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Formulation And Evaluation Of Proliposomal Powder For Hypertension Management By Pulmonary Drug Delivery System

Sajiya Bee[*1], Shivangni Rathore[2], Revathi A Gupta[3]

1.Student, Institute of Pharmacy, Dr. APJ Abdul Kalam University, Indore (M.P.)
2. Assist, Professor, Institute of Pharmacy, Dr. APJ Abdul Kalam University, Indore (M.P.)
3. Principal, Institute of Pharmacy, Dr. APJ Abdul Kalam University, Indore (M.P.)

Abstract—

The beta-2 receptor blocking drug atenolol is frequently used to treat coronary artery disease and hypertension. An example of a hydrophilic β-adrenoceptor blocking medication is atenolol (4-[2-hydroxy-3-isopropylaminopropoxy-] phenyl-acetamide). According to the biopharmaceutics categorisation system (BCS), it is categorised as a class III drug. This indicates that atenolol has limited permeability but high solubility when taken orally. Drugs that are hydrophilic have trouble passing through cell membranes and are often not well absorbed from the stomach. About 50% of the beta blockers are eventually accessible because atenolol is not fully absorbed from the gut after oral treatment. For AT pills, a dosage of 25–100 mg taken orally twice a day is advised.

It is a suitable candidate for proliposomes due to these drawbacks. Pro-liposomes are free-flowing, dry granular materials that form liposomal dispersion when hydrated or in contact with bodily fluids. They are made of phospholipid and a porous powder that dissolves in water. Many medications' issues with solubility and bioavailability can be resolved by creating pro-liposomal formulations. The shelf life of liposomal solution may be limited, and in order to address the stability problem with liposomes, a unique formulation technique known as "pro-liposome" has been created that may swiftly and minimally manipulate liposomes as necessary.

The pulmonary epithelium is extensively vascularised and comparatively large. Additionally, efflux transporters, which promote medication absorption, are not very prevalent. Through this route, atenolol may be administered less often, medications with varied physicochemical qualities can be absorbed, metabolic enzyme levels are lower than hepatic ones, and the beginning of action can be relatively quick. Therefore, the new powder liposome formulation offers a great deal of potential for pharmacological exploration.

Keywords: Atenolol , Proliposome, quality, Pulmonary drug delivery system, BCS class III medicine.

1. Introduction

Liposomes are small spherical vesicles made up of one or more concentric lipidic bilayers enclosing an interior aqueous compartment. Natural and/or manufactured lipids, which are comparatively biocompatible, biodegradable, and non-immunogenic, make up the membrane of liposomes. Liposomes are utilised as carriers for both water-soluble and lipophilic compounds due to their special bilayer-structure characteristics. The inner watery compartments contain hydrophilic compounds. Lipid bilayers are the primary entrapment site for lipophilic medicines [1].

The biocompatibility and biodegradability of liposomes are two of its appealing biological characteristics. Their potential to improve encapsulant effectiveness by boosting drug solubility and stability, delivering encapsulated pharmaceuticals to particular target areas, and offering sustained drug release makes them promising as active vectors. Additional benefits of liposomes include minimal toxicity because of their phospholipid content, good encapsulation efficiency despite drug solubility, protection of the medication from degradation factors including light and pH, and less tissue irritation. However, liposomes in aqueous dispersion have significant stability issues related to phospholipid hydrolysis, fusion, and aggregation, which may shorten their shelf life. Even while freeze drying is now the most widely used method for potentially enhancing liposome durability, it still has several drawbacks, such as residual water content and issues with chemical stability brought on by the lyoprotectants utilised.

Compared to other methods, this one uses more energy throughout the production process and is much more expensive because of the lyophilization stage. The efficiency and viability of the manufacturing process should be acknowledged in addition to the intended product qualities for large-scale liposome manufacture.

In comparison to freeze-dried goods, dry liposomal formulations in the form of proliposomes seem to be the most promising option in terms of their ease of use and practicality. The medication, phospholipid, and water-soluble porous powder make up proliposomes, which are obviously dry, free-

flowing particles that get hydrated when water is added to create a liposomal dispersion. Proliposomes could be fabricated into several dose forms, including as vaginal administration, transdermal delivery methods, and tablets or capsules.

