Physicochemical and Biopharmaceutical Assessment of Diclofenac-Loaded Liposomes

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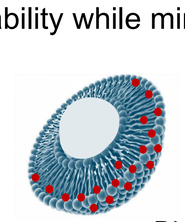
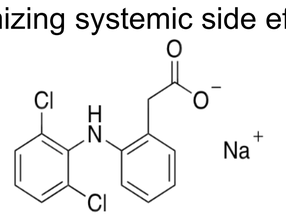
OVERVIEW

The main objective of the study was to develop and optimize a small unilamellar vesicle (SUV)-based liposomal formulation (50–100 nm) to improve topical delivery for inflammatory skin conditions. The study aimed to characterize the physicochemical properties of the formulation, assess encapsulation efficiency, and evaluate its release profile, skin permeation, and anti-inflammatory activity *in vitro*. The formulation was optimized and purified by ethanol evaporation for 72h, achieving high encapsulation efficiency (88.8 ± 8.2%) and stable vesicles (size: 86.4 ± 8.4 nm; PDI: 0.145; Z-potential: –17.9 mV). A fluorescence quenching assay revealed diclofenac localization near the lipid bilayer surface. In vitro release and permeation were assessed using Franz diffusion cells. Anti-inflammatory activity was confirmed via qPCR after LPS-induced inflammation, showing a 70% reduction in TNF-α and CXCL1 expression.

INTRODUCTION

Inflammatory skin disorders require effective and safe treatments. Diclofenac, a widely used non-steroidal anti-inflammatory drug (NSAID), faces challenges in topical administration due to its poor skin penetration. Encapsulation in liposomal drug delivery systems represents a promising strategy to enhance local bioavailability while minimizing systemic side effects.





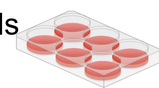
• Diclofenac

METHODS

Formulation of liposomes: Liposomes were prepared by dissolving phosphatidylcholine, hydrogenated phosphatidylcholine, Tween 80, α-tocopherol, cholesterol, and sodium diclofenac in ethanol (10% w/w). The organic phase and phosphate buffer were heated to 75 °C, then mixed (final buffer content: 85.5% w/w) and vortexed for 1 min. The dispersion was sonicated (20% amplitude, 15 s, 2000 Ws), and preservatives (sodium nipagin and nipasol) were added. Empty liposomes (without diclofenac) were also prepared as controls.

Characterization of liposomes:

1. Size distribution and charges of the liposomes by dynamic light scattering (DLS)
2. Morphology by cryogenic transmission electron microscopy (cryo-TEM)
3. Encapsulation efficiency by HPLC-UV
4. Ethanol elimination by evaporation
5. Determination of ethanol by gas chromatography
6. Localization of diclofenac by Stern-Volmer fluorescence quenching assays
7. *In vitro* permeation and release profiles by Franz cells
8. Anti-inflammatory activity evaluation in HaCaT cells
9. *In vitro* cytotoxicity evaluation in HaCaT cells



RESULTS

SIZE DISTRIBUTION AND CHARGES (DLS), %EE (HPLC) AND MORPHOLOGY (CRYO-TEM)

Method	Parameter	Non-loaded Liposomes	DCF-Loaded liposomes
Theoretical	Theoretical DCF concentration (mg/mL)	0.00	5.00
HPLC-UV	Experimental encapsulated DCF concentration (mg/mL)	-	4.34
	%EE	-	93.35
DLS	Diameter (nm)	87.84	102.20
	PDI	0.27	0.22
	Z-Potential (mV)	-14.26	-17.93
Cryo-TEM / ImageJ	Nº of analyzed nanoparticles	107	111
	Diameter (nm)	94.59	114.47
	PDI	0.21	0.22

