การวิเคราะห์คุณภาพวิตามินซีโดยการเคลือบท่อนาโนคาร์บอนบนอิเล็กโทรดใช้งานด้วยวิธีทางเคมีไฟฟ้าแบบอัตโนมัติ

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บทคัดย่อ

การศึกษานี้รายงานเกี่ยวกับการดัดแปลงท่อนาโนคาร์บอนบนไส้ดินสอให้เป็นอิเล็กโทรดใช้งานโดยวิธีดิฟเฟอเรนเชียล พัลส์ โวลแทมเมตรีแบบอัตโนมัติเพื่อตรวจหาวิตามินซี เมื่อเปรียบเทียบพบว่าประสิทธิภาพการวัดด้วยไส้ดินสอเปลือยและไส้ดินสอดัดแปลงด้วยท่อนาโนคาร์บอนโดยการวิเคราะห์ทางเคมีไฟฟ้าในไมโครไตเตอร์เพลท 24 หลุม ให้สัญญานศักย์ไฟฟ้าที่ต่ำกว่าและช่วงความสูงของกระแสที่กว้างขึ้น ช่วงของความเป็นเส้นตรงที่กว้างขึ้น และความไวของการทดสอบวิตามินซีแบบโรโบติกบนกราฟมาตรฐานขึ้นอยู่กับศักย์ไฟฟ้าออกซิเดชันที่เปลี่ยนแปลงและการขยายสัญญาณที่ดีกว่า สำหรับการวิเคราะห์หาค่าการละลาย ของวิตามินซี ที่เติมลงไปในตัวอย่างที่ทราบค่าความเข้มข้นเทียบกับยาเม็ดวิตามินซี พบว่าอัตราการกลับคืนใกล้เคียงร้อยละ 100 แสดงให้เห็นว่าการเคลือบท่อนาโนคาร์บอนเป็นตัวตรวจวัดที่ดีน่าเชื่อถือมีคุณภาพสูงในการนำไปใช้งานที่ต้องการการวิเคราะห์ตัวอย่างวิตามินซีครั้งละจำนวนมากๆ

คำสำคัญ : วิตามินซี / การวิเคราะห์ทางเคมีไฟฟ้า / อัตโนมัติ / โวลแทมเมทรี

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Robotic Ascorbic Acid Electroanalysis: Quality Gain via Carbon Nanotube Working Electrode Coatings

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Abstract

This study reports advanced automated ascorbic acid (AA) differential pulse voltammetry (DPV) at carbon nanotube (CNT)-modified pencil lead working electrodes (PLEs). Compared to the performance of the bare equivalents, CNT-modified PLEs offered in the electrochemical 24-well microtiter plate assay noticeably improved voltammograms with the analyte current peaks appearing at lower anodic potentials and with larger amplitudes. In robotic AA calibration trials the practical cathodic peak shift and signal amplification were associated with a wider linear range and better sensitivity. In addition, when pre-recorded calibration curves were used for AA determinations in spiked model and dissolved Vitamin C tablet samples, the reproducibly gained recovery rates were very close to 100%. Apparently, the CNT sensor coating is an analytical asset for the assay and this modification is recommended, particularly when the aim is to reliably achieve a high-quality robotic AA electroanalysis on larger sample numbers.

Keywords : Ascorbic acid / Vitamin C / Electroanalysis / Automation / Voltammetry

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1. Introduction

Water-soluble ascorbic acid (AA), also known as drug Vitamin C, is a highly regarded redox-active natural compound with antioxidant activity [1] and a strong involvement in a variety of processes within human physiology and pathology [2]. Because of the essential functions in the body it is generally agreed that our daily nutrition should supply sufficiently with AA in order to assure preservation of health [3]. Knowledge about the AA content of eatable fruits, vegetables and herbs and pharmaceutical supplementations is thus an important aspect for guiding personal diet choice and a systematic AA analysis of the products on the food market a definite requisite. Successful for accurate AA screens in foodstuff are spectroscopic, photometric, fluorimetric and electrochemical detection schemes and the actual user selection depends on the available technical equipment and expertise in a particular laboratory setting. We recently introduced automated AA electroanalysis in a specially designed robotic electrochemical microtiter plate well workstation as promising complementary option for a non-manual assessment of multiple samples. A pronounced level of convenience was gained as a consequence of the elimination of time-consuming repetitive electrochemical cell and electrode cleaning steps in between individual measurements [4].

