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Developing the solid lipid nanoparticles of Losartan using the stearic acid and glyceryl monostearate as the carrier matrices

Divya R.
Department of Pharmaceutics SRM College of Pharmacy SRM Institute of Science and Technology, Kattankulathur, India

Remya P. N.
Department of Pharmaceutics SRM College of Pharmacy SRM Institute of Science and Technology, Kattankulathur, India
*Corresponding author email: remyamathavan@gmail.com

N. Damodharan
Department of Pharmaceutics SRM College of Pharmacy SRM Institute of Science and Technology, Kattankulathur, India

***Abstract*---**This research work deals with the design and development of the solid lipid nanoparticles of Losartan to improve solubility of poorly soluble drug. Losartan solid lipid nanoparticles are formed using hot homogenization technique followed by ultrasonication technique. Experiment trend is predicted using the standard calibration curve. The parameters studied are the percent drug release, entrapment efficiency, particle size of SLN and the in-vitro drug release of prepared SLN. The results obtained in this research work clearly indicated that the developed solid lipid nanoparticle delivery system for the highly lipophilic drug, Losartan using stearic acid, glyceryl monostearate as carrier matrices can be used effectively for hypertension.

***Keywords*---**entrapment efficiency, hot homogenization technique, hypertension, losartan, invitro drug release, scanning electron microscopy (SEM), solid lipid nanoparticles (SLN), ultrasonication technique.

Introduction

Losartan drug is used to treat hypertension. Its poor solubility in water is reducing its efficiency in curing the hypertension. Design and development of

solid lipid nanoparticles (SLNs) is an effective technique to safely carry the drugs. SLNs will act as carriers for the loaded drugs. Loading the poor soluble drugs in the SLNs improved the efficiency of the drugs[1][2]. Drug efficiency improvement studies can be handled by considering the various critical factors and weak points in solid lipid nanoparticle formulations[3]. The cutting-edge technologies for the solid lipid nanoparticles, incorporation, loading and the drug release mechanisms are proposed by various researchers[4]. High-pressure homogenization[5], Micro-emulsion technique[6], Lipid nano-pellets[7] and Lipospheres[8], Precipitated lipid particles[9] are some of the states of the art available techniques for the preparation of lipid particles.

Incorporation of various drugs in solid lipid nanoparticles along their loading capacity are studied by[10][11] and they proposed the optimum parameters for the loading capacity of the drug in solid lipid nanoparticles (SLN). Studies on the mechanism of drug release[12][13] from SLN are useful in improving the releasing rate of the drug. Analytical characterization of an SLN is a challenging task because of its nano size particles and the complex nature of the system. Stability and release kinetics of the drug is controlled by various parameters, like the co-existence of the colloidal structures, crystallinity, lipid modification, size of the particle and the zeta potential[14]. The recent studies on the preparation of SLNs[15] motivated the present investigation of preparing and evaluating the performance of the Losartan loaded solid lipid nanoparticles. The results of this study are useful in preparing efficient Losartan drug which cures hypertension in an effective way.

Materials and Methods

Materials

Equipment used in formulation development are Weighing balance (AY-220), Ultrasonic processor, U.V. Visible spectrophotometer, Dissolution Test Apparatus 8000, Water bath, Refrigerator, and Centrifuge. Materials used in the formulation are, Losartan, Polysorbate 20, Polysorbate 40, Polysorbate 80, Glyceryl monostearate and Soya lecithin.

Procedure for the preparation of standard curve for Losartan

Losartan of 100 mg was transferred to a 100ml volumetric flask, dissolved using methanol as solvent and volume is made up to 100ml to get the stock solution of 1000µg/ml. Then 1ml was pipetted out from the stock solution I and the volume were made up to 10ml with methanol. This gives the stock solution II (100µg/ml). Then a volume of 0.5,1.0,1.5,2.0 and 2.5 ml was pipetted into 5 separate 10ml volumetric flasks from the stock solution II, then make up the volume to the mark to give 5,10,15,20 and 25µg/ml concentration solution and taking methanol as the blank. The absorbance was measured exactly at 234 nm and then the graph was plotted against concentration Vs absorbance.

Preparation of Solid lipid nanoparticles (SLNs)

The SLNs has been prepared by the hot homogenization technique which was then followed by the ultra-sonication technique[16]. Briefly, lipid (steric acid/Glycerol monostearate) was melted in a boiling tube by heating. Then lecithin (soya lecithin/ egg; lecithin) was added. Then the drug Losartan was incorporated into this lipid lecithin melt. Then it was maintained at 5^0C which is above the melting point of lipids. Simultaneously, polysorbate 80/ polysorbate 40/polysorbate20 was dissolved in distilled water in another beaker. Then it was heated to the temperature equal to that of the lipid phase. Later the aqueous phase was transferred to the lipid phase. The product was homogenized at 20,000 rpm for 15 minutes. Then it was placed in a Probe Ultrasonicator at the 75% amplitude for 25 minutes. Formulation data for solid lipid nanoparticles are given in Table 1.

