

Determination of analgesics in pharmaceutical formulations and urine samples using nano polypyrrole modified glassy carbon electrode

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Abstract Three analgesics (acetaminophen, acetylsalicylic acid, and dipyrrone) were determined electrochemically using nano polypyrrole 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 dipyrrone were observed in the cyclic voltammograms. The influence of pH, scan rate, and concentration reveal the irreversible diffusion controlled loss of $4e^-$ in acid and neutral media and $2e^-/1H^+$ in basic medium for acetaminophen. In the case of acetylsalicylic acid, $2e^-$ and $1H^+$ loss in acidic and neutral pH and $2e^-$ and $2H^+$ loss in basic pH were observed and the oxidation was irreversible and controlled by adsorption. Irreversible removal of $2e^-$ and $1H^+$ in all pH media was observed for dipyrrone and the oxidation was controlled by diffusion. A systematic study of the experimental parameters that affect the differential pulse stripping voltammetric response was carried out and optimized conditions which yield maximum peak current were arrived at. Scanning electron microscopy (SEM) analysis confirms good accumulation of the drugs on the electrode surface. The calibration was made under optimum conditions. The range

of study for acetaminophen, acetylsalicylic acid, and dipyrrone was 50–250, 40–300, and 100–400 ng/mL and the lower limit of determination was 45, 25, and 70 ng/mL respectively. 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 · Dipyrrone · Nano polypyrrole · Voltammetry · Stripping voltammetry

1 Introduction

Use of electrochemical methods in pharmaceutical analysis attracted attention due to their accuracy, sensitivity, and cost effectiveness [1]. The electroanalytical features and performances of carbon paste electrodes for fabrication of chemo- and biosensors are well documented [2–4]. The modification of electrode surface assumes considerable importance because of the remarkable improvement in sensitivity. Tan et al. [5] compared the electrochemical oxidation of L-cysteine on C60-modified carbon electrode with that on bare glassy carbon electrode and reported significantly better results for C60-modified electrode.

Analgesics are a diverse group of drugs used to relieve pain. Acetaminophen (AAP) is a common analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains. AAP acts as painkiller by inhibiting prostaglandin synthesis in the central nervous system and relieves fever by sedating hypothalamic heat-regulating center [6]. Generally AAP does not exhibit any harmful side-effects, but hypersensitivity or overdoses in few cases leads to formation of some liver and nephrotoxic metabolites [7]. Many assays have been described for

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AAP, including titrimetry, chromatography, fluorometry, colorimetry ultraviolet (UV) spectrophotometry, and various modes of electrochemistry [8]. Determination of AAP using spectrophotometric methods and pulse perturbation technique was also described [9–11]. Electrochemical detection and cyclic voltammetric determination of AAP were reported [8, 12]. The differential pulse voltammetric behavior of some drugs, including AAP at various conducting polymers [13] and at pumice mixed carbon electrodes [14], have been examined and reviewed [15]. AAP was determined by voltammetric method using C60-modified glassy carbon electrode [1].

Acetylsalicylic acid (ASA) is a remedy with anti-inflammatory, analgesic, and antipyretic properties. When its mechanism of action began to be understood [16], the possibility for use against cardiac and circulatory disturbances became evident [17]. The partial least-squares algorithm, reverse-phase sequential injection chromatography technique was described for determination of ASA [18, 19]. Acid–base titrimetric and spectrophotometric methods exploiting Trinder's reaction were followed after the hydrolysis of ASA [20]. Some spectrophotometric flow injection methods, using online hydrolysis, were also developed [21]. Ultraviolet, fluorescence, and infrared spectrophotometric methods were described for determination of ASA and salicylic acid in pharmaceutical preparations [22, 23]. However their frequent usage was restricted, possibly due to the difficulty and tedious preparations of the samples. Chromatographic procedures, mainly high-performance liquid chromatography (HPLC) and atomic absorption spectrometric methods, were also used [24]. These methods, however, involved expensive apparatus and usually need extraction procedures. Ion-selective electrodes were developed showing high specificity, good detection limits, and relatively low cost [25]. 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 [26].

