Platinum and Cobalt hydroxide – modified Platinum Electrode as Sensor for Electrochemical Oxidation of Amoxicillin

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Abstract. Antibiotics has recently increased due to their use in the medical treatment process and the process of their disposal into the environment. One of the antibiotics that represents the research subject is amoxicillin. The scan rate and pH in the electrochemical oxidation of amoxicillin on Pt disc electrode and cobalt hydroxide modified-Pt electrode has been studied. The current peak from voltammetry measurement indicated that modification on electrode surface could improve the electrochemical response and sensitivity of the working electrodes because of its electrocatalytic effect. The Pt/Co(OH)\textsubscript{2} modification working electrode displayed high sensitivity in electro-oxidation determination of amoxicillin with a linear range of the sensor was from 20 to 80 µM, with the limit of detection 7.15 µM for Pt disc electrode and 3.64 µM for Pt/Co(OH)\textsubscript{2} electrode. The comparing method with HPLC shows that the results of determination in real samples with the electro-oxidation using a modified electrode were in good agreement with a confidence level at 95%.

Keywords: Amoxicillin, voltammetry, electrochemical oxidation, electro-catalytic, platinum

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Introduction

Intensive use of antibiotics and their benefits in health services to treat diseases and to enhance the animal growth give some problems, namely the accumulation of water pollution in the environment. The residual of antibiotics appears in the water as wastewater, as a result of ineffective wastewater treatment. This antibiotic wastewater can pose a risk to the environment due to its wide use, resistance to natural biodegradation, and bacterial resistance. The most widely used antibiotic to treat various kinds of bacterial infections is amoxicillin which has a beta-lactam bond and one of the semisynthetic penicillin antibiotics which is stable in acidic conditions has been detected on the surface of the water and shows resistance to biological waste treatment [1]–[6].

In some research, various analytical methods for determining amoxicillin in the different matrix such as micellar electro kinetics chromatography [7], spectrophotometry [3], [8], [9], and high-performance liquid chromatography (HPLC) [10]–[13] have been reported. Although these methods very sensitive and accurate with a lower detection limit but the operation of the instrument quite complicated, long analysis time, expensive, and complicated for on-site measurements, and sample preparation is complicated, need a large quantity of high purity organic solvents, long equilibration, and derivation treatment. Furthermore, the low solubility of amoxicillin in organic solvents makes developing extraction and pre-concentration procedures difficult, thus need to develop an alternative and efficient method.

Voltammetry, is one of electrochemical methods which the analysis completed in a short analysis time, determines the analyte directly, and does not require any particular pre-treatment of the sample. The determination of amoxicillin using voltammetry techniques has already been described [14]–[18] with the goal of improving the electrode's selectivity and sensitivity. The electrocatalytic process at the surface of modified electrode increases the reaction kinetics chemically [19]–[22].

Amoxicillin electrocatalytic oxidation using a mediator on metal electrodes is still uncommon; most electrocatalytic oxidation processes use carbon paste electrodes modified by polymer compounds in the form of molecularly imprinted polymers. According to the literature, cobalt hydroxide has a high conductivity and can act as an electron transport mediator in electrocatalytic properties [19]–[21]. So, the goal of this work is to modify the electrode and focus on its performance in

![Figure 1. EDS characterization of (a) Pt and (b) Pt/Co(OH)$_2$ electrode](image-url)
Electrochemical oxidation to determine amoxicillin at the electrode surface. The method’s practical application is demonstrated by determining the concentration of amoxicillin in wastewater and comparing the results to those obtained using HPLC methods.

### Experimental

#### Material

Amoxicillin trihydrate was obtained from Kalbe Farma pharmaceuticals, along with potassium dihydrogen phosphate (> 99.5 percent), potassium hydrogen phosphate (> 98.5 percent), sodium sulfate, cobalt chloride, methanol HPLC grade (Merck), and other chemicals available as analytical grade and pro analysis grade (Merck and Sigma Aldrich), and was used as received for the experiment with no further treatment. All stock amoxicillin and buffer solutions, as well as all solutions used in this study, were prepared with bidistilled water. All of the experiments were conducted at room temperature (25.8°C). Wastewater containing amoxicillin was obtained from a pharmaceutical facility in Bandung, Indonesia.

