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DESIGN, CHARACTERIZATION AND IN VITRO EVALUATION OF AMIODARONE ENCAPSULATED SOLID LIPID NANOPARTICLES

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Abstract:

The purpose of this study was to design; characterization and in vitro evaluation of Amiodarone encapsulated solid lipid nanoparticles. Amiodarone loaded SLN, was prepared by pre-emulsion followed by probe sonication method. Box- Behnken design was introduced to optimize the formulation of solid lipid nanoparticles Results: Fourier transform infrared spectroscopy studies indicate that no interaction or minor at molecular level suggest the excipients added were compatible with the drug. The value of zeta potential of optimized formulation of Amiodarone-SLN was found to be +75.9 mV which is sufficient to keep the particles stable. The optimized formulation has particle size of 233 ±3 nm and entrapment efficiency of 87.4 ± 1.29%, which were in good agreement with the predicted values. The scanning electron microscopy (SEM) analysis revealed distinct and mono-dispersed SLN with spherical shape.

Key words: Solid lipid nanoparticles, probe sonication method, Box-Behnken design & Fourier transform infrared spectroscopy.

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INTRODUCTION:

Among all the routes in use for the systemic delivery of drug by means of different dosage forms, oral drug delivery is recognized for decades as the most commonly utilized route of administration. The popularity of the oral route may be in part attributed to its ease of administration and the belief that drug administered orally is well absorbed1. The oral route of drug delivery is naturally considered the preferred and most patient-convenient way of drug administration[1-3].

To achieve a systemic approach to the successful development of an oral dosage form it is essential to develop the product within the intrinsic characteristics of gastrointestinal physiology, pharmacokinetics and pharmacodynamics as formulation design[4-6].

Researchers mostly focus on development of drug delivery system for lipophilic drugs, as these drugs mainly suffer from oral bioavailability problems due to hydrophilic environment of gastrointestinal tract. Numerous drug discovery approaches have been screened to overcome barriers and to enhance the bioavailability of drugs viz. modification of chemical entity, use of permeation enhancers, use of inhibitors of proteolytic enzymes to lower their activity, modulation of GI transit time, reduction of hepatic first pass elimination and design of novel drug delivery systems. Targeted systems release drugs at the specific site of the gut, where proteolytic activity is relatively low, protecting drugs from luminal proteolytic degradation, resulting into enhanced absorption and improved bioavailability[5-9].

Lipid-based drug delivery systems are promising, since lipids are known oral drug absorption enhancers and can be prepared with low particle size. Lipid-based delivery systems comprises of a range of products from simple oil solutions to complex mixtures of oils, surfactants, co-surfactants and co-solvents[3]. Lipid formulations used for oral delivery of drugs usually consist of a drug dissolved in a blend of two or more excipients, such as triglyceride oils, partial glycerides, surfactants or co-surfactants[10]. In the oral delivery of poorly water soluble, lipophilic drugs, lipid based delivery systems are finding increasing application. Improved oral bioavailability of lipidic dosage forms may be due to several mechanisms[10-16]. The primary mechanism is typically partial or complete avoidance of the slow dissolution process responsible for limited bioavailability of hydrophobic drugs from solid dosage forms. Preferably the lipidic formulation allows the drug to remain in a dissolved state during its transit through the gastrointestinal tract.

MATERIALS AND METHODS:

Materials

Amiodarone was received from Lupin Ltd., Pune, India, Compritol 888 ATO, Precirol ATO 5, Gelucire 44/14, Emulcire etc was purchased from Loba Chemie Pvt. Limited, Mumbai.

