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Research Article

DETERMINATION OF METRONIDAZOLE IN BIOLOGICAL SAMPLES EMPLOYING DIFFERENTIAL PULSE AND SQUARE WAVE POTENTIAL-WAVEFORMS STRIPPING VOLTAMMETRIC METHODS

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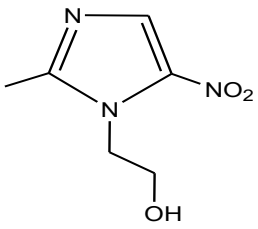
ABSTRACT

Metronidazole is an anti-microbial drug. In the pharmaceutical industry, Electro chemical analysis is an effective analytical method that is being more widely used. In Cyclic voltammetry glassy carbon electrodes is very crucial for the determination of pharmaceuticals. This research may lead to the establishment of a highly efficient analytical approach for determining Metronidazole in pharmaceutical as well as biological samples. Cyclic voltammetric investigation of Metronidazole tablets was carried out by using a three-electrode cell system, which were activated glassy carbon electrode as working electrode, Ag/AgCl as the reference electrode and a platinum wire as the auxiliary electrode for the analysis of Urine and Blood serum samples. Metronidazole exhibited one distinct and well-defined cathodic peak in the potential range -0.5 to -1Vvs Ag/AgCl/KCl reference electrode, at all concentrations. Mean percentage recoveries and relative standard deviations of 98.54 (1.117) (DP-CAdSV) and 99.54 (1.541) (SW-CAdSV) were achieved based on replicate measurements of 1×10^{-6} mol L^{-1} spiked human urine. Similarly on replicate measurements of $2x10^{-6}$mol L^{-1} Metronidazole in spiked human serum, mean percentage recoveries and relative standard deviations of 99.95 (0.722) (DP-CAdSV) and 99.69 (1.238) (SW-CAdSV) were obtained This procedure requires no pre-treatment, sensitive and time saving therefore it can be adopted for the pharmacokinetic studies.

Keywords: Metronidazole, Differential pulse, Square wave Voltammetry.

1. INTRODUCTION

Metronidazole (2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol) is a nitro-imidazole (Scheme) derivative and has been widely used for the treatment of protozoal diseases including trichomoniasis and giardiasis [1-3]. This drug is effective against trichomonas, Vincent's organisms, and anaerobic bacteria. Veterinarians also use metronidazole to treat bacterial infections as well as giardia in dogs and cats [4]. Metronidazole contains a nitro group which is the electrochemically active reducible center. In the absence of oxygen the reduction process for nitroimi-dazoles is similar to that for nitrobenzene [5, 6].



Scheme: Chemical Structure of Metronidazole

Metronidazole has yet to be identified on an ultra trace graphite electrode (UTGE) based on its extensive electrochemical reductive action. As a result, the properties of the reduction mechanism and the determination of metronidazole in tablet dosage form using a UTGE were deemed of concern [7-15]. This work was directed to study the voltammetric behavior of Metronidazole, owing to the high sensitivity and simplicity of the voltammetric techniques, and lack of literature data on the electrochemical behavior of Metronidazole [16-25].

The present investigation described a fully validated, simple, rapid and more sensitive developed procedure for the determination of Metronidazole in pharmaceutical formulations and serum employing differential pulse and square wave at the glassy carbon electrode. As an advantage the determination procedure did not require sample pretreatment or any time-consuming extraction step prior to the drug assay.

2. MATERIAL AND METHODS

2.1. Apparatus and Instrumentation

A CHI instrument, Model 1230A (SR 400) electro-

chemical analyzer Connected with PC was used for cyclic voltammetry analysis. A three-electrode cell device was used, with the operating electrode being an activated ultra trace glassy carbon electrode (ø = 3 mm, CHI), the reference electrode being Ag/AgCl (3 M KCl), and the auxiliary electrode being a platinum wire. A digital pH-meter (CHINO- DB-1011) was used for measurement of pH values of the investigated solutions. The mass of various chemicals and samples was measured using a digital weighing balance. For adjusting the pH, a magnetic stirrer with a hot plate was used.

2.2. Reagents and Chemicals used for analysis

Metronidazole was purchased from Sun Pharma India Limited and was used without further purification. Britton Robinson, acetate, borate, citrate, and phosphate buffers, Acetonitrile, CHI polishing kit with alumina paste, and Wattmann filter paper were used. Double Distilled water is used during the entire process.

2.3. Pre-treatment of the Ultra trace Glassy Carbon Electrode

The Ultra trace glassy carbon electrode was polished to a mirror finish using a CHI polishing kit with alumina paste and thoroughly washed with double distilled water before measurements

2.4. Preparation of standard solution of Metronidazole

A stock standard solution of bulk Metronidazole (1×10^{-3} mol L^{-1}) was prepared in distilled water and stored at 4°C until assay. One tablet of Metronidazole was weighed and crushed to a homogeneous fine powder in a mortar. A portion of this crushed material equivalent to 100mg of Metronidazole accurately weighed and transferred into a 10 ml calibrated flask containing 8 ml distilled water. The content of the flask was sonicated for 12 min and then make up to volume with distilled water. Subsequently the solution was filtered through a 0.45 µm-pore filter (Wattmann filter paper). A series BR buffer of pH values 3 to 11 was prepared and used as a supporting electrolyte.

2.5. Analysis of Spiked Urine and Blood Serum Samples

Human blood was collected from healthy donors and centrifuged at room temperature for 30 minutes at 4000 rpm and the obtained serum samples (supernatant) were stored frozen until the assay. An aliquot volume of serum sample (1.0 ml each) were fortified with various concentrations of Metronidazole ($1 \times 10^{-7}$to 1×10^{-3}mol L^{-1}) in tubes then each was mixed with a 1.0 ml volume Acetonitrile to denature and precipitate proteins. After vortexing for 30s, the mixture was then centrifuged for 10 min at 5000 rpm to get rid of serum protein residues and supernatant was taken. Quantifications were performed by means of the calibration curve method. A portion of a human urine sample was taken and examined as serum sample.

2.6. Analytical procedure

BR buffer of pH 7 and the appropriate concentration of the analyte (bulk Metronidazole) were transferred into the electrochemical cell, through which a pure deoxygenated nitrogen stream was passed for 10 min to remove the oxygen gas before measurements. The square wave voltammetric stripping was initiated in the cathodic direction over the range -0.5 to -1 V vs Ag/AgCl/KCl reference electrode at room temperature. Differential pulse and cyclic voltammetric measurements were carried out under similar conditions. Quantification of Metronidazole was performed by means of both calibration curve and standard addition methods. The reversibility of the reduction process was investigated by using CV.

3. RESULTS AND DISCUSSION

On a glassy carbon electrode, cyclic voltammetry, differential pulse cathodic adsorptive stripping (DP-CAdSV) voltammetry, and square wave cathodic adsorptive stripping (SW-CAdSV) voltammetry were used to investigate Metronidazole's electrochemical activity. Metronidazole recorded a single clearly identified reduction peak in BR buffer in all the electrochemical methods.

