

FORMULATION AND EVALUATION OF NEBIVOLOL PRONIOSOMAL GEL

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ABSTRACT

The aim of the study is to develop a proniosomal gel for Nebivolol used for the treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The results showed that the type of lipid incorporated altered the entrapment efficiency of proniosomal gel and higher entrapment efficiency of 63.6 ± 2.5 % was obtained with the proniosomal gel prepared from Span 40. Different formulations of Proniosomal gel using Span 40 as surfactant were prepared by changing the ratios of surfactant: lecithin and the optimized formulations F1, F2, F3, F4, F5, F6 and F7 were further characterized. SEM studies revealed uniform size and spherical shape of proniosomal gel, FTIR studies revealed that there was no interaction between the drug and excipients. *In-vitro* experiments of the F1, F2, F3, F4, F5, F6 and F7 formulations showed a release rate of 73.2, 51.8, 52.7, 78.3, 65.8, 71.4, 58.2 respectively. Hence formulation F4 was optimized as the drug release was found to be highest i.e., 78.3 % in 7 hours.

Keywords: Nebivolol, Proniosomal gels, Anti hypertensive.

INTRODUCTION

The traditional colloidal systems like microspheres and emulsions appeared in 1950's, out of which emulsions has been primarily used by the cosmetic industry in the topical delivery of cosmetic agents. In 1960's liposomes were discovered, and the introduction of liposomes in cosmetic market was in 1986 by company Dior. From a long time liposomes were considered as the main innovative contributors in the dermal area for both pharmaceutical and cosmetic products. Due to some drawbacks like high cost, variable purity of natural phospholipids and unstable nature, surfactant based vesicles 'Niosomes' came into existence.

From early 1980's, Niosomes have gained wide attention by researchers for their use as drug targeting agents, drug carriers to have variety of merits while avoiding demerits associated with conventional form of drugs. Niosomes were studied as better alternatives to liposomes for entrapping both hydrophilic and hydrophobic drugs.

Niosomes are microscopic lamellar structures, which are formed on the admixture of non-ionic surfactants with or without incorporation of cholesterol or other lipids. Niosomes are widely studied as an alternative to liposomes. These vesicles appear to be similar to liposomes in terms of their physical properties. From a technical point of view, Niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associate with liposomes. These vesicular delivery systems have attracted considerable attention in topical drug delivery for many reasons. These penetration enhancers are biodegradable, non-toxic, amphiphilic in nature, and effective in the modulation of drug release properties. Their effectiveness is strongly dependent on their physiological properties, such as composition, size, charge, lamellarity and application conditions.

The advancement in the niosomes leads to the evolution of proniosomal delivery systems. Proniosomes are non-ionic based surfactant vesicles, which may be hydrated immediately before use to yield aqueous niosome dispersions. Proniosomes are now a days are used to enhance drug delivery in addition to conventional niosomes.

Disadvantages of Niosomes

- Physical instability
- Aggregation
- Fusion
- Leaking of entrapped drug
- Hydrolysis of encapsulated drugs limiting the shelf life of the dispersion

To overcome these disadvantages, proniosomes are prepared and reconstituted into niosomes.

Advantages of proniosomes over the niosomes

- Avoiding problems of physical stability like aggregation, fusion, leaking.
- Avoiding hydrolysis of encapsulated drugs which limits the shelf life of the dispersion.

PRNOSIOMES

Proniosomes are vesicular systems, in which the vesicles are made up of non-ionic based surfactants, cholesterol and other additives which may be hydrated immediately before use to yield aqueous niosome dispersions.

