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PHARMACOKINETIC MODIFICATION: ANTI-HYPERTENSIVE ISRADIPINE DRUG LOADED CONTROL RELEASE SOLID LIPID MICROPARTICLES

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Abstract

Purpose: Isradipine (ID), a DHP derivative Calcium Channel blocker, has a high therapeutic potential but with very short biological $t_{1/2}$ and low bio-availability of 15-24%. To overcome these drawbacks, Controlled Release SLM dosage form was developed; to improve the bio-availability and to reduce the dosing frequency to reduce the associated toxicity of Isradipine; **Materials and Methods:** Solid Lipid Microparticles (SLM) are micro-scale drug carriers possessing a matrix made from fatty acid, glyceride, fatty alcohol, and solid wax (0.75, 1.00 and 1.5%) with high melting points combining many advantages of drug carrier systems. The prepared IDS-SLMs were characterized for particle size, PDI, zeta potential, drug content, entrapment efficiency (EE %). *In vitro* drug release studied using dialysis bag method in 0.1N HCl and pH 6.8 phosphate buffer solutions. The optimized formulation was stable at refrigerated and room temperature for 6 months.

Results and Discussion: The standard stock solution was prepared and scanned by UV spectrophotometer showed absorption maxima at 330 nm. FTIR and DSC studies revealed that no interaction between the drug and lipids. ID-SLMs prepared using Dynasan-116 (IDS8 – SLM), with Particle size of 614.44 nm, PDI of 0.415, ZP of -26.2 mV with 83.88±2.40% of EE was optimized and was stable for 6 months. SEM studies showed nearly spherical shaped particles. The kinetic drug release study data of IDS1 to IDS9 formulations fitted best into Higuchi equation with diffusion mechanism. The result shows that, drug release rate for the F8 formulation followed zero order mechanism. The selected IDS8-SLM formulation was subjected to six months stability studies at Accelerated six months $40^0C \pm 2^0C$/75%RH±5%RH and showed stability with

respect to release pattern and all physical parameters. **Conclusion:** Taken together, the results, it is indicative that lipid based SLMs are suitable carrier system for improving the oral bioavailability of ID-SLMs.

Key word: Isradipine, Solid Lipid Microparticles (SLM), Scanning electron microscopic (SEM), Fatty acid, Glyceride, Fatty alcohol, Solid wax.

Introduction

Hypertension, the elevated Blood Pressure (BP), is a major risk factor that pre-disposes to cardiovascular disorders and responsible for most of the morbidity and mortality[1]. Nowadays, patients are considered hypertensive if their BP reach or exceed 90/140mmHg[2]. High BP affects about 20% of the world's adult population and is a serious condition that can lead to coronary heart disease, heart failure, stroke, kidney failure and other health problems[3]. This drug delivery system is an innovative and appealing way to deliver drugs and bioactive compounds in a controlled fashion. It consists of particles (dimensional range microns) composed of a solid hydrophobic fat matrix in which the active drug compound is dissolved or dispersed.SLM is usually prepared by different methods which including hot melt emulsification, solvent evaporation, high pressure homogenization, membrane emulsification and spray cooling/congealing[4]. Hypertension condition affects 16 to 37% of the world population and accounts for 16 - 18% deaths globally. Medication therapy for the management of Hypertension includes use of various class of drugs, like Class - I to IV, and the class III drugs (Calcium Channel blockers) has an important role in the management of Hypertension.Isradipine, a DHP derivative belonging to class of Calcium Channel blockers, is an effective therapeutic agent used for management of Hypertension condition but the drug has few drawbacks like extensive susceptibility to first pass metabolism resulting in reduced bio-availability of 15 - 24%. Also, the compound exhibits toxicity of overdosing due to the frequency of dosing[5]. Thus, the need of overcoming these drawbacks related to medication with Isradipine lead to this work of developing a Controlled Release SLM dosage form to improve the bio-availability and to reduce the dosing frequency to reduce the associated toxicity of Isradipine.IDS-SLMs Isradipine administration offers number of advantages in therapeutics, where the controlled releases of drug delivery as well as predictable and reproducible drug release of kinetics are important features of them.The objectives of the present study were to develop and investigate controlled release solid lipid microparticles of drug to improve the bioavailability, reduce the dose and frequency of drug administration[6-7].

Materials

Isradipine was obtained as a gift sample from Dr. Reddy laboratory, Hyderabad, India. Stearic acid, Imwitor-900, Dynasan-112 and Egg Lecithin were purchased from Sigma-Aldrich Chemicals, Hyderabad, India.Methanol, acetonitrile, chloroform were of HPLC grade (Merck, India). Centrisart filters (molecular weight cut off to 20,000) were purchased from Sartorius, Goettingen, Germany.

Methods

Preparation of Standard Stock Solution of Drug

100mg of Drug (Isradipine) transferred into 100ml volumetric flask was dissolved in 0.1N HCL & Phosphate buffer pH 6.8 and volume was made up to the mark with same buffer medium. This gives the stock solution of1000mg/ml concentration and from the stock solution 10ml was withdrawn and diluted to 100ml to get a concentration of 100mg/ml. this stock solution was used for spectral studies.

Standard curve plotting forIsradipine

From thestandard stock solution, aliquots of 1 to 10ml were withdrawn and made up to10ml using pH 6.8 buffer solution and 0.1N HCl to give a concentrations of 2, 4, 6, 8, 10, 12 and 14μg/ml and concentrations of 2, 4, 6, 8, 10, 12, 14, 16 and 20 μg/ml, respectively. UV absorption spectrums for these solutions were measured at 330 nm.The results are shown in the Table No.: 2 & 3 and Figure No.: 1 & 2.

Infra-Red Spectrophotometric Analysis[5]

The pellets were made with mixing 1gm of drug and 100gm of dried potassium bromide powder. Mixer was then compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The thin pallet was put on pellet disc to get IR Spectra studied.The results were mentioned in the Figure No.: 5.

Preparation of IDS-SLMs

ID loaded SLMs were prepared by hot homogenization followed by the ultrasonication method and further, subjected to freeze drying[8-9]. Drug, solid lipid and emulsifier (egg lecithin) were dissolved in 10ml of a mixture of methanol and chloroform (1:1). Organic solvents were completely removed using a rotary flash evaporator.The embedded lipid layer was moltened by heating to 5°C above the melting point of the lipid. An aqueous phase prepared by dissolving the stabilizer (poloxamer 188) in distilled water (1.5%w/v) and heated to the same temperature as that of the oil phase was

added to the oil phase and homogenized using a homogenizer (DIAX 900, Germany) for 4 minutes at 12000 rpm. The coarse oil in water emulsion so obtained was then sonicated using a probe soincator (Vibracell, USA; 12T-probe) for 20 min.