3.1. Cyclic Voltammetric Studies

In the potential range of -0.5 to -1 Vvs, Metronidazole showed one clearly identified cathodic peak, Ag/AgCl/KCl reference electrode, at all concentrations.

3.2. Effect of pH

This study was made in the pH range 3-11 at a target concentration of 2µg/ml Metronidazole solution. CV, DP-CAdSV, and SW-CAdSV records showed one reduction wave with half-wave potential -0.85 V at pH 7. With the rise in pH the peak potential (fig.1) shifted in the more negative direction which shows that protons participate directly in the reduction process. Peak

current is clearly influenced by the pH value and the nature of the buffer, as seen by the plot of i_p vs pH. The peak current reached the highest value at around pH 7 in BR buffer (fig. 2).

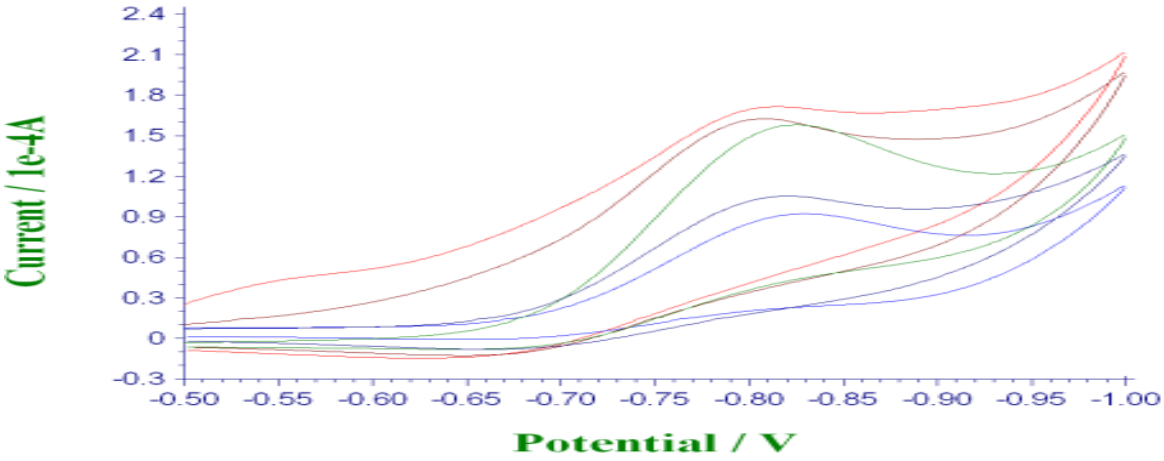


Fig. 1:Plot of peak current (i_p) vs pH for the Cyclic Voltammograms of 1×10^{-5} mol L^{-1} Metro-nidazole in BR buffer (a) pH -3(b) pH -5 (c) pH -7 (d)pH-9 (e)pH-11

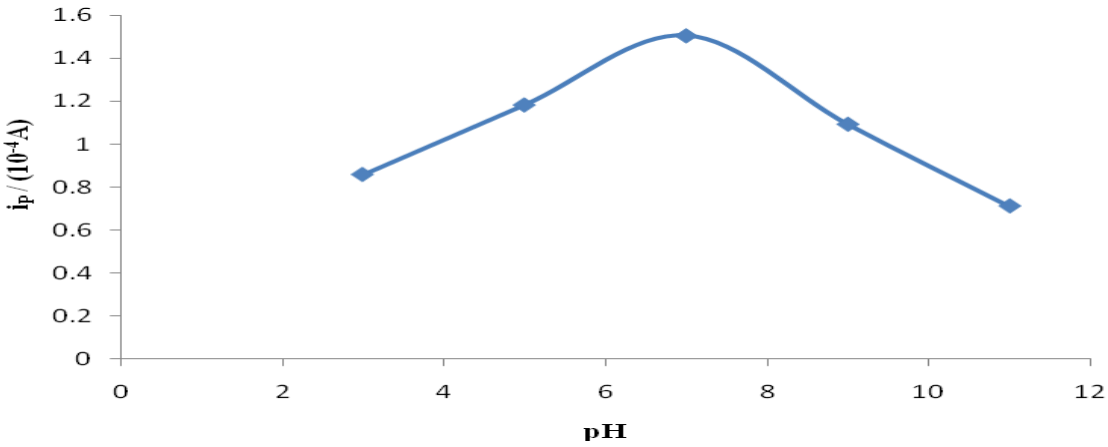


Fig. 2: Plot of peak current (i_p) vs pH for the Cyclic Voltammograms of 1×10^{-5} mol L^{-1} Metro-nidazole in BR buffer (a) pH -3(b) pH -5(c) pH -7 (d)pH-9 (e)pH-11

3.3. Effect of Scan Rate

Using a solution with various concentrations of $1x10^{-5}$, $2x10^{-5}$, $3.0x10^{-5}$, $4x10^{-5}$& $5.0x10^{-5}$ mol L^{-1} and recording CVs at 50, 75, 100, 125,150, and 175 mV sec^{-1} scan rate, the effect of scan rate(ν) on the cathodic peak current was calculated.

Cyclic voltammograms (fig. 3) revealed that there was a well identified reduction peak at -0.85 V. The reverse scan revealed no peaks, showing that the electrode processes are reversible in nature. The given equation represents the relation between the cathodic peak current $i_p/10^{-4}$A, the diffusion coefficient of the electro active species, D_o/ cm^2 s^{-1}, and the scan rate, ν / mVs^{-1} [26].

$i_p = 2.99x10^5\, n\, (\alpha n_\alpha)^{½}\, ACD^{½} \upsilon^{½}$ ..... (1)

Where n is the number of electrons exchanged in reduction, α is the transfer coefficient, **A** is the apparent surface area of the electrode (cm^2), and $\mathbf{C_o}$ is the concentration of the electro active species (m mol dm^{-3}). For an irreversible process the transfer coefficient α can be calculated from [27].

$(E_p - E_{p/2}) = 47.7/n\alpha$ ..... (2)

Where $E_{p/2}$ is the potential at which the current equals one-half of the peak current. According to a close

review of data on the effect of scan rate, the linearity of the correlation is actually realized up to scan rate,

The relationship between the peak potential (Ep) and scan rate (υ) for a completely irreversible electrode process is given below [28-32]:

$$Ep = \frac{2.303RT}{\alpha nF}\log\frac{RTKf}{\alpha nF} - \frac{2.303RT}{\alpha nF}\log v \cdot \quad \text{..... (3)}$$

E_p (v) = 0.094(log υ) + 0.657, (r^2= 0.981) ..... (4)

As peak potential (-Ep) was plotted against logarithm of scan rate (log υ) (Fig. 4) at a specific concentration at pH 9, a straight line was observed, which can be represented as

A straight line is obtained when peak current (i_p) is plotted against square root of scan rates ($\upsilon^{1/2}$) (fig. 5), which may be expressed by the equation

$i_p(10^{-4}\ A) = 0.105\upsilon^{1/2}(mV/s)^{1/2} + 0.739$ (r^2=0.984) ..(5)

The plot of logarithm of peak current ($\log i_p$) versus logarithm of scan rate (log ν) gave a straight lines (fig. 6) with slope 0.284, which is less then theoretical value of 0.5 that is expected for ideal reaction of solution species. The lower experimental slope (0.284) may be attributed to the partial involvement of the diffusive drug molecules in the electrode reaction of the adsorbed ones. The overall electrode process is mainly diffusion-controlled with adsorption of the drug molecules at the electrode surface.

