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The Inkjet Printing of Reducible AgNPs as Amperometric Glucose Biosensor Electrodes

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ABSTRACT

The enzymes immobilization is a crucially effective factor for biosensor preparation. Metal nanoparticles are potentially able to immobilize the enzymes due to their unique properties including large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency, and strong adsorption ability. A novel and highly sensitive amperometric glucose biosensor was obtained using the inkjet printing of reducing agents and metal salts. In this method, AgNPs are reduced from silver nitrate using ascorbic acid solutions on paper as substrate. Inkjet deposited patterns are utilized as electrodes in different electrochemical experiments and their morphology were investigated by SEM imaging. Results of cyclic voltammetry tests revealed that the glucose oxidase could be highly stabilized and immobilized on the surface of silver nanoparticles. Furthermore, the biosensor has a very high sensitivity of 8 μA/ mm^2 and a low detection limit of about 5 mM. These results demonstrate that inkjet printing AgNPs have potential applications in glucose biosensors. Prog. Color Colorants Coat. 7(2014), 285-294 © Institute for Color Science and Technology.

1. Introduction

The great demand for the development of glucose biosensor is increasing due to importance of glucose concentration and electro catalytic oxidation of glucose in clinical applications [1, 2]. The immobilization of the enzymes is one of the crucial factors in biosensor preparation. Different methods have been used to immobilize enzymes and improve the enzymatic activity. Metal nanoparticles as potential candidates for the immobilization of the enzymes have many unique properties such as large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency, and strong adsorption ability [3]. Currently, the glucose oxidase (GOx) is widely employed in most of the glucose biosensors, especially the amperometric

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glucose biosensors due to its stability and high selectivity to glucose. It contains two flavine adenine dinucleotide (FAD) cofactors and catalyzes the oxidation of glucose according to reaction of glucose and oxygen in the presence of GOx to produce gluconolactone and oxygen peroxide.

Since the amount of glucose is proportional to the produced H_2O_2, the glucose concentration can be readily determined through measuring the current derived from the electrochemical reaction of H_2O_2 [4]. In the vast majority of these applications, GOx attaches on different supports to yield derivatives with catalytic activities [5]. The support material, which plays an important role for the immobilized enzyme, should be readily available, non-toxic and avoid causing any deleterious alteration of the enzyme.On the other hand, the effect of nanoparticles on the GOx activity is important in biosensor and nano-bioelectronics research area. Many researchers showed that the GOx activity can be largely enhanced by adding gold nanoparticles (AuNPs) on the GOx electrode. In addition, several mechanisms for the effect of AuNPs on the GOx activity in terms of electron donation and accepting were reported [6]. However, silver is much cheaper and prevalent than gold. Thus, silver nanoparticles (AgNPs) have great potential applications in the immobilization of GOx. Nanoparticles of Different kinds, and sometimes the same kind, may have different effects on the properties of enzymes compared to microscale particles. Recently, controversial reports have appeared in the literature, some works reported the enhancement effect of AgNPs on the GOx activity [7]. Silver nanoparticles have numerous applications in diverse fields due to their catalytical, antibacterial and biosensing properties. Several methodologies have been reported for preparation of nanosized silver particles [8].

Moreover, it is well known that silver is one of the best conductors among metals and so its nanoparticles may facilitate efficient electron transfer in biosensors. Recently, Ren and his coworkers reported the feasibility of an amperometric glucose biosensor based on immobilization of glucose oxidase (GOx) in silver sol for the first time. GOx was simply mixed with silver nanoparticles and cross-linked with a polyvinyl butyral (PVB) medium by glutaraldehyde [9]. Then a platinum electrode was coated with the mixed solution.

The effects of the amount of the silver particles were studied with respect to the current response for enzyme electrodes.In addition, the one-step synthesis of silver nanoparticles/carbon nanotubes/chitosan film (Ag/CNT/CHT) as a novel immobilization matrix for the enzymes proposed to fabricate sensitive glucose biosensors. The biosensor was prepared by embedding horseradish peroxidase (HRP) and glucose oxidase (GOx) in Ag/CNT/CHT hybrid films based on layer-by-layer technique [10].

