

Determination of three analgesics in pharmaceutical and urine sample on nano poly (3, 4-ethylenedioxythiophene) modified electrode

Gopalakrishnan Gopu ·
Baladhandapani Muralidharan · Chinnapiyan Vedhi ·
Paramasivam Manisankar

Received: 3 November 2010 / Revised: 28 July 2011 / Accepted: 28 August 2011 / Published online: 25 September 2011
© Springer-Verlag 2011

Abstract Three analgesics, acetaminophen, acetylsalicylic acid, and dipyron were determined by stripping voltammetry using nanosized poly(3,4-ethylenedioxythiophene)-modified glassy carbon electrode. The cyclic voltammetric behavior of the three analgesics was studied in aqueous acid, neutral, and alkaline conditions. One well-defined oxidation peak each for acetaminophen and acetylsalicylic acid and three oxidation peaks for dipyron were observed in the cyclic voltammograms. The influence of pH, scan rate, and concentration revealed irreversible diffusion controlled reaction. A systematic study of the experimental parameters that affect the differential pulse stripping voltammetric response was carried out. Calibration was made under maximum peak current conditions. The scanning electron microscope analysis confirmed good accumulation of the drugs on the electrode surface. The range of study for both acetaminophen, acetylsalicylic acid were 0.015–0.4 and dipyron was 0.025–0.4 µg/ml. The lower limit of determination for both acetaminophen,

acetylsalicylic acid was 0.01 µg/mL and for dipyron was 0.02 µg/mL. The suitability of the method for the determination of the three analgesics in pharmaceutical preparations and urine samples was also ascertained.

Keywords Acetaminophen · Acetylsalicylic acid · Dipyron · Nano Poly (3,4-ethylenedioxythiophene) · Cyclic voltammetry · Stripping voltammetry

Introduction

In the past decades, conducting polymer-modified electrodes have received great attention due to their excellent characteristics, including high stability and selectivity, good reproducibility and conductivity, more active sites and good homogeneity. They are widely applied in many areas, such as molecule or ion recognition [1], electrocatalysis, electron transfers, and sensors [2].

Generally, acetaminophen (AAP) does not exhibit any harmful side effects but hypersensitivity or overdoses in few cases leads to the formation of some liver and nephrotoxic metabolites [3]. Many assays have been described for AAP including titrimetry, chromatography, fluorometry, colorimetry UV spectrophotometry, and various modes of electrochemistry [4]. Determination of AAP using spectrophotometric methods and pulse perturbation technique are also described [5–7]. Derivative ratio spectrophotometric method for the determination of ternary mixture of aspirin, paracetamol, and salicylic acid was reported by Yazbi et.al [8]. Electrochemical detection and cyclic voltammetric determination of AAP were also reported [9]. The differential pulse voltammetric behavior of some drugs including AAP at various conducting polymers [10, 11] and at nanoparticle-modified carbon paste electrode [12] have been examined and reviewed [13]. AAP was

Electronic supplementary material The online version of this article (doi:10.1007/s11581-011-0619-2) contains supplementary material, which is available to authorized users.

G. Gopu (✉) · P. Manisankar
Department of Industrial Chemistry, Alagappa University,
Karaikudi 630 003 Tamil Nadu, India
e-mail: nggopi79@yahoo.com

B. Muralidharan
Department of Chemistry,
Birla Institute of Technology and Science-Pilani,
Dubai, United Arab Emirates

C. Vedhi
Department of Chemistry, V.O. Chidambaram College,
Tuticorin 628 008 Tamil Nadu, India

determined by voltammetric method using C60-modified glassy carbon electrode [14], polyaniline-multiwalled carbon nanotube film–multiwalled carbon nanotubes composite-modified electrode [15] and nickel magnetic nanoparticles modified electrodes [16].

Acetylsalicylic acid (ASA) is a remedy with anti-inflammatory, analgesic, and antipyretic properties. Determination of nonsteroidal anti-inflammatory drugs in pharmaceuticals and human serum by dual-mode gradient high-performance liquid chromatography (HPLC) and fluorescence detection was reported [17]. Simultaneous determination of acetylsalicylic acid, paracetamol, and caffeine using solid-phase molecular fluorescence and parallel factor analysis was also discussed [18]. The acid-base titrimetric and spectrophotometric methods exploiting Trinder's reaction were followed after the hydrolysis of ASA [19]. A reverse-phase HPLC method for the simultaneous analysis of paracetamol, ASA, and ascorbic acid was developed [20]. Ultraviolet, fluorescence, FT-Raman spectroscopy and infrared spectrofluorimetric methods were described for the determination of ASA and salicylic acid in pharmaceutical preparations [21, 22]. Simultaneous determination of acetylsalicylic acid and caffeine in pharmaceutical formulation were reported by first derivative synchronous fluorimetric method [23]. The electrochemical oxidation of salicylic acid in pharmaceutical formulations of ASA was studied on a glassy carbon electrode using cyclic voltammetric and differential pulse voltammetric method [24]. Salicylic acid determination in cow urine and drugs was carried out using a bienzymatic sensor [25].