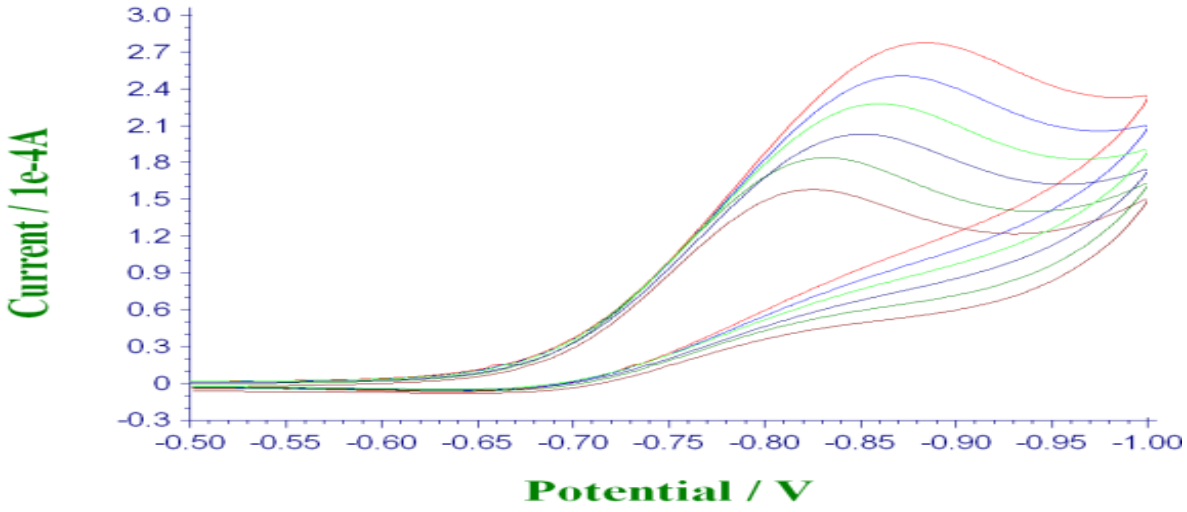


Fig. 3: Cyclic voltammograms of 1×10^{-5} mol L^{-1} Metronidazole in BR buffer pH 7 at different scan rates: (a) 50 mVs^{-1} (b) 75 mVs^{-1} (c) 100 mVs^{-1} (d) 125 mVs^{-1}(e) 150 mVs^{-1} (f) 175 mVs^{-1}

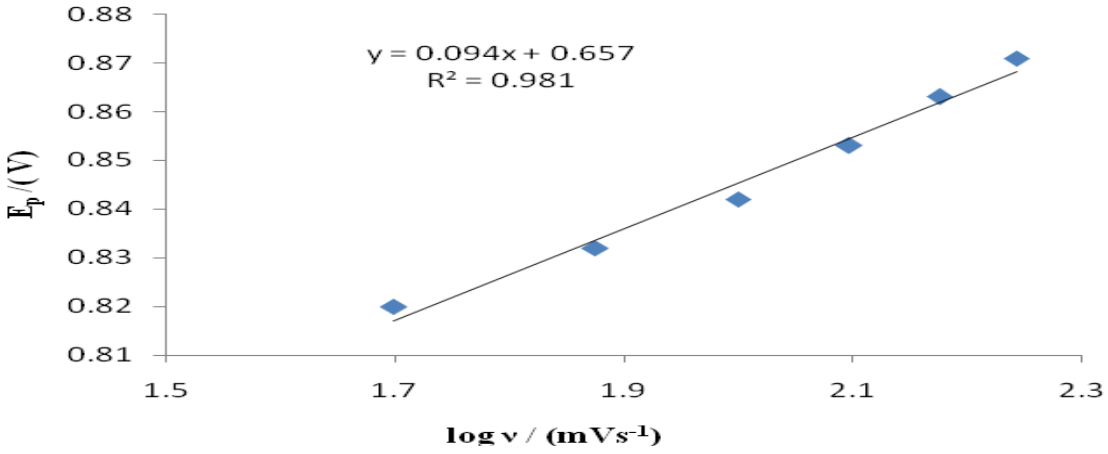


Fig. 4: Plot of peak potential ($-E_p$) vs logarithm of scan rate (log ν) for the Cyclic voltammograms of 1×10^{-5} mol L^{-1} Metronidazole in BR buffer at pH 7

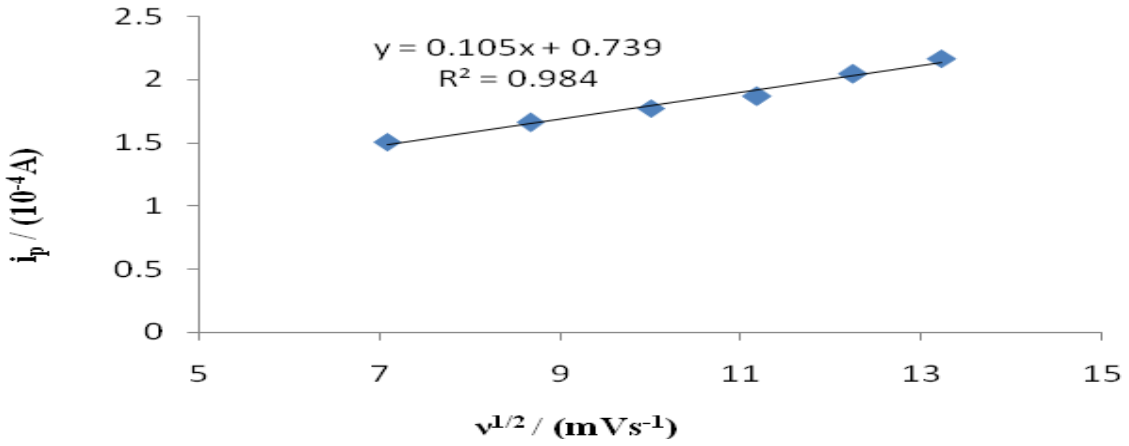


Fig. 5: Plot of peak current (i_p) vs square root of scan rates ($v^{1/2}$) for the Cyclic voltammograms of 1×10^{-5} mol L^{-1} Metronidazole in BR buffer at pH 7

