

An Electrochemical Approach for the Determination of the Fruit Juice Freshness Using Carbon Paste Electrode Modified with ZnO Nanorods

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The presence of citric acid in food products is directly related to their color and taste, leading to the freshness of the products. Here, detection of citric acid in fruit juice were investigated using ZnO nanorods based carbon paste electrode (Z-CPE) through the electrochemical methods. A facile aqueous solution technique was used to synthesize ZnO nanorods and Z-CPE was prepared by dropping the dispersed ZnO nanorods in methanol on the surface of CPE. X-ray diffraction, transmission electron microscopy and field emission scanning electron microscopy analyses of prepared ZnO nanorods showed the ZnO nanorods were synthesized in high density of hexagonal phase. Cyclic voltammetry, differential pulse voltammetry, and electrochemical impedance spectroscopy techniques were conducted for electrochemical study of Z-CPE. Electrochemical analyses showed charge transfer behavior was facilitated due to the presence of high aspect ratio and high effective surface area of ZnO nanorods in the electrode surface. Study on the concentration effect of citric acid on Z-CPE indicated a linear relationship between peak current and citric acid concentration (0.5 mM to 6 mM as calibration range). The detection limit and sensitivity of citric acid sensor were estimated 0.998 $\mu\text{A}/\mu\text{M}$ and 3 nM, respectively. The results show that ZnO nanostructures can be of great help to enhance electrochemical sensors for detection of citric acid as an important element in juices, especially citrus fruits.

Keywords: ZnO nanorods; Carbon paste electrode; Citric acid; Electrochemical technique; Cyclic voltammetry; Electrochemical impedance spectroscopy

1. INTRODUCTION

Citric acid (2-hydroxy-1,2,3-propanetricar-boxylic acid) as a natural and weak acid finds out in citrus fruits. It is used as an additive in various foods and drinks in the food industry because of its sour taste and preservative properties. Aroma and flavor of food are preserved with citric acid additive which helps for its freshness. In the medicine industry, many nutritional and pharmaceutical supplements are

made from citrate salts. Consumption of citric acid in foods and beverages increases the body's urinary excretion. Thus, it can be effective in the treatment of kidney disease through calcium urolithiasis. Therefore, study and detection of citric acid can be considered as an effectual and pivotal investigation to recognize and optimize the quality of products and consumption patterns of foods and medicines.

Many studies have been performed to determine the citric acid content of juices, especially in citrus fruits and lemons. Most of these studies had been conducted through methods such as chemiluminescence method [1] electrochemical impedance spectroscopy [2], chronoamperometry [3], cyclic voltammetry, reversed-phase liquid chromatography [4], high-performance liquid chromatography [5], ion chromatography method [6], Raman spectroscopy [7], isotope ratio mass spectrometry [8], pyrolysis mass spectrometry [9] and potentiometry [10]. Among these methods, the practical methods based on electrochemical techniques such as electrochemical impedance spectroscopy and cyclic voltammetry are precise, inexpensive and fast methods that can be used to determine analytes in solutions. Electrochemical studied had been developed based on modification sensor and application of nanocatalysts and nanostructures on electrode surface to improve the stability and sensitivity of sensors. Zinc oxide (ZnO) nanostructure is one of important semiconductor for electrochemical investigations in the past decade because of its outstanding properties such as easiness of synthesis, nontoxicity, low cost, wide-bandgap, large exciton binding energy, photoconductivity, photochemical stability, optical and catalytic properties [11, 12]. Moreover, these properties of ZnO lead to applications in electronic and optoelectronics industry, medicine, gas sensors, solar cells and wastewater treatment [13]. ZnO nanostructures are synthesized in different morphology such as nanowires, nanorods, nanobowls, nanopyramid, nanotubes and nanoparticles [14, 15]. Nowadays, numerous physical and chemical approaches are presented for synthesis of different nanostructures of ZnO such as physical and chemical vapour deposition, atomic layer deposition, pulsed laser deposition, thermal oxidation, sol-gel and spray pyrolysis [16-18].