In the initial work, bare pencil lead electrodes (PLEs) served as detecting component of the three-electrode assembly that travelled in the robotic system in computer-controlled fashion through the microtiter plate well solutions to ultimately test at halts of certain length their AA levels via voltammetry. Carbon nanotube (CNT) sensor coatings, on the other hand, are well-known for their capability to improve the electrochemical detection of organic molecules including our AA [5]. Here, the apparently beneficial CNT surface adaptation was tried on the PLEs of the system for robotic AA voltammetry, anticipating an enhancement of the analytical response of the arrangement. Changes of the properties of anodic AA detection due to CNT placement were assessed via cyclic voltammetry (CV), differential pulse voltammetry (DPV), calibration curve acquisition and model or tablet sample recovery rate measurements. Demonstrated below will be that the desired effect indeed was obtained and a better quality of microtiter plate-based AA voltammetry was reached.

2. Materials and Methods

2.1 Chemicals and samples

Chemicals: Ultrapure deionized water was the solvent of aqueous solutions and liquid for electrode rinsing. 1 M stocks of AA (Italmar (Thailand) Co., Ltd., Thailand, A.C.S. reagent) were freshly prepared in advance of the analytical trials and stored throughout at 4 °C in a capped dark volumetric flask. Electrolyte for AA voltammetry was 0.1 M potassium chloride (Italmar (Thailand) Co., Ltd., Thailand, reagent grade). A manual or robotic PLE dip-cleaning procedure used a 4 M dilution of 65% nitric acid (Italmar (Thailand) Co., Ltd., Thailand, reagent grade).

Samples: 1 mM model samples were prepared in the desired wells of a microtiter plate by mixing 3 μL 1 M AA/0.1 M KCl stock solution with 2997 μL 0.1 M KCl. Tablet samples were made by dissolving one vitamin C tablet, here Hicee with 500 mg per pill (Takeda, Bangkok, Thailand), at room temperature in 50 mL of water. 60 μL aliquots of the obtained aqueous AA tablet solution were during microtiter plate loading added in the sample wells to 2940 μL of 0.1 M KCl to set the analyte level to about 1 mM concentration.

2.2 Instrumentation for manual/robotic voltammetry

The technical details of the employed robotic electrochemical workstation have been described earlier [4]. In brief, it used a computer-controlled low-noise potentiostat (PalmSens, Palm Instruments BV, Netherland) and had incorporated three computer-controlled micropositioning devices for precise vertical (z) positioning of a working-counter-reference electrode assembly and horizontal (x/y) movements of a standard 24-well microtiter plate. All voltammetric AA measurements experiments used the potentiostat in three-electrode configuration. The workstation allowed manual and, through utilization of specially designed software scripts, automated voltammetry experiments, and calibration or sample measurements.

The three electrode assembly for robotic AA voltammetry involved PLEs as working electrodes (WEs) together with a coiled platinum wire counter electrode (CE) and a silver/silver chloride wire pseudo-reference electrode (RE). A special holder kept the electrode wires sufficiently close to avoid damage when moved into microtiter plate wells.

2.3 Working electrode preparation and CNT modification

6 cm long pieces of 0.5 mm diameter pencil leads (Pentel® HB, Pentel Co., Ltd., Japan) were tightly sealed into heat shrinking tubes but with leaving 2 mm at the bottom and 4 cm at the top end exposed as small cylindrical sensor and large alligator clip contact area, respectively. Completed PLEs were dip-cleaned for 1 min in 4 M HNO_3 and then water rinsed. Next was electrochemical CNT modification of the PLE surface. The voltage-induced placement of the desired CNT layers followed recently reviewed literature procedures [6] and used a 20 mg/mL water suspension of a carboxylic acid-functionalized version of CNTs (P3-SWCNT, Carbon Solutions, Inc., USA) as electrolyte and a function generator with DC option (Agilent® 3322A 20 MHz function/arbitrary wave form generator) as power supply. In a two electrode cell arrangement with a platinum wire in use as inert counter electrode a 10 minute application of a positive DC voltage of 1 V to the PLE precursor electrodes induced an electrophoretic attraction and subsequent firm deposition of the negatively charged CNTs on the positively charged PLE surface. Treated PLEs were gently sprayed with water and then allowed to dry in air. Scanning electron microscope (SEM) inspections of identically prepared PLE sensors in another study proved presence of a very thin but homogeneous nanoporous CNT surface linings as result of the electrodeposition procedure [7]. Prior to use for voltammetry trials CNT-modified PLEs were again rinsed with water.

