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Original Article

Green synthesis of silver nanoparticles using onion extract and their application for the preparation of a modified electrode for determination of ascorbic acid



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ARTICLE INFO

Article history:

Received 24 November 2015

Received in revised form

26 April 2016

Accepted 3 May 2016

Available online 21 June 2016

Keywords:

Allium cepa L.

ascorbic acid

biosynthesis

onions

silver nanoparticles

voltammetry

ABSTRACT

A high-quality method for one-pot biosynthesis of silver nanoparticles (AgNPs) using onion extracts as reductant and stabilizer is reported. The synthesized AgNPs were characterized by ultraviolet-visible spectroscopy (UV-Vis), X-ray powder diffraction (XRD), and transmission electron microscopy (TEM). UV-Vis spectroscopy results showed that the AgNP absorption band was located at a peak of 397 nm in aqueous solution. Both XRD and TEM results confirmed that the AgNPs were mainly spherical with average diameters of 6.0 nm by TEM and about 5.3–10.2 nm calculated using XRD data. The ability of AgNPs to reduce charge transfer resistance was also investigated using electrochemical impedance spectroscopy. Finally, the effect of synthesized NPs on ascorbic acid signal was investigated by square wave voltammetry. The peak current of square wave voltammograms of ascorbic acid increased linearly with its concentration in the range of 0.4–450.0 μM. The detection limit for ascorbic acid was 0.1 μM.

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1. Introduction

Nanotechnology deals with biotic and abiotic materials that vary in size from 1 nm to 100 nm [1–5]. Nanoparticles (NPs) have novel properties, which depend on specific characteristics such as size, morphology, and distribution [6–12]. Nowadays, inorganic NPs and their nanocomposites are extensively used in various industries, biomedicines, and

catalysis reactions [13–15]. In particular, silver NPs (AgNPs) have various important applications. Historically, silver has been known to have disinfecting effects and is used in a broad range of applications from traditional medicines to culinary items. It has been reported that AgNPs are nontoxic to humans and most effective against bacteria, viruses, and other eukaryotic microorganisms at low concentrations without any side effects [16].

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<http://dx.doi.org/10.1016/j.jfda.2016.05.004>

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AgNPs play a significant role in the field of diagnostic medicine [17], antimicrobial synthesis, and therapeutics [18,19]. Ag^+ contributes to the antimicrobial ability of AgNPs under various hypothesis including Ag^+ adherence, break-down, permeabilization of cell membrane, and interaction with proteins and DNA; however, reactive oxygen species are reported to interfere with its physiological functions [20,21].

Stable AgNPs can be synthesized by chemical methods such as chemical reduction, electrochemical techniques, and photochemical reduction [22–24]. Meanwhile, the use of biosynthetic methods has attracted much attention because of their safe conditions in synthesis procedures, good distribution of synthesized NPs, use of nontoxic solvents, etc. [25].

Many biotechnological applications such as remediation of toxic metals use microorganisms such as bacteria [26] and yeast [27] for the synthesis of NPs. Nair and Pradeep [28] have synthesized NPs of gold, silver, and their alloys by treating the corresponding metal ions within cells of lactic acid bacteria present in buttermilk.

In recent years, plant-mediated biological synthesis of NPs is gaining importance due to its simplicity and eco-friendliness [29]. Moreover, plant extracts could be advantageous over microorganism synthesis because there is no need to expand the process of culturing and maintaining cell lines, and they are of low cost, fast, efficient, and generally lead to the formation of crystalline NPs with a variety of shapes and sizes [30–36].

Allium cepa L. is one of the most widely cultivated and used plants, and its bulb (onion) is used as both food and medicine. Onions (*A. cepa* L.) possess strong, characteristic aromas and flavors, which have made them important ingredients of various food items. Onions and onion flavors (essential oil) are important seasonings widely used in food processing. It has been shown that onion possesses various biological properties, including antibiotic, antidiabetic, antioxidant, anti-atherogenic, and anticancer effects [37].

