AMERICAN JOURNAL OF PHARMTECH RESEARCH

Design, Development and Evaluation Of Anti-Hypertensive Drug Solid Lipid NanoParticles

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ABSTRACT

Recently, solid lipid Nano-particles have received much attention by the researchers owing to its biodegradability, biocompatibility and the ability to deliver a wide range of drugs. The aim of the present study was to design Diltiazem solid lipid Nano-particles and to evaluate them. Diltiazem solid lipid Nano-particles were prepared by hot homogenization technique using different lipids (Tristearin, GMS and Comprital), soy lecithin as stabilizers and tween 80, Poloxamer as surfactants. The Nano-particles were evaluated for particle size & PDI, zeta potential, entrapment efficiency and *in vitro* drug release. The particle size ranged from 49.7 to 523.7 nm. PDI of all formulations were good within the range of 0.189 to 0.427. The zeta potential ranged from -10.5 to -29.6 Mv, Entrapment efficiency of all formulations were observed was in the range of 78.68 to 95.23 %. The cumulative percentage release of Diltiazem from different Diltiazem Nano-particles varied from 53.36 to 88.74% depending upon the drug lipid ratio and the type of lipid used. The average percentage of drug released from different SLNs after 24 hours showed in the following order: F9 (53.35%) < F6 (56.75%) < F4 (61.74%) < F7 (63.8%) < F5(67.77%) < F8(69.04%) < F3(75.31%) < F1(79.36%) <F2 (88.74%) respectively. The release kinetic studies showed that the release was first order diffusion controlled and the n values obtained from the Korsmeyer-Peppa's model indicated the release mechanism was Quasi-Fickian type (n-value of 0.47).

Keywords: Diltiazem, solid lipid Nano-particles, FTIR, *in vitro* drug release.

*Corresponding Author Email: ashokkumarscp@gmail.com
Received 10 July 2021, Accepted 07 August 2021

Please cite this article as: Kumar PA *et al.*, Design, Development and Evaluation Of Anti-Hypertensive Drug Solid Lipid Nano Particles. American Journal of PharmTech Research 2021.

INTRODUCTION

Convenient route of drug administration because of higher compliance, lesser the conventional drug delivery system (oral route) is considered to be the most complications and lower cost as compared to the novel drug delivery system. But due to poor stability, poor Permeability they lack in the oral bioavailability. The physicochemical and metabolic instability in the stomach and the liver negatively influences the drug concentration in the blood.

Due to degradation of the drug (via hepatic first pass metabolism) there is chance of increased toxicity and the desired concentration of drug may not be able to reach the site of action (target site). Hence to overcome these problems associated with the oral route, the new colloidal drug delivery system was developed which provide controlled drug delivery system and the targeted drug delivery system.

Colloidal carrier drug delivery system such as emulsions, liposomes and polymeric micro and Nano-particles. The colloidal particles ranging in size between 10 and 1000 nm are known as Nanoparticles. They are usually manufactured from synthetic or natural polymers. And ideally suited to optimize drug delivery and reduce toxicity.[1,2]

The Nano-particles have the ability to penetrate through the several anatomical barriers due to their nanometre size and they can release the drug in the sustained manner.

Nano-particles for Drug Targeting

Nano-particles can be defined as solid, sub-micron, colloidal particles ranging in size from 10 nm to 1000 nm in diameter, generally but not necessarily made of natural or synthetic polymers, in which drugs can be adsorbed, entrapped, encapsulated or covalently attached and are produced by mechanical or chemical means. Nano-particles have become one of the most active 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 the right dosage. They have received considerable attention over the past 20 years due to their advantages[3] compared to other drug delivery systems.

Depending on the type of material or carrier used, four broad classes of Nano-particles are Recognized:

- ✓ Polymeric Nano-particles,
- ✓ Lipid based Nano-particles,
- ✓ Metal based Nano-particles and
- ✓ Biological Nano-particles.[4]

Solid Lipid Nano-particles[1]

Solid lipid Nano-particles introduced in 1991, represent an alternative carrier system to traditional colloidal system such as emulsions, liposomes and polymeric micro and nano-particles. SLN's are attracting major attention as novel colloidal drug carrier for intravenous application. SLN's are the submicron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid dispersed in water or in aqueous surfactant solution.

SLN's offer unique properties such as small size, large surface area, high drug loading and the interaction of phase at the interface and are attractive for their potential to improve performance of pharmaceuticals.

In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid Nano-particles.[5]

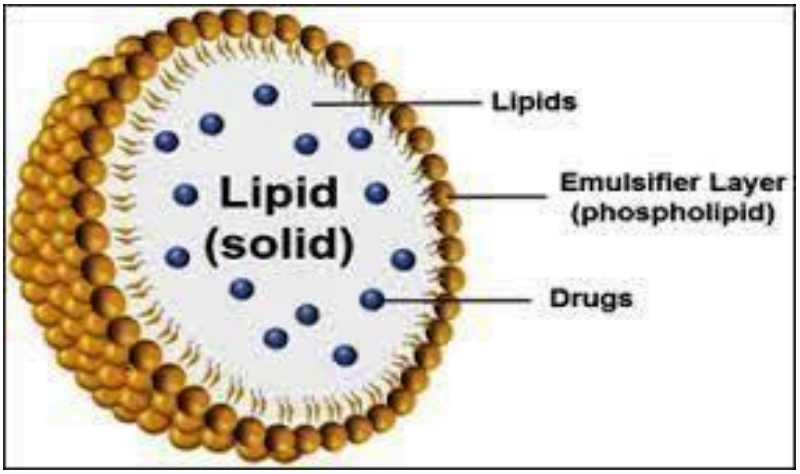

Figure 1: The structure of SLNs.

COMPOSITION OF NANO-PARTICLES[7-11] .

SLN's are the dispersions containing about 70-99.9% of water and about 0.1% - 30% w/w solid lipid dispersed in aqueous medium and if necessary stabilized with preferably 0.5 to 5% w/w of surfactant.

LIPID PHASE

The lipid matrix is composed of highly tolerable and biocompatible lipids possessing excellent biodegradability and low toxicity. The lipids used should possess the ideal characteristics.

