Talanta 83 (2010) 149–155

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A comparison of edge- and basal-plane pyrolytic graphite electrodes towards the sensitive determination of hydrocortisone

Rajendra N. Goyal*, Sanghamitra Chatterjee, Anoop Raj Singh Rana

Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, Uttranchal, India

ARTICLE INFO

Article history:
Received 2 August 2010
Received in revised form 26 August 2010
Accepted 27 August 2010
Available online 25 September 2010

Keywords:
Pyrolytic graphite electrode
Hydrocortisone
Square-wave voltammetry
Pharmaceuticals
Human plasma

ABSTRACT

Electrochemical sensor employing edge-plane pyrolytic graphite electrode (EPPGE) for the sensitive detection of hydrocortisone (HC) is delineated for the first time. The electrochemical properties are investigated exercising the cyclic voltammetry and square-wave voltammetry (SWV). When equating with the bare basal-plane pyrolytic graphite electrode (BPPGE), the EPPGE gave better response towards the detection of HC both in terms of sensitivity and detection limit. The voltammetric results indicated that EPPGE remarkably enhances the reduction of HC which leads to considerable amelioration of peak current with shift of peak potential to less negative values. The difference in the surface morphology of two electrodes has been studied. Also, the EPPGE delivered an analytical performance for HC with a sensitivity of 45 nA nM^{-1} and limit of detection of 88 nM in the concentration range 100–2000 nM. The method has been utilized for the determination of HC in pharmaceuticals and real samples. The electroanalytical method using EPPGE is the most sensitive method for determination of HC with lowest limit of detection to date. The major metabolites present in blood plasma did not intervene with the present investigation as they did not exhibit reduction peak in the experimental range used. A comparison of results with high performance liquid chromatography (HPLC) signalizes a good agreement.

1. Introduction

Corticosteroids are widely applied for their anti-inflammatory and anti-allergic outcomes in human and veterinary medicine. Hydrocortisone, HC (I) commonly known as cortisol (11,17,2l-trihydroxy-4-pregnene-3,20-dione) is the main glucocorticosteroid exuded by the adrenal cortex gland and acts to limit the body's response to stress. HC in its salt form is soluble in aqueous solution and is widely used in aseptic formulations. It also has an attenuating, as opposed to the suppressing effect of synthetic glucocorticoids on the immune response which is regarded beneficial and utile in curing septic shocks [1]. HC cream produced a significantly rapid clinical improvement and is the mainstay of treatment for mild to moderate eczema [2]. HC is also increasingly being practiced for prevention or treatment of vasopressor resistant hypotension in neonatal medicine and in addition to that it is also useful in treating chronic lung diseases in neonates [3–5]. HC in its acetate salt form is employed for the healing of different rheumatoid, allergic pathologies and dermatitis, both by systemic and topical way [6,7]. The keto group at position 3, the double bond between carbons 4 and 5 and the hydroxyl group at 11 are crucial for its therapeutic activity [8]. Hydrocortisone is easy to use, possesses clinical efficacy that compares well with much more potent preparations for the doctoring of oral diseases [9]. The innovative hydrocortisone butyrate emulsion is an efficient addition to topical corticosteroid therapy of psoriasis and also suitable for the treatment of the scalp and other hairy parts of the body [10]. It is well known that during Addisonian crisis the privation of cortisol leads to life-threatening hypotension. James et al. demonstrated the beneficial role of HC in a 75% burned patient with longstanding Addison's disease [11]. The HC administration seems to mark the turning-point from septic state to the recovery phase of the patient [12]. HC, as a glucocorticosteroid, is on the World Anti-Doping Agency's 2008 prohibited list and its use by athletes necessitates a therapeutic use exemption [13].

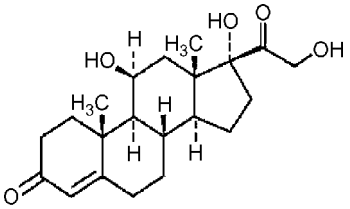
In view of the prominence of HC in clinical and doping applications, there are many methods for the determination of HC which includes radio immuno assay [14], LC–mass spectrometry or LC–tandem mass spectrometry [15,16], micellar electrokinetic capillary chromatography [17], fluorimetric derivation before HPLC analysis [18,19], HPLC–UV [20–22] and gas chromatography combined with mass spectrometry [23]. Most of these methods need internal standards [24,25] and the need for derivatization. The immunological methods are sensitive, but have cross-reactivity with other steroids and give relatively high concentrations. The

* Corresponding author. Tel.: +91 1332 285794 (O)/274454 (R); fax: +91 1332 273560.
E-mail addresses: rngcyfcy@iitr.ernet.in, rngcyfcy@rediffmail.com (R.N. Goyal).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.08.054

HPLC method using column switching shows interference by coexisting material. Although the LC/MS method is highly selective and sensitive, it requires evaporation to dryness of sample prior to analysis. Literature review unveils that very few attempts have been made to determine HC using voltammetric techniques. HC has been previously determined using differential pulse voltammetry (DPV) at carbon paste electrode [26]. The determination was not carried out in biological fluids. Thus, the aim of this study was to develop a simple and rapid method for the analysis of HC in blood samples and also to quantitate the compound in marketed formulations. The square-wave voltammetry (SWV) is a pulse technique that offers the advantage of great speed and sensitivity. The method has proved to be highly sensitive for the analysis of organic molecules including drugs owing to its simplicity, low cost and relatively short analysis time as compared to the other routine analytical techniques including chromatography.