There are many well-established manufacturing techniques for the fabrication of biosensors such as photolithography [11], screen printing, nano-imprinting [12], micro-contact printing [13] and dip-pen lithography [14]. More recently, ink-jet printing (IJP) has been rapidly developed for the display and semiconductor industry with a great potential to be used in biomedical and biosensor applications [15-18].

Inkjet printing is an attractive method for patterning and fabricating objects directly from design or image files with no needs for masks, patterns, or dyes, hence reducing the time, cost and space consumed and the toxic waste created during the manufacturing process. In this way, the employment of inkjet printing technique can solve many problems in a facile and effective manner. Overall, this technique offers benefits such as speed, flexibility, creativity, cleanliness and eco friendliness. In principle, inkjet printing technology appears to be the simplest printing method, but it demands multi-disciplinary skills to precisely control the solid/liquid/gas interface [16]. Inkjet printing technique allows for the patterning of conductive traces onto a substrate in a one-step process [19, 20]. Silver as a best conductor is studied most extensively for metal deposition by inkjet printing. The organo-silver compounds or silver nanoparticle suspensions have been used for inkjet-printing of silver conductors. However, both of these materials are expensive and the deposition process needs to be promoted for a cheaper protocol [21].

In this paper, the possibility of inkjet printing of AgNPs on a flexible substrate was studied to fabricate glucose biosensors. A conventional drop-on-demand inkjet printer was utilized to inkjet salt solution containing silver ions to make an in situ chemical reduction of conductive silver nanoparticles on the substrate. The electrochemical performance of the electrode was investigated by cyclic voltammetry and differential potential voltammetry.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, from *Aspergillus niger*; 128,000 unit g^{-1}) was purchased from Sigma. β-D glucose was obtained from Merck. Both ascorbic acid and silver nitrate were used as analytical grade chemicals (99.5% purity, Merck). A4 copying paper (80 GSM) was used as substrate in the inkjet metal deposition experiments. HCl, NaOH and KCl were of analytical grade purchased from Merck Company. Double-distilled water was used in all the experiments for cleaning and solution preparation. A buffer solution was prepared from phosphate buffered saline (PBS, Sigma). Water repellent agent was obtained from Supros Company and the PVC adhesive was commercial PVC glue normally uses in piping.

2.2. Instruments

A Hewlett Packard (*hp)* single head office inkjet printer (Apollo 1200) and one black (*hp*26) cartridge was used to print metallic salt and/or reducing agent solutions in separate runs. Usually the substrate was first printed with the reducing solution and then with metal precursor ink. Microsoft Word was employed as the printer controlling software. A four-contact method was used for measuring the electrical conductivity of the deposited samples in preference to the four-point probe method usually employed in the electrical assessment of electrodes [20]. Cyclic voltammetric measurements were performed using an electrochemical analyzer CHI 660 (CH Instruments, Austin, TX) connected to a personal computer.

A three-electrode configuration was employed, consisting of an inkjet deposited silver electrode serving as working electrode (AgNPs electrode), whereas Ag/AgCl (3M KCl) and platinum electrode served as the reference and counter electrodes, respectively. All electrochemical experiments were carried out at room temperature. Scanning electron microscopy (SEM) with an XL30 scanning electron micro analyzer (Philips, Netherlands) at an acceleration voltage of 15 kV was used to capture proper images to show the morphology of silver nanoparticles.

2.3. Inkjet printing of Silver salt and reducing agent

A 30% w/v solution of ascorbic acid (adjusted at pH=5.5 by addition of NaOH solution) and a 50.25% w/v silver nitrate solution (pH=3.5) were used in the inkjet silver deposition electrode. Usually, the reducing ink was printed first and then the silver nitrate solution was overprinted after an intermediate drying at room temperature for 5 minutes (Figure 1).

