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Carbon nanotube embedded poly 1,5-diaminonapthalene modified pyrolytic graphite sensor for the determination of sulfacetamide in pharmaceutical formulations

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An electrochemically conductive single-walled carbon nanotube (SWCNT) embedded poly 1,5-diaminonapthalene (DAN) modified sensor has been developed for the determination of sulfacetamide (SFA). The surface morphology of the modified sensor has been characterized by FE-SEM, which revealed good dispersion of the carbon nanotube in polymer matrix. SFA was quantified using square wave voltammetry in phosphate buffer of pH 7.2, which acted as supporting electrolyte during analysis. The modified sensor exhibited an effective catalytic response towards the oxidation of SFA with excellent reproducibility and stability. The peak current of SFA was found to be linear in the concentration range of 0.005–1.5 mM and detection limit and sensitivity of 0.11 μM ($S/N=3$) and 23.977 μA mM^{-1}, respectively were observed. The analytical utility of method was checked by determining the SFA in various pharmacological dosage forms. The results obtained from the voltammetry were validated by comparing the results with those obtained from HPLC. The proposed method is sensitive, simple, rapid and reliable and is useful for the routine analysis of SFA in pharmaceutical laboratories.

1. Introduction

Sulfonamides, commonly known as sulfa drugs, are benzenoid amino compounds derived from the parent molecule sulfanilic acid that acts as bacteriostatics by inhibiting the formation of dihydrofolic acid, which is essential for the development of bacterial cells [1]. Sulfonamides are the most widely prescribed antimicrobial agents to cure human and veterinary infections due to their low cost and capability for remedy of various bacterial infections [2].

Sulfacetamide (SFA, Structure I), an important member of sulfonamide group is a synthetic antibacterial agent, employed for the treatment of numerous dermatological aberrations [3]. SFA is a highly potent drug and its sodium salt is effective in the curing of conjunctivitis [4], pityriasis versicolor [5] and acne [6]. The combination of sodium sulfacetamide with sulfur has been found to provide adjunctive effect for the treatment of rosacea armamentarium due to its dual uses as topical therapy and therapeutic cleansers [7]. It is suggested that SFA in combination with sulfathiazole may be used for healing of T-47D breast cancer cells [8]. In addition to its various uses as medicines, SFA has many side-effects including rashes on the skin, fever, vasculitis, anaphylaxis, cutaneous drug reaction, fixed drug eruption and hypersensitivity reactions like erythema and Stevens-Johnson syndrome [3]. SFA has also been found to cause toxic epidermal necrolysis, which is a more severe form of Stevens–Johnson syndrome [9].

The extensive clinical applications and side-effects of SFA render it necessary to develop a highly sensitive, fast and reliable approach to determine SFA in pharmaceutical formulations. Owing to concerns over the analytical determination of SFA, several methods have been developed for the analysis of SFA employing various techniques. Jen et al. reported the use of high performance liquid chromatography (HPLC) method to determine several sulfonamides in highly complex swine waste water [10]. The gas chromatography–mass spectrometric (GC–MS) method was utilized to analyze six sulfonamides in animal tissues [11]. Other kinds of detection such as spectrophotometric determination [12,13], photochemically induced fluorescence [14], flow injection analysis with fluorimetric [15] or chemiluminescence detection [16] and FT-IR spectroscopy have also been employed [17]. Other separation techniques, e.g. capillary zone electrophoresis with amperometric [18] or spectrophotometric detection [19,20], micellar electro kinetic capillary chromatography [21,22] and gas chromatography with atomic-emission detector were also used [23]. Nevertheless, most of these techniques often suffer from several disadvantages regarding to the cost, complex sample preparation due to tedious extraction and separation steps, use of various organic solvents and long analysis time, which make them practically unhelpful in

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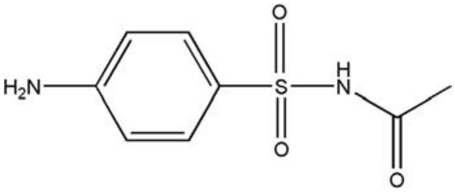
routine analysis. However, electroanalytical methods have attracted much attention of researchers towards the monitoring of drugs and biomolecules, owing to their low cost, ease of instrumentation, high sensitivity and selectivity and possibility of analysis without complicated sample pretreatment [24]. In the last decade although, there are several reports available concerning the electrochemical properties of sulfonamides using different types of electrodes [25–31], but very few electroanalytical methods have been developed for the determination of SFA due to the problem associated with electrode surface fouling. Fogg et al. have determined SFA by cathodic stripping voltammetry using hanging mercury drop electrode [32], but this method is not much beneficial due to the environmental toxicity of mercury.