In this study, we wish to report a green synthesis of AgNPs by reduction of silver ions using aqueous and alcoholic onion extracts for facile and fast phytosynthesis of AgNPs. The ability of AgNPs to reduce charge transfer resistance and the effect of these extracts on ascorbic acid signal using electrochemical impedance spectroscopy (EIS) and voltammetric methods is also investigated. To the best of our knowledge, only one report describing the synthesis of AgNPs using water extracts of onion can be found in the literature [38] and no further reports pertaining to the use of methanolic onion extract for green synthesis of AgNPs and its effect on ascorbic acid signal by square wave voltammetry (SWV) have been published.

2. Methods

2.1. Materials and procedure for the preparation of onion extracts

Pure silver nitrate (AgNO_3), ascorbic acid, and $\text{K}_4[\text{Fe}(\text{CN})_6]$ were purchased from Merck (Tehran, Iran). NaOH was purchased from Sigma-Aldrich (Tehran, Iran). Onions (*A. cepa*) were

purchased from a local store in Babol, and these onions were reportedly collected from Mazandaran Province (Qaemshahr, Iran). Double-distilled water was used in all experiments. A certain weight of onions (*A. cepa*; 10 g) was washed 10 times with double-distilled water and boiled with 100 mL water for 45 minutes and then filtered through a Whatman Number 1 filter paper. The filtrate was used as a reducing and stabilizer agent for the synthesis of AgNPs.

2.2. Synthesis of AgNPs

A certain volume of the onion extract (10 mL) was added to a 10 mL silver nitrate solution ($1 \times 10^{-2}\text{M}$) and the volume was adjusted to 40 mL with deionized water. The flask was then incubated at room temperature. The measured pH was 5.42. Different conditions were used for the optimization of AgNPs synthesis in this work.

2.3. Ultraviolet-visible spectroscopy

The formation and stability of AgNPs were investigated by ultraviolet-visible (UV-Vis) spectrophotometry (Cary, UV-500, Japan). The absorption spectrum of reaction solutions as a function of reaction time, different biomaterials used, and AgNO_3 dosage were recorded at same time and at wavelengths ranging from 300 nm to 600 nm.

2.4. Microscopic investigations

Transmission electron microscopy (TEM) was applied for the morphological analysis of AgNPs; 3 μL of the sample was placed on a carbon-coated copper grid and allowed to dry at room temperature. The TEM images were obtained using a Philips cm10HT version of TEM, which was operated at 100 kV.

2.5. X-ray diffraction measurements

Crystalline metallic pattern of AgNPs powder was analyzed using X-ray diffraction (XRD). To obtain a pellet of pure NPs for XRD analysis, the reaction medium was centrifuged by five cycles at 18,000 rpm for 20 minutes followed by redispersion in deionized water. The XRD patterns were recorded on an X'Pert Pro MPD, which was operated at a voltage of 40 kV and current of 40 mA with Cu- K_α radiation. The scanning was done in the region of 2θ from 20° to 80° .

2.6. Electrochemical investigation

SWV was performed using a potentiostat/galvanostat connected to a three-electrode cell and Metrohm Model 663 VA stand linked with a computer (Pentium IV, 1200 MHz) running Autolab software. The system was run on a personal computer using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 100 kHz to 0.1 Hz was used. A conventional three-electrode cell assembly consisting of a platinum wire as an auxiliary electrode and an Ag/AgCl (KCl_{sat}) electrode as a reference electrode was used.

AgNPs carbon paste electrode (AgNPs/CPE) was prepared by mixing of 0.1 g of AgNPs and 0.90 g of graphite powder in

the presence of a suitable amount of liquid paraffin as a binder. The mixture was then mixed well for 70 minutes until a uniformly wetted paste was obtained. A portion of the paste was filled firmly into one glass tube as described earlier to prepare AgNPs/CPE and was used as a working electrode for electrochemical investigation.

2.7. Official method

Indophenol solution was standardized by titrating it with 2.0 mL of standard ascorbic acid solution and 5 mL of $\text{HPO}_3 + \text{HOAc}$ solution to the end point (a persistent rosy pink color) [39]. The consumption of the blank was determined by titrating the indophenol solution with 7 mL of $\text{HPO}_3 + \text{HOAc}$ solution plus a given amount of water equivalent to the volume indophenol solution used in the previous standardization titration.