Dipyron is widely used in many countries, and in others it has been restricted or banned because of the alleged risks

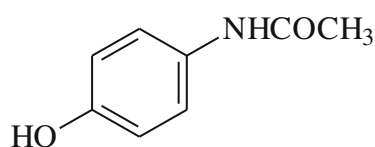
of adverse reactions, in particular agranulocytosis [26]. The drug can cause occasional or rare reactions as transitory disturbances and inflammation of the renal tissue, mainly in patients with renal disease history or in cases of overdose [27]. Flow injection amperometric, biampereometric, liquid chromatography–diode array, reflectometric and liquid chromatography/mass spectrometry methods described for the determination of dipyron (DP) [28–32]. Determination of dipyron and acetaminophen in pharmaceutical preparations by cyclic voltammetry at a copper(II) hexacyanoferrate(III)-modified carbon paste electrode were also studied by Teixeira et al. [33].

The adverse effect of the common drugs either in small doses or larger doses necessitate the development of newer analytical techniques or modification in the existing methods for improved sensitivity. Thus, it is planned to utilize conducting polymer-modified electrode for the electroanalysis of the three drugs. This work presents the electrochemical determination of analgesic drugs on conducting nanosized poly(3,4-ethylenedioxythiophene)-modified glassy carbon electrode.

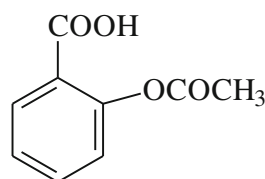
Experimental

Apparatus and reagents

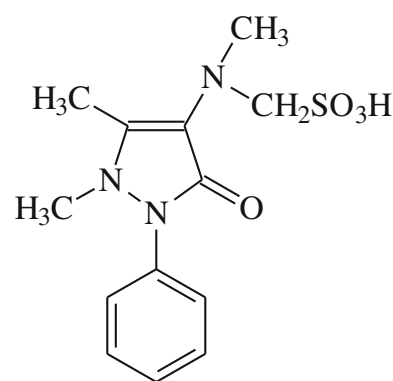
EG&G M 273A Electrochemical Analyzer–Princeton Applied Research Corporation was employed mainly for carrying out electroanalytical studies. The three analgesic drugs were purchased from SIGMA and used as such. The analgesic structures are given below.



Acetaminophen



Acetylsalicylic acid



Dipyron

The stock solutions were made up in double-distilled TKA-LAB purified water. For the electrochemical studies, Britton Robinson buffers, 0.1 mol dm⁻³ KOH, KCl, and

H₂SO₄ were used as the medium for the analysis. 3,4-Ethylenedioxythiophene (Bayer) and tetra butyl ammonium perchlorate (Sigma) were used for electropolymerisation.

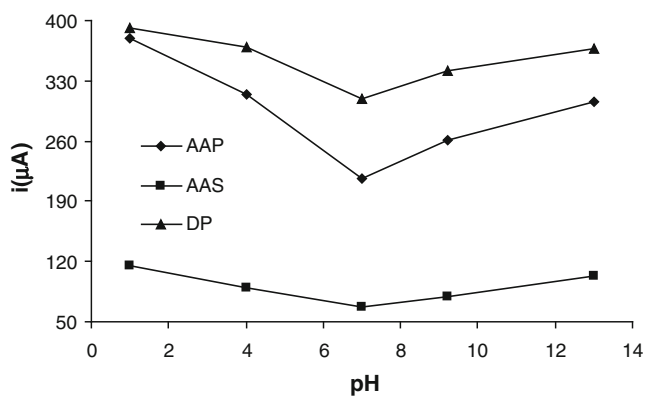


Fig. 1 Plot of current vs pH

Procedure

Purging of nitrogen was done for analyte solution placed in the electrochemical cell of 15-ml capacity for 20 min under stirred conditions. Various voltammograms were recorded while nitrogen gas was blanketed. To get reproducible results, great care was taken in the electrode pretreatment. The glassy carbon electrode was pretreated in two ways: mechanical polishing over a velvet microcloth with an alumina suspension and electrochemical treatment by applying a potential of 1.5 V for 2 s in 0.1 M sulphuric acid.

Preparation of nano poly(3,4-ethylenedioxythiophene)-coated glassy carbon electrode

Nano poly(3,4-ethylenedioxythiophene) coated glassy carbon electrode (PEDOT/GCE) was prepared by potentiodynamic method [34]. Nanosized poly(3,4-ethylenedioxythiophene) film was deposited on GCE by the electrooxidation of 0.01 M 3,4-ethylenedioxythiophene in acetonitrile containing 0.1 M tetra butyl ammonium perchlorate. The polymerisation of this monomer was carried out voltammetrically by giving multicycle in the potential range between -0.2 and 1.2 V at 50 mV/s using Ag/AgCl reference electrode [35]. Thickness

