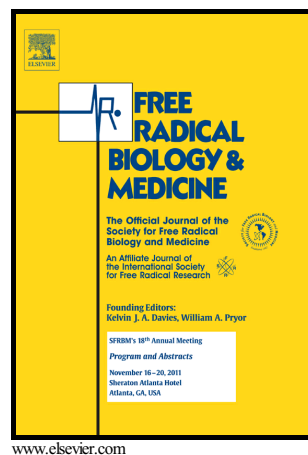


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Coordination and redox interactions of β -lactam antibiotics with Cu^{2+} in physiological settings and the impact on antibacterial activity

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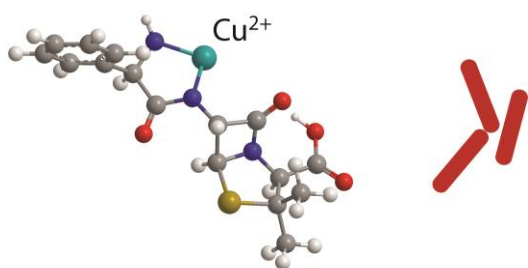
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Abstract

An increase in the copper pool in body fluids has been related to a number of pathological conditions, including infections. Copper ions may affect antibiotics via the formation of coordination bonds and/or redox reactions. Herein, we analyzed the interactions of Cu^{2+} with eight β -lactam antibiotics using UV-Vis spectrophotometry, EPR spectroscopy, and electrochemical methods. Penicillin G did not show any detectable interactions with Cu^{2+} . Ampicillin, amoxicillin and cephalexin formed stable colored complexes with octahedral coordination environment of Cu^{2+} with tetragonal distortion, and primary amine group as the site of coordinate bond formation. These β -lactams increased the solubility of Cu^{2+} in the phosphate buffer. Ceftazidime and Cu^{2+} formed a complex with a similar geometry and gave rise to an

organic radical. Ceftriaxone-Cu²⁺ complex appears to exhibit different geometry. All complexes showed 1:1 stoichiometry. Cefaclor reduced Cu²⁺ to Cu¹⁺ that further reacted with molecular oxygen to produce hydrogen peroxide. Finally, meropenem underwent degradation in the presence of copper. The analysis of activity against *Escherichia coli* and *Staphylococcus aureus* showed that the effects of meropenem, amoxicillin, ampicillin, and ceftriaxone were significantly hindered in the presence of copper ions. The interactions with copper ions should be taken into account regarding the problem of antibiotic resistance and in the selection of the most efficient antimicrobial therapy for patients with altered copper homeostasis.

Graphical abstract:



Keywords:

Antibiotic; Complex; Copper; EPR spectroscopy; Free radicals

1. Introduction

Copper is a redox-active metal with capacity to form 4-6 coordinate bonds. The concentration of labile copper (various redox-active Cu^{2+/1+} complexes with small ligands) in human blood plasma and other fluids is tightly regulated. However, copper is mobilized in infections, inflammation, and tissue damage [1,2], as well as in a number of other acute and chronic conditions, such as Wilson's disease, Alzheimer's disease, diabetes, and neonatal jaundice [3–5]. The potential role of copper in the well-known problem of antibiotics resistance has been pointed out recently [6]. Copper ions may directly affect the activity of antibiotics through coordination and/or redox interactions [6,7]. It has been observed that Cu²⁺ ions suppress the intestinal transport of some cephalosporins [8]. On the other hand, it has been reported that Cu²⁺ may promote antimicrobial and anti-inflammatory effects of cephalexin [9]. These effects have been

related to the formation of complexes. Interactions of Cu^{2+} with penicillins at low pH have also been reported [10,11]. It is worth mentioning that several studies have synthesized complexes of cephalosporins and penicillins with Cu^{2+} in organic solvents, with variable successes regarding the antimicrobial activity [12–16]. However, the interactions between copper ions and antibiotics under physiological conditions are still poorly understood. Detailed information on such interactions may be essential for elucidating the underlying mechanisms of antibiotic resistance and for selecting the most efficient therapy for bacterial infections, particularly in patients with conditions that are related to increased labile copper pool.

