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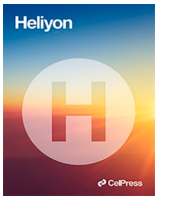

Nano-encapsulated Cu(II) complex as a promising insecticidal for *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Nanoparticle (NP) research is an area of scientific interest with high potential for application in biomedical, optical, and electronic fields. Due to their relatively large surface area compared to their mass, NPs can be more chemically reactive and change their reactive strength or other properties. NP-based drug delivery systems provide transport and an effective and controlled way to release the drugs. This work aimed to study the solubility and biological activity of nano-encapsulated copper metal complexes for the induction of toxicity and mortality in larvae of *Aedes aegypti* mosquitoes. After the nano-encapsulated metal complexes were prepared, the efficiency of this incorporation was determined by electron paramagnetic resonance, and toxicity bioassays were performed. The polymeric-based PLGA NPs encapsulating metal complexes exhibited high toxicity and specificity for target organisms (insect vectors, i.e., *A. aegypti*), with relatively less environmental impact and long-term control of their breeding.

1. Introduction

Aedes aegypti is an adaptable, resilient, and proper vector for several viruses responsible for epidemics in Brazil and worldwide, such as Mayaro, dengue, zika, yellow fever, and chikungunya [1]. Insecticides are the main instrument to control the mosquito population and restrain disease transmission. However, several factors have been contributing to the inefficiency of insecticides, such as mosquito resistance [2]. Thus, there is a need for the development of new molecules with broader biological activity, mainly as insecticides, with greater efficiency and lower environmental impact [3].

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The use of unconventional, available, and low-cost metallic molecules/ions is proposed based on a strategy to enhance biological effects and, possibly, reduce resistance to conventional insecticides through the use of glycerol (hydroxylated carrier) and copper sulfate (Cu(II)), as insecticidal mixture. It is considered that glycerol (Gly)in the concentrations used, does not have relevant biological activity. However, this molecule can interact/coordinate with Cu(II) for cellular permeation and induce the oxidative reaction because it produces free radicals (ROS) *in situ* using the insect's metabolism for cellular damage and insect death, preferably from the digestive system (mesenterium).

The Cu (II) ions are considered biological catalyst and micronutrients essential for the metabolism of plants, fungi, and animals [4]. Metal complexation has reduced reactivity [5], enhanced solubility and mobility, and easy removal of metals in living tissues [6]. The ethylenediaminetetraacetic acid (EDTA) has been used to regulate the absorption of Cu(II) in the form of acetate Cu (II) and stands out as a chelating agent [7]. Copper is used in the composition of several products and has been used for a long time as an agricultural fungicide [5]. Besides, the Bordeaux mixture has been used as an insecticide [6]. Other studies with metal complexes and vectors such as *Aedes aegypti*, *Aedes albopictus*, *Anopheles subpictus,* and *Culex quinquefasciatus* have shown that transition metals are toxic, safe for use in appropriate concentrations and conditions and have the potential to be used as insecticides [8–19].

Glycerol (propane-1,2,3-triol) is a small organic molecule with an essential function in cells and industrial applications. Glycerol has three hydroxyls in its structure. Hydroxyl groups are responsible for water solubility, hygroscopicity, interactions and/or coordination with divalent and trivalent metals. Glycerol is a flexible molecule that can perform different coordination geometries through intra and intermolecular hydrogen interactions. Theoretical studies using density functional theory (DFT) have shown that 126 conformers for glycerol may be possible. The studies indicate the importance of enthalpic and entropic contributions as expressed in Gibbs free energy for determining the conformational and energetic preferences of the glycerol molecule. This geometry can be used to understand mechanisms and conformations in various chemical reactions and practical applications. The combination of intramolecular hydrogen bonding and intermolecular solvation of hydroxyls in the aqueous phase stabilizes the glycerol molecule [8].