In considering the importance of citric acid, the present study was performed to determine the citric acid and freshness of fruits using ZnO nanorods based carbon paste electrode (Z-CPE). First, ZnO nanorods were synthesized and X-ray diffraction (XRD) and field emission scanning electron microscopy (FESEM) analyses were applied to structural characterization of prepared ZnO nanorods. Then, the CPE electrode was prepared and modified by the ZnO nanorods. The electrochemical techniques were conducted for electrochemical study of Z-CPE electrodes.

2. MATERIALS AND METHOD

Zinc nitrate, sodium hydroxide, ethanol, graphite powder, and citric acid were purchased from Sigma-Aldrich. The phosphate buffer solution consisted of a potassium phosphate solution (KH_2PO_4 and K_2HPO_4 from Merck; 0.1 M total phosphate). The pH of solution was adjusted at 6.0 by pH meter (Sigma, P1367 MSDS). All other chemicals were of analytical grade and were used without further purification. All solutions were made up of double-distilled water.

In order to prepare the ZnO nanorods, a 0.5 M aqueous solution of zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) and 1 M aqueous solution of sodium hydroxide (NaOH) were mixed in distilled water. Then, the beaker

containing NaOH solution was heated at the temperature of about 55°C. The Zn (NO₃)₂ solution was added dropwise (slowly for 60 minutes) to the above-heated solution under high-speed stirring. The beaker was sealed at this condition for 100 minutes. The precipitated ZnO nanorods were cleaned with deionized water and ethanol. Finally, the ZnO nanorods were dispersed in methanol in room temperature.

A bare CPE was fabricated by grinding and mixing graphite powder with paraffin oil in a ratio of 75:25 at an agate mortar till a homogeneous paste was gained, then the paste was pressed into a standard Teflon tube with a 3 mm internal diameter and smoothed by a weighing paper to attain a uniform surface. A pencil core was inserted into the tube as an electrical contact.

Z-CPE Electrode was prepared by dropping the dispersed ZnO nanorods in methanol on surface of carbon paste electrode. In order to form a strong link between carbon paste surface and ZnO nanorods, the carbon paste electrode was placed in an oven under 70 °C for 2 minutes. Then, electrode was washed with deionized water. To assurance of modification of carbon paste electrode, the dropping of dispersed ZnO nanorods on electrode surface and the thermal process was carried out three times.

Cyclic voltammetric and Electrochemical impedance spectroscopy (EIS) experiments were performed with a model EA-201 Electro Analyzer (chemilink systems), equipped with a personal computer that was used for electrochemical measurement and treating of data. A conventional three-electrode cell was employed throughout the experiments, with bare or ZnO nanorods modified carbon paste electrode as a working electrode, a saturated calomel electrode as a reference electrode, and a platinum electrode as a counter electrode. XRD was performed within 2θ range of 25 – 75° at 40 keV, by XRD, Bruker D 8, Advance, Germany using CuKα as radiation (1.5406 Å) in configuration. Morphology and shape of ZnO nanorods were studied by FESEM (Hitachi-3000). Samples of commercial lemon juice were acquired from local grocery stores. 20 mL lemon juice were stirred for 30 min in a glass vessel. Then, cleaned using a syringe filter with 0.45 mm porous membranes. Next, the samples were properly diluted in the supporting electrolyte before analysis.

3. RESULTS AND DISCUSSION

Fig. 1 shows the XRD patterns of the synthesized ZnO nanorods. According to the JCPDS card No. 361451 the patterns demonstrated by ZnO nanorods were grown in hexagonal phase. The space group P63 mc and lattice constants of ZnO structure are $a = 3.24982 \text{ \AA}$ and $c = 1.6021 \text{ \AA}$, respectively [19]. The strongest peak at $2\theta = 36.20^\circ$ is corresponding to the reflection from the (1 0 1) plane.

In order to study the shape and morphology of synthesized ZnO nanorods, FESEM and TEM images of prepared sample were provided. The top-view FESEM images of the ZnO nanorods is shown in Fig 2a which shows a hexagonal-faceted morphology. The diameter of the obtained ZnO nanorods was approximately 60 nm. The nanorods covered the entire surface of the substrate. Figure 2b indicates TEM image of ZnO nanorods. As shown, the average lengths of ZnO nanorods are approximately 500 nm and the surface of ZnO nanorods are very smooth.

