Talanta 120 (2014) 1–9

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Voltammetric determination of sumatriptan based on a graphene/gold nanoparticles/Nafion composite modified glassy carbon electrode

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ARTICLE INFO

*Article history:*
Received 29 August 2013
Received in revised form
25 November 2013
Accepted 26 November 2013
Available online 3 December 2013

*Keywords:*
Sumatriptan
Graphene
Gold nanoparticles
Electro-reduction
Nafion

ABSTRACT

A mixture of graphene oxide and tetrachloroauric acid was electrochemically co-reduced directly on a glassy carbon electrode (GCE) surface via cyclic voltammetry so as to form a graphene (Gr)–gold nanoparticles (AuNP) composite. This nanocomposite was then coated with nafion (NAF) film so as to form Gr/AuNP/NAF/GCE. Sumatriptan (SUM) is a drug belonging to the triptan class, used for the treatment of migraine headaches. In this work, an electrochemical method based on the adsorptive stripping differential pulse voltammetry (AdSDPV) employing Gr/AuNP/NAF/GCE has been proposed for the subnanomolar determination of SUM. Characterization of the electrode material has been carried out by UV–visible spectrophotometry, X-ray diffraction and scanning electron microscopy. Also the electrode surface has been characterized by means of cyclic voltammetry, electrochemical impedance spectroscopy, chronocoulometry. By employing Gr/AuNP/NAF/GCE at pH 7.0 phosphate buffer, a 20-fold enhancement in the AdSDPV signal was observed as compared to GCE. Under the optimized conditions, $I_p$ (μA) was proportional to the SUM concentration in the range of $1.0\times10^{-6}$–$4.12\times10^{-5}$ M ($R^2$=0.9991) and $2.14\times10^{-9}$–$1.0\times10^{-6}$ M ($R^2$=0.9954) with a detection limit ($3\times SD/s$) of $7.03\times10^{-10}$ M. The practical analytical utilities of the modified electrode were demonstrated by the determination of SUM in pharmaceutical formulations, human urine and blood serum samples. This proposed method was validated by HPLC and the results are in agreement at the 95% confidence level.



## 1. Introduction

Sumatriptan (SUM) is a synthetic drug belonging to the triptan class, used for the treatment of migraine headaches [1]. It is structurally similar to Serotonin and is its agonist [2]. The specific receptor subtypes it activates are present on the cranial arteries and veins. Acting as an agonist at these receptors, SUM reduces the vascular inflammation associated with migraines. However, an overdose is toxic and leads to several side effects viz., paresthesia, warm/cold sensations, chest pain, fatigue and vertigo. Thus, its determination is of importance.

Literature reports a few analytical methods viz., high-performance liquid chromatography [3], ultraperformance liquid chromatography–tandem mass spectrometry [4,5], high performance thin layer chromatography [6] and electrochemistry [7–10] for determination of SUM. However, the chromatographic methods are time consuming, expensive, require complicated preconcentration processes and need complicated instruments. On the other hand, electrochemical methods have been employed in the present work for determination of SUM due to their high simplicity, high sensitivity, good stability and low cost.

Electrochemical analysis based on chemically modified electrodes has proved to be a sensitive and selective method for the determination of various organic molecules as well as metal ions [11–13]. These electrodes are inexpensive and possess many advantages such as low background current, wide range of potential windows, rapid surface renewal and easy fabrication. Nanomaterials [14–18], metal complex [19,20], macrocycles [21–23], etc. are some of the modifiers employed to fabricate chemically modified electrodes. Glassy carbon electrodes (GCEs) are very versatile as electrode material for trace level determination of organic molecules as they provide high sensitivity, negligible porosity, and good mechanical rigidity. GCEs have been modified by means of different modifiers [24,25].

Graphene (Gr) has triggered a new genre for the development of novel electrode materials due to its amazing structural, mechanical, electrical and physical properties [26–31]. Gold nanoparticles (GNPs), on the other hand, due to their large aspect ratio (surface area to volume), biocompatibility and high electrical

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http://dx.doi.org/10.1016/j.talanta.2013.11.077

conductivity have also been widely employed as a modifier in voltammetry for analysis of various species [32,33]. The introduction of metal nanoparticles into the dispersion of graphene sheets could inhibit the aggregation of graphene sheets and result in a mechanically jammed, exfoliated graphene agglomerate with very high surface area [34]. Nafion, a perfluorinated sulphonated cation exchanger with properties of excellent antifouling capacity, chemical inertness and high permeability to cations, has been extensively employed as an electrode modifier for organic molecules [35,36]. Thus, a synergistic effect of Gr, AuNP and NAF composite film modified GCE can enable a sensitive determination of SUM.

In this article, a graphene/gold nanocomposite/Nafion film modified glassy carbon electrode has been used to determine SUM. The reduction reactions of both graphene oxide (GO) and $HAuCl_4$ occured under cathodic conditions and thus a mixture of GO and $HAuCl_4$ is electrochemically co-reduced directly on the glassy carbon electrode through cyclic voltammetry (CV). The loading amount of deposits is controlled by the number of potential circle. This composite film modified GCE was used for the subnanomolar determination of SUM employing adsorptive stripping differential pulse voltammetry (AdSDPV). The surface characterization of the electrodes has been carried out using a scanning electron microscope (SEM), UV–visible spectrophotometry (UV–vis) and X-ray diffraction analysis (XRD). The electrochemical surface characterization of the modified electrodes has been carried out using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and chronocoulometry (CC). Employing the proposed method, determination of SUM has been carried out in pharmaceutical formulations, urine and blood serum samples. Moreover, the proposed voltammetric method was validated by HPLC and the results obtained were in good accordance with those obtained by the proposed method. To the best of our knowledge, only three voltammetric methods are available for the determination of SUM employing chemically modified electrodes [8–10].

## 2. Experimental

### 2.1. Chemicals and instrumentation

All chemicals were of A.R. grade and were used as received without any further purification. Sumatriptan (Sigma Aldrich; >98 HPLC grade) was used as received without further purification. Graphite powder (99% trace metals basis) was purchased from S.D. Fine (India). Chloroauric acid ($HAuCl_4$) was from obtained Sigma Aldrich. Nafion (NAF, 1100EW, 5 wt% aqueous alcoholic solution, Aldrich) was prepared as 0.1% solution by dilution with ethanol. All solutions were prepared using double distilled water of specific conductivity (0.3–0.8 μS). Phosphate buffer solution (PBS; 0.1 M, pH 7.0) was employed as a supporting electrolyte. The developed method was employed for analysis of the following pharmaceuticals: Imitrex (25.0 mg and 50.0 mg; Glaxo Smith Kline) and Treximet (85 mg; Glaxo Smith Kline).

