Translating Applied Aquatic Chemistry Research to Introductory High School Chemistry Coursework: Developing Methodology to Determine Copper Speciation

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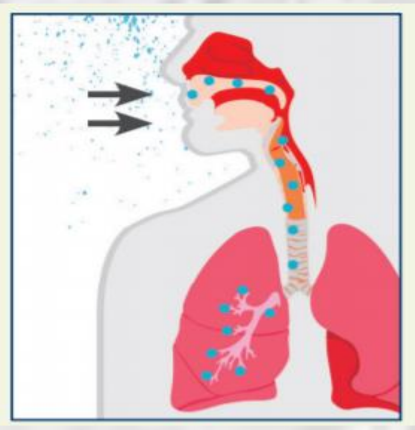
Potential Impacts of Aqueous Copper in Public Water Sources

Harnessing the disinfectant properties of an existing installed resource, such as copper (Cu) pipe, is an attractive practical engineering solution for "passive" control of opportunistic pathogens (OPs), such as *Legionella*, because Cu often acts as a natural biocide. However, Cu is not consistently effective in practice. Background water chemistry likely directly and indirectly influences Cu toxicity towards *Legionella* due to the different species [i.e., Cu(I) vs Cu(II)] and complexes that form. However, methods to determine and quantify copper speciation in drinking water have not been developed. The specific objective of this study was to adapt existing chelating and spectrophotometric methods to determine Cu speciation in drinking water.



Source: CDC Legionnaire's disease fact sheet

Fig. 1. Inhalation exposure route of *Legionella*, the leading cause of drinking water associated disease in US

- Cu(II): • Can act as biocide, but may be rendered inert due to water chemistry forming biounavailable complexes
- Cu(I): • Can deposit one lesser noble metals (e.g., Al anode rods in water heaters) and initiate H_2 gas evolution (Fig. 3)
- HOB: • Autotrophic Hydrogen-Oxidizing Bacteria (HOB) use H_2 as an electron donor and fix organic carbon
- OP: • HOBs serve as either a direct or indirect carbon source for opportunistic pathogens (OPs) such as *Legionella*

Fig. 2. Mechanisms of microbial growth dependent on Cu-speciation

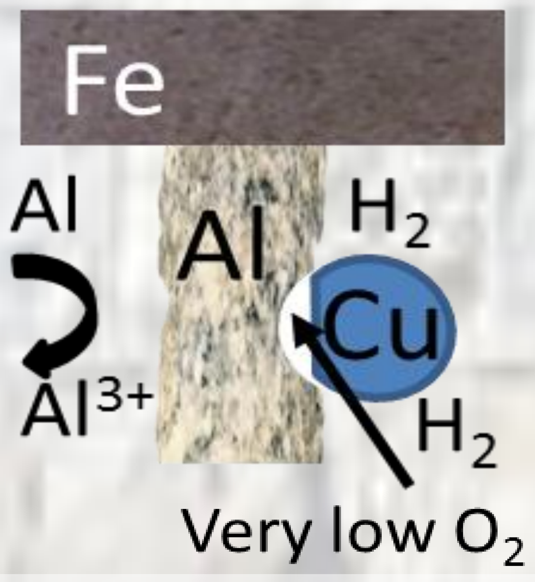


Fig. 3. Cu(I) deposition onto less noble metals creating H_2 nutrients for HOBs

Experimental Determination of Copper Speciation

Cu(II) was measured by quantifying the difference between total Cu and Cu chelated with Chelex-100 sodium form resin. Cu(I) was determined by complexing it with bathocuproine (BCP), which has an orange hue, and measuring absorbance at 484 nm.

