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Formulation and Optimization of Liposomes for Antihypertensive Drugs

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Abstract

The basic purpose of this study to formulate and Characterized Verapamil loaded liposomes. a drug which having 20% oral bioavailability , biological half-life is short and extensive first pass metabolism. Verapamil loaded liposomes were prepared by using film lipid hydration technique. Liposomes were prepared by using soya lecithin and Cholesterol; liposomes were characterized for various methods such as shape, size and, entrapment efficiency and Zeta Potential, Drug excipients compatibility which is determined by FTIR. Mean particle size, entrapment efficiency, zeta potential was analyzed and found to be 463 nm,89 %,25 mV respectively. FTIR shows there was no additional peak means no interaction between drug and excipient and they are compatible with each other. By using film hydration method verapamil loaded Liposomes was successfully prepared and evaluated. Which have good particle size, EE% and zeta potential, so by doing further investigation as *in vitro* and *in vivo* study it could be good choice for conventional drug delivery system?

Keywords: Liposomes, Solubility, Verapamil , Nano carriers

Introduction

Verapamil hydrochloride (VP) is one of the most commonly used calcium channel blockers in the pharmaceutical market (1). It is effectively used as an antiarrhythmic drug to suppress arrhythmia. Its strong vasodilation and negative isotopic effects support myocardial infarction and cardiomyopathy (2). According to clinical studies, oral administration of VP is not associated with common side effects such as reflex tachycardia and resistance seen in 90% of other treatments, in patients with essential hypertension. It has been shown to be one of the first wave treatments. The absorbed dose is recorded. However, it is extensively metabolized in the liver, accounting for only 20-30% of the amount present. Because it short half life is given three times daily at a dose of 80 mg that is suitable for the patient (3,4). Short biological half-life, high hepatic release, and low biological utility impede patient tolerance. Verapamil hydrochloride appears to be a good candidate for transdermal drug delivery. Therefore, there is no invasive delivery via the transdermal route. H. Elastic liposomes may be a better option for effective and sustained delivery of verapamil hydrochloride. Liposomes are biocompatible and biodegradable total monomers or multi-layered phospholipid bilayers or multi-layered phospholipid bilayers and self-loading spherical vesicles (5,6). They can load hydrophilic, hydrophobic, or amphipathic APIs containing peptides, biomolecules, and nucleotides that passively accumulate in target tissues and

organs after administration, and the payload is released through a variety of local processes. And thereby achieves a high concentration of active substance locally. For example, liposomes are sensitive to pH or temperature and can be designed to allow controlled release of their payloads when exposed to specific environmental conditions of target tissues and organs. Liposomes can also be loaded with multiple APIs, each targeting a variety of metabolic pathways that reduce tissue damage and facilitate repair. Liposomes are colloidal particles formed as concentric bio molecular layers that can encapsulate a drug (7). They are lipid sac that completely surrounds the amount of water. These lipid molecules are usually phospholipids with or without additives (8). Cholesterol is included to improve the properties of the liposome bilayer, increase the micro viscosity of the bilayer, reduce the permeability of the membrane to water-soluble molecules, stabilize the membrane, and increase the rigidity of the vesicles. Can be (9-12). Phospholipids such as soy lecithin cholesterol have been selected for the formation of drug-incorporated liposomes. Phospholipids are amphipathic molecules because they have a hydrophobic tail and a hydrophilic or polar head. Cholesterol acts as a “liquid buffer”. Cholesterol itself does not form a bilayer, but it can be incorporated into phospholipid membranes at high concentrations with different molar ratios of cholesterol to soy lecithin (13). Thin film hydration technology was used to prepare the liposomes. This is superior to solid lipid nanoparticles, which require longer and more complex procedures.

Materials and Method

Materials

Verapamil was obtained a gift sample from Emcure Pharmaceuticals in Pune. Cholesterol and soy lecithin purchased from Thermosil Fine ChemPvt. Pune. All reagents were of the highest and pure quality and solvents were of analytical grade, and deionized distilled water was used to prepare all solutions.

