Preparation and Characterization of Rizatriptan Loaded Solid Lipid Nanoparticles

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Abstract:
The aim of the present study is to prepare and characterize Rizatriptan loaded solid lipid nanoparticles (SLNs). Rizatriptan loaded SLN were prepared by modified solvent injection method and characterized for shape, surface morphology, particle size, and drug entrapment. These SLNs were spherical in shape with smooth surface and possesses particles of size range 141.1 to 185.7nm. Fourier transformed infrared spectroscopic analysis and differential scanning colorimetry studies were performed to study the presence of interaction between the drug and excipients and the state of drug and lipid modification respectively. The drug release from SLNs formulation was studied which shows the sustained release of drug. The drug release kinetics were analysed for the prepared formulations and the best fitting model was ascertained. In conclusion, SLN with small particle size and sustained release for rizatriptan from SLNs can be obtained by this method.

Keywords: Solid lipid nanoparticles, rizatriptan, solvent injection method, zeta potential.

1. INTRODUCTION:

In recent years, it has become more and more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. A promising strategy to overcome these problems involves the development of suitable drug carrier systems. SLNs were introduced at the beginning of the 1990s, as an alternative to solid nanoparticles, emulsions and liposomes in cosmetic and pharmaceutical preparations [1–3]. The SLN were realised by exchanging the liquid lipid (oil) of the emulsions by a solid lipid, which means lipids being solid at room temperature but also at body temperature. The use of solid lipids instead of liquid oils is a very attractive idea to achieve controlled drug release, because drug mobility in a solid is considerably lower compared with liquid oil.

SLN usually consists of physiologically well-tolerated ingredients already approved for pharmaceutical application in humans, can readily be produced in large scale, have good storage capabilities including freeze-drying, can be sterilised and show low cytotoxicity, when injected intravenously [4,5]. . SLN formulations have been developed for various routes of application (parenteral, oral, dermal, ocular, pulmonary, and rectal) and are thoroughly characterized and evaluated.

Rizatriptan benzoate, is a selective 5-hydroxytryptamine 1B/1D (5-HT1B/1D) receptor agonist commonly prescribing for treatment of migraine headache.[6,7] Theories on the etiology of headache suggest that symptoms are due to local cranial vasodilatation and/or to the release vasoactive and pro-inflammatory peptides from sensory nerve ending in an activated trigeminal system. Rizatriptan benzoate directly and selectively constricts intracranial, extracerebial blood vessels and inhibits the release of the sensory neuropeptides from perivascular nerves to prevent neurogenic vasodilationand extravasation in the dura matter. [8,9] Rizatriptan benzoate has been shown to relieve migraine within 2 hr in 67% to 77% of patients. [10,11] Oral bioavailability of rizatriptan benzoate is 40-45%. [12] Rizatriptan benzoate undergoes metabolism by monoamine oxidase A isoenzyme (MAO-A) to an inactive indole acetic acid metabolite. In addition, several other inactive metabolites are formed. An active metabolite, N-monodesmethyl-rizatriptan, with pharmacological activity similar to that of the parent compound has been identified in small concentrations which may result in less bioavailability. Hence in the present study an attempt is being made to maximize bioavailability of this drug by preparing chitosan based microparticles for nasal drug delivery system using spray drying.

The preparation of SLNs suffers from the drawback of poor incorporation of water-soluble drugs. In our investigation, rizatriptan has been used as a model drug as it is a poorly water soluble drug.

2. MATERIAL AND METHODS:

2.1. Materials:

Rizatriptan was obtained as a gift sample from Matrix laboratories (Hyderabad, India). Phospholipon 80 was a gift from Lipoid (Ludwigshafen, Germany). Tristearin was procured from TCI Chemicals (India) Private Ltd. Cellophane membrane (molecular weight cut-off,

3500 dalton) was procured from Himedia (Mumbai, India). All other reagents used in this study were of analytical grade.