2.4 Data analysis and statistics

Two types of samples were analysed in this study: (1) A 0.1 M KCl solution that was spiked with 1 mM ascorbic acid to make model sample) and (2) a liquid prepared by the dissolution of Vitamin C tablets in 0.1 M KCl. The known AA content of the samples supported the determination of % recovery rates as ([AA]measured/[AA]adjusted) × 100.

In one single microtiter plate run both model and tablet samples were jointly quantified, each in six fold repetition (see Figure 4 for the corresponding plate load); n was thus equal to 6 per sample for such an automated analytical trial; microtiter plate runs have been repeated three times with the aim to evaluate the quality of the reproduction of sample concentration assessments with different plates. Average recovery rates and standard deviations were computed for the six measurements within one microtiter plate run and values were compared in terms of reproducibility for the triplicate plate run repetitions.

3. Results and Discussion

Cyclic (CV) as well as differential pulse (DPV) voltammograms were recorded at bare and CNT-modified PLEs in 5 mM AA/0.1 M KCl solutions to electrochemically reveal the expected differences in the faradaic charge transfer behavior between the two types of sensors. Fig. 1A compares the two observed CVs and it clearly can be seen that the onset of the electrode-induced oxidation of AA appears for a CNT-modified PLE at significantly lower anodic potential than for the bare version. And the rise in the faradaic redox current occurs for the CNT-PLE with a noticeably steeper slope. Both observations together are good evidence for the achievement of a facilitated charge transfer interaction of the dissolved analyte at the surface of a CNT-PLE composite electrode. The favorable AA electrochemistry at CNT-modified PLEs led in DPV recordings to a substantial cathodic shift of the AA electrooxidation peak (Fig. 1B).

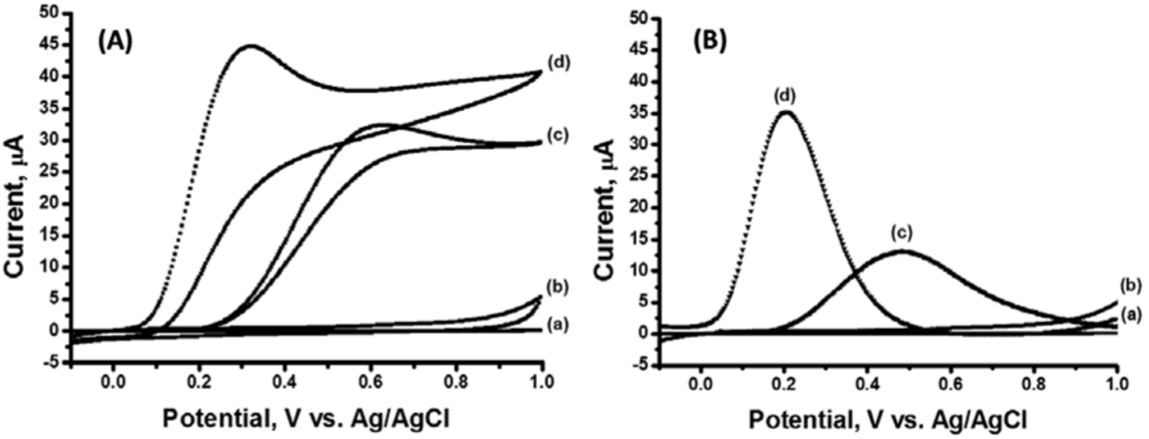

Figure 1 (A) Cyclic voltammograms (CVs) of a bare (a, c) and CNT-modified (b, d) PLE in 0.1 M KCl (a, b) and in 5 mM AA in 0.1 M KCl (c, d). All CVs were recorded with 100 mV s^{-1} as scan speed. (B) Differential pulse voltammograms (DPV) of a bare (a, c) and CNT-modified (b, d) PLE in 0.1 M KCl (a, b) and in 5 mM AA in 0.1 M KCl (c, d). All DPVs were acquired with an E_{step} of 4 mV, E_{pulse} of 50 mV, and t_{pulse} of 0.07 s.