The pyrolytic graphite electrode material comprises both edge-plane and basal-plane graphite, with the basal/edge ratio and graphite monocrystal size depending on the quality of the pyrolytic graphite used [27]. For a large variety of redox couples, electron-transfer rate constants at basal-plane graphite have been found to be over 10^3 times slower than for edge-plane graphite [27]. Compton et al. [28] and Goyal et al. [29] recently reported the analytical use of a carbon electrode populating largely of edge-plane-active sites for electroanalytical detection of thiols and steroids, respectively. The better analytical performance of EPPGE with faster electrode kinetics in comparison to BPPGE and other carbon electrodes has been previously reported in literature for several compounds [30–35]. The EPPGE gave low background currents and improved electrocatalytic signals compared with those obtained by use of BPPGE. Hence, in the present method, EPPGE has been used to attain a very low detection limit, high sensitivity, and decreased over potentials coupled with increased current values by using SWV.



(I)

2. Experimental

2.1. Chemicals

HC was acquired from Sigma–Aldrich, Steinheim. It was employed without further purification. HC containing ointment and injections marketed by different pharmaceutical companies were purchased from the local market of Roorkee. Phosphate buffer solutions (PBS) of 1 M were prepared according to the method of Christian and Purdy [36]. Double distilled water was used throughout the investigation. All other reagents and solvents used in this section were of analytical grade.

2.2. Apparatus

The voltammetric experiments were accomplished using a three electrode single compartment cell equipped with EPPGE or BPPGE (~6 mm^2) as the working electrode, platinum wire as the counter electrode and an Ag/AgCl (3 M NaCl) reference electrode (BAS Model MF-2052 RB-5B). The edge- and basal-plane pyrolytic graphite pieces were obtained from Pfizer Inc., New York, USA. Experiments were carried out using a BAS CV-50W voltammetric analyzer (Bioanalytical Systems, West Lafayette, IN, USA). All the potentials quoted are versus Ag/AgCl electrode at an ambient temperature of $25 \pm 2\,^{\circ}C$. The surface morphology of the electrodes was characterized by recording field emission scanning electron microscopy (FE-SEM) using Quanta 200 FE-SEM instrument. HPLC studies were performed on Shimadzu LC-2010A HT system with RP-18e (5 μm) column. The mobile phase used for HPLC experiment was a mixture of methanol:water (60:40) at a flow rate of 1.0 mL min^{-1} and detection was carried out at 254 nm.

2.3. Procedure

HC was insoluble in water, hence, the stock solution of HC (1 μM) was prepared by dissolving the required amount of the compound in minimum amount of methanol and then diluting it with double distilled water. The solution was ultra sonicated for 30 min. Required amount of the stock solution was added to 2 mL of phosphate buffer solution (1.0 M, pH 7.2) and the total volume was made to 4.0 mL with double distilled water. Since oxygen is a major interference at negative measurement potential, high-purity nitrogen was passed for 12–15 min to deoxygenate the solutions before recording each voltammogram. The electrode surface was cleaned after each run by abrading it on an emery paper. Instrumental conditions for square-wave voltammetry were: initial E: −1000 mV, final E: −1800 mV, square wave amplitude (E_{sw}): 25 mV, potential step (E): 4 mV and square wave frequency (f): 15 Hz. Cyclic voltammograms were recorded in the sweep range 10–1000 mV s^{-1} with initial sweep to negative potentials. Human blood plasma samples of normal person were obtained from the Indian Institute of Technology Hospital, Roorkee and used as control. The plasma samples of patients undergoing treatment with HC were obtained from IIT hospital, Roorkee after centrifuging the whole blood with EDTA as anticoagulant for 15 min. The samples were obtained after 2 h of administration of injection of HC. The biological samples did not exhibit any reduction peak on scanning from −1.0 to −1.8 V indicating thereby the absence of other reducible compounds in this potential window. The samples were therefore used for analysis without any dilution.

2.4. Preparation of electrode

A pyrex glass tube of appropriate length and diameter was cleaned thoroughly and dried. Thin glass rod was used to apply epoxy resin (Araldite) inside the one end of pyrex glass tube. An edge-plane pyrolytic graphite piece (1 mm × 1 mm × 3 mm) was then slided carefully from the other open end of the tube with the help of wire till it gets covered with epoxy resin to avoid any air pocketing between the tube and the graphite piece. The electrode is then left for drying for 24 h at room temperature after which the glass tube end was rubbed on an emery paper till the graphite piece was exposed. The electrode surface is then washed with distilled water several times in order to remove fine carbon particles, adhered to the surface of pyrolytic graphite electrode. A sufficient amount of mercury was then placed into the glass tube and a platinum wire of appropriate length was inserted to make proper contact of electrode to the outer circuit. The electrode surface was cleaned after each run by rubbing it on an emery paper followed by washing with a jet of distilled water and touching with soft tissue paper. As the electrode surface area gets changed each time due to the cleaning process, hence, voltammetric measurements were performed in triplicate and an average value of the current is reported. The surface morphology of basal and edge planes of PGE was studied by recording FE-SEM image as shown