Nanotechnology is vital in creating innovative solutions and providing new tools for various applications, such as electronics, optical, and nanomedicine. The proteins (typical sizes of 5–10 nm) and nanoparticles (NPs) have the same order of size magnitude, which facilitates the encapsulation, making it feasible to engage the cell through specific interactions [9]. Nanoparticles are colloidal transport systems that can encapsulate, protect, permeate, and deliver functional components with greater safety and stability [10,11].

The aims of this work are evaluating the biological activity of the combination of glycerol (Gly), copper sulfate ($CuSO_4$), and ligands (citric acid (CA); ethylenediaminetetraacetic acid (EDTA); iminodiacetic acid (IDA)) polymerized by encapsulation as a non-conventional insecticide for population control of *A. aegypti* and other Culicidae insects. The study sought to study the use of the complex mixture based on the solubility, stability, cellular permeation, and increase in the biological activity of nanoencapsulated copper Cu(II) for immature forms of the insect to induce toxicity due to oxidative stress reaction, free radicals, ROS species, cellular damage and mortality in larvae of *A. aegypti* mosquitoes [12].

2. Materials and procedures

2.1. Reagents

Purasorb@ PDLG 5002 (PLGA 50:50) was purchased from Corbion Purac (Gronigen, Netherlands); Pluronics F127 (BASF, Ludwigshafen, Germany); ethyl acetate (Merck Millipore, Darmstadt, Germany); glycerol (Carlo Erba, Milan, Italy); and Copper(II) sulfate pentahydrate (Sigma-Aldrich, Algés, Portugal). The solutions were prepared with ultrapure water ((Synergy 185, Merck Millipore, Darmstadt, Germany). All reagents and chemicals were used without any further purification. The methodology was used to prepare glycerol-copper nanoparticles with PLGA by double emulsion and solvent evaporation.

2.2. Equipment

Vortex Shaker (25.000 rpm); Ultrasonic probe (Vibra Cell, Sonics & Materials Inc., Danbury, Connecticut, USA); microcentrifuge (5417R, Eppendorf AG Barkhausenweg, Hamburg, Germany); ZetaSizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK); magnetic bars (Multistirrer 15, VELP Scientifica, New York, USA).

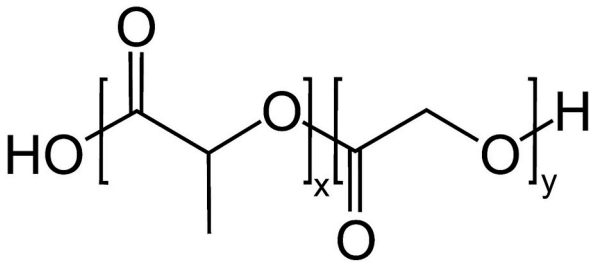

Fig. 1. Structure of poly(lactic-*co*-glycolic acid). x = number of lactic acid units; y = number of glycolic acid units.

2.3. Preparation of metal complexes (MCs) and nano-encapsulated MCs

Metal complexes (MCs) were prepared in a molar ratio 1:1 (ligand:metal) after mixing and stirring the metal ion in the ligand solution. The reaction mixture was left under slow stirring overnight. All metal complexes were loaded into poly(lactic-*co*-glycolic acid) (PLGA) (Fig. 1). NPs were prepared by a double emulsion/solvent evaporation method [19]. A copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) solution was prepared at a concentration of 1000 mM. Solutions of different MC concentrations were prepared at the solubility limit, namely: citric acid at 500 mM [CA-Cu(II)], ethylenediaminetetraacetic acid at 250 mM [EDTA-Cu(II)], iminodiacetic acid at 250 mM [IDA-Cu(II)], and glycerol at 100 mM [Gly-Cu(II)].

Efforts were dedicated to determining the best conditions to ensure that NPs were synthesized with high reproducibility. Briefly, PLGA and a specified quantity of complexes were dissolved in ethyl acetate and agitated at full speed for 30 s. Next, 10 mL of Pluronic F127 0.5 % (w/v) was added to the previous solution and sonicated for 60 s. Finally, it was transferred to an additional 10 mL of Pluronic F127 0.5 % (w/v) under magnetic stirring in a fume hood at room temperature (RT) for 20 h until complete evaporation of the organic solvent. NPs alone (i.e., free from complexes) were prepared according to the same procedure.

