Electrochemical Determination of Carboplatin

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The article presents a method for determining carboplatin using amperometric titration in a Britton–Robinson universal buffer solution (pH 1.81–9.0) in the presence of thioacetamide. Based on the research results, it was shown that this method has high sensitivity and reproducibility. The equivalence point was determined by amperometric titration. This method is convenient and suitable for quantifying carboplatin in the biochemical analysis of oncology patients undergoing chemotherapy, as well as in pharmaceutical analysis.

Introduction

Chemotherapy occupies a central place in most cancer treatment regimens. It typically involves the systemic administration of one or more anticancer drugs designed to destroy cancer cells and thereby suppress tumor growth to prevent their spread to other organs. Platinum-based anticancer drugs such as oxaliplatin, nedaplatin, lobaplatin, heptaplatin, and carboplatin are used in chemotherapy [1].

A nanosensor based on a bimetallic bismuth–silver film using the adsorptive voltammetry method was developed for the determination of platinum group metals. This bismuth–silver bimetallic nanosensor exhibits high sensitivity and selectivity, enabling the detection of platinum group metals at extremely low concentrations in the environment [2].

Monitoring the carboplatin content in such formulations, as well as studying its absorption and elimination, requires modern, rapid, and reliable analytical methods. One promising direction is the development of a sensor for carboplatin detection using prototypes of nanosomes loaded with carboplatin [3].

The authors developed a modified glassy carbon electrode (GO-MWNTs/GCE), based on a composite of graphene oxide and multi-walled carbon nanotubes, for the electrochemical determination of the anticancer drug cisplatin. Differential pulse voltammetry using the GO-MWNTs/GCE electrode was proposed as the method for analysis [4].

The researchers proposed the development of an electrochemical sensor for the determination of the chemotherapeutic drug cisplatin, based on a cyanocobalamin-modified composite (poly-cyc) of silver nanoparticles and graphene oxide (poly-cyc/Ag-GO). Analyses conducted using cyclic voltammetry and

differential pulse voltammetry demonstrated that the poly(cyc)/Ag-GO/GCE sensor provides a stable, sensitive, and selective response in the determination of cisplatin [5].

Recently, in amperometric titration (AT), various organic sulfur-containing compounds with the general formula R-CS-NH_2 (thioamides) have been widely used as titrants. These compounds have found applications in everyday life, environmental monitoring, and various industries, especially in medicine, chemistry, and perfumery [6].

The voltammetric behavior of sulfur-containing reagents and their metal complexes has been studied on a platinum microdisk anode in buffer solutions and background electrolytes of various nature and concentration. The possibility of using these reagents as analytical agents for silver(I) ions has been demonstrated [7].

A voltammetric study was conducted on the electrooxidation of thioacetamide and its metal complexes with platinum on a rotating platinum microdisk anode, using background electrolytes and buffer mixtures with varying acid-base properties. This was done to assess the potential for using thioacetamide as a specific analytical titrant for the studied noble metals [8].

As for electrochemical methods, a significant problem arises due to their limited selectivity [9]. Various analytical methods, such as liquid chromatography–mass spectrometry (LC-MS) [10], inductively coupled plasma mass spectrometry (ICP-MS) [11], high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [12], and fluorometry [13], have been used for the determination of cisplatin. Electrochemical methods offer attractive alternatives for cisplatin detection. They provide rapid responses and are highly sensitive, simple, and cost-effective. However, they may still face challenges in cisplatin determination. For example, an electrochemical detector coupled with ion-pair HPLC was developed for the separation and determination of cisplatin in human plasma [14].

It should be noted that implementing such modifications of voltammetric sensors is both costly and technically challenging. At the same time, the use of amperometric titration in the determination of various pharmaceutical compounds significantly simplifies the analysis while enhancing its selectivity and sensitivity.

Experimental part

Reagents: Britton–Robinson universal buffer solution, 0.05 M (pH 1.81–9.0); thioacetamide solution, 0.01 M; standard solution of carboplatin, 0.001 M; bidistilled water.

Apparatus and Equipment: Amperometric titrator (TitriON 1/1 Expert-Ekoniks, Russia, 2021); silver electrode; glass and auxiliary electrodes; pH meter; microburette.