In the present work we explored the use of carbon nanotube (CNT) embedded conductive polymer composite coated surface for the anodic analysis of SFA and to the best of our knowledge such type of sensor has not been used till now. Carbon nanotubes possess exceptional mechanical, thermal and electrical properties with their very high aspect ratio (length to diameter ratio > 1000), hence they are considered as an ideal additives for the formation of conductive network within a polymer matrix [33,34]. By now, several papers have reported certain aspects of mechanical enhancement of polymer system by the incorporation of CNT [35]. This composite has been found to exhibit unique properties due to the electronic interaction between two components, therefore many applications have been reported, such as capacitors, actuators, electrodes, schottky diodes and organic light emitting diodes [36]. Both nanotubes and conductive polymer possess conjugated π-electron system and interaction is likely to occur through π–π stacking [37,38]. It is well reported that properties of CNT/polymer composite strongly depend on the extent of CNT dispersion in monomer solution, hence insufficient dispersion of CNT is established as a diminishing factor for the composite's physical properties. Poor dispersion of CNT is due to their strong agglomeration tendency which is caused by intermolecular van der Waals interactions and entanglement between them [39]. Sonication is the most ubiquitous method as it is associated with cavitation forces which results in localized heating followed by separating nanotube from each other. However, intense mixing of CNT can induce breakage of CNT that drives to poor electrical properties in the nano composite. In most cases mild sonication conditions (low power, short time) in the presence of an appropriate solvent allows agglomerates to be separated from each other due to some degree of interaction between CNT and solvent and this results in disaggregation [40].

Poly 1,5-diaminonapthalene (p–DAN) which has received an increasing interest due to its unique electroconductive, electroactive and electrocatalytic properties [41], is selected in the present study for embedding CNT. The carbon nanotube/conductive p-DAN composite was prepared by electro-polymerization of monomer (DAN) in the presence of suspended nanotube (in ethanol) at the edge plane surface of pyrolytic graphite (EPPG).The composite film was found to exhibit effective catalytic response towards the electro-oxidation of SFA. The developed method is simple, sensitive, low cost and free from requirement of sample pretreatment and is successfully applied for the determination of SFA in pharmaceutical formulations. Moreover, the results obtained from electrochemical sensing of SFA in pharmaceuticals have been successfully validated using HPLC (Scheme 1).



Scheme 1. Sulfacetamide (I).

2. Experimental

2.1. Chemicals and reagents

Sulfacetamide was purchased from Sigma Aldrich, USA and used without further purification. Single walled carbon nanotube (SWCNT) of purity > 98% were obtained from Bucky, USA. Pyrolytic graphite pieces were obtained from Pfizer, USA as a gift. Perchloric acid, potassium chloride, potassium ferricyanide and 1,5-diaminonapthalene were purchased from Sigma Aldrich, USA. All the chemicals used to prepare phosphate buffers in the pH range 2.4–11.0 were obtained from E. Merck (India) Ltd., Mumbai. Phosphate buffers were prepared according to the method reported by Christian and Purdy [42]. The ionic strength was maintained at 1.00 mol L^{-1} during all the study. All the reagents used were of analytical grade and double distilled water was used throughout the experiments.

2.2. Apparatus

All electrochemical measurements were carried out with the help of computer controlled model (Epsilon-EC USB, ver. 2.00.71) electrochemical work station at room temperature. The electrochemical cell set-up included Ag/AgCl (saturated 1 M KCl, CH Instruments) as reference electrode, platinum wire as counter electrode and EPPG as working electrode. Field emission scanning electron microscopy (FE-SEM, JEOL-JSM 7400) instrument was used to study the surface morphology of bare and modified sensors. HPLC studies were performed using a Shimadzu (LC-2010 HT) instrument equipped with C_{18} reverse phase column. A mixture of acetonitrile and water (ratio of 20:80) was used at a flow rate of 1 ml/min and the absorbance of eluent was monitored at 254 nm. The AC impedance of the modified sensor was measured using a potentiostat/galvanostat (PAR, model; versastat 3) linked to a personal computer.

2.3. Fabrication of SWCNT/p-DAN composite film covered pyrolytic graphite

The pyrolytic graphite sensor was prepared according to the previously reported method [43]. A piece of pyrolytic graphite was fixed into one end of the hollow glass tube with araldite adhesive so that its edge plan side is exposed. The glass tube was then kept undisturbed for about 24 h, at room temperature, so that adhesive becomes hard and holds the graphite piece tightly. The glass tube was rubbed on an emery paper till the surface of edge plane of graphite was exposed. The glass tube was then filled with mercury and the contact was made by means of copper wire.

The SWCNT/polymer composite film was grown using an electrochemical method in which SWCNT and polymer are simultaneously deposited on the edge plane surface of pyrolytic graphite sensor. First, purified SWCNT (2.5 mg) was dispersed in 5 ml ethanol under ultrasonic agitation for 20 min to achieve well dispersed suspension. 10 mM solution of monomer 1,5-DAN was prepared in 1 M $HClO_4$. Then, suspended SWCNT was mixed with monomer solution and solvent (ethanol) was evaporated by mild heating. The fabrication process used was as reported by Silva et al. [44]. Prior to the fabrication, the EPPG was first cleaned by rubbing it on an emery paper and dried under nitrogen flow. Then, electrochemical polymerization of 1,5-DAN monomer was carried out using cyclic voltammetry (CV); the potential range was varied from − 0.1 V up to $+1.0$ V at a scan rate of 0.1 V s^{-1}. The SWCNT/p-DAN composite film was produced by sweeping continuously for 50 cycles.