For sample titration, a 100 mL portion of the juice was mixed with an equal volume of $\text{HPO}_3 + \text{HOAc}$ solution before filtering. A volume of the filtrate equivalent to approximately 250 mg of ascorbic acid was then titrated with indophenol solution using the same procedure as described earlier including the titration of the blank.

3. Results and discussion

3.1. Synthesis optimization

The color change from watery to brown due to the reduction of silver ion can be used as an indicator, which suggests the formation of AgNPs (Figure 1 inset). In addition, the UV-Vis spectra were used for further investigation of AgNPs synthesis in this work (Figure 1). As can be seen in Figure 1, the maximum absorbance band appears at approximately 397 nm, which is related to the synthesis of Ag^0 (AgNPs). To obtain the best conditions for the synthesis

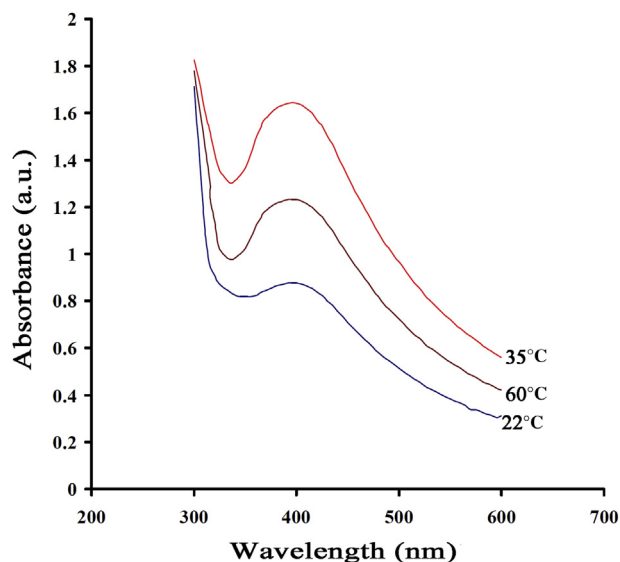


Figure 2 – Ultraviolet-visible spectrum of silver nanoparticles at temperatures of 22°C, 35°C, and 60°C, respectively.

of AgNPs, some factors were optimized in the synthetic procedure [40].

At first, the effect of temperature on the formation of AgNPs was investigated. The UV-Vis spectra of AgNPs at three different temperatures (22°C, 35°C, and 60°C) are shown in Figure 2. As can be seen, the formation rate of AgNPs was increased by increasing the temperature up to 35°C and then decreasing it.

To determine the effect of pH, we studied the UV-Vis spectra of AgNPs in the pH range of 4.0–11 at 35°C. The UV-Vis spectra of AgNPs in three different conditions including acidic, neutral, and basic (pH = 4.0, 7.0, and 10.0) are shown in

