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CHARACTERIZATION AND DEVELOPMENT OF ATORVASTATIN LOADED LIPOSPHERES TO TREAT HYPERLIPIDEMIA

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ABSTRACT

Atorvastatin calcium belongs to class of HMG-COA reductase inhibitors. It is one of the commonly used anti-hyperlipidemic drug for the treatment of hyperlipidemia. It is a BCS class II drug (low solubility and high permeability). Its bioavailability is low due to poor aqueous solubility. The objective of this work is to prepare and evaluate Atorvastatin calcium loaded lipospheres for sustained release of drug. The optimized Atorvastatin calcium loaded lipospheres were later compressed into tablet and compared with marketed product. Stability studies are conducted suitably for optimized lipospheres. Atorvastatin calcium loaded lipospheres was prepared using stearic acid and soyabean-phosphatidylcholine(soya-lecithin) as a core and coat by melt-dispersion method. The formulation F-14 was found to be the better formulation, thus considered as optimized and it is further compressed to tablet. The obtained results suggested that a lipospheres are potentially promising formulation for the efficient delivery of poorly water-soluble drugs by oral drug delivery system. The prepared lipospheres proved to be potential for oral sustained drug delivery.

KEYWORDS: Lipospheres; Atorvastatin calcium; tablet; Stearic acid, soya-phosphatidylcholine and Tween-80.

1. INTRODUCTION

Multiple-unit drug delivery systems such as nanoparticles, microparticles, microemulsions and liposomes offer more advantages than the single-unit systems with respect to their uniform distribution in the gastrointestinal tract resulting in uniform absorption of the drug.

The drawback of these particulate systems, being the degradation of the polymer and organic solvent residues present in the delivery system which could result in severe acceptability and toxicity problems. To resolve these issues, lipid microspheres, often called lipospheres, have been proposed as a new type of fat-based encapsulation system for drug delivery of bioactive compounds.

Lipospheres are lipid-based water-dispersible solid particles bearing particle size between 0.01 and 100 µm in diameter, composed of a solid hydrophobic lipid core containing active drug moiety dissolved or dispersed in a solid fat matrix, which is stabilized by a layer of phospholipid molecules as an external coat.

Improved stability of the drug in the formulation, freeze-dry and reconstitution properties, controlled particle size, high-drug load, well-controlled drug release, and no carrier toxicity made liposphere systems superior over other particulate delivery systems such as emulsions, liposomes, and microspheres.

In addition, lipospheres protect the drug candidates from hydrolysis, corroborating the shelf life and facilitating high bioavailability and prolonged plasma levels. The lipospheres offer well-controlled delivery to a variety of drug candidates.[1]

Applications of lipospheres include parenteral drug delivery, transdermal drug delivery, oral drug delivery, nasal drug delivery, ocular drug delivery, protein and peptide drug delivery, gene delivery, and brain targeting, etc.

Hyperlipidemia defines an elevated level of lipids- like cholesterol and triglycerides in the blood. This condition is linked to atherosclerosis, heart attack, stroke and peripheral artery disease. Lifestyle modification is suggested along with medication to lower the lipid levels.

Statins are one of the most commonly used drugs for the management of hyperlipidaemia and act by inhibiting the (HMG-CoA) reductase enzyme, which is the rate-controlling enzyme of the mevalonate pathway.

Atorvastatin calcium (ATR) is a synthetic statin belongs to BCS Class-II that works by lowering TGs and LDL and raising HDL levels. Besides possessing anti-inflammatory and antioxidant properties, which makes it one of the most studied drugs in the pharmaceutical field. Atorvastatin calcium can cure high cholesterol compare to other statins and also its availability as dosage form in the market is more. It can be administered any time in a day show greater efficacy in lowering LDL.[2]

2. MATERIALS AND METHODS

List of Materials

Table 2.1: List of Materials used in development and evaluation of Atorvastatin calcium loaded Lipospheres.

S.No	MATERIALS	MANUFACTURER
1.	Atorvastatin Calcium	Microlabs pvt ltd
2.	Tween 80	S. D. Fine Chemicals Ltd. Mumbai
3.	Methanol	Karnataka chemicals, Bangalore.
4.	Poly- vinyl Alcohol	S. D. Fine Chemicals Ltd. Mumbai
5.	Soya-lecithin	S D Fine- chem (LTD), Mumbai.
6.	Stearic-Acid	Sisco Research Laboratories pvt.ltd
7.	Paraffin wax	Nice chemicals pvt ltd
8.	Cetyl alcohol	S D Fine- chem (LTD), Mumbai.
7.	Potassium dihydrogen phosphate	S. D. Fine Chemicals Ltd. Mumbai.
8.	Sodium hydroxide	S. D. Fine Chemicals Ltd. Mumbai.
9.	Magnesium stearate	Rolex chemical industries
10.	Talcum Powder	S D Fine- chem (LTD), Mumbai.
11.	Lactose Monohydrate	S D Fine- chem (LTD), Mumbai.

a) Pre - formulation studies

Preformation study gives direction for development of Formulation In choice of drug form, composition, physical structure, helps in adjustment of pharmacokinetic and biopharmaceutical properties. It establishes the physical characteristics of the drug and finds out the compatibility of drug with commonly used recipients. The preformation stage is an integral part of pharmaceutical product development process, which supports the dosage form design of new drug and its quality control.

b) Identification of the pure drug

Identification of the atorvastatin calcium was carried out by FTIR Spectrophotometry.

