Formulation Development, *In Vitro* And *In Vivo* Studies of Eprosartan Mesylate Loaded Solid Lipid Nanoparticles for Improved Bioavailability

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Abstract*— Eprosartan mesylate (EM) is an angiotensin receptor blocker (ARB) used to treat hypertension. It has an oral bioavailability of 13%. The work intends to evolve eprosartan mesylate of solid lipid nanoparticles (EM-SLNs) to enhance bioavailability. EM-SLNs were developed using hot homogenization followed by ultrasonication by using lipids dynasan 118, dynasan 116, and dynasan 114. The optimized formulation EM-SLNs (F7) showed the particle size is 151.3 nm, poly disperse index (PDI) shows 0.210 and zeta potential (ZP) values were -31.74mV, which indicated the stability of developed EM-SLNs. The entrapment efficiency (EE) was found to be 85.10%. EM-SLNs were established as likely spherical with a lustrous exterior, as making do with scanning electron microscope (SEM). Relative bioavailability of the optimized EM-SLNs (F7) was increased by 1.84 times, differentiated with the coarse suspension of pure drug.

Indexed Terms- Bioavailability, solid lipid nanoparticles, homogenization and eprosartan mesylate

I. INTRODUCTION

Hypertension (HTN) is a growing global problem associated with numerous underlying pathophysiological conditions. Eprosartan mesylate (EM) is used to treat high blood pressure (HTN). This drug works by relaxing blood vessels so blood can flow more easily. It belongs to a class of drugs called angiotensin receptor blockers (ARB).[1]

Solid lipid nanoparticles (SLNs) are about their nano size, higher surface area and extensive drug loading proportions. Similarly, it promotes drug consistency and can potentially enhance the oral bioavailability of water-insoluble drugs.[2]

The key objective of the existent research work was to develop the EM-SLNs, to avoid the pre-systemic metabolism and to enhance the oral bioavailability of EM. It has a low oral bioavailability of only 13%. It is a biopharmaceutical classification system (BCS) class II drug with a partition coefficient of 3.9 and a half-life of 5 to 9 hrs.[3] These characteristic properties of EM make it an appropriate candidate for the design and development of SLN formulation for the management of hypertension.

II. MATERIALS AND METHODS

2.1. Materials

Eprosartan mesylate was acquired as a gift sample from Sai Ram Organics Pvt Ltd, Hyderabad, India. Dynasan 114, Dynasan 116 and Dynasan 118 were procured from Hi Media Labs, Mumbai. Lipoid E80 was procured from Lipoid, Germany respectively. Pluronic F 68 was a gift sample from Aurobindo Labs, Hyderabad, India. Methanol and chloroform were of HPLC grade Merck, Mumbai.

2.2. Methods

2.2.1. Formulation development of EM-SLNs

The EM-SLNs were formulated by using homogenization fallowed by ultrasonication technique. EM (API), solid lipid and lipoid E80 were liquefied in a 15 mL combination of chloroform and methanol (1:1). The organic solvents were separated by a rota evaporator (Heidolph, Germany) Drug encapsulated lipid covering layer was molten by the heating system at 5°C over the melting point of lipid used in the formulation. The aqueous phase was developed by using surfactant pluronic F 68. This surfactant dissolves in double distilled water and is heated to a similar temperature to the oil phase. The hot aqueous phase was added to the oil phase, and submitted to the homogenization cycle process was conducted (at 12,000 rpm) by using a homogenizer for 5min. Coarse heated oil in water (O/W) emulsion

attained was ultrasonicated for 10 min. The SLNs were developed by permitting heated nanoemulsion to cool to room temperature.[4] The diverse formulation components are presented in Table 1.