Herein, we analyzed coordination and redox interactions of Cu^{2+} with β -lactam antibiotics from three different classes (Fig. 1): penicillins (penicillin G, ampicillin, and amoxicillin), cephalosporins (cephalexin, cefaclor, ceftriaxone, and ceftazidime) and carbapenems (meropenem), in the phosphate buffer at physiological pH 7.4. In addition, we applied minimum inhibitory concentration (MIC) test to examine the impact of copper ions on the performance of β -lactam antibiotics against *E. coli* and *S. aureus*.

2. Materials and methods

2.1. Chemicals

$\text{CuCl}_2 \times 2\text{H}_2\text{O}$, antibiotics (reference standard purity), and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). All experiments were performed using bidistilled deionized ultrapure (18 M Ω) water. Phosphate buffer (50 mM) was prepared using KH_2PO_4 and KOH to adjust pH to 7.4. Stock solutions of antibiotics in the buffer were prepared daily, and kept light-protected on ice. Incubation and measurements were conducted in the dark at 293 K.

2.2. UV-Vis spectrophotometry

UV-Vis absorption spectra were obtained using 2501 PC Shimadzu spectrophotometer (Kyoto, Japan). Sample volume was 1 mL. Scan time was 50 s. Samples were freshly prepared and immediately scanned at wavelengths from 800 to 200 nm.

2.3. Oximetry

$[\text{O}_2]$ was determined using a Clark type oxygen electrode (Hansatech Instruments Ltd., King's Lynn, UK), operating with Lab Pro interface and Logger Pro 3 software (Vernier, Beaverton, OR, USA). All systems were stirred and recorded for 2–5 min before Cu^{2+} addition to establish

the stability of baseline and zero rate of O₂ change. Decrease in [O₂] was monitored for 30 min before the addition of catalase (Sigma-Aldrich; 100 IU/mL).

2.4. EPR spectroscopy

Low-T EPR spectra of Cu²⁺ were recorded under non-saturating power conditions, on a Bruker Elexsys II E540 spectrometer operating at X-band (9.4 GHz), using the Bruker N₂ Temperature Controller ER4131VT to maintain T at 110 K. The experimental parameters were: microwave power, 3.17 mW; scan time, 100 s; modulation amplitude, 0.5 mT; modulation frequency, 100 kHz; number of accumulations, 4. All spectra were baseline corrected. Samples were placed in quartz cuvettes (Wilmad-LabGlass, Vineland, NJ, USA), and frozen in cold isopentane after 5 min incubation period. The concentration of copper was 0.2 mM, whereas concentrations of antibiotics were 0.2 mM and 0.4 mM.

2.5. Cyclic voltammetry

The voltammetric measurements were performed using a potentiostat/galvanostat CHI 760b (CH Instruments, Inc, Austin, TX, USA). The electrochemical cell was equipped with: a boron-doped diamond electrode (inner diameter of 3 mm; Windsor Scientific LTD, UK), resistivity of 0.075 Ω cm, and a boron doping level of 1000 ppm as declared by supplier (working electrode); Ag/AgCl (3 M KCl) (reference electrode); and Pt wire of large surface area (counter electrode).

2.6. MIC assay

Bacterial strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were obtained from American Type Culture Collection. Agar plates (Torlak, Belgrade, Serbia) were supplemented with antibiotics or antibiotics with equimolar concentration of Cu²⁺. The dilutions were prepared in 0.9% NaCl and mixed with agar in v/v 1:9. Final concentrations of antibiotics were: 6.25, 12.5, 25, 50, 62.5, 125, 250, 500, and 1000 µg/mL. The plates were inoculated with a microplate replicator. The dilutions were designed to provide an inoculum in the range of 50–200 CFU. MIC was defined as the lowest concentration at which no growth was observed following 24 h incubation under aerobic conditions at 37°C. All experiments were performed in triplicate.

