Organic and Medicinal Chemistry
International Journal
ISSN 2474-7610

Research Article
Volume 14 Issue 4 - July 2026
DOI: 10.19080/OMCIJ.2026.14.555891

Organic & Medicinal Chem IJ
Copyright © All rights are reserved by Bakakhonov Anvar Akabirovich

# Determination of Doxorubicin Using Amperometric Titration

**Bakakhonov Anvar Akabirovich[1]* and Gaziev Laziz Turgunovich[2]**

[1]*PhD in Chemical Sciences, Jizzakh State Pedagogical University, Uzbekistan*

[2]*PhD in Medical Sciences, Jizzakh State Pedagogical University, Uzbekistan*

**Submission**: May 06, 2025; **Published:** July 14, 2026

***Corresponding author:** Bakakhonov Anvar Akabirovich, Jizzakh State Pedagogical University, PhD in Chemical Sciences, Jizzakh, Uzbekistan

**Abstract**

The study aimed to analyze doxorubicin in blood serum, urine, and biological fluids as a potential new marker of toxicity, particularly in overweight patients receiving doxorubicin. The study involved 10 cancer patients receiving palliative treatment with doxorubicin. Amperometric titration was used to determine doxorubicin levels in urine, blood, and biological samples.

**Keywords:** Cancer; Chemotherapy; Doxorubicin; Amperometric titration

## Introduction

The concentration of doxorubicin in the samples was determined based on data obtained from a calibration curve. Determining doxorubicin levels is a complex process. The half-life in one group was observed to be nearly twice as long as in other patients. The results indicate that doxorubicin concentration in urine is higher than in blood. Doxorubicin clearance, as determined by amperometric titration, is reduced in individuals with increased fat mass. The study data suggest that excess body weight affects the elimination of doxorubicin from the body. Higher fat mass may contribute to a more significant increase in serum triglyceride levels. Plasma glycerides may serve as an additional indicator of elevated doxorubicin toxicity as a toxicity marker.

## Literature Review

Cancer remains one of the leading health challenges worldwide. Each year, tens of millions of people globally are diagnosed with cancer, and more than half of these patients ultimately succumb to the disease. In many countries, cancer ranks as the second leading cause of death after cardiovascular diseases [1]. The rising incidence of cancer is driven by an aging population (tumors are most commonly diagnosed in older individuals) and the high prevalence of risk factors. Key risk factors significantly impacting mortality from malignant tumors include elevated body mass index (BMI), insufficient intake of fruits and vegetables, low physical activity, tobacco and alcohol use, air pollution, and various procedures performed in inappropriate settings or circumstances (e.g., preventive and therapeutic injections) [2]. According to the World Health Organization (WHO), 35% of cancer-related deaths are attributable to these risk factors [2]. Survival rates for cancer patients are influenced by various factors, including tumor location, clinical and morphological risk factors (patient age, tumor morphology, degree of tumor differentiation, disease stage, reproductive factors, etc.), the patient's overall health, comorbidities, and specific anticancer treatment modalities.

Chemotherapy is a cornerstone of cancer treatment. It involves the use of anticancer drugs to destroy cancer cells in the body. Even when cancer is surgically removed, cancer cells may persist in nearby tissues or metastasize other parts of the body. The goals of chemotherapy include reducing tumor size before surgery or radiation, eliminating cancer cells that may have spread to surrounding tissues or organs post-surgery (adjuvant chemotherapy), alleviating cancer symptoms (e.g., pain), or controlling tumor growth in cases of metastasis (palliative therapy).

Each chemotherapeutic drug has an internationally established dosage, calculated based on body surface area in square meters.

The drug dose is tailored to each patient depending on their weight, height, overall health, and comorbidities. Responses to chemotherapy can vary significantly between individuals, which has important clinical implications. Accurate dose calculation reduces the likelihood of toxic drug reactions [3]. Five key conditions guide treatment decisions: the right drug, the right patient, the right dosage, the right administration route, and the right timing [4]. This framework helps clinicians optimize therapy and avoid errors, as excessively low doses lack therapeutic efficacy and impact overall survival and recurrence-free periods, while excessively high doses increase toxicity, potentially leading to fatal outcomes.

