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ANBOLIC (ANBOLIC (AN), FUROSEMIDE (FU) AND METHANDIENONE (ME)) COMPONENTS IN SPORTS BY CHEMICAL METHOD

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ABSTRACT

Analysis of doping compounds is a matter of concern to scientists. Graphene oxide (GO) was electrochemically reduced on a glassy carbon electrode (GCE) surface (ERGO/GCE). Under the optimized conditions, the method allowed simultaneous determination of anabolic (AN), furosemide (FU) and Methandienone (ME). The results showed that the denatured electrode for the dissolved signal of all three analytes with anabolic value: 0.36 μM and quantitative limit of 1.07 to 1.42 μM ; furosemide: 0.25 μM and quantitative limits from 0.75 to 1.00 μM ; Methandienone: 0.23 μM and quantitative limit from 0.70 to 0.93 μM .

POSTING ISSUE

Doping and anti-doping are one of the topical issues in sports and in sports medicine in specific. In the world and perhaps in our country, the abuse of doping in sports training and competition is more and more and more sophisticated. The sociological research of doping use cases has found the following four reasons: For the sake of the resident, the governmental; Because of the expectations of the sports fanatic mass; With the view of winning at any cost; Because of the high bonuses. Because of doping, many athletes suffer from dangerous illnesses, their bodily and psychological health has declined. Some athletes died from doping use. Many meetings of the directors of the Olympic Committees of the countries, the leaders of the International Sports Confederation have discussed and proposed immediate, special steps to check and have religious sanctions. With athletes using catalysts, to protect the cleanliness of the Olympic Charter, to protect the lofty purposes of sport. The International Olympic Committee's Sports Medicine Council has tried to come up with a detail of banned drugs. Doping test analysis is time-consuming and costly, requires modern scientific equipment

and a range of doping compounds. Therefore, it is required that the detailed chemistry industry must develop precise analytical techniques with sound sense, great selectivity, and low detection limits to identify organic compounds and doping compounds in specific. Many multi-functional analysis techniques were introduced and used, such as atomic absorption spectroscopy (UV-Vis) analysis, high-performance liquid chromatography (HPLC), mass spectrometry (GC-MS), and electrochemical analysis. However, the UV-Vis, HPLC, and GC-MS methods reveal many limitations, the very high cost of material and analysis, while the electrochemical analysis approach is common. Dissolved von-amperometric (SV) methods offer many improvements such as high sensitivity, accuracy, selectivity, and low detection limit, low cost of equipment, and analysis. and therefore, well suited for the direct analysis of some organic compounds. Stemming from the above problems shows that the decision of organic compounds by SV method using ERGO changed electrodes is friendly and is a new research direction in electrical analysis. domestic and in the world. At the same time, it is useful in workshops in Vietnam equipped with multifunctional electrochemical analyzers. That is the reason to choose the topic:

“Research and analyze some doping compounds in sports by electrochemical methods”

In the analysis process, we use the following groups of research approaches: Methods of analysis and document synthesis; Chemical analysis method; statistical mathematical method.

Use of chemicals and material in the analysis process: The chemicals used in research are simple chemicals (PA): graphite, boric acid, acetic acid, phosphoric acid, disodium hydrophosphate, mononatri orthophosphate, anbolic (AN), furosemide (FU) and Methandienone (ME).

The equipment used is: Electrochemical analyzer CPA - HH5; Glassy carbon electrodes and electrolysis vessels; Precisa XB 220A analyties, Switzerland; Aquatron double distillation machine (Bibby Sterilin, UK); Micropipet Labpette types: of Labnet, USA; Magnetic stirrer Velp Scientifica; Universal Centrifuge 320R; Cole - Parmer 8890 ultrasound machine.

RESEARCH RESULTS

Selection of the working electrode

Electrochemical properties of anbolic (AN), furosemide (FU), and Methandienone (ME)

The dissolving ring von-ampe (CV) system was used to study the electrochemical properties of three analytes, anbolic (AN), furosemide (FU), and Methandienone (ME) on ERGO / GCE electrodes. The dissolving sugar results shown in Figure 1 show that anbolic (AN) and furosemide (FU) are inevitable, methandienone (ME) is the reversible pseudo-agent.