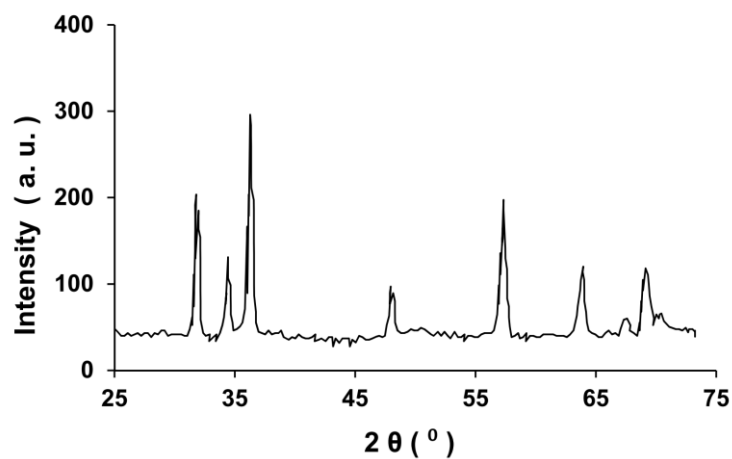


Figure 1. XRD patterns of synthesized ZnO nanorods.

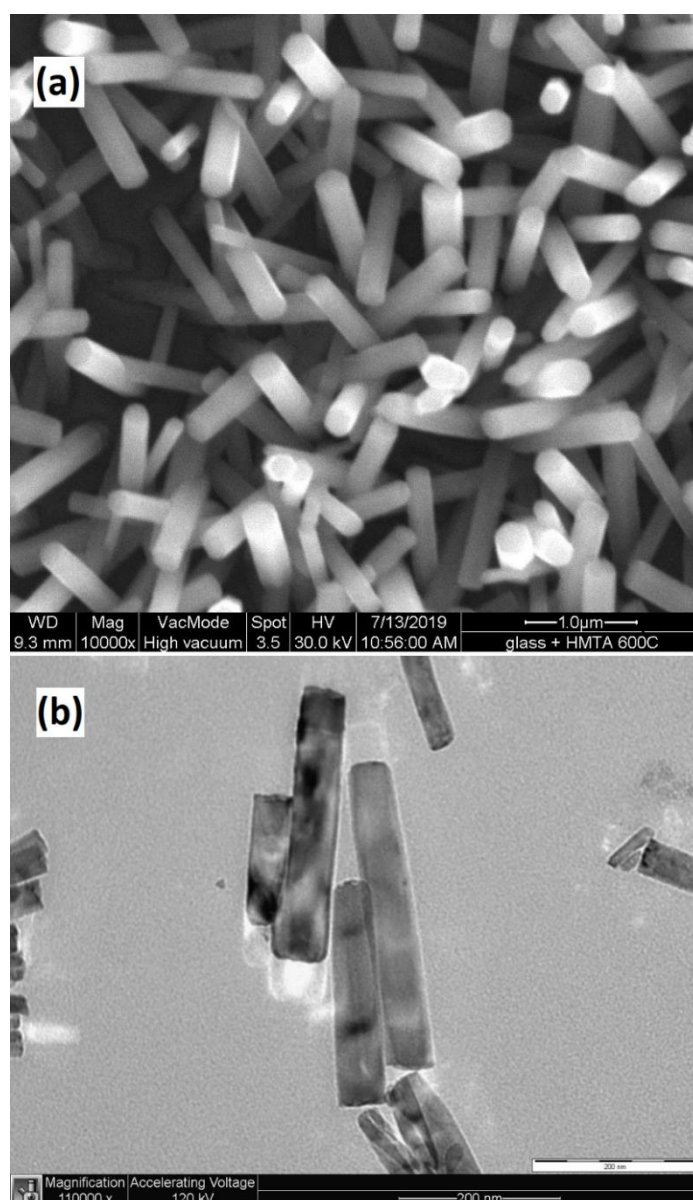


Figure 2. (a) SEM and (b) TEM images of synthesized ZnO nanorods

EIS was applied to study the electron transfer on the Z-CPE electrode. The analysis was conducted in the presence of citric acid. Nyquist plots of the samples is indicated in Figure 3. The obvious difference is seen the Nyquist plots of bare CPE electrode and Z-CPE electrode which indicate that the charge transfer process had improved due to the presence of ZnO nanorods on the electrode surface. A small and big semicircle appear in high and low-frequency regions, respectively. The first semicircle is attributed to the bulk resistance (R_1) which is parallel to the constant phase element (C_1) which generally enumerates conductive pathways across the carbon electrode [20, 21]. The second semicircle is also attributed to the charge transfer resistance (R_2) that is parallel to the second constant phase element (C_2), being as the electrochemical characterization of the double-layer structure, formed at the interface of electrode and solution. R_s is the resistance of electrolyte [19]. The comparison of the second semicircle diameters in the Nyquist plots of the two electrodes indicates that the charge transfer resistance of the citric acid is noticeably reduced in the Z-CPE electrode. This means clearly that ZnO nanorods can act as the electro-catalyst material for electro-oxidation of citric acid when existing in the electrode surface.

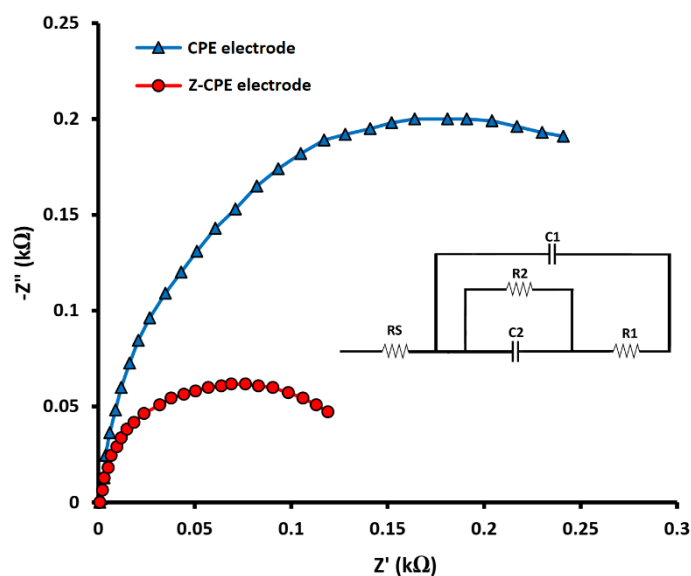