2.4. Determination of efficiency of Cu incorporation

At RT, the different NPs were incubated in an aqueous solution for 20 h. Afterward, they were concentrated by centrifugation (4300 rpm for 15 min) using paper filters (Millipore). The filtered fluid was collected and stored for further analysis. The NPs were resuspended in water, and this procedure was repeated twice. The absorbance of all samples was measured using a microplate reader (BioTek) at 310 nm (i.e., UV-A range), and the complexes encapsulation (CE, %) was measured using equation (1).

$$CE\ (\%) = ((total\ MCs\ - non\ encapsulated\ MCs)\ /\ total\ MCs)\ x\ 100 \quad (1)$$

2.5. Physicochemical characterization (Particle size, zeta potential)

Zeta potential and the diameter of the MCs-PLGA NPs in an aqueous solution were measured through a zetasizer nano ZSP. All measurements were performed after NPs were dispersed in purified water.

2.6. Electron paramagnetic resonance

To better know the Gly-Cu(II) complexes formed in solution, electron paramagnetic resonance (EPR) analysis was performed in two pH values and different proportions of metal:ligand. Aqueous solutions of $CuSO_4.5H_2O$ and glycerol in the ratio of 1:2 and 1:10 were prepared by dissolving the copper salt in water and adjusting the pH value to 5 and 11, which is observed in the insect digestion system. Afterward, the solutions were transferred to a capillary tube and placed in the EPR quartz tube.

EPR spectra were recorded in frozen solution using an X-band (9 GHz) Bruker ELEXSYS E500 spectrometer equipped with a variable temperature unit (ER 4B1 VT) and obtained in the following general experimental conditions: microwave frequency of 100 kHz, microwave power of 20 mW, modulation amplitude of 8 G, gain of 60 dB, acquisition time of 600 s at 100 ± 1 K. The spectra were simulated with the computer suite program Bruker WinEPR/SimFonia.

2.7. Insecticide bioassays

Bioassays of toxicity using larvae at 3rd/4th (L3/L4) instar of *A. aegypti* (Rockefeller strain) were performed to analyze the insecticidal activity. The concentrations were prepared according to the LC_{50} (33 ppm) [20] for Cu(II) ions. Aliquots of nano-encapsulated MC were prepared in distilled water concentrations from 1.9 to 132 mg mL^{-1}. Groups of 20 larvae were placed in 50 mL vials with distilled water at 25 ± 2 °C in quadruplicate. Mortality rates were analyzed 24, 48, and 72 h after the application. The experiments were performed with negative (distilled water) and positive (organophosphate temephos; 1 ppm) controls. For each concentration, three independent bioassays were performed on three different days. It was considered dead larvae that did not show any movement after stimulation by the touch of a brush. The lethal concentrations of LC_{10}, $LC_{50,}$ and LC_{99} were calculated by Probit (95 % confidence intervals) using the software StatPlus™ Professional (version 5).

3. Results and discussion

3.1. Nano-encapsulated MCs

The synthesized PLGA NPs encapsulating MCs were characterized. The properties of materials change with size as they approach the nanoscale and as the percentage of atoms on the surface increases. NPs exhibit unique properties when compared to bulk material. Due to their size, they are more chemically reactive and change their strength and properties, which makes them extremely important in the biological field.

3.2. Cu-loading efficiency

The dispersion quality was evaluated through polydispersity, zeta potential, and size (diameter). A homogenous distribution between 100 and 150 nm was observed in all the encapsulated Cu(II) complexes (Table 1). The low polydispersity index (PDI) shows that all investigated NPs were monodisperse in water, and no strong aggregation could be detected (Table 1).

The zeta potential is the charge measured at the material's surface, including the most strongly associated counterions. Results showed that NPs were negatively charged, with different values for the Cu(II) complex (Table 1).