Table 1
Formulation of solid lipid nanoparticles

	F1	F2	F3	F4	F5	F6
Drug	20	40	20	40	20	40
Soya lecithin	10	10	10	10	10	10
Glyceryl monostearate	-	-	-	10	10	10
Stearic acid	10	10	10	-	-	-
Tween 80	10	20	-	-	-	-
Tween 40	-	-	10	20	-	-
Tween 20	-	-	-	-	10	20
Distilled water	80	80	80	80	80	80

Evaluation of Losartan SLNs

The prepared Losartan solid lipid nanoparticles were analysed for surface morphology and the size distribution, entrapment efficiency, in-vitro drug release and the zeta potential[17][18].

Morphology

SEM was used to examine the shape and surface morphology (i.e., smoothness, roundness, aggregation formation, and size distribution) of solid lipid nanoparticles loaded with Losartan (SEM). The samples of SLNs for SEM study were sprinkled on the double adhesive tape which was affixed on aluminium stub. The aluminium stub was placed in the high-vacuum chamber of an SEM.

Measuring the Entrapment efficiency (EE) of the drug

To determine the entrapment efficiency of the drug the free drug content was obtained after centrifuging the solid lipid nanoparticles sample in the high-speed centrifuge at 15000 rpm for a time period of 30 min using the Remi centrifuge (Mumbai, India). The entrapment efficiency was given by equation (1).

$$\text{Entrapment efficiency (\%)} = \frac{Total\ Losartan\ drug\ content - free\ Losartan\ drug\ content}{Total\ Losartan\ drug\ content} \times 100 \quad \text{----(1)}$$

In-vitro drug release

The in vitro drug release studies of Losartan were conducted for the all six solid lipid nanoparticle dispersions using the dialysis bag diffusion technique. The sample of Losartan loaded solid lipid nanoparticle dispersions having 2.5 mg of Losartan was used for the test. The sample were transferred in the dialysis bag and then the sample was sealed in the bag. This bag was completely immersed in a beaker having the 900 ml of phosphate buffer with pH of 6.8. The sample was subjected to stirring at a constant speed of 50 rpm at 37^0C. An aliquot part of 1 ml sample was periodically withdrawn up to 6 hours and analysed for the drug content. For the analyzation of drug content UV-vis spectrophotometer was used at 234 nm.

Zeta potential

The zeta potential for each sample of solid lipid nanoparticle loaded with Losartan was measured using the Zeta-sizer. To safeguard the light intensity of each sample in the range of the sensitivity of the instrument, the sample was then diluted to 1:9 v/v with distilled water.**3.**

Results and Discussion

Standard curve of Losartan

The experimental system behaviour was predicted by plotting the calibration curve of Losartan drug. The standard curve of Losartan is shown in Figure 1. The R^2 value from the plot was 0.99417 and the intercept was 0.3334. This curve was useful to simulate the experimental values of the present system.

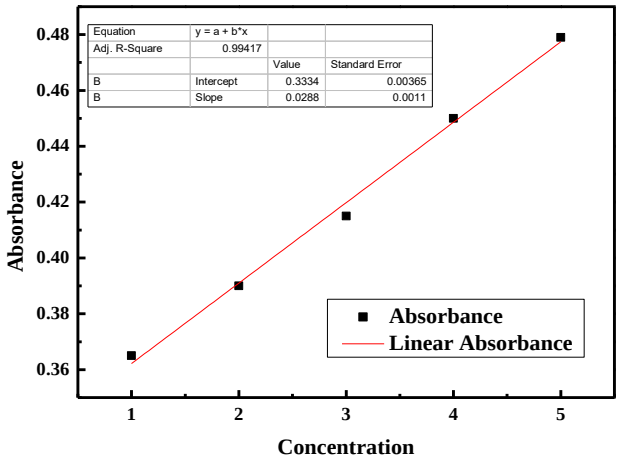

Figure 1. Calibration curve of Losartan

Morphology

Scanning electron microscopy diagrams for the prepared formulations is given in Figure 2. It was observed that the surface of the solid lipid nanoparticles loaded with Losartan drug was rough due to the higher percentage of the drug uniformly distributed on the carrying matrices.