Doxorubicin (DOX), chemically known as (2R,4S)-4-(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyloxy)-2-hydroxyacetyl-1,2,3,4-tetrahydro-2,5,12-trihydroxy-7-methoxynaphthacene-6,11-dione, is an anthracycline antibiotic produced by Streptomyces peucetius var. caesius. It is one of the most critical anticancer drugs in clinical practice due to its broad spectrum of antineoplastic activity [5-8].

## Materials and Methods

**Patients:** To determine doxorubicin levels in biological fluids (blood and urine), a group of 10 lung cancer patients undergoing palliative treatment with doxorubicin was formed.

**Sample collection:** Blood plasma (2 ml, purple-top tube) was collected at the following time intervals: 0 (before treatment), 20, 40, 60, 120, and 180 minutes, and 24, 48, and 72 hours after infusion completion (doxorubicin infusion lasted 1 hour). Blood samples were allowed to clot at room temperature for 30 minutes, then centrifuged at 2500rpm for 10 minutes and frozen at –20°C until analysis.

**Doxorubicin analysis by Amperometric Titration (AT):** AT was employed to detect doxorubicin in blood and urine samples. All solvents were of analytical grade. The following reagents were used: **Doxorubicin hydrochloride** (Doxorubicin HCl, ≥98%, Irvin 2, Russia);

i. **Titrants:** iron (III) chloride ($FeCl_3$, 0.01–0.1 M), cerium (IV) sulfate ($Ce(SO_4)_2$, 0.01–0.1 M), potassium permanganate ($KMnO_4$, 0.01–0.1M);

ii. **Buffer solutions:** phosphate buffer (PBS, 0.1M, pH 4.0, 7.0, 9.0, prepared using $NaH_2PO_4$ and $Na_2HPO_4$), acetate buffer (0.1 M, pH 4.0–5.5, based on acetic acid $CH_3COOH$ and sodium acetate $CH_3COONa$);

iii. **Electrolytes:** KCl (3.0 M) for the Ag/AgCl electrode, $Na_2SO_4$ (0.1 M) for conductivity;

iv. **Additional media:** urine (pH 6.0–7.5), cerebrospinal fluid (CSF, prepared under sterile conditions), cell culture medium (DMEM + 10% FBS);

v. **Auxiliary reagents:** distilled and deionized water, ethanol ($C_2H_5OH$, 96%) for electrode surface cleaning, $H_2SO_4$ and NaOH (0.1 M) for pH adjustment.

**Sample and standard preparation:** Biological samples (blood plasma, urine and other fluids) were obtained from clinical volunteers at the Jizzakh Branch of the Republican Scientific and Practical Medical Center of Oncology and Radiology. Informed consent was obtained from patients prior to sample collection, based on previous studies [9,10]. Patients (average weight 60±2 kg) received doxorubicin at a dose of 40 mg via slow intravenous infusion. A control blood sample was collected before drug administration (0 hours). Subsequent blood samples containing doxorubicin were collected at 0.5, 1, and 2 hours post-administration. Samples were diluted with PBS buffer (pH 4) from 1.0 ml to 2.0 ml for electrochemical analysis without pretreatment. Urine was collected at 6, 12, 24 (Day 1), 48, 72 (Day 3), and 96 hours (Day 5) post-infusion and stored at –20°C.

## Methodology of Amperometric Titration of Doxorubicin

### Equipment and reagents

i. Electrochemical measuring device (Titrion 1/1 potentiometer)

ii. **Working electrode:** platinum, graphite, or mercury-coated electrode

iii. **Auxiliary electrode:** platinum wire or graphite electrode

iv. **Reference electrode:** Ag/AgCl or calomel electrode

v. **Titrant:** oxidizing agents such as permanganate ($KMnO_4$), ferric ions, or iodate ($IO_3^-$)

vi. **Electrolytic solution:** phosphate buffer (pH 7.4) or acetate buffer (pH 4.5);

vii. **Doxorubicin solution:** standard solution of known concentration (0.1–1.0mM).

### Experimental conditions

i. **Temperature:** 25°C ± 2°C

ii. **pH:** 4.5–7.4 (selected based on titrant)

iii. **Light exposure:** Doxorubicin may degrade under light, so experiments were conducted in a light-protected environment

iv. **Stirring:** Constant stirring was maintained using a magnetic stirrer.