ID loaded SLMs were finally obtained on cooling the hot nanoemulsion room temperature. The prepared SLM formulation containing 10% w/v mannitol was prepared and kept in a deep freezer (Sanyo, Japan) at -40˚C,overnight. The frozen samples were then transferred into freeze-dryer (Lyodel, Delvac Pumps Pvt. Ltd, India). Vacuum was applied for about 48hrs to get SLM powdered lyophilized product[10-14].

Table No.1: Composition of IDS-SLMs with different Surfactant concentrations.

Ingredients	Formulation								
	IDS1	**IDS2**	**IDS3**	**IDS4**	**IDS5**	**IDS6**	**IDS7**	**IDS8**	**IDS9**
Isradipine(mg)	10	10	10	10	10	10	10	10	10
Stearic acid (mg)	150	150	150	-	-	-	-	-	-
Imwitor-900	-	-	-	150	150	150	-	-	-
Dynasan-112 (mg)	-	-	-	-	-	-	150	150	150
Egg Lecithin (mg)	75	100	150	75	100	150	75	100	150
Poloxamer-407 (%)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Double dis. Water (mL)	10	10	10	10	10	10	10	10	10
Chloroform:Methanol (1:1) (mL)	10	10	10	10	10	10	10	10	10

Characterization of Solid Lipid Microparticles

Particle Size, PDI and Zeta PotentialMeasurement

The prepared free flowing powder was used for measurement of size, PDI, ZP after reconstitution with double distilled water (1:50 ratio) and size was measured at 90° angles by Malvern zeta sizer (Nano ZS90). The average particle size, polydispersity index and zeta potential were obtained directly from the instrument[15].The results were mentioned in the Table No.: 4.

Measurement of Entrapment Efficiency (EE)

Entrapment efficiency was determined by measuring the concentration of free drug (un-entrapped) in aqueous medium (Narendar et al., 2017). The aqueous medium was separated by ultra-filtration using centrisart tubes (Sartorius, USA) which consisted of filter membrane (M.Wt. cut off 20,000 Da) at the base of the sample recovery chamber. About 2.5 mL of the formulation was kept in the outer chamber and sample recovery chamber was placed over the sample and centrifuged at 4000 rpm for 15 min. The SLM along with the encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of DS in the aqueous phase was estimated by HPLC[15].The results were mentioned in the Table No.: 5.

Determination of Total Drug Content

About 100 µL of the formulation was dissolved in 0.9 mL of chloroform and methanol mixture (1:1) and then further dilutions were made with mobile phase. The diluted samples were estimated by HPLC for the amount of IDS presents[15].The results were mentioned in the Table No.: 5.

***In vitro*Drug Release Studies**

Dialysis method was used to perform *in vitro* release studies. Dialysis membrane (Himedia, India) having pore size 2.4 nm and molecular weight cut-off between 12,000 -14,000 was used for the release studies. Dialysis membrane was soaked overnight in double distilled water prior to the release studies[13-15]. Hydrochloric acid (0.1N) followed by phosphate buffer pH 6.8 were used as release media. The experimental unit had donor and receptor compartments. Donor compartment consisted of a boiling tube which was cut open at one end and tied with dialysis membrane at the other end into which reconstituted IDS-SLM formulation equivalent of 1mg/mL dispersion was taken for release study.

Receptor compartment consisted of a 250 mL beaker which was filled with 100 mL release medium and the temperature was maintained at 37±0.5°C. At 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours time points, 1 mL samples were withdrawn from receiver compartment and replenished with the same volume of release medium. The collected samples were suitably diluted and analyzed by UV-Visible Spectrophotometer (SL-150, ELICO) at 330 nm.The results were mentioned in the Table No.: 6 and Figure No.: 3.

Solid state characterization

Drug-Excipient compatibility studies by Differential Scanning Calorimeter (DSC)

DSC techniques was used to investigate drug-excipient compatibility. DSC of pure drug, pure lipids and lyophilized optimized formulation was performed by Mettler-Toledo DSC 821e (Columbus, OH, USA) instrument at a heating rate of 10˚C/min in temperature range 60-300˚C. DSC was calibrated by using indium[13-15]. The results were mentioned in the Figure No.: 6.

Powder X-ray diffractometry (PXRD)

Powder XRD studies were performed on Multiflex X-ray diffractometer (M/s. Rigaku, Japan) for the samples by exposing them to nickel filtered CuKα radiation (40kV, 30mA) and scanned from 2˚ to 70˚, 2θ at a step size of 0.045˚ and step time of 0.5s. Samples used for PXRD analysis were IDS, lipid, physical mixture of drug with lipid (1:1) and lyophilized IDS loaded solid lipid microparticles[13-15].The results were mentioned in the Figure No.: 7.

Surface morphology study by Scanning Electron Microscopy (SEM)

The size and morphology of microparticles was studied by Scanning Electron Microscope (SEM, Hitachi, Japan). Lyophilized solid lipid microparticles of IDS were suitably diluted with double distilled water (1 in 100) and a drop of microparticle dispersion was placed on sample holder and air dried. Then the sample was observed at accelerating voltage at various magnifications. Imaging was carried out in high vacuum[13-15]. The results were mentioned in the Figure No.: 8.

Kinetic modeling of drug release[16-17]

There are number of kinetic models, which describe the overall release of drug from the dosage forms. Model dependent methods like zero, first order, Higuchi and Peppas were studied to elucidate the release rate and its mechanics.

a) Zero Order Release Kinetics

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation;

$$Q = K_0 t$$

Where, Q = fraction of drug released at time‘t’

K_0 = Zero order release rate constant

A plot of fraction of drug released against time will be linear if the release obeys zero order release kinetics.

b) First order release kinetics

The first order equation describes the release from system where release rate is concentration dependent. The first order kinetic can be expressed by the following equation;

$$\ln (1-Q) = - K_1 t$$

Where, Q = Fraction of drug released at time‘t’

K_1 = First order release rate constant

A plot of logarithm of the fraction of drug remained against time will be linear if the release obeys first order kinetics.

c) Higuchi equation:

Higuchi proposed the first example of kinetic model aimed to describe drug release from a matrix system. The simplified Higuchi equation is as follows,

$$Q = K_2 t^{1/2}$$

Where K_2 = release rate constant

A plot of fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. This equation describes drug release as a diffusion process based on Fick’s law, square root of time dependent.

