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FORMULATION AND EVALUATION OF ORLISTAT SOLID LIPID NANOPARTICLES

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ABSTRACT

Anti-obesity medications are pharmacological agents that reduce or control body weight.The aim of the present study is to design Orlistat solid lipid nanoparticles and to evaluate them. Orlistat solid lipid nanoparticles were prepared by using the different lipids like Tristearin, GMS and Compritol with Poloxamer and tween 80 as surfactants in combination with soy lecithin as a stabiliser by hot homogenization technique. No interaction was found between the drug and the lipids by the FTIR and DSC studies.The solid lipid nanoparticles were evaluated for particle size & PDI, zeta potential, entrapment efficiency and *in vitro* drug release. The particle size of the formulations ranged from 67.65 to 462.6 nm. PDI of all the formulations were good within the range 0.197 to 0.523. The zeta potential ranged from -15.2 to -43.42mV. Entrapment efficiency observed was in the range of 77.25 to 97.34%. The cumulative percentage release of optimized formulation F10 showed 93.73% drug release after 24hrs. The percentage release kinetic studies showed that the release was first order diffusion controlled and the n value (0.5206) obtained from the Korsmeyer-Peppas model indicated Anamolous (non-Fickian) diffusion type of release mechanism.

INTRODUCTION:

SLNs are the novel drug delivery system in which the active drug is incorporated into lipid carriers with the help of the stabilizers.[1] They are the submicron colloidal carriers ranging from 50 to 1000nm possessing various unique properties such as small size, large surface area, high drug loading, attractive for their potential to improve performance of pharmaceuticals.[2] SLNs avoid the limitations of polymeric nanoparticles, fat emulsions and liposomes.[3] Obesity is defined as an excess of body fat (increased fat cell size and number) relative to lean body mass. Clinically, obesity is defined on the basis of BMI.[4] Any individual with BMI 30kg/m^2 or more is classified as obese. Orlistat, a first in a class of anti-obesity agent which has been shown to reduce absorption of dietary fat by an average of 30% at a dose of 120mg 3 times daily. The resulting caloric deficit has a positive effect on weight control. The aim of the present study was to develop Orlistat nanoparticles using solid lipids and developed solid lipid nanoparticles provide sustained release of drug, thereby increases treatment efficiency.[5]

MATERIALS AND METHODOLOGY

Materials

Orlistat was procured from the swapanroop drug and pharmaceuticals,

Ahmadabad. Tristearin was purchased from sasaol, Germany. Compritol was obtained from Gattefose, France. Glycerylmonostearate was purchased from lab Fine Chem Industries, Mumbai. Methanol, Tween 80 and Chloroform were purchased from SD fine Chem limited, Bengaluru. Soy lecithin and Poloxamer were obtained from HI Media laboratories Pvt ltd, Bengaluru. All the reagents used were of analytical grade.

Methods

Determination of λ_{max} of Orlistat in phosphate buffer of pH 6.8

Accurately weighed quantity(100mg) of Orlistat was taken in 100ml volumetric flask and was dissolved by using phosphate buffer of pH 6.8 and the volume was made up to 100ml with phosphate buffer of pH 6.8 to produce 1000µg/ml solution. From the above stock solution, 10 µg/ml solution was prepared and scanned between 200nm and 400nm by using phosphate buffer of pH 6.8 as blank.[6] The absorption maxima obtained (218nm) is used for further studies.

Preparation of calibration curve in phosphate buffer of pH 6.8

Accurately weighed quantity(100mg) of Orlistat was taken in 100ml volumetric flask and was dissolved in phosphate buffer of pH 6.8. Finally, the volume was made up to 100ml with phosphate buffer of pH 6.8 to produce 1000µg/ml solutions (stock solution-I). 1, 2, 3, 4, 5, 6 and 7ml of stock Solution-I was taken and transferred to 10ml volumetric flask and volume was made up to 10 ml using phosphate buffer of pH 6.8. The absorbance of these solutions was determined in UV spectrophotometer at 218nm and the calibration curve was plotted.