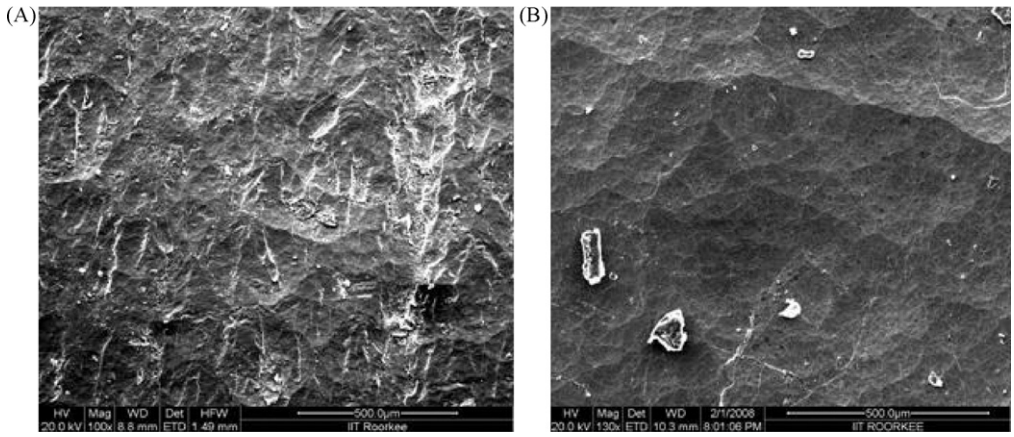


Fig. 1. A comparison of FE-SEM images of (A) basal plane of PGE and (B) edge plane of PGE.

in Fig. 1. It was found that basal plane is rough and non-smooth as compared to edge plane at which edges of layers are clearly visible.

3. Results and discussion

3.1. Determination of surface area

The surface area of bare EPPGE and BPPGE was calculated. For this purpose, cyclic voltammograms were recorded for 1 mM $K_3Fe(CN)_6$ using 0.1 M KCl as the supporting electrolyte at different scan rates. Well-defined redox couple was observed at both the electrodes due to the presence of Fe^{3+}/Fe^{2+}. For a reversible process, the following equation applies at 25 °C:

$$i_p = 2.69 \times 10^5 A n^{3/2} D_R^{1/2} C_o v^{1/2}$$

where i_p refers to the peak current in Ampere and A is the electrode surface area in cm^2. For 1 mM $K_3Fe(CN)_6$, $n = 1$, $D_R = 7.6 \times 10^{-6}\ cm^2\ s^{-1}$, C_o is the concentration of $K_3Fe(CN)_6$ in M and v is the scan rate in V s^{-1}. The slope of i_p versus $v^{1/2}$ plot was then used to calculate the surface area of bare EPPGE and BPPGE which was found as 0.08172 cm^2 and 0.07981 cm^2, respectively. Thus, it was found that the effective working area of both the electrodes is same.

3.2. Voltammetric behavior of hydrocortisone

3.2.1. Cyclic voltammetry

The cyclic voltammograms recorded for 1000 nM HC at the bare EPPGE and BPPGE in 1 M phosphate buffer solution at pH 7.2 are illustrated in Fig. 2. A well-defined single reduction peak at ~−1386 mV was obtained at EPPGE which shifted to more negative potentials (~−1469 mV) with a marked decrease in peak current at the bare BPPGE. These results clearly reveal that edge-plane sites act as a very efficient promoter to enhance the kinetics of the electrochemical reduction of HC. The absence of anodic peaks in the reverse scan clearly indicated that the reduction of HC at this particular voltammetric sensor is irreversible in nature. To ascertain the nature of electrode reaction, sweep rate studies were performed in the range 10–1000 mV s^{-1}. The peak current was found to increase with increasing sweep rates and the plot of $i_p/v^{1/2}$ versus log v clearly indicated that the electrode process is adsorption controlled [37,38]. As SWV is considered to be a more sensitive technique in comparison to cyclic voltammetry, it is used for the further determination of HC.

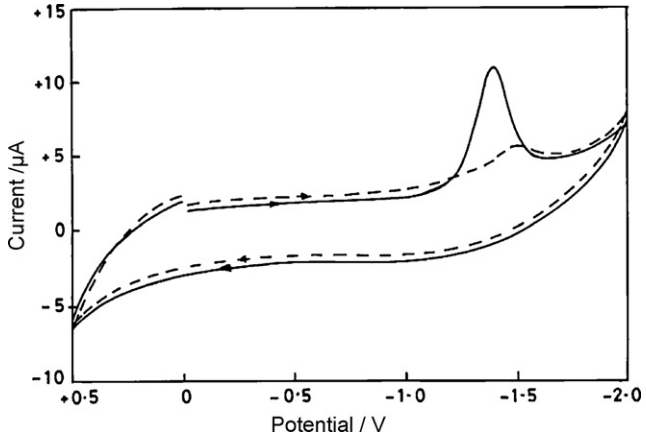


Fig. 2. Typical cyclic voltammograms observed for 1000 nM HC in phosphate buffer solution of pH 7.2 at bare EPPGE (—) and bare BPPGE (- - -) at 20 mV s^{-1}.

