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Research Article
Effect of Thiadiazole Substituent and its Copper Nanocomposite Catalyst for Biological Activity of Metalworking Fluids

[1]Seham A. Shaban, [2]Heba M.F. Abdelhakim, [3]Hussein A.M. Mustafa and [2,4]Abdelfatah M. Badawi

[1]Laboratory of Catalysts, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt
[2]Ministry of Military Production, Center of Scientific Excellence, Cairo, Egypt
[3]Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt
[4]Laboratory of Petrochemical, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt

Abstract
Biocompatible compound 1,3,4-thiadiazole substituent X1 and its copper nano composite analog X2 have been prepared. The compound X1 and X2 were tested as antimicrobial agents against gram-positive and gram-negative bacteria. The compound X1 showed lower antimicrobial activity against the gram-positive bacteria while the of copper nanocomposite X2 showed moderate antimicrobial activity against the gram-positive bacteria. The effect of bacterial growth for the new products against industrial biocides used in metal-working fluids was evaluated. The thermal stability of the metal-working oil formulation in the presence of X1 and X2 are studied by applying the standard test methods (IP-311, IP-263 and IP-125). The specific surface area, average particle size pore volume and pore diameter of the catalyst samples prepared were determined on a Quantachrome 3200 and determination of morphology and particle size using Transmission Electron Microscopy (TEM). The equilibrium geometry and ground state properties of X1 were calculated using Hartree Fock (HF) and Density Functional Theory (DFT) methods employing various combinations of exchange and correlation functions (B3-LYP) with 6-31G* basis set. The active centers and charge density maps (HOMO and LUMO) of catalyst samples were analyzed.

Key words: Density functional theory, biological activity, metalworking, nanocomposite catalyst

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Corresponding Author: Seham A. Shaban, Laboratory of Catalysts, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacterial contamination of large circulating oil systems, similar systems is a growing and expensive problem. The bacterial colonies, once established, clog control systems, quickly degrade oil quality and oil performance and produce corrosive byproducts. If not detected early, the problem will manifest itself into expensive repairs, extended downtime and a significant expenditure of scarce resources (Richard *et al*., 2005).

Traditional enumeration methods for bacteria in MWFs include direct and indirect detection technologies (Wang *et al*., 2007). Due to the slow growth rate and aggregate-forming characteristics of mycobacteria, these approaches may either underestimate their cell density or are too time-consuming to be effectively used for process control (Chang and Adriaens, 2006).

Recently, some molecular biological approaches have been tried, such as polymerase chain reaction (Elbir *et al*., 2008; Veillette *et al*., 2008; Girish *et al*., 2005; Koch *et al*., 2014) still, these methods mostly focused on identification and indirect quantification instead of on direct enumeration and may not be readily applied to the field due to lack of disaggregating measures. These limitations necessitate the development of a rapid, sensitive and accurate enumeration method to effectively quantify mycobacteria in MWFs. In the past decade, flow cytometry (FCM) has been increasingly used for direct optical detection of bacteria in aquatic systems and in environmental samples with complex matrix characteristics (Koch *et al*., 2014; Pan *et al*., 2014).

Nano particles were found to be superior compared to microbeads (MB), since reaggregation of the particles is limited, which improves detection and enumeration accuracy (Arruebo *et al*., 2009). Antibody conjugated MB have been applied to separation of a wide range of bacteria (Niederweis *et al*., 2010), including mycobacterium tuberculosis (Hermon-Taylor, 2009), *M. paratuberculosis* (Mazurek *et al*., 2010) and *M. ulcerans* (Balada-Llasat *et al*., 2013) in clinical matrices. Traditional immunofluorescence (IF) has been applied to detect mycobacteria in clinical matrices (Selvaraju *et al*., 2008) and also in metal-working fluids (Gajjar *et al*., 2009).

Nano particles action may be due in part to their release of free ions. Heavy metal ions have diverse effects on bacterial cell function. For Cu ions, the mechanism may involve oxidative stress (Malachova *et al*., 2011). The redox cycling of Cu ions results in depletion of glutathione and affects the sulfhydryl groups of proteins causing DNA damage and lipid oxidation. The Cu ions inactivate proteins with SH groups and prevent the ability of DNA to replicate (Gajjar *et al*., 2009).

The aim of this study was to evaluate the biological activity of metalworking fluids of 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2. The equilibrium geometry and ground state properties of X1 and its copper nanocomposite catalyst X2 were calculated using Hartree-Fock (HF) and Density Functional Theory (DFT) methods employing various combinations of exchange and correlation functions of 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2.