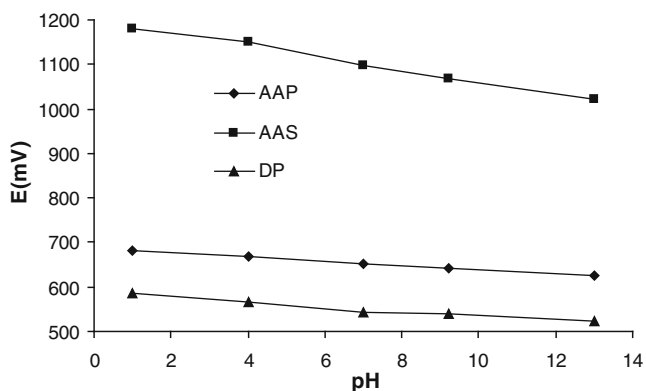


Fig. 2 Plot of potential vs pH

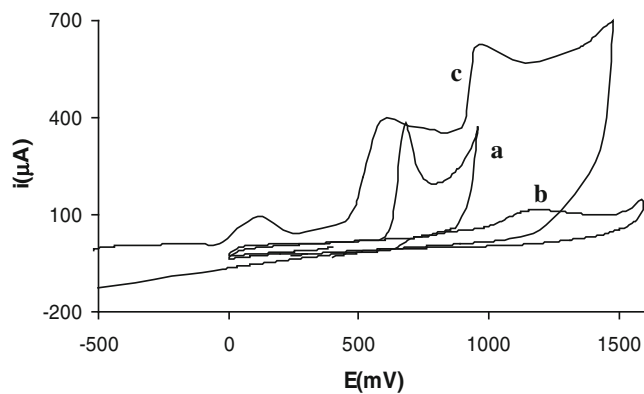


Fig. 3 Cyclic voltammogram of 250 mg/mL; a AAP, b ASA, and c DP on nanoPEDOT at pH 1.0; scan rate 100 mV/s

of the film was controlled coloulometrically and 0.1 µ thick films were used in all cases.

Care was taken to remove the coating and clean the glassy carbon electrode after every experiment in 1:1 HCl/water and 1:1 H₂O₂/acetic acid mixture before usual surface treatment. Nitric acid (6 M) solution was used to clean the cell.

The electrode stability of PEDOT-modified electrode is of prime importance in these studies. The electrode was prepared quickly and found to be stable in the medium. It showed slight decrease in peak current after 15 days of its preparation and thus it is recommended that it should not be used after 15 days. The response time of the electrode was very fast and all measurements were carried out easily and quickly.

Results and discussions

Effect of pH

The pH of the supporting electrolyte has a significant influence on the electrooxidation of analgesics at the

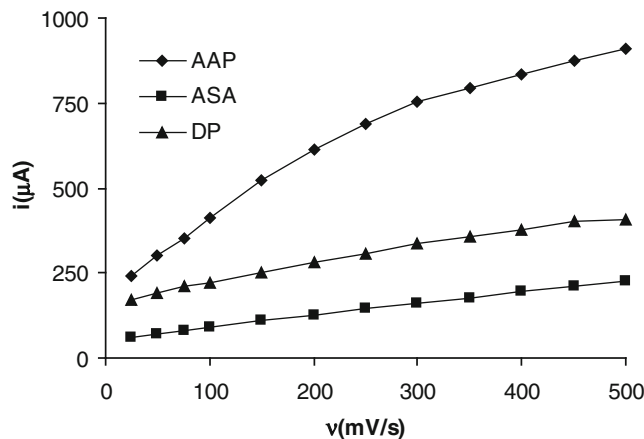


Fig. 4 Plot of peak current vs. scan rate

modified electrode. The electrooxidation of 250 $\mu\text{g/mL}$ electroactivity of acetaminophen, acetylsalicylic acid, and dipyrone was studied over pH range 1.0–13.0 in aqueous medium using cyclic voltammetry. The peak potential and current were measured from the cyclic voltammogram obtained at sweep rate, 100 mV/s on nanoPEDOT/GCE. The plot of peak currents vs. pH was given in Fig. 1. The potential of the oxidation peak shifted to lower side with increase in pH (Fig. 2). Eventhough higher potential was observed at pH 1.0, maximum peak current was obtained for all the three analgesics at this pH, 1.0. Hence from analytical point of view, it was considered as the most suitable pH for further studies of drugs.

Cyclic voltammetric study of analgesics at pH 1.0

Cyclic voltammetric studies of acetaminophen, acetylsalicylic acid, and dipyrone were carried out at the optimized pH 1.0 using nanoPEDOT/GCE system. A representative cyclic voltammogram is presented in Fig. 3. All scan rates and concentration varied, only one oxidation peak for AAP and ASA, and three-oxidation peaks for DP were observed.

The influence of scan rate on the electrode reaction was studied by varying the scan rate from 25 to 500 mV/s at a concentration 250 $\mu\text{g/mL}$. The plot of peak current vs. scan rate resulted in curved line (Fig. 4) whereas the plot between the peak current and square root of scan rate resulted in a straight line ($i_p=41.174 \nu^{1/2}+13.901$, $R^2=0.9937$ for AAP; $i_p=10.218 \nu^{1/2}-11.408$, $R^2=0.9937$ for ASA; and $i_p=14.24 \nu^{1/2}+88.13$, $R^2=0.9923$ for DP). The lower slope value (0.4853 for AAP, 0.3012 for ASA, and 0.3067 for DP) obtained from the linear plot, log peak current vs. log scan rate suggested that the oxidation was diffusion controlled (Fig. 5). The peak potential correlates with log scan rate (Fig. 6) and resulted in a straight line. The fractional αn value (0.7105 for AAP, 0.5679 for ASA,