### Procedure

#### Electrode preparation

i. The working electrode was washed with distilled water and ethanol.

ii. For platinum electrodes, cleaning was performed with an $H_2SO_4/HNO_3$ mixture followed by water rinsing.


How to cite this article: Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891

iii. For mercury-coated electrodes, a fresh mercury layer was applied.

**Doxorubicin solution preparation**

i. Doxorubicin hydrochloride or its base was dissolved in 0.1M phosphate buffer.

ii. M KCl or NaCl was added as an electrolyte.

**Titration process**

i. The electrode system was placed in an electrolytic cell.

ii. Electrode potential was set at 0.2–0.8 V (for oxidizing titrants) or -0.2 to -0.8V (for reducing titrants).

iii. Titrant ($KMnO_4$, $Fe^{3+}$, or $IO_3^-$) was added slowly using a micropipette.

iv. Current changes were measured and recorded after each titrant addition.

**Determination of Titration Endpoint**

i. An amperometric titration curve was plotted.

ii. The point of sharp current change was taken as the equivalence point.

iii. Doxorubicin concentration was calculated based on the titrant volume at the equivalence point.

## Analysis and Results

When studying the amperometric titration curve of doxorubicin Figure 1, a change in current strength was observed depending on the titrant volume, with the equivalence point (approximately 0.75 ml) considered the point where doxorubicin fully reacted. The influence of pH on doxorubicin under different conditions—amperometric titration at pH 4.0, 7.0, and 9.0—was also investigated. Electrodes used included glassy carbon electrode (GCE), boron-doped diamond (BDD), and platinum electrodes.

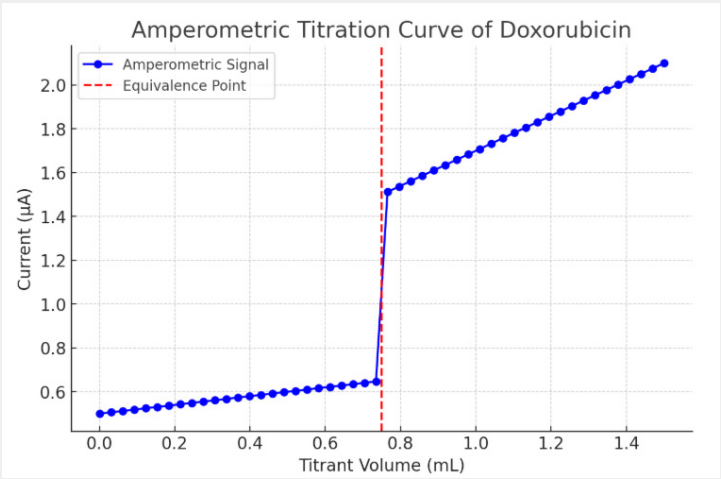


**Figure 1:** Amperometric Titration Curve of Doxorubicin.

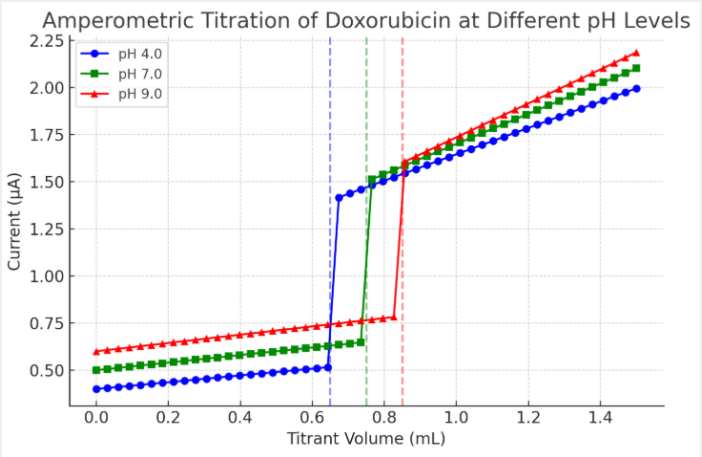


**Figure 2:** Amperometric Titration Curves of Doxorubicin at Different pH Levels (4.0, 7.0, 9.0).


How to cite this article: Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891