3.2.2. Square-wave voltammetry

Initially, square-wave voltammograms were recorded for 1000 nM HC at pH 7.2 at the bare EPPGE and bare BPPGE. The working electrodes had almost the same surface area exposed to solution. Fig. 3 represents a comparison of square-wave voltammograms observed for the drug at different working electrodes. In the case of bare BPPGE, a small peak is observed at ~−1443 mV

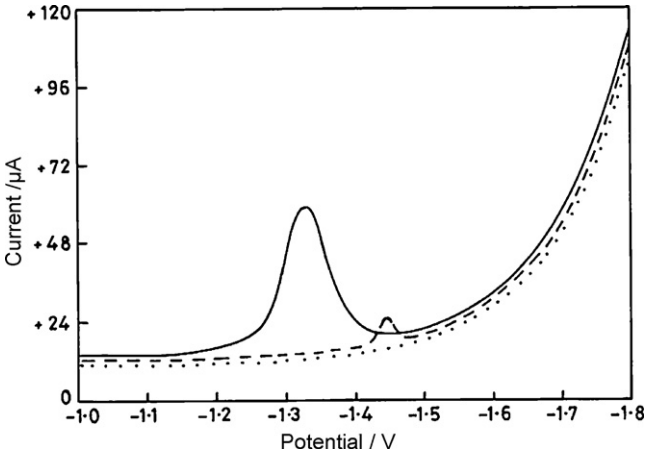


Fig. 3. Comparison of square-wave voltammograms of 1000 nM HC (pH 7.2) at (a) bare EPPGE (—), (b) bare BPPGE (- - -), and (c) background PBS at pH 7.2 at bare EPPGE (···).

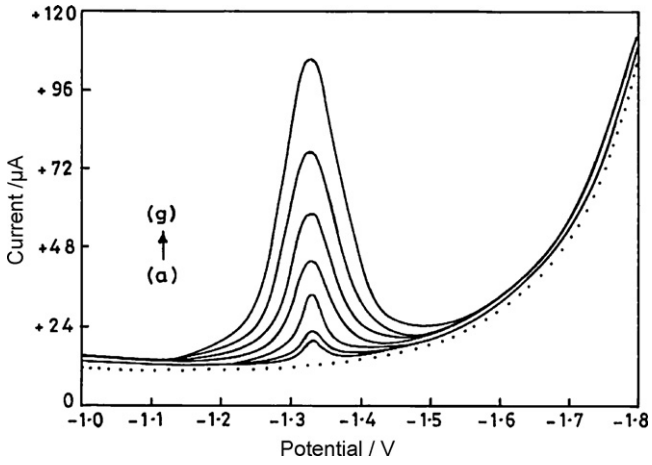


Fig. 4. Osteryoung square-wave voltammograms recorded for (a) PBS (background) at the bare EPPGE (···) and (b) increasing concentration of HC at the bare EPPGE electrode (—) [curves were recorded at (a) 100 nM, (b) 250 nM, (c) 500 nM, (d) 750 nM, (e) 1000 nM, (f) 1500 nM and (g) 2000 nM concentration in PBS of pH 7.2].

which shifts to ~−1330 mV at bare EPPGE, thus indicating that the exposed edge-plane sites in bare EPPGE contribute in making it a better substrate for sensing than bare BPPGE. The increase in peak current confirms that the adsorption is much stronger on bare EPPGE than bare BPPGE. Thus, the increase in current response along with the lowering of peak potential is a clear evidence of the electrocatalytic activity of edge-plane sites of EPPGE towards the reduction of HC. The different behavior of the HC reduction on the edge and basal orientations reflects the difference in the surface electrocatalytic properties. A strong interaction between the reactant and the electrode surface is required to facilitate the electron transfer and to provide the adsorption sites for the intermediates. The reduction on the basal plane is inhibited due to the fact that a significant fraction of the applied potential falls across the space charge region [39,40] and there are no unsatisfied valences on the basal plane to provide the sites for the adsorption of the intermediates and the functional groups.

3.3. Concentration study

The quantitative analysis of the drug was based on the dependence of the peak current on the concentration of HC. Typical square-wave voltammograms depicting the systematic increase in the peak current values with an increase in the concentration in the range 100–2000 nM at bare EPPGE are presented in Fig. 4. The linear calibration plot at bare EPPGE along with error bars is presented in Fig. 5(A). While in the case of bare BPPGE, the observed concentration range is 500–10000 nM. The peak current is found to increase linearly with increasing concentration of HC and the linear regression equation is expressed as:

$$i_p\,(10^{-5}\,A) = 0.0045C\,(nM) + 0.0816 \quad \text{at bare EPPGE}$$

$$i_p\,(10^{-5}\,A) = 0.0006C\,(nM) + 0.0963 \quad \text{at bare BPPGE}$$

where C is the concentration of HC. The correlation coefficients for the expressions were 0.9943 and 0.9919 for bare EPPGE and BPPGE, respectively. The current values are obtained by subtracting the background current and are reported as an average of at least three replicate determinations. A comparison of the calibration characteristics at bare EPPGE and BPPGE electrodes is presented in Table 1. The accuracy and precision data have been calculated at 500 nM concentration of HC. The detection limit of the proposed method