Table 1: Formulation components of EM-SLNs

Formulation Ingredients (mg)	Formulation codes								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Organic phase									
Eprosartan mesylate	10	10	10	10	10	10	10	10	10
Dynasan 118	50	100	150	-	-	-	-	-	-
Dynasan 116	-	-	-	50	100	150	-	-	-
Dynasan 114	-	-	-	-	-	-	50	100	150
Lipoid E 80	1000	1000	1000	1000	1000	1000	1000	1000	1000
Chloroform: Methanol (1:1) (mL)	15	15	15	15	15	15	15	15	15
Aqueous phase									
Pluronic F 68	1000	1000	1000	1000	1000	1000	1000	1000	1000
Double distilled water (mL)	10	10	10	10	10	10	10	10	10

2.3 Characterizations of EM-SLNs

A) Determination of particle size, PDI and ZP

The mean size of particles and ZP of EM-SLNs were measured by employing a Malvern Zetasizer (Nano ZS90). The developed EM-SLNs were 100µl diluted to 5mL with the help of double distilled water to achieve ideal kilo counts per second (KCPS) of 50-200 for measurements.[5]

B) Surface morphology studies by SEM studies

The surface morphology of SLN formulations was ascertained by SEM (Hitachi200, Japan) under a high vacuum of 40kv. In the earlier examination, the formulated samples were fixed on a double adhesive carbon tape, which was stuck on aluminium stubs and then covered with gold beneath an argon atmosphere.[6]

C) Determination of drug content

About 0.1 ml of EM-SLNs preparation was collected and diluted pertinent with chloroform: methanol (1:1) and then the drug content of diluted samples was examined by HPLC.

D) Determination of entrapment efficiency (EE)

The EE of developed formulations was certainty by assessing the concentration of free drug (unentrapped) in the aqueous phase. The aqueous phase was partitioned by ultrafiltration utilising centrisort tubes which are composed of a filter membrane (MWCO 20kDa) at the base of the test sample regeneration chamber, was placed on the topmost of the sample and centrifuged around at 4000 rpm for the time of 30 min. The developed EM-SLNs assisting with the encasing drug stay on in the exterior chamber and the aqueous phase is transferred into the sample recovery chamber with a filter membrane. The appropriate amount of EM in the aqueous solution phase was measured by HPLC.[7]

E) *In vitro* studies of EM-SLNs

The release studies were executed using a dialysis membrane having an adequate pore size of 2.4nm and it was immersed overnight in double distilled water. The drug release of formulations was carried out successively for 1st 2 hrs in 0.1N HCl, subsequently by using pH6.8 in phosphate buffer for the remaining 22 hrs by open tube approach. In which dialysis membrane was fixed to an open tube (SLNs dispersion) as the donor compartment and buffer (100mL) containing 200mL beaker as receptor compartment and the temperature was maintained at 37±0.5°C, 2mL sample was withdrawn at the different time interval sampling points up to 24 hrs and replaced with an equal proportion of fresh buffer. The samples were collected, and examined by UV-visible spectrophotometer at λmax 233 nm, to find out the extent of the drug released.[8]

2.4 Pharmacokinetic study

Animals

Healthy male Wistar rats (weighing 200-230gm) were exploited for the pharmacokinetic study of the developed formulation. The animals were starved overnight and had available access to water. The animal studies were carried out with earlier acceptance by the Institutional Animal Ethical Committee (IAEC).

Study protocol

The animals were separate into 2 groups (n=6) and were orally administered with a developed formulation of EM-SLNs (F7) and coarse suspension of a pure drug at a dose level of 10mg/kg body weight. All the formulations were administered orally with the rat oral feeding tube. At suitable specified time intervals after oral administration, blood samples were collected at different time interval points by retro-orbital venous plexus puncture method. The blood samples were permitted to clot and centrifuged for 10 min at 12000 rpm. The serum was isolated and conveyed into clean micro centrifuge tubes and stored at -20°C until HPLC studies.[9]

Calculation of pharmacokinetic parameters

The concentration of EM-SLNs in rat serum samples was attained from the calibration curve prepared. The pharmacokinetic parameters like C_{max}, T_{max}, AUC and $t_{1/2}$ were measured by Kinetica (2000) software.[10] The relative bioavailability was determined by the following equation; % Relative BA= (AUC_{SLN} X Dose control / AUC control X Dose ^{SLN}) X100.

