Int. J. Electrochem. Sci., 3 **(2008)** 509-518

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Determination of Triamcinolone Acetonide Steroid on Glassy Carbon Electrode by Stripping Voltammetric Methods

C. Vedhi, R. Eswar, H. Gurumallesh Prabu and P. Manisankar[*]

Department of Industrial Chemistry, Alagappa University, Karaikudi –630 003,Tamil Nadu, India
[*]E-mail: pms11@rediffmail.com or pmsankarsiva@yahoo.com

Received: 9 December 2007 / *Accepted:* 6 February 2008 / *Online published:* 20 February 2008

A corticosteroid, triamcinolone acetonide was determined by stripping voltammetric procedure using glassy carbon electrode. Cyclic voltammetric behaviour of steroid was studied in 50% aqueous methanol at acid, neutral and alkaline pH conditions. Influence of pH, sweep rate and steroid concentration were studied. An irreversible and adsorption-controlled well-defined reduction peak was observed in all pH conditions. The reduction peak potential shifted cathodically with change in pH. Controlled potential coulometry revealed two-electron reduction of the α,β-unsaturated carbonyl function present in the steroid. A systematic study of the experimental parameters that affect the differential pulse / square wave stripping voltammetric response was carried out and optimized conditions were arrived at. Under optimum conditions, differential pulse and square wave adsorptive stripping voltammetric procedures were developed for the determination of triamcinolone acetonide steroid at pH 13.0. A calibration plots was derived and the lower limit of determination observed are 0.1 μg/mL from DPSV and 0.01 μg/mL from SWSV.

Keywords: Triamcinolone acetonide steroid, Cyclic Voltammetry, Stripping Voltammetry, Glassy carbon electrode.

1. INTRODUCTION

Corticosteroids are hormones produced naturally by the adrenal glands, which have many important functions on every organ system. Corticosteroids decrease inflammation by acting within cells to prevent the release of certain chemicals that are important in the immune system. These chemicals are normally involved in producing immune and allergic responses, resulting in inflammation. The use of corticosteroids in combination with other hormonal substances has long been known to result in increased mass gain with bovines [1]. The developments of drug analysis for steroidal hormones during the last 15 years have clearly revealed the significance and use of high-performance liquid chromatography (HPLC) related [2] and electroanlytical techniques for routine

analysis. A HPLC method was reported for the determination of estriol 16-glucuronide and 17-glucuronide steroids in bile and urine. The electrochemical detector was found to be superior to the UV detector with respect to selectivity and sensitivity and therefore it was more suitable for the determination of estrogen conjugates in biological fluids. This study revealed that both estriol 16-glucuronide and 17-glucuronide were excreted in rat bile, while only the former was present in human pregnancy urine [3]. A novel reversed-phase HPLC method was reported for the determination of active component triamcinolone acetonide and its degradation product triamcinolone [4]. A study of HPLC method for the analysis of related substances in triamcinolone acetonide was also described. Several systems of solvents and samples of different lots and preparative origins were examined and a rapid-scanning diode array UV detector was particularly useful [5,6]. High performance thin layer chromatography (HPTLC) system for quantitative determination of androgens, corticosteroids, mineralocorticoids and gestagens on silica gel KG-60 HPTLC-plates with different solvent systems were reported [7]. New techniques such as capillary electrophoresis, micellar (or microemulsion) electrokinetic chromatography and capillary electrochromatography also attracted wide interest in steroid analysis. Determination of betamethasone and triamcinolone acetonide by GC-NCI-MS in excreta of treated animals and development of a fast oxidation procedure for corticosteroids was also reported [1]. The colorimetric method for the determination of triamcinolone, triamcinolone acetonide and fluocinonide in tablets, ointments and cream using isonicotinic acid hydrazide provided precise and reproducible results (recovery ranged within 97.42-101.75%, variation coefficient ranged within 0.99-2.56%) [8]. Simultaneous determination of terbinafine HCl and triamcinolone acetonide by UV derivative spectrophotometry and spectrodensitometry were also developed [9]. Electroanalytical methods in steroid analysis were reviewed in the year 1989 [10]. These methods have never played very important role in this field but sufficiently sensitive and selective methods are often published up to the present time mainly for the assay of formulations. In the majority of the cases differential pulse polarography, based on the reduction of the 4-ene-3-oxo group was used. Stripping voltammetric determination of spironolactone[11], Beclomethasone dipropionate[12], Dexamethasone sodium phosphate[13] Betamethasone valerate [14] and Gestodene[15] were also reported. Utilisation of the simple, easy, lesser time consuming and cost effective electroanalytical methods for the steroidal analyses assumes significance now a days. This along with the non-availability of electroanalytical determination for triamcinolone acetonide initiated the development of stripping analysis of the steroid.