Methods

For preparation of Amiodarone loaded SLN, pre-emulsion followed by probe sonication method was selected. Briefly, lipid phase consisted of Amiodarone, lipid and lipid phase surfactant maintained at 70°C. An aqueous phase was prepared by dissolving aqueous surfactant in distilled water (sufficient to produce 50 ml of preparation) and heated to same temperature as of oil phase. Hot aqueous phase was added to oil phase and homogenization was carried out at 70°C using high speed homogenizer at different speeds for 30 min. Coarse hot 'oil in water emulsion' so obtained was subjected to further size reduction using ultrasonic Probe sonicator for 10-30 min..[17]

Optimization

In simultaneous optimization, the experimentation is completed before the optimization takes place. In simultaneous methods, usually called as response surface methodology (RSM), one or more selected experimental responses are recorded for a set of experiments, carried out in a systematic way, to predict the optimum and the interaction effects124-127. In this study, a Box- Behnken design was introduced to optimize the formulation of solid lipid nanoparticles. Initial studies were undertaken to decide the excipients and their levels in the experimental design. The choice of lipid was done on the basis of solubility and partitioning of Amiodarone in the lipid. Aqueous phase surfactant and lipid phase surfactant were selected on the basis of stability of dispersion prepared by using different surfactants. Three factors, the drug: lipid ratio (X1), concentration of Span 80 (lipid phase surfactant) (X2) and sonication time (X3) were used in the design and the responses were the average particle size (PS) (Y1) and % Entrapment Efficiency (EE) (Y2). These three factors that might affect the designed characteristic of nanoparticle formulation were varied over three levels (Table 5.6) and arranged according to a Box-Behnken[18] .

Drug Entrapment Efficiency (DEE)

The entrapment efficiency of prepared SLN was calculated by centrifugation method123. About 2 ml of dispersion of SLN and 5 ml of methanol was taken in centrifuge tube and further it was centrifuged at 13,000 rpm for 1 hour. After

centrifugation the supernatant was removed and diluted with appropriate solvent. The concentration of drug (free drug) in supernatant layer was determined by using UV visible spectrophotometer..[15].

$$DEE\ (\%) = \frac{\text{Actual amount of drug encapsulated x100}}{\text{Theoretical drug content}}$$

In vitro dissolution:

The in vitro drug release from optimized batch of drug loaded SLN and drug suspension was performed in phosphate buffer pH 4.2 buffer using the dialysis bag method23, 123. Phosphate buffer pH 4.2 was prepared as per USP. Dialysis membrane having molecular weight cut off 12,000–14,000 Da was used. Membrane was soaked in double- distilled water for 24 h before use. Two milliliters of dispersion was poured into the bag with the two ends fixed by clamps. The bags were placed in a conical flask and 50 ml receiving phase was added. The conical flasks were placed into a thermostatic shaker at 37 °C at a rate of 140 times per min. At 0.5, 1, 2, 4, 6, 8, 12 and 24 h, 5 ml aliquots were removed and were replaced with fresh dialysis medium. Samples were analyzed by using UV-Visible spectrophotometer. All the operations were carried out in triplicate[16-17].

Fourier transforms infra-red spectroscpy (FT-IR)

The FT-IR spectrum were measured at 4000 cm-1 to 500 cm-1 using BRUKER-FTIR spectrophotometer. Small amount of finely ground solid samples under the study were added to 100 times of its weight of KBr and compressed using hydraulic press to get a thin transparent pellet. These pellets are transferred to FT-IR instrument to determine the spectra.

Particle size

The mean particle size and polydispersity index of the optimized batch of drug loaded SLN was measured using particle size analyzer based on the dynamic light scattering method. The SLN formulations were dispersed in distilled water at appropriate concentrations. All measurements were performed in triplicate. The polydispersity index (PI) indicates the width of the size distribution..[19]

Scanning Electron Microscpy (SEM)

The surface of optimized mucoadhesive microspheres was analysed by SEM. Mucoadhesive microspheres were adhered on aluminium studs and coated with gold using a sputter coater SC 502, using vacuum (0.1 mm Hg) and then analysed by SEM.[17].

Zeta potential

The zeta potential of optimized batch of drug loaded SLN was measured by zeta potential analyzer based on the Laser Doppler Micro-electrophoresis. An electric field was applied to the dispersion of particles, which then move with a velocity related to their zeta potential. This velocity was measured using laser interferometric technique which enables the calculation of electrophoretic mobility, and from this, the zeta potential.

Field emission scanning electron microscopy (FE-SEM)

The surface morphology of optimized SLN formulation was observed using field emission scanning electron microscope at 25 ± 2°C. The SLN dispersion was placed on aluminum foil and air dried for 24 h. Further, it was analyzed at 3000× magnification with accelerating voltage of 10 kV.

