Design, development and evaluation of ketotifen fumarate solid lipid nanoparticles.

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Abstract

The aim of the present study is to develop Ketotifen Fumarate solid lipid nanoparticles and to evaluate them. Formulations were formulated by using three lipids at various proportions. Solid lipid nanoparticles were prepared by hot homogenization followed by ultra-sonicationtechnique. The nanoparticles were evaluated Suitable lipids (Triastearin, GMS and Compritol), stabilizer (Soy lecithin) and surfactant (Poloxamer) were selected. FT-IR studies were carried to check drug-excipient compatibility. In the present study nine studies, release kinetics for particle size, PDI, zeta potential Drug content, percentage drug entrapment efficiency, *in vitro* drug release.

FT-IR drug-excipient compatibility studies were revealed that there was no interaction between drug and selected lipids. The particle size ranged from 192.7 to 359.5 nm, PDI of all formulations were good within the range of 0.432 to 1.000, zeta potential ranged from -3.25 mV to -26.7 mV, Percentage drug entrapment efficiency of all formulations were observed were in the range of 78.88% to 93.67%. The cumulative percentage release of Ketotifen Fumarate from different formulations varied from 76.61% to 93.88%. Among all formulations, the formulation F1 showed highest drug release of 93.88% and considered as optimized formulation. The release kinetic studies showed that the release was first order (R2=0.956) diffusion controlled and the 'n' value obtained from the Korsmeyer-Peppas model indicated the release mechanism was Anomalous diffusion (non-fickian type) (n-value of F1 was 0.613). The developed SLNs were able to sustain the drug release for 12 hrs.

Keywords: Ketotifen fumarate, Solid lipid nanoparticles, FT-IR, *In vitro* drug release.

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Introduction

Optical Coherence Tomography (OCT) is a non-invasive, noncontact technique that visualizes the retina and has increasingly been used in ophthalmology [1,2]. It is a new imaging technology which is based on the Laser Interferometry principle. Image capture is fast and painless.

Retinal thickness can be measured accurately by OCT due to its high depth resolution (10 microns). Comparison of OCT and histological images from prototype devices has shown good correlation between real measurements of Retinal Nerve Fibre Layer (RNFL) thickness and OCT estimates. The third-generation instrument, Stratus OCT (Carl Zeiss Meditec, Dublin, CA, USA), relies on time-domain technology (TD-OCT). This technology recently was superseded by new instruments that use Spectral-Domain Technology (SD-OCT), such as Cirrus OCT (Carl Zeiss Meditec).

Spectral Domain OCT (SD-OCT) provides approximately twice the axial resolution and 45 times to 100 times the scanning speed and can reveal the three-dimensional configuration of the retina in comparison with time-domain OCT (TD-OCT) [3].

SD-OCT significantly increases the amount of data acquired during each session; the motion artefacts are significantly reduced; and better repeatability and reproducibility and an increased signal-to-noise ratio are achieved compared with TD-OCT. Cirrus HD-OCT (Cirrus Version 6.0; Carl Zeiss Meditec, Dublin, CA) is a commercially available SD-OCT with a scan speed of 27,000 axial scans per second and an axial resolution of 5 μm [4].

The diagnosis and follow-up of children with an ocular disease is more difficult than that of adults because of the challenge in obtaining reliable and reproducible visual examinations. Important diagnostic tools used in adults such as visual fields, require their cooperation. For children, such tools are often impractical because the results are unreliable, and hence difficult to interpret However, OCT provides objective measurements of the affected structures. Generally, children older than 3 years or 4 years of age can cooperate sufficiently. Macular measurements are even easier to obtain than those of the optic nerve, making OCT particularly suitable for use with uncooperative children or those with poor fixation [5].

SD-OCT is progressively being used to evaluate paediatric macular diseases, childhood glaucoma, and non-glaucomatous optic neuropathies. Additionally, it is used for monitoring changes in the progression of the disease and assessing the efficacy of current and novel treatments for eye diseases in paediatric population [6].