#### Preparation of working electrode

The working electrode used is a Pt electrode that has been sanded and soaked for about 3 minutes in concentrated nitric acid, then rinsed with bidistilled water and sonicated for 15 minutes in alcohol then rinsed again and dried. Meanwhile, the Pt/Co(OH)₂ electrode was prepared by coating Pt wire with cyclic voltammetry in a solution 0.1 M Na₂CO₃, 40 mM sodium-potassium-tartrate, and 4 mM CoCl₂ at pH 11 in a 0.1 M NaOH as supporting electrolyte solution. [20], [23], [24] The auxiliary electrode was a Pt wire and the reference electrode (RE) was Ag/AgCl electrode. Both working electrode were characterized in K₃[Fe(CN)₆]/K₄[Fe(CN)₆] solution with NaCl as supporting electrolyte.

#### Sample preparations and measurements

Amoxicillin wastewater was centrifuged at 600 rpm for 15 minutes before being vacuum filtered through a PTFE membrane filter pore size 0.45 μm and diluted with pyrogen-free aqua pro injection. The sample was dissolved in a supporting electrolyte 0.1 M Na₂SO₄ and transferred to the electrochemical cell for voltammetry analysis, and the amoxicillin wastewater was determined using the standard addition method, which involved the addition of 10 - 20 M of amoxicillin standard. Sample then directly analyzed using DPV on a Potensiostat eDAQ 410 and Echem version 2.1.5 software in the potential range of 0 to 1200 mV at a scan rate of 100 mV/s with a Pt disc electrode and a Pt/Co(OH)₂ electrode. Measurement of amoxicillin wastewater was carried out by HPLC Infinity Agilent Tech. 1260 series with detector G1315 5D (1260 QUAT Pump VL), Phenomenex LC column Gemini 5u C18 110 A 150 x 4.6 mm, manual injector. The pump type is G7111A (1260QUAT Pump VL). Determination amoxicillin with HPLC was carried out using ammonium acetate 20 mM pH 4 and methanol with a ratio of 50:50 and a flow rate of 1.0 mL/min.

![Cyclic voltammogram of a mixture of K₃[Fe(CN)₆] and K₄[Fe(CN)₆] 0.01 M in 0.1 M NaCl with a scan rate of 100 mV/s using Pt and Pt/Co(OH)₂ as working electrode, Ag/AgCl electrode as reference electrode, and Pt wire as auxiliary electrode](image-url)
Results and Discussion

Characterization of Working Electrodes in Supporting Electrolyte

The surface of the disc Pt and Pt/Co(OH)₂ electrode were characterized using EDS. Pt disc electrode shows the composition of Pt 90.27%, and other components, namely C and O, respectively 8.44% and 1.29%. The presence of C and O in the composition of Pt wire because it was obtained from its supplier. Generally Pt wire was made by melting metal and twisted. At that time there may be CO₂ gas trapped in the Pt. EDS of the Pt/Co(OH)₂ electrode in the Figure 1 below shows the result with a percentage of Co elemental composition of 8.97%; Pt was 60.11% and other impurities such as C, O, Na and Si were 12.40%, 16.28%, 1.43% and 0.81%, respectively. This indicates that the cobalt has coated Pt. The presence of Si comes from the initial process of sanding the Pt wire with sandpaper and is still attached to the surface of the Pt wire during the cobalt coating process.

On Figure 2, Pt disc, the voltammogram shows Ipa value is 55.61 μA at Epa 0.2760 V and the Ipc value is 47.06 μA at Epc 0.1360 V. On Pt/Co(OH)₂ electrode, the voltammogram of Ipa value is 114 μA with Epa 0.2740 V and Ipc 112 μA and Epc 0.1340 V. Ipa/Ipc ratio for the disc electrode Pt is 1.18 and the Ipa/Ipc ratio for the disc electrode Pt/Co(OH)₂ is 1.01. A comparison of the two working electrode ratios of both the Pt and Pt/Co(OH)₂ disc electrodes shows that Ipa/Ipc ratio reaches a value of one indicating that the electrons involved in the reaction [Fe(CN)₆]³⁻/[Fe (CN)₆]⁴⁻ are one and that type of reaction is a reversible reaction. The formal potential difference between the two working electrodes of Pt and Pt/Co(OH)₂ is 0.0020 V, this shows that the working electrode of the Pt/Co(OH)₂ disc has the same performance as the working electrode of the Pt disc with a higher response increase in the working electrode of the Pt/Co(OH)₂ disc.