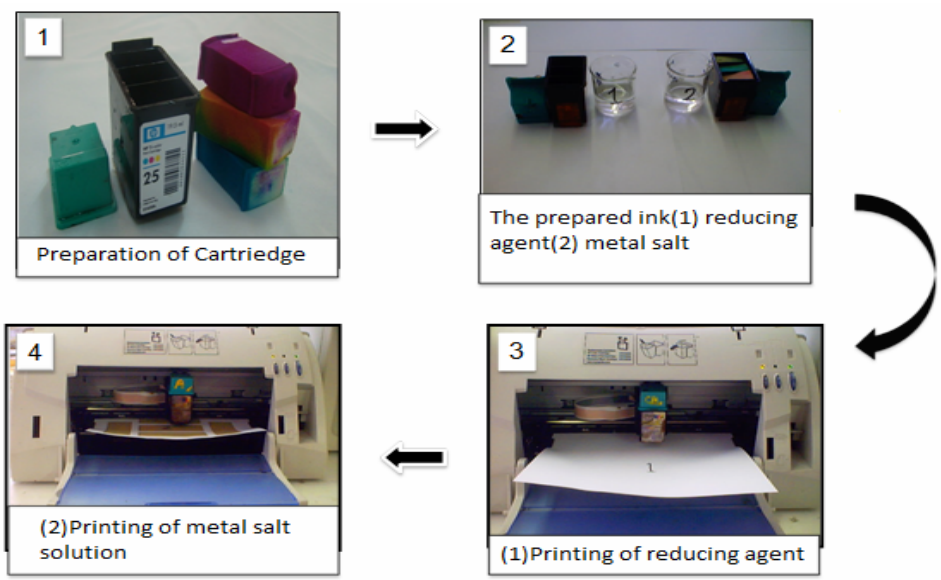

Figure. 1. The steps of inkjet printing by reducing of silver nanoparticle on paper substrate.

To extract the unreacted chemicals and their residues trapped among the deposited metallic particles during the redox reaction, the silver deposited pattern should be rinsed with water (wet extraction) and then hot pressed against a clean sheet of paper at 150 °C (hot extraction). Some yellow residual compounds was extracted from the printed pattern during rinsing and hot pressing procedures, respectively. Printed patterns (0.2 cm × 1.5 cm) were then glued to a polyester film for better handling during the electrochemical tests using PVC paste.

Deposited electrodes were cut in small pieces and coated with waterproof agent (Bit-guard Fc) with one end kept out of paste to allow enough contact between the testing solution and the printed surface.

2.4. Fabrication of GOx electrodes

The GOx was dissolved in a phosphate buffer solution to give a final concentration of 0.6mgmL^{-1}. For the fabrication of the enzyme biosensor, 10µL of GOx solution was dropped onto the surface of the AgNPs/ paper electrode and the solvent was allowed to evaporate at room temperature for 4h.

2.5. Amperometric measurement

The sensitivity of the glucose biosensor was tested by measuring the current response. The experiment was carried out by a three-electrode cell consisting of an enzyme electrode, a reference electrode of Ag/AgCl and a platinum electrode as the counter electrode. Measurements were conducted in a 5mL phosphate buffer (pH=7.0) cell at room temperature. Potential window of −0.4V to +0.65V versus the reference Ag/AgCl electrode was used in these DPV experiments. When background current reached a constant value, different concentrations of β-D glucose solution were added. Response current was then recorded and the correlation between response currents and different GOx solutions was obtained. The results were highly repeatable.

3. Results and discussion

3.1. Morphology of inkjet printed layers of AgNPs

The growth of deposited silver particles on the paper substrates were studied by considering letters A and G (respectively stands for inkjet printing of ascorbic acid (A) and silver nitrate (G) inks) in the deposition sequence. Figure 2 shows the SEM images of grown silver particles at AAAG which means printing of three layers of ascorbic acid ink followed by one layer of silver nitrate solution according to the conductivity of 5.54 × 10^5 S/m.

The inkjet printing of silver nitrate based on AAAG (Figure 2-a) protocol demonstrates a superior distribution of G nanoparticles on the continuous matrix of A fully spread on the paper substrate.

