Voltammetric Determination of Cyproterone Acetate in Pharmaceutical Preparations

Nahed El-Enany¹, Dina El-Sherbiny², Fathalla Belal¹

¹Department of Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt; ²Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

ABSTRACT

The voltammetric behaviour of cyproterone acetate (CPA) was studied using direct current (DCt) and differential pulse polarography (DPP). The drug manifests cathodic waves over the pH range of 4-11.8. In Britton-Robinson buffer (BRb) of pH 10, the diffusion current-concentration relationship was found to be rectilinear over the range 3.2-32 µg/mL and 0.5-14 µg/mL using DCt and DPP modes, respectively, with minimum limits of detection (LOD) of 0.13 µg/mL using the DDP. The diffusion-current constant (Ids) was 9.29 ± 0.046 (n=9). The proposed method was successfully applied to the determination of the studied compound in its formulations. The mean percentage recoveries in tablets were 99.48 ± 1.25 and 100.01 ± 1.07 (n=4) using DCt and DPP modes, respectively. The results obtained were in agreement with those of the reference method. A proposal for the electrode reaction was postulated. (Int J Biomed Sci 2010; 6 (2): 128-134)

Keywords: cyproterone acetate; Direct Current time controlled (DCt); differential pulse (DPP); pharmaceutical dosage forms

INTRODUCTION

Cyproterone acetate (CPA), 6-chloro-1β,2β-dihydro-17-hydroxy-3'H-cyclopenta[1,2]pregna-4,6-diene-3,20-dione 17- acetate (Fig. 1) is a progestogen with antiandrogenic properties. It is used for the control of libido in severe hypersexuality or sexual deviation in men. It is also used for the palliative treatment of prostatic carcinoma. CPA is used with ethinylestradiol in woman for the control of acne and hirsutism and provides also contraception in those women (1).

Figure 1. Structural Formula of cyproterone acetate.
ity control laboratories. Therefore, there is a need for an alternative substitute to these techniques for the routine quality control analysis of CPA, and polarography was a promising substitute. Review of literature revealed that, up to the present time, there have been no reports concerning the electrochemical behavior of CPA. The molecular structure of cyproterone acetate reveals the presence of carbonyl group conjugated with a double bond, which initiated the present study. A simple, specific and sensitive method was developed for the determination of cyproterone in its dosage forms, based on the reduction of the keto group into the corresponding hydroxyl, at the dropping mercury electrode (DME).

EXPERIMENTAL

Apparatus

The polarographic study, DC, and the DPP measurements were carried out using the Polarecord E 506 Metbrohm (Herisau, Switzerland). The mercury drop-time of 1 sec was electronically controlled using the 663 VA Stand from the same company. The polarograms were recorded using a potential scan rate of 10 mV/sec. A three-electrode electrochemical cell comprising a Dropping Mercury Electrode (DME) as the working electrode, an Ag/AgCl reference electrode, and a graphite rod as the auxiliary electrode, was used. Phase selective AC polarograms were recorded using the same instrument; the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

Materials and reagents

All materials and reagents used were of analytical reagent grade.

- Cyproterone acetate reference standard was kindly supplied by Glaxo SmithKline.
- Tablets containing Cyproterone acetate (Diane-35® tablets labeled to contain 2 mg Cyproterone acetate +0.035 mg ethynylestradiol per tablet) (Batch # 502 B) were purchased from a local pharmacy.
- Britton-Robinson Buffers (BRb): 0.08 M solution covering the pH range 4.0-11.8 (a mixture of each of acetic, orthophosphoric and boric acids, adjusted to the required pH with 0.4M sodium hydroxide (10).
- Methanol (Sigma, St. Louis, MO, USA).

A stock solution containing 100 µg/mL of cyproterone acetate was prepared in methanol, and further diluted with the same solvent as appropriate. The stock solutions were stable for 10 days when kept in the refrigerator.

GENERAL PROCEDURE

Aliquots of the stock solution were transferred into a set of 25 mL volumetric flasks so that the final concentration is in the range of 3.2-32 and 0.5-14 µg/mL for the DC, and DPP modes, respectively. The solution was completed to the volume with BRb of pH10.0. The whole contents of the flasks were transferred into the polarographic cell, nitrogen gas was passed for 5 min, then the polarograms were recorded in both the DC and DP polarographic modes respectively over the potential range -0.8 to -1.6 V versus Ag/AgCl. The current (µA) of each of DC or DPP were plotted versus the concentration (µg/mL) to get the calibration graphs. Alternatively, the corresponding regression equations were derived.

