Chapter 5

Electrochemical sensing of ascorbic acid using immobilized silver nanoparticles on self-assembled L-cysteine monolayer modified gold electrode

5.1. Introduction

Ascorbic acid (AA), the most ubiquitous vitamin present naturally in fruits and vegetables, is important in forming the protein collagen, and plays a paramount role as an antioxidant and preservative. Ascorbic acid has been widely used in food industry, pharmaceutical formulation and cosmetic applications. Therefore, an accurate, reliable and rapid method is needed for accurate determination of ascorbic acid. Different methods can be used for the quantitative estimation of AA such as volumetric titration,[1,2] spectrophotometry,[3,4] fluorimetry,[5,6] high performance liquid chromatography,[7,8] flow analysis,[9,10] terbidimetry,[11] etc. The detection of AA by voltammetric methods has received much attention in recent years.[12] Using a conventional electrode it is very difficult to determine AA accurately due to its high over-potential, poor reproducibility, low selectivity and sensitivity. Therefore, different approaches have been used to modify the electrode surface such as ion-exchange polymer,[13] inorganic and organic materials[14,15] and self-assembled monolayers terminated with different functional groups.[16]

Recently, modification of electrode surface with noble metal nanoparticles has received much attention mainly due to their interesting electrocatalytic and biosensing applications.[17-23] Detection of ascorbic acid using gold nanoparticles attached to glassy carbon (GC) electrode modified with 4-aminobenzoic acid followed by coupling with 4-aminothiophenol has been reported with the detection limit 2.8 μM AA.[24] The detection limit of 0.1 mM AA was reported at multilayers of AuNPs/redox polymers immobilized on GC electrode.[25] Electrocatalytic oxidation of AA by the immobilised citrate capped AuNPs on 1,6-hexanedithiol (HDT) modified Au electrode [26] has also been reported with a detection limit of AA of 1 μM.

Among all metal nanoparticles, silver nanoparticles (AgNPs) are of high interest because of their catalytic properties.[27] Traditionally, AgNPs have been employed as catalyst in different reactions. Also, Ag exhibits the highest electrical and thermal conductivity among all metals.[28-30] Herein, we present the electrocatalytic oxidation of AA in phosphate buffer solution by the positively charged silver nanoparticles (Ag@CTAB) immobilised on L-cysteine modified gold electrode (Au/L-cysteine/AgNPs) using cyclic voltammetry. The detection limit was calculated from

differential pulse voltammetric studies and to the best of our knowledge is lowest detection limit reported for ascorbic acid using metal nanoparticles. The Au/L-cysteine/AgNPs modified electrode is very stable and provides a very sensitive, selective, reliable, easy to perform and fast method for AA determination. The present modified electrode has been applied for the determination of AA present in fruit and vegetable juices.

5.2. Experimental

5.2.1. Synthesis of silver nanoparticles

CTAB capped Ag nanoparticles (Ag@CTAB) were prepared by adding 2 mL of ice cold solution of 0.1 M $NaBH_4$ to 1.25 ml of 10^{-2} M $AgNO_3$ prepared in 48.75 mL of 0.01 M CTAB solution under vigorous stirring for eight hours. The colorless solution changed slowly to yellow, indicating the formation of AgNPs.[32] The solution was stored at 4 ^{o}C in a dark bottle until further use.

5.2.2. Electrode modification

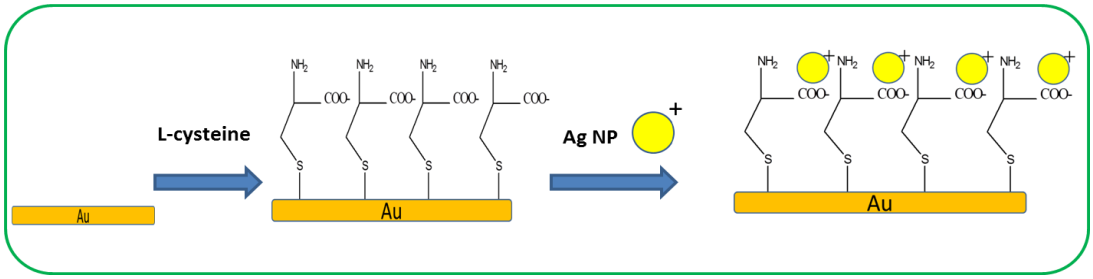

Fig. 5.1. Schematic representation of the electrode modification process.

