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Sensitive determination of amlodipine besylate using bare/unmodified and DNA-modified screen-printed electrodes in tablets and biological fluids

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ABSTRACT

The screen-printed technique is widely used as an efficient tool for electrochemical analysis in environment, clinical and agri-food areas. Significantly, it has the ability to transfer electrochemical laboratory experiments into the field. In the present work, we report a highly sensitive, simple, low-cost protocol for determination of amlodipine (AML) using bare/unmodified and DNA-modified screen-printed electrodes (SPEs). The immobilization of DNA molecules onto SPE offers promising robust and chemically stable molecular wires, which provides a unique opportunity for charge transfer processes. Consequently, the electroanalytical sensing of AML was explored at bare/unmodified and DNA-modified SPEs in a linear range between 0.066–1.0 μM and 0.066–2.0 μM with the detection limit (3σ) found to be 20.70 nM and 14.94 nM, whilst corresponding sensitivities of: 0.43 A L mol^{-1} and 4.23 A L mol^{-1} respectively. Although, the superior electrochemical signature of bare SPEs is evident, the immobilization of DNA onto SPEs enhances the sensitivity 10-times more than the bare SPEs. Furthermore, the optimized electroanalytical protocol using the unmodified SPEs, which requires no pre-treatment and electrode modification step, was then further applied to the determination of AML in real samples.

1. Introduction

Amlodipine (AML) is chemically designated as 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid, 3-ethyl, 5-methylester, besylate (Scheme 1). AML is a dihydropyridine derivative with calcium-channel blocker (CCB) activity, widely used in the management of hypertension caused by coronary artery disease, chronic stable angina pectoris and prinzmetal's variant angina [1]. It inhibits selectively the arterial vascular smooth muscle cell proliferation resulting in prevention of the progressive narrowing of the arteries as well as preventing the coronary spasms resulting in increased blood flow with myocardial oxygen supply [2]. The control of high blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions. Therefore, the development of an analytical procedure for AML determinations with high sensitivity and selectivity is not only of pharmaceutical significance for point-of-care detection, but also important for industrial purposes.

Several analytical techniques including spectrophotometry [3–5], high performance liquid chromatography (HPLC) [6–10], high performance thin layer chromatography (HPTLC) [10], gas chromatography (GC) [11,12], capillary electrophoresis (CE) [13,14] flow injection [15], and enzyme-linked immune-sorbent assay [16] have been reported for AML determination in pharmaceutical formulations and biological fluids. Although these methods are sensitive for determination of AML, they require expensive instruments, laborious sample pre-treatment, highly skilled technicians, long analysis time and generate large amount of wastes, which make them unsuitable in quality control laboratories. Taking the above-mentioned lacuna and the electroactivity of AML into consideration, electrochemical methods have been widely explored because of their merits to provide an accurate, sensitive and yet

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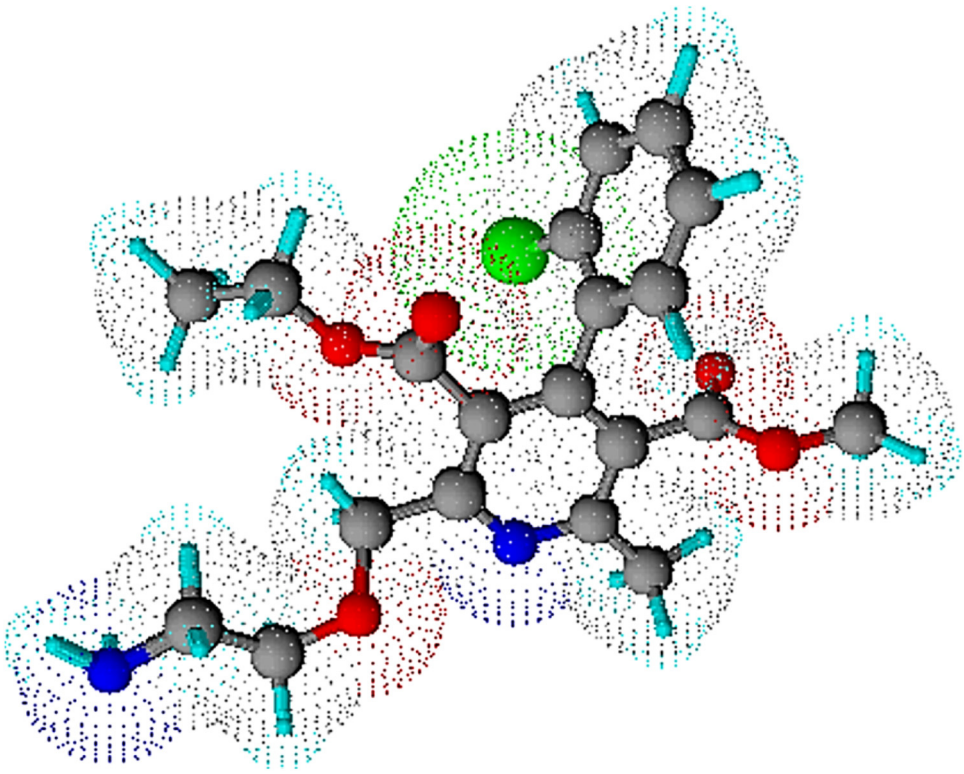