Determination of Cyproterone acetate in Diane-35™ tablets

Ten tablets were weighed and pulverized well. A weighed quantity of the powder equivalent to 10.0 mg of the drug was transferred into a small conical flask. The drug was extracted three times each with 30 mL of methanol, the extracts were filtered through Whatman filter paper into a 100 mL volumetric flask. The conical flask was washed with few mls of methanol and the washings were passed into the same volumetric flask. Then, the solution was completed to the volume with methanol. Aliquots containing the working concentration range was transferred into 25 mL volumetric flasks. Complete as described under “General procedure”. The nominal content of the tablets (concentration found) was determined either from the calibration graph or using the corresponding regression equations adopting either of the DC or DPP modes.

DISCUSSION

Figure 2 shows the typical (DC and DPP) polarograms of CPA in BRb of pH10.0 containing 20% methanol. Methanol was added as a solubilizer for CPA, it also decreased the adsorption of the drug likely to occur at the surface of DME. Cyproterone acetate produces well-defined cathodic waves over the pH range of 4-11.8 in BRb (Fig. 3). Reduction of CPA at the dropping mercury electrode was found to be pH dependent, as the E½ values were shifted to more negative values upon increasing the pH (Fig. 3). A plot of E½ versus pH gave two straight lines with one break at pH8 (Fig. 4). The relation between E½ values and the pH of the solution is represented by the following equations:
E_0 = 480 + 80 pH
over the pH range 4-7 and
E_0 = 1050.4 + 11.36 pH
over the pH range 8-11.8

The number of electron transfer at the rate determining
step (α_n) were calculated from the equation of Meites and
Israel (11).

E = E_0 - (0.059/α_n) log[i/id-i]
where id is the diffusion current and α is the transfer coef-
ficient. Logarithmic analysis of the reduction waves ob-
tained in BRb of different pH values resulted in straight
lines. The α_n values were calculated according to the
treatment of Meites and Israel (11) and are listed in Table
1, at pH10.0, α_n was 0.885. Assuming that the rate-de-
termining step involves the transfer of two electrons, the
value of α_n point out to the completely irreversible nature
of the reduction process.

Study of the wave characteristics
Changing the buffer concentration over the range 6 × 10^-3 M to 6 × 10^-2 M was found to yield a negligible effect
on the wave height of CPA. This indicates a diffusion-con-
trolled wave, partially affected by adsorption phenomenon.

Table 1. Effect of pH on the development of the polarographic
waves of cyproterone acetate

| pH  | -E_0 (mV) | -ΔE_0 / ΔpH | W_½ (mV) | α_n |
|-----|-----------|-------------|----------|-----|
| 4.0 | 800       | 80.0        | 50       | 0.66|
| 5.0 | 880       | 90.0        | 40       | 0.738|
| 7.0 | 1040      | 100.0       | 50       | 0.404|
| 8.0 | 1140      | 10.0        | 60       | 0.450|
| 9.0 | 1150      | 20.0        | 35       | 0.501|
| 10.0| 1170      | 8.3         | 30       | 0.885|
| 11.2| 1180      | 50          | 0.786    |
| 11.8| 1180      | 60          | 0.710    |

W_½, Half-peak width in the DPP mode; n_a: Number of electrons
transferred in the rate-determining step; α: Transfer coefficient.
Cyproterone acetate was found to be stable in BRb of pH10.0 (the analytical pH) for about one and half hour at room temperature, after which the peak height began to decrease slowly.

The diffusion current constant (Id) was calculated according to Ilkovic equation (12) for varying concentrations of the drug using the following equation:

$$Id = i/d / C \cdot m^{2/3} \cdot t^{1/6}$$

and was found to be 9.29 ± 0.046 (n=9). The results are shown in Table 2.

**Mechanism of electrode reaction**

The number of electrons consumed during the reduction process was accomplished through comparison of the waveheight of cyproterone with that obtained from an equimolar solution of a previously studied, structurally related, compound and of nearly identical value of diffusion coefficient, namely Spironolactone (13). In BRb of pH10.0, both compounds gave one wave, of the same height. Hence, it is concluded that 2 electrons are involved in the reduction process. Based on the presence of carbonyl group, and by analogy to the reported mechanism of reduction proposed for Spironolactone, the following pathway is postulated (Figure 5).

**Analytical performance**

Under the described polarographic conditions, at pH10.0, CPA exhibits a well defined diffusion controlled cathodic wave and sharp differential pulse peak, both are suitable for analytical applications. No polarographic maximum was developed; hence no maximum suppressor was needed.

Plots representing the relationship between the diffusion current of both the DC and DPP modes versus the concentration of CPA gave straight lines over the concentration ranges of 3.2-32 and 0.5-14 µg/ml using DC and DPP modes respectively, with minimum limits of detection (LOD) of 0.15 and 0.13 µg.ml⁻¹ using DC and DPP modes, respectively (Table 3).

