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Original Article

A COMPARATIVE ASSESSMENT OF VESICULAR FORMULATIONS: TRANSFERSOMES AND CONVENTIONAL LIPOSOMES LOADED IVABRADINE HYDROCHLORIDE

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ABSTRACT

Objective: Ivabradine hydrochloride (IH), a benzazepine derivative used to treat cardiovascular disease angina pectoris. In this study IH-loaded novel carrier systems transfersomes (TFs) and conventional liposomes (CLs) were developed and compared for their efficacy to enhance the stability of drugs from degradation.

Methods: TFs formulations (TF-1, TF-2 and TF-3) were prepared by using different biocompatible surfactants; tween-80 (TW), span-80(S) and sodium deoxycholate (SC) in the concentration ratio of 15 parts with 85 parts of soy phosphatidylcholine as phospholipid by thin-film hydration method. These vesicles were compared with CLs formulation (L-1) prepared in 7:3 molar ratio of soy phosphatidylcholine: cholesterol by following the same method. These vesicles were compared for physical appearance, vesicle shape, and size, percentage drug entrapment efficiency (%DEE), deformability index (DI), *in vitro* percentage cumulative drug release study, and physical stability studies. The chosen optimized novel carriers were observed under scanning electron microscopy.

Results: The compared data demonstrated that the physical appearance for all vesicles was turbid and had a spherical shape. The size distribution was in the range of 129.0 nm to 273.5 nm in vesicles. The %DEE (79.0±0.94) and DI (35.0±1.9) was found maximum in TF-1 formulation that was 2.3 times higher than L-1 formulation. The *in vitro* percentage cumulative drug release study followed second-order polynomial kinetics that was 2.0 times higher than L-1and 2.9 times higher than the plain drug in 30 min (90.4±0.06%) from TF-1. The vesicles were found to be stable at refrigeration conditions.

Conclusion: Thus, amongst of all vesicles TW loaded TFs (TF-1) was chosen as an excellent novel vesicular carrier for hydrophilic drugs due to its higher deformability behavior than CLs that protects the certain drugs from biodegradation and provides stability.

Keywords: Ivabradine, Ivabradine hydrochloride, Vesicles, Conventional liposomes, Transfersomal preparations

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INTRODUCTION

IH, an inhibitor of the *If* pacemaker current, selectively targets the heart rate and to beta-blockers that does not reduce myocardial contractility or relaxation [1]. It is a hydrophilic drug (≥5 mg/ml) and having short biological half-life used for patients with normal sinus rhythm and heart rate ≥ 70 beats per minute and who are contraindicated to the use of beta-blockers. Results of a post-marketing surveillance study in thousands of patients, where therapy with Ivabradine tablets was associated with a significant reduction in the frequency of angina attacks and consumption of short-acting nitrates of 87%. This includes an increase in myocardial diastolic perfusion time [2], enhancement in coronary flow reserve [3], and improvement in endothelial function [4] in patients with chronic stable coronary artery disease [5]. IH is effective and safe in all subpopulations of angina patients seen in clinical practice, independent of age, comorbidities, and use of beta-blocker in tolerated doses but treatment was withdrawn because of adverse effects in 7% of patients. The most common adverse effects responsible for withdrawal were luminous phenomena (14.5%), bradycardia (2%), headaches (2.6-4.8 %) [6]. Thus, to overcome these problems a carrier-based system loaded with the drug was proposed. The vesicular carriers TFs and CLs were developed and compared for their effectiveness in the present work. Vesicles consist of hydrophobic and hydrophilic moieties together and can accommodate drug molecules of higher molecular weight. These bi-layer vesicles have the ability to overcome the permeation difficulty by squeezing themselves along the inter-cellular sealing lipid of the stratum corneum [7]. TFs having flexible membrane by phospholipid and surfactants in the proper ratios that minimize the risk of complete vesicle rupture. They can deform and pass through narrow constriction from 5 to10 times less than their own diameter. These are the biocompatible and biodegradable carrier for low and high molecular weight drugs e. g. analgesic, anesthetic, corticosteroids, hormones, anticancer, insulin, gap junction protein, and albumin [8]. The CLs are microscopic spherical particles in which membranes having one or more lipid bilayer and encapsulate a fraction of drug dispersion [9]. These have high membrane fluidity, which promotes penetration of the drug into deeper layers of the skin and can deliver a large quantity of hydrophilic and lipophilic drugs, proteins, and macromolecules through the skin [10]. Thus, the present study was aimed at the preparation and comparison of IH loaded TFs and CLs vesicular system.