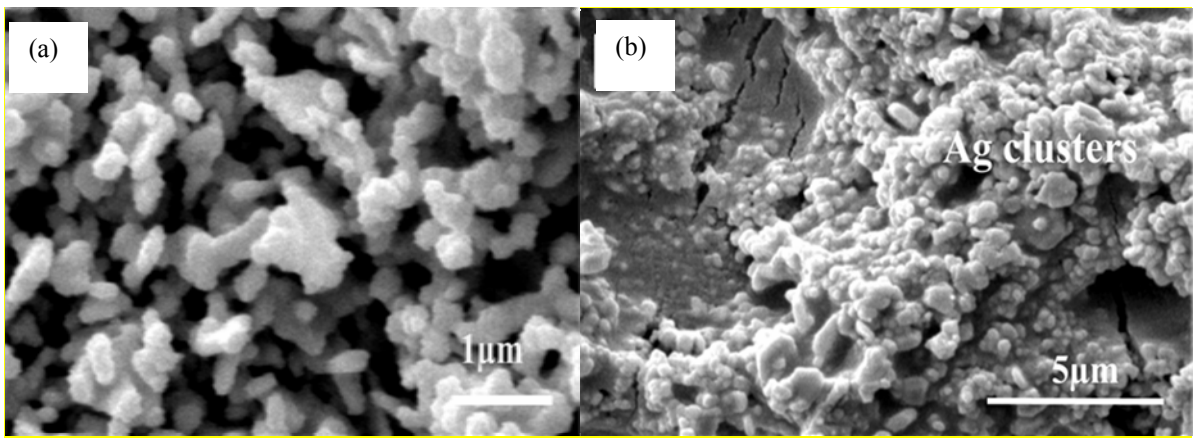

Figure 2. The SEM image of inkjet deposited silver pattern on paper substrate with AAAG protocol (X5000). The SEM image of same sample after hot press at 150 °C for 20 second and cleaning with double distilled water(X15000).

The change in morphology of inkjet printed layers occurs while the concentration of reducing agent (A) reaches to an appropriate level. Rinsing the printed surfaces with distilled water could also lower the final resistivity by extracting the reactants residue left on the surface and between the silver particles formed by the redox reaction, but its effect was not in all cases as significant as the effect of heat treatment process. Removal of these impurities was proved by SEM observations. Typical morphology of a deposited pattern is shown in magnified SEM images taken after hot extraction and rinsing procedures (Figure 2-b).

3.2. Direct electrochemistry of GOx /AgNPs/paper electrode

Cyclic voltammetry (CV) is a sophisticated potential scanning technique for clarifying the reaction mechanism to imply the degree of electrochemical reversibility in a specific process on electrode surface. The standard potential (E°) for half reduction reaction of silver is about 0.799 volt.

$$Ag^{+} + e^{-} \rightarrow Ag(s) \qquad E^{\circ}=0.799 \qquad (1)$$

Therefore, the scanning potential range should be less than the standard potential to inhibit the silver overoxidation in potential sweeping. CV is a simple and easy mean to show the changes of electrode behavior after each assembly step, because the electron transfer between the solution species and the electrode must occur by tunneling through either the barrier or the defects in the barrier [16]. Figure 3 shows the cyclic voltammograms (CVs) of AgNPs/paper and GOx/AgNPs/paper electrodes in 0.1M PBS solution (pH=7.0) at $20mVs^{-1}$. A pair of well-defined and nearly symmetric redox peaks was observed at the AgNPs/paper electrode. A broad oxidation peak and nearly sharp reduction peak is observed at 0.6 V and -0.1 V for AgNPs/paper electrode respectively. For AgNPs /paper electrode the potential scanning reveals anodic peak around 0.63 V for oxidation of Ag to Ag^{+}. The cathodic peak also enhanced at -0.1 V which can be attributed to reduction of Ag^{+} to Ag. The significant difference between redox peak voltages is a reliable evidence for irreversible cathodic reaction during oxidation path. The glucose recognition is achieved using enzyme technique based on the H_2O_2 release. The sensor sensitivity depends on the electrochemical response as a function of H_2O_2 concentration. This biosensor detects the H_2O_2 released from enzymatic reaction of glucose and GOX by utilizing AGPs/paper as working electrode.

$$\begin{aligned} &Glu\cos e + O_2 + H_2O \xrightarrow{Glu\cos eOxidase} \\ &Gluconicacid + H_2O_2 \\ &H_2O_2 \rightarrow O_2 + 2H^{+} + 2e^{-} \end{aligned} \qquad (2)$$

