Published online 2022 April 20 **Original Article**

Physicochemical Evaluation and Formulation of Propranolol Niosomal Gel with a Potential Effect on Infantile Hemangioma

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Received 2021 September 12; **Revised** 2021 December 06; **Accepted** 2022 April 28.

Abstract

Background: Propranolol is a non-specific beta-blocker that is used to treat hypertension, angina, arrhythmia, tremor, and manage thyrotoxicosis. Based on the results of various studies, propranolol can control infantile hemangioma by vasoconstriction, apoptosis induction, and inhibition of cell proliferation signals.

Objectives: The aim of this study was to evaluate the physicochemistry and formulation of propranolol niosomal gel for infantile hemangioma.

Methods: The conventional film hydration method was used to prepare medium size (2-6 m) multilamellar vesicles (MLVs) containing propranolol. At the lipid phase, sorbitan esters (Spans) and their polyethoxylated derivatives (Tweens) were combined with cholesterol, and deionized water was utilized as a hydration medium. Laser light scattering was used to perform the size analysis, and the Franz diffusion cells were utilized to investigate the release of propranolol from niosomal suspensions and carbomer-based niosomal gels. The vesicles were assessed for their stability within six-month storage at 4°C, and ultraviolet spectrophotometer and centrifuge technique were employed to measure the efficiencies of encapsulation.

Results: Based on the findings, the best niosomes were obtained at 40 and 60 spans in the presence of Tween 40 and 60; however, Span/Tween 20 and 80 were not able to form propranolol niosomes. The selected formulations had an MLV appearance and size distribution of 5 µm. Encapsulation efficiency and release rate of selected niosomes were optimal. Niosomes had good stability during six months of storage at refrigerator temperature.

Conclusion: Based on the obtained results in this study, the application of a new topical dosage form of propranolol showed promising results for the treatment of infantile hemangioma.

Keywords: Infantile hemangioma, Niosome, Particle size analysis, Propranolol, Stability

1. Background

Infantile hemangioma describes a benign disease that interferes with cutaneous endothelial cells and causes angiogenesis. This is the most common tumoral disease that might happen after birth, and these proliferative lesions occur eighty percent of the time on the neck and head. Within the first three months, they grow to eighty percent of their maximum size and their growth can continue up to 18 months (1).

A few medications have been known for the medical treatment of clinically significant hemangioma, namely glucocorticosteroids (oral, intralesional, and topical), interferon alfa, and, seldomly, vincristine and topical imiquimod (2). Accidentally, it has been revealed that beta-blockers, in particular propranolol (3), induce the involution of infantile hemangioma (4). Beta-blockers, most specifically propranolol and more recently topical timolol, have been prescribed for infants with severe or disfiguring haemangiomas and propranolol oral solution (Hemangeol) was approved by the FDA in March 2014 (5).

Niosomes are vesicles with a unilamellar or multilamellar structure that are created from synthetic non-ionic surfactants, which can be an alternative to liposomes as drug carriers (6). The surfactant molecules are usually oriented in such a way that would eventually lead to the outward point of the hydrophilic end of the non-ionic surfactant, whereas the bilayers are formed by the hydrophobic ends facing each other (7). The review of articles and clinical studies have demonstrated that such beta-blockers as propranolol can be potent, efficient, and safe drugs that can be adopted for the treatment and management of infantile hemangioma. This new drug delivery system could be used for topical delivery of propranolol in infantile hemangioma.

2. Objectives

The aim of this study was to evaluate the physicochemistry and formulation of propranolol niosomal gel for infantile hemangioma.

3. Methods

3.1. Gel construction

Polymer diffusion in the liquid phase is done in two stages. The first one refers to the fast penetration

of solvent molecules to the solid phase of the polymer, which is accompanied by the slow swelling of particles and eventually the conversion to gel. The second stage is the release of solvated polymer molecules and their slow penetration into the solvent phase, which can be fastened with stirring. Stirring should be done before the polymer is swollen and has become sticky, so that the polymer particles are dispersed in the dissolution environment, otherwise all will stick together. Two methods can be used to prevent this problem: 1) To change the solvent temperature as if the particlesbecome go wet and dispersed prior to occurrence of swelling and dissolution. 2) To wet and deflocculate the polymer particles by miscible solvent with water which is not possible to dissolve polymers and then add the solvent. Making the polymer solution as a thickener is the first step for gel preparation.

