

Scientific paper

# Cathodic Adsorptive Stripping Voltammetric Determination of Prednisolone in Pharmaceutical Preparation and Human Urine

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## Abstract

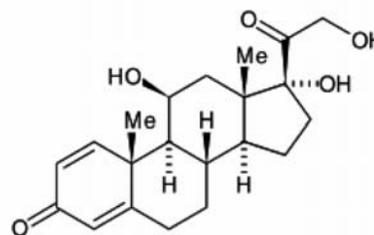
Differential pulse cathodic adsorptive stripping voltammetric method was developed for the determination of prednisolone based on electrochemical reduction of the drug at a hanging mercury drop electrode (HMDE) in 0.04 M Britton-Robinson buffer pH 3.5. The peak current varied linearly over the concentration range 7.21–144.2 ng/ml prednisolone with limit of detection and limit of quantification of 3.95 and 13.17 ng/ml prednisolone, respectively. The proposed method was successfully applied for the determination of the drug in commercial tablets and spiked human urine samples.

**Keywords:** Prednisolone, Differential pulse cathodic adsorptive stripping voltammetry, HMDE. Pharmaceutical dosage form, Human urine.

## 1. Introduction

Prednisolone, 11 $\beta$ ,17 $\alpha$ ,21-Trihydroypregna-1,4-diene-3,20-dione, [50-24-8], is a corticosteroid drug. It is used in treatment of a wide range of inflammatory and autoimmune conditions such as asthma,<sup>1</sup> rheumatoid arthritis,<sup>2</sup> ulcerative colitis and Crohn's disease.<sup>3</sup> Several analytical methods have been employed for the determination of prednisolone including, high performance liquid chromatographic (HPLC),<sup>4–13</sup> gas chromatographic (GC),<sup>14–16</sup> micellar electrokinetic capillary chromatographic,<sup>17–19</sup> micellar electrokinetic chromatographic,<sup>20–22</sup> spectrophotometric,<sup>23–27</sup> spectrofluorimetric,<sup>28</sup> chemiluminometric,<sup>29–31</sup> polarographic<sup>32–34</sup> methods. Also differential pulse stripping voltammetric method with the aid of chemometrics<sup>35</sup> was used for simultaneous determination of prednisolone, prednisone and dexamethasone. Recently four voltammetric methods were developed for the determination of the drug based on oxidation of the drug at glassy carbon electrode,<sup>36</sup> oxidation at fullerene-C<sub>60</sub>-modified gold electrode and gold nanoparticles modified indium tin oxide electrode,<sup>37</sup> reduction using  $\beta$ -cyclodextrin modified carbon paste electrode<sup>38</sup> or reduction at single wall carbon nanotube modified edge plane pyrolytic graphite electrode.<sup>39</sup> In this work the electrochemical behaviour of prednisolone at a hanging mercury drop electrode (HMDE) was investigated

and a differential pulse cathodic adsorptive stripping voltammetric method (DPCAdSV) was developed for determination of this drug.



Structural formula of prednisolone

## 2. Experimental

### 2.1. Apparatus

All voltammetric measurements were performed using Metrohm 757 VA Computrace (Herisau, Switzerland) equipped with a Metrohm VA 694 stand. Three electrodes assembly cell consisted of hanging mercury drop electrode (HMDE) as working electrode, an Ag/AgCl in 3 mol/l KCl (Metrohm 6.0728.000) as a reference electrode and platinum wire (Metrohm 6.0343.000) as an auxiliary

electrode. The pH measurement were carried out with Jenway model 3305 pH meter.

## 2. 2. Reagents and Materials

Prednisolone acetate was obtained from Misr Co. for Pharm. Ind., Cairo, Egypt, and the pharmaceutical product Predilone tablets 5 mg/tablet was obtained from Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt.