Procedure

Weighed amount of drug was mixed with IR grade KBr (1:10) Compressed under 10-ton pressure in a hydraulic press to form transparent pellet. The pellet was scanned by IR spectrophotometer over a range of 4000 to 500 range.

c) Preparation of calibration curve of atorvastatin calcium

Determination of Lambda Max.

To determine the wavelength of maximum absorption atorvastatin calcium standard solution was prepared using methanol and scanned in UV wavelength range of 200-400 nm utilizing methanol as a blank. The absorption maxima obtained in the spectrum graph was considered as a lambda max for the pew drug solution in methanol.

d) Calibration curve of Atorvastatin calcium

Method

Stock-1 solution: 50mg of Atorvastatin Calcium was weighed accurately and transferred in 50ml volumetric flask and dissolved in methanol and the volume is made up to the mark with methanol (1000ug/ml).

Serial dilution: From stock-1 solution ranging from 0.5, 1, 1.5, 2 and 2.5ml were transfer to 10ml volumetric flask and were diluted up to the mark with methanol to get drug solution concentration ranging from 10-100ug/ml. Absorbance was measured at 246 nm against water as a blank. The calibration curve was plotted using concentration of drug on X-axis and absorbance on Y-axis. The mean absorbance of 3 samples was considered for plotting (n=3).[3]

e) Drug-Excipients Compatibility study

Drug excipients compatibility testing at an early stage helps in selection of recipients that increase the probability of developing a dosage form to assess the drug excipients compatibility, the analytical technique Fourier transform infrared spectroscopy (FT-IR) was adopted.

FTIR

Compatibility of the drug with the excipients is determined by subjecting the physical mixture of the drug and the excipients of the main formulation to infrared absorption spectral analysis (FT-IR). Any change in chemical composition of the drug after combining it with excipients was investigated in IR spectral analysis.

Procedure

Weighed amount of drug atorvastatin calcium and other excipients like steric acid, tween 80 soya-lecithin and polyvinyl alcohol were physically mixed. The mixture is then blended with IR grade KBR and compressed under 10-ton pressure in a hydraulic press to form transparent pellet. The pellet was scanned by IR spectrophotometer over a range of 4000 cm^{-1} to 500 cm^{-1} range.[4]

f) Solubility studies of pure drug and Lipospheres

Procedure: Solubility of pure drug

10mg of pure drug is dissolved in 10ml of 7.4ph phosphate buffer. 1ml from this solution is diluted to 10ml and absorbance was recorded using UV-spectrophotometer at 246nm. The obtained absorbance is used to identify the concentration of pure drug.

Solubility of Lipospheres

Procedure: Solubility of Lipospheres (optimized formula)

30mg of liposphere (equivalent to 10mg of pure drug) is dissolved in 10ml of 7.4ph phosphate buffer. 1ml from this solution is diluted to 10ml and absorbance was recorded using UV-spectrophotometer at 246nm. The obtained absorbance is used to identify the concentration of pure drug.

g) Formulation of Lipospheres

Lipospheres can be prepared from various methods like melt dispersion technique. Solvent evaporation technique, co solvent method, ultrasonication method, Etcetera. The most convenient method chosen for the preparation of liposphere was melt- dispersion technique.[5]

h) Preparation of Lipospheres[6]

MELT-DISPERSION TECHNIQUE

In this method, the lipid or lipid mixture is melted and maintained at a temperature slightly above the melting point of the lipid, in which the drug is dispersed. This mixture is emulsified with an external aqueous phase containing a suitable surfactant and phospholipids and it's maintained at a temperature nearly or slightly higher than the lipid phase. The formed emulsion is kept in a sonicator for about 15mins then the formulation is immediately cooled by submerging it in an ice bath with continuous stirring to produce a uniform dispersion of lipospheres. The obtained dispersion is filtered in a Whatman filter paper and the lipospheres are collected kept for drying in a room temperature.

Fig. 4.1 Digital Bath Type Sonicator.

SOLVENT-EVAPORATION TECHNIQUE

Accurately weighed amounts of the drug, lipid core and phospholipids were dissolved in chloroform. The organic solvent was slowly evaporated under reduced pressure at 50– 60°C using a rotary evaporator. The resulting solid was mixed with 10 ml PBS at 50–60°C with continuous mixing till the formation of a homogenous dispersion. The temperature was then reduced to 10°C with continuous rotation at 150 rpm for 5 min. The system was sonicated for 15 min in a bath type sonicator then cooled down to 20°C with continued shaking for another 5 min.

Fig. 4.2 Rotary Vacuum Evaporator.

i) COMPOSITION OF BLANK LIPOPHERES

Nine formulations (F-1 to F-9) were prepared using three different lipids in order to select the best lipid for proceeding to further studies.