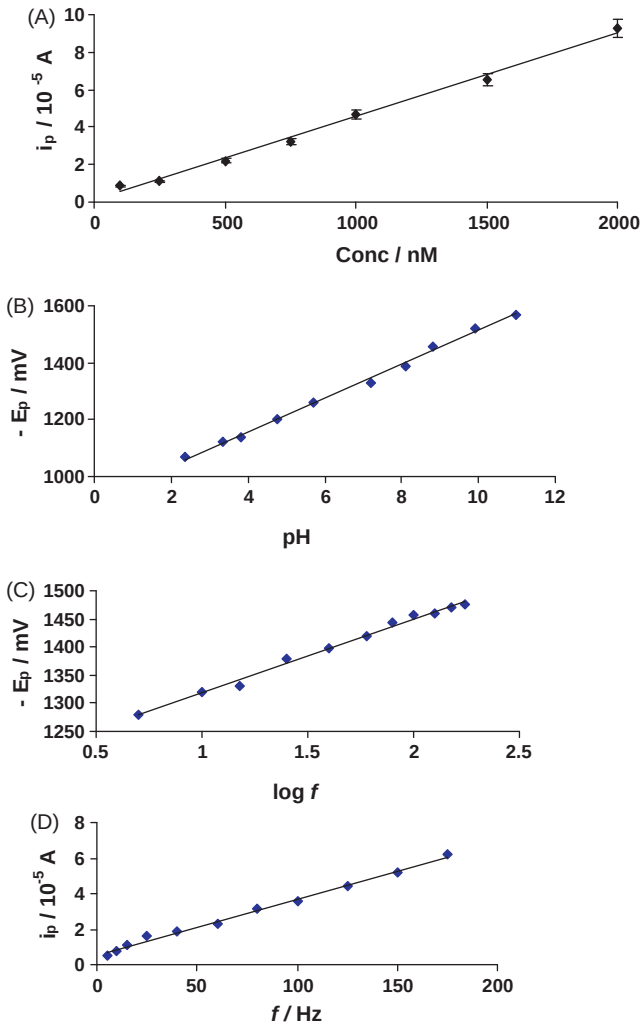


Fig. 5. (A) Linear calibration plot observed for HC at bare EPPGE at pH 7.2. (B) Observed dependence of peak potential ($-E_p$) on pH for 250 nM hydrocortisone at bare EPPGE. (C) $-E_p$ versus log f plot observed for 250 nM hydrocortisone at bare edge-plane pyrolytic graphite electrode at pH 7.2. (D) Linear dependence of peak current (i_p) on square-wave frequency for 250 nM hydrocortisone at pH 7.2 at bare EPPGE.

was calculated by using the formula $3\sigma/b$, where σ is the standard deviation of the blank and b is the slope of the calibration curve and it was found as 88×10^{-9} M and 49.1×10^{-8} M for bare EPPGE and BPPGE, respectively. The sensitivity was estimated to be 45 nA nM^{-1} and 6 nA nM^{-1} for bare EPPGE and BPPGE, respectively. Since, bare EPPGE exhibited better analytical performance in comparison to bare BPPGE hence, further investigations were carried out at bare EPPGE.

Table 1
Comparison of the calibration characteristics at bare EPPGE and BPPGE.

	EPPGE	BPPGE
Calibration range	100–2000 nM	500–10000 nM
Limit of detection	88×10^{-9} M	49.1×10^{-8} M
Limit of quantification	29.3×10^{-8} M	16.4×10^{-7} M
Sensitivity	45 nA nM^{-1}	6 nA nM^{-1}
Intercept	0.0816	0.0963
RSD of slope	1.6%	3.2%
RSD of intercept	1.9%	3.6%
Accuracy	1.2%	2.9%
Precision	0.8	1.4

Table 2
Determination of HC in pharmaceutical preparations using EPPGE.

Sample	Stated content	Detected content[a]	Error (%)	Recovery (%)
Lycor	1.0% (w/w)	0.98% (w/w)	−2.0	98.00
Efcorlin	50 mg/mL	48.53 mg/mL	−2.9	97.06
Primacort-100	50 mg/mL	49.05 mg/mL	−1.9	98.10

[a] RSD value for determination was less than 2.1% for $n = 3$.

3.4. Influence of pH and square-wave frequency

The electrochemical behavior of HC at different pH values and square-wave frequencies was then studied at bare EPPGE. The voltammetric reduction of 250 nM HC was studied in the range 2.4–11.0 in phosphate buffer solution. The pH value strongly affects the peak potential (E_p) of HC, with the E_p shifting linearly towards more negative potential values with increase in pH as shown in Fig. 5(B). The relationship between $-E_p$ and pH is represented by the following equation:

$$-E_p\,(\text{pH}\,2.4\text{–}11.0) = [919 + 59.471\,\text{pH}]\ \text{versus Ag/AgCl}$$

having correlation coefficient of 0.9962. The slope of 59 mV/pH indicates that the number of protons and electrons involved in the reduction process is equal.

The effect of square-wave frequency on peak potential was examined in the range 5–175 Hz at pH 7.2 at bare EPPGE. The plot of $-E_p$ versus $\log f$ was linear and is found to shift towards more negative potentials with increase in square-wave frequency as seen in Fig. 5(C). The variation of $-E_p$ with $\log f$ can be expressed by the equation:

$$-E_p\,(\text{mV}) = 130.38 \log f + 1188.9$$

with a correlation coefficient of 0.9926. The peak current (i_p) for the reduction of HC was found to increase linearly with the increase in square-wave frequency in the range 5–175 Hz as seen in Fig. 5(D) and the relation between i_p and f can be expressed by the relation:

$$i_p\,(10^{-5}\,\text{A}) = 0.0314f + 0.5463$$

having a correlation coefficient of 0.9942. These observations are in agreement with the properties of irreversible electrochemical process which is adsorption controlled [41–43]. The results assisted the inferences obtained from cyclic voltammetry studies.