A stock standard solution  $1 \times 10^{-3}$  M prednisolone acetate was prepared by dissolving the required amount of the drug in methanol (Sigma). More dilute solutions were prepared daily in methanol. Britton-Robinson (BR) buffer, acetate buffer, phthalate buffer, citrate buffer and sodium perchlorate ( $\text{NaClO}_4$ ) were prepared and tested as supporting electrolytes. All reagents used were of analytical reagent grade.

## 2. 3. Procedure

A known amount of the drug solution was pipetted into 10 ml measuring flask and completed to the mark by 0.04 M Britton-Robinson buffer pH 3.5. The solution was transferred into the voltammetric cell and deaerated with pure nitrogen for 3 min. in the first cycle and 30 s for each successive cycle; the nitrogen was then kept over the solution during measurements. The accumulation potential  $E_a$  at  $-400$  mV was applied to a new mercury drop (drop area  $0.3 \text{ mm}^2$ ) whilst still stirring the solution (2000 rpm); following the accumulation period (40 s); the stirring is stopped and allowed to equilibrium for 10 s. The differential pulse voltammogram was obtained by applying a negative going potential scan from  $-400$  to  $-1200$  mV with scan rate of 50 mV/s and pulse amplitude of 50 mV.

## 2. 4. Determination of Prednisolone in Predilone Tablets

Ten tablets (Predilone tablets, 5 mg/tablet) were accurately weighed and powdered in a mortar, the required amount from the crushed tablets powder was dissolved in about 20 ml methanol and filtered in 50 ml measuring flask. The residue was washed three times with methanol and the volume was completed to the mark by the same solvent. A suitable volumes of the above tablet solution is pipetted into 10 ml measuring flask, completed to the mark by 0.04 M Britton-Robinson buffer pH 3.5 and the procedure is repeated as described. The nominal contents of the tablets is calculated using standard addition technique.

## 2. 5. Determination of Prednisolone in Spiked Human Urine

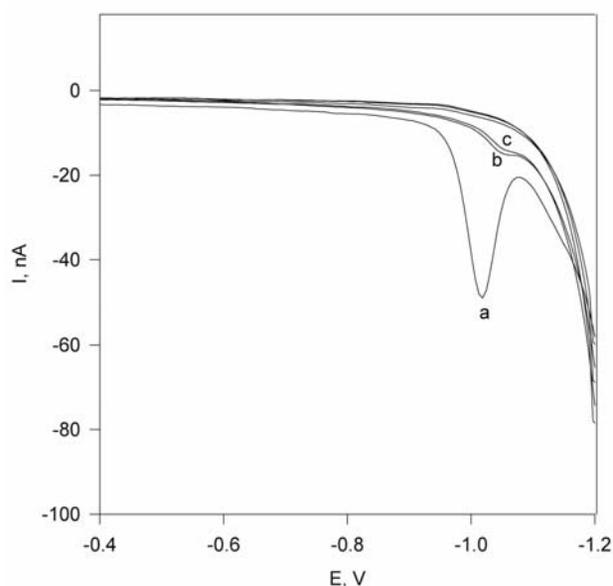
1 ml of  $1 \times 10^{-3}$  M prednisolone acetate and 0.5 ml urine of a healthy person were completed to 50 ml to pre-

pare  $2 \times 10^{-5}$  M prednisolone in spiked urine sample, different volumes of the above spiked urine sample (20–30  $\mu\text{l}$ ) are pipetted into a 10 ml measuring flask and completed to the mark by 0.04 M Britton-Robinson buffer pH 3.5 and the procedure is repeated as described. The amount of prednisolone is calculated using standard addition technique.