Figure 2 shows the amperometric titration curves of doxorubicin at various pH levels (4.0, 7.0, 9.0): at pH 4.0, the equivalence point was observed at 0.65ml; at pH 7.0, at 0.75ml; and at pH 9.0, at 0.85ml. As pH increased, more titrant was required to reach the equivalence point, indicating varying electrochemical reaction behavior of doxorubicin under different pH conditions. At pH 4.0, titration required less titrant and showed lower current strength. At pH 9.0, doxorubicin was less prone to oxidation or reduction, resulting in a stronger signal.

The amperometric titration curves of doxorubicin with different electrodes (GCE, BDD, Pt) Figure 3 were analyzed as follows: with the glassy carbon electrode (GCE), the equivalence point was at 0.70ml (current growth was slower with excess titrant); with the boron-doped diamond electrode (BDD), it shifted to 0.80 ml (offering a strong current signal and high sensitivity); with the platinum electrode (Pt), it was at 0.75 ml (showing hybrid properties with moderate sensitivity and stability). The BDD electrode provided high sensitivity and accuracy due to its wide potential range. The GCE produced lower current but was easier to handle. The platinum electrode (Pt) was versatile, offering stable results but at a higher cost.

Amperometric titration of doxorubicin with different titrants ($Fe^{3+}$, $Ce^{4+}$, $MnO_4^-$) Figure 4 showed: with $Fe^{3+}$ (iron(III) ion), the equivalence point was at 0.70 ml (moderate oxidative effect, lower current signal); with $Ce^{4+}$ (cerium(IV) ion), it was at 0.75 ml (moderate current signal due to higher oxidative capacity); with $MnO_4^-$ (permanganate ion), it was at 0.80 ml (strongest oxidant, higher current growth and sensitivity). $Fe^{3+}$ was a mild oxidant requiring less titrant volume. $Ce^{4+}$ was a balanced oxidant suitable for universal use. $MnO_4^-$, the strongest oxidant, provided a high current signal and sensitivity.

In biological media (blood and urine) Figure 5, the equivalence point for blood samples was 0.85 ml (proteins and biocomponents slowed titration), while for urine, it was 0.75 ml (titration occurred faster due to degradation processes). Titration in blood required more titrant due to biological molecule interference. In urine, it proceeded more quickly and accurately. The influence of biocomponents must be considered, as doxorubicin molecules may dissolve differently in blood and urine.

Results of amperometric titration at different doxorubicin concentrations (10μM, 50μM, 100μM) Figure 6 showed: at 10μM, the equivalence point was 0.65ml (slow and low current growth); at 50μM, it was 0.75 ml (moderate signal with high sensitivity); at 100μM, it was 0.85ml (faster and more intense current growth). As concentration increased, current growth dynamics intensified, and the titration point shifted. At 100μM, a strong signal indicated high electrode interaction. At 10μM, sensitivity was lower, with the titration point reached earlier

At very low concentrations (1 μM, 5 μM, 10 μM) Figure 7: at 1μM, the equivalence point was 0.50 ml (very weak current signal, minor changes); at 5 μM, it was 0.60 ml (moderate current response, better sensitivity); at 10μM, it was 0.70ml (more noticeable and stable current increase). At 1μM, current change was minimal with low accuracy. Between 5–10μM, titration was more detectable with better current expression. Highly sensitive electrodes (BDD, modified GCE) are recommended for very low concentrations. At high concentrations (100μM, 250μM, 500μM) Figure 8: at 100μM, the equivalence point was 0.85 ml (moderate current signal, noticeable oxidation); at 250μM, it was 1.10ml (stronger current signal, stable titration response); at 500μM, it was 1.50ml (sharp current increase, high sensitivity). At higher concentrations, the titration point shifted right, requiring more titrant. The current signal increased significantly, indicating doxorubicin oxidation at high concentrations. Electrode passivation is likely, so frequent cleaning or rotating electrodes are recommended.