The efficiency of PLGA NPs encapsulation (CE) of the Cu(II) complexes showed completely different yields (Table 1), with the salt ($CuSO_4$) presenting the lowest efficiency. Nevertheless, the efficiency of encapsulation for all the other complexes showed exciting and promising values.

3.3. Identification of glycerol-Cu(II) complexes in solution

As explained before, EPR spectra were performed at different aqueous solutions in the ratio of 1:2 and 1:10 at pH 5 and 11. The EPR spectra of aqueous solutions of $CuSO_4.5H_2O$ and a mixture of $CuSO_4.5H_2O$ and glycerol in the ratio of 1:2 obtained at pH 5 are shown in Fig. 2A. A comparison of the spectra showed that at pH 5, adding glycerol in a 1:2 M ratio did not significantly change the spectrum profile. However, an indication of an additional interaction could be seen in the lower field region. Computer simulation of the spectra allowed the identification of two species in the $CuSO_4.5H_2O$ and glycerol (1:2) solution, characterized by the spin-Hamiltonian parameters presented in Table 2. The presence of a species with different values of the spin-Hamiltonian parameters upon adding glycerol at pH 5 suggests an interaction of the ligand with the copper (II) center.

At pH 11, the situation was identical, as seen in Fig. 2B, in which the spectra showed that adding glycerol promoted the formation of a different species in the solution. The values of the spin-Hamiltonian parameters calculated by computer simulation are registered in Table 2 and suggest that glycerol also interacted with the Cu(II) center at pH 11. The values of the spin-Hamiltonian parameters of all species are characteristic of oxygen coordination spheres; the values obtained by simulation are characteristic of the coordination of Cu (II) by two oxygen ligands [21].

To assess the influence of the glycerol excess in the formation of Cu(II):glycerol complexes, a titration was performed using a $CuSO_4.5H_2O$ solution at pH 5 and glycerol in increasing amounts from 1:0.5 to 1:10. The spectra obtained by 1:2 and 1:10 M ratios are shown in Fig. 2C. Comparison of the two spectra indicates that no significant changes occurred after this procedure.

Glycerol is a simple polyol compound, an alcohol (1,2,3-propanetriol), and a sub-product of biodiesel widely used in different industries, such as cosmetics, food, and others [22]. Biodiesel is produced from animal fats and vegetable oils. It has become an attractive option as fuel because it is obtained from renewable sources, so its production can be exponentially increased by transesterifying vegetable oils and fats [23]. Glycerol is the main by-product of biodiesel, up to 10 % by weight of the total amount, and is seen as an environmental problem or a significant opportunity for use in different technological applications [22,23].

When in an aqueous solution, glycerol can change thermodynamic parameters by increasing the interaction of the target species with water. The association between α-cyclodextrin and three mono alcohols (1-pentanol, 1-hexanol, and 1-heptanol) in glycerol plus water was already observed by titration microcalorimetry at 288.15, 298.10, and 308.15 K [22].

The results herein showed that the glycerol linkage to the monoalcohols binding to α-cyclodextrin partially explains the change in the observed parameters, indicating that glycerol also acts as a co-solvent, thus affecting the solution interactions. Recently, glycerol was used for structural modification and conformational restriction of lysophosphatidylserine (LPS) to increase potency and receptor selectivity [23]. LPS is an endogenous lipid mediator that precisely activates membrane proteins of the P2Y and its related families of G protein-coupled receptors (GPCR) GPR34 (LPS1), P2Y10 (LPS2), and GPR174 (LPS3) [24].

3.4. Insecticide bioassays

Insecticide assays in larvae of *A. aegypti* were performed, and all the encapsulated MCs in PLGA NPs showed relevant toxicity except EDTA-Cu(II). Mortality results for nano-encapsulated metal complexes are shown in Table 3. The negative control (PLGA NPs in water) did not present mortality, while the positive control (temephos) caused 100 % mortality after 24 h. Only Gly-Cu(II) caused mortality after 24 h, with the other complexes showing signs of mortality after 48 h, with the full extension of insecticide observed 72 h later.