3. Results and discussion

Fig. 2 presents UV-Vis spectra of eight β-lactam antibiotics in the absence and presence of Cu²⁺. The spectrum of penicillin G did not change in the presence of Cu²⁺ (Fig. 2A), whereas ampicillin and amoxicillin showed an intensive absorption at approximately 310 nm, and a weak

peak at 645 nm (Fig. 2B, C). The latter was assigned to d-d transition in the coordinate-bound Cu^{2+} [17]. The $\lambda_{\text{max}} \sim 310$ nm peak was also observed for cephalexin and cefaclor. The λ_{max} at 273 nm for amoxicillin, which arises from the forbidden $\pi \rightarrow \pi^*$ transition in the phenolic ring [18], was not affected by copper ions. The key structural difference between penicillin G and these four antibiotics is the NH_2 group on side-chain. Therefore, UV-Vis results imply that this group represents a site of coordinate bonding to Cu^{2+} . Another probable binding site is N in the amide group. Coordinate bonds result in ligand-to-metal (Cu^{2+}) charge transfer transition at ~ 310 nm [13,19], which is in line with the observed absorption maxima. It has been proposed previously that Cu^{2+} forms a tridentate complex with cephalexin via amine, carboxylate, and carbonyl groups [13]. However, this complex has been synthesized in methanol, and it was water-insoluble. It is important to note that cefaclor and cephalexin underwent degradation in the presence of Cu^{2+} , as concluded from the disappearance of λ_{max} at ~ 265 nm and the rise of a broad peak at ~ 400 nm (Fig. 2D, E). The former peak has been attributed to $\text{O}=\text{C}-\text{N}-\text{C}=\text{C}-$ group in the cephalosporin ring system [20]. The degradation of cefaclor was at least five times faster than cephalexin. Furthermore, UV-Vis spectra of ceftriaxone and ceftazidime did not change in the presence of Cu^{2+} (Fig. 2F, G). This implies that Cu^{2+} did not interact with the strongest chromophores in these antibiotics, and that transitions between Cu^{2+} and donor atoms show lower extinction coefficients (ϵ) than the chromophores. Previous studies have shown that ϵ for absorption maxima of ceftriaxone at 240 nm and 268 nm are $\sim 30000 \text{ M}^{-1} \text{ cm}^{-1}$, whereas ceftazidime showed $\epsilon \sim 8500 \text{ M}^{-1} \text{ cm}^{-1}$ for the maximum at ~ 260 nm [21,22]. The absorbance of ceftriaxone is most likely related to the cephalosporin ring system and triazine dione moiety. In addition, aminothiazole group shows λ_{max} at 253 nm, but ϵ ($\sim 8000 \text{ M}^{-1} \text{ cm}^{-1}$) is significantly lower than ceftriaxone absorption maxima [23]. On the other hand, λ_{max} at 255 nm for ceftazidime may be attributed to both, aminothiazole group and pyridinium cation [23,24]. It appears that Cu^{2+} did not interact with the aminothiazole moiety (at least in ceftazidime; UV-Vis data were inconclusive for ceftriaxone). Ketoxime, a group that is also common for these two antibiotics and may form coordinate bonds with Cu^{2+} via N atom [25], shows λ_{max} at ~ 190 nm [23], and could not be observed here. Finally, UV-Vis spectrum of meropenem with λ_{max} at 298 nm was not initially changed by Cu^{2+} (Fig. 2H). However, the signal showed a gradual decrease in the presence of copper ions, which implies that degradation of meropenem took place.

Interactions between organic molecules and transition metals may involve redox reactions, such as the reduction of Cu^{2+} to Cu^{1+} . Given that Cu^{1+} rapidly reacts with molecular oxygen at physiological pH to produce the superoxide radical anion which is further dismutated to H_2O_2 [26,27], the reduction of Cu^{2+} may be monitored by oximetry. Figure 3 shows that a significant O_2 consumption was induced by the addition of Cu^{2+} to cefaclor and that H_2O_2 was accumulated in the system. No significant change was observed for any of the other examined antibiotics. The initial rate of O_2 consumption by cefaclor/copper system was $\sim 35 \mu\text{M}/\text{min}$. This rate reflects the kinetics of cefaclor degradation, taking into account the stoichiometry of the overall reaction: $\text{cefaclor} + \text{Cu}^{2+} + \text{O}_2 \rightarrow \text{cefaclor}^{\cdot+} + \text{Cu}^{2+} + \text{O}_2^{\cdot-}$ (Cu^{1+} represents an intermediate). It is important to point out that meropenem- Cu^{2+} system did not show detectable O_2 consumption, which implies that meropenem does not undergo oxidation or that it may stabilize Cu^{1+} .