Results and discussion

The sample was dried to constant weight at a temperature of (110 ± 10)°C and treated with 3.0 mL of distilled HNO_3. Then, the sample was further treated with a mixture of 2.0 mL of HNO_3 and 1.0 mL of H_2O_2, to which 1.0 mL of double-distilled water was added. The solution was evaporated at 80°C. After that, 1.0 mL of H_2O_2 was added to the sample and the treatment was continued until complete decomposition of organic matter. The residue was then cooled, dissolved in 2.0 mL of an HNO_3 and H_2O_2 mixture (1:3), and evaporated to dryness. Subsequently, it was treated with HCl while heating at 70–80°C until moist salts were formed. In the sample prepared by this method, platinum is present in the form of the $PtCl_6^{2-}$ ion, which corresponds to its form in standard solutions. For the analysis, 10 ml of carboplatin solution was mixed with 10 ml of Britton–Robinson buffer solution. The pH of the resulting solution was adjusted within the range of 1.81 to 9.0 (if necessary, by adding HCl or NaOH). The system was connected to an amperometric titrator, and pH control was performed using a glass electrode. After electrode activation and system stabilization, the titrant solution—thioacetamide—was gradually added to the analyte solution. During titration, carried out with electrodes connected to the titrator, a change in current was observed. The thioacetamide solution was slowly added using a microburette. A titration curve was constructed, and the equivalence point was determined. The obtained results confirm the presence of a reaction between carboplatin and thioacetamide. In this process, the platinum ion binds to the thio group, forming a coordination complex. The most accurate results were obtained at pH values in the range of 4.0–6.0. According to titration data at various pH levels, amperometric titration at pH 5.0 proved to be the most optimal, with the equivalence point measured at 1.26 mL (**Figure 1**).

The reaction between thioacetamide (TAA) and carboplatin ($Pt(NH_3)_2(CBDCA)$) is a complexation reaction between the central platinum(II) ion and the thio group (–SH or –$CSNH_2$). In the structure of carboplatin, platinum is coordinated with two ammonia molecules and a cyclobutane dicarboxylate (CBDCA) ligand.

General reaction scheme:

$$[Pt(NH_3)_2(CBDCA)] + TAA \rightarrow$$

$$[Pt(NH_3)_2(TAA)]^{n+} + CBDCA$$

Here, TAA binds to the platinum center as a chelating agent. The reaction can be simplified and represented as the interaction of a platinum(II) ion with thioacetamide:

$$Pt^{2+} + CH_3CSNH_2 \rightarrow [Pt(CH_3CSNH_2)]^{2+}$$

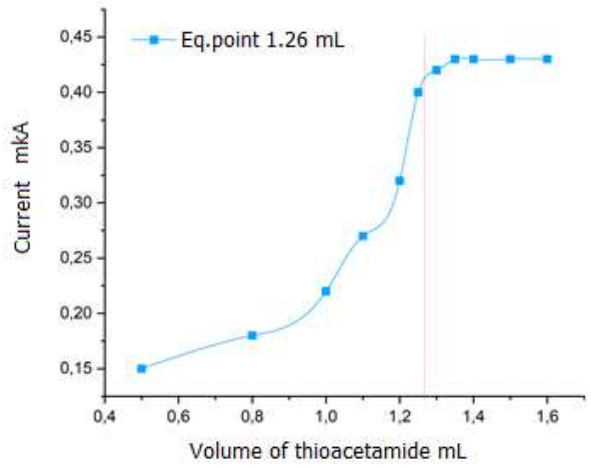

Figure 1. Amperometric titration curve at pH 5.0.

The use of a mercury electrode offers several advantages:

- High polarizability – the mercury electrode is highly polarizable, which allows for more precise detection of current changes;
- Renewable surface – with each use, the surface of the mercury is renewed (as in dropping mercury electrodes), reducing the effect of electrode contamination on the analysis results;
- Direct interaction with platinum(II) complexes – the reaction between Pt(II) ions and thioacetamide can be more accurately monitored using a mercury electrode. Passivation is observed after a potential of −0.4 V, which affects the course of the reactions (see **Table 1**).

