

A Further Study on the Use of Monocopper (II) Citrate as an Antimicrobial Agent in Metalworking Fluid[©]

L. PIET and H. W. ROSSMOORE (Member, ASLE)
Wayne State University
Detroit, Michigan 48202



VOLUME 41, 2, 103-105

LUBRICATION ENGINEERING

A further evaluation of the potential antimicrobial properties of monocopper (II) citrate (MCC) was undertaken to determine its suitability for use in metalworking fluid (MWF). The results presented here are an extension of earlier work on the activity of this compound.

*The effects of MCC against *Candida tropicalis*, a representative fungal contaminant of MWF, at the alkaline pH of MWF are inhibitory only at high concentrations and the inhibition is transient. The antimicrobial activity of MCC can be completely reversed by the presence of soluble iron (III) ions and this has direct significance for its use in MWF, where soluble iron is commonly present. In addition, a preliminary study on the use of MCC as a nucleophilic binding agent to intensify the action of other biocides was also performed.*

INTRODUCTION

Monocopper (II) citrate (MCC) is claimed by a recent US patent to be an effective antimicrobial agent (4). A second, subsequent patent also claimed metalworking fluid (MWF) stabilizing activity for MCC (5). The former patent states that the compound is effective against microorganisms growing in alkaline environments (pH 8-12) due to the stability of the metal complex form only at high pH, with dissociation into toxic copper ions occurring upon encountering the lower pH (7.0) within microbial cells.

Initial studies on MCC (6) have shown that it can temporarily inhibit the growth of *Pseudomonas aeruginosa* in laboratory media and transiently reduce the cell count in MWF contaminated with *Pseudomonas* spp. Since the use of MCC as a MWF additive is becoming more widespread, a further investigation of its ability to influence microbial growth seemed warranted.

Although bacteria are highly important in the biodegradation of MWF, fungi and yeast can play a major role as well, especially in the synthetic fluids (2), (9). *Fusarium* and

Cephalosporium are prominent fungal contaminants, and among the yeasts, *Candida* and *Trichosporon* spp. are often isolated (7). Fungi and yeast are known to be sensitive to the toxic effects of Cu ion (3) and consequently the effect of MCC at high pH on a representative yeast, *Candida tropicalis*, was studied.

As a result of the machining operation itself, MWF can become contaminated with selectively large concentrations of soluble iron. The high stability constant of ferric citrate can allow exchange reactions between the ferric and copper ions in binding to the citrate ligand (1). Such reactions may destroy the antimicrobial activity of MCC in alkaline environments. The effects of added iron salts on the activity of MCC in synthetic laboratory media were also studied so that its practical use could be better judged if iron is also present in the system.

Due to the fact that certain biocides can be inactivated by nucleophilic compounds present in the MWF environment (e.g., amines and sulfides), the possibility exists for using MCC as a nucleophilic binding agent (via its electrophilic Cu atom) and thus achieving an intensification of biocide activity. Biocide A is an example of a compound that is sensitive to nucleophiles, and the possible synergistic mixture of this compound with MCC against the bacterial flora of spoiled MWF was also studied.

MATERIALS AND METHODS

Monocopper (II) citrate was available as a concentrated commercial preparation. Analysis via the iodide reduction method revealed a concentration of 1 mole/liter of Cu ion. The preparation was sterilized by passage through an 0.22 μ membrane filter (6).

Effects on Yeast

A. C. tropicalis species isolated from spoiled MWF and kept on Sabouraud Dextrose Agar was used. The test medium was of the following composition per liter: sucrose 0.5 percent (w/v); NH_4Cl 0.2 percent; KNO_3 0.1 percent; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 percent; yeast extract 0.1 percent; β -glycerophosphate 0.033 mole; and pH adjusted to 8.8 using KOH.

Presented at the 39th Annual Meeting
in Chicago, Illinois,
May 7-10, 1984

Final Manuscript Approved March 1, 1984.