- Stability and compatibility with drug molecule.
- Biocompatible and biodegradable.
- Ease of manufacture on a large scale.
- Flexibility to yield multiple release profiles.

Lipid biocompatibility and lack of toxicity are important consideration in the design of a drug delivery system. Especially those designed for systemic application.

SURFACTANTS

The type of the surfactant or surfactant mixture and their concentration used plays a major impact on the quality of SLN, since surfactants affects surface properties of SLN. The best choice contributes to higher stability by preventing particle aggregation more efficiently. In SLN the classes of surfactants used are (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion including phospholipids, bile salts, poloxamers and other ionic and non-ionic surfactants.

Table 1: The composition of SLNs

Lipids	Surfactants
Triacylglycerols:	Phospholipids:
Trimyristin	Soya lecithin
Comprital	Egg lecithin
Tristearin	
Aclyglycerol:	Ethylene oxide /propylene oxide
Glycerol monostearate	copolymer:
Glycerol behenate	Poloxamer188
	Poloxamer182
Fatty acids: Stearic acid	Sorbitan Ethylene oxide /propylene
	oxide copolymer:
Palmitic acid	Polysorbate 20
	Polysorbate 60
Waxes:	Alkylarylpolyehter alcohol polymers:
Cetylpalmitate	Tyloxapol

OTHER INGREDIENTS:

SLN dispersions, which are stabilized by electrical charge, are sensitive to the addition of electrolytes. Whereas, high salt concentrations induce an increase in viscosity and lead to formation of gels, low salt concentrations, in contrast reduce the dispersion viscosity. The observed viscosity effect in dispersions with ionic stabilizers is probably due to a shrinking of the particle electrical double layer by the influence of added electrolytes and corresponds to the reduction of the particle's effective volume fraction.

Methods of Preparation and principles for solid lipid Nano-particles.

SLNs are produced by several methods extensively described in the literature. The principle of all methods is based on generation of Nano emulsions by substituting oil for melt lipid using both high- and low-energy methods. Then additional cooling gives rise to lipid crystallization and the generation of SLNs.

The high energy approach for SLN production, in general consists in (i) keeping the lipid phase (plus potentially solubilized drug) 5–10 °C above its melting point, (ii) premixing it with the aqueous surfactant solution at the same temperature, (iii) Nano- emulsifying the pre-emulsion using a high-energy method (high pressure homogenizer or high shear sonication and finally, (iv) cooling it down to room temperature to crystallize the lipids. The low energy approaches for SLN are either based on generation of Nano-emulsions by spontaneous emulsification (lipid melt instead of oil) generated by solvent evaporation or diffusion so rapid displacement of the surfactants from the oily to aqueous phase, or other based on formation of a micro-emulsion above melting point of the lipid (lipid melt/SAA/coSAA/water system) followed by cooling to precipitate the Nano-particles.

In brief, methods used for SLN production include:

1. Homogenization followed by Ultrasonication
2. High pressure homogenization
    - Hot homogenization
    - Cold homogenization
3. Ultrasonication/high speed homogenization
    - Probe ultrasonication
    - Bath ultrasonication
4. Solvent evaporation method.
5. Solvent emulsification-diffusion method
6. Supercritical fluid method
7. Micro emulsion-based method
8. Spray drying method
9. Double emulsion method

MODEL OF DRUG RELEASE.[6,10]

The general principles of drug release from lipid Nano-particles are as follows:

1. There is an inverse relationship between drug release and the partition coefficient of the drug.
2. Higher surface area due to smaller particle size in nanometre range gives higher drug release.
3. Slow drug release can be achieved when the drug is homogenously dispersed in the lipid matrix. It depends on type and drug entrapment model of SLN. Crystallization behaviour of

the lipid carrier and high mobility of the drug lead to fast drug release. There is an inverse relationship between crystallization degree and mobility of drug.

The drug incorporation model of SLN is crucial to the drug release pattern. It is related to the composition and production method of SLN as explained above. For instance, the drug-loaded lipid phase remains mainly in the solid state in the case of production by cold homogenization technique. The solid solution drug incorporation model appears here. Drug release is prolonged over several weeks since mobility of the drug molecularly dispersed in colloidal particles is very limited.

Fast initial drug release (burst effect) exists in the first 5 minutes in the drug-enriched shell model (i.e. about 100% within <5 min) as a result of the outer layer of the particles due to the large surface area of drug deposition on the particle surface. The burst release is reduced with increasing particle size and prolonged release could be obtained when the particles were sufficiently large, i.e. lipid Nano-particles. The type of surfactant and its concentration, which will interact with the outer shell and affect its structure, should be noted as the other important factor, because a low surfactant concentration leads to a minimal burst and prolonged drug release.

The particle size that affects drug release rate directly depends on various parameters such as composition of SLN formulation (such as surfactant/surfactant mixture, amount of drug incorporated, structural properties of lipid and drug), production methods and conditions (such as time, production temperature, equipment, sterilization and lyophilization).

MECHANISM OF ABSORPTION OF SLN.[13, 14, 15]

The general property of Nano-particles is that they are adhesive (this is a general behaviour of all Nano-particles, not specific for lipid Nano-particles). The adhesiveness of particles to a surface increases with the increase of the surface area of the particles and after adhesion to the gut wall the drug is exactly released at its place of absorption. The lipid absorption enhancing effect can be explained by the degradation of the lipids by enzymes in the gut leading to the formation of surface-active mono and di-glycerides on the surface of the lipid Nano-particles. These molecules detach and form micelles. During the detachment and micelle forming process, the drug dissolved in the lipid is taken up in the micelle (solubilized). The formed micelles interact with surface-active bile salts leading to the formation of the so-called "*mixed micelles*." In the subsequent absorption process of the lipid degradation product, the drug is simultaneously absorbed. These two mechanisms are exemplified in Figure 2