Materials and Methods

Ketotifen Fumarate was purchased from Fleming laboratories limited, Hydreabad, Tristerin from Sasol Germany, Glycerol mono stearate from Research-Lab Fine chem. Industries, Compritol from Gattefosse-France, Soy lecithin was purchased

from HiMedia Laboratories Pvt. Ltd, and Poloxamer, chloroform and methanol were purchased from SD Fine-Chem limited. All the reagents used were of analytical grade.

Fourier-Transform Infrared Spectroscopy (FT-IR)

Drug-polymer interactions were studied by FTIR spectroscopy. Pure drug, excipients, and physical mixture of drug and excipients were subjected to FTIR studies. The spectra were recorded by scanning in the wavelength of 400 cm-1 –4000 cm-1 in an FTIR spectrophotometer [7]. The samples analyzed by FT-IR include

- Pure drug (Ketotifen Fumarate).
- Physical mixture of drug+Tristearin (1:1).
- Physical mixture of drug+Poloxamer (1:1).
- Physical mixture of drug+Compritol (1:1).

Preparation of solid lipid nanoparticles with Ketotifen Fumarate using lipids (Tristearin, GMS and Compritol)

SLNs were prepared by hot melt homogenization followed by ultra-sonication technique. The lipid was first melted in a boiling tube using a water bath and then the soy lecithin and drug was added into the lipid melt (lipid phase). Heating was continued until soy lecithin and drug are miscible with lipid melt. Solvents like methanol and chloroform in the ratio 1:1 are used for complete miscibility, later these solvents were completely evaporated using water bath. Simultaneously in another beaker Poloxamer was dissolved in water (aqueous phase) and heated to the same temperature as that of lipid phase. Then the aqueous phase was transferred slowly into the lipid phase while homogenizing the mixture at 20,000 rpm for 5 min using high speed homogenizer and then immediately the mixture was sonicated using probe ultra sonicator at 75% amplitude for 20 min, temperature was maintained above 5°C of melting point of lipid throughout process (Table 1) [8].

Table 1. *Composition of different formulations of Ketotifen Fumarate SLNs Prepared with Tristearin, GMS and Compritol using Poloxamer.*

Formulation code	Drug (mg)	TS	GMS	CM	Polx (mg)	Soy (mg)	Water (ml)
		(mg)	(mg)	(mg)			
F1	10	50	-	-	25	25	10
F2	10	100	-	-	50	50	10
F3	10	150	-	-	75	75	10
F4	10	-	50	-	25	25	10
F5	10	-	100	-	50	50	10
F6	10	-	150	-	75	75	10
F7	10	-	-	50	25	25	10
F8	10	-	-	100	50	50	10
F9	10	-	-	150	75	75	10

TS-Tristearin, GMS-Glycerol monostearate, CP-Compritol, Polx-Poloxamer, SL-Soy Lecithin

Particle size, polydispersity index and zeta potential particle size analysis

The particle size was determined by dynamic light scattering, using a Malvern zetasizer with vertically polarized light supplied by an argon-ion laser (Cyonics). Experiments were performed at a temperature of 25.0 ± 0.1°C at a

Polydispersity index

Polydispersity Index; a parameter calculated from a Cumulants analysis of the DLS-measured intensity auto correlation function. Polydispersity index are determined by the same instrument i.e., Malvern zetasizer [9].

Zeta potential

Zeta potential was measured using Malvern zetasize, nanoparticles were diluted with distilled water and placed in a clear disposable zeta cell at 25°C. The sample was subjected for two zeta runs to determine both size and potential [10].

Drug content

About 0.2 ml of drug-loaded SLNs was added into 5 ml of methanol in the centrifuge tube. The solution was vortexed for 10 min and then centrifuged at 5000 rpm for 30 min. The supernatant was collected. The drug content in the supernatant was analyzed by UV spectrophotometer for Ketotifen Fumarate at 300 nm [11].

Percentage Drug Entrapment Efficiency (%DEE)

About 2 ml of Ketotifen Fumarate loaded solid lipid nanoparticles was taken and placed in outer chamber of the centrisart device and the sample recovery chamber is placed on the top of the sample. The unit is centrifuged at 5000 rpm for 20 min. The solid lipid nanoparticles along with the encapsulated drug remained in the outer chamber and the aqueous phase is moved into the sample recovery chamber through filter membrane (molecular weight cut-off 20,000 daltons). The resulting aqueous phase was analyzed by UV-Spectrophotometer for Ketotifen Fumarate at 300 nm. The % Drug entrapment efficiency was calculated by using the followingrelationship.