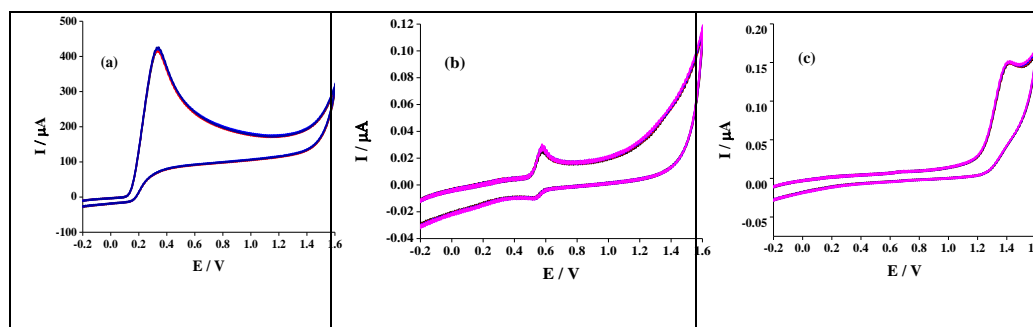


Figure 1. CV curves of AN (a), FU (b), and ME (c) with combinations of 10-2, 10-4, and 10-3 M in a B-RBS buffer of 0.2 M used ERGO / GCE electrodes (with a GO amount of 5 μ g, the number of contraction rings is 5).

The CV method, ERGO / GCE electrodes gave the dissolved peak current signals of all three analytes; however, only two peaks of anbolic (AN) and furosemide (FU) appear in the GCE and GO / GCE electrodes, which shows that reduced graphene oxide has a principal advantage over graphene oxide when applied. anbolic (AN), furosemide (FU), and Methandienone (ME). Therefore, ERGO / GCE electrodes exhibiting superiority over GCE and GO / GCE electrodes should be selected for more research.

Optimize experimental conditions

In the ASV method, a mixture of dissolving voltmeter signal recording systems can be used such as linear potential scan von-ampe technique (LS), various pulse voltammetry (DP) technique, and square wave von-ampe (SW), ... In these approaches, two techniques DP and SW are used popularly. Therefore, in this study, the anodic dissolved square wave voltmeter technique (DP-ASV) was chosen to investigate analyte systems including AN (5.10-5 M), FU (5.10-6 M). and ME (5.10-5 M) in buffer Britton-Robinson 0.1 M (pH = 3).

Investigate enrichment capacity

When choosing the enrichment capacity (Eacc), the enrichment potential should be chosen to ensure that it enriches only the analyte on the electrode surface, minimizing enrichment or other electrode reactions that affect the electrode. affects the sense and accuracy of the test. The enrichment potential needs to be more negative or equal to the depolarization potential of the materials to be determined to reduce them on the electrode surface. Thus, to investigate the effect of the enrichment potential on the analytic process, the experiment was conducted at different enrichment potentials (from -0.2V to 0.3V). Record dissolved current by DP-ASV method, potential scanning range from -0.2V to +1.6 V. The results are shown in figure 2.