Mechanism of drug release

The mechanism by which drug is released from swellable polymer-based matrices is a very complex process. In case of some polymeric matrix systems the release mechanism may be classified either as diffusion or erosion controlled, whereas most of the systems exhibit a combination of both these mechanism of drug transport through the polymer. The ‘n’ takes various values depending on the geometry of release device. Peppas derived a simple relationship, which described drug release from a polymeric system equation,

$$M_t / M_\infty = K t^n$$

Here, M_t / M_∞ are the absolute cumulative amount of drug release at a time‘t’ and infinite time respectively. ‘K’ is a constant incorporating structural and geometrical characteristic of the polymer matrix system and ‘n’ is the release exponent that specifies mechanism of drug release from swellable matrix devices as described below. To elucidate

drug release mechanism, 60 % of drug release data were fitted in above equation and data were plotted as log cumulative percentage of drug release *vs* time, and the exponent 'n' was calculated from the slope of the straight line. Kinetic constant incorporates structural and geometrical characters of the drug/polymer system. For non Fickian release, the n value falls between 0.5 and 1.0 ($0.5< n < 1.0$), whereas in the case of Fickian diffusion, n= 0.5; for zero-order release (case transport), n=1, and for Supercase II transport, n>1. The values of n as estimated by linear regression of log (M_t/ M_∞)*vs* log (t) of different formulations were calculated Kinetic constant incorporates structural and geometrical characters of the drug/polymer system. For non Fickian release, the n value falls between 0.5 and 1.0 ($0.5< n < 1.0$), whereas in the case of Fickian diffusion, n= 0.5; for zero-order release (case transport), n=1, and for Supercase II transport, n>1. The values of n as estimated by linear regression of log (M_t/ M_∞)*vs* log (t) of different formulations were calculated.

All the eight formulation of prepared matrix tablets of Isradipine were subjected to in-vitro release studies except batch IDS8-SLM these studies were carried out using Electrolab TDT 08L dissolution apparatus (USP). The dissolution medium consisted of 250 ml of Hydrochloric acid (0.1N) followed by phosphate buffer pH 6.8 for 24 hrs. The results were mentioned in the Table No. 7 to 9 and Figure No. 4

Stability studies[18-19]

IDS8-SLM loaded Solid Lipid Microparticles were stored at Accelerated six months ($40^0C\pm2^0C$/75%RH±5%RH). The average size, PDI, ZP, assay and entrapment efficiency were determined periodically after 1st day, 15 days, one Month, Two, Three and Six months (Vinay Kumar et al., 2012). The results were mentioned in the Table No. 10.

Statistical analysis

As found in earlier works reported no experiment is complete without statistical approaches to present results. In present investigation entire process was also comprised with several interbatch comparisons of different parameters monitored. Data was generated after suitable mathematical calculations and corresponding statistical interpretation accessed from MS Excel 2007.

The results were expressed in mean ± SEM. whereas comparison between two means was performed for studying the statistical significance using unpaired student 't' test in Microsoft office excel 2007.In each aspect values of p= 0.05 were considered to be significant.

Results and Discussion

Solubility Studies of Isradipine in Various Media

Solubility studies were performed in distilled water, 0.1N HCl, pH 6.8 phosphate buffer by shaking flask method. To each of these Medias an excess amount of drug was added. Then these solutions were kept for shaking on shaker for 72 hours. After 72 hours samples were centrifuged the supernatant was suitably diluted and estimated for Isradipine concentration using UV spectrometer at 330 nm.

Media	Solubility (mg/mL)
Water	0.0098 (98µg/mL)
0.1N HCl	0.0162
pH 6.8 PB	0.0282

(Mean±SD, n=3)

Determination of λ_{max}

Standard Calibration Graph of Isradipine

Weighed amount of drug (100mg) was taken in volumetric flask (100 ml) and it was dissolved in small amount of methanol. Finally the volume was made up to the mark with 0.1NHCL and pH 6.8 phosphate buffer (Stock 1 i.e., 1mg/ml). From which secondary stock was prepared by taking 1ml of primary stock in 10ml of volumetric flak and the volume was made up to mark. (Stock II, 100µg/ml). From the above stock solutions different concentrations of solutions were prepared (i.e. 2, 4, 6, 8, 10, 12 and 14 µg/ml for pH 6.8 buffer and 2, 4, 6, 8, 10, 12, 14, 16 and 20 µg/ml for 0.1N HCL) which were analyzed by UV-visible spectrometer at 330 nm.

Table No. 2:Standard Graph of Isradipine in pH 6.8 Phosphate Buffer at 330 nm

Concentration (µg/mL)	Absorbance
0	0
2	0.166
4	0.327
6	0.453
8	0.602
10	0.731
12	0.882
14	0.993

(Mean±SD, n=3)

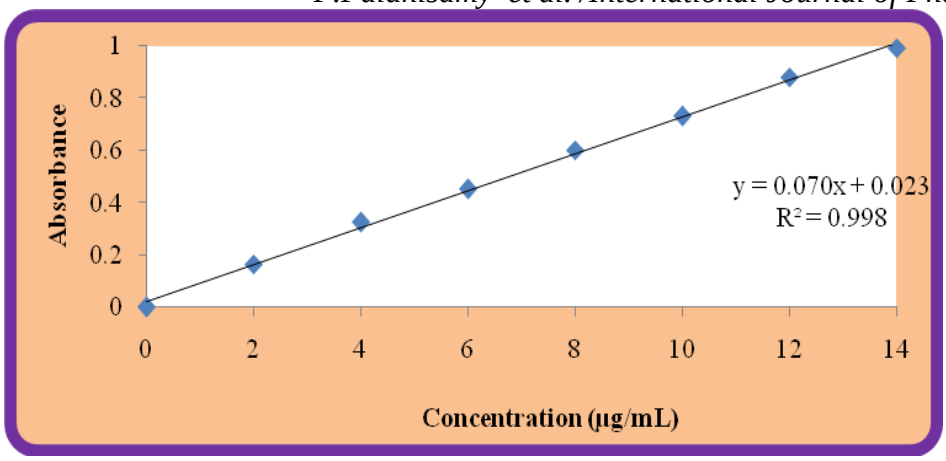


Figure No.1: Standard Graph of Isradipine in pH 6.8 Phosphate Buffer at 330 nm.