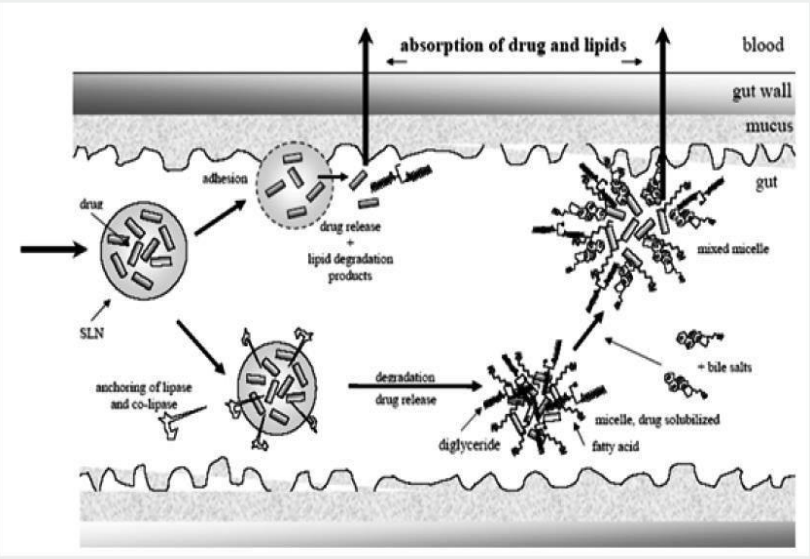

Figure 2: Mechanism: Lipid Nanoparticles for oral bioavailability enhancement [13].

Figure 2: Mechanism of absorption promotion effect of lipid being formulated as lipid Nanoparticles.

STABILITY.[6, 16, 17]

The physical properties of SLN's during prolonged storage can be determined by monitoring changes in zeta potential, particle size, drug content, appearance and viscosity as the function of time. External parameters such as temperature and light appear to be of primary importance for long term stability. The zeta potential should be in general, remain higher than -60mV for a dispersion to remain physically stable. Common disadvantages of SLN include particle growth, unpredictable gelation tendency, unexpected dynamics of polymorphic transitions, inherently low incorporation capacities due to crystalline structure of solid lipid, drug expulsion after polymorphic transition during storage the dispersions (70-99.9%) have been observed. The preparation, method of choice of lipids and surfactants could be optimized to increase their stability.

TOXICOLOGICAL CONCERNS:

To formulate parenteral lipid- based carriers, surfactant of qualified status must be employed, e.g. lecithin, Tween 80, Poloxamer 188, Span 85 and Sodium glycocholate. It can be assumed that the cytotoxicity of the **SLN** can be mainly attributed to components of the aqueous phase, especially to non-ionic emulsifiers and preservatives that have eventually been used. The interactions of SLN and their respective cytotoxicity were studied with human granulocytes, showing low uptake by phagocytosis resulting in prolonged duration time in blood. Furthermore, when performing bolus injections into mice good tolerability was found for most of the surfactants coating SLN. After autopsy and histopathology no significant evidence was documented that SLN were acutely toxic to tested animals.

Advantages of SLNs.[6]

Control and / or target drug release.

- Excellent biocompatibility
- Improve stability of pharmaceuticals.
- High and enhanced drug content.
- Easy to scale up and can be subjected to commercial sterilization Procedures.
- Better control over release kinetics of encapsulated compounds.
- Enhanced bioavailability of entrapped bioactive compounds.
- Chemical protection of labile incorporated compounds.
- Conventional emulsion manufacturing methods applicable.
- Very high long-term stability.
- Application versatility.

Disadvantages of SLN's

- Particle growth.
- Unpredictable gelation tendency.
- Unexpected dynamics of polymeric transitions.

APPLICATIONS OF SOLID LIPID NANO-PARTICLES.[11]

A) Oral SLNs in anti-tubercular chemotherapy

Anti-tubercular drugs such as Rifampicin, Isoniazid, Pyrazinamide-loaded SLN systems, were able to decrease the dosing frequency and improve patient compliance. The nebulization in animal by incorporating the above drugs in SLN also reported for improving the bioavailability of the drug.

B) SLNs for topical use

SLNs have been used for topical application for various drugs such as Tropolide, Imidazole, Flurbiprofen and Glucocorticoids. The penetration of Podophyllotoxin-SLN into stratum corneum leads to the epidermal targeting.

C) SLNs as a targeted carrier for anticancer drug to solid tumour's

SLNs have been reported to be useful as drug carriers to treat neoplasms. Tamoxifen, an anticancer drug incorporated in SLN to prolong release of drug after i.v administration in breast cancer and to enhance the permeability and retention effect.

D) SLNs in breast cancer and lymph node metastases

Mitoxantrone-loaded SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of drug. Efficacy of Doxorubicin (Dox) has been reported to be enhanced by incorporation in SLNs. The system has enhanced its efficacy and reduced breast cancer cells.

E) SLNs as gene vector carrier

SLN can be used in the gene vector formulation. In one work, the gene transfer was optimized by incorporation of a diametric HIV-1 HAT peptide (TAT 2) into SLN gene vector. There are several recent reports of SLN carrying genetic/peptide materials such as DNA, plasmid DNA and other nucleic acids.

F) SLNs as cosmeceuticals

The SLNs have been applied in the preparation of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers. The in vivo study showed that skin hydration will be increased by 31% after 4 weeks by addition of 4% SLN to a conventional cream.

Hypertension[18]

High blood pressure (hypertension) is a common condition in which the long-term force of the blood against your artery walls is high enough that it may eventually cause health problems, such as heart disease. Blood pressure is determined both by the amount of blood your heart pumps and the amount of resistance to blood flow in your arteries. The more blood your heart pumps and the narrower your arteries, the higher your blood pressure

Signs and Symptoms[19]

Most people with high blood pressure have no signs or symptoms, even if blood pressure readings reach dangerously high levels. A few people with high blood pressure may have headaches, shortness of breath or nosebleeds, but these signs and symptoms aren't specific and usually don't occur until high blood pressure has reached a severe or life-threatening stage.

There are two types of high blood pressure.

Primary (essential) hypertension

For most adults, there's no identifiable cause of high blood pressure. This type of high blood pressure, called primary (essential) hypertension, tends to develop gradually over many years.

Secondary hypertension

Some people have high blood pressure caused by an underlying condition. This type of high blood pressure, called secondary hypertension.