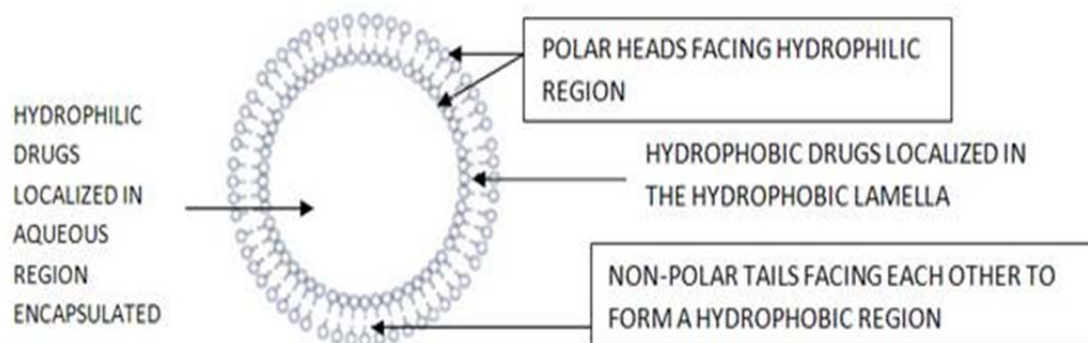


Fig 1: Structure of proniosomal vesicle

Proniosomes exist in two forms depending on their method of preparation.

1. Semisolid liquid crystal gel
2. Dry granular powder

Out of these two forms, the Proniosomal powders are mainly used for oral delivery of drugs. Proniosomal powders are dry formulations containing water soluble carrier particles imbibed with surfactants which can be measured as needed and dehydrated to form niosomal dispersions immediately before use on brief agitation in hot aqueous media within minutes. The resulting niosomes are very similar to conventional niosomes and more uniform in size. The proniosomal approach minimizes the problems associated with liposomes and niosomes by using dry free flowing product, which is more stable during sterilization and storage. Ease of transfer, accurate dosing, processing, distribution, measuring and storage make proniosomes a versatile delivery system with potential for use with a wide range of active compounds. The provesicular powders previously prepared by loading water soluble sorbitol powder with an organic solvent and cholesterol and then mixed with spans followed by vacuum evaporation of solvents. Several studies have been reported which prove utility of oral proniosomal powder in providing enhanced solubility and bioavailability for poorly soluble drugs.

MATERIALS AND METHODS

Materials:

Nebivolol was received as gift sample from Hetero Drugs, Hyderabad. Cholesterol and Span 60 are obtained from S.D.Fine Chemicals, Mumbai. Methanol and Chloroform are from Merck Specialities Pvt. Ltd.,

ANALYTICAL METHODS

Development of UV spectroscopic method

Preparation of calibration curve: The standard curve was prepared in the concentration range of 20 - 80 $\mu\text{l/ml}$. Different volumes of standard stock solutions, containing 20 - 80 $\mu\text{g ml}^{-1}$ of drug were transferred to 10 ml volumetric flasks and volume was made up with methanol. The absorbance was measured at 284 nm against the corresponding reagent blank. The drug concentrations of Nebivolol were analyzed by UV-Spectrophotometer at 284 nm.

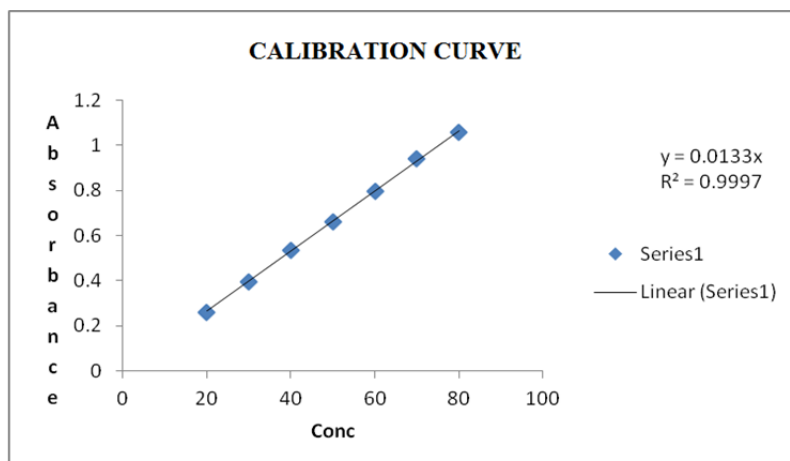
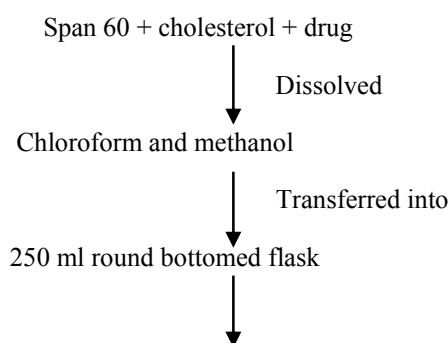