The present work reports the cyclic voltammetric behaviour of triamcinolone acetonide (TA), a corticosteroid medicine [16] and stripping voltammetric procedures for the determination of this steroid.

2. RESULTS AND DISCUSSION

2.1. Cyclic Voltammetric Behaviour of Triamcinolone Acetonide

Cyclic voltammetric studies of triamcinolone acetonide (TA) were carried out at pH 1.0 to 13.0 in 50% aqueous methanol medium. Cyclic voltammograms of 400 μg/mL TA at five different pHs,

1.0, 4.0, 7.0, 9.2 and 13.0 are presented in figure 1. The cyclic voltammograms exhibited one well-defined reduction peak at all pH media. The effect of sweep rate was studied between 25 to 500 mV/s. The peak current increased linearly with sweep rate (Fig.2) with good correlation and non-linear correlation was observed for the plot i_p Vs. $\nu^{1/2}$. The slope of the straight line obtained from the plot log i_p vs. log ν was between 0.54 to 0.72 at various pHs. As the concentration of the compound increased from 100 to 700 µg/mL at a constant sweep rate of 100 mV/s, there was a gradual increase in the peak current up to 400 µg/mL and then a decrease (Fig 3). These facts reveal diffusionless adsorption-controlled reduction. The fractional αn around 0.4 and absence of oxidation peak in the reversible scan show irreversible electronation.