GOx adsorbed on Ag nanoparticles exhibits direct electron transfer towards the oxidation of glucose. In the absence of GOx, the less significant redox peaks were noticed in this potential range for bare AgNPs/paper electrodes. However, a pair of well-defined redox peaks was noticed on the AgNPs/GOx modified electrode in the same potential window. The GOx inclusion causes an enhancement of electrochemical responses on the GOx/AgNPs/paper. Both oxidation and reduction peak positions shift to lower voltages with higher intensity, obviously, regarding the influence of glucose oxidase on electrochemical activity of AgNPs/paper electrodes. It can be concluded that the redox process should be described extensively to GOx which is characteristic of reversible electron transfer process of redox active center in the AgNPs/ GOX and plays a key role in facilitating the direct electron transfer of GOx and the electrode surface. Direct Electrochemistry of GOx at reduced graphene oxide/zinc oxide composite modified electrodes show a similar behavior for glucose sensor compared to AGNPs/GOx electrodes [22]. After successive scannings, no noticeable changes in CVs of this enzyme electrode were observed. It can be referred as great potential of deposited electrode for being as a stable electrochemical sensor.

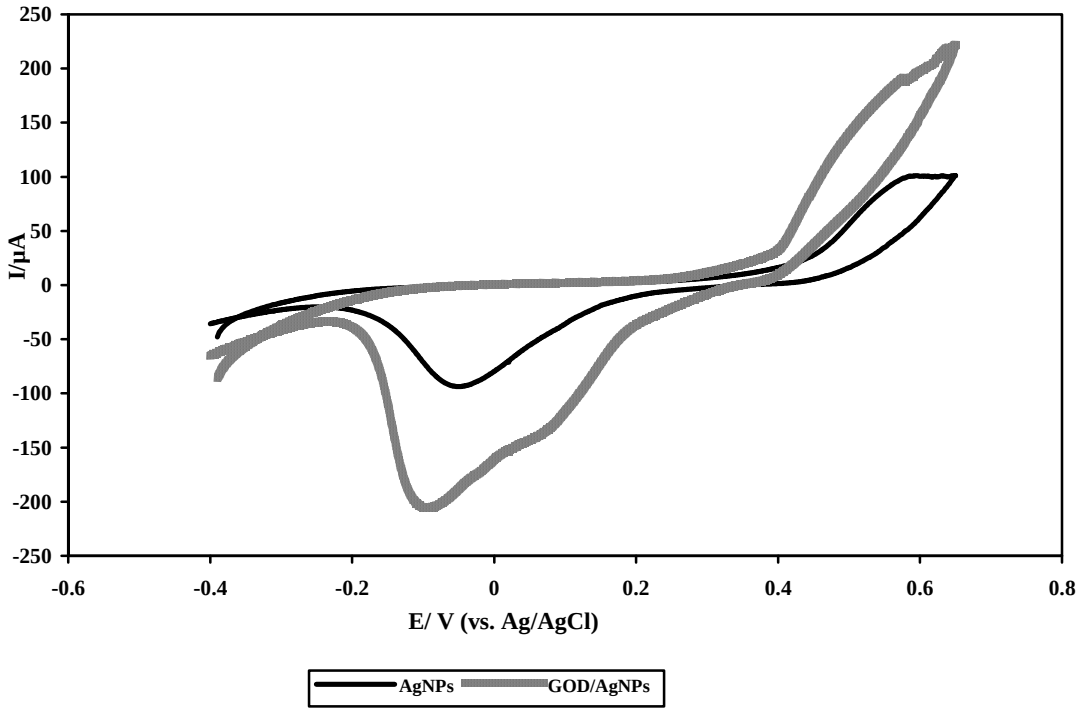

Figure 3: Cyclic Voltammograms of AgNPs/paper (a), and GOX/AgNPs/paper (b) electrodes in 0.1M PBS solution (pH=7.0) at 20mVs^{-1}.