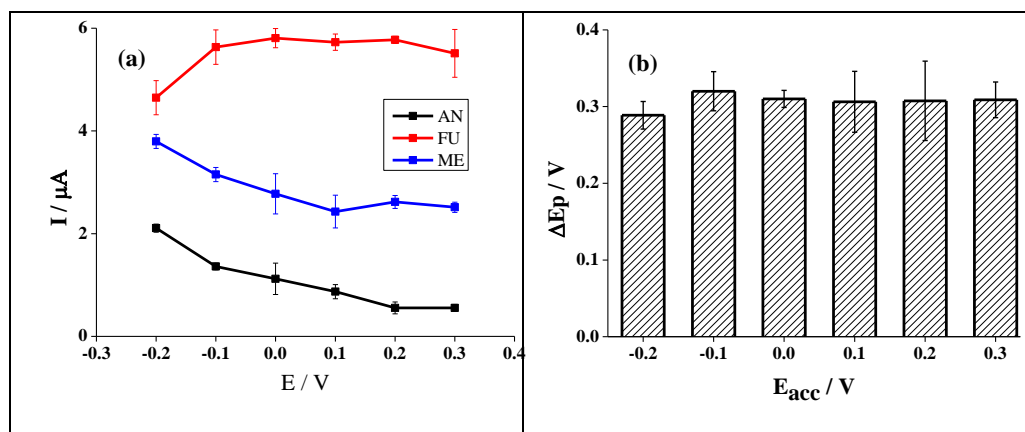


Figure 2. The fluctuation of I_p (a) and probable distance of vertices AN and FU (b) in other forms of getting rich.

From the results in Figure 2, it shows that: $E_{\text{acc}} = -0.1 \text{ V}$, the peak separation capability is the best, and the failure of the signal I_p corresponds to the small analyte item. Therefore, the enrichment capacity of -0.1 V is chosen for the next course.

The enrichment time survey

The enrichment time has an important effect on the termination signal of the analyte. When the enrichment time increases, the dissolving signal rises, at the time of large enhancement, it saturates the electrode surface with the analyte, the termination signal rises. Therefore, the analysis of the enrichment time aims to choose the time at which the required return is the high dissolution signal, but the analysis time is not too much. To conduct the enrichment time analysis, we proceed: record the dissolved voltages of the analytes at different enrichment times (0, 15, 30, 45, 60, 75, 90 s). We show the results in figure 3.

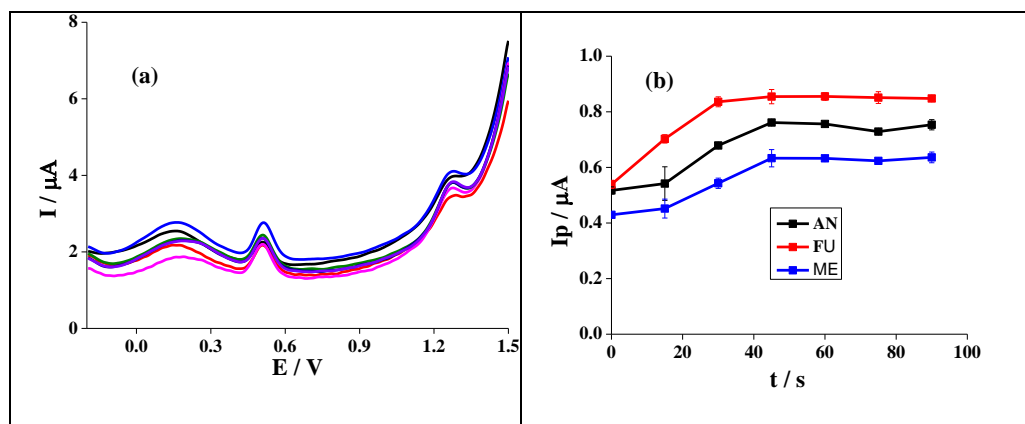


Figure 3. Curves DP-ASV (a) and curves showing variations of I_p (b) with other enrichment times. We measured values, $C_{\text{AN}} = 5.10 \cdot 10^{-5} \text{ M}$, $C_{\text{FU}} = 5.10 \cdot 10^{-6} \text{ M}$, $C_{\text{ME}} = 5.10 \cdot 10^{-5} \text{ M}$ in buffer B-RBS 0.2 M (pH = 3).

From the results of Figure 3 shows: When the enrichment time extends from 0 s to 45 s, the peak current intensity increments. Continuing to increase the

enrichment time, the dissolution peak current is almost constant. Therefore, to save 45 s of analysis time, it was selected for the next course.