In vitro drug release study

In vitro drug release studies were carried out in Franz diffusion cell; 2 ml of nanoparticles dispersion was used for diffusion study. Nanoparticles containing Ketotifen Fumarate were placed in donor compartment while the receiver compartment consists of 22 ml of diffusion medium Phosphate buffer pH 6.8 maintained at 25 ± 2°C in Franz diffusion cell. The rpm of the magnetic bead was maintained at 50 rpm. 2 ml of the aliquot was withdrawn at predetermined intervals. The samples were analyzed for the drug content by UV-Spectrophotometer at 300

nm. Equal volume of the diffusion medium was replaced in the vessel after each withdrawal to maintain sink condition. Three trails were carried out for all formulations. From data obtained percentage drug release was calculated and plotted against function of time to study the pattern of drug release.

Results and Discussion

Determination of λ max of ketotifen fumarate in methanol and phosphate buffer of pH 6.8

A solution of Ketotifen Fumarate was scanned in UV spectrophotometer between 200 nm-400 nm and Ketotifen Fumarate shows absorbance max at 298 nm for methanol and at 300 nm for phosphate buffer pH 6.8 and respectively

Standard plot of Ketotifen fumarate in methanol

Standard solutions of ketotifen fumarate in methanol (10 µg/ml-80 µg/ml) were prepared and measured at 298 nm using UV-Spectrophotometer. The standard plot of ketotifen fumarate was as shown. The obtained correlation coefficient was 0.999 and the regression equation y=0.044, x+0.009 was used to calculate the concentration of unknown samples of drug content estimation

Standard plot of ketotifen fumarate in phosphate buffer

Standard solutions of ketotifen fumarate in phosphate buffer of pH 6.8 (10 µg/ml-70 µg/ml) were prepared and measured at 300 nm using UV-Spectrophotometer. The standard plot of ketotifen fumarate was as shown. The obtained correlation coefficient was 0.996 and the regression equation y=0.039 x +0.009 was used to calculate the concentration of unknown samples of %DEE and *in vitro* studies (Figures 1a and 1b).

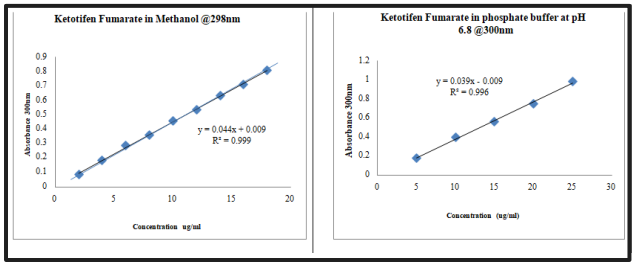

Figure 1. a) *Standard gragh of Ketotifen Fumarate in Methanol,* ***b)*** *Standard graph of Ketotifen Fumarate in Phosphate buffer pH 6.8.*

FT-IR study

The FTIR spectrum analysis was used to know the drug – excipients compatibility. The FTIR was performed for the drug ketotifen fumarate, lipids (TS, GMS and CP), and physical mixture of drug and lipids. The FTIR spectra of pure drug and the mixture of drug and lipids. Interpretation of the spectrum is shown in Figure 2 and Table 2.

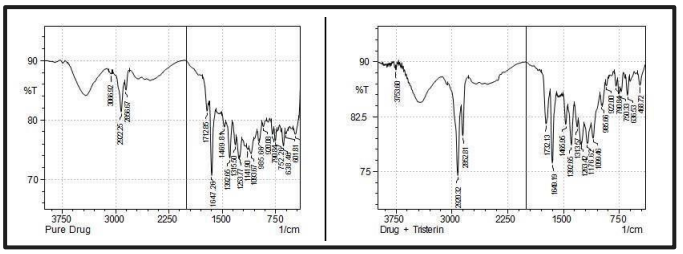

Figure 2. *FT-IR spectrum of physical mixture of ketotifen fumarateandpolaxomer.*