Anti-Hypertensive drugs[20]

Antihypertensive drugs comprise several classes of compound with the therapeutic intention of preventing, controlling, or treating hypertension. The classes of antihypertensive drug differ both structurally and functionally. They are important in anesthetic practice because they are commonly prescribed to the general population, with the overall prevalence of hypertension being 31% in the UK [defined by the National Institute for Health and Care Excellence (NICE) as a measurement of 140/90 mm Hg or higher in clinic, with subsequent ambulatory or home measurement of 135/85 mm Hg or higher]. Antihypertensive drugs are used frequently in other unrelated conditions, for example, β-blockers in thyrotoxicosis and anxiety, or angiotensin-converting enzyme inhibitors (ACEIs) in heart failure. Hence both the drug and its indication are relevant to the conduct of anesthesia.

MATERIALS AND METHOD

Diltiazem was purchased from Yarrow chemicals, Mumbai, 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 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.

The samples analyzed by FT-IR include

a. Pure drug (Diltiazem)

b. Physical mixture of drug + Tristearin (1:1).

c. Physical mixture of drug + GMS (1:1).

d. Physical mixture of drug + Compritol (1:1).

Preparation of solid lipid nanoparticles with Diltiazem 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 tween 80 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.

Table 1: Composition of different formulation of Diltiazem SLN's prepared with Tristearin, GMS and Comprital using Tween-80.

Formulation Code	Drug (mg)	TS (mg)	GMS (mg)	CM (mg)	Soy (mg)	Tw 80(mg)	DM 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

Particle Size, Polydispersity index and Zeta potential:

Particle size analysis The particle size was determined by dynamic light scattering, using a Malvern zeta sizer 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.

Polydispersity index:

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

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.

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 Diltiazem at 231nm.

Drug content was calculated using the following formula

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

Percentage Drug entrapment efficiency (%DEE):

About 2ml of Diltiazem 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 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 Diltiazem at 229nm. The % Drug entrapment efficiency was calculated by using the following relationship.

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

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 Diltiazem 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 229nm. 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.

Determination of λ max of Diltiazem in methanol

Accurately weighed quantity of 100 mg of Diltiazem was taken in 100 ml volumetric flask and it was dissolved in methanol and made up to 100 ml using methanol.

Scanning: From the above stock solution, 10 µg/ml solution was prepared and scanned between 200-400 nm by keeping methanol as blank. The absorption maxima of 237 nm for Diltiazem was obtained and used for further studies.

Standard plot of Diltiazem in methanol:

Standard solutions of Diltiazem in methanol (10-80µg/ml) were prepared and measured at 237nm using UV-Spectrophotometer. The standard plot of Diltiazem was as shown in (Figure). The

obtained correlation coefficient was 0.9981 and the regression equation y = 0.0111 x + 0.0409 was used to calculate the concentration of unknown samples of Drug content estimation.

Preparation of calibration curve in phosphate buffer of pH 7.4 and methanol

Accurately weighed quantity of 10 mg of Diltiazem was taken in two 100 ml volumetric flasks and it was dissolved in phosphate buffer and methanol separately (Stock Solution-I 100µg/ ml). From Stock Solution I, 1,2,3,4,5 and 6ml was taken and transferred to 10 ml volumetric flasks and volume was made up to 10 ml using volume with phosphate solution and methanol respectively. 10, 20, 30,40,50and 60 µg/ml solutions respectively. The aliquots were analysed at 237 nm. The plot of concentration v/s absorbance was plotted.

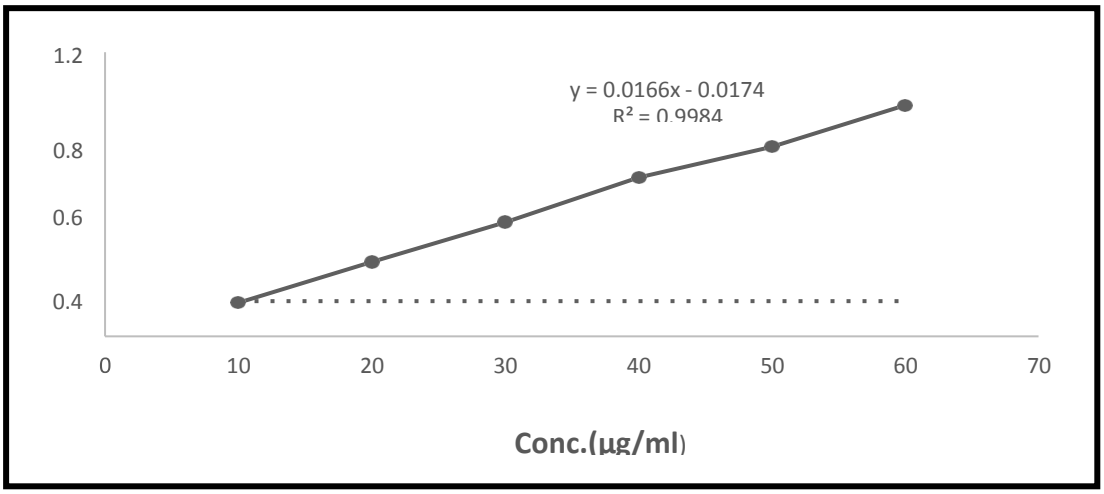

Figure 3: calibration curve

FT-IR Study The FTIR spectrum analysis was used to know the drug – excipients compatibility. The FTIR was performed for the drug (DPH), lipids (TS, GMS and CP), and physical mixture of drug and lipids. Figures 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 Diltiazem so there is no interaction between the drug and selected lipids.

