

ANTI-OXIDANT COATED LIPOSOME AS THE DELIVERY SYSTEM FOR PAPAINE BASED NATURAL COSMETICS

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OBJECTIVE

Liposome is a topical delivery system that has evoked a considerable interest in the cosmetic industry. Liposome is biocompatible, biodegradable, exhibits low toxicity and has the ability to entrap both hydrophilic and lipophilic active materials. Over the last decades, liposome has been intensively studied as carriers for transdermal administration of drugs and skin care active ingredients. Papain, a protease, is an exfoliative agent which has also been touted to be able to reduce the signs of aging. The aim of this study was to evaluate the potential of liposome loaded with papain in natural cosmetics. The liposome is expected to enhance the transdermal penetration of papain into the skin and also to stabilize papain and reduce skin damage due to direct exposure to papain. In this study, L- α -phosphatidylcholine from soybean was used to produce liposome. This biological source of lipid typically is easier to be oxidized due to the unsaturated fatty acid. To overcome this problem, an antioxidant is added to decrease oxidation.

METHODOLOGY

Multilamellar liposome vesicles encapsulated papain were prepared using soy lecithin (L- α -phosphatidylcholine). The lipids in chloroform were dried in rotary evaporator for 8 hours, the thin film was then hydrated with 0.1mM buffer, pH 6.5 containing 0.05g dissolved papain followed by sonication. The mixture was then extruded through 0.45 μ m nylon filter membrane, 0.2 μ m nylon filter membrane and 0.1 μ m PTFE filter membrane to obtain a final vesicle size of approximately \pm 100nm. Two types of liposome - papain vesicles were prepared; coated and uncoated with antioxidant (L-ascorbate). Untrapped papain was removed by column chromatography in Sephadex G-50 gel, equilibrated with pH 6.5 buffer and encapsulation efficiency was calculated using the following formula:

$$\text{Encapsulation efficiency \%} = [1 - (\text{encapsulated papain} / \text{total papain})] \times 100$$

(1)

In - vitro permeation study was performed on abdominal skin of rat and reconstructed human skin from EPISKIN® in order to observe the permeation of liposome-papain through the stratum corneum of the skin. Rat abdominal skin was chosen as a model based on the similarity in permeability and morphology to human skin. The rat was anesthetized using chloroform. Abdominal skin of rat was shaved and cut into 2.1 cm \times 2.1 cm \times 0.23 cm (thickness) and afterwards stored in a freezer at -10°C until used. Two hours prior to the diffusion experiment, the samples were thawed and soaked in a pH 6.5 buffer. EPISKIN® 1.2

cm² skin equivalents were purchased from Episkin SNC (Lyon, France). The permeation study was done using a side-by-side diffusion cells equipment with a diffusion area of 1.07 cm². The skin was clamped between the donor and the receptor chamber. The receptor compartment was filled with 2.5 ml of buffers (pH, 6.5). The diffusion cells were thermostated at human body temperature of 37°C and the solution was stirred by magnetic bar. 2 ml of liposome-papain and buffers were placed in a donor chamber. Samples of 1000 µl were removed at defined time intervals for analysis and replaced immediately by an equal volume of fresh buffers. Each sample was subjected to papain assay to determine papain concentration.

Mixture design was applied to develop a base natural cream formulation. The experimental design and statistical analysis were performed using Design Expert version 6.0.8 (Stat. Ease, Inc). Eleven independent variables were chosen to evaluate the combined effects of three responses. The response values were viscosity, stability and pH. The apparent viscosity of the formulations was measured using a rotational viscometer (Fungi Lab). pH was measured using a standard laboratory pH meter from HANNA Instrument. Stability test was performed using freeze-thawing method. The formulation was placed at -10°C for 24 hours followed by thawing at room temperature, 25°C for 24 hours. The cycle was repeated three times.

RESULTS

The liposome, which was at pH 6.5, was stored at 4°C and the stability of the liposome was monitored up to sixty days. Coated liposome was found to be more stable than uncoated liposome. After two months, the encapsulation efficiency of the coated liposome was 80%, whilst the encapsulation efficiency of the uncoated liposome was 74%. The antioxidant was used in this study to minimize the oxidation of lipid. In addition, pH of 6.5 was selected as it has been reported that rate of lipid hydrolysis is lowest at this pH. The results show that the antioxidant coating, together with the pH 6.5 environment, resulted in the reduction in liposome breakage.

Percutaneous penetration implies several steps. The release of papain through skin will depend on the physicochemical properties of papain itself combined with the influence of the vehicle to alter the substance's penetration profile. Results obtained from the analysis showed that liposome-papain effectively penetrated the skin. Compared to rat skin, the level of papain penetration increased in EPISKIN[®]. Various studies have suggested that liposomes increase percutaneous penetration of compounds through interaction of vesicles with the stratum corneum and that this penetration is responsible for the greater concentration of the active ingredient in the skin.

The last part of the study was to formulate a stable natural base cream formulation. The papain carrier will be incorporated in this base cream. Design Expert was used to formulate the base cream. In formulating a skin care product, the product must be sufficiently homogenous in order to be economically attractive and must be physically stable to avoid changes in appearance during storage. Viscosity, pH and stability are important not only from the consumer's standpoint but also for processing. The modern formulator usually uses a multifactorial approach to formulate a product and their typical product performance objectives are: formulation is cosmetically elegant and user-friendly, formulation is physically stable and formulation can be scaled up and produced commercially.

The Scheffe model was used to fit and interpret the data and the results signified that the linear model was the most suitable for all responses. The results of the optimisation were presented in Table 1 and Table 2. Final formulation from the optimisation of experimental design showed that all formulations were stable physically and chemically (data from microbiological assay not shown).

Table 1: Cream formulation after Mixture Design Optimisation (weight percentages)

Cream	VCO	Palm Oil	Beeswax	Glycerin	Stearic acid	Tween 80	Soy lecithin	Distilled water	Xanthan gum	Acacia gum	Potassium sorbate
1	4.61	4	2.93	0.01	0.23	1.87	1.67	77.31	3.42	2.16	1.6
2	4.90	4.04	2.65	2.05	0.01	1.94	2.66	74.03	3.72	2.21	1.6
3	4.58	4.75	3.00	2.03	1.68	1.48	3.86	72.21	3.71	0.91	1.6
4	3.98	3.65	2.75	2.12	0.45	0.99	2.91	76.57	3.61	1.16	1.6

Table 2:

Response	Viscosity (cPa.s)		Stability (%)		Predicted	pH	Observed
	Predicted	Observed	Predicted	Observed			
Cream 1	21 564.6	19 359	100	100	6.5	6.499	
Cream 2	21 771.6	17 227	100	100	6.5	6.5	
Cream 3	21 564.1	23 918	100	100	6.5	6.5	
Cream 4	19 489.6	16 209	99.998	100	6.4449	6.498	

