Application of Hierarchical CuO Bowl-like Array Film to Amperometric Detection of L-Ascorbic Acid

Min ZHAO, Jianwei ZHAO, Lirong QIN,† Zhengyan JIANG, and Hongliang JIA

School of Physical Science and Technology, Southwest University, Chongqing 400715, China

A hierarchical CuO array film was fabricated by electrochemical deposition with the aid of a colloidal monolayer as a template, followed by oxidation at high temperature. The prepared CuO film with a granular structure was arranged with ordered, hexagonal close-packed, and bowl-like pores. Combined with a gold layer sputtered on a glass substrate, this CuO array film was used as an electrochemical electrode in the amperometric detection of L-ascorbic acid. The array film exhibited a high sensitivity of 3464 \( \mu \text{A\,mM}^{-1}\text{cm}^{-2} \), a wide linear range from 1 \( \mu \text{M} \) to 7 mM, and a detection limit of 0.2 \( \mu \text{M} \). Excellent stability was also achieved. The results demonstrate that the hierarchical CuO bowl-like array film is a promising new platform for the construction of non-enzymatic ascorbic acid sensors.

Keywords Array film, hierarchical structure, non-enzymatic, sensor, ascorbic acid

(Received February 5, 2018; Accepted June 13, 2018; Published November 10, 2018)

Introduction

L-Ascorbic acid (AA), or vitamin C, is one of the important water-soluble compounds existing in various vegetables, fruits, and soft drinks,1,2 but it cannot be artificially synthesized. AA plays a key role in maintaining good health, such as in cancer prevention, immunity boosting, and oxidation resistance.3,4 Scurvy is caused by vitamin C deficiency. The concentration of AA in the central nervous system is on the millimolar level, which is much less than that in other humoral fluids. Thus, it is highly necessary to develop a rapid and simple method to determine AA with high sensitivity and low detection level. In recent years, many analytical methods, such as electrochemical methods, indirect spectrophotometric and solid-phase iodine methods, and liquid chromatography have been developed for determination of the AA content in foods, drugs, and plants.5–9 Electrochemical methods are particularly promising because of their simple operation, fast response, high sensitivity, and low cost. In contrast, enzymatic sensors show poor reproducibility and are unstable due to their intrinsic nature,10,11 and the preparation and cost of enzymatic sensors are respectively difficult and expensive. Therefore, the preparation of non-enzymatic AA sensors with good stability, reproducibility, and low cost is desired. Non-enzyme sensors based on metal or oxide nanomaterials, in which the nanomaterials are bound to enzymatic adhesives, as modified electrodes, have found widespread use. In this case, the interface resistances are increased, and the nanomaterials on the electrode are easily detached after the experiment. Therefore, it is a challenge to prepare new electrodes with good conductivity, hydrophilicity, high surface areas, and low interface resistance. In view of this, methods of directly depositing metals onto the surface of the electrodes to further obtain a nanofilm structure have been studied. This type of electrode is termed a free-standing electrode. In this way, it is easy to obtain array structures with large surface areas and avoid the defects associated with modified electrodes.

Cupric oxide (CuO), as an important p-type semiconductor, shows good electrochemical activity and has been applied in many fields, such as conductors, batteries, sensors, catalysis, and electrochemical materials.12–16 Compared with Cu, CuO exhibits better chemical stability and is easier to recover. CuO nanomaterials are desirable electrode candidate materials for electrochemical sensors. In recent years, research on the fabrication and properties of CuO nanomaterials have increased steadily. CuO nanostructures with various morphologies have been employed for the construction of electrochemical sensors, including nanowires,17,18 nanosheets,19 nanoflowers,20,21 nanoparticles,22,23 and nanobelts.24 The shape and size of the nanomaterials also have great influence on their properties. There are many reports of CuO-based sensors for glucose and hydrogen peroxide detection, where the large surface area, high stability, and remarkable sensitivity of these systems are assets,25,26 but there are few such systems for ascorbic acid assay. Electrochemical deposition is one of the most promising approaches for the fabrication of CuO nanostructures with low cost, simple operation, and high sensitivity. Among these techniques, the template method with simple operation and high repeatability has received widespread attention.