MATERIALS AND METHODS

Materials

The IH drug was generously gifted by Ind swift Ltd., Chandigarh, India. Cholesterol (From CDH fine chemicals), Soy phosphatidylcholine (from bright laboratories) and tween-80 (TW), span-80 (S), sodium deoxycholate (SC) from Merck specialist, Mumbai was purchased. All other chemicals and solvents used in this study were of analytical grade. Freshly prepared distilled water was used throughout the studies.

Methods

Estimation of IH

The drug IH was estimated by ultraviolet (UV) spectrophotoscopic method (UV-2400 PC series, Shimadzu, Japan) at 286.0 nm absorbance in phosphate buffer (pH 6.8) [11]. The calibration curve was prepared in the concentration range 10-55 µg/ml that followed Lambert-beer law.

Preparation of vesicles

Vesicles TFs were prepared by a thin-film hydration method [12] with some modification. Precisely, the 85 parts of surfactants; TW, S, and SC were mixed separately with 15 parts of soy phosphatidylcholine in 3 clean, dry round bottom flasks marked as

TF-1, TF-2, and TF-3 respectively. The mixture of chloroform: methanol (2:1 v/v) 20 ml was added gradually in each flask by shaking slowly to prepare a suspension. The organic solvent was evaporated by a rotary evaporator and under vacuum overnight. The 10% v/v ethanol in phosphate buffer solution (pH 6.8) was used as hydrating media to hydrate the dried thin film by rotation at 60 rpm for 1h at room temperature. The resulting vesicles were swollen for 2 h at room temperature to get large multi-lamellar vesicles. This thick suspension was broken by shaker (Jyoti scientific industry, India), sonicated (Soniwel, India) for half an hour at 4 °C and extruded through 200 nm and 450 nm polycarbonate membranes (Millipore). The saturated concentration of the drug was incorporated in prepared vesicles.

The CLs were prepared by dissolving Soy phosphatidylcholine: cholesterol (7:3 molar ratios) by the same method as described above for comparison in the present work and marked as L-1.

Evaluation of prepared vesicles

Morphology and mean vesicle size

For this study, a thin film of prepared vesicles was spread on a slide and a coverslip was placed over it and observed under the optical microscope (Olympus, India) using a 45X lens in triplicate [13].

Physical appearance

The vesicular sample (10 ml) from each formulation was diluted with 5 ml of distilled water and sonicated for 5 min and observed against the black background and reported turbidity as dense and colloidal form.

Percentage of drug entrapment efficiency

The percentage drug entrapment efficiency (%DEE) was analyzed by centrifugation of prepared vesicles at 22000 rpm at 4 °C for 2 h to obtain the pellets of vesicles and the supernatant containing the free drug. These pellets were washed with distilled water to remove any unentrapped drug by centrifugation. The drug content was analyzed in the supernatant after suitable dilution with phosphate buffer solution (pH 6.8) by measuring absorbance at 286 nm. The same procedure adopted for CLs drug entrapment as control. The %DEE was calculated statistically in three trial by following the equation- $\%DEE = \frac{D1-D2}{D1}100$, Where, D_1= Initial amount of drug added,

D_2= Amount of drug in the filtrate, (D_1-D_2) = Amount of entrapped drug [14].

Deformability index

Deformability Index (DI) was measured in all vesicles by the extrusion method by using a filter membrane having a pore size diameter of 100 nm. A stainless steel filter holder having a 50 mm diameter has been used at 2.5 bar pressure. The extruded quantity of vesicles suspension in 5 min was measured by using equation [15] $Ev = J(\frac{vs}{pd})^2$. Where, Ev=Elasticity of vesicles membrane, J = Extruded amount of suspension in 5 min, vs = Vesicles size, pd = pore diameter. Data were recorded statistically to follow mean±SD in triplicate.

***In vitro* percentage cumulative drug release studies**

Prepared formulations TF-1, TF-2, and TF-3 were investigated under *In vitro* percentage cumulative drug release studies. In this study treated cellophane membrane was used as a dialysis membrane. It was tied on the mouth of each dialysis tube having a surface area of 4.50 cm^2 and diffusion conditions were maintained and set up the assembly on a magnetic stirrer at 100 rpm. Phosphate buffer (pH 6.8) was used as receptor media at 37 °C. Each 10 mg vesicle sample was placed on the cellophane membrane and this assembly was touched onto the dissolution medium surface. Samples were withdrawn 2 ml at regular intervals of 10 min and replaced with the same amount of receptor media. The same procedure was adopted for plain drug solution and L-1 formulations as control. Collected samples were suitably diluted with buffer solution up to 3 h and analyzed at 286.0 nm using phosphate buffer (pH 6.8) as blank. The study was conducted in the statistical significance of mean±SD in each of the three trials.

