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Physicochemical aspects of liposomes encapsulating the hrEGF-rP64k conjugate

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Short Communication

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Abstract

The therapeutic vaccine CIMAvax-EGF® is formulated with the hrEGF-rP64k conjugate and Montanide ISA 51 VG®, an oil-based adjuvant associated with granuloma formation. In this work, dehydration–rehydration vesicles (DRVs) composed of DPPC:Cholesterol (1:1) were physiochemically characterized as an alternative antigen delivery system, evaluating the effect of sucrose on vesicle size, encapsulation efficiency, and storage stability.

Keywords: Dehydration; Biodegradable; Proteins; Encapsulation; Immunotherapy

Introduction

The therapeutic vaccine CIMAvax-EGF® is based on the conjugate hrEGF-rP64k formulated with Montanide ISA 51 VG®, a mineral oil adjuvant that induces granulomas at injection sites and is not metabolized. These limitations motivate the exploration of biodegradable antigen delivery systems [1].

Liposomal vesicles represent biocompatible and low-immunogenic nanoparticles capable of storing significant amounts of antigen and are not associated with granuloma formation when composed of natural or synthetic phosphatidylcholine [2]. Among preparation methods, dehydration–rehydration vesicles (DRVs) allow efficient encapsulation of labile macromolecules such as proteins due to their large internal aqueous compartment and high material-to-lipid ratio.

In this study, liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (1:1 molar ratio) were prepared following the dehydration–rehydration methodology originally described by Kirby and Gregoriadis [2]. Proteins were incorporated at 30 µg per 6,4 µmol of total lipids. In selected formulations, sucrose was added before lyophilization at a sugar: phospholipid mass ratio of 3:1, reaching final concentrations of 14 mM or 40 mm

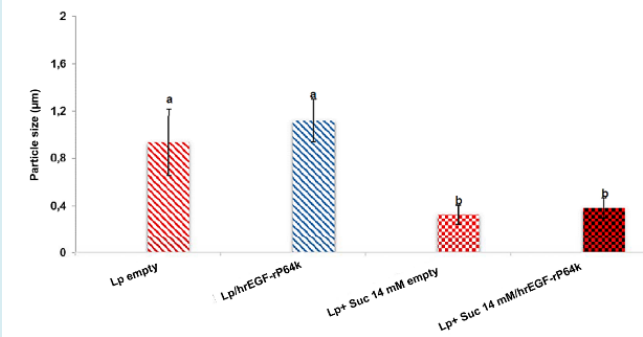


Figure 1: Size Of Liposomal Preparations in the Presence or Absence of Sucrose.

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Baseline DRVs (without sucrose) were moderately polydisperse, approximately 1 μm in diameter, and encapsulated around 27% of hrEGF-rP64k as determined by fluorimetry and UMELISA-EGF. These values are consistent with previous encapsulation efficiencies reported for similar systems [3].

The size of the vesicles (Lp) wasdetermined by DLS after rehydration. Empty Lp: control vesicles to which no proteins were added. Data from three replicates of the experiment are shown; the bars correspond to the standard deviation. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

Sucrose addition significantly modified vesicle properties. At 14 mM, vesicle size decreased approximately threefold, while encapsulation efficiency was reduced by roughly half (~12%) compared to formulations without sugar. In contrast, 40 mM sucrose-maintained encapsulation efficiency at values comparable to sugar-free preparations.

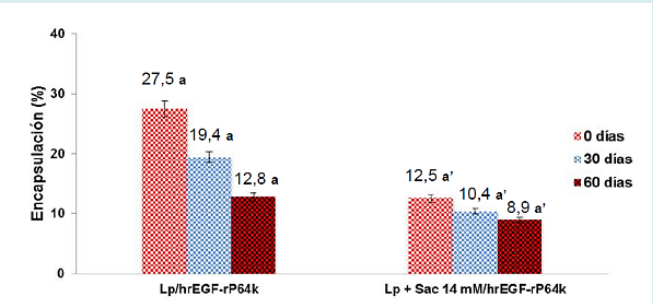


Figure 2: Encapsulation Efficiency of the Hregf-Rp64k Conjugate in Liposomes with or without Sucrose Stored in the Lyophilized State.

The encapsulation of hrEGF-rP64k after Lp rehydration was determined by UMELISA-EGF. The bars correspond to the standard deviation (n=3). The EE (%) shown at the top of the bars were transformed by calculating their square root; identical letters indicate that there are no significant differences according to ANOVA ($p>0.05$).

Storage stability was evaluated after 30 and 60 days at −20 °C in the lyophilized state. Liposomes without sucrose showed a significant increase in size and polydispersity after storage, likely associated with vesicle fusion events, which are thermodynamically favourable processes. Vesicles containing 14 mM sucrose exhibited a less pronounced size increase during storage.

The stabilizing effect of sucrose is attributed to its interaction with the lipid bilayer. During dehydration, sucrose solutions become supersaturated and may form a vitreous, amorphous solid matrix that physically separates vesicles, limits mobility, and increases the activation energy of fusion processes. After rehydration, sugars remain intercalated between phospholipids both below and above the gel-to-liquid crystalline transition temperature (Tc), reducing solute leakage and stabilizing membrane structure [4].

Together, these results demonstrate that sucrose concentration critically modulates vesicle size, encapsulation efficiency, and long-term stability of DPPC:Cholesterol DRVs encapsulating hrEGF-rP64k. The 40 mM condition preserves antigen loading, while 14 mM enhances storage stability despite reducing encapsulation efficiency.

This physicochemical characterization provides a foundation for considering liposomal DRVs as a biodegradable alternative platform for antigen delivery in anti-EGF immunotherapy.

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