All voltammetric, chrono and electrochemical impedance studies (EIS) measurements have been performed on Eco Chemie, Electrochemical Work Station, model Autolab PGSTAT 30 using GPES software, version 4.9.005 and Frequency Response Analyzer, software version 2.0, respectively. A three-electrode system employing, a GCE (diameter=3 mm) was used as a working electrode, platinum wire and Ag/AgCl (sat. KCl) were used as counter and reference electrodes, respectively. The pH measurements were performed using ELICO LI 120 pH meter. HPLC used for validating the method was an Agilent model 1100. The HPLC analysis was carried out on a C18 column using phosphate buffer (0.05 M): acetonitrile (80:20, v/v; pH adjusted to 6.0) as a mobile phase at a flow rate of 1 mL/min and wavelength of 214 nm [37]. XRD analysis was carried out on an X-ray diffractometer (Shimadzu 7000S, Shimadzu Analytical, Japan) equipped with $CuK_\alpha$ radiation ($\lambda$=0.154 nm). The UV–visible spectroscopy was carried out on a Shimadzu UV-2450 spectrophotometer with samples in a quartz cuvette operated from 200 to 800 nm. The scanning electron microscope employed for surface characterization of the electrodes was a FEI Quanta-200 model with an operating voltage of 20 kV.

### 2.2. Preparation of the graphene/AuNP/Nafion/GCE

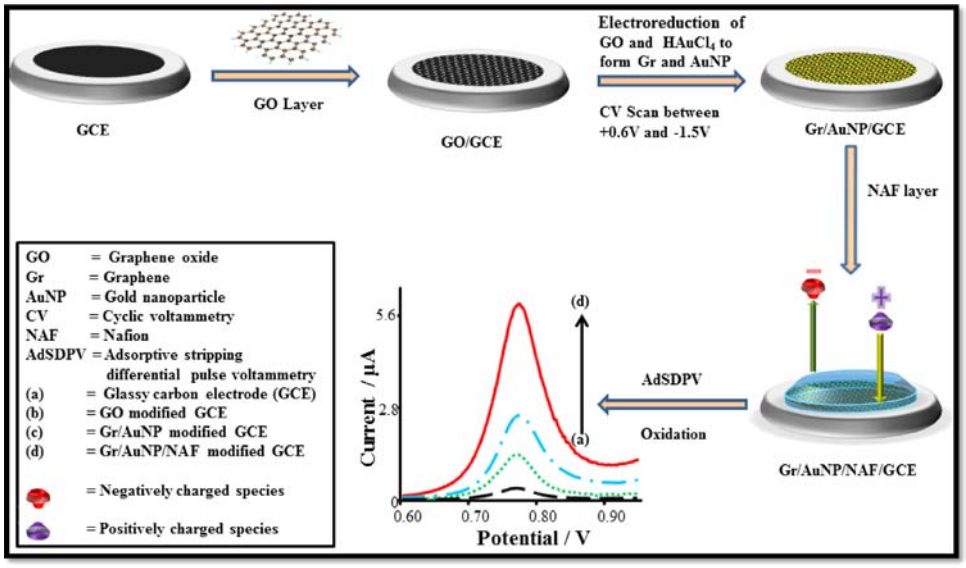
The GCE was pretreated by abrading its surface with aqueous slurries of alumina powders (1.0 μm and 0.3 μm α-$Al_2O_3$) on polishing cloth and carefully rinsed with water to give a smooth and clean electrode surface. After that, the electrode was ultrasonicated in distilled water for about 30 s, and finally allowed to dry under infrared lamp.

GO was synthesized directly from graphite by Hummers method [38]. The synthesized graphite oxide powder was exfoliated in doubly distilled water by ultrasonication for 2 h to form homogeneous GO dispersions with a concentration of 1.0 g $L^{-1}$. Graphene/AuNP composite was prepared according to a literature procedure [39]. This procedure for the fabrication of modified electrode is depicted pictorially in Scheme 1. For the preparation of graphene–gold nanocomposites, a mixture solution containing 10.0 mg $L^{-1}$ GO and 0.24 mM $HAuCl_4$ was prepared. The electrochemical co-reduction was performed in the mixture solution under magnetic stirring using cyclic voltammetry. Here, the CV scan was performed between −1.5 and 0.6 V at a rate of 25 mV $s^{-1}$ (Fig. S1). The thickness of the nanocomposite film was controlled by five potential cycles. It was observed that after electro-reduction, the surface of the GCE changed from black to bright red (color of AuNPs), which indicated that AuNPs were deposited onto the electrode surface.

After electrochemical co-reduction, the working electrode was washed with doubly distilled water and dried under I.R. lamp. Finally, NAF modification was carried out by drop casting (7.0 μL, 0.1%) onto the surface of the GCE and the solvent was allowed to evaporate at room temperature.

### 2.3. Experimental procedure

For stripping voltammetric analysis of SUM, appropriate quantities of the analyte solution was placed into a 25 mL standard volumetric flask and then diluted to the mark with PBS, pH 7.0 (0.1 M). The solution was then transferred into the electrochemical cell where the measurements were carried out. A magnetic stirrer (Expo Hi-Tech, India) with a stirring bar was used to provide the convective transport of the analyte during its accumulation onto the GCE surface. An accumulation potential of −0.5 V was applied to the Gr/AuNP/NAF/GCE for 60 s while the solution was stirred at 400 rpm with the magnetic stirrer. At the end of the accumulation period, the stirring was stopped, and a 15 s rest period was allowed for the solution to become quiescent. The voltammogram was then recorded by scanning the potential towards the positive direction from +0.6 to +0.95 V using the differential pulse mode employing a step potential of 5 mV and a modulation amplitude of 50 mV. When necessary, renewal of the electrode surface was easily accomplished by soaking the modified electrode into the supporting electrolyte and cycling the potential between −1.5 V and +0.6 V (vs. Ag/AgCl) in PBS (pH 7.0) buffer solution five times before use so as to renew the electrode surface. The cyclic voltammetric experiments were carried out by scanning the potential from 0.6 V to +1.0 V. Double potential step chronocoulometry was carried out with a pulse period of 5 s from +0.5 V to +1.0 V vs. Ag/AgCl. SEM images were obtained by removing the surface layers of GO, Gr/AuNP and Gr/AuNP/NAF from their




**Scheme 1.** Schematic illustration of stepwise electrode modification.

respective electrodes and dropping on carbon-coated aluminum grids for SEM imaging since the modified electrodes could not be inserted directly into the SEM.

### 2.4. Treatment and determination of samples

Analysis of SUM was carried out in pharmaceutical formulations and synthetic samples. Twenty tablets of SUM were weighed and ground to a fine powder using a mortar and pestle. For all of these experiments, the samples were diluted to 100 mL with pH 7.0 phosphate buffer solution. Recovery tests were performed for determination of SUM by spiking standard solutions of these molecules into pharmaceutical formulations. The urine and blood serum samples were collected from healthy volunteers. For the determination of SUM in urine samples, no pre-treatment step was carried out. Blood serum samples were obtained from a local pathology clinic and stored under refrigeration. To avoid interferences occurring from the serum matrix, a 50 µL serum sample was added to the electrochemical cell containing 25 mL of buffer solution. The cleaning of all the samples was done by filtering through a 0.22 µm PVDF syringe filter (Millex, Millipore Corporation) before voltammetric measurements.