Dipyron (DP) is another analgesic, antipyretic, and anti-inflammatory drug. After oral administration it is hydrolyzed to 4-methylaminoantipyrine and further metabolized to 4-aminoantipyrine, 4-formylaminoantipyrine, and 4-acetylaminoantipyrine. Although the drug is widely used in many countries, in others it has been restricted or banned because of the alleged risks of adverse reactions, in particular agranulocytosis [27]. 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 [28]. Flow injection amperometric and liquid chromatography/mass spectrometry methods are described for determination of DP [29–31]. Spectrophotometric methods such as

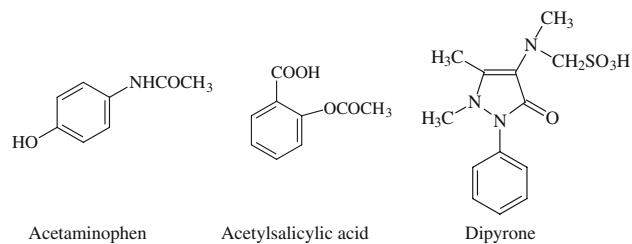
ultraviolet–visible absorption, fluorescence, and chemiluminescence were frequently reported for DP determination [32]. Electrochemical determination of dopamine, norepinephrine, valproate, isoniazid, and hydrazine was reported on polypyrrole-modified electrode [33–35].

The adverse effects of the common drugs either in small or larger doses necessitate the development of newer analytical techniques or modification of 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 polypyrrole modified glassy carbon electrode.

2 Experimental

2.1 Apparatus and reagents

EG&G M 273A Electrochemical Analyzer—Princeton Applied Research Corporation (PARC) was employed mainly for carrying out electroanalytical studies. The three analgesic drugs were purchased from Sigma and used as such.



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. Pyrrole (AR—Merck) and tetrabutylammonium perchlorate (Sigma) were used for electropolymerization.

2.2 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 (1.0, 0.3, and 0.05 μm) and electrochemical treatment by applying a potential of 1.5 V for 2 s.

2.3 Preparation of nano polypyrrole coated glassy carbon electrode (Ppy/GCE)

Polypyrrole films were deposited on GCE by electrooxidation of 0.1 M pyrrole in acetonitrile containing 0.1 M tetrabutylammonium perchlorate at 0.90 V (versus Ag/AgCl) applied potential [36]. Thickness of the film was controlled coulometrically and 0.1- μm -thick films were used in all cases. The SEM photograph (Fig. 6a) reveals the deposition of nanosize (100-nm) Ppy on GCE.

Care was taken to remove the coating and clean the glassy carbon electrode after every experiment in 1:1 HCl/water and 1:1 H_2O_2 /acetic acid mixture before usual surface treatment. Nitric acid (6 M) solution was used to clean the cell.

The electrode stability of polypyrrole 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 (6%) after 15 days after 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.

3 Results and discussions

3.1 Electrochemical studies of analgesics on polypyrrole modified electrode

Cyclic voltammograms of three analgesic drugs were recorded using nano polypyrrole modified glassy carbon electrode in acid, neutral, and alkaline conditions at 100 mV/s scan rate. Figure 1 represents the cyclic voltammogram of 300 $\mu\text{g}/\text{mL}$ AAP at pH 1.0. The cyclic voltammogram exhibited one oxidation peak in all three pHs. Good peak response with maximum peak current was