Scheme 1. Chemical structure of AML.

simple, inexpensive, compact and low-power platform for on-site determinations. Pyrolytic graphite (PG) [17], glassy carbon (GC), carbon paste [18,19] and gold electrodes [20] have been used. However, these macro-electrodes were poisoned quickly and their sensitivity and reproducibility were significantly decreased. Therefore, considerable attention has been focused on the chemical modification of macro-electrodes in order to enhance electroanalytical performance. Graphene–chitosan nanocomposite/GC [21], and multi-walled carbon nanotubes modified carbon paste [22] have been developed recently. In addition, boron doped diamond (BDD) electrode is particularly attractive in determination of AML [23,24] and other pharmaceutical compounds because it has long-term stability, inertness, good resistance to passivation, high chemical stability and lower residual current but it requires multi-treatment and polishing steps to improve its response.

Molecular wires provide a unique charge transfer rate, and consequently many efforts have been directed to fabricate functional molecular wires with precisely defined, uniform lengths and functionalized terminal groups bonded to electrode surface and/or electroactive moieties. The deoxyribonucleic acid (DNA) molecules might satisfy these synthetic requirements of robust and stable functional molecular wires; hence, base-paired DNA facilitates electronic charge transfer process through the DNA π-stacking [25]. Such DNA-mediated charge transfer mechanism offers high sensitivity and simple assay without labelling. The DNA-based electrochemical sensing has a variety of possible applications in detection of small molecules (*e.g.* drugs, pollutants, carcinogens, *etc.*) due to their binding ability with DNA structures [26]. Recently, *Svítková* et al. employed a BDD electrode to explore DNA damage by antihypertensive AML. The presence of the AML molecules caused intensive damage of the DNA layer on the BDD electrode [27].

The development of screen-printed electrodes (SPEs) has become a major revolution in the construction of electrochemical sensors/biosensors [28]. These commonly graphite screen-printed electrodes are constructed of employed inks consisting of graphite and carbon black particles with a polymeric binder which are screen-printed onto a suitable substrate an then cured at a suitable temperature. These simple manufacturing steps of the electrode allow the transfer of electrochemical laboratory experiments to the market for reproducible and disposable on-site detection of various analytes [29]. In the present work, the bare/unmodified and DNA-modified SPEs were used for the first time as a simple, inexpensive, rapid and sensitive electrochemical biosensor for determination of AML in pharmaceutical formulations and human fluids. The SPEs showed high sensitivity, excellent long-term stability and inter- and intra-day repeatability for determination of AML real samples.

2. Experimental section

2.1. Reagents and materials

All chemicals were of the highest analytical grade available and were used as received without further purification from Sigma-Aldrich. All solutions were prepared using doubly distilled water of resistivity more than 18.2 MΩ cm. Amlodipine besylate (AML) was kindly supplied by Glopal Napi Pharmaceutical Co. (6th of October, Egypt) and it's purity value was (98.21% $\pm$ 1.22). Amlodipine tablet was labelled to contain 5.0 mg AML was purchased from Amriya pharmaceutical industries, Cairo, Egypt. ct-DNA was purchased from Sigma Aldrich and used as received without further purification. Buffer solutions were prepared from boric acid/citric acid/phosphoric acid (0.05 mol L^{-1}) and the pH adjustments were made by the addition of sodium hydroxide solution (1.0 mol L^{-1}).