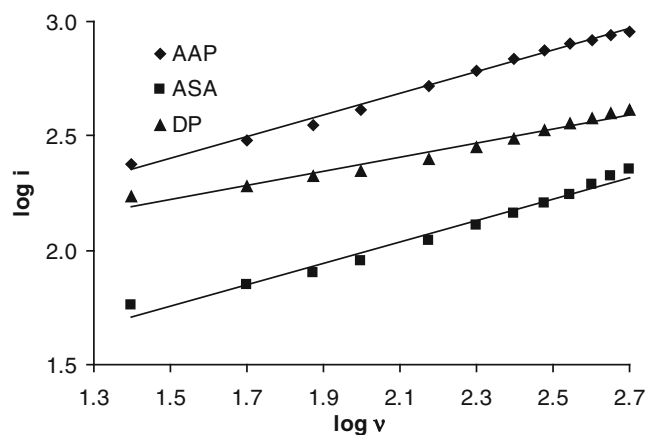


Fig. 5 Plot of log peak current vs. log scan rate

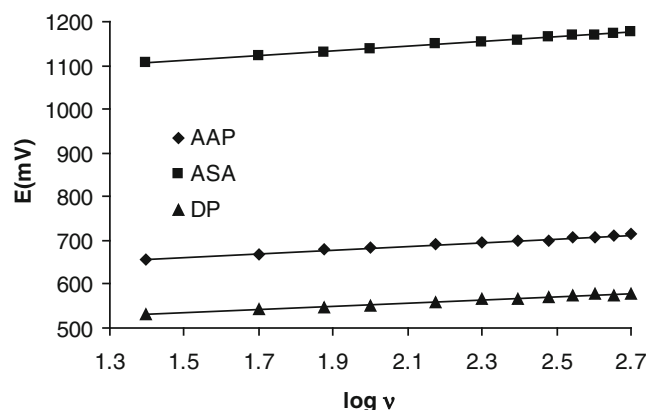


Fig. 6 Plot of peak potential vs. log scan rate

and 0.8333 for DP) calculated from the slope, along with the absence of peak in the reverse scan suggested irreversible electron transfer. The effect of concentration was studied between the concentration range 25 and 350 $\mu\text{g/mL}$. The plot of peak current vs. concentration ($y=1.0342x+52.764$, $R^2=0.9935$ for AAP; $y=0.2764x+7.549$, $R^2=0.9955$ for ASA; and $y=0.3867x+35.923$, $R^2=0.9915$ for DP) also resulted in a straight line.

The number of electron transferred was calculated from controlled potential coulometric studies at pH 1.0 from the charge consumed for the electrolysis. The number of electrons transferred in the oxidation was found to be a 2 for all the three analgesics. The standard rate constant, k_s was calculated from the intercept value of $\log i_p$ vs. $E-E_i$ plot derived from the following equation.

$$i_p = nFAck_s \exp[-\alpha n/RT - (E_i - E_o)]$$

where,

- i_p peak current
- n number of electrons transferred
- F Faraday constant, 96,487 C
- A area of the electrode, cm^2
- C concentration of the electrolyte, moles/ cm^3
- k_s standard rate constant, cm/s
- αn transfer coefficient
- R gas constant, 8.314
- T temperature, K
- E_o peak potential, V
- E_i potential at the foot of the response, V

The value of k_s is $2.672 \times 10^{-5} \text{ cm s}^{-1}$ for AAP, 4.023×10^{-5} for ASA, and 3.772×10^{-5} for DP. The lower value of the rate constant k_s confirms that the electron transfer is irreversible.

Table 1 Optimum experimental conditions in DPSV

Variable	Range studied			Optimum value		
	AAP	ASA	DP	AAP	ASA	DP
pH	1 to 13	1 to 13	1 to 13	1.0	1.0	1.0
Accumulation potential (mV)	500 to 900	800 to 1,100	200 to 700	600	900	400
Accumulation time (s)	10 to 60	10 to 60	10 to 60	40	20	30
Initial scan potential (mV)	0 to 500	300 to 900	−100 to 400	400	600	0
Pulse Height (PH) (mV)	25 to 150	25 to 150	25 to 150	50	50	50
Pulse width (PW) mSec	25 to 150	25 to 150	25 to 150	50	50	50
Scan Increment (SI) mV	2 to 20	2 to 20	2 to 20	4	6	6
Scan rate (SR) mV/s	10 to 100	10 to 100	10 to 100	40	40	40
Stirring rate (rpm)	50 to 250	50 to 250	50 to 250	250	250	250
Rest period (Sec)	2 to 10	2 to 10	2 to 10	5	5	5

DPSV differential pulse stripping voltammetry

Differential pulse stripping voltammetric analysis of analgesics

Cyclic voltammetric results revealed the electroactive nature of the three analgesics in the nanoPEDOT-modified glassy carbon electrode at pH 1.0. Hence, differential pulse stripping voltammetric study was carried out and it performed well in the determination of all the three analgesic drugs. Experiments were carried to find out the best accumulation conditions in the chosen pH 1.0 with solution containing 0.3 $\mu\text{g/mL}$ of AAP, ASA, and DP.

For a 0.3- $\mu\text{g/mL}$ solution, the anodic peak current remained almost constant with accumulation time increasing, indicating accumulation time had no effect and diffusion dominated the electrode process. However, when the concen-

tration of acetaminophen, acetylsalicylic acid, and dipyron was much lower, the electrode process might exhibit adsorptive characteristics and suitable accumulation could contribute to the enhancement of sensitivity. Therefore, considering the need for the detection of lower concentration samples, 10 s was chosen as accumulation time. In addition, when accumulation potential changed from 500 to 900 mV for AAP, 800 to 1,100 mV for ASA, 200 to 700 for DP, the maximum responses were obtained at 600, 900, and 400 mV, respectively (Table 1). Deposition time was varied from 10 to 60 s for three drugs. The maximum current response was observed at 40 s for AAP, 20 s for ASA, and 30 s for DP. The initial scan potential (IP) is also an important parameter like accumulation potential. The initial scan potential was varied between 0 and 500 mV for AAP, 300 and 900 mV for

Fig. 7 SEM photographs of **a** nanoPEDOT, **b** AAP on nanoPEDOT, **c** ASA on nanoPEDOT, and **d** DP on nanoPEDOT