The electrochemically cleaned electrode was immersed in 10 mM L-cysteine solution containing 0.1 M $HClO_4$ for 24 h to allow the chemisorption of the reagent on to the gold. The modified gold was then rinsed thoroughly with ethanol and water. Immobilization of Ag nanoparticles on Au/L-cysteine modified SAM electrode was done by dipping the Au/L-cysteine electrode into an Ag colloidal solution for 4 h (Fig. 5.1). The resultant electrode was washed with doubly distilled water and used for electrochemical measurements of AA.

5.3. Results and discussion

5.3.1. Characterization of the silver nanoparticles

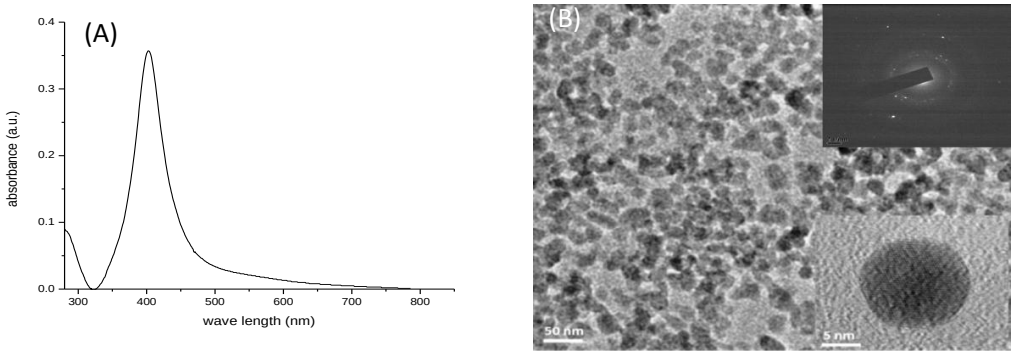

Fig. 5.2. (A) UV-VIS spectra and (B) TEM [HRTEM and SAED (inset)] images of CTAB stabilized silver nanoparticles.

Characteristic UV-VIS absorption of the CTAB capped Ag nanoparticles, with the sharp peak appearing at 410 nm was observed (Fig. 5.2A). From TEM analysis, it was found that the nanoparticle was of around 20 nm (Fig. 5.2B).

5.3.2. Electrochemical verification of the modified electrode

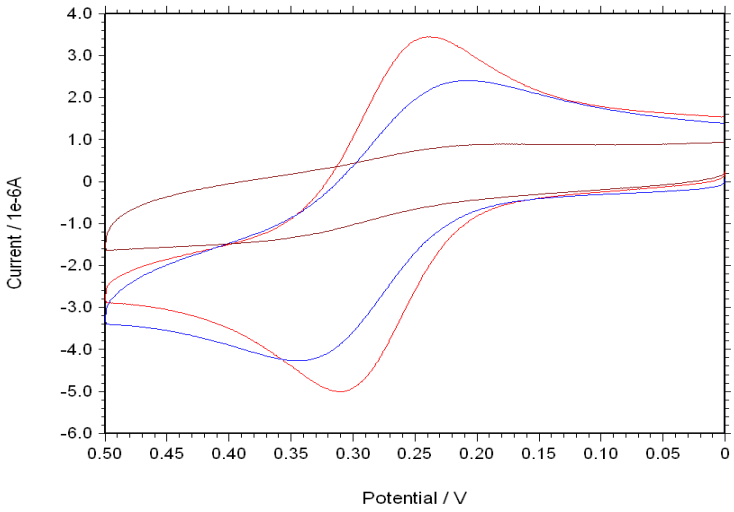

Fig. 5.3. Cyclic voltammograms of 0.5 mM $K_4[Fe(CN)_6]$ at pH 7 using different working electrodes. [(Blue) bare Au electrode, (brown) modified Au/L-cysteine electrode, (red) modified Au/L-cysteine/AgNPs electrode]