III. RESULTS AND DISCUSSION

3.1. Determination of particle size, PDI, ZP, drug content and EE of EM-SLNs (n=3)

All the developed formulations were studied in sequence to influence their particle size distribution, ZP, PDI, drug content and EE. The expected outcomes are stated in below Table 2.

Table 2: Size, PDI, ZP, drug content and EE of EM-SLNs

Formulations	Size (nm)	PDI ± SD	ZP(mv) ± SD	Drug content (mg) ± SD	EE (%) ± SD
F1	194.3 ±2.49	0.216 ±0.03	-30.43± 2.42	87.79± 2.01	81.75 ±1.05
F2	211.4 ±3.15	0.243 ±0.05	-32.51± 2.26	90.61± 2.11	83.64 ±1.94
F3	226.9 ±2.71	0.258 ±0.07	-33.16± 2.45	94.01± 3.00	84.17 ±2.01
F4	172.2 ±3.17	0.198 ±0.11	-28.91± 2.74	90.06± 1.10	79.51 ±1.75
F5	188.4 ±2.59	0.205 ±0.04	-29.25± 3.28	92.41± 2.05	80.75 ±2.13
F6	203.9 ±2.48	0.213 ±0.06	-32.40± 2.79	94.42± 2.10	81.49 ±1.97
F7	151.3 ±2.60	0.210 ±0.08	-31.74± 2.65	96.16± 2.17	85.10 ±1.58
F8	164.2 ±3.08	0.221 ±0.02	-30.92± 3.86	100.01 ±2.06	86.09 ±1.42
F9	187.5 ±2.51	0.216 ±0.07	-32.57± 2.72	108.02 ±0.20	86.75 ±2.35

The ZP of formulations was in the extent of -33.16 to -28.91mV; particle sizes ranged from 151 to 226nm and PDI was within the permissible range (below 0.3) respectively. EE was estimated to be 79 to 86%. Drug content was determined. The particle size of EM-SLNs depends on the alkyl chain extent of lipids, the higher the alkyl chain length higher the particle size. The particles having smaller particle sizes have more stability. The surfactant pluronic F68 lowered the electrostatic repulsion among the particles following sterical stabilization of the nanoparticles by producing a coat over their surface for maintaining stability.[11]

3.3 SEM studies of EM-SLNs

The external morphology of optimized formulation of EM-SLNs (F7) outcomes presented that the particles were circular with a constricted size allocation and had flat uniform surfaces. The samples were analyzed in SEM under a high vacuum of 20kV.[12] There is no agglomeration of SLNs existence in the sample and the SEM photograph is shown in Fig 1.

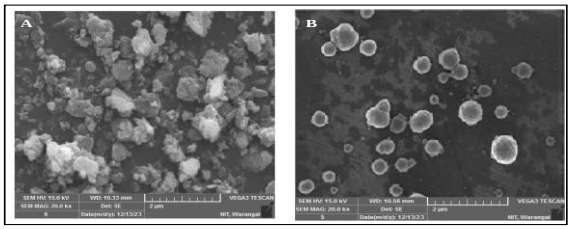

Fig 1: SEM image of a pure drug (A) and EM-SLNs F7 (B)

3.4 *In vitro* release studies of EM-SLNs

The formulations containing dynasan 118, dynasan 116, and dynasan 114 exhibited drug release extending from 69.31 to 79.16%, 71.05 to 83.26%, and 81.73 to 92.86% respectively for 24 h (Fig 2). The developed formulation F7 showed higher drug release was determined to be 92.86% and release of drug in a sustained manner. The lipid content in formulations gradually; the percentage of drug release were decreased. The release pattern of all formulations presented a conventional biphasic arrangement with a primary expeditive phase accompanied by a slow phase release pattern observed in phosphate buffer. The primary rapid phase can be because of the burst release of a drug.[13]