Stearic acid was selected as best lipid out of three and further nine formulations were designed by varying amount of drug, volume of aqueous phase, sonication time, core: coat ratio and method of preparation.[7]

Table 2.2 COMPOSITION OF LIPOSPHERES

Formulation	Stearic acid	Paraffin wax	Cetyl alcohol	Soya-lecithin	Poly-vinyl alcohol	Tween-80	Distilled water	Time
F-1	400			200	5	1	20	15
F-2		400		200	5	1	20	15
F-3			400	200	5	1	20	15
F-4	600			200	5	1	20	15
F-5		600		200	5	1	20	15
F-6			600	200	5	1	20	15
F-7	800			200	5	1	20	15
F-8		800		200	5	1	20	15
F-9			800	200	5	1	20	15

Based on the results obtained stearic acid was selected as best lipid for further studies

Table 2.3 COMPOSITION OF DRUG LOADED LIPOSPHERES.

Formulation	Drug	Stearic acid	Soya-lecithin	Poly-vinyl alcohol	Tween-80	Distilled water	Time
F-10	40	400	200	5	1	20	15
F-11	40	600	200	5	1	20	15
F-12	40	800	200	5	1	20	15
F-13	10	400	200	5	1	20	15
F-14	80	400	200	5	1	20	15
F-15	80	400	200	5	1	25	15
F-16	80	400	200	5	1	10	15
F-17	80	400	200	5	1	20	10
F-18	80	400	200	5	1	20	20

j) EVALUATION OF LIPOSPHERES

All formulations are evaluated for particle size, entrapment efficiency, drug content and drug release studies.

i) Physical Appearance

Visual examination of the generated lipospheres was done to check the shape, size and colour.

ii) Particle size

It was determined by optical microscopy method. The optical microscope with stage micrometer was used to determine particle size of lipospheres. Calibration of microscope was done and the particles were kept on the slide, distance covered by the particle is multiplied by 1 division of eye-piece micrometer to get size of the particle size.[8]

iii) Drug content

The drug content was determined by dissolving 10mg of lipospheres in 7.4Ph phosphate buffer. 1ml from the above solution is again dissolved in 10ml of phosphate buffer. The solution was filtered and resulting solution was analyzed at 246nm in the UV-Visible spectrophotometer.[9]

$$\%\text{Drug Content} = \frac{\text{Amount of drug in lipospheres}}{\text{Amount of lipospheres}} \times 100$$

iv) Entrapment Efficiency

The entrapment efficiency was determined by indirect method using filtrate. 1ml of filtrate in 10ml of phosphate buffer again 1ml from this solution was diluted to 10ml. The resulting diluted solution was analyzed at 246nm in the UV-Visible spectrophotometer.[10]

$$\%\ \text{EE} = \frac{(\text{Total drug} - \text{Amount of drug entraped})}{\text{Total drug}} \times 100$$

v) In-vitro drug release study

In – vitro drug release study was performed by using USP-II (Paddle type) apparatus. Weighed amount of lipospheres were placed in three different baskets in dissolution medium containing 900ml of phosphate buffer(7.4Ph). The apparatus was rotated at a speed of 100 rpm by maintaining temperature at 37±1ºC in each test. Samples were withdrawn at regular interval of 5 minutes as 5 ml which was predetermined and this was continued up to 40 minutes by replacing equal quantity of fresh dissolution medium. The filtered samples were analysed by using UV Spectrophotometer.[11]

vi) Scanning Electron Microscopy

The powders were imaged by a scanning electron microscope (SEM) run at an accelerating voltage of 10kV using Hitachi SU 3500. The powder in few µg were fixed on to stub by a double-sided sticky carbon tape and kept inside the SEM chamber and analysed at different magnification such as 60X, 200X, 500X. 1.10X and 2.50X respectively to obtain better clarity on the particle morphology/ topology.[12]

vii) Stability studies

The formulation F-14 was selected for long-term stability studies and kept in room temperature 25^{0}C for 3 months as per ICH guidelines and was analyzed.

k) FORMULATION OF LIPOSPHERES LOADED TABLET[13]

Lipospheres loaded tablet was prepared by dry granulation method. Lactose monohydrate as binder, magnesium stearate as lubricant and talc as glidant.

Table: 2.4

Formulation code	Lipospheres	Lactose monohydrate	Magnesium stearate	Talc
F-14	200mg	30mg	10mg	10mg

l) EVALUATION OF LIPOSPHERES LOADED TABLET

i) Weight variation test

Weight variation test is the test by which variation of weight from tablet to tablet may be determined. Weight variation was conducted to ensure that, each of tablets contains the right amount of drug. The test was performed by weighing the 10 tablets individually using analytical balance, then calculating the average weight, and comparing the individual tablet weights to the average. The deviation of the weight of the tablets from the average weight was calculated as the weight variation.[14]

$$\text{Weight variation} = \frac{\text{Initial weight of tablet} - \text{Final weight of tablet}}{\text{Initial weight of tablet}} \times 100$$

ii) Hardness test

Hardness of formulated liposphere loaded tablets was determined using 'Monsanto' type hardness tester.

