P-087	**Liposomal Drug Delivery Systems : Approaching Fluoroquinolone Encapsulation**	

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INTRODUCTION AND OBJECTIVES

Quinolones are a well know class of antibacterial agents, and one of the most prescribed drugs in medicine, having evolved from the simple treatment of urinary tract infections to a wide use in the treatment of various bacterial infections (Ball 2000). This wide use seems to be the main cause for bacterial resistance and, with increasing menace of bacterial resistance, development of new drugs and strategies to increase efficacy is of great importance.

Through the years, Quinolones have been structurally altered to overcome part of their adversities, such as toxicity and to obtain new improved drugs, with less toxic effects and broader spectrum of action, thus becoming drugs of first choice in treatment of bacterial infections.

Quinolones can be divided into 4 generations, according to the structural changes made to their basic nucleus such as introduction of a fluorine atom in position 6, a piperazinyl group in position 7, insertion of alkyl groups in the *para* position of the piperazinyl group and a nitrogen atom in position 1.. These changes allowed better clinical efficacy and reduced toxicity. (Ball 2000; Appelbaum 2000; De Souza 2005)Although changes have been made and new compounds produced, these drugs are still known for their various side effects and toxicity, and some of the agents have even been withdrawn or not approved for use.

Due to the need to increase drug efficacy, for the past few years, drug delivery systems (DDS's) have been the target of intense research because they aim to improve efficacy in the site of action as well as to improve aspects such as pharmacokinetics and/or minimizing side effects.

Liposomes are frequently used due to their high versatility and biocompatibility in terms of composition, size and physicochemical properties, allowing a wide range of applications in different areas, such as Biophysics or Chemistry (Lasic 1995). They are considered for DDS's when therapeutic agents are toxic, have high potency and low blood circulation times. Encapsulation of drugs, such as antifungal agents, has been reported and commercialized, but research regarding quinolones and liposomes, consists, mainly, in membrane permeability and physicochemical studies.

The main goal of this work was to encapsulate fluoroquinolones in SPPC:Cholesterol liposomes with high encapsulation efficiency, determine which pH gradient leads to a higher encapsulation of drug inside the vesicles and synthesis of Copper(II) ternary complexes for subsequent encapsulation in liposomes.

MATERIALS AND METHODS

Materials $(NH_4)_2SO_4$, $Cu(NO_3)_2.3H_2O$, NaOH and 1,10-Phenanthroline were purchased from Merck, CH_3Cl, Sucrose, Chloroform, all Quinolones, Cholesterol and Dialysis tubing were purchased from Sigma-Aldrich (Madrid, Spain) and Ethanol, *p.a.* from Panreac.
Lipid SPPC (1-Steroyl-1-Palmitoylphosphatidylcholine) was purchased from Avanti Polar Lipids.

SPPC vesicles (~20mM) were prepared by the extrusion technique. Briefly, SPPC and Cholesterol (1:1) were dissolved in CH_3Cl, dried under a stream of Argon, and liposomes were formed by hydration of the lipid film with $(NH_4)_2SO_4$, pH 5,5, and extrusion through 200 and 100nm (2 and 10 cycles, respectively) polycarbonate filters. Size and polidispersity were determined by Dynamic Light Scattering (DLS).

Encapsulation studies consist of **two steps:**

Step 1 **-** Formation of a pH gradient between inner and outer liposome.

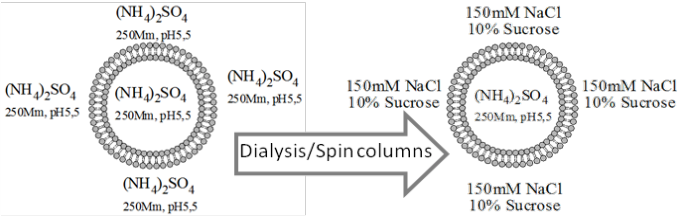

Figure 1: pH gradient formation

The pH gradient is formed by passing the liposome suspension through a Sephadex G-50 column equilibrated either with 150mM NaCl or 10% Sucrose (spin column) or by dialysis of the suspension against either one of the mentioned solutions, to exchange external buffer.

Step 2 **-** Incubating Fluoroquinolones with liposomes above its phase transition temperature.

