รายงานวิจัยฉบับสมบูรณ์

โครงการ อนุภาคนาโนซิลเวอร์ซัลไฟด์ที่ใช้ไคโตซานเป็นตัวหุ้มในการปรับแต่งคาร์บอนอิเล็กโทรดในการวิเคราะห์ วิตามินซี อาร์บูติน ไฮโดรควิโนนและอนุพันธ์บางตัวพร้อมกัน

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เดือน เมษายน ปี 2562 ที่เสร็จโครงการ

สัญญาเลขที่6080145

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและต้นสังกัด

Project Code : MRG6080145

Project Title : Silver sulfide nanoparticles using chitosan as stabilizer modified glassy carbon electrode for simultaneous determination of ascorbic, arbutin, hydroquinone and their derivatives

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Abstract

A stable complex of silver nanoparticles (Ag NPs) and silver sulfide nanoparticles (Ag_2S NPs) together with multi-walled carbon nanotube (MWCNTs) was used to modify a glassy carbon electrode (GCE) for simultaneous detection of hydroquinone (HQ), arbutin (Ar) and ascorbyl glucoside(AA2G). The modified electrode (AgNPs/Ag_2S NPs/MWCNTs/GCE) showed excellent electrocatalysis towards all analytes. The AgNPs/Ag_2S NPs/MWCNTs/GCE show the electrocatalytic activity by the anodic peak potential of all analytes shifted to a negative direction with ≈ 5-fold higher anodic current, compared with those of the unmodified MWCNTs/GCE and AgNPs/MWCNTs/GCE. The anodic peak currents obtained for HQ, Ar and AA2G exhibited linearly from sub-micromolar to several-hundred micromolar concentrations without any cross-interference. The modified electrode possessed a very large active surface area with a detection limit (S/N= 3) of 0.2 μM for HQ, 0.1 μM for Ar and 0.25 μM for AA2G. The sensor was applied to determine HQ, Ar and AA2G in whitening essence sample with the satisfactory recoveries.

Keywords : Ag_2S nanoparticles, Hydroquinone, Arbutin, Ascorbyl glucoside

1. Introduction

Skin lightening products are used to lighten hyperpigmentation, such as age spots and dark spots related to pregnancy or inhibiting melanin formation. The famous bleaching agents which containing in skin lightening product are ascorbyl glucoside (AA2G) or ascorbyl phosphate (MAP), kojic acid, arbutin (Ar) and glutathione (GSH). Several whitening agents such as hydroquinone (HQ) and ammonium mercury are banned or limited in numerous countries because of their negative effects on human health. However, the HQ derivatives including Ar may release HQ when subjected to conditions of high temperature, ultraviolet (UV) radiation or dilute acid [1].

Due to the similar electroactive nature of AA and HQ, may resulting in an overlapping voltammetric response in complex media such as cosmetic cream where interferences are usually possessing. The different modalities of sensor development are still required to diminishes the peak potential corresponding to the oxidation of Ar, AA2G, HQ and their derivatives and solves peak overlapping problems in complex samples. Therefore, chitosan is an interested choice for using as Ag nanoparticles (Ag NPs), Ag_2S-chitosan and multiwall carbon nanotubes (MCNTs) for simultaneous determination of Ar, AA2G, HQ and their derivatives because their unique properties that mentioned above. Based on the synergetic effect of Ag_2S-chitosan, Ag NPs and CNTs, the simultaneous determination of AA2G, HQ and Ar is possible.

2. Experimental Section

2.1 Reagents and Materials

Arbutin, hydroquinone, ascorbyl glucoside, chitosan, multi-walled carbon nanotubes and remaining chemicals were purchased from Sigma-Aldrich (USA) except for silver nitrate as a product of Riedel-de Haën (Germany). All the chemicals were of analytical grade and used as received. Aqueous solutions were prepared by deionized water. Different pH-phosphate buffer solutions (0.1 $molL^{-1}$) were prepared from 0.1 $molL^{-1}$ NaH_2PO_4 and 0.1 $molL^{-1}$ Na_2HPO_4 with the pH adjusted by 1$molL^{-1}$ H_3PO_4 or 1 $molL^{-1}$ NaOH.

2.2 Apparatus and Instruments

Voltammetric experiments were performed using an Autolab PGSTAT204 potentiostat (Metrohm Autolab B.V., Utrecht, Netherlands) operated with the NOVA 2.1 software from the same manufacturer. The electrochemical cell included a three-electrode system with a modified or unmodified GCE as working electrode, an Ag/AgCl (vs. Ag/AgCl, 3 mol L^{-1} KCl) as the reference, and a platinum wire as the auxiliary electrode. All pH values were measured with a digital pH meter (model 1230, Orion).

The UV-2450 UV-vis spectrophotometer (Shimazu, Japan) and Zetasizer Nano ZS (Malvern, MA, USA) were used for the characterization of Ag_2S-chitosan. The modified materials were examined by the ZEISS GeminiSEM – Field Emission Scanning Electron Microscope (SEM) and Oxford Instruments X-act EDX system (Zaventem, Belgium) the Transmission Electron Microscope (TEM) FEI Tecnai G^2 20 TWIN Transmission Electron Microscope (Málaga, Spain)

2.3 Synthesis Ag_2S NPs capped by Chitosan

The chitosan suspension was prepared by solubilizing chitosan (1.0 g) in acetic acid (50 mL, 1.0 wt %) solution. Then, $AgNO_3$ (5 mL, 0.01 M) was added immediately into the suspension under constant stirring for 30 min for preparation of the $AgNO_3$ in chitosan suspension. Then, 5.00 mL of 8 mg mL^{-1} freshly prepared $Na_2S.9H_2O$ was added quickly to the above mixture and then stirred for another 90 min. The resulting Ag_2S NPs were kept in a refrigerator at 4 °C before use.

2.4 The Fabrication of the Ag@Ag_2S/ MWCNTs/glassy carbon electrode

The glassy carbon as working electrodes were polished with alumina powder (0.5 um and 0.03 um), then washed with DI water, ethanol and dried at room temperature. Then 3.0 μL of the 0.1%(w/v) MWCNT in DMF was dropped onto the surface of the GCE, left to dry for 3 hours in air. Subsequently, 3.0 μL of Ag_2S NPs was dropped onto the modified electrode. The electrodeposition process was performed by applying a constant potential range of -0.8 to 1.35 V (Ag/AgCl) at a scan rate of 20 mV/s on a MWCNT/GCE for 15 cycles in 5.0 mL of mixture of 1%(*v/v*) ascorbic acid in 0.1 M KCl containing 0.5 $mmolL^{-1}$ $AgNO_3$. Then, the electrodes were thoroughly washed with water and dried in air. Finally, Ag@Ag_2S/MWCNTs/GCE electrode was obtained.

2.5 Electrochemical Measurement

The CV experiments were carried out from -0.3 to 0.5 V at different scan rates (10 to 400 mVs^{-1}) in 0.1$molL^{-1}$ phosphate buffer (pH7) containing appropriate amounts of the two analytes. Differential pulse voltammetry (DPV) was performed with a scan rate of 10 mVs^{-1}; pulse amplitude = 50 mV; pulse width = 250 ms; sample width = 0.0167 s; pulse period = 1.0 s; quiet time = 2 s and range potential = -0.10 to 0.45 V vs Ag/AgCl. The impedance measurements were performed in 0.1 $molL^{-1}$ KCl containing the $Fe(CN)_6^{3-/4-}$ redox couple (5.0 $mmolL^{-1}$ of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$) with the frequency varying from $1.0x10^{-3}$ to $1.0x10^{5}$ Hz and a signal amplitude of 5.0 mV.

2.6 Sample Preparation

The commercial essence and cosmetic cream (Nakornratchasima, Thailand) samples were used to demonstrate the applicability of the developed sensors. For the cosmetic cream, 0.5 g of the sample was sonicated in 5.0 mL of 10%(v/v) ethanol in 0.1 M phosphate buffer (pH 7) for 15 min. A portion of the supernatant (0.5 mL) was diluted to 5 mL in 0.1 molL^{-1} PBS and used for the measurement without filtration.

2.7 Electrochemical behavior of analytes at Ag@Ag_2O/MWCNTs/GCE

The Ag@Ag_2S/MWCNTs/GCE were connected to the respective terminals of the AUTOLAB electrochemical analyzer for measuring voltammetric response. In all such experiments, 5.0 mL of supporting electrolyte solution was placed in the electrochemical cell. Cyclic voltammetric (CV) experiments were performed in the potential range -0.8 V to +1.0 V with a scan rate of 100 mVs^{-1} unless otherwise stated. Differential pulse voltammograms (DPVs) were recorded between -0.1 and +1.0 V for different concentrations of HQ, Ar and AA2G standard solutions in 0.1 molL^{-1} phosphate buffer (pH 7.0) under following instrumental parameters as mention above. The oxidation peak of HQ at +0.05 V, Ar at +0.51 V and AA2G at +0.77 V obtained in DPV was used for quantification. All electrochemical measurements were carried out at room temperature (25 ± 2 ˚C). The analyte quantitation achieved by measuring the peak current and the standard addition method was used to evaluate the content of all analytes in real samples.

3. Results and Discussion

3.1 Silver sulphide stabilized by chitosan characterization

According to the study of the optimum condition of Ag_2S stabilized by CS, it has been identified by the transmission electron microscope (TEM) and UV-vis spectrophotometer. The synthesized Ag_2S stabilized by chitosan has the average size ≈ 600 nm and with a negative charge (measured with the Zetasizer Nano ZS), which indicates that the surface area of AgS does not have Ag^+ remaining. Based on the study of Ag_2S / CS synthesis conditions, it was found that The optimum condition for the synthesis of Ag_2S/ CS is the mole ratio of Ag:S is 2:1, temperature 40 ºC, based on the study of the amount of chitosan (CS) in the range of 0.1-1.0% (w/v) and taken to measure the spectrum. The absorption of light showed that Ag_2S had a peak shoulder at 300 nm, which related to the amount of Ag_2S. The changing the amount of CS from 0.25-1.0% (w/v) did not affect the particle size and amount of Ag_2S. Ag_2S stabilized by chitosan showed a peak at 300 nm, indicating Ag_2S nanoparticles with small particles. After added ascorbic acid at different concentration level, the Ag_2S spectrum exhibited a surface plasmon band position at ~402 nm (Figure 2), resulted from the generated of Ag nanoparticles on Ag_2S surface [2]

The absorption spectrum of Ag_2S, it was found that it was similar to previous research and when adding ascorbic acid to Ag_2S, it was found that the absorbance spectrum of

Ag_2S had a peak of 390 nm, indicating ascorbic can reduce Ag_2S to nano-silver (AgNPs) as shown in Figure 3.