Linear regression analysis of the data gave the following equations:

$$id = 8.97 \times 10^{-4} + 0.027 C$$

using DC mode…… and

$$ip = 1.355 \times 10^{-3} + 0.026 C$$

using DPP mode.

where C is the concentration in µg/ml, id is the diffusion current in µA in the DC mode and ip is the current in µA in the DPP mode.

**Method validation**

The proposed method was validated using the following criteria; linearity, sensitivity, intra-day and inter-day precision, accuracy, specificity, and robustness.

**Linearity**

Linearity was evaluated by calculation of the regression equations over the ranges given in Table 3.

The sensitivity of the method was evaluated by determining the limit of detection (LOD) according to ICH Q2B guidelines (14).

$$LOD=3.3 S_y/b$$

where the standard deviation of the intercept of the regression lines and b=the slope of the calibration curve. Statistical evaluation of the regression lines regarding standard deviation of the residual ($S_{y/x}$), standard deviation of the intercept ($S_{a}$) and standard deviation of the slope ($S_{b}$) is given in Table 3. The small value of the figures indicates the high accuracy and high precision of the method (15).

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| No | Concentration (mM) | Current (µA) | id/C (µA/mM) | Id = id/Cmn²/₃t¹/₆ |
|----|--------------------|--------------|--------------|----------------------|
| 1  | 0.00768            | 0.0864       | 11.25        | 9.282                |
| 2  | 0.0096             | 0.1080       | 11.25        | 9.282                |
| 3  | 0.0192             | 0.2160       | 11.25        | 9.282                |
| 4  | 0.0240             | 0.2730       | 11.375       | 9.385                |
| 5  | 0.0288             | 0.3240       | 11.25        | 9.282                |
| 6  | 0.0384             | 0.4290       | 11.172       | 9.218                |
| 7  | 0.0480             | 0.540        | 11.25        | 9.282                |
| 8  | 0.0576             | 0.650        | 11.28        | 9.307                |
| 9  | 0.0768             | 0.8610       | 11.211       | 9.250                |

Mean = 9.29 ± 0.046
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Each result is the average of three separate determinations. Id, Limiting diffusion current constant; id, Limiting diffusion current (µA); C, Concentration in m mole/L; M, Flow rate in mg/ sec; T, Drop time in second.

$$id = 8.97 \times 10^{-4} + 0.027 C$$

using DC mode…… and

$$ip = 1.355 \times 10^{-3} + 0.026 C$$

using DPP mode.

Figure 5. Postulated pathway.
To test the validity of the proposed method it was applied to the determination of authentic sample of CPA over the concentration range cited in Table 3. The results obtained were in good agreement with those obtained using a reference UV derivative spectrophotometric method (2). Using Student’s t-test and variance ratio F-test (15) revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 3).

**Accuracy**

The repeatability was performed through analysis of two concentrations of CPA in pure forms adopting the two polarographic modes (DPP and DCₜ) on three successive times, and the results are listed in Table 4.

Intermediate precision. It was performed through repeated analysis of CPA in pure form applying the proposed method, using the concentrations showed in Table 4, for a period of three successive days.

The repeatability and reproducibility in both modes were fairly good, as indicated by the small values of standard deviation (SD), relative standard deviation (RSD), and percentage error (% Er).

**Precision**

**Repeatability.** The repeatability was performed through analysis of two concentrations of CPA in pure forms adopting the two polarographic modes (DPP and DCₜ) on three successive times, and the results are listed in Table 4.

Intermediate precision. It was performed through repeated analysis of CPA in pure form applying the proposed method, using the concentrations showed in Table 4, for a period of three successive days.

**Robustness**

The robustness of the method is demonstrated by the consistency of the diffusion current with minor changes in concentration range (µg/ml) 3.2-32 0.5-14

Limit of detection (LOD) (µg/ml) 0.15 0.13

Correlation coefficient (r) 0.9999 0.9999

Values between parentheses are the tabulated t and F values respectively, at p=0.05 (15). Sₓₓ, standard deviation of the residual; Sₓ, standard deviation of the intercept of the regression line; Sᵧ, standard deviation of the slope of the regression line; % Error, %RSD/√ n.