To decrease water loss in monophase gels, adding the humctants like propylene glycol, glycerol and sorbitol is common. This prevents the solvent evaporation and leads to a cooling sensation on the skin after usage. It also prevents the shrinkage of product surface after packing.

3.2. Determination of IR spectrum

At first, to determine the purity of propranolol and identify the original one, the IR spectrum was used, whose spectrum can be seen in the results section.

3.3. Determination λ_{max} of propranolol

The used λ_{max} for propranolol assay was determined in ethanol 96% by scanning the fresh propranolol solution (40 µg/ml) between 200 and 400 nm.

3.4. Standard curve of propranolol

To depict the standard curve of propranolol, some concentrations of it were prepared in ethanol 96%.

3.5. Preparation of niosomes

The conventional film hydration method (i.e., handshaking method) was employed for vesicular formulations. In a round-bottomed flask, 300 µmol of surfactants (equal molar percent of Tween (T): Span (S) with the same hydrocarbon chain type and length) /cholesterol were dissolved in the chloroform. The organic solvent was evaporated at 70°C under reduced pressure. Then in a vacuum incubator oven, residual chloroform was evaporated for 6-8 h at room temperature. Afterward, the hydration of thin lipid film, formed on the inner wall of the flask, was accomplished with 5 ml deionized water in a water bath with 70°C temperature rotating gently for 30 min. To conduct further studies, the final formulations were wrapped in foil and stored in the refrigerator (4-8°C).

3.6. Preparation of propranolol containing niosomes

Selected niosomes were used to make propranolol niosomes. In this process, propranolol, which was dissolved in 5 ml of warm water, was added in the hydration phase of niosome preparation.

3.7. Optical microscopy

The formation of vesicles, the estimated number of vesicles, the different shapes of them (e.g., multilamellar vesicle [MLV], small unilamellar vesicle, round, and tubular), the presence of vesicle aggregation, the constituent cholesterol crystals/ surfactant particle/droplet separation, and the phase separation were investigated microscopically.

3.8. Measurement of *vesicle size*

A static laser light scattering method was employed to measure the particle size distributions of vesicles. The vesicle size was assessed one week and one, three, and six months (stored at refrigerator) after preparation.

3.9. Determination of propranolol encapsulation efficiency

The vesicle suspensions were centrifuged at 20,000 rpm at 25°C for 15 min to separate the non-entrapped propranolol from the conventional niosomes. Following the disruption of the niosomes by ethanol 96%, the amount of propranolol was analyzed in the supernatant and pellets.

3.10. Evaluation of propranolol release

To study propranolol release from niosomes at 37±1°C, a vertical all-glass Franz-type diffusion cell was used with a surface area of 1.5 cm^2 and a volume of 15 ml for the receptor phase.

3.11. Study of vesicle stability

The vesicles were kept in glass vials in the refrigerator at 4°C temperature for six months. The morphology of MLVs, modifications in the size, and separation of the constituents were evaluated by a particle size analyzer (Malvern)after one, three, and six months.

4. Results

4.1. Spectrophotometry of infrared

The IR absorption spectrum of propranolol HCl exhibited the maximum rate at the same wavelengths of a similar preparation of the reference.

4.2. Determination λ_{max} of propranolol

The determined λ_{max} of propranolol was 291 nm in ethanol.

4.3. Standard curve of propranolol

Table 1 tabulates the data of the ultraviolet absorption standard curve of propranolol in ethanol 96% at 291 nm.