$$\text{Hardness test} = \frac{\text{Total Hardness of tablet}}{\text{Number of tablets}}$$

iii) Thickness test

Thickness of liposphere loaded tablet was determined using vernier caliper.[15]

iv) Friability test

In this investigation, friability was determined by using Electro lab EF-2 Friabilator (USP) and the values of friability were presented as percentage (%). Ten tablets from each formulation were individually weighed and transferred into friabilator which was operated at 25 rpm and continued up to 4 minutes (100 revolutions). Then, the tablets weights were measured again and the percent (%) of friability was determined using following formula.[16]

v) Disintegration test

The disintegration test was carried out using disintegration tester in distilled water medium. To determine disintegration time, three tablets of formulation were placed in each tube and the basket rack is positioned in a 1 litre beaker of water at 37 ± 0.5^{0}c. The time required to break of each tablet into small particles and move through the mesh was recorded. Then, the mean disintegration time was determined for each formulation of tablet.

vi) Dissolution test

The dissolution test was conducted using Dissolution Tester – USP Apparatus-II (Paddle type). Three tablets of each formulation were placed in 3 different beakers in dissolution medium containing 900 ml of phosphate buffer (pH 7.4). The apparatus was rotated at a speed of 100 rpm by maintaining temperature at 37±1ºC in each test. Samples were withdrawn at regular interval of 5 minutes as 5 ml which was predetermined and this was continued up to 40 minutes by replacing equal quantity of fresh dissolution medium. The filtered samples were analysed by using UV Spectrophotometer and percentage (%) of drug release was determined by measuring the absorbance.

m) COMPARITIVE STUDY OF PREPARED TABLET WITH MARKETED PRODUCT

Marketed product of Atorvastatin calcium (Lipitor-80) was compared with the liposphere loaded tablet. Evaluation parameters like hardness, thickness, friability, disintegration and dissolution studies were conducted.

n) Mathematical modelling of drug release profile: (for liposphere and compressed tablets)

The drug release data from the optimised nano emulsion and nanoemulgel formulation were fitted into the zero order, first order, Higuchi matrix, Korsmeyer-Peppas and Hixon Crowell models. The regression coefficient was used to estimate the optimum model for drug release, with the value of the regression coefficient being closer to 1 indicating a better fit for the release mechanism.

Zero order kinetics

It describes the system in which the drug release rate is independent of its concentration.

Qt = Q0 + K0t

Where, Qt = Amount of drug dissolved in time t

Q0 = Initial amount of drug in the solution, which is often zero and K0 = zero order release constant.

If the zero-order drug release kinetic is obeyed, then a plot of Qt versus t will give a straight line with a slope of K0 and an intercept at zero.

First order Kinetic

It describes the drug release from the systems in which the release rate is concentration dependent.

Log Qt = log Q0+ kt / 2.303

Where, Qt = amount of drug released in time t. Q0 = initial amount of drug in the solution

k = first order release constant.

If the first order drug release kinetic is obeyed, then a plot of log (Q0-Qt) versus t will be straight line with a slope of kt/ 2.303 and an intercept at t=0 of log Q0.

Higuchi model

It describes the fraction of drug release from a matrix is proportional to square root of time.

$M_t / M_\infty = kt^{1/2}$

Where,

Mt and M ∞ are cumulative amounts of drug release at time t and infinite time

k = Higuchi dissolution constant reflects formulation characteristics.

If the Higuchi model of drug release (i.e., Fikian diffusion) is obeyed, then a plot of Mt/ M∞ versus t1/2 will be straight line with slope of k.

Korsmeyer - Peppas model

The power law describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation.

$M_t/ M_\infty = kt^n \quad \log [M_t/ M_\infty] = \log k + n \log t$

Where,

Mt and M∞ are cumulative amounts of drug release at time t and infinite time (i.e., fraction of drug release at time t).

k = constant incorporating structural and geometrical characteristics of CR device, n = diffusion release exponent indicative of the mechanism of drug release for drug dissolution.

A plot of log {Mt/ M∞} versus log t will be linear with slope of n and intercept gives the value of log k.

Antilog of log k gives the value of k.[17]

Table 2.5 Mechanism of drug release as per korsmeyer equation/peppas model.

Release exponent (n)	Drug release mechanism
0.5	Fickain diffusion
>0.5 -< 1.0	Anomalous transport, swelling and diffusion (non- Fickian)
1.0	Swelling and erosion (case I)
>1.0	Super -case-II transport

3) RESULTS AND DISCUSSION

a) PREFORMULATION-STUDIES

I) Identification of pure drug by FTIR

The IR spectrum of pure drug was found to be similar to the standard spectrum of Atorvastatin calcium. The spectrum of Atorvastatin calcium shows the functional group as per the reference peak shows below.