2.2. Fabrication of screen-printed electrodes (SPEs)

The SPEs were fabricated in-house with appropriate stencil using a DEK 248 screen-printing machine (DEK, Weymouth, U.K.). These electrodes have been used extensively in previous studies for their fabrication, first, a carbon-graphite ink formulation (prod-

uct code C2000802P2; Gwent Electronic Materials Ltd., U.K.) was screen-printed onto a polyester (Autostat, 250 μm thickness) flexible film (denoted throughout as standard-SPE); these electrodes have been used extensively in other work [30]. This layer was cured in a fan oven at 60 °C for 30 min. Next, a silver/silver chloride reference electrode was included by screen-printing Ag/AgCl paste (product code C2040308D2; Gwent Electronic Materials Ltd., U.K.) onto the polyester substrates and a second curing step was undertaken where the electrodes were cured at 60 °C for 30 min. Finally, a dielectric paste (product code D2070423D5; Gwent Electronic Materials Ltd., U.K.) was then printed onto the polyester substrate to cover the connections. After a final curing at 60 °C for 30 min these SPEs are ready to be used. These SPEs have been reported previously and shown to exhibit a heterogeneous electron transfer (HET) rate constant, k^o, of *ca.* 10^{-3} cm s^{-1}, as measured using the $[Ru(NH_3)_6]^{3+/2+}$ redox probe.

2.3. DNA modified SPEs

The SPEs were modified with DNA molecules by a simple drop-casing process. The DNA stock solution was prepared by dissolving 10.0 mg of DNA in 10.0 mL doubly distilled water and stored at 4 °C. A measured volume of DNA solution was dropped onto the SPE surface and left it to dry for 30 min in desiccator. Then, the DNA-SPEs are ready to use for further experiments.

2.4. Apparatus

The voltammetric experiments were performed using an Autolab 302N potentiostat/galvanostat workstation. All measurements were conducted using a screen-printed three-electrode configuration. Connectors were used for the efficient connection of the screen-printed electrochemical sensors.

2.5. Determination of AML in real samples

Ten tablets were accurately weighed and finely powdered. An amount of powder equivalent to 10 mg of amlodipine besylate was transferred into a 25 mL volumetric flask containing 20 mL ethanol, the solution was sonicated for 30 min, and then completed with the ethanol to the mark and filtered carefully. The first portion of the filtrate was rejected. Then, the solution was diluted by ethanol to reach a final concentration of 6×10^{-5} μM. An accurate measured volume of the stock solution (100 μL) was quantitatively added into 15 mL of B.R. buffer solution of pH 9 to yield a sample solution having a final concentration of 0.4 μM. Then, consecutive additions of AML standard solution (0.2, 0.4 and 0.6 μM) were injected to the electrochemical cell. The differential pulse voltammetric (DPV) responses were recorded and a standard addition method was used to determine the concentration of AML in tablet.

The urine samples were collected from three volunteers in Hospital of Sohag University. A 0.5 mL of urine sample was injected into 14.5 mL of B.R. buffer solution, pH 9. The DPV response was recorded and a standard addition method was used to determine the concentration of AML in urine samples.

3. Results and discussion

The electrochemical characteristics of bare/unmodified and DNA-modified screen-printed electrodes (SPE) were firstly investigated using electrochemical impedance spectroscopy. Fig. 1 shows a complex plane plot of 20 μM AML in B.R. buffer solution of pH 9 at bare SPE and DNA-modified SPE; the applied DC-voltage is about 0.7 V (*vs.* Ag/AgCl), the excitation voltage applied to the electrochemical cell was 5 mV (*peak to peak separation*), and the frequency range was from 100 kHz to 0.1 Hz. The impedance plot consists of

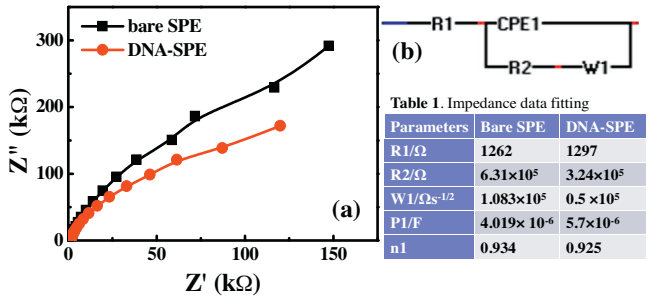

Table 1. Impedance data fitting

Parameters	Bare SPE	DNA-SPE
R1/Ω	1262	1297
R2/Ω	6.31×10^5	3.24×10^5
W1/Ωs$^{-1/2}$	1.083×10^5	0.5×10^5
P1/F	4.019×10^{-6}	5.7×10^{-6}
n1	0.934	0.925

Fig. 1. Complex plane plots of 20 μM AML in B.R. buffer pH 9 at bare SPE and DNA-modified SPE and in-set the corresponding equivalent circuit model that describe the electrochemical process [The frequency range100 kHz and 0.1 Hz, the excitation voltage was 0.005 V; peak to peak].

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Impedance data fitting.

