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Design, development and evaluation of amlodipine besylate solid lipid nanopaticles

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Abstract
The aim of the present study is to develop Amlodipine Besylate solid lipid nanoparticles and to evaluate them. Recently, solid lipid nanoparticles have gained much attention of the researchers due to its reduced toxicity, improved stability. Amlodipine Besylate solid lipid nanoparticles were prepared by hot homogenization technique for the treatment of angio vascular disease. Amlodipine Besylate is a second generation angio selective calcium channel blocker. The design of Amlodipine Besylate solid lipid nanoparticles and their evaluation which would attenuate the problems associated with the oral administration of Amlodipine Besylate, and also easy to maintain the constant drug level in the blood. Amlodipine Besylate solid lipid nanoparticles were prepared by hot homogenization technique using different lipids (Tristearin, Glycerol monostearate and Compritol 888), soy lecithin used as a stabilizer and (tween 80, poloxamer-188) chosen as surfactants.
The nanoparticles were evaluated for particle size & PDI, zeta potential, entrapment efficiency and *in vitro* drug release. The particle size ranged from 96.37 to 812.2nm. PDI of all formulations were good within the range of 0.015 to 0.576. The zeta potential of blank SLN was -15.2 mV whereas drug loaded SLN showed zeta potential from -3.19 mV to -45.8mV. Entrapment efficiency observed was in the range of 82 to 95 %.
The cumulative percentage release of Amlodipine Besylate from different Amlodipine Besylate nanoparticles varied from 66 to 82 % depending upon the drug-lipid ratio and the type of lipid and surfactant used.
The release kinetic studies showed that the release was First order and the release mechanism was non-Fickian type.

Keywords: amlodipine besylate, solid lipid nanoparticles, FTIR, *in vitro* drug release

Introduction
Most of the people prefer oral route of administration as the major route of administration of pharmaceuticals having the advantage of being pain free, convenient to handle, noninvasive. However, oral dosage form has several disadvantages. Before the orally applied drug is able to reach its target, in most instances it needs to overcome multiple compartments of the human body, which is challenging for a broad spectrum of pharmaceuticals, especially for protein or peptide-based ones [1].
To overcome the disadvantages of oral route of administration, many new powerful drug substances have been found due to new technologies employed in drug discovery. The development of new drugs alone is not sufficient to ensure progress in drug therapy. The low bioavailability of the drug molecules due to poor permeability is the common problem. Drug targeting, remaining at the target site to deliver the drug are the several challenges that are faced during the development of an efficient drug delivery system. Therefore, there is a need to develop a drug carrier system that have no toxicity (acute and chronic), have sufficient loading capacity, drug targeting and controlled release characteristics. It should process good physical and chemical stability [2].
According to NNI (National Nanotechnology Initiative), Nanoparticles are defined as the solid colloid having the size in nanometers that ranges from 10-1000 nm atleast in one dimension (generally 50-500 nm). In which drugs can be adsorbed, entrapped, encapsulated or covalently attached and are produced by mechanical or chemical means. Nanoparticles can be formulated using lipid (solid at room temperature), natural polymer (chitosan, guar gum, gelatin, bovine serum albumin, human serum albumin, sodium alginate), synthetic polymer (poly d lactide, poly- E-caprolactane, polymethacrylate), semisynthetic polymer and protein. The materials for formulating the nanoparticles are selected according to their encapsulation capacity, drug stability, drug release pattern and their targeting capacity [3].
Nanoparticles have become one of the most important areas of research in the field of drug delivery due to their ability to deliver drugs to the right place, at appropriate times and in right dosage. The small sizes of nanoparticles give them unique physicochemical and biological properties which make it a suitable candidate for biomedical applications. Smaller the size of nanoparticles, macrophage uptake can be avoided due to which circulatory life span of drug is enhanced and suitable amount of drug can be delivered to a particular target site by active or passive transport mechanism [4].
Amlodipine Besylate is an anti-hypertensive drug. Systemic arterial hypertension is the most important modifiable risk factor for all-cause morbidity and mortality worldwide and is associated with increased risk of cardiovascular disease (CVD). Amlodipine Besylate is a second generation angio selective calcium channel blocker and inhibits the movement of calcium ions into vascular smooth muscles. Also used in the treatment of cerebrovascular stroke, neurodegenerative diseases, leukemia and breast cancer [5].

