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Formulation Of Atenolol Novel Cardiovascular Lipid Nanocarrier Transferosome.

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A B S T R A C T

The beta-blocker atenolol has a wide variety of uses in the management of cardiovascular disease. An enhanced atenolol delivery system was developed and optimized in this study using lipid nanocarrier transferosomes. A nanostructured lipid carrier formulation was prepared by a number of techniques. The optimized formulation was then assessed for particle size, zeta potential, scanning electron microscopy, and drug encapsulation efficiency. Atenolol-loaded cardiovascular lipid nanocarrier transferosomes demonstrate a promising potential for enhanced drug delivery. Physicochemical properties and sustained release characteristics of the optimized formulation suggest improved efficacy and compliance for patients. The developed transferosomal formulation must undergo further preclinical and clinical studies to determine its therapeutic potential and pharmacokinetic profile.

Keywords: Lipid, Atenolol, Solid, Transferosome.

Introduction

There are two types of drug carriers: solid lipid nanoparticles (SLNs) and nanostructured lipid carriers. A NLC carrier is arranged spatially with solid lipids and liquid lipids. As solid and liquid lipids are arranged spatially alternatively, drug loading efficiency is enhanced, as is the ability to overcome the crystallinity of the lipid matrix, which easily crystallizes solid lipids [1, 2].

In NLC, lipid chains and blocks form amorphous particles which are not reconstituted during storage. A method such as this prevents drugs from ejecting during storage. NLC can be used to transport solutions, suspensions, and ointments in lieu of conventional carriers [3, 4]. The term micellar colloidal drug carriers (NLCs) refers to dispersions of nanoparticles with a size range of 10 nanometers to 500 nanometers. It is necessary to combine solid lipids with liquid lipids in order to produce a good matrix for NLC. The melting point of NLC will be lowered due to the presence of oil, as compared to that of SLN [5, 6].

Lipids can enhance the solubility and bioavailability of some drugs that are less water soluble or lipophilic

(BCS Class III such as atenolol) in drug delivery systems that provide greater solubility and bioavailability than water. A potential carrier mode is provided by lipid nano-particles such as NLC, which differ from existing lipid particulate systems like Liposomes and Polymeric Nanoparticles [7-9].

Materials

The atenolol was purchased from Aurobindo Pvt Ltd in Hyderabad. The following components are included in Compatrol 888AT0: Acconon C-44 EP/NF, Dynasan 114, Softigen, Witepsol H32, Steric acid, Poloxamer, Cholesterol, Tween-20, Tween-80, Span-20, Span-80, Polysorbate 20, Polysorbate 80, Manittol, PVA, Polyvinylpyrolidine, DMSO, PEG, KH_2PO_4, NaOH, Methanol (HPLC Grade), Ammonium Acetate, Formic Acid, Trifluro acetate, Perchloroacetic Acid, ACN.

Methods

Preparation of Nanostructured lipid carriers by various techniques

Hot homogenization Technique

Hot homogenization was used to prepare NLCs. A lipid matrix was formed by blending and melting different proportions of liquid and solid lipids at 75°C. Weighed amount of sample (10 mg) was homogeneously dispersed in derived lipid matrix. In preparation of 0.25 to 1% surfactant and co-surfactant solution, 75°C was maintained under hot conditions. A homogenizer was used to homogenize the hot aqueous phase [10]. In order to generate a monodisperse NLC, dispersion phase was simultaneously mixed with a surfactant solution and homogenized for 15000 RPM for about 30 minutes with a high speed homogenizer (CAT, Germany) whiles the temperature was maintained at 80°C.

Formulation of NLC

Trail formulation for the selection of product and process variables

Initially, a hot homogenization method was used to prepare a trial NLC formulation to confirm its formulation. Based on the formulation variables like the composition of lipid (Witepsol, campritol), surfactant(poloxamer) and process variables (homogenization time, ultrasonication time) and nine formulations have been prepared. According to the formulation, lipid is weighed according to the ratio of 1:5 & 1:10 i.e., the proportion between the drug & lipid. Lipid mixtures were melted at 75°C to get clear, viscous liquids. After that, the Atenolol (10 mg) drug was dispersed into the melted lipid with constant stirring to obtain a homogeneous mixture. As shown in formulation table 1, then these lipid mixtures was added drop wise to a beaker containing surfactant solution. (0.25-0.5% of Tween80). The NLC suspension formed a milky white color.