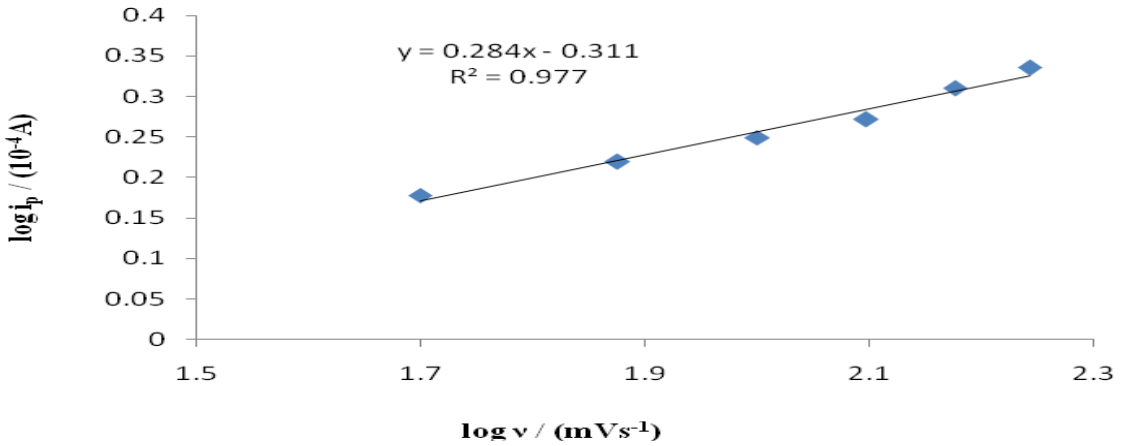


Fig. 6: Plot of logarithm of peak current ($logi_p$) Vs logarithm of scan rate (log ν) for the Cyclic voltammograms of 1×10^{-5} mol L^{-1} Metronidazole in BR buffer at pH 7

3.4. Effect of Concentration

The influence of Metronidazole concentration on the expression of cyclic voltammograms can be observed in fig. 7.

The Randles-Sevick equation stated that peak current (i_p) is directly proportional to concentration (C_O.). A plot of peak current (i_p) vs concentration (C_0) for Metronidazole yields a straight line (fig. 8).

i_p (10^{-4} A)=0.226 xC (10^{-5} $molL^{-1}$)+0.947, (r^2=0.998) .(6)

3.5. Stripping Voltammetric Studies

Using pulse and square wave potential-waveforms, stripping voltammetric methods were designed for trace determination. On its pre-concentration onto the glassy carbon electrode, differential pulse and square wave voltammetry is used to record stripping voltammograms of bulk Metronidazole in the Britton Robinson (BR) universal buffer (pH 3 to 11). At pH 7, a well-defined single irreversible cathodic peak with a better enhanced peak current magnitude was observed after adsorptive accumulation for 15 seconds. As a result, in the rest of the sample, a Britton Robinson (BR) universal buffer with a pH of 7 was used as a supporting electrolyte.

3.6. Differential Pulse Cathodic Adsorptive Stripping Voltammetry (DP-CAdSV) Method

The appropriate pulse-height scan rate and preconcentration parameters for aggregate Metronidazole determination using differential pulse cathodic adsorptive stripping voltammetry (DP-CAdSV) at the GCE and voltammograms recorded under optimum condition are shown in fig. 9.

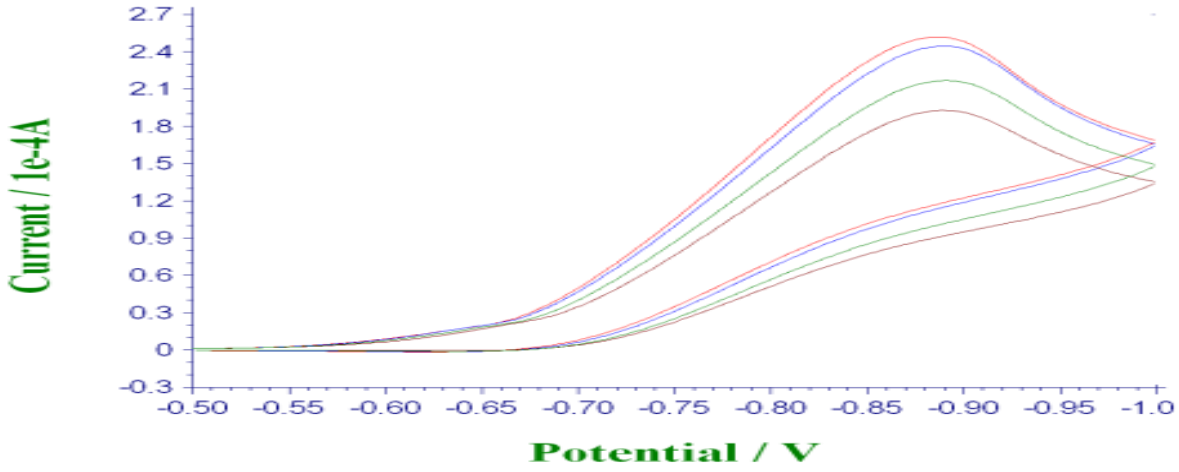


Fig. 7: Cyclic voltammograms of Metronidazole in BR buffer pH 7 at scan rate 100 mV^{-1} in different concentrations: (a) 1×10^{-5} mol L^{-1} (b) 2×10^{-5} mol L^{-1} (c) 3×10^{-5} mol L^{-1} (d) 4×10^{-5} mol L^{-1}

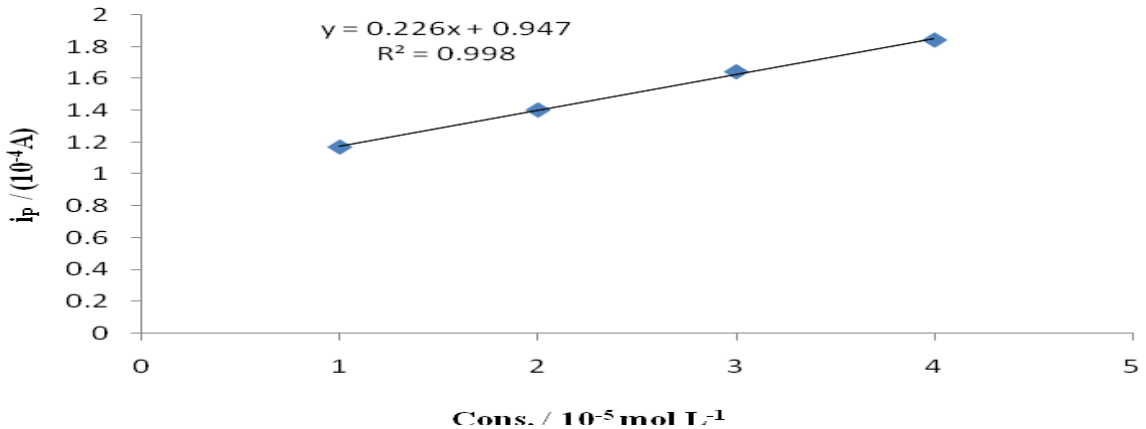


Fig. 8: Plot of peak current (i_p) vs concentration (C_o.) for the Cyclic voltammograms of Metro-nidazole in BR buffer of pH 9 at scan rate 100 mVs^{-1} in different concentrations