Matierals and methods
Amlodipine Besylate was purchased from Balaji drugs. Tristearin 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 Tween 80, 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

a. Pure drug (Amlodipine Besylate)
b. Physical mixture of drug + Tristearin (1:1).
c. Physical mixture of drug + GMS (1:1).

Preparation of solid lipid nanoparticles with Amlodipine Besylate using lipids (Compritol, Tristearin and Glycerol monostearate)

Solid lipid nanoparticles were prepared by using lipids (Compritol 888, Tristearin and glycerol monostearate) and surfactants (Tween 80 and Poloxomer 188). Lipid was first melted by heating in a boiling tube and then soy lecithin and drug was added to the lipid melt which was then heated to the temperature 5°C above the melting point of the lipid. Simultaneously, surfactant (poloxamer 188/tween 80) was dissolved in water in a test tube and heated to temperature equal to that of lipid phase. This aqueous phase was transferred to lipid phase in small quantities by continuous homogenization. This mixture was homogenized at 20,000 rpm for 15mins and then immediately placed in probe ultrasonicator at 75% amplitude for 15mins. Blank nanoparticles were prepared in a similar manner omitting the Amlodipine Besylate in the preparation [7].

Table 1: The composition of different formulations of Amlodipine Besylate SLNs prepared with GMS, Tristearin and Compritol.

Formulation number	Drug (mg)	GMS (mg)	TS (mg)	CM (mg)	Tween 80(mg)	Polaxomer (mg)	Soyalecithin (mg)	DW (ml)
F1	10	50	--	--	50	--	50	10
F2	10	100	--	--	50	--	50	10
F3	10	150	--	--	50	--	75	10
F4	10	--	50	--	50	--	50	10
F5	10	--	100	--	50	--	50	10
F6	10	--	150	--	50	--	75	10
F7	10	--	--	50	50	--	50	10
F8	10	--	--	100	50	--	50	10
F9	10	50	--	--	--	50	50	10
F10	10	--	--	150	50	--	50	10
F11	10	100	--	--	--	50	50	10
F12	10	150	--	--	--	50	50	10

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±0.1ºC at a measuring angle of 90º to the incident beam.[8]

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.[8]

Zeta potential

Zeta potential was measured using Malvern zeta sizer. Nanoparticles were diluted with distilled water and placed in a clear disposable zeta cell at 25 °C. The sample was subjected for 3 zeta runs to determine both size and zeta potential.[9]

Drug content

About 0.2 mL of drug loaded solid lipid nanoparticles was added into 5 mL of methanol in centrifuge tube. The solution was vortexed for 10 min and then centrifuged at 5000 rpm for 30 min. The supernatant was collected. Drug content in the supernatant was analyzed by UV- Spectrophotometer for Amlodipine Besylate at 238 nm.[10]

Drug content was calculated using following formula.

$$\% \text{ drug content} = \frac{\text{Practical amount of drug obtained}}{\text{Theoretical amount of drug}} \times 100$$

Percentage Drug entrapment efficiency (%DEE)

About 2 mL of solid lipid nanoparticles loaded with Amlodipine Besylate was placed in outer chamber of the centrisart device and the sample recovery chamber was placed on the top of the sample. The unit was 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 was 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 Amlodipine Besylate at 238nm. The entrapment efficiency was calculated by using the following relationship.[10]

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

Percentage Drug Loading Efficiency

Loading efficiency help us to deal with SLN's after their separation from the medium and to know their drug content.

$$\% \text{ Loading efficiency} = \frac{\text{Amount of drug in lipid phase}}{\text{Amount of lipid taken}} \times 100$$

***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 drug 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 sample was withdrawn at predetermined intervals. The samples were analyzed for the drug content by UV-Spectrophotometer at 238 nm. Equal volume of the diffusion medium was replaced in the receiver compartment after each withdrawal to maintain sink condition. Three trails were carried out for all formulations. From data obtained percentage cumulative drug release was calculated and plotted against function of time to study the pattern of drug release [11].