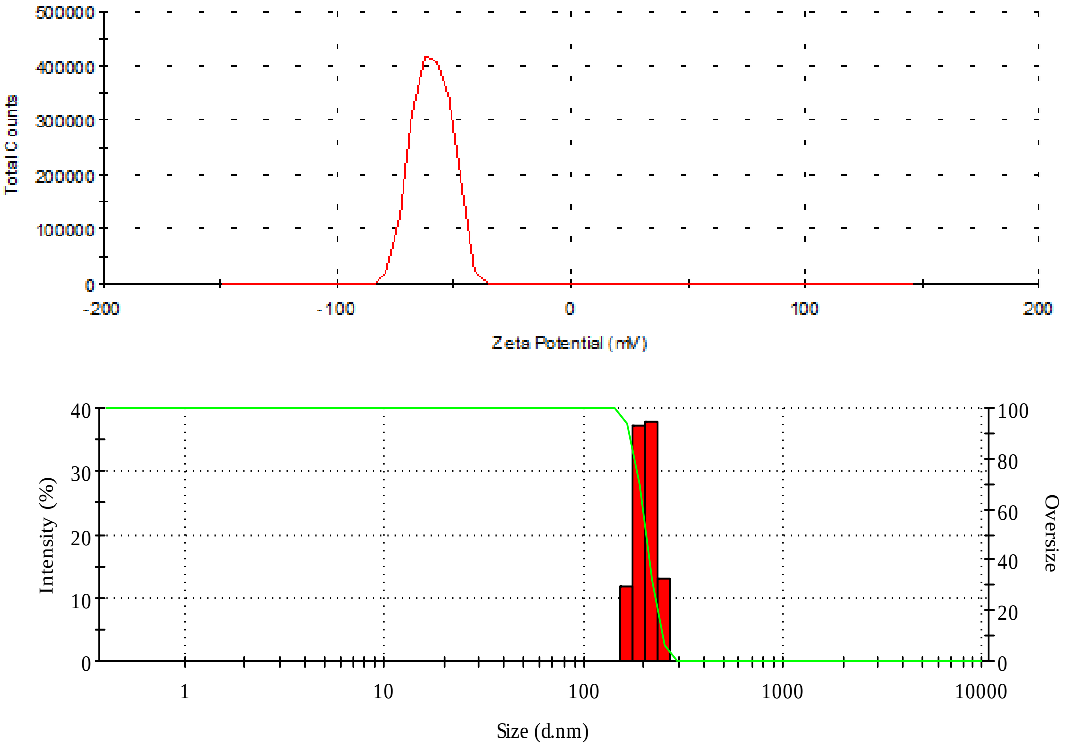

Figure 1 The zeta potential of Ag_2S stabilized by chitosan The size distribution of Ag_2S stabilized by chitosan

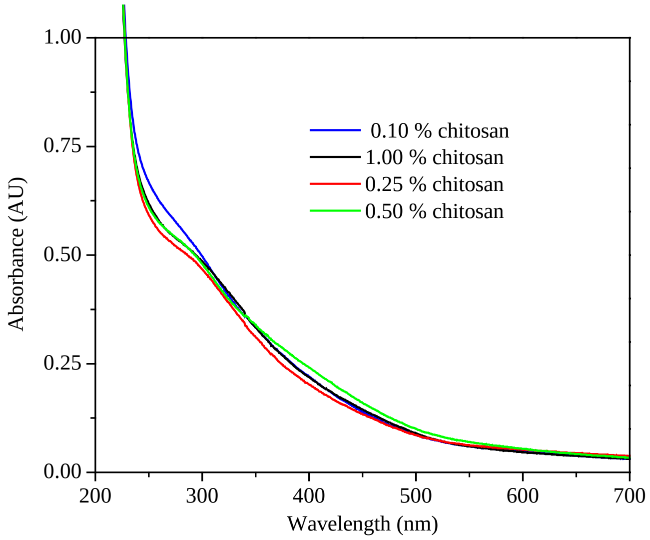

Figure 2 The absorption spectra of Ag_2S using different concentration of chitosan

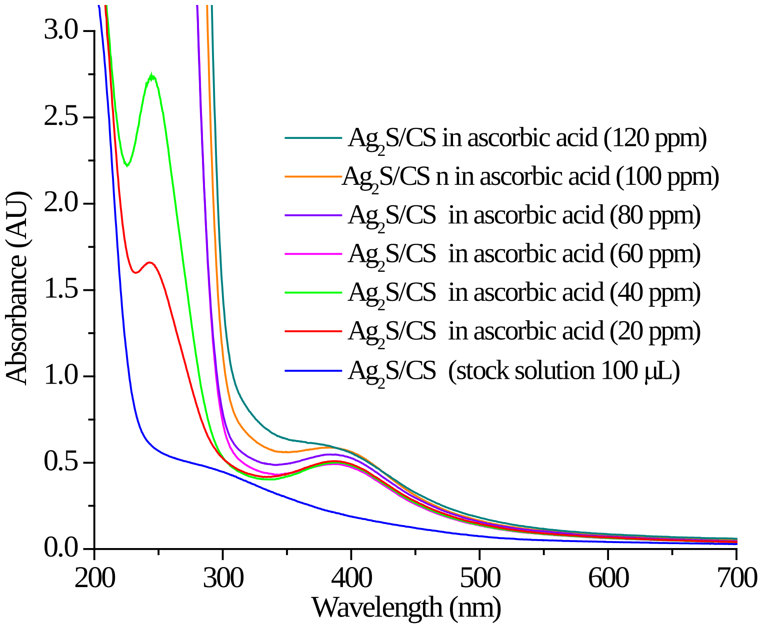

Figure 3 The absorption spectra of Ag_2S (10 times dilution) and Ag_2S in the presence of ascorbic acid at different concentrations.

Conferring to TEM, it was found that Ag_2S has a round shape with a particle size of 2-10 nm (Fig. 4). The Ag_2S stabilized by chitosan have been successfully synthesis, confirming by all characterization results.

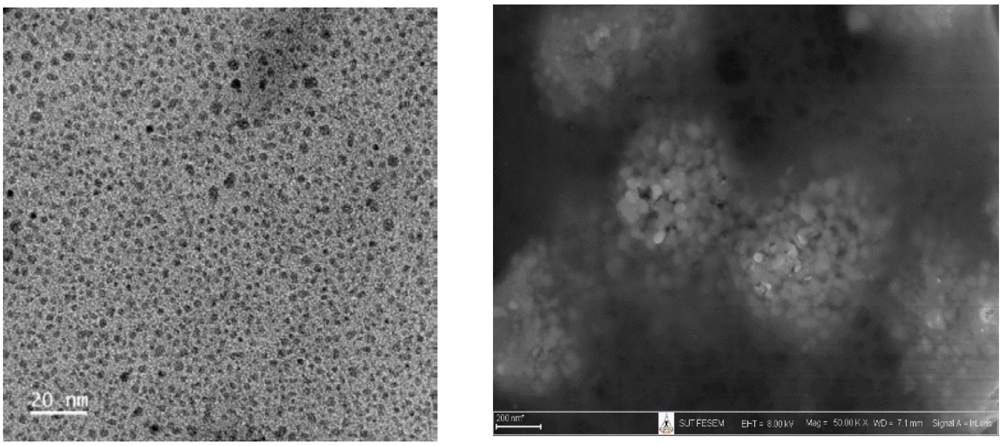

Figure 4 Picture of TEM showing the characteristics of synthetic Ag_2S (diluted 20 times) and SEM image of AgNPs/A_2S/MWCNTs

3.2 Electrochemical Behavior of the modified electrode

Normally Ar and AA2G could not detected by MWCNTs and bare GCE, therefore different approaches have been proposed to solve this problem. In this work, the GCE are modified with various materials (as shown in Fig. 5) by the layer by layer drop casting method. Then, the modified electrodes were used for studying the electrochemical behavior of the standard solution of HQ, AR and AA2G measuring the cyclic voltammetry (CV). The oxidation peaks of HQ and AR was 0.3 V and 0.7 V, respectively while no oxidation of peak of AA2G for all modified electrode. Beside, both Ag NPs and Ag_2S NPs affected the signal of HQ and AR by their catalytic property, but the oxidation peak of HQ and Ar shifted towards more positive. These should be derived from the influence of electrical conductivity properties that are less than Ag NPs and chitosan on the electrode surface. In the case of Ag_2O, it was found that the current signal obtained from both HQ and AR decreased with no peak of AA2G as shown in Figure 5.