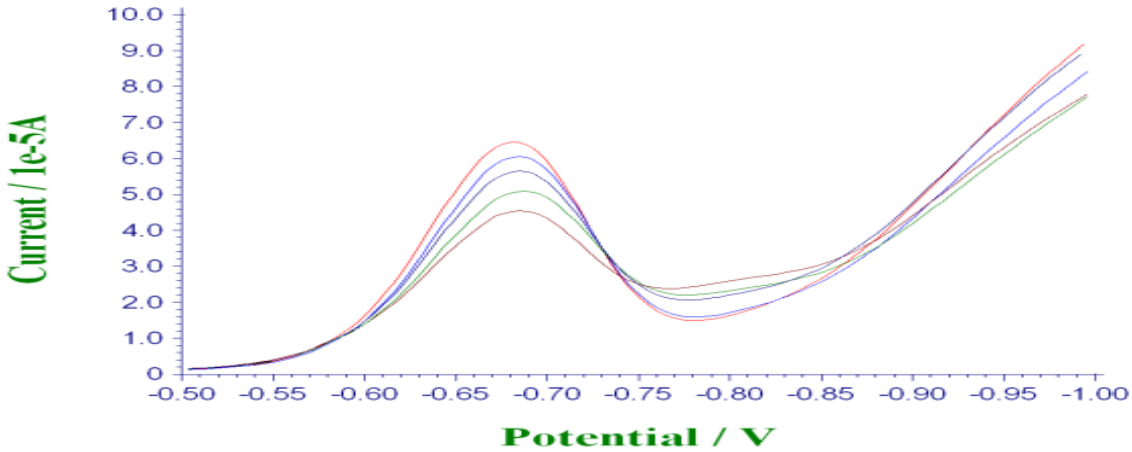


Fig. 9: The DP-CAdS voltammograms for increased concentrations of Metronidazole in bulk samples: (a) $1x10^{-6}$ mol L^{-1} (b) $2x10^{-6}$ mol L^{-1} (c) $3x10^{-6}$ mol L^{-1} (e) $4x10^{-6}$ mol L^{-1} (g) $5x10^{-6}$ mol L^{-1}

3.7. Square Wave Cathodic Adsorptive Stripping Voltammetry (SW-CAdSV) Method

The optimal pulse-height scan rate and preconcentration parameters for mass Metronidazole determination using square wave cathodic adsorptive stripping voltammetry (SW-CAdSV) at the GCE were expressed in fig. 10 as recorded SW-CAdSV voltammograms at pH 7.

3.8. Validation of the Procedure

Validation of the proposed procedure for assay of the drug at trace levels was examined via evaluation of the limit of detection (LOD), limit of quantization (LOQ), reproducibility, recovery, selectivity, robustness and ruggedness.

LOD and LOQ

The Limits of detection (LOD) and quantification (LOQ) of Metronidazole were calculated using the following equations [30, 33].

$$LOD = 3s/b \quad \ldots\ldots (7)$$

$$LOQ = 10s/b \quad \ldots\ldots (8)$$

Where s is the standard deviation of the intercept and b is the slope of the calibration curve reproducibility, accuracy and precision of results applying the described stripping voltammetric methods and were examined by performing five replicate analyses of standard solutions of bulk Metronidazole.

A LOD of 2.484 x10^{-7}mol $L^{-1}$and a LOQ of 8.280x10^{-7} mol L^{-1} bulk were achieved by applying the described DP-CAdSV method. On other hand a LOD of 5.177x10^{-7} mol L^{-1} and a LOQ of 1.725x10^{-6} mol L^{-1} bulk Metronidazole were achieved applying the described SW-CAdSV method (Table 1). The obtained results confirmed the reliability of the described stripping voltammetric methods for assay of Metronidazole.

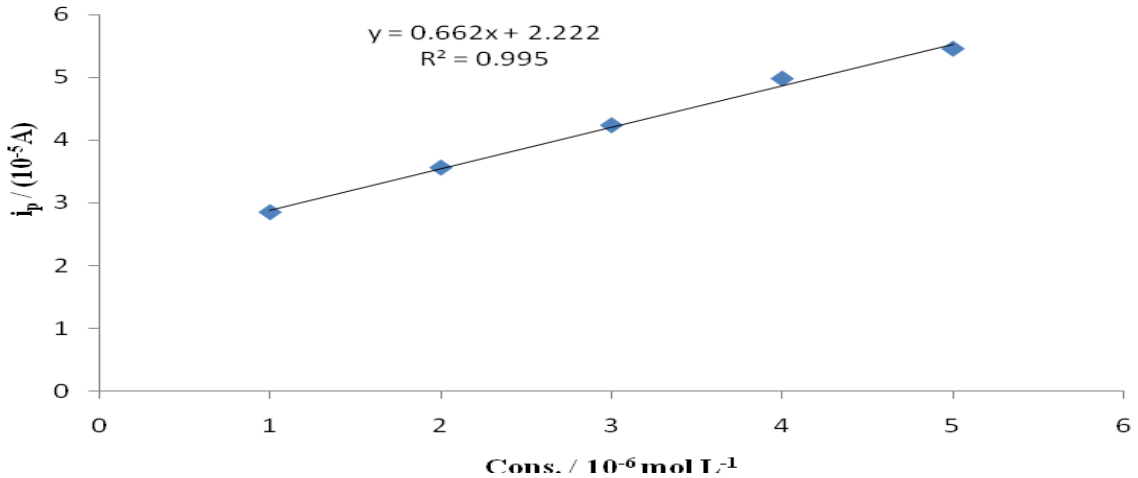


Fig. 10: Plot of peak current (i_p) vs concen-trations of Metronidazolne in BR buffer of pH 7 bulk samples from DP-CAdS voltammograms.

Table 1: Stripping voltammetric determination of Metronidazole in, Bulk form, Urine and serum using DP-CAdSV and SW-CAdSV Modes

Techniques	Bulk Form		Urine		Serum	
	DP-CAdSV	SW-CAdSV	DP-CAdSV	SW-CAdSV	DP-CAdSV	SW-CAdSV
Linearity range (mol L^{-1})	1x10^{-6} -1.4x10^{-5}	1x10^{-6} -1.4x10^{-5}	1x10^{-6} -1x10^{-5}	1x10^{-6} -1x10^{-5}	1x10^{-6} -1x10^{-5}	1x10^{-6} -1x10^{-5}
Slope (A/M)	0.662x10^{-6}	0.544 x10^{-6}	0.454 x10^{-6}	0.406 x10^{-6}	0.394×10^{-6}	0.525×10^{-6}
Intercept (µA)	2.222	2.512	0.908	2.579	0.3400	2.665
Correlation Coefficient (r^2)	0.995	0.997	0.995	0.994	0.996	0.995
Variance ratio (F)	0.548	0.138	0.101	0.0641	0.0432	0.1172
LOD (mol L^{-1})	2.484 x10^{-7}	5.177 x10^{-7}	6.683x10^{-7}	4.742 x10^{-7}	3.293×10^{-7}	6.700×10^{-7}
LOQ (mol L^{-1})	8.280 x10^{-7}	1.729 x10^{-6}	2.221 x10^{-6}	1.580 x10^{-6}	1.097×10^{-6}	2.233×10^{-6}

3.9. Assay of Metronidazole in Spiked Human Urine

Metronidazole in spiked human urine was successfully analyzed by the voltammetric methods DP-CAdSV and SW-CAdSV without the necessity for extraction of the drug prior to the analysis. Figs. 11 and 13 indicate descriptive DP-CAdSV and SW-CAdS voltammograms of Metro-nidazole in spiked human urine recorded under the ideal operating conditions of the mentioned stripping voltammetric methods. As shown in figs. 12 and 14 (curve) respectively, no interfering peaks were observed in the blank human urine sample within the studied potential range.