Table 1. Data of ultraviolet absorption standard curve of propranolol in ethanol 96% at 291 nm

Concentration (ppm)	Abs1	Abs2	Abs3	Mean	SD[1]	RSD%[2]
5	0.081	0.085	0.083	0.083	0.002	2.410
10	0.183	0.194	0.182	0.186	0.007	3.573
20	0.363	0.358	0.372	0.364	0.007	1.947
40	0.757	0.769	0.741	0.756	0.014	1.859
50	0.906	0.958	0.941	0.935	0.027	2.836

Standard deviation; 2. Relative standard deviation

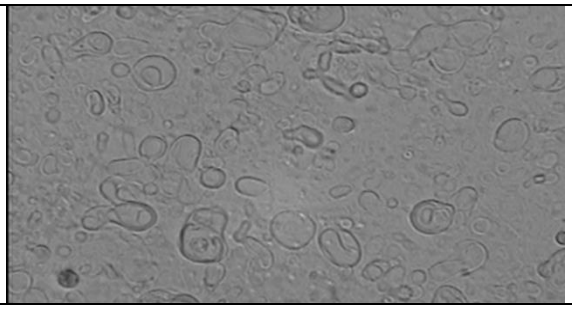

Figure 1. Micrograph (40×10 magnifications) of Span40/Tween40/Cholesterol (35:35:30 m.r)

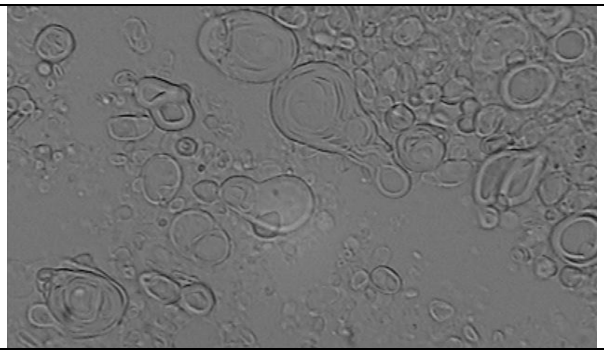

Figure 2. Micrograph (40×10 magnifications) of Span60/Tween60/Cholesterol (35:35:30 m.r)

4.4. Optical microscopy

The micrographs (40×10 magnifications) of Span40/Tween40/Cholesterol (35:35:30 m.r.) and Span60/Tween60/Cholesterol (35:35:30 m.r.) revealed the different shapes and sizes of niosomes (Figures 1 and 2).

4.5. Measurement of vesicle size

The size distribution curves of the selected niosomal formulations were analyzed and all showed bell-shaped patterns indicating a log-normal size distribution (Figures 3 and 4).

4.6. Encapsulation efficiency of propranolol

Table 2 presents the encapsulation efficiencies percentage (EE %) of different formulations of propranolol .

4.7. Release of propranolol

Figure 5 depicts the total percentage of propranolol released from the formulations containing a 30-molar ratio of cholesterol during four

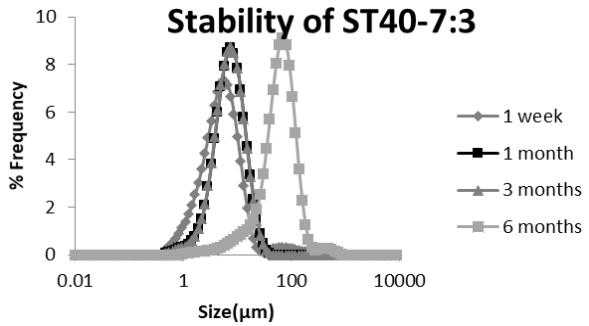

Figure 3. Size distribution curve of span40/Tween40/Cholesterol (35:35:30 m.r.) containing propranolol

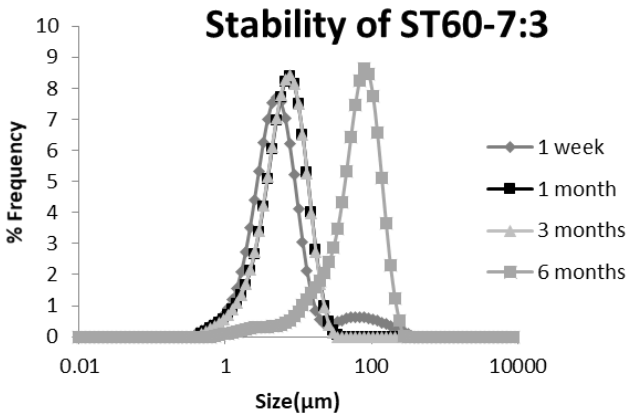

Figure 4. Size distribution curve of Span60/Tween60/Cholesterol (35:35:30 m.r.) containing propranolol