2. Material and Methods

Apparatus and chemicals: Atenolol by Softgel Healthcare Pvt, Ltd, Chennai, Soya phosphatidylcholine by Dr. Reddy's Laboratories, Hyderabad, Cholestrol, ehanol, and starch by Ranchem Ltd., India,

Methods: Preparation of Atenolol loaded Proliposomes via the slurry method

Starch was used as a carbohydrate carrier in the slurry technique to create proliposomes. As the lipid phase, cholesterol and soy phosphatidylcholine (SPC) were used in a 1:1mol ratio. A component of the lipid phase was atenolol. Absolute ethanol was used to dissolve the lipid phase that included SPC, cholesterol, and atenolol. To ensure equal distribution of the lipid phase and drug across the carrier particles, 100ml of starch was put in a glass beaker, and the ethanolic solution was poured onto the carbohydrate carrier to create a slurry. The organic solvent's evaporation was maintained for one hour by rotating the beaker at 270 RPM using a magnetic stirrer in a water bath that had been previously set to 45°C.

Table 1: The formulation design matrix for proliposomes

Formulation	Drug (mg)	SPC	Cholesterol	Starch	RPM
F1	25	2	2	25	220
F2	25	6	2	25	220
F3	25	2	6	25	220
F4	25	6	6	25	220
F5	25	2	4	25	170
F6	25	6	4	25	170
F7	25	2	4	25	270
F8	25	6	4	25	270
F9	25	4	2	25	170
F10	25	4	6	25	170
F11	25	4	2	25	270
F12	25	4	6	25	270
F13	25	4	4	25	220

3. Experimental work

3.1 Preformulation Studies

The study of a medical ingredient's physical and chemical properties, both alone and in conjunction with excipients, is known as preformulation. Preformulation studies aim to identify the physicochemical properties and excipients that may affect the manufacturing process, formulation design, and pharmacokinetic-biopharmaceutical aspects of the final product.

3.2 Determination of Solubility

Tamoxifen solubility studies were conducted to determine the impact of pH. Ten milligrammes of the medication were taken in separate test tubes, and then one millilitre of solvent was added. The solvent was added continuously until the sample was fully dissolved. The solvent needed to solubilise the medication powder was used to record solubility.

3.3 UV and FTIR Spectroscopy

The wavelength of maximum absorbance was then found by scanning the solution containing 10µg/ml between 224 nm. The reference standard FT-IR spectra of Atenolol and the acquired FT-IR spectrum of formulation were compared.

4. Result and discussion

4.1 Preformulation Study

4.1.1 Description

Atenolol is white solid powder.

4.1.2 Result of Solubility

Atenolol is soluble in water.

4.2 Result of Melting Point

The medicine's melting point was determined to be comparable to the stated value, confirming that the drug samples that were received met the stated specifications. The melting point of a particular pharmacological ingredient will vary depending on any impurities that may be present. Atenolol has a reported melting point of 158°C. When the capillary technique was used to determine the medication's melting point, the substance began to melt at 158°C and melted fully at 160°C. The aforementioned test revealed that the sample medication satisfies the Atenolol standard test.

4.3 Calibration curve

The concentration and associated absorbance were used to create the atenolol curve. According to the results of linear regression analysis, y = 0.0784x + 0.0642 is the equation for the line of best fit. In the concentration range of 10–50 µg/ml, linearity was noted.

Table: 2- Calibration curve value

S. No	Concentration	Absorbance
1	10	0.023
2	20	0.085
3	30	0.164
4	40	0.251
5	50	0.332

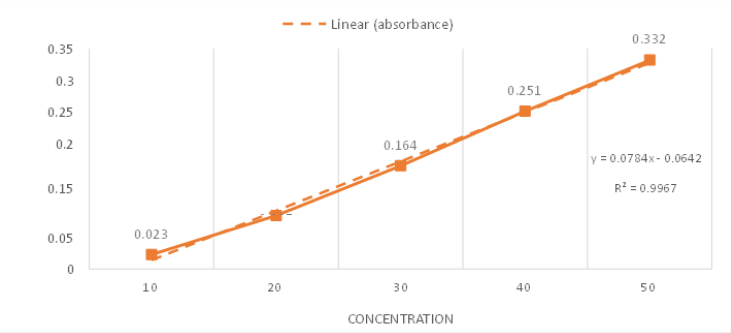

Fig 1. Calibration of Atenolol

4.3.1 FT-IR Spectroscopy

The interaction of proliposomes, cholesterol, Atenolol, and soy phosphatidylcholine is displayed in the FTIR spectrum. The 400–4000 cm-1 spectrum is included. By comparing the FT-IR spectra of the pure medicine with the FTIR of its formulation, the medication's compatibility in the formulation was verified.