Linear variations of the peak current (i_p) with concentration of Metronidazole in spiked human urine were obtained within the concentration ranges of $1x10^{-6}$ -$1x10^{-5}$mol L^{-1}(DP-CAdSV) and $1x10^{-6}$ -$1x10^{-5}$mol L^{-1} (SW-CAdSV). The linear regression equation is expressed as:

For DP-CAdSV:

$i_p(10^{-5}A)=0.454x10^{-6}$ (mol L^{-1})+0.908; r^2 =0.995 .. (9)

and for SW-CAdSV;

$i_p(10^{-5}A)=0.406x10^{-6}$ (mol L^{-1})+2.579; r^2=0.994 ..(10)

Detection limits of $6.683x10^{-7}$ and $4.742\ x10^{-7}$mol L^{-1} and quantification limits of $2.221\ x10^{-6}$ and $1.580\ x10^{-6}$ mol L^{-1} Metronidazole was obtained using the DP-CAdSV and SW-CAdSV methods respectively. Mean percentage recoveries and relative standard deviations of 98.54 (1.117) (DP-CAdSV) and 99.54 (1.541) (SW-CAdSV) were achieved based on replicate measure-ments of 1×10^{-6} mol L^{-1} (table 2) Metronidazole in spiked human urine. The reliability of the stated strip-ping voltammetric methods for assaying Metronidazole in human urine was verified by these findings.

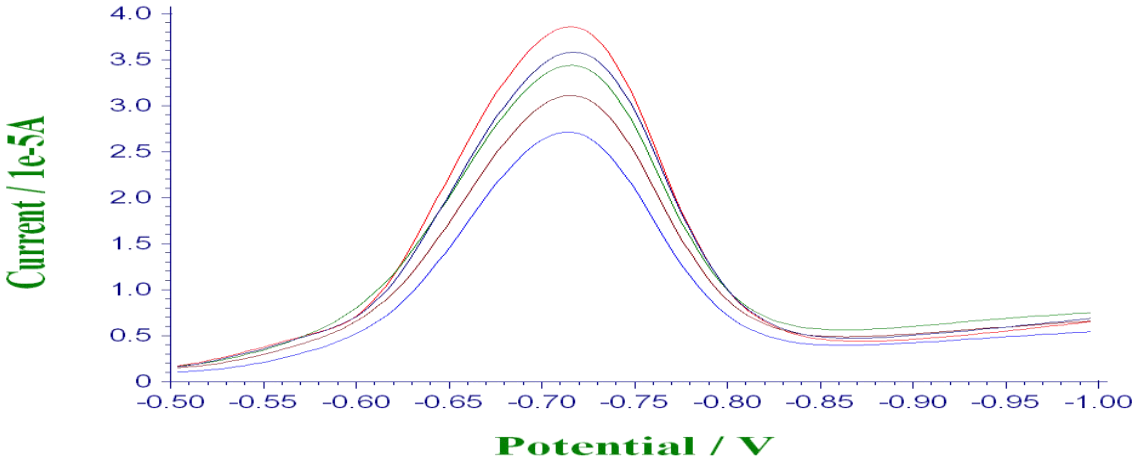


Fig. 11: The DP-CAdS voltammograms for increased concentrations of Metronidazole in spiked urine samples: (a) $1x10^{-6}$ mol L^{-1} (b) $2x10^{-6}$ mol L^{-1}(c) $3x10^{-6}$mol L^{-1}(*d*) $4x10^{-6}$mol L^{-1} (e) $5x10^{-6}$ mol L^{-1}

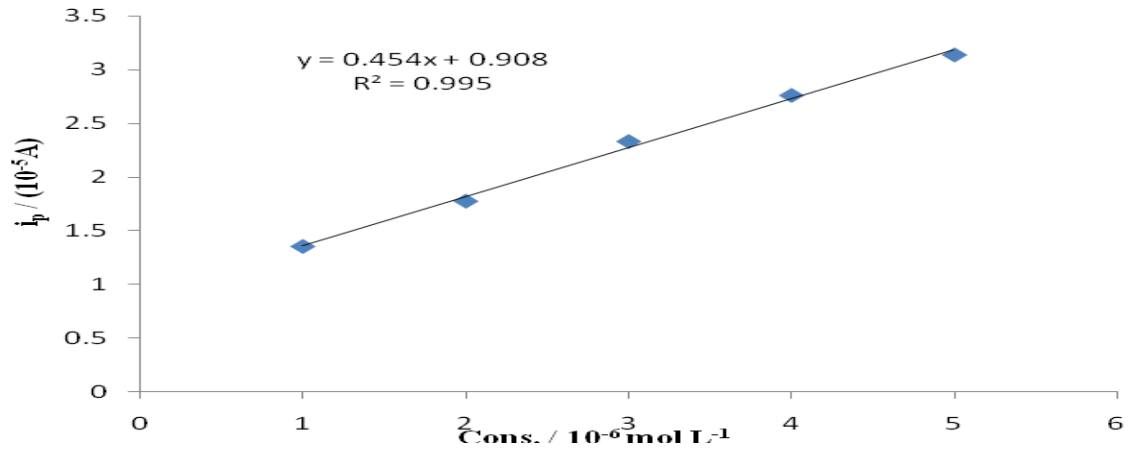


Fig. 12: Plot of peak current (i_p) vs concen-trations of Metronidazole in BR buffer of pH 7 from DP-CAdS voltammograms in spiked urine sample

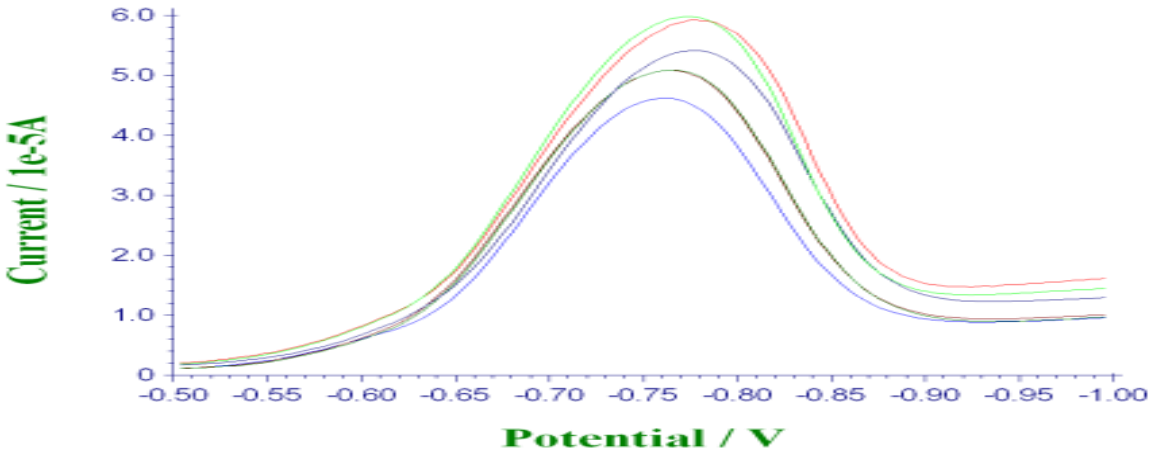


Fig. 13: The SW-CAdS voltammograms for increased concentrations of Metronidazole in spiked urine samples: (a) 1x10^{-6} mol L^{-1} (b) 2x10^{-6}mol L^{-1} (c) 3x10^{-6} mol L^{-1} (d) 4x10^{-6} mol L^{-1} (e) 5x10^{-6} mol L^{-1}