Table 2. Propranolol encapsulation efficiency percent in niosomal formulations

	Abs	Concentration	Amount of drug (µg)	Encapsulation efficiency
Supernatant ST40 (35:35:30)	0.900	50.278	1675.926	73.368
Sediment ST40 (35:35:30)	1.090	60.833	608.333	26.620
Supernatant ST60 (35:35:30)	0.746	41.722	1390.741	75.576
Sediment ST60 (35:35:30)	0.804	44.944	449.444	24.424

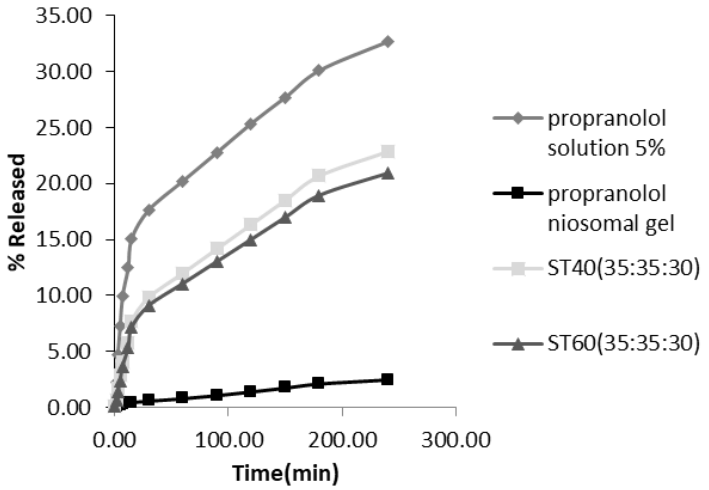

Figure 5. Released profile of prepared formulations

hours in ethanol 96% buffer phosphate (60:40) at temperature of 37°C.

5. Discussion

Sorbitan esters (or Spans) and their ethoxylated derivatives (or Tweens) were assessed in this study for the entrapment of propranolol. None of the used lipid compositions formed the niosomes (Table 2). The liquid-state surfactants (Span 20 and 80) yielded many separated crystals with no or low number of vesicular structures.

Span/Tween 80 have unsaturated alkyl chains ($C_{9=9}$)and there are some reports about the instability of their niosomes (8,9). In addition, as a lipophilic molecule, vitamin E competes with cholesterol by intercalating into bilayers. This behavior was also reported for lyophobic substances, such as acetazolamide (10), at optimal cholesterol concentrations and for some water-soluble molecules, such as ciprofloxacin HCl (11). The overall result is the separation of cholesterol molecules, which are mainly insoluble in aqueous media and form large and flat crystals.

The niosomes, almost at one main form (i.e., MLVs), were obtained with the gel-state utilized surfactants (Span 40 and 60) and were nearly spherical and uniform in size. The micrographs (40×10 magnifications) of Span40/Tween40/ Cholesterol (molar ratio [m.r] 35:35:30) and Span60/Tween60/Cholesterol (m.r 35:35:30) are depicted in figures 1 and 2, demonstrating the

formation of these vesicular structures. The structure of the surfactant determined the ability of surfactants to form the vesicles. The packing properties of surfactants are dependent on the balance between hydrocarbon chain volume (V), hydrophilic surface area (a_0), and the maximum length (Lc) that can be assumed by chains.

The niosomal systems are typically enriched with cholesterol as an additive, which is known to abolish the gel to the liquid phase for transition of liposomal and niosomal systems; as a result, vesicles leak lesser and the niosomes are more stable (12). The surface pressure on monolayers of nonionic surfactant/cholesterol mixtures was assessed and showed a condensing effect of cholesterol.