Low-T EPR spectroscopy was used to investigate the stoichiometry and structure of complexes of Cu^{2+} with antibiotics (Fig. 4). Only antibiotics that did not decompose in the presence of Cu^{2+} (as determined from UV-Vis spectra; Fig. 2) were examined. It is important to point out that the EPR spectra of samples containing antibiotics and copper ions in 1:1 and 2:1 molar ratios were practically identical (not shown), implicating that the formed complexes show 1:1 stoichiometry. Cu^{2+} in the phosphate buffer exhibited an anisotropic EPR signal with one strong $g_{\perp} = 2.05$ line and four weak lines coming from hyperfine coupling with $^{63}\text{Cu}/^{65}\text{Cu}$ nuclei ($I = 3/2$) along $g_{\parallel} = 2.38$ [28,29]. The g -values ($g_{\parallel} > g_{\perp} > g_e$ (g -value for free electron = 2.0023)), and the spectral shape imply that Cu^{2+} is octahedral coordination environment with tetragonal distortion [30,31]. The addition of penicillin G did not change the signal (Fig. 4A), further confirming that this β -lactam does not form a complex with Cu^{2+} under physiological settings. Similar structures of complexes of ampicillin, amoxicillin, and cephalexin with Cu^{2+} were implicated by next-to identical EPR spectra (Fig. 4B). EPR spectra of complexes of these three antibiotics with Cu^{2+} showed g -values that were similar to Cu^{2+} in antibiotic-free phosphate buffer - $g_{\parallel} = 2.38$ and $g_{\perp} = 2.07$ (Fig. 4A), but the double integral of the signal was $1.5\times$ higher. This implies that Cu^{2+} in the complex with antibiotics preserved octahedral geometry with tetragonal distortion. This is in line with a previous report on Cu^{2+} complexes with ampicillin and amoxicillin [16]. A larger g_{\perp} value implicates that anisotropy has decreased. Higher signal intensity may be explained by an increased solubility of Cu^{2+} in the presence of ampicillin, amoxicillin, and cephalexin. According to the speciation diagram for Cu^{2+} in phosphate buffer, Cu^{2+} is present in two forms at pH 7.4 -

the insoluble $\text{Cu}_3(\text{PO}_4)_2$ and the soluble $\text{Cu}(\text{HPO}_4)_2^{2-}$ (Supplemental Fig. S1). The tri-nuclear molecule shows a very broad EPR signal due to intra-molecular spin-spin interaction between Cu^{2+} centers [32,33]. Therefore, $\text{Cu}(\text{HPO}_4)_2^{2-}$ represents the main 'EPR-active' Cu^{2+} species in the control system. Ampicillin, amoxicillin, and cephalexin appear to convert some of the insoluble tri-nuclear form into a soluble mono-nuclear complex with two coordinate bonds with the antibiotics, and the two other sites probably covered by phosphate ions. This is in accordance with the UV-Vis data and with the 1:1 stoichiometry. These antibiotics may increase labile copper pool in body fluids.

