Synthesis, encapsulation and antibacterial studies of ternary copper(II) complexes of fluoroquinolone Levofloxacin.

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INTRODUCTION AND OBJECTIVES

Fluoroquinolones (FQ) are an important class of antimicrobial agents and due to their pharmacological properties they are drugs of first choice in the treatment of most bacterial infections and one of the most successful in clinical practice (Efthimiadou, 2008). Unfortunately, this extensive use has led an emergence of microbial resistance, compromising its efficacy.

The lack of new drugs due to the long process of synthesis and approval has pushed forward the concept that metal complexes of antibiotics might play an important role. It is known that metal ions are present in several biological processes and that drugs possess modified pharmacological/toxicological properties when administered in the form of metal complexes. (Wu, 2003) The choice of Cu(II) lies in the fact that it forms stable complexes at physiological concentrations.

Due to a possible replacement of copper by other physiological divalent ions the encapsulation of these novel compounds may result in a more effective and direct action in the target site.

The main purpose of this work was to synthesize a ternary copper (II) levofloxacin 1,10-phenanthroline complex, the determination of its antimicrobial susceptibility and to proceed to study its encapsulation into liposomes.

MATERIALS AND METHODS

Materials

Levofloxacin (lvx), 1,10-phenanthroline (phen), $Cu(NO_3)_2$, $(NH_4)_2SO_4$, 1-steroyl-2-palmitoyl-sn-3-phosphocholine (SPPC), Cholesterol. All other reagents were of analytical grade and used with no further purification.

Synthesis of the ternary copper (II) complex

To a solution of lvx, NaOH (1M) was added, followed by the addition of phen and $Cu(NO_3)_2$ under constant stirring and solvent Ethanol:H_2O (1:1). The green solution was then concentrated on a rotovapor and left to stand at room temperature after which green crystals were collected.

Antimicrobial susceptibility testing

Minimal Inhibitory Concentrations (MICs) were determined in Iso-Sensitest broth following a standard micro-dilution technique.

Stock solutions of the drug, lvx:phen:Cu(II) (1:1:1 mixture) and synthesized ternary complex were prepared in 10 mM Hepes buffer pH 7.4 (I=0.10 M NaCl), stored at 4 ºC and protected from light. (Sousa 2012). 11 concentrations were tested in each assay. Microplates were incubated at 37 ºC and read after 18-24h. Each assay was repeated at least six times. (Sousa 2012)

Liposome preparation

Briefly, SPPC and Cholesterol were dissolved in CH_3Cl, dried under a stream of Argon and hydrated to produce liposomes. Extrusion was performed through filters of 200 and 100 nm (2/10 cycles respectively).

Encapsulation studies require a 2 step process: pH gradient formation between inner and outer liposome and incubation of drug with liposomes above their phase transition temperature.

RESULTS AND DISCUSSION

Synthesis

The ternary complex was characterized by UV/visible and infrared spectroscopy, elemental analysis and X-ray crystallography (figure 1). The results show that lvx is coordinated to the metal via the carbonyl and one of the oxygen atoms from the carboxylate group and that the coordination compound has a nitrate group as a counterion.

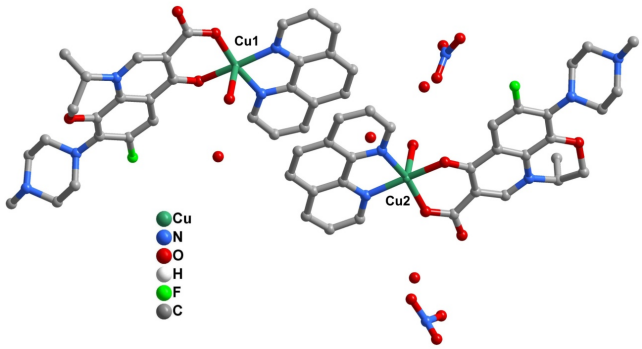

Figure 1 - Ball-and-Stick representation of asymmetric unit of the crystal structure of compound $[Cu(lvx)(phen)(H_2O)](NO_3)\cdot 2H_2O$.

Minimum Inhibitory Concentrations

Table 1 - MIC results obtained in *E. coli* strains.

	ATCC 25922	JF568	JF701	JF703
lvx	0.05 ± 0.02	0.09	0.09	0.36
Synthesized complex	0.07	0.08 ± 0.02	0.12 ± 0.03	0.19 ± 0.05
Cu(II):lvx:phen solution (1:1:1)	0.07	0.13 ± 0.03	0.11 ± 0.04	0.31 ± 0.08

***JF701 and JF703 are OmpC and OmpF porin deficient strains, respectively.**

MIC's data, for FQ, show that for the OmpF deficient strains (JF703) there is a significant increase in the MIC, which points to a porin dependent pathway. Nevertheless, for the complex the increase is not as pronounced suggesting an additional route, revealing that this compound can be an interesting candidate in the fight against antibiotic resistant microorganisms. (Sousa, 2012)

Encapsulation

Encapsulation of lvx into SPPC:Chol vesicles was possible through an $(NH_4)_2SO_4$ gradient with a encapsulation percentage of ~54%.

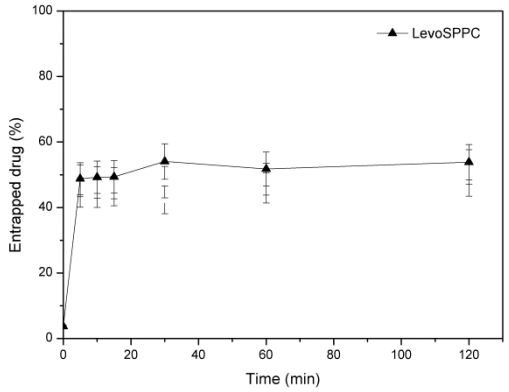

Figure 2 – Encapsulation profile of levofloxacin into SPPC:Chol vesicles

Loading of Culvxphen into SPPC:Chol liposomes was performed by establishment of $(NH_4)_2SO_4$ gradient and lipid film hydration with Hepes pH 7.4, Tris-Cl pH 8.0 and Culvxphen.

Table 2 – Encapsulation percentages of metal complex into SPPC:Chol liposomes

Gradient	**E (%)**
$(NH_4)_2SO_4$	15
Lipid film hydration	**E (%)**
Hepes buffer pH 7.4	6
Tris-Cl buffer pH 8.0	4
Culvxphen	2

CONCLUSIONS

A ternary Culvxphen complex was successfully synthesized and characterized by several techniques.

The results obtained for various *E.coli* strains show that the metal complex has an antimicrobial effect comparable to that of the free drug and shows that there is a different penetration route. These observations support its suitability for further biological testing.

Encapsulation data confirms loading of lvx although at a relatively low percentage (10% leakage after 4 weeks).

Regarding the encapsulation of the metal complex, with an $(NH_4)_2SO_4$ gradient, although a 15% loading was achieved, the procedure used for complex quantification was not suitable since extreme pH's are used (pH>11) and there is no complex formed in this range. The obtained value is that of the free drug and not the ternary complex. The low percentages obtained (table 2) have no significant meaning since they are below the determined experimental error (10%) and are probably due to copper associated positive charge which prevents membrane permeation.

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ACKNOWLEDGMENTS

This work was partially funded through project PTDC/SAU-FAR/111414/2009. I. Sousa thanks FCT (Lisboa, Portugal) for her PhD grant (SFRH/BD/47486/2008).