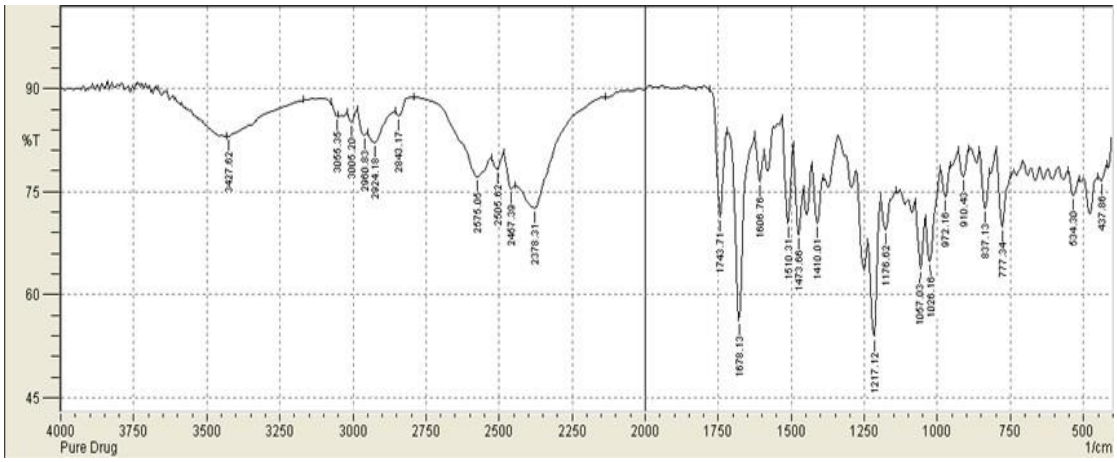

Figure 4: **The FTIR spectrum of Pure Diltiazem**

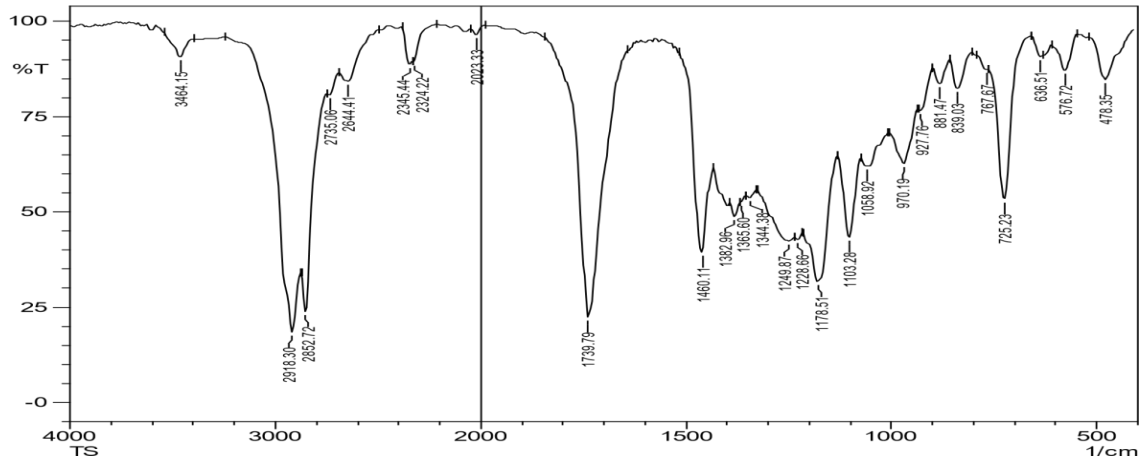

Figure 5: The FTIR Spectrum of Tristearin

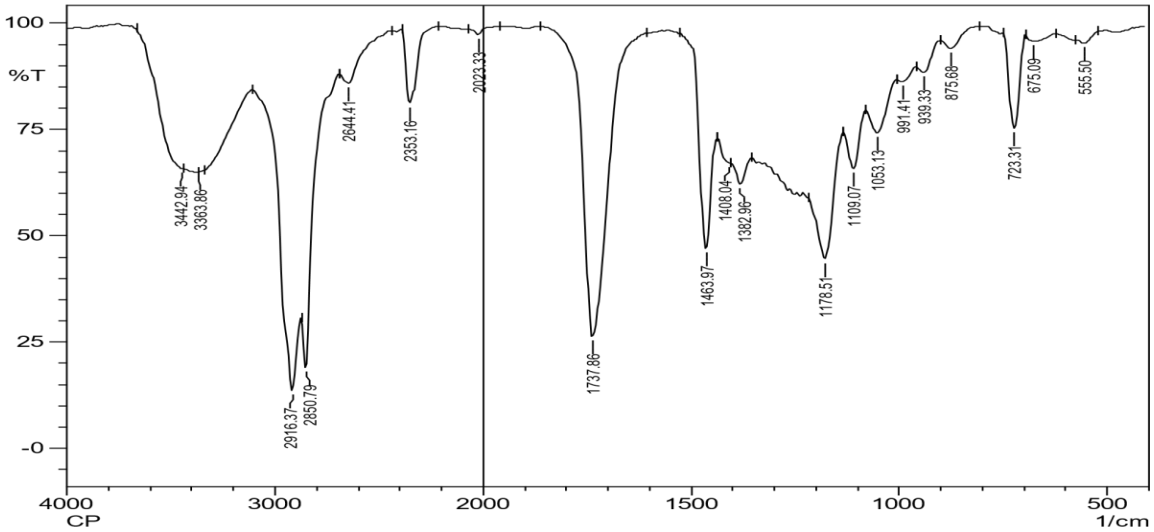

Figure 6: The FTIR Spectrum of Comprital.

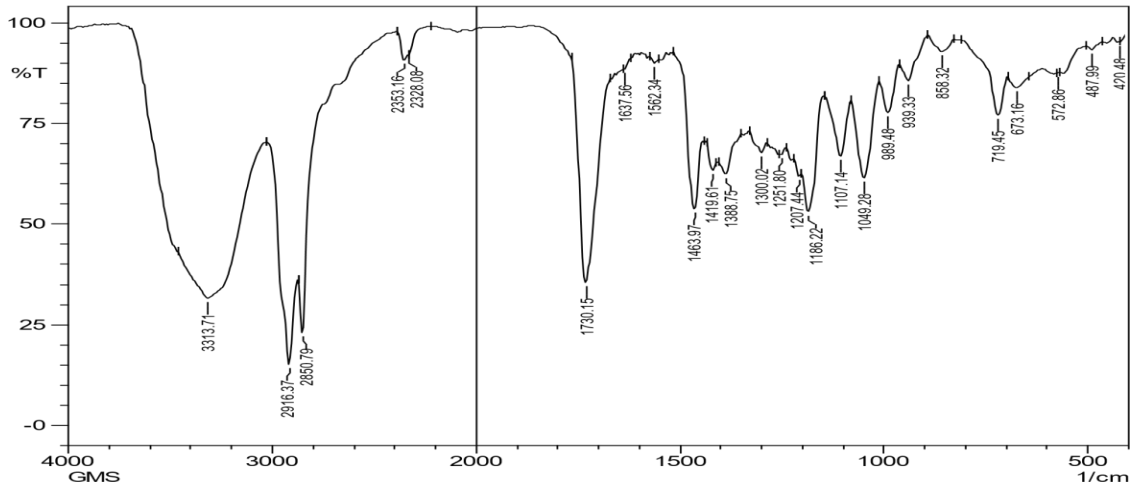

Figure 7: The FTIR Spectrum of GMS

Table 2: Interpretation of FTIR spectrum of above figures.