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semicircle followed by straight line, which reveal direct electron transfer of the SPEs and AML [30]. It was attempted to imitate the impedance spectra by an equivalent circuit, which makes physical sense to the electrochemical system simulation (Fig. 1b). As shown in Table 1, the ohmic resistance (R_1) was slightly increased in the presence of the DNA layers, indicating that a good electron conductor covers the SPE surface. This also confirmed by the numerical values of charge transfer resistance (R_2) which decrease by half of its original value of bare/unmodified SPE. Consequently, the Warburg impedance value of DNA-SPE was decreased and capacitance was slightly increased. This result indicates an effective charge transfer at SPE surface *via* DNA-mediated mechanism which is in good agreement with the previous reports [26].

Next, the electrochemical oxidation behaviour of AML at bare screen printed-graphite electrodes was carefully investigated over a range of pH values (2.0–12.0) as shown in Fig. 2(a, and b). The cyclic voltammetric measurements were carried out in 0.05 M B.R. buffer solutions containing 30.0 μM of AML at a scan rate of 100 mVs^{-1}. From inspection of Fig. 2a, it shows a well-defined voltammetric signal shifts toward less positive potential values, indicating the participation of protons in the electrode process (Fig. 2b). The slope of the linear relationship between the peak potential E_p *vs.* pH equation was found to be 38 mV/pH. This value is lower than that of Nernstian slope (59 mV/pH at 298 K) for a process that involves the transfer an equal number of protons and electrons. Therefore, we propose that the electrochemical oxidation of AML at SPEs likely involves a two-electron and one-proton transfer through oxidation of 1,4-Dihydropyridines to pyridine cation followed by formation of aromatic pyridine ring *via* losing of another proton. Overall, AML undergoes an anodic process that includes $2e^-/2H^+$ and produces the corresponding pyridine derivative. This statement is in good agreement with the result reported for the oxidation process of AML on BDD [23]. Given the pH dependence of the voltammetric response, an increase in the wave intensity of the anodic peak current was observed in basic solutions. Based on the above results, B.R. buffer solution of pH 9 was chosen as supporting electrolyte for determination of AML in further experiments (see Fig. 2).

Fig. 3a, presents the cyclic voltammetric responses in the absence and presence of 5.0 μM of AML in B.R. buffer of pH 9 at

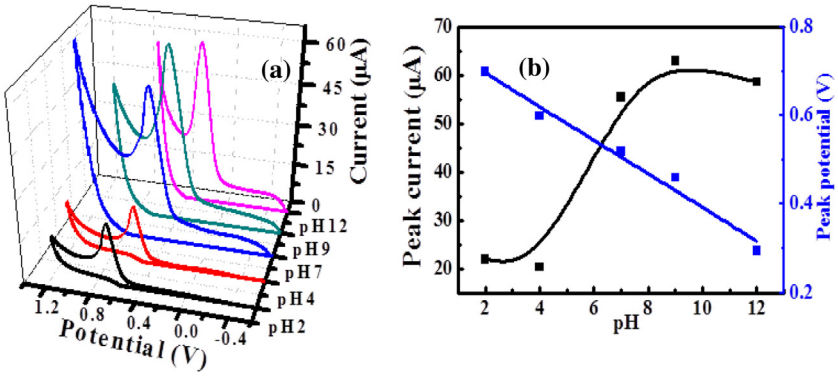

Fig. 2. (a) Cyclic voltammograms of 30 μM of AML at SPEs in different pH of B.R. buffer solutions; scan rate 100 mVs^{-1}, (b) Analysis of oxidation peak current and peak potential of AML as a function of pH variations.