Kinetic Modelling of Drug Dissolution Profiles

The results of in vitro release profile obtained for all the formulations were plotted in modes of data treatment as follows:-

1. Zero order kinetic model – Cumulative % drug released versus Time.
2. First order kinetic model – Log cumulative percentage drug remaining versus Time.
3. Higuchi's model – Cumulative percentage drug released versus square root of Time.
4. Korsmeyer equation / Peppas model – Log cumulative percentage drug released versus log time.[12]

Results and discussion

Drug-polymer interaction study by FT-IR spectrophotometer

An FT-IR spectroscopy study had been carried out separately to check the compatibility between the drug (Amlodipine Besylate) and the lipids (TS and GMS) used for the preparation of nanoparticles. The FT-IR was performed for drug, lipids and physical mixture of drug and lipids. The spectra obtained from FT-IR spectroscopy study at wave number from 4000 to 400 cm-1 are shown below.

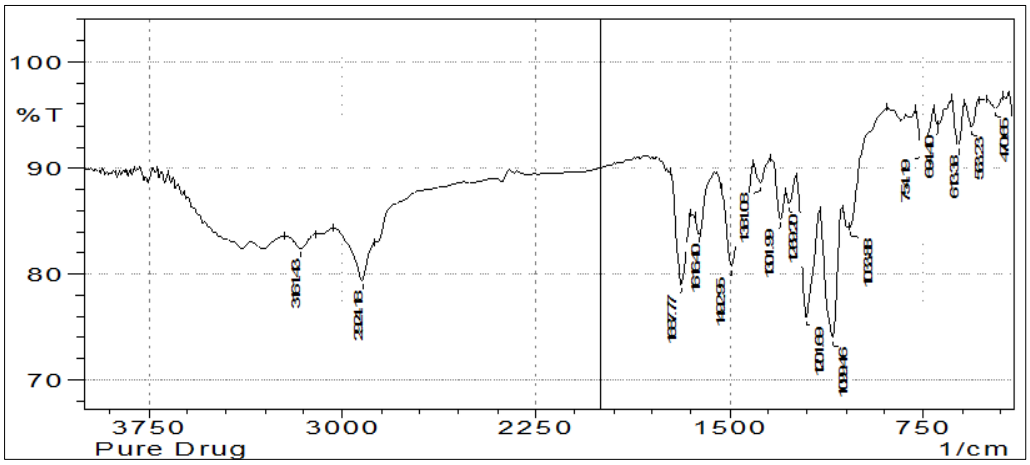

Fig 1: The FTIR spectrum of pure Amlodipine Besylate

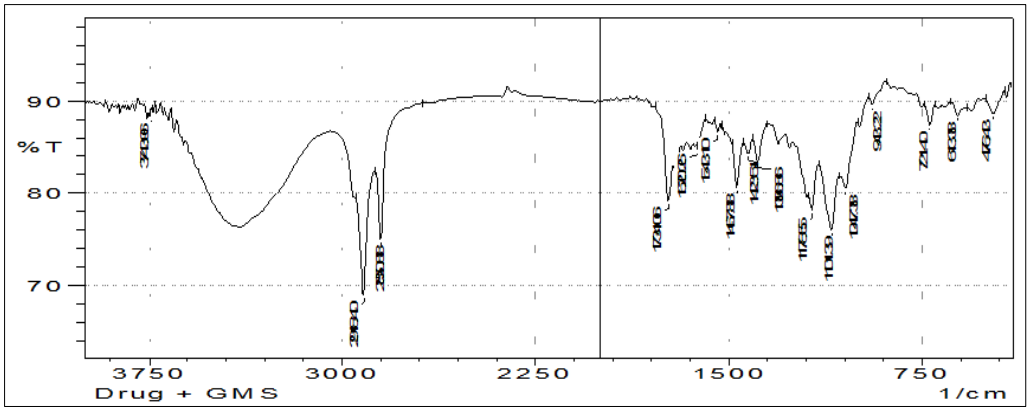

Fig 2: FTIR spectrum of Amlodipine Besylate +GMS.