The supporting electrolyte function is to control the potential that applied in the measurement by reducing the electromigration effect of the solution and maintaining a constant ionic strength. Supporting electrolytes are inert can be derived from inorganic salts, mineral acids, or buffers, which are pure and should not be easily oxidized or reduced in the vicinity of the analyte potential [25], [26].

Pt disc and Pt/Co(OH)₂ electrode were characterized in supporting electrolyte solutions 0.1 M Na₂SO₄ in the potential range -200 mV to 1200 mV. The results in the Na₂SO₄ solution gives the highest anodic peak current response to amoxicillin electro-oxidation, which is shown in the Figure 1 (a) and 1(b). Results showed that oxidation potential of amoxicillin at Pt electrode have potential at 574 mV for Pt disc and 560 mV for Pt/Co(OH)₂ electrode vs. Ag/AgCl in 0.1 M Na₂SO₄. Therefore, the oxidation of amoxicillin at the surface of the Pt/Co(OH)₂ electrode occurs at

![Figure 3](image-url)  
*Figure 3.* Cyclic voltammogram of amoxicillin (AMX) for (a) Pt disc working electrode and (b) Pt/Co(OH)₂ electrode in 0.1 M Na₂SO₄, auxiliary electrode Pt wire, reference electrode Ag/AgCl, and scan rate 100 mV/s
a potential about 14 mV less positive than the Pt electrode disc shows the electrocatalytic in Pt/Co (OH)_2 electrode.

The current peak of oxidation from the cyclic voltammogram is increased in Figure 3a and 3b from 20.7 µA using Pt disc electrode to 79.3 µA with Pt/Co(OH)_2 electrode. From the voltammogram in Figure 3, the reduction current peak formed 3.4 µA at 140 mV for Pt disc electrode and 49.4 µA at 160 mV. This reduction current peak generated from the electro-oxidation product of amoxicillin at both electrode surfaces [27].

Based on the value of 0.059/(n) < ΔEp < 0.212 V, it can be concluded that the amoxicillin reaction using Pt disc electrode and Pt/Co(OH)_2 electrode in Na_2SO_4 solution is a type of irreversible reaction.[15]–[17], [28] For irreversible reactions, the electron transfer process is slow while for a quasi-reversible system the current is controlled both by electron transfer and mass transfer [25], [26]. Voltammograms both of the quasi-reversible and irreversible system type are described with a greater separation of potential peaks compared to reversible systems.

In an irreversible system, it will only form one current peak in the voltammogram. This is due to the slow transfer of electrons and slow equilibrium. Irreversible processes can occur in the reduction or oxidation of the analyte which produce non-electroactive compounds. The irreversible process is characterized by a value of ΔEp that is greater than 59/n mV where the value will increase according to the increase in the scan rate.

Figure 4. Voltammogram cyclic of 50 µM amoxicillin (AMX) in 0.1 M Na_2SO_4 using Pt disc electrode with different scan rate, Ag/AgCl as a reference electrode and Pt wire as an auxiliary electrode.

Figure 5. (a) Effect of a square root of scan rate to current response and (b) the relationship between log v and log I_p for working electrode Pt disc

\[
y = 0.9984 v^{1/2} + 0.030 \\
R^2 = 0.9912
\]

\[
y = 0.9984 v^{1/2} - 0.1257 \\
R^2 = 0.9683
\]
From the results of electroactivity investigations on electro-oxidation from Pt disc and Pt/Co(OH)$_2$ electrodes by cyclic voltammetry, it was obtained that amoxicillin oxidation peaks at 574 mV and 560 mV peaks in Na$_2$SO$_4$ and reduction peaks at 140 mV and 160 mV. As well as Chiu et al. [29] showed the voltammogram of amoxicillin oxidation with MnO$_2$ have a oxidation peak at 0.6 V and a reduction peak at +0.1 V on a screen-printed carbon (SPC) electrode in 0.1 M H$_3$PO$_4$.