Table 2. *Interpretation of FT-IR spectra of dothiepin HCl and its physical mixtures.*

Sl No	Sample name	Functional group	Actual value (cm^{-1})	Value obtained (cm^{-1})
1	Ketotifen Fumarate	N–H Stretching S=O Stretching O–H Stretching C=O Stretching C–H Stretching	3000 – 2800	3066.9
			1350 – 1300	1315.5
			1390 – 1380	1392.7
			1725 – 1705	2856.7
			2830 – 2695	
2	Ketotifen Fumarate +Tristearine (1:1)	S=O Stretching O–H Stretching C=O Stretching C–H Stretching	1350 – 1300	1315.5
			3700 – 3584	3753.6
			1740 – 1720	1732.1
			2830 – 2695	2862.8
3	Ketotifen Fumarate +Polaxomer (1:1)	S=O Stretching O–H Stretching C=O Stretching C–H Stretching	1350 – 1300	1315.5
			1390 – 1380	2920.3
			1740 – 1720	1710.9
			2830 – 2695	2850.9
4	Dothiepin HCl +Campritol (1:1)	N–H Stretching S=O Stretching O–H Stretching C=O Stretching C–H Stretching	3000 – 2800	3066.92
			1350 – 1300	1315.50
			3300 – 2500	1390.72
			1725 – 1705	1736.99
			2830 – 2695	2850.88

Particle size, PDI and zeta potential

The Particle size of ketotifen fumarate SLNs prepared with lipids (Tristearin, GMS and Compritol) using Polaxomer were in the range of 192.7 nm to 359.5 nm. PDI of all formulations were good within the range of 0.432 to 1.000. The zeta potential ranges from -3.25 mV to –26.7 mV were shown in Table 3. Similarly, dothiepin HCl loaded SLNs of optimized formulation F1 shows size particle size of 57.42 nm with 0.406 PDI and -20.9 mV zeta potential are shown and sizes was in nano range and zeta potential obtained was optimum for good stabilization (Table 3).

Table 3. *The particle size, PDI and zeta potential of Ketotifen Fumarate SLN's.*

Serial No	Formulation code	Particle size(nm)	PDI	Zeta potential(mV)
1	F1	192.7	0.856	-7.7
2	F2	2.04	1	-3.25
3	F3	123.8	0.758	-20.5
4	F4	147.5	0.691	-25.1
5	F5	198.2	0.569	-26.7
6	F6	271.3	0.515	-19.3
7	F7	141.6	0.623	-21.9
8	F8	155.4	0.432	-24.5
9	F9	359.5	0.587	-16.8

Drug content and %drug entrapment efficiency

The drug content of formulations was carried out by extraction with methanol as mentioned in the methodology section. The drug content results were ranged between 76.61% to 93.88%. Percentage drug entrapment efficiency for ketotifen fumarate loaded SLNs was determined by measuring the concentration of entrapped drug in aqueous medium by centrifugation method. From the results it has been observed that, the high lipid concentration containing formulations have higher entrapment efficiency compared to other formulations.

Percentage drug entrapment efficiency of ketotifen fumarate loaded SLNs was good in the range of 84.42% to 93.79% (Table 4).

Table 4. *Percentage entrapment efficiency, and percentage drug content.*

Sl No	Formulation code	%Drug content	Amount of ketotifen fumarate		%Entrapment efficiency
			In aqueous phase (mg)	In lipid phase (mg)	
1	F1	76.61	1.132	8.905	89.05
2	F2	79.37	0.874	9.123	91.23
3	F3	81.68	0.44	9.367	93.67
4	F4	83.79	1.915	8.765	87.65
5	F5	88.91	1.501	8.312	83.12
6	F6	77.45	1.899	8.765	87.65
7	F7	82.96	1.677	8.679	86.79
8	F8	93.88	2.165	7.888	78.88
9	F9	87.92	1.982	8.541	85.41

In vitro drug release study

Further for the selection of optimized formulation, prepared SLNs of ketotifen fumarate were subjected to in vitro drug release study by Franz diffusion method for 12 hrs.

The drug released from all the formulations are shown and were in the range of 56.89% to 87.90% at 12 hrs and all formulation exhibited sustained release of drug.

