

Development and *In Vitro* Characterization of Peptide-Loaded Niosomes for Topical Delivery

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Antimicrobial resistance is progressively reducing antibiotic efficacy against skin infections, renewing interest in antimicrobial peptides as broad-spectrum alternatives with lower propensity to induce resistance [1]. Insect salivary glands harbor promising peptides such as Hyp13pro, recently identified from *Anopheles coluzzii*, whose antibacterial potential is limited by poor stability [2]. Niosomes, vesicular nanocarriers composed of non-ionic surfactants, can overcome this limitation by protecting the peptide, improving skin retention and enabling controlled release at the infection site. In this project, Hyp13pro was encapsulated in niosomes prepared by thin-film hydration using Tween 85 and Span 20. Empty (Nio) and loaded (Nio13) niosomes were characterized by DLS to determine size, polydispersity index (PDI), and ζ -potential, while vesicle morphology was studied by TEM. Bilayer fluidity was evaluated by fluorescence anisotropy using the DPH probe [3]. Niosomal entrapment efficiency (EE) and peptide stability were assessed by UV–Vis spectrophotometry. *In vitro* release and Franz cell permeation studies (employing Strat-M[®] membrane) were also performed. Antimicrobial activity was tested against *S. epidermidis* and *S. aureus*, and cytocompatibility on HaCaT keratinocytes. Both formulations showed suitable hydrodynamic diameters (100 nm for Nio and 120 nm for Nio13), low PDI, negative ζ -potential (-25mV) and an EE of 55%. Anisotropy remained unchanged after peptide loading, suggesting Hyp13pro localization in the aqueous core. Both formulations were stable for up to 90 days, and encapsulation markedly enhanced peptide stability. Nio13 provided a high controlled release (~60% at 8 h) and promoted peptide retention within the Strat-M[®], supporting local skin delivery. Nio13 also enhanced antibacterial activity against both strains compared with the free peptide, while remaining biocompatible. These findings support niosomal encapsulation for topical skin infection treatment.

[1] Li X, Zuo S, Wang B, Zhang K, Wang Y. Antimicrobial mechanisms and clinical application prospects of antimicrobial peptides. *Molecules*. 2022;27(9):2675.

[2] Bevivino G, Maurizi L, Ammendolia MG, Longhi C, Arcà B, Lombardo F. Peptides with antimicrobial activity in the saliva of the malaria vector *Anopheles coluzzii*. *Int J Mol Sci*. 2024;25(10):5529.

[3] Rinaldi F, Del Favero E, Rondelli V, Pieretti S, Bogni A, Ponti J, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem*. 2017;32(1):538-546. doi:10.1080/14756366.2016.1268607.