2.4. Experimental procedure

The stock solution of sulfacetamide (2 mM) was prepared by dissolving the required amount of the compound in double distilled water. In order to prepare desired concentration of SFA, required amount of this solution was added to the cell containing 2 ml of buffer and total volume was made to 4 ml using double distilled water. Blank experiment was run in presence of buffer and water. Solutions were purged with high purity nitrogen for 15 min to remove oxygen before recording each voltammogram. Phosphate buffer of pH 7.2 was used as supporting electrolyte during the experiments. The optimized operating conditions for square wave voltammetry (SWV) were set with initial potential at 500 mV, final potential 1200 mV, square wave frequency 15 Hz, square wave amplitude 25 mV and potential step 4 mV. The surface of the modified sensor was cleaned after each run using a time base technique by applying a constant potential (-100 mV) for 60 s in buffer.

3. Results and discussions

3.1. Electrochemical synthesis of polymer nano composite thin film

Electrochemical polymerization of 1,5-DAN was carried out using cyclic voltammetry in the presence of SWCNT. The detailed explanation of several redox peaks observed in cyclic voltammograms during the growth of SWCNT/p-DAN composite film, at the surface of EPPG during polymerization of DAN, has been presented earlier [45]. In brief, the initial scan to positive potentials gives an oxidation peak corresponding to the oxidation of monomer at 665 mV. One cathodic peak is obtained at 475 mV in the reverse sweep. The peak current of the oxidation peak decreases, whereas, the reduction peak increases with increasing potential cycles. In the second scan, two additional anodic peaks are obtained at 311 mV and 505 mV and one cathodic peak at 145 mV. These peaks were absent in the first cycle. The current of anodic and cathodic peaks increases with the time which reflects the growth of polymer film. In addition, simultaneously the incorporation of SWCNT in polymer matrix provides the nano structured composite frame work to enhance electro catalytic property of the sensor.

3.2. Effect of number of cycles during the growth of thin film

In order to check the effect of number of polymerization cycles upon the deposition of polymer nano composite thin film on the EPPG surface, electropolymerization of 1,5-DAN in presence of nanotube was carried out by varying the number of cycles ranging from 20 to 50. It was observed that on increasing the number of cycles, more nanotube was found to embed into the polymeric structure. This was confirmed by the characterization of thin films with the help of FE-SEM analysis. Before characterization, SWCNT/p-DAN composite film was first rinsed with double distilled water and then with ethanol. Fig. 1 compares the SEM images of bare EPPG (a), p-DAN modified EPPG (b) and SWCNT/p-DAN composite modified EPPG after 30th (c) and 50th (d) polymerization cycle. A clear surface can be seen at bare EPPG (a) and sponge like structure is observed in case of p-DAN. From the comparison of SEM micrographs of image (c) and image (d), it can be clearly seen that the nanotubes are more effectively embedded in polymer structure after 50th cycle in comparison to 30th cycle. It is indicated that the embedment of nanotube in polymer framework is an important issue for interfacial bonding between polymer and nanotube, which provides a surface with high surface area and good conductivity.

3.3. Electrochemical reactivity in terms of surface area

Impact of modification on electrode surface area was calculated by recording cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ at different scan rates using 0.1 M KCl as supporting electrolyte at unmodified EPPG, p-DAN/EPPG and SWCNT/p-DAN modified EPPG. A redox couple was observed due to the Fe^{+3}/Fe^{+2} conversions at unmodified and modified pyrolytic graphite. It was noticed that in case of SWCNT/p-DAN modified EPPG, an increase in peak current for Fe^{+3}/Fe^{+2} system was dependent on number of cycles used during polymerization. Fig. 2 clearly shows that the peak current for Fe^{+3}/Fe^{+2} system increases from 20 cycles to 50 cycles and then became constant at higher number of cycles. Thus, polymerization of 1,5-DAN at the pyrolytic graphite surface was carried out for 50 cycles and used in further studies.

At unmodified pyrolytic graphite surface high peak-to-peak separation ($\Delta E_p=0.165$, peak a) and low value of peak current were observed for Fe^{3+}/Fe^{2+} of redox couple. In comparison to unmodified surface, polymer modified surface exhibited lower peak separations ($\Delta E_p=0.145$, peak b) and higher peak current. The best results were obtained for SWCNT/p-DAN modified EPPG with lowest peak-to-peak separation ($\Delta E_p=0.120$ V, peak d; 50th cycle) and highest peak current showing the enhancement in the reversibility of Fe^{3+}/Fe^{2+} of redox couple on embedment of nanotube in polymer matrix, as depicted in Fig. 2. The peak current for a reversible process follows the relation:

$$i_p = 0.4463\left(F^3/RT\right)^{1/2} An^{3/2} D_R{}^{1/2} C_0 \nu^{1/2}$$

where i_p refers to the peak current (Ampere), F is Faraday's constant (96,485 C mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (298 K), A is the surface area of electrode (cm^2), $n=1$ for $K_3Fe(CN)_6$, D_R is diffusion coefficient (7.6×10^{-6} cm^2 s^{-1}), ν is scan rate (Vs^{-1}) and C_0 is the concentration of $K_3Fe(CN)_6$ in mol L^{-1}. The surface area was calculated from the slopes of i_p versus $\nu^{1/2}$ plots and found as 0.084 cm^2, 0.297 cm^2 and 0.679 cm^2 for the unmodified, p-DAN modified EPPG and SWCNT/p-DAN modified EPPG, respectively, exhibiting significant improvement in surface area after modification with polymer and nanotube.