Figure 3. Nyquist plots of electrochemical impedance spectroscopy analysis obtained from (a) CPE and Z-CPE electrodes in 0.1 mM citric acid and 0.1 mM phosphate buffer solution at pH = 6.0. The inset shows equivalent electrical circuit.

Figure 4 shows the recorded cyclic voltammograms “a” and “b” as the responses of CPE and Z-CPE electrodes at 0.1M phosphate buffer solution and pH 6.0. It exhibits that no redox peaks are observed for both of electrodes due to the absence of citric acid in phosphate buffer solution [9]. The background current of Z-CPE electrode is more than CPE electrode because of high aspect ratio and high effective surface area of ZnO nanorods in the electrode surface. The recorded cyclic voltammograms “c” and “d” indicate the responses of CPE and Z-CPE electrodes in 0.5 mM citric acid within 0.1M phosphate buffer solution at pH 6.0. It can be seen that citric acid exhibits no oxidative peak at CPE electrode. But Z-CPE electrodes show the oxidative peak at potential of +1.0 V as the responses of 0.5 mM citric acid in 0.1 M phosphate buffer solution. It can be concluded that the ZnO nanorods are

responsible for the facilitated electro-oxidation of citric acid on the modified carbon paste electrode. It should be emphasized that, while citric acid is not electroactive in a CPE electrode, a well-defined electro-catalytic peak at +1.0 V can be obviously seen in Z-CPE electrode. This electrochemical behavior makes ZnO nanostructures an important electron mediator for electro-catalysis of citric acid. The results obtained herein are in good accordance with the EIS results in Fig. 3.