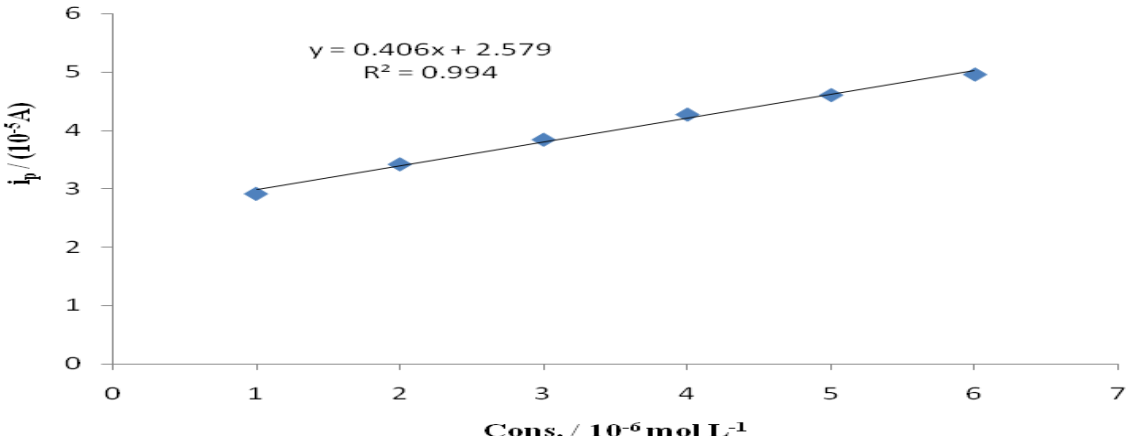


Fig. 14: Plot of peak current (i_p) vs concen-trations of Metronidazole in BR buffer of pH 7 from SW-CAdS voltammograms in spiked urine sample.

3.10. Assay of Metronidazole in Spiked Human Blood Serum

The described voltammetric methods (DP-CAdSV and SW-CAdSV) successfully analyzed metronidazole in spiked human serum without the need for drug extraction prior to examination. Figs. 15 and 17 demonstrate representative DP-CAdSV and SW-CAdS voltammograms of Metronidazole in spiked human serum recorded under the ideal operating conditions of the mentioned stripping voltammetric methods. Within the studied potential spectrum, no intervening peaks were found in the blank human serum, as seen in figs. 16 and 18 (curve). For the stripping voltammetric method, the calibration plot of peak current versus concentration was recorded to be linear over the range 1x10-^{6} to 1x10^{-5} mol L^{-1}.

The linear regression equation is expressed as:

For DP-CAdSV:

$i_p(10^{-4}\,A)=0.394x10^{-6}\,(mol\,L^{-1})+0.340$; $r^2=0.996$.(11)

for SW-CAdSV:

$i_p(10^{-5}\,A)=0.525x10^{-6}\,(mol\,L^{-1})+2.665$; $r^2=0.995$.(12)

Detection limits of 3.293×10^{-7} and 6.700×10^{-7} mol L^{-1} and quantization limits of 1.097×10^{-6} and 2.233×10^{-6} mol L^{-1} Metronidazole were obtained by the mentioned DP-CAdSV and SW-CAdSV methods respectively.

Focused on replicate measurements of 2x10^{-6} mol L^{-1} Metronidazole in spiked human serum, mean percentage recoveries and relative standard deviations of 99.95 (0.722) (DP-CAdSV) and 99.69 (1.238) (SW-CAdSV) were obtained (table 2). The accuracy of the mentioned stripping voltammetric methods for analysing Metro-nidazole in human serum was confirmed by these results.

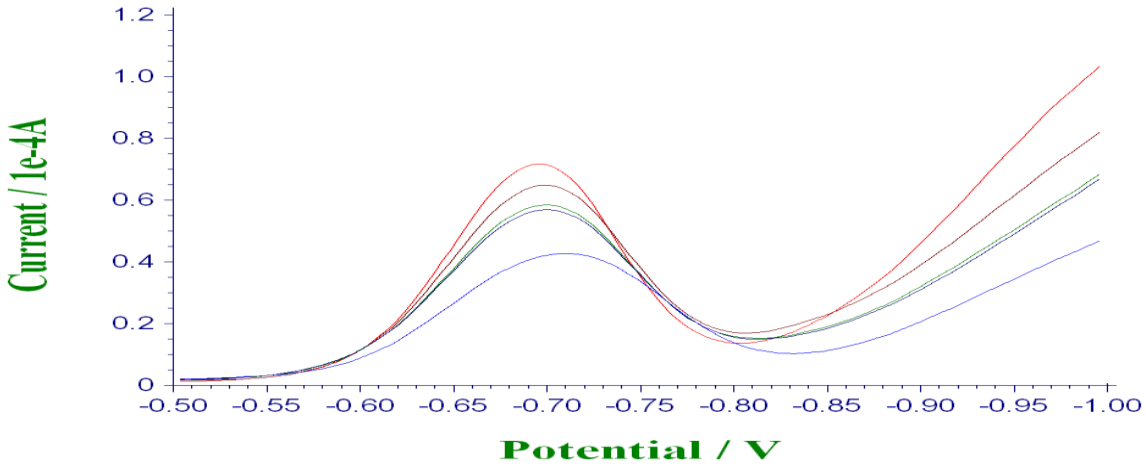


Fig. 15: The DP-CAdS voltammograms for increased concentrations of Metronidazole in human serum samples: (a)1x10^{-6} mol L^{-1}(b) 2x 10^{-6} mol L^{-1}(c) 3x10^{-6} mol L^{-1}(*d*) 4x10^{-6} mol L^{-1} (e) 5x10^{-}mol L^{-1}