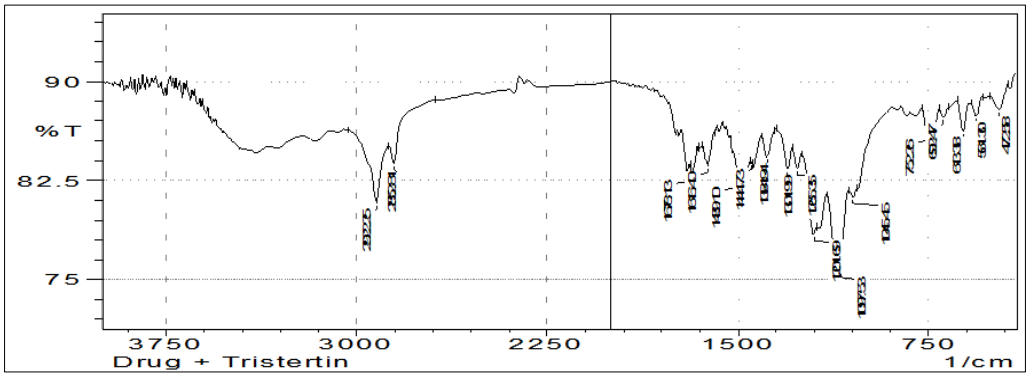

Fig 3: FTIR spectrum of Amlodipine Besylate+ Tristearin.

Table 2: Interpretation of FTIR spectrum

SI.NO	Name of the sample	Functional group	Characteristic Absorption (Cm^{-1})	Observed Peak(Cm^{-1})
1	Amlodipine Besylate	CH-stretching C=O-stretching =CH-stretching CN-stretching =CH-bending C-cl-stretching	3100-2900 1740-1600 2000-1650 1650-1500 1465-1150 750-600	2924.18 1616.40 1687.77 1492.96 1201.69 754.19
2	Amlodipine Besylate: GMS (1:1)	CH-stretching C=O-stretching =CH-stretching CN-stretching =CH-bending C-cl-stretching	3100-2900 1740-1600 2000-1650 1650-1500 1465-1150 750-600	2918.40 1734.06 1620.25 1467.88 1178.56 721.40
3	Amlodipine Besylate: TS (1:1)	CH-stretching C=O-stretching =CH-stretching CN-stretching =CH-bending C-cl-stretching	3100-2900 1740-1600 2000-1650 1650-1500 1465-1150 750-600	2922.25 1678.13 1616.40 1489.10 1201.69 752.26

The above table shows the interpretation of FTIR spectrum of drug Amlodipine Besylate. For example, the characteristic absorption of CH- stretching ranges from3100-2900 but the spectrum of pure drug with lipid mixture shows 2918-2924. Similarly the remaining absorption peak of different functional groups of drugs are retained in the formulation. Hence there is no interaction between drug and the lipid used in the formulation.

Determination of ƛmax of Amlodipine Besylate in methanol and phosphate buffer of pH6.8

A solution of Amlodipine Besylate was scanned in UV spectrophotometer between 200-400nm and Amlodipine Besylate shows absorbance max at 239nm for methanol and at 238nm for phosphate buffer pH6.8 and respectively.

Standard graph of Amlodipine Besylate using methanol and phosphate buffer of pH 6.8

Standard graphs of Amlodipine Besylate plotted in 2 to 25µg /ml concentration range in potassium dihydrogen orthophosphate buffer of pH-6.8 at 238nm using UV-Spectrophotometer. Regression equation obtained is Y=0.041x-0.042 and R2 = 0.998. This equation was used for estimation of in case of release studies, drug content estimation and determination of entrapment efficiency.