2.2. Preparation of SLNs:

The SLNs were prepared by modified solvent injection method. Briefly, tristearin, phospholipon 80 and drug rizatriptan were dissolved in ethanol in a definite ratio and warmed to 70 ± 2°C. Tween 80 in a definite amount was dissolved in PBS solution (pH 7.4) to prepare aqueous phase. The organic phase, i.e. alcoholic solution containing lipid mixture, was added dropwise with stirring to prewarmed aqueous solution (70 ± 2°C) with the help of a hypodermic needle. [13] The mixture was then sonicated for varying time periods to obtain nanoparticles with optimum size.

2.3 Fourier transformed infrared (FTIR) spectroscopic analysis:

Fourier transform infrared (FT-IR) spectra of the samples were obtained in the range of 400 to 4,000 cm−1. IR spectral analysis of pure rizatriptan, tristearin, phospholipon 80, physical mixture of rizatriptan with tristearin, phospholipon 80 and rizatriptan SLN formulation were carried out. The peaks and patterns produced by the pure drug were compared with physical mixture and formulation.

2.4 Scanning electron microscopy:

Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the SLNs. The samples were mounted on alumina stubs using double adhesive tape, coated with gold in HUS-5GB vaccum evaporator. Then the sample was observed in Hitachi S-3000N SEM at an acceleration voltage of 10KV and a magnification of 5000X.

2.5 Differential scanning colorimetry:

Differential Scanning Calorimetry (DSC) analysis was performed using Netzsch DSC 200PC (Netzsche, Selb, Germany). The instrument was calibrated with indium (calibration standard, >99.999%) for melting point and heat of fusion. A heating rate of 10^0C/min was employed in the range of 25–200^0C. Analysis was performed under nitrogen purge (20 mL/min). The samples were weighted into standard aluminium pans and an empty pan was used as reference.

2.6 Particle size determination:

The average particle size, polydispersity index and zeta potential of the lipid particulate dispersions were determined using a Zetasizer (DTS Ver. 4.10, Malvern Instruments, UK). The sample of dispersion was diluted to 1:9 v/v with double distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range. Double distilled water was filtered through 0.45 μm membrane filters (Pall Life sciences, Mumbai, India) prior to particle size determination.

2.7 Determination of Entrapment Efficiency (EE):

The EE was determined by analyzing the free drug content in the supernatant obtained after centrifuging the SLN suspension in high speed centrifuge at 16000 rpm for 30 min at 30°C using Remi cooling centrifuge (Mumbai, India).

The EE was calculated as follows:

$$EE= \left\{ \frac{\text{Total drug(assay) - free drug}}{\text{total drug}} \right\} \times 100$$

2.8 In vitro drug release:

Drug release from the formulations was studied in vitro using dialysis membrane (Himedia, India; molecular cut-off point 3500 Da; Gupta et al. 2005). Membrane was soaked in double-distilled water for 12 h before mounting in a diffusion cell. SLN suspension (1 mL) free from any unentrapped drug was placed in dialysis tube, which was suspended in a beaker containing 20mL PBS (pH 7.4) containing 50% v/v ethanol. The contents of the beaker were stirred using a magnetic stirrer at 37 ± 2^0C. Samples were withdrawn periodically and replaced with the same volume of fresh elution solution to keep the volume in the receptor compartment constant. The samples were analysed for drug content using UV-visible spectrophotometry.

2.9 Drug Release Kinetics:

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), korsmeyer-peppas model and Hixson crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values (r2) for correlation coefficients for formulations, the best-fit model was decided.

Table 1: Composition of various SLNs formulations

Formulation	Rizatriptan	Tristearin	Phospslipon 80H	Tween 80	Sonication time
R-1	50mg	50mg	50mg	0.5 ml	6 min
R-2	50mg	50mg	50mg	0.5 ml	12 min
R-3	50mg	50mg	50mg	0.5 ml	15 min
R-4	50mg	50mg	50mg	1 ml	6 min
R-5	50mg	50mg	50mg	1 ml	12 min
R-6	50mg	50mg	50mg	1 ml	15 min

3. RESULTS AND DISCUSSION:

SLNs with a narrow size distribution were prepared by modified solvent injection method using tristearin as a solid lipidThe small and uniform sized SLNs can be prepared by this method without using any sophisticated instruments.

