

Preparation and Characterization of Liposome Containing Minoxidil and Rosemary Essential Oil

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Abstract

Minoxidil and Rosemary essential oil have been used for several years to stimulate hair growth. Therefore, co-administration of both Minoxidil and Rosemary essential oil could enhance hair growth. The chemical/biological characteristics of liposomes, which encapsulate both hydrophobic and hydrophilic drugs, can be utilized to encapsulate the herbal and chemical drug concoction concomitantly. A thin-film hydration method was used to prepare the liposomes. The entrapment efficacy of the liposomes was determined for Minoxidil and Rosemary essential oil using UV spectrophotometry and hydrodistillation; as dictated in the European pharmacopeia. Furthermore, a dynamic light scattering (DLS) analysis was conducted to determine the particle size and zeta potential of the prepared liposome. In addition, the storage stability of the liposome was checked after 60 days. The results showed that co-encapsulation of Minoxidil and Rosemary essential oil increased the encapsulation efficacy of Minoxidil while the entrapment efficacy of essential oil was not significantly influenced. In addition, according to the DLS results, the particle size and zeta potential of prepared liposomes didn't change significantly during 60 days of storage.

Keywords:

Liposomes; Hydrodistillation; Spectrophotometry; Co-encapsulation

attribute to the Minoxidil mechanism of action [3]. One of the biochemical actions of Minoxidil for hair growth is its stimulatory effect on prostaglandin and Vascular endothelial growth factor (VEGF) synthesis [4]. However, a limitation to the application of Minoxidil is its poor skin penetration ability and water solubility [5]. In order to enhance Minoxidil's penetration and solubility it has to be formulated in an ethanol based solution [5] which has been known to cause dermatitis irritation, pruritus, erythema, scaling and dryness of skin [6]. In addition, Terpene compounds such as cineol, limonene, and Nerolidol have been observed to improve the penetration of Minoxidil in the skin [7]. These Terpene compounds, especially cineol constitute the major fraction of Rosemary essential oil [8-10]. Therefore, co-administration of Minoxidil and Rosemary essential oil can lead to the high potency for a hair growth formulation.

The advents of novel drug delivery system provide the appropriate means to encapsulate both hydrophilic and hydrophobic compounds [11]. Liposomes have capability of increasing the concentration of topically applied drugs in the dermis while reducing the unfavourable risk by restricting the absorbance of systemic drug [12]. Moreover, the similarity of lipid composition of liposomes and membranes of intercellular lamellae and keratinocytes render the improvement in drug release properties and skin compatibility [13]. Therefore, liposomes appear to be an appropriate vehicle to co-encapsulate Minoxidil and the Rosemary essential oil [14].

Furthermore, previous studies of liposomal encapsulation of Minoxidil has led to the higher concentration of drugs in the pilosebaceous units in comparison to conventional Minoxidil formulations [15]. In addition, the organic solvent deletion of conventional formulations reduces the adverse side effects of long term application. Moreover, liposomal encapsulation of Rosemary essential oil could protect the essential oil against degradation factors such as pH and light, and increase its stability [16].

Introduction

Minoxidil and Rosemary essential oil have been known for their hair growth stimulatory properties for several years (Article 1999) [1,2] and there are several explanations that

Therefore, the aim of this study is to prepare liposomes containing Minoxidil and rosemary essential oil that presented acceptable physicochemical properties.

Materials and Methods

Materials

The Rosemary essential oil was purchased from Barij essence company and the other consumed substances were purchased from Merck Company.