The feature of modified sensor was also probed using electrochemical impedance spectroscopy over the frequency range 0.1–10^5 Hz at 10 points per decade in 5 mM solution of $K_3Fe(CN)_6$. Fig. 3 shows a Nyquist plot obtained for bare EPPG, p-DAN/EPPG and SWCNT/p-DAN/EPPG. The charge transfer resistances for bare EPPG, p-DAN/EPPG and SWCNT/p-DAN/EPPG were 3518 Ω, 854 Ω and 353 Ω, respectively. A semicircle showing the maximum resistance was obtained for unmodified EPPG. In the case of modified sensors, significant decrease in resistance was observed. The SWCNT/p-DAN/EPPG exhibited lowest resistance, which may be attributed to the unique characteristics of polymer and CNT, such as good electrical conductivity, high surface area and high chemical stability. This indicates the capability of polymer nano composite to facilitate the electron transfer at the surface of sensor [46].

3.4. Cyclic voltammetric studies

Cyclic voltammograms were recorded for 1 mM sulfacetamide at SWCNT/p-DAN modified EPPG at pH 7.2 using a sweep rate of 0.1 V s^{-1} as shown in Fig. 4. A well-defined peak at (E_p) ~900 mV (I_a) was observed, when the sweep was initiated in the positive direction. In the reverse sweep two cathodic peaks II_a and II_b were noticed.