The equation $\%\text{Drug released} = \frac{\text{released amount of drug at time t}}{\text{Initial amount of drug}}100$, was used to estimate percentage drug release kinetics quantitatively.

Stability studies

A protocol of stability study was carried out with some modifications to examine the aggregation and leakage from prepared vesicles formulations throughout storage [16]. Vesicle formulations were stored in airtight amber colored glass vials (Three each) at refrigeration temperature (4±2) °C, room temperature (25±2) °C and body temperature (37±2) °C for a period of at least 4 mo. After every 15 d, time interval up to 4mo vesicle formulations were examined for physical appearance, mean vesicle size, and mean %DEE of the drug. Statistical estimation was done as mean±SD [17].

Scanning electron microscopy study

The morphology of the drug IB and selected optimized vesicular carrier (TF-1) were observed by scanning electron micrographs (SEM) using Joel Scanning electron microscopy JSM-840 with a 10 kV accelerating voltage. The sample was put on the grid and fixed by adhesive and used for photomicroscope, without metal coating.

RESULTS AND DISCUSSION

The TFs vesicular formulation (TF-1, TF-2 and TF-3) were developed with the incorporation of different kinds of surfactants (TW, S and SC): soy phosphatidylcholine at the ratio of 15:85. These have different hydrophilic-lipophilic balance values like 15, 4.3, and 16, respectively. The CLs formulation L-1 was developed with cholesterol with Soy phosphatidylcholine phospholipid. Table 1 showed the formulation compositions of vesicular preparations.

Table 1: Formulation composition of TFs and CLs*

Formulation	TW (%w/w)	S (%w/w)	SC (%w/w)	CHL (%w/w)	SPC (%w/w)	CF: MT mixture (2:1) ml	IH (mg)
TF-1	15.0	-	-	-	85.0	20.0	300.0
TF-2	-	15.0	-	-	85.0	20.0	300.0
TF-3	-	-	15.0	-	85.0	20.0	300.0
L-1	-	-	-	3.0	07.0	20.0	300.0

*All formulations were prepared in 10% v/v ethanol in Phosphate buffer (pH 6.8) q. s. to produce 100%w/w, TW= Tween-80, S= Span-80, SC= sodium deoxycholate, SPC= Soy phosphatidylcholine, IH= Ivabradine hydrochloride, CHL=Cholesterol, CF: MT=Chloroform: Methanol

The characterization of prepared vesicular formulations was shown in table 2. It was found that all vesicles formulations were turbid (off-white precipitate) and colloidal against the black background. Microscopically spherical morphology was observed in the suitably diluted samples of each vesicle. The size distribution of vesicles was observed in the range of 129.0±0.21 nm to 273.5±0.20 nm. This related to increment in the surface free energy and hydrophilicity or lipophilicity of the surfactant molecules [18]. The particle size of vesicles was depended on the presence of surfactant and phospholipid concentration. Data revealed that the %DEE of prepared vesicles TF-1, TF-2, TF-3, and L-1 were found to be 79.0±0.94, 47.3±0.83, 61.7±0.95, and 34.3±1.21, respectively. Drug encapsulation in TF-1 was found to be 2.3 times higher than L-1 formulation. The drug distribution coefficient between the lipid and

aqueous phase affects the entrapment of lipophilic and hydrophilic drugs into lipid vesicles [19]. Drug encapsulation was increased due to affinity towards hydrophilic surfactant of TW, which was suitable to entrap hydrophilic drug IB [20].

TFs showed deformability character due to having a flexible phospholipid bi-layer membrane. This property was not present on CLs. When surrounding stress was enforced on the vesicle for penetration, spontaneously TFs undergo deformation to avoid the risk of structure rupture. The comparative graph had been prepared between % DEE and DI (fig. 1). The maximum DI was found in formulation TF-1 that is 12.5 times than CLs formulation. Deformability of TFs is related to membrane fluidity, which was made up of phospholipid and surfactant. They transform bilayer into a single layer because having a non-bulky hydrocarbon chain and almost linear structure of TW [21].