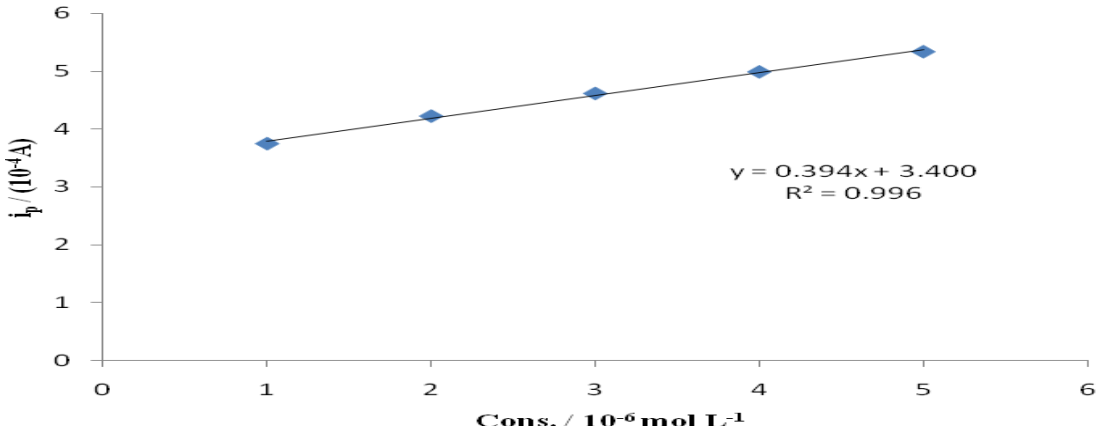


Fig. 16:Plot of peak current (i_p) vs concentrations of Metronidazole in BR buffer of pH 7 from DP-CAdS voltammograms in human serum sample

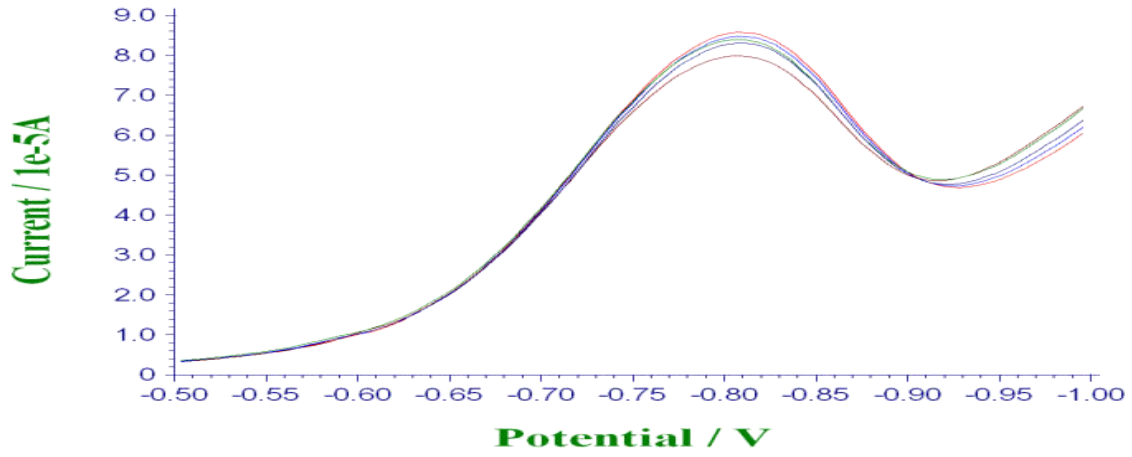


Fig. 17: The SW-CAdS voltammograms for increased concentrations of Metronidazole in human serum samples: (a)1x10^{-6} mol L^{-1} (b) 2x10^{-6} mol L^{-1} (c) 3x10^{-6} mol L^{-1} (d) 4x10^{-6} mol L^{-1}

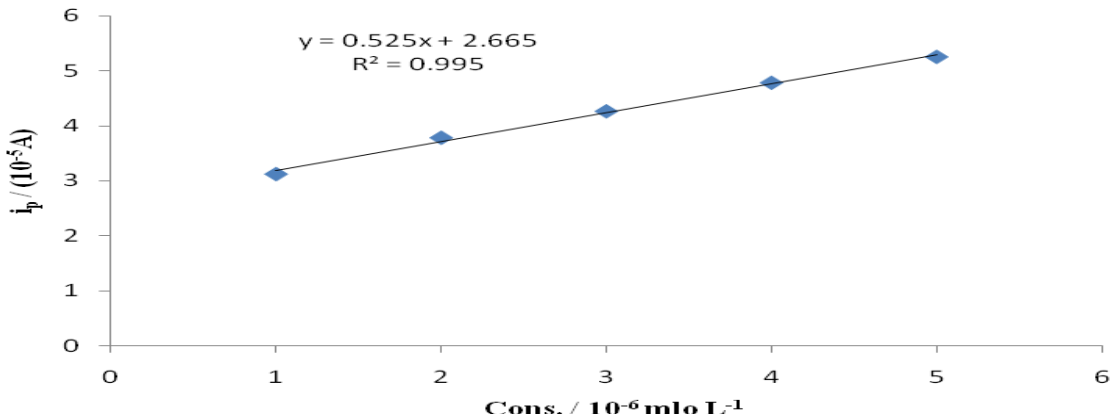


Fig. 18: Plot of peak current (i_p) vs concentrations of Metronidazole in BR buffer of pH 7 from SW-CAdS voltammograms in human serum sample

Table 2: Application of the stripping voltammetric determination of Metronidazole drug in Bulk form, urine and Serum using DP-CAdSV and SW-CAdSV Modes

Techniques	Bulk Form		Urine		Serum	
	DP-CAdSV	SW-CAdSV	DP-CAdSV	SW-CAdSV	DP-CAdSV	SW-CAdSV
Added (µ mol L^{-1})	2	2	2	2	2	2
	4	4	4	4	4	4
	6	6	6	6	6	6
	8	8	8	8	8	8
	10	10	10	10	10	10
	12	12	12	12	12	12
Found (µ mol L^{-1})	2.01	1.99	2.01	1.94	1.99	2.03
	3.89	3.87	3.90	4.03	3.95	4.01
	5.84	5.92	5.88	6.07	6.04	6.02
	7.94	7.85	7.82	7.89	8.01	7.89
	10.02	10.04	9.85	9.95	10.05	9.93
	11.93	11.96	11.88	12.04	12.02	11.78
N	3	3	2	2	2	2
Average recovery %	100.5	99.5	100.5	97.0	99.5	101.5
	97.25	96.75	97.5	100.75	98.75	100.25
	97.33	98.66	98.0	101.1	100.66	100.33
	99.25	98.12	97.75	98.62	100.125	98.625
	100.2	100.4	98.5	99.5	100.5	99.3
	99.41	99.66	99.0	100.3	100.16	98.16
Mean	98.99	98.84	98.54	99.54	99.95	99.69
S.D.	1.397	1.300	1.1001	1.5345	0.7123	1.233
RSD %	1.423	1.389	1.1172	1.5412	0.7226	1.238

4. CONCLUSION

The electrochemical behavior of Metronidazole on GCE was established. Metronidazole is irreversibly reduced at negative potentials. This work shows that the Metronidazole concentration in human serum and in pharmaceutics can be determined by using voltammetric techniques on the basis of their reduction process on GCE in BR buffer. This behaviour makes it possible to identify and quantify drugs in biological samples at low concentrations. The procedure showed clear advantages such as no pretreatment or time-consuming extraction steps and could be applied in both pharmacologic and quality control research studies. This study shows that there is a great possibility of monitoring Metronidazole makes the method useful for pharmacokinetic and pharmaco dynamic purposes. The described methods

could be recommended for use in trace analysis, quality control and clinical laboratories.

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Conflict of interest

There is no conflict of interest in publication of this paper.

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