## 3. Results and discussion

### 3.1. Effect of pH and supporting electrolyte

Standard solution of SUM ($5.2\times10^{-6}$ M) was used to find the optimum pH of supporting electrolyte which is best suited for its determination employing bare GCE. The influence of the pH on the oxidation peaks current of SUM was investigated employing Britton–Robinson (B.R.) buffer in the pH range of 2.0–11.0. It was observed that as pH of the medium was gradually increased, the potential kept on shifting towards less positive values, suggesting an involvement of proton in the reaction. Over the pH range 2.0–11.0, the peak potential ($E_p$) for SUM is a linear function of pH. This relationship can be described by the following equation:

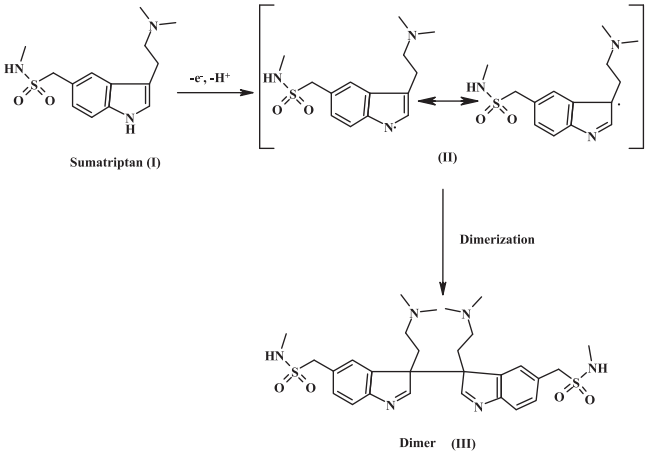
$$\text{SUM}: \quad E_{p,a}\ (\text{mV}) = -61.8\ \text{pH} + 1140.4 \quad (R^2 = 0.9939) \qquad (1)$$

A slope of −61.8 (close to the theoretical value of −59 mV/pH) reveals that an equal number of protons and electrons are involved in the oxidation reaction of SUM. It was further observed that the peak current for SUM was maximum at pH 7.0 (Fig. S1). Thus, this pH was employed for further studies. Various buffers, viz., phosphate, tris, citrate–phosphate and HEPES buffers were then employed at pH 7.0 (Fig. S2). Out of these, pH 7.0 phosphate buffer solution (PBS) gave the best response in terms of peak current and peak shape and hence was employed as supporting electrolyte for further studies. In the next step, optimization of buffer concentration was carried out by varying its concentration in the range of 0.01–1.0 M. The best peak response was observed for 0.1 M of PBS and hence was used for further studies.

### 3.2. Effect of Gr, AuNP and Nafion on the oxidation peak of SUM

The amount of modifier can change the properties and functions of the electrode surface. With regards to this, thickness of graphene–gold nanocomposite film was a crucial control factor for the determination of SUM. A dispersion containing 10.0 mg $L^{-1}$ GO and $2.4\times10^{-4}$ M $HAuCl_4$ was prepared for electrochemical co-reduction synthesis of graphene–gold nanocomposites. During the cathodic sweep of CV between +0.6 V and −1.5 V, the reduction of both GO and $HAuCl_4$ occurred. The loading amount of deposits was controlled by the number of potential cycles ($N$). The relationship between oxidation peak current and the number of potential circle is shown in Fig. S3. It can be seen that a sharp increase in the oxidation peak current was observed up to seven CV cycles. Further increasing the loading amount of deposits caused a decrease in the peak current which suggested that nanocomposite film turned thicker and hence the electron transfer rate was hindered. Therefore, seven CV cycles were chosen as an optimum number for the electro-reduction experiments.

SUM has four p$K_a$ values: 4.21 and 5.67 for succinic acid part; 9.63 for tertiary amine group and 12.0 for sulfonamide group [7]. As shown in Scheme 2, the tertiary amine part is involved in the oxidation of sumatriptan. Thus, when pH 7.0 PBS is employed as a supporting electrolyte, the reaction site of SUM exists in cationic form. This positively charged SUM exchanges with the $H^+$ from NAF, thus facilitating its accumulation onto the electrode surface. Thus, optimizing the amount of nafion as a modifier is necessary. The relationship between peak currents and the amount of 0.1% NAF is shown in Fig. S4. Initially, the peak current increases with increasing amount of 0.1% Nafion. However, when the amount




**Scheme 2.** Probable mechanism of SUM oxidation.

exceeds 7 μL, the peak current starts to decrease. On increasing the amount of NAF from 1 to 7 μL, the sites of ion exchange increases, and the adsorption of SUM on the Nafion-modified electrode is enhanced. Hence, the peak current increases. However, when the amount of Nafion is increased beyond 7 μL, a decrease in peak current is observed. This is because an increased Nafion film thickness will cause a higher resistance for the electrochemical process, which in turn hinders the electron exchange between SUM and protons of NAF. Thus, 7 μL of Nafion was used to prepare the modified electrode. Gr/AuNP/NAF/GCE was prepared by dropping 7 μL of NAF onto the Gr/AuNP/GCE surface and the solvent was allowed to evaporate under IR lamp.

### 3.3. Cyclic voltammetry (CV)

The cyclic voltammograms of SUM ($5.5\times10^{-6}$ M) at GCE, GO/GCE, Gr/NAF/GCE, Gr/AuNP/GCE and Gr/AuNP/NAF/GCE are given in Fig. 1(A). It can be observed from the figure that moving from GCE to Gr/AuNP/NAF/GCE, the anodic peak current of SUM increases. It can also be observed that the background current is higher for Gr/AuNP/NAF/GCE. This is due to the increased surface area of the electrode surface. Thus, the oxidation of SUM becomes facile on Gr/AuNP/NAF/GCE.

The effect of potential scan rate on the peak current of SUM was also studied. It can be seen that the oxidation of SUM is completely irreversible in nature. From Fig. 1(B), it can be seen that the oxidation peak shifted to a more positive value with increasing scan rates along with a concurrent increase in current. The CV results indicated that the anodic peak currents ($I_p$) of SUM increased linearly with the scan rate ($\nu$) in the range from 10 mV $s^{-1}$ to 1000 mV $s^{-1}$ (Fig. 1(C)). This finding implied that the oxidation of SUM is an adsorption controlled process on the Gr/AuNP/NAF/GCE.