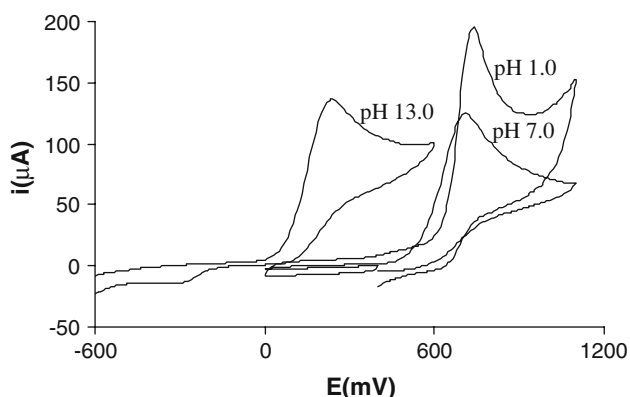


Fig. 1 Cyclic voltammogram of 300 mg/mL acetaminophen on Ppy/GCE at pH 1.0, 7.0, and 13.0; scan rate 100 mV/s

observed at acidic pH, whereas in neutral and basic medium lesser current response compared with at acid pH was observed. The typical cyclic voltammogram of 300 $\mu\text{g}/\text{mL}$ ASA (Fig. 2) shows oxidation in all the three media exhibiting higher peak current at pH 1.0. The cyclic voltammogram observed at pH 1.0 of 500 $\mu\text{g}/\text{mL}$ DP for the scan rate of 100 mV/s is presented in Fig. 3. Three oxidation peaks are observed in acidic and neutral pH, whereas two peaks are observed at basic pH. The main oxidation peak observed around 750 mV for AAP, 1,170 mV for ASA, and 590 mV for DP has maximum current response and hence for further probing these peaks were considered.

The effect of pH was studied in detail by choosing one pH difference between pH 1.0 and 13.0. Figures 4 and 5 show the variation of peak potential and current, respectively, with pH. For AAP, two linear portions are distinguished, the first one at lower pH to neutral and the second one at neutral to basic pH. The first portion has slope 12.779 mV/pH, which is close to the theoretical

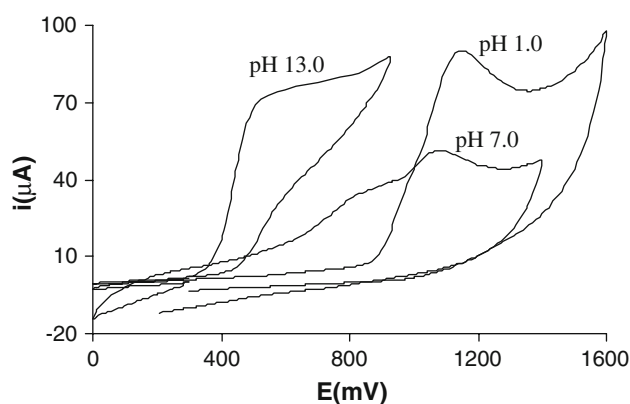


Fig. 2 Cyclic voltammogram of 300 mg/mL acetylsalicylic acid on Ppy/GCE at pH 1.0, 7.0, and 13.0; scan rate 100 mV/s

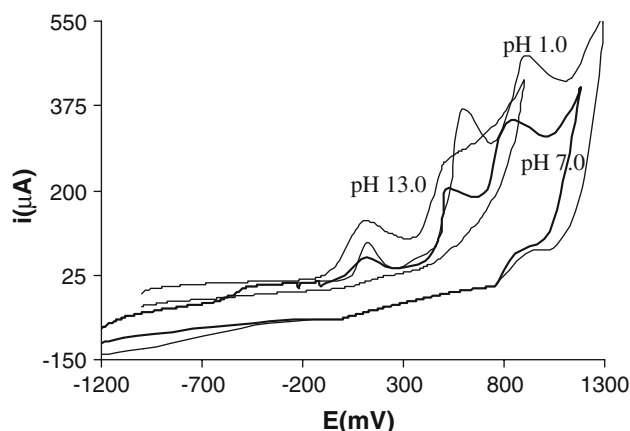


Fig. 3 Cyclic voltammogram of 500 mg/mL dipyron on Ppy/GCE at pH 1.0, 7.0, and 13.0; scan rate 100 mV/s