Table 1: Preparation of Trial NLC formulation by Hot Homogenization Method

Ingredients		**T N1**	**T N2**	**T N3**	**T N4**	**T N5**	**T N6**	**T N7**	**T N8**	**T N9**
Drug	Atenolol (mg)	10	10	10	10	10	10	10	10	10

Product variable	Witepsol (mg) – Solid Lipid	50	50	100	100	-	-	-	-	25
	Campritol (mg) – Solid Lipid	-	-	-	-	50	50	100	100	25
	Softigen (ml) – Liquid Lipid	5	5	5	5	5	5	5	5	5
	Polaxomer (%)- Surfactant	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.5
Vehicle	Water (ml)	50	50	50	50	50	50	50	50	50
Process variable	15000 RPM Homogenization (min)	10	15	10	15	10	15	10	15	15
	Ultrasonication time (min)	10	5	10	5	10	5	10	5	5

Table 2: Box-Behnken – (MLR) Multiple Linear Regression- factorial designing of Atenolol NLC optimization

Run	**Independent variables**					
	Factor A (Witepsol :Softigen) X1(mg)	**Factor B Surfactant (Polaxomer) X2 (mg)**	**Factor C Homo-Speed (15000 RPM) in time X3 (min)**	**Factor A X1 (mg)**	**Factor B X2 (%)**	**Factor C X3 (min)**
AN1	-1	0	-1	7:3	0.5	5

AN2	0	0	0	8:2	0.5	10
AN3	1	0	-1	9:1	0.5	5
AN4	-1	-1	0	7:3	0.25	10
AN5	0	0	0	8:2	0.5	10
AN6	-1	0	1	7:3	0.5	15
AN7	0	1	1	8:2	1.0	15
AN8	0	0	0	8:2	0.5	10
AN9	1	-1	0	9:1	0.25	10
AN10	0	0	0	8:2	0.5	10
AN11	1	1	0	9:1	1.0	10
AN12	0	1	-1	8:2	1.0	5
AN13	0	-1	-1	8:2	0.25	5
AN14	1	0	1	9:1	0.5	15
AN15	0	0	0	8:2	0.5	10
AN16	-1	1	0	7:3	1.0	10
AN17	0	-1	1	8:2	0.25	15

Design optimization using Box Behnken (BBD)

According to Table 2, the previously optimized variables were fixed in BBD, which was designed using software like Design Expert 9 and Stat-ease Inc. In total, 17 formulations for four centric factorial points & 12 factorial runs were generated [11]. By these, we determined how the dependent variables corresponding to the independent variables were affected by changes in the independent variables. With the aid of a 33 factorial design, a 1st order response surface model was implemented and the outcome effects were elucidated. Selected independent variables from previously optimized parameters were given as X1 for solid-lipid: liquid-lipid ratio i.e., Witepsol: Softigen ratio; X3 for mixing rate was at 15000 rpm for alternative periods; X2 for various degree of surfactant (Polaxomer) at various levels code such as low (-1), medium (0) & high (+1). In order to evaluate the effect of the NLC formulation on dependent variables, the following

variables were used: (Y1-Particle size in mm, Y2- Zeta potential in mV and Y3-% EE).

Analysis of NLC formulation

Particle Size (PS) & Particle Size Distribution <0.3

A nanoparticle size analyzer (Horiba-SZ-100 Nanopartica series) was used to measure Particle size distribution, mean PS in nm and PDI of NLCs. In order to prepare the samples, the NLC dispersion was diluted twice with distilled water and deionized twice. A 0.45µ membrane filter was used to filter the solution. Based upon viscosity of medium, a 90º light scattering intensity for samples of low viscosity and a 170º light scattering intensity for samples of high viscosity were automatically detected. PI should equal <0.3, indicating uniform monodisperse size distribution of NLC particles between 10 and 1000 nm. A triplicate sample was taken for all measurements (n=3).