Organophosphates act as inhibitors of acetylcholinesterase, an essential enzyme for the central nervous system, with their toxic action being fast and efficient [25]. On the other hand, metal complexes target the insect's digestive system cells, causing slow

Table 1
Characteristics of different Cu(II) complexes-loaded PLGA NPs prepared by a double/solvent evaporation method.

Parameter	$CuSO_4$	CA-Cu(II)	IDA-Cu(II)	EDTA-Cu(II)	Gly-Cu(II)	Control
Size (nm)	111.73	124.07	126.53	121.20	116.33	141.43
PDI	0.07	0.08	0.12	0.09	0.05	0.23
Zeta potential (mV)	−1.60	−0.24	−17.43	−5.99	−2.20	−12.90
CE (%)	6.18	52.53	35.46	75.30	32.27	–

CE - Complexes encapsulation; PDI - Polydispersity index; CA - citric acid; EDTA - ethylenediaminetetraacetic acid; IDA - iminodiacetic acid; Gly – glycerol. Ultrapure water - Control.

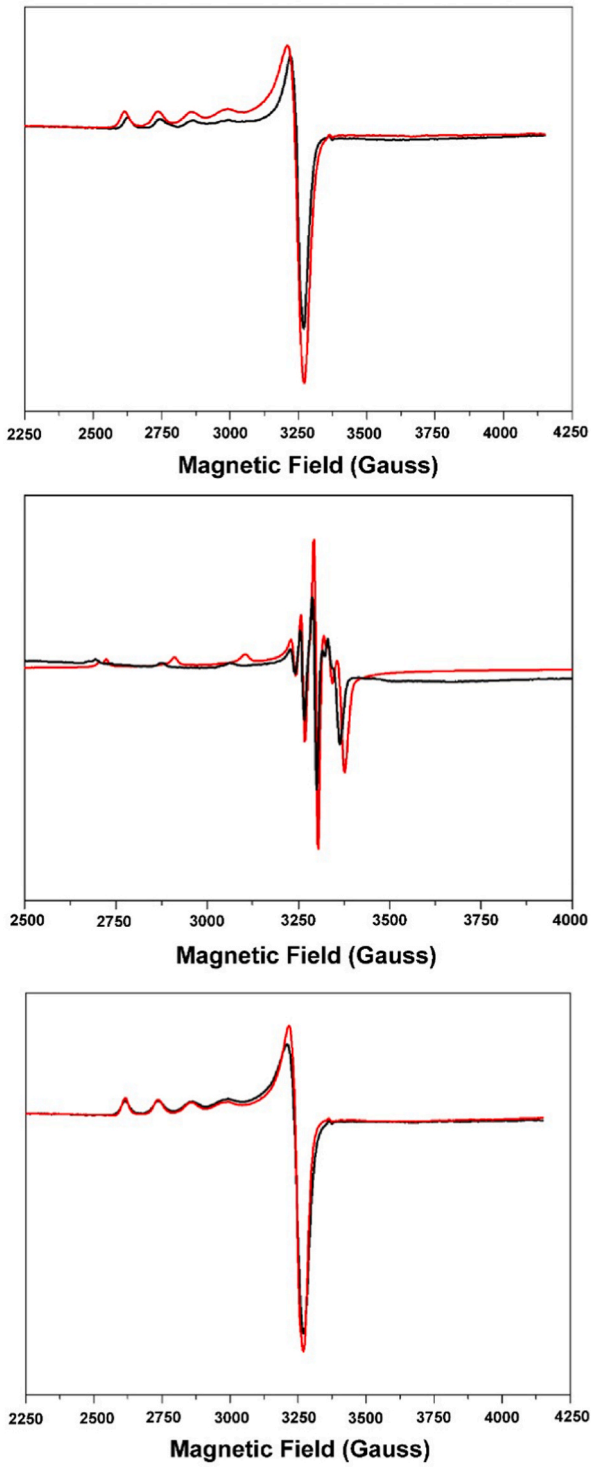

Fig. 2. EPR spectra. $CuSO_4.5H_2O$ (black line) and $CuSO_4.5H_2O$ and glycerol (1:2) (red line), at 100 K, A) pH = 5, B) pH = 11; and C) Cu(II):glycerol in the ratios 1:2 and 1:10, at 100 K and pH 5.

mortality [26]. This could be observed by the continuous increase in the mortality of the larvae between 24 and 72 h. It is considered that for approval of the metal insecticides as a commercial product, to prove the efficacy of the compound, the larvae should be dead at 72 h [27].