Liposome preparation

Liposomes were prepared by a thin-film hydration method as reported in the literature accurately weighed quantities of the Egg Phosphatidyl choline (EPHC) and Cholesterol (CHOL) with the molar ratio of 7:3 were dissolved in methanol: Chloroform (1:3) mixture. The solution was placed in a rotary evaporator (Rotavapor R 200/205, Buchi) at 55°C until a thin lipid film on the wall of a round-bottomed flask was obtained. The resulting lipid film was kept under a vacuum overnight in order to eliminate traces of organic solvents. The lipid film was then hydrated with 10 mL of the aqueous solution described in (Table 1) for one hour. Afterwards the homogenous suspension of the liposome was spun in the centrifuge for 45 minute at 15000 rpm. Following the separation of the supernatant, the sediment was rehydrated with distilled water and it was sonicated for 10 min by using an ultrasound bath (Transonic 460 H, Singen), and finally the liposome mixture was extruded with a 400 nm filter.

Table 1: Content of aqueous solution for hydrating a thin-film layer.

	Formulation n 1	Formulation n 2	Formulation n 3	Formulation n 4
Minoxidil e	-	1 mg/ml	-	1 mg/ml
Essential oil	-	-	300 µl	300 µl

Determination of Minoxidil-entrapment efficacy

The supernatant of centrifuged suspension containing liposome were analyzed at 285 nm spectrophotometrically. The percent drug entrapment for the prepared liposome was calculated by using equation (1).

$$\frac{(\text{Total drug added} - \text{non entrapped drug})}{\text{Total drug added}} \times 100 \quad (1)$$

Determination of Rosemary essential oil-entrapment efficacy

The quantity of entrapped essential oil in the liposome was determined via a method introduced in the European pharmacopeia [17]. The method was carried out by means of

steam distillation by placing the prepared liposomal solution in the flask described below and heat it for 30 min (Figure 1). After 30 min, heating was stopped, and following 10 min of cooling a room temperature the volume of the essential oil was read off. The drug entrapment percentage was calculated using equation (2).

$$\frac{(\text{The volume of essential oil in the tubed after 30 minutes of heating})}{\text{Total volume of added essential oil}} \times 100 \quad (2)$$



Figure 1: Hydro distillation method: Clevenger type apparatus.

Size and zeta potential

The vesicle size and zeta potential analysis of the liposomes were carried out by using a Malvern Zetasizer 2000 HS (Malvern instrument limited, Malvern, UK, NIPER, SAS Nagar, Punjab).

Storage stability studies

In order to determine the physical stability of the liposomes, size of the particle and polydispersity index (PDI) were measured by Malvern Zetasizer. The vesicles were stored at 4°C for up to 2 months under light protection [18]. In predetermined time intervals, vesicles were characterized for their vesicle size and PDI.

Results and Discussion

The influence of rosemary essential oil on Minoxidil encapsulation efficacy

The Minoxidil encapsulation efficacy (EE) with and without the essential oil was 73% and 64% respectively. Based on our results, the liposome formulation containing essential oil represented larger EE% of Minoxidil which was accompanied by increase in the particle size and zeta potential of the vesicles. The high zeta potential frequently led to an increase in the repulsion forces of the bilayer structure of the vesicles

which consequently increased the size of the liposomes. Minoxidil, being partially hydrophobic, was expected to be localized in the membrane compartment of lipid vesicles [13]. In Baranl et al. [10] study it was shown that Terpen compounds such as cineol increased the entrapment efficacy of hydrophobic drugs [10], thus the increase in EE could be related to Terpen compound of essential oil.

The influence of Minoxidil on Rosemary essential oil encapsulation efficacy

The essential oil encapsulation efficacy with and without Minoxidil was 50% and 55% respectively. There was no significant change to the encapsulation efficacy of Rosemary essential oil accompanied with Minoxidil.

Size and Zeta potential of liposomes

Zeta potential and particle size of the formulated vesicles after probe sonication is presented in **Table 2**. The results showed that the average size of liposomes containing Minoxidil was 169 nm with a PDI of 0.261, while the average size of the liposomes containing Minoxidil and essential oil was 183 nm with a PDI of 0.174. In cases of vesicles containing solely essential oil, the vesicle size was 187 nm with a PDI of 0.105. The particle size of free-drug liposomes was 118 nm. The increasing of particle size of liposomes containing Terpene was observed. These findings are in agreement with previous study [10]. The PDI of the investigated formulations was below 0.3, which indicates the homogeneity of the prepared liposomes [19]. Regarding the zeta potential measurements, all liposomal dispersions had a negative surface charge indicating that the formulations were more stable and homogeneous in distribution. Moreover, liposomes containing Terpene are more negative than conventional liposomes. These negative charge values of the obtained liposomes are attributed to the presence of ethanol [20].