In the last few years, due to the unique characteristics and superior performances of porous films with an ordered arrangement of pores and a large active area, these materials have received considerable attention.27,28 In this paper, a hierarchical bowl-like CuO array film is successfully fabricated on a substrate and is used as an electrochemical sensor to detect ascorbic acid. Different from the traditional modified electrodes, our product can be seen as a “two-dimensional” array film deposited directly on the electrode without using any conductive...
Experimental

Chemicals and reagents

For the present experiment, the monodispersed polystyrene (PS) spheres (diameter: 0.5 μm) were purchased from Alfa Aesar Corp. Dopamine, ascorbic acid, and uric acid were obtained from Sigma-Aldrich. All other chemicals were of analytical grade and were purchased from Chongqing Chuandong Chemical Co., Ltd. NaOH solution (0.5 M) was the supporting electrolyte and deionized water was used throughout. The products were investigated by scanning electron microscopy (SEM, JEOL JSM-7100F) equipped with an energy dispersive X-ray spectroscopy (EDS), and X-ray photoelectron spectroscopy (XPS, Thermo Scientific ESCALAB 250). Electrochemical measurements were performed on a CHI 660E electro-chemical workstation (CH Instruments, China) with a conventional three-electrode system. A CuO array, an Ag/AgCl electrode, and a platinum electrode were employed as working, reference and counter electrodes, respectively. All experiments were carried out at ambient temperature (20 ± 1°C).

Preparation of CuO array film

As a useful template, the colloidal monolayer has been proved to be effective for fabricating periodic pore arrays. In this study, the monodisperse polystyrene (PS) templates were homemade. Prior to fabrication, the monolayer PS sphere template was prepared on a clean substrate according to an ethanol-assisted self-assembly method at the air/water interface similar to Dai’s report. Preparation of the CuO array film is illustrated in Scheme 1. Specifically, the PS spheres were transferred to a gold-plated glass substrate (2.5 × 0.5 cm) and then dried at ambient temperature. Using a two-electrode system with a carbon counter electrode, electrodeposition of Cu array film was performed under a constant potential of –1.3 V for 15 min. The electrolyte comprised 0.2 M CuSO₄·5H₂O and 0.1 M H₃BO₃, with a pH of 3.5. After electrodeposition, the semi-finished product was soaked in dichloromethane for 5 min to dissolve the PS spheres to obtain an ordered Cu array film. Finally, the Cu array film was oxidized in a tubular furnace at 450°C for 1 h to obtain the CuO array film. An effective area of
the CuO array film, ∼0.25 cm², was retained by coating with a quantity of epoxy resin. Comparatively, the developed product can overcome the defects of traditional modified electrodes and the active material deposited on the electrode is not easily detached.

Results and Discussion

Characterization of the array film

The PS spheres and the semi-finished product were analyzed by SEM and EDS. The typical SEM image of the top view of the monolayer (Fig. 1(a)) shows that the PS spheres were organized into a close-packed arrangement with long-range order. The distance between the center of two adjacent spheres was ∼500 nm. After dissolving the PS spheres, the Cu array film (as shown in Figs. 1(b) and 1(c)) is also arranged in an ordered, hexagonal closed-packed, bowl-like, and porous array with a very smooth surface, where the geometrical ordering of the PS template is retained. The data reveals that the PS sphere templates produce good repeatability and regularity. The element type and percentage were analyzed using EDS. The results (Fig. 1(d)) show that the semi-finished product is composed of Cu. The Au signal arises from the gold-plated glass substrate.

After annealing, the final product is still arranged in an ordered, hexagonal closed-packed, and porous array (Fig. 2(a)). However, in contrast with the semi-finished product, the enlarged SEM image (Fig. 2(b)) shows that the final porous film is undulating with peaks and troughs on the surface, similar to a series of bowls. It also should be noted from Fig. 2(c) that the final array film consists of fine grains, leading to a very rough and loose surface topography, which increases the surface area greatly. The average atomic ratio of Cu:O (Fig. 2(d)). The average atomic ratio of Cu:O is ∼1:1, which is consistent with CuO. In order to further analyze the chemical composition, the CuO array film was characterized by XPS (inset of Fig. 2(d)). Two characteristic Cu 2p peaks are observed at ∼933.6 eV (2p₃/2) and 953.5 (2p₁/2), indicating the presence of Cu²⁺. This again demonstrates that the final product is composed of CuO.