value of 15 mV/pH for the involvement of $4e^-$ transfer in the rate-determining step, whereas the second one has a slope of 36.717 mV/pH, which is close to 30 mV/pH for $2e^-/1H^+$. Usually in acidic medium the proton availability is excess and hence protonations become fast. The $4e^-$ in acidic medium may lead to formation of quinone, while in neutral and basic media the mechanism suggested by Goyal et al. [1] can be considered. In the case of ASA also two portions are found in Fig. 4. The slope of the first portion, 27.0 mV/pH, is nearer to 30 mV/pH, suggesting the removal of $2e^-$ and $1H^+$. The slope of the second portion, 66 mV/pH, is close to 60 mV/pH, indicating the removal of $2e^-$ and $2H^+$. Taking into account the previous results for phenol and related compounds, here also the complex mechanism proposed for the electrooxidation of ASA by Torriero can be considered. DP exhibited decreasing trend in peak potential with increasing pH. A straight line with slope closer to 30 mV/pH is obtained for DP in the pH range 1.0–10.0, suggesting the loss of $2e^-$ and $1H^+$. Hence a mechanism proposed for DP already [32] holds well here also. At basic pH, only two peaks are observed and the second peak is broad; hence a complex mechanism complicated by chemical steps may be possible. The peak current showed decreasing trend with increasing pH up to neutral pH and then showed increasing trend to basic pH (Fig. 5). This again confirms the operation of two different mechanisms in acidic and basic conditions. In all three drugs, the peak current exhibits maximum value at pH 1.0. From an analytical point of view, pH 1.0 was chosen for the development of electroanalytical determination procedure for the said drugs on polypyrrole modified glassy carbon electrode owing to maximum peak current responses. The increase in peak current may be due to the increase in the electroactive surface area in modifying the glassy carbon surface with doped polypyrrole and higher electrocatalytic activity at pH 1.0.

Peak current dependence on scan rate was studied by varying the scan rate from 25 to 500 mV/s at concentration