From *in vitro* drug release study we concluded that formulations with low drug to lipid ratio (F1, F2 and F8) show better *in vitro* drug release than other drug lipid ratios.

The formulation F1 containing Tristear in with low drug to lipid ratio (1:1) showed highest drug release of 87.90% at 12 hrs, hence it was considered as optimized formulation among all other formulations.

Further the *in vitro* drug release data obtained from optimized formulation F1 was subjected to kinetic study to understand the release mechanism.

Obtained *in vitro* drug release data were processed and plotted as zero order, first order, higuchi model and korsmeyer-peppas model.

Considering the regression values and ' ' value the *in vitro* drug release from the nanoparticles of formulation F1 follows a First order kinetics and the release mechanism was anomalous diffusion (non- Fickian) type (Tables 5 and 6) (Figure 3).

Table 5. *The processing of in vitro drug release data of formulation F1 into different Kinetic models.*

Time (Hours)	Log time	SQRT	%CDR	Log %CDR	CRR	Log % CRR
1	0	1	10.9	1.0374	89.1	1.9498
2	0.301	1.4142	16.52	1.218	83.48	1.9215
3	0.4771	1.732	22.78	1.3575	77.22	1.8877
4	0.602	2	27.21	1.4347	72.79	1.862
5	0.6989	2.236	31.95	1.5044	68.05	1.8328
6	0.7781	2.4494	39.73	1.5991	60.27	1.7801
8	0.903	2.828	55.02	1.7405	44.98	1.653
10	1	3.162	64.78	1.8114	35.22	1.5467
12	1.0791	3.4641	77.85	1.891	22.15	1.3453

Table 6. *The regression (R2) and (n) values from the above plots.*

Formulation code	Regression factor			Peppas model	
	Zero order	First order	Higuchi model	R2	n-value
F1	0.996	0.956	0.961	0.985	0.613

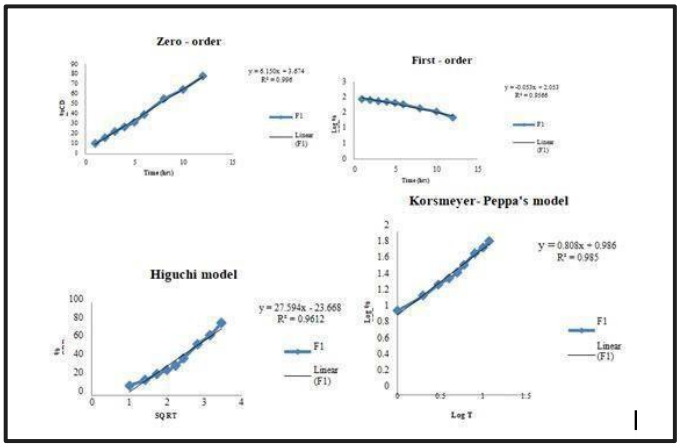

Figure 3. *The plots of in vitro drug release data of formulation F1 into different Kineti models.*

CONCLUSION

In this study, an attempt was made to formulate solid lipid nanoparticles of ketotifen fumarate were using three lipids Tristearin, GMS and compritol, poloxamer as a surfactant, Soy lecithin as a stabilizer. From the FT-IR studies it was confirmed that there is no interaction between the drug and the selected lipids. Ketotifen fumarate solid lipid nanoparticles were prepared by hot homogenization followed by ultra-sonication technique. This method was able to produce nanoparticles of acceptable range and stability. All the formulations showed good particle size, PDI, zeta potential, %drug entrapment efficiencies, and *In vitro* drug release studies. By considering that, the *in vitro* drug release of formulation F1 was good compared to all other formulations, F1 is considered as optimized formulation. The *in vitro* drug release kinetics of F1 formulation revealed that the drug release follows first-order kinetics and from Korsmeyer-Peppas plot indicates that the release was anamolus diffusion (non-fickian type). It can be concluded that the formulated SLNs of Ketotifen Fumarate with physiologically safe lipids was capable of exhibiting sustained release properties for a period of 12 hrs and they may be thus used to reduce dosing frequency, thereby minimize the occurrence of side effects, improving bioavailability and increase the effectiveness of the drug.

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