



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-sonication Suitable lipids (Triastearin, GMS and technique. The nanoparticles were evaluated 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 ($R^2 = 0.956$) 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 12hrs.

Keywords: Ketotifen Fumarate; Solid lipid nanoparticles; FT-IR; In vitro drug release

Introduction

Solid lipid nanoparticles [SLNs] are the newer approach gaining popularity nowadays,

As it is the most desirable and easy to manufacture formulation for the enhancement of solubility of poorly water-soluble drugs. The formulation of nanoparticles provides a higher efficacy and lesser toxicity for the cure of numerous diseases. SLNs were introduced in 1991 and they provide delivery systems for attractive, less toxic drugs

compared to polymer systems that combine the advantages of polymeric nanoparticles, lipid and liposomal emulsions.[1]

Recently, important effort have been devoted to use the potentials of nanotechnology in drug delivery systems since it offers a suitable means of site-specific and/or time- controlled delivery of small or large molecular weight drugs and other bioactive compounds. Pharmaceutical nanotechnology focuses on designing therapeutically active and biocompatible molecules in different forms such as nanoparticles, nanocapsules and micellar systems. These systems offer many advantages in drug delivery, mainly improving bioavailability, providing targeted delivery of drugs, extending drug or gene effect in target tissue and preventing chemical or enzymatic degradation. The nanoscale size of these delivery systems is the basis for all these advantages.[2]

SLNs are attractive submicron colloidal carriers (50-1000nm) used for both lipophilic and hydrophilic drugs. Drugs are entrapped in the heart of the biocompatible lipid and the surface agent in the outer layer. SLN can be used to improve biological availability and achieve continuous release of the drug. They provide benefits such as the lack of acute and chronic toxicity of carrier, good tolerability, and biodegradability. SLNs can be efficiently used to improve the oral bioavailability of drugs either by increasing the drug permeability or by overcoming the first-pass effect. [3]

Ketotifen Fumarate (IUPAC name: (E)-but-2-enedioic acid; 2-(1-methylpiperidine-4-ylidene)-6-thiatricyclo[8.4.0.03, 7] tetradeca-1(14),3(7),4,10,12-pentaen-8-one. (Molecular formula: C₂₃H₂₃NO₅S). Ketotifen Fumarate is the fumarate salt of ketotifen, a Cycloheptathiophene derivative with Anti-allergic activity. Ketotifen selectively blocks histamine (H₁) receptors and prevents the typical symptoms caused by histamine release. A cycloheptathiophene blocker of histamine H₁ receptors and release of inflammatory mediators. It has been proposed for the treatment of asthma, rhinitis, skin allergies and anaphylaxis. This agent also interferes with the release of inflammatory mediators from mast cells involved in hypersensitivity reactions, thereby decreasing chemotaxis and activation of eosinophils. [4]

Materials and Methods

Ketotifen Fumarate was purchased from Fleming laboratories limited, Hyderabad, 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 400–4000cm⁻¹ in an FTIR spectrophotometer. [6] 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 ultrasonication 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 20,000rpm for 5min using high speed homogenizer and then immediately the mixture was sonicated using probe ultra sonicator at 75% amplitude for 20min, temperature was maintained above 5°C of melting point of lipid throughout process.[8]

Formulation code	Drug (mg)	TS (mg)	GMS (mg)	CM (mg)	Polx (mg)	Soy (mg)	Water (ml)
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

Table1: Composition of different formulations of Ketotifen Fumarate SLNs Prepared with Tristearin, GMS and Compritol using Poloxamer.

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 (Cyomics). Experiments were performed at a temperature of 25.0±0.1°C at a measuring angle of 90° to the incident beam [9].

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

Drug content was calculated using the following formula

$$\text{Drug content (\%)} = \frac{\text{Practical amount of drug (mg)}}{\text{Theoretical amount of drug (mg)}} \times 100$$

Percentage Drug entrapment efficiency (%DEE)

About 2ml of Ketotifen Fumarate loaded solid lipid nanoparticles was taken and placed in outer chamber of the centrifuge device and the sample recovery chamber is placed on the top of the sample. The unit is centrifuged at 5000rpm for 20min. 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 20,000daltons). The resulting aqueous phase was analyzed by UV-Spectrophotometer for Ketotifen Fumarate at 300 nm. The % Drug entrapment efficiency was calculated by using the following relationship. [12]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Drug content (mg)} - \text{Amount of drug in aqueous phase (mg)}}{\text{Drug content (mg)}} \times 100$$