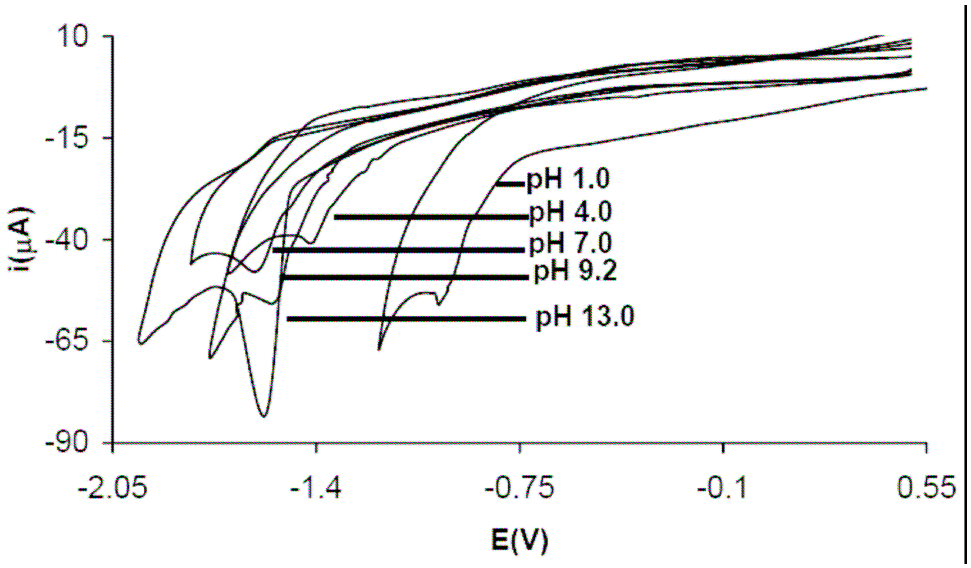


Figure 1. Cyclic voltammograms of 400 µg/mL TA on GCE at five different pHs

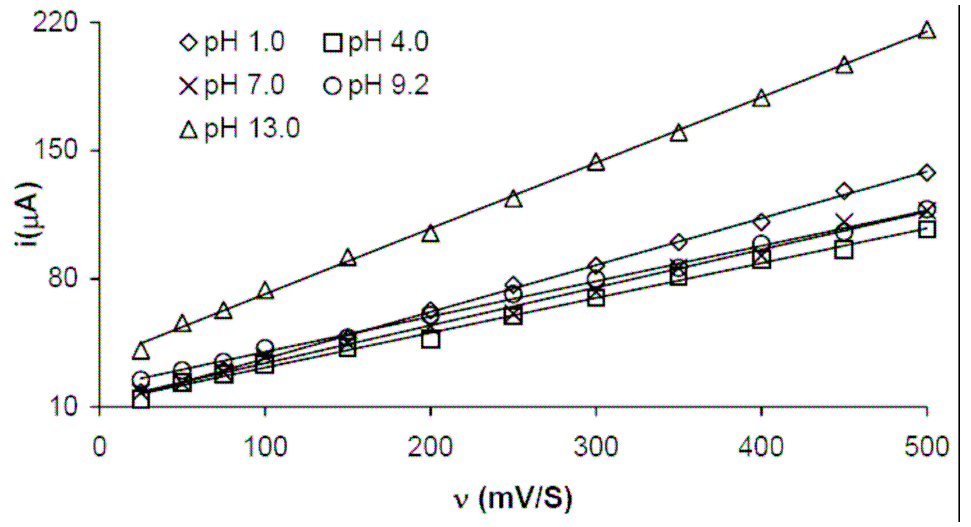


Figure 2. Plot of peak current vs scan rate of TA at various pHs

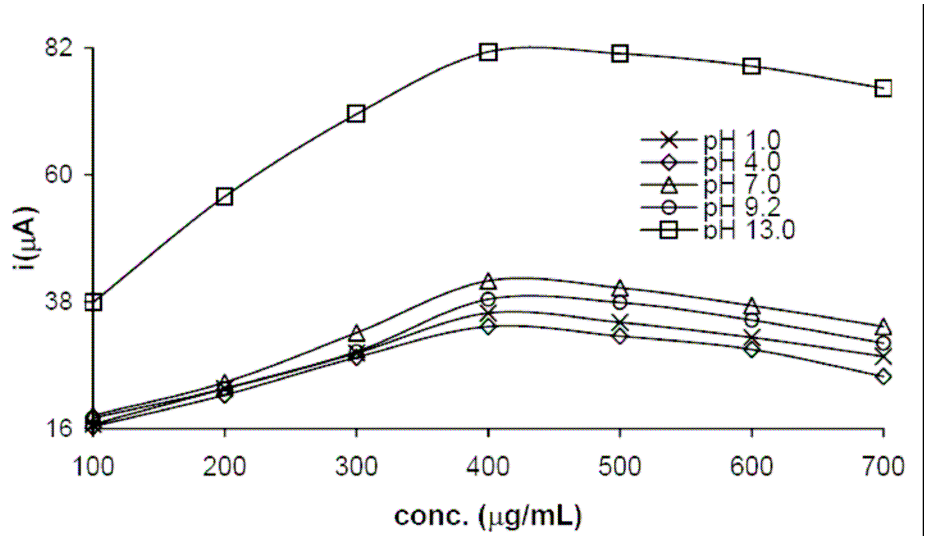


Figure 3. Plot of peak current vs concentration

Under identical conditions the cyclic voltammetric results are compared at various pH. For analytical consideration, the variation of peak current with pH was studied (Fig.4). Appearance of well-defined sharp peak and maximum current at pH 13.0 suggests the selection of the pH for further electroanalytical studies. The higher current at pH 13.0 may be due to faster electron transfer rate at this pH.

