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Electrochemical Sensor Constructed by Carbon Paste Electrode Modified with Mesoporous Silica Encapsulating PANI Chains Decorated with GNPs for Detection of Ascorbic Acid

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Abstract

In this work, an electrochemical sensor constructed by carbon paste electrode (CPE) modified with mesoporous silica encapsulating polyaniline (PANI) decorated with gold nanoparticles (GNPs) was presented and applied to detect ascorbic acid (AA). First of all, PANI latex was synthesized by emulsion polymerization. Moreover, the acid-catalyzed sol-gel solutions of tetraethyl silicate (TEOS) was prepared in the presence of D-glucose, followed by encapsulation of previous PANI latex into asprepared sol-gel solution. Upon formation of silica frameworks, electroactive composite powders were obtained. Subsequently, the as-prepared composite powders were subjected to Soxhlet extraction to remove D-glucose and the final product denoted by PM silica. Furthermore, PGM silica was obtained by immersing PM silica in 5 mL of 0.10 mM HAuCl₄ solution for 6 h, which reduce the gold nanoparticles (GNPs) onto the surface of PANI chains inside the nanochannels of wormhole-like mesoporous silica framework. The redox reactions that mediated between EB form of PANI and aqueous HAuCl₄ solution were monitored and confirmed by FTIR spectra. The appearance of GNPs inside the PGM silica was characterized by TEM, XPS, XRD, and BET analyses. PGM silica was found to reveal higher redox capability as compared to that of PM silica based on the electrochemical CV studies.

It should be noted that PGM silica-modified CPE was found to exhibit high sensitivity, a relatively low limit of detection, excellent sensing repeatability and a broader linear dynamic range for AA detection than those of bare CPE or PM silica-modified CPE. Moreover, A electrochemical sensor constructed from PGM silica-modified CPE was found to reveal better selectrivity for a tertiary mixture of AA/DA/UA as compared to that of PM silica-modified CPE based on a series of studies of

differential pulse valtammograms. The real commercial product for AA detection was also investigated in this work.

Keywords: electroactive; polyaniline; mesoporous; gold; ascorbic acid; sensor.

1. Introduction

Ascorbic acid (AA) is found in many foods/drinks and is well known for its anti-oxidant properties. In addition, it plays a significant role in many human metabolic processes because of its redox properties and can be used in the prevention and treatment of diseases[1] such as infertility, mental illness, AIDS, cancer, and the common cold. Thus, the development of a simple, rapid, highly selective, and sensitive method for the determination of AA is desirable, having diagnostic medical and food safety applications. Several techniques have been explored for the determination of AA, including spectroscopic, [2] chromatographic, [3] enzymatic, [4] and electroanalytical [5] methods. Among these various methods, electrochemical methods using various chemically modified electrodes (CMEs) are considered the best because of their simplicity and high sensitivity for the determination of AA. In addition, for the development of electrochemical sensors, materials with redox capabilities are desired. In recent years, many researches have focused on electrochemical biosensing because of ease of tailoring properties of conducting polymers, such as their reversible redox capabilities. Polyaniline (PANI) is an electroactive polymer with outstanding electrochemical properties toward biologically important molecules; consequently, it is quite suitable for use in electrochemical sensors.[6-14]

Moreover, several research studies[15-26] have reported the encapsulation of polymers and/or oligomers within the channels of mesoporous hosts in the past decades. For example, in 1994, Bein *et*

al.[15] reported the preparation of encapsulation of PANI chains in MCM-41 hosts with a channel diameter of \sim 3 nm. This channel size permitted encapsulation of several PANI chains, and the resulting filaments showed microwave conductivity. In an effort to apply PANI in the field of electrorheological (ER) fluids, Choi *et al.*[16] synthesized a PANI/mesoporous SBA-15 nanocomposite. Moreover, Yeh *et al.*[22] explored PANI encapsulated within mesoporous silica, prepared by a non-surfactant templating route, and found that the materials having electroactive and had a high surface area simultaneously.

Several types of nanoparticles, including metals, oxides, and semiconductor nanoparticles, have also been applied in biosensing. For example, gold and silver nanoparticles or silver–silica hybrid nanostructures have been used as biosensor substrates.[27-29] Among the nanomaterials used as components in biosensors, gold nanoparticles (GNPs) have received the greatest interest because they have several interesting properties.[30, 31] GNPs with diameters of 1 - 100 nm have a high surface-tovolume ratio and a high surface energy, providing stable immobilization of a large number of biomolecules, thus retaining their bioactivity. Moreover, GNPs permit the fast and direct electron transfer between a wide range of electroactive species and electrode materials.