Sr. no	Name of the Compound	Wave number (cm^{-1})	Functional group
1	Diltiazem	1026,16.1176.62 and 1217.2	C-O Stretching
		1410.01 and 1476.66	C-H BendingN-H
		1510.31 and 1678.13	Bending
		2843.17 and 2924.18	C-H Bending
		3055.35 and 3427.62	O-H Stretching
2	Diltiazem :Tristearin	1026.16 and 1176.62	C-O Stretching
	(1:1)	1402.30 and 1467.88	C-H Bending
		1512.24 and 1678.13	N-H Bending
		2850.88 and 2920.32	C-H Stretching
3	Diltiazem : GMS	1176.62 and 1219.05	C-O StretchingC-H
	(1:1)	1408.08 and 1465.96	Bending N-H Bending
		1508.38 and 2920.32	C-H Stretching
		2850.88 and 2920.32	O-H Stretching
		3043.77 and 3421.83	
4	Diltiazem :	1024.24 and 1219.05	C-O StretchingC-H
	Compritol (1:1)	1408.08 and 1467.88	Bending N-H Bending
		1512.24 and 1678.20	C-H Stretching
		2850.88 and 2920.37	O-H Stretching
		3039.91 and 3417.98	

Particle size, PDI and Zeta Potential:

Particle size analysis of the Diltiazem solid lipid Nano particles was done by theMalvern system.

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

Formulation Code	Particlesize (d. nm)	PDI	Zeta potential (mV)
F1	433.9	0.189	-22.6
F2	523.7	0.487	-37.4
F3	49.7	0.204	-8.85
F4	119.7	0.256	-23.9