Construction of standard calibration curve for verapamil hcl

Establish a standard calibration curve for verapamil in 6.8 phosphate buffer. Preparation of standard solution: 100 mg of verapamil HCl was accurately weighed into a 100 mL volumetric flask and dissolved in a small amount 6.8 phosphate buffer. Volume was adjusted to mark with 6.8N phosphate buffer. To obtain a concentration of 1000 µg / ml (SSI). From this, 1 ml was taken and diluted to 100 ml to a concentration of 10 µg / ml (SSII). Preparation of Working Standard Solutions: (SSII) Pipette 1 ml, 2 ml, 3 ml, 4 ml and 5 ml aliquots into a 10 ml volumetric flask. Volumes consisted of 6.8 phosphate buffers at final concentrations of 1, 2, 3, 4 and 5 µg/ml, respectively. The resultant solutions were scanned in the range of 278nm using UV spectrophotometer.

Compatibility studies drug and excipients compatibility studies by ftir spectrophotometer

The test samples were dispersed in potassium bromate powder and analyzed. FTIR spectra were utilized to study compatibility between the drug and polymer. The position of FTIR bands of important functional groups of drugs was identified. (14)

Method of preparation of verapamil liposomes

Film hydration method (bangham method)

Thin film hydration is used to formulate liposomes. The Soya lecithin and cholesterol have been dissolved in 10 ml of chloroform: methanol of various ratios (1: 1) and 80 mg of Verapamil changed into delivered to the solution, after which evaporated from a rotary evaporator for rotating the aggregate at one hundred rpm for 15 mins. The thin film formed on a flask at a round bootom flask, after which hydrated to the phosphoric acid buffer (pH 6.8). The suspension is agitated for half-hour and decreases the size for 1 hour. Initially 9 bathes of Different

concentration of Cholesterol: Lecithine w/w (F1-F9) was prepared. Based in Particle size and entrapment performance F4, F5, F6 confirmed the least particle size and most entrapment capacity as proven in table 5.For optimization of bath 3^2 factorial design was applied.

Table 1: Experimental designing by 3^2factorial

Formulation Variables	Levels coded			Dependent Variable
	-1	0	+1	
X1= Cholesterol: Lecithin w/w ratio	1:2.5	1:3	1:3.5	Y1=Particle size(nm)
X2= Rotations per minute (RPM)	50	80	120	Y2=Entrapment Efficiency (%)
				Y3= Zeta Potential(mV)

Statistical design study

A central composite experiment design expert software(Version 12) used for studying the effect of various variables used in manufacturing. Independent variables are X1 cholesterol: lecithin w / w and X2 = ratio of (RPM) per minute. The dependent variable is selected by the particle size (Y1: PS), Entrapment efficiency (Y2: EE), and Zeta potential (Y3: ZP).

Table 2: A 3^2 Full Table 2: A 3^2 Full factorial Experimental Design Layout

Formulation Code	Coded Factor Levels	
	X1	X2
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

Characterization of liposomes

Particle size analysis

The determination of the average particle size of liposomes was very important. Malvern Instruments, of DRSSK Labs Pvt LTD. was used to determine particle size.

Entrapment efficiency

The liposome Entrapment efficiency is determined by Ultracentrifuge by 30,000 to 40,000 rpm and 4 ° C for 1 hour .The following centrifugation of supernatants and vesicles will be divided. The supernatant is removed and the amount of drug is analyzed by the analysis method. The percentage of encapsulated drugs was determined after dissolution of liquid prepared for 10 minutes with absolute alcohol . The concentration of the Verapamil in absolute alcohol was determined spectrophotometrically at 278 nm using a UV-visible spectrophotometer. The effect of encapsulation expressed in a percentage of Entrapment is calculated through the following relationships (15-18).

Zeta potential

The Zeta potential drug of samples was performed on the three independent samples of the cavity in each of the formulas, and the average SD value was calculated after performing a physical measurement of stability.

Result and Discussion

Construction of standard calibration curve for verapamil hcl in 0.1n hcl

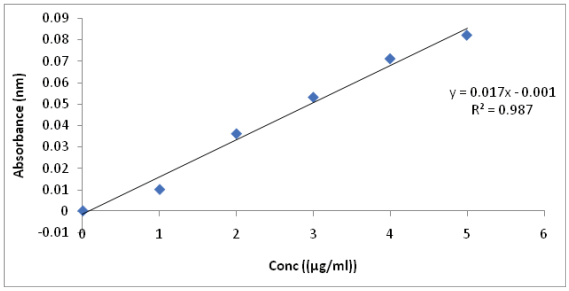
Calibration curve for verapamil HCl at 6.8 phosphate buffer are shown in the figure 1. The curve was linear over the 1-5 µg/ml concentration range with a regression value of 0.987. A high regression value indicates that the

calibration curve obeys Beer's law.