On the other hand, the EPR spectrum of Cu^{2+} was significantly altered in the presence of ceftriaxone (Fig. 4C), implicating the formation of coordinate complex with a different geometry than Cu^{2+} that is coordinated by phosphate ions. The N atoms in amide and ketoxime groups are most likely the sites of coordinate bond formation [7]. The shape and a broader g_{\perp} line ($\Delta B_{\perp} = 7.8$ mT; for Cu^{2+} in the control system $\Delta B_{\perp} = 4.9$ mT), imply that the complex might have a different geometry compared to other complexes here. This may be related to the rigidity of ketoxime group. It is worth mentioning that ceftriaxone has shown potent anticancer effects [34], which may be related to the formation of the complex with Cu^{2+} [7]. Finally, the sample containing Cu^{2+} and ceftazidime, exhibited an EPR signal with $g_{\parallel} = 2.39$ and $g_{\perp} = 2.07$, and an additional signal at $g = 1.99$ (Fig. 4D). The main signal was assigned to Cu^{2+} in octahedral coordination environment with tetragonal distortion. Ceftazidime may act as a tridentate ligand with N atoms in ketoxime and amide groups and O in the carboxylate group (on the side chain) being a sites of coordinate bonds formation. The $g = 1.99$ signal is more narrow compared to other lines ($\Delta B = 1.4$ mT vs. $\Delta B \sim 4.9$ mT), and comes from an organic radical that is produced in the reaction with Cu^{2+} . Cu^{2+} may also bind to O atoms in cephalosporin ring which has deprotonated carboxyl group. Such binding may destabilize and open the ring resulting in radical formation.

Cyclic voltammetry was applied to examine redox properties of copper ions in the presence of antibiotics at equimolar concentrations. Except meropenem, other β -lactam antibiotics did not show detectable oxidation/reduction currents in the applied scan range. Cyclic voltammograms (CV) of Cu^{2+} showed very weak currents (Fig. 5A), which is in line with the presence of insoluble $\text{Cu}_3(\text{PO}_4)_2$ at pH 7.4, and with the previous observations that phosphate buffer attenuates copper-related currents [27]. The formation of complexes with ampicillin, amoxicillin,

and cephalexin resulted in a significant increase of peak currents (i_p) (Fig. 5A, B), confirming that these antibiotics increased Cu^{2+} solubility in the phosphate buffer at physiological pH. Only a slight increase of oxidation/anodic (E_{pa}) potentials was observed. Further, ceftriaxone and ceftazidime did not show a significant impact on i_p (Fig. 5B). In the presence of cefaclor, a strong i_p coming from the oxidation of Cu^{1+} to Cu^{2+} was observed. The Cu^{1+} current showed fast increase over 5 min incubation period (Fig. 5C). This further confirms that cefaclor reduced Cu^{2+} to Cu^{1+} . It is important to note that the chlorine on the cephalosporin ring represents the sole difference between cephalexine and cefaclor. Highly electronegative Cl atom withdraws electrons from the ring, which may lead to the destabilization of β -lactam ring in the presence of Cu^{2+} and to Cu^{2+} reduction. Finally, CV of copper/meropenem system was ligand centered (Fig. 5D). Meropenem showed E_{pa} at 1060 mV, which could be assigned to the irreversible oxidation of sulfide moiety [35]. A similar high E_{pa} has been previously reported for methionine [36]. In the presence of Cu^{2+} , E_{pa} was shifted to 985 mV, implying that the sulfide group was more susceptible to oxidation. More importantly, i_p showed a time-dependent decrease that resembled the kinetics of meropenem degradation as determined by UV-Vis spectroscopy. It is noteworthy that S in sulfide group shows higher affinity for Cu^{1+} than Cu^{2+} [37].

The results of MIC analysis are presented in Table 1. Copper induced a drastic decrease in the activity of meropenem against *E. coli* and *S. aureus*, which is in line with the observed degradation of this antibiotic in the presence of copper ions. The formation of complexes with Cu^{2+} suppressed the activity of amoxicillin against *E. coli* and *S. aureus*, and the activity of ampicillin against *E. coli*. For ampicillin and *S. aureus*, MIC remained below the detection range in the presence of copper. In contrast, the activity of cephalexin against *E. coli* was slightly increased in the presence of copper, which is in accordance with previous reports [38]. MIC for ceftriaxone against both strains was increased, implying that the formation of the complex with Cu^{2+} may decrease its activity. Penicillin G also showed decreased activity against *S. aureus* in the presence of copper. This may be related to a very slow copper-induced degradation [10], since penicillin G and copper ion did not show coordinate or redox interactions here. Cressman *et al.* have reported that penicillin G can undergo rapid hydrolysis (half-life 50-100 s) in the presence of copper in slightly acidous conditions (pH 5.5) at $t = 30^\circ\text{C}$ [10]. Taking into the account that MIC assay involves 24 h incubation at higher temperature (37°C), it is plausible that some degradation of penicillin G could take place. In contrast, MIC for cefaclor against *E. coli*