Fig. 5.3 shows the cyclic voltamograms of 0.5 mM $[Fe(CN)_6]^{3-/4-}$ obtained for bare Au, Au/L-cysteine and Au/L-cysteine/AgNPs electrodes in PBS at pH7. The bare Au electrode exhibits a quasi-reversible voltammetric response for $[Fe(CN)_6]^{3-/4-}$ redox couple with a peak separation of 116 mV at a scan rate of 100 mV/s. The cathodic peak current was significantly decreased (2.4×10^{-6} A to 8.6×10^{-7} A) in the case of Au/L-cysteine SAM modified electrode as compared to bare Au electrode. This suggests that the monolayer of L-cysteine was densely packed on Au electrode surface and thus effectively blocked the electronic communication between $[Fe(CN)_6]^{3-/4-}$ in solution and the underlying gold electrode surface. After the immobilization of AgNPs on Au/L-cysteine electrode the cathodic current peak increased from 8.6×10^{-7} A to 3.5×10^{-6} A with a peak separation of 67 mV, indicating that the Ag nanoparticles were successfully immobilized on the L-cysteine modified gold electrode and a good electronic communication was achieved between the redox species $[Fe(CN)_6]^{3-/4-}$ in solution and the underlying Au electrode through AgNPs.

5.3.3. Effect of pH on modified electrode

The influence of *p*H of electrolyte solution on the electrochemistry of immobilized AgNPs over Au-L-cysteine SAM was studied. The cathodic peak current reached the maximum value at pH 7.0. Beyond this *p*H, the cathodic peak current decreased. It is known that the isoelectric point of native cysteine is 5.06 and with a negative charge on cysteine in the pH domain of 6.0−7.5. In other words, the L-cysteine coated gold electrode carries negative charge in this pH range. At pH 7.0, the interaction between positively charged Ag nanoparticles and the negatively charged L-cysteine reached a maximum as is observed from cathodic peak current. At low pH, a poor response was obtained, as shown by low currents. This is due to poor interaction between the carboxyl group with positively charged Ag nanoparticles.

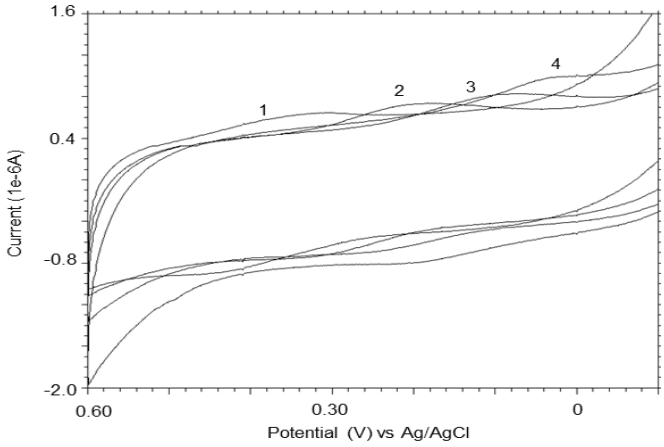

Fig. 5.4. Cyclic voltammogram of AgNPs, immobilized on Ag/L-cysteine electrode,in 0.1 M PBS solution [(1) pH 2.0, (2) pH 4.0, (3) pH 6.0, (4) pH 7.0].

A representative cyclic voltamogram at varying pH (2.0 to 7.0) is given in Fig. 5.4. At the higher pH range (7.5−10.0), the free amino group may interact with the gold surface and weaken the interaction between amine group and AgNPs, showing low current height. At high pH (> 10.0) the signal was unstable and disappeared.