The SEM study revealed that most of the SLNs were fairly spherical in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the nanometric range, as depicted in Figure 1.
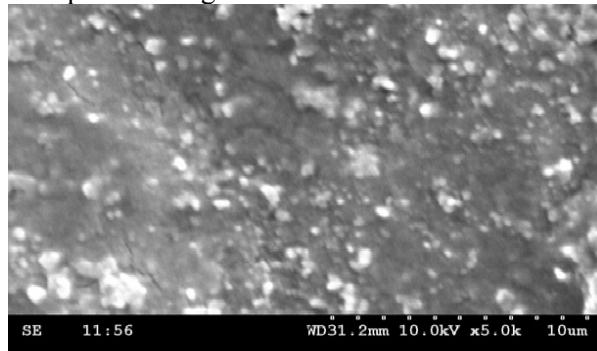

**Figure 1:** SEM image of rizatriptan loaded SLNs
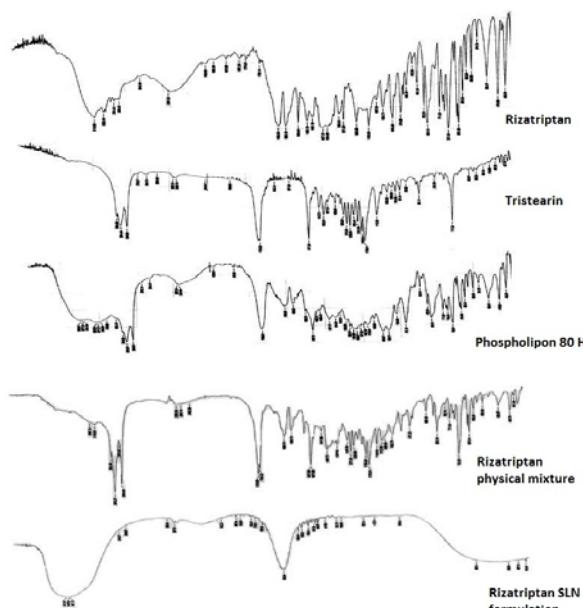
Figure 2: Overlaid FTIR graphs

IR spectroscopic studies were conducted to determine possible drug: lipid interactions. IR spectra of pure drug rizatriptan, tristearin, phospholipon 80 H and physical mixture of rizatriptan with tristearin and phospholipon 80 H were obtained and shown in figure 2. The characteristic peaks of rizatriptan and lipids were present in the physical mixture, thus indicating that there is no significant evidence of chemical interaction between drug and lipid, which confirms the stability of drug. The major peaks CH3, CH2 stretch at 2915cm-1, C=C stretch at

1604cm-1, NH bend at 1271cm-1, C-N stretch at 1139 cm-1 which were present in drug rizatriptan are also present in physical mixture which indicates that there is no interaction between rizatriptan benzoate and the solid lipid.
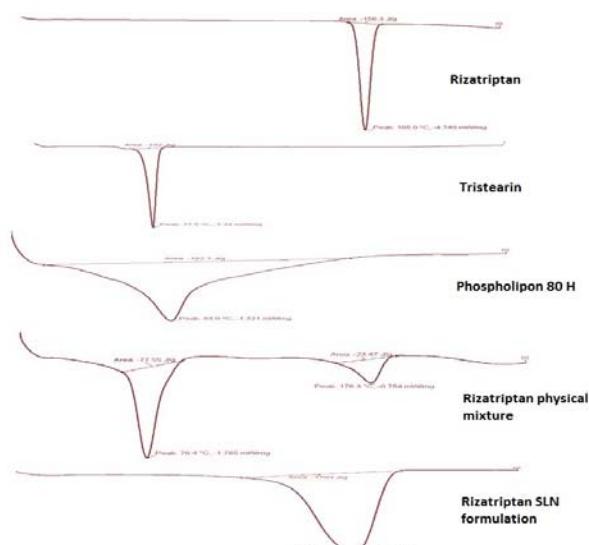