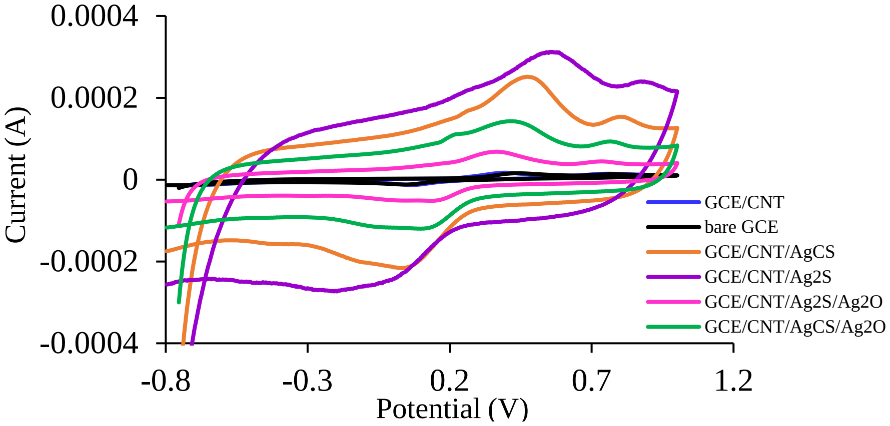

Figure 5 Cyclic voltammogram compares signals obtained from the simultaneously analysis of HQ AR and AA2G using various modified electrodes in 0.1 M phosphate buffer (pH 7)

From the results of Ag NPs effect and the possible to generate Ag NPs on Ag_2S NPs by ascorbic acid reducing, it was interested to modify the electrode by drop casting Ag_2S and MWCNTs first and then using the electrodeposition to modify it with Ag NPs by running CV from - 0.8 to 1.2 V in 1 %(*w/v*) ascorbic acid, comparing with 1 %(*w/v*) ascorbic acid solution containing 10 mM $AgNO_3$. The obtained CV of the modified electrodes in simultaneously analyze HQ, AR and AA2G are shown in Fig. 6, it is found that all 3 analytes can be measured in both different modification methods. However, it was found that the peak of AR and AA2G increased while the peak of the HQ decreased after added Ag NPs on Ag_2S NPs, indicating the modified material has an effect on memory because of the specificity of the Ag NPs. Therefore, the AgNPs/Ag_2S/MWCNTs/GCE was chosen as the electrode for simultaneous determination of HQ, Ar and AA2G.

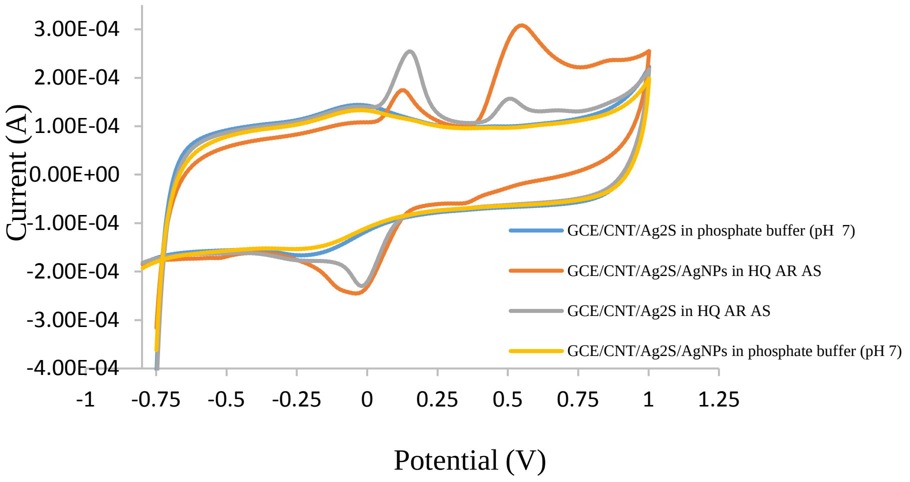

Figure 6 CV response of HQ, AR and AA2G analysis using electrodes AgNPs/Ag_2S /MWCNTs/GCE compared with Ag_2S /MWCNTs/GCE

The peak potentials of HQ and Ar shifted towards more negative when the pH increased from 4.0 to 8.0, confirming the oxidation of these compounds involved the proton-transfer process while smaller effect was observed in AA2G (Fig. 7A and B). The slope of the oxidation potential versus pH plot was 38.4 VpH^{-1} for HQ and 40 VpH^{-1} for Ar, respectively. Such values were slightly different from the theoretical value of 58.5 $mVpH^{-1}$ for the redox reaction of a two-electron coupled two proton transfer process [3]. Meanwhile, the AA2G is the adsorption mechanism due to its poor electrochemical activity. The oxidation potential versus pH plot was 21 VpH^{-1} which related to one proton transfer process [4].

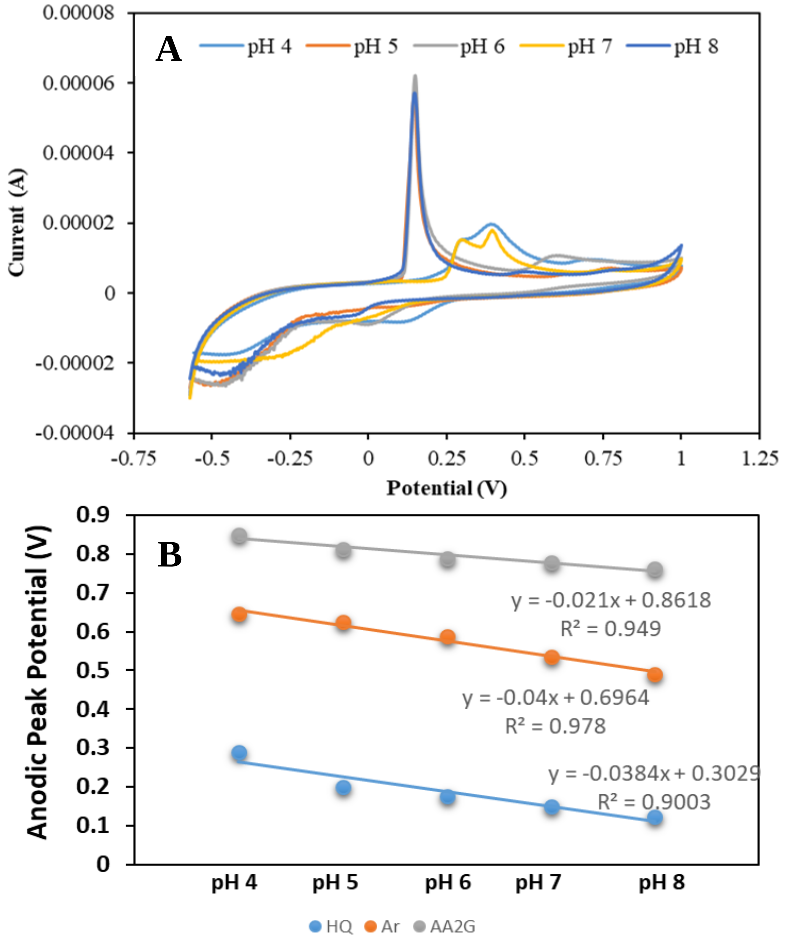

Figure 7 Influence of pH on the oxidation peak current and potential of 400 ppb of all analytes with pH values ranging from 4.0–8.0.

The resistance of the charge transfer on the electrode surface (R_{ct}) is represented as a semicircle diameter in the Nyquist diagram. The EIS of all modified electrode compared with those bare GCE in in 0.1 molL^{-1} KCl containing the $Fe(CN)_6^{3-/4-}$ redox couple (5.0 mmolL^{-1} of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$) are shown in Fig 8. The bare GCE presented a semicircle (~290 W) domain as expected for a very low electron transfer resistance to the redox probe (Figure 5A). After modification with CNTs, the slope of the line part increased drastically together with a small semicircle (about 63.6 W). Such behavior evinced the presence of a high electron conduction pathway between the electrode-electrolyte interface to enable the diffusion of an electron on the electrode surface. The semicircle was hardly visible for the AgNPs/CNTs/GCE with R_{ct} of ~33.5 W while the semicircle was very broad in Ag_2S/CNTs/GCE which can be attributed to the presence of Ag_2S-chitosan. After decorated with the AgNPs, the AgNPs/Ag_2S/CNTs/GCE exhibited a pronounced decrease in the interfacial resistance with 2 semicircles. Apparently, two semicircles observed in the high frequency domain describes the resistive/capacitive behavior associated with the electron transfer (faradaic process) coupled with the double layer charging process [5].

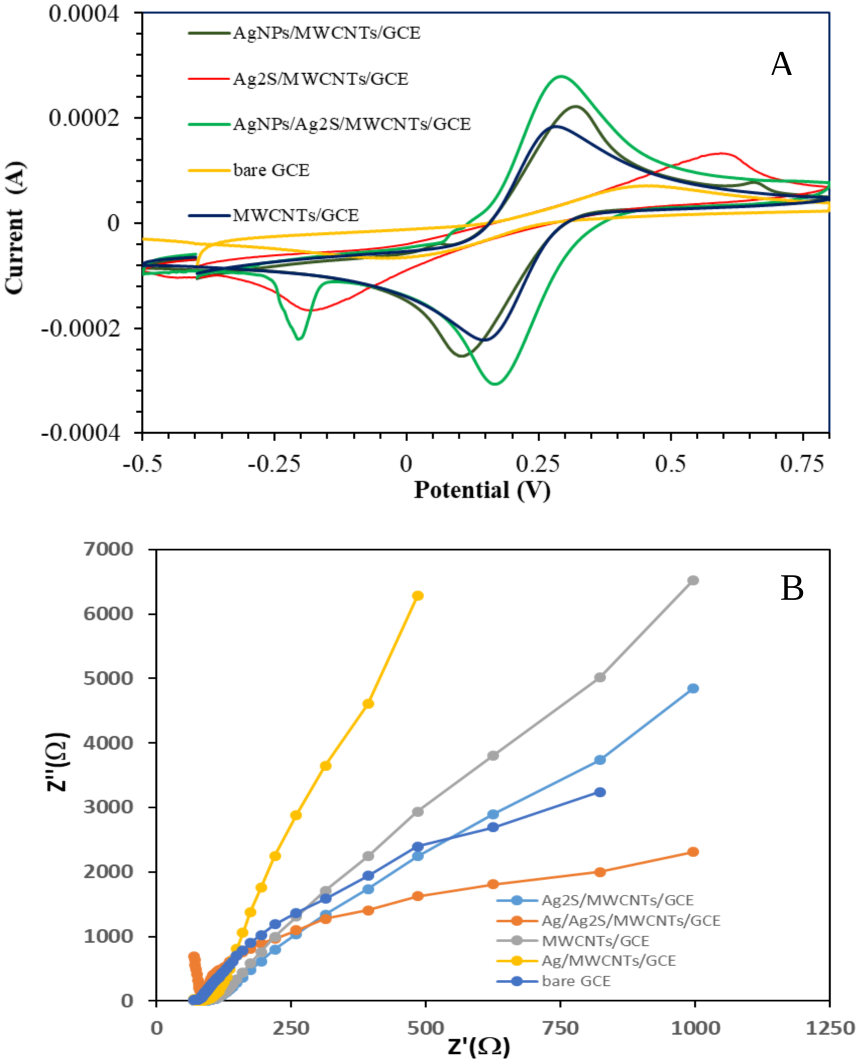

Figure 8 Cyclic voltammograms (A) and nyquist plot (B) of the bare GCE and the modified GCEs in 0.1 M KCl solution containing 5.0 mM $Fe(CN)_6^{3}$/$Fe(CN)_6^{4}$ (1:1).