5.3.4. Electrocatalytic oxidation of ascorbic acid

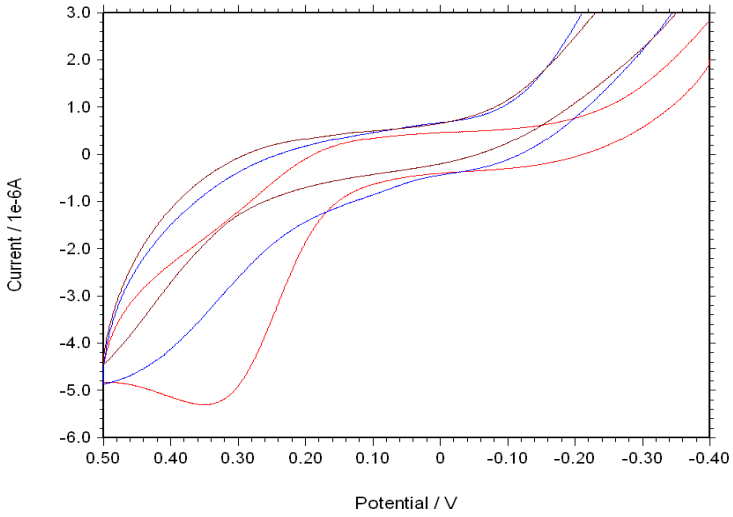

Fig. 5.5. Cyclic voltammogram of 0.5 mM ascorbic acid in 0.1 M PBS solution at pH 7 using different working electrodes. [(Brown) bare Au electrode, (blue) modified Au/L-cysteine electrode, (red) modified Au/L-cysteine/AgNPs electrode].

The cyclic voltammograms obtained in the presence of 0.5 mM ascorbic acid, 0.1 *M* PBS and at pH 7, on bare Au, Au/L-cysteine SAM electrode, and, Au/L cysteine/AgNPs modified electrode are represented in Fig. 5.5. An irreversible oxidation of AA occurred at 0.34 V at Au/L-cysteine/AgNPs modified electrode, which was 100 mV less positive than the oxidation of AA (0.44 V) at Au/L-cysteine electrode; the oxidation current of AA increased from 4.6 μA to 5.4 μA. This behavior demonstrates electrocatalytic activity for Au/L-cysteine/AgNPs electrodes towards AA oxidation. No reduction peak appeared for ascorbic acid on bare or modified electrodes. This confirms the data reported in literature that electrochemical oxidation of ascorbic acid is an irreversible process.[35]

Electrochemical impedance spectroscopy (EIS) was carried out in 0.5 M ascorbic acid at pH 7.0 (PBS buffer) using modified working electrodes (bare Au electrode, Au/L-cysteine SAM electrode and Au/L-cysteine/AgNPs modified electrode) where the frequency range was 0.01 Hz to 100000 Hz and E_{ac} = 10 mV. The diameter of the semicircle observed in the Nyquist plot corresponds to the charge transfer resistance, R_{ct}; the smaller the semi-circle, faster is the charge or electron transfer.[33]

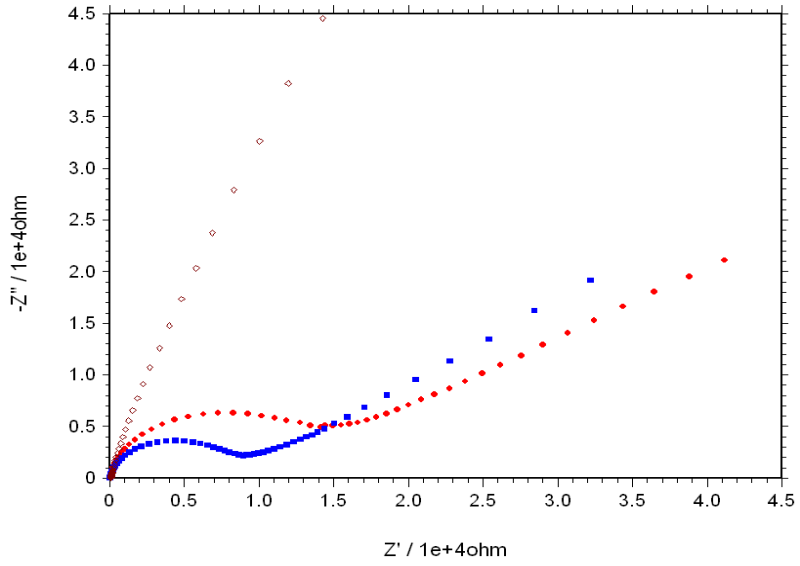

Fig. 5.6. Nyquist plot ($-Z^{//}$ versus $Z^{/}$) of 0.5 mM ascorbic acid in 0.1 M PBS solution at pH 7 using various working electrodes obtained from impedance measurements. [(Brown) bare Au electrode, (red) modified Au/L-cysteine electrode, (blue) modified Au/L-cysteine/AgNPs electrode].