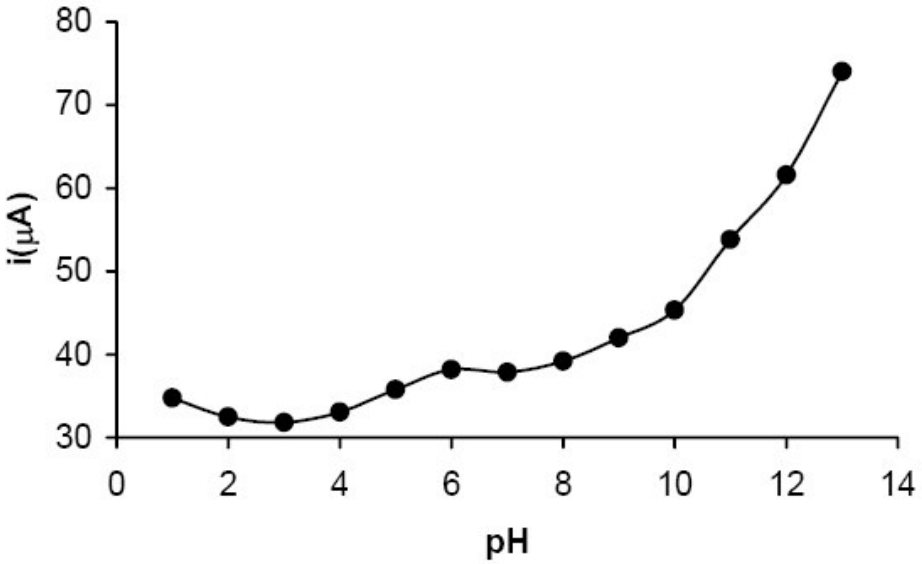
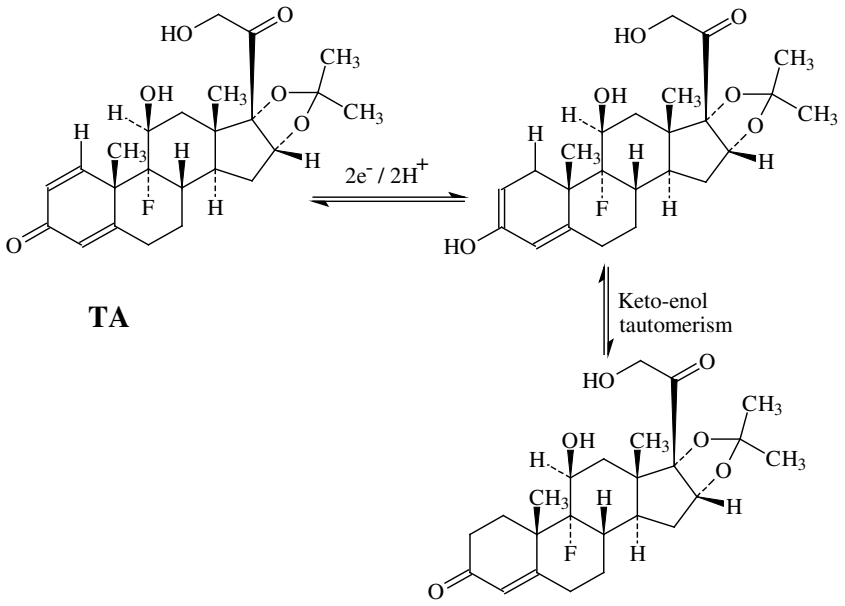


Figure 4. Plot of peak current vs. pHs

Controlled potential coulometry was carried out for 1.3 μg/mL of TA at pH 13.0 and the coulometric number of electrons in the reduction was determined to be 2. TA contains a α,β-

unsaturated carbonyl system, which undergoes easier reduction than other groups. The two electron transfer leads to the saturation of the double bond through the reduction of α,β-unsaturated carbonyl function (Scheme 1).



Scheme 1. Reduction mechanism of TA

2.2. Electroanalytical determination of triamcinolone acetonide

Adsorptive stripping voltammetry involves two steps in which the first step is accumulation of the substrate on the electrode and the second step involves stripping. More and more accumulation of the substrate results in higher stripping peak current, which leads to an increase in the sensitivity of the determination. Cyclic voltammetric results revealed good adsorption of the substrate on electrode at pH 13.0 and hence adsorptive stripping voltammetric studies performed well in the determination of TA. Differential pulse and square wave strippings were chosen for the study.

2.3. Differential Pulse Stripping Voltammetry (DPSV)

Experiments were carried out to find the best accumulation parameters in the chosen pH medium 13.0 with solution containing 50 μg/mL of TA. Upon observing the preconcentration–stripping voltammograms performed for accumulation potentials (Eacc) varying from –50 to -800 mV at accumulation time (DT) of 60 seconds, maximum peak current was found at -50 mV Eacc. Maximum peak current was observed only at 60 second accumulation time and above this the peak current was found to decrease. The optimum Eacc -50 mV and an accumulation time 60 seconds may be due to maximum accumulation of the substrate resulted in sufficient surface coverage of the electrode. The stripping parameters such as initial scan potential (IP), pulse height (PH), pulse width

(PW) and potential scan increment (PI) were varied between –100 and –900 mV, 25 and 150 mV, 25 and 125 msec, and 2 and 15 mV respectively and the optimum conditions yielding maximum peak current were identified. The optimum IP, PH, PW and PI values are –100 mV, 50 mV, 50 msec and 6 mV respectively. The effect of scan rate in the range between 10 and 80 mV/s was carried out and the stripping voltammetric signal at 80 mV/s showed the maximum peak current. The results are presented in table 1.