Depending on the actual parameter settings of a particular trial, a complete robotic voltammetry run through a fully loaded microtiter plate well may need time up to about 3 hours. Obligatory is for a period of such a length a degradation resistance of the dissolved AA and the faradaic response stability of the CNT-modified sensors of the non-manual assay. Fig. 2A and B present a display of a collection of seven DPVs that were recorded with a CNT-modified PLE in a microtiter plate well containing 5 mM AA in 0.1 M KCl at intervals of 30 min and a plot of the observed seven AA peak currents as function of time. All voltammograms were well proportioned bell-shaped *I/E* curves with the current maxima gathered round 0.3 V vs. reference electrode and, more important with respect to AA and electrode stability, all tracks overlaid almost perfectly and peak amplitudes had insignificant deviances. Apparently, a degradation of the labile AA and an unfavourable CNT-PLE fouling due to surface adsorption of products of the interfacial electrochemical AA oxidation did not take place at disturbing level, at least not over the 3 hour period of inspection

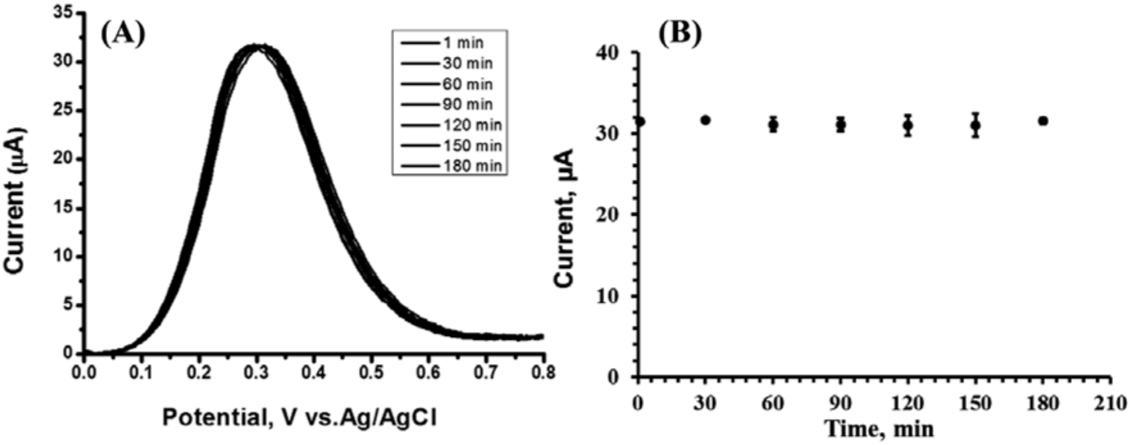

Figure 2 (A) Overlay of seven differential pulse voltammograms (DPVs) as recorded in course of an AA/ CNT-PLE-WE stability test in a 5 mM AA/ 0.1 M KCl test solution in a microtiter plate well with a three-electrode assembly holding a CNT-PLE as WE. DPVs were recorded over a period of 3 hours every 30 minutes. The DPV parameters were as defined in previous Fig.1. (B) Plot of DPV peak currents as function of time of recording for the seven traces superimposed in (A).