Investigate the effect of vibration amplitude

The vibration amplitude has an important effect on the termination signal of the analyte. If the vibration amplitude is small, the dissolved peak current will be low, the amplitude of the vibration is large, the dissolved peak current is high, but when the amplitude of the vibration is large, the climax is expanding, increasing the effect of other factors on the analyte. Therefore, choosing the vibration amplitude will determine the analytical capacity of the approach. Because of this, we investigated the vibration amplitude by recording the dissolving peak floods of the tests at different vibration amplitudes. We show the results in Table 1.

Table 1. Influence of vibration amplitude on the discharged current signal according to DP-ASV method

Vibration amplitude ΔE (V)	AN		FU		ME	
	$I_{p,TB}(\mu A)$	RSD(%)	$I_{p,TB}(\mu A)$	RSD(%)	$I_{p,TB}(\mu A)$	RSD(%)
0,04	0,598	2,49	1,247	3,47	1,202	2,90
0,05	0,726	1,94	1,349	0,80	1,140	1,45
0,06	0,887	0,61	1,613	0,40	1,356	0,37
0,07	1,002	0,93	1,821	1,44	1,602	0,41
0,08	1,119	0,98	1,962	1,15	1,838	0,27
0,09	1,231	0,65	2,058	1,56	2,061	1,32
0,10	1,326	1,18	2,069	1,06	2,115	1,30

From the results in Table 1 shows, when the vibration amplitude is 0.05 and 0.06 V, the peak separation capacity is best, however, the failure is shorter at 0.06 V pulse amplitude. Therefore, a pulse amplitude of 0.06 V (60 mV) is chosen for more research.

Effects of scanning speed

In the dissolved volt-ampere method, the potential scanning rate has a significant effect on the dispersed signal of the analyte. If the scanning velocity is fast, it shortens the test stage, the dissolved signal is high, but the rate of the dissolved signal is reduced or loss of dissolved signals may occur. Conversely, when the potential scanning rate is slow, the measurement repeatability is high, the resulting dissolved signal has a proportional shape, but the dissolved signal is low. Therefore, an appropriate rate of potential sweep must be chosen to reduce the recording time while ensuring measurement accuracy and smoothness and symmetry of the von-ampe curve.

To conduct the scan speed survey, we recorded dissolved voltages of three analyses simultaneously AN, FU, and ME at different scanning speeds: 5, 10, 15, 20, 25, 30. mVs^{-1} . It shows the scanning speed survey results in Figure 4.

From the results of Figure 4, it shows that when the scanning rate is $20 \text{ mV}\cdot\text{s}^{-1}$, the dissolving signal of AN and FU is highest (compared with the survey area) and RSD in repeated measurements of the fertilizers. small area. Therefore, a scanning speed of $20 \text{ mV} / \text{s}$ is chosen for the next study.

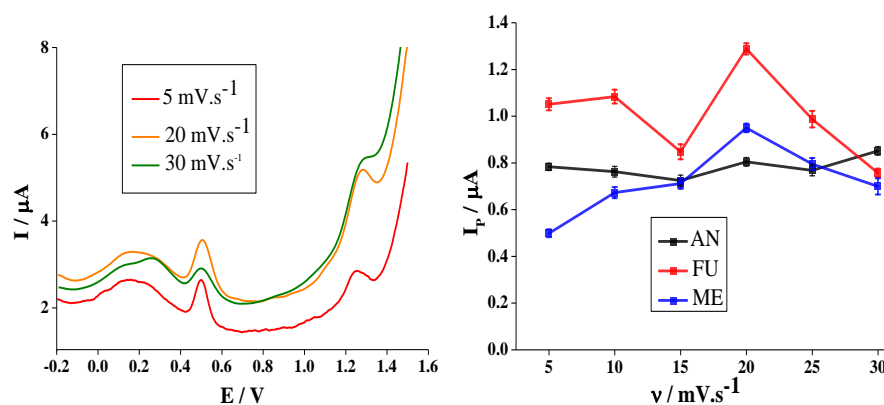


Figure 4. DP-ASV curves (a) and groups representing IP (b) changes at various scanning speeds. Values were calculated, CAN = $5 \cdot 10^{-5} \text{ M}$, CFU = $5 \cdot 10^{-6} \text{ M}$, CME = $5 \cdot 10^{-5} \text{ M}$ in buffer B-RBS 0.2 M ($\text{pH} = 3$).