Table 2: Size and Zeta potential of Formulation (1): Liposome without drugs; Formulation (2): Minoxidil loaded liposome; Formulation (3): Rosemary essential oil loaded liposome; Formulation (4): Minoxidil and Rosemary essential oil loaded liposome formulation.

	Particle size	Zeta potential
Formulation 1	118	-18.8
Formulation 2	169	-34
Formulation 3	187	-37.5
Formulation 4	183	-37

Stability studies

The physical stability of the four liposome formulations which were stored at 4°C for 60 days presented in **Figures 2 and 3**. The stability results showed minimal changes of particle size and PDI of the investigated liposomes. The particle size and the PDI of liposomal dispersions had slightly increased

after 60 days of storage. These results showed that the co-existence of the Minoxidil and Rosemary essential oil in the vesicular formulations did not affect the vesicle's stability during time.

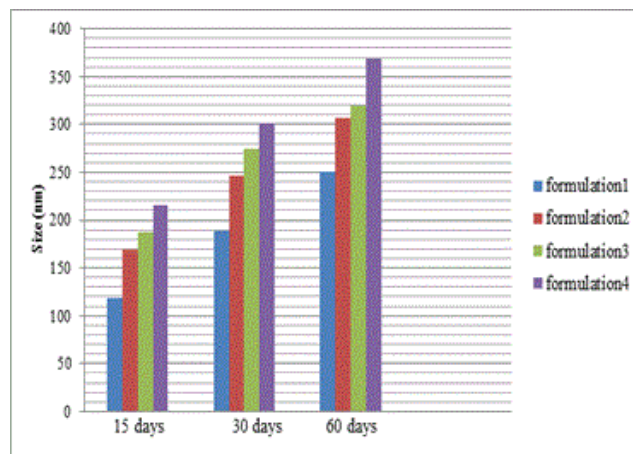


Figure 2: Size of liposome particle in 60 days of storage for Formulation (1): Liposome without drugs; Formulation (2): Minoxidil loaded liposome; Formulation (3): Rosemary essential oil loaded liposome; Formulation (4): Minoxidil and Rosemary essential oil loaded liposome formulation.

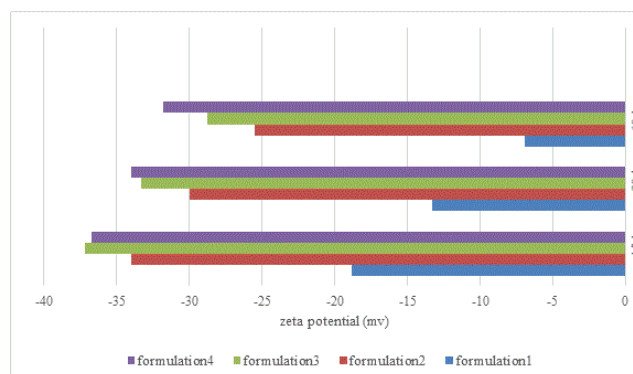


Figure 3: Zeta potential of liposome particle in 60 days of storage for Formulation (1): Liposome without drugs; Formulation (2): Minoxidil loaded liposome; Formulation (3): Rosemary essential oil loaded liposome; Formulation (4): Minoxidil and Rosemary essential oil loaded liposome formulation.

Conclusion

Minoxidil and Rosemary essential oil successfully entrapped in the liposome with appropriate size and entrapment efficacy which possible its consumption as the future hair growth stimulator formulation. The stability of formulation in terms of size and zeta potential remained appropriate during 60 days of storage.

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