Zeta (surface charge) Potential (ζ)

Here Horiba Particle Size analyzer (Nanopartica series- SZ-100) was used to determine the surface charge potential (Table.3). Dilute form of NLC dispersions were injected into an electrophoretic cell having electrical field of 80 mV. A triplicate of each measurement was carried out at 25ºC.**Table 3: Colloid's Surface charge Potential and stability behavior**

Surface charge Potential	Stability behavior of the colloid
0 to ±5	Rapid Coagulation /flocculation
±10 to ±30	Incipient instability
±30 to ±40	Moderate stability
±40 to ±60	Good Stability
±61	Excellent Stability

Here we used Smolochowski's equation to determine the Zeta potential directly from the equation. A liquid's viscosity can be calculated by taking the following into account: µ - Electrophoretic mobility; ζ- Zeta Potential; η is its viscosity and Ɛ - Electric permittivity.

Microscopical studies of surface morphology

A SEM (Hitachi S-3000N) was used to observe the surface morphology of selected optimized NLC formulations. A sputter coater was used to coat stabilized NLC samples (powder) with Pt. of 600 Aº and then it was examined by an SEM. A sample holder with coated NLC was then scanned using an electron beam. Upon striking the NLC particles, the beam of electrons emits secondary electrons, which are used to generate the image of the surface characteristics of the NLC. Then measuring average Particle Size of NLC derived from the SEM having the size of NLC derived from the Horiba Nanoparticle size analyzer.

STUDY ON ENCAPSULATION EFFICIENCY

To assess encapsulation effectiveness, centrifugation was used. An ml of NLC Nano dispersion with a molecular weight of 12000 to 14000 Daltons and the Pore size of 2.4 nanometer has been taken in the dialysis bags (Himedia). A centrifuge tube was filled with the prepared dialysis membrane bag. In a REMI centrifuge, centrifugal tube was pre-filled with phosphate buffer of pH 7.4 (9 ml), centrifuged for 1 hour at 15000 RPM to exclude free drug. 5ml of sample was withdrawn from phosphate buffer saline after one hour. UV Spectrophomotometer at 224 nm was used to determine the concentration of Atenolol in the withdrawn sample. Same ingredients were used to prepare a blank solution (without Drug). A triplicate evaluation was performed (n=3).

%EE= (Xs-Xt)/s X 100

As a result of the equation below, we were able to calculate the percentage entrapment efficiency; in this equation, Xs =

The optimization of the formulation of atenolol NLC

Table 4: Effect of variables on the NLC of atenolol by Boxbehnken design

Batch	Independent variables			Dependent variables			Further Variables
	X1	X2	X3	Y1	Y2	Y3	
	Factor A: (Witepsol : Softigen) X1 (mg)	Factor B: X2 (mg) Surfactant (Polaxomer)	Factor C: Homo. Speed (15000 RPM) time X3 (min)	PS nm*	ZP mV*	EE %*	% Drug Content*
AN1	-1	0	-1	288.6 ± 3.88	-34.6 ± 3.22	72.38 ± 2.58	73.24 ± 3.28
AN2	0	0	0	243.2 ± 4.66	-42.6 ± 4.2	76.58 ± 2.85	80.56 ± 2.68

					2		
AN3	1	0	-1	489.2 ± 4.22	-30.6 ± 3.72	50.72 ± 3.26	60.14 ± 3.56
AN4	-1	-1	0	289.6 ± 3.56	-28.6 ± 2.86	67.41 ± 3.24	78.34± 3.54
AN5	0	0	0	318.4 ± 3.86	-38.6 ± 2.40	68.66 ± 2.57	70.34 ± 2.58
AN6	-1	0	1	**288.1 ± 3.26**	**-46.3 ± 3.42**	**85.68 ± 2.54**	**82.38 ± 2.36**
AN7	0	1	1	402.6 ± 4.22	-28.6 ± 3.66	60.54 ±2.88	58.22 ± 3.38
AN8	0	0	0	340.4 ± 4.78	-36.6 ± 3.54	66.46 ± 5.42	70.24 ± 3.28
AN9	1	-1	0	562.8 ± 4.68	-20.4 ± 3.66	54.56 ± 4.58	60.26 ± 2.36
AN10	0	0	0	334.6 ± 4.42	-40.	65.8 ± 2.78	70.24 ± 2.38