MATERIALS AND METHODS

Preparation of 1,3,4-thiadiazole substituent and its copper nanocomposite catalyst: The 1,3,4-thiadiazole substituent (X1) was synthesized according to the Fig. 1 (Abdelhakim, 2011). The copper nanoparticles with the average size of 10 nm were prepared by common chemical precipitation as described elsewhere (Li *et al*., 2001; Shaban *et al*., 2009). After the eluate was washed to neutral, 0.97 g of copper nanoparticles were transferred to a suitable organic solvent and then were slowly mixed with 0.03 g of X1 dissolved in N, N-dimethyl formamide (DMF). The mixture was stirred for 24 h at room temperature and then dried under vacuum. After the removal of DMF, the nanoparticle composite were formed (X2). The composite were washed with DMF and almost no free compound X1 could be found in the solution, indicating that almost all of the compound X1 had been combined with the copper nanoparticle.

Geometries: Initial geometries were optimized at Hartee Fock (HF) level of theory and further reoptimized using DFT methods to include correlation. In this study, an exchange functional which was proposed by Becke *et al*. (1993) in 1988 using a gradient-corrected correlation functional of Lee, Yang and Parr (Sharkas *et al*., 2011; Miehlich *et al*., 1989) was employed. All calculations were performed using the Gausian 94 (Frisch *et al*., 1998).

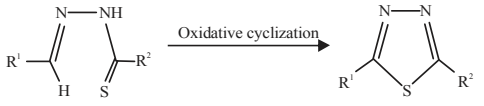

Fig. 1: 1,3,4-thiadiazole substituent

Instruments:

- The TEM image and Selected-area Electron Diffraction (SAED) was taken with a JEOL JEM-2000 EX model transmission electron microscope, using an accelerating voltage of 100 kV. The sample of TEM was prepared by 2 h ultrasonic dispersion of 0.2 g of product in 50 mL ethanol. Then, a drop of the solution was placed on a copper micro grid and dried in air before performance
- Surface area, pore volume, average pore diameter and pore size distribution of catalyst sample were analyzed by means of nitrogen adsorption using Nova 3200, Quanta Chrome (commercial BET unit)

Biological activity: The synthesized X1 compound and its copper nanocomposite analog were screened for their biological activity using the diffusion agar technique (Zhu, 2000). For bacteria, the broth media were incubated for 24 h. As for fungus, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile Sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation. The tested microorganisms were gram-positive bacteria (e.g., *Staphylococcus aureus*, *Streptococcus faecalis* and *Bactecillus subtilis*), gram-negative (e,g., *Escherichia coli*, *Neisseria gonorrhea* and *Pseudomonas aeruginosa*) and fungi (e.g., *Aspergillus flavus* and *Candida albicans*).

Evaluation of the synthesized compounds as biocides for metal-working oils: The synthesized X1 compound and its copper nanocomposite X2 dissolved in imported naphthenic base oil (MVIN 40) with ratio 1:3, i.e., 25% then mixed with neutral base oil 140/160 produced from Amerya refinery.

Preparation of the metal-working oil formulation: Metal-working oil formulation was prepared by blending 77% wt. of neutral base oil 140/160 produced from Amerya refinery, 20% wt. of commercial imported emulsifier EM 3154 and 3% of each of the dissolved synthesized X1 compound or its copper nanocomposite X2 in naphthenic base oil.

Determination of thermal stability of water mix metal-working fluids: The thermal stability of the metal-working oil formulation in presence of the selected synthesized compounds X1, X2 were studied by applying the standard test method (IP-311, 1994).

This method covers the determination of the thermal stability of water mix metal-working fluids over the range of temperatures (0-50°C) at which the fluids would normally be stored. The method describes the tests of high temperature stability and low temperature stability.

Determination of frothing characteristics of water mix metal-working fluids: The determination of frothing characteristics of water mix metal-working oil formulation in presence of the selected synthesized compounds X1, X2 were studied by applying the standard test method (IP-312, 1992). This method assesses the frothing characteristics of water mix metal-working fluids used in the form of aqueous dilutions.

RESULTS AND DISCUSSION

Transmission Electron Microscopy (TEM): Figure 2 is a TEM image of copper nanocomposite well dispersed in organic media. The particles have very narrow particle size distribution from 5-7 nm. It is a perfect anti-wear and friction reducing additive in lubricants and worn surface. copper nanocomposite can be seen that the uniform nanocrystalline had sphere shapes with weak agglomeration. The particles with a small and narrow size distribution can be obtained in Fig. 2.