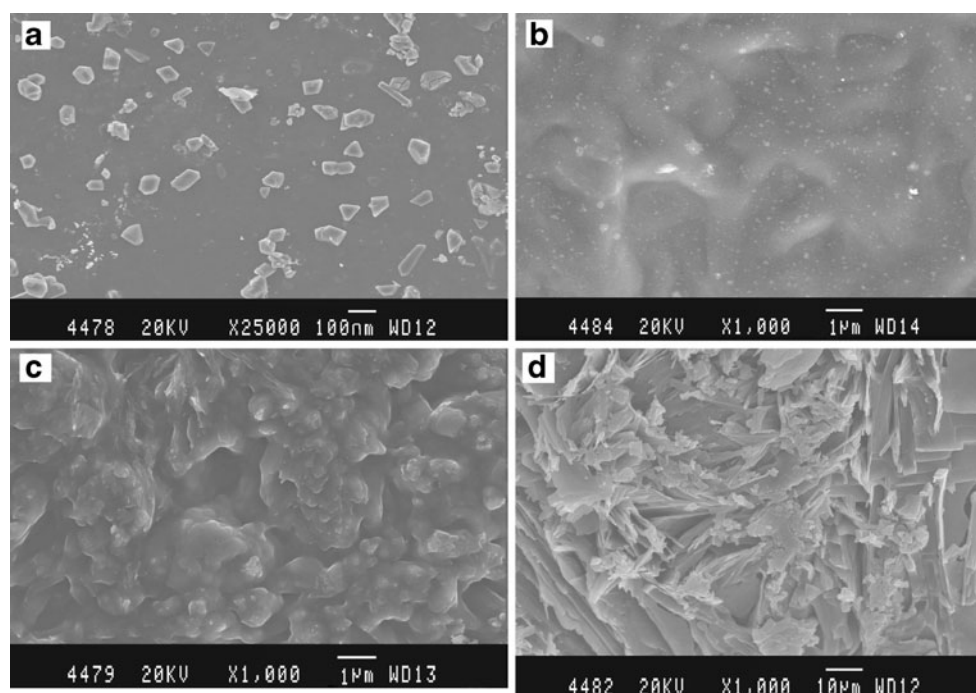
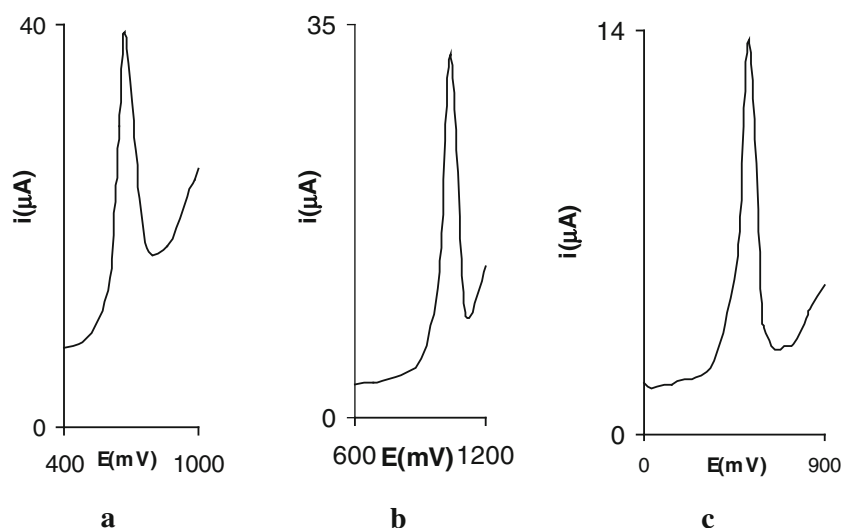


Fig. 8 DPSV of **a** AAP, **b** ASA, and **c** DP under optimum experimental condition



ASA, -100 and 400 mV for DP and the stripping peak current was measured maximum at 400 , 600 , and 0 mV IP, respectively.

The accumulation of the three analgesics on the modified electrode surface under the optimum accumulation conditions was understood from the changes in the electrode surface before and after accumulation. Scanning electron microscope (SEM) was employed to study the surface morphology of the three accumulated analgesics on nanoPEDOT-coated glassy carbon electrode. Figure 7a shows the small ununiform granular nanoPEDOT surface. The drug AAP adsorbed on nanoPEDOT electrode during accumulation and exhibited bigger void plate-like structure (Fig. 7b). ASA exhibited nodule-like structure (Fig. 7c) and DP exhibited broken leaves structure (Fig. 7d). Of the three analgesics, AAP resulted in lesser accumulation. This could be understood from the lesser stripping current for AAP. Because of better accumulations, stripping leads to good results and hence stripping parameters were optimized.

The factors affecting the stripping step, i.e., primary oxidation process that were responsible for the determination were varied and optimum conditions were arrived at. The influences of pulse height, pulse width, scan increment and scan rate were studied by varying their values and the maximum peak current conditions were found out. The range of study and optimized conditions are presented in Table 1. The optimum conditions that resulted in maximum peak current response were used to study the effect of analyte concentration.