					2 ± 2.58		
AN11	1	1	0	586.4 ± 4.66	-38.6 ± 4.36	46.92 ± 3.54	56.62 ± 3.12
AN12	0	1	-1	442.6 ± 4.28	-38.6 ± 3.66	54.8 ± 2.46	66.58 ± 2.14
AN13	0	-1	-1	388.6 ± 3.98	-18.6 ± 3.68	66.56 ± 2.86	66.24 ± 2.16
AN14	1	0	1	598.6 ± 4.72	-12.2 ± 3.28	46.08 ± 2.68	46.92 ± 2.18
AN15	0	0	0	356.8 ± 3.76	-16.8 ± 2.54	73.46 ± 3.34	67.80± 2.26
AN16	-1	1	0	256.2 ± 4.66	-29.2 ± 4.22	80.54 ± 4.33	80.34 ± 3.28
AN17	0	-1	1	487.6 ± 6.86	-12.4 ± 2.68	60.62 ± 6.44	64.20 ± 3.34

* Note:-1: Low level; 0: moderate level; +1: high level (mean ± SD, n=3)

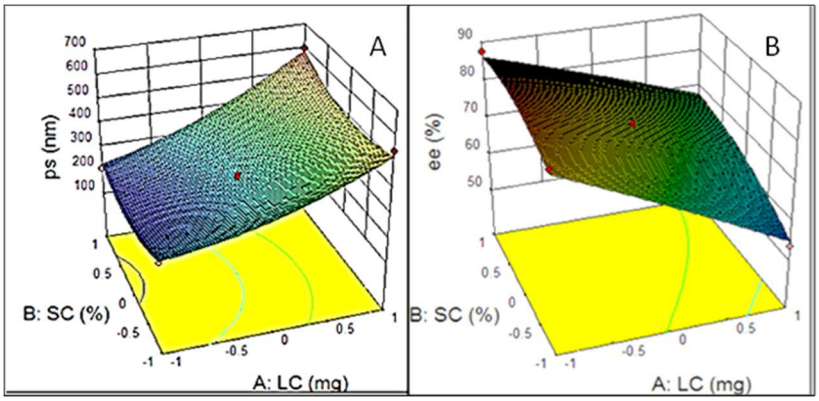

Figure 1: (A) 3-D surface design showing effect of concentration of lipid (X1) and concentration of surfactant (X2) concentrations on particle size; (B) Surface plot showing Entrapment efficiency (%).

As a result of the Box-Benken optimization Table 4, it was revealed that independent variables had a significant impact on dependent variables throughout the formulaiton of Atenolol NLC. We concluded that particle size and lipid concentration are strongly correlated based on our data [12]. Lipid concentration and particle size were found to be strongly correlated (r2 = 0.992). As concentration of lipid was improved, particle size of NLC getting bigger. The particle size decreased from -1 to 0 when the surfactant concentration and homogenization time were increased. In addition, particle size increased as the level increased from 0 to +1.As a result of executing an ANOVA between lipid concentration and PS, the 'P' value was found to be * 0.0001. This indicates that PS increased significantly when the lipid concentration was increased. When the surfactant concentration and homogenization time were increased, the PS increased significantly as well. Based on analysis of all formulations (AN1-AN17), formulation AN6 was required to have a size of particle about 288.1 ± 3.26nm at low -1 level lipid (7:3 Solid lipid: Liquid lipid ratio). Zeta potential on particles increased with increasing surfactant concentration. Although the particles were able to achieve one of their maximum conductivity abilities. As the surfactant level increased after the 0 level, the Zeta potential remained unchanged (r2 = -0.993 negative linear regression). Increasing lipid concentrations may cause this effect. When surfactant concentrations were increased during NLC preparation, particle size decreased simultaneously, which confirmed NLC was extremely stable on its phase, leading to maximum particle conductivity. This was confirmed by maintaining the particle's motion without sedimentation at the desired surface charge potential. As compared to the rest of the NLC formulations (AN1-AN17), Atenolol AN6 exhibited a zeta potential of -46.3 ± 3.42mV at low surfactant concentrations (0.5%) and moderate homogenization times (10 minutes). The %EE increases at the same time as surfactant concentration and homogenization time increase. At moderate 1 homogenization time (15 minutes) and high 0 level (0.5 % surfactant concentration), AN6 had the highest entrapment efficiency of all Atenolol NLC formulations (AN1-AN17). AN6 was found to be the optimal formulation for Atenolol loaded NLC based on the optimization data. Based on the Box-Behnken design coefficient, the polynomial equations were derived as follows based on the variations in dependent variables on independent variables. By applying the Box-Behnken design to the response, the polynomial equation confirms what independent variables affect

dependent variables as follows:

PS (nm) =232.42 A + 28.6 AB - 18.42 BC + 32.86 A2 + 42.60 B2 + 24.62 C2 + 246.12

ZP (mV) = 5.420 A - 7.843 B - 3.642 C + 6.66 AB + 8.40 B2 + 5.30C2 - 42.24

EE (%) = -12.36 A + 4.264 B + 1.642 C + 4.266 AB - 2.265 B2 + 68.24

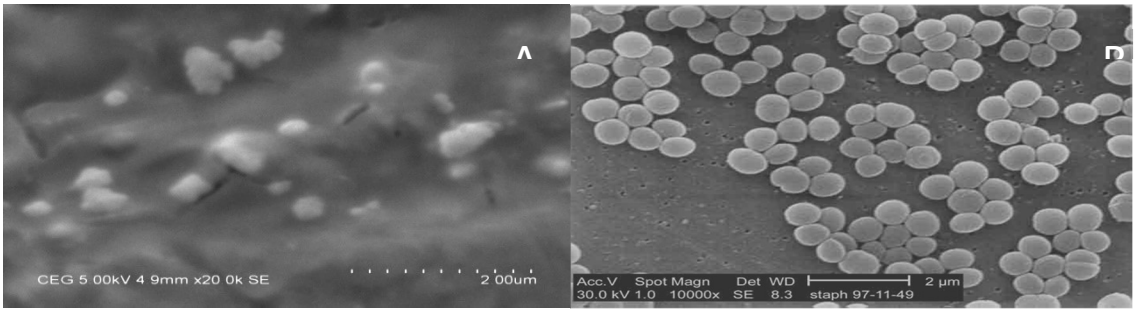

Figure 2: SEM studies of optimized Atenolol NLC formulation – AT6

)Photomicrographs of Enlarged Nanostructured Lipid Carriers; (B) Nanostructured Lipid Chains; (C) Functional Blocks of the Nano Structured Lipid Carrier; (D) Nanostructured Lipid Carriers as a Group [13]. SEM images of the Optimized formulation, where the NLC were visualized as blocks of spherical particles bound together by spherical shapes. As a result, the drug penetration through physiological barriers was enhanced and the drug loading efficiency was improved. Based on the drug content and entrapment efficiency of Atenolol NLC (AN6), the formulation demonstrated 82.38 ± 2.36 (%DC) and 85.68 ± 2.54 (%EE).

Discussion

A number of techniques were used to prepare nanostructured lipid carriers (NLCs) for atenolol novel cardiovascular lipid nanocarrier transferosomes, including hot homogenization. It involves melting the lipid components and dispersing the drug within the lipid matrix using high shear forces and high temperatures. Solid lipids and liquid lipids are typically used as excipients in the formulation of NLCs, and surfactants and cosurfactants stabilize the nanoparticles. We used a trail formulation approach to select the optimal variables for the formulation process, including the composition of the lipid, the concentration of the surfactant, and the homogenization speed. To optimize particle size, zeta potential, and encapsulation efficiency, NLC formulation parameters were iteratively optimized.

NLC formulation characteristics were systematically evaluated by Box-Behnken design (BBD) using multiple variables. By analyzing how particle size, zeta potential, and encapsulation efficiency interact with formulation parameters, BBD was able to determine optimal conditions. Physical stability and colloidal properties of NLC formulations were assessed using particle size and zeta potential measurements. NLC nanoparticles were examined using scanning electron microscopy (SEM) to determine their surface morphology and structural integrity.

Based on the results of the encapsulation efficiency study, it was determined how much atenolol was effectively encapsulated within the lipid matrix of the NLC formulations. To maximize drug payload and

minimize drug leakage during storage, high encapsulation efficiency is desirable. To achieve optimal drug delivery performance and therapeutic efficacy, the discussion emphasizes the importance of formulation techniques, optimization strategies, and characterization methods in developing novel cardiovascular lipid nanocarrier transferosomes of atenolol.

CONCLUSION

In conclusion, novel cardiovascular lipid nanocarrier transferosomes incorporating atenolol present a promising strategy to enhance drug delivery. The formulation we developed has the optimal physicochemical properties and drug encapsulation efficiency after systematic optimization studies. With this innovative approach, the limitations of traditional formulations can be addressed, which may lead to improved cardiovascular drug therapy. To improve cardiovascular patient outcomes, more studies are needed to assess atenolol-loaded transferosomes' clinical efficacy and safety.

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