In physiological conditions (blood plasma and buffer) Figure 9: in blood plasma, the equivalence point was 0.90ml, with slower current increase due to protein and biocomponent effects on the electrode surface. In buffer, it was 0.75ml, with faster and clearer current increase due to an alkaline environment. Titration in plasma was slower due to biomolecule adsorption. In buffer, it was faster and more accurate, lacking excess proteins or biocomponents. BDD or modified GCE electrodes yield better results in plasma.

In various biological media (urine, cerebrospinal fluid (CSF), cell culture) Figure 10: in urine, the equivalence point was 0.80ml with a moderate current response, affected by ions and metabolites. In CSF, it was 0.70ml (high sensitivity, fewer biomolecules, stable ionic composition). In cell culture, it was 0.85ml with the highest current due to vitamins, glucose, and buffer systems. Titration in CSF was clear and stable with sharp current increase. In cell culture, doxorubicin interacted with components, affecting the titration point. In urine, titration was more complex due to variable composition and metabolites.

The effect of temperature on doxorubicin titration Figure 11 showed: at 25°C (standard lab temperature), the equivalence point was 0.85ml with slow current growth and low reaction dynamics. At 37°C (body temperature), it was 0.75ml with a stronger current signal and faster titration. At 50°C (high temperature), it was 0.65ml with significantly accelerated reaction, though electrode passivation or solution instability was possible. Higher temperatures shifted the equivalence point left due to faster reaction kinetics. 37°C was optimal, aligning with physiological conditions. At 50°C and above, undesirable effects like electrode passivation or oxidation disruptions may occur, requiring surface cleaning and parameter control.


How to cite this article: Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891