The number of electrons ($n$) involved in the reaction was calculated from the cyclic voltammetry. $E_p - E_{p/2}$ value was calculated to be 94.2 mV. This value was then substituted in the following equation to obtain '$n$' value:

$$E_p - E_{p/2} = 47.7/\alpha n_a \text{ mV at } 25\ ^\circ\text{C} \quad (2)$$

Solving this Eq. (2), the $\alpha n_a$ value is found to be 0.506. Now, for a totally irreversible reaction, the electron transfer coefficient ($\alpha$) is assumed to be 0.5. Therefore, by substitution of the value of $\alpha$ in the above equation provides the value of n to be ca. 1 for the oxidation of SUM.

In order to verify the exact value of $\alpha$, we made use of the Laviron equation for irreversible electrode process [40]:

$$E_{pa} = E^0 + (RT/\alpha nF)\ln(RTk^0/\alpha nF) + (RT/\alpha nF)\ln\nu \quad (3)$$

where $\alpha$ is the transfer coefficient, $k^0$ is the electrochemical rate constant, $n$ is the number of electrons, $\nu$ is the scan rate and $E^0$ is the formal potential. Other symbols have their ususal meanings.

The value of αn can be calculated from the slope of $E_{pa}$ vs. ln $\nu$. In this system, a slope of 0.0516 was obtained. Now substituting $R$, $T$ and $F$ values of 8.314 J/K mol, 298 K and 96,500 C and $n=1$, the value of $\alpha$ was calculated to be 0.498 (ca. 0.5).

The probable electron transfer mechanism is as given in Scheme 2. The oxidation process takes place at the indole moiety of SUM. As can be seen from Scheme 2, one electron and one proton oxidation of SUM (I) rapidly gives a free radical (II) which on combining with another SUM molecule gives a dimer (III) in which two units are joined at β position.

### 3.4. Chronocoulometry (CC)

Electro-oxidation of $5.0\times10^{-5}$ M SUM at the GCE, GO/GCE, Gr/AuNP/GCE and Gr/AuNP/NAF/GCE was investigated by employing chronocoulometry for the determination of the kinetics and mechanisms of electrode reactions. Employing double-potential step chronocoulometry, after point-by-point background subtraction, the plot of charge ($Q$) vs. the square root of time ($t^{1/2}$) showed a linear relationship. According to the integrated Cottrell equation, the diffusion coefficient and $Q_{ads}$ of SUM could then be estimated from the slope and intercept, respectively, of the plot of total $Q$ vs. $t^{1/2}$, given by the Anson equation [41]. The resulting calculated parameters are presented in Table 1. As can be seen from the table, the value of the slope and the $Q_{ads}$ for the Gr/AuNP/NAF/GCE were more than that for other electrodes, confirming that NAF along with Gr/AuNP makes the accumulation of SUM onto the electrode surface more effective.

The surface coverage ($\Gamma^0$) for all four electrodes was calculated using the following relationship:

$$Q_{ads} = nFA\Gamma^0 \quad (4)$$

and the results are given in Table 1. From these values, it was observed that the surface coverage was maximum in the case of the Gr/AuNP/NAF/GCE. Thus, due to the synergistic effect of Gr, AuNP, NAF, the electrode surface coverage by SUM drastically increased and the kinetics of oxidation became more facile, confirming the results obtained from CV.

### 3.5. Electrochemical impedance spectroscopy (EIS)

In an attempt to clarify the differences among the electrochemical performance of the GCE, GO/GCE, Gr/AuNP/GCE and Gr/AuNP/NAF/GCE, electrochemical impedance spectroscopy (EIS) was employed as a technique for the characterization of each electrode surface. As such, the Nyquist plots for $K_3[Fe(CN)]_6$/$K_4[Fe(CN)]_6$ showed a significant difference in responses for all four electrodes, as shown in Fig. 1(D). A semicircle with a large diameter was observed for the GCE in the frequency range $10^{-2}$–$10^6$ Hz. However, the diameter of the semicircle diminished when Gr/AuNP/NAF/GCE was employed. Furthermore, the charge transfer resistance ($R_{ct}$) values obtained from Fig. 1(D) for $K_3[Fe(CN)]_6$/$K_4[Fe(CN)]_6$ ($1\times10^{-3}$ M) at the GCE, GO/GCE, Gr/AuNP/GCE and Gr/AuNP/NAF/GCE were 0.9, 0.55, 0.37 and 0.15 KΩ, respectively. This observation implied that the charge transfer resistance of the electrode surface decreased and the charge transfer rate increased upon employing the Gr/AuNP/NAF/GCE. A Warburg at 45° was also observed for all the electrodes of interest. The $R_{ct}$ value for the NAF modified electrodes was less than that of GCE