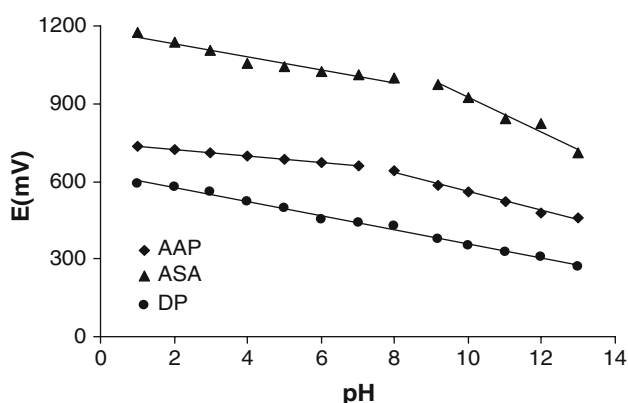


Fig. 4 Plot of peak potential versus pH for AAP, ASA, and DP

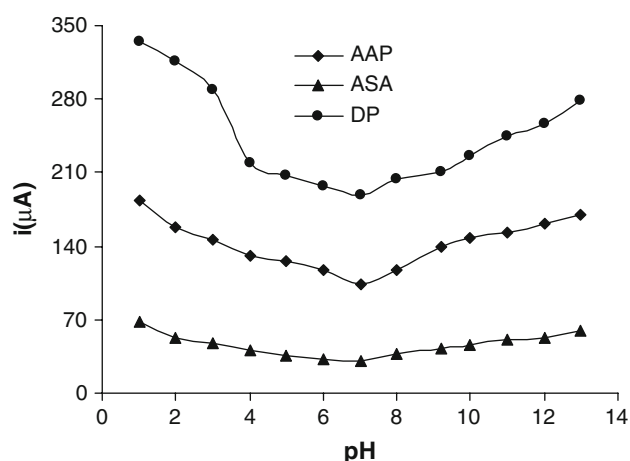


Fig. 5 Plot of peak current versus pH for AAP, ASA, and DP

of 300 $\mu\text{g/mL}$ of AAP and ASA. Similar dependence of peak current on the scan rate was studied for 500 $\mu\text{g/mL}$ DP. In the case of AAP and DP, peak current against scan rate resulted in curved lines while peak current versus square root of scan rate led to straight lines with good correlation ($i_p = 10.465v + 74.664$, $r^2 = 0.997$ for AAP and $i_p = 26.659v + 66.758$, $r^2 = 0.998$ for DP). This suggests diffusion-controlled electron transfer in both cases. Log values of peak currents were plotted with log scan rates and straight lines were obtained for AAP and DP ($i_p = 0.309v + 1.6454$, $r^2 = 0.997$ for AAP and $i_p = 0.3964v + 1.7406$, $r^2 = 0.992$ for DP). The fractional slope values confirm diffusion-controlled oxidation process for AAP and DP. Linearity was observed when peak current was correlated with the scan rate ($i_p = 0.3041v + 38.11$, $r^2 = 0.994$) for ASA. Curved line was obtained for the plot of peak current versus the square root of scan rate. The slope of the linear plot of log peak current with $\log v$ ($i_p = 0.5322v + 0.8054$, $r^2 = 0.983$) was 0.532, confirming adsorption-controlled reaction.

The absence of reduction peaks in all the drugs reveals irreversible oxidation. As reported earlier [37], the standard rate constant k_s was calculated from the slope of $\log i_p$ versus $E-E_i$ plot. The value of k_s is 8.576×10^{-5} cm/s for AAP, 1.097×10^{-6} for ASA, and 4.692×10^{-6} for DP. The lower value of rate constant k_s confirms irreversible electron transfer.

3.2 Differential pulse stripping analysis of analgesics

Cyclic voltammetric results revealed the electroactive nature of the three analgesics in the polypyrrole modified glassy carbon electrode at pH 1.0. Hence differential pulse stripping voltammetric study was carried out and performed well in the determination of all the three analgesic drugs. Experiments were carried out to find out the best

accumulation potential in the chosen pH 1.0 with solution containing 50 ng/mL for both AAP and ASA and 100 ng/mL of DP for stripping analysis.

The accumulation potentials (E_{acc}) were varied from 100 to 500 mV for AAP, 100 to 800 mV for ASA, and –600 to 200 for DP at deposition time (DT) 10 s. This showed the maximum peak current for an accumulation potential in 500, 800, and 100 mV, respectively, for AAP, ASA, and DP (Table 1). Deposition time was varied from 10 to 60 s for the three drugs. The maximum current response was observed at 10 s for AAP, 45 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 300 mV for AAP, 0 and 400 mV for ASA, and –1000 and –200 mV for DP and the stripping peak current was measured maximum at 100, 200, and –600 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. SEM was employed to study the surface morphology of the three accumulated analgesics on nano polypyrrole coated glassy carbon electrode. Figure 6a shows the small uniform granular nano Ppy surface. The drug ASA adsorbed on nano Ppy electrode during accumulation and exhibited bigger void plate-like structure (Fig. 6b). DP exhibited broken leaves-like structure (Fig. 6c) and AAP exhibited granular structure (Fig. 6d). Of the three analgesics, AAP resulted in less 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 potential 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.