Preparation of solid lipid nanoparticles of Orlistat

SLNs were prepared by hot homogenization technique. The lipid was first melted in a boiling tube using waterbath and then soy lecithin and drug was incorporated in to the melted lipid.Heating is continued till the compounds in the boiling tube melt. If they doesn't melt organic solvents like methanol and chloroform in the ratio 1:1 is added and heating is continued until all the organic solvents evaporates. Simultaneously in another beaker tween80 or poloxamer is taken and dissolved in 10 ml water (aqueous phase) and heated to temperature equal to that of lipid phase. Then the aqueous phase is transferred slowly into the lipid phase while homogenising the mixture at 20000rpm and homogenised for 5 mins under high speed homogenizer and then and then immediately sonicatedin probe ultrasonicator at 75% amplitude for 20 mins.[7]

Characterization of prepared solid lipid nanoparticles

Excipients Compatibility Study

Compatibility Study was carried out by use of IR spectroscopy and Differential Scanning Calorimetry (DSC) for the confirmation of any interaction between Orlistat and excipients.

Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR was performed using shimadzu FTIR 8300 spectrophotometer. An FTIR spectroscopy study has been carried out separately to check the compatibility between the drug (Orlistat) and the lipids (TS, GMS, CM) used for the preparation of SLNs. The spectra were recorded by scanning between frequency ranges of 4000-500cm^{-1} in a FTIR spectrophotometer.[8]

Differential Scanning Calorimetry

The melting point of the pure drug and compatibility of the drug with the lipids were studied by differential scanning calorimeter. [9] DSC was performed using Shimadzu DSC-60.

Size, Zeta potential and Polydispersity index

The mean particle size, Polydispersity index, zeta potential was analysed for each formulation by using laser diffraction principle in Malvern Zetasizer. These studies were performed at 25°C with an angle of detection 90°.[10]

Drug Content

About 0.2ml of drug loaded solid lipid nanoparticle dispersion was added into 5ml of methanol in centrifuge tube and vortexed for 15 minutes. Then centrifuged at 5000rpm for 30 mins and supernatant was collected. The supernatant was analysed for concentration of orlistat by UV spectrophotometer at 218nm. Drug content was calculated using following formula. [11]

$$\text{Drug content} = \frac{\text{Practical amount of the drug obtained}}{\text{Theoritical amount of drug added}} \times 100$$

Percentage Drug Entrapment Efficiency (%DEE)

About 2ml of drug loaded SLNs was taken and placed in outer chamber of the centrisart tube and the sample recovery chamber was placed on the top of sample. The unit was centrifuged at 5000rpm for 20 minutes. The SLNs along with encapsulated drug remains in the outer chamber and the aqueous phase was moves into the sample recovery chamber through filter membrane (molecular weight cut-off 20,000 Daltons). The resulting aqueous phase was analysed by UV spectrophotometer for Orlistat at 218nm. The entrapment efficiency was calculated by using the following equation. [10]

$$\%DEE = \frac{\text{Total amount of the drug} - \text{Amount of the drug in aqueous phase}}{\text{Total amount of the drug}} \times 100$$

***In vitro* Drug Release Study**

In vitro Drug Release Studies were carried out in *Franz diffusion cell* 2ml of drug loaded SLNs was placed in donar compartment while the receiver compartment consists of 22ml of diffusion medium, phosphate buffer of pH 6.8 maintained at 37±1℃ in Franz diffusion cell at 50 rpm. 1ml of aliquots was withdrawn at predetermined intervals. The samples were analysed for drug content by UV spectrophotometer at 218nm. Equal volumes of the diffusion medium were replaced into receiver compartment after each withdrawal to maintain sink condition. Three trails were carried out for all formulations. From the data obtained, the percentage drug release was calculated and plotted against function of time to study the pattern of drug release. Obtained in vitro drug release data was processed into kinetic models to study the mechanism of drug release.[11]

Results and Discussion

Preparation of standard graph of Orlistat using phosphate buffer at pH 6.8

Standard graph of Orlistat using phosphate buffer of pH 6.8 was plotted by taking concentration (µg/ml) on x-axis and absorbance on y-axis. Standard graph is shown in Fig-1. The regression equation obtained is y = 0.0115x – 0.0029 and R^2 = 0.9984

FTIR

Drug-lipid interactions if any, were studied by FTIR spectroscopy. The spectras obtained from FTIR spectroscopy study are shown in fig 2. Wavenumbers peaks of corresponding functional groups of Orlistat are shown in Table 2. Perusual to the FTIR spectra, the absorption peaks of Orlistat were retained in the physical mixture of drug with various lipids. The spectra of physical mixtures did not show the absence of vibration bands of Orlistat. It indicates that there was compatabilitybetween the drug and lipids.[12]