In this work, the insecticidal activity of nano-encapsulated Gly-Cu(II), which caused 11 % mortality within 24 h, clearly

Table 2
Spin-Hamiltonian parameters of the Cu(II) species at pH 5 and 11.

Complex	g-value			A-value (10^{-4} cm^{-1})		
	g_{zz}	g_{xx}	g_{yy}	A_{zz}	A_{xx}	A_{yy}
$CuSO_4.5H_2O$, pH = 5	2.408	2.080	2.080	134.0	6.0	6.0
$CuSO_4.5H_2O$, pH = 11[a]	2.274	2.050	2.054	194.0	30.0	26.0
	2.284	2.050	2.054	196.0	30.0	26.0
Cu(II)-glycerol, pH = 5	2.412	2.086	2.086	140.0	6.0	6.0
Cu(II)-glycerol, pH = 11[a]	2.262	2.050	2.054	196.0	30.0	26.0
	2.262	2.050	2.054	198.0	30.0	30.0

[a] At pH 11, spin-Hamiltonian parameters allow the two Cu isotopes to be observed.

Table 3
Mean percentage ± standard deviation of mortality of *A. aegypti* larvae undergoing treatment with nano-encapsulated metal complexes after 24, 48, and 72 h of application.

Time (h)	$CuSO_4.5H_2O$ (mg mL^{-1})				
	8.25	16.50	33.00	66.00	132.00
24	0	0	0	0	0
48	5.67 ± 4.98	11.88 ± 5.62	19.45 ± 7.67	20.06 ± 6.31	40.75 ± 8.89
72	14.04 ± 14.12	28.56 ± 17.72	30.08 ± 12.17	39.68 ± 14.49	59.46 ± 11.87
Time (h)	**IDA-Cu(II) (mg mL^{-1})**				
	6.08	12.15	24.30	48.65	97.30
24	0	0	0	0	3.12 ± 6.25
48	0	0	0	0	9.37 ± 18.75
72	0	0	0	0	10.93 ± 21.87
Time (h)	**CA-Cu(II) (mg mL^{-1})**				
	8.25	16.50	33	66	132
24	0	0	0	0	0
48	0	0	0	0	0
72	8.38 ± 11.42	1.75 ± 3.04	3.44 ± 2.98	6.22 ± 7.76	28.35 ± 18.05
Time (h)	**Gly-Cu(II) (mg mL^{-1})**				
	1.90	3.83	7.67	15.35	30.70
24	9.08 ± 5.93	9.52 ± 1.83	28.93 ± 11.10	34.51 ± 10.06	11.25 ± 6.67
48	28.63 ± 13.69	38.56 ± 10.02	54.63 ± 10.60	53.58 ± 4.73	57.29 ± 6.80
72	50.74 ± 12.68	58.30 ± 9.44	69.40 ± 10.32	70.47 ± 6.07	79.76 ± 8.34

Mortality results for nano-encapsulated metal complexes in three different time points (24, 48 and 72 h) to compound and metal complexes ($CuSO_4$ and ligands-Cu(II) (IDA - iminodiacetic acid, CA - citric acid and Gly – glycerol). The EDTA-Cu(II) PLGA NPs did not present relevant toxicity.

demonstrated that the encapsulation of complexes decreased the time of action. The concentrations used for CA-Cu(II) and $CuSO_4$ did not cause significant mortality, showing that higher concentrations are needed to achieve 100 % mortality. However, in the case of the Gly-Cu(II), high toxicity was observed, even when lower concentrations were used, causing 50 % mortality for the lowest concentration and 80 % for the highest concentration after 72 h of exposition. The range of toxicity of the Cu(II) ion is narrow despite both higher or lower doses presenting the potential to cause pathologic damage. Although we can hypothesize that the NPs carried the complexes into the cell's digestive system of larvae with high efficiency, additional studies need to be performed, especially at the histological and molecular levels, to elucidate the mechanism of release of complexes into the cells. After 24 h, a decrease in the mortality percentage was observed with the highest concentration used (30.70 mg mL^{-1}), indicating that some NPs agglomerated.