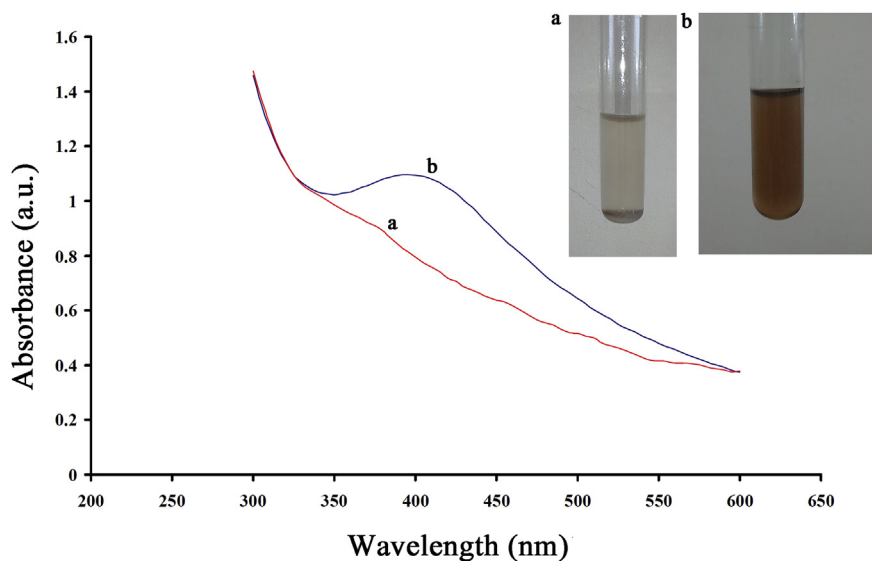


Figure 1 – Absorption spectrums of silver nitrate (a) and silver nanoparticles (b). Inset: silver nitrate (a) and silver nanoparticles (b) at the optimal condition ($T = 35^\circ\text{C}$, $\text{pH} = 10.0$, and $\text{time} = 18$ hours).

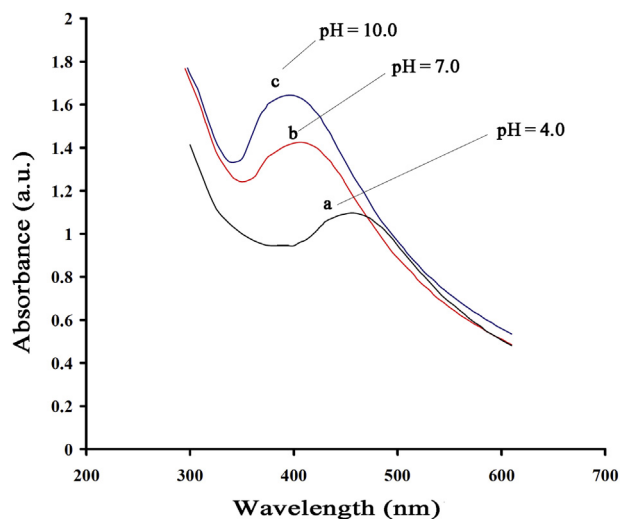


Figure 3 – Ultraviolet-visible spectrum of silver nanoparticles at different pH values: 4.0, 7.0, and 10.0.

Figure 3. The results obtained showed that the synthesis of AgNPs can be partially completed under a neutral condition and it is completed by raising the pH value up to 10.0. Further increasing the pH leads to a decrease in the absorbance band of UV-Vis spectrum that could be related to the oxidation of Ag in the basic media. By contrast, under acidic conditions, no absorbance band for AgNPs was observed. The aggregation of AgNPs to form larger NPs was believed to be favored over the nucleation.

Reaction time had a great effect in the biosynthesis of AgNPs and higher yields of AgNPs were obtained by increasing the reaction time to 24 hours. Because of the instability of the AgNPs formed, an optimum duration is required, as

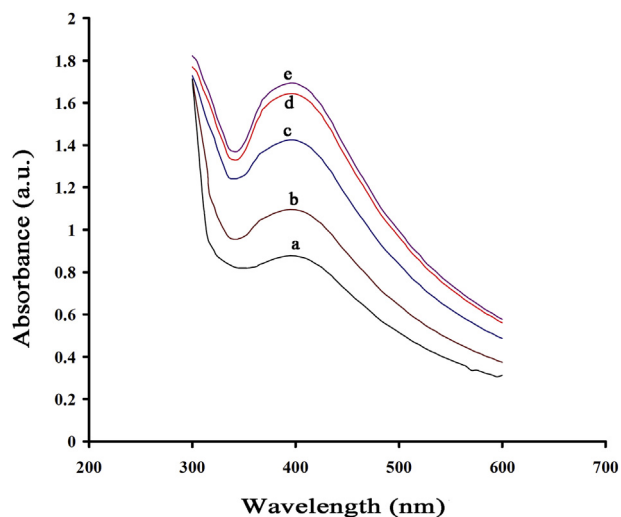


Figure 4 – Ultraviolet-visible spectrum of silver nanoparticles at different reaction times: (a) 3 hours; (b) 10 hours; (c) 14 hours; (d) 18 hours; and (e) 24 hours ($T = 35^{\circ}\text{C}$ and $\text{pH} = 10.0$).

agglomeration of AgNPs after the optimal duration results in larger particle sizes. The optimal time required for the completion of the reaction was determined to be 18 hours (**Figure 4**).