In vitro Drug Release Study

In vitro drug release studies were carried out in Franz diffusion cell; 2ml of nanoparticles dispersion was used for diffusion study. Nanoparticles containing Ketotifen Fumarate were placed in donor compartment while the receiver compartment consists of 22ml of diffusion medium Phosphate buffer pH6.8 maintained at 25±2°C in Franz diffusion cell. The rpm of the magnetic bead was maintained at 50rpm. 2ml 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. [13]

Results and Discussion

Determination of λ_{max} of Ketotifen Fumarate in methanol and phosphate buffer of pH6.8 A solution of Ketotifen Fumarate was scanned in UV spectrophotometer between 200-400nm and Ketotifen Fumarate shows absorbance max at 298 nm for methanol and at 300 nm for phosphate buffer pH6.8 and respectively.

Standard plot of Ketotifen Fumarate in methanol

Standard solutions of Ketotifen Fumarate in methanol (10-80µg/ml) were prepared and measured at 298 nm using UV-Spectrophotometer. The standard plot of Ketotifen Fumarate was as shown in (Figure 1A). The obtained correlation coefficient was 0.999 and the regression equation $y = 0.044x + 0.009$ was used to calculate the concentration of unknown samples of Drug content estimation.

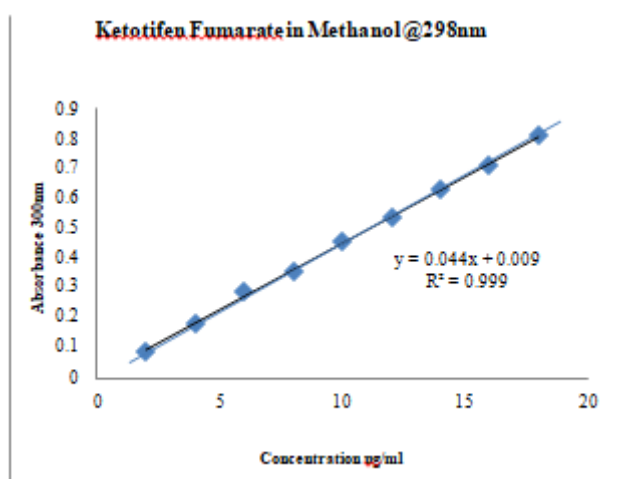


Figure1A: Standard graph of Ketotifen Fumarate in Methanol

Standard plot of Ketotifen Fumarate in Phosphate buffer

Standard solutions of Ketotifen Fumarate in Phosphate buffer of pH6.8 (10-70µg/ml) were prepared and measured at 300 nm using UV-Spectrophotometer. The standard plot of Ketotifen Fumarate was as shown in (Figure 1B). The obtained correlation coefficient was 0.996 and the regression equation $y = 0.039x + 0.009$ was used to calculate the concentration of unknown samples of %DEE and in vitro studies.

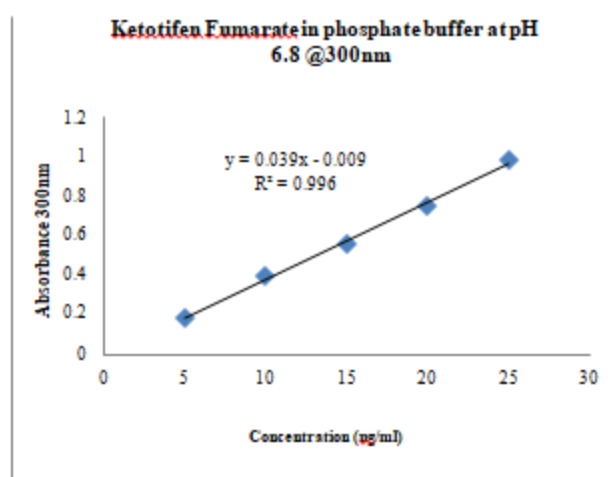


Figure1B: Standard graph of Ketotifen Fumarate in Phosphate buffer pH6.8

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. Figures 2A, 2B, 2C, 2D shows the FTIR spectra of pure drug and the mixture of drug and lipids. Interpretation of the spectrum is shown in Table 2. The FTIR spectrum of physical mixtures did not show any significant shift in the vibration bands of Ketotifen Fumarate so there is no interaction between the drug and selected lipids.