In this paper, we attempts to construct an electrochemical sensor for AA detection by preparing sensing materials integrating both conducting polymer and metallic nanoparticles. First of all, mesoporous silica encapsulating PANI chains (denoted by PM silica) was prepared by a non-surfactant-template route with D-glucose as pore-forming agent. Subsequently, reducing the GNPs onto the surface of PANI chains inside the nanochannels of mesoporous silica through the redox reactions between the emeraldine base (EB) form of PANI and an aqueous HAuCl₄ solution. The materials (denoted by PGM silica) with reduction of GNPs upon the surface of PANI inside the nanochannels of

PM silica was found to promote electrocatalytic properties of PM silica, indicating that the PGM silica can be potentially applied in electrochemical sensor. The appearance of GNPs onto the surface of PANI chains inside the nanochannels of PGM silica was characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and Brunauer-Emmett-Teller (BET) analyses. The oxidation transition of the EB form toward the pernigraniline base (PB) form of PANI, accompanied by the reduction of the aqueous HAuCl₄ solution, forming GNPs, was confirmed by the observation of Fourier-transformation infrared (FTIR) spectroscopy. Redox capability of PM silica and PGM silica was further investigated by electrochemical cyclic voltammetry (CV) studies. The electrochemical snsor for the sensitivity studies of AA detection constructed by PGM-modified CPE, PM silica-modified CPE and bare-CPE was performed and compared. The selectivity studies of electrochemical sensor of PGM silica and PM silica- modified CPE was also investigated by differential pulse voltammograms (DPV) for the tertiary mixture of AA/UA/DA. Moreover, the detection of AA in real commercial products of vitamin C tablets was also carried out in this work.

2. Experimental section

2.1 Chemicals

Aniline (Fluka, 99%) was purified prior to use. Gold(III) chloride trihydrate (HAuCl₄·3H₂O; Alfa Aesar, 99%), tetraethyl orthosilicate (TEOS; Acros, 97%), D-(+)-glucose (Acros, 99%), sodium dodecyl benzene sulfonate (DBSA; Fluka, 98%), dimethylformamide (Macron, 99%), ammonium peroxydisulfate (APS; J.T. Baker, 99%), *n*-butyl alcohol (Sigma-Aldrich, 99.5%), *N*,*N*-diphenyl-*p*-phenylenediamine (Merck, 99%), 4,4-diaminodiphenylamine sulfate (Aldrich, 99%), L-Ascorbic acid

(Sigma, 99%), Dopamine hydrochloride (Sigma, 99%), Uric acid (Sigma, 99%), Vitamin C Tablet (Standard Chem. & Pharm. Co. & Yungshin Pharm. Ind. Co.) and hydrochloric acid (HCl; Riedel-de Haën, 37%) were used without purification. Methanol (MeOH, Macron, 98%) was used as a solvent for Soxhlet extraction. All reagents were reagent grade unless otherwise stated.

2.2 Instrumentation

FTIR spectroscopy was performed using an FT/IR-4100 instrument (JASCO) using KBr pellets of the samples. N₂ adsorption–desorption isotherms were recorded at 77 K on a Micromeritics ASAP 2010 automated analyzer. The surface area was obtained by the BET method, and the pore size distributions were calculated by applying the Barrett–Joyner–Halenda (BJH) model to the adsorption data. XPS analysis was performed on an ESCALAB MK II X-ray photoelectron spectrometer. XRD diffraction patterns were recorded with Cu Ka ($\lambda = 0.15418$ nm) using a Philips X'pert Pro. The morphology of the PGM silica was observed using TEM (JEOL JEM-1400). Cyclic voltammetry (CV) was conducted on a VoltaLab 40 (PGZ 301) electrochemical workstation in 20 mL of 0.1 M phosphate buffer solution (PBS). A three-electrode system was used, consisting of PM silica and PGM silica-modified carbon paste electrodes (CPEs) as the working electrodes. A platinum foil served as the auxiliary electrode and a Ag/AgCl (3 M NaCl solution) electrode was used as the reference electrode, respectively.

2.3 Preparation of PANI latex and aniline pentamers

In this work, PANI latex was synthesized by emulsion polymerization with APS as chain initiator. Moreover, an aniline pentamer (AP) was also synthesized as control experiment. A typical procedure to prepare PANI latex is given as follows: DBSA (0.5 g) and 10 mL of n-butyl alcohol were mixed with

22.5 g of aniline in 100 mL of distilled H₂O for 1 h. Then, 100 mL of an aqueous solution containing 27.5 g of APS was added dropwisely. After 24 h, polymerization of PANI was complete, and a stable green-colored PANI latex was obtained. The number-average molecular weight (M_n) and weight average molecular weight (M_w) of the as-prepared PANI obtained using emulsion polymerization were determined to be ~ 6600 and ~ 8600, respectively, based on gel permeation chromatography (GPC) with polymethylmethacrylate (PMMA) as the standard. A representative procedure to synthesize the AP used in the control experiment is given as follows: first of all, 2.6 g of N,N-diphenyl-pphenylenediamine and 3.5 g of 4,4-diaminodiphenylamine sulfate were dissolved in a solvent mixture containing 15 mL of a 1.0 M HCl aqueous solution, 20 mL of distilled H₂O and 100 mL of dimethylformamide, followed by stirring at -5 °C for 30 min. Subsequently, a solution of 2.28 g of APS in 50 mL of a 1.0 M HCl solution was added via a dropping funnel into the as-prepared mixture to give a AP-containing solution. The reaction mixture was further stirred for 1 h at -5 °C prior to adding 300 mL of distilled H₂O. The resulting solution containing precipitate of AP was filtered out. The obtained precipitate was subsequently washed with 200 mL of 1.0 M aqueous HCl solution to remove any unreacted monomer. For the de-doping process, the as-received solid product was washed thoroughly with 100 mL of a 10 wt-% of NH4OH solution, followed by washing with excess amount of distilled H₂O. The final product was then dried in a vacuum oven at 50 °C for 24 h. The yield of obtained AP fine green powder was calculated at ~ 57%.