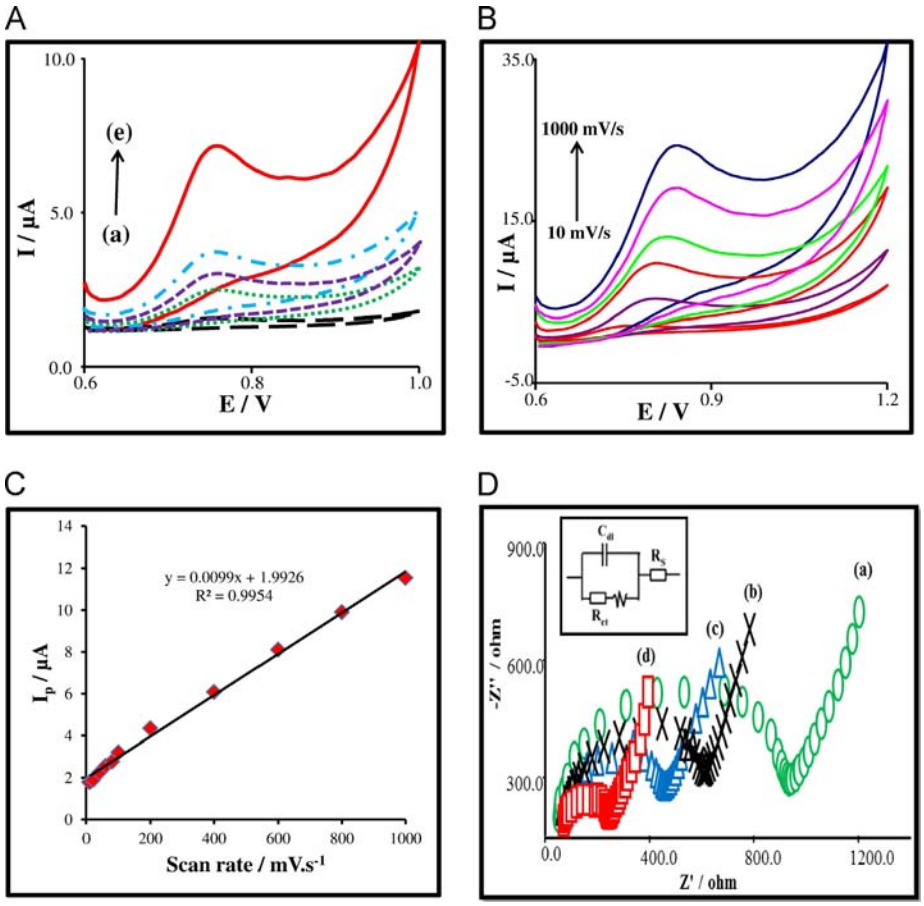



**Fig. 1.** (**A**) Cyclic voltammograms of $5.5 \times 10^{-6}$ M SUM at four different electrodes: (a) GCE (- - -), GO/GCE (• • • • • • •), Gr/NAF/GCE (— — —), Gr/AuNP/GCE (—•—•—) and Gr/AuNP/NAF/GCE (————). Voltammetric conditions: scanning electrode potential with a scan rate of 50 mV $s^{-1}$ between +0.6 and +1.0 V in pH 7.0 phosphate buffer (0.1 M); (B) Cyclic voltammograms of SUM ($5.0 \times 10^{-6}$ M) obtained in phosphate buffer (pH 7.0) employing varying scan rates (mV $s^{-1}$): (1–6) 10, 60, 100, 400, 600 and 1000. (C) $I_p$ vs. scan rate plot for the data obtained from (B); (D) Nyquist plots for EIS measurements ($1 \times 10^{-3}$ M $K_3[Fe(CN)]_6/K_4[Fe(CN)]_6$) at GCE (OOO), GO/GCE (× × ×), Gr/AuNP/GCE (ΔΔΔ) and Gr/AuNP/NAF/GCE (□□□). In the box on the right upper side is the equivalent circuit used for data fitting.

**Table 1**
Chronocoulometry of $5.0 \times 10^{-5}$ M SUM at four electrodes.

| Electrode | Slope ($\mu C/s^{-1/2}$) | $Q_{ads}$ ($\mu C$) | Surface coverage ($10^{-10}$ mol/cm$^2$) | Diffusion coefficient ($10^{-6}$ cm$^2$/s) |
|---|---|---|---|---|
| GCE | 0.19 | 0.23 | 01.61 | $5.71 \pm 0.11$ |
| GO/GCE | 1.11 | 3.95 | 04.82 | $5.74 \pm 0.07$ |
| Gr/AuNP/GCE | 1.37 | 7.99 | 07.89 | $5.68 \pm 0.10$ |
| Gr/AuNP/ NAF/GCE | 3.25 | 75.08 | 31.12 | $5.75 \pm 0.03$ |

only till 7 μL of NAF was coated onto the electrode surface. Beyond this volume of NAF, the $R_{ct}$ value for NAF coated electrodes increased as compared to GCE. The double layer capacitance ($C_{dl}$) was then obtained at the maximum frequency in the Nyquist plot. The $C_{dl}$ values for GCE, GO/GCE, Gr/AuNP/GCE and Gr/AuNP/NAF/GCE were 0.044, 0.073, 0.108 and 0.267 μF, respectively.

The Kramers–Kronig transformation test was carried out to test the validity of the impedance data. The Kramers–Kronig transformation gave a $\chi^2$ (chi square) of $2.83 \times 10^{-6}$. Therefore, the system satisfied all the conditions for very good impedance data (i.e., linearity, causality, stability and finiteness of the system). Thus, the test implied that the impedance data were validated with respect to impedances over a wide frequency range and were of very good quality.

### *3.6. UV–visible spectrophotometry, X-ray diffraction and scanning electron microscopy*

Characterization of electrode material was further carried out by UV–visible spectrophotometry (UV–vis), X-ray diffraction (XRD) and Scanning electron microscopy (SEM). UV–vis spectra of graphene oxide and reduced graphene oxide are shown in Fig. 2(A). The electrochemical reduction of graphene oxide to graphene and $HAuCl_4$ to AuNPs was verified by scraping off the Gr/AuNP composite from the GCE surface and monitoring it by measuring the UV–visible spectra of the solutions after diluting the sample with deionized water. Graphene oxide shows strong absorption peak at ca. 230 nm (Fig. 2(A), curve (b)) which corresponds to the π to $\pi^*$ transition of the aromatic C–C bond. After electrochemical reduction, the disappearance of peak at 230 nm and appearance of a peak at 275 nm corresponds to the complete reduction of graphene oxide to graphene [42]. Color of gold is attributed to its surface plasmon resonance (SPR).The SPR band of gold appears at 539 nm [43]. In Fig. 2(A), curve (c), the peaks at ca. 270 nm and 540 nm correspond to the presence of graphene and AuNP in the composite due to the electro-reduction process. Further confirmation of the electro-reduction process was carried out by carrying out the XRD analysis for Gr/AuNP. As can be seen from plot Fig. 2(B), Gr shows peaks at $2\theta = 24.54^\circ$ and $43.54^\circ$, corresponding to its (002) and (111) reflections [42]. On the other hand, AuNPs give five peaks at 38.1°, 43.80°, 64.50°, 77.50°and 81.66° which correspond to (111), (200), (220),

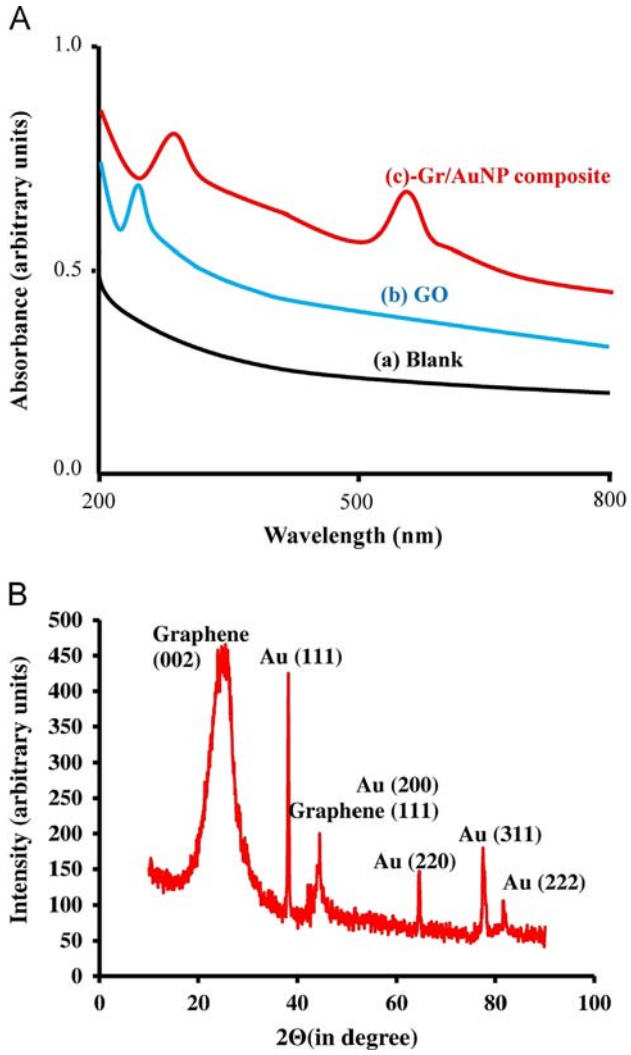



**Fig. 2.** (A) UV–vis spectra for (a) blank, (b) graphene oxide and (c) graphene/AuNP composite and (B) representative XRD patterns of Gr/AuNP composite.