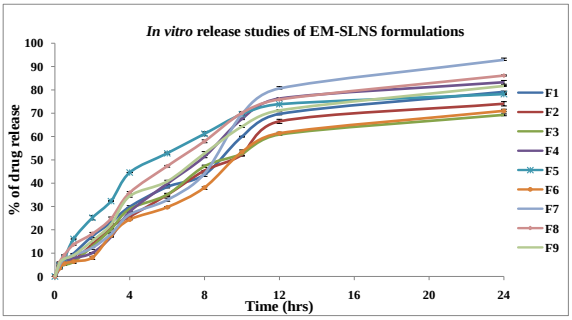

Fig 2: Drug release profile of EM-SLNs formulations (n=3)

3.5: Stability studies

The EM-SLNs were preserved at room and refrigerated temperatures for 90 days and the characteristic size of particles, ZP and PDI were studied and results are shown in Table 3. There was no considerable difference found during stability studies.[14]

Table 3: Stability studies of formulation EM-SLNs (F7)

Day	At room temperature (25°C) (n=3)			At refrigerated temperature (4°C) (n=3)		
	Size (nm)	PDI	ZP (mV)	Size (nm)	PDI	ZP (mV)
1	151.3 ±2.60	0.210 ±0.08	-31.74 ±2.65	151.9 ±3.04	0.211 ±0.06	-32.18± 1.84
30	157.9 ±3.14	0.219 ±0.10	-32.59 ±2.74	158.7 ±2.85	0.220 ±0.08	-33.52± 2.81
60	160.5 ±2.46	0.223 ±0.07	-33.07 ±2.18	161.9 ±3.12	0.224 ±0.04	-34.06± 2.29
90	163.1 ±3.06	0.228 ±0.12	-33.95 ±2.56	164.5 ±4.16	0.229 ±0.07	-35.21± 2.73

3.6: Pharmacokinetic study

The pharmacokinetic parameters were determined by non-compartmental conceptions by using Kinetica 2000 software. The parameters like AUC, Cmax, Tmax, MRT and $t_{½}$ were attained for optimized EM-SLNs (F7) and differentiated with that of coarse suspension. The results of pharmacokinetic parameters are calculated and stated in Table 4 and Fig 3. The bioavailability of EM-SLNs (F7) is relatively higher than that of the coarse suspension. This is due to enhancing the surface area of the drug available for release, which would increase the solubility of the drug.[15] When compared, to administering the EM-SLNs and coarse suspension, there were 1.84-fold increases in relative bioavailability to the coarse suspension.

Table 4: Pharmacokinetic studies of EM-SLNs and coarse suspension

Parameters	Optimized EM-SLNs (F7)	Coarse suspension (EM)
C_{max}(µg/mL)	259.15±9.12***	197.93±8.51
T_{max} (hr)	2	2
AUC(µg/mL).h	2701.74±30.26**	1468.34±27.14
$T_{1/2}$(hr)	19.38±2.54**	7.13±2.46
MRT	23.69±2.81**	9.78±2.48

***significance, **less significance

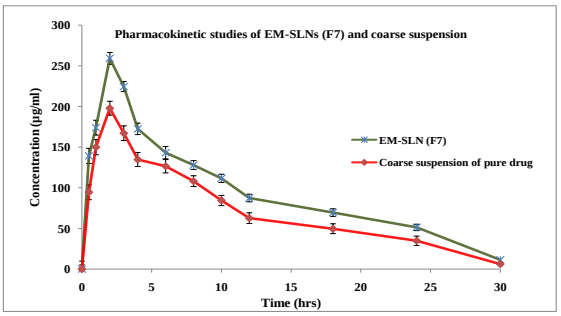

Fig 3: Pharmacokinetic profile of EM-SLNs (F7) and coarse suspension

CONCLUSION

The study of this work that SLN carriers may help enhance the bioavailability of EM for the treatment of hypertension. The poor absorption of EM was enhanced by formulating into EM-SLNs with the use of lipids and further examined *in vitro* and *in vivo* studies. The optimized EM-SLNs (F7) showed good ZP, PDI, particle size and drug release was found to be 92.86% in 24 hrs and exhibited a prolonged manner release pattern. In pharmacokinetic studies, the EM-SLNs (F7) showed a 1.84-fold increase in relative bioavailability compared to the coarse suspension in Wistar rats.