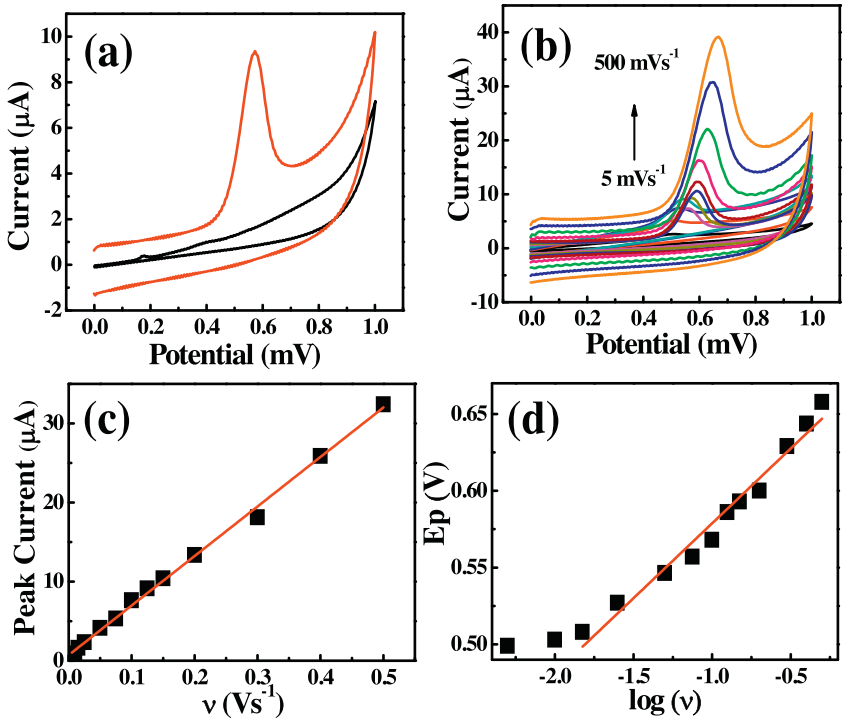

Fig. 3. (a) Cyclic voltammetric profiles of SPEs in B.R. buffer, pH 9 in absence and presence of 5.0 μM AML at a scan rate 100 mVs^{-1}, (b) CVs responses for the electrochemical oxidation of AML at different scan rates, (c) Dependence of the electrochemical oxidation peak current upon the voltammetric scan rate, (d) the relation between the peak potential and the logarithm of scan rate.

a scan rate of 100 mVs^{-1}. The effect of scan rate (υ) upon the electrochemical oxidation of AML at SPEs was examined in the range of 5–500 mV/s as shown in Fig. 3b. The sole presence of an electrochemical oxidation peak at all scan rates suggests an irreversible electrochemical reaction. The linear plot of the voltammetric peak height against scan rate (υ) was constructed; I_{AML} (μA) = 62.6 υ (Vs^{-1}) − 0.71 μA; R^2 = 0.997, which indicates that, the electrochemical oxidation of AML is governed by adsorption mechanism (Fig. 3c). This evidence was also noted by analysing the data through a plot of log I_{AML} *vs.* log υ, which reveals a slope of 0.85. This slope is higher than the theoretical value (0.50) for a diffusion-controlled process [17,23]. A small shift in the peak potential toward positive direction was also observed which confirms the irreversibility of the electrode process. The number of electrons consumed in oxidation of AML on SPEs can be also estimated by applying the following equation: $E_p - E_{p/2}$ = 47.7 mV/αn. Using the cyclic voltammogram in Fig. 3a, the value of $E_p - E_{p/2}$ is about 0.52 V, the number of electrons transferred (n) was estimated to be equals to 2. The oxidation peak potential also increased with scan rate and the relationship between peak potential and logarithm of scan rate (Fig. 3d) can be expressed by the following equation;

$$E_p(V) = 0.079\,log\,(\nu/Vs^{-1}) + 0.65\,V, R^2 = 0.99$$

These observations suggest that the adsorption plays a significant role in the irreversible electrode reaction of AML at the SPEs. As for an irreversible electrode process, the peak potential E_p is defined by the following equation;

$$E_p = E^o + \left(\frac{2.303RT}{\alpha nF}\right)\log\left(\frac{RTk^o}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right)\log \nu$$

where α is the electron transfer coefficient, k^o is the standard heterogeneous rate constant of the reaction, *n* is the number of electron transferred, *ν* is the scan rate, and E^o is the formal redox potential. Thus, the value of k^o can be estimated to be 5.98×10^3 s^{-1} from the intercept of this linear plot. These results indicate the direct electron transfer on the surface of the SPE and AML. Therefore,

Table 2
Comparison of various voltammetric methods for the determination of AML with recently published work.

Electrode	Linear range (μM)	LOD (μM)	Sensitivity (A L mol^{-1})	pH	Refs.
GC	8.1–41	12	0.015	5.5	[19]
MWCNTsP/CTAB	0.58–5.9	0.049	0.45	6.0	[24]
GC	1–35	0.31	……	5.5	[18]
EPPGE/ SWCNT	0.005–1	0.001	4.7	7.2	[17]
AuE/ oMWCNT	24–34	4.2	3.4	11.0	[20]
Graphene–chitosan/GC	1–70	0.6	0.296	7.3	[21]
BDD	0.2–6, 6–38	0.07	----	5.0	[23]
BDD	0.497–28.0	0.0764	0.215	5.0	[24]
Bare SPE	0.066–1.0	0.0207	0.43	9	This
DNA-modified SPE	0.066–2.0	0.0149	4.23		work

GC, glassy carbon electrode; MWCNTsP/CTAB, multi-walled carbon nanotubes paste in the presence of cationic surfactant cetyltrimethylammonium bromide; PG/SWCNT, pyrolytic graphite electrode modified single- walled carbon nano tube; AuE(oMWCNT) gold electrode modified oxidized multi-walled carbon nano tube; BDD, boron-doped diamond electrode; Graphene–chitosan composite film modified GC.