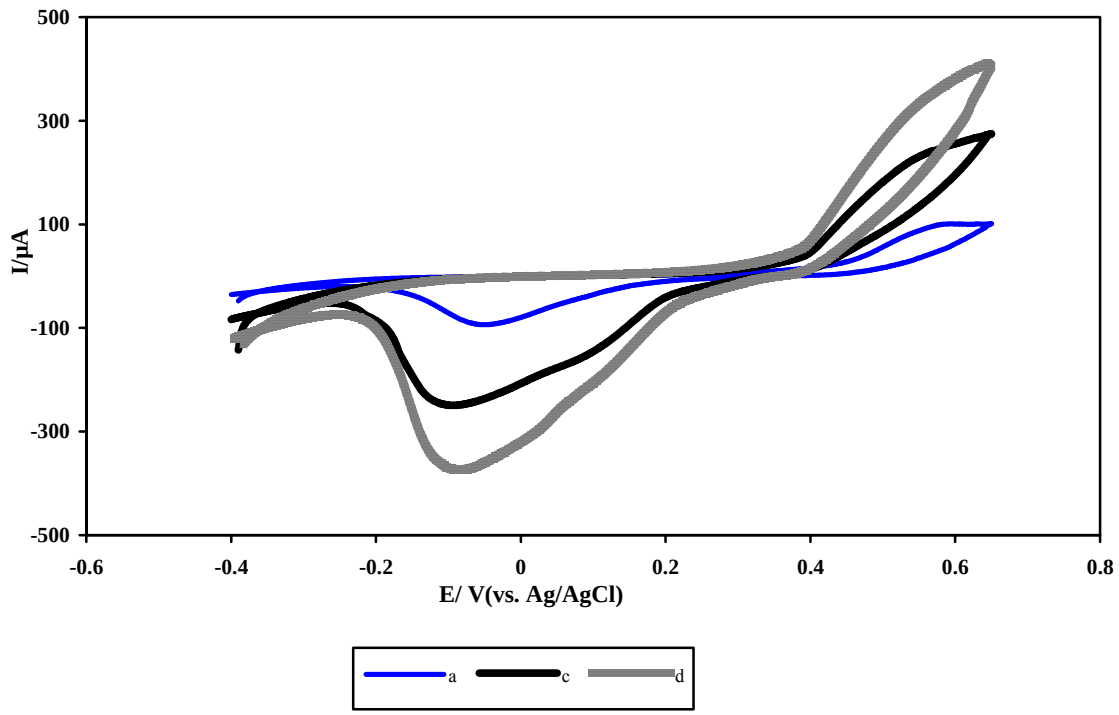

Figure 4: (a) Cyclic voltammograms of the biosensor without, with glucose (40mM), (b) glucose (8omM) and (c) in PB solution (0.1M, pH= 7.0) at scan rate of 20mV/s.

3.3. Electrochemical characterization of GOx/AgNPs electrode

The electrochemical characterization of the biosensor is investigated by cyclic voltammetry between −0.4V and 0.65V versus Ag/AgCl in PB solution (pH= 7.0) at scan rate of 20 mV/s. Figure 4 shows the cyclic voltammogram of the biosensor without glucose (curve a) and after addition of glucose (40mM) (curve b) and glucose (80mM) (curve c).

It can be found that the reduction current is suppressed while the oxidation current increases significantly which relates to the oxidation of glucose by GOx catalysis. Moreover, a broad shoulder peak shows up apparently at around 0.6V which can be ascribed to H_2O_2 generated during the oxidation of glucose [4]. These results indicate that the GOx can be well defined to AgNPs and the fabricated biosensor has good detection ability to glucose. This result is attributed to the redox characteristics of the electroactive centers of the immobilized GOx, indicating enhanced improved electron transfer between GOx and AgNPs.

During a reverse scan (in the presence of glucose), two cathodic peaks (b_1 and b_2) appeared at 0.18 and -0.11V respectively, as reported in previous studies. These cathodic current peaks are due to the reduction of Ag_2O to Ag in two steps [23]. The first cathodic peak (b_1) is due to the electroformation of the Ag monolayer. The second cathodic peak (b_2) is caused by the formation of the Ag multilayer.