Dissolved Cu

CuVer1
ICP-MS (filter 0.45 µm)

Cu(I) Free or complexed

Spectrophotometric detection of Cu(I)-BCP complex
Goals:
Optimize BCP concentration
Monitor Cu(I) Kinetics

Cu(II) Free or complexed

Quantification of Cu(II) with Chelex
Vary pH
Vary Cu(II) concentration

Free Cu(II)

Ion Specific Electrode (future work)

-Current methods for drinking water only quantify total and "soluble" Cu
-Cu(II) is always the thermodynamically favored form of Cu, making stable Cu(I) stock solutions difficult to create

Quantifying Cu(II) with Chelex[1]

Cu(II) Stock

- 5.0×10^{-5} M/ 3.2 mg/L as $Cu(NO_3)_2$
- pH 5.31

Total Cu measured by Hach method 8026 directly from stock solution

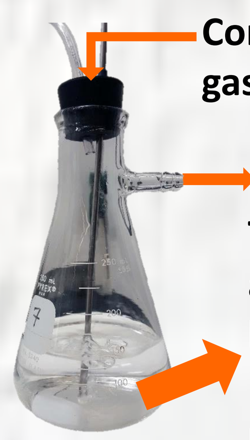
Cu(II) chelated by Chelex resin washed in 0.5 M $NaC_2H_3O_2$ buffer and removed by 0.45 µm filters

Cu(II) = total Cu – resin treated Cu

- Concentration of Cu(II) solution varied from 1.6-3.2 mg/L, diluted with nanopure water
- pH of stock varied from 5.31-6.07 adjusted with NaOH

Bathocuproine Complex[2]

Cu(I) Stock created from Cu_2O in 0.1 M HCl and 1 M NaCl



Continuously purged with N_2 gas to remove O_2

N_2 gas exit

To quantify Cu(I): 10 mL aliquots reacted with BCP buffered to pH > 6 with Na_2CO_3 and absorbance measured at 484 nm

Experimental

- Addition of 5.6-39.4 mg BCP per 10 mL sample to determine optimal dose
- Monitor absorbance over time to determine stability of Cu(I) solution
- Create dilution curve to approximate detection limit

Fig. 4. Summary of methods to determine speciation of Cu (left) and a summary of experimental methods to quantify Cu(II) (middle) and Cu(I) (right)

Experimental Results

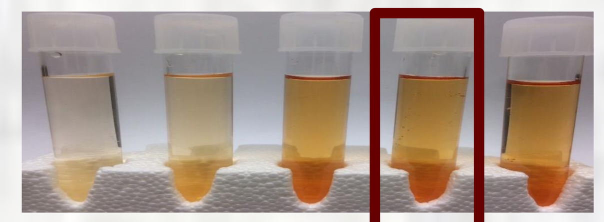
Confirming functionality of Chelex resin for different water chemistries

Solution	% Removed
3.2 ppm Cu(II)*	99.2
1.6 ppm Cu(II)	98.9
3.2 ppm Cu(II) pH 6.1	98.9
3.2 ppm Cu(II) pH 5.3	98.1
3.6 ppm Cu(I)**	0.0

$^*Cu(NO_3)_2$; $^{**}Cu_2O$

- Chelex resin consistently removed all Cu(II) present
- Chelex does not remove Cu(I) in stock solution, thus it may not interfere with measuring Cu(I) in environmental matrices

Determining optimal BCP dose



BCP	40	80	120	160	200	mg
Abs	0.64	1.58	2.14	2.19	2.20	@484 nm

Optimum BCP dose confirmed at 1.6 and 0.32 mg/L Cu(I)