Table 3
A comparison of observed concentration of HC in human plasma after 2 h of HC administration at bare EPPGE and by using HPLC.

Spiked (μM)	Observed (μM) by SWV	Actual concentration (μM)[a] by	
		Bare EPPGE	HPLC
Sample 1			
0.0	0.83	0.83	0.85
1.0	1.85	0.85	0.88
3.0	3.82	0.82	0.80
Sample 2			
0.0	0.78	0.78	0.76
1.0	1.77	0.77	0.80
3.0	3.80	0.80	0.83
Sample 3			
0.0	0.86	0.86	0.89
1.0	1.89	0.89	0.91
3.0	3.83	0.83	0.81

[a] RSD value for determination was less than 3.7% for $n = 3$.

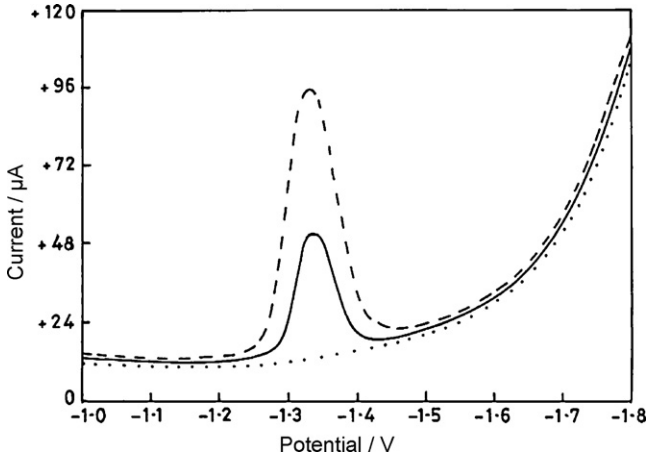


Fig. 6. A comparison of voltammograms observed for blood plasma sample of patient being treated with HC (—) and the patient sample spiked with 1 μM HC (- - -) at pH 7.2 at bare EPPGE. Normal human blood sample is represented as (· · ·).

3.5. Pharmaceutical formulations

The method was successfully implemented for the analysis of three commercial medicinal samples containing HC, viz Efcorlin (Glaxo SmithKline Pharmaceuticals Limited, Ahmedabad), Primacort-100 (Macleods Pharmaceuticals Limited, Daman) and Lycor (Micro Labs limited, Bangalore). Solutions obtained by dissolution of HC ointments and injections were subsequently diluted so that HC concentration lies in the range of calibration plot. Square-wave voltammograms were then recorded and the concentration of HC in the pharmaceutical preparations was ascertained. Results summarized in Table 2 show that the content for all assayed ointments and injection falls within the tagged amount indicating the good agreement with the proposed voltammetric method.

3.6. Quantification in real samples

The effectiveness of the proposed approach has also been evaluated on human blood plasma samples obtained from patients undergoing treatment with HC. The blood sample with EDTA as anticoagulant was centrifuged and the supernatant was taken for analysis. The samples were used for analysis without any dilution as the major metabolites present in human plasma such as ascorbic acid, uric acid and dopamine did not exhibit reduction peak in the range −1.0 to −1.8 V. Fig. 6 represents a typical voltammogram which depicts human blood plasma of normal person used as control. The human blood plasma of patient obtained after 2 h of administration of 1.0 mL hydrocortisone sodium succinate injection and the blood plasma of patient spiked with HC are also included in Fig. 6. A well-defined peak of HC was observed at ∼−1330 mV in the patient sample which increased on spiking. The results obtained are tabulated in Table 3 which clearly indicates that HC can be easily determined in blood samples using this method.

3.7. Comparison by HPLC

To prove the reliability of data obtained, the results obtained by the voltammetric method were compared with HPLC analysis. The system suitability parameters of HPLC are mentioned in Table 4. Initially, various concentrations of HC were analyzed using HPLC and the peak area was calculated. A well-defined peak is obtained at $R_t \sim 4.545$ min in the standard sample of HC as shown in Fig. 7(A). The plasma samples were used for HPLC analysis after filtration

Table 4
System suitability parameters for HPLC studies.

Repeatability	0.37% for $n = 3$
Capacity factor (k')	2.5
Resolution (R_s)	2.5
Tailing factor (T)	2.0
Theoretical plates (N)	2100

with 0.47 micron filter paper. Blood plasma sample of normal person used as control was then injected in HPLC and the peak of HC was found to be absent as depicted in Fig. 7(B). Finally, the concentration of the drug was determined in the human blood plasma of patients undergoing treatment with HC. Fig. 7(C) shows a typical HPLC chromatogram of Sample 1 of patient plasma. The peak at $R_t \sim 4.545$ min is due to HC. However, no attempt was made to identify the rest of the peaks which are likely to be due to uric acid, ascorbic acid, etc. The plasma sample of the patient was then spiked with HC which is presented in Fig. 7(D). The chromatogram clearly indicates the increase in peak at $R_t \sim 4.545$ min. A calibration curve was obtained by plotting the peak area of the analyte peaks against the analyte concentration. The resulting calibration plot was linear. A comparison of the values obtained by HPLC and the proposed voltammetric method (as listed in Table 3) clearly indicated that the results obtained by two methods are in good agreement. The calculated values obtained from *F*-test and paired *t*-test are 1.63 and 1.26, respectively, which are less than their tabulated values thereby indicating that there is no significant difference between the precision of both the methods at 10% level.