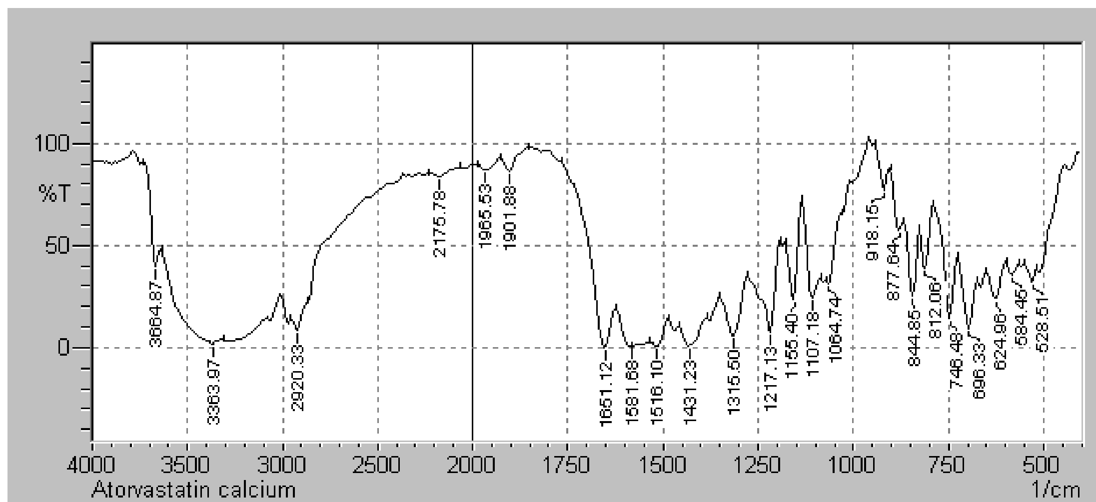


Fig. 3.1: FTIR Spectra of Atorvastatin Calcium.

Table 3.1: Characteristic peaks of FTIR-spectra OF Atorvastatin Calcium.

S. No	Functional group	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})
1	O-H bending	3400-3200	3363.97
2	C-N stretching	1350	1315.50
3	-NH stretching	3600-3100	3363.97
4	C=O stretching	1680-1550	1651.12

The FT-IR spectrum of pure drug was studied. It was observed that all the characteristic peak of Atorvastatin calcium were similar to the standard peaks. Thus, it was concluded that the drug was pure.

ii) Determination of λ max

Determination of λ max of Atorvastatin calcium standard solution was scanned in UV spectrophotometer with wavelength range of 200-400nm. The absorption maximum was found to be at 246 nm.

iii) Standard calibration curve of Atorvastatin calcium in pH 7.4 phosphate buffer.

Table 3.2 Calibration curve of Atorvastatin calcium.

S. No	Concentration	Absorbance at 246nm (mean ± SD)
1	5	0.15 ± 0.0110
2	10	0.33 ± 0.0219
3	15	0.49 ± 0.0035
4	20	0.65 ± 0.0063
5	25	0.78 ± 0.0424

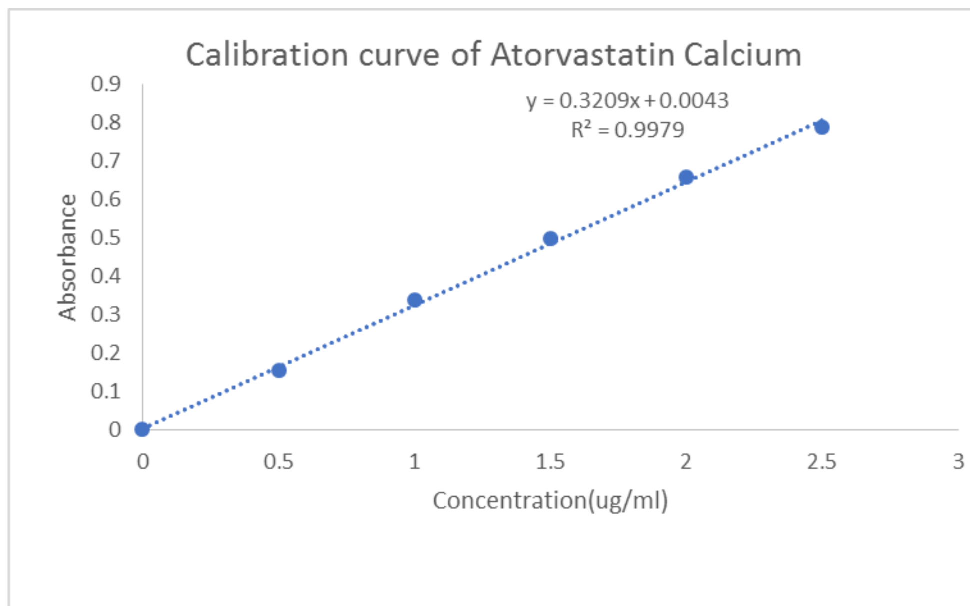


Fig 3.2 Standard calibration curve of Atorvastatin calcium.

Calibration curve of Atorvastatin calcium

Determination of (λ max)

Scanning of drug solution was carried out in UV region (200-400nm) by using UV-Visible spectrophotometer to find out wavelength of maximum absorption (λ max). The λ max was found to be 246 nm.