(311) and (222) planes. XRD pattern further shows that gold nanoparticles are crystalline in nature and are face-centered cubic (fcc) in structure [43].

Fig. 3 compares the morphological features of the different electrode materials using SEM. Fig. 3(a) shows sheets of graphene oxide. Fig. 3(b) is the SEM image for graphene and AuNPs showing the presence of AuNPs as a cluster along with graphene. Fig. 3(c) is the image of the final composite employed in the present work viz., Gr/AuNP/Nafion. The SEM image of final composite shows that the nafion film is uniformly coated onto the graphene and AuNPs surface. Fig. 3(d) is the energy-dispersive X-ray spectrum for the final composite which shows the following elements: C from graphene and nafion; Au from AuNPs and F, O, S from Nafion.

### 3.7. Adsorptive stripping differential pulse voltammetry (AdSDPV)

AdSDPV was employed to study the influence of the accumulation potential ($E_{acc}$) and the accumulation time ($t_{acc}$) on the oxidation peak current of SUM (Fig. S5) employing Gr/AuNP/NAF/GCE. After optimization of the experimental conditions, $E_{acc}$ of $-0.5$ V and $t_{acc}$ of 60 s were selected as the optimum accumulation potential and time where SUM could be determined with good sensitivity.

A comparative study was then carried out, employing AdSDPV for $6.0\times10^{-6}$ M SUM (Fig. 4(A)) on the GCE, Gr/GCE, Gr/NAF/GCE, Gr/AuNP/GCE and Gr/AuNP/NAF/GCE. From these experiments, it could be observed that the best results in terms of peak current were obtained employing Gr/AuNP/NAF/GCE. The reasons for the notable sensitivity of SUM determination at the Gr/AuNP/NAF/GCE may be summarized as follows: (a) Gr/AuNP/NAF/GCE contains the cation exchanger, NAF, which has a selective cation exchange enriching property due to the electrostatic interaction and (b) electrochemically deposited Gr/AuNP displays attractive characteristics, such as high electrical conductivity, larger specific surface area, excellent adsorptive ability and catalytic ability. Hence, the synergetic effect of Gr/AuNP and Nafion and contributes to a higher current response of SUM. Thus, it can be concluded that the electro-oxidation of SUM became facile at the surface of Gr/AuNP/NAF/GCE.

### 3.8. Determination of SUM

Based on the above findings, an analytical method was proposed for determining concentrations of SUM employing AdSDPV at Gr/AuNP/NAF/GCE. The optimized conditions were applied for finding the limit of detection (LOD; $3\times SD/s$ where SD is the standard deviation for the intercept of the regression line and 's' is the slope of the linear calibration plot), linear working range (LWR), linear regression equation (LRE) and correlation coefficient ($r$). Validation of the proposed procedure for assay of standard SUM was examined via evaluation of limit of detection (LOD), limit of quantitation (LOQ), reproducibility, precision, selectivity and robustness. Under the optimized conditions, $I_p$ (μA) was proportional to the SUM concentration in two concentration ranges (Fig. 4(B)):

$$(a)\ \text{LWR}: \ 2.14\times10^{-9}-1.0\times10^{-6}\ \text{M}$$
$$: I_p\ (\mu\text{A}) = 1.8659\ [\mu\text{M}] + 0.1129\ (R^2 = 0.9954) \quad (5)$$

$$(b)\ \text{LWR}: \ 1.0\times10^{-6}-4.12\times10^{-5}\ M$$
$$: I_p\ (\mu\text{A}) = 0.4824\ [\mu\text{M}] + 1.7519\ (R^2 = 0.9991) \quad (6)$$

with a detection limit ($3\times SD/s$) of $7.03\times10^{-10}$ M (% RSD=3.22).

A break in the calibration curve of SUM probably reflects the formation of a sub-monolayer in the first range of calibration and formation of a monolayer in the second range [44]

### 3.9. Interference studies

Under optimal experimental conditions, the interference from selected organic compounds and metal ions was evaluated. The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error less than $\pm$ 5.0% at a concentration of $4.3\times10^{-8}$ M. Ascorbic acid (AA), uric acid (UA), citric acid, glucose and urea are the most common constituents found with SUM in biological fluids. AA ($pK_a$=4.17) and uric acid ($pK_a$=5.7) exist in anionic (negatively charged) form at pH 7.0 PBS. Thus, they get repelled by NAF (a cation exchanger) from the electrode surface and thus do not interfere with the analysis of SUM. Furthermore, interference studies were carried out employing molecules viz., indole-3-acetic acid, indole-3-pyruvic acid, indole-3-lactic acid and 5-hydroxy indole-3-acetic acid which have active group similar to SUM. All these analogs have a $pK_a$ value between 3.0 and 4.7 [45,46]. This indicates that these analogs are negatively charged in pH 7.0 PBS. Thus, they are repelled by the electrode surface and do not interfere with the analysis of SUM. A 500 fold excess of glucose, citric acid, urea did not interfere with the analysis of SUM. A 1000-fold excess of $Na^+$, $K^+$, $NH_4^+$ or $NO_3^-$ had no effect on the $I_p$ of SUM. These results suggested that the determination of SUM in pharmaceutical formulations and biological samples at Gr/AuNP/NAF/GCE is not