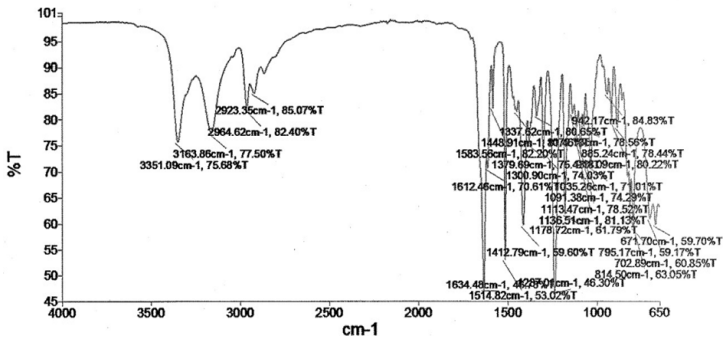

Fig 2. FT-IR of Atenolol

4.4 Drug - Excipients Compatibility Study

FT-IR Spectroscopy of Formulation

The atenolol proliposome's FTIR spectroscopy analysis result is shown in Fig. 6. Atenolol's spectra displays distinctive N-H stretching bond peaks at 653.75 cm-1 and 3340.1 cm-1. The stretching between the carbonyl group's C=O bond was the cause of the stretching band peak at 1647.88 cm-1.

Stretching between O-H bonds was the cause of the spectrum's peak value of 919 cm-1. The N-O bond was the cause of the spectrum's peak value of 1332.57 cm-1. The C-H bond was the cause of the stretching of 1238 and 1141.65 cm-1. The C-N of the amine group was the cause of the peak value 1092.48 cm-1's stretching. The table displays each peak that corresponds to the appropriate bond.

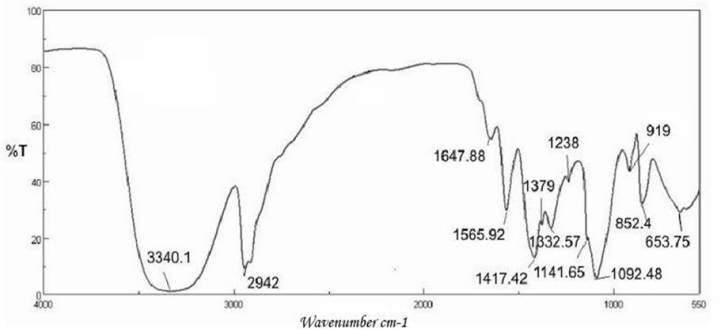

Figure 3 FT-IR Spectra of Atenolol Proliposome

Table. 3 Optimization of Atenolol proliposome

Std	Run	Factor 1 A: SPC (g)	Factor 2 B: cholesterol (g)	Factor 3 C: stirring speed (rpm)	Response 1 Particle size (nm)	Response 2 % Drug permeated
12	1	4	6	270	2014	51.4
2	2	6	2	220	1554	62.8
11	3	4	2	270	2764	56.5
3	4	2	6	220	1658	48
10	5	4	6	170	2984	54.2
8	6	6	4	270	1982	72.31
9	7	4	2	170	3630	49.1
1	8	2	2	220	1761	55
15	9	4	4	220	683	93.78
4	10	6	6	220	1313	78.2
7	11	2	4	270	2764	42.14
5	12	2	4	170	5650	45.8
13	13	4	4	220	683	93.78
6	14	6	4	170	3201	65.25
14	15	4	4	220	683	93.78

4.5 Characterization of Proliposomes of Atenolol

4.5.1 Anova

After obtaining and adjusting the responses, an ANOVA was computed and the expected R2 was ascertained using the Box Behnken Design. Table 19 provides the P values, accuracy, %CV, adjusted, and anticipated R2.