**Table 3. Analytical parameters for the polarographic determination of cyproterone acetate using DCₜ and DPP modes**

| Parameter                        | DCₜ mode          | DPP mode         | Reference Method (2) |
|----------------------------------|-------------------|------------------|----------------------|
| No. of experiments               | 9                 | 9                | 3                    |
| Mean found (%) ± SD              | 99.96 ± 0.61      | 100.02 ± 0.94    | 100.49 ± 0.49        |
| Variance                         | 0.372             | 0.24             |                      |
| Student’s t-value                | 1.37 (2.23)       | 0.81 (2.23)      | 0.24                 |
| Variance ratio F-test            | 1.55 (4.46)       | 3.68 (4.46)      |                      |
| Concentration range (µg/ml)      | 3.2-32            | 0.5-14           |                      |
| Limit of detection (LOD) (µg/ml) | 0.15              | 0.13             |                      |
| Correlation coefficient (r)      | 0.9999            | 0.9999           |                      |
| Intercept                        | 8.97 × 10⁻⁴       | 1.36 × 10⁻³      |                      |
| Slope                            | 0.027             | 0.026            |                      |
| Sₓₓ                              | 2.03 × 10⁻³       | 1.47 × 10⁻³      |                      |
| Sₓ                              | 1.25 × 10⁻³       | 9.67 × 10⁻⁴      |                      |
| Sᵧ                              | 7.48 × 10⁻⁵       | 1.23 × 10⁻⁴      |                      |
| % Error                          | 0.20              | 0.31             |                      |

**Table 4. Validation of the proposed method for the determination of cyproterone acetate in pure form**

| Precision           | DCₜ mode | DPP mode | Reference Method (2) |
|---------------------|----------|----------|----------------------|
| Repeatability       | 99.63    | 100.93   | 101.16               |
|                     | 100.04   | 101.31   | 99.61                |
|                     | 100.75   | 100.75   | 99.42                |
| Mean ± S.D.         | 100.14   | 100.99   | 100.06               |
| % RSD               | 0.57     | 0.29     | 0.95                 |
| % Error             | 0.33     | 0.17     | 0.55                 |

Intermediate precision

| Mean ± S.D.         | 101.88   | 101.89   | 100.77               |
| % RSD               | 0.57     | 0.29     | 0.95                 |
| % Error             | 0.33     | 0.17     | 0.55                 |

Robustness

The robustness of the method is demonstrated by the consistency of the diffusion current with minor changes in concentration range (µg/ml) 3.2-32 0.5-14

Limit of detection (LOD) (µg/ml) 0.15 0.13

Correlation coefficient (r) 0.9999 0.9999

Values between parentheses are the tabulated t and F values respectively, at p=0.05 (15). Sₓₓ, standard deviation of the residual; Sₓ, standard deviation of the intercept of the regression line; Sᵧ, standard deviation of the slope of the regression line; % Error, %RSD/√ n.
Polarographic determination of cyproterone acetate in its tablets using the proposed and the reference methods

| Pharmaceutical preparation                      | DCt mode | DPP mode | Reference Method |
|------------------------------------------------|----------|----------|-----------------|
| Labeled amount (mg) | % recovery | Labeled amount (mg) | % recovery | % Recovery |
| Diane-35™ tablets* | 2 | 101.33 | 2 | 98.99 | 100.54 |
| (cyproterone acetate 2mg + 0.035 mg ethynylestradiol/tablet) | 2 | 98.61 | 2 | 99.19 | 99.43 |
| (Batch # 502 B) | 2 | 98.90 | 2 | 100.91 | 101.59 |
| Mean | 99.48 | 100.01 | 100.52 |
| ± SD. | 1.25 | 1.07 | 1.08 |
| Student’s t-value. | 1.15 | 0.62 |
| Variance ratio F-test. | 1.34 | 1.02 |

The tabulated values of t and F are (2.57) and (9.55) respectively, at p=0.05 (15). ‘product of Schering AG Germany.

Applications
Both DCt and DPP modes were successfully applied to the assay of CPA in its commercial tablets (Diane-35™). The percentage recovery based on 4 separate determinations are abridged in Table 5. The results are in agreement with those obtained using a reference UV derivative spectrophotometric method (2), where the first derivative of the methanolic solution was measured at 303 nm. Statistical analysis of the results using the Student’s t-test and the variance ratio F-test (15) revealed no significant difference between the performance of the two methods regarding accuracy and precision, respectively (Table 5).

Specificity
The specificity of the method was investigated by observing any interference encountered from the common excipients, such as talc, lactose, magnesium stearate, aviol and starch. These excipients did not interfere with the proposed methods.

CONCLUSION
A simple and sensitive method was developed for the determination of CPA in formulations. It has distinct advantages over other existing methods regarding sensitivity, saving time. Moreover, the proposed method does not require elaborate treatment for the sample or prior extraction for pure form. As well as, the method is sensitive enough for the analysis of lower concentration of CPA as low as 0.5 µg/ml.

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