT/SPE was prepared as follows: a mixture containing 2 mg carbon nanotube powder and 1-mL ethanol was first sonicated for 10 min, and then 5 μL of the mixture was dip-coated onto a clean SPE. The CNT/SPE was then equilibrated in a carrier solution at 0.75 V vs. Ag/AgCl until the current became constant in FIA, which normally took around 10 min. The quantification of the kojic acid was achieved by measuring the oxidation peak current in FIA at room temperature (25°C). After experiments, the electrodes were washed thoroughly with distilled water and stored in a closed container at ambient atmosphere.

Two skin-lightening creams (#1 & #2) were supported from Hsin Lan Chemical Co. Ltd (Taichung, Taiwan). The formulation was PCA-Na, squalane, stearic acid, α -tocopherol, cetyl alcohol, triglyceride, steareth-2 and steareth-4 with addition of 0.5 or 1% kojic acid. Prior to the analysis, 0.05 g samples were diluted in 50 mL of distilled water and the solution was filtered through a Lida filter (Lida, Nylon-66 and 0.22 μm).

RESULTS AND DISCUSSION

The ability to oxidize kojic acid was used to assess the effects of the CNT/SPE and bare SPE. The cyclic voltammetry (CV) response of 20 ppm kojic acid on unmodified SPE and CNT/SPE in the 0.12 M phosphate solution (pH 12) is shown in Figure 1. These results

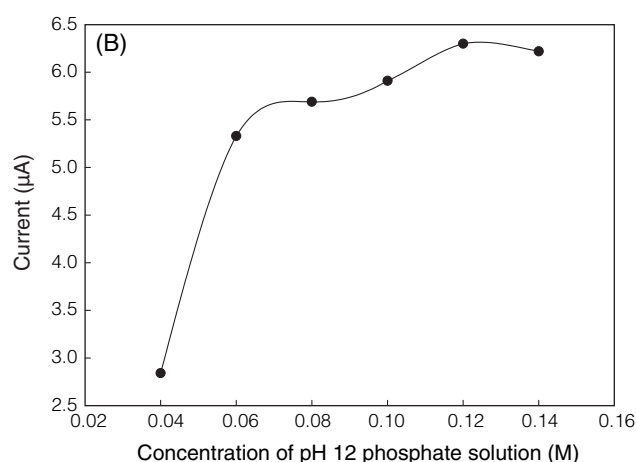


Figure 3. The effect of phosphate solution (A) pH and (B) concentration on the detection of 20 ppm kojic acid at the CNT/SPE. Other FIA conditions were the same as in Figure 2.