The amounts of these factors that yield the best answer were identified after examining how the independent variables affected the replies. It is clear that the drug permeability and proliposome particle size are influenced by the ratio of SPC, cholesterol, and stirring speed. As a result, the ideal level for the ratio of SPC, cholesterol, and stirring speed was determined to be medium. The ideal formulation produces proliposomes with a reduced particle size and a high drug permeability value. The theoretical values of 93.76% and 683 nm were obtained by selecting a level of 4 for SPC and cholesterol and a level of 220 rpm for stirring speed using a computer optimisation procedure. Lower levels result in a lower percentage of drug penetration and larger particles, whereas higher levels result in poor drug permeability and larger particles. Therefore, the ideal level was determined to be medium.

Table. 4 Optimization of Atenolol proliposome

RESPONSE	Adjusted R2	Predicted R2	Model P value	Adequate precision	%CV
Particle size (nm)	0.8110	-0.0802	0.0055	9.1906	26.08
% Drug permeated	0.9843	0.9102	<0.0001	26.93	3.55

4.5.2 Microscopic Observation of Hydrated Proliposomes

Hydrated optimised proliposome formulation by light photomicrography. The proliposomes transform into liposomes when they get moist. We can see in the picture how proliposome hydration results in the development of a tiny bilayer vesicle.

4.5.3 Scanning Electron Microscopy

It was discovered that all of the formulations had pH values between 4.2 and 6.4. It was determined that the pH range was suitable for preventing skin irritation. Skin irritation may result from formulations with higher or lower pH values.

4.5.4 Drug Entrapment Efficiency

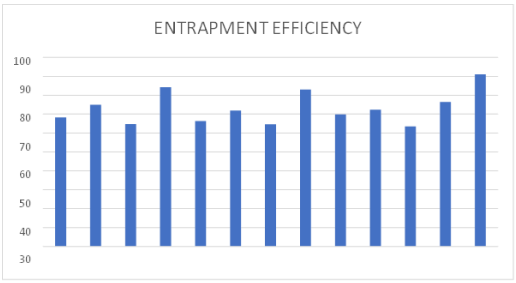

Fig: 4; Entrapment efficiency

The percentage of the total medication entrapped was used to determine the atenolol proliposomes' entrapment efficiency. The ratio of SPC, cholesterol, and stirring speed all affected the proliposome formulation's entrapment effectiveness. The entrapment efficiency is impacted when the SPC to cholesterol ratio and stirring speed drop. With an entrapment effectiveness of 91.04%, they have created the optimised formulation (F13) at moderate levels of SPC, cholesterol, and stirring speed (rpm). Table 20 presents the findings of the study on the entrapment efficiency of atenolol proliposomes..

4.5.5 In-vitro release of study

Table: 5; Drug release in permeation study

Time (hr)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.85	1.02	0.02	12.86	0.01	2.67	0.006	9.26	0.21	0.16	1.04	1.22	17.39
2	3.01	4.3	1.2	19.72	0.89	6.2	0.4	13.5	1.02	0.98	3.44	4.2	25.27
3	7.41	9.6	5.1	27.54	4.51	11.8	2.05	22.5	3.65	2.92	8.09	10.2	33.24
4	11.6	16.2	9.6	36.71	8.4	18.54	7.08	32.11	5.88	6.21	13.4	16.4	35.78
5	17.29	21.8	17.18	43.25	15.47	20.5	11.2	38.7	18.08	19.8	19.6	20.5	41.32
6	26.6	27.33	21.88	49.62	21.01	26.25	17.5	47.51	23.9	24.91	29.07	28.12	48.78
7	33.48	38.1	29.14	56.84	28.1	32.48	24.12	51.42	30.18	31.09	37.21	34.28	62.02
8	36.21	42.62	32.48	59.98	30.65	36.64	29.46	56.28	32.58	34.92	39.28	46.78	71.52
9	39.71	49.5	36.22	60.85	34.24	48.65	31.02	59.4	36.44	37.4	40.8	42.6	76.62
10	43.56	52.37	41.05	63.48	36.28	52.68	36.54	62.84	39.62	40.29	43.85	44.69	80.15
11	46.72	54.2	43.58	68.21	40.5	56.15	38.4	64.27	41.68	44.81	48.2	46.25	83.91
12	55	62.8	48	78.2	45.8	65.25	42.14	72.31	49.1	54.2	56.5	51.4	93.78