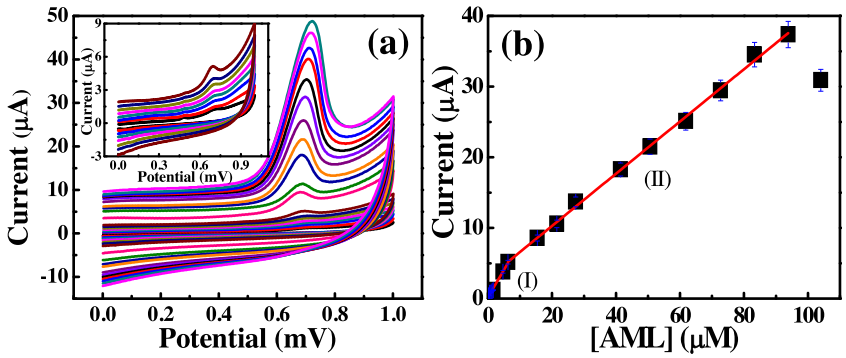

Fig. 4. (a) Cyclic voltammograms resulting from increasing additions of AML concentrations (0.166–104 μM) using bare SPEs, scan rate = 50 mVs^{-1} into B.R. buffer solution pH 9, (b) Analysis of the voltammetric profiles in terms of the peak height as a function of AML concentrations.

SPEs are highly recommended for the determination of AML in real samples compared to the recently published pre-treated boron-doped diamond electrode (BDD) [23,24]. Such SPEs offer some advantages over conventional solid electrodes such as low-cost, no-pre-treatment step, reproducible and can be used as a single-use sensor.

Fig. 4, shows the cyclic voltammetric curves resulting from increasing additions of AML concentrations in the range of 0.166–104 μM into buffer solution of pH 9 at scan rate of 50 mVs^{-1}. The bare SPE shows significant enhancement of oxidation peak current upon AML additions. The analysis of the voltammetric peak current reveals two linear responses (Fig. 4b). The first is over the range 0.166 μM–6.23 μM, (I_P (μA) = $0.77C_{AML}$ ($\mu molL^{-1}$) + 0.23 μA; R^2 = 0.99; N = 3) and the second from 6.23 μM to 93.8 μM, (I_P (μA) = $0.369C_{AML}$ ($\mu mol\,L^{-1}$) + 2.8 μA; R^2 = 0.998; N = 3); The limit of detection was determined to be 80.85 nM based on the standard deviation of the intercept and the average slope of the first linear calibration range that was obtained. The differential pulse voltammograms obtained utilizing the bare SPEs through the additions of AML into buffer solution of pH 9 over the concentration range of 66 nM–1.0 μM are shown in Fig. 5 (a, and b). The addition of AML increases the current intensity of the respective oxidation peak at 0.6 V (*vs.* Ag/AgCl). The linear calibration plot was obtained from the voltammograms over concentration range of 0.066 μM–1.0 μM (I_P (μA) = $0.46C_{AML}$ ($\mu mol\,L^{-1}$) + 0.0022 μA; R^2 = 0.998; N = 3). The limit of detection (3σ) was found to correspond to 20.7 nM, whilst the limit of detection using bare SPEs is comparable with the previously reported method using BDD or GC electrodes (Table 2). Since, the SPEs are preferable because they do not require any a pre-treatment and polishing steps.

In attempts to further enhance the electroanalytical performance of the bare SPE, DNA molecules were simply immobilized onto SPE surfaces. A solution of 1.0 g/L DNA was prepared in deionized water, then, particular volume has been immobilized onto the bare SPE electrode *via* drop-casting process. Consequently, DNA-modified SPEs were next used to explore the potential electrochemical improvements towards AML determination over bare SPEs. Fig. 6a shows the differential pulse voltammograms of 0.2 μM of AML in B.R. buffer of pH 9 using bare SPE and various volumes of DNA loaded onto SPEs. Analysis of the voltammetric peak current as a result of increasing amounts of DNA on SPE where it can readily be observed that the current was increased in magnitude. Comparison of a bare SPE with that of DNA-modified SPEs revealed that the current response of 5 μL DNA was the optimal electrode for determination of AML (Fig. 6b). This interesting enhancement of the oxidation current of AML was related to the mediation of DNA molecular wires to charge transport [26,27]. Fig. 6c shows the differential pulse voltammetric response of AML onto DNA modified SPE over a concentration range of 0.066–4.0 μM in B.R. buffer of pH 9. Analysis of the voltammograms peak height reveals linear response over the range of 0.066–2.0 μM; I_P(μA) = $4.23C_{AML}$ ($\mu mol\,L^{-1}$) + 0.15 μA; R^2 = 0.998; N = 3 (Fig. 6d). The limit of detection was calculated to be 14.94 nM. Interestingly, the sensitivity of the DNA-SPE toward oxidation of AML was enhanced 10-times more than bare SPEs. Whilst the limit of detection using SPEs is comparable with the DNA-modified SPE, the bare SPEs are preferable in determination of AML in tablet since they do not require a pre-treatment step.