Figure 3: Overlaid DSC thermograms

Figure 3 reveals the thermal behaviors of the pure components rizatriptan, tristearin, phospholipon 80 H, together with the thermal behavior of the final SLNs prepared and the melting point peaks were tabulated in table 2. The DSC curve of pure drug rizatriptan benzoate exhibits a sharp endothermic peak at 185^0C. The thermograms of Phospholipon 80 H and tristearin displayed broad endothermic peaks at 83 and 77.5 ^0C. On the other hand, the SLNs thermogram displayed complete disappearance of characteristic peak of rizatriptan; a fact that the drug was molecularly dispersed within the lipid matrix. That was accompanied by the formation of a new endothermic peak at 126.5 ^0C.

Table 2: DSC data of pure components, physical mixture and SLN formulation

S.No	Sample	Melting peak
1.	Rizatriptan	185
2.	Phospholipon 80 H	83
3.	Tristearin	77.5
4.	Rizatriptan physical mixture	76.4, 176.3
5.	Rizatriptan SLN formulation	176.5

The SLNs were characterized for average particle size, PDI, zeta potential and percentage drug entrapment efficiency. The particles of size range 141.1 to 185.7nm were obtained for rizatriptan loaded SLNs. Zeta potential of various rizatriptan loaded SLNs varies from -2.3 to -7.4 and polydispersity index was found to be between 0.220 to 0.348 (table 3). Despite of the low zeta potential the prepared SLNs were stable.

Table 3: Average particle size, zeta potential and PDI of rizatriptan loaded SLN formulations

Formulation	Average particle size (nm)	Zeta potential	Polydispersity index
R-1	185.7	-2.3	0.348
R-2	179.6	-4.12	0.298
R-3	161.1	-4.28	0.309
R-4	168.7	-5.56	0.250
R-5	154.3	-5.82	0.268
R-6	141.1	-7.40	0.292

Table 4: Total drug content and entrapment efficiency of rizatriptan loaded SLN formulations

Formulation	Total drug content (mg)	Entrapment efficiency
R-1	37.1	13.4%
R-2	41.1	18.9%
R-3	40.2	25.1%
R-4	38.6	19.9%
R-5	39.1	24.0%
R-6	42.2	27.0%

Table 5: % Drug release data of rizatriptan loaded SLNs formulation

Time	% Drug Release					
	R-1	R-2	R-3	R-4	R-5	R-6
0	0.00	0.00	0.00	0.00	0.00	0.00
1	9.89	3.92	2.41	3.62	12.76	9.21
2	13.84	8.75	7.96	7.81	13.70	16.71
3	18.07	14.06	12.84	12.64	16.51	18.62
4	19.32	16.75	17.71	16.62	21.50	23.04
5	19.81	20.48	20.11	19.69	21.62	24.76
6	22.76	22.65	24.63	22.20	22.65	26.90
7	23.20	23.57	25.90	24.63	25.26	29.28
8	23.95	24.34	27.78	25.82	24.69	30.63
24	37.88	41.32	48.30	40.01	44.81	50.25

These SLNs exhibited drug entrapment efficiency of 13.4% to 27.0% which is due to its hydrophilic nature (table 4). In vitro drug release study were performed for all the formulations R-1 to R-6 which shows the sustained release of drug 37.38%, 41.32%, 48.30%, 40.01%, 44.81% and 50.25% respectively after 24 h. The results are shown in Table 5.