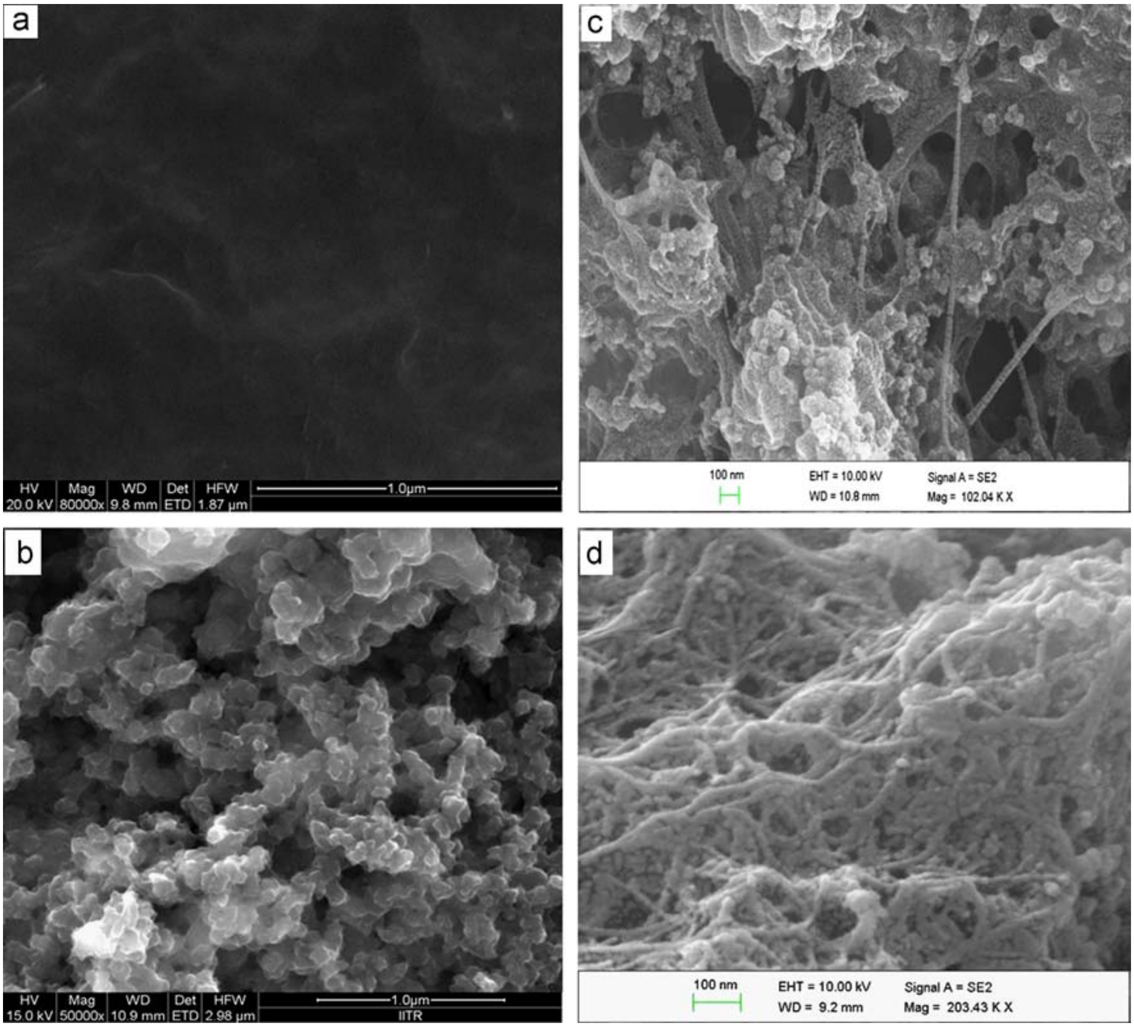


Fig. 1. FE-SEM images of (a) bare EPPG, (b) p-DAN/EPPG and SWCNT/p-DAN/EPPG after 30th (c) and 50th (d) polymerization cycle.

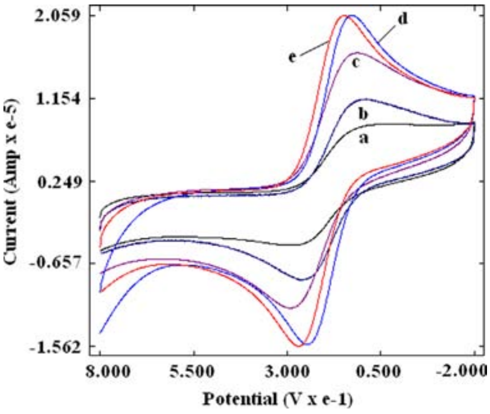


Fig. 2. Comparison of the cyclic voltammograms of 1 mM K_3 [Fe(CN)$_6$] in 0.1 M KCl at (a) bare EPPG, (b) polymer modified EPPG and SWCNT/p-DAN/EPPG during 30th cycle (c), 50th cycle (d) and 70th cycle (e).

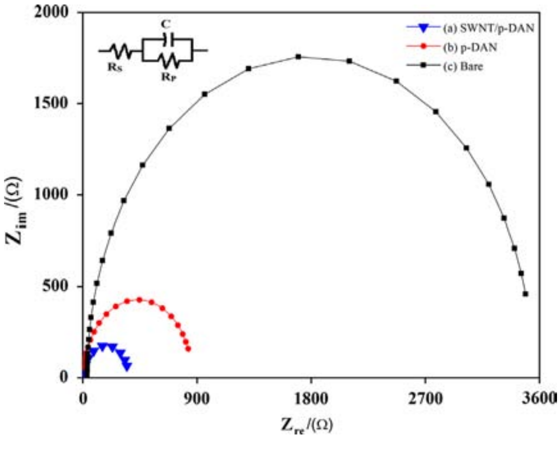


Fig. 3. The impedance changes of different modified pyrolytic graphite sensor. SWCNT/p-DAN modified pyrolytic graphite sensor (a), p-DAN modified pyrolytic graphite sensor (b) and bare pyrolytic graphite sensor (c). Electrolyte composition of EIS measurement: 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ and 100 mM KCl. Frequency range: 0.1–10^5 Hz.

These cathodic peaks formed a quasi-reversible couples with peaks $II_{a'}$ and $II_{b'}$, respectively, on the second sweep in the positive direction. Cyclic voltammograms were also recorded by initiating the sweep first in the negative direction, to check whether the peaks II_a and II_b are due to the reduction of the species formed in the oxidation reaction of peak I_a or are due to the independent reduction of sulfacetamide. The absence of reduction peaks clearly indicated that sulfacetamide does not undergo reduction and peaks II_a and II_b are related to the reduction of species generated in the peak I_a. The separation of peak potentials between $II_a/II_{a'}$ and $II_b/II_{b'}$ couples increased with increasing the sweep rate, indicating the quasi-reversible nature of these couples [47].

To find out the nature of the reaction, sweep rate studies were performed in the range 10–800 mV/s using SWCNT/p-DAN/EPPG sensor. The analyte peak current (i_p) was found to increase with increasing sweep rate and the plot of peak current versus scan rate showed straight line as shown in the inset of Fig. 4. The linear dependence of the peak current on scan rate can be represented by the relation

$$i_p(\mu A) = 0.126\ v(\mathrm{mV/s}) + 7.029$$

having $R^2 = 0.987$. The linearity of above graph reveals that the oxidation reaction of SFA, occurring at the surface of modified EPPG, involves adsorption complications [47]. Further, to prove the electrode reaction mechanism, a graph was plotted between log i_p and log v. The equation arising from this plot is

$$\log\ i_p = 0.970 \log\ v - 0.7363, \quad (R^2 = 0.975)$$

The slope of this linear plot is larger than expected value 0.53 for purely diffusion controlled process confirming the electrode reaction as adsorption controlled [48]. A graph between peak current and square root of scan rate was also plotted and the equation can be represented as

$$i_p(\mu A) = 4.14\ v^{1/2} + 18.32\ (R^2 = 0.984)$$

It further confirms that the oxidation of sulfacetamide follows adsorption complications.