Finally, the reaction of silver nitrate solution with different wt% of onion extract was optimized. Different concentrations of silver nitrate solutions were used to obtain the highest yield of AgNPs. In this study, we achieved a maximum yield with 5mM silver nitrate solution (**Figure 5**). By contrast, a 17% wt% onion extract was found to be the best to achieve the maximum yield (data not shown).

3.2. AgNPs characterization

AgNPs powders were analyzed by XRD analyses. The XRD pattern of AgNPs nanopowders, in the 2θ range of 25° – 80° , is shown in **Figure 6A**. Intense peaks of NPs (1, 1, 1), (2, 0, 0), (2, 2, 0), and (3, 1, 1) appeared, which are indexed as crystalline silver face-centered cubic phase. The mean particle size of AgNPs calculated by the Scherrer equation ($D = K\lambda/\beta\cos\theta$) was about 5.3–10.2 nm. This value is close to particle size obtained from TEM investigation. The minor difference in particle size evaluation by the two methods is related to the aggregation and limitation in Scherrer equation for the XRD method.

In continuation, we characterized the morphology of the as-grown nanostructures by the TEM method. The obtained micrograph of the NP is shown in **Figure 6B**. It is clear that AgNPs were successfully prepared in spherical shape. In addition, the histogram of size distribution of AgNPs is shown in **Figure 6C**. This good distribution helps to reproduce signals in the electrochemical investigation.

EIS was also used to investigate the electron transfer ability of AgNPs. **Figure 7** presents the Nyquist diagrams of

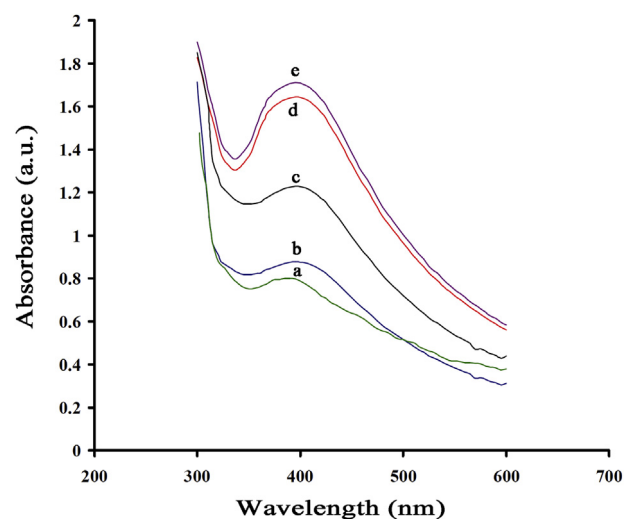


Figure 5 – Ultraviolet-visible spectrum of silver nanoparticles at different concentrations of silver nitrate solution: (a) 1.0mM; (b) 2.0mM; (c) 3.5mM; (d) 5.0mM; and (e) 6.0mM of silver nitrate ($T = 35^{\circ}\text{C}$, $\text{pH} = 10.0$, and time = 18 hours).