Preparation of calibration curve of Atorvastatin calcium

The standard calibration curve of Atorvastatin calcium was developed at the λ max of 246nm. The calibration curve was linear between the concentration ranges of 5-25µg/ml.

iv) DRUG-EXCEPIENT COMPATIBILITY STUDIES

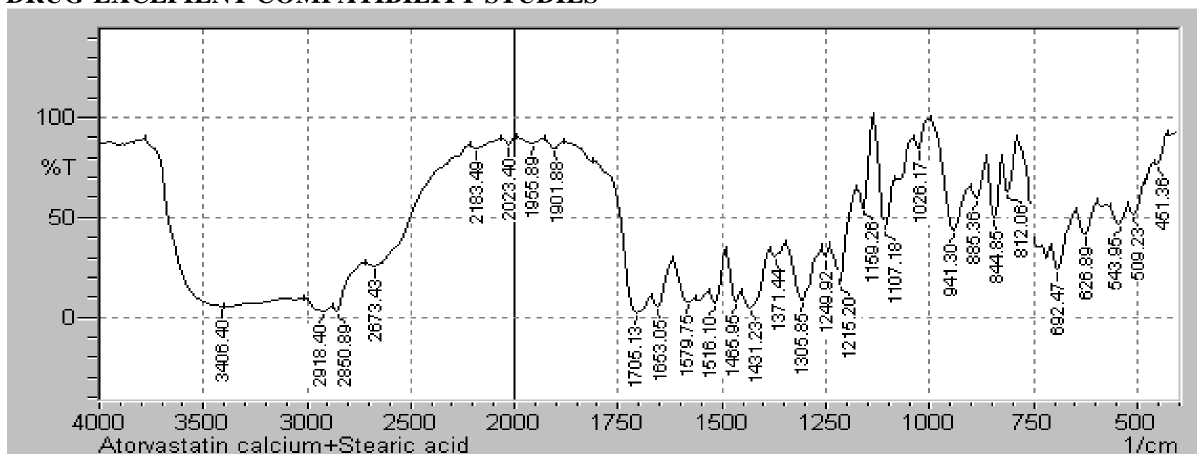


Fig. 3.3 FTIR Spectra of Atorvastatin calcium + Stearic Acid.

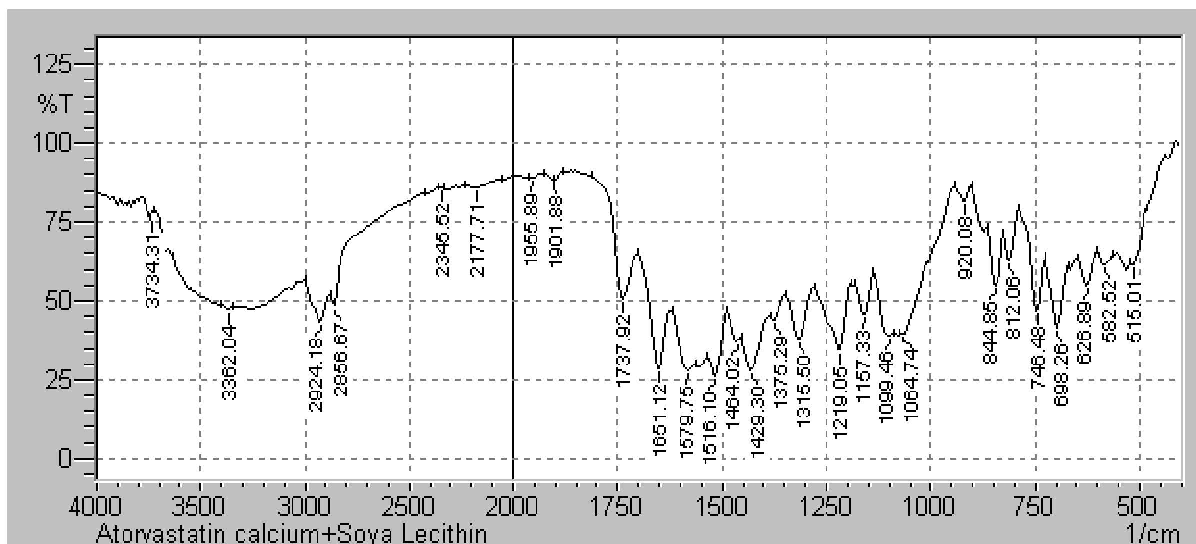


Fig.3.4 FTIR Spectra of Atorvastatin calcium + Soya Lecithin.