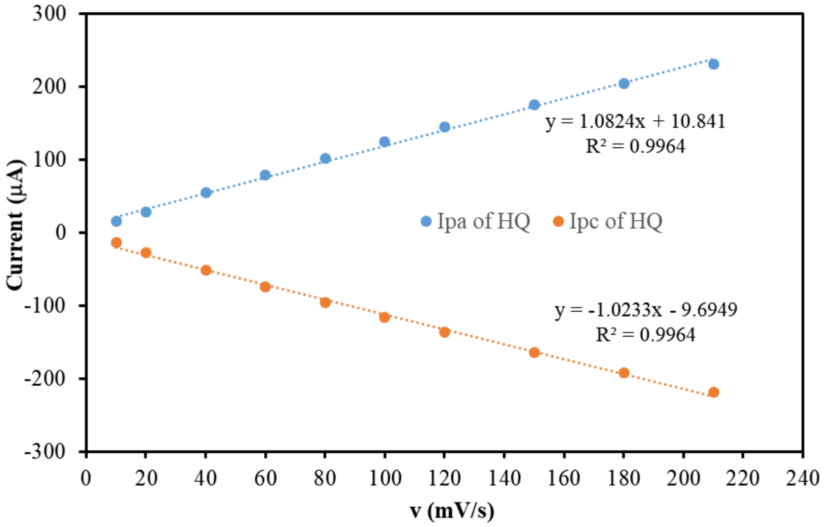

Figure 9 Effect of the scan rate influence on the current response of hydroquinone using AgNPs/Ag_2S/MWCNTs/GCE

The influence of the scanning rates on CV response of Ag/Ag_2S/MWCNT/GCE in 0.1 $mmolL^{-1}$ phosphate buffer solution (pH 7) containing HQ, AR and AA2G (200 ppb) was also evaluated (see Fig. 10). Only HQ shows the reversible redox reaction, therefore, it has been used to evaluate the reversible electron transfer phenomenon in more detail. Figure 9 is the corresponding calibration curve and linear plot of the peak current (I_p) versus the scanning rates on HQ, peak. The peak current I_p is proportional to scanning rate **V**, signifying the dominance of the adsorption process.

$$I_p = \frac{n^2F^2vA\tau_c}{4RT}$$

where *A* is the electrode-area; *n* is the number of electrons; *R* is an ideal gas constant, *T* is the temperature in Kelvin; *F* is Faraday's constant; *V* is the scan rate and τ_c is the surface-coverage of the Ag/Ag_2S/MWCNT on the electrode-surface. the τ_c for Ag/Ag_2S/MWCNT was calculated to be 4.08×10^{-2} mol cm^{-2} which indicated that Ag/Ag_2S/MWCNT owns much larger surface coverage, so it has a bigger area to come into contact with the analytes.

3.3 Electrocatalytic oxidation of Hydroquinone, Arbutin and Ascorbyl glucoside at Ag NPs/Ag_2S/MWCNTs/GCE

The influence of the scanning rates on CV response of Ag/Ag_2S/MWCNT/GCE in 0.1 mmolL-1 phosphate buffer solution (pH 7) containing HQ, AR and AA2G (200 ppb) was also evaluated (see Fig. 10). It shows that HQ, AR and AA2G anodic peak current increased when the scanning rates ranged from 10 to 210 mV s^{-1}. Increasing the potential scan rate shifts the peak towards the anodic potentials. A linear evolution of the peak potential versus the logarithm of the product “electrode surface area/thin layer volume/potential scan rate was observed (Fig. 10), showing the irreversible behavior of the system, excepted HQ.

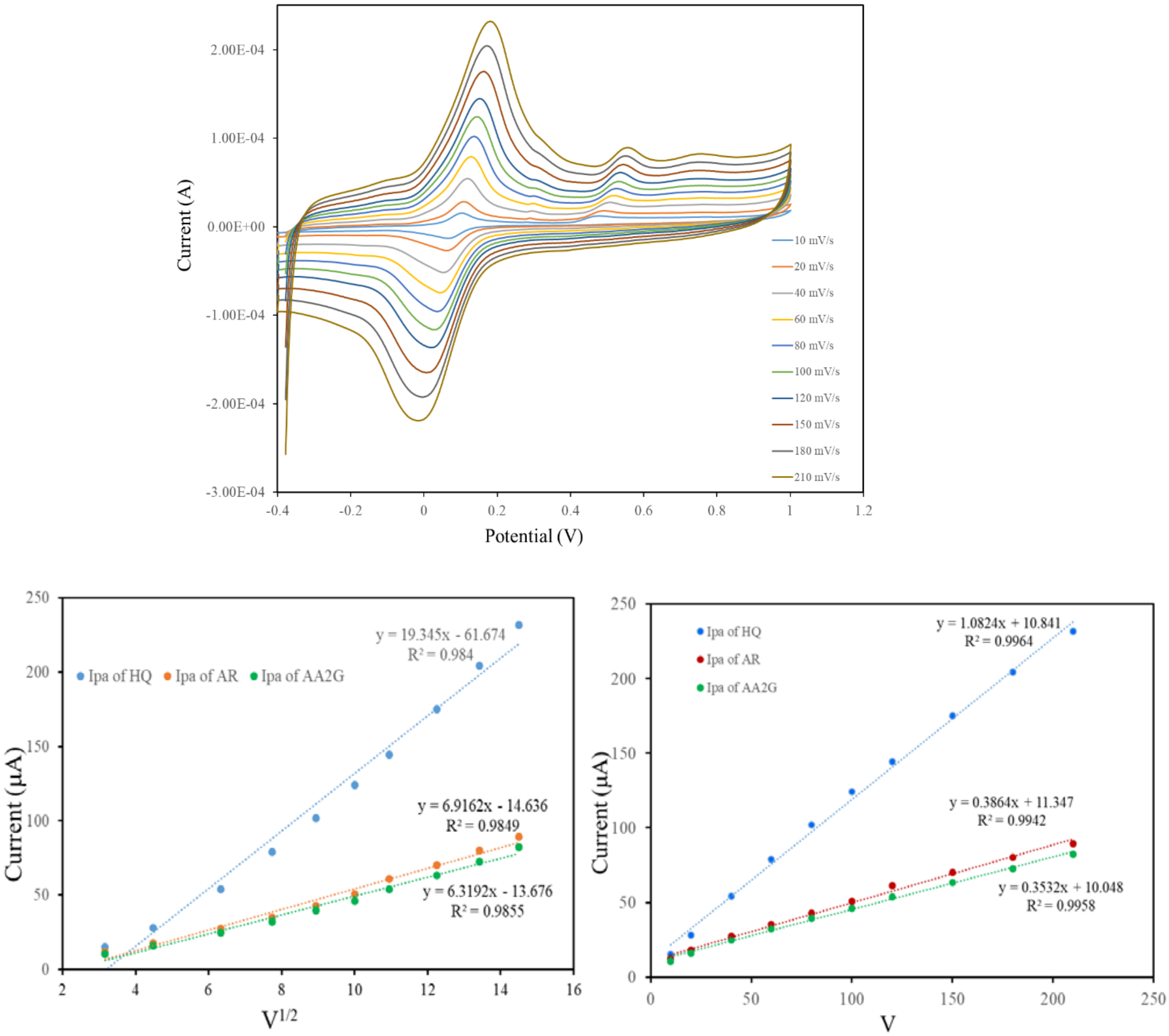

Figure 10 Cyclic voltammograms of the sensor at different scan rates (10-210 mVs^{-1}) in 5.00 mL of 0.1 M PBS (pH 7.0) containing 200 ppb of HQ, Ar and AA2G at room temperature and the relationship between I_{pa} versus V and $V^{1/2}$

Peak potential E_p was proportional to the logarithm of scan rate for $v \geq 0.1$ V s^{-1} (Fig. 11), indicating the limitation of charge-transfer kinetics. Based on the Laviron theory [6], the electron transfer coefficient α could be calculated. For anodic peaks, the slopes of E_p versus log V were 0.155 for HQ, 0.0596 for Ar and 0.0332 for AA2G, respectively. The calculated value of α were 0.19 for HQ, 0.99 for Ar and 1.78 AA2G which is assumed to be 0.5 in a totally irreversible electrode process. Thus Ar and AA2G were irreversibility while HQ was reversible process. According to the following equation

$$E_p = E^{\emptyset} + \frac{2.303RT}{(1-\alpha)nF}\log v$$

Irreversibility was also confirmed by the absence of cathodic signals for Ar and AA2G during the reverse scan in the potentials range of -0.8 to 1.0 V.