2.4 Preparation of PM silica

The conventional preparation process for PM silica was given as follows: 5.20 g of TEOS, 3.45 g of EtOH, and 1.8 g of 1 M HCl aqueous solution were mixed in a 50-mL flask at 85 °C for 2 h. The

solution was combined with an appropriate quantity of a solution containing 0.75 g D-glucose to a prehydrolyzed solution of TEOS. After 0.5 h of magnetic stirring, 6 g of PANI latex was added to the as-prepared sol–gel solution, followed by stirring for an additional 1 h. The obtained solution was then covered with a layer of paraffin film pierced with ~15 - 20 pinholes to allow the evaporation of solvent and reaction by-products. After ~ 2 weeks, an electroactive silica composite including pore-forming agent (*i.e.*, D-glucose) was produced. To remove D-glucose, the as-synthesized composite sample was grounded into fine powders and followed by extracting the remaining components with excess MeOH/H₂O (1:2 v/v) at 120 °C for 7 days. After dynamic drying at 100 °C for 24 h, fine powder of composite in dark green color, denoted PM silica, was obtained.

2.5 Synthesis of PGM silica

A representative procedure to prepare PGM silica is given as follows: the PGM silica sample was prepared by immersing as-synthesized PM silica material into aqueous solution of 0.10 mMHAuCl₄·3H₂O at room temperature for 6 h. After centrifugal filtration and washing with excess amount of distilled H₂O, the PGM silica was collected and dried overnight under vacuum at 50 °C.

2.6 Preparation of PM silica/PGM silica-modified CPE

A modified carbon paste electrode (CPE) for the electrochemical sensor was fabricated by homogenization followed by mixing using agate pestle and mortar. First of all, carbon paste (75 mg) was prepared by thoroughly mixing graphite powder (50 mg), PM silica or PGM silica (15 mg) and paraffin oil (10 mg). A Teflon tube, open at one end (3 mm diameter), was filled with the prepared carbon paste and served as electrode body. Electrical contact was made with a copper wire through the

center of tube. Suitable packing was obtained compact electrode surface toward bond paper until surface was smooth.

3. Results and Discussion

The base form of PANI can be schematically illustrated in the following manner:[32]



The *y* value ranges from 0 for a fully oxidized polymer (so-called pernigraniline) to 0.5 for the half-oxidized polymer (emeraldine) to 1 for the fully reduced polymer (leucoemeraldine).

3.1 Redox behavior of PANI and AP

In this study, the redox capability of as-prepared PANI and AP was determined by cyclic voltammetry (CV) using a $1.0 \ M H_2SO_4$ aqueous solution, a scan range from -0.2 to +1.0 V, and a scan rate of 50 mV·s⁻¹. Cyclic voltammogram of neat CPE contains no redox peaks, as shown in Figure 1(a). Moreover, the voltammogram of PANI-modified CPE contains a pair of redox peaks at 192 and 778 mV, as shown in Figure 1(b). The pair of redox peak that appeared in PANI-modified CPE was similar to that of AP, which exhibited a pair of redox peaks at 261 and 542 mV, as shown in Figure 1(c). The as-prepared PANI having longer conjugated length as compared to that of AP, implying that superior electroactivity of PANI as compared to that of AP. For example, the PANI was found to exhibit a higher current density of 917 μ A cm⁻² in the first oxidation wave compared to 316 μ A cm⁻² for AP.

Therefore, for the subsequent experiments, PANI was chosen as the potential candidate for the following electrochemical sensor for AA detection.

3.2 Characterization of PM and PGM silica

PM and PGM silica materials were prepared by acid-catalyzed sol-gel process in the presence of PANI latex using a non-surfactant template, as shown in Scheme 1. For the preparation of PGM silica, the PM silica was immersed in 5 mL of a $0.1 \, mM$ HAuCl₄·3H₂O aqueous solution for 6 h at room temperature. The GNPs can be obtained after the reduction of AuCl₄⁻, mediated by the redox change of the PANI chains encapsulated in the nanochannels of wormhole-like mesoporous silica frameworks from the EB to PB form.

The redox reactions occurring simultaneously between the EB form of PANI and the HAuCl₄·3H₂O aqueous solution were confirmed by the FTIR spectra of PM silica and PGM silica, as shown in Figure 2. In Figure 2(a), the benzenoid (B) diamine and quinoid (Q) diimine units of the EB form of the PANI chains encapsulating in the PM silica are indicated by the transmission bands appeared at wavenumber of 1503 and 1596 cm⁻¹, respectively. After immersing PM silica into aqueous HAuCl₄·3H₂O solution for 6 h, the spectrum [Figure 2(b)] of the as-obtained PGM silica showed a characteristic band corresponding to the increase in Q unit of EB form of PANI at wavenumber of 1596 cm⁻¹, indicating that EB form of PANI had been partially oxidized toward the PB form. The spectral changes also implied that GNPs were reduced and formed upon the surface of PANI chains inside the nanochannels of mesoporous silica frameworks at the same time. In addition, the appearance of GNPs inside PGM silica was further confirmed by transmission electron

microscopy (TEM), X-ray diffraction pattern (XRD), X-ray photoelectron spectroscopy (XPS) and Brunaeur-Emmett-Teller (BET) analyses.