Analytical characteristics

The experimental results showed that the peak current increased with the increase in concentration of drugs. A representative differential pulse stripping voltammogram is given in Fig. 8. A calibration plot was made and shown in

Fig. 9, which indicated the linear dependence of peak current with concentration under optimum experimental condition that led to maximum peak current. The limit of detection (LOD) was found 0.01 $\mu\text{g/mL}$ for both AAP and ASA, and 0.02 $\mu\text{g/mL}$ for DP. The reproducibility of the stripping signal was realized in terms of relative standard deviation (2.2% for AAP, 2.7% for ASA, and 2.1% for DP) for seven identical measurements carried out at a concentration level of 0.05 $\mu\text{g/mL}$. The LOD values obtained from this study for the three analgesics were compared with that reported already and the details are presented in Table 2. The table shows that the differential pulse stripping voltammetric method using PEDOT-modified electrode for the determination of the analgesics is superior to the already available methods.

Determination of AAP generally suffered from the interference of *p*-aminophenol as well as ascorbic acid, caffeine, glucose, and urea [14]. Hence, a systematic study of interference due to these compounds was carried out for all three analgesics. Specificity of the nanoPEDOT-

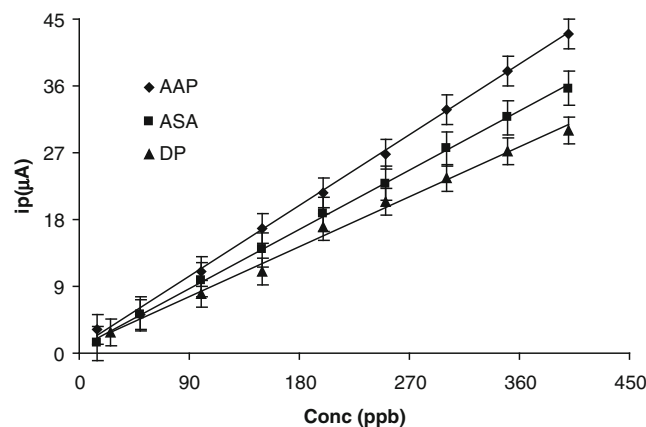


Fig. 9 Calibration plot of peak current vs. concentration

Table 2 Comparison LOD values of available methods

Methods	LOD		
	AAP	ASA	DP
Voltammetric determination [24, 36, 37]	0.09–0.93 mg L ⁻¹	1–0 µg mL ⁻¹	2241.8 µg mL ⁻¹
Flow injection–FT-IR Spectrometric [38]	8 µg mL ⁻¹	–	–
HPLC method [17, 39]	31.6 µg mL ⁻¹	2,502 µg mL ⁻¹	–
Spectrophotometric determination [7, 40]	4 µg mL ⁻¹ , 0.5–20 µg mL ⁻¹	1–40 µg mL ⁻¹	–
Flow injection amperometric determination [29, 32, 41]	20.54 µg mL ⁻¹	–	644.5 µg mL ⁻¹ 10–50 mg L ⁻¹

modified electrode to 0.05 µg/mL of AAP and ASA, and 0.1 µg/mL of DP in the presence of ascorbic acid, caffeine, glucose, *p*-aminophenol, and urea were checked by recording differential pulse stripping voltammograms for oxidation of AAP, ASA, and DP after addition of varying concentration of each interferent (0.01–0.1 µg/mL). Ascorbic acid, glucose, caffeine, and urea do not affect the peak current of drugs even up to tenfold excess. However, in the case of AAP, oxidation peak due *p*-aminophenol started merging with that of AAP from 0.04 µg/mL concentration of the interferent.

Proposed method for the determination of drugs in pharmaceutical and urine samples

The pharmaceutical samples having AAP, ASA, and DP were collected from medical shops at Karaikudi and analyzed. The tablets were powdered, dissolved, and subsequently diluted to a required concentration. Differential pulse stripping voltammograms of the three analgesics at pH 1.0 were recorded under optimum experimental conditions arrived. By substituting the

peak current in the calibration plot and keeping dilution factor in to consideration, the amount of analgesic present in the tablet was determined. The results are presented in Table 3 and a good agreement with the reported value was observed. Concentration range studied and LOD of drugs on PEDOT/GCE

Measurement of the analgesics in urine samples collected after 8 h of administration. Of the urine sample, 1.0 ml was mixed with 0.1 M H₂SO₄ solution and the pH was brought to 1.0. This experiment was repeated for five times and the average weight of drugs in 1.0 ml of urine sample is found to be 0.1 µg for AAP, 0.23 µg for ASA, and 0.27 µg for DP with relative standard deviation 1.9%, 2.5%, and 2.3%, respectively. There is no appreciable interference due to the presence of small amount urine present in the electrolyte hence the same calibration plot was used. There was no degradation of the analyte in solution during experiment. The other matters present in tablets and urine samples are not interfering with the study. This method is simple and suitable for the determination of the said drugs. Repetition rate is found to be high. Hence, the proposed method can be used as

Table 3 The amount of compounds are determined by DPSV in tablets

Brand name	Company name	Tablets (mg)	Experimental value (mg)
Acteaminophen			
Calpol	Glaxosmithkline	500	499
Crocin	Glaxosmithkline	500	499
Colimex tabs	Wallace	500	499
Doliprane	Nicholas piramal	500	500
Cyclopam	Indoco	500	498
Acetyl salicylic acid			
Disprin	Reckitts	350	349
Mycopyrin	Nicholas piramal	350	348
Asa50	German remedies	50	49
Colsprin100	Reckitts	100	97
Ecosprin	Sidmak	150	149
Dipyrone			
Novalgin	Aventis	500	499

DPSV differential pulse stripping voltammetry

Table 4 Concentration range studied and LOD of drugs on PEDOT/GCE

Drugs	Range studied ($\mu\text{g/mL}$) DPSV	LOD ($\mu\text{g/mL}$) DPSV	% of RSD DPSV
AAP	0.015–0.4	0.010	3.1
ASA	0.015–0.4	0.008	2.7
DP	0.025–0.4	0.015	2.5

DPSV differential pulse stripping voltammetry

a better alternative to spectrophotometric or chromatographic methods.