3.3 Analytical characteristics

The experimental results showed that peak current increased with increasing concentration of drugs. A representative differential pulse stripping voltammogram is given in Fig. 7. A calibration plot ($y = 0.1864x - 0.1267$; $R^2 = 0.9999$ for AAP, $y = 0.0848x + 0.9425$; $R^2 = 0.9966$ for DP, $y = 0.1173x + 7.2844$; $R^2 = 0.9964$ for ASA) was made and is shown in Fig. 8, 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 to be 45, 25, and 70 ng/mL for AAP, ASA, and DP, respectively. The reproducibility of the stripping signal was realized in terms of relative standard deviation for ten identical measurements carried out at a concentration level of 200 ng/mL and the values are presented in Table 2. The LOD values obtained from this study for the three analgesics were compared with that reported already and the details are presented in Table 3. The table shows that the differential pulse stripping voltammetry (DPSV) method using polypyrrole 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 [1]. Hence, a systematic study of interference due to these compounds was carried out for all three analgesics. Specificity of the nano polypyrrole

Table 1 Optimum experimental conditions in DPSV

Variable	Range studied			Optimum value		
	AAP	ASA	DP	AAP	ASA	DP
pH	1–13	1–13	1–13	1.0	1.0	1.0
Accumulation potential (mV)	100–500	100–800	–600–200	500	800	100
Accumulation time (s)	10–60	10–60	10–60	5	45	30
Initial scan potential (mV)	0–300	0–400	–1000 to –200	100	200	–600
Pulse height (PH) (mV)	25–150	25–150	25–150	50	75	50
Pulse width (PW) (ms)	25–150	25–150	25–150	50	50	50
Scan increment (SI) (mV)	2–20	2–20	2–20	4	6	6
Scan rate (SR) (mV/s)	10–100	10–100	10–100	50	70	60
Stirring rate (rpm)	50–250	50–250	50–250	250	250	250
Rest period (s)	2–10	2–10	2–10	5	5	5

AAP acetaminophen, ASA acetylsalicylic acid, DP dipyrone

Fig. 6 SEM photographs of (a) polypyrrole (b) ASA/Ppy (c) DP/Ppy, and (d) AAP/Ppy

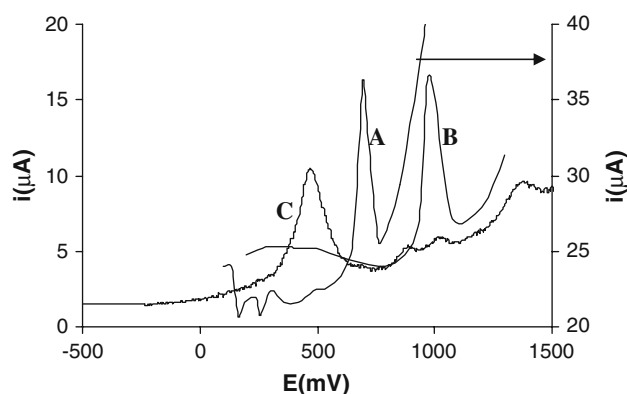
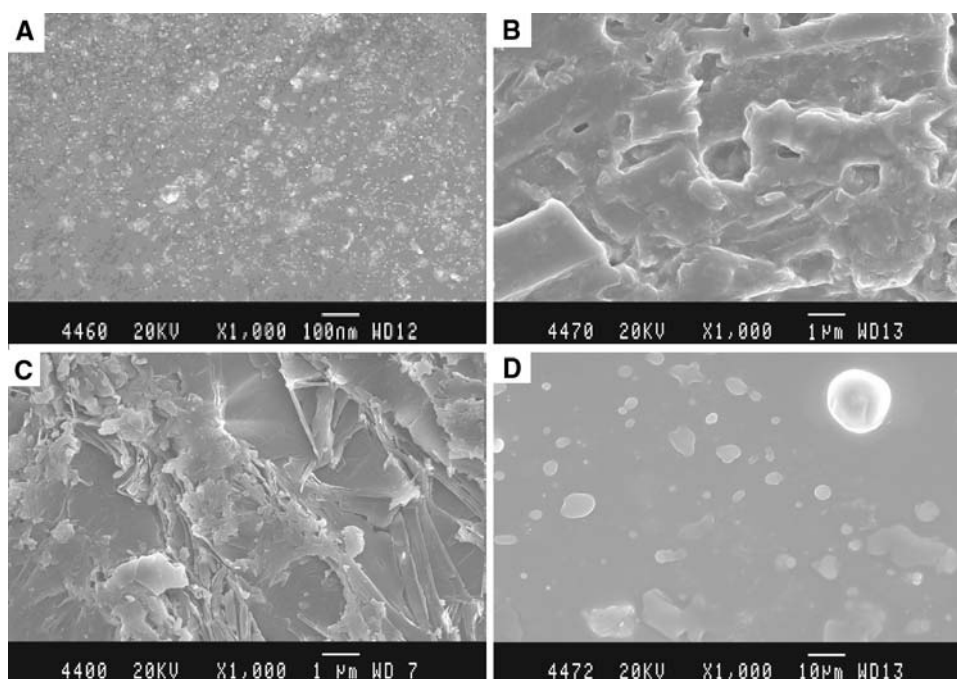


