Impact of dissolved copper on photodegradation of enrofloxacin and ciprofloxacin and removal of residual antimicrobial activity

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Abstract

Antibiotics are commonly used to treat bacterial infections in humans and promote animal growth in agriculture. With increased consumption of antibiotics in recent decades, antibiotic resistance has become a global public health concern. To minimize the usage of antibiotics, some industries have adopted metals with antibacterial and antifouling properties. For example, copper alloys have been integrated into aquaculture operations. The objective of this study was to investigate the effect of copper on the photochemical fate and residual antimicrobial activity of a key antibiotic used in aquaculture, enrofloxacin. The specific objectives were as follows: (1) to determine the photodegradation kinetics of enrofloxacin and production of ciprofloxacin with and without dissolved copper; and (2) to assess the impact of dissolved copper on the removal of antimicrobial activity associated with enrofloxacin and its photoproducts. Photodegradation experiments were conducted in Rayonet reactors with 254 nm (engineered processes) or 365 nm (natural systems) lamps. Antimicrobial activity was measured with *Escherichia coli* (ATCC 25922) growth inhibition assays. Antibiotics were measured by high performance liquid chromatography (HPLC) with fluorescence detection. Our study found that copper inhibits photodegradation of enrofloxacin and, thereby, slows the removal of residual antimicrobial activity for irradiation at 254 nm and 365 nm.

Materials and Methods

Figure 1. Photograph of the Rayonet reactor (right). The merry-go-round reactor included eight 254/365 nm lamps and eight quartz tubes, which contained 10 mL of experimental solutions. During operation, the tubes were sequentially removed at specific time intervals to collect samples for analysis of antibiotics and residual antimicrobial activity: 254 nm, 4-min intervals up to 32 min; and 365 nm, 15-min intervals up to 2 h.

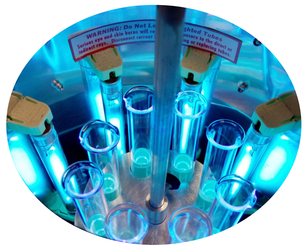
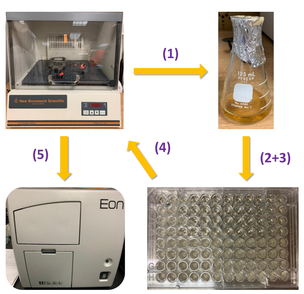


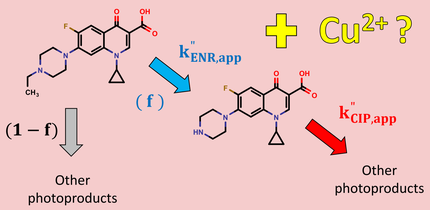
Figure 2. Photograph of the HPLC (left). The UV detector was set to record at 275 nm. The fluorescence detector used an excitation wavelength of 275 nm and an emission wavelength of 447 nm. The HPLC column was an Accucore pentafluorophenyl column with a particle size of 2.6 µm, length of 150 mm, and diameter of 4.6 mm. The mobile phase was comprised of 20% acetonitrile and 80% 20 mM phosphate buffer (pH ~2.5). The total run time was 7 min.

Figure 3: Antimicrobial activity assay (right). The protocol was as follows: (1) *E. coli* was cultured in the exponential growth phase; (2) 100 µL of solutions containing antibiotic standards or irradiated solutions were added to microplate wells; (3) 100 µL of an *E. coli* inoculum was added to the wells; (4) microplates were incubated for 16-20 h; and (5) the optical density at 625 nm was recorded and converted to growth inhibition.



Results and Discussion

Hypotheses



Cu^{2+} will inhibit (i) ENR and CIP photodegradation and (ii) the removal of residual antimicrobial activity associated with antibiotic photoproducts

Developing the calibration curves for ENR and CIP

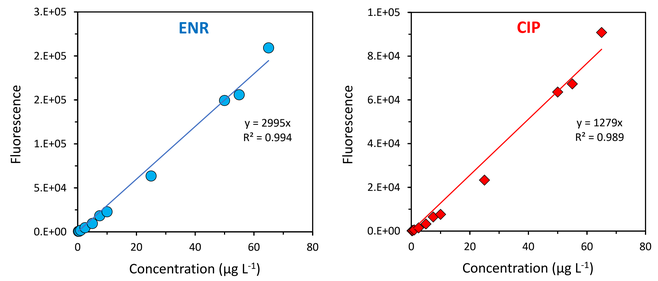


Figure 4. Calibration curves for ENR and CIP using the HPLC with fluorescence detection method. The high coefficients of determination (R^2) values enabled accurate analysis of antibiotic concentrations in experimental samples.

Measuring the antimicrobial activity of ENR and CIP

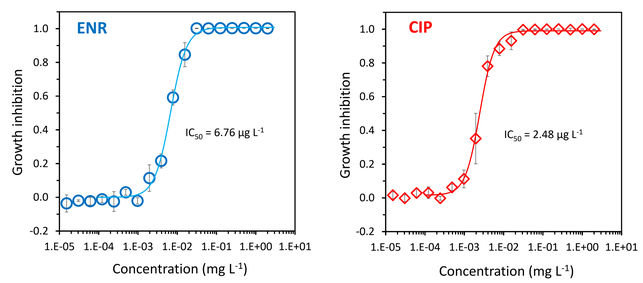


Figure 5. Dose-response curves for ENR and CIP with the *E. coli*-based antimicrobial activity assay. The half-maximal inhibitory concentrations (IC_{50} values) suggested that ENR and CIP were similarly potent antibiotics.