Table 2: Characterization of vesicles*

Formulation	Mean vesicle size (nm)	%DEE	DI	Physical appearance
TF-1	129.0±0.21	79.0±0.94	35.0±1.9	+++
TF-2	273.5±0.20	47.3±0.83	16.8±1.6	++
TF-3	184.8±0.54	61.7±0.95	31.7±2.1	+++
L-1	198.4±0.25	34.3±1.21	2.8±1.3	++

*All values were expressed as (mean±SD, n=3) observations,+= Slightly colloidal,++=Turbid and colloidal,+++=Dense and colloidal

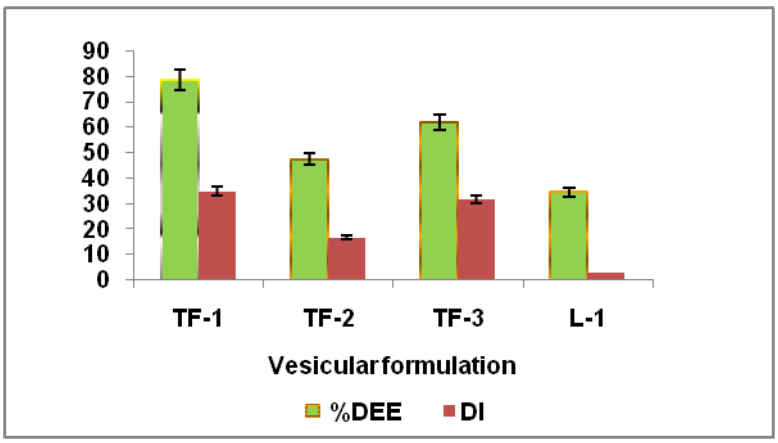

Fig. 1: Comparative study of %DEE and DI on vesicular formulations (mean±SD, n=3)

The *In vitro* percentage cumulative drug release studies through dialysis membrane on vesicular formulations were carried out by comparing with the plain drug formulation. Data from fig. 2 showed that it followed polynomial second order kinetics. The percentage of cumulative drug released was found maximum (90.4±0.06%) from TF-1 in 30 min. It was 2.0 times higher than L-1and 2.9 times higher than the plain drug. Smaller the diameter of the vesicle larger would be the surface area. It created a higher concentration of entrapped drug release from prepared vesicles. Due to the smaller size of prepared TFs of TW with IH and more affinity towards hydrophilicity showed higher drug release profile than liposomal vesicular formulation.

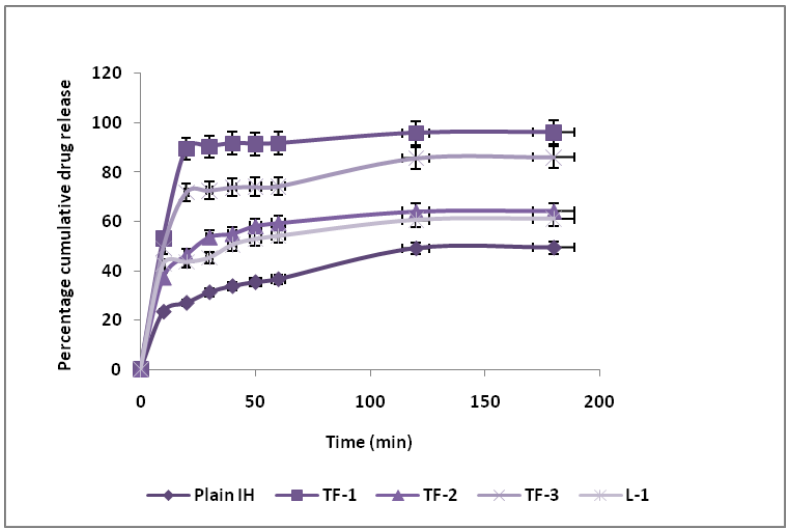

Fig. 2: *In vitro* percentage cumulative drug released from vesicular formulations (mean±SD, n=3)

The physical stability studies of vesicular formulation (TF-1, TF-2, TF-3 and L-1) were investigated for %DEE, mean vesicle size, and physical appearance up to 4 mo. Table 3 indicated that in refrigeration condition (4±2 °C), insignificant changes (p>0.05) in % DEE and vesicular size of stored TFs and CLs were detected. The color remained as same as off white precipitate. No color changes were observed. But, It was found that there were significant changes (P<0.05) in %DEE and vesicle size of the vesicular formulations stored at (25±2) °C and (37±2) °C. The vesicles were swelled or aggregated in the vehicle during the storage period. At higher temperature leakage of encapsulated drug from vesicles were detected. At higher temperature, pale yellow color and slight sedimentation were observed. This could be explained by enhanced fluidity of the lipid bilayer of vesicles at the higher temperatures. This led to the chemical degradation of phospholipids membrane packaging arrangement. Temperature 25 °C or less was found to be suitable storage of vesicular formulations. All parameters were evaluated statistically.