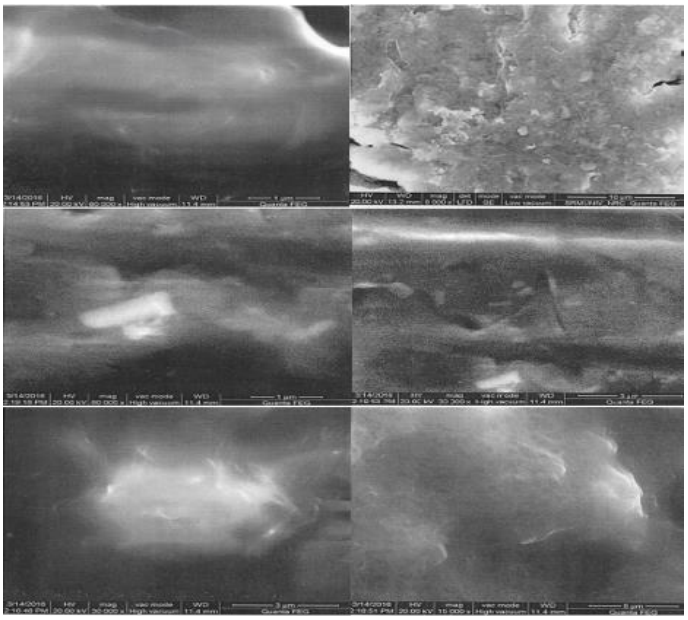

Figure 2. Scanning electron microscopy of Losartan solid lipid nano particles F1 (Containing Tween 80 and drug concentration- 20 mg); F2 (Containing tween 80 and drug concentration- 40 mg); F3 (Containing tween 40 and drug concentration- 20 mg); F4 (Containing tween 40 and drug concentration- 40 mg); F5 (Containing tween 40 and drug concentration- 20 mg); F6 (Containing tween 20 and drug concentration- 40 mg)

In-vitro drug release studies

The invitro release of Losartan for the dispersion of solid lipid nanoparticles was given in table 2. It was found that the invitro release of the drug Losartan was in the range of 60.05% to 78.51% at the end of 3 hours.

Table 2
In-vitro release profile of Losartan for different batches loaded with tween 80, tween 40 and tween 20

Time in minutes	Formulation					
	Tween 80		Tween 40		Tween 20	
	F1	F2	F3	F4	F5	F6
15	37.20	35.12	32.37	40.52	26.02	30.01
30	43.20	37.44	35.21	41.25	33.89	37.72
45	46.45	40.60	40.11	43.51	38.33	42.41

60	55.13	45.21	47.05	46.35	44.68	53.06
90	60.12	55.22	51.45	52.55	47.14	58.39
120	73.33	65.89	57.16	68.24	51.92	63.11
180	78.51	72.12	60.05	71.56	69.34	72.88

Influence of surfactants on in-vitro drug release

Figure 3 gives the in-vitro release profile of Losartan for different batches loaded with tween 80. The formulation F1, F2 were prepared using steric acid as a lipid matrix with tween 80 as a stabilizer. For F1, F2 formulations the maximum drug release from Losartan - loaded SLN was 78.51% and 72.12% respectively. Figure 4 gives the in-vitro release profile of Losartan for different batches loaded with tween 40. The formulations were F3 and F4 and the maximum drug release from these formulations were 60.05% and 71.56%. Similarly for the formulations of F5 and F6 from Figure 5 it was observed that the maximum drug release was 69.34% and 72.88%. In summary the formulations F1 and F2 were shown the maximum drug release.

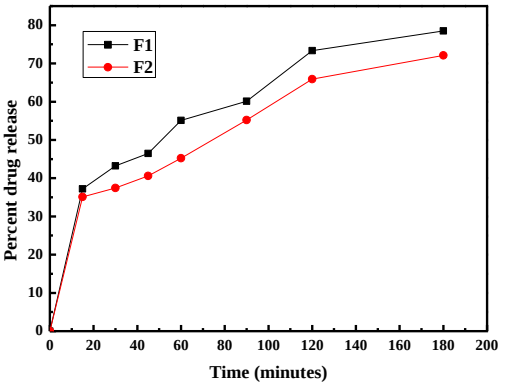

Figure 3. In-vitro release profile for tween 80 batches (F1 and F2)

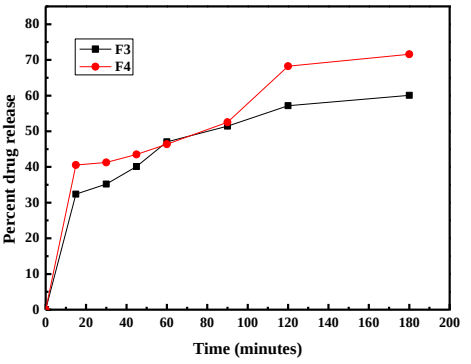

Figure 4. In-vitro release profile for tween 40 batches (F3 and F4)