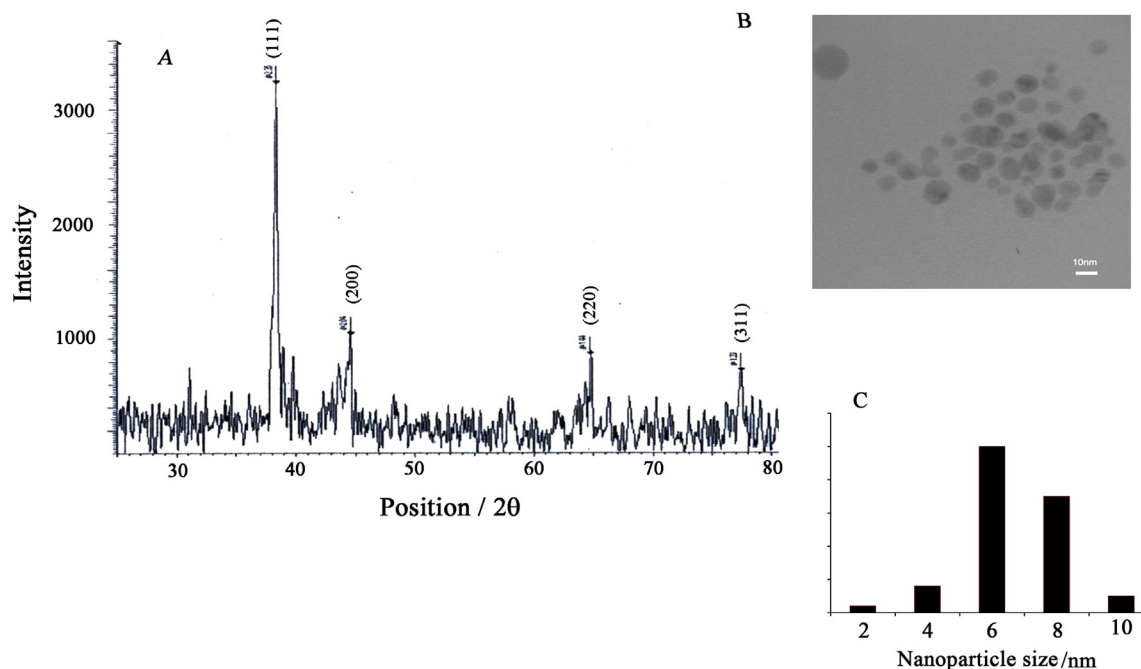


Figure 6 – (A) X-ray powder diffraction patterns of silver nanoparticles synthesized by treating *Allium cepa* with silver nitrate aqueous solution. (B) Transmission electron microscopy micrograph of silver nanoparticle synthesized in this work. (C) Histogram of size distribution of gold nanoparticles vs. nm.

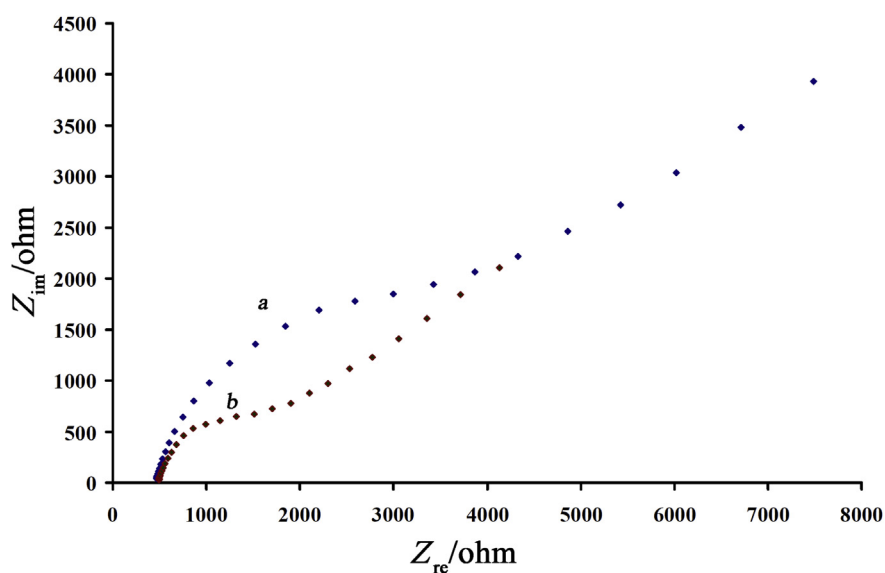


Figure 7 – Impedance spectra of bare carbon paste electrode (a) and silver nanoparticles/carbon paste electrode (b) in 1.0 mmol/L $\text{Fe}(\text{CN})_6^{4-/3-}$ containing 0.10 mmol/L KCl. Conditions were as follows: E_{dc} , +0.45 V versus Ag/AgCl; E_{ac} , 5 mV; frequency range: 0.1–100,000 Hz.

the imaginary impedance (Z_{im}) versus the real impedance (Z_{re}) of the EIS obtained at a surface of CPE (Figure 7, red squares) and AgNPs/CPE (Figure 7, blue squares) in the presence of 1.0 mM $\text{K}_4[\text{Fe}(\text{CN})_6]/\text{K}_3[\text{Fe}(\text{CN})_6]$. The result obtained showed that at the surface of AgNPs/CPE, the semi-circle diameter is lower than that of CPE, and this result

suggests that the electrical conductivity of AgNPs/CPE can be comparable with CPE; besides, it also confirmed that, in the presence of AgNPs, charge transfer resistant is reduced at the surface of CPE. Therefore, these results can be applied for AgNPs as a modified sensor for electrochemical analysis systems.