Fig. 7 DPSV of (a) 50 ng/mL AAP, (b) 50 ng/mL ASA, and (c) 100 ng/mL DP on Ppy/GCE

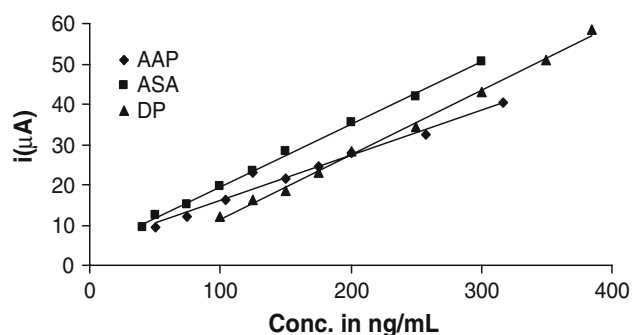


Fig. 8 Calibration plot for acetaminophen (AAP), acetylsalicylic acid (ASA), and dipyrone (DP)

modified electrode to 50 ng/mL of AAP and ASA and 100 ng/mL of DP in the presence of ascorbic acid, caffeine, glucose, *p*-aminophenol, and urea was checked by

Table 2 DPSV behavior of analgesic drugs

Drugs	Range studied (ng/mL)	LOD (ng/mL)	Relative standard deviation (RSD) value (%)
Acetaminophen	50–250	45	2.8
Acetylsalicylic acid	40–300	25	2.1
Dipyrone	100–400	70	2.6

recording differential pulse stripping voltammograms for oxidation of AAP, ASA, and DP after addition of varying concentration of each interferent (10–100 ng/mL). Ascorbic acid, glucose, caffeine, and urea did not affect the peak current of drugs even up to tenfold excess. However in the case of AAP, oxidation peak due to *p*-aminophenol started merging with that of AAP from 40 ng/mL concentration of the interferent.

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

Pharmaceutical samples containing 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. By substituting the peak current into the calibration plot and considering the dilution factor, the amount of analgesic present in the tablet was determined. The results are presented in Table 4; good agreement with the reported value was observed.

Table 3 Comparison values of available methods

Methods	LOD		
	AAP	ASA	DP
Voltammetric determination [25, 26, 38]	0.09–0.93 (mg L ⁻¹)	1–60 (μg mL ⁻¹)	7.2 × 10 ⁻⁶ (M L ⁻¹)
Flow injection–FT-IR spectrometric [39]	8 (μg mL ⁻¹)		
HPLC method [40]	31.6 (μg mL ⁻¹)		
Spectrophotometric determination [10, 41]	4 (μg mL ⁻¹), 0.5–20 (μg mL ⁻¹)	1–40 (μg mL ⁻¹)	
Flow injection amperometric determination [30]			2.07 × 10 ⁻⁶ (M L ⁻¹)