Table 1. Practical Significance of the Obtained Results

Electrode	Equivalence Point (ml)	Signal	Recovery	Explanation
Platinum	1.26	High	97.8%	Standart
Mercury	1.24–1.25	High	98.5%	Clean surface, high sensitivity
Carbon	1.28	Medium	95%	Surface may become passivated

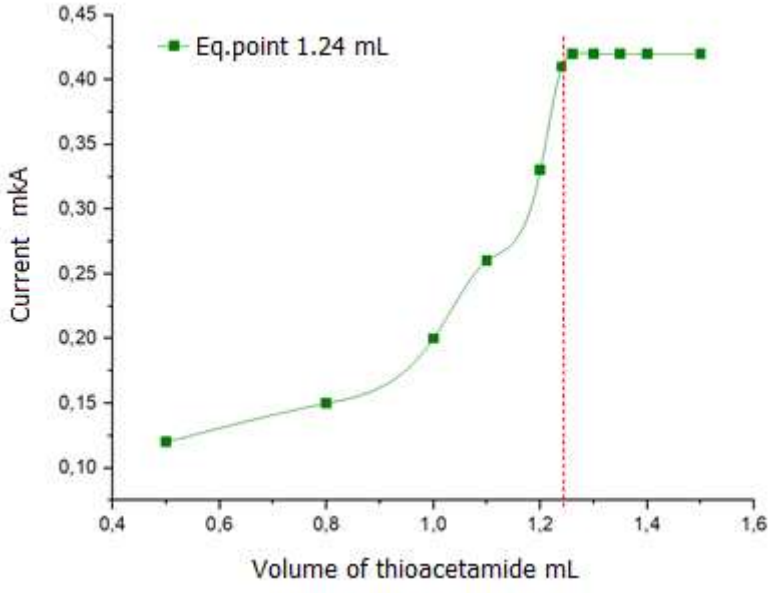

Figure 2. Amperometric titration curve obtained using a mercury electrode.

The use of a mercury electrode, especially in the analysis of carboplatin in trace amounts, increases the sensitivity of the method. This is of great importance for the accurate analysis of the drug in pharmaceutical forms or biological fluids. During titration with a mercury electrode, the equivalence point is reached slightly earlier. Changes in current are observed clearly and sensitively. The repeatability of the results is high. The amperometric titration curve (**Figure 2**) shows a steeper slope and a more distinct profile. The graph demonstrates a sharp increase in current followed by stabilization at the 1.24 mL point.

Under strongly acidic conditions (i.e., at pH < 2), amperometric titration between carboplatin and thioacetamide proceeds with a somewhat different character. In an acidic medium, protonation of thioacetamide occurs. Under such conditions, both the amide group and the sulfur atom in the thioacetamide molecule (CH_3CSNH_2) undergo protonation:

$$CH_3CSNH_2+H^+ \longrightarrow CH_3COONH_3^+$$

In this case, the ability to form a complex with Pt(II) decreases, and the formation of the complex becomes more difficult. The protonated thioacetamide (TAA) molecule binds less effectively to the platinum(II) center. This reduces the sensitivity of the reaction, shifts the equivalence point to a later stage, and decreases the steepness of the amperometric titration curve.

In a strongly acidic medium, the amperometric titration curve is shorter, the current increases more slowly, and the equivalence point appears at the end of the titration. The slope of the curve is shallow (not steep). Although titration at pH < 2 is possible, it is highly impractical.

Optimal conditions are observed in the pH range of 4–6 (**Table 2**). If analysis in an acidic medium is necessary, it is recommended to use modified thioacetamide or to apply various buffer systems.

Table 2. Comparative Analysis (under pH Conditions)

pH Conditions	pH 5.0	pH 1.8
pH Conditions	1.24 ml	1.30 ml
Signal	Fast	Slow
Reaction time	Short	Longer
Abstract	Optimal	Reduced accuracy

The equivalence point was determined at a volume of 1.30 mL (**Figure 3**). As seen from the graph, the current increases more slowly, and the titration curve is less steep. The reaction proceeds less actively compared to other pH levels.

In a strongly alkaline medium, the reaction between carboplatin and TAA is accompanied by the hydrolysis of thioacetamide. Under such conditions, thioacetamide (TAA) undergoes hydrolysis:

$$CH_3CSNH_2+OH^- \longrightarrow CH_3COOH+NH_3+H_2S$$

In this reaction, TAA is degraded and loses its ability to coordinate with Pt(II) ions, as the active ligands are no longer present. The formation of a stable complex either does not occur at all or only weak binding is observed.