## 3. Results and Discussion

### 3. 1. Cyclic Voltammetric Studies

Fig. 1 shows the cyclic voltammograms for  $6 \times 10^{-7}$  M prednisolone in 0.04 M Britton Robinson buffer pH 3.5, scan rate of 50 mV/s and accumulation potential of  $-0.4$  V. A reduction peak appears at  $-1.02$  V which may be due to the reduction of C=O double bond of the drug molecule and no oxidation peak is observed in the anodic branch which suggests that the process is irreversible. The figure shows also that the second and third cycles at the same mercury drop exhibited lower peak current intensity



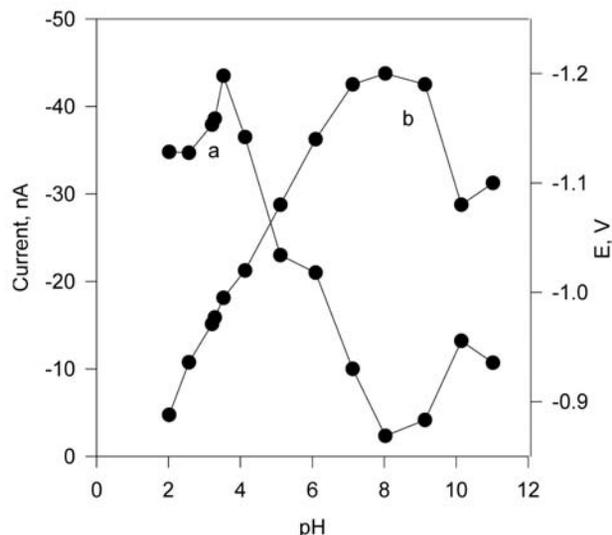
**Fig. 1** Successive cyclic voltammograms of  $6 \times 10^{-7}$  M prednisolone solution in 0.04 M BR buffer pH 3.5,  $E_a = -0.400$  V and scan rate of  $50 \text{ mVs}^{-1}$  after an accumulation of 40 s. curve a, first cycle; curve b, second cycle and c, third cycle.

which may be due to desorption of the drug species out of the mercury drop surface. This behaviour indicated the interfacial adsorptive character of the drug onto the mercury drop surface. A plot of logarithm of the peak current versus logarithm of the scan rate gave a straight line relation with a slope of 1.09 which is close to the theoretically expected 1.0 for an ideal reaction of surface species,<sup>40</sup> this also confirm the adsorptive character of the drug.

### 3. 2. DP Voltammetric Studies

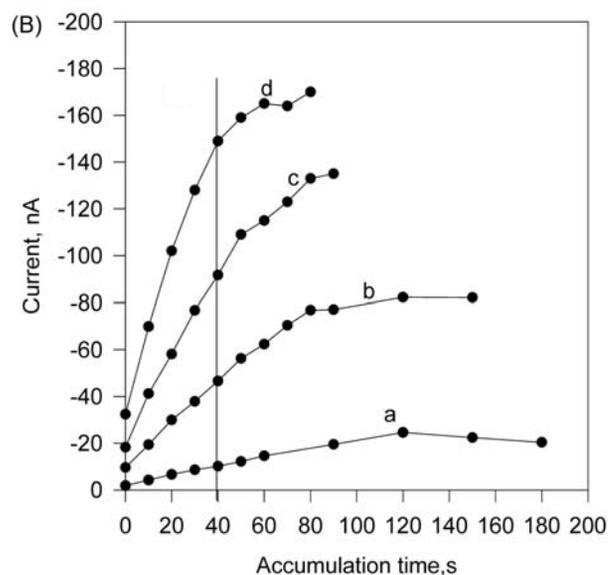
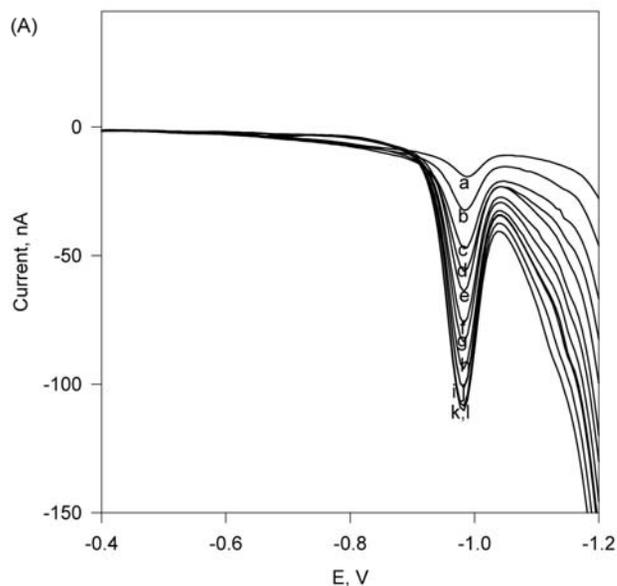
Different electrolytes such as sodium perchlorate, acetate buffer, phthalate buffer, citrate buffer and BR buffer were tested in the presence of  $2 \times 10^{-7}$  M prednisolone at accumulation potential of  $-0.400$  V and 30 s accumulation time. Both the peak height and peak shape were taken in consideration during choosing the supporting electrolyte. The results showed that BR buffer give the best background current and signal response. The effect of pH on the peak current and peak shape was studied over the pH range 2–12. (Fig. 2). The peak current has its maximum value at pH 3.5 and the potential is shifted to more negative values indicating that the protons are involved in the electrode reaction process. The study of the BR buffer concentration (0.02, 0.04 and 0.1 M) indicated that the highest peak current was obtained at 0.04 M BR buffer.