Properties of the CuO array film

The prepared CuO array film electrode was directly used as a non-enzymatic sensor to detect AA without further transfer processing. Figure 3(a) displays the results of cyclic voltammograms (CVs) of the CuO array film in the presence and absence of AA in 0.5 M NaOH solution, acquired at a scan rate of 100 mV s⁻¹. In the absence of AA, a pair of redox peaks can be observed near −0.1 and −0.6 V (curve a), which might be attributed to the quasi-irreversible reaction of Cu(II)/Cu(I) redox couple.³¹ The involved chemical reactions are as follows:

\[
\begin{align*}
2\text{CuO} + \text{H}_2\text{O} + 2\text{e}^- & \rightarrow \text{Cu}_2\text{O} + 2\text{OH}^- \quad (1) \\
\text{Cu}_2\text{O} + 2\text{OH}^- & \rightarrow 2\text{Cu}^+ + \text{H}_2\text{O} + 2\text{e}^- \quad (2)
\end{align*}
\]

Comparatively, when AA was added into the NaOH solution (curve b), an increase in the intensity of the broad oxidation peak near 0.6 V was observed. The bare Au/glass electrode was also tested (Fig. S1 in Supporting Information) and there were no change of redox peaks with the addition of AA. By contrast, it could be concluded that the CuO array film is electrocatalytically active towards the oxidation of AA. Many researchers have studied the action of the electrode material during the oxidation of organic substrates and it seems that the formation of Cu(III) species at high pH may have an effect on the catalytic process. The exact mechanism for the oxidation of AA in alkaline medium on the modified electrode was not studied in detail, but it might be a reasonable explanation that the Cu(III) species act as an electron-transfer mediator.³²
The possible catalytic mechanism of the CuO array film electrode for AA oxidation can be explained by the following scheme:

\[
\begin{align*}
\text{CuO} + \text{OH}^- & \rightarrow \text{CuOOH} + e^- \quad (3) \\
2\text{CuOOH} + \text{C}_6\text{H}_8\text{O}_6 & \rightarrow 2\text{CuO} + \text{C}_6\text{H}_6\text{O}_6 + 2\text{H}_2\text{O} \quad (4)
\end{align*}
\]

It should be emphasized that the above reactions typically take place under two electrochemical conditions, which include the presence of sufficient OH\(^-\) ions and a proper applied potential.\(^{33,34}\) In this reaction, the transfer of electrons results in a significant increase of the peak current. Moreover, in a recent report, Barragan et al.\(^{35}\) presented a new explanation that Cu(III) species do not participate in the oxidation mechanism, and that the semiconductive properties of the material and hydroxyl ion adsorption play important roles.\(^{35}\) Further theoretical and experimental studies should be performed to give more insight into the electro-oxidation mechanism of AA by CuO materials; corresponding work is already underway. On the other hand, although the Cu compounds are not electro-conductive, the thinner array film here would shorten the electron transfer paths and contribute to the transport of carriers to the Au electrode. The potential effect of the scan rate was also studied in 0.5 M NaOH solution with 1 mM AA (Fig. 3(b)). The oxidation and reduction peak currents both increased linearly relative to the square root of the scan rate in the range of 30 - 250 mV s\(^{-1}\), indicating that a diffusion-controlled process occurs in the CuO array film electrode.