Conclusions

A selective and sensitive method has been developed for the determination of analgesics of AAP, ASA, and DP in pharmaceutical and urine sample using differential-pulse stripping voltammetry based on their electrochemical behavior. Electrooxidation of analgesics were showed irreversibly on nanoPEDOT modified glassy carbon electrode in the pH range 1.0–13.0 and the oxidation was controlled by diffusion. The influence of pH revealed the loss of $2e^-$ in all pH media was observed. From analytical point of view, pH 1.0 was found suitable for differential pulse stripping voltammetric studies. Optimum accumulation and stripping conditions were arrived at the calibrations were made. The accumulation of the drugs was understood from the SEM studies. The lower limit of determination for both the acetaminophen and acetylsalicylic acid was $0.01 \mu\text{g/mL}$ where as for the dipyron was 0.02. The% of RSD was 2.2% for AAP, 2.7% for ASA and 2.1% for DP (Table 4). Thus this method can very well be used for the determination of three drugs in real samples also. This technique is simple and easy to carry out. Lower detection limit was obtained from this study for all the three analgesics compared to the reported values and hence the proposed method is better than the available methods.

References

- Jin GY, Zhang YZ, Cheng WX (2006) Poly(*p*-aminobenzene sulfonic acid)-modified glassy carbon electrode for simultaneous detection of dopamine and ascorbic acid. *Sens Actuat B* 107:528–534
- Chen JH, Zhang J, Zhuang Q, Zhang SB, Lin XH (2007) Electrochemical study of bergenin on a poly(4-(2-pyridylazo)-resorcinol) modified glassy carbon electrode and its determination in tablets and urine. *Talanta* 72:1805–1810
- Patel F (1992) The fatal paracetamol dosage—how low can you go? *Med Sci Law* 32:303–310
- Tungkananuruk K, Tungkananuruk N, Burns DT (2005) Cyclic voltammetric determination of acetaminophen in paracetamol tablets. *KMITL Sci Tech J* 5:547–551
- Lavorante AF, Pires CK, Reis BF (2006) Multicommutated flow system employing pinch solenoid valves and micro-pumps. spectrophotometric determination of paracetamol in pharmaceutical formulations. *J Pharm Biomed Anal* 42:423–429
- Zarei AR, Afkhami A, Sarlak N (2005) Simultaneous spectrophotometric determination of paracetamol and salicylamide in human serum and pharmaceutical formulations by a differential kinetic method. *J AOAC Int* 88:1695–1701
- Pejic N, Kolar-Anic L, Anic S, Stanisavljev D (2006) Determination of paracetamol in pure and pharmaceutical dosage forms by pulse perturbation technique. *J Pharm Biomed Anal* 41:610–615
- El-Yazbi FA, Hammud HH, Assi SA (2007) Derivative-ratio spectrophotometric method for the determination of ternary mixture of aspirin, paracetamol and salicylic acid. *Spectrochim Acta Part A* 68:275–278
- Silva MLS, Garcia MBQ, Lima JLFC, Barrado E (2006) Flow system with electrochemical detection for determination of paracetamol in pharmaceutical formulations. *Port Electrochim Acta* 24:261–271
- Ozcan L, Sahin Y (2007) Determination of paracetamol based on electropolymerized-molecularly imprinted polypyrrole modified pencil graphite electrode. *Sens Actuat B* 127:362–369
- Shahrokhian S, Asadian E (2010) Simultaneous voltammetric determination of ascorbic acid, acetaminophen and isoniazid using thionine immobilized multi-walled carbon nanotube modified carbon paste electrode. *Electrochim Acta* 55:3621–3627
- Mazloum-Ardakani M, Beitollahi H, Kazem Amini M, Mirkhalaf F, Abdollahi-Alibeik M (2010) New strategy for simultaneous and selective voltammetric determination of norepinephrine, acetaminophen and folic acid using ZrO_2 nanoparticles-modified carbon paste electrode. *Sens and Actuat B* 151:243–249
- Ozkan SA, Uslu B, Aboul-Enein HY (2003) Analysis of pharmaceuticals and biological fluids using modern electroanalytical techniques. *Crit Rev Analyt Chem* 33:155–181
- Goyal RN, Singh SP (2006) Voltammetric determination of paracetamol at C_{60} -modified glassy carbon electrode. *Electrochim Acta* 51:3008–3012
- Li M, Jing L (2007) Electrochemical behavior of acetaminophen and its detection on the PANI–MWCNTs composite modified electrode. *Electrochim Acta* 52:3250–3257
- Wang SF, Xie F, Hu RF (2007) Carbon-coated nickel magnetic nanoparticles modified electrodes as a sensor for determination of acetaminophen. *Sens Actuat B* 123:495–500
- Ibrahim H, Boyer A, Bouajila J, Couderc F, Nepveu F (2007) Determination of non-steroidal anti-inflammatory drugs in pharmaceuticals and human serum by dual-mode gradient HPLC and fluorescence detection. *J Chromatogr B* 857:59–66
- Alves L, Poppi J (2009) Simultaneous determination of acetylsalicylic acid, paracetamol and caffeine using solid-phase molecular fluorescence and parallel factor analysis. *Anal Chim Acta* 642:212–216
- Trinder P (1954) Rapid determination of salicylate in biological fluids. *Biochem J* 57:301–303
- Akay C, Degim T, Sayal A, Aydin A, Ozkan Y, Gul H (2008) Rapid and simultaneous determination of acetylsalicylic acid, paracetamol, and their degradation and toxic impurity products by HPLC in pharmaceutical dosage forms. *Turk J Med Sci* 38:167–173
- De Beer TRM, Baeyens WRG, Vander Heyden Y, Remon JP, Vervaet C, Verpoort F (2007) Influence of particle size on the quantitative determination of salicylic acid in a pharmaceutical ointment using FT-Raman spectroscopy. *Eur J Pharm Sci* 30:229–235
- Martos NR, Diaz AM, Vallvey LFC (2001) Spectrofluorimetric determination of acetylsalicylic acid and codeine mixtures in pharmaceuticals. *Anal Lett* 34:579–595
- Karim MM, Jeon CW, Lee HS, Alam SM, Lee SH, Choi JH, Jin SO, Das AK (2006) Simultaneous determination of acetylsalicylic