DSC

The thermal measurements of pure Orlistat and physical mixtures of Olistat and Tristearin, GMS and Compritol were carried out using DSC. The pure drug Orlistat showed peak at 41.79℃. In physical mixture of Orlistat andTristearin, Orlistat at 41.75℃. In physical mixture of Orlistat and GMS, Orlistat showed peak at 41.69℃. In physical mixture of Orlistat and Compritol, Orlistat showed peak at 41.54℃. It shows that the drug is stable with different lipids at different conditions. (fig 3 shows the DSC curve pure drug and the physical mixture of drug and lipids)

Table 1: Formulation design of SLNs using lipids Tristearin, GMS and Compritol.

Formulation No	Drug (mg)	TS (mg)	GMS (mg)	CM (mg)	Tw80 (mg)	Polx (mg)	Soy (mg)	Water (ml)
F1	10	100			50		50	10
F2	10	150			75		75	10
F3	10		100		50		50	10
F4	10		150		75		75	10
F5	10			100	50		50	10
F6	10			150	75		75	10
F7	10	100				50	50	10
F8	10	150				75	75	10
F9	10		100			50	50	10
F10	10		150			75	75	10
F11	10			100		50	50	10
F12	10			150		75	75	10

Drug – Orlistat, TS – Tristearin, GMS – Glycerylmonostearate, CM - Compritol, Tw 80 – Tween 80, Polx – Poloxamer, Soy – Soy lecithin

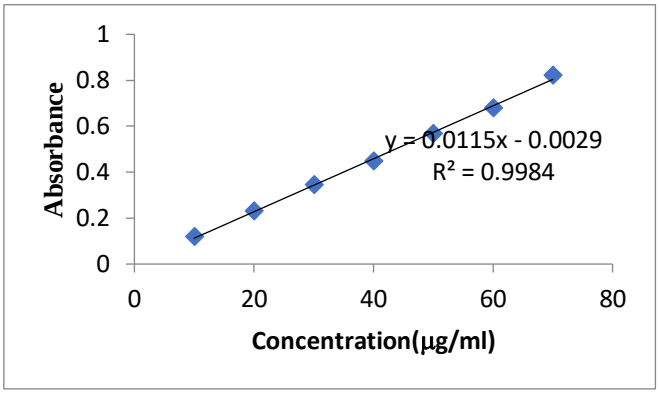
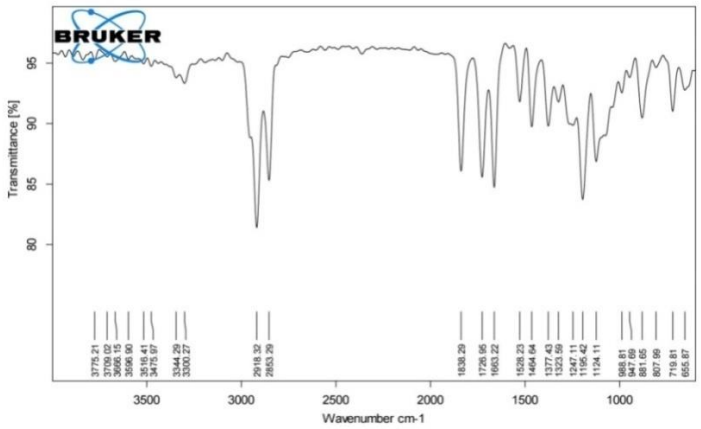
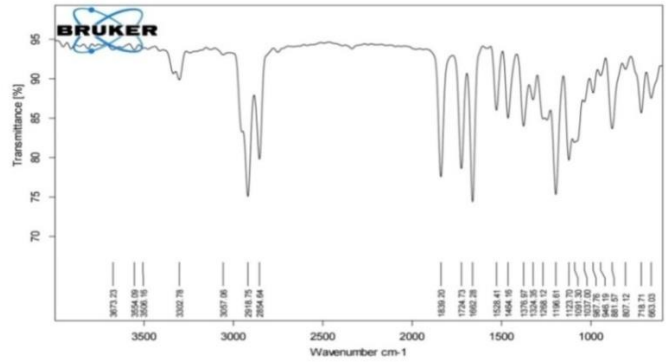