Amperometry was also used for studying the properties of the CuO array film electrode. Figures 4(a) and 4(b) illustrate the constant potential (0.6 V) amperometry responses of the modified electrode when AA was successively added into 0.5 mM NaOH solution, where the solution was stirred constantly by a magnetic stirrer. When the AA is injected, the increased current and the response time of less than 5 s show the sensitive response to the concentration change of AA. In contrast, the gold-plated glass substrate shows no activity for AA oxidation in our experiment. Some current fluctuation was evident with the increase of the AA concentration because of the effect of the stirring when the local concentration was increased, similar to the case in previous reports.\(^{36,37}\) The linear relationship between the current and the concentration (left), the amperometric response of the CuO electrode to the addition of AA, glucose, uric acid, dopamine, and cysteine in 0.5 M NaOH solution (right).

\[
I \ (\text{mA}) = 0.871C_{\text{AA}} \ (\text{mM}) + 0.254
\]

The correlation coefficient is 0.9991. The electrode had a wide linear response range of AA concentrations (1 mM to 7 mM). The sensitivity was determined to be up to 3484 \(\mu\text{A mM}^{-1}\text{cm}^{-2}\). It is also found from the inset in Fig. 4(a) that the small amount of AA added would cause an obvious
current response, corresponding to a detection limit of 0.2 μM at a signal-to-noise ratio of 3. Table 1 presents a comparison of the performance of the CuO array film electrode presented herein with that of other AA sensors prepared based on micro/nanomaterials. The comparison reveals that the present electrode material shows excellent performance in terms of the very high sensitivity, wide linear range, and low detection limit. These perfect characteristics of the developed electrode material may be related to the specific structure of the CuO array film produced herein. Firstly, due to the proper redox potentials of CuO, it is an ideal sensor material. Many sensitive sensors have been developed by using copper and its oxide materials as electrodes.38–40 Secondly, the specific surface area of the bowl-like array film is a key factor. Obviously, a larger contact area between the sensed species and sensing materials can be provided by the hierarchical bowl-like structure, thus leading to excellent sensing performance. Thirdly, the CuO array film was constructed directly on the Au/glass substrate and the resulting tight contact can enhance electron transfer between the Au electrode and the active sites of CuO. Thus, the simple fabrication of the bowl-like CuO array film and its good electrocatalytic ability make it an excellent material for AA detection in alkaline medium.

Aiming to study the applicability of the bowl-like CuO array film, the content of AA in vitamin C tablets was analyzed. The vitamin C tablets were purchased from a local drugstore. According to the label, each tablet contained 100 mg of AA. In the analysis, the standard addition approach was applied, by which a known amount of AA in water was added to the test solution. The recoveries for the determination of AA were in the range of 96.7 – 101.6%, as shown in Table 2. These results suggest the high potential practicability of the CuO array film for the determination of AA. The effect of interfering substances commonly coexisting with AA was also examined. The inset of Fig. 4(b) displays the amperometric response (at a potential of 0.6 V applied) of the CuO array film electrode to the addition of AA, glucose, uric acid, dopamine, and cysteine in 0.5 M NaOH solution. The normal physiological level of AA is generally much higher (100 – 1000 times) than that of dopamine.41,42 Thus, the concentration of interfering substances added was one-tenth of the ascorbic acid concentration. Compared with the strong current response achieved with AA, obvious fluctuation was not observed with the injection of interferents as shown in Table 3. Because the CuO array film sensor prepared herein was enzyme-less, its long-term stability was evaluated by measuring the sensitivity for 0.5 mM AA using the sensor stored under dry conditions. After 3 weeks of storage, the current response showed that the electrode maintained 97.5% of its initial response for AA. After 3 months, the current signals showed a less than 10% decrease relative to the initial response. And a year later, the current response still remained about 70% of its initial response for AA, revealing extremely good long-term stability of the developed CuO non-enzymatic sensor.

### Conclusions

In this work, a hierarchical CuO bowl-like array film was fabricated by electrochemical deposition and subsequent oxidation at high temperature using the colloidal monolayer as a template. The prepared CuO array film as an effective non-enzymatic sensor was directly used for the detection of ascorbic acid. The array film shows remarkable electrochemical properties with high sensitivity, a wide linear range, a low detection limit, and long-term stability. The hierarchical CuO array film introduced in this study is a potential candidate strategy for the development of novel AA detection methods.

### Acknowledgements

This work was supported by the Chongqing Research Program of Basic Research and Frontier Technology (Grant No. cstc2016jcyjA0125) and the Fundamental Research Funds for the Central Universities of China (Grant No. XDJK2016C063).