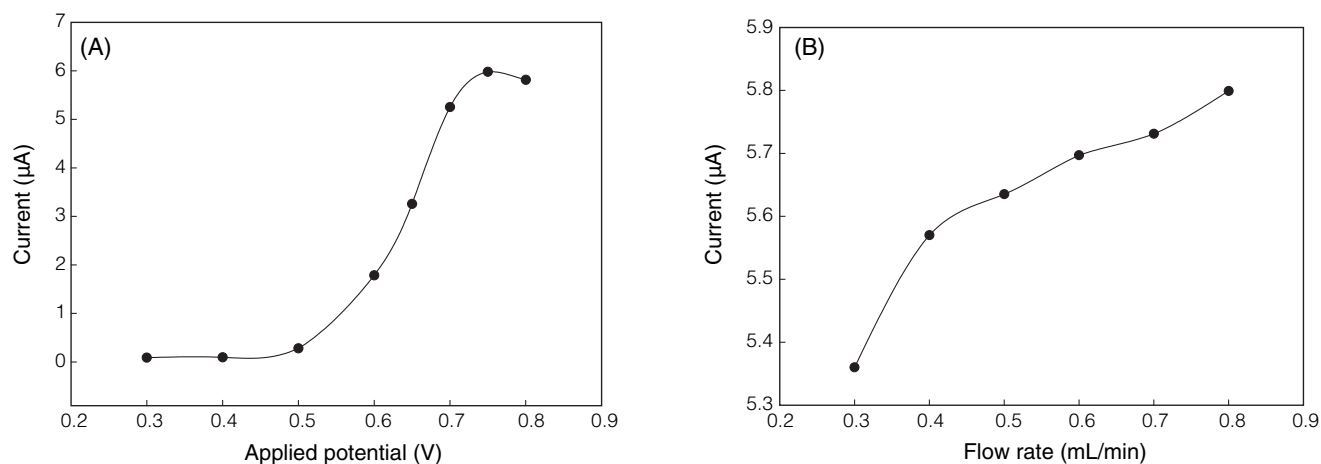


Figure 4. The effects of (A) applied potential (E_{app}) and (B) flow rate on the detection of 20 ppm kojic acid at the CNT/SPE. Other FIA conditions were the same as in Figure 2.

demonstrate that the CNT/SPE exhibits greater electrochemical activity towards the kojic acid oxidation than the bare SPE electrode does. The enhanced catalytic activity was presumably due to the favorable surface functional group orientation of CNT's towards kojic acid as well as the working surface area enhancement.

Analytical parameters of the system were optimized by first examining the effect of the CNT coating volume in the range of 1-6 μL on the detection of 20 ppm kojic acid by FIA. As seen in Figure 2, an optimal coating volume of 5 μL was obtained in this study. The concentration and pH value of supporting electrolytes are another important factors for the sensitivity of the working system. The effects of the pH value and concentration of phosphate solution on the FIA response for kojic acid were thus examined and a maximum reached at the phosphate solution of pH 12 and 0.12 M, as shown in Figure 3(A) and (B).

Individual hydrodynamic parameters in FIA were systematically optimized and revealed in Figure 4. The applied potential (E_{app}) was varied from 0.3 to 0.8 V vs. Ag/AgCl, and the current response reached the highest point at 0.75 V vs. Ag/AgCl as shown in Figure 4(A). Presumably, the observed maximum peak current with slight decrease above 0.75 V corresponds to the mass transfer limited region with regular catalysis. For 20 ppm kojic acid, the hydrodynamic flow rate (H_f) was applied from 0.3 to 0.8 mL/min, as shown in Figure 4(B). The FIA current value rose as the H_f increased. The current at $H_f = 0.8$ mL/min presented only about 8% increase in magnitude than that at $H_f = 0.3$ mL/min. However, current signal fluctuated more while higher H_f was applied. So, the $H_f = 0.5$ mL/min was chosen as the optimum for further analyses.

Under the optimized FIA condition, the CNT/SPE exhibited a linear relationship between the current response and the kojic acid concentration. In Figure 5(A) and (B), high and positive correlation coefficients ($r = 0.9971$ and 0.9989) was obtained for the standard kojic

acid solutions of 0.1-30 ppm and 30-120 ppm, respectively, indicating the capability of electrode surface renewing and mass transfer controlled electron transfer. Extending of saturation limit of kojic acid concentration can occur by this way. Further, twelve continuous injections of 1.0, 10, 40, and 80 ppm kojic acid resulted in RSD values of 1.54, 1.54, 0.67, 0.58%, respectively, ensuring no fouling effect of proposed electrode in the detection of kojic acid. This linear range has been significantly extended in comparison with our previous study (up to 36 ppm)⁽⁶⁾. The enhanced signals were coupled with low noise levels, hence permitting convenient monitoring of sub-ppm changes in the levels of the respective kojic acid. Furthermore, in comparison with our previous pre-anodized SPE (detection limit: 0.024 ppm)⁽⁶⁾, the detection limit was as low as 0.01 ppm (based on $S/N = 3$), which suggested that CNT layer had a positive effect on SPE performance. Meanwhile, taking into account the fact that five different CNT/SPEs for detecting 20 ppm kojic acid yielded a RSD value of 4.43%, the reproducibility and sensitivity of the present analytical assays are acceptable for the electrochemical measurements in the flow injection system.