After analyte and working electrode stability was proven as appropriate for the purpose of the study, an automated AA calibration curve acquisition was carried out in the robotic workstation for sixteen analyte concentrations in the microtiter plate sample wells spread over the range of 0.1 to 18 mM. The corresponding set of DPVs is shown in Fig. 3A while Fig. 3B is the resulting calibration curve construction as plot of the extracted DPV peak currents, I_p, versus the actual AA level in the particular sample well. Actually I_p increased linearly with the AA concentration up to 18 mM ($R^2 = 0.9954$) but above 18 mM good linearity was lost (not shown). Important to mention at this stage that the linear range of robotic AA DPV at CNT-PLEs was similar to the outcome of their equivalent manual assessment (data not shown) but about twice as wide as valid for robotic or manual calibration trials with bare PLEs [4]. And the sensitivity of the robotic electrochemical AA assay with CNT-PLEs, about 5.1 μA/mM in the particular case shown in Figure 3, was throughout the trials a minimum of five times higher than the about 1.0 μA/mM at best possible with bare PLEs in charge of detection.

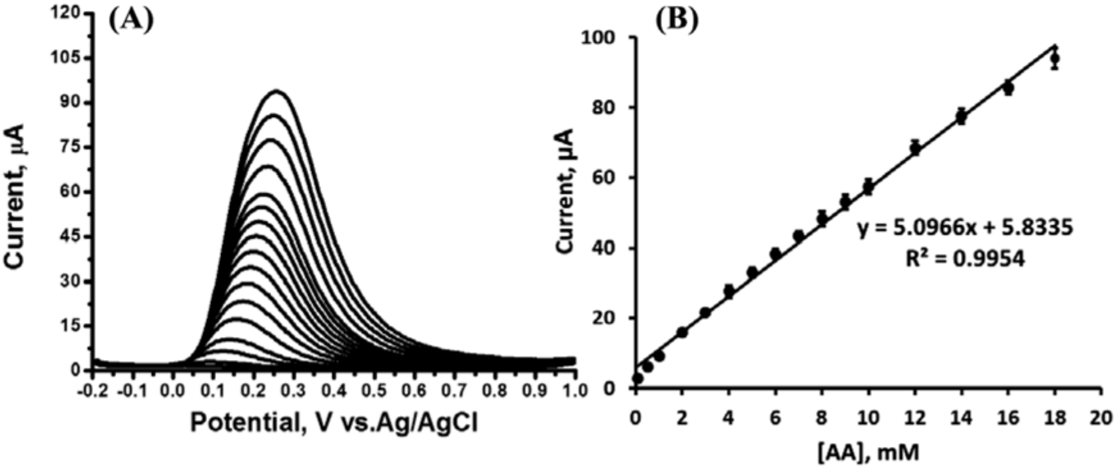

Figure 3 (A) Robotic AA calibration run of a CNT-PLE sensor through sixteen wells of a microtiter plate filled with analyte solutions of 0.1 to 18 mM level. Differential pulse voltammograms (DPVs) were recorded automatically one after the other using the parameter set as mentioned in Fig. 1. The DPV parameters were as defined in previous Fig. 1. (B) The peak currents of the sixteen AA DPVs in (A) plotted as a function of AA concentration. Linearity of the voltammetric response extends to 18.0 mM with a good R^2 value of 0.9954.

Finally, pre-calibrated CNT-PLEs were used for the robotic voltammetric measurements of AA in spiked model samples and in samples that were dilutions of a solution of a synthetic AA tablet. Fig. 4 provides an illustration of the microtiter plate load as used for the automated quantification trials. For electrode dip-cleaning, wells A1 and (A2, B1, and C1) were filled with 4 M HNO_3 and water, respectively. Well A3 had then an AA-free 0.1 M KCl filling for base line recording while the wells A4-6 and B2-4 carried the 1 mM spiked model sample, and wells B5-6 and C2-5 were loaded with dissolved tablet solutions for which the AA concentration was pre-adjusted via dilution to reach a final concentration of an about 1 mM. Fig. 4A and 4B display two representative DPVs measured in the wells with spiked model (A) and a dissolved tablet (B) sample, respectively. When the pre-calibration curve valid for the CNT-PLE of the trial (not shown) and the ΔI values of the DPVs of 4A and 4B were used for the computation of sample AA levels, 0.96 and 1.12 mM (= 495.62 mg/tablet, taking dilution factors into account) were revealed for the spiked model and the dissolved tablet samples, presenting recoveries of 96.62 and 98.97%, respectively. Table 1 is a summary of the outcome of the triplicate automated analysis of the two sets of six samples by the merger of robotic AA DPV at CNT-PLEs with the pre-calibration method. For both the model and real samples the recoveries of the eighteen measurements reached values close to 100%, which is clear sign of the outstanding performance level of the assay.