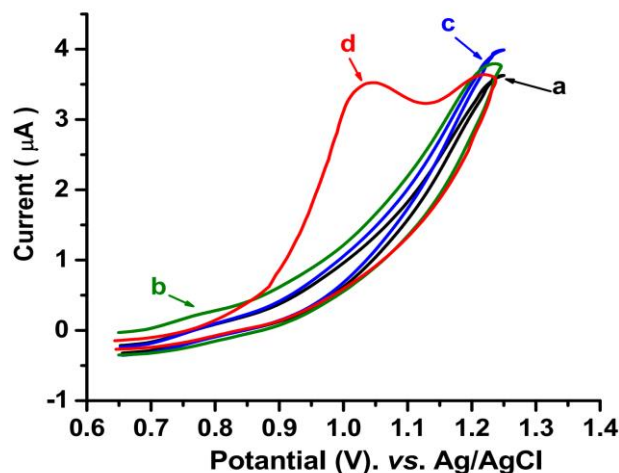


Figure 4. Recorded cyclic voltammograms (a) CPE electrode and (b) Z-CPE electrode in 0.1 M phosphate buffer solution at pH 6.0 and 20 mV s^{-1} . Recorded cyclic voltammograms (c) CPE electrode and (d) Z-CPE electrode in 0.5 mM citric acid in 0.1 M phosphate buffer solution at pH 6.0 and 20 mV s^{-1} .

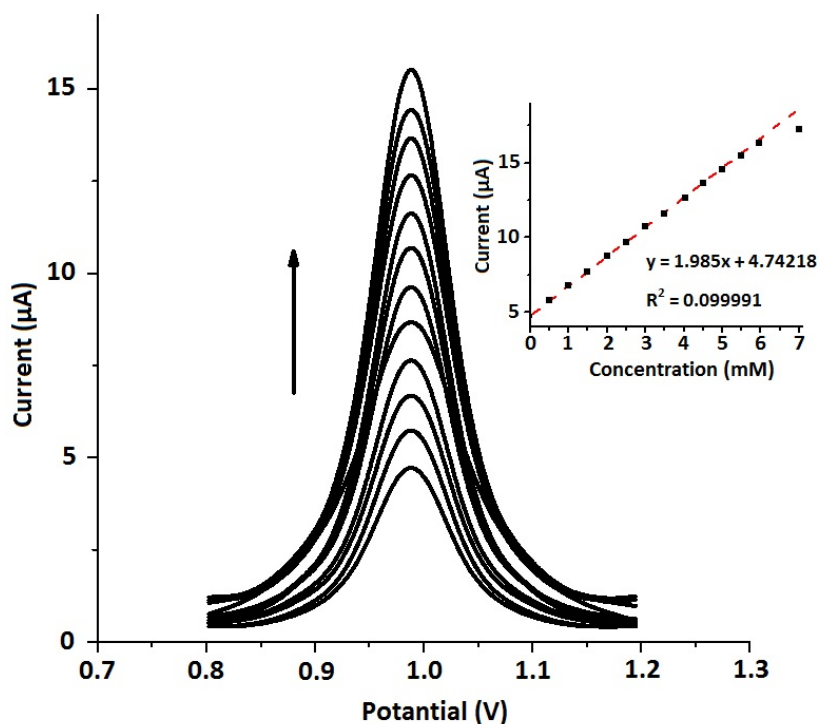


Figure 5. Recorded cyclic voltammograms of Z-CPE electrode as a response to successive injection of 0.5 mM citric acid in 0.1 M phosphate buffer solution at pH 6.0 and 20 mV s^{-1} . The inset shows plot of catalytic peak currents vs. citric acid concentration.