Test the reliability of the method

To apply the ERGO / GCE changed electrode to the test of actual samples, the accuracy of the system must be assessed early. The numerical quantities used for assessment include repeatability, diameter range, subtlety, LOD, LOQ.

Test the signal repeatability

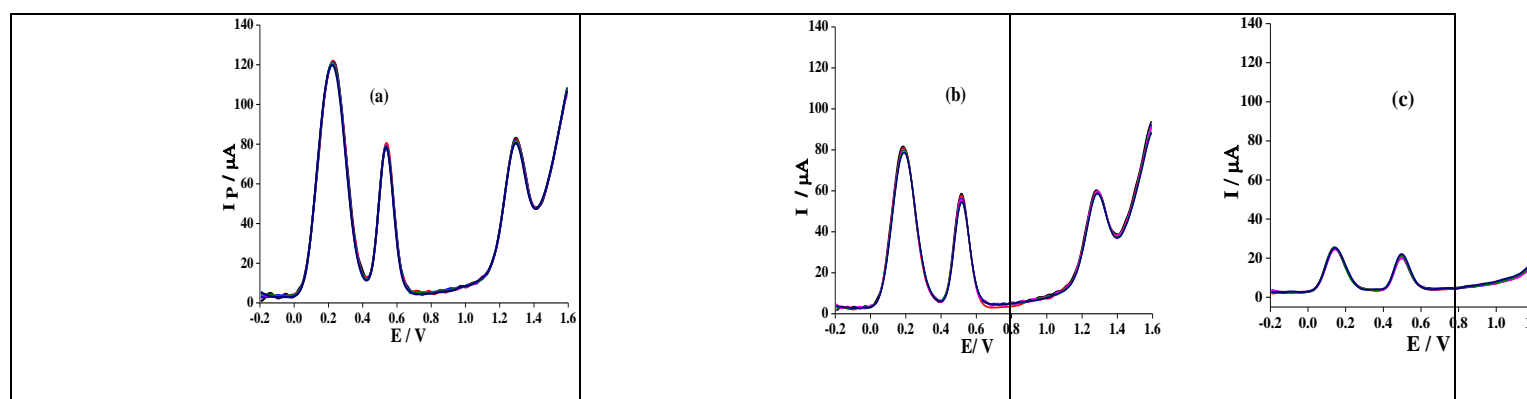
I_p repeatability on the ERGO / GCE electrode is measured by relative standard variation (RSD). To determine the repeatability of I_p , we performed repeat measurements 6 times with 3 different combinations. We show the results in Table 2 and Figure 5.

Based on the results in Table 2 and Figure 5, we can assume that the repeatability of IP for the three substances AN, FU, and ME is very valuable, ranging from 0.76% to 3.03%. When measuring the RSD in the experiments with $\frac{1}{2}\text{RSDH}$ at the respective concentrations were smaller and thus, the repeatability of the IP in the DP-ASV method using ERGO / GCE changed electrodes was fully fair.

Table 2. I_p , TB, SD, RSD values when renewed at 3 different combinations corresponding to the DP-ASV method

	C (M)	$I_{p,TB}(\mu A)^{(b)}$	SD	RSD, (%)	$\frac{1}{2}.RSD_H$ (%)
TN 1 ^(a)	$C_{AN}=10^{-2}$	117,2	0,89	0,76	2,00
	$C_{FU}=10^{-4}$	74,48	0,75	1,00	4,00
	$C_{ME}=10^{-3}$	48,32	0,88	1,83	2,83
TN 2	$C_{AN}=5.10^{-3}$	21,92	0,53	2,01	2,22
	$C_{FU}=5.10^{-5}$	17,03	0,87	2,14	4,44
	$C_{ME}=5.10^{-4}$	12,00	0,52	2,35	3,14
TN 3	$C_{AN}=10^{-3}$	76,85	0,98	1,28	2,83
	$C_{FU}=10^{-5}$	51,92	1,57	3,03	5,66
	$C_{ME}=10^{-4}$	32,03	0,35	1,08	4,00

Average I_p value after those 6 repeats; B-RBS buffer 0.2 M (pH = 3).