3.5. Square wave voltammetric studies

The electrocatalytic activity of the SWCNT/p-DAN modified pyrolytic graphite sensor was manifested by the comparison of the square-wave voltammograms of 0.5 mM SFA recorded at unmodified and modified EPPG. A well-defined sharp oxidation peak (peak b) with increased current response at peak potential ~850 mV was observed for SFA at SWCNT/p-DAN modified EPPG in comparison to the unmodified surface. Under similar conditions, a small peak (peak a) is obtained at E_p ~870 mV at unmodified EPPG. The remarkable increment of peak current together with shift in the E_p to a lower potential value clearly demonstrates that SWCNT/p-DAN modified EPPG acts as an efficient electron promoter to enhance the rate of the electrochemical reaction which can be assigned to the catalytic effects of SWCNT and p-DAN. As SWV is a more sensitive analytical technique, hence, further voltammetric studies of SFA were carried out using this technique.

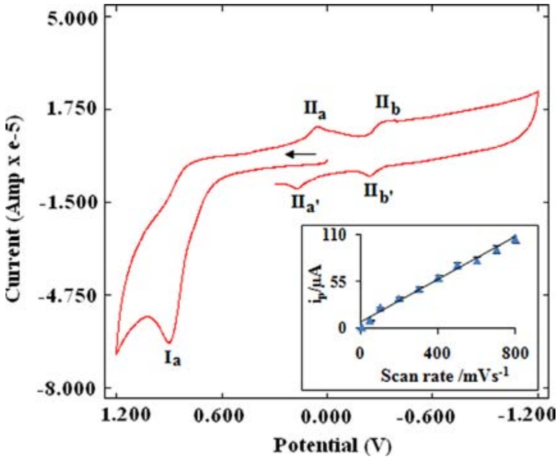


Fig. 4. Cyclic voltammogram observed for 1 mM SFA at scan rate 0.1 V s^{-1} using SWCNT/p-DAN/EPPG; inset is showing a graph between i_p and v.

3.5.1. Effect of pH of supporting electrolyte

The pH of the supporting electrolyte plays an important role during any electrochemical reaction. In order to check the effect of pH on peak potential of SFA, pH of phosphate buffers was varied in the range 2.4–11.0 using unmodified and SWCNT/p-DAN modified EPPG. The peak potential of the SFA shifted towards less positive potentials with increase in pH (Fig. 5(a)). The dependence of the peak potential on pH can be represented by the relations

$$E_p/\mathrm{mV}\ (\mathrm{pH}\ 2.4-11.0) = -59.87\mathrm{pH} + 1302 \quad \text{at unmodified EPPG}$$

$$E_p/\mathrm{mV}\ (\mathrm{pH}\ 2.4-11.0) = -59.35\mathrm{pH} + 1261 \quad \text{at modified EPPG}$$

with coefficient of determination of 0.994 and 0.997 for unmodified and modified EPPG, respectively. The identical value of the slope of these plots (0.059 V) indicates that equal number of protons and electrons are involved in the oxidation of sulfacetamide at unmodified and modified surfaces. SFA can undergo oxidation in two different ways: a single electron oxidation or a one electron process followed by subsequent electron transfer to form nitroso or azo products, respectively. Nitroso and azo compounds are found to be responsible for the formation of quasi reversible couples II_a–$II_{a'}$ and II_b–$II_{b'}$, respectively [49] and hence, it is concluded that oxidation of SFA occurs in 2e$^-$ oxidation at SWCNT/p-DAN modified EPPG.

3.5.2. Effect of square wave frequency

The dependence of peak current of 0.5 mM sulfacetamide on the square wave frequency (f) was studied in the range 5–35 Hz. The peak current was found to increase linearly with square wave frequency (Fig. 5(b)) and the linear relation between i_p and f can be expressed by the following equations:

$$i_p/\mu A = 0.053f + 0.975 \quad \text{at unmodified EPPG}$$

$$i_p/\mu A = 0.420f + 7.751 \quad \text{at modified EPPG}$$

with R^2 of 0.996 and 0.988 for unmodified and SWCNT/p-DAN modified EPPG, respectively. These observations are in agreement with the results obtained from cyclic voltammetric analysis and

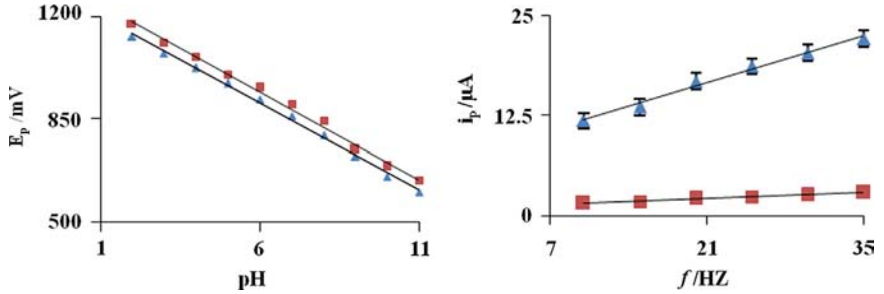


Fig. 5. (a) Effect of pH of supporting electrolyte on peak potential of SFA at bare EPPG (■) and SWCNT/p-DAN/EPPG (▲), (b) Variation of i_p with f at bare EPPG (■) and (▲).

confirm that electrochemical process involves adsorption complications.