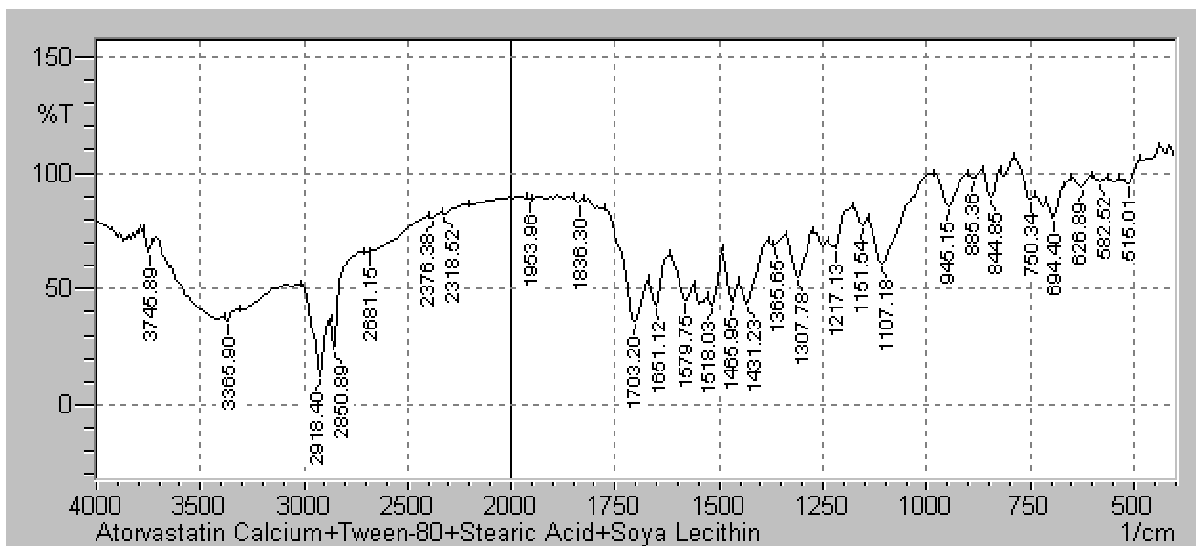


Fig.3.5 FTIR Spectra of Atorvastatin calcium + Tween-80 + Stearic acid+ Soya lecithin.

Table 3.3: FTIR data of drug and excipient.

Excipient nme	Drug (Atorvastatin calcium) characteristic Peaks of observed	Drug + Excipient Peak	Observation
Stearic acid	3363.97 cm^{-1} 1315.50 cm^{-1} 3363.97 cm^{-1} 1651.12 cm^{-1}	3406.40 cm^{-1} 1305.85 cm^{-1} 3406.40 cm^{-1} 1653.05 cm^{-1}	No Interaction
Soya-phosphatidyl choline	Same as above	3362.04 cm^{-1} 1375.29 cm^{-1} 3362.04 cm^{-1} 1579.75 cm^{-1}	No Interaction
All	Same as above	3365.90 cm^{-1} 1307.78 cm^{-1} 3365.90 cm^{-1} 16615.12 cm^{-1}	No Interaction

The compatibility studies were carried out by FTIR spectrophotometer. The FT-IR spectrum of pure drug and physical mixture of drug and other excipients was studied. The FT-IR spectrum of Atorvastatin calcium indicates the characteristic peaks at 3363.97cm-1 (N-H Stretching), 3363.97cm-1 (O-H bending), 1651.12cm-1 (C=O Stretching), 1315.50cm-1 (C-N Stretching). It as been observed that there is no interaction between the drug and the excipients used in the formulation as shown in the table. There are no changes in the characteristic peaks of FTIR spectra of drug after mixed with excipients which indicates that the drug was compatible with the formulation components.

iv) IMAGES OF LIPOSPHERE

Fig. 3.6 LIPOSPHERES OBTAINED BY USING STEARIC ACID AS LIPID.

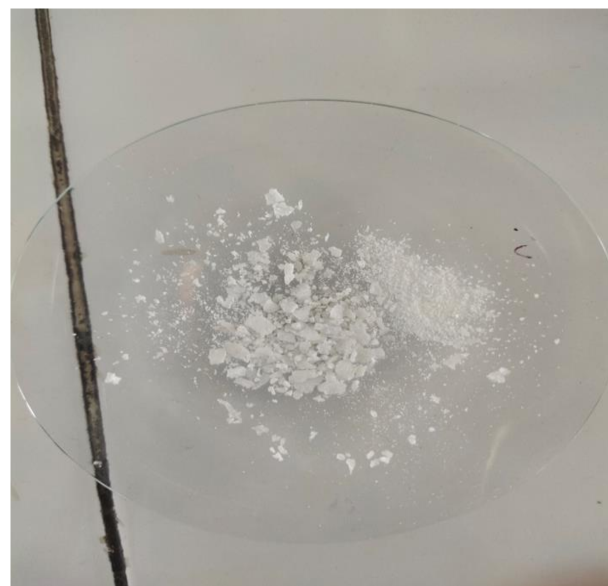


Fig 3.7 LIPOSPHERS OBTAINED BY USING PARAFFIN WAX AS LIPID.

Fig.3.8 EMULSION OBTAINED BY USING CETO-STEARYL ALCOHOL AS LIPID.