The release kinetics was evaluated by fitting the data into first order, zero order, Higuchi, Peppas, and Hixon–Crowell equations using PCP Disso software v3 Pune, India. The drug release kinetic data of different SLN formulations was depicted in table 6. Based on the results, the release of rizatriptan from SLNs best-fitted Higuchi equation and the possible mechanisms for the drug release might be diffusion of the drug from the matrix and matrix erosion resulting from degradation of lipids.

From the results it was observed that, larger particles showed slower release compared to smaller particles. This may be due to the larger surface area of smaller particles leading to faster drug release. In general the drug release from all formulation followed a steady pattern. The drug release may be mainly controlled by drug diffusion through the lipid matrix.
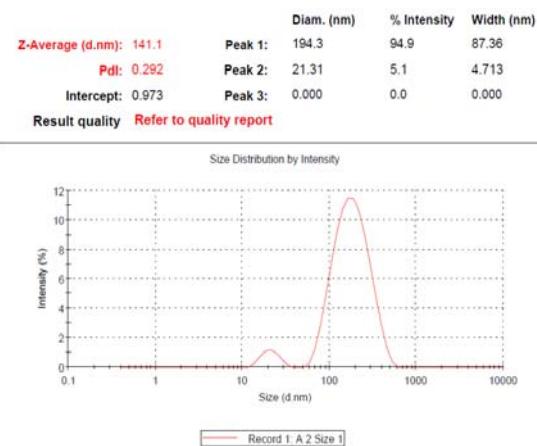
Figure 4: Average particle size of formulation R-6
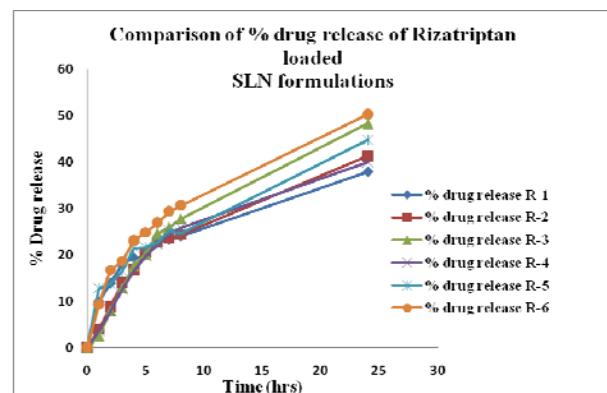
Figure 5: % Drug release graph of different SLN formulations

Table 6: Drug release kinetic data of rizatriptan loaded SLN formulations

		R-1	R-2	R-3	R-4	R-5	R-6
Zero order model	R^2	0.0049	0.5547	0.7466	0.5406	0.2877	0.3448
	K	2.1381	2.2055	2.4856	2.1723	2.3968	2.7330
First order model	R^2	0.3239	0.7396	0.8740	0.7104	0.6160	0.6690
	K	-0.0256	-0.0269	-0.0317	-0.0264	-0.0298	-0.0353
Higuchi matrix model	R^2	0.9541	0.9692	0.9442	0.9545	0.9791	0.9912
	K	8.5690	8.4843	9.3390	8.3728	9.3957	10.7114
Peppas model	R^2	0.9846	0.9291	0.9072	0.9262	0.9533	0.9769
	K	10.5045	5.3608	3.9268	4.8867	11.3256	10.5705
Hixson crowell model	R^2	0.2234	0.6829	0.8281	0.6575	0.5184	0.5782
	K	-0.0080	-0.0084	-0.0097	-0.0082	-0.0092	-0.0108

CONCLUSION:

Rizatriptan, a hydrophilic drug was successfully incorporated into SLNs by modified solvent injection method. The formulated rizatriptan loaded SLNs were in nanometric range with spherical structure. The release of drug from SLNs best-fitted Higuchi equation and the possible mechanisms for the drug release might be diffusion of the drug from the matrix and matrix erosion resulting from degradation of lipids. This experiment confirmed the evidence that solvent injection technique was a simple, available and effective method to prepare SLNs loading hydrophilic drugs.

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