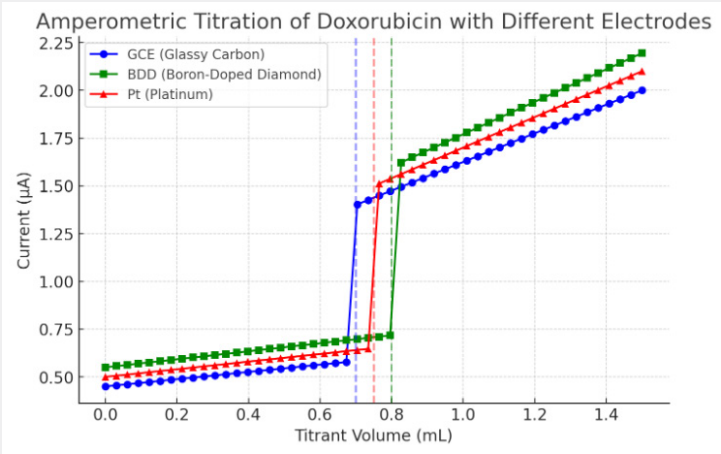


**Figure 3:** Amperometric Titration Curves of Doxorubicin with Different Electrodes (GCE, BDD, Pt).

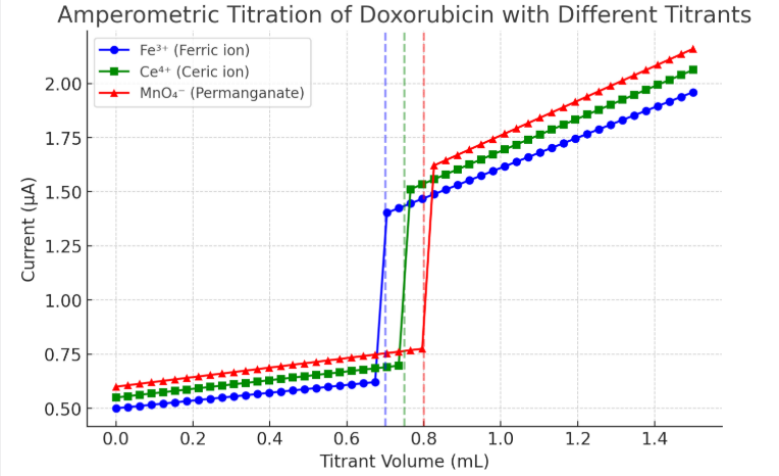


**Figure 4:** Amperometric Titration Curves of Doxorubicin with Different Titrants (Fe³□, Ce⁴□, MnO□□).

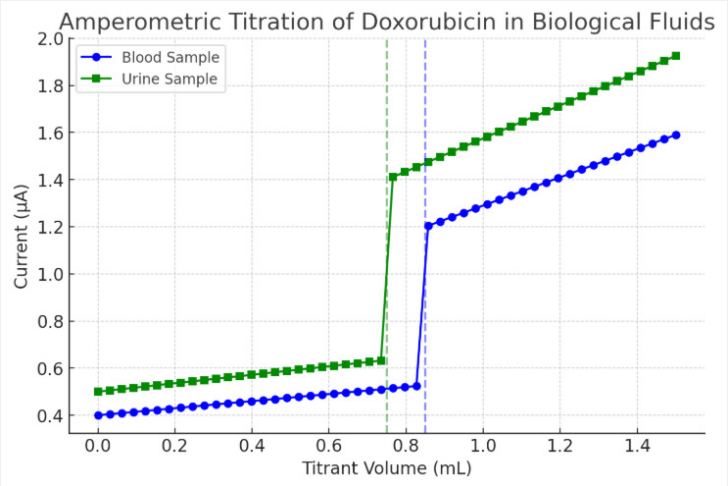


**Figure 5:** Amperometric Titration Curves of Doxorubicin in Biological Media (Blood and Urine).


How to cite this article: Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891