Table 4
Median lethal concentration (LC_{50}) (mg mL^{-1}) and upper and lower confidence intervals ($CI_{0.05}$) of $CuSO_4.5H_2O$ and Gly-Cu(II) at 24, 48, and 72 h.

Time (h)	LC_{50} ($CI_{0.05}$)	B ± SD	X^2	DF
$CuSO_4.5H_2O$				
24	347.707 (237.324–770.718)	2.650 ± 114.295	0.293	3
48	147.318 (130.269–164.367)	2.244 ± 0.075	1.172	3
72	94.825 (53.365–449.289)	3.007 ± 0.145	7.343	3
Gly-Cu(II)				
24	22.303 (19.966–24.639)	2.966 ± 0.705	0.061	2
48	4.960 (3.509–6.411)	4.283 ± 0.045	0.916	1
72	0.744 (0.148–1.323)	5.101 ± 0.242	0.181	1

B - Slope; DF - Degrees of freedom; SD - Standard deviation.

The median lethal concentration (LC_{50}) for $CuSO_4$ and Gly-Cu(II) complexes are shown in Table 4. There were no significant differences between 48 (t = −7.567) and 72 h (t = −9.473). Overall, we can conclude that Gly-Cu(II) is the complex showing higher insecticide activity to the larvae of *A. aegypti*.

Some results in the literature showed that the transition elements are toxic, especially Cu(II) and Fe(III), when used at the median lethal concentration (LC_{50}) of 33 mg L^{-1} they can cause physical and metabolic damage in mosquito larvae and eggs [20]. Other related effects include microtubule hyperpolymerization, followed by aggregation and cell mass apoptosis between 0.75 and 1.00 mM Cu(II) in C6/36 cells of *A. albopictus*, with higher concentrations of Cu(II) leading to necrotic cell death and apoptosis [26].

When complexed, Cu(II) metal has decreased reactivity, and the complex can induce toxicity in *A. aegypti* larvae. Copper is used in the composition of several products and has been used for a long time as an agricultural fungicide [5]. The same compound $CuSO_4$ in Bordeaux mixture was used as an insecticide [6]. Da Silva et al. evaluated the toxicity of Bordeaux mixture in larvae of *A. aegypti* and proposed an LC_{50} of 3.06 mg mL^{-1} (2.73–3.35) during 24 h [9], which is much lower than that obtained in the present study (94.825 mg mL^{-1}). These LC_{50} results can be compared to other cupric compounds, confirming their toxicity to mosquitoes. Several LC_{50} for larvae of *A. aegypti* at 3rd and 4th instars are available (in mg L^{-1}): 3.06 for Cu(II) (confidence interval 2.73–3.35) [9], 26.91 for copper acetate [28], 32.65 for Na_2[Cu(II)(EDTA)] [29], 33 for $CuSO_4$ [20], in the range from 10.0 to 0.625 to *Anopheles subpictus* and *Culex quinquefasciatus* [10], and intervals 120 to 160 for $CuSO_4$ (0.75–1.00 mM) for *A. albopictus* C6/36 cells [26].