Table 3: Standard readings of Verapamil HCl in 6.8 Phosphate Buffer

Concentration(µg/ml)	Absorbance at 278nm
0	0
1	0.010
2	0.036
3	0.053
4	0.071
5	0.082

Fig 1: Standard graph of Verapamil HCl in 6.8



Phosphate Buffer

CompatibilityStudies Drug and excipients compatibility studies by FTIR spectrophotometer

Fourier transform infrared spectroscopy (FTIR) was used to study the excipient interactions and the results are shown in Figure 2. 3, 4, 5, 6. Verapamil HCl shows the characteristic peak of FTIR at 2949 cm−1 (due to its N-H stretching) and 1728 cm−1 (due to Amino N-H bending) 1460 cm−1 (due to CH3 bending alkanes) 1060 cm−1 (due to 813 cm−1 due to Alkene C-H bending -1) (19). In this study, a mixture of HCl Verapamil prepared and filler prepared (Soya lecithin and cholesterol) showed a characteristic peak of the same wavelength. Indicates that certain functional groups based on these studies and graphs were not obtained by additional peaks, so there is no significant interaction between the drugs and fillers.

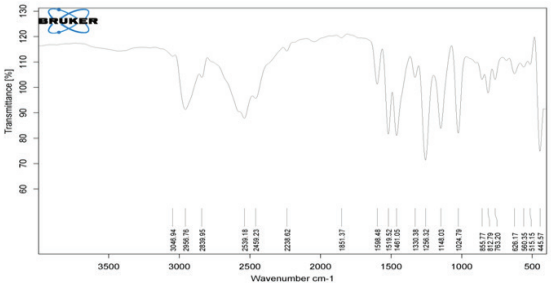


Fig.2: FTIR of Pure Verapamil Hcl

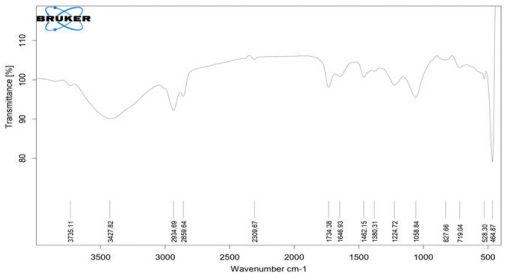


Fig.3: FTIR of Soya lecithin

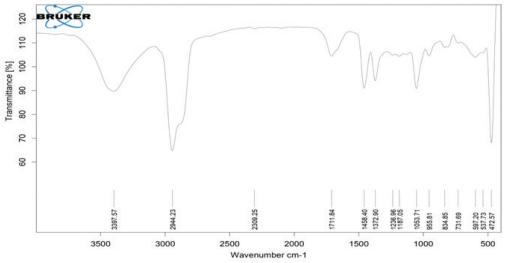


Fig.4: FTIR of Cholesterol

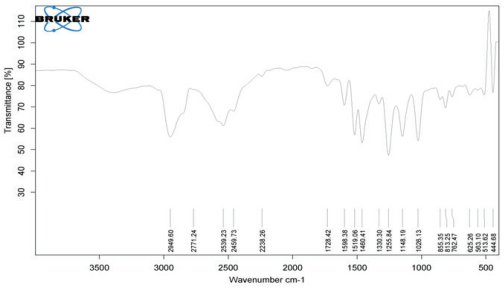


Fig 5: FTIR of Pure Verapamil+Cholesterol+Soya lecithin

Characterization of prepared liposomes

Analysis of particle size, entrapment efficiency and zeta potential

Particle size

Verapamil liposomes were prepared using cholesterol and other proportions of lecithin, and the particle sizes of the liposomes ranged from 417 nm to 786 nm. Table 5 shows the effect of various factors on mean PS. It is clear that these investigative factors significantly affected X1 = cholesterol:lecithin W/W ratio and X2 = mean PS values.It was evident that increasing the amount of lecithin for factor (X1) and increasing the rpm for factor (X2) significantly decreased the average PS produced by liposomes with smaller particle sizes.