Fig 2: Standard graph of Nebivolol

FORMULATION OF PRNIOSOMES

Proniosomes were prepared by using slurry method. In brief, accurately weighed amounts of lipid mixture comprising of span 60 and cholesterol as per formulation ratios were dissolved in 20 ml of solvent mixture containing chloroform and methanol (2:1). The resultant solvent solution was transferred into a 250 ml round bottom flask. The flask was attached to a rotary flash evaporator (Hei-VAP advantage/561 - 01300, Heidolph, Germany) and the organic solvent was evaporated under reduced pressure at a temperature of $45 \pm 2^{\circ}\text{C}$. The obtained proniosomes were stored in a tightly closed container for further evaluation.



The flask was attached to a Rotary flash evaporator and organic solvent was evaporated under reduced pressure at a temperature of $45 \pm 2^{\circ}\text{C}$.

After ensuring the complete removal of solvent, the resultant proniosomes were collected and stored in a closed container.

EVALUATION

1. Morphological evaluation of prepared proniosomes by Scanning Electron Microscopy

The surface morphology of the proniosomes was evaluated by scanning electron microscopy. The proniosome gel was placed on a cavity glass slide and little water was added drop wise along the side of the cover slip. The formation of vesicles was monitored through a microscope and photomicrograph was taken.

2. Percentage Drug Entrapment

The PDE of Nebivolol proniosomes was calculated after determining the amount of untrapped drug by dialysis. The dialysis was performed by adding the niosomal dispersion to a dialysis tube (donor compartment) and then dipping the tube into a beaker containing 200 ml of PBS pH 6.8 (receptor compartment) on a magnetic stirrer, rotated at a speed of 80 to 120 rpm for 7 hours. After 3 hours, the solution in the receptor compartment was estimated for untrapped drug at 284 nm by using a UV spectrophotometer.

$$\text{Percent Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} * 100$$

3. *In vitro* diffusion study

In vitro dissolution study of proniosomal gel was performed by using franz diffusion cells by taking phosphate buffer 6.8 pH. The volume of diffusion medium used was 20 ml and maintained at a temperature of $37 \pm 0.5^\circ\text{C}$ with paddle speed set at 50 rpm throughout the experiment. An aliquot of 5 ml was collected at predetermined time intervals 30 min, 1, 2, 3, 4, 5, 6, 7 hrs respectively and replaced with fresh buffer to maintain constant volume⁴⁰. Samples were analysed for Nebivolol using UV-Visible spectrophotometer at 284 nm.

4. Fourier transform infrared (FTIR) spectroscopy

Infrared spectra of pure drug, and optimized proniosome formulation were obtained using FT-IR spectrophotometer (Bruker, Alpha-T, Lab India) by the conventional KBr pellet method

5. Determination of drug content in Proniosomes

Drug content was determined immediately after converting niosomes to proniosomes and it was found to be 80 % and for the optimized formulation (F4).