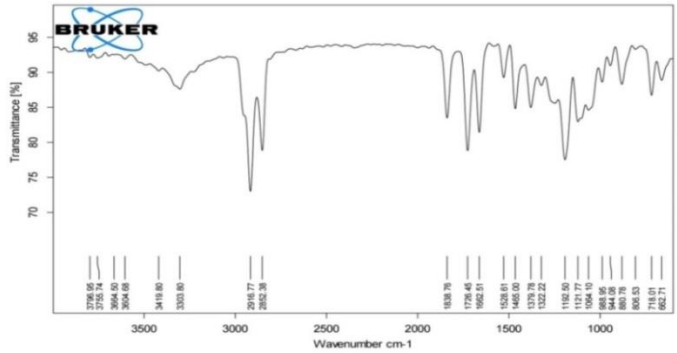
Figure 1: The standard graph of Orlistat using phosphate buffer of pH 6.8



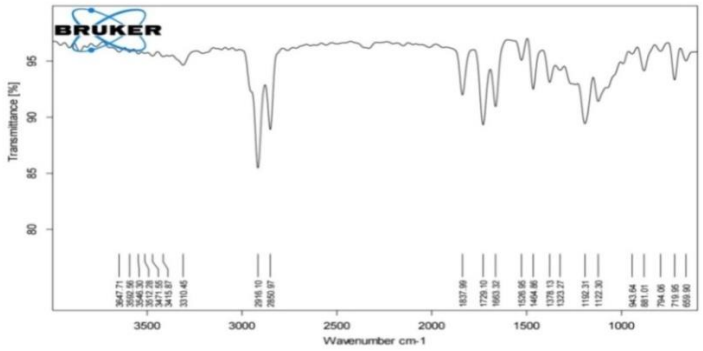
A) Pure drug



B) Physical mixture of Orlistat and Tristearin

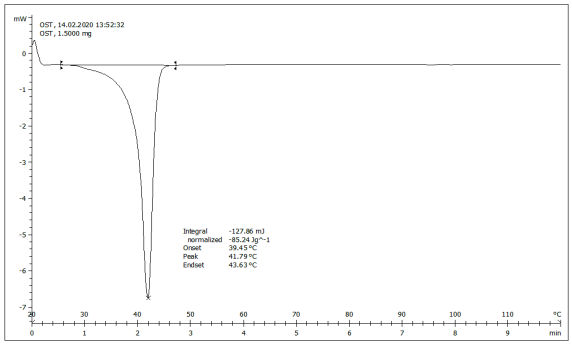


C) Physical mixture of Orlistat and GMS



D) Physical mixture of Orlistat and Compritol

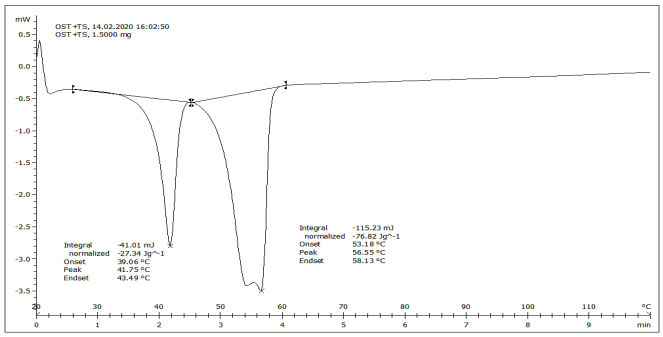
Figure 2 : FTIR spectra of A) Orlistat; B) Physical mixture of Orlistat and Tristearin:C) Physical mixture of Orlistat and GMS; D) Physical mixture of Orlistat and Compritol.



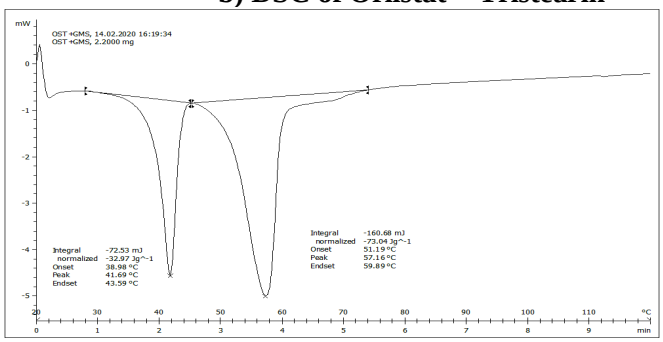
a) DSC of Pure drug

Table 2: Functional group and their wave number by FTIR of pure drug and its physical mixtures.