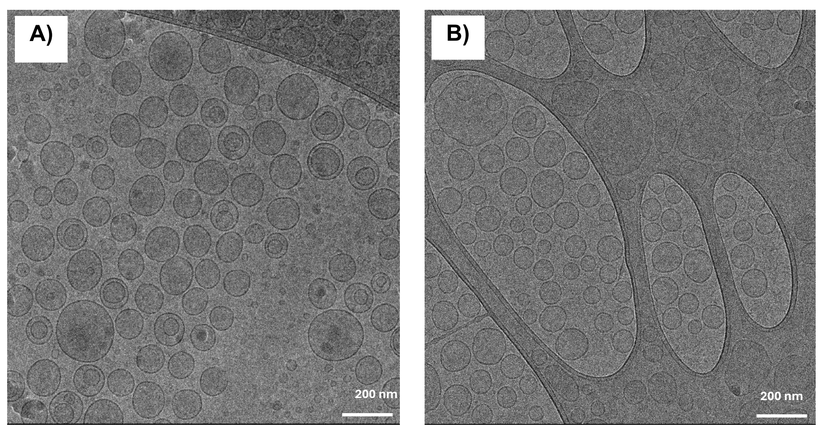


Figure 1. A) and B) are representative Cryo-TEM images of non-loaded and loaded liposomes respectively. The captures show well-defined liposomal structures with a spherical morphology, monolamellarity and uniform size.

LOCALIZATION OF DICLOFENAC WITHIN THE LIPOSOME SYSTEM

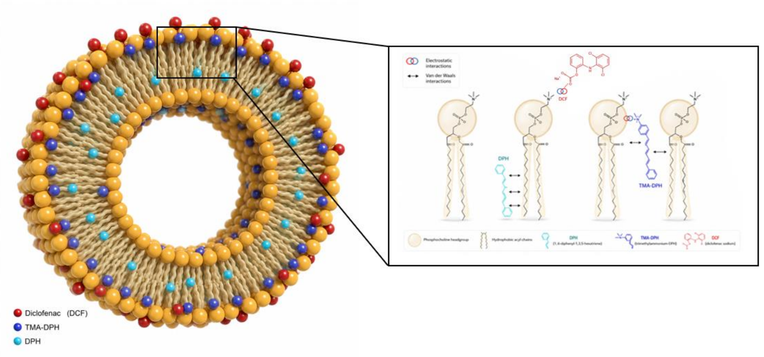
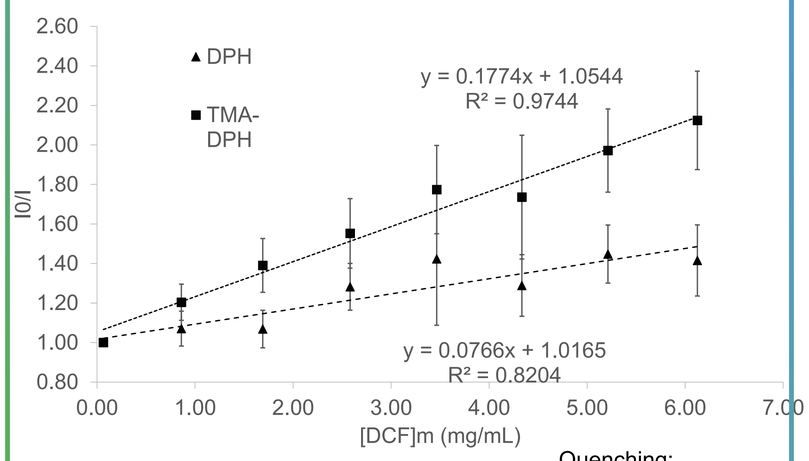


Figure 2. Graphical representation of the fluorescence quenching system and the probable locations and interactions with the liposome system for each compound



$$\frac{I0}{I} = K_{sv} \times [DCF]_m + 1$$

Quenching: $\frac{K_{SV}\ TMA-DPH}{K_{SV}\ DPH}$

2.32 TMA-DPH/DPH

Figure 3. Stern-Volmer plots for fluorescence quenching assays featuring DPH and TMA-DPH as fluorescent probes, and DCF as quencher.