was slightly decreased (*i.e.* antibacterial activity was promoted by copper), which may be explained by the production of hydrogen peroxide. Finally, MIC values for ceftazidime were not significantly affected by copper.

4. Conclusions

The coordinate interactions of ampicillin, amoxicillin, cephalexin, ceftriaxone and ceftazidime with copper show 1:1 stoichiometry. Structural difference between penicillin G, which does not interact with Cu^{2+} , and ampicillin, amoxicillin, and cephalexin, clearly mark amine group on the side chain as the site of coordinate bond formation. The later three antibiotics increased the solubility of copper in the presence of phosphates and may contribute the labile copper pool in body fluids. Cefaclor showed the capacity to reduce Cu^{2+} , leading to the production of hydrogen peroxide that may contribute to the antimicrobial effects of this drug. The degradation of meropenem in the presence of copper ions and significantly lower antimicrobial activity of amoxicillin, ampicillin, and ceftriaxone that may be related to the complex formation, call for caution in the application of these antibiotics to the patients with conditions that are related to increased labile copper pool. In addition, copper ions may be involved in the underlying mechanisms of antibiotic resistance. Pertinent to this, our results point out the necessity of investigating antibiotic resistance in cohorts of patients with conditions that are linked to the increased labile copper pool.

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Declarations of interest

None

Appendix A. Supplementary Material

Supplementary material is available.

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Fig. 1. Structural formulas of β -lactam antibiotics. Penicillins: penicillin G, ampicillin, and amoxicillin; Cephalosporins: cephalexin, cefaclor, ceftriaxone, and ceftazidime; Carbapenems: meropenem.

Fig. 2. UV-Vis spectra of β -lactam antibiotics in the absence or the presence of copper at equimolar concentration, in 50 mM phosphate buffer, pH 7.4. (A) Penicillin G (0.2 mM); (B) Ampicillin (0.5 mM); (C) Amoxicillin (0.5 mM); (D) Cephalexin (0.2 mM); (E) Cefaclor (0.2 mM); (F) Ceftriaxone (0.05 mM); (G) Ceftazidime (0.2 mM); (H) Meropenem (0.1 mM). The spectra of ceftriaxone- Cu^{2+} and ceftazidime- Cu^{2+} systems represent a sum of spectra of these antibiotics and Cu^{2+} , taken separately. All antibiotics were stable in copper-free solutions.

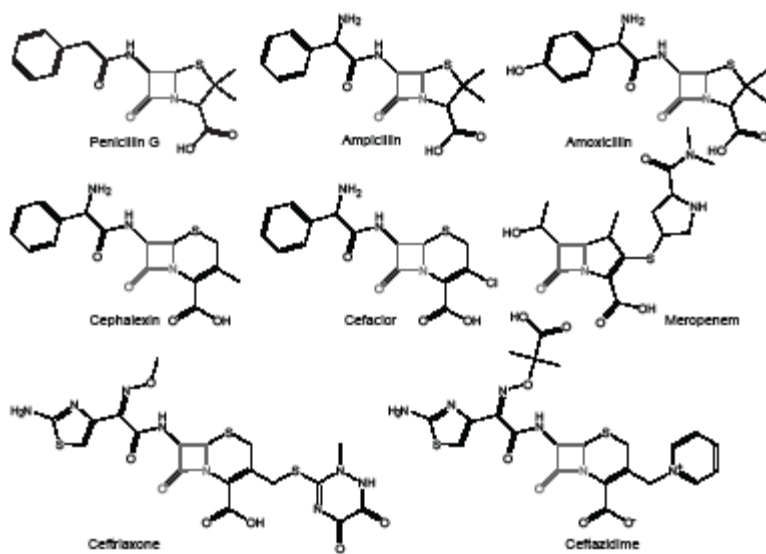
Fig. 3. The consumption of molecular oxygen in systems with cefaclor (0.2 mM) and Cu^{2+} (0.2 mM) in 50 mM phosphate buffer, pH 7.4. Changes in O_2 concentration (left panel), and O_2 consumption rate (right panel) induced by the addition of Cu^{2+} to the equimolar solution of cefaclor. H_2O_2 accumulation was quantified 30 min after the addition of antibiotic to the system, by catalase-induced O_2 release ($2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$).