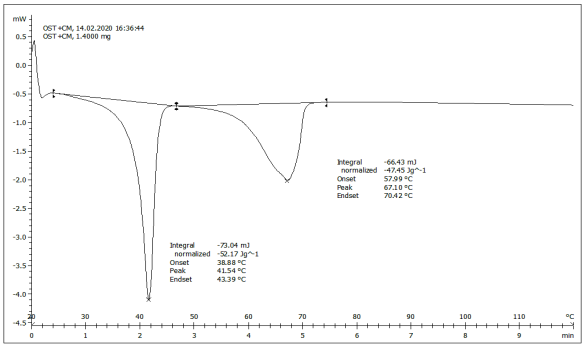
Sl.No	Name of the compound	Wave number (cm^{-1})	Functional group
1	Orlistat	1726.95 2918.32 3300.27 881.65 1464.64	C=O stretching C-H stretching N-H stretching C-H bending C=C stretching
2	Orlistat : Tristearin (1 : 1)	1724.73 2918.75 3302.78 881.57 1464.16	C=O stretching C-H stretching N-H stretching C-H bending C=C stretching
3	Orlistat : GMS (1 : 1)	1726.45 2916.77 3303.80 880.78 1465.00	C=O stretching C-H stretching N-H stretching C-H bending C=C stretching
4	Orlistat : Compritol (1 : 1)	1729.10 2916.10 3310.45 881.01 1464.86	C=O stretching C-H stretching N-H stretching C-H bending C=C stretching



b) DSC of Orlistat + Tristearin



c) DSC of Orlistat + GMS



d) DSC of Orlistat + Compritol

Fig 3 : Differential Scanning Calorimetrythermogram

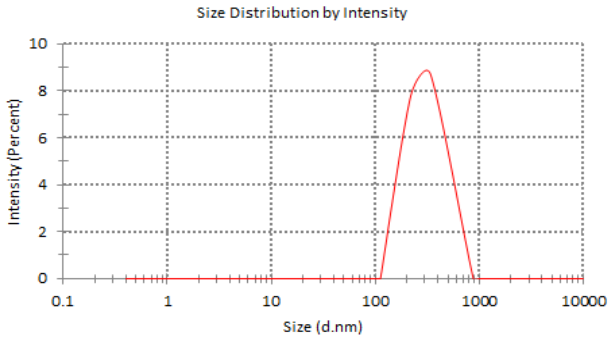


Figure 4 :Size distribution profile of optimized formulation F10

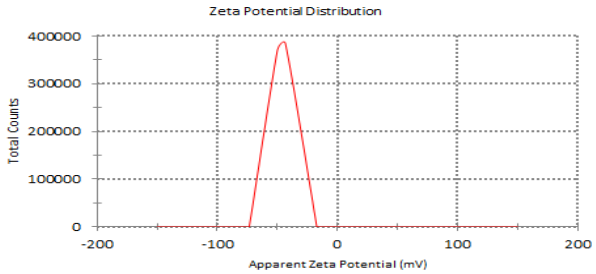


Figure 5 : Zeta potential profile of optimized formulation F10

Table 3 :The particle size, PDI and zeta potential of Orlistat prepared with Tristearin, GMS and Compritol using Tween 80 and poloxamer

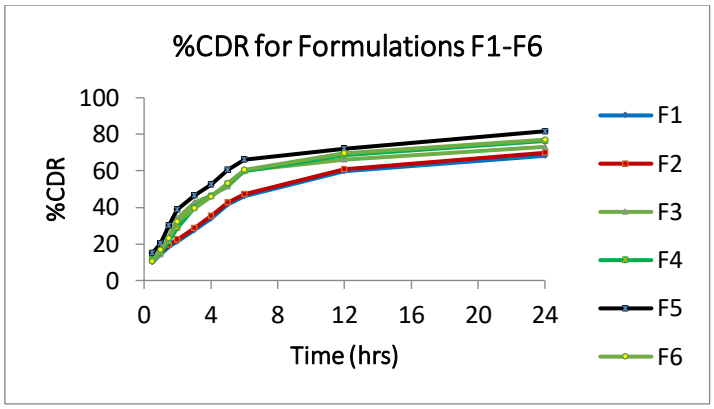
Formulation No	Particle size (d.nm)	PDI	Zeta potential (mV)
F1	98.8	0.211	-15.2
F2	67.65	0.308	-16.7
F3	112.5	0.228	-30.3
F4	106.3	0.364	-33.6
F5	313.2	0.197	-18.37
F6	418.4	0.286	-24.1
F7	114.54	0.223	-29.8
F8	131.9	0.352	-31.5
F9	462.6	0.245	-36.8
F10	339.72	0.373	-43.42
F11	389.4	0.523	-31.7
F12	445.1	0.478	-36.7