Table. No: 3 Standard Graph of Isradipine in 0.1N HCl at 330 nm.

Concentration	Absorbance
0	0
2	0.112
4	0.203
6	0.31
8	0.41
10	0.498
12	0.592
15	0.736
20	0.936

(Mean±SD, n=3)

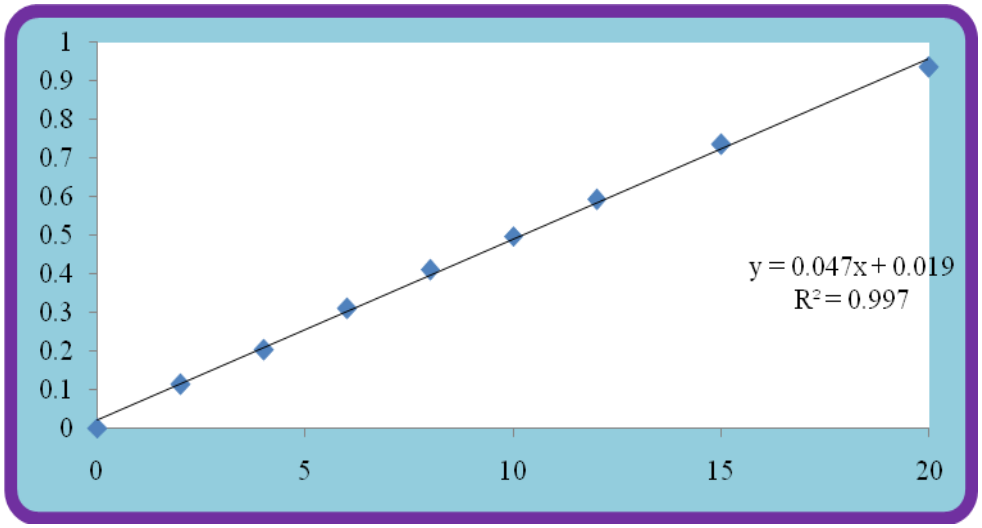


Figure No. 2: Standard Graph of Isradipine in 0.1N HCl at 330 nm.

Characterization of ID-SLMs

Measurement of Particle size, PDI, Zeta potential

The surfactant plays major role in the formation of mciroparticles and subsequently to microparticles by freeze drying process. The concentrations used were in the ranging of 0.75, 1.00 and 1.5% of total formulation. Results of the formulations were showed in Table. No: 3.From the results, size, PDI and ZP of formulations were decreased along with increased concentration for 0.75 to 1% range. Then with 1.5% increased in the size and PDI was observed. But, no changes in the ZP were noticed. Hence, selected 1% (100 mg) of surfactant for the preparation of SLMs.

The solid lipid microparticles were prepared by hot homogenization followed by the ultrasonication for different formulations i.e., IDS1 – IDS9. All the prepared samples were analysed in order to determine their particle size, zeta potential and PDI values.

The results are presented in Table. No: 4 the particle size of the formulations prepared with stearic acid (IDS1 – IDS3) range from 1388.1±8.52nm to 1020.24±2.52nm, PDI 0.28±0.009 to 0.738±0.003 and zeta potential from -23.7±1.45mV to -20.2±2.84 mV respectively, Formulations with Imwitor-900 (IDS4 – IDS6) exhibited 1364.71±4.86nm to 937.74±4.61nm, PDI of 0.894±0.008 to 0.458±0.021 and ZD of -17.6±1.63 mV to -20.2±2.74mV respectively, Formulations with Dynasan-114 (IDS7 – IDS9) exhibited 1184.91±4.72nm to 782.86±3.79nm, PDI of 0.738±0.007 to 0.563±0.008 and ZD of -19.8±1.68 mV to -22.6±2.81Mv respectively. From the result obtained, formulations containing Dynasan-114 (IDS7 – IDS9) showed relatively lower particle size, but the PDI was higher and zeta potential was lower, whereas formulations containing Stearic acid & Imwitor-900 higher particle size, lower PDI and higher zeta potential(IDS1 – IDS6) when compared to Dynasan-114 formulations.

The SLM formulations exhibited negative surface charge with the inclusion of ID which clearly suggested the orientation of IDS in the lipid matrix.

The surface charge is a key factor for the stability of colloidal dispersion. In our case, the zeta potential values of SLM formulations were found to be in between -17.6±1.63 to -26.2±2.73 mV. It is currently admitted that zeta potential -30 mV is required for electrostatic stabilization (Narendar and Kishan, 2017). However, many experiments demonstrated that not only electrostatic repulsion, but also the steric stabilizer could impart stability to the SLM dispersion. Poloxamer-407 was used in the formulation as surfactant.

It is a nonionic surfactant and decreased the electrostatic repulsion between the particles following sterical stabilization of the nano/microparticles by forming a coat around their surface for maintaining the stability of SLM.

A surfactant mixture i.e., phosphatidylcholine and poloxamer-407 was employed in the formulations because SLMs stabilized by combination of surfactants were reported to have lower particle size and higher storage stability when compared to formulations stabilized with only one surfactant.

Table No. 4: Effect of surfactant on Size, PDI and ZP of IDS-SLMs.