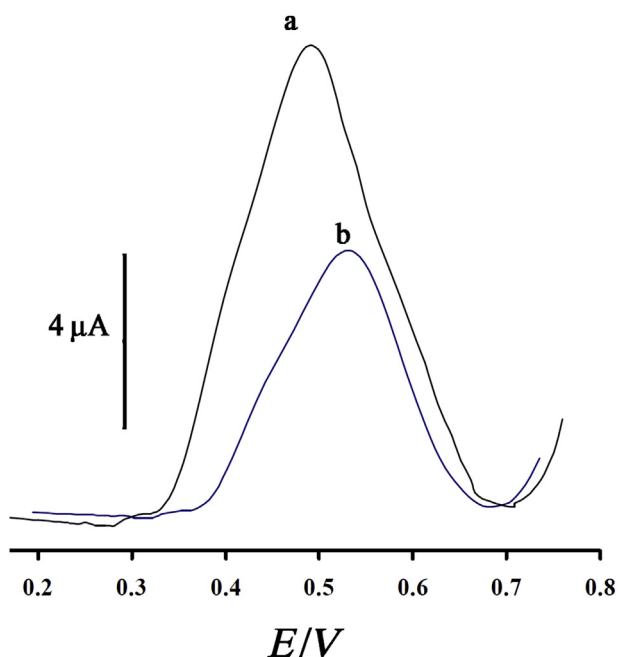


Figure 8 – Square wave voltammograms of AgNPs/CPE (a) and CPE (b) in the presence of 50 μM ascorbic acid at pH 7.0, respectively. Conditions were as follows: amplitude potential of 50 mV and frequency of 10 Hz. AgNP = silver nanoparticle; CPE = carbon paste electrode.

3.3. Application of AgNPs for ascorbic acid determination

AgNPs as a conductive NP can be used for modification of electrochemical sensors for ascorbic acid determination at a

trace level. **Figure 8** (Curves a and b) shows the electrochemical responses of AgNPs/CPE and CPE in 50 μM ascorbic acid in phosphate-buffered saline solution (pH 7.0), respectively. For AgNPs/CPE and CPE, ascorbic acid showed an oxidation peak potential (E_{pa}) of 0.483 V and 0.553 V, respectively. However, the peak current of ascorbic acid for AgNPs/CPE was much larger than that for CPE; it was approximately 2.0 times larger than CPE by SWV. Thus, AgNPs exhibited a catalytic activity toward the oxidation of ascorbic acid.

The square wave voltammograms clearly show that the plot of peak current versus ascorbic acid concentration is linear for 0.4–450.0 μM of ascorbic acid, with the regression equation being $I_p (\mu\text{A}) = (0.0639 \pm 0.0033) C_{\text{ascorbic acid}} + (1.0731 \pm 0.2631)$ ($r^2 = 0.9963$, $n = 9$), where C is micromolar concentration of ascorbic acid and I_p is the peak current (**Figure 9**). The detection limit was 0.1 μM ascorbic acid according to the definition of $Y_{LOD} = Y_B + 3\sigma$.

Finally, we have examined the applicability of the modified electrode for determination of ascorbic acid in natural samples. **Table 1** shows the ascorbic acid content of some fresh fruit and vegetable juices determined by the proposed method and other published methods [41]. The results of statistical calculation shown in **Table 1** indicate a good precision and good agreement between the repeatability of the proposed and official methods (F test) and the mean values obtained (t test).