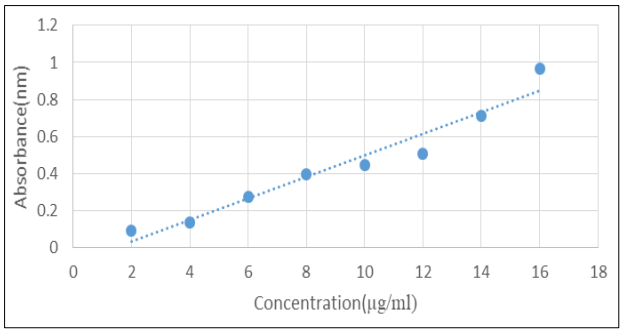

Fig 4: The Standard graph of Amlodipine Besylate in phosphate buffer of pH 6.8

Size, Pdi and Zeta Potential

Amlodipine Besylate SLNs prepared with lipids (Tristearin, Glyceryl monostearate and Compritol) using soy lecithin as stabilizer and tween 80, polaxomer 188 as surfactant are evaluated for their Particle size, PDI and zeta potential. Report of size and zeta potential was obtained from the zetasizer. Blank solid lipid nanoparticle was prepared using tristearin and polaxomer. The particle size of blank nanoparticles was found to be 91.5 nm whereas drug loaded particles were in the range of 96.37nm to 812.2nm.

PDI of all formulations were good within the range of 0.015 to 0.984.

The zeta potential of blank SLNs was -13.8, whereas drug loaded SLN showed zeta potential from -3.19 to -45.8mV.

Table 3: The particle size, PDI and zeta potential of the formulations.

Formulation Code	Particle Size(nm)	PDI	Zeta Potential (mV)
F1	478.9	0.471	-3.19
F2	250.8	0.356	-25.6
F3	532.7	0.481	-18.3
F4	96.37	0.576	-24.8
F5	812.2	0.548	-15.8
F6	801.7	0.527	-14.1
F7	71.07	0.345	-29.7
F8	127.89	0.015	-45.8
F9	146.15	0.485	-39.3

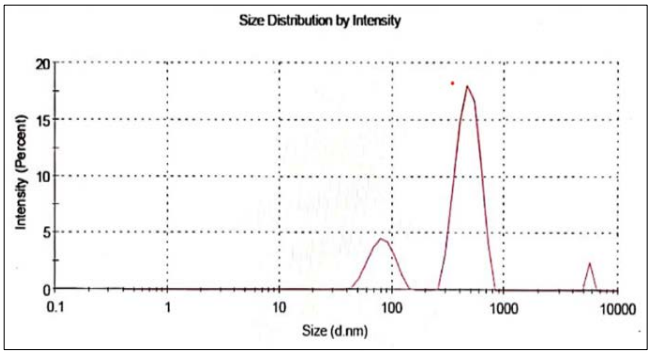

Fig 5: The size distribution of F1 formulation

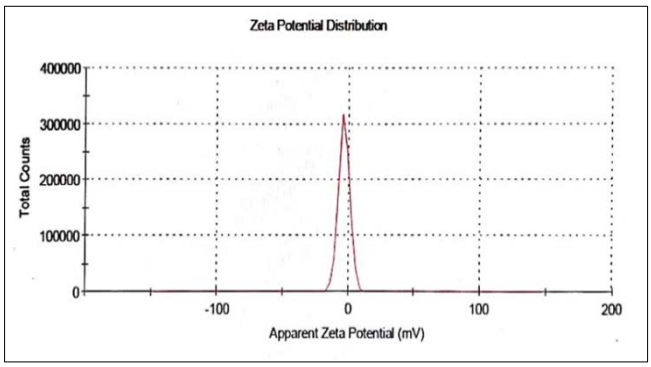

Fig 6: The Zeta potential of F1 formulation

Drug content

Drug content of prepared SLN of were done by extracting drug with methanol. The drug content results were ranged from 91.6% to 97.6 %. The formulation F6 containing GMS as lipid and Tween as surfactant shows highest drug content.

Entrapment efficiency (EE)

Average entrapment efficiency of Amlodipine Besylate SLNs was found to be in the range of 82% to 95% in all formulations using Tristearin, GMS and Compritol as lipid material. The formulation F7 shows (82.36%) lowest and formulation F6 shows (95.12%) entrapment efficiency.

Table 4: Entrapment Efficiency and Loading Efficiency of Amlodipine Besylate solid lipid nanoparticles.