Fig. 5.6 shows that the semi-circle decreases upon immobilization of AgNPs on Au/L-cysteine SAM electrode surface. The decrease in semi-circle (R_{ct}) shows the following trend: bare Au (2.518×10^{6} Ω) > Au/L-cysteine (13.776×10^{3} Ω) > Au/L-cysteine/AgNPs (7.574×10^{3} Ω). The observed trend is due to the fact that the modified electrodes facilitate electron transfer rate for the oxidation of ascorbic acid to dehydroascorbic acid. Impedance measurements clearly show that the Au/L-cysteine/AgNPs exhibits lower resistance as compared to the bare Au and Au-L-cysteine SA modified electrodes. This study shows that the Au/L-cysteine/AgNPs modified electrode is an efficient electrocatalyst for AA oxidation.

5.3.5. Determination of ascorbic acid

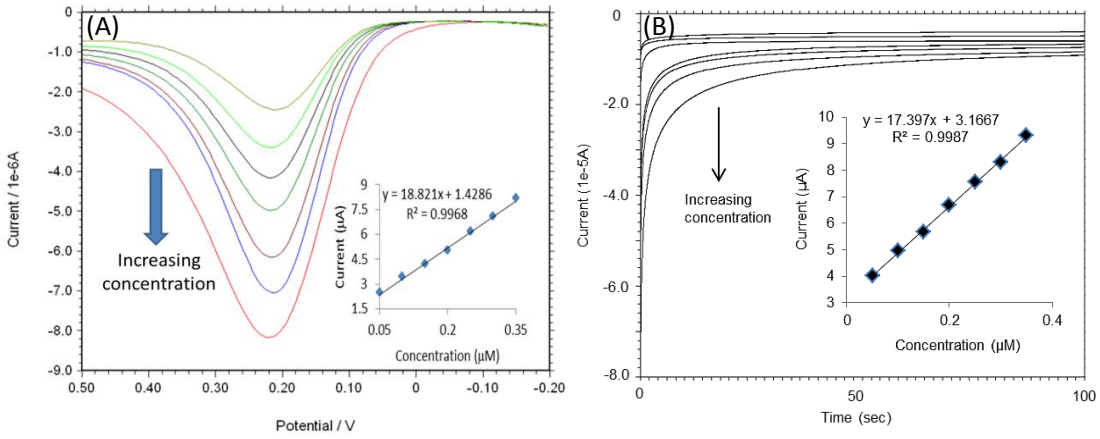

Fig. 5.7. (A) Overlaid DPV and (B) CA (at the potential level, + 0.34 V *vs* Ag/AgCl, corresponding to AA anodic oxidation) with increasing ascorbic acid concentration (0.05—0.35 µM) in 0.1 M PBS (pH 7) at Au/L-cysteine/AgNPs modified electrode. [Inset: Plot of current as a function of concentration of ascorbic acid with linear trend line (R^2 > 0.99)]. LOD = 3σ/m = (3 × 1.174 × 10^{-11}) / 17.397 = 2.0 × 10^{-12} M [where σ is the standard deviation of the peak current in blank solution (n= 25), and m is the slope of the linear calibration graph]

The dependence of voltammetric response on the ascorbic acid concentrations at Au/L-cysteine/AgNPs modified electrode was studied using DPV. Fig. 5.7A shows the DPV responses of the modified electrode towards AA at varying concentrations. The

oxidation current of AA increases linearly in the range of 0.05−0.35 μ*M*, (linear regression equations: I_p (μA) = 18.821 *C* (μ*M*) +1.4286 with a correlation coefficient of 0.996, as shown in Fig. 5.7A (inset)). Detection limit of 2×10^{-12} *M* for AA was obtained using 3σ/*m* (where σ is the standard deviation (1.259×10^{-11} A) of the peak current in blank solution, *n* = 25 and *m* (18.826 μA/μ*M*) is the slope of the calibration curve). The detection limit was further confirmed by using chronoamperometry (Fig. 5.7B).