RESULTS AND DISCUSSION

1. Scanning Electron Microscopy

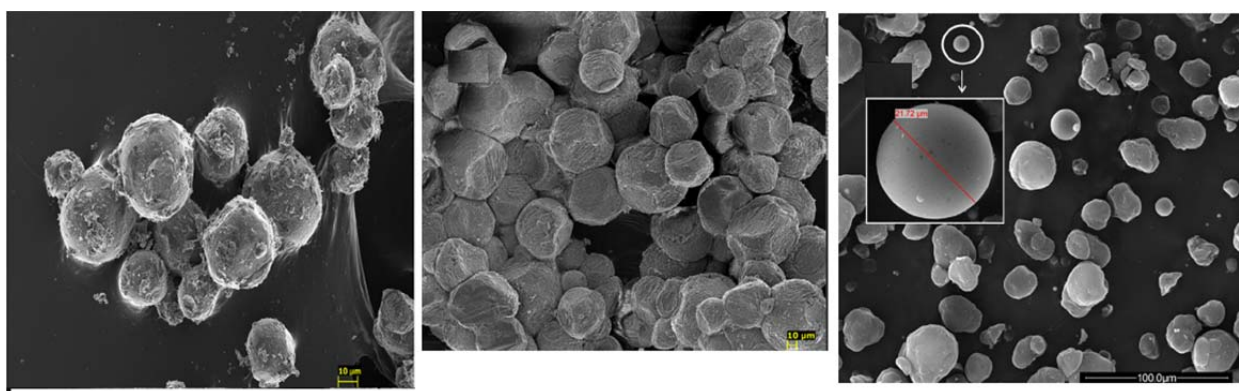


Fig 3: SEM analysis of F4 formulation

2. Entrapment Efficiency

Proniosomes prepared with non-ionic surfactants of alkyl ester including Span (sorbitan esters) and Tween (polyoxyethylene sorbitan esters) were utilized to determine the encapsulation of associated Nebivolol and vesicle size. As shown in Table 1, encapsulation efficiency of proniosomes formed from formulation PNGF1, PNGF3, PNGF4, PNGF5 proniosome gel exhibit lower encapsulation efficiency when compared to PNGF2. The results of entrapment efficiency are shown in the fig 4. Nebivolol was best encapsulated by niosomal gel prepared using Span 40 when compared to other grades and this was attributed to the fact that S40 is solid at room temperature, showed higher phase transition temperature and low permeability. The encapsulation efficiency of S40 at 59.50% was much higher than S20, T60 and T80 at 23.84%, 28.84%, 17.24% and 15.84%. Furthermore S40 was optimized based on the encapsulation efficiency by taking different ratios of surfactant and lecithin and encapsulation percentage is determined.

Table 1: Encapsulation percentage of various Proniosomal Gel Formulations

Sl. No	Niosomal code	Encapsulation percentage (%)
1.	PNG F1	21.64 \pm 1.2
2.	PNG F2	57.3 \pm 2.5
3.	PNG F3	21.78 \pm 1.4
4.	PNG F4	27.76 \pm 1.9
5.	PNG F5	16.21 \pm 1.7

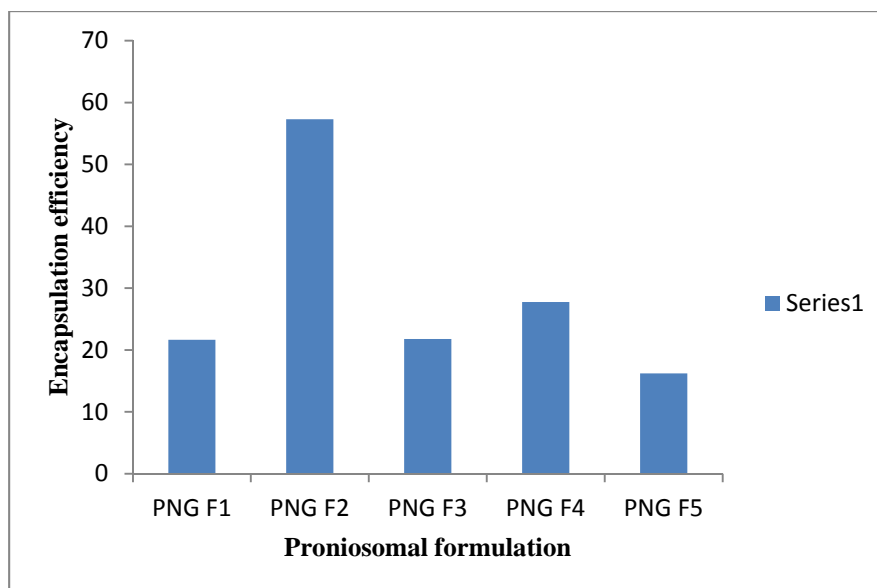


Fig 4: Encapsulation percentage of various Proniosomal Gel Formulations

Table 2: Proniosomal gel formulations with various ratios of sorbitan fatty acid esters and lecithin