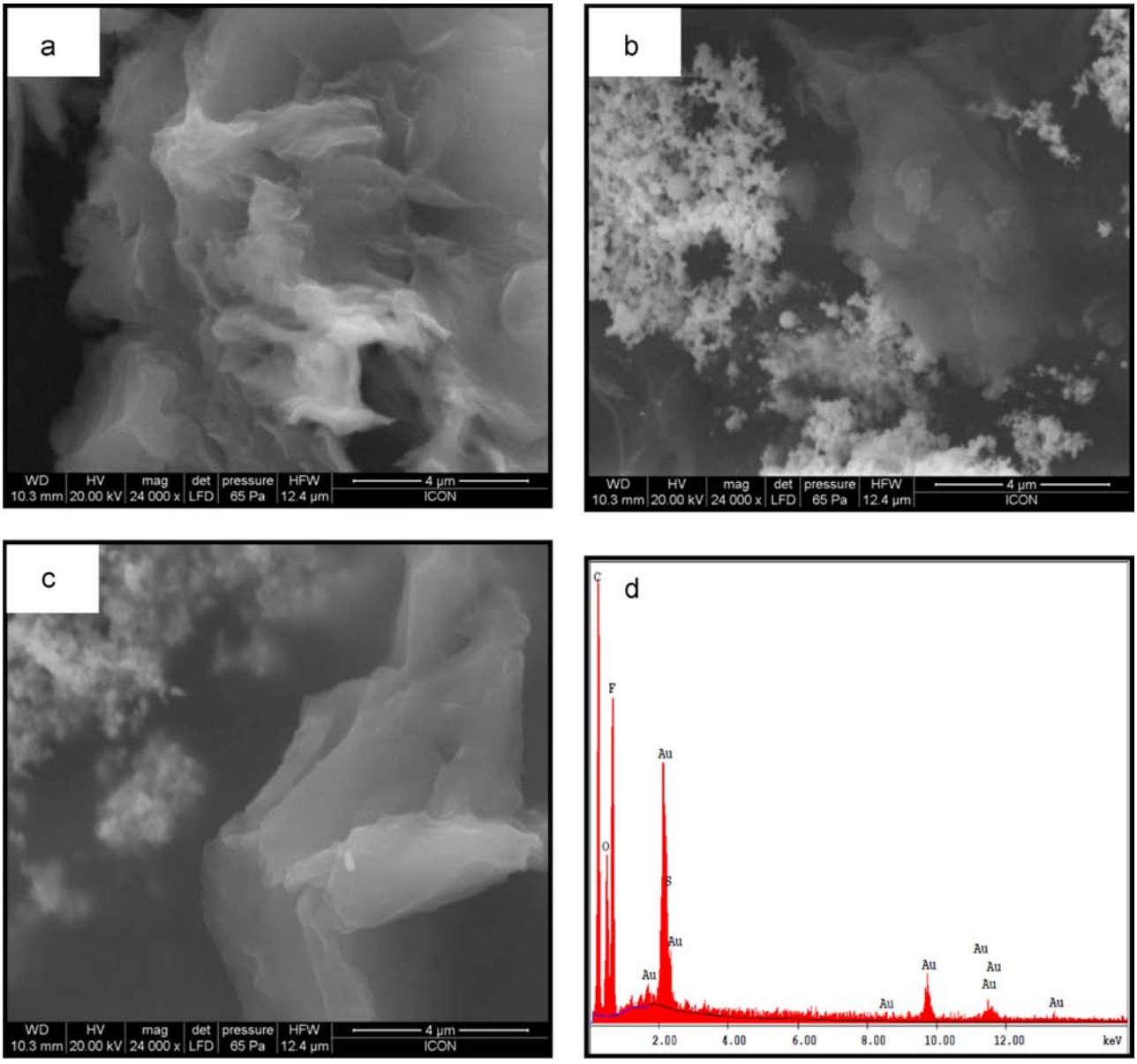



**Fig. 3.** SEM images of (a) graphene oxide, (b) Gr/AuNP, (c) Gr/AuNP/NAF composite film and (d) energy-dispersive X-ray spectrum for Gr/AuNP/NAF.

significantly affected by the most common interfering species and thus this method is selective in nature.

### 3.10. Validation studies and analytical applications

For validation of the proposed method, various parameters, such as repeatability, reproducibility, precision and accuracy of analysis were obtained by performing five replicate measurements for $4.55 \times 10^{-8}$ M standard SUM over a single day (intraday assay, $n=5$) and for five days over a period of one week (interday assay). Satisfactory mean percentage recoveries (% $R$) and relative standard deviations (% RSD) were obtained and are reported in Table S1. The recoveries obtained confirmed both the high precision of the proposed procedure and the stability of SUM solutions.

The robustness of the proposed procedure (Table S2) was also examined by studying the effect of small variations in pH, $E_{acc}$ and $t_{acc}$ on the recovery of SUM. As can be seen from Table S2, % $R$ was in the range of 98.5–101.5% under all variable conditions and did not show any significant change when the critical parameters were varied which implies that the method is robust in nature.

For further evaluation of the validity of the proposed method, recovery tests were carried out in pharmaceutical formulations, urine and human serum samples. Recovery tests were performed on pharmaceutical formulations, as mentioned in Table S3. These tests gave % $R$ values in the range of 98.5–100%. Similarly, recovery tests were performed on morning urine samples collected from healthy volunteers. The % $R$ obtained for these samples were in the range of 98.5–99%. Additionally, recovery tests were also performed on human blood serum. The % $R$ obtained in this case was in the range of 98.0–99% (Table S3). Based on these results, recovery of SUM was not affected significantly, and consequently, the described method is accurate for its assay in complex matrices. For analytical applications, the determination of the amount of SUM in all samples has been carried out by the standard addition method. The amount of SUM obtained in the pharmaceutical formulations by the proposed method was found to agree well with the label contents. The results also showed that interferences from the matrix were negligible.

The proposed method was further validated by employing HPLC (Table 2). This table shows that the amount of SUM obtained

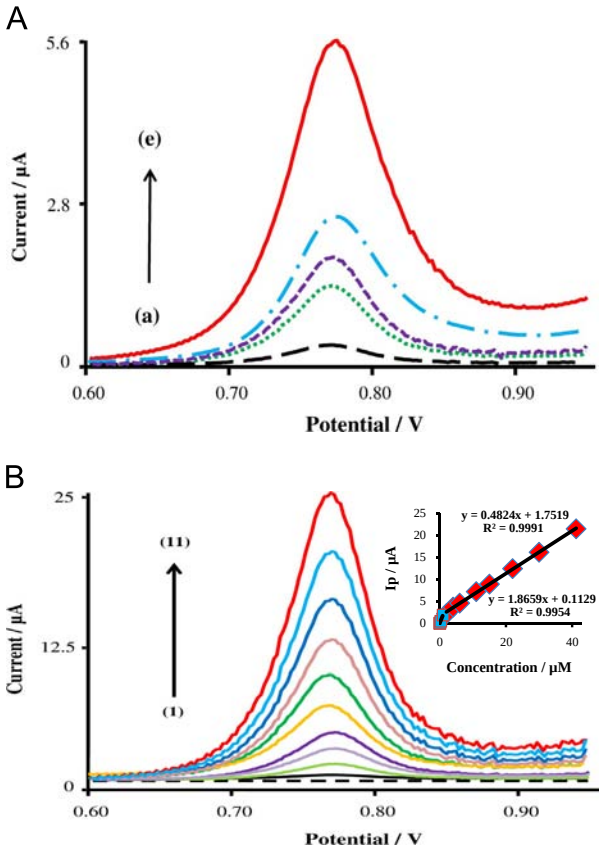



**Fig. 4.** (A) AdSDPV of $6.0\times10^{-6}$ M SUM at four different electrodes: (a) GCE (- - -), GO/GCE (• • • • • • •), Gr/NAF/GCE (▬ ▬ ▬), Gr/AuNP/GCE (▬•▬•▬•) and Gr/AuNP/NAF/GCE (——). Voltammetric conditions: $E_{acc}=-0.5$ V, $t_{acc}=60$ s, in phosphate buffer (pH 7.0), step potential=5 mV and modulation amplitude=50 mV. (B) AdSDPV curves obtained at Gr/AuNP/NAF/GCE for SUM at different concentrations: in the range from (1) blank, (2) $2.14\times10^{-9}$, (3) $1.0\times10^{-8}$, (4) $3.0\times10^{-7}$, (5) $1.0\times10^{-6}$, (6) $4.0\times10^{-6}$, (7) $6.0\times10^{-6}$, (8) $1.5\times10^{-5}$, (9) $2.2\times10^{-5}$ (10) $3.0\times10^{-5}$ and (11) $4.12\times10^{-5}$ M.

