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# Adsorptive Voltammetry of 4-chlordehydromethyltestosterone

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**Abstract**

The article presents the results of the study of the electrochemical behavior of the anabolic steroid 4-chlordehydromethyltestosterone. The studies detected operating conditions for the voltammetric determination of anabolic glassy carbon electrode. The influence of such factors as pH, supporting electrolyte, the electrolysis and adsorption component on the analytical signal of the analyte was studied. The algorithm of sample preparation is presented and conditions for the quantitative determination of the active ingredient of the drug "Turinabol" by voltammetry is proposed. The evaluation of the proposed method accuracy was conducted.


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*Keywords:* anabolic steroids, 4-chlordehydromethyltestosterone electrochemical methods of analysis, adsorptive voltammetry.

## 1. Introduction

The study of pharmaceuticals by physico-chemical methods of analysis is widely used in pharmacology, toxicology and forensic chemistry. This is due to an active medical and non-medical use of drugs in today's society. The constant development of new physiologically active substances of natural or synthetic origin is the basis for the development of methods of analytical chemistry in this direction. Numerous experiments on the synthesis of new derivatives of previously studied psychoactive substances, both natural and synthetic origin contribute to the expansion of these processes. In this regard, the importance of the monitoring of the consumption of anabolic steroids takes qualitative and quantitative physical-chemical analysis which requires the continuous development of new, more sensitive, rapid and selective methods. Chromatographic[1,2], spectral methods[3] and preferably

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chromatography-mass spectrometry[4-7] are widely used for analysis of anabolic steroids. In this case the objects of study are medicines.

4-chlordehydromethyltestosterone is oral anabolic steroid that was developed in the early 1960s at the firm "Yenafarm" and is included to the Prohibited List of the World Anti-Doping Code[8]. To date, the definition of 4-chlordehydromethyltestosterone is a subject of a number of scientific publications where the main methods of determining this steroid in biological fluids, food additives and food, at concentrations ranging from $10^{-8}$ to $10^{-5}$ g/ml, are the gas and liquid chromatography, preferably with mass spectrometry[9-12].

An apparatus solution of the problem of the steroids quantitative chemical determination by these methods requires significant financial investments. Its use at mobile laboratories is impractical.

Application of voltammetry for analysis of 4-chlordehydromethyltestosterone is not described in literature. But there is information of the detection by this method of other anabolic steroids. Thus, the square-wave voltammetry on graphite electrode and its analogue, a modified single-walled carbon nanotubes is applied for the simultaneous determination of corticoids in the urine of two isomers of testosterone and epitestosterone. At that, the calibration curves are linear within the concentration range of 5-1000 nM for both steroids with a detection limit of $2.8 \cdot 10^{-9}$ M and $4.1 \cdot 10^{-9}$ M, and testosterone to epitestosterone respectively[13]. The absorptive stripping voltammetry using clad lead film electrode is used for the testosterone determination in pharmaceuticals and human urine samples without separation steps. In this case, the accumulation of testosterone occurs in acetate buffer with pH 5.2 with a current at a potential of -1.1 V, the detection limit is $9 \cdot 10^{-9}$ M, and the relative standard deviation in measurements of testosterone with concentration of $1 \cdot 10^{-7}$ M is 3.8 [14]. For the analysis of human serum the electrochemical immunosensor for testosterone was developed. The design of which provides direct attachment to antitestosterone composite PTFE electrode attached to the surface of gold nanoparticles and carbon nanotubes[15]. The investigation of the electrochemical behavior of nandrolone decanoate in biological fluids showed that the peak current of oxidation on the electrode with gold nanoparticles modified indium tin oxide, is registered on the background of phosphate buffer in the range pH 2.1-9.2 in the potential 0.4-0.7 V.

The advantage of this method over the chromatographic and spectroscopic, is a combination of versatility, selectivity, sensitivity and ease of hardware design, as well as the presence at the market of high-quality electrochemical instrumentation devices of the domestic production.

In this connection it can be argued that voltammetry is a promising method of analysis of anabolic steroids, which has not previously been used for the determination of 4-chlordehydromethyltestosterone and its electrochemical behavior has not previously been studied.