Morphology of GNPs onto the surface of PANI chains inside the nanochannels of PGM silica was observed by TEM at magnification of 40k, as shown in Figure 3. For example, the electronic images of TEM with scale bar of 50 nm was found to show black spots with irregular shape of ~ 1 nm in diameter can be identified as GNPs, as shown in Figure 3 (a). Moreover, the electron diffraction pattern of GNPs in PGM silica was shown in Figure 3 (b). The XRD diffraction pattern of PM silica and PGM silica was illustrated in Figure 4. For example, the XRD of PM silica (*i.e.*, without incorporating GNPs) was shown in Figure 4(a). A clear broad diffraction pattern appeared at the location of $2\theta = 25.04^{\circ}$ was ascribed to the amorphous structures of PANI polymer chains.[33] However, after immersing the PM silica in 5 mL of a 0.1 mM aqueous HAuCl₄·3H₂O solution, the as-obtained PGM silica showed four additional clear diffraction peaks at the position of $2\theta = 38.01, 43.95, 64.52$, and 77.43°, indicating Bragg reflections from the Au planes of (111), (200), (220), and (311), respectively, as revealed in Figure 4(b).[34] The formation of GNPs inside the nanochannels of PGM silica was also confirmed by analysis of XPS spectra, as shown in Figure 5. For example, the binding energies of doublet observed for Au 4f_{7/2} and Au 4f_{5/2} were consistent with those of Au⁰ oxidation state, located at the position of 83.4 and 86.9 eV, respectively, as shown in Figure 5(A).[35] These results clearly indicated that the reduction of Au(III) to Au⁰ was achieved. As shown in Figure 5(B), three characteristic peaks for nitrogen of PANI are present, representing three different electronic states. Binding energy of nitrogen of benzenoid was observed at the value of 399.5 eV, whereas that of quinoid was seen at 398.6 eV, and that of e cationic radical (N^+) was observed at 400.6 eV.[36]

It should be noted that the formation of uniform, ultrafine GNP distribution in the PGM silica plays a vital role in exposing AuCl₄⁻ to the PM silica. Scheme 2 shows a possible mechanism for the reduction of GNPs onto the surface of PANI chains inside the nanochannels of mesoporous silica frameworks. In this mechanism, the AuCl₄⁻ ions are selectively adsorbed onto the surface of PANI chains inside the nanochannels of PANI chains inside the nanochannels of PANI chains inside the nanochannels of PM silica and the protonated imine sites of PANI by electrostatic attraction, facilitating complexation [37]. These complexes serve as the specific active sites for the formation of Au⁰ nuclei because of electron transfer from the imine sites to AuCl₄⁻,while PANI inside the nanochannels of the PM silica matrix is oxidized, simultaneously reducing AuCl₄⁻ [38]. Subsequently, AuCl₄⁻ ions are further reduced on the surface of Au⁰ nuclei by autocatalytic surface reduction, leading to the growth of Au⁰ nuclei and a uniform and ultrafine GNP distribution.

After the identification of the EB form of PANI chains and characterization of GNPs, the surface area, pore volume, and pore size of the as-prepared mesoporous silica (MS), PM silica and PGM silica were also investigated. The adsorption isotherms of the samples show type-IV-like isotherms, with a sharp increase in the absorbed volume of N₂ at the relative pressure range of $P/P_0 = 0.4-0.6$. The N₂ adsorption–desorption isotherms for the MS, PM silica, and PGM silica samples at an operating temperature of 77 K are shown in Figure 6. The data of composition, pore parameters of MS, PM silica and PGM silica obtained from BET studies are summarized in Table 1. For example, BET surface area (SA) of mesoporous silica (MS) was found to be 1145 m² g⁻¹, whereas its pore volume and pore diameter were 0.941 cm³ g⁻¹ and 2.9 nm, respectively. Moreover, BET SA of PM silica decreased to 630 m² g⁻¹, and the corresponding pore volume and pore diameter of PM silica was found to decrease to 0.275 cm³ g⁻¹ and 2.19 nm, respectively. After formation of GNPs inside the nanochannels of PM silica, the

SA, pore volume, and pore size of PGM silica was found to be further decreased to $\sim 624 \text{ m}^2 \text{ g}^{-1}$, 0.272 cm³ g⁻¹, and 2.13 nm, respectively. These results indicated that the GNPs were formed onto the PANI chains inside the nanochannels of wormhole-like frameworks of PM silica.