Surface area and pore volume: The surface area, pore volume and pore diameter results are presented in Table 1. The surface area results show that copper nanocomposite catalyst has a uniform covering of the porous surface. The same

Table 1: Surface properties of the compound X1 and its copper nanocomposite X_2

Parameters	SSA ($m^2 g^{-1}$)	Pore volume (cc g^{-1})	Pore radius Å
X1	80.24	0.034	4.83
X2	175.53	0.074	8.83

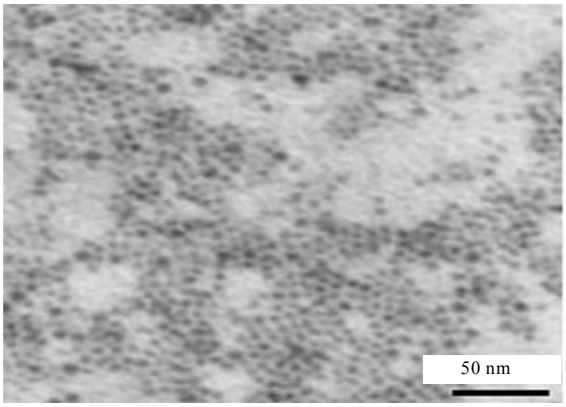

Fig. 2: TEM image of the substituent copper nanocomposite

behavior is observed with respect to the average volume and diameter of pores (Koizumi *et al*., 1997). Modification of X1 with copper nanocomposite resulted in an increase in the pore volumes and surface areas.

Different surface characteristics, namely the specific surface area (S_{BET}), total pore volume (Vp) and mean pore radius (ŕ) of various solids were determined from N_2 adsorption isotherms conducted at/-196°C. All the isotherms displayed common characteristics, being similar in the shape to type II of Brunauer's classification (Brunauer, 1945; Brunauer *et al*., 1938). Representative adsorption/desorption isotherm for nitrogen on copper nanocomposite catalyst is shown in Fig. 3. The adsorption/desorption isotherm exhibited closed hysteresis loops over the high-pressure section of the isotherm. The existence of such hysteresis loop may be explained in terms of capillary condensation in micropores.

More detailed information on the pore size distribution is available from Fig. 3 constructed by plotting DVp/Dr against the pore radius (Mikhail *et al*., 1968). It is seen from Fig. 3 that the investigated solid exhibited distribution in which most of the pores was located in the micropore range. However, the maxima of the pore volume distribution curve are located at range of 18-50 Å. On other hand, the high surface area facilitates to the high dispersion of copper nanocomposite active components, consequently, promotes the increase of biocide activity (Radheshkumar and Munstedt, 2006; Sanvicens and Marco, 2008; Egger *et al*., 2009; Saber *et al*., 2009).

Geometries: The ultimate goal of the present work is to pinpoint those electronic structural and the biological activity of the X1 and X2 compounds studies. To achieve this goal, we start by establishing a good state of geometry as reliable as possible and compare it (X1) with the available experimental data. Table 2 presents the ionization potential, electron affinity, energy gap, dipole moments of the studied 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2.

The computed energy gap ($\Delta\epsilon = E_{LUMO} \times E_{HOMO}$) for 1,3,4-thiadiazole (X1) is 5.63 eV, which is higher than its copper nanocomposite catalyst X2 (3.46 eV). Therefore, compound X2 is more reactive than X1 and the presence of copper nanocomposite are responsible for the reactivity of copper nanocomposite thiadiazole derivatives. The computed dipole moment of X2 is 4.232 D, i.e., more than twice that of 1,3,4-thiadiazole substituent (X1). The addition of copper nanocomposite has a pronounced effect not only on the magnitude but also on the direction of the computed dipole moment.

Biological activity of the synthesized X1 compound and its copper nanocomposite X2: The biological activity of the synthesized 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2 against gram-negative bacteria (*Pseudomonae aeruginosa*), gram-positive bacteria

Table 2: Ground state properties of 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2

	Ground state properties	
Parameters	X1	X2
Total energy (au)	-586.259	-1348.600
Nucleus-electron potential energy (au)	-1.827	-6.915 D+03
Nucleus- nucleus potential energy (au)	2.216	1.881 D+03
I.P = -E(HOMO) (eV)	5.657	5.624
E.A = E (LUMO) (eV)	-0.0291	-2.163
Δε = [E (LUMO) -E (LUMO)] (eV)	5.628	3.461
Electronic moment (μ), Debye	1.846	4.232