The voltammetric determination of AML using SPEs was validated according to ICH [31] guidelines and complied with USP [32] on the validation of analytical methods (see Tables 3–6). Intra-day repeatability was determined by measurement of six replicates of three concentration levels covering the low, medium, and high ranges of AML calibration plot. Further, inter-day variation was also evaluated repeatedly in the same concentration ranges over a period of three days. Intra-day and inter-day precision were

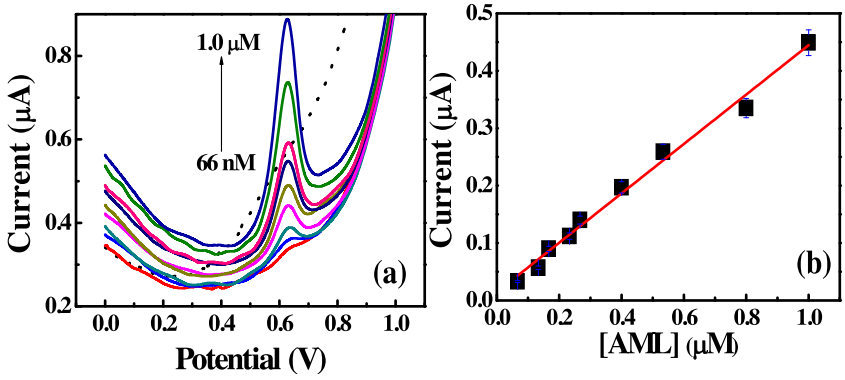

Fig. 5. (a) Differential pulse voltammograms recorded over a range of AML concentrations (0.066–1.0 μM) in a pH 9 of B.R. buffer solution using bare SPEs, (b) linear correlation of oxidation peak current and concentration of AML [DPV parameters; step potential 0.005 V, modulation time 0.05 s, modulation amplitude 0.025 V, interval time 0.5 s and scan rate 0.01 Vs^{-1}].

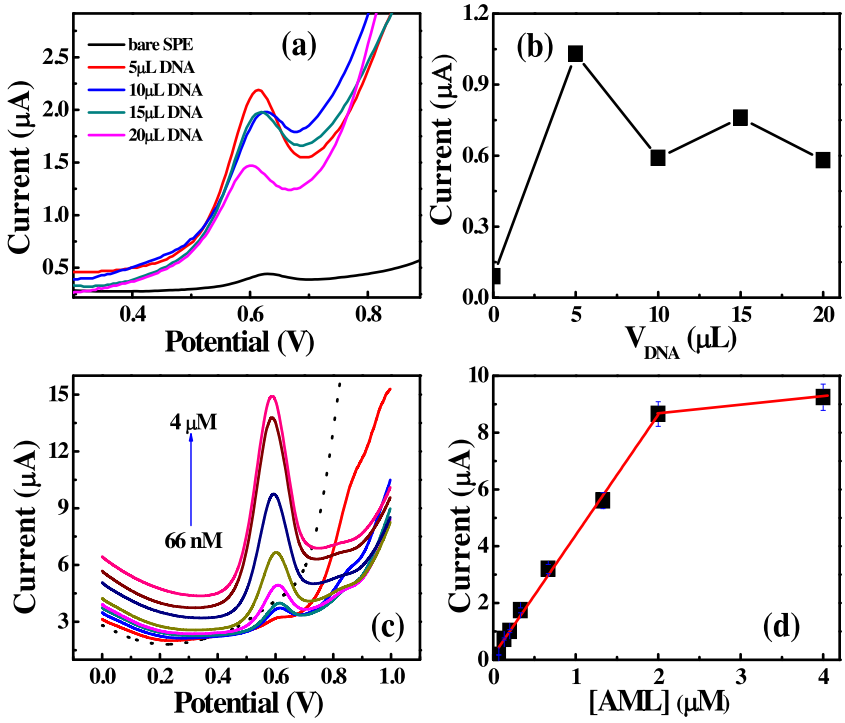

Fig. 6. (a) Differential pulse voltammograms of 0.2 μM AML in B.R. buffer solution of pH 9 using bare and DNA-modified SPEs. (b) The current responses as a function of the volume of DNA loading on SPEs. (c) DPV responses recorded over a range of AML concentrations (0.066–4.0 μM) in a pH 9 solution using 5 μL DNA modified SPE. (d) linear correlation of oxidation peak current and concentration of AML on DNA-modified SPEs [DPV parameters; step potential 0.005 V, modulation amplitude 0.025 V, modulation time 0.05 s, interval time 0.5 s, scan rate 0.01 Vs^{-1}].