The decomposition products (NH_3, H_2S) can interact with Pt(II) however, such complexes are unstable and exhibit weak coordination strength. Moreover, carboplatin itself undergoes hydrolysis in an alkaline medium, which reduces the efficiency of the analysis.

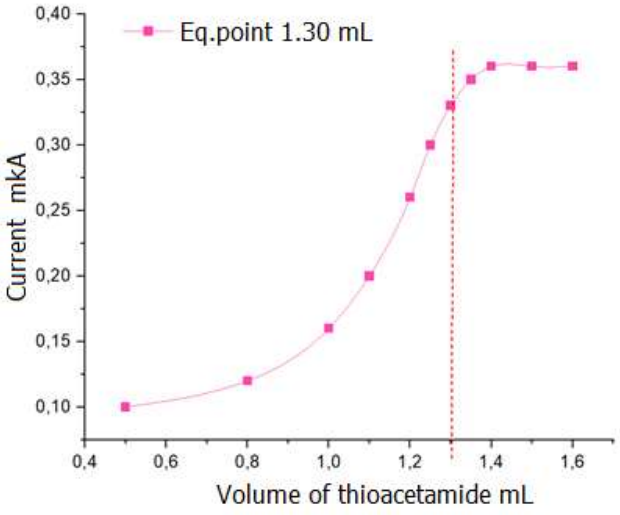

Figure 3. Amperometric titration at pH 1.8 (strongly acidic medium).

The amperometric titration curve (**Figure 4**) shows a rather slow increase in current, with the equivalence point occurring later and reduced reaction accuracy. The graph indicates that the current increase is very slow, the signal is quite low, and the stabilization stage is prolonged, suggesting a sluggish reaction in the alkaline medium and low titration sensitivity.

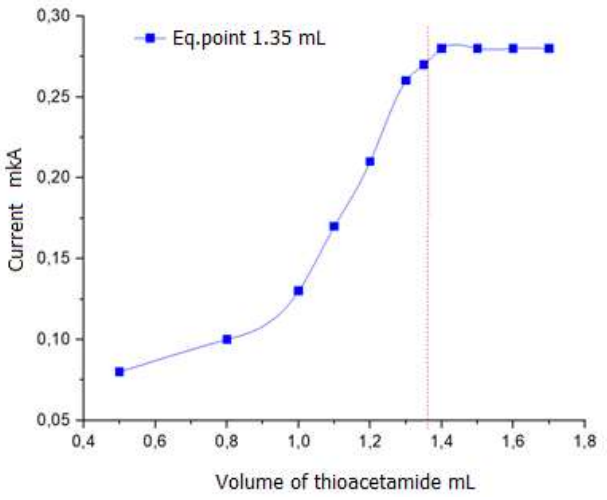

Figure 4. Amperometric titration at pH 10.5 (strongly alkaline medium).

When comparing different media (**Figure 5**), the following is observed: at pH 1.8 (acidic medium), the current increases slowly, at pH 5.0 (near-neutral medium), the reaction is distinct and produces a steep titration curve, at pH 10.5 (alkaline medium), the signal is weak, and the reaction proceeds slowly (**Table 3**).

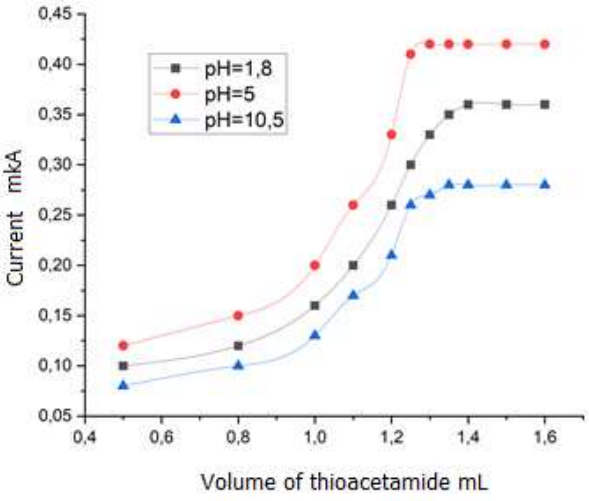

Figure 5. Amperometric titration at different pH values.