3.3 Cyclic voltammetry of PM and PGM silica

After the characterization of as-prepared materials, the important physical property of materials used in electrochemical sensor application need to examined is redox capability. In this study, the redox capability of MS, PM silica and PGM silica was identified by electrochemical cyclic voltammetry (CV) studies. Based on the proposed redox mechanism of PANI,[39] electrochemical CV measurements were performed in a 1.0 M H₂SO₄ solution at a scan rate of 50 mV·s⁻¹. The voltammograms of MS, PM silica and PGM silica using a Ag/AgCl electrode as reference electrode were shown in Figure 7.

As shown in Figure 7(a), the curve of raw MS without incorporating PANI is a straight line without any redox peak, indicating that no redox behavior occurred in the MS-modified electrode. Moreover, the curve of PM silica revealed a pair of oxidation peaks at the potential of 243 and 512 mV, corresponding to the change in oxidation state from LB to EB form of PANI, as well as from EB form to PB form of PANI,[20, 40] as shown in the right-hand side of Figure 7. This observation indicated that encapsulation of PANI chains into the nanochannels of wormhole-like mesoporous silica framework may introduce redox capability to MS sample. For example, PM silica exhibited first oxidation peak at current density of 337 μ A cm⁻², as shown in Figure 7(b). Furthermore, by reduction of tiny number of GNPs onto the surface of PANI chains inside the nanochannels of PM silica, the voltammogram of PGM silica was found to display first oxidation peak at current density of 834 μ A cm⁻², as shown in Figure 7(c). This result indicated that the reduction of GNPs onto the surface of PANI

chains inside the worm-like channels of PM silica may facilitate the oxidation of PANI from EB form towards PB form, which was encapsulated in PGM silica.

3.4 Electrocatalytic activity of PM silica/PGM silica CPE for the detection of AA

Electrocatalytic activity of as-prepared materials was used to detect AA by CV and amperometry. Figure 8 shows the voltammograms corresponding to bare CPE, PM silica, and PGM silica-modified CPE in 0.1 M PBS (pH = 7) in the absence and presence of 2 mM AA, with a scan rate at 50 mVs⁻¹. Platinum foil and Ag/AgCl were used as the counter and reference electrodes, respectively. When there was no AA appeared in PBS solution, no oxidation current appeared in voltammograms.

However, In Figure 8 (A), in the presence of 2 mM AA in PBS, a small oxidation current of bare CPE occurred at a more positive potential of 670 mV, as shown in Figure 8 (A) (a), as compared to that of the PM silica (E = 390 mV) and PGM silica-modified CPE (E = 390 mV), as shown in Figure 8 (A) (b) and Figure 8 (A) (c), respectively. For example, the voltammogram of PM silica has a maximum current density for first oxidation transition at 193 μ A cm⁻², which was lower than that of PGM silica, which was 338 μ A cm⁻².

Furthermore, the repeatability of constructed electrochemical sensor for AA detection was also investigated, as shown in Figure 8 (B). The long-term stability of PGM silica-modified CPE and PM silica modified CPE was studied by storing the electrode at room temperature for 2 weeks in 0.1 M PBS solution at pH = 7.0. It should be noted that, by detection of 2 *mM* of AA, PGM-modified CPE was found to reveal good repeatability at the stable current density of ~ 340 μ A/cm² with the mininum relative standard derivatives of 0.43%, as shown in 8(B) (b) However, the PM silica modified CPE was found to exhibit stable current density of ~ 190 μ A/cm² with the mininum relative standard derivatives

of 0.59%, as shown in Fig. 8 (B) (a). After this testing period, the constructed electrochemical sensor was found to retain ~ 93 - 94 % of its initial response, indicating the both constructed electrochemical sensors reveal excellent long-term stability.

Effect of scan rate on performance of PGM silica modified electrode towards oxidation of AA was also studied for kinetic parameters. The different of scan rate (10 to 500 mV s⁻¹) was studied at PGM silica-modified CPE in 0.1 M PBS (pH = 7) in the 400 μ M AA, and their results are shown in Figure 9. The oxidation current of AA was increased when the scan rate rises from 10 to 500 mV s⁻¹. Additionally, the linear relationship was plotted between peak current (I_p) and square roots of scan rates (ν ^{1/2}), the observed linear regression equation is; I_p (μ A) = 0.248 ν ^{1/2}(Vs⁻¹)^{1/2} + 16.084.951, R² = 0.9976. Meaning that the electrode reaction of AA oxidation is controlled by diffusion based electron transfer reaction, as presented in the Figure 9 (B). Moreover, the plots of peak potential (E_p) versus log ν (Figure 9(C)), the linear equation of this straight line is obtained as ; $E_p = 0.1077 \log (\nu) 0.5662$, R² = 0.9955, was use to further investigate the mechanism of electrocatalysis of AA on the modified electrode, Using Eq. (1), for a fully irreversible diffusion-controlled system. The anodic peak potential can be evidenced by [41]:

$$E_p = \frac{2.303\text{RT}}{2(1-\alpha)n_a F}\log\nu + k$$

Where, α is the electron transfer coefficient, R (R = 8.314 J K⁻¹ mol⁻¹) is the rate constant, F (F = 96485 C mol⁻¹) is Faraday constant and T (T = 298 K) is room temperature and n_a is the number of electrons involved in the rate-determining step. Herein, the Tafel slope of 0.215 V/decade was calculated from Figure 9(C), which indicates that single-electron transfer was involved in the rate-limiting step assuming a transfer coefficient of $\alpha = 0.73$.