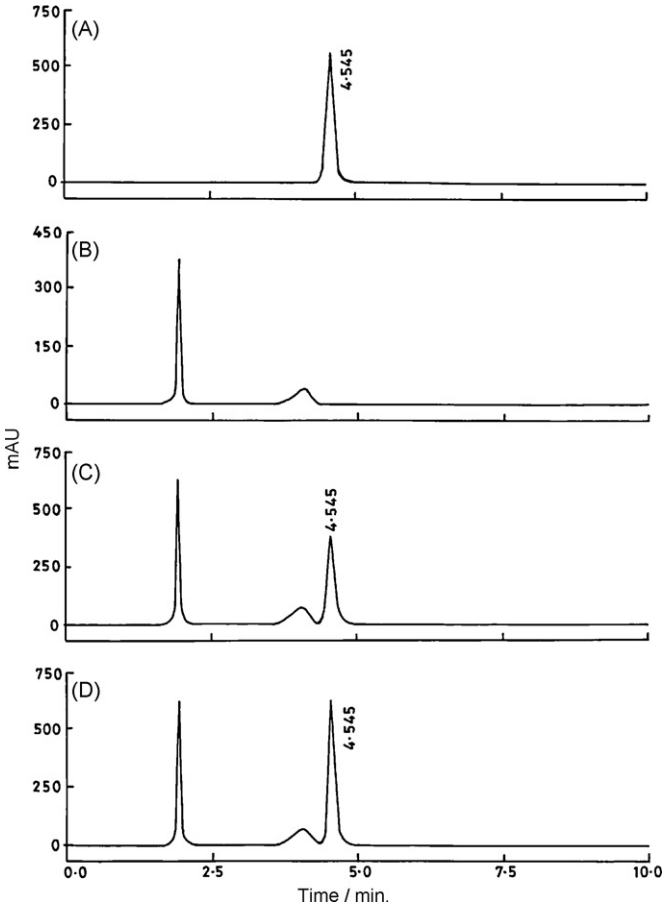


Fig. 7. Observed HPLC chromatograms of (A) standard HC, (B) normal human blood used as control, (C) blood sample of patient (Sample 1) being treated with HC and (D) 1 μM HC spiked in blood sample 1.

4. Conclusions

The purpose of the present investigation was to develop a robust method for the ratiocination of HC in pharmaceutical formulations and real samples. It has been unfold that EPPGE displays an appealing voltammetric response in comparison to BPPGE due to its low signal to noise ratio, increased sensitivity, low detection limit and large potential window. The peculiar catalytic activity of an EPPGE relies on the crystal orientation on its surface. The high percentage of the edge orientation results the high catalytic activity. The edge orientation on the surface of an EPPGE serves as an active site for the reduction of HC. Highly ordered pyrolytic graphite (HOPG) is used to fabricate the edge- and basal-plane electrodes. Edge-plane pyrolytic graphite electrode is an electrode constructed from HOPG where the graphite layers are perpendicular to the disc surface and are separated with an interlayer spacing of ~3.35 Å. Surface defects occur in the form of steps exposing the edges of the graphite layers. Conversely a basal-plane pyrolytic graphite electrode is fabricated such that the layers of graphite lie parallel to the surface [44]. The difference between the edge and basal orientations may account for the fact that functional groups are easier to adsorb on the edge plane.

Thus, the EPPGE shows electrocatalytic activity towards the reduction of HC leading to an increment in the current response and a change over of the reduction potential to lower values in comparison to bare BPPGE. The developed protocol has lower detection limit (88×10^{-9} M) as compared to the previously notified at carbon paste electrode modified with β-cyclodextrin (4.2×10^{-7} M) [26] using DPV. The available sites for reduction in HC are the keto groups present at positions 3 and 20. It is clearly stated in literature that in ketosteroids the carbonyl group conjugated with a double bond undergoes reduction preferably in comparison to the isolated keto group [45]. Thus, the most probable site for reduction in HC is at position 3 where the keto group undergoes reduction in $2e^-$, $2H^+$ process to give a hydroxyl group. Thus, the present method has been satisfactorily employed to the voltammetric determination of HC in pharmaceuticals and human blood plasma samples obtained from patients being treated with the drug using SWV. A comparison of the results with HPLC indicates that the method is sensitive and the results are comparable. The developed protocol is simple, rapid, reproducible and sensitive for the determination of HC at bare EPPGE.

Acknowledgements

Authors (SC and ARSR) are thankful to the Council of Scientific and Industrial Research, New Delhi and Department of Science and Technology, New Delhi for the award of Senior and Junior Research Fellowship, respectively. Thanks are due to Dr. Vandana Khobragade, Chief Medical Officer, IIT Hospital, Roorkee for providing the samples of blood plasma of persons undergoing treatment with Hydrocortisone. Financial assistance for this work was provided by the Department of Science and Technology vide grant no. SR/S1/IC-21/2007.