3.5.3. *Effect of concentration*

The effect of concentration of SFA on peak current was studied at optimized parameters of SWV in the concentration range 0.005–1.5 mM using unmodified and SWCNT/p-DAN modified sensor (Fig. 6). The peak current was found to increase with increase in concentration of SFA. The current values are reported as an average of at least three replicate determinations and are obtained by subtracting the background current. Linear calibration plots (i_p vs. concentration) for unmodified (0.3–1.5 mM) and modified EPPG (0.005–1.5 mM) are illustrated in inset of Fig. 6. The linear relation between i_p and concentration obeys the following regression equations:

$$i_p / (\mu A) = 3.4647\ \mathrm{C\ (mM)} + 0.069 \quad \text{at unmodified EPPG}$$

$$i_p / (\mu A) = 23.977\ \mathrm{C\ (mM)} + 3.353 \quad \text{at modified EPPG}$$

with R^2 of 0.992 and 0.993 for unmodified and modified EPPG, respectively, where C is the concentration of sulfacetamide. The sensitivity of SFA is found to be 3.4647 and 23.977 μA mM^{-1}. The detection limits were calculated using the formula $3\sigma/b$, where σ is the standard deviation of blank solutions and b is the slope of calibration plots and were found to be 10.00 ± 0.02 and 0.11 ± 0.01 μM for unmodified and modified EPPG, respectively. The calibration outputs obtained using unmodified and modified EPPG were validated using analytical parameters (Table 1) as per ICH guidelines [50,51]. The results obtained using SWCNT/p-DAN modified EPPG indicate that the addition of SWCNT to polymer matrix enhances the electrochemical properties by promoting the charge transfer process. Low detection capability of SWCNT/p-DAN modified EPPG is found useful for the detection of SFA in pharmaceutical formulations up to low concentration.

Table 1
The calibration characteristics for the determination of sulfacetamide using unmodified and modified EPPG.

Analytical Parameters	Unmodified	Modified
Concentration range (mM)	0.3–1.5	0.005–1.5
Coefficient of determination (R^2)	0.992	0.993
Detection limit (μM)	10	0.11
Limit of quantification (μM)	33	0.37
RSD % of peak current	1.02%	1.97%
Bias % of peak current	0.484%	2.62%
Sensitivity (μA/mM)	3.4647	23.977
Standard error of slope (α, 0.05)	± 0.343	± 2.64
Standard error of intercept (α, 0.05)	± 0.285	± 1.80
Precision of peak current	0.70	0.89

3.6. *Stability and reproducibility*

The long-term stability of the SWCNT/p-DAN modified EPPG was checked by measuring the peak current response of fixed concentration (0.1 mM) of SFA. Modified EPPG was stored for two weeks and response was checked again. There was no significant difference in current response even after two weeks. The peak current was slightly decreased with relative standard deviation (RSD) of 1.52%, which can be assigned to the excellent stability of the modified sensor. Furthermore, modified EPPG was stored for a period of one month and was found to exhibit good stability retaining ~94% of its initial response to the oxidation of SFA.

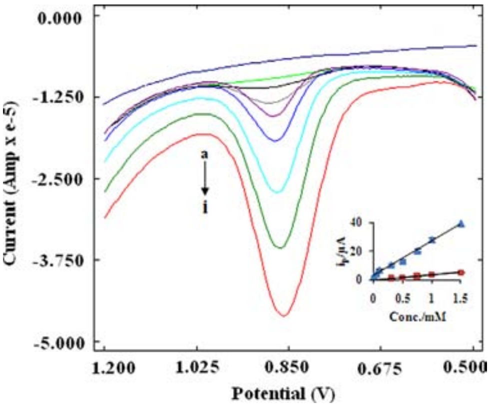


Fig. 6. Square wave voltammograms observed for blank phosphate buffer (a) and increasing concentration of SFA at 0.005 mM (b), 0.05 mM (c), 0.1 mM (d), 0.3 mM (e), 0.5 mM (f), 0.75 mM (g), 1 mM (h) and 1.5 mM (i) using SWCNT/p-DAN/EPPG; inset is depicting the calibration plot at bare EPPG (■) and SWCNT/p-DAN/EPPG (▲), with error bars of 5% value.

To investigate the reproducibility of the modified electrode, repetitive determinations of SFA were carried out at fix concentration (0.1 mM). The intra-day precision of the method was evaluated by repeating six experiments in the same solution containing 0.1 mM of SFA using the modified EPPG. The RSD for oxidation peak was found to be 1.40%, indicating excellent intra-day reproducibility of the modified electrode. Inter-day precision was investigated by recording the peak current value of the modified electrode for eight consecutive days for the same concentration of SFA and the relative standard deviation was found to be 1.53% for oxidation of SFA, demonstrating the excellent reproducibility of modified EPPG.