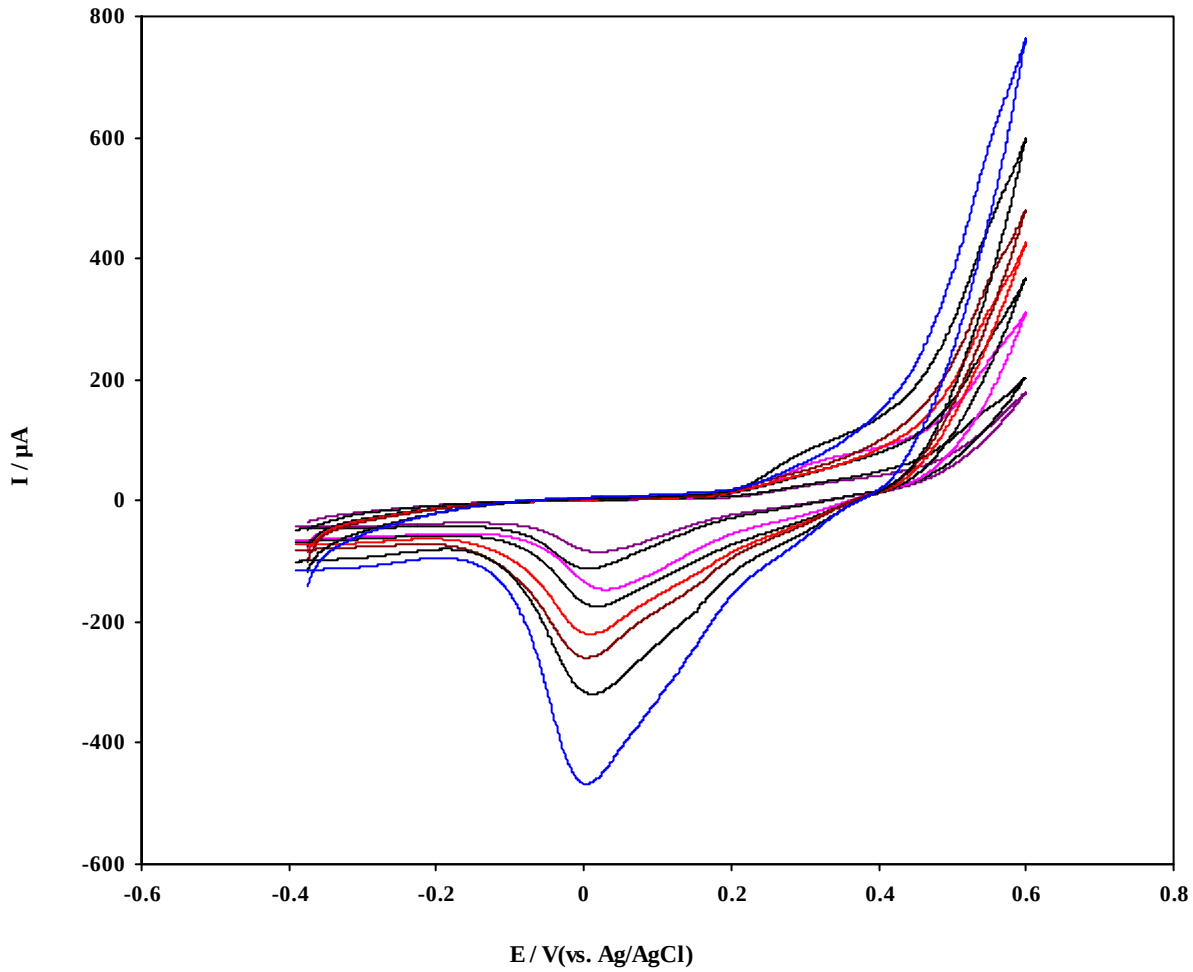

Figure 5: CVs of the biosensor in PBS (0.1M, pH= 7.0) at various scan rates. The scan rate (from inner to outer) is 10, 20, 40, 60, 80, 100, 150 and 200 mVs^{-1}, respectively. Inset: plot of currents versus scan rate.

3.4. Effect of scan rates

Typical CVs of the enzyme electrode in PBS (0.1M, pH=7.0) at different scan rates are presented in figure 5. It can be seen that the potential and peak currents are dependent on the scan rates. The peak-to-peak separation widens with the increased scan rate. In the scan rate of 10-200 mVs^{-1}, the anodic and cathodic peak potentials shift slightly to the positive and negative directions, respectively. Consequently, the peak potential separation between the anodic and cathodic increases with the potential scan rate. The anodic and cathodic peak currents (Ip_a and Ip_c) versus scan rate are displayed in the inset. They are both linearly proportional to the scan rate (v) ranging from 10 to 200mVs^{-1} with a correlation coefficient of 0.987 (inset in Figure 5). This suggests that the whole process of the reaction is a typical surface-controlled process, as expected for immobilized systems.