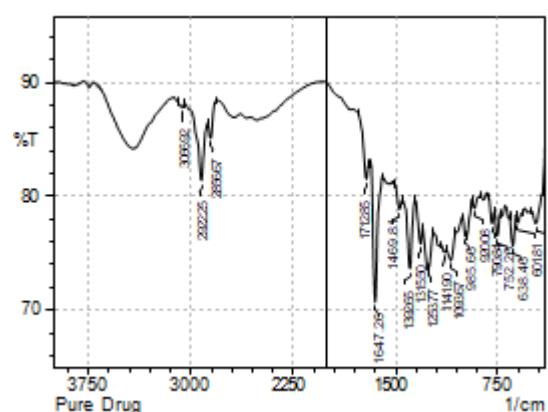


Figure2A: FT-IR spectrum of pure Ketotifen Fumarate.

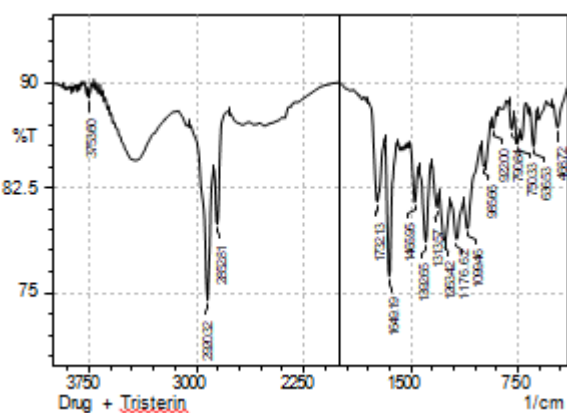


Figure2B: FT-IR spectrum of physical mixture of Ketotifen Fumarate and Tristearin.

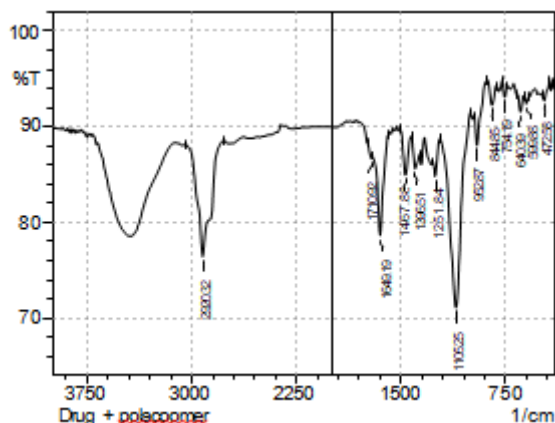


Figure 2C: FT-IR spectrum of physical mixture of Ketotifen Fumarate and Polaxomer

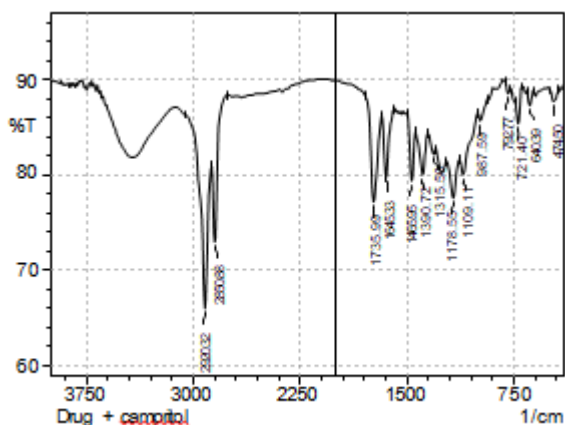


Figure 2D: FT-IR spectrum of physical mixture of Ketotifen Fumarate and Compritol

Sl No	Sample Name	Functional Group	Actual value (cm-1)	Value obtained (cm-1)
1	Ketotifen Fumarate	N - H Stretching	3000 - 2800	3066.92
		S = O Stretching	1350 - 1300	1315.5
		O - H Stretching	1390 - 1380	1392.65
		C = O Stretching	1725 - 1705	2856.67
		C - H Stretching	2830 - 2695	
2	Ketotifen Fumarate + Tristearin (1:1)	S = O Stretching	1350 - 1300	1315.5
		O - H Stretching	3700 - 3584	3753.6
		C = O Stretching	1740 - 1720	1732.13

3	Ketotifen Fumarate + Polaxomer (1:1)	C - H Stretching	2830 - 2695	2862.81
		S = O Stretching	1350 - 1300	1315.5
		O - H Stretching	1390 - 1380	2920.32
		C = O Stretching	1740 - 1720	1710.92
4	Dothiepin HCl + Compritol (1:1)	C - H Stretching	2830 - 2695	2850.88
		N - H Stretching	3000 - 2800	3066.92
		S = O Stretching	1350 - 1300	1315.5
		O - H Stretching	3300 - 2500	1390.72
		C = O Stretching	1725 - 1705	1736.99
		C - H Stretching	2830 - 2695	2850.88

Table 2: Interpretation of FT-IR spectra of Dothiepin HCl and its physical mixtures.