## 2. Experimental part

### *2.1. Apparatus and reagents*

We used an analytical voltammetric complex STA (TU 4215-001-20694097-98), consisting of an electronic unit and a measuring unit with three electrochemical cells. Data processing was carried out using the software "STA" version 2.0.1.8834. The three-electrode electrochemical cell was used at the paper version. The glassy carbon electrode was used at the research of voltammetric behavior as an indicator electrode. This electrode is a glassy carbon rod with a diameter of 2 mm and a length of 12 mm, pressed into a plastic housing with current supply, and has a high chemical and electrochemical stability, a wide area of working potentials in both aqueous and non-aqueous media, mechanical simplicity and security updates surface application. The silver/silverchloride saturated electrode was used as an auxiliary electrode and a reference electrode. Detections of the pH of the solution were performed using a portable pH meter-ionomer with an error of less than ± 0.1%.

Twice-distilled water was used for the preparation of solutions. The disodium hydrogenphosphate and potassium dihydrogenphosphate in the pH range from 3.70 to 11.30 were selected as the supporting electrolyte solution. The standard solution 4-chlordehydromethyltestosterone a concentration of $2.4 \cdot 10^{-3}$ M was prepared by dissolving a sample substance in the mass of 0.020 g of water- ethanol mixture (4:1) 25 ml. Working solutions were prepared by serial dilution of a standard solution of bidistilled water immediately before the experiment.

## 3. The experiment

An important factor in determining of the organic substance is the pH of the medium, which affects not only the

rate of the electrode process, but its mechanism. Aqueous supporting electrolytes such as sodium phosphate, potassium phosphate, buffer Britton-Robinson, and other solutions having a pH slightly alkaline component were primarily considered as possible backgrounds. However, not all of these electrolytes are allowed to receive direct calibration curve over a wide range of concentrations of 4-chlordehydromethyltestosterone Fig. 1.

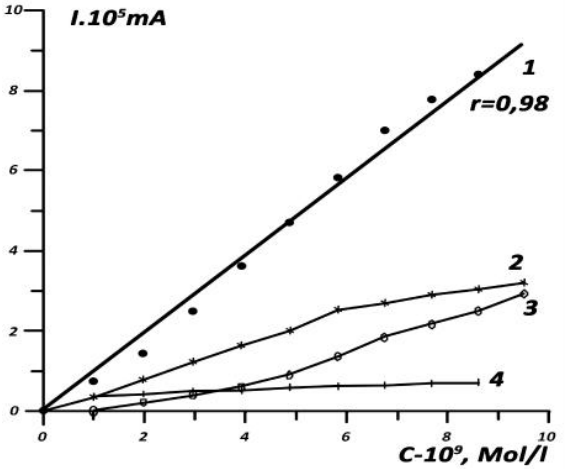


Figure 1. Dependence of anodic current of 4-chlordehydromethyltestosterone on its concentration in different background electrolytes: 1 - phosphate buffer pH 5.3; 2 – 0.01 M HCl; 3 - universal buffer mixture of pH 5.3; 4 – 0.05 M $Na_2B_4O_7$, τ = 30 s

The dependence of the potential maximum of anodic peak 4-chlordehydromethyltestosterone pH is complex. Increasing the pH of the medium led to a shift of the potential to more negative indexes, i.e., to the embarrassment of the oxidation of 4-chlordehydromethyltestosterone (Fig. 2), which appears to be associated with the previous protolytic reaction of deprotonation of the protonated forms of 4-chlordehydromethyltestosterone.

The study on the dependence of the current 4-chlordehydromethyltestosterone from electrolysis potential showed (Fig. 3), that the limit of current in the electrolysis process is achieved by electrolysis at a potential in the range of 0.3-0.5 V. In addition, it was found that the absence of a mechanical procedure purifying of electrode by filter paper decreases the peak current value and increases width of half-peak in the specified range of the electrolysis potential.

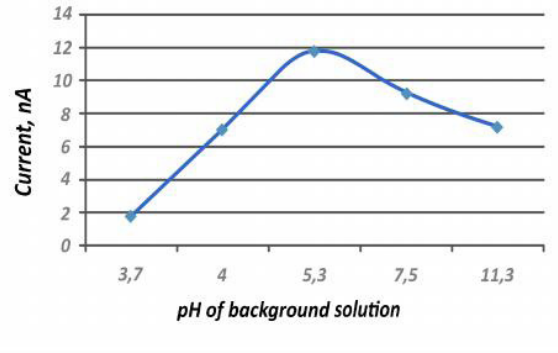


Fig. 2. Dependence of the current of peak on pH in the background solution (τe = 15s; w = 20 mV/s; Ee = 0.4 V; CDMT C = 2.4 • $10^{-6}$ M).