C. tropicalis cells were grown in this medium for 36 hours to produce the experimental inoculum. A series of test flasks, each containing 100 ml of the same medium at pH 8.8, were each inoculated with 0.2 ml of the cells. Monocopper (II) citrate was added before inoculation at the following concentrations, expressed as ppm of Cu: 0, 3.3, 6.6, 33, 66, and 330. The flasks were incubated at 30°C in a rotary shaker at 200 rpm. Growth of the cells was assessed by a visual estimation of the turbidity of the cultures at various intervals. The measurement of the increase in turbidity of a microbial culture is both a rapid and a reliable method for evaluating the increase in biomass (10).

MCC and Biocide A

A sample of contaminated soluble oil (5 percent v/v) (pH 8.5) containing approximately 10^8 cells/ml and positive for sulfate reducing bacteria was used as the test system. The bacterial contaminants were largely *Pseudomonas* sp. Biocide

A was added to test flasks containing 30 ml of the contaminated fluid to achieve a final concentration of 30 ppm. Monocopper (II) citrate was also added at the following levels: 0 ppm, 200 ppm, and 500 ppm. The effects on bacterial cell growth were determined by performing standard plate counts at regular intervals using 1 percent peptone (pH 7.0) as a diluent.

RESULTS AND DISCUSSION

The activity of MCC against *Ps. aeruginosa* as determined from a previous study is summarized in Table 1. The compound is indeed effective at alkaline pH but this effectiveness is of a temporary nature.

The effect of MCC on *C. tropicalis* at alkaline pH is seen in Table 2. Significant inhibition occurs only with the highest concentration employed (330 ppm), but even this is overcome after 50 h of incubation.

TABLE 1—THE ACTIVITY OF MONOCOPPER (II) CITRATE AGAINST *PSEUDOMONAS AERUGINOSA* IN VARIOUS SYSTEMS [MODIFIED FROM (6)]

TABLE 1—THE ACTIVITY OF MONOCOPPER (II) CITRATE AGAINST <i>PSEUDOMONAS AERUGINOSA</i> IN VARIOUS SYSTEMS [MODIFIED FROM (6)]					
A. Alkaline Soy Casein Digest Medium					
	TIME (h)	CONTROL	650 ppm		
	0	-	-		
	5	-	-		
	17	+	-		
	22	+	-		
	48-72	+	+		
B. Alkaline Synthetic Medium					
	TIME (h)	CONTROL	6.5 ppm	33 ppm	
	0	-	-	-	
	10	+	-	-	
	27	+	-	-	
	30	+	+	-	
	48	+	+	+	
C. Contaminated 5% Soluble Oil Emulsion					
		BACTERIAL COLONY-FORMING UNITS/ml			
	TIME (h)	CONTROL	13 ppm	65 ppm	325 ppm
	0	2.7×10^7	2.7×10^7	2.7×10^7	2.7×10^7
	16	5.4×10^7	3.9×10^7	5×10^6	5.2×10^5
	18	14.0×10^7	1.9×10^8	9×10^6	1.1×10^6
	30	12.0×10^7	9.0×10^7	9.7×10^7	1.0×10^7
	48	1.7×10^7	1.2×10^7	6.0×10^7	1×10^8

+ = growth
- = no growth

TABLE 2—EFFECT OF MONOCOPPER (II) CITRATE ON *CANDIDA TROPICALIS* (INORGANIC SALTS MEDIUM, pH 8.8)

TIME	CONTROL	3.3 ppm (as Cu)	6.6 ppm	33 ppm	66 ppm	330 ppm
0 h	--	--	--	--	--	--
26 h	+	+	+	+	+	--
37 h	++	++	++	++	+	--
50 h	++	++	++	++	++	+
74 h	++	++	++	++	++	++

-- = no growth
+ = light growth
++ = heavy growth

TIME	CONTROL	33 ppm MCC (as Cu)	WITH 0.18 mM FeCl ₃	WITH 0.44 mM FeCl ₃	WITH 1.3 mM FeCl ₃
0 h	—	—	—	—	—
18 h	+	—	—	—	+
36 h	+	—	—	+	+
56 h	+	—	+	+	+
64 h	+	+	+	+	+