Table 1. Range studying and optimum parameters condition of stripping voltammetry of triamcinolone acetonide on glassy carbon electrode at pH 13.0

Parameters	DPSV		SWSV	
	Range examined	Optimized value	Range examined	Optimized value
Accumulation potential (mV)	-50 to -800	- 50	000 to - 1000	- 200
Deposit time (sec)	60 to 300	60	60 to 300	60
Initial scan potential (mV)	-100 to -900	-100	500 to -700	500
Pulse height (mV)	25 to 150	50	-	-
Pulse width (msec)	25 to 125	50	-	-
Scan rate (mV/sec)	20 to 80	80	-	-
Square wave amplitude (mV)	-	-	20 to 60	40
Frequency (Hz)	-	-	20 to 100	60
Scan increment (mV)	2 to 15	6	2 to 10	6
Stirring rate (rpm)		250		250
Rest period (Sec)		5		5

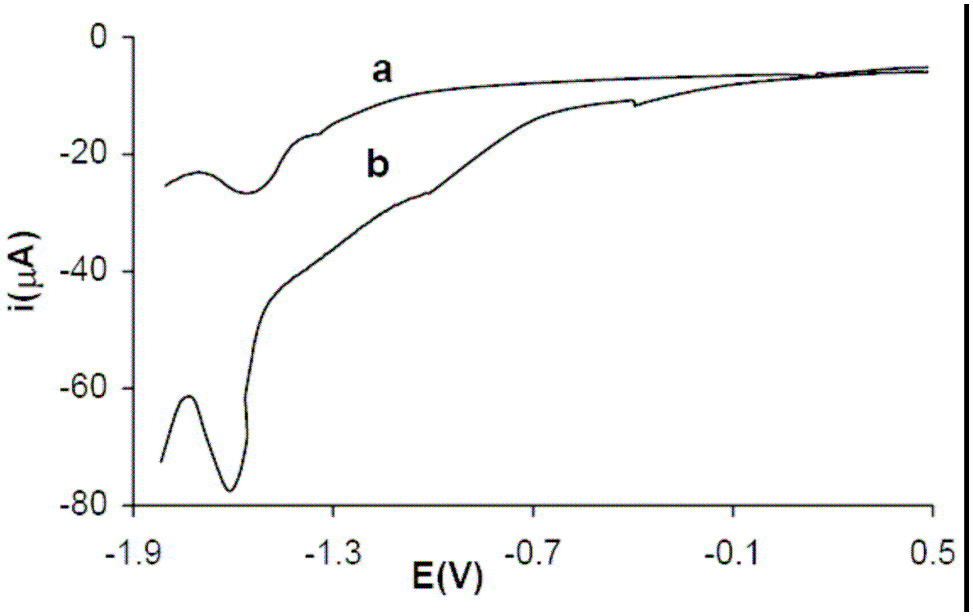


Figure 5. a) DPSV b) SWSV of 50 μg/mL of TA at pH 13.0

Under these optimum experimental conditions, the influence of TA concentration on the stripping signal was studied. The experimental results showed that the peak current increased with the increase in concentration of TA. A representative differential pulse stripping voltammogram is given in figure 5a. A calibration was made, which indicated the linear dependence of peak current with concentration ($ip = 0.0004C + 4.1841$; $R^2 = 0.998$) in the range of determination and it was found to be good in between 0.2 and 50 μg/mL. The limit of detection was 0.1 μg/mL. The reproducibility of the stripping signal was realized in terms of relative standard deviation for 6 identical measurements carried out at a concentration level of 10 μg/mL and found to be 2.6 %.

2.4. Square Wave Stripping Voltammetry (SWSV)

Optimization of accumulation and stripping parameters were done as in DPSV experiments. In the first stage, the accumulation potential and time and in the second stage, initial scan potential, square wave amplitude, frequency and scan increments were optimized. The entire study was carried out in the pH 13.0 with 50 μg/mL TA concentration. The range of study of various parameters and optimum values are presented in table 1. The optimum conditions that resulted in good peak response were used to study the effect of concentration. The stripping signal obtained under optimum experimental conditions was influenced by the concentration of TA. As the concentration of TA increased from 0.015 μg/mL, the stripping peak current also increased linearly. A representative square wave stripping voltammogram is given in figure 5b. A calibration between the concentration of TA and peak current was made. The linear dependence of peak current (ip) with concentration (C) was understood from the straight-line equation, $ip = 0.0026C + 17.351$ with good correlation, $R^2 = 0.990$. The range of determination was found in between 0.015 and 50 μg/mL. The limit of detection was 0.01 μg/mL. The reproducibility of the stripping signal was understood from the relative standard deviation (2.8%) calculated for 6 identical measurements at a concentration level of 10 μg/mL.