Formulation code	Size (nm)	PDI	ZP (mV)
IDS1	1388.1±8.52	0.28±0.009	-23.7±1.45
IDS2	720.84±2.73	0.647±0.032	-20.3±1.84
IDS3	1020.24±2.52	0.738±0.003	-20.2±2.84
IDS4	1364.71±4.86	0.894±0.008	-17.6±1.63
IDS5	677.74±1.64	0.458±0.021	-22.8±2.07
IDS6	937.74±4.61	0.629±0.002	-20.2±2.74
IDS7	1184.91±4.72	0.738±0.007	-19.8±1.68
IDS8	619.44±3.71	0.415±0.006	-26.2±2.73
IDS9	782.86±3.79	0.563±0.008	-22.6±2.81

(Mean±SD, n=3)

Total drug content and Entrapment efficiency

Formulations containing stearic acid (IDS1 – IDS3) showed total drug content ranging from 9.63±0.07mgto 9.79±0.07mg and entrapment efficiency 60.83±1.85% to 70.77±1.72% respectively. Formulations prepared with Imwitor-900 (IDS4 – IDS6) showed total drug content ranging from 9.81±0.04 mgto 9.88±0.07 mg and entrapment efficiency 64.83±156% to 74.83±2.36% respectively. Formulations developed with Dynasan-112 (IDS7 – IDS9) showed total drug content ranging from 9.63±0.07 mgto 9.89±0.05 mg and entrapment efficiency 71.54±2.13to 80.64±2.73 % respectively. High lipophilicity of Isradipine resulted in high entrapment efficiency of drug in triglyceride microparticles (Dynasan-112). This might be because of the long chain fatty acids attached to the glyceride resulting in increased accommodation of lipophilic drugs. The less ordered lipid matrix created imperfections leading to void spaces in which drug molecules could be entrapped. In this method of preparation, drug

was dissolved in molten lipid at temperature above the melting point of lipid and there was no drug leakage or precipitation of drug during the preparation. High encapsulation efficiency of drug in lipid microoparticles can cause high amount of drug to pass through the lymphatic transport, which in turn bypasses the first pass metabolism.

Table. No: 5Effect of surfactant on Surfactant Entrapment efficiency and drug content of IDS-SLMs.

Formulation code	Total drug content (mg)	Entrapment efficiency (%)
IDS1	9.63±0.07	60.83±1.85
IDS2	9.96±0.04	74.82±2.86
IDS3	9.79±0.07	70.77±1.72
IDS4	9.81±0.04	64.83±156
IDS5	9.99±0.06	79.37±3.75
IDS6	9.88±0.07	74.83±2.36
IDS7	9.63±0.07	71.54±2.13
IDS8	9.97±0.06	83.88±2.48
IDS9	9.89±0.05	80.64±2.73

(Mean±SD, n=3)

***In-vitro* dissolution studies**

24 hrs *In vitro* release studies of Isradipine from IDS-SLMs were performed for all formulations for 2 hrs in 0.1N HCl followed by 22 hrs in pH 6.8 phosphate buffer by dialysis method (Figure 3). From the results,*in-vitro* release was observed to be prolonged both in pH 6.8 and in 0.1NHCl. Formulations containing stearic acid, Imwitor-900 and Dynasan-112showed drug release 82.89±1.74, 85.92±3.85 and 94.85±3.61 from IDS2, IDS5 and IDS8 formulations, respectively in 0.1N hydrochloric acid followed by pH 6.8 phosphate buffer.

Release studies of ID from IDS-SLMs were conducted for a period of 24 h (2h in 0.1N HCl at pH 1.2 and 22 h in pH 6.8 phosphate buffer), since intestinal transit is much slower than gastric transport. Isradipine belongs to BCS class-II, and is a lipophilic drug and this may be a reason for the slow release from the lipid matrix. Isradipine showed more solubility in pH 6.8 phosphate buffer than in 0.1N HCl. Hence, initially the release was found to be less. But, the un-entrapped drug in aqueous portion of the SLM dispersion got released very quickly. During the initial period, the release rate was found to be affected by the concentration of lipid and surfactant in aqueous phase. The release rate increased with the increasing of lipid concentration. This could be due to the Isradipine location within the outer shell

of lipid rather than in the inner core. On the other hand, the increase of lipid concentration prolonged the Isradipine release from SLM, attributed to the good solubility of Isradipine in all formulations and to the homogenous distribution of drug within the lipid matrix. Similarly, the surfactant with 100mg formulations also showed superimposed release from the SLMs. Further, the suspension formulation showed more than 99 % of drug release during 24 h time period and also lyophilized suspension exhibited similar pattern of drug release. In comparison, ID8-SLM formulation exhibited reasonably good Particle size, PDI, high Zeta potential value, and the higher Entrapment efficiency with better release of drug from the lipid matrix in pH 6.8 phosphate buffer, hence it was considered as the optimized formulation.

TableNo. 6: *In-vitro* drug release of ID from IDS-SLMs – Effect of surfactant concentration.

Time (h)	IDS2	IDS5	IDS8
0	0	0	0
0.5	10.74±1.53	14.10±1.88	19.03±1.07
1	15.83±1.73	19.92±2.68	24.91±2.83
1.5	18.11±2.18	22.84±2.05	28.73±1.64
2	23.34±2.11	28.10±2.84	32.84±3.10
3	30.54±2.93	32.81±1.95	42.00±2.51
4	42.01±2.74	43.91±2.11	49.05±2.64
6	51.73±2.11	55.01±2.51	57.71±2.88
8	61.83±3.29	62.91±3.04	65.93±3.00
12	71.83±2.74	73.82±2.83	77.03±2.74
24	83.72±2.82	86.44±3.54	95.83±3.15

(Mean±SD, n=3)

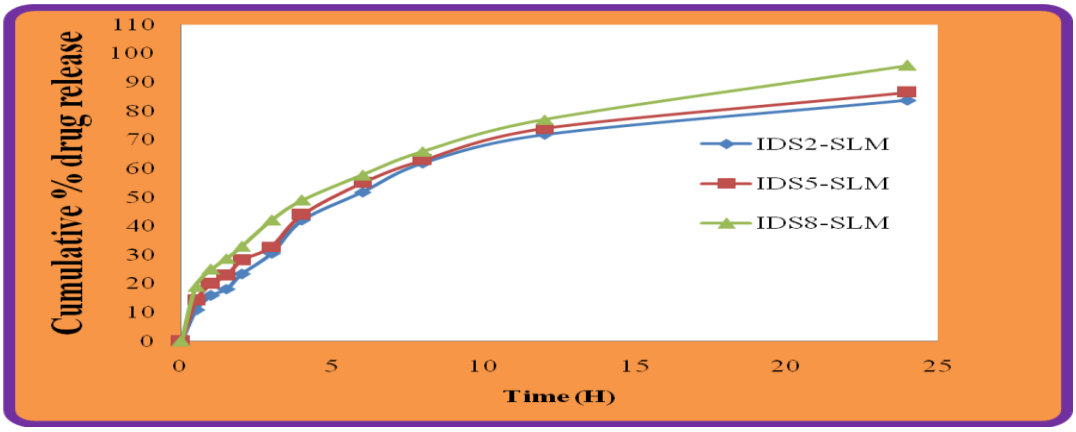


Figure No. 3: *In-vitro* drug release of ID from IDS2, IDS5 & IDS8 – SLMs Effect of surfactant concentrations.