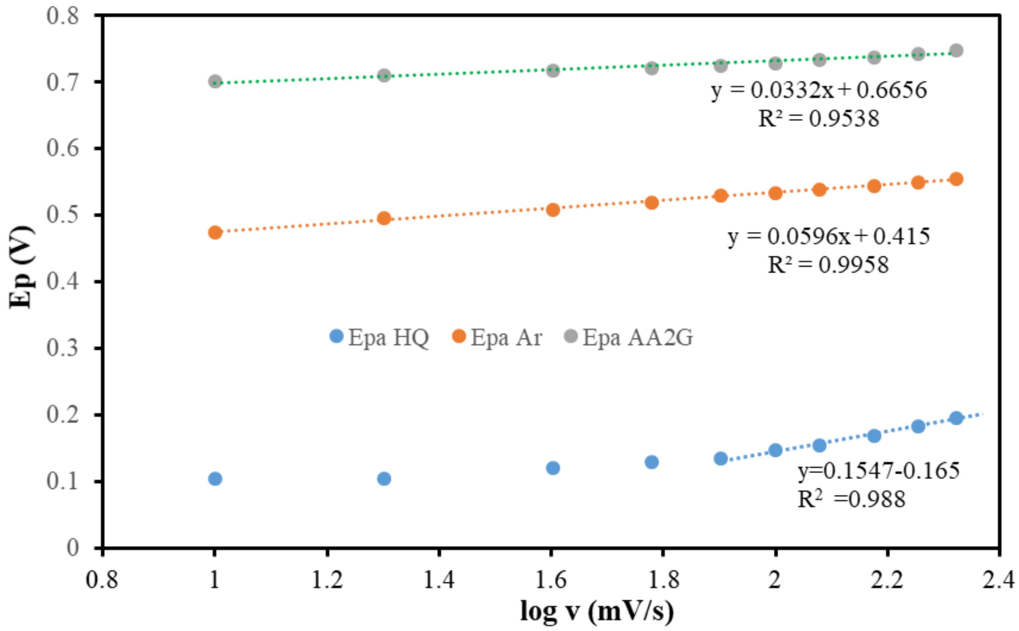

Figure 11 Laviron's plot for the dependence of peak potentials of each analyte on logarithm of scan rate for cyclic voltammograms presented in Fig. 10.

3.4 Analytical performance of the modified electrode

Modified electrodes were studied by DPV and the individual peak of the standard HQ (0.08 V), Ar (0.47 V) and AA2G (0.76 V) at 50 ppb concentration level are shown in Fig. 12 (upper). The electrochemical behaviors of the analytes using different modification electrode substrates was also studied to explain several phenomena occurring at chemically modified electrodes. As expected, the anodic peak of HQ, Ar and AAG peaks occurred in the AgNPs/MWCNTs/GCE and AgNPs/AgS/MWCNTs/GCE. However, the peak currents increased a 5-fold on the AgNPs/Ag_2S/MWCNTs/GCE, compared to the Ag_2S/MWCNTs/GCE, indicating the assisted electron transfer of Ag_2S. In addition, the anodic peak potential of HQ also shifted from 200 mV to 80 mV when using the combined of AgNPs and Ag_2S to modify the GCE (Fig. 12). Albeit Ar and AA2G peak was not observed in both MWCNTs alone and bare GCE. After modified MWCNTs by Ag NPs or chitosan it was found that Ar and AA2G can be detected but AgNPs/MWCNTs is much more effective than chitosan/MWCNTs.

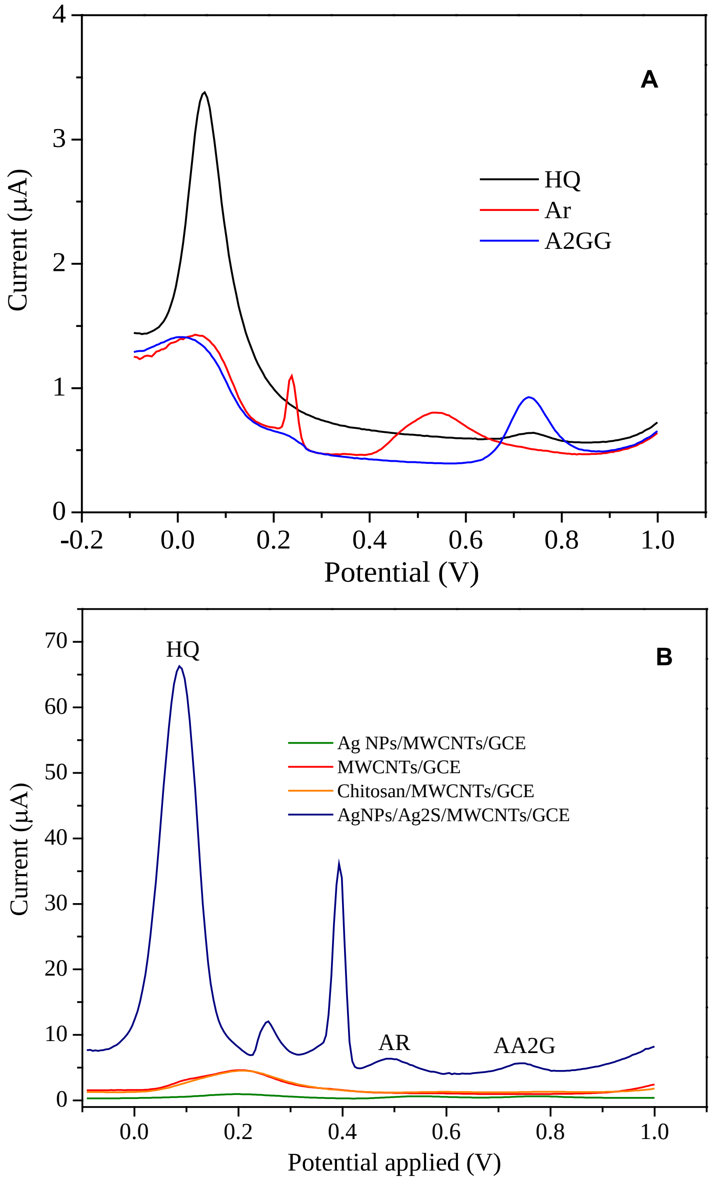

Figure 12 DPV of HQ Ar and AAG using AgNPs/AgS/MWCNTs/GCE and DPV obtained from the simultaneous analysis of HQ Ar and AA2G (concentration 100 ppb) using the different surface-modified substrates on GCE.

The effect of pH on the anodic peak current of all analytes in differential pulse voltammetry (DPV) mode was also studied as shown in Fig. 12A. Notice also that the anodic peak currents after the blank subtraction of HQ and Ar increased with an increase in pH (Fig. 12B). Whereas, the peak current of AA2G was quite stable at pH 4 to 7 and then sharply decreased with increasing pH from 7.0 to 8.0. It was reasoned that the AA2G was adsorbed on electrode surface at high pH [7]. Consequently, the pH 7.0 was chosen for the simultaneous detection of HQ and CT to achieve the compromised between sensitivity and selectivity of all analytes.

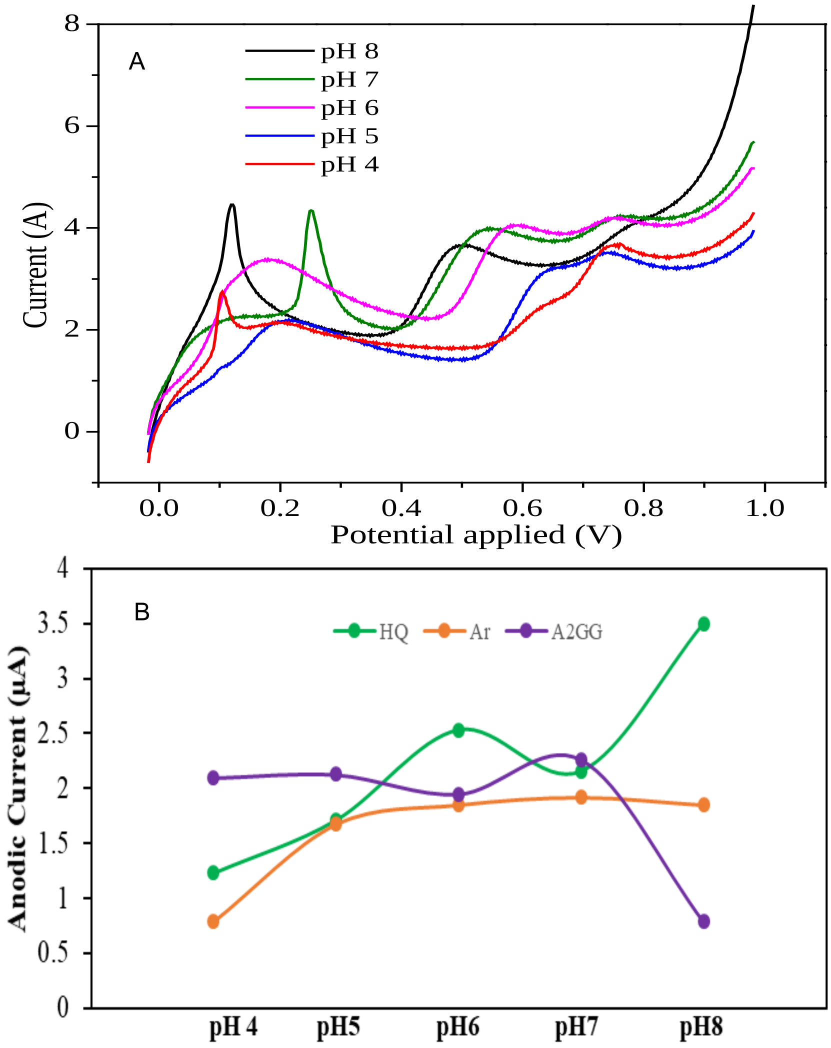

Figure 13 Influence of pH on the oxidation peak current and potential of 50 ppb of all analytes with pH values ranging from 4.0–8.0.

Based on the voltammetric results and the described DPV above, DPV response was recorded to estimate the calibration curve and the detection limit for all analytes determination at AgNPs/Ag_2S/MWCNTs/GCE. The typical analytes DPV during the successive addition of standard solution of analyte into 5.0 mL 0.1 M phosphate buffer solution (pH 7) is shown in Fig. 13. As shown, a well-defined DPV response of each analyte was observed, demonstrating stable and efficient catalytic ability of AgNPs/Ag$_2$S/MWCNTs/GCE. The anodic current was linear with the concentration from ppb to hundred-ppb level with a slope and linear correlation coefficient as shown in Table 1.