Formulation No	%Drug Content	%Entrapment Efficiency	%Loading Efficiency
F1	91.6	91.25	8.16
F2	94.8	85.3	5.28
F3	93.6	92.64	4.12
F4	95.8	85.23	7.15
F5	92.4	83.69	6.69
F6	97.6	95.12	5.79
F7	95.6	82.36	6.48
F8	92.4	85.70	5.16
F9	92.6	87.37	4.25

Loading Efficiency

Loading efficiency of Amlodipine Besylate SLN prepared with Tristearin as lipid was decreased for formulation F1 (8.16) to F3 (4.12). This is because the amount of drug added in formulation is constant (10mg), the amount of lipid increased to 50mg to 150mg. As the lipid concentration increased, the projected loading efficiency obviously decreases. The formulation F3 also may have good loading efficiency i.e., obtained by increasing the amount of drug in the formulation. Similarly the loading efficiency decreased in case of GMS as the percentage increased the formulation F4 to F6. However the highest loading efficiency is observed for F1.

Table 5: Loading efficiency

Formulation Code	Amount of Amlodipine Besylate		Loading Efficiency (%)
	In Aqueous Phase (mg)	In Lipid Phase (mg)	
F1	0.702	9.298	8.98
F2	0.986	9.014	6.45
F3	0.572	9.45	4.72
F4	1.23	8.77	8.77
F5	2.25	7.75	5.16
F6	1.96	8.04	4.02
F7	1.52	8.48	8.48
F8	1.93	8.07	5.35
F9	1.41	8.59	4.29

Release studies

The drug releases from the nanoparticles were studied by Franz diffusion method. The *in vitro* release profiles of Amlodipine Besylate from Amlodipine Besylate nanoparticles. The cumulative percentage release of Amlodipine Besylate from different Amlodipine Besylate nanoparticles varied from 68.12 % to 85.8 % depending upon the drug, surfactant and the type of lipid used.

Table 6: Table showing the percentage cumulative drug release of F1-F9 at 12 hrs.

SL NO	TIME (HRS)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0.5	14.98	17.2	15.36	11.9	10.58	12.44	9.96	10.23	12.96
2	1.0	18.08	20.36	19.98	13.49	14.12	15.23	13.5	11.24	13.58
3	1.5	29.68	25.27	30.26	15.20	20.56	22.15	18.36	15.34	16.37
4	2.0	33.98	30.15	35.14	25.14	24.13	25.48	25.41	24.78	26.34
5	3.0	38.05	35.26	39.22	35.14	38.12	34.12	37.21	35.74	38.33
6	4.0	45.34	40.28	42.05	44.28	48.23	45.45	44.23	42.85	41.27
7	6.0	48.99	44.32	47.12	51.34	52.46	55.28	47.25	46.28	47.44
8	8.0	52.67	54.39	54.33	59.23	57.12	60.39	52.49	54.59	56.12
9	10	59.61	60.81	63.24	65.12	61.58	68.46	69.68	68.56	65.46
10	12	68.12	66.25	72.65	78.23	77.56	80.78	82.12	83.45	78.12