These results show the high toxicity of the Cu(II) complexes for *A. aegypti,* which can be explained by cellular permeation, quantity, and Cu(II) availability. Thus, the manner of application is essential for the insecticide activity and an important part of the vector control strategy. For instance, the LC_{50} of $(Cu(H_2NTA)_2).2H_2O$ is 146.11 mg L^{-1} (132.18–160.10) [30], while when complexed with amino acids as L-glutamic acid-Cu(II), it is 53.401 mg L^{-1} (31.124–94.546), and L-aspartate-Cu(II) is 108.647 mg L^{-1} (62.106–144.984) [17]. Bordeaux mixture was evaluated, and the LC_{50} is 3.06 mg L^{-1} (2.73–3.35) [9]. Copper causes mortality in other aquatic organisms through apoptosis by reacting to reactive oxygen species (ROS) that trigger oxidative stress in intertidal Copepod *Japonicas tigriopus* [30]. In addition, de Arruda et al. [29] showed that Cu(II) in the metal complex [Na_2 (EDTA-Cu(II)] caused mortality with an LC_{50} of 32.65 mg L^{-1}.

4. Conclusions

Metal complexes were successfully encapsulated in PGLA nanoparticles, showing high monodispersity and no aggregation. The encapsulation efficiencies varied for Cu(II) complexes-loaded PLGA NPs prepared by a double/solvent evaporation method: 52.53 % for CA-Cu(II), 35.46 % for IDA-Cu(II), 75.30 % for EDTA-Cu(II), and 32.27 % for Gly-Cu(II). $CuSO_4$ presented a low efficiency of encapsulation (6.18 %). These efficiencies could not be correlated to the zeta potential and suggest that polymer modification processes may encapsulate metal complexes of Cu.

Insecticidal bioassays were performed to analyze and compare the metal complexes (metal insecticides). Gly-Cu(II) complexes showed the highest insecticidal activity. The nanoencapsulation with PLGA provides increased insecticidal activity over time of exposure since there was a decrease in the values of LC_{50} (in mg mL^{-1}): 22.303 at 24 h, 4.960 at 48 h, and 0.744 at 72 h.

Electron paramagnetic resonance (EPR) data showed that the spin-Hamiltonian values obtained by simulation are characteristic of the coordination of Cu(II) by two oxygen ligands and by spectra simulations. EPR allowed us to distinguish two species: isolated copper sulfate and the presence of glycerol at pH 5 and 11. The data suggest that two glycerol molecules are attached to the metal, and thus, we can conclude that the glycerol interacts and coordinates with Cu(II) at pH 11. Metal-ligand interactions and coordination processes can occur at different intensities over a wide pH range. These interactions/coordinations may allow the carrier molecule (Gly) to transport the metal to the intracellular environment.

Metal complexes with different ligands can be proposed as an alternative insecticide for immature insects since to create a coordination complex it is necessary that the carrier ligands formed by atoms, molecules or ions with unbound electrons and bonded to the central atom of a transition metal form the coordinate bonds. It can be speculated that metals interfere with their food chain, attractiveness, and reproductive process through toxic effects on breeding. In addition, the ligands can reduce reactivity, increase security, and facilitate cell permeation for enhancing biological effects in bioactive compounds, including insecticides. The insecticidal activity of a specific ligand could increase by the metal complexation and enable the conduction of the metal ions in a vector, which will be more appropriate for carrying/permeating copper ions across biological membranes, potentiating toxicity by oxidative stress using insect metabolism. Cellular and metabolic damage is caused by the production of free radicals with the formation of oxidizing species *in situ* in the target organism.

We propose applying glycerol and Cu(II) mixture to form Gly-Cu(II) complex in the insect digestive system, which has a pH gradient from 5 to 11, to control immature forms of vector insects, eggs, and larvae.

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Data availability Statement

Data will be available on request.

CRediT authorship contribution statement

Eduardo José de Arruda: Writing - review & editing, Writing - original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Glenda Biasotto:** Writing - review & editing, Writing - original draft, Visualization. **Marisa Masumi Beppu:** Supervision. **Fernando Jorge Monteiro:** Supervision. **Pedro L. Granja:** Writing - review & editing, Writing - original draft. **Maria Rangel:** Writing - original draft, Investigation. **Andreia Leite:** Writing - review & editing, Writing - original draft. **Isaías Cabrini:** Conceptualization. **Tiago Santos:** Investigation. **Daniel A. Gonçalves:** Writing - review & editing, Writing - original draft. **Herintha Coeto Neitzke Abreu:** Writing - review & editing, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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