PERMEATION AND RELEASE PROFILES

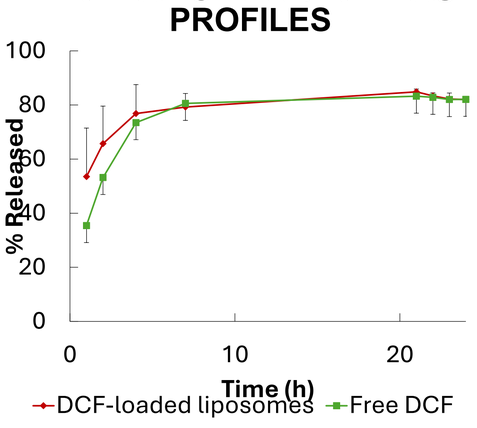


Figure 4. Mean release data obtained from the DCF-loaded liposomes, Free DCF, and Commercial reference gel after 24 hours of the in-vitro release test.

IN VITRO CYTOTOXICITY EVALUATION IN HACAT CELLS

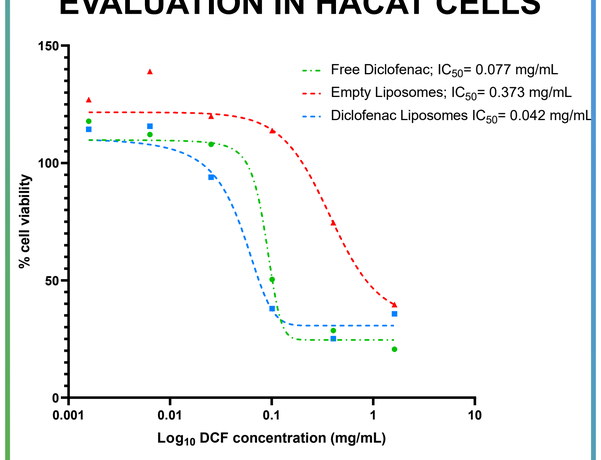


Figure 5. IC 50 analysis for diclofenac loaded liposomes, empty liposomes and free diclofenac

ANTI-INFLAMATORY ACTIVITY EVALUATION IN HACAT CELLS

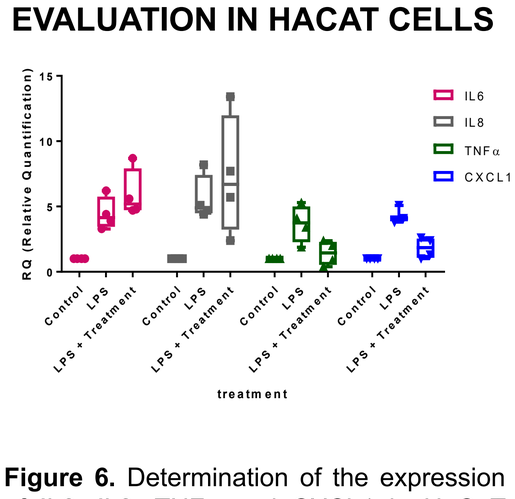


Figure 6. Determination of the expression of IL6, IL8, TNFα and CXCL1 in HaCaT cells.

CONCLUSIONS

Based on the results obtained, the following conclusions can be drawn:

- Diclofenac-loaded small unilamellar vesicles (SUVs) were successfully developed with a narrow size distribution (~86 nm) and high encapsulation efficiency (~89%)
- Fluorescence quenching studies suggested that diclofenac is mainly located near the polar head groups of the lipid bilayer.
- The diclofenac-loaded liposomes demonstrated superior performance compared to the commercial reference gel in both *in vitro* release and permeation studies.
- *In vitro* assays demonstrated significant anti-inflammatory activity, with a marked reduction of TNF-α and CXCL1 expression levels.

Overall, these results support the potential of liposomal diclofenac as a promising topical treatment for inflammatory skin conditions.

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