**Scan rate**

The scan rate is carried out to determine the electrochemical mechanism that occurs on the electrode surface, whether it is controlled by diffusion or adsorption process. The effect of the scan rate was carried out by measuring 50 μM of amoxicillin using cyclic voltammetry in the potential range of -200 mV to 1200 mV with varied scan rates. The cyclic voltammogram for this measurement was carried out in a supporting electrolyte Na$_2$SO$_4$ solution using a Pt working electrode and a Pt/Co(OH)$_2$ electrode can be seen in the Figure 2.

From data in Figure 4, for the Pt disc electrode, the effect of the anodic peak current on the scan rate of amoxicillin shows a linear relationship ($R^2 = 0.9605$) as shown in Figure 5a. A linear relationship is obtained from the anodic peak current to the root of scan rate ($R^2 = 0.9910$), shown in Figure 5b. This shows that the electro-oxidation

![Figure 7. Voltammogram cyclic of 50 μM amoxicillin (AMX) in Na$_2$SO$_4$ 0.1 M using Pt/Co(OH)$_2$ electrode with different scan rate, Ag/AgCl as a reference electrode and Pt wire as an auxiliary electrode.](image)

![Figure 8. (a) Effect of a square root of scan rate to current response and (b) the relationship between log $v$ and log $I_p$ for working electrode Pt/Co(OH)$_2$](image)
reaction of amoxicillin on the surface of the Pt disc electrode is controlled by the diffusion process. The plot of the logarithm of the scan rate against the logarithm of the anodic peak current of amoxicillin gives a slope of 0.5003 with $R^2 = 0.9930$ where the slope value approaches the theoretical value of 0.5 for a process controlled by the diffusion process. [14], [15], [30] This re-affirms that the amoxicillin oxidation reaction on the working electrode surface of the Pt disc is controlled by the diffusion process.

For the generated of electro-oxidation amoxicillin, reduction process on the working electrode surface of the Pt disc, the effect of the cathodic peak current on the scan rate of amoxicillin shows a linear relationship ($R^2 = 0.9558$) as shown in Figure 5a. However, the plot between the peak cathodic currents against the root of scan rate ($R^2 = 0.9680$) shows a more linear relationship in the scan rate range of 10 - 100 mV/s as shown in Figure 5b. This shows that the generated electro-oxidation amoxicillin reaction on the surface of the Pt disc electrode is controlled by the diffusion process. The plot of the logarithm of the scan rate against the logarithm of the cathodic peak current of amoxicillin gives a slope of 0.5353 with $R^2 = 0.9637$ (Figure 3b) where the slope value approaches the theoretical value of 0.5 for a diffusion-controlled process.

The kinetics of the electrodes verify that diffusion is the controlling factor for mass transport. Where diffusion flow control is a process where there is a difference in concentration between the electrode surface and the concentration in the body of the solution. Observations show that in the electro-oxidation of amoxicillin using a Pt disc working electrode, as the scan rate increases, the redox peak current also increases gradually, as shown in the voltammogram in Figure 4.

For the modified working electrode, Pt/Co(OH)$_2$, it showed that as the scan rate increases, the anodic peak potentials shift to positive value and the cathodic peak potentials are converted to a negative value. The kinetics of the amoxicillin reaction at the Pt/Co(OH)$_2$ electrode which is the controlling factor for mass transport is the diffusion process on the surface of the Pt/Co(OH)$_2$ electrode as seen in the voltammogram in Figure 7.

The effect of anodic current on the scan rate of amoxicillin shows a linear relationship ($R^2 = 0.9615$) shown in Figure 8a. Meanwhile the plot between the anodic peak current to the root of scan rate ($R^2 = 0.9517$) in range of 10 - 100 mV/s is shown in Figure 8b. This shows that the electro-oxidation reaction of amoxicillin on the surface of the Pt/Co(OH)$_2$ electrode is controlled by the diffusion process.