2.5. Comparison

The results of DPSV and SWSV studies were compared and the better stripping voltammetric method was identified. The relative standard deviations calculated from DPSV and SWSV techniques for 6 identical measurements are well within the accepted limit. Hence both the methods can be applied for the determination of TA steroid. Of the two methods, the range of determination is wider and limit of detection is on the lower side when SWSV method was employed. Hence SWSV method is found to be the most suited method for the trace determination of TA steroid.

2.6. Determination of TA steroid in pharmaceutical and assay in urine samples

The commercial sample of Kenacort (Sarabhai Piramal Pharmaceutical Ltd., Vadodara, India) containing TA was made up to 100 ml using 50% aqueous methanol and kept as stock solution for the real analyses. 1.0 ml of the TA solution was pipetted out into a cell containing 9.0 ml of pH 13.0 solution. Under the optimum experimental conditions arrived for SWSV, the stripping voltammograms

were recorded (Fig. 6a) and the peak current values were noted. By substituting the peak current in the calibration plot, the amount of TA present in 10 ml of the cell solution was found out and in turn the amount of TA present in the whole of the commercial sample (40.0 mg) was calculated (38.9 mg). The same experiment was repeated for 5 times and the relative standard deviation was determined to be 2.2%.

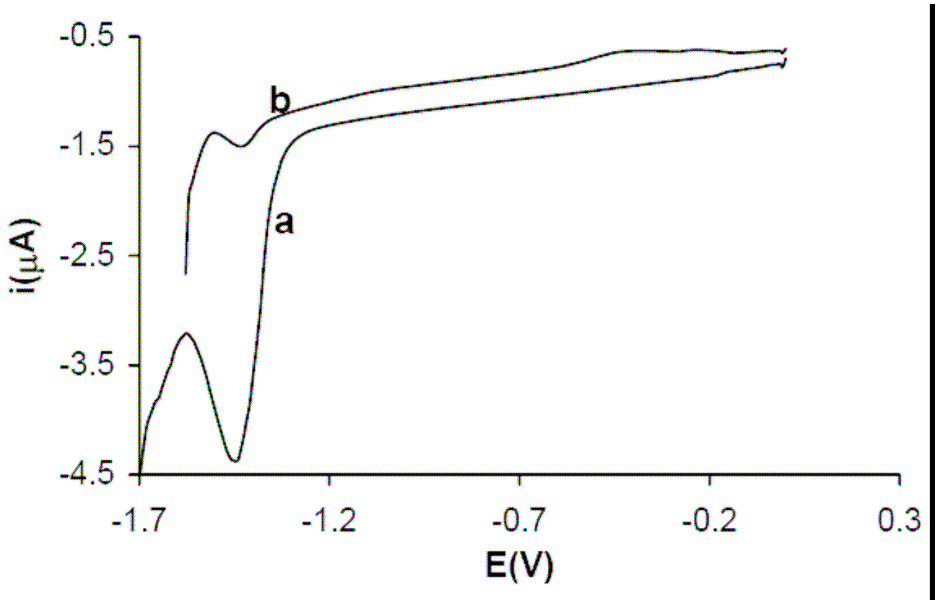


Figure 6. a) SWSV of TA injection b) SWSV of TA urine sample at pH 13.0

Measurement of TA in a urine sample collected after 8 h from administration of the drug. 1.0 ml of the urine sample was mixed with NaOH solution and the pH was brought to 13.0. The SWSV was carried out under the optimum experimental conditions and the resultant voltammogram is presented in Figure 6b. This experiment was repeated for 5 times and the average weight of TA in 1.0 ml of urine sample is found to be 0.05 μg with relative standard deviation 2.4%. There is no appreciable interference due to the presence of small amount urine present in the electrolyte hence the same calibration plot can be used. There is 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 a stable and suitable method for the determination of TA. Repetition rate is found to be high. Hence the proposed method can be used as a better alternative to spectrophotometric or chromotographic methods.