3.7. *Analytical utility*

In order to evaluate the analytical applicability of the present methodology, the modified EPPG was applied for the determination of SFA in commercially available SFA containing eye drops viz. locula 20% and 30% (East India Pharmaceutical works ltd.), and albucid (Allergan India pvt. ltd.). Locula is the ophthalmic preparation of SFA which is the most prescribed eye drop for the treatment of common inflammatory disorders of eye. Prior to the determination of SFA in eye drops, the samples were dissolved in water and then diluted by required amount of the buffer solution of pH 7.2, so that the concentration of SFA was in the working range. Square wave voltammograms were recorded using SWCNT/p-DAN modified EPPG under exactly same conditions that were applied while recording the voltammogram during concentration study. Keeping dilution factor in consideration, the concentration of SFA was calculated using regression equation which was compared with the labeled amount printed on the eye drops. The results, as demonstrated in Table 2 showed that the content of SFA in eye drops were within the claimed amount with error in the range 3.0–4.0%, revealing the good accuracy of proposed method.

3.8. *Method validation with HPLC*

To prove the validity of the developed method, the results obtained from pharmaceutical analysis of SFA, were compared with HPLC analysis. For this purpose different concentrations of SFA were analyzed using HPLC and a well-defined peak was obtained at R_t~3.57 min in the standard solution of SFA. A calibration curve was obtained by plotting the peak area of the analyte peaks against the analyte concentration. The resulting calibration plot was linear. Then the concentration of drug was determined in the pharmaceutical samples (eye drops). The samples were sufficiently diluted so that their concentration was

Table 2
Determination of SFA in different pharmaceutical samples at SWCNT/p-DAN composite modified EPPG using SWV and validation of these voltammetric results with HPLC.

Sample	Stated content (/10ml)	Content determined by	
		SWV	HPLC
Locula	3 mg	2.91 mg (3.0%)	2.89 mg
Locula	2 mg	1.93 mg (3.5%)	1.94 mg
Albucid	2 mg	1.92 mg (4.0%)	1.90 mg

The RSD values were in the range 1.05–3.01% for $n=5$ determinations. The values in bracket denote the errors found during SWV observations.

in the working range of SFA. The HPLC chromatogram of SFA in pharmaceutical sample showed a peak at $R_t\sim3.57$ min due to SFA. However, no attempt was made to identify the rest of the peaks which are likely to be due to added components in the drug. In order to confirm that this peak is due to SFA, sample was then spiked with a known concentration of standard solution of SFA. The chromatogram obtained for spiked sample indicates the increase in peak at $R_t\sim3.57$ min, confirming that this peak corresponded to SFA. A comparison of the values, obtained by HPLC and the developed voltammetric method has been shown in Table 2, which indicates a good agreement in between the two methods with RSD values in the range 1.05–3.01% for $n=5$.

The values obtained for the determination of sulfacetamide in pharmaceutical tablets using electrochemical and HPLC methods were compared in terms of Student's *t*-test and *F*-test. It was found that in all samples the observed values of *t* and *F* were much smaller than their tabulated values and also the observed values of probability factor for *t* and *F*-test were lower than their significance levels. For example, the results obtained from the determination of sulfacetamide in pharmaceutical sample of Locula (3 mg) using electrochemical and HPLC methods for $n=3$ measurements were compared by carrying out the Student's *t*-test and *F*-test. The calculated *t* and *F* values were 0.248 and 10.23, respectively which were lower than their tabulated values, showing that the results obtained by both the methods are not significantly different from each other at $\alpha=0.05$, confirming the high probability of acquiring these results.

4. Conclusion

In summary, this method provides a simple and quick tool for the direct anodic voltammetric determination of SFA using SWCNT/p-DAN modified EPPG. The conductivity of the SWCNT/p-DAN modified EPPG was improved significantly in comparison to only p-DAN host owing to the excellent properties of carbon nanotubes [52], which were further reflected in the form of sensitivity of the sensor during voltammetric measurements. Modified EPPG exhibited ~8 times more sensitivity together with low detection capability in comparison to the unmodified EPPG. The high background current observed using conventional electrode causes a problem for the electrochemical oxidation of sulfacetamide in achieving high sensitivity and reproducibility. The EPPG sensor with its excellent properties, modified with nanotube and polymer is observed to be a most suitable sensor for the detection of this drug. Low detection of SFA using proposed method indicates that this approach could be successfully applied to the trace analysis of SFA in pharmaceutical industries. The method was successfully employed for the determination of SFA in different pharmaceutical samples and the results obtained showed good accuracy with error in the range 3.0–4.0%. The results were validated in term of HPLC showing a good agreement between the two methods. Furthermore, the SWCNT/p-DAN modified EPPG has long-term stability and good reproducibility with benefits of fast response time, ease of preparation and regeneration of the surface that makes the proposed platform very useful in many other sensing applications.

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