F5	97.67	0.278	-30.7
F6	133.0	0.279	-42.0
F7	108.0	408.0	-15.2

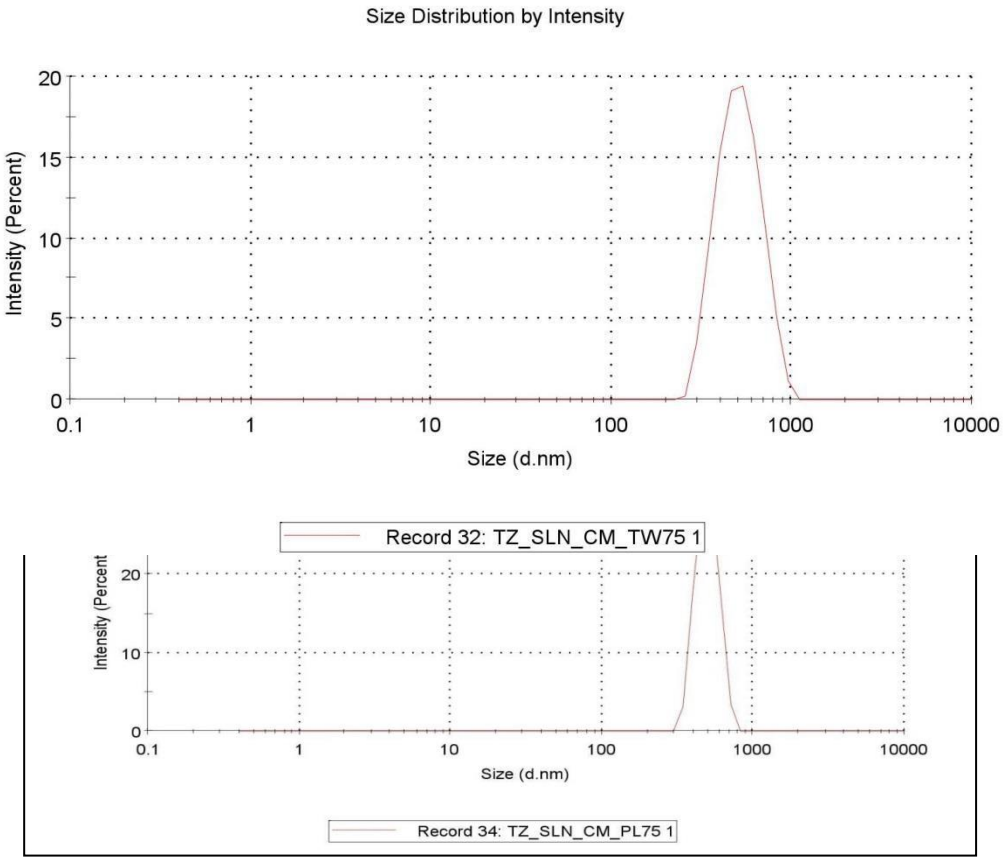

Figure 8: Size distribution profile of F2 formulation

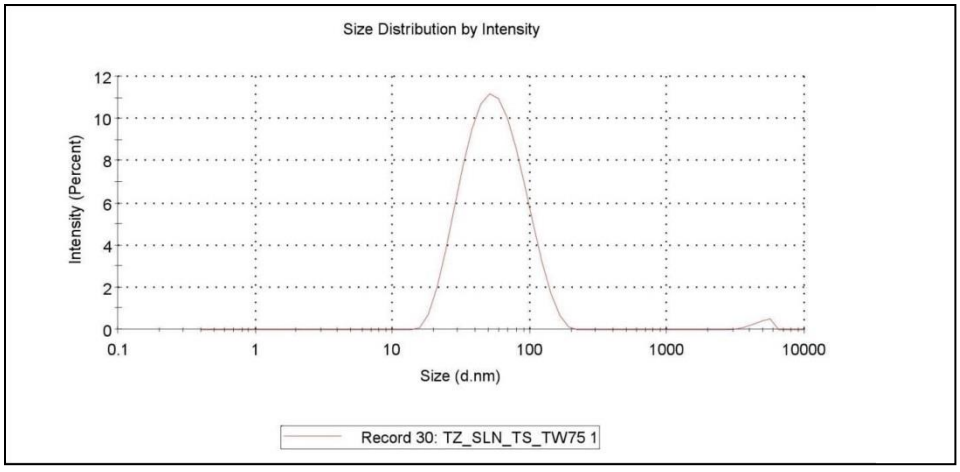

Figure 9: Size distribution profile of F3 formulation

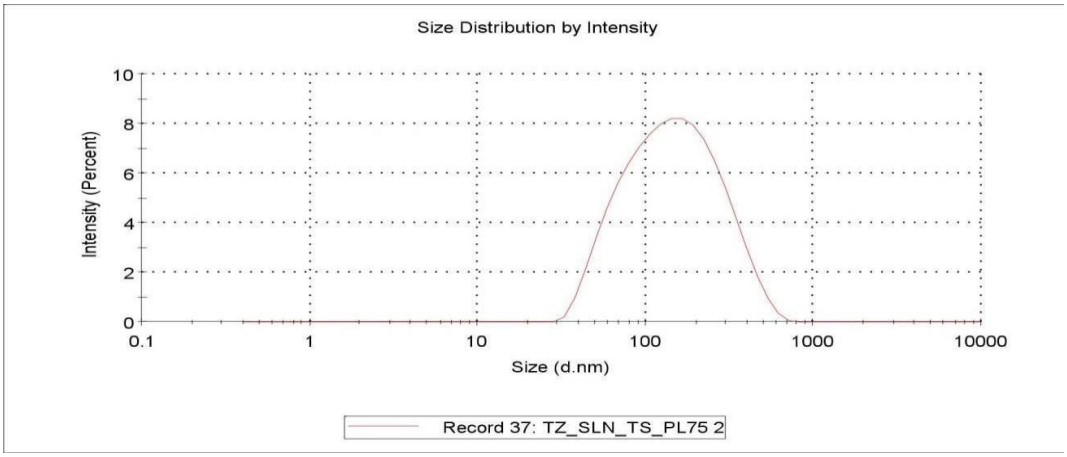

Figure 10: Size distribution profile of F4 formulation

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 95.85 to 99.98%. Percentage drug entrapment efficiency for Diltiazem 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 Diltiazem loaded SLNs was good in the range of 84.42 to 93.79%.

Table 4: Drug content:

Formulation code	Drug content
F1	96.58
F2	97.88
F3	96.50
F4	94.34
F5	97.79
F6	99.08
F7	95.06
F8	94.38
F9	96.21

Table 5: Entrapment efficiency:

Formulation Code	Amount of drug in aqueous phase	Amount of drug in lipid Phase	Entrapment efficiency	Loading efficiency
F1	0.990	9.009	90.09	18.01
F2	0.713	9.287	92.86	9.28
F3	0.476	9.524	95.23	6.34
F4	2.053	7.947	79.46	15.89
F5	1.519	8.481	84.80	8.48
F6	1.296	8.704	87.03	5.80

F7	2.131	7.869	78.68	15.73
F8	1.911	8.089	80.88	8.08
F9	1.423	8.577	85.76	5.71

***In vitro* drug release study:**

The drug releases from the Nano-particles were studied by *Franz* diffusion method. The *in vitro* release profiles of Diltiazem from Diltiazem Nano-particles are shown in Table. The cumulative percentage release of Diltiazem from different Diltiazem Nano-particles.

Table 6: Percentage drug released from formulations (F1-F9) during 24 Hours.

Time (Hours)	Formulations F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	9.18	16.79	8.64	6.25	6.68	5.75	4.4	11.66	8.22
1	18.24	23.77	17.11	10.53	14.4	12.45	9.82	20.19	15
1.5	20.43	26.13	19.95	13.39	17.11	15.74	14.5	23.48	19.2
2	24.52	28.83	23.64	17.16	22.02	18.55	16.81	26.82	21.35
3	36.6	36.51	28.35	24.14	27.8	26.55	22.12	33.89	30.42
4	43.09	46.26	44.78	32.36	36.4	32.85	27.82	41.73	38.64
5	50.07	57.99	53.28	38.67	44.15	39.84	32.85	47.91	45.24
6	60.76	66.28	58.72	49.53	51.02	45.39	39.9	55.26	51.8
12	68.07	78.06	66.58	53.35	59.35	51.79	47.35	63.43	57.45
24	79.36	88.74	75.31	61.74	67.77	56.75	53.35	69.04	63.8

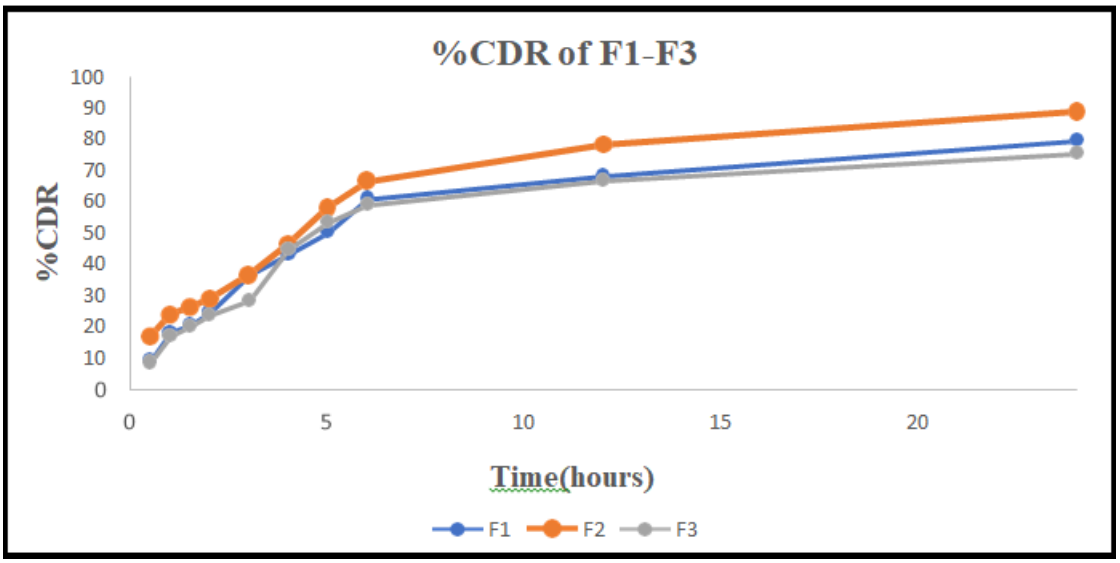

Figure 11: **The comparison of % cumulative drug release profile of Diltiazem loaded SLNs (F1-F3).**