**Figure 5.** DP-ASV curves at three concentrations AN, FU, and ME with three model experiments (TN1, TN2, and TN3).

Thus, if the repeatability of the dissolved RSD signal (%) $< 1/2$ RSDH, then it can be considered that the repeatability of the analysis results is good.

Linear range

Results of the decision of the linear dimension of the DP-ASV method using changed ERGO / GCE electrodes for AN, FU and it presents me in Figure 6.

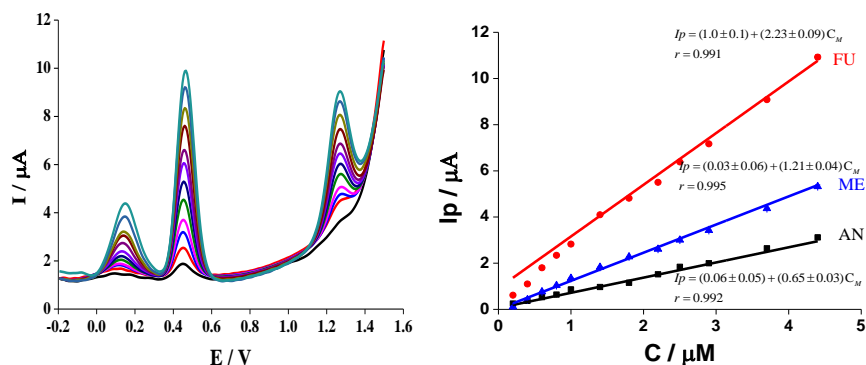


Figure 6. DP-ASV curves of AN, FU, and ME at various co-standard additive concentrations (A), Linear regression curves representing the relation between IP and accumulations of AN, FU, and ME (B).

When conducting a further standard examination at the same time AN, FU, and ME found that for each item there is only a defined linear range (Figure 6) with large correlation coefficients. We define the linear regression equations as follows:

$$\text{AN: } IP(A) = (0,06 \pm 0,05) + (0,65 \pm 0,03) C_{AN} \quad r = 0,999$$

$$\text{FU: } IP(A) = (1,0 \pm 0,1) + (2,23 \pm 0,09) C_{FU} \quad r = 0,994$$

$$\text{ME: } IP(A) = (0,03 \pm 0,06) + (1,21 \pm 0,04) C_{ME} \quad r = 0,995$$

In figure 6, it shows that AN, FU, and ME can be determined in the survey sample.

Detection limit and sensitivity

Derived from the result of the linear range analysis, we depend on the detection limit through the formula $3S_y / x / b$ where S_y / x is the measured variance of the measure and b is the slope of the regression equation. AN: 0.36 μM and limit quantification from 1.07 to 1.42 μM ; FU: 0.25 μM and differ from 0.75 to 1.00 μM ; ME: 0.23 μM and limit quantification from 0.70 to 0.93 μM .

CONCLUDE

The anodic dissolve square wave voltmeter (DP-ASV) approach is used to examine the causes that affect (such as enhancement, enrichment time, pulse amplitude, and potential sweep rate) on the signal of anabolic (AN), furosemide (FU), and Methandienone (ME). GCE changed electrodes by reducing (electrochemical reduction) graphene. The results showed that the denaturing electrode provided the termination signal of all three tests with An value: 0.36 μM and a significant limit of 1.07 to 1.42 μM ; FU: 0.25 μM and differ from 0.75 to 1.00 μM ; ME: 0.23 μM and limit quantification from 0.70 to 0.93 μM .

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