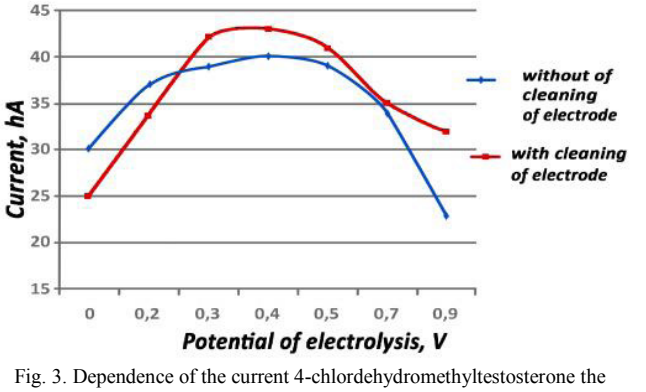


Fig. 3. Dependence of the current 4-chlordehydromethyltestosterone the potential electrolysis in the presence of phosphate buffer and electrode cleaning procedures.

It was also found that changing the electrolysis time does not significantly affect the magnitude of the analytical signal, and the operating value electrolysis time (τe) was 10-30 s. When τe is more 30 s, the degree of adsorption of

the substance on the electrode increases, which leads to a displacement of the peak capacity and, consequently, poor reproducibility, and the signal deviation from linearity calibration curve. The influence of electrochemical adsorption is confirmed by the fact that by increasing the potential speed changing the limiting current decreases (Fig. 4). From the resulting dependence it is seen the maximum current limit at rates of capacity 15 mV/s. Also the increase in peak current of chlordehydromethyltestosteron with increasing exposure time of the electrode in a solution of the supporting electrolyte without supplying a polarizing voltage (Fig. 5) was observed.

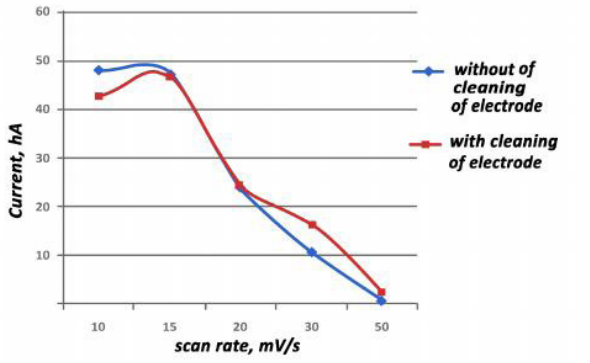


Figure 4. Dependence of the peak current of 4-chlordehydromethyltestosterone on the scan rate (Ee = 0.4 V; τe = 15s; 4-CDMT C = 2.4 • $10^{-6}$ M).

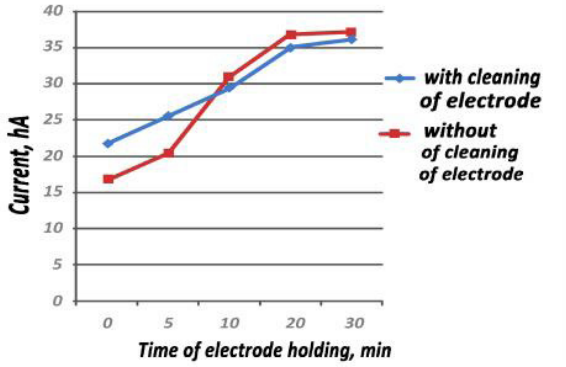


Fig. 5. Dependence of current peak 4-chlordehydromethyltestosterone from the holding time electrode in phosphate buffer (w = 20 mV/s; 4-CDMT C = 2.4 • $10^{-6}$ M).

During the procedure of choosing working conditions for the voltammetric determination of 4-chlordehydromethyltestosterone the potential electrolysis (Ee) 0.4 V and rate of change of potential sweep (w) 20 mV/s were chosen. Figure 6 shows a 4-chlordehydromethyltestosterone voltammogram obtained under the chosen conditions . The analytical signal of the analyte was observed at a potential of 1.12 V.

We have obtained the dependence of the peak current of 4-chlordehydromethyltestosterone on its concentration in solution (Fig. 7). It has been established that the linearity of the calibration curve is stored in the concentration range of 0.89 • $10^{-7}$-0.71 • $10^{-5}$ M with a correlation coefficient of 0.992 and a coefficient of sensitivity 22.54.