Table 3: Stability studies of prepared vesicles after four mo

Formulation	Mean %EE at			Mean vesicle size (nm) at		
	(4±2) °	(25±2) °	(37±2) °	(4±2) °	(25±2) °	(37±2) °
TF-1	78.4±0.94	75.4±0.24	74.1±0.17	128.6	134.1	145.3
TF-2	47.1±0.86	33.6±0.92	29.7±0.81	282.7	294.6	298.1
TF-3	78.4±0.83	71.1±0.86	69.8±0.82	189.2	193.5	208.7
L-1	26.4±0.74	20.1±0.99	16.7±0.04	224.5	236.8	239.9

* Results expressed as (mean±SD, n=3)

The SEM study of drug IB showed crystalline morphology of the drug (fig. 3). On the basis of maximum %DEE, maximum DI, maximum percentage cumulative drug release, smaller vesicle size and stability than CLs formulation, the optimized TFs carrier from formulation TF-1 was selected for SEM that showed spherical shape (fig. 4).

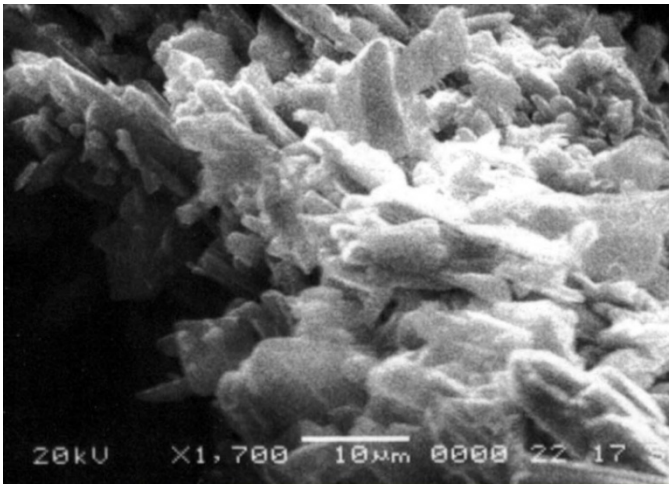

Fig. 3: SEM of IB expressed as mean±SEM, n=3

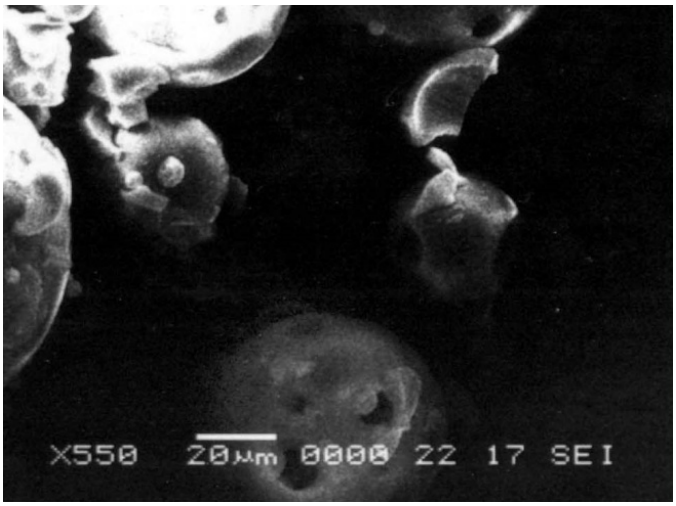

Fig. 4: SEM of TF-1 expressed as mean±SEM, n=3

CONCLUSION

It was concluded that TFs are excellent drug carriers than CLs due to their higher deformability behavior. These are phospholipid vesicles, self-optimized aggregates, more elastic with the ultra-flexible membrane, and are able to deliver the drug reproducibly either into or through the skin with high efficiency than CLs. In the present study, the encapsulated drug IB was incorporated with phospholipid bi-layer and surfactant that enhanced the stability and efficacy of the drug. Thus, TFs were the novel vesicular carrier for various drugs and served as a potential tool in future dosage development.

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Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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