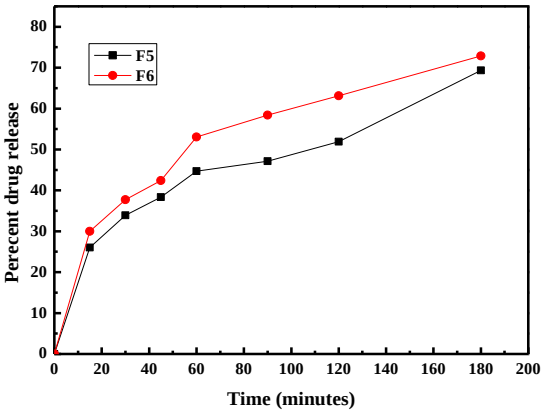

Figure 5. In-vitro release profile for tween 20 batches (F5 and F6)

Entrapment efficiency

The entrapment efficiency values for the formulations were given in the table 3. The overall entrapment efficiencies were found to be in the range of 76.2% to 87.5%.

Effect of surfactants on entrapment efficiency

In the table 3 the entrapment efficiencies of all the surfactants were given. It was observed that the entrapment efficiencies for the formulations with higher surfactant concentrations were higher for F2 was 78%, for F4 was 72%, and for F6 was 87.5%. In summary, increasing the surfactant content enhanced the entrapment efficiency. It might be because raising the concentration of the surfactant causes an increase in the solubility of the medication in the lipid.

Table 3
Entrapment efficiency of the Losartan drug

Formulation Code	Drug Concentration (mg)	%Entrapment Efficiency
F1	20	76.2
F2	40	78
F3	20	67
F4	40	72
F5	20	80
F6	40	87.5

Stability studies

Stability of the formulated SLNs loaded with Losartan was determined using the zeta potential of the sample. Zeta potential values for the samples were given in Figure 6 and in Figure 7.

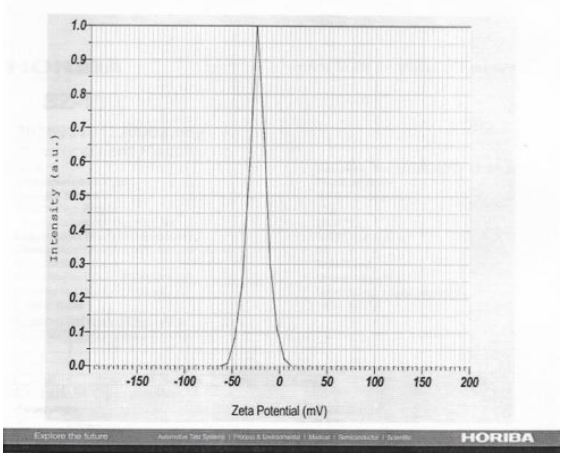

Figure 6. Zeta potential graph for the sample

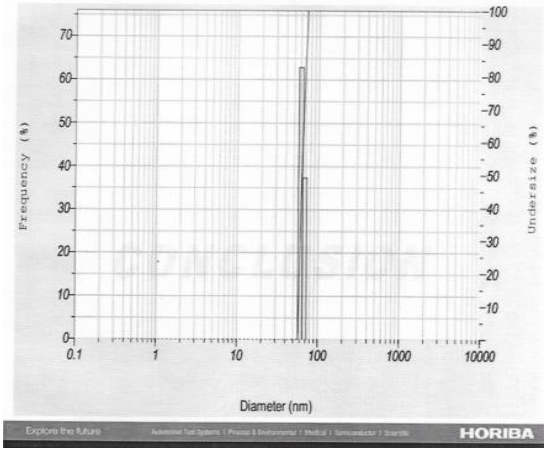

Figure 7. Zeta potential graph for the sample

Conclusion

In this study, the solid lipid nanoparticles delivery system was developed for the highly lipophilic drug, Losartan using stearic acid, glyceryl monostearate as carrier matrices which is meant to be intended for hypertension in an effective means. SLNs produced by using hot homogenization technique and ultra-sonication technique produced nanoparticles of acceptable range. All of the formulation had extremely showed very high entrapment efficiencies. Among the different batches of formulations, F1 and F2 were selected as the optimized formulation. The produced particles were nanosized and had a negative surface charge, according to particle size analyses. Based on the findings, it is possible to

infer that the Losartan lipid nanoparticulate delivery system developed using generally acknowledged and physiologically safe lipids was capable of demonstrating sustained release qualities.

As a result, by selecting a good lipid carrier, Losartan-loaded nanoparticles may be able to sustain blood pressure for the course of the study (12 hours). The findings can be ascribed to the efficacy of formed Losartan nanoparticles in treating hypertension, as well as their involvement in sustaining therapeutic levels over time. These findings might also be explained by the fact that new carriers, such as lipids, could entrap lipophilic medications like Losartan, improving its solubility, absorption, and allowing it to stay in the systemic circulation for a longer period of time.

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Conflict of interest statement

The authors declared no conflict of interest.

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