CV responses of PGM silica-modified CPE with various concentrations of AA in PBS were shown in

Figure 10. The responses indicate that an increase in the concentration of AA corresponds to a rise in the oxidation current. The concentration of AA in the buffer varied from 0.0 to 10.0 *mM*. The inset shows the peak current plotted against the amount of AA added. A linear plot of oxidation current density versus the concentration of AA revealed a slope of 70.81 μ A·mM⁻¹ ($R^2 = 0.929$), suggesting the efficient electrocatalytic oxidation of AA by the PGM silica-modified CPE[42, 43].

Figure 11 (a) showed the typical amperometric responses to AA by bare CPE, PM silica-, and PGM silica-modified CPE at an applied potential of 390 mV in a 30 mL PBS solution (pH = 7) under constant stirring. The current response increased gradually, accompanied by an increase in AA concentration. It should be noted that the GNPs formed/reduced onto the surface of PANI chains inside the nanochannels of wormhole-like mesoporous silica framework resulted in a sudden increase in current response of AA. For example, sensitivity of PGM silica-modified CPE was calculated to be 95.95 μ A·mM⁻¹, which is 6.4-fold and 34.6-fold higher than those of PM silica-modified CPE and bare CPE, respectively, as shown in Table 2.

At low concentrations, the bare CPE, PM silica-, and PGM silica-modified CPE all displayed a direct linear relationship between the current response and AA concentration at a constant potential of 390 mV (Figure 11(b)). However, slope for PGM silica-modified CPE was steep compared to those of other electrodes. On the gradual injection of different quantities of AA into a strongly stirred PBS solution equipped with PGM silica-modified CPE, AA was found to detect with high sensitivity (95.95 μ A mM⁻¹) and a high correlation coefficient (0.995). The defined sensitivity of PGM silica-modified CPE was 95.95 μ A·mM⁻¹, which was found to be 6.4-fold higher than that of PM silica-modified CPE (15.13 μ A·mM⁻¹) and almost 34.6-fold higher than that of bare CPE (2.76 μ A·mM⁻¹). The linear dynamic range (LDR) and limit of detection (LOD) (S/N = 3) of the PGM silica-modified

CPE were found to be 0.005–13.3 mM and 0.97 μ M, respectively. This electrode can be seen easily in the magnified image figure in the upper left corner of Figure 11(A).

In contrast, at higher AA concentrations (0.1–57.4 mM), the relationship between the responses and the concentration of AA can be described by a two-parameter sigmoidal equation:[44]

$$j = j_{max}[1 - \exp(-c/b)],$$

where *j* represents the oxidation current, j_{max} is the maximum value obtained at a sufficiently high concentration of AA, c is the concentration of AA, and b is an empirical coefficient characterizing the steepness of *j*, *c* transient, where $j_{max} = 1421.52 \text{ }\mu\text{A} \text{ }\text{cm}^{-2}$ and b = 16.05 mM with a correlation coefficient of r = 0.9937. However, such a fixed equation for *j* vs. *c* is inconvenient to use; therefore, the logarithm of the abscissa of the calibration curve was used to generate a linear relationship. The slope of the linear curve represents the sensitivity of the electrode. The defined sensitivity of PGM silicamodified CPE, PM silica-modified CPE and bare CPE were 62.94 µA cm⁻² (lg(mM))⁻¹,17.16 µA cm⁻² $(\lg(mM))^{-1}$ and 3.22 µA cm⁻² $(\lg(mM))^{-1}$, respectively. Moreover, the oxidation current increased with the addition of AA and achieved a steady state after 2 s. This result indicated that the proposed PGM silica-modified CPE sensor is a rapid-response sensor with high sensitivity, wide LDR, and low LOD. A comparison of the sensitivity, LOD, and LDR for the other samples is given in Table 2. As seen in this comparison, the designed sensor exhibited high sensitivity, a relatively LOD, and a broad LDR. Moreover, the corresponding calibration plots for current density vs. concentration of AA of Bare CPE, PM silica-modified CPE and PGM silica-modified CPE was also studied, as shown in Figure 11 (B).

3.5 Differential pulse voltammetric responses for tertiary mixture of AA, DA and UA

For the selectivity studies of constructed electrochemical sensor, a tertiary mixture of 20 μ M ascorbic acid (AA)/dopamine (DA)/uric acid (UA) was employed. Differential pulse voltammograms (DPV) were recorded for a mixture of 20 μ M of DA, 20 μ M of AA and 20 μ M of UA for bare CPE, PM silica-modified CPE and PGM silica- modified CPE in 0.1 *M* PBS (pH = 7), the experiments were carried out using DPV in the range of - 0.3 to 0.7 V, as shown in Figure 12. The Figure 12 (a) - (c) were graphs containing a tertiary mixture of AA/DA/UA by different modified CPEs and the Figure 12 (d) - (f) were three individual peaks for AA, DA and UA combined into one graph by PGM silica-modified CPE. It should be noted that the the selectivity of bare CPE (Figure 12(a)) and PM silica-modified CPE (Figure 12 (b)) is poor for the tertiary mixture of AA/DA/UA. In contrast, the PGM silica-modified CPE exhibited excellent selectivity and three distinct voltammetric peaks can be resolved at the potential of - 0.01, 0.11, and 0.33 V (Figure 12 (d) - (f)), which corresponds to the oxidation of AA, DA, and UA, respectively.