Phototransformation kinetics of ENR, production of CIP, and removal of residual antimicrobial activity

254 nm (engineered treatment processes)

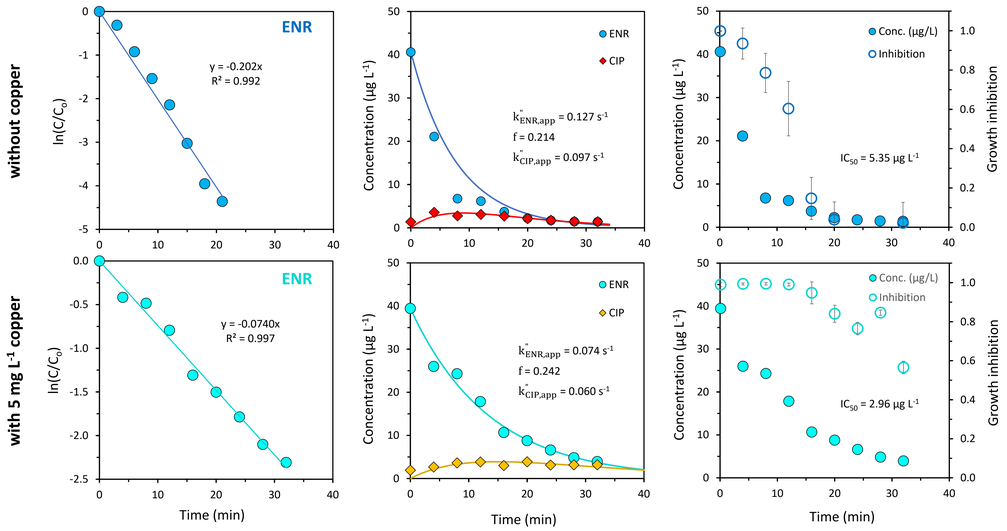


Figure 6. (left) The first-order rate constant for ENR photodegradation at 254 nm, (center) kinetic model for ENR degradation, CIP formation, and CIP degradation, (right) concurrent removal of ENR and antimicrobial activity for the (top) no copper and (bottom) 5 mg L^{-1} Cu^{2+} scenarios. The kinetic parameters and IC_{50} values are overlaid on the plots. The pH was 6.3-6.4 for (top) and 5.0-5.8 for (bottom).

365 nm (natural systems)

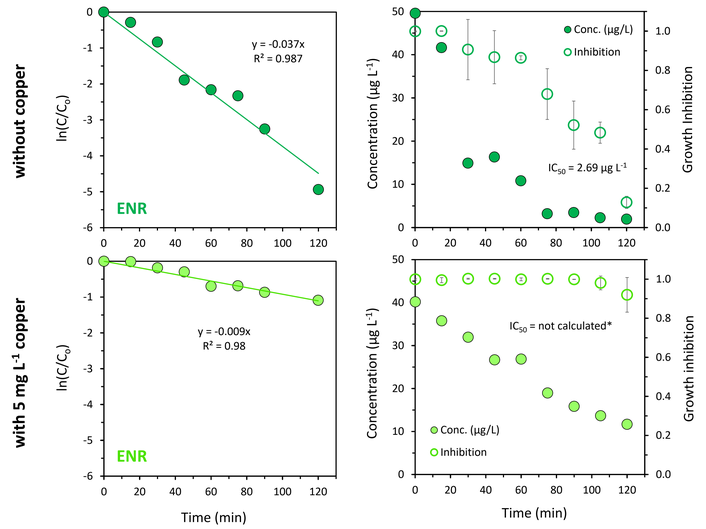


Figure 7. (left) The first-order rate constant for ENR photodegradation at 365 nm and (right) concurrent removal of ENR and antimicrobial activity for the (top) no copper and (bottom) 5 mg L^{-1} Cu^{2+} scenarios. The pH was 5.4-5.7 for (top) and 5.4-5.7 for (bottom).

Conclusions

- In the presence of copper, the rate constants for ENR ($k''_{ENR,app}$) and CIP ($k''_{CIP,app}$) decreased by 42% and 38%, respectively. However, the percent of ENR that was converted to CIP slightly increased from 21% to 24%. The results indicate that dissolved copper inhibited photodegradation of fluoroquinolone antibiotics, although the specific mechanisms require further investigation. This outcome may have important consequences for the fate of antibiotics in aquaculture systems.
- At 254 nm, the apparent IC_{50} for irradiated samples (*i.e.*, those containing ENR, CIP, and other photoproducts) samples decreased in the presence of copper. The results suggest that the relative concentrations of ciprofloxacin and other antimicrobially active photoproducts were higher in this scenario. This outcome aligns with the slower degradation kinetics (above) and may have crucial implications for development of antimicrobial resistance in aquaculture systems.

References

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