Table 4 : Drug content and Entrapment efficiency of all formulations

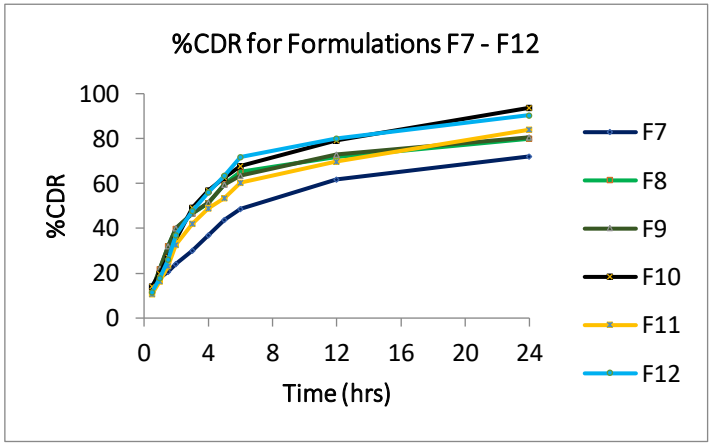
Formulation No	Drug Content	Amt of drug in Aqueous phase	Amt of drug in Lipid phase	Entrapment Efficiency
F1	96.72	1.065	8.935	89.35
F2	97.66	0.737	9.263	92.63
F3	98.41	0.532	9.468	94.68
F4	98.78	0.314	9.686	96.86
F5	94.41	2.042	7.958	79.58
F6	95.07	1.284	8.716	87.16
F7	98.38	1.007	8.993	89.93
F8	99.85	0.658	9.342	93.42
F9	97.54	0.488	9.512	95.12
F10	97.82	0.266	9.734	97.34
F11	96.36	2.275	7.725	77.25
F12	97.29	1.658	8.342	83.42

Table 5 : % Cumulative drug release for all formulations after 24 hrs

Formulation No	% CDR @ 24 hours
F1	68.23
F2	69.89
F3	73.16
F4	76.37
F5	81.68
F6	77.05
F7	72.07
F8	79.94
F9	80.58
F10	93.73
F11	83.96
F12	90.48



Percentage Cumulative Drug Release for formulations F1 - F6

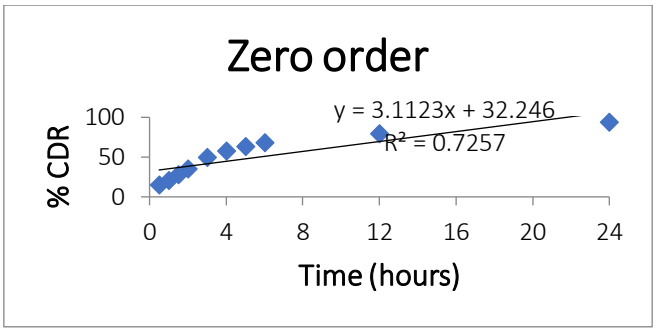


Percentage Cumulative Drug Release for formulations F7- F12

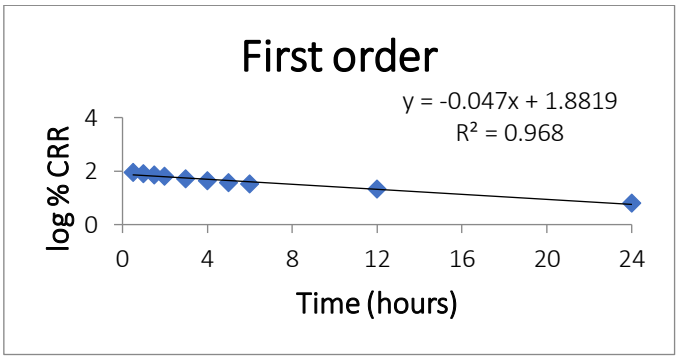
Figure 6: In vitro drug release profiles of Orlistat SLN formulations prepared with three different lipids and two surfactants.