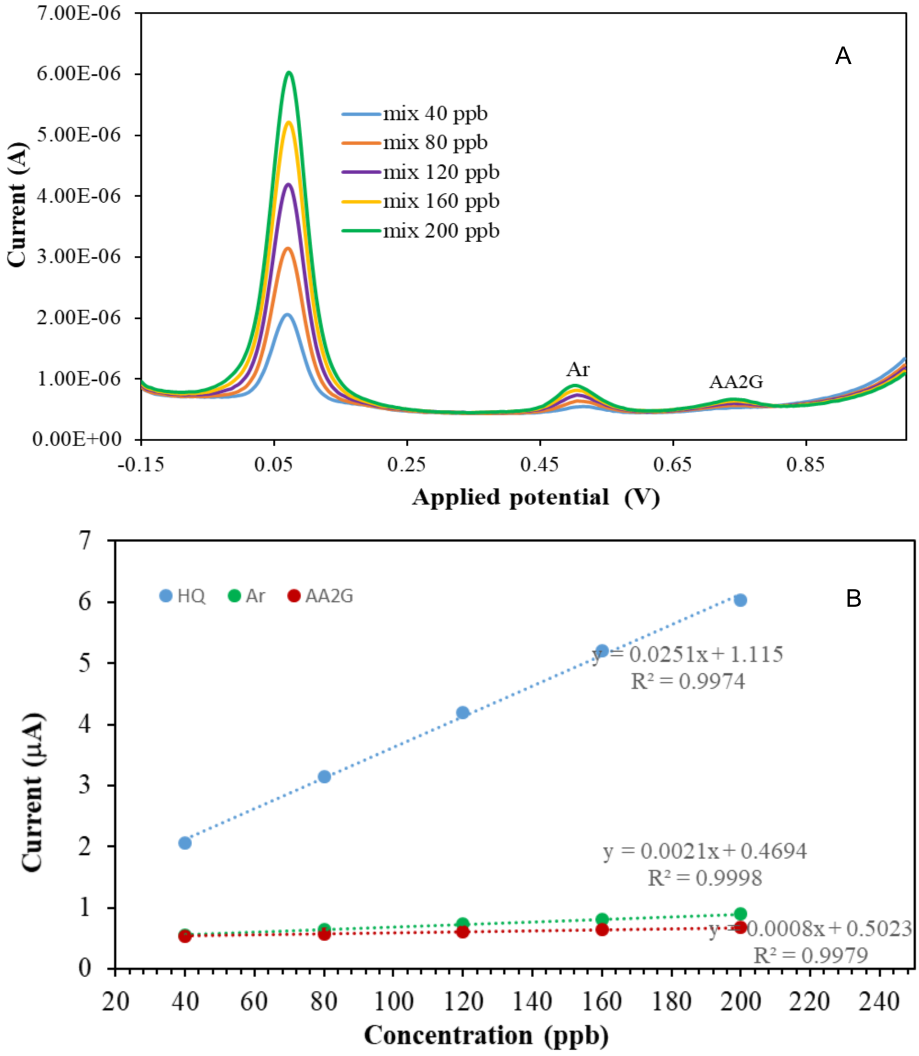

Figure 14 DPV of AgNPs/Ag$_2$S/MWCNTs/GCE vs. Ag/AgCl/KCl upon successive determination of different concentration of analyte in 0.1 M phosphate buffer, pH 7.0 (A) andanodic current responses vs. analyte concentration (B)

Table 1. Quantitative analytical parameters

Analyte	**Linear range**	**slope**	**R^2**
Hydroquinone	40-300	0.0251	0.9974
Arbutin	40-350	0.0021	0.9998
Ascorbyl glucoside	40-350	0.0008	0.9976

The oxidation peak currents of all analytes were linear with the concentration in the range of sub ppm to ppm as shown in Figure 15. The detection limit (S/N=3) for 0.2 μM for HQ, 0.1 μM for Ar and 0.25 μM for AA2G.

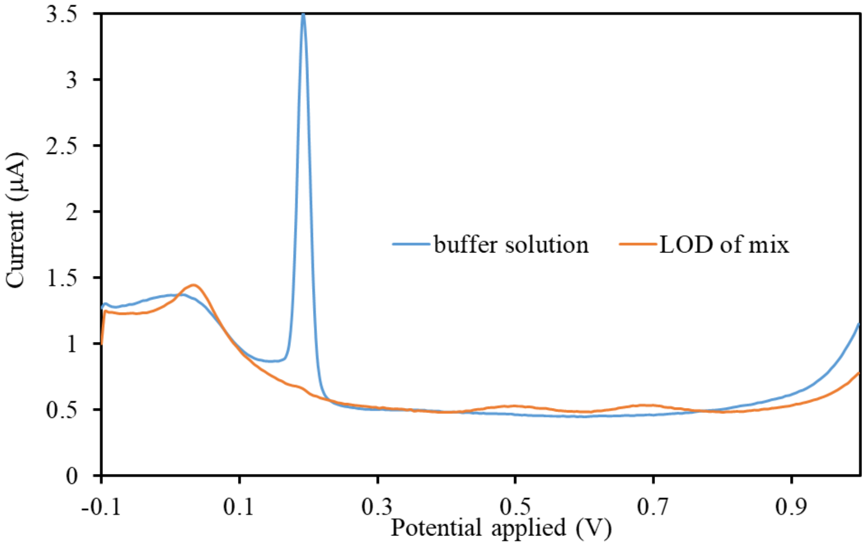

Figure 15 Limit of detection for simultaneous determination of all analytes

Repeatability for the simultaneous analysis of HQ, Ar and AA2G (each 100 ppb) was studied for 3 replicates (Fig. 16). The relative standard deviation (RSD) of the anodic peak current was 6.2% for HQ, 6.3% for Ar and 5.8 for AA2G. The stability of the AgNPs/Ag_2S/MWCNTs/GCE was also examined using the DPV signals of HQ and CT (each 40 ppb) as shown in Fig. 17. The signals from the analysis of the three substances was changed. While the other two types are reduced when reusing the electrode, the signal of HQ increased due to its adsorption onto the electrode surface.

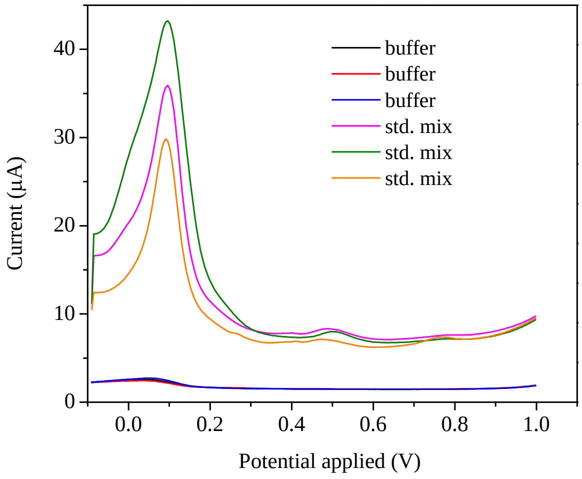

Figure 16 DPV of three replicated determination of mix standard solution of the same electrode

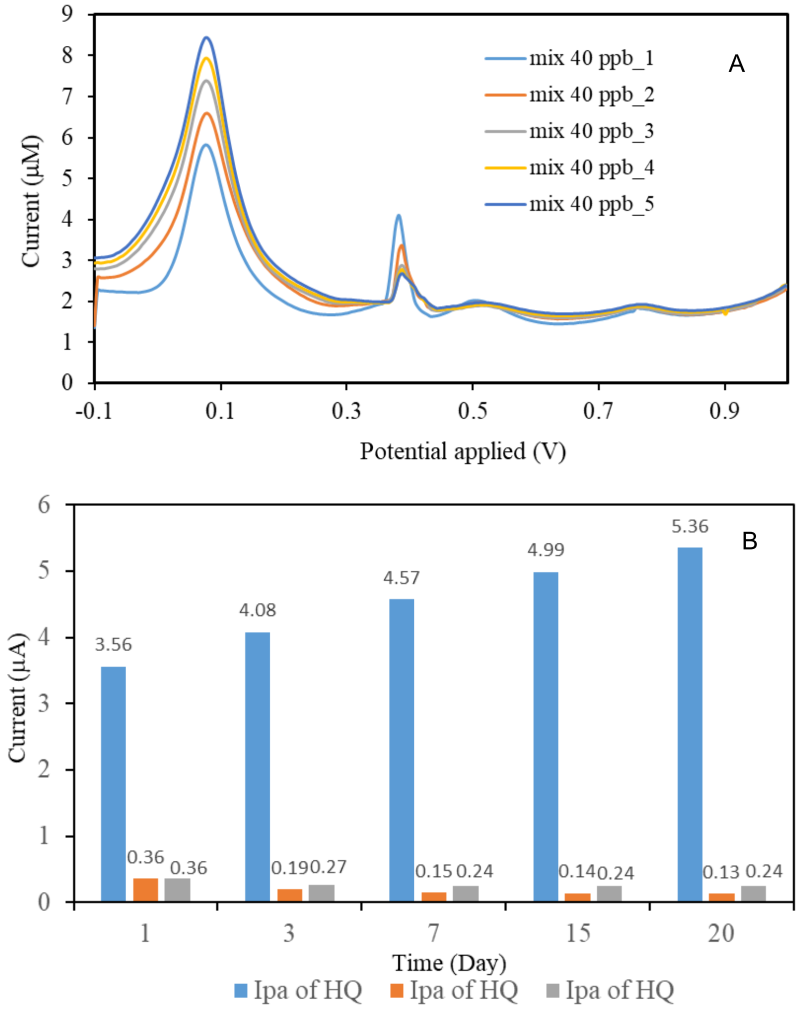

Figure 17 Stability of the electrode that used within 20 days (50 ppb in each analyte)

The stability of the analyte signal when changing the concentration of a particular substance are important in confirming that this method can analyze all three substances simultaneously. According to the results of changing the concentration of the substance while the concentration of the other two substances is fixed as shown in Fig. 18 and Table 2. When varied concentration of HQ, the Ipa of other was not significantly changed. With the increasing in concentration of Ar, the I_{pa} of HQ gradually increased while AA2G decreased. In the case of AA2G, only the Ipa of Ar was significantly increased.