3.6 Real sample of vitamin C tablets for the detection of AA

To validate the reliability of PGM silica-modified CPE in real samples, the proposed method was applied to directly detect AA in real commercial products of vitamin C tablets. The results of tests using two kinds of commercial vitamin C tablets spiked with 20 -50 μ M of AA were summarized in Table 3, and these data were found in good agreement with those added with quantitative recoveries from 95.7-104.7%, indicating that proposed method is quite reliable for detection of AA in real commercial samples.

4. Concluding remarks

In this paper, an electrochemical sensor, constructed by CPE modified with PGM silica, with high sensitivity, excellent sensing repeatability, a relatively low limit of detection, a broader linear dynamic range and high selectivity for the detection of AA was successfully presented. First of all, the PANI with $M_w = 8,600$ and $M_n = 6,600$, respectively, was prepared by emulsion polymerization based on the studies of gel permeation chromatography (GPC). The PM silica was prepared by performing the acidcatalyzed sol-gel reactions of TEOS in the presence of suitable amount of D-glucose (used as poreforming agent) and previous PANI latex, followed by removal of D-glucose through Soxhlet extraction. Subsequently, PGM silica was obtained by immersing suitable amount of PM silica in 5 mL of 0.1 mM HAuCl₄ solution for 6 h. The redox reactions that mediated between EB form of PANI and HAuCl₄ aqueous solution were monitored and confirmed by FTIR spectroscopy. The appearance of GNPs onto surface of PANI chains inside the nanochannels of PGM silica was characterized by TEM, XRD, XPS and BET analyses. The appearance of GNPs in the nanochannels of PGM silica was clearly observed in TEM image. Moreover, XRD pattern of PGM silica was found to exhibit four additional diffraction peaks as compared to that of PM silica. The four diffraction peaks found at the position of $2\theta = 38.01$, 43.95, 64.52, and 77.43°, which are characteristic Bragg reflections from the plane of Au (111), (200), (220), and (311) planes, respectively. Furthermore, the XPS spectrum of PGM silica was found to show binding energies of Au 4f7/2 and Au 4f5/2 doublet, which were consistent with oxidation state of Au⁰ located at the position of 83.4 and 86.9 eV, respectively, indicating the appearance of GNPs.

After reduction/formation of GNPs inside the nanochannels of PM silica, the corresponding surface area, pore volume, and pore size of PGM silica was found to decrease as compared to that of PM silica based by the studies of BET. The redox capability of PGM was found to be higher than that of PM silica based on the electrochemical CV studies. The higher redox capability of PGM silica as compared

to that of PM silica implied that the electrochemical sensor constructed by CPE modified with PGM silica may reveal better sensitivity.

The as-prepared PGM silica-modified carbon paste electrode (CPE) was found to exhibit higher sensitivity, a better sensing stability, a relatively low limit of detection, and a broader linear dynamic range for AA detection as compared to that of bare CPE and PM silica-modified CPE. For example, a electrochemical sensor constructed by PGM silica-modified CPE was found to demonstrate ~ 34.6-fold and ~ 6.4-fold increase of AA detection as compared to that of bare CPE and PM silica-modified CPE, respectively. Moreover, the PGM silica-modified CPE revealed an excellent electrochemical response toward AA at a lower oxidative potential of 390 mV, with excellent stability and sensitivity of 95.95 μ A·mM⁻¹, a relatively low detection limit of 0.97 μ M, and a wide linear dynamic range from 5 μ M to 13.3 mM. The electrochemical sensor constructed by CPE modified with PGM silica was found to reveal excellent selectivity for the tertiary mixture of 20 µM AA/DA/UA as compared to that of bare CPE and CPE modified with PM silica. The electrochemical sensor constructed by this work for AA detection of real commercial samples of vitamin C tablets was investigated and the results was quite reliable.

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Figure Captions

Fig. 1. Cyclic voltammograms of (a) CPE, (b) PANI and (c) aniline pentamer as measured in 0.1 M of H₂SO₄ aqueous solution with scan rate of 50 mV·s⁻¹.

Fig. 2. Fourier-Transformation Infrared Spectroscopy of (a) PM silica and (b) PGM silica.

B: benzenoid diamine; Q: quinoid diimine

Fig. 3. TEM images of (a) PGM silica, (b) an electron diffraction pattern of GNPs inside PGM silica.

Fig. 4. Relative intensity of XRD diffraction patterns of (a) PM silica and (b) PGM silica.

Fig. 5. XPS spectrum of (a) PGM silica and (b) N(1S) spectra of PGM silica.