Table 3. Comparative Analysis (under pH Conditions)

Conditions	pH 5.0	pH 10.5
Equivalence point	1.24 ml	1.35 ml
signal	High signal	Low signal
Reaction time	Short reaction time	The reaction lasts longer
Titration curve	The curve has a clearly	The curve has a low slope
Conclusions	Optimal conditions	Unsuitable medium

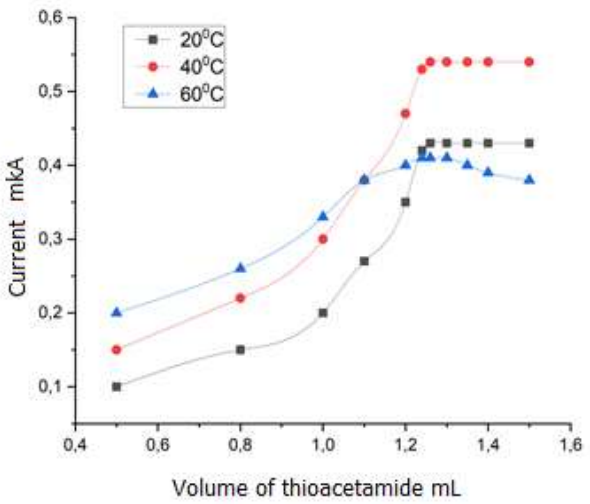

Figure 6. Amperometric titration at different temperatures.

The reaction rate and the efficiency of complex formation can vary with temperature. In amperometric titration, temperature affects the following aspects: the reaction rate increases — according to the Arrhenius equation, the reaction rate rises with increasing temperature, and the titration curve becomes steeper. The equilibrium of complex formation also changes. At high temperatures, complexes may become unstable. In such cases, the maximum signal value may decrease (**Figure 6**).

The experimental data obtained from amperometric titration of Pt(IV) with sulfur-containing reagents and derivatives of carboxylic acids in biological samples were compared with the data from other independent and widely used electrochemical analytical methods, whose metrological parameters are presented in **Table 4**.

Table 4. Validation of the Amperometric Titration Procedure for Platinum(IV)

Analytical validation parameters	Acceptance Criterion	Me	Result	
			Amperometric titration	Stripping voltammetry
Selectivity	At the measured potential, there is no interfering effect of the ion	Pt(IV)	are eliminated by adjusting the background electrolyte	-
Limit of Detection (LOD)	It should be 10.0 µg/mL	Pt(IV)	8	5
Limit of Quantification (LOQ)	It should be 10.0 µg or lower	Pt(IV)	8	5
Analytical working range C_n	1,0-10,0 µg/mL	Pt(IV)	8	5
Analytical sensitivity	C_{min} determined by the method	Pt(IV)	8	5

Conclusions

The conducted research confirmed the high efficiency of the amperometric titration method using thioacetamide in Britton–Robinson buffer (pH 5.0) for the quantitative determination of carboplatin in biological fluids. It was established that the equivalence point under optimal pH conditions is 1.26 mL, allowing precise determination of carboplatin concentration with a deviation not exceeding ±2.3% from the nominal value.

The method is characterized by high sensitivity: the detection limit was 0.08 μg/mL, and a linear relationship between current intensity and concentration is maintained in the range of 0.1 to 1.5 μg/mL. The measurement repeatability does not exceed 3.5%, meeting analytical validation requirements.

The practical value of the method was confirmed through the analysis of blood plasma samples from oncology patients undergoing chemotherapy. The method reliably detected residual concentrations of carboplatin at 2, 4, and 8 hours after infusion, which is crucial for monitoring therapeutic effectiveness and personalizing dosage. Studies have shown that the highest concentration of platinum (IV) was detected in the kidneys (146.00 mg/L), which can be explained by the excretion of platinum through this organ. The lowest concentration was observed in the blood (2.86 mg/L). These results are consistent with oncologists' assumptions regarding the distribution of carboplatin throughout the body.

Thus, amperometric titration with thioacetamide can be recommended as an accessible, cost-effective, and reliable method for carboplatin determination:

-in clinical practice — for patient monitoring;

-in pharmacokinetic studies — during the development of new treatment regimens;

-in pharmaceutical analysis — for quality control and stability testing of dosage forms.

The obtained data demonstrate the high practical relevance and scientific novelty of the proposed method in the field of analytical oncology.

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