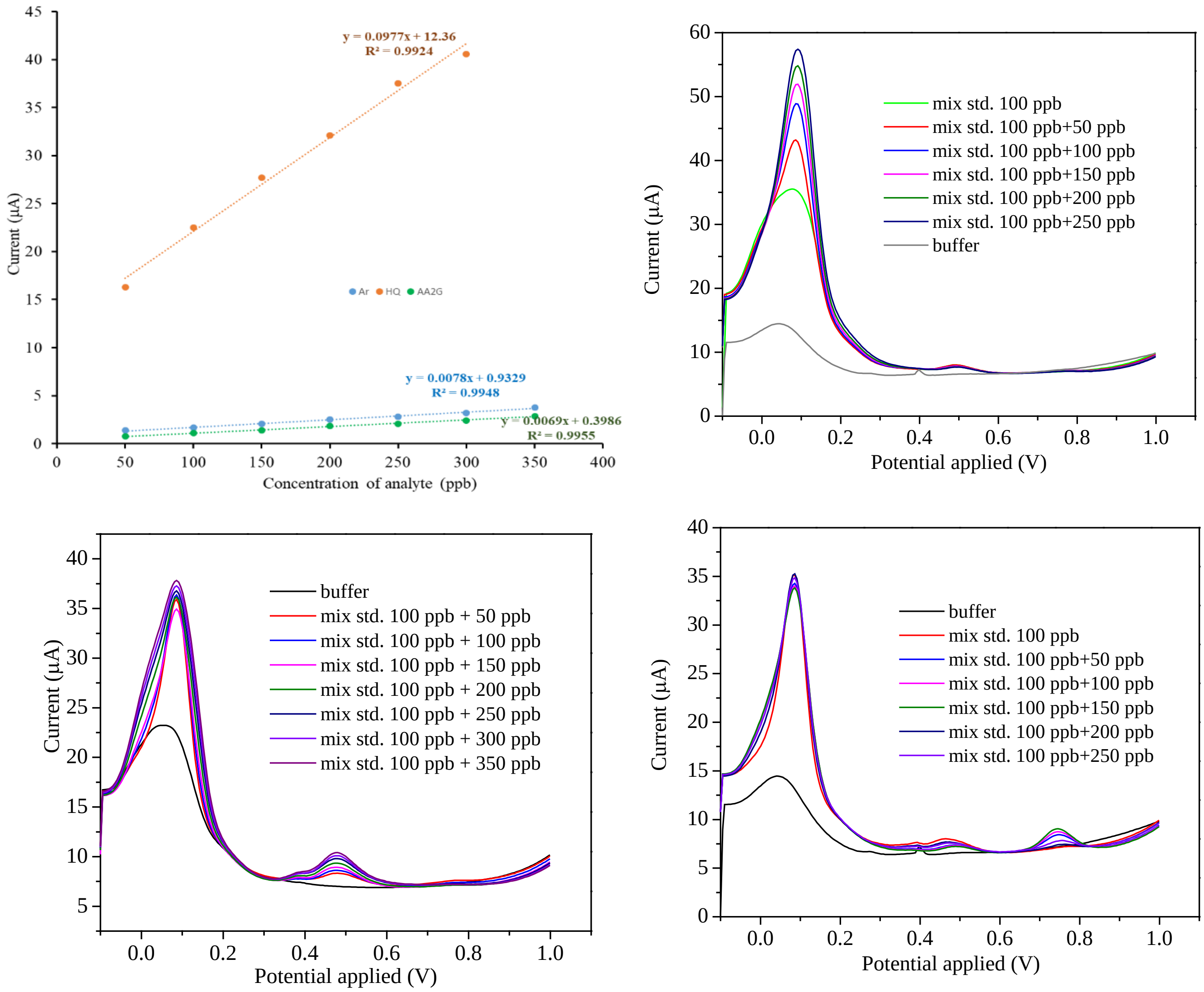

Figure 18 The stability of current signal while varied concentration of an analyte (continue).

Table 2 The changing of the anodic current of the analytes while varied concentration of an analyte

Varied HQ concentration			
I_{pa} of Ar at 50 ppb	**change of signal**	**I_{pa} of Ar at 50 ppb**	**change of signal**
0.43	0.2	0.43	0.01
0.33	-0.1	0.33	-0.01
0.32	-0.11	0.32	0.12
0.24	-0.19	0.24	-0.1
0.23	-0.2	0.23	-0.18
Average = 0.33 (±0.07)		Average = 0.34 (±0.05)	
Varied Ar concentration			
I_{pa} of HQ at 50 ppb	**change of signal**	**I_{pa} of AA2G at 50 ppb**	**change of signal**
18	-1.3	0.36	0.01
18.8	-0.5	0.34	-0.01
20	0.7	0.47	0.12
20.4	1.1	0.25	-0.1
20.9	1.6	0.17	-0.18
Average = 19.8 (±1.16)		Average = 19.8 (±1.16)	
Varied AA2G concentration			
I_{pa} of HQ at 50 ppb	**change of signal**	**I_{pa} of Ar at 50 ppb**	**change of signal**
19.7	-2.6	0.44	-0.03
19.3	-3	0.42	-0.05
19.1	-3.2	0.37	-0.1
20.7	-1.6	0.37	-0.1
20.3	-2	0.36	-0.11
Average = 20.23 (±1.18)		Average = 0.40 (±0.04)	

3.5 Effect of interference

It is well known that hydroquinone has a lot of derivatives in whitening cosmetic cream and ascorbic acid is the product of ascorbyl glucoside denaturation This research selects Kojic as a representative of the derivative of hydroquinone, because it is a whitening substance that have the properties similar to arbutin. The effect of these species was conducted by determination the current change of all analytes after spiking the standard Kojic acid (4 ppm) and ascorbic acid (4 ppm) As shown in Table 2, there is no significant change of current response of HQ and AA2G (less than ±5%) in both interferences, excepted Ar. At high concentration of kojic acid and ascorbic acid caused the Ar response significantly change; however, it takes place in 40 times concentrated of interference.

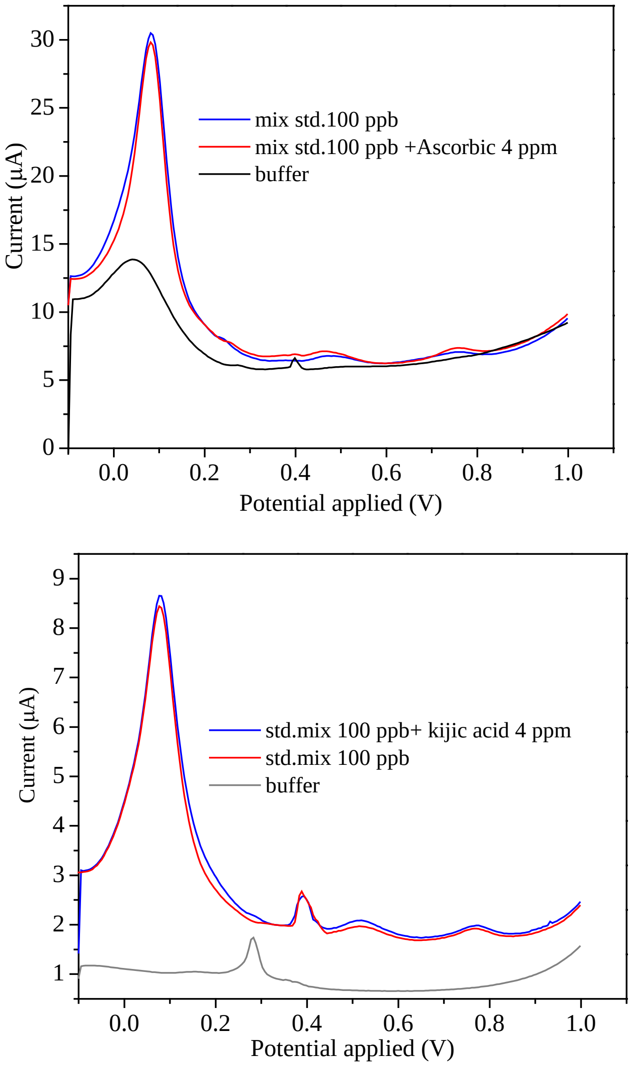

Figure 19 DPV of mix standard solution in the presence of kojic acid and ascorbic acid

Table 3 The changing of the anodic current of the analytes (100 ppb) the presence of kojic acid and ascorbic acid

Effect of Ascorbic acid at 4 ppm					
HQ	%change of I_{pa}	Ar	%change of I_{pa}	AA2G	%change of I_{pa}
17.9	+0.56	0.7	+50	0.35	2.86
Effect of Kojic acid at 4 ppm					
HQ	%change of I_{pa}	Ar	%change of I_{pa}	AA2G	%change of I_{pa}
5.56	+3.96	0.16	+25	0.2	-8.3

3.6 Application to in real sample analysis

The application of the proposed method for HQ, Ar and AA2G determination was established by determine them in cosmetic whitening cream and essence sample. A 0.5 g of cream and 0.5 mL of essence sample was diluted by 5 mL of 10%(v/v), 50%(v/v) and 75%(v/v) of ethanol in 0.1 M phosphate buffer solution (pH 7). The standard solution of all analytes were spiked into the samples. Then, the mixtures were sonicated for 15 minutes and then pipetted the supernatant to dilute 5 times by 0.1 phosphate buffer (pH 7) before determination by DPV and the results compared to the reference standard. From studying the optimum conditions for extracting HQ Ar and AA2G simultaneously in the cream sample, the recovery of the analyte was very poor, especially HQ. The best results for cream sample obtained in 75%(v/v) of ethanol in 0.1 M phosphate buffer solution (pH 7). Whereas, the essence sample has the highest percentage recoveries when using the solvent to extract 10% (v/v) ethanol in 0.1 M phosphate buffer solution as shown in Fig. 20.