Fig. 4. 110 K EPR spectra of 0.2 mM Cu^{2+} in the absence or the presence of 0.2 mM β -lactam antibiotics in 50 mM phosphate buffer, pH 7.4. (A) Cu^{2+} without (gray) and with penicillin G (black). (B) Cu^{2+} in the presence of ampicillin, amoxicillin or cephalexin; (C) Cu^{2+} in the presence of ceftriaxone; (D) Cu^{2+} in the presence of ceftazidime. Samples were incubated for 5 min at 298 K and quickly frozen. EPR parameters were: microwave power, 3.17 mW; scan time, 100 s; modulation amplitude, 0.5 mT; modulation frequency, 100 kHz; number of accumulations, 4

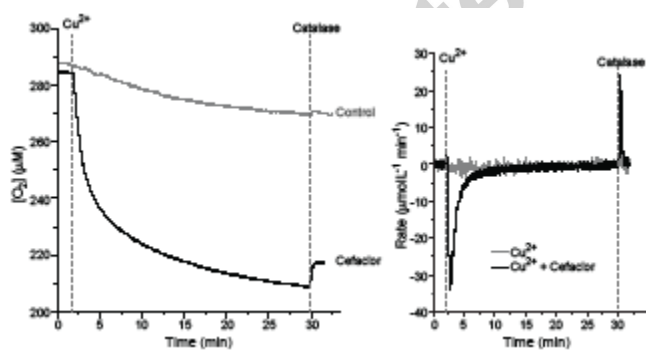
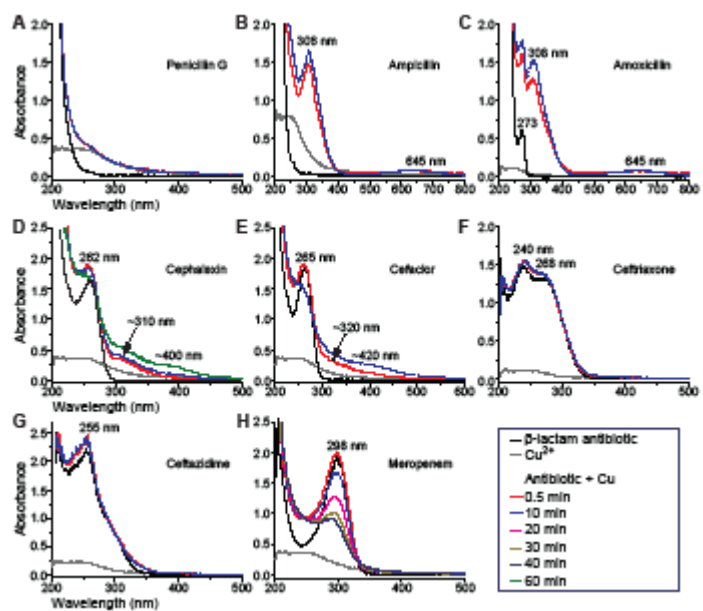
Fig. 5. Cyclic voltammograms (CV) of copper and antibiotics in 50 mM phosphate buffer, pH = 7.4, at boron-doped diamond electrode. (A) CVs of Cu²⁺ (2 mM) in the absence and the presence of 2 mM penicillin G, ampicillin, or amoxicillin. (B) CVs of Cu²⁺ (2 mM) in the absence and the presence of 2 mM cephalexin, ceftriaxone, or ceftazidime; (C) Changes in CV of copper (0.2 or 2 mM) during incubation with equimolar concentration of cefaclor; (D) CV of Cu²⁺ (0.5 mM), meropenem (0.5 mM), and copper + meropenem system. Voltammograms were collected immediately following the addition of CuCl₂. Scan rate was 0.1 V/s. Scan range was -1 to 1 V (-1 to 1.5 V for meropenem). Peak current oxidation/anodic potentials (E_{pa}) are presented. Arrows point out the change in currents.