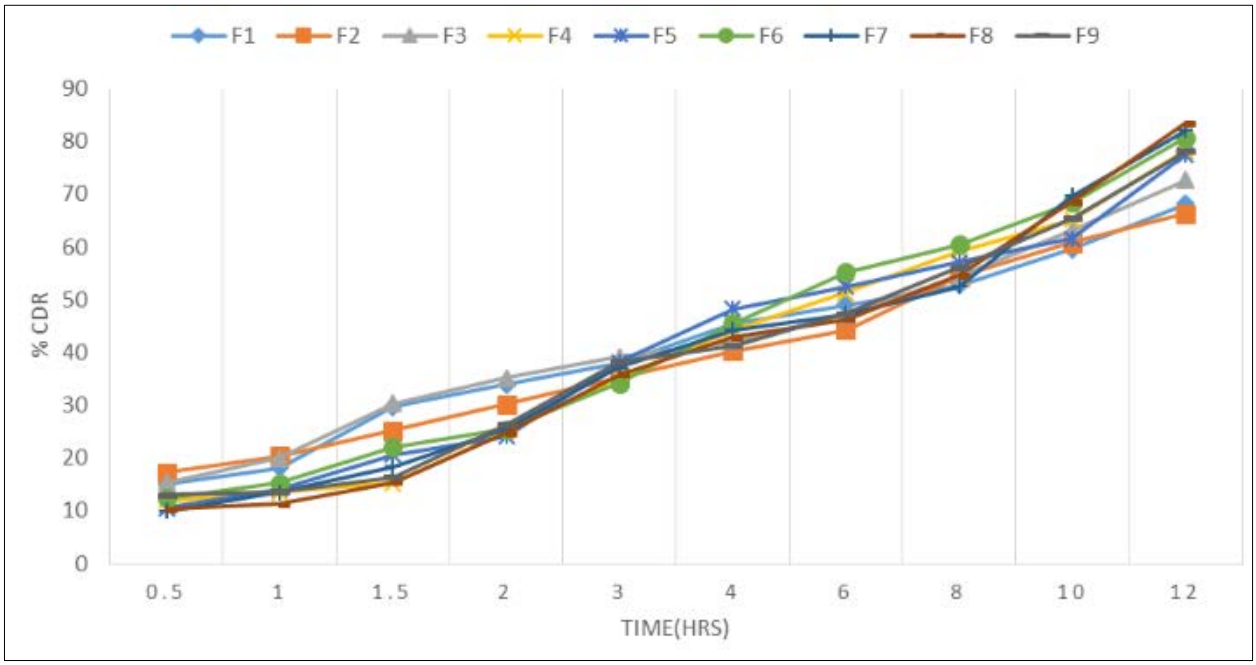

Fig 7: Percentage cumulative drug release profile of Amlodipine Besylate F1 to F9

Release kinetics

In vitro release data obtained from F1 to F9 formulations were fitted to various kinetic models. R^2 values for zero order kinetics were ranged from 0.812 to 0.975 and for the first order kinetics ranged from 0.836 to 0.909 and for the Higuchi model ranged from 0.960 to 0.987. Data were fitted to Higuchi model better with higher R^2 values, which indicate the drug release was Higuchi model. Since, the "n" value obtained from the Korsmeyer-Peppas model in case of optimized formulations was more than 0.5 hence the mechanism of drug release from these SLNs was anomalous i.e. non-Fickian.

The mechanism of drug release as per Korsmeyer Equation / Peppas model is shown below.

Table 7: The releases kinetic values for optimized F6 formulation

Time	log time	SQRT	% CDR	LOG % CDR	% CRR	% Log Unreleased
1	0	1	15.23	2.72	84.77	4.43
2	0.69	1.41	25.48	3.23	74.72	4.31
3	1.09	1.73	34.12	3.52	65.88	4.18
4	1.38	2	45.45	3.81	54.55	3.99
6	1.79	2.44	55.28	4.01	44.72	3.80
8	2.07	2.82	60.39	4.10	39.61	3.67
10	2.30	3.16	68.46	4.22	31.54	3.45
12	2.48	3.46	80.78	4.39	19.22	2.95

Conclusion

In this study, an attempt was made to formulate Amlodipine Besylate solid lipid nanoparticles using compritol, tristearin and glyceryl monostearate as carrier matrices, tween 80 and polaxomer 188 as surfactants, soy lecithin as stabilizer.

FT-IR were carried out to find out the possible interaction between the selected drug and lipids

(TS, and GMS). It revealed that there was no interaction between the selected drug and lipids.

Amlodipine Besylate solid lipid nanoparticles were prepared by hot homogenization technique. The method was able to produce nanoparticles of acceptable range and stability. All the formulations from F1 to F9 showed high entrapment efficiencies. SLNs were developed by taking three different types of lipids and with two different types of surfactants. Among the all batches, in case of F6, lipid and surfactant was optimized after considering their particle size, zeta potential and *in vitro* drug release profile.

Size, PDI and zeta potential of F1 to F9 formulations developed were in the acceptable and suitable range. Average entrapment efficiency of F1 to F9 formulations was found to greater than 70%.

The release kinetics revealed that the drug release follows Higuchi model. The release from Amlodipine Besylate nanoparticles from Korsmeyer-Peppas equation indicates the release mechanism was non-Fickian. Based on the observations, it can be concluded that the formulated lipid nanoparticulate delivery system of Amlodipine Besylate using widely accepted and physiologically safe lipids was capable of exhibiting sustained release properties for a period of 12 hours. They may be thus used to reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug.

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