5.3.6. Interference and selectivity study

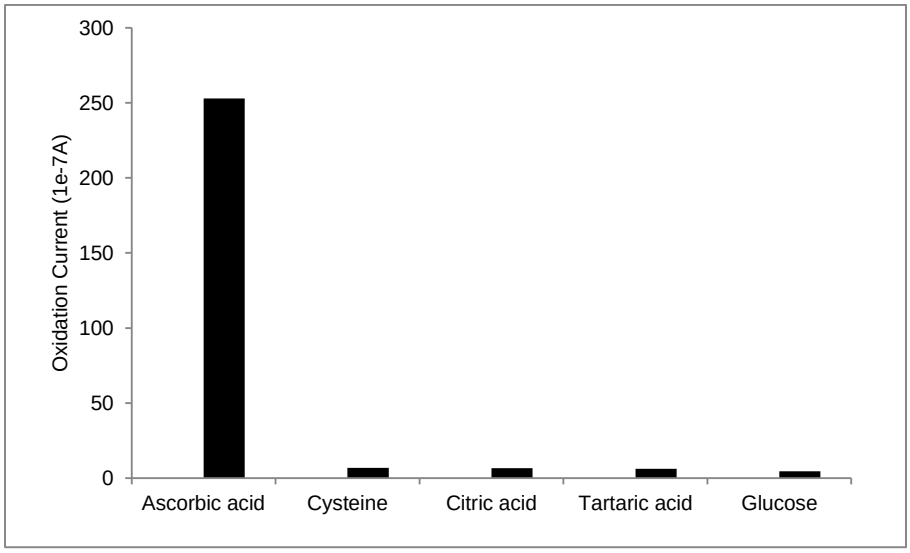

Fig. 5.8. Bar diagram of oxidation current for 0.5μM ascorbic acid and 0.5 mM cysteine, citric acid, tartaric acid and glucose.

The application of Au/L-cysteine/AgNPs modified electrode for the determination of AA at 0.5 μ*M* concentration was also checked in the presence of 0.5 mM concentration of glucose, tartaric acid, citric acid, and cysteine as interferents in PBS solution (pH 7). In the presence of interferents, the oxidation peak potential of AA was stable and the current response of AA was also not affected (Fig. 5.8). Thus, Au/L-cysteine/AgNPs modified electrode may be successfully used to determine the concentration of AA in the presence of physiologically common interferents.

5.3.7. Real sample analysis

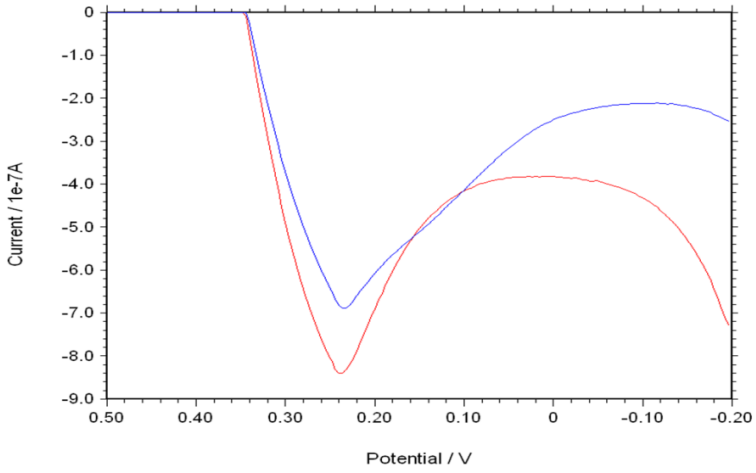

Fig. 5.9. Overlaid DPV of orange juice before (blue) and after addition of ascorbic acid (red) in 0.1 M PBS.