The differential pulse voltammograms as the response to successive injection of 0.5 mM citric acid in 0.1 M phosphate buffer solution at pH 6.0 were done to study the concentration effect of citric acid for Z-CPE electrode. As shown in Fig. 5 the current of electro-catalytic peak at +1.0 V has increased with the increase in the citric acid concentration. Thus, there is a linear relationship between peak current to citric acid concentration between 0.5 mM to 6 mM ($R^2 = 0.9988$), indicating the linear response of to study the concentration effect of citric acid for Z-CPE electrode to determination of citric acid (Inset Fig. 5). When the citric acid concentration is more than 6 mM, the linear relationship appears due to the saturation of the active site of ZnO nanorods in electrode surface. Therefore, linear response range of Z-CPE electrode for determination of citric acid is from 0.5 mM to 6 mM.

In order to determine the detection limit and sensitivity of citric acid sensor, differential pulse voltammetric studies of Z-CPE electrode was carried out in 0.1 M phosphate buffer solution at pH 6.0 and injection low concentration of citric acid. Fig. 6 demonstrates the square wave voltammetric response to successive injection of 0.1 μM citric acid. The inset figure 6 exhibits the plot of catalytic peak currents vs. citric acid concentration that showed the linear relationship and linear response of sensor to detection of citric acid. The sensitivity and detection limit of sensor were estimated 0.998 $\mu\text{A}/\mu\text{M}$ and 3 nM, respectively. Table 1 shows the comparison of different methods to determine the citric acid as mentioned in the literature [1, 5, 22-24].

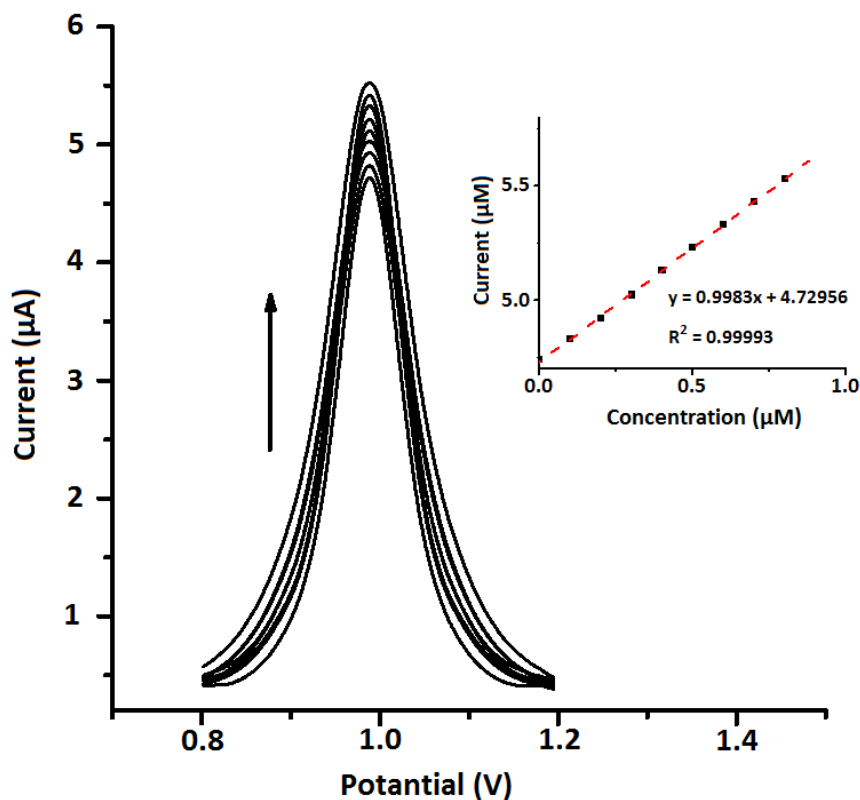


Figure 6. Differential pulse voltammetric response of Z-CPE electrode to successive injection of 0.1 μM citric acid in 0.1 M phosphate buffer solution at pH 6.0 and scan rate is 20 mV s^{-1} . The inset shows plot of catalytic peak currents vs. citric acid concentration.

Table 1. Comparison of methods for detection of citric acid.