Entrapment efficiency

The EEs of all formulated liposomal formulations containing verapamil ranged from 69.6% to 89% as shown in Table 5. X1 = weight ratio of cholesterol to lecithin and X2 = revolutions per minute (rpm) significantly affected the Entrapment efficiency of lipid:surfactant by weight ratio. Increasing the weight ratio decreased the Entrapment efficiency. Formula (F6) showed the maximum Entrapment efficiency at a cholesterol:lecithin ratio of 1:3.5 and a rotation speed of 80 rpm (20).

Zeta potential

The ZP value of the prepared composition is 25.7 to 51.2 mV as shown in Table 5.It has been previously mentioned that high values of the absolute zeta potential ensure good stability of nanoparticles (21). From the result of the zeta potential, it can be seen that the obtained value will indicate a good degree of stability for all liposome compositions prepared.

Table 4: Particle size and entrapment efficiency of Liposomes prepared using Film Hydration method

Formulations	Cholesterol : Lecithin w/w	Drug (mg)	Chloroform: methanol (v/v)	Particle size (nm)	Entrapment efficiency (%)
F1	1:1	80	1:1	478±1.5	51±1.2
F2	1:1.5	80	1:1	500 ±1.8	55 ±1.8
F3	1:2	80	1:1	429 ±3.4	68 ±3.1
F4	1:2.5	80	1:1	434 ±6.6	74 ±3.6
F5	1:3	80	1:1	345 ±9.5	84 ±1.6
F6	1:3.5	80	1:1	494 ±9.4	65±2.8
F7	1:4	80	1:1	613 ±1.6	47±1.9
F8	1:4.5	80	1:1	729 ±8.2	54±1.3
F9	1:5	80	1:1	627 ±1.7	59 ±3.9

Values represent mean±SD (n =3)

ptimization of liposomes:

Verapamil Liposome prepared by film hydration method. In this study, it shows that the particle size and Entrapment efficacy markly affected by cholesterol to lecithin ratio and stirring time. Therefore, the optimal formulation (F6) with cholesterol to lecithin ratio (1:3.5), chloroform to methanol ratio (1:1) and stirring speed (80 rpm) has maximum Entrapment Efficiency (89%) and a minimum particle size (463 nm),so on this basis it could be a optimized batch for further study.

Table 5: Experimental runs, independent variables and measured responses of the 3^2 full factorial experimental designs.

For-mulae	X1 Cholesterol: Lecithin w/w ratio	X2= Rotations per minute(RPM)	Y1= Particle size (nm)	Y2= Entrapment efficiency (%)	Y3= Zeta poten-tial(mV)
F1	1:2.5	50	417±2.4	47±3.2	-46.1 ± 1.3
F2	1:3	50	551 ±2.8	55 ±1.6	51.2 ± 1.1
F3	1:3.5	50	667 ±5.4	61 ±2.7	-46.0 ± 1.5
F4	1:2.5	80	462 ±7.6	63 ±3.9	-42.1 ± 0.4
F5	1:3	80	459 ±11.4	76 ±1.1	-39.2 ± 0.8
F6	1:3.5	80	463 ±9.4	89±2.7	-25.7 ± 1.8
F7	1:2.5	120	602 ±1.6	59±2.1	-36.4 ± 0.3
F8	1:3	120	719 ±10.3	45±1.1	-41.1 ± 1.0
F9	1:3.5	120	786 ±1.23	49 ±4.2	-37.2 ± 0.7

Values represent mean±SD (n =3)

CONCLUSION:

Verapamil loaded Liposomes using cholesterol and lecithin were prepared and evaluated. we developed verapamil loaded liposomes by using film hydration method. As a result, optimized liposomes are prepared by selecting the selected optimal factor, and the evaluation is to develop a successful composition that has very excellent compatibility, convenient particle size and high drug Entraptment efficiency. However, they say that liposomal formulations are used as a better choice for traditional drug delivery systems in hypertension therapy. Additional studies should be done in the test pipe and in vivo.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS:

The author has no conflict of interest to declare.

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