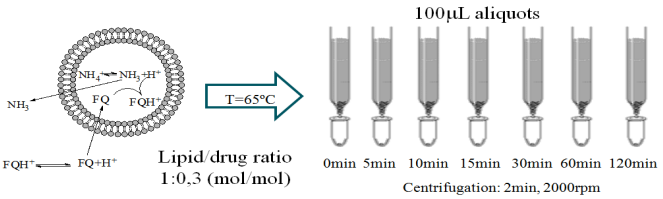

Figure 2: Fluoroquinolone loading/encapsulation in liposomes

Incubation is performed at a lipid/drug ratio of 1:0.3 mol/mol, at 65ºC for 2h. 100µL aliquots are removed at appropriate time points and passed through a Sephadex G-50 column to remove unencapsulated drug.

Copper (II)/Fluoroquinolone/1,10-Phenanthroline synthesis

0,5mL of NaOH (1M) was added to 0,5mmol Fluoroquinolone (Norfloxacin; Sparfloxacin, Ofloxacin; Levofloxacin) and 0,5mmol 1,10-Phenanthroline. To this mixture, 0,5mmol of $Cu(NO_3)_2.3H_2O$ solution was added and left to react with continuous stirring, for aprox 2h. Solvent used was Ethanol:Water (1:1).

RESULTS AND DISCUSSION

Fluoroquinolone quantification is performed by an absorbance assay, following removal of encapsulated drug by a Bligh-Dyer extraction procedure. Spectra of the different aqueous phases were traced and drug concentration and % encapsulation were determined.

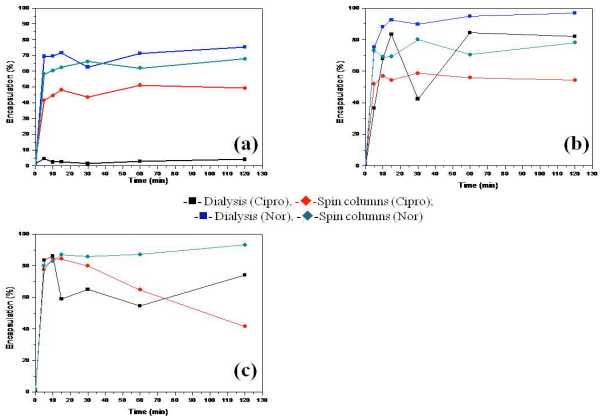

Figure 3 - Encapsulation (%) *vs* time (min) for different pH gradients **(a)** 150mM NaCl/ 150mM NaCl; **(b)** 10% Sucrose/10% Sucrose; **(c)** 150mM NaCl/10% Sucrose.

After synthesis, complexes were allowed to dry under vacuum and sent to elemental analysis.

Table 1 – Elemental analysis of synthesized ternary complexes

		Calculated	Found
Levofloxacin	**C (%)**	**48.1**	**47.5**
	H (%)	**4.59**	**4.26**
	N (%)	**11.6**	**11.9**
Norfloxacin	**C (%)**	**44.3**	**44.3**
	H (%)	**4.51**	**4.02**
	N (%)	**12.9**	**12.6**
Ofloxacin	**C (%)**	**46.4**	**46.1**
	H (%)	**4.8**	**4.49**
	N (%)	**9.31**	**8.57**
Sparfloxacin	**C (%)**	**48.4**	**48.4**
	H (%)	**4.85**	**4.71**
	N (%)	**12.8**	**12.7**

CONCLUSIONS

Encapsulation:

Several pH gradient were tested: Dialysis of liposomes against NaCl and Sucrose solutions and spin columns equilibrated with the same solutions. Preliminary results show that highest encapsulations occur for pH gradients formed by Sucrose (b) and NaCl (c), followed by centrifugation of the recovered aliquots in sucrose equilibrated spin columns. In all studies, maximum encapsulation occurred, predominantly, between 10 and 30min of incubation, reaching a maximum peak at 15min. It is also possible to observe that, when using the Sucrose/Sucrose system, encapsulations change significantly but, for NaCl/Sucrose, encapsulations at the maximum peak are close, thus indicating that this system will probably yield better results in the future.

Synthesis:

Regarding the synthesis of the Copper (II) ternary complexes, elemental analysis as well as infrared spectra (not shown) reveals that, although needing further purification, the complexes have been produced.

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