- **Fig. 6.** (A) Nitrogen adsorption–desorption isotherms for (a) MS, (b) PM silica and (c) PGM silica. (B) Pore-size distribution curves for (a) MS, (b) PM silica and (c) PGM silica.
- Fig. 7. Cyclic voltammograms of (a) MS, (b) PM silica and (e) PGM silica as measured in $1.0 M H_2SO_4$ aqueous solution with scan rate of 50 mV·s⁻¹. The proposed reversible redox mechanism of PANI was shown to the right-hand side.
- Fig. 8. (A) Cyclic voltammograms of (a) bare CPE, (b) PM silica and (c) PGM silica modified CPE in 2 mM of AA with scan rate of 50 mV·s⁻¹. (B) The repeatability of (a) PM silica (b) PGM silica modified CPE in proposed sensor for fourteen successive measurements in 2 mM AA.
- Fig. 9. (A) Cyclic voltammograms obtained of PGM silica-modified CPE in PBS (pH~7) at different scan rates (curves a to g; 10, 20, 40, 100, 200, 300, 500 mVs⁻¹) in the presence of 400 μM AA.
 (B) The plot between *I*_p vs. square roots of scan rates (ν^{1/2}) (C) The plot of *E*_p vs. log ν.
- **Fig.10.** Cyclic voltammograms of PGM silica-modified CPE in PBS (pH~7) with different concentration of AA: (a) 0 mM, (b) 2 mM, (c) 4 mM, (d) 6 mM, (e) 8 mM and (f) 10 mM with scan rate of 50 mV·s⁻¹. Inset shows the corresponding calibration curve.

- Fig. 11. (A) Typical amperometric response obtained with (a) bare CPE, (b) PM silica and (c) PGM silica-modified CPE at 390 mV with successive additions of AA into 30 mL of PBS. (B) The corresponding calibration plot for current density vs. [AA] of the (a) bare CPE, (b) PM silica-modified CPE and (c) PGM silica-modified CPE.
- Fig. 12. Differential pulse voltammograms obtained with (a) bare-CPE, (b) PM silica and (c) PGM silica-modified CPE for a tertiary mixture of 20 μM AA/DA/ UA in 0.1 M PBS. (d) 20 μM AA, (e) 20 μM DA and (f) 20 μM UA were the individual peak obtained from PGM silica-modified CPE in 0.1 M PBS, followed by combined into one graph.
- Scheme 1. Flowchart for the synthetic route of PM silica and PGM silica.
- Scheme 2. Possible mechanism for the formation of PGM silica.





































Table 1. Compositions and pore parameters of MS, PM silica, and PGM silica materials.

				BET	Pore		Particles
Sample	PANI	TEOS	HAuCl ₄	surface	volume	BJH pore size	size of
code	(g)	(g)	(mM)	area	$(cm^3 \cdot g^-)$	(nm)	GNPs
				$(m^2 \cdot g^{-1})$	¹)		(nm)
MS	0	5 21		1120 /7	0.941	2 90	
IVIS	0	5.21	-	1127.47	0.741	2.90	
PM silica	6	5.21	-	630.41	0.275	2.19	
PGM	6	5.21	0.1	623.79	0.272	2.13	1
silica							

Table 2. Comparison for the sensitivity, LOD and LDR of electrodes modified with different

sensing materials for AA detection.

Materials/Electrode type	Sensitivity (µA·mM ⁻¹)	Limit of detection (LOD) (µM)	Linear dynamic range (LDR) (mM)	Ref.
Silica mesochannels/ITO	-	11	0.026-2.651	[45]
Mesoporous silica film/GNP/ITO	6.13	-	0.05-10	[46]
Mesoporous silica film/PANI/ITO	0.12	-	0.1-10	[47]
Silica mesochannel array/ITO	23.5	0.52	0.01-2	[48]
NMB-AIMCM-41/CPE		10	0.01-0.5	[49]
Pd/SBA-15/CPE	20.4	0.4	0.01-0.14	[50]
DBSA-doped PANI NPs/CPE	10.75	8.3	0.500-8	[51]
PANI/CE	17.7	30	0.030-0.27	[52]
AuNPs@aniline pentamer silica	78.3	1.54	0.100-1	[53]
CNF/Pdnano/CPE		15	0.05-4	[54]
Cu NPs/PANI/GCE		2	0.05-3.5	[55]
Au-PANI/GCE	25.69	8.2	0.01-12	[56]
PM silica/CPE	15.13	6.76	0.025-0.8	This work
PGM silica/CPE	95.95	0.97	0.005-13.3	This work

Table 3. Detection of AA in commercial products of Vitamin C tablets.

	Sample detected	Added	found	Recovery	RSD
Samples	(µM)	(µM)	(µM)	(%)	(N=3, %)
1 ^a	20.17	20	39.31	95.7	2.36
		50	71.45	102.56	1.85
2 ^b	20.32	20	39.79	97.35	2.84
		50	72.65	104.66	1.21

a Vitamin C Tablet, purchased from Standard Chem. & Pharm. Co., Ltd.

b Vitamin C Tablet, purchased from Yungshin Pharm. Ind. Co., Ltd.