3. CONCLUSIONS

TA is cathodically reduced irreversibly on glassy carbon electrode in the pH range 1.0 to 13.0 and the reduction is controlled by adsorption. Two electrons are involved in the reduction. Effect of pH leads to the conclusion that pH 13.0 is suitable for analytical studies. Employing DPSV and SWSV

techniques carried out the adsorptive stripping voltammetric studies. Optimum conditions are arrived at, the influence of concentration was studied and a calibration plot was obtained. This was taken as a standard and used to find out the amount of TA present in the pharmaceutical tablet and urine samples. Alessia Panusa et.al. performed the detection of TA by HPLC-ESI-MS [17] and observed good correlation between peak areas and solution concentrations in the range 0.05–10.0 μg/mL. Compared to this a better determination range between 0.03 and 50 μg/mL for TA is obtained from the proposed SWSV method. This method can very well be used for real samples also. This technique is simple and easy to carry out. Once just changing the analyte and polishing the electrode, within a few minutes, the amount of TA can be determined. Hence stripping voltammetry is a better technique over chromatography technique.

4. EXPERIMENTS

EG&G M 273A Electrochemical Analyzer (Princeton Applied Research Corporation) was employed mainly for the electroanalytical techniques. The triamcinolone acetonide was purchased from Sigma. The stock solution and supporting electrolytes were prepared in doubly distilled and deionised water using TKA water purification system. 0.1M H_2SO_4 (Merck-AR) solution (pH 1.0), Britton Robinson buffer (Merck) (pH 4.0, 7.0 and 9.2) and 0.1M KOH (Merck- AR) solution (pH 13.0) were used as the medium for the analysis. Electrochemical method was adopted using a three-electrode system. Glassy Carbon (GC) working electrode, Pt foil counter electrode and saturated calomel (SCE) reference electrode were used. Purging and blanketing of nitrogen were done for analyte solution placed in the electrochemical cell of 15-ml capacity for 15 minutes under stirred conditions.

References

1. D. Courtheyn, J. Vercammen, M. Logghe, H. Seghers, K. De Wasch, H. De Brabander, *Analyst* 123 (1998) 2409.
2. S. Gorog, *Analytical Sci*, 20 (2004) 767.
3. K. Shimada, F. Xie, T. Nambara, *J Chromatogr B*, 232(1982)13.
4. L. Matysova, R. Hajkova, J. Sicha, P. Solich, *Anal Bioanal Chem*, 376(2003)440.
5. G. Cavina, R. Alimenti, B. Gallinella, L. Valvo, *J Pharm Biomed Anal*., 10(1992)685.
6. S. Sudsakorn, L. Kaplan, D.A. Williams, *J Pharm Biomed Anal*, 40(2006)1273.
7. S.W. Golf, V. Graef, J.T.Schiller, H. Hischer, W. Funk, *Biomed Chromatogr*, 2 (1987) 189.
8. D. Agbaba, D. Zivanov-Stakic, S. Vladimirov, K. Zubac, *Acta Pol Pharm*., 47(1990) 15.
9. Y.S. El-Saharty, N.Y. Hassan, F.H. Metwally, *J Pharm Biomed Anal.*, 28(2002) 569.
10. P. M. Bersier, J. Bersier, in S. Görög (Hrsg.) Steroid Analysis in the Pharmaceutical Industry, Ellis Horwood Ltd, S.Chichester, (1989)
11. F. Belal, *Microchim Acta*, 107(1992)11.
12. M. I. Walash, F. Belal, M. E-S. Metwally, M. Hefnawy, *Microchim Acta*, 112(1994) 217.
13. C. Jeyaseelan, A. P. Joshi, *Anal Bioanal Chem*, 373(2002)772.
14. J-E. Belgaied, *Anal Bioanal Chem*, 376(2003)706.
15. J.J. Berzas, J. Rodríguez, G. Castañeda, *Electroanalysis*, 11(1999)268.

16. T. Mitsui, H. Azuma, M. Nagasawa, T. Iuchi, M. Akaike, M. Odomi, T. Matsumoto, *Journal of Neurology*, 249(2002)1004.
17. A. Panusa, M. Ottaviani, M.Picardo, E. Camera, L. Gagliardi, P. Chimenti, A. Granese, D. Tonelli, *Analyst*, 129(2004)719.