MCC = monocopper (II) citrate

— = no growth

+ = heavy growth

TIME	CONTROL	30 ppm A	30 ppm A + 200 ppm MCC (as Cu)	30 ppm A + 500 ppm MCC (as Cu)
0 h	2.2×10^8	2.2×10^8	2.2×10^8	2.2×10^8
6 h	N/A	1.3×10^8	4.4×10^7	2.5×10^7
12 h	N/A	1.2×10^8	6.8×10^5	5.3×10^5
20 h	N/A	2.8×10^8	7.8×10^3	5.2×10^3
33 h	9.4×10^8	4.2×10^8	$<10^2$	$<10^2$
50 h	7.1×10^8	7.5×10^8	$<10^2$	$<10^2$

MCC = monocopper (II) citrate

N/A = counts not available

Table 3 shows the effects of soluble iron on the activity of MCC in alkaline solution. The flask containing the highest iron concentrations behaves identically to the control, indicating that ferric ions can successfully compete with the citrate ligand and abolish the growth-suppressing effect of MCC. Only diminishing the amount of added FeCl₃ will restore the full activity of this level of MCC. The presence of the ferric ion had no appreciable effect on the pH of the medium.

These results may have a deep impact on the effectiveness of MCC in controlling bacteria in MWF. Since dissolved iron is always expected in ferrous operations, MCC could easily be rendered ineffective in those systems.

The results of MCC in combination with Biocide A are shown in Table 4. The enhancement of activity against the viable cell count in this fluid is marked. The level of Biocide A was deliberately chosen below the normal recommended level to insure that any synergism, if expressed, could be clearly seen. Monocopper (II) citrate at comparable levels in another MWF system did not produce a comparable reduction in numbers (Table 1).

CONCLUSIONS

Although the first report of MCC (4) was based on its antimicrobial activity, it should be stressed that subsequent commercialization was directed toward its use as a MWF stabilizer, especially for soluble oils (5). The preliminary

nature of these results should be emphasized; however, they do indicate a potentially fruitful area of investigation since MCC does exhibit some interesting microbial properties. Whether or not these are sufficiently attractive economically for commercial pursuit remains unanswered.

REFERENCES

- (1) Ashcroft, S. J. and Mortimer, C. T., *Thermochemistry of Transition Metal Complexes*, Academic Press, New York (1970).
- (2) Bennett, E. O., "The Deterioration of Metal Working Fluids," *Prog. Indust. Microbiol.*, **13**, p 121 (1974).
- (3) Hugo, W. B. and Russell, A. D., "Types of Antimicrobial Agents," in: *Principles and Practices of Disinfection, Preservation and Sterilisation*, Russell, A. D., W. B. Hugo, and G. A. J. Ayliffe (Eds.), Blackwell Scientific Publications, Boston, p 69 (1982).
- (4) Maurer, G. L. and Shringapurey, S. K., "Complexes of Heavy Metal Ions and Polyfunctional Organic Ligands as Antimicrobial Agents," *US Patent No. 4,055,655* (1977).
- (5) Maurer, G. L. and Shringapurey, S. K., "Metalworking Fluid Compositions and Methods of Stabilizing Same," *US Patent No. 4,129,509* (1978).
- (6) Piet, L. and Rossmore, H. W., "Evaluation of Monocopper (II) Citrate as a Potential Antimicrobial Metal Complex," *Dev. Indust. Microbiol.*, **25**, in press (1984a).
- (7) Rossmore, H. W., "Microbial Degradation of Water-Miscible Metalworking Fluids" in *Comprehensive Biotechnology*, **3**, Chapter 47, Robinson, Campbell W. and John A. Howell (Eds.), in press (1984b).
- (8) Rossmore, H. W., "Nitrogen Compounds," in *Disinfection, Sterilization and Preservation*, 3rd Ed., Lea and Febiger, Philadelphia, Block, Seymour S. (Ed.), pp 271-308 (1983).
- (9) Rossmore, H. W. and Holtzman, G. H., "Growth of Fungi in Cutting Fluids," *Dev. Indust. Microbiol.*, **15**, pp 273-280 (1974).
- (10) Spooner, D. F. and Sykes, G., "Laboratory Assessment of Antibacterial activity," in *Methods in Microbiology*, **7B**, Academic Press, New York, Norris, J. R. and D. W. Ribbons (Eds.), pp 211-276 (1972).