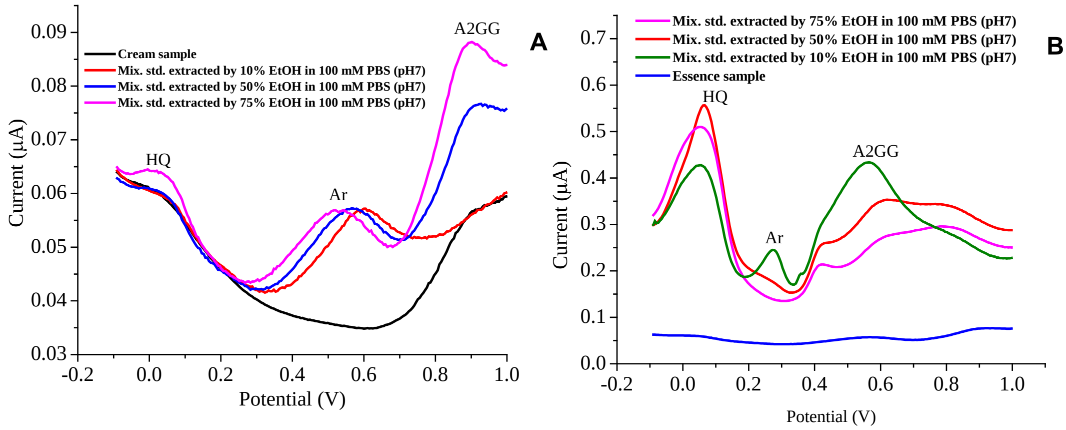

Figure 20 DPV signal obtained from the analysis of HQ, Ar and AA2G (100 ppb) in the cream sample and essence sample extracted under different concentation of ethanol in 0.1 phosphate buffer (pH 7)

The standard solution spiking technique with the background subtraction was used to determine the recovered amount of HQ, Ar and AA2G in essence and cream sample matrices and the recovery ranged from 94.8–106.3% for essence sample while the recovery of HQ in cream sample was very low because of the complicated matric in cream sample (Fig. 21 and Table 4).

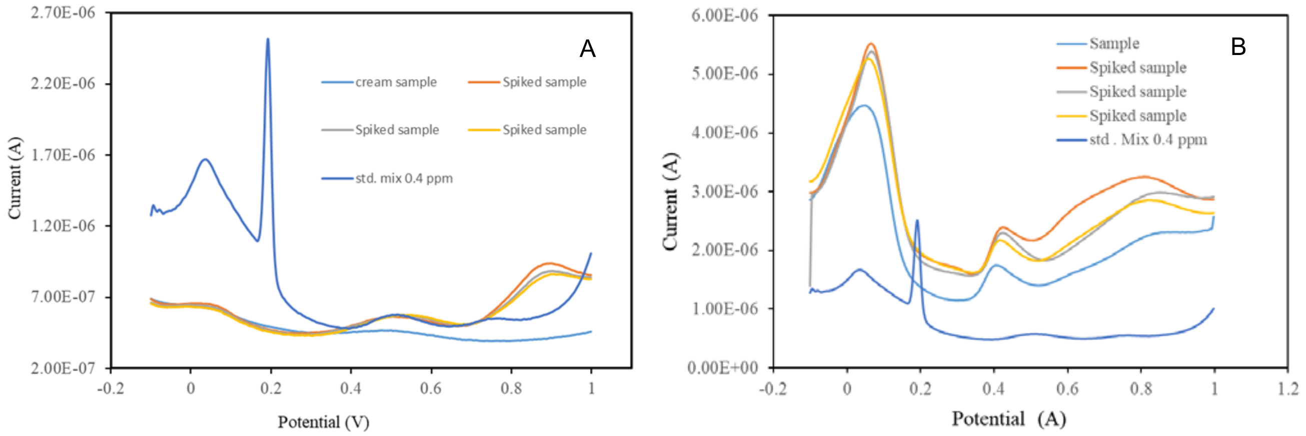

Figure 21 DPV signal obtained from the analysis of HQ, Ar and AA2G (100 ppb) in the cream sample and essence sample extracted under different concentation of ethanol in 0.1 phosphate buffer (pH 7)

Table 4 Percent recovery of analytes in cream and essence samples

Analyte	Recovery (Cream Sample)	Recovery (Essence sample)
HQ	9.19 (±0.27)	94. 8 (±8.07)
Ar	77.3 (±6.99)	105.9 (±1.83)
AA2G	86.1 (±8.81)	106.3 (±5.44)

According to the recovery study results, the cosmetic cream sample without any preconcentration and cleaning step was too complicated matric for this method. Therefore, the application of this proposed method was carried on the essence sample. The standard addition method (3 replicates) was performed for the quantitative analysis. From studying the optimum conditions for extracting HQ, Ar and AA2G simultaneously. Therefore, the response signal obtained for analytes at this concentration range had to be subtracted by the blank signal. The 3 replicate of determination essence sample are shown in Fig. 22. Unfortunately, HQ peak was intefere by masking with some reagents in the essence sample while the current response of Ar and AA2G can be determine but Ar peak shifted from 0.47 V to 0.3 V. The concentration of Ar and AA2G from the standard addition method was 15.63 (±1.55) ppb and. 45.09 (±3.44) ppb, respectively. The essence sample tested contained arbutin at a concentration 78.18 μg /100 mL sample and 225.43 μg /100 mL sample of ascorbyl glucoside.

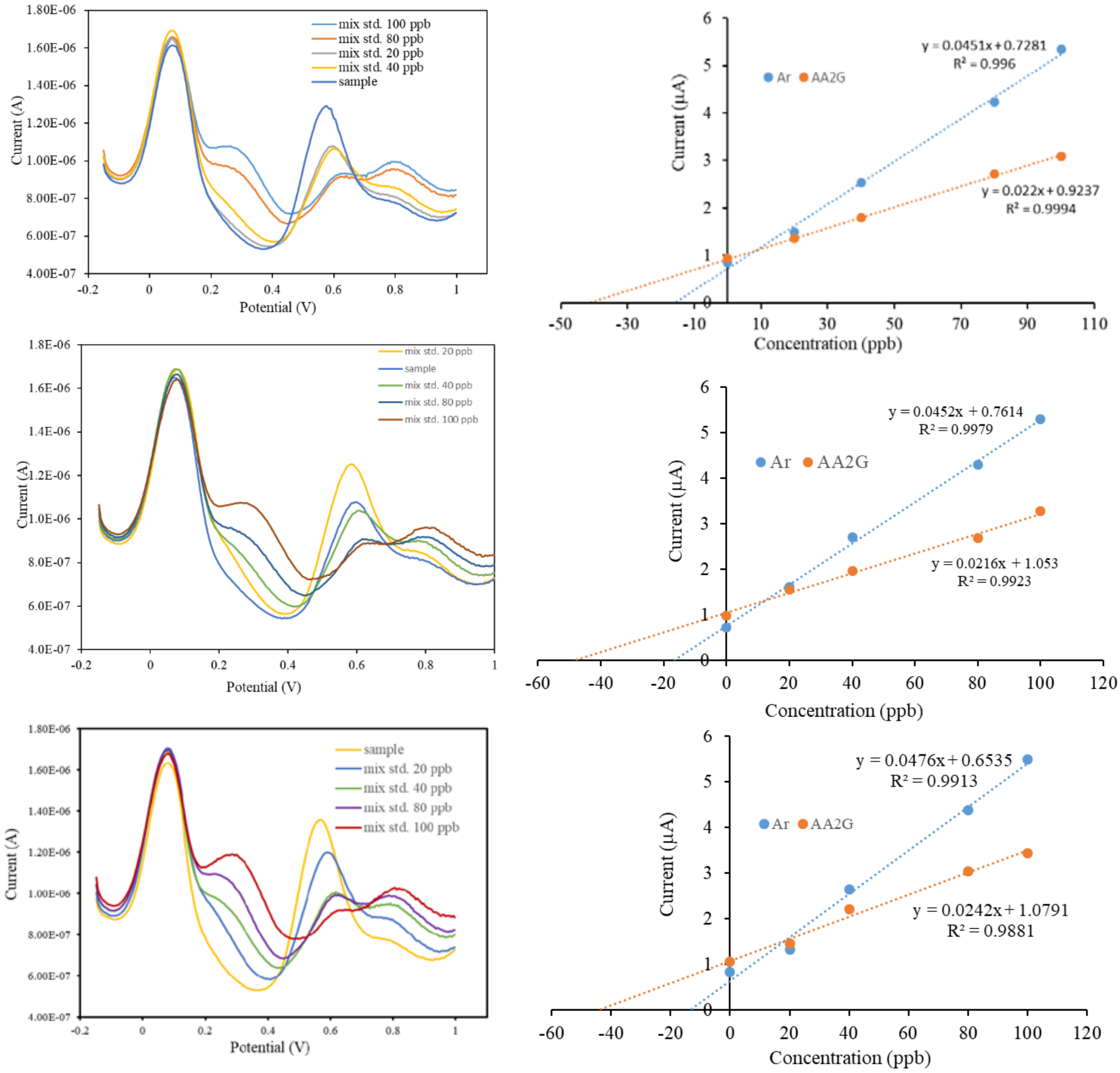

Figure 22 The standard addition curve (3 replicates) of the determination all analyte in the essence sample

4. Conclusion

This work describes a simple strategy for the fabrication of AgNPs/Ag$_2$S-chitosan/MWCNTs modified GCE by exploiting the synergetic effect of Ag NPs, Ag2S-chitosan and MWCNTs to provide excellent electrocatalysis for HQ, Ar and AA2G. The applicability of the newly modified procedure was demonstrated for the simultaneous determination of Ar and AA2G with good reproducibility in commercial essence sample.

RECOMMENDATION

When a high matrix background is present in the sample, the sample cleaning step is needed before determination by the proposed method such as solid phase extraction (SPE) based methods.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ

   (1) Nutthaya Butwong*, Supalax Srijaranai, Jeremy D. Glennon, John H. T. Luong, Cysteamine Capped Silver Nanoparticles and Single-Walled Carbon Nanotubes Composite Coated on Glassy Carbon Electrode for Simultaneous Analysis of Hydroquinone and Catechol, Electroanalysis (2018) doi: 10.1002/elan.201700704

   (2) Nutthaya Butwong*, Pimpanitpa Kunthadong, Phimpha Soisungnoen, Chatrachatchaya Chotichayapong, Supalax Srijaranai, John H. T. Luong, Silver-doped CdS quantum dots incorporated into chitosan-coated cellulose as a colorimetric paper test stripe for mercury, Microchimica Acta 185 (2018) 126.

2. การนำผลงานวิจัยไปใช้ประโยชน์

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3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

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