Table 6 : The regression values of kinetic models of different formulations of Orlistat

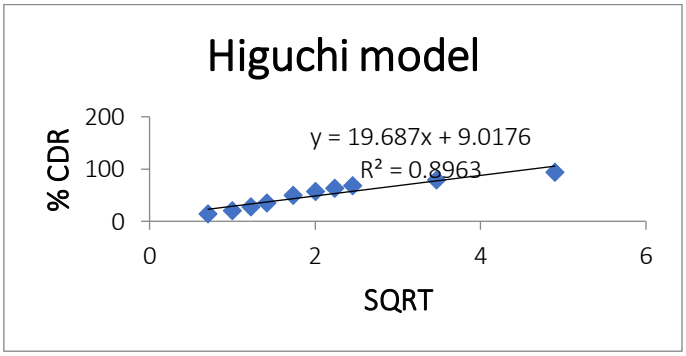
Formulation No	Regression factor			Korsmeyer – peppa's	
	Zero Order	First Order	Higuchi model	R^2	n values
F1	0.802	0.8956	0.9423	0.973	0.5389
F2	0.8065	0.9032	0.945	0.9754	0.5146
F3	0.643	0.7832	0.8358	0.8904	0.5346
F4	0.6888	0.8317	0.8706	0.9272	0.5461
F5	0.6572	0.8358	0.8469	0.9132	0.4656
F6	0.69	0.8364	0.8734	0.9237	0.5434
F7	0.8197	0.9193	0.9515	0.978	0.4748
F8	0.6412	0.8157	0.836	0.8877	0.4786
F9	0.6587	0.8369	0.8506	0.8985	0.4695
F10	0.7257	0.968	0.8963	0.9365	0.5206
F11	0.738	0.9231	0.9049	0.9304	0.5545
F12	0.6685	0.9049	0.8564	0.91	0.5628



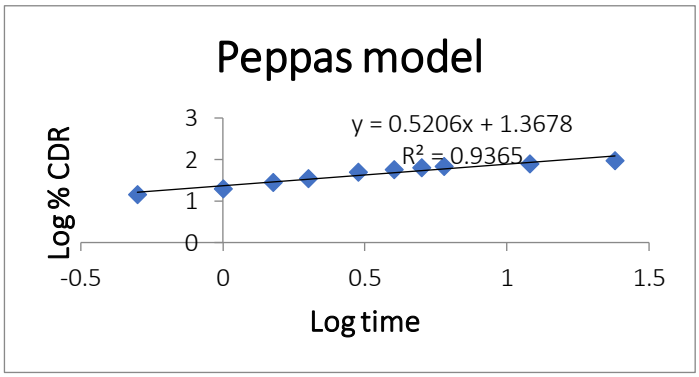
a) Zero order kinetics of optimised formulation F10



b) First order kinetics of optimised formulation F10



c) Higuchi model of optimised formulation F10



d) The peppas model plot of optimised formulation F10

Figure 7 :The kinetic models of optimized formulation F10

Particle size, PDI and Zeta potential

Size of Orlistat SLNs prepared with lipids Tristearin, GMS and Compritol using Poloxamer and tween 80 were in the range 67.65 to 462.6d.nm. PDI of all the formulations were good within the range 0.197 to 0.523. The zeta potential ranges from -15.2 to -43.42mV were shown in Table 3. Similarly, formulation F10 showed 167.82d.nm particle size with 0.211 PDI and –27.94 mV zeta potential as shown in fig 4 and figure 5.sizes were in nano range and zeta potential obtained was optimum showing good stabilization hence the formulation F10 is considered for optimized formula.

Drug Content and Entrapment Efficiency

The drug content estimation of formulations was carried out by extraction with methanol as mentioned in the methodology section. The drug content results were ranged between 94.41 to 99.85%. Average percentage entrapment efficiency of orlistat SLNs was good in the range of 77.25 to 97.34% and the loading efficiency was found to be in the range of 5.56 to 9.51% was shown in Table 4.

Release studies

The drug release from the nanoparticles was studied by Franz diffusion method. The cumulative percentage release of Orlistat from different orlistat nanoparticles i.e. F1 to F12 varied from 68.23 to 93.73% depending upon the drug, surfactant and the type of lipid used. Table5 and fig 6shows the percentage of cumulative drug release of all the formulations.

The experiment showed that the drug release from F10 formulations showed maximum drug release at 24 hrs.