Ref.	Method	Detection limit	Linear range
[1]	Chemiluminescence method	3.0×10^{-8} M	3.0×10^{-8} to 6.0×10^{-6} M
[22]	Electrochemical impedance spectroscopy	0.01 mM	0.05 to 1.0 mM
[5]	Chronoamperometry	40 μ M	50 μ M to 2.5 mM
[23]	Pyrolysis mass spectrometry	1 ng/ ml	10 ng/ml to 100 mg/ml
[24]	Cyclic voltammetry	1.17×10^{-4} M	1.0 to 9.0 mM
This work	Electrochemical analysis	3 nM	0.5 mM to 6 mM

The results indicate that the detection limit, linear range, and sensitivity of Z-CPE electrode for detection of citric acid are comparable and better than the other methods obtained from the literature. It can be attributed to the high aspect ratio and high effective surface area of ZnO nanorods in the electrode surface.

In the test of real samples, the differential pulse voltammetric response of Z-CPE electrode was recorded in 0.1 M phosphate buffer solution at pH 6.0 for 5.0 mL⁻¹ commercial lemon juices. Fig. 5 shows two typical series of the flow injection response of the 0.5 mM citric acid. As shown, the sensor indicates an increase of catalytic current in the existence of citric acid.

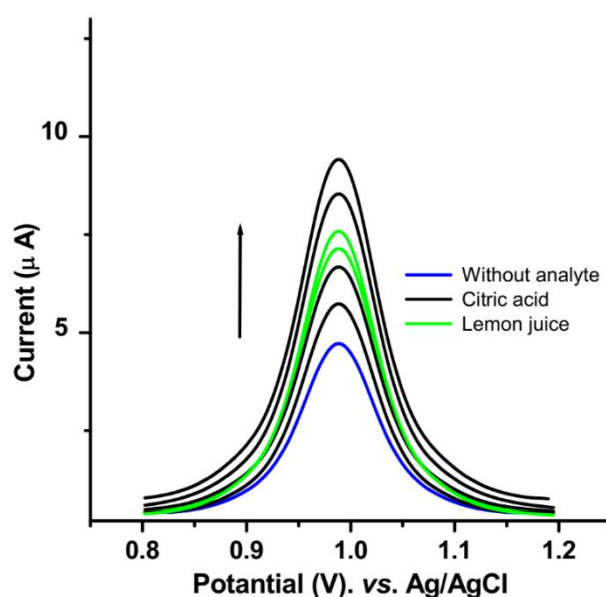


Figure 7. Differential pulse voltammetric response of Z-CPE electrode to 0.5 mM injection of citric acid and 5 mL⁻¹ lemon juice solutions, in 0.1 M phosphate buffer solution at pH 6.0 and scan rate is 20 mV s⁻¹.

Then, two successive injections of lemon juice were performed in the solution. It was found that there was still an increase in the catalytic current but this increase was lower than the catalytic current of citric acid. Finally, to investigate the stable behavior of the sensor, two consecutive injections of citric acid were done. Comparison between the catalytic current showed that Lemon juice is a rich source of citric acid, containing 1.40 g/oz which is in agreement with the previous researches [25].

4. CONCLUSION

An electrochemical study on the determination of citric acid as an important indicator in detecting freshness of fruit juices was performed using ZnO nanorods based carbon paste electrode. The carbon paste electrode was prepared and modified by the synthesized ZnO nanorods. Cyclic voltammetry, differential pulse voltammetry, and EIS techniques were applied for electrochemical analysis. XRD, TEM and FESEM analyses of prepared ZnO nanorods showed that the synthesized ZnO nanorods were grown in high density of hexagonal phase. EIS analysis showed that charge transfer behavior facilitated due to the presence of ZnO nanorods in the electrode surface. The cyclic voltammetry study showed oxidative peak as a response to the presence of citric acid was stable for Z-CPE electrode due to the high aspect ratio and high effective surface area of ZnO nanorods on the surface of the electrode. The differential pulse voltammetry technique was conducted to study the detection limit, sensitivity and freshness of citric acid sensor. The detection limit and sensitivity of citric acid sensor were estimated 0.998 $\mu\text{A}/\mu\text{M}$ and 3 nM, respectively. The results show that ZnO nanostructures can be of great help to enhance electrochemical sensors for detection of citric acid as an important element in juices, especially citrus fruits.