### Supporting Information

CV curves of the bare Au/glass electrode in the presence and absence of AA in NaOH solution are listed in Supporting Information. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

---

**Table 1** Comparison of different electrode materials for determination of ascorbic acid

| Type of electrode                  | Sensitivity/μA mM⁻¹ cm⁻² | Linear range/μM | Detection limit/μM | Reference |
|-----------------------------------|--------------------------|-----------------|---------------------|-----------|
| CuO NWs modified electrode        | 1660                     | 0.1 - 3100      | 0.095               | 38        |
| CuO hierarchical nanostructures   | 533                      | 100 - 7000      | 90                  | 39        |
| 3DGF/CuO nanoflowers              | 2060                     | 0.43 - 200      | 0.43                | 40        |
| Nafion/CuO nanowires/Au film      | 766.5                    | 0.25 - 2500     | 0.05                | 41        |
| Nanoporous Fe₂O₃/Au film          | 1281.9                   | 25 - 10000      | 1                   | 42        |
| Hierarchical CuO array film       | 3484                     | 1 - 7000        | 0.2                 | This work |

**Table 2** Determination of AA in vitamin C tablets (n = 3)

| Sample | Detected/μM | Added/μM | Found/μM | Recovery, % |
|--------|-------------|----------|----------|-------------|
| 1      | 192.6       | 200      | 379.6    | 96.7        |
| 2      | 388.6       | 200      | 597.8    | 101.6       |

**Table 3** Interference measurements of the hierarchical CuO array film electrode with the successive additions of AA and other interferents

| Addition | Concentration/μM | Current relative value, % |
|----------|------------------|---------------------------|
| AA       | 0.5              | 100                       |
| Glucose  | 0.5              | 7.4                       |
| Uric acid| 0.05             | 3.2                       |
| Dopamine | 0.05             | 1.7                       |
| Cysteine | 0.05             | 2.6                       |
References

1. P. M. Chavhan, V. Reddy, and C. Kim, *Int. J. Electrochem. Sci.*, 2012, 7, 5420.

2. M. J. Villanueva, M. D. Tenorio, M. Sagardoy, A. Redondo, and M. D. Saco, *Food Chem.*, 2005, 81, 609.

3. P. Kalimuthu and S. A. John, *Bioelectrochemistry*, 2009, 77, 13.

4. Z. Yu, H. Li, J. Lu, X. Zhang, N. Liu, and X. Zhang, *Electrochim. Acta*, 2015, 158, 264.

5. A. G. Frenich, M. E. H. Torres, and A. B. Vega, *J. Agric. Food Chem.*, 2005, 53, 7371.

6. F. O. Silva, *Food Control*, 2005, 16, 55.

7. M. J. R. Lima, I. V. Toth, and A. O. S. S. Rangel, *Talanta*, 2005, 68, 207.

8. M. Noroozifar and M. Khorasani-Motlagh, *Talanta*, 2003, 61, 173.

9. N. B. Li, W. Ren, and H. Q. Luo, *J. Solid State Electrochem.*, 2008, 12, 693.

10. H. Wei and E. K. Wang, *Chem. Soc. Rev.*, 2013, 42, 6060.

11. Z. Yu, H. Li, J. Lu, X. Zhang, N. Liu, and X. Zhang, *Electrochim. Acta*, 2015, 158, 264.

12. D. Wen, S. J. Guo, S. J. Dong, and E. K. Wang, *Biosens. Bioelectron.*, 2010, 26, 1056.

13. B. Liao B, Q. Wei, K. Y. Wang, and Y. X. Liu, *Sens. Actuators, B*, 2001, 80, 208.

14. W. Zhang, S. Ding, Z. Yang, A. Liu, Y. Qian, S. Tang, and S. Yang, *J. Cryst. Growth*, 2006, 291, 479.

15. V. Chowdhuri, V. Gupta, K. Sreenivas, R. Kumar R. S. Mozumdar, and P. K. Patanjali, *Appl. Phys. Lett.*, 2004, 84, 11801182.