**Table 2**
Comparison between the proposed method and the HPLC method for sample analysis.

| Sample | SUM | | | *F*-test | *t*-Test |
|---|---|---|---|---|---|
| | a | b | c | | |
| **Imitrex** | 25 | 24.55 ± 2.1 | 24.01 ± 2.73 | 0.877 | 0.423 |
| **Imitrex** | 50 | 49.73 ± 1.9 | 48.82 ± 2.41 | 0.832 | 0.371 |
| **Treximet** | 85 | 84.93 ± 1.7 | 74.24 ± 2.33 | 0.935 | 0.356 |

Theoretical *F*-value=6.39 and *t*-test value=2.77 at 95% confidence limit for $n_1=5$ and $n_2=5$.

[a] Amount of SUM in a tablet (mg).
[b] Amount of SUM obtained by the proposed method (mg) ± % RSD ($n=5$).
[c] Amount of drug obtained by the HPLC method (mg) ± % RSD ($n=5$).

by the proposed method agreed well with the amount obtained by the HPLC method. Applying a paired *F*- and *t*-tests on the results obtained by the proposed procedure and those obtained by the standard method, it was found that all results are in agreement at the 95% confidence level. The experimentally calculated values of *F*- and *t*-tests were less than that of theoretical values for *F*- (6.39) and *t*-(2.77) tests (Table 2).

Also, it is observed from the table that there was no significant difference between the amount of SUM obtained by the proposed procedure and the HPLC method showing the validity of the developed method. Thus, determination of SUM can be carried out with great confidence in pharmaceutical formulations, urine and blood serum samples by the proposed method.

### 3.11. Stability and reproducibility of the Gr/AuNP/NAF/GCE

Stability of the Gr/AuNP/NAF/GCE was tested by keeping the electrode in pH 7.0 PBS for 10 days and then the CVs were recorded and compared with the CVs obtained before immersion. The results indicated that the peak current decreased by 1.24% for Gr/AuNP/NAF/GCE, which indicates that the electrode has good stability. The stability of the Gr/AuNP/NAF/GCE was also tested by storing it in air for two months, the electrode retained 99.1% of its initial peak current response for a SUM concentration of $5.2\times10^{-8}$ M by the end of one month, which shows the long-term stability of thin-film modifier on the surface of GCE during the determinations in aqueous solutions. A small loss in the sensor response was observed after a period of one month (Fig. S7). The results indicate a good stability of the sensor and capacity for repeated measurement to be performed on the same electrode.

In order to study the reproducibility of the electrode preparation procedure, five modified electrodes based on the same fabrication procedure were prepared and used for the determination of $5.2\times10^{-8}$ M SUM solution. The RSD for the between electrode peak currents (average of five determinations on each electrode) was calculated to be 3.4%. Using AdSDPV, the RSD for 10 replicate measurements on a single electrode in $5.2\times10^{-8}$ M SUM was 2.3%. The results indicate that the modified electrode has high reproducibility and repeatability in both the preparation procedure and the voltammetric determinations.

### 3.12. Comparison of proposed method with literature methods

Table 3 shows the comparison between the analytical performance of the present method and previous literature methods for the determination of SUM [7–10]. The electrodes used for the determination of SUM are: glassy carbon electrode (GCE) [7], MWCNT/AgNP/pyrolytic graphite electrode [8], MWCNT/polypyrrole doped with new coccine/GCE [9] and MWCNT/cobalt-schiff base/carbon paste electrode [10]. Out of all these electrodes, the limit of detection obtained for SUM is the lowest by the proposed method. These results reveal that the proposed Gr/AuNP/NAF/GCE has a large advantage over other reported methods in terms of

**Table 3**
Comparison between various electroanalytical methods for the determination of SUM with the proposed method.

| Electrode | Linear working range (M) | Limit of detection (M) | Samples analyzed | Ref. |
|---|---|---|---|---|
| Glassy carbon electrode (GCE) | $1.0\times10^{-6}$–$8.0\times10^{-6}$ | $0.5\times10^{-6}$ | Pharmaceutical formulations | [7] |
| MWCNT/AgNP/Pyrolytic graphite electrode | $8.0\times10^{-8}$–$1.0\times10^{-4}$ | $4.0\times10^{-8}$ | Pharmaceutical formulations | [8] |
| MWCNT and polypyrrole doped with new coccine/GCE | $0.02\times10^{-6}$–$10\times10^{-6}$ | $6.0\times10^{-9}$ | Pharmaceutical formulations | [9] |
| MWCNT/cobalt-schiff base/carbon paste electrode | $1.0\times10^{-6}$–$1.0\times10^{-3}$ | $0.3\times10^{-6}$ | Pharmaceutical formulations, serum | [10] |
| Gr/AuNP/NAF/GCE | $2.14\times10^{-9}$–$1.0\times10^{-6}$ and $1.0\times10^{-6}$–$4.12\times10^{-5}$ | $7.03\times10^{-10}$ | Pharmaceutical formulations, urine, blood serum | This work |

linear working range, limit of detection and number of analyzed samples.

## 4. Conclusion

Combining unique properties of electrochemically generated Gr/AuNP composite such as high specific surface area, electrocatalytic and adsorptive properties, with the cation selectivity of the Nafion film, a Gr/AuNP/NAF/GCE was developed for the determination of SUM. Adsorptive stripping voltammetry at Gr/AuNP/NAF/GCE has been shown to be suitable for the determination of sub nanomolar levels of SUM. The sensitivity was enhanced significantly by preconcentration of the drug on the modified electrode surface due to the presence of NAF. The NAF film provides an excellent natural barrier to interferences from negatively charged compounds and thus many interferences could be avoided. The method has been employed for the determination of SUM in pharmaceutical formulations, urine and blood serum samples. Since SUM is used for treatment of migraine headaches, it is expected that the proposed method will be useful in its determination in biological fluids as well as pharmaceutical formulations and thus would be of great help to both clinical as well as pharmaceutical industries.

## Acknowledgments

The funding for this work is partly by the University Grants Comission, New Delhi, India and partly by the US Army International Technology Center, Tokyo, Japan through contract number FA2386-12-1-4086.

## Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.077.

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