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References

1. H. Zhike, G. Hua, Y. Liangjie, L. Shaofang, M. Hui, L. Xiaoyan and Z. Yun'e, *Talanta*, 47 (1998) 301.
2. M. Husairi, J. Rouhi, K. Alvin, Z. Atikah, M. Rusop and S. Abdullah, *Semiconductor Science and Technology*, 29 (2014) 075015.
3. H. Chen, S. Zhang, Z. Zhao, M. Liu and Q. Zhang, *PROGRESS IN CHEMISTRY*, 31 (2019) 571.
4. F. Khosravi, *International Journal of Life Sciences*, 9 (2015) 50.
5. P.G. ErGoNuL and C. Nergiz, *Czech. J. Food. Sci.*, 28 (2010) 202.
6. S. Tang, N. Li, D. Yuan, J. Tang, X. Li, C. Zhang and Y. Rao, *Chemosphere*, 234 (2019) 658.
7. J. Rouhi, C.R. Ooi, S. Mahmud and M.R. Mahmood, *Materials Letters*, 147 (2015) 34.
8. E. Jamin, F. Martin, R. Santamaria-Fernandez and M. Lees, *Journal of Agricultural and Food Chemistry*, 53 (2005) 5130.
9. J. Liu, R. Jin, Y.W. YinaQiao, X. Wang and Y. Wang, *International Journal of Electrochemical Science*, 13 (2018) 10415.
10. D. Yuan, C. Zhang, S. Tang, X. Li, J. Tang, Y. Rao, Z. Wang and Q. Zhang, *Water research*, 163

- (2019) 114861.
11. A. Burgos, R. Schrebler, G. Caceres, E. Dalchiele and H. Gomez, *International Journal of Electrochemical Science*, 13 (2018) 6577.
 12. P. Shao, J. Tian, F. Yang, X. Duan, S. Gao, W. Shi, X. Luo, F. Cui, S. Luo and S. Wang, *Advanced Functional Materials*, 28 (2018) 1705295.
 13. J. Rouhi, C.R. Ooi, S. Mahmud and M.R. Mahmood, *Electronic Materials Letters*, 11 (2015) 957.
 14. H. Savaloni and R. Savari, *Materials Chemistry and Physics*, 214 (2018) 402.
 15. P. Shao, J. Tian, X. Duan, Y. Yang, W. Shi, X. Luo, F. Cui, S. Luo and S. Wang, *Chemical Engineering Journal*, 359 (2019) 79.
 16. S. Chaudhary, A. Umar, K. Bhasin and S. Baskoutas, *Materials*, 11 (2018) 287.
 17. F. Khurshid, M. Jeyavelan, M.S.L. Hudson and S. Nagarajan, *Royal Society Open Science*, 6 (2019) 181764.
 18. Y. Zhang, M.K. Ram, E.K. Stefanakos and D.Y. Goswami, *Journal of Nanomaterials*, 2012 (2012) 22.
 19. N. Naderi, M. Hashim, K. Saron and J. Rouhi, *Semiconductor Science and Technology*, 28 (2013) 025011.
 20. F. Husairi, J. Rouhi, K. Eswar, A. Zainurul, M. Rusop and S. Abdullah, *Applied Physics A*, 116 (2014) 2119.
 21. X. He, F. Deng, T. Shen, L. Yang, D. Chen, J. Luo, X. Luo, X. Min and F. Wang, *Journal of colloid and interface science*, 539 (2019) 223.
 22. T. Alizadeh, S. Nayeri and A. Habibi-Yangjeh, *Sensors and Actuators B: Chemical*, 279 (2019) 245.
 23. A. Ghassempour, N.M. Najafi and A.A. Amiri, *Journal of Analytical and Applied Pyrolysis*, 70 (2003) 251.
 24. S. Awasthi, A. Srivastava and M. Singla, *Synthetic Metals*, 161 (2011) 1707.
 25. K.L. Penniston, S.Y. Nakada, R.P. Holmes and D.G. Assimos, *Journal of Endourology*, 22 (2008) 567.