3.4. Conclusion

The high phenolic content of the water extract of onions having strong properties helps to reduce the Ag cations to AgNPs. The AgNPs synthesized using onions were characterized by EIS and XRD, and confirmed by TEM methods. UV-Vis showed peaks at a wavelength of 397 nm, thus proving the formation of spherical AgNPs. Finally, we studied the effect of

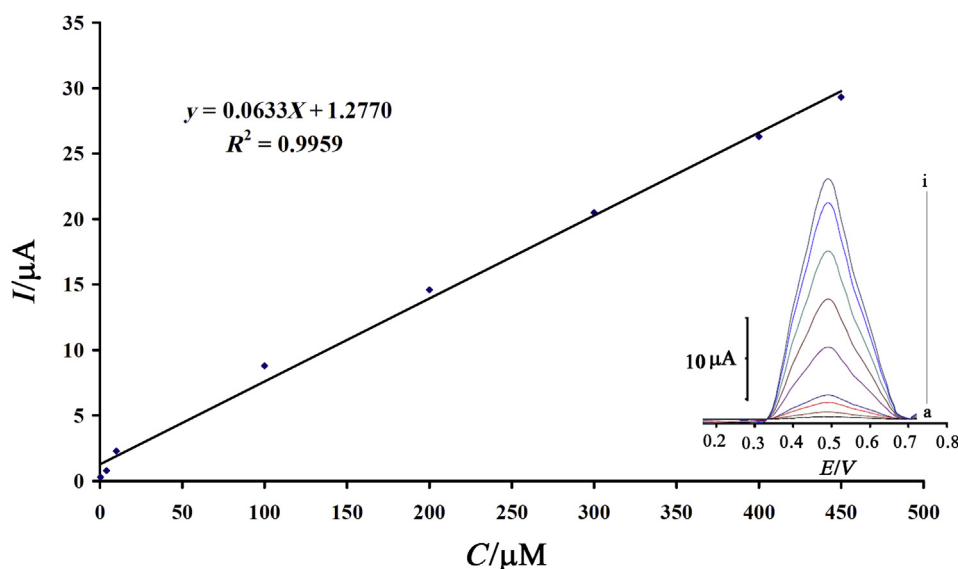


Figure 9 – Plots of the electrocatalytic peak current as a function of ascorbic acid concentration. The inset shows the square wave voltammograms of silver nanoparticles/carbon paste electrode in 0.1 mmol/L phosphate-buffered solution (pH 7.0) containing different concentrations of ascorbic acid: a–i correspond to 0.4 $\mu\text{mol/L}$, 5.0 $\mu\text{mol/L}$, 20.0 $\mu\text{mol/L}$, 30.0 $\mu\text{mol/L}$, 100.0 $\mu\text{mol/L}$, 200.0 $\mu\text{mol/L}$, 300.0 $\mu\text{mol/L}$, 400.0 $\mu\text{mol/L}$, and 450.0 $\mu\text{mol/L}$ of ascorbic acid.

Table 1 – Determination of ascorbic acid in real samples (n = 3).

| Sample | Vitamin C added ($\mu\text{mol/L}$) | Found (vitamin C); proposed method ($\mu\text{mol/L}$) | Found (vitamin C); published method ($\mu\text{mol/L}$) | F_{ex} | F_{tab} | t_{ex} | $t_{\text{tab}(95\%)}$ |
|---------------|---------------------------------------|--|---|-----------------|------------------|-----------------|------------------------|
| Orange juices | — | 135.75 \pm 1.31 | 136.61 \pm 1.75 | 11.78 | 19.0 | 3.2 | 3.8 |
| Kiwi juices | — | 53.22 \pm 0.73 | 52.87 \pm 0.82 | 9.2 | 19.0 | 2.7 | 3.8 |
| Apple | — | 11.25 \pm 0.45 | 12.02 \pm 0.85 | 7.1 | 19.0 | 2.1 | 3.8 |

Data are presented as means \pm standard deviation.

AgNPs for the preparation of electrochemical sensor for determination of ascorbic acid in the dynamic range of 0.4–450.0 μM .

Conflicts of interest

All contributing authors declare no conflicts of interest.

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