REFERENCES

[1] Delie F, Blanco-Príeto MJ. Polymeric particulates to improve oral bioavailability of peptide drugs. *Molecules*. 2005; 10:65–80.

[2] Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: A modern formulation approach in drug delivery system. *Indian J Pharm Sci*. 2009; 71:349–58.

[3] Ahad A, Al-Saleh A, Al-Mohizea AM, Al-Jenoobi FI. Pharmacodynamic study of eprosartan mesylate-loaded transfersomes Carbopol gel under Dermaroller on rats with prednisolone acetate-induced hypertension. *Biomed Pharmacother* 2017; 89:177–84.

[4] Akanksha Garud, Deepti Singh, Navneet Garud. Solid Lipid Nanoparticles (SLN): Method, Characterization and Applications. *International Current Pharmaceutical Journal* 2012; 1(11):384- 393. doi: 10.3329/icpj.v1i11.12065.

[5] Jana Pardeike, Aiman Hommoss, Rainer H Muller. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International Journal of Pharmaceutics* 2009;366(1-2):170-184.

[6] Ekambaram P, Sathali AA. Formulation and evaluation of solid lipid nanoparticles of ramipril. *J Young Pharmacists* 2011; 3(3):216-220.

[7] JS Mulla, IM Khazi, Naveen Kumar Sharma, SP Hiremath, VG Jamakandi. Solid lipid nanoparticles: methods of preparation. *Indian Journal of Novel Drug delivery* 2011;3(3): 170-175.

[8] Hu FQ, Yuan H, Zhang HH, Fang M. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. *International Journal of Pharmaceutics* 2002;239(1-2):121-128.

[9] Daniela Chirio, Marina Gallarate, Elena Peira, Luigi Battaglia, Loredana Serpe and Michele Trotta. Formulation of curcumin-loaded solid lipid nanoparticles produced by fatty acids coacervation technique. *Journal of Microencapsulation* 2011; 28(6): 537-548.

[10] Olbrich, Oliver Kayser and Rainer Helmut Muller. Enzymatic degradation of Dynasan 114 SLN effect of surfactants and particle size. *Journal of Nanoparticle Technology and Applications Research* 2002;4(1):121–129.

[11] Arjun Narala and Kishan Veerabrahma. Preparation, characterization and evaluation of

quetiapine fumarate solid lipid nanoparticles to improve the oral bioavailability. *Journal of Pharmaceutics* 2013; 1-7.

[12] Shuyu Xie, Luyan Zhu, Zhao Dong, Yan Wang, Xiaofang Wang, and WenZhong Zhou. Preparation and evaluation of ofloxacin-loaded palmitic acid solid lipid nanoparticles. *International Journal of Nanomedicine* 2011;6: 547–555.

[13] L. Hu, Q. Xing, J. Meng, and C. Shang. Preparation and enhanced oral bioavailability of cryptotanshinone-loaded solid lipid nanoparticles. *AAPS PharmSciTech* 2010;11(2):582- 587.

[14] G Suresh, K Manjunath, Vobalaboina Venkateswarlu and V Satyanarayana. Preparation, characterization, and in vitro and in vivo evaluation of lovastatin solid lipid nanoparticles. *AAPS PharmSciTech*, 2007;8(1):162-170.

[15] Jawahar N, Eaggapanath T, Nagaswamy V, Samantha JS. Preparation and characterization of PLGA nanoparticles containing a hypertensive agent. *Int J Pharm Tech Res* 2009; 2:390-393.