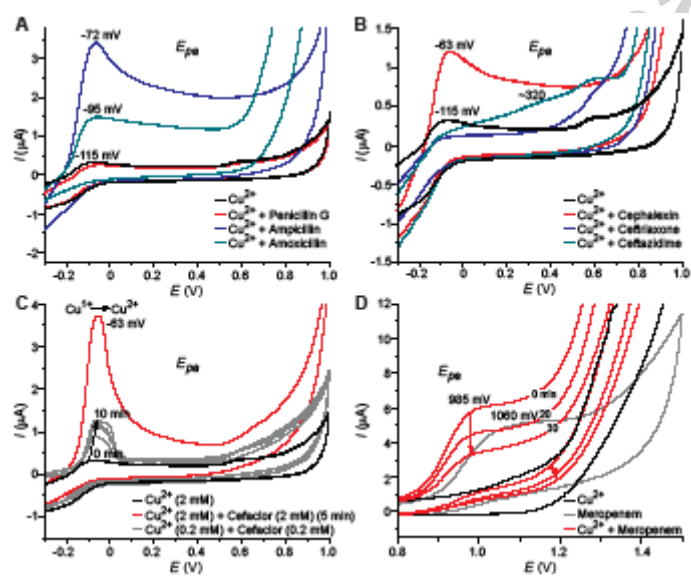
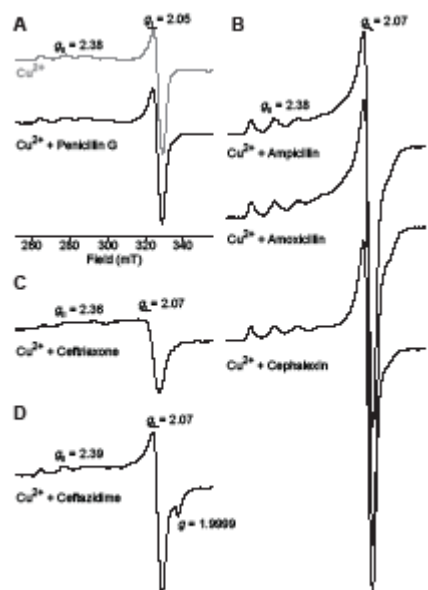
Table 1. Minimal inhibitory concentration (MIC; $\mu\text{g/mL}$) of β -lactam antibiotic in the absence or the presence of equimolar Cu²⁺ for *E. coli* and *S. aureus*. Copper did not show detectable inhibitory effects in the concentration range applied here. Penicillin G is ineffective against Gram-negative bacteria, such as *E. coli*.

Antibiotic	without copper		with copper	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
Penicillin G	-	<6.25	-	62.5
Ampicillin	25	<6.25	125	<6.25
Amoxicillin	62.5	<6,25	1000	62.5
Cephalexin	125	<6.25	25	<6.25
Cefaclor	50	<6.25	25	<6.25
Ceftriaxone	<6.25	<6.25	12.5	12.5
Ceftazidim	12.5	12,5	12.5	25
Meropenem	12.5	<6.25	>1000	>1000



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Highlights:

- β -Lactam antibiotics showed coordination/redox interactions with Cu
- Ampicillin, amoxicillin, cephalixin and ceftriaxone formed stable Cu^{2+} complexes
- Cefaclor reduced Cu^{2+} to Cu^{1+} , whereas meropenem underwent degradation
- Cu^{2+} decreased antimicrobial effects of meropenem, amoxicillin, and ceftriaxone

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