The effect of accumulation potential on differential pulse cathodic adsorptive stripping peak current height was examined for  $2 \times 10^{-7}$  M prednisolone at 30 s accumu-



**Fig. 2.** Effect of pH on the differential pulse cathodic adsorptive stripping peak current, a and peak potential, b of  $2 \times 10^{-7}$  M prednisolone in 0.04 M BR buffer,  $E_a = -0.4$  V,  $t_a = 30$  s, scan rate =  $50$  mVs $^{-1}$  and pulse amplitude =  $50$  mV.

lation time, the current peak was nearly constant on changing the accumulation potential ( $E_a$ ) from  $-0.4$  to  $-0.8$  V, but it decreased by increasing accumulation potential more than  $-0.8$  V. The effect of accumulation time  $t_a$  on the adsorptive stripping peak current was studied at four different concentration ranges:  $5 \times 10^{-8}$ ,  $2 \times 10^{-7}$ ,  $5 \times 10^{-7}$  and  $1 \times 10^{-6}$  M prednisolone, Fig. 3. The current increases linearly with increasing the accumulation time indicating the longer the accumulation time, the increase the drug concentration at the electrode surface and the larger the peak current, then as the accumulation time increases the peak current



**Fig. 3** A) Voltammograms for  $2 \times 10^{-7}$  M prednisolone solution after an accumulation of a, 0; b, 10; c, 20; d, 30; e, 40; f, 50; g, 60; h, 70; i, 80; j, 90; k, 120 and l, 150 s B) Effect of accumulation time on the peak current for a;  $5 \times 10^{-8}$  M, b;  $2 \times 10^{-7}$  M, c;  $5 \times 10^{-7}$  M and d;  $1 \times 10^{-6}$  M prednisolone in 0.04 M BR buffer pH 3.5 at  $E_a = -0.400$  V, scan rate  $50$  mVs $^{-1}$  and pulse amplitude  $50$  mV.