Kinetic Studies

Table No.7: *In-vitro* kinetic release profiles of Solid Lipid Microparticles of Isradipine IDS2-SLM formulation.

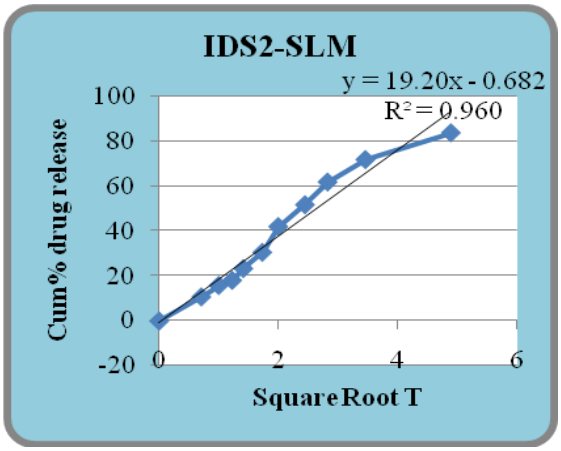
Time(hrs)	%Drug release	% Cum.drug remaining	Log % cum.drug remaining	Square root of time	Log time	Log % cum.drug release	Cube root of % drug remaining
0	0	100	2	0	0	0	4.6415
0.5	10.74	89.26	1.9506	0.7071	-0.310	1.0310	4.4690
1	15.83	84.17	1.9251	1	0	1.1994	4.3824
1.5	18.11	81.89	1.9132	1.2247	0.1760	1.2579	4.3425
2	23.34	76.66	1.8845	1.4142	0.3010	1.3681	4.2480
3	30.54	69.46	1.8413	1.7320	0.4771	1.4848	4.1106
4	42.01	57.99	1.7633	2	0.6020	1.6233	3.8706
6	51.73	48.27	1.6836	2.4494	0.7781	1.7137	3.6410
8	61.83	38.17	1.5817	2.8284	0.9030	1.7911	3.3669
12	71.83	28.17	1.4497	3.4641	1.0791	1.8563	3.0427
24	83.72	16.28	1.2116	4.8989	1.3802	1.9228	2.5344

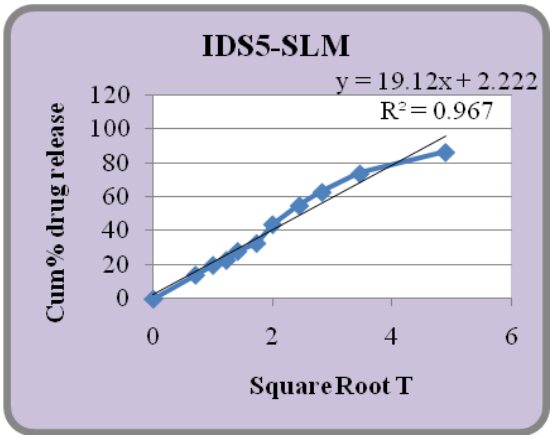
Table No. 8:*In-vitro* kinetic release profiles of Solid Lipid Microparticles of Isradipine IDS5-SLM formulation

Time(hrs)	%Drug release	% Cum.drug remaining	Log % cum.drug remaining	Square root of time	Log time	Log % cum.drug release	Cube root of % drug remaining
0	0	100	2	0	0	0	4.6441
0.5	14.10	85.9	1.9339	0.7071	-0.310	1.1492	4.4122
1	19.92	80.08	1.9035	1	0	1.2992	4.3103
1.5	22.84	77.16	1.8873	1.2247	0.1760	1.3586	4.2572
2	28.10	71.9	1.8567	1.4142	0.3010	1.4487	4.1582
3	32.81	67.19	1.8273	1.7320	0.4771	1.5160	4.0653
4	43.91	56.09	1.7488	2	0.6020	1.6425	3.8279
6	55.01	44.99	1.6531	2.4494	0.7781	1.7404	3.5566
8	62.91	37.09	1.5692	2.8284	0.9030	1.7987	3.3349
12	73.82	26.18	1.4179	3.4641	1.0791	1.8681	2.9693
24	86.44	13.56	1.1322	4.8989	1.3802	1.9367	2.3846

Table No.9: *In-vitro* kinetic release profiles of Solid Lipid Microparticles of Isradipine IDS8-SLM formulation

Time(hrs)	%Drug release	% Cum.drug remaining	Log % cum.drug remaining	Square root of time	Log time	Log % cum.drug release	Cube root of % drug remaining
0	0	100	2	0	0	0	4.6415
0.5	19.03	80.97	1.9083	0.7071	-0.310	1.2794	4.3262
1	24.91	75.09	1.8755	1	0	1.3963	4.2188
1.5	28.73	71.27	1.8529	1.2247	0.1760	1.4583	4.1460
2	32.84	67.16	1.8271	1.4142	0.3010	1.5164	4.0647
3	42.00	58.00	1.7634	1.7320	0.4771	1.6232	3.8708
4	49.05	50.95	1.7071	2	0.6020	1.6906	3.7072
6	57.71	42.29	1.6262	2.4494	0.7781	1.7612	3.4840
8	65.93	34.07	1.5323	2.8284	0.9030	1.8190	3.2418
12	77.03	22.97	1.3611	3.4641	1.0791	1.8866	2.8426
24	95.83	4.17	0.6201	4.8989	1.3802	1.9815	1.6095





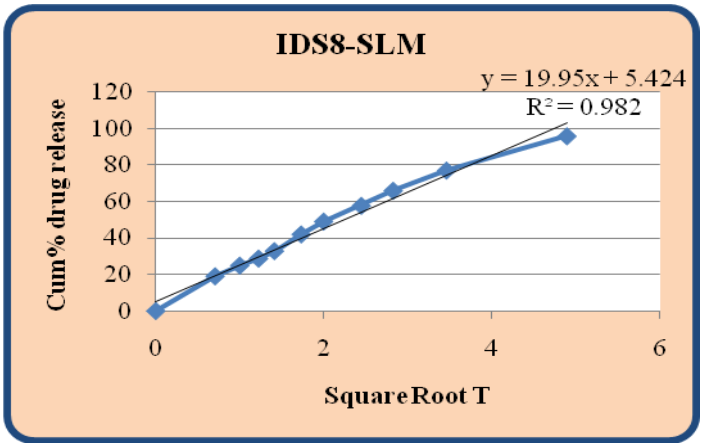


Figure No. 4: *In-vitro* kinetic studies Solid Lipid Microparticles of Isardipine IDS2-SLM, IDS5-SLM & IDS8-SLM.