RESULTS & DISCUSSION:

Table 1 Particle size and entrapment efficiency of Amiodarone loaded SLN (R_1- R_{13}) as per Box Behnken design

nulation code	icle size (nm)	Entrapment Efficiency
R_1	590	88.3
R_2	346	88.8
R_3	680	88.7
R_4	589	85
R_5	375	83.8
R_6	699	87.7
R_7	433	83.5
R_8	510	83
R_9	285	84.5
R_{10}	451	84.5
R_{11}	422	85.5
R_{12}	264	81.5
R_{13}	427	83

The cellular uptake of drug-loaded SLN by absorptive enterocytes is influenced by particle size. Particle size increased with the increasing lipid concentration which may be attributed to higher viscosity of dispersion medium at higher lipid concentrations, leading to increased particle size with wide size distributions. Moreover, the surfactants cannot completely cover the surface of the SLN, at higher lipid content, resulting into an increase in particle agglomeration.

The increase in the sonication time resulted in an appreciable decrease in the particle size. The reduction of particle size is attributed to the development of cavitational forces during the sonication step, resulting in reduction of lipid droplets to nano meter size with enhanced surface area.

Optimization and validation

The criteria for selection of optimized formulation were primarily based upon the highest possible values of % EE (>85%) and values of particle size which are less than 250 nm. Amongst several solutions obtained using numerical optimization tool of the Design Expert software, three formulations (D1, D2, and D3) with the highest desirability value were prepared. The selected factor combinations with desired responses were selected and actually prepared. Table 6.23 compares the predicted and experimental values. The low value of percentage error underlines prognostic ability of selected design.

Table 2 Comparison of experimental results with predicted responses

Batch Code	Composition X_1/ X_2/ X_3	Response	redicted value	perimental value	Percent error
D1	**0.96/ 1/0.96**	PS (nm)	241	248	0.0061
		EE (%)	88.0	87.2	0.0226
D2	**1/ 1/ 1**	PS (nm)	231	233	-0.0165
		EE (%)	88.0	87.4	0.0085
D3	**0.98 / 1/ 1**	PS (nm)	236	242	0.0130
		EE (%)	88.0	86.3	0.0071

The optimized formulation has particle size of 233 ±3 nm and entrapment efficiency of 87.4 ± 1.29%, which were in good agreement with the predicted values. Particle size distribution curve of optimized batch of Amiodarone-SLN.

In vitro drug release study

In vitro drug release study was performed to ensure the retarded release of drug in the upper gastrointestinal tract so as to minimize the potential passive absorption of premature released Amiodarone from SLN before the cellular uptake of SLN by Peyer's patches takes place. The results of release studies are represented graphically as cumulative % drug release-time (h). In present investigation Amiodarone loaded SLN dispersion of optimized formulation showed significantly low release of Amiodarone (38.6% in 24 h) than dispersion of pure drug (89.3 % in 24 h). This is due to the entrapment of drug in the lipid matrix of SLN & the erosion of lipid has to take place for drug to be released.

Fourier transform infra-red spectroscopy

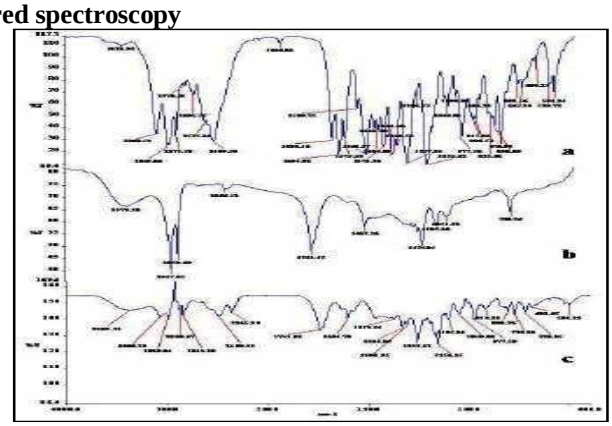

Figure 1 FTIR spectra of (a) Amiodarone, (b) GMS and (c) physical mixture of Amiodarone and GMS

Zeta potential

Laser Doppler Micro-electrophoresis was used to measure zeta potential. Zeta potential is a key factor for evaluation of the stability of colloidal dispersion. Research has revealed that zeta potentials above ±30 mV offer full electrostatic stabilization148. The value of zeta potential of optimized formulation of Amiodarone-SLN was found to be +75.9 mV which is sufficient to keep the particles stable.