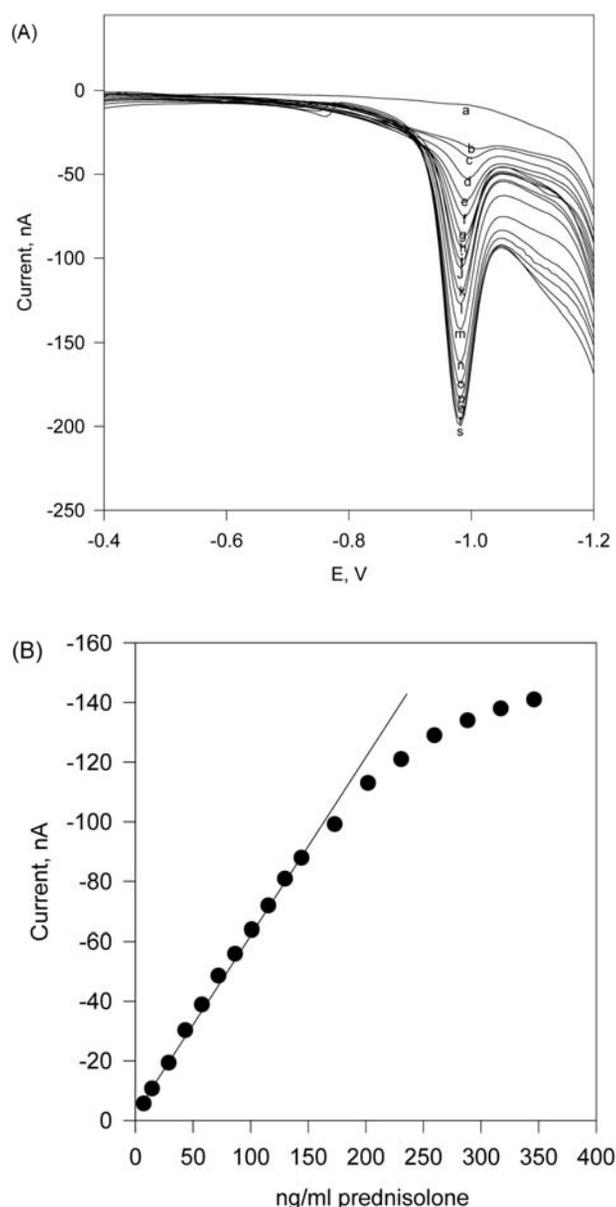
tends to level off. Increasing the accumulation time leads to an increase in sensitivity and decrease in linear concentration range. Obviously the choice of accumulation time requires compromise between sensitivity, linear concentration range and speed. The optimum accumulation time were 120, 80, 50 and 40 s for  $5 \times 10^{-8}$ ,  $2 \times 10^{-7}$ ,  $5 \times 10^{-7}$  and  $1 \times 10^{-6}$  M prednisolone, respectively. 40 s accumulation time was generally used for the subsequent studies.

Instrumental parameters such as pulse amplitude and scan rate were also optimized. Variations of pulse amplitude ( $10$ – $100$  mV) and scan rate ( $10$ – $80$  mVs $^{-1}$ ) at  $2 \times$

$10^{-7}$  M prednisolone at 40 s accumulation time were examined. The results shows that a pulse amplitude of 50 mV and a scan rate of  $50 \text{ mVs}^{-1}$  produced the best peak in intensity and shape.

### 3. 3. Calibration graph, Limit of Detection and Limit of Quantitation

Under the optimized conditions of a accumulation potential of  $-0.4 \text{ V}$ , scan rate of  $50 \text{ mVs}^{-1}$ , pulse amplitude of 50 mV and 40 s accumulation time, the peak current of the differential pulse cathodic adsorptive stripping vol-



**Fig. 4.** (A) Differential pulse cathodic adsorptive stripping voltammograms for solutions of prednisolone of increasing concentrations over the range 7.21–346.1 ng/ml and (B) their regression line in 0.04 M BR buffer pH 3.5 at  $E_a = -0.400 \text{ V}$ ,  $t_a = 40 \text{ s}$ , scan rate =  $50 \text{ mVs}^{-1}$  and pulse amplitude = 50 mV.

tammograms was found to be linearly related to the prednisolone concentration in the linear range 7.2–144.2 ng/ml prednisolone according to the regression equation of the calibration curve:  $I(\text{nA}) = -2.961 - 0.602 C (\text{ng/ml})$  Fig. 4. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the relation  $(k(\text{SD})/s)^{41}$  where  $k = 3$  for LOD and 10 for LOQ, SD is the standard deviation of the of the intercept and  $s$  is the slope of the calibration curve, were found to be 3.95 and 13.17 ng/ml.

### 3. 4. Reproducibility and Robustness

The intra-day and inter-day (day-to-day) precision expressed by relative standard deviation were 0.680 and 2.14% ( $n = 8$ ).