This finding was evidenced by reduction in the effective area per molecule and elevation of the cholesterol content of the monolayer. This effect can be due to the cholesterol accommodation in the molecular cavities that are created by the assemblage of surfactant monomers into vesicles and accounts for the observed reduction in the permeability of cholesterol-containing membranes, compared to cholesterol-free membranes (13). In general, it has been found that a molar ratio of 1:1 between cholesterol and non-ionic surfactants is an optimal ratio for the formulation of physically stable niosomal vesicles.

The stable niosome dispersions demonstrate an invariable level of entrapped drug, a permanent size of the particle, and the lack of separation and precipitation in the membrane components. However, in literature, in the numerous cases , the formulation of non-ionic surfactant vesicles with various charges is reported, inducing such amphiphiles as positively charged stearylamine (12) and negatively charged dicetyl phosphate (14).

In some studies, this type of charged vesicles has been so called as "ionic-nonionic" or "hybrid" niosomes (15). Regardless of the different nomenclature found in the literature, the main reason for including the ionic molecules is the achievement of greater protection against flocculation in vesicle suspensions due to the electrostatic repulsion between charged bilayers.

Another common procedure for enhancing the stability of the niosomal suspensions is "steric" stability achievement, in which some polyoxyethylenes contain amphiphiles or moieties, such as Tween 40 (11). In the current research, all prepared gel-state niosomal formulations had good stability, in which the change in vesicular stability following three months storage in 2-8°C was negligible (P>0.05).

Cholesterol can induce vesicle formation in different types of surfactants. Cholesterol is expected to act in a twofold manner, similar to liposomes, meaning that cholesterol both increases and reduces the chain order above and below the phase transition temperature, respectively. On the contrary, it is expected to boost the planarity of bilayers. The orientation will be as if the single hydroxyl group is placed in or near the head group lattice and the relative bulky lipophilic part is buried in the hydrocarbon core (16).

Generally, an increase in the cholesterol content led to a decrease in the mean volume diameter of niosomes. This effect was significantly detectable in Span40/Tween40 (P<0.05). The nature and intensity of the interaction of a drug with the lipid bilayer depend primarily on the three-dimensional chemical structure, lipophilicity, and dipole moment or charge of the molecule (17).

In the present study, the entrapment of propranolol was evaluated by the spectrophotometry method. However, no significant differences were observed among the different prepared formulations for entrapment of propranolol (P>0.05). A 30-50-m.r elevation in the cholesterol amount led to a reduction in the entrapment efficiency (EE%) of propranolol for the ST40 niosomes. Likewise, an increase in cholesterol content in ST60 resulted in the increment of propranolol (EE%) (P>0.05).

An optional delivery system having desired release characteristics will be achieved by determining the rate of drug release. Additionally, in-vitro release studies are commonly conducted to predict the possible performance of a delivery system in ideal situations, which may be indicative of its in-vivo performance. However, the drug retention and release rate of the vesicular formulations are drug-dependent and can vary dramatically. The anticancer medications, namely doxorubicin and epirubicin, are largely retained inside liposomes, whereas other anticancer medication (i.e, vincristine) and the antibiotic such as ciprofloxacin tend to leak out rapidly (18).

An increase in the amount of cholesterol in both niosomal formulations resulted in a decrease in the released drug. The reason for this might be due to the fact that lipid bilayers are less permeable when the cholesterol level is high.

5. Conclusion

This study reported the successful encapsulation of propranolol in the vesicles composed of different types of Spans and Tweens. It is known that many factors influence the size of lipid vesicular systems. In the niosomes, the cholesterol content of used surfactants is the major determinant of the niosomal dimensions. However, in this study, the non-ionic vesicular systems were prepared for propranolol, which were capable of transporting the drug through rat abdominal skin. This new drug delivery system might be used for topical delivery of propranolol in infantile hemangioma. It is recommended to perform future studies to evaluate our findings in clinical or

in-vivo experiments.

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