Fruit (orange, lemon, apple, and grape) and vegetable (tomato, cabbage, and cauliflower) juices were obtained by pressing. The stock solution (1 ml juice was diluted with 9 mL PBS buffer (pH=7) solution) was prepared and DPV was recorded in each

Table 5.1. Determination of ascorbic acid content in various fruit and vegetable juices using DPV.

Sample (juice)	Initial current(μA)	Current after addition of standard ascorbic acid solution (μA)	Ascorbic acid conc. (mg/100 mL juice)	Added ascorbic acid ($\times 10^{-3} M$)	Found($\times 10^{-3} M$)	Recovery (%)
Orange	-0.6891	-0.8395	39.91	1	1.0585	105.85
Lemon	-0.8171	-0.9855	47.52	1	0.9996	99.96
Apple	-0.6566	-0.8335	34.41	1	0.9995	99.95
Grape	-0.6523	-0.8480	51.05	1	0.9991	99.91
Tomato	-0.6603	-0.7552	29.18	1	1.0003	100.03
Cabbage	-0.4534	-0.5227	18.59	1	0.9996	99.96
Cauliflower	-0.6538	-0.7922	26.00	1	0.9992	99.92

case with the Au/L-cysteine/AgNPs modified electrode. The ascorbic acid content was calculated by measuring the peak currents obtained for sample solutions and after

addition of standard AA solution, using the equation $I = KC$, where I is the current obtained for sample solution and K is the increased amount of current after addition of standard AA solution and C is the unknown concentration. The obtained results are presented in Table 5.1 and agree with the data reported in literature.[34-36] The accuracy of the method was also verified by recovery studies adding standard ascorbic acid solutions to samples. Recoveries of 99.91−105.85 % were achieved. A representative DPV for AA in orange juice solution and after addition of standard AA solution is shown in Fig. 5.9. Also, recovery and reproducibility of these measurements were satisfactory.

5.4 Conclusions

The present study demonstrates an excellent approach for the development of a novel silver nanoparticles/L-cysteine modified gold electrode for the electrocatalytic oxidation of ascorbic acid. Fast electron transfer and high stability for the oxidation of ascorbic acid were achieved at the Au/L-cystine/AgNPs modified electrode. The electrocatalytic oxidation of AA showed the following order of the studied electrode: Au/L-cysteine/AgNPs > Au/L-cysteine > bare Au. EIS results are consistent with the CV responses. The DPV results indicate that the Au/L-cysteine/AgNPs modified electrode has a superior detection limit (2.0×10^{-12} M) than earlier reported gold nanoparticles modified electrode systems. This electrode selectively determines ascorbic acid in presence of physiologically common interferents and has been successfully used for analysis in fruit and vegetable juices with good recovery. The sensor displays good storage stability if kept in aqueous medium at room temperature. The Au/L-cysteine/AgNPs modified electrode retained its initial activity after one–two weeks of storage.

5.5. References

1. Lenghor N, Jakmunee J, Vilen M, Sara R, Christian G D & Grudpan K, *Talanta*, 2002, **58**, 1139.

2. H. Rajantie, J. Strutwolf and D. E. Williams, *J. Electroanal Chem.*, 2001, **500**, 108.

3. I. O. Serrano, T. H. Jover and O. M. Belloso, *Food Chem.*, 2007, **105**, 1151.

4. H. S. O. Chen, S. C. Ng and S. H. Seow, *Synth. Met.*, 1994, **66**, 177.

5. S. P. Arya, M. Mahajan and P. Jain, *Anal. Chim. Acta*, 2000, **417**, 1.

6. I. Tetsuharu, H. Shuuji, Y. Masatoshi, N. Masaru and O. Yosuke, *Chem. Pharm. Bull.*, 1985, **33**, 3499.

7. T. N. Shekhovtsova, S. V. Muginova, J. A. Luchinina and A. Z. Galimova, *Anal. Chim. Acta*, 2006, **573-574**, 125.

8. J. Lykkesfeldt, *Anal. Biochem.*, 2000, **282**, 89.

9. A. P. S. Paim, C. M. N. V. Almeida, B. F. Reis, R. A. S. Lapa, E. A. G. Zagotto and J. L. F. C. Lima, *J. Pharm. Biomed. Anal.*, 2002, **28**, 1221.