The release kinetics data was fitted into various mathematical models to evaluate the kinetics and mechanism of Isradipine release. All the formulations in this investigation could be best expressed by Higuchi's classical diffusion equation, as the plots showed high linearity (r^2: 0.960 to 0.982) indicating that the drug release followed the zero order controlled release pattern.

Stability Studies

TableNo.10: Stability studies of Optimized Formulation IDS8-SLM at Accelerated six months ($40^0C\pm2^0C$/75%RH±5%RH)

Time	Size (nm)		PDI		ZP (mV)	
	At 4˚C	At 25 ˚C	At 4˚C	At 25 ˚C	At 4˚C	At 25 ˚C
Initial	619.44±3.7	619.5±3.7	0.415±0.006	0.415±0.005	-26.2±2.7	-25.8±2.7
15 days	620.14±2.9	620.03±2.7	0.416±0.004	0.417±0.006	-25.6±2.3	-25.8±2.4
30 days	621.31±2.8	621.84±2.9	0.417±0.004	0.418±0.003	-25.7±2.7	-25.8±2.8
45 days	621.82±2.9	622.12±3.1	0.418±0.003	0.418±0.005	-25.7±2.7	-25.8±2.9
60days	629.82±3.1	629.91±3.3	0.419±0.005	0.419±0.007	-25.8±2.8	-25.9±2.8
90days	631.20±2.1	631.34±2.3	0.419±0.005	0.420±0.003	-25.9±3.1	-25.9±3.7
180days	632.18±2.9	632.89±3.1	0.421±0.003	0.423±0.004	-26.9±4.1	-27.2±4.3

(Mean±SD, n=3)

Stability studies for the finally optimized formulation of IDS8-SLMwere performed at room temperature and refrigerated temperature for 6 months. Changes were observed in Particle size and Zeta potential values, but statistically insignificant, as shown in Table No, :10, indicating the resistance for instability problems during storage at room temperature and 4^oC of the SLMs.

Solid State Characterization

Drug and Excipient compatibility studies

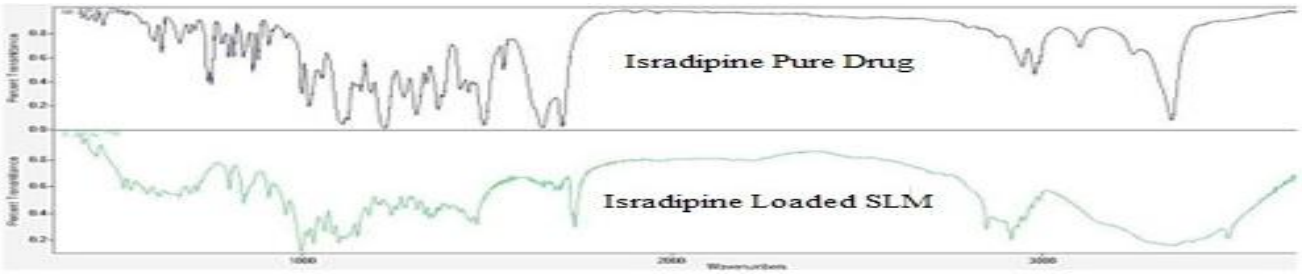
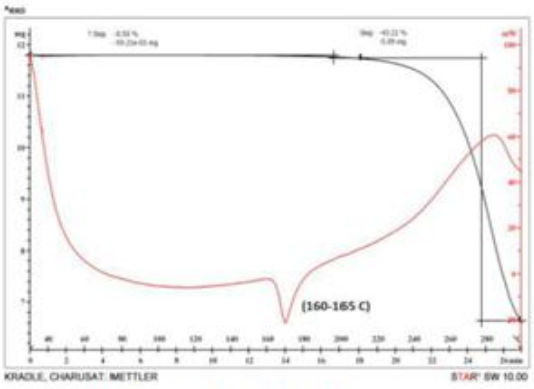
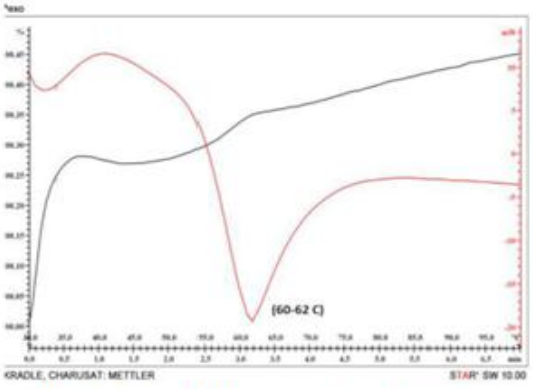


Figure. No: 5 FTIR Spectarum of Pure Isradipine and Physical Mixture of Isradipine and Optimized Formulation IDS8-SLM.



DSC Graph of Isradipine



DSC Graph of Physical mixture of Isradipine with Dynasan 112

Figure No. 6: DSC Thermograms of Pure Isradipine and Physical Mixture of Isradipine and Optimized Formulation IDS8-SLM

Figure No. 7: XRD Spectra of Pure Isradipine and Physical Mixture of Isradipine and Optimized Formulation IDS8-SLM.