Table 1 AA recovery of model and Vitamin C tablet samples for determinations with a CNT-modified PLE via robotic DPV in a 24-well microtiter plate.

	Sample (n=6)	Adjusted	Measured	Recovery %
1st plate run	Spiked solution, mM	1	0.97 ± 0.01	96.60 ± 0.90
	Vitamin C tablet, mg)	500	494.88 ± 7.80	98.97 ± 1.55
2nd plate run	Spiked solution, mM	1	0.90 ± 0.04	90.83 ± 3.97
	Vitamin C tablet, mg)	500	495.98 ± 8.60	99.20 ± 1.20
3rd plate run	Spiked solution, mM	1	0.92 ± 0.05	92.33 ± 5.37
	Vitamin C tablet, mg)	500	489.77 ± 8.65	97.95 ± 1.73

Well 13 H_2O		**Well 7** H_2O		**Well 1** HNO_3	
Well 14 (Sample 9)		**Well 8** (Sample 4)		**Well 2** H_2O	
2940 μL buffer	60 μL AA-TS	2997 μL buffer	3 μL AA-SS		
Well 15 (Sample 10)		**Well 9** (Sample 5)		**Well 3**	
2940 μL buffer	60 μL AA-TS	2997 μL buffer	3 μL AA-SS	3000 μL buffer	
Well 16 (Sample 11)		**Well 10** (Sample 6)		**Well 4** (Sample 1)	
2940 μL buffer	60 μL AA-TS	2997 μL buffer	3 μL AA-SS	2997 μL buffer	3 μL AA-SS
Well 17 (Sample 12)		**Well 11** (Sample 7)		**Well 5** (Sample 2)	
2940 μL buffer	60 μL AA-TS	2940 μL buffer	60 μL AA-TS	2997 μL buffer	3 μL AA-SS
Well 18		**Well 12** (Sample 8)		**Well 6** (Sample 3)	
X		2940 μL buffer	60 μL AA-TS	2997 μL buffer	3 μL AA-SS

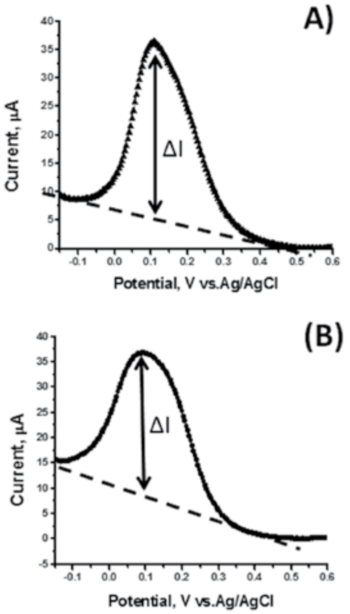

Figure 4 Schematic representation of a typical microtiter plate load for a robotic analytical voltammetry run on two samples, actually a spiked model and a dissolved tablet sample (Left). (A) One of the six differential pulse voltammograms (DPVs) as recorded in the wells with spiked model samples. (B) One of the six DPVs as recorded in the microtiter plate wells with dissolved tablet sample. (AA-SS: 1 M ascorbic acid stock solution; AA-TS: one Vitamin C tablet in 50 mL water). The DPV parameters were as defined in previous Fig. 1.

4. Conclusion

Evidence has been provided that a thin-film CNT surface modification is considerably improving the DPV response of a PLE working electrode for the analyte AA. Operated in a robotic electrochemical device for automated voltammetric AA quantifications in a 24-well microtiter plate format CNT-PLEs delivered a twice as wide linear range as their bare equivalents, an at minimum five times higher sensitivity and, last but not least, more reliably recovery rates for model and real sample AA analysis within a good ± 5% reach of the ideal value of 100%. Firm placement of the supportive CNT working electrode coating was possible via simple electrophoretic deposition, which makes the modified PLEs readily available and an optimal choice for routine microtiter plated-based AA screening in large sample libraries as faced in food and pharmaceutical industry in course of vitamin C screening trials.

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