3.5. Sensitivity of biosensor

Figure 6 shows the calibration curve of GOx–AgNPs biosensor. This calibration curve shows a good linearity in the range of 5 -11 mM with a detection limit of 5 mM. The sensitivity of AgNPs based glucose biosensor is calculated to be around 8$\mu A/cm^2$ when a glucose oxidase concentration of 3 mg/mL was used. The regression equation based on the acquired data was evaluated using least square method. The regression represents by y=7.875x+111.2, where y is the current in µA and x is the glucose concentration in mM with the R^2 value of 0.951. The linear response of the glucose biosensor to glucose is up to about 80 mM of glucose, which is higher than the 15 mM required for practical use in the detection of blood glucose [24-25].

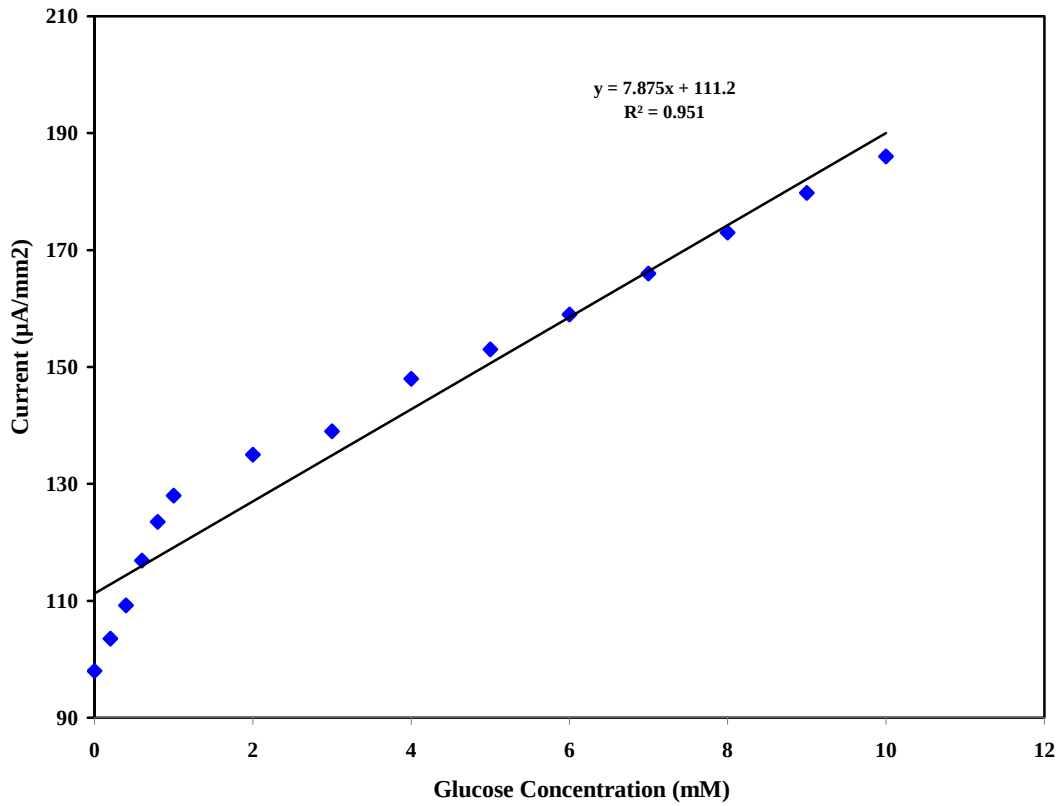

Figure. 6: The calibration curve (R^2= 0.951) corresponding to amperometric responses at -0.4 V to 0.65V. Scan rate: 20mVS^{-1}.

4. Conclusions

Current study depicts the possibility of using inkjet printing technology to fabricate AgNPs-based glucose biosensors. Deposited silver patterns suitable for use as electrochemical electrodes were fabricated using silver nitrate and ascorbic acid as metal salt and reducing ink, respectively. Their reaction on the surface of the substrate could grow silver nano clusters of 50-200 nm. The biosensor shows linear response over a wide linear range from 5 to 11 mM and detection limit shows a value around 5 mM. The good electronic properties and biocompatibility of AgNPs electrode provides the direct electron transfer of redox enzyme and therefore maintain its bioactivity. Finally, we have successfully fabricated the electrochemical biosensor based on inkjet printing of AgNPs and a potential possibility for the further fabrication of biosensors.

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