16. X. P. Gao, J. L. Bao, G. L. Pan, H. Y. Zhu, P. X. Huang, F. Wu, and D. Y. Song, *J. Phys. Chem. B*, 2004, 108, 5547.

17. Z. Zhuang, X. Su, H. Yuan, Q. Sun, and D. Xiao, *Analyst*, 2008, 133, 126.

18. G. Wang, Y. Wei, W. Zhang, X. Zhang, B. Fang, and L. Wang, *Microchim. Acta*, 2010, 168, 87.

19. Z. Yang, J. Xu, W. Zhang, A. Liu, and S. Tang, *J. Solid State Chem.*, 2007, 180, 1390.

20. M. J. Song, S. W. Hwang, and D. Whang, *Talanta*, 2010, 80, 1648.

21. A. X. Gu, G. F. Wang, X. J. Zhang, and B. Fang, *Bull. Mater. Sci.*, 2010, 33, 17.

22. L. C. Jiang and W. D. Zhang, *Biosens. Bioelectron.*, 2010, 25, 1402.

23. J. F. Ping, S. P. Ru, K. Fan, J. Wu, and Y. B. Ying, *Microchim. Acta*, 2010, 171, 117.

24. T. K. Huang, K. W. Lin, S. P. Tung, T. M. Cheng, I. C. Chang, Y. Z. Hsieh, C. Y. Lee, and H. T. Chiu, *J. Electroanal. Chem.*, 2009, 636, 123.

25. H. Luo, F. Su, C. Liu, J. Li, R. Liu, Y. Xiao, Y. Li, X. Liu, and Q. Cai, *Talanta*, 2011, 86, 157.

26. K. Zhang, N. Zhang, H. Cai, and C. Wang, *Microchim. Acta*, 2012, 176, 137.

27. Z. C. Ruan and M. Qiu, *Phys. Rev. Lett.*, 2006, 96, 233901.

28. C. T. Sousa, D. C. Leitão, M. P. Proenca, J. Ventura, A. M. Pereira, and J. P. Araujo, *Appl. Phys. Rev.*, 2014, 1, 031102.

29. Y. Yang, J. Zhao, L. Qin, Y. Yin, and L. He, *Mater. Lett.*, 2016, 179, 27.

30. Z. Dai, Y. Li, G. Duan, L. Jia, and W. Cai, *ACS Nano*, 2012, 6, 6706.

31. H. Razmi and H. Nasiri, *Electroanalysis*, 2011, 23, 1691.

32. L. Zhang, Y. H. Ni, and H. Li, *Microchim. Acta*, 2010, 171, 103.

33. M. Khairy and B. G. Mahmoud, *Electroanalytical Chemistry*, 2016, 28, 2606.

34. B. Wang, J. He, F. Liu, and L. Ding, *J. Alloys Compd.*, 2017, 693, 902.

35. J. T. C. Barragan, S. Kogikoski, E. T. S. G. da Silva, and L. T. Kubota, *Anal. Chem.*, 2018, 90, 3357.

36. P. V. Suneesh, V. S. Vargis, T. Ramachandran, B. G. Nair, and T. G. Satheesh Babu, *Sens. Actuators, B*, 2015, 215, 337.

37. Y. Sun, H. Yang, X. Yu, H. Meng, and X. Xu, *RSC Adv.*, 2015, 5, 70387.

38. C. Wang, J. Liu, X. Huang, H. Wang, Y. Zheng, L. Lin, S. Wang, S. Chen, and Y. Jin, *Appl. Surf. Sci.*, 2014, 292, 291.

39. B. G. Mahmoud, M. Khairy, F. A. Rashwan, C. W. Foster, and C. E. Banks, *RSC Adv.*, 2016, 6, 14474.

40. Y. Ma, M. Zhao, B. Cai, W. Wang, Z. Ye, and J. Huang, *Biosens. Bioelectron.*, 2014, 59, 384.

41. J. Zhao, Z. Yan, L. Qin, X. Feng, and P. Wang, *Chem. Lett.*, 2014, 43, 814.

42. Y. Yin, J. Zhao, L. Qin, Y. Yang, and L. He, *RSC Adv.*, 2016, 6, 63358.