FT-IR Fourier-transform infrared

Table 4 The amount of compounds determined by DPSV in tablets

Brand name	Company name	Tablets (mg)	Experimental value (mg)
Acetaminophen			
Calpol	Glaxosmithkline	500	498
Crocic	Glaxosmithkline	500	499
Colimex tabs	Wallace	500	499
Doliprane	Nicholas piramal	500	499
Cyclopam	Indoco	500	498
Acetylsalicylic acid			
Disprin	Reckitts	350	349
Mycropyrin	Nicholas piramal	350	348
Asa50	German remedies	50	48
Colsprin100	Reckitts	100	98
Ecosprin	Sidmak	150	149
Dipyron			
Novalgin	Aventis	500	498

Analgesics were also measured in urine samples collected 8 h after administration. One milliliter of urine sample was mixed with 0.1 M H₂SO₄ solution and pH was brought to 1.0. DPSV was carried out under the optimum experimental conditions and the resultant voltammogram is presented in Fig. 9. This experiment was repeated five times and the average weight of drugs in 1.0 mL urine sample was found to be 0.07 μg for AAP, 0.13 μg for ASA, and 0.17 μg for DP with relative standard deviation 2.3%, 2.2%, and 2.9%, respectively. There is no appreciable interference due to the small amount of urine present in the electrolyte, hence the same calibration plot was used. There was no degradation of the analyte in solution during the experiment. The other materials present in the tablets and urine samples do not interfere with the study. This method is simple and suitable for the determination of the said drugs. Reproducibility is found to be high. Hence the proposed method can be used as a better alternative to spectrophotometric or chromatographic methods.

4 Conclusion

Analgesics AAP, ASA, and DP were anodically oxidized irreversibly on polypyrrole modified glassy carbon electrode in the pH range 1.0–13.0; oxidation was controlled by diffusion for AAP and DP and by adsorption for ASA. The influence of pH revealed the loss of 4e⁻ in acid and neutral media and 2e⁻/1H⁺ in basic medium for AAP. Loss of 2e⁻ and 1H⁺ in acidic and neutral pH and 2e⁻ and 2H⁺ loss in basic pH were observed for ASA. Removal of 2e⁻ and 1H⁺ in all pH media was observed for DP. The lower values of standard rate constant calculated confirmed irreversible oxidations in all three analgesics. From an analytical point of view, pH 1.0 was found to be suitable for differential pulse stripping voltammetric studies. Optimum accumulation and stripping conditions were arrived and the calibrations were made. Accumulation of the drugs was understood based on SEM studies. Lower limit of detection and percentage RSD were observed. The developed

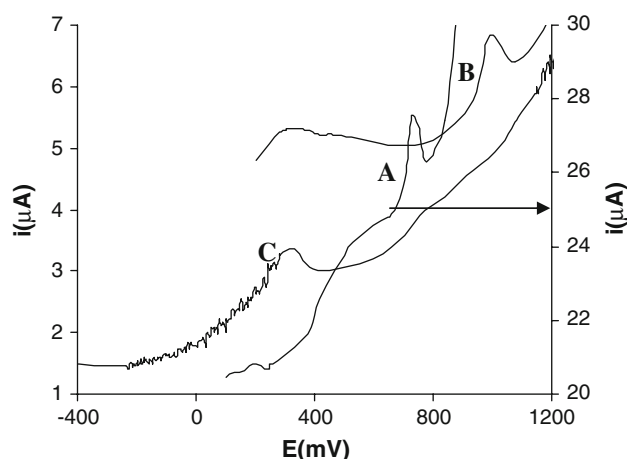


Fig. 9 DPSV of urine samples on Ppy/GCE: **a** acetaminophen, **b** acetylsalicylic acid, and **c** dipyron

method was used to determine the analgesics present in pharmaceutical formulations and urine samples. Thus this method can very well be used for determination of the three drugs in real samples also. This technique is simple and easy to carry out. Lower detection limit was obtained from this study for the three analgesics and compared with reported values, showing that the proposed method is better than the available methods.

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