Two different cosmetic skin-lightening creams were analyzed for the kojic acid. The typical chromatograms of FIA obtained in the real sample analysis by standard addition method are shown in Figure 6. The analytical data for the assays were summarized in Table 1. The labeled amount of kojic acid is close to the detected values by CNT/SPE/FIA and the calculated recoveries all fell within the range of 93.67-106.86. The results validate the usefulness of the CNT/SPE for the practical analysis of kojic acid by FIA.

CONCLUSIONS

A carbon-nanotube modified screen-printed elec-

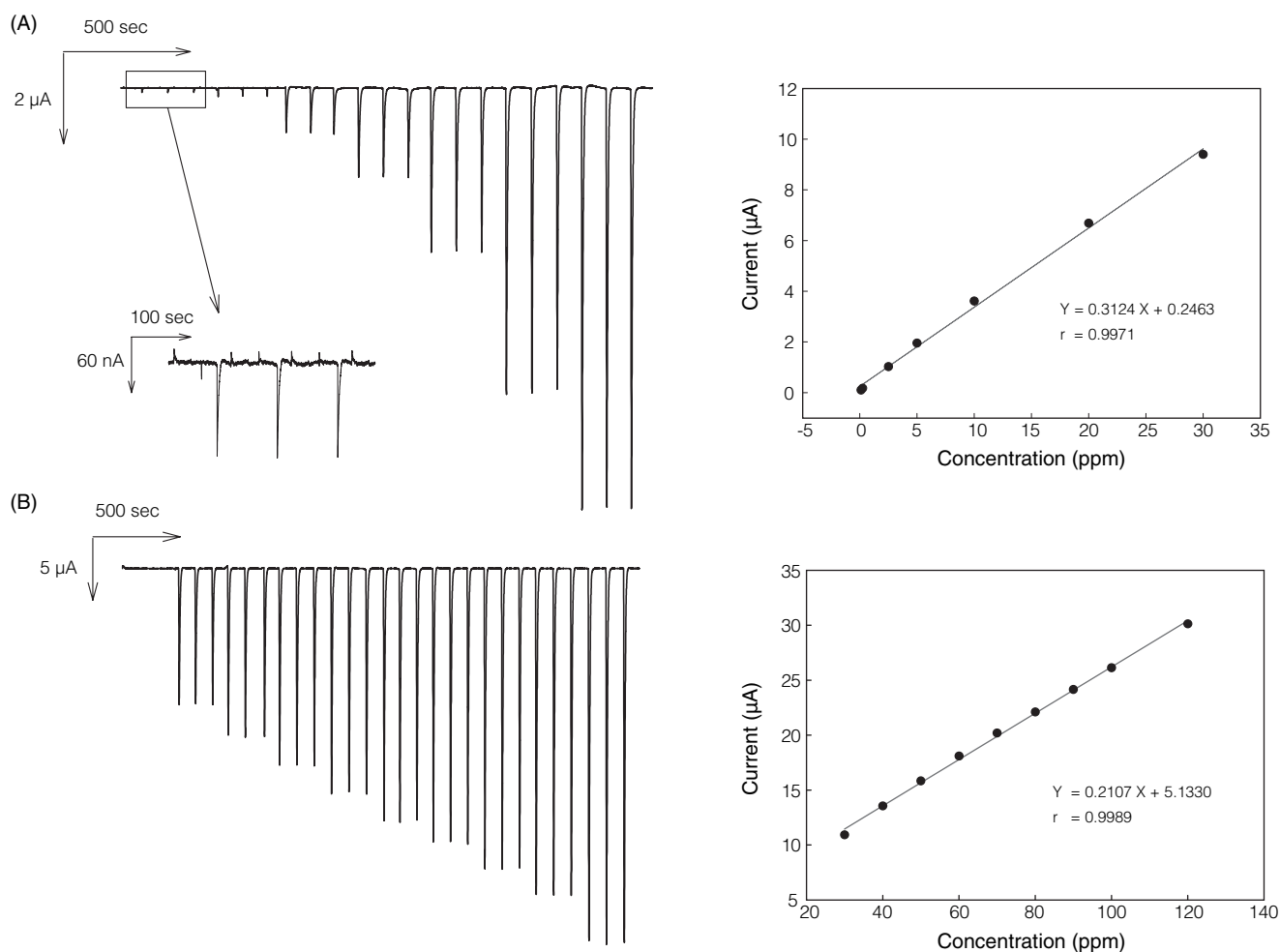


Figure 5. FIA peak responses of CNT/SPE with increasing standard kojic acid (A) from 0.1 to 30 ppm, (B) from 30 to 120 ppm, using 0.12 M phosphate as a carrier solution at an applied potential of 0.75 V vs. Ag/AgCl. Flow rate = 0.5 mL/min.

Table 1. Results for the assay of kojic acid in cosmetic skin-lightening cream with the CNT/SPE by FIA

Sample	Kojic acid (ppm)				Recovery
	Basal label	1st detected	Std. injected	2nd detected	