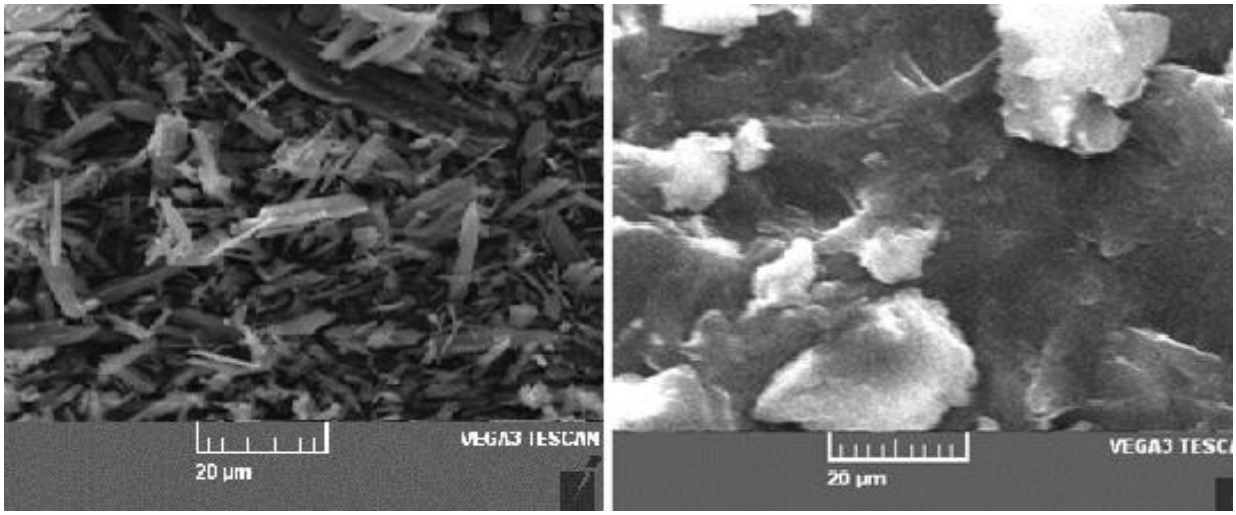


Figure No. 8: SEM Images Isradipine and Physical Mixture of Isradipine and Optimized Formulation IDS8-SLM - X20, X40, X60, X190 and X1k, magnifications.

Summary and Conclusion

SLMs of Isradipine, a poorly water soluble drug with oral bioavailability of 15-24%, were prepared by two step method, containing hot homogenization step followed by ultra-sonication method. The Nine IDS-SLMs were prepared with three lipids (Stearic acid, Imwitor-900 and Dynasan-112), each at three different concentrations. Particle Size, PDI, Zeta potential, Entrapment efficiency,Total drug content, *in-vitro* drug release and kinetic studies, stability studies and Solid state characterization and evaluation of the prepared SLMs were performed.Drug release kinetics study indicates the formulation following Higuchi drug release model with diffusion mechanism and also indicates that the lipid Dynasan-112 proved to be suitable release retarding material.The Isradipine as IDS-SLMs was shown to be the suitable for oral delivery of IDS which could also help in overcoming of reduced oral bioavailability due to first-pass metabolism byfollowing lymphatic transport pathway.

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Conflict of Interest: We declared that this review does not have any conflict of interest.

Reference

1. Kannel, W. B., "Elevated systolic blood pressure as a cardiovascular risk factor", Am. J. Cardiol., Vol. 85, No. 2, pp. 251-255, 2000.
2. Kannel, W. B., "Hypertensive risk assessment: Cardiovascular risk factors and hypertension", J. Clin. Hypertens., Vol. 6, No. 7, pp. 393-399, 2004.
3. European Society of Hypertension. "European Society of Cardiology guidelines for the management of arterial hypertension", J. Hypertens., Vol. 21, No. 6, pp. 1011-1053, 2003.
4. Richard, E. K., "A text book of cardiovascular physiology concept", 2nd Ed., Lippincott Williams and Wilkins, Philadelphia, 2001.
5. Nandita, G. D. and Sudip, K. D., "Controlled release of oral dosage form", Formulation, Fill and Finish., pp. 10-16, 2003.

6. Cortesi, R., Esposito, E., Luca, G. and Nastruzzi, C. "Production of lipospheres as carriers for bioactive compounds", Biomaterials., Vol. 23, No. 11, pp. 2283-2294, 2002.
7. Reithmeier, H., Herrmann, J. and Gopferich, A. "Lipid microparticles as a parenteral controlled release device for peptides", J. Control. Release., Vol. 73, No. 2, pp. 339-350, 2001a.
8. Jaspart, S., Piel, G., Delattre, L. and Evrard, B. "Solid lipid microparticles: Formulation, preparation, characterisation, drug release and applications", Expert Opin. Drug. Deliv., Vol. 2, No.1, pp. 75-87, 2005.
9. Doria, C., Charcosset, C., Barresi, A. A. and Fessi, H. "Preparation of solid lipid particles by membrane emulsification: Influence of process parameters", Colloids Surf., Vol. 338, No.1-3, pp. 114-118, 2009.
10. Passerini, N., Qi, S., Albertin, B., Grassi, M., Rodrigue, L. and Craig, D. Q. "Solid lipid microparticles produced by spray congealing: Influence of the atomizer on microparticles characteristics and mathematical modeling of the drug release", J. Pharm. Sci., Vol. 99, No. 2, pp. 916-931, 2010.
11. Marja, S., Cynthia, K., Hakan, G., Carina, D. and Anne, M. J. "Evaluation of controlled-release polar lipid microparticles", Int. J. Pharm., Vol. 244, No. 1, pp. 151-161, 2002.
12. Amal, H. E., Iman, M. A. and Ibrahim, A, A. "Testosterone solid lipid microparticles for transdermal drug delivery: Formulation and physicochemical characterization", J. Microencapsulation., Vol. 24, No. 5, pp. 457-475, 2007.
13. NarendarDudhipala and KishanVeerabrahma, "Candesartan cilexetil loaded solid lipid nanoparticles for oral delivery: characterization, pharmacokinetic and pharmacodynamic evaluation", Drug Delivery, 2016, 23(2): 395–404
14. NarendarDudhipala, KishanVeerabrahma, Improved anti-hyperlipidemic activity of Rosuvastatin Calcium via lipid nanoparticles: Pharmacokinetic and pharmacodynamic evaluation, European Journal of Pharmaceutics and Biopharmaceutics 110 (2017) 47–57.
15. Thirupathi.G, Swetha.E and D.Narendar, role of Isradipine Loaded Lipid Nanoparticles on the Pharmacodynamic effect in rats, Drug Research, 2017, 67; 163-167.
16. Haung Y., Khanvilkar KH, Moore AD, Hilliard Lott M. in "Drug Development and Industrial Pharmacy", 2003, 29 (1) 79- 88.

17. Pandey VP, Manavalan R, Sunder Rajan T, Ganesh KS, in "Indian Journal of Pharmaceutical Science", 2003, 65 (1), 44-48.

18. Ahlneck, C., and Zografi.1990. The molecular Basis of moisture effects on the physical and chemical stability of drugs in the solid state, Int.J. Pharm.62:87-85.

19. ICH Guideline Published by Europian Medicines agency CAMP /ICH/ 2736/99 August 2003.

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