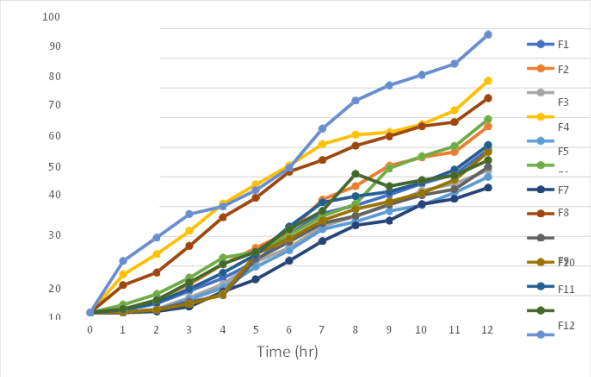

Fig:5; Invitro drug permeability plot

The drug permeability profile of Atenolol Proliposome formulations is displayed in Fig. 5.

The cumulative percentage of drug diffusion for formulations F2, F5, F7, and F9 was 48, 45.8, 42.14, and 49.1%, respectively.

The cumulative percentage of drug diffusion for formulations F1, F10, F11, and F12 was found to be between 55, 54.2, 56.5, and 51.4%.

The cumulative percentage of drug diffusion for formulations F2 and F6 was determined to be 78.2 and 72.31%, respectively.

F3 for formulation was 93.78%, indicating that the ratio of SPC (4g), cholesterol (4g), and a reduction in proliposome particle size enhances drug release.

4.5.6 In- Vitro Drug Release Kinetics:

Table: 23; Drug release kinetics values of r2 for optimized formulation

OPTIMIZED FORMULATION	Zero-order kinetics		First-order kinetics		Higuchi		Korsmeyer- Peppas	
	K	r2	K	r2	K	r2	K	N
	11.29	0.9396	-0.139	0.067	51.72	0.9919	1.98	0.6299

4.6 Stability Studies of best formulation

Table 5: Stability study of selected formulation of emulgel

FORMULATION	TEST CONDITION	TEMPERATURE	%EE	PHYSICAL APPEARANCE
OPTIMIZED FORMULATION	INITIAL	4°C	91.04±2.16	Yellowish fine powder
	1 MONTH	4°C	90.02±0.65	Yellowish fine powder
		24°C	88.26±2.61	
		37°C	85.82±3.15	Yellowish clumps

The proliposome formulations' physical characteristics and entrapment effectiveness were assessed after one month of storage at 4°C, 24°C, and 37°C. The proliposomes kept at 4°C and 24°C were stable after a month. However, there was a noticeable difference in appearance for the formulations that were kept at 37°C. Proliposome formulations held at 4°C had a 1.12% loss in entrapment effectiveness, whereas those stored at 24°C and 37°C experienced 3.15% and 5.73% decreases, respectively. Table 21 displayed the entrapment efficiency result.

Conclusion

Designing and developing a proliposomal dry powder inhalation for pulmonary delivery of an antihypertensive medication was the aim of the current investigation. For AT tablets, a dosage of 25–100 mg taken orally twice a day is advised. Due to Atenolol's decreased permeability, conventional oral administration typically produces unpredictable drug concentrations in plasma, which can result in dosage dumping, a decrease in the pharmacological efficacy, or the emergence of unwanted side effects. Because of these drawbacks, inhaling atenolol is regarded as one of the primary non-invasive drug delivery methods, and it is especially intriguing for cardiac targeting. This is due to the fact that the pulmonary vein is primarily used to deliver medications to the heart during absorption.

Therefore The use of Box-Behnken Design to create and optimise atenolol proliposomes is justified. In order to generate atenolol proliposomes, several concentration ratios of SPC (2, 4 & 6g) and cholesterol (2, 4 & 6g) were used, along with stirring speeds (170, 220 & 270 rpm) that responded to particle size and drug penetration percentage.

The optimal formulation was chosen based on the atenolol proliposomes' drug permeability and particle size measurements. To ascertain the impact of each parameter, the data gathered from the invitro drug permeability was examined using RSM. The impacts of the several factors involved were then evaluated. Out of the several ratios, the optimal SPC and cholesterol composite was chosen. 4 g of SPC, 4 g of cholesterol, and 220 rpm stirring speed.

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