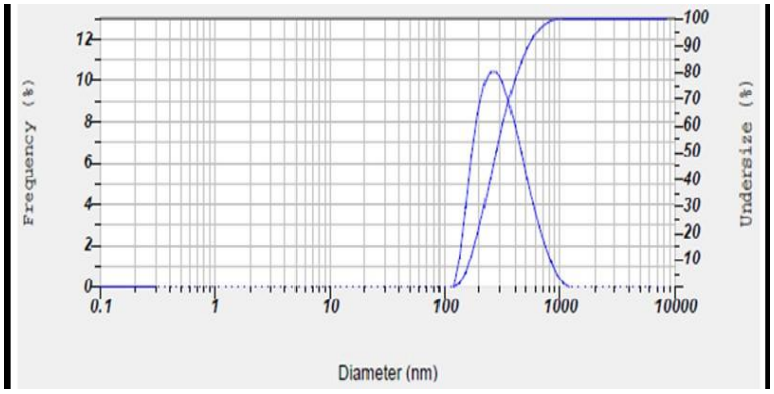

Figure 2 Particle size distribution curve of optimized batch of Amiodarone loaded SLN

Field emission scanning electron microscopy

As spheres are symmetric in all three dimensions, spherical particles can be more easily transported across the epithelia than ellipsoidal particles. SEM image revealed distinct and mono-dispersed SLN with spherical shape (Figure 6.28). Due to lyophilization, although the particles are present as agglomerates, the size ranged between 150–300 nm with a smooth surface.

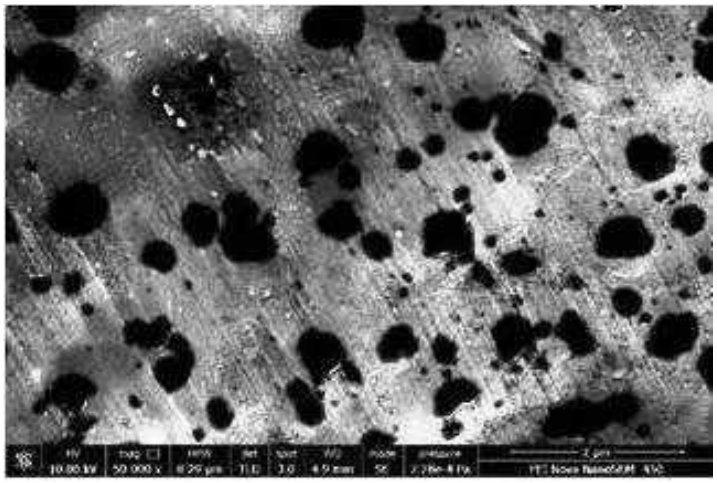

Figure 3 SEM image of optimized batch Amiodarone loaded SLN

CONCLUSION:

From the findings of the experimental work carried out following final conclusions can be drawn

•SLN could be looked upon as new patient friendly nanolipid formulation for oral delivery of the selected drugs.

•Amiodarone loaded SLN were an effective approach in improving its oral bioavailability and may prove valuable in treatment of arrhythmia.

•SLN could be an effective drug delivery system for the treatment of psychiatric conditions like schizophrenia via oral route.

•Reduction in the cost is possible due to better bioavailability and reduction of dose.

•Conclusively extending the concept- intestinal lymphatic uptake using novel lipid based drug nanocarrier will improve the bioavailability of drugs.

•The presence of lipid in the formulation appears to have a potential in the development of perorally administered drug products.

•Development of oral lipid based nanocarriers of drugs undergoing extensive pre- systemic hepatic metabolism, can be a novel, cost effective, industrially scalable and effective alternative to the conventional oral dosage forms available in the market.

•This approach will open the doors to oral delivery of BCS class II drugs as well as improve the therapeutic efficacy of drugs with poor brain permeation.

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Conflict of Interest
The authors declare no conflict of interest, financial or otherwise.

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