Sl. No	Proniosomal code	Ratios		Nebivolol (mg)	Cholesterol (mg)
		Span 40	Lecithin		
1.	F2	2	1	10	20
2.	F3	1	2	10	20
3.	F4	3	1	10	20
4.	F5	1	3	10	20

The encapsulation percentage obtained by different ratios of sorbitan fatty acid esters and lecithin were almost same with slight difference that is formulations having more of surfactant have encapsulation slightly higher than those with higher lecithin ratio. Table 3 shows the encapsulation percentage of different formulations. The percentage encapsulation of different formulations of Span 40 is shown in the figure 5.

Table 3: Encapsulation percentage of different formulations

Sl. No	Proniosomal code	Ratios		Encapsulation percentage (Percentage)
		Span 40	Lecithin	
1.	F2	2	1	63 ± 2.1
2.	F3	1	2	55.8 ± 1.9
3.	F4	3	1	63.6 ± 2.5
4.	F5	1	3	56.8 ± 1.2

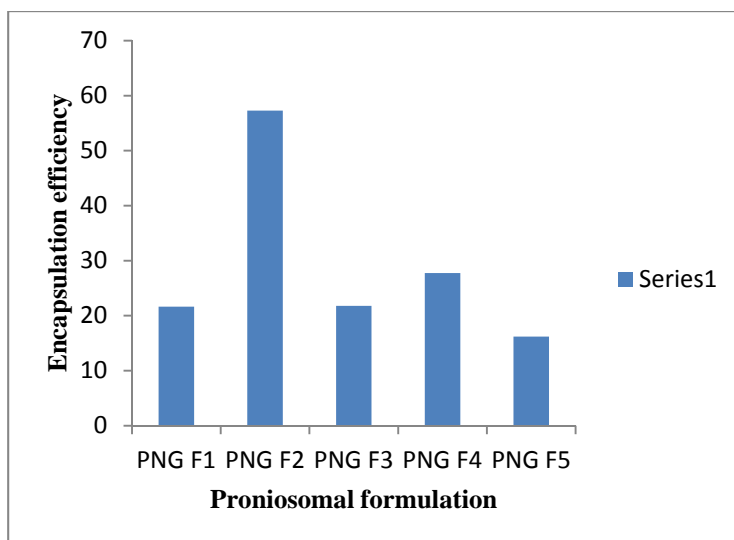


Fig 5: Encapsulation percentage of different formulations

3. In-vitro Studies

Among all the formulations, F4 showed greater amount of drug release after 7 hours.

Table 4: In-vitro diffusion studies

S.No	Formulation	Cumulative % drug release
1	F1	73.2
2	F2	51.8
3	F3	52.7
4	F4	78.3
5	F5	65.8
6	F6	71.4
7	F7	58.2