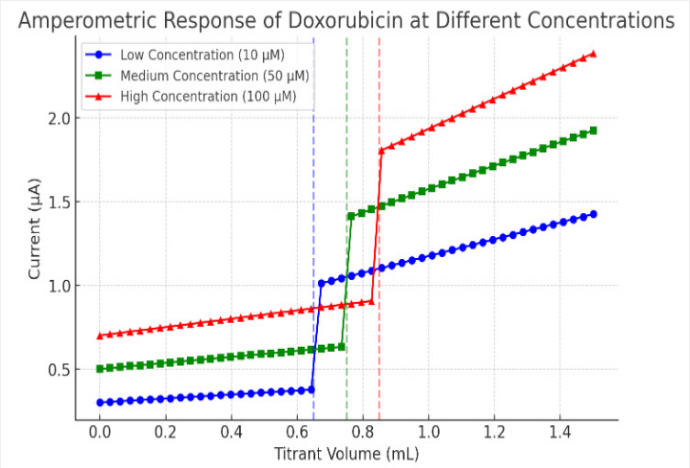


**Figure 6:** Amperometric Titration Curves of Doxorubicin at Different Concentrations (10µM, 50µM, 100µM).

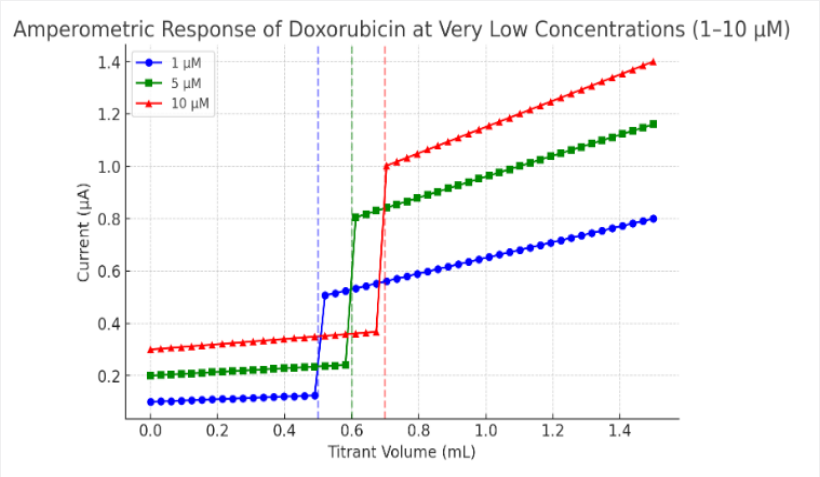


**Figure 7:** Amperometric Titration Curves of Doxorubicin at Very Low Concentrations (1µM, 5µM, 10µM).

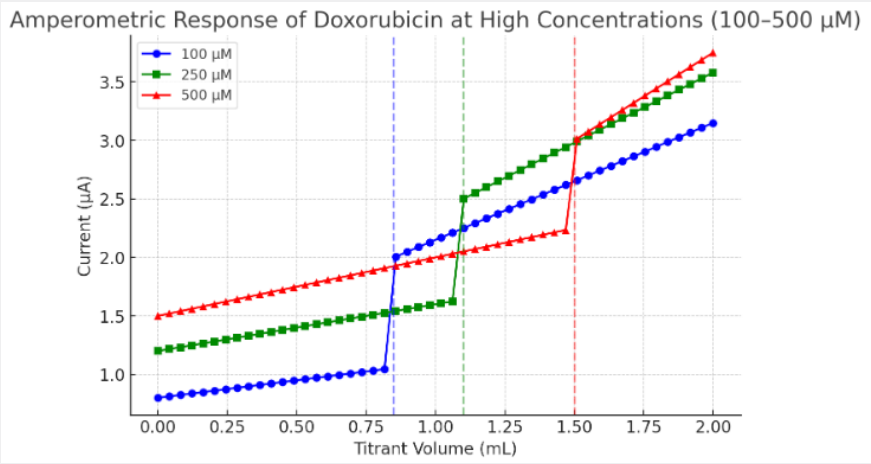


**Figure 8:** Amperometric Titration Curves of Doxorubicin at High Concentrations (100µM, 250µM, 500µM).



**How to cite this article:** Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891

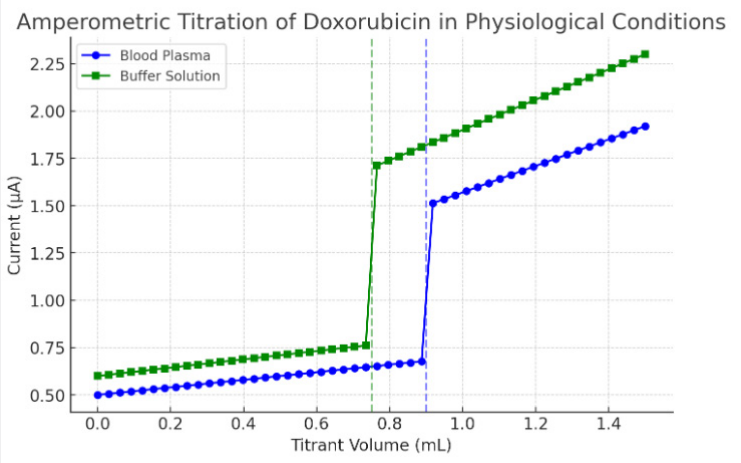


**Figure 9:** Amperometric Titration Curves of Doxorubicin in Physiological Conditions (Blood Plasma and Buffer).

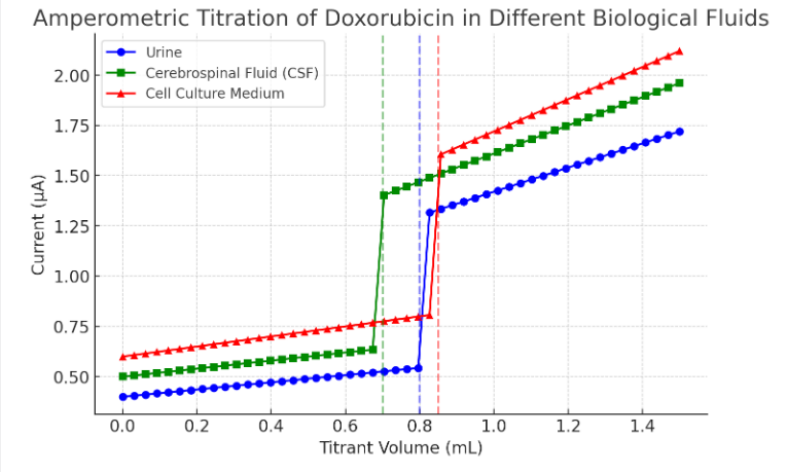


**Figure 10:** Amperometric Titration Curves of Doxorubicin in Various Biological Media (Urine, CSF, Cell Culture).

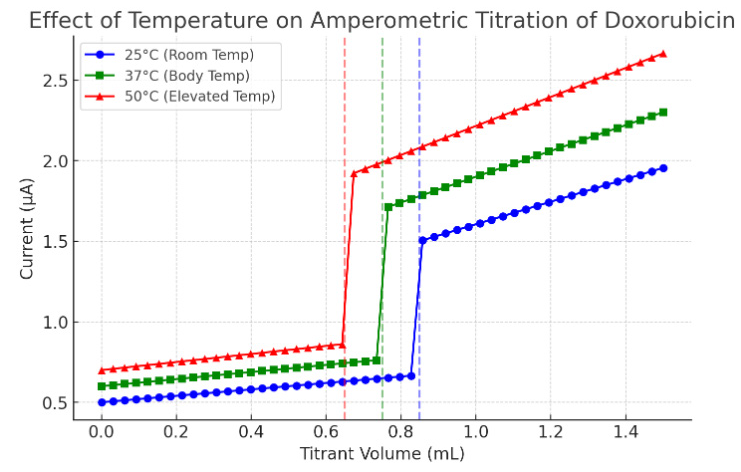


**Figure 11:** Effect of Temperature on Amperometric Titration of Doxorubicin.


How to cite this article: Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891

## Conclusion

Accurate chemotherapeutic drug dosing is critical, as excessively high doses can cause severe side effects or death, while overly low doses reduce treatment efficacy and survival rates. Precise dose determination remains controversial, whether based on established formulas or considering the described toxicity of chemotherapeutic drugs when patients receive inadequate doses. Studies on obese patients often avoid calculated doses due to fear of side effects, though this is not always justified. This underscores the need for new, precise methods to calculate chemotherapeutic drug dose.

## References


1. Parkin DM, Bray F, Ferlay J, (2005) Global cancer statistics, 2002. CA Cancer 55(2): 74-108.
2. World Health Report (2009) Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: World Health Organization.
3. Favier M, de Cazanove F, Saint Martin F (1994) Preventing medication errors in antineoplastic therapy. Am J Hosp Pharm 51(6): 832-833.