Release kinetics

In vitro release data obtained from various formulations were fitted to various kinetic models to explore the kinetics and mechanism of drug release. R^2values for zero order kinetics were ranged from 0.643 to 0.8197 and for the first order kinetics ranged from 0.7832 to 0.968 and for Higuchi model ranged from 0.8358 to 0.9515. The regression values for Higuchi model is greater than zero and first order kinetic models. The regression value for Korsmeyer – peppas ranged from 0.8877 to 0.978. Data were fitted to Korsmeyer- peppas better with higher R^2 values, which indicate the drug release was Korsmeyer – Peppa's. Since, the n value obtained from Korsmeyer– peppa's model was more than 0.5. Hence the mechanism of drug release from these SLNs was Anomolous (non - Fickian) diffusion mechanism. The regression values of the formulations are listed in Table 6. The kinetic models of optimized formulation F10 are shown in fig 7.

CONCLUSION

SLNs containing Orlistat was prepared successfully using three lipids tristearin, GMS and Compritol by hot melt homogenization technique. The FTIR and DSC studies revealed that there is no chemical interaction between drug and selected lipids. In vitro release studies confirmed that the suitable percentage of drug released from the formulation F10 which indicates GMS and poloxamer as surfactant in the concentration of 1.5%. In addition, the developed SLNs have good particle size, PDI and zeta potential with good entrapment efficiency. Release kinetics studies showed that Orlistat released from the nanoparticles follows Quasi- Fickian diffusion mechanism. Based on the observations, it can be concluded that the formulated solid lipid nanoparticulate delivery system of Orlistat using widely accepted and pharmacologically safe lipids was capable of exhibiting sustained release properties for a period of 24 hours. Thus SLN formulations may reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug

REFERENCES

1. Kushwaha AK, Vuddanda PR, Karunanidhi P, Singh SK, Singh S. Development and evaluation of solid lipid nanoparticles of raloxifene hydrochloride for enhanced

bioavailability. Bio. Med. Res. Int., 2013; 2013; 1-9.
2. Ekambaram P, Sathali AA, Priyanka K. Solid lipid nanoparticles: areview. Sci.Rev. Chem. Commun, 2012;2(1): 80-102.
3. Madan JR, Khude PA, Dua K. Development and evaluation of solid lipid nanoparticles of mometasonefuroate for topical delivery. Int. J. Pharmaceu.Inve, 2014;4(2): 60-4
4. Ahirwar R, Mondal PR. Prevalence of obesity in India: A systematic review. Diabetes Metab Syndrome: Clin Res Rev. 2019;13(1):318-21.
5. Drew BS, Dixon AF, Dixon JB. Obesity management: update on Orlistat. Vasc Health Risk Manag. 2007:3(6)817-21
6. Shakir BS, Manikanta S, Jahnavi T. UV spectrophotometric determination of rupatadinefumerate in bulk tablet dosage form by using single-point standardization method. Int J Curr Pharm Sci 2019;11:120-4
7. Khames A, Khaleel MA, El-Badawy MF, El-Nezhawy AO. Natamycin solid lipid nanoparticles-sustained ocular delivery system of higher corneal penetration against deep fungal keratitis: preparation and optimization. Int. J. Nanomed, 2019; 14: 2515-31.
8. Nair R, Kumar AC, Priya VK, Yadav CM, Raju PY. Formulation and evaluation of chitosan solid lipid nanoparticles of carbamazepine. Lipids in health and disease, 2012; 11(1):72.
9. Srinivas P, Preeti S. Formulation and evaluation of gemcitabine hydrochloride loaded solid lipid nanoparticles. J Global Trends in Pharmaceu Sci., 2017; 5(4):2017-23.
10. Cirri M, Maestrini L, Maestrelli F, Mennini N, Mura P, Ghelardini c et al. Design, characterization and in vivo evaluation of Nanostructured Lipid Carriers(NLC) as a new drug delivery system for hydrochlorothiazide oral administration in pediatric therapy. *Drug delivery, 2018;25*(1): 1910-21.
11. Gouda R, Baishya H, Qing Z. Application of mathematical models in drug release kinetics of Carbidopa and levodopa ER tablets. J Dev Drugs, 2017; 6(02): 1-8.
12. Pawar AR, PravinDigambarChoudari, AshwinBalasahebNidhane, Oral bioavailability enhancement of sertraline hydrochloride by nanoprecipitation and solvent diffusion techniques for stable nanosuspension. Asian J. Pharmaceu, 2016; 10(03): 250-58.