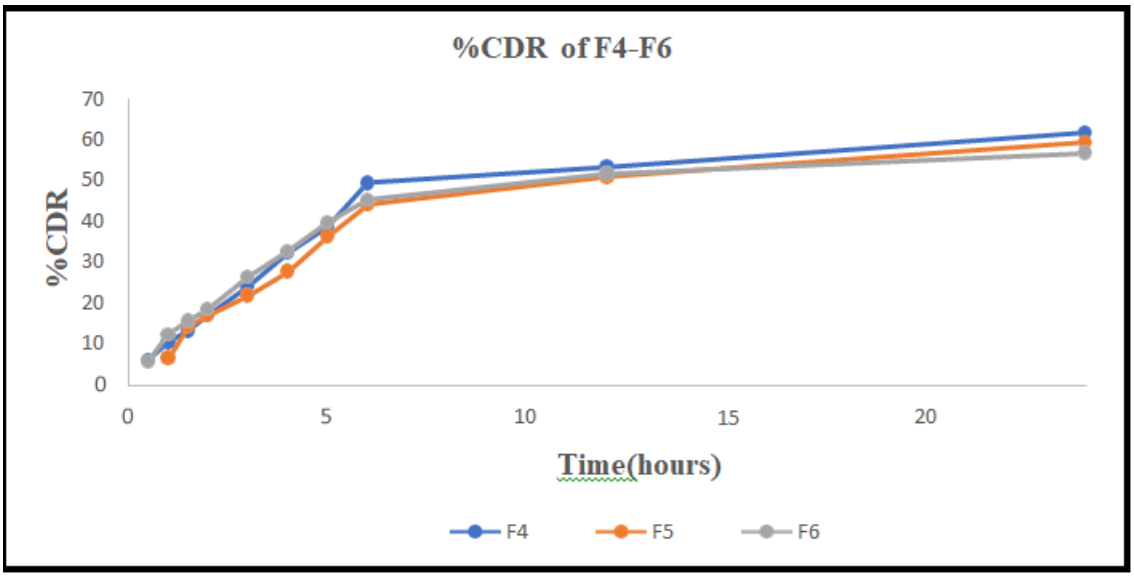

Figure 12: The comparison of % cumulative drug release profile of Diltiazem loaded SLNs (F4-F6).

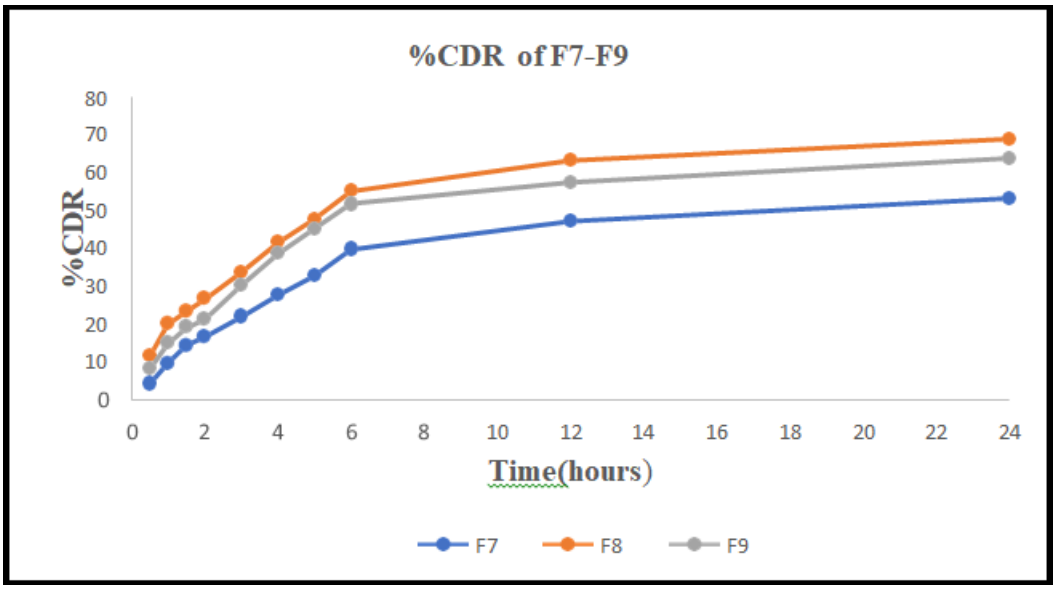

Figure 13: The comparison of % cumulative drug release profile of Diltiazem loaded SLNs (F7-F9).

CONCLUSION

In the present study, an attempt was made to develop solid lipid Nano particulate delivery system for lipophilic drug Diltiazem using Tristearin, GMS and Comprital as carrier matrices, which are meant to be used for better anti-psychotic action. FT-IR studies were carried out to find out the possible interaction between the selected drug and lipids. FT-IR studies revealed that there was no interaction between the selected drug and lipids. Diltiazem solid lipid Nano-particles were prepared by hot homogenization technique. The method was able to produce Nano-particles of acceptable range and stability. All the formulations showed very high entrapment efficiencies.

SLNs were developed by taking 0.5 to 1.5% w/v of lipids. Among the all batches, in case of F_1, 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 F5 formulations developed were in the acceptable and suitable range. Average entrapment efficiency of F1 to F5 formulations was found to greater than 70%.

Average entrapment efficiency most of Diltiazem SLNs was found to be greater than 80 % whereas the optimized formulations F2 was shown 92.72 % entrapment.

Release kinetics studies showed that Diltiazem release from the nano-particles fallows quasi-Fickian diffusion. Based on the observations, it can be concluded that the formulated lipid nano particulate delivery system of Diltiazem using widely accepted and physiologically safe lipids was capable of exhibiting sustained release properties for a period of 24 hours. They are thus may reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug.

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