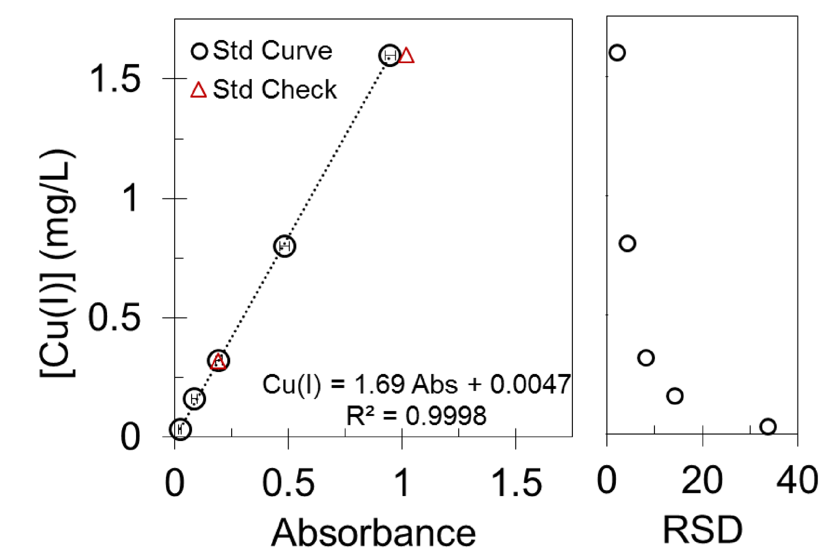


Fig. 5. Determining detection limit of BCP method (RSD = Relative standard deviation)

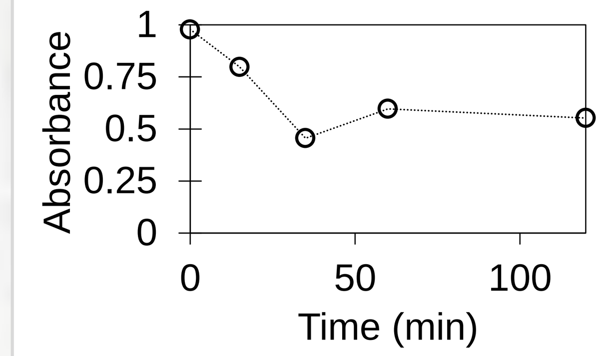


Fig. 6. Determining stability of Cu(I) stock solution over time

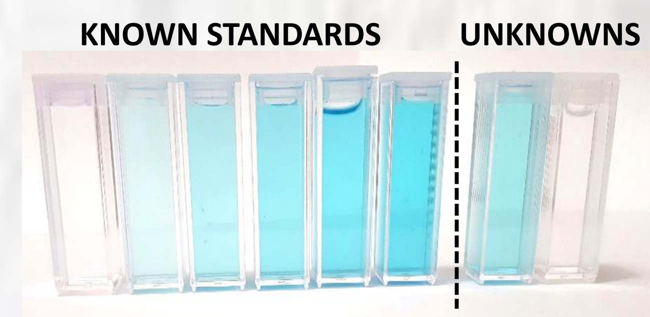
- The optimum dose of BCP is 16 mg/L
- Strong linear correlation was observed down to 0.32 mg/L Cu(I), relative standard deviation increased at low concentrations, requiring further validation
- Cu may convert to Cu(II) in the Cu(I) stock solution over time

Translating Research to the High School Classroom

The concepts explored in this research can be applied to the foundations of general chemistry curriculum, and will be woven into the existing curriculum as a common ribbon to unify concepts throughout the school year. Examples include Solution Chemistry, Equilibrium, Acids and Bases, Reaction Rates, and Redox.

Laboratory Exercise

Students will also apply similar detection methodology (using a Vernier Colorimeter and solutions of copper(II) sulfate) to construct a Beer's Law calibration curve and determine concentrations of unknown solutions.



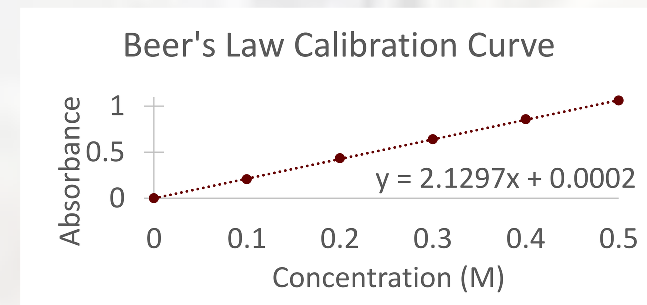


Fig. 7. High School Lab Exercise

Future Work Includes…

Testing functionality of both methods in environmental sample matrices.

Apply these methods to bench- and pilot-scale experiments to determine the effects of Cu speciation on *Legionella* growth in pure culture and environmental samples

Acknowledgments

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