10. T. Prez-Ruiz, C. Martinez-Lozano, A. Sanz and A. Guillen, *J. Pharm. Biomed. Anal.*, 2004, **34**, 551.

11. M. Spickenreither, S. Braun, G. Bernhardt, S. Dove and A. Buschauer, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5313.

12. Jr D. W. Martin, in *Harper's Review of Biochemistry*, edited by Martin Jr D W, Mayes P A & Rodwell V W, 19th Ed. (Lange Los Altos, CA) 1983.

13. J. M. Zen, Y. J. Chen, C. T. Hsu and Y. S. Ting, *Electroanalysis*, 1997, **9**, 1009.

14. R. S. Freire and L. T. Kubota, *Analyst*, 2002, **127**, 1502.

15. C. R. Raj and T. Ohsaka, *J. Electroanal. Chem.*, 2003, **540**, 69.

16. C. R. Raj, K. Tokuda and T. Ohsaka, *Bioelectrochem.*, 2001, **53**, 183.

17. A. S. Rad, A. Mirabi, E. Binaian and H. Tayebi, *Int. J. Electrochem. Sci.*, 2011, **6**, 3671.

18. M. Chao and X. Ma, *Int. J. Electrochem. Sci.*, 2012, **7**, 6331.

19. L. Wang, H. Zhu, H. Hou, Z. Zhang, X. Xiao and Y. Song, *J. Solid State Electrochem.*, 2012, **16**, 1693.

20. R. W. Murray, *Chem. Rev.*, 2008, **108**, 2688.

21. E. J. Nam, E. J. Kim, A. W. Wark, S. Rho, H. Kim and H. J. Lee, *Analyst*, 2012, **137**, 2011.

22. M. Campas, D. Garibo and B. P. Simon, *Analyst*, 2012, **137**, 1055.

23. B. J. Sanghavi, S. M. Mobin, P. Mathur, G. K. Lahiri and A. K. Srivastava, *Biosens. Bioelectron.*, 2013, **39**, 124.

24. L. Zhang and X. Jiang, *J. Electroanal. Chem.*, 2005, **583**, 292.

25. L. Qian, Q. Gao, Y. Song, Z. Li and X. Yang, *Sens. Actuators B*, 2005, **107**, 303.

26. A. Sivanesan, P. Kannan and S. A. John, *Electrochim. Acta*, 2007, **52**, 8118.

27. Y. G. Sun and Y. N. Xia, *Science*, 2002, **298**, 2176.

28. X. E. Verykios, F. P. Stein and R. W. Coughlin, *Catal. Rev-Sci. Eng.*, 1980, **22**, 197.

29. T. Sun and K. Seff, *Chem. Rev.*, 1994, **94**, 857.

30. A. Russell & K. L. Lee, *Structure-Property Relations in Nonferrous Metals*, (John Wiley & Sons) 2005, p 322.

31. J. F. Smalley, K. Chalfant, S. W. Feldberg, T. M. Nahir and E. F. Bowden, *J. Phys. Chem. B*, 1999, **103**, 1676.

32. W. Wang & B. Gu, in *Concentrated Dispersions: Theory, Experiment, and Applications*, edited by P Somasundaran & B Markovic, *ACS symposium Series* 878, (Oxford University Press) 2004, Chap. 1, P 1.

33. P. N. Mashazi, P. Westbroek, K. I. Ozoemena and T. Nyokong, *Electrochim Acta*, 2007, **53**, 1858.

34. K. Matsumoto, K. Yamada, and Y. Osajima, *Anal. Chem.*, 1981, **53**, 1974.

35. A. M. Pisoschi, A. F. Danet and S. Kalinowski, *J Automat Meth Manag Chem.*, 2008 (2008) 937651 and references therein.

36. A. M. Pisoschi, A. Pop, G. P. Negulescu and A. Pisoschi, *Molecules*, 2011, **16**, 1349.