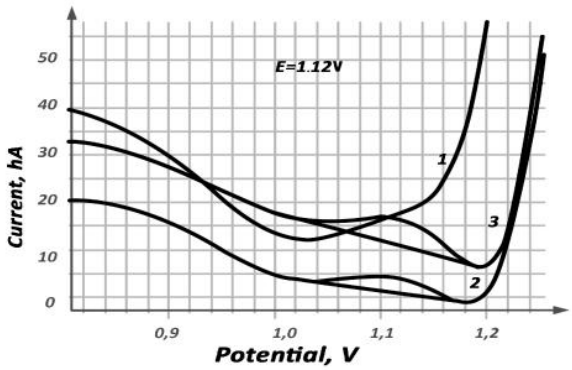


Fig. 6. Voltammograms of 4-chlordehydromethyltestosterone at glassy carbon electrode.
τe = 15; w = 20 mV/s; Ee = 0.4 V:
1 - Phosphate buffer at pH = 5.3;
2 - $C_{4\text{-HDMT}}$ = 1.43 • $10^{-6}$ M;
3 - $C_{4\text{-HDMT}}$ = 2.87 • $10^{-6}$M.

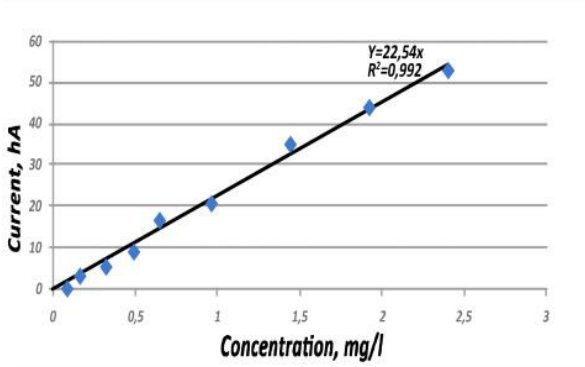


Fig. 7. Dependence of the peak current of 4-chlordehydromethyltestosterone at glassy carbon electrode in phosphate electrolyte upon the concentration. (τe = 15s; w = 20 mV/s; Ee = 0.4 V).

According to the results obtained at working conditions, we have developed the algorithm for the determining of the quantitative sample preparation and chemical analysis of pharmaceuticals based on the active ingredient 4-chlordehydromethyltestosterone. Sample preparation includes removing of the gelatin shell from tablets and their grinding. Further from 0.1 to 1.0 g of sample previously powdered in quartz dish or porcelain crucible were added

into measuring flask with the volume of 100 $cm^3$. Then we added 20 $cm^3$ of ethanol and allowed to stand it for 10-20 minutes, stirring occasionally. The content of the flask was adjusted to the mark with twice distilled water and mixed thoroughly. The obtained solution was filtered through a double paper filter for analysis conduction. The filtrate is the prepared sample for voltammetric measurements.

Validation procedure was carried out by the method of additives of certified mixtures. For this purposes the preparation under the trademark “Turinabol” was analyzed. The obtained active substance content is shown in the table 1:

Table 1 Results of the voltammetric determination of 4-chlordehydromethyltestosterone in tablets "Turinabol" and verification of correctness (P = 0.95), n = 7

| Sample | Content of 4-chlordehydromethyltestosterone, mg/tab. | | | | Sr |
|---|---|---|---|---|---|
| | By prescription | Probe | Added | Found | |
| Tablets " Turinabol" | 10.0 | 10.2 ± 1.0 | 10.0 | 19.8 ± 1.7 | 0.01 |

Then we calculated the content of active ingredient per tablet, which contained 10 mg of 4-chlordehydromethyltestosteronee. As seen from the table, obtained results did not exceed more than 2.75% error.

## 4. Results and discussion

As the result of studies there were established working conditions of the quantify 4-chlordehydromethyltestosteronee by voltammetry using a glassy carbon electrode , in which the detection limit and lower limit defines the contents are, respectively, $0.17 \cdot 10^{-7}$ M and $0.91 \cdot 10^{-7}$ M, a linear dependence of the calibration curve stored in the concentration range of $0.89 \cdot 10^{-7}$-$0.71 \cdot 10^{-5}$ M.

The study at the operating conditions of the dependence of the standard deviation of determined content of 4-chlordehydromethyltestosterone resulted in the calculation of the determination limit ( Cd), which was 0.03 mg/l or $0.91 \cdot 10^{-7}$ M (Sr = 0.33), as well as the detection limit ( Cmin ), which amounted to $0.17 \cdot 10^{-7}$ M.

## Acknowledgments

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