#1 (0.5%)	4.86	5.04 ± 0.02	5	9.89 ± 0.28	96.88 ± 1.42
			10	14.62 ± 0.39	95.79 ± 0.97
			20	23.78 ± 0.07	93.67 ± 0.13
#2 (1%)	9.54	10.30 ± 0.15	5	15.65 ± 0.16	106.86 ± 0.80
			10	20.64 ± 0.16	103.29 ± 0.41
			20	29.39 ± 0.27	95.41 ± 0.54

trode (CNT/SPE) has been demonstrated highly efficient as the flow detector for kojic acid in alkaline condition. An improved signal was observed at the CNT/SPE compared to a bare SPE. Extended flow injection analysis with the CNT/SPE was found to be highly reproducible with promising real sample analyses. Future efforts will be aimed to a deep understanding of the electrocatalytic activity of CNT/SPE, at exploiting it for monitoring

electroactive cosmetic ingredients following chromatographic and electrophoretic separations.

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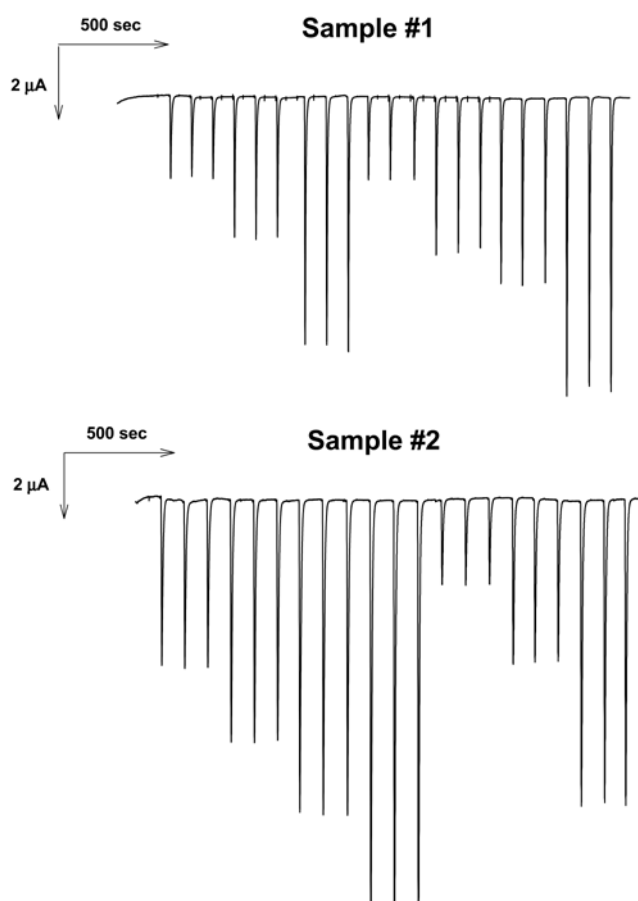


Figure 6. Real sample analysis of kojic acid with two different brands of skin-lightening cosmetic creams at the CNT/SPE by FIA. Other FIA conditions were the same as in Figure 5.

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