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 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 57.42nm with 0.406PDI and -20.9mV zeta potential are shown in and sizes was in nano range and zeta potential obtained was optimum for good stabilization.

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

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

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%.

SI 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

Table4: Percentage Entrapment Efficiency, and Percentage Drug Content.

In Vitro Drug Release Study

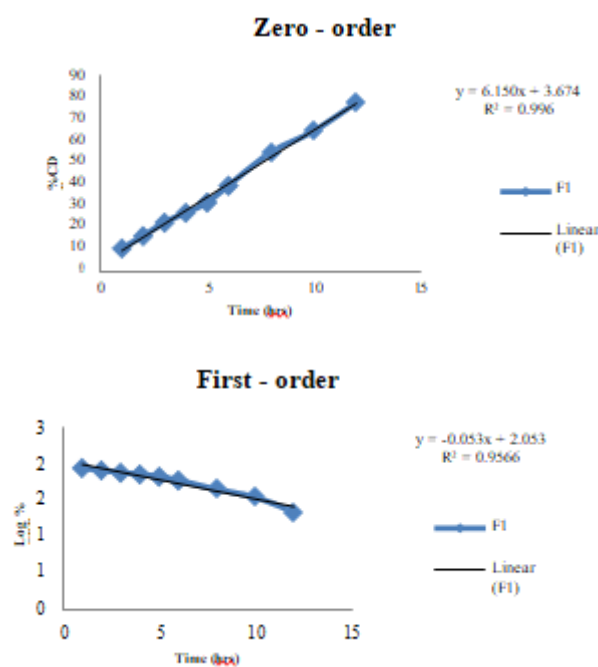
Further for the selection of optimized formulation, prepared SLNs of Ketotifen Fumarate were subjected to in vitro drug release study by Franz diffusion method for 12hrs. The drug released from all the formulations are shown in Table 5 and were in the range of 56.89 to 87.90% at 12hrs and all formulation 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 Tristearin with low drug to lipid ratio (1:1) showed highest drug release of 87.90% at 12hrs, 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 (Table 5) and plotted as zero order, first order, Higuchi model and Korsmeyer - Peppas model (Figure 5). The regression values (R²) from the plots were tabulated in the Table 7. Considering the

regression values and 'n' 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.

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

Table5: The processing of in vitro drug release data of formulation F1 into different Kinetic models.



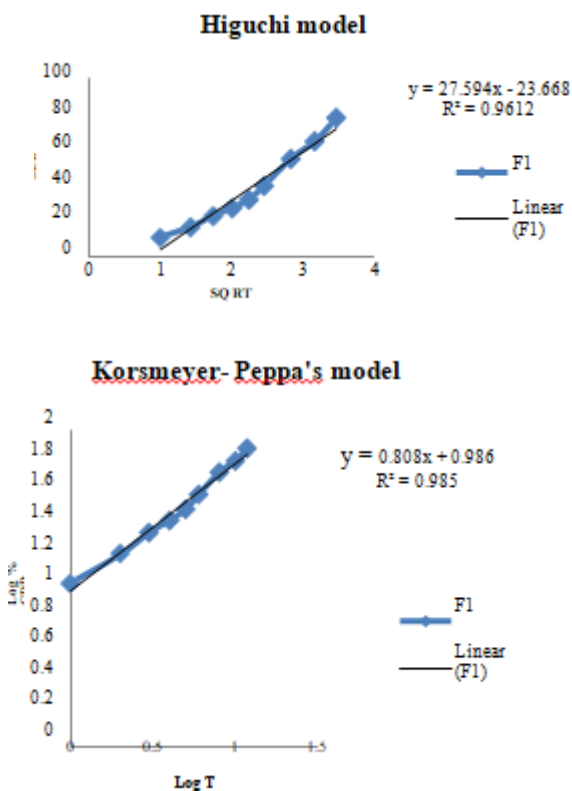


Figure6: The plots of in vitro drug release data of formulation F1 into different Kinetic models.

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

Table6: The regression (R2) and (n) values from the above plots.

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 Anomalous 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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