Table 3
Inter- and Intra-day precision of the proposed method.

Concentration ((μM))	Intra- day RSD(%)[a]	Inter- day RSD(%)[b]
0.2	2.78	2.91
0.4	1.84	2.31
0.6	3.28	3.52

[a] Estimated from 6 determinations at each concentration level.
[b] Estimated from 18 determinations at each concentration level over three days.

expressed as relative standard deviation (RSD%) and the results are depicted in Table 3. The RSD% values didn't exceed 3.52% indicating that the proposed method for voltammetric determination of AML using screen-printed electrodes is highly applicable with excellent precision. Furthermore, the robustness of the analytical protocol has been evaluated (Table 4). The robustness is a measure of the analytical protocol capacity to be constant by small but deliberate variation in the method parameters, which provides indication of its reliability during normal usage. As shown in Table 4, a small variation in the differential pulse voltammetric parameters did not affect significantly the current response. These results show that the SPEs are highly promising electrode material for determination of AML in real samples.

3.1. Determination of AML in real samples

To verify the applicability of the SPEs and standard addition method, the SPE was applied for the analysis of AML in dosage forms (tablet, containing 5 mg AML) and real-life sample (urine sample). Each experiment was performed in six replicates by the standard addition protocol. The recovery experiments were carried out by

Table 4
Robustness of the proposed voltammetric determination of AML.

Experimental parameter	Recovery (%) ± SD[a]
Optimal parameters	99.93 ± 1.96
Starting Potential	
−0.02	98.45 ± 1.56
0.02	100.98 ± 2.45
Modulation Amplitude	
0.02495	97.96 ± 2.45
0.02505	101.53 ± 1.58
Modulation Time	
0.0495	98.67 ± 1.97
0.0505	99.45 ± 2.25
Step Potential	
0.0495	103.97 ± 2.79
0.0505	98.15 ± 1.69
Interval Time	
0.495	98.29 ± 1.88
0.505	97.64 ± 2.26

[a] Average of three determinations of 0.6 μM Amlodipine.

Table 5
Determination of AML in Tablet sample.

Amount taken (μM)	Amount added (μM)	Total Amount found (μM)	Recovery (%) ± SD[a]
0.4	0.2	0.615	102.5 ± 2.69
0.4	0.4	0.825	103.12 ± 1.89
0.4	0.6	1.043	104.3 ± 3.34

[a] Average of six determinations.

Table 6
Determination of AML in Urine sample.

Concentration Added [μM]	Concentration Found [μM]	% Recovery ± SD[a]
0.3	0.27	89.93 ± 3.56
0.45	0.41	92.09 ± 2.31
0.6	0.49	81.25 ± 2.67

[a] Average of six determinations.

adding 0.4 μM of AML solution prepared from commercial product to 15 mL of B.R. buffer of pH 9 followed by successive additions of 0.2 μM of AML. The DPV responses were recorded and the data is illustrated in Table 5.

As well as, human urine sample was analysed using the same parameters of DPV experiment. A 0.5 mL urine sample was injected into 14.5 mL of B.R. buffer solution of pH 9 in order to obtain an optimal electrochemical response. The data of the recovery experiment was presented in Table 6, which clarify that, the SPE is highly sensitive for detecting low and higher concentrations of AML in real samples.

4. Conclusion

The bare/unmodified and DNA-modified graphite screen-printed electrodes (SPEs) were firstly used for determination of amlodipine antihypertensive drug. A good linearity in B. R. buffer (pH 9) in wide concentration range with corresponding sub-nanomolar detection limits of amlodipine was obtained. The immobilized DNA molecules onto SPEs significantly mediate charge transfer process. Consequently, the sensitivity of the SPE was enhanced 10-times compared to bare SPE. The developed protocol shows low detection limit, relative simplicity, low-cost compared to previously reported macroelectrodes. Due to the applicability of unmodified SPEs and cancellation of pre-treatment step, it has been employed for the determination of amlodipine in pharmaceutical formulations and urine samples. Finally, the proposed voltammetric method using bare SPEs does not need sophisticated instruments or any separation step and so can be used as alternative to chromatography methods in laboratories lacking the required facilities for these techniques.

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