![Figure 9](image_url) **Figure 9.** Effect of the pH to current response of 50 μM amoxicillin in 0.1 M phosphate buffer solution using working Pt disc electrode using cyclic voltammetry with scan rate 10 mV/s, Ag/AgCl as a reference electrode and Pt wire as an auxiliary electrode.

![Figure 10](image_url) **Figure 10.** Effect of pH to current response of 50 μM amoxicillin in 0.1 M phosphate buffer solution using working Pt disc electrode using cyclic voltammetry with scan rate 10 mV/s, Ag/AgCl as a reference electrode and Pt wire as an auxiliary electrode.
log Ipa (µA) = 0.6814 log v (mV.s⁻¹) – 2.5674 (Eq.1)

The plot of the logarithm of the scan rate against the logarithm of the anodic peak current of amoxicillin gives a slope of 0.6814 with R² = 0.9682 where the slope value approaches the theoretical value of 0.5 for processes controlled by the diffusion process [14] [21].

For the generated electro-oxidation of amoxicillin in reduction process on the working electrode surface of the Pt/Co(OH)₂, the effect of the cathodic peak current on the scan rate of amoxicillin shows a linear relationship (R² = 0.9911) shown in Figure 8a. However, from the plot between the anodic peak current to the root trace rate (R² = 0.9503) shows a linear relationship in the scan range of 10 - 100 mV/s as shown in Figure 8b. This shows that the the generated electro-oxidation of amoxicillin on Pt/Co(OH)₂ electrode surface controlled by the adsorption process. The plot of the logarithm of the scan rate against the logarithm of the cathodic peak current of amoxicillin gives a slope of 1.2297 with R² = 0.9952 (Figure 8b) where the slope value shows the process controlled by the adsorption process.

The electrochemical response of Pt/Co(OH)₂ electrode in 0.1 M Na₂SO₄ shows anodic and cathodic peaks (Figure 8) there is an increase in the anodic peak current and a decrease in the cathodic peak current indicated that the mediated oxidation happens. When the scan rate increased, the peak potential for the catalytic oxidation of amoxicillin shifts to the positive potentials, suggesting a kinetic limitation in the reaction between the redox sites of the Pt/Co(OH)₂ electrode surface and amoxicillin.

**Effect of pH**

The effect of pH gave a significant effect to oxidation peak current and oxidation potential of amoxicillin molecule on modified electrode. Figure 9 shows the dependence of peak current on the pH in the range 2-7 due to amoxicillin’s pKa value. [31], [32] The influence of pH is known that anodic potential is shifted linearly toward the less positive to the increasing of pH value, giving rise to a slope of -58 mV/pH unit for Pt electrode disc and -60.6 mV/pH for Pt/Co(OH)₂ electrode (Figure 10) and it indicated that the oxidation of amoxicillin process involves 1e⁻. This slope approximately suggests that the number of electron transfer is equal to hydrogen ion that taking part in the electrode reaction.

The increase in peak current and decrease in oxidation potential indicates the electrocatalytic effect of the working electrode on the amoxicillin oxidation process. The generated product of amoxicillin oxidation is maintained on the surface of the electrode, [15][24] so there is its reduction of around a potential of 0.20 V for the

**Figure 11.** Voltammogram of amoxicillin (AMX) in different concentration with DPV scan rate 50mV/s, Pt wire as an auxiliary electrode and Ag/AgCl electrode as a reference electrode and current response at the range concentration of amoxicillin at 20 - 80 µM using Pt disc electrode.

**Figure 12.** Voltammogram of amoxicillin (AMX) in different concentration with DPV 50mV/s, Pt wire as an auxiliary electrode and Ag/AgCl electrode as a reference electrode and current response at the range concentration of amoxicillin at 20 - 80 µM using Pt/Co(OH)₂ electrode.
Pt disc electrode and 0.35 V for the Pt/Co(OH)$_2$ electrode when the reverse scan is performed as in previous voltammogram in Figure 2 and Figure 4.