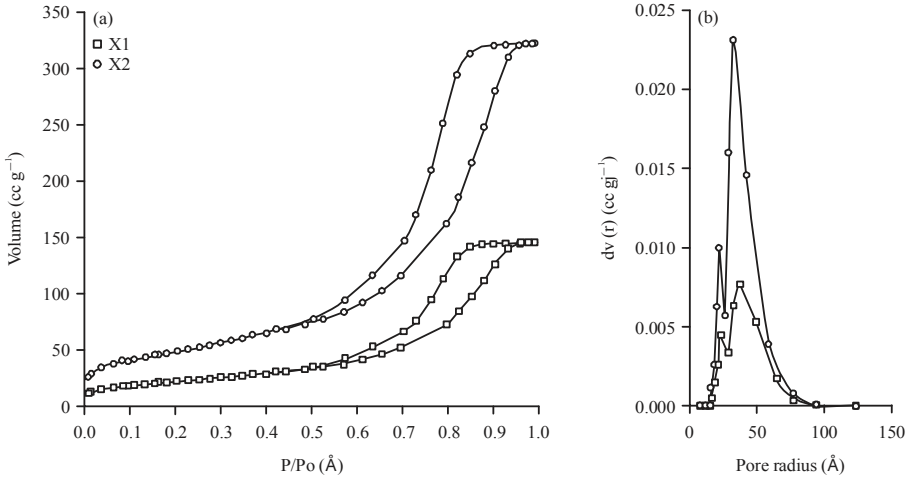

Fig. 3(a-b): Textural characteristics of the compound, (a) X1 and (b) Copper nanocomposite X2

Table 3: Biocidal activity of the compound X1 and its copper nanocomposite X2

	Inhibition zone diameter (mm mg^{-1} per sample)			
Samples	*Pseudomonas aeruginosa* (G^-)	*Aspergillus flavus* (G^+)	*Staphylococcus aureus* (Fungus)	*Candida albicans* (yeast)
Control	0.0	0.0	0.0	0.0
X1	4	6.0	0.0	7.0
X2	14	14.0	0.0	13.0

Table 4: Evaluation of the physical specification limits of the compound X1 and its copper nanocomposite X2 compared with the commercial biocide (Glokill-77)

Sample tests	Specification limit	Glokill-77	X1	X2
Blend stability (IP-311)	Clear and homogenous	Clear and homogenous	Clear and homogenous	Clear and homogenous
Emulsification stability (IP-263)				
Oil separation	Nil	Nil	Traces	Nil
Total separate material (max)	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Frothing characteristics (IP-312)				
0 min	Zero	Zero	Zero	Zero
5 min	Zero	Zero	Zero	Zero
Corrosion (IP-125)	(%-0)	(%-0)	(%-0)	(%-0)
Alkalinity, PH at water dilution 20:1	8.5 (min)	8.5	8	8.5

(*Staphylococcus aureus*), *Aspergillus flavus* fungus and *Candida albicans* yeast are represented in Table 3. The biocidal activity of the compounds under investigation X1 and X2 agrees with their ability for adsorption at the water/cell membrane interface. This adsorption increases solubility through the cell membrane increasing its permeability towards the media ingredients. Thus biological reactions disturb within the cell cytoplasm. Table 3 shows that, the synthesized 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2 have a good biocidal activity against bacteria, fungi and yeast used in this investigation. Table 3 shows that, compounds X2 give relatively better inhibition zones against bacteria and fungi than compound X1. It is noticed that, compounds X1 and X2 show negative biological activity against *A. flavus* fungus. The comparison of the data in Table 3 indicates that, the copper nanocomposite catalyst X2 have a better biological activity towards bacteria, fungi and yeast under study than their parent 1,3,4-thiadiazole substituent X1 (El-Sukkary *et al*., 2009).

The increase in biocidal activity of the copper nanocomposite catalyst X2 is attributed to the lower electronegativity and large volume of copper ions, which increase their molecular surface area. Hence, the effective area of the complex molecules on the cell membrane increases resulting in a higher biocidal action. The type of copper nanoparticles plays an important role in the activity of the different copper nanocomposites (Negm *et al*., 2009; Morsy *et al*., 2009).

Evaluation of the copper nanocomposites as biocides for metal-working oils: The copper nanocomposite X2, which has the highest biological activity compared with the parent compound X1 was evaluated as biocide for metal-working oils Table 4. The tests were applied according to the standard test methods IP-311, IP-263 and IP-125 at water dilution of 20:1. From the study of the obtained results compared to the specification limits we can be concluded:

- The results of the copper nanocomposite X2 meet the specification limits for all tests, so it can be recommended for use as biocide for metal-working oil formulations with dosage 3% weight
- On the other hand, the copper nanocomposite X1 failed in both IP-263 and IP-125 test methods, it gives (oil traces) instead of (Nil) oil separation as in the specification limit and gave pH at 8.0 instead of 8.5 (which is minimum) in the specification limit. Such as fluid have very good cooling power in which very fine droplets of oil are suspended in water (Anyaogu *et al*., 2008)

CONCLUSION

The copper nanocomposite X2 have more effectiveness towards adsorption at air/water interface than their parent 1,3,4-thiadiazole substituent X1. The 1,3,4-thiadiazole substituent X1 and its copper nanocomposite catalyst X2 have a good biological activity against gram-positive and gram-negative bacteria, fungi and yeast. The biocidal activity of the copper nanocomposite catalyst X2 is greater than that of their parent 1,3,4-thiadiazole substituent X1.

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