4. Hilan Dandan R, Brunton LL, Goodman LS (2014) Goodman Gilman's Manual of Pharmacology and Therapeutics. McGraw Hill, New York.
5. MR Alcaráz, AV Schenone, MJ Culzoni, HC Goicoechea (2014) Modeling of second-order spectrophotometric data generated by a pH-gradient flow injection technique for the determination of doxorubicin in human plasma. Microchemical Journal 112: 25-33.
6. J Hofman, A Skarka, J Havrankova, V Wsol (2015) Pharmacokinetic interactions of breast cancer chemotherapeutics with human doxorubicin reductases. Biochemical Pharmacology 96(3): 168-178.
7. RA El Awady, MH Semreen, MM Saber Ayad, F Cyprian, V Menon, et al. (2016) Modulation of DNA damage response and induction of apoptosis mediates synergism between doxorubicin and a new imidazopyridine derivative in breast and lung cancer cells. DNA Repair 37: 1-11.
8. SA Moosavian, K Abnous, A Badiee, MR Jaafari (2016) Improvement in the drug delivery and anti-tumor efficacy of PEGylated liposomal doxorubicin by targeting RNA aptamers in mice bearing breast tumor model. Colloids and Surfaces B: Biointerfaces 139: 228.
9. De Jongh FE, Verweij J, Loos WJ (2001) Body surface area-based dosing does not increase accuracy of predicting cisplatin exposure. J Clin Oncol 19(17): 3733-3739.
10. Drott C, Persson H, Lundholm K (1989) Cardiovascular and metabolic response to adrenaline infusion in weight-losing patients with and without cancer. Clin Physiol 9(5): 427-439.


This work is licensed under Creative Commons Attribution 4.0 License
DOI: 10.19080/OMCIJ.2026.14.555891

**Your next submission with Juniper Publishers will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attai nment for your research
- Manuscript accessibility in different formats
  **( Pdf, E-pub, Full Text, Audio)**
- Unceasing customer service

**Track the below URL for one-step submission**

https://juniperpublishers.com/online-submission.php



**How to cite this article:** Bakakhonov Anvar A, Gaziev Laziz T. Determination of Doxorubicin Using Amperometric Titration. Organic & Medicinal Chem IJ. 2026; 14(4): 555891. DOI:10.19080/OMCIJ.2026.14.555891