Reproducibility

The repeatability test of one working electrode, both Pt and Pt/Co(OH)$_2$, was carried out by measuring 100 µM amoxicillin solution 10 times. The two working electrodes showed good repeatability with %RSD of 4.259 for the Pt working electrode and 6.279 for the Pt/Co(OH)$_2$ working electrode. The results of the repeatability test of one Pt/Pt/Co(OH)$_2$ working electrode showed that the working electrode had good stability and repeatability on amoxicillin measurements. The difference that occurs at the Pt/Co(OH)$_2$ working electrode is influenced by the thickness of the cobalt hydroxide layer attached to the surface of the Pt wire.

Linear range and limit of detection of working electrode

The linear range was studied by DPV under optimized condition. From the experimental, it was found that Pt disc electrode has a linear range concentration at 20 – 80 µM (Figure 11) and furnished a linear model of $i_{pa}$ (µA) = (0.0138 [amoxicillin] (µM) + 6.10913 x 10$^{-5}$) + (0.3825 + 0.0329) (R$^2$ = 0.9902). In the range concentration, the largest gradient value is obtained. The obtained gradient shows the measurement sensitivity, the smaller gradient shows that the sensitivity is lower, while the larger gradient show the better working electrode sensitivity. The LOD of modified electrode was found at 7.15 µM.

For Pt/Co(OH)$_2$ electrode has a linear range concentration at 20 – 80 µM (Figure 12) and give a linear model of $i_{pa}$ (mA) = 0.0002 [amoxicillin] (µM) ± 4.5062 x 10$^{-5}$ + 0.0011 ± 2.4267 x 10$^{-4}$ (R$^2$=0.9935). In the range concentration, the largest gradient value is obtained. The obtained gradient shows the measurement sensitivity. And for the sensitivity it shows that Pt/Co(OH)$_2$ electrode higher than the Pt electrode. The LOD of modified electrode, Pt/Co(OH)$_2$, was found at 3.64 µM.

Real sample analysis

The modified electrode was finally applied to the amoxicillin analysis in pharmacy wastewater. Table 1 shows the content of amoxicillin in spiked wastewater sample, obtained by the standard addition method using 2 different electrodes. And the comparison of amoxicillin measurements between voltammetry and HPLC methods.

Ojani et.al [22] used Ni/Curcumin/CPE modified electrodes showing LOD values of 5 µM for a working area of 8 to 100 µM with Amperometry and cyclic voltammetry techniques and provided measurement results for the determination of amoxicillin as shown in Table 2.

As a modified electrode result, the average recovery of detection amoxicillin ranged between 97.6 % and 100.1 %. Table 1 shows that the sample t test values for amoxicillin wastewater have a smaller value than the t table value at the 95% confidence level. (t exp for Pt disc electrode 1.00 and for Pt/Co(OH)$_2$ is 1.25, where the value of t table at the 95% confidence level (n = 3) is 2.776). Based on these results, it can be concluded that the measurement of amoxicillin in wastewater using Pt disc and Pt/Co(OH)$_2$ electrode and comparison methods did not provide a significant difference of the two methods. These results indicated that the prepared censor could be employed for trace analysis of amoxicillin in real samples.

Conclusion

The results show that Pt disc and Pt modified cobalthydroxide electrode can be electro-oxidized the amoxicillin on the surface electrodes. The performance of the modified electrode was significantly better than Pt disc electrode because it proves more sensitive. The

<table>
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<th>Samples (µM)</th>
<th>Voltammetry (WE: Pt)</th>
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<td>Found (µM)</td>
<td>Recovery %</td>
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<td>36.7 ± 0.1</td>
<td>99.5 ± 0.3</td>
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A = amoxicillin (AMX) wastewater

Table 1. The comparing results of determination of amoxicillin in samples
comparaison results of voltammetric determination of amoxicillin in real sample were in good agreement with HPLC methods. It is proving that modified electrode could be used as a good electrode for determination of amoxicillin by voltammetric methods.

Acknowledgements

The authors appreciatively acknowledge The Ministry of Research and Higher Education Republic of Indonesia for their grants and Analytical Research Group Laboratory, Institute Technology of Bandung (ITB) for analytical instrumentation facilities.

References


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<tr>
<th>Sample</th>
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<th>Amount found</th>
<th>Recovery (%)</th>
<th>RSD (%) (n=3)</th>
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<td>21.12 μM</td>
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