OIL-FROZEN W1/O/W2 DOUBLE EMULSIONS FOR TRANSDERMAL BIOMACROMOLECULAR DELIVERY CONTAINING ETHANOL AS CHEMICAL PENETRATION ENHANCER.

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INTRODUCTION
Skin may be a potent immunological induction site since it also plays a role as an active immune barrier by containing immunocompetent cells. Transdermal vaccination using a cream-like formulation is a very attractive goal for both developing countries and emergency mass vaccinations (as a rapid response to pandemics or bioterrorist attacks) so that vaccination could be logistically simpler and cost-efficient since fewer medical personnel would be required. In this work, oil-frozen water-in-oil-in water (W1/O/W2) double-emulsion formulations were studied for transdermal delivery of a model biomacromolecule.

METHODS
W1/O/W2 double emulsions (DE) were prepared by a two-step emulsification procedure, with a volumetric ratio 2:2:1. Optical and fluorescence microscopy were employed to verify double emulsion structure. To quantify the amount of protein released from the emulsion, quantitative UV-Vis spectrophotometry was performed.

Skin penetration studies of FITC-BSA containing formulations were performed. A Franz diffusion glass cell apparatus was used to hold epidermis samples during the in vitro penetration experiments. FITC-BSA skin penetration was studied using an inverted Confocal laser scanning microscope.

A sandwich-type enzyme-linked immunosorbent assay (ELISA) was employed to determine in-vitro the effect of exposure of BSA released from W1 to W2 (which contained ethanol at varying concentrations up to 40% w/v) on the ability to be recognized by a specific antibody.

RESULTS
Oil-frozen W1/O/W2 double emulsions containing ethanol up to 40% in the external W2 phase were successfully prepared and exhibited external coalescence upon thawing of the oil phase, releasing up to 85% of the protein encapsulated in the internal aqueous phase. Oil-frozen W1/O/W2 double emulsions were studied in-vitro as potential transdermal macromolecular delivery formulations, achieving FITC-BSA penetration of up to 91 µm into porcine skin. ELISA studies were performed to observe the effect of the emulsification process and ethanol content on the ability of BSA to form antigen-antibody complexes; results indicated that ethanol content and the emulsification process did not diminish the BSA-antibody complex formation when compared to a BSA standard aqueous solution.

CONCLUSION
It was shown that ethanol content in W2 improved proportionally the release of W1 droplets upon thawing of oil-frozen double emulsions. Biomacromolecule penetration into viable epidermis was achieved when incorporating ethanol in the oil-frozen double emulsion. It is shown that oil-frozen W1/O/W2 double emulsions can potentially be used for transdermal vaccine delivery formulations.

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