The robustness<sup>41</sup> of the proposed method was examined by evaluating the effect of small changes in some of the most important procedure parameters including, pH of the Britton-Robinson (BR) buffer (3.3–3.7) and the accumulation potential  $E_{\text{acc}}$  ( $-0.35 - -0.5$ ). None of the changes significantly affects drug recovery and consequently the optimized procedure was reliable for the assay of prednisolone, and it could be considered robust.

The influence of excipients usually present in the pharmaceutical formulations was studied. No interferences ( $< 1.9\%$  change) were observed in the presence of a 100 fold excess of talc, starch, lactose or magnesium stearate

### 3. 5. Determination of Prednisolone in Predilone Tablets and Urine Samples

The proposed DPCAdSV method was applied successfully to the determination of prednisolone in Predilone tablets 5 mg/tablet, using standard addition technique. The obtained results were compared with those obtained by the official HPLC method<sup>42</sup> Table 1. Student's  $t$ - and  $F$ -tests (at 95% confidence level) were applied.<sup>43</sup> The results showed that the calculated  $t$ - and  $F$ - values did not exceed the theoretical values, from which it can be concluded that the accuracy and precision of the proposed procedure did not differ significantly from the official HPLC method.

**Table 1:** Statistical comparison between the results of Predilone tablets using the proposed DPCAdSV method and HPLC official method.

Parameter	Proposed method	Official HPLC method <sup>42</sup>
Mean recovery, %	99.38	99.97
SD	1.106	0.271
F-ratio (6.59) <sup>a</sup>	5.514	
t-test ( 2.365) <sup>b</sup>	1.090	

Average of four determinations for the proposed method and five determinations for the HPLC official method.

<sup>a</sup> Tabulated F-value at 95% confidence level.

<sup>b</sup> Tabulated t-value at 95% confidence level and 7 degrees of freedom.

The determination of prednisolone in spiked urine samples at two different concentration levels (14.42 and 21.63 ng/ml) was also carried out using standard addition technique (Table 2). The mean recovery for the two concentrations were 97.85 and 99.26% with relative standard deviations of 1.42 and 0.75%, respectively.

**Table 2:** Determination of prednisolone in spiked urine samples using the proposed method

Taken (ng/ml)	Found (ng/ml)	Recovery, %	RSD
14.42	14.11	97.85	1.42
21.63	21.47	99.26	0.75

Average of four determinations

## 4. Conclusion

The proposed DPcAdSV method provides a fast, sensitive, accurate and simple approach to the determination of prednisolone in commercial tablets and spiked human urine samples. The developed method is about 31 times more sensitive than the reported voltammetric method based on oxidation of the drug at glassy carbon electrode,<sup>32</sup> 2 and 8 times more sensitive than the methods<sup>33</sup> based on the oxidation of the drug at fullerene C<sub>60</sub> modified gold electrode or nanogold modified indium tin oxide electrode, respectively, 44 times more sensitive than the method using  $\beta$ -cyclodextrin modified carbon paste electrode,<sup>34</sup> and 2.1 times more sensitive than differential pulse stripping voltammetric method with the aid of chemometrics<sup>35</sup> Although the square wave voltammetric method at single wall carbon nanotube modified edge plane pyrolytic graphite electrode,<sup>35</sup> is some more sensitive than the developed method, but it is time consuming method, the fabrication of the modified electrode in this method take 8–10 hours.

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## Povzetek

Razvita je bila metoda za določevanje prednizolona z diferencialno pulzno adsorpcijsko katodno stripping voltametrijo. Metoda temelji na elektrokemijski redukciji substance na stacionarni živosrebrni elektrodi v 0,04 M Britton Robinsonovem pufru pri pH 3,5. Zveza med merjenim maksimalnim tokom in koncentracijo prednizolona je linearna v območju od 7,21 do 144,2 ng/ml. Meja zaznave razvite metode je 3,95 ng/ml, meja kvantifikacije pa 13,17 ng/ml. Metoda je bila uspešno uporabljena za določevanje učinkovine v komercialnih tabletah in humanem urinu.