References

[1] L.A. Druce, C.M. Thorpe, A. Wilton, Med. Hypotheses 70 (2008) 56–60.
[2] T.F. Poyner, B.K. Dass, J. Eur. Acad. Dermatol. Venereol. 7 (1996) 23–30.
[3] P.C. Ng, C.H. Lee, F.L. Bnur, Pediatrics 117 (2006) 367–375.
[4] I. Seri, Pediatrics 117 (2006) 516–518.
[5] S.W. Aucott, J. Perinatol. 25 (2005) 77–78.
[6] Martindale, The Complete Drug Reference, Pharmaceutical Press, London, 1999, pp. 1043–1045.
[7] H.W. Sears, J.W. Bailer, A. Yeadon, Clin. Ther. 19 (1997) 710–719.
[8] R. Bruggemeir, M.E. Wolff, Burger's Med. Chem. Drug Discov. 3 (1996) 445–510.
[9] T. Kristmundsdottir, T. Loftsson, W.P. Holbrook, Int. J. Pharm. 139 (1996) 63–88.

[10] G.E. Pierard, J.M. Lachapelle, G. Frentz, E. Schopf, E. Stolz, J. Eur. Acad. Dermatol. Venereol. 6 (1996) 11–14.
[11] S.E. James, S.J. Ghosh, J. Montgomerie, B.M. Philp, P. Dziewulski, Burns 28 (2002) 391–393.
[12] G. Bouachour, P. Tirot, J.P. Gouello, E. Mathieu, J.F. Vincent, P. Alquier, Intensive Care Med. 21 (1995) 57–62.
[13] http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf.
[14] C. Lee, D.E. Goeger, Clin. Biochem. 31 (1998) 229–233.
[15] V.A. Frerichs, K.M. Tornatore, J. Chromatogr. B 802 (2004) 329–338.
[16] H. Shibasaki, T. Furuta, Y. Kasuya, J. Chromatogr. B 692 (1997) 7–14.
[17] L.V. Rao, J.R. Petersen, M.G. Bissell, A.O. Okorodudu, A.A. Mohammad, J. Chromatogr. B: Biomed. Appl. 730 (1999) 123–128.
[18] E. Neufeld, R. Chayen, N. Stern, J. Chromatogr. B 718 (1998) 273–277.
[19] N. Shibata, T. Hayakawa, K. Takada, N. Hoshino, T. Minouchi, J. Chromatogr. B 706 (1998) 191–199.
[20] U. Turpeinen, U.H. Stenman, Scand. J. Clin. Lab. Invest. 63 (2003) 143–150.
[21] R. Gatti, E. Cappellin, B. Zecchin, G. Antonelli, P. Spinella, E. Mantero, E.F. De Palo, J. Chromatogr. B 824 (2005) 51–56.
[22] E. Rouits, M. Boisdron-Celle, A. Morel, E. Gamelim, J. Chromatogr. B 793 (2003) 357–366.
[23] T. Furuta, M. Matsuzawa, H. Shibasaki, Y. Kasuya, J. Chromatogr. B: Biomed. Sci. Appl. 738 (2000) 367–376.
[24] S.S. Tai, M.J. Welch, Anal. Chem. 76 (2004) 1008–1014.
[25] P.W. Tang, W.C. Law, T.S.M. Wan, J. Chromatogr. B 754 (2001) 229–244.
[26] K. Balaji, G.V.R. Reddy, T.M. Reddy, S.J. Reddy, Afr. J. Pharm. Pharmacol. 2 (2008) 157–166.
[27] R.L. McCreery, A.J. Bard, M. Dekker, Electroanal. Chem. 17 (1990) 221–374.
[28] R.R. Moore, C.E. Banks, R.G. Compton, Analyst 129 (2004) 755–758.
[29] R.N. Goyal, S. Chatterjee, A.R.S. Rana, Talanta 80 (2009) 586–592.
[30] C.E. Banks, R.G. Compton, Anal. Sci. 21 (2005) 1263–1268.
[31] D.S. Shishmarev, N.V. Rees, R.G. Compton, Electroanalysis 22 (2010) 31–34.
[32] R.T. Kachoosangi, C.E. Banks, R.G. Compton, Electroanalysis 18 (2006) 741–747.
[33] C.M. Welch, C.E. Banks, S. Komorsky-Lovric, Croat. Chem. Acta 79 (2006) 27–32.
[34] F. Wantz, C.E. Banks, R.G. Compton, Electroanalysis 17 (2005) 1529–1533.
[35] F. Wantz, C.E. Banks, R.G. Compton, Electroanalysis 17 (2005) 655–661.
[36] G.D. Christian, W.C. Purdy, J. Electroanal. Chem. 3 (1962) 363–367.
[37] R.H. Wopshall, I. Shain, Anal. Chem. 39 (1967) 1514–1527.
[38] R.N. Goyal, A. Sangal, J. Electroanal. Chem. 521 (2002) 72–80.
[39] D.Y. Qu, J. Power Sources 109 (2002) 403–411.
[40] E. Yeager, Electrochim. Acta 29 (1984) 1527–1537.
[41] A. Radi, M.A. EL Ries, G.E. Bekhiet, Anal. Lett. 32 (1999) 1603–1612.
[42] M.R.C. Massaroppi, S.A.S. Machado, L.A. Avaca, J. Braz. Chem. Soc. 14 (2003) 113–119.
[43] S. Komorsky-Lovric, S. Gagic, R. Penovski, Anal. Chim. Acta 389 (1999) 219–223.
[44] C.E. Banks, R.G. Compton, Analyst 131 (2006) 15–21.
[45] P. Zuman, I.M. Kolthoff, Progress in Polarography, vol. 2, Interscience Publishers, New York, 1962.