- acid and caffeine in pharmaceutical formulation by first derivative synchronous fluorimetric method. *J Fluoresc Sep* 16:713–721
24. Matias FAA, Vila MMDC, Tubino MJ (2004) Quantitative reflectance spot test for the determination of acetylsalicylic acid in pharmaceutical preparations. *J Braz Chem Soc* 15:327–330
 25. Campanella L, Gregori E, Tomassetti M (2006) Salicylic acid determination in cow urine and drugs using a bienzymatic sensor. *J Pharm Biomed Anal* 42:94–99
 26. Torriero AAJ, Luco JM, Sereno L, Raba J (2004) Voltammetric determination of salicylic acid in pharmaceuticals formulations of acetylsalicylic acid. *Talanta* 62:247–254
 27. Jones SL (1996) Dipyrone into the nucleus raphe magnus inhibits the rat nociceptive tail-flick reflex. *Eur J Pharmacol* 318:37–40
 28. Matos RC, Angnes L, Araujo MCU, Saldanha TCB (2000) Modified microelectrodes and multivariate calibration for flow injection amperometric simultaneous determination of ascorbic acid, dopamine, epinephrine and dipyrone. *Analyst* 125:2011–2015
 29. Medeiros EP, Castro SL, Formiga FM, Santos SRB, Araujo MCU, Nascimento VBA (2004) Flow injection method for biamperometric determination of dipyrone in pharmaceuticals. *Microchem J* 78:91–96
 30. Senyuva HZ, Aksahin I, Ozcan S, Veli Kabasakal B (2005) Rapid, simple and accurate liquid chromatography–diode array detection validated method for the determination of dipyrone in solid and liquid dosage forms. *Anal Chim Acta* 547:73–77
 31. Weinert PL, Pezza L, Pezza HR (2007) A Simplified reflectometric method for the rapid determination of dipyrone in pharmaceutical formulations. *J Braz Chem Soc* 18:846–854
 32. Penney L, Bergeron C, Wijewickreme A (2005) Simultaneous determination of residues of dipyrone and its major metabolites in milk, bovine muscle, and porcine muscle by liquid chromatography/mass spectrometry. *J AOAC Int* 88:496–504
 33. Teixeira MFS, Marcolino-Junior LH, Fatibello-Filho O, Moraes FC, Nunes RS (2009) Determination of Analgesics (dipyrone and acetaminophen) in pharmaceutical preparations by cyclic voltammetry at a copper(II) hexacyanoferrate(III) Modified carbon paste electrode. *Curr Anal Chem* 5:303–310
 34. Vasantha VS, Chen S-M (2005) Electrochemical synthesis and application of PEDOT/ferrocyanide film modified glassy carbon electrode. *Electrochem Acta* 51(2):347–355
 35. Manisankar P, Vedhi C, Gurumallesh SG, Prabu H (2007) Influence of surfactants on the electrochromic behaviour of poly(3,4-ethylenedioxythiophene). *J Appl Polym Sci* 104:3285–3291
 36. Ni Y, Wang Y, Kokot S (2004) Differential pulse stripping voltammetric determination of paracetamol and phenobarbital in pharmaceuticals assisted by chemometrics. *Anal Lett* 37:3219–3235
 37. Pirola R, Bareggi SR, De Benedittis G (1998) Determination of acetylsalicylic acid and salicylic acid in skin and plasma by high-performance liquid chromatography. *J Chromatogr A* 705:309–315
 38. Bouhsain Z, Garrigues S, De la Guardia M (1996) Simultaneous stopped-flow determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations by Fourier transform infrared spectrometry with partial least-squares data treatment. *Analyst* 121:635–638
 39. Qi ML, Wang P, Leng YX, Gu JL, Fu RN (2002) Simple HPLC method for simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in tablet formulations. *Chromatographia* 56:295–298
 40. Andres C, Soledad C, Mercedes G, Miguel V (2000) Continuous flow spectrophotometric determination of paracetamol in pharmaceuticals following continuous microwave assisted alkaline hydrolysis. *Talanta* 53:417–423
 41. Felix FS, Brett CMA, Angnes L (2007) Carbon film resistor electrode for amperometric determination of acetaminophen in pharmaceutical formulations. *J Pharm Biomed Anal* 43:1622–1627