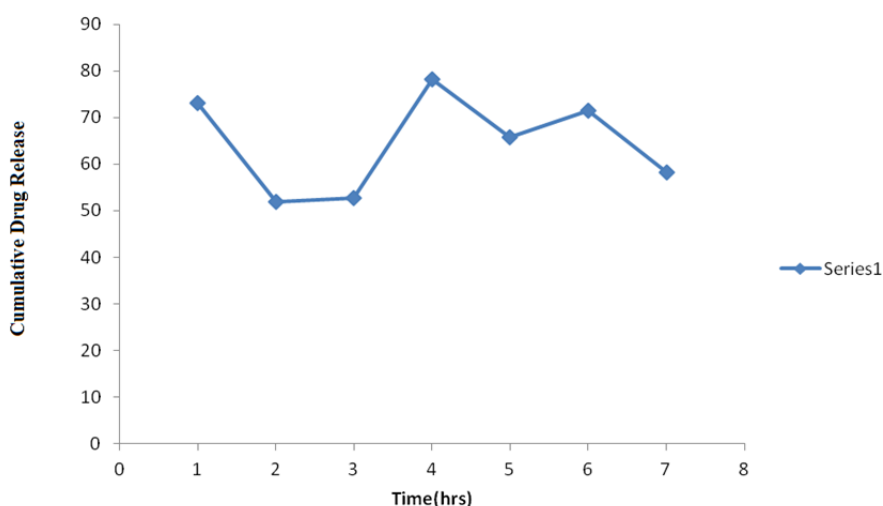


Fig 6: In-vitro drug release profile for F4 Proniosomal Gels

Release kinetics

The Release kinetics of the optimized formulations studied in *in-vitro* drug release is given in the tables. Different Kinetic model of the Formulations F2, F3, F4 and F5 are shown above.

Table 5: Release kinetics of optimized formulations in *in-vitro* drug release

Sl.No	Formula	Zero Order	First Order	Hixson-Crowell (R ²)	Higuchi (R ²)	Korsmeyer-Peppas	
						(R ²)	n
1	F2	0.9764	0.6821	0.9153	0.7948	0.9762	0.6643
2	F3	0.9737	0.7518	0.9286	0.7878	0.9734	0.5341
3	F4	0.9822	0.6938	0.9262	0.8073	0.9823	0.6572
4	F5	0.9672	0.7736	0.9245	0.7756	0.9677	0.5758

4. Fourier transform infrared spectroscopy

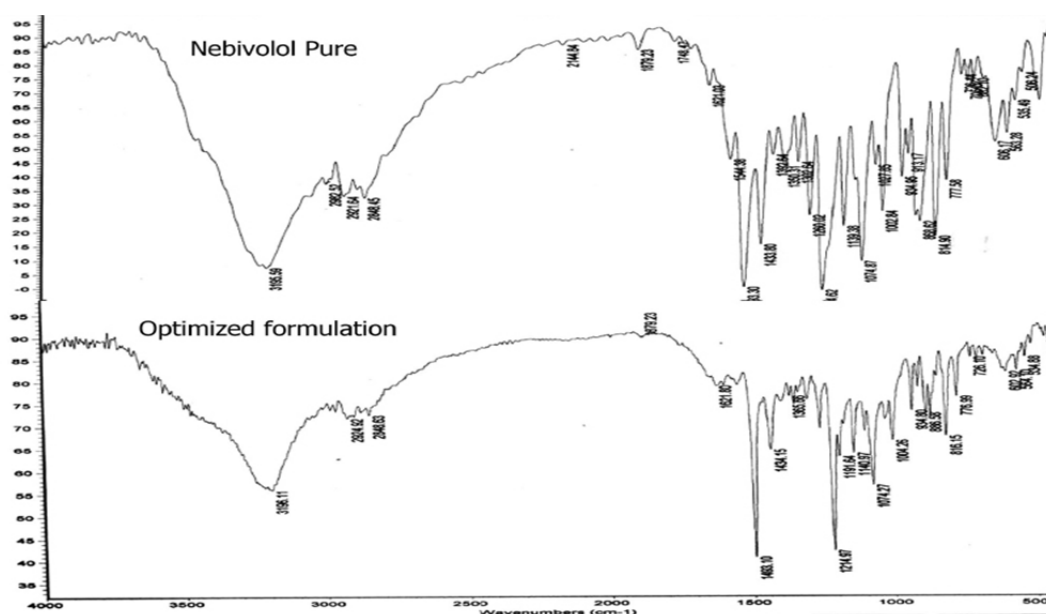


Fig 7: Infrared spectrum of F4 formulation

5. Determination of drug content in Proniosomes

Drug content was determined immediately after converting niosomes to proniosomes and it was found to be 80 % and for the optimized formulation (F4).

CONCLUSION

Studies were conducted with various levels of amount of cholesterol and span 60 to optimize Proniosomes. All formulations were evaluated for the different Physico-chemical characteristics. Formulated proniosomes gave satisfactory results for entrapment efficiency. *In-vitro* drug release behavior was improved. There is no significant difference between the FTIR patterns of the optimized formulation of proniosomal powder and to that of the pure drug.

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