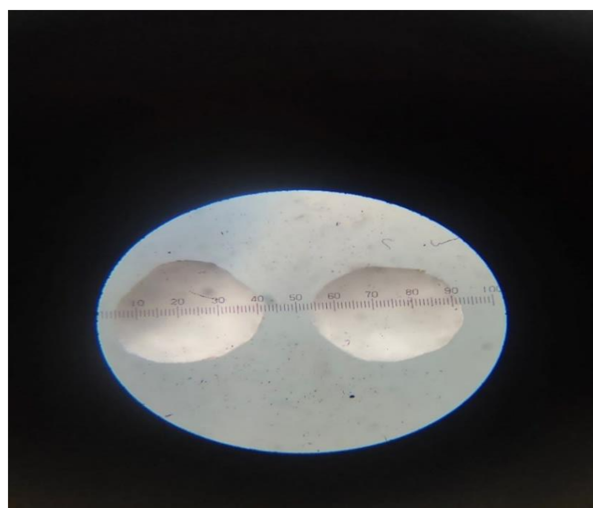


Fig 3.9 LIPOSPHERES OF F-14 FORMULATION.

The formulations from F-1 to F-9 were prepared by varying the lipids. The lipids were stearic acid, paraffin wax and Ceto-stearyl alcohol. Formulations F-1, F-4 and F-7 contained stearic acid at different amount/ratio. Liposphere obtained were free flowing, non-sticky and spherical in shape. Formulations F-2, F-5 and F-8 contained paraffin wax obtained lipospheres were sticky, not free flowing and also irregular in shape. Formulations F-3, F-6 and F-9 contained Ceto-stearyl alcohol and the resulting emulsions were viscous unable to filter to obtain the product. By reviewing the above results stearic acid was selected as a best lipid and further studies were carried out using stearic acid as lipid.[18]

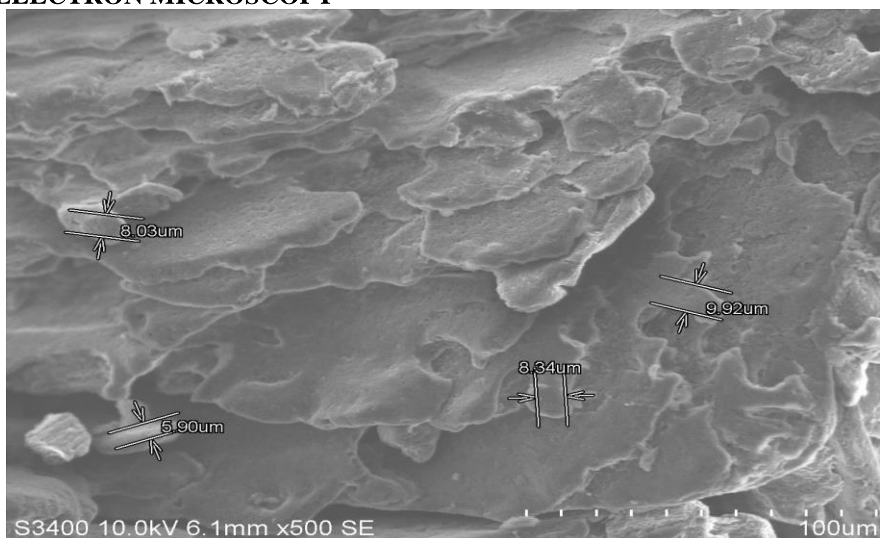
v) Solubility test for pure drug and lipospheres.

Table 3.4 Solubility data of pure drug and Lipospheres.

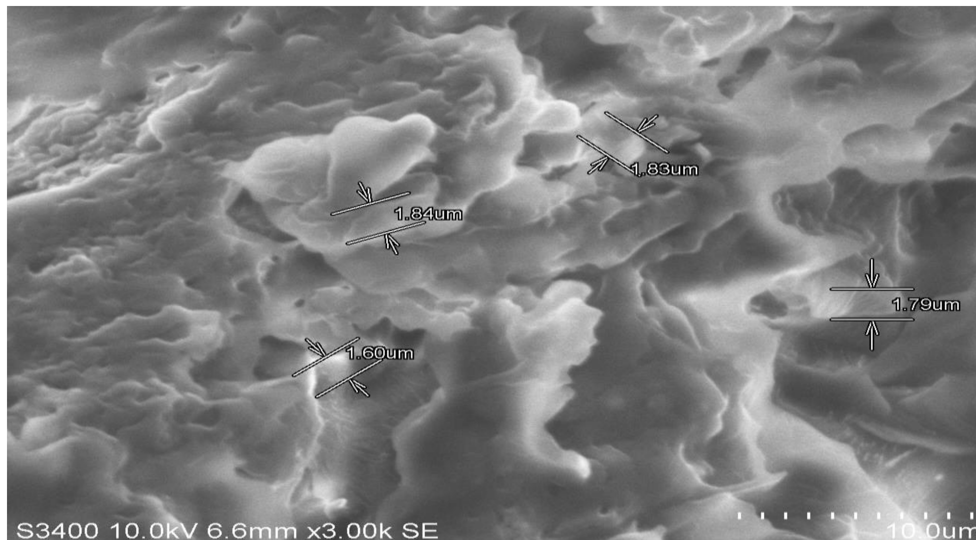
Solubility	Absorbance (246nm)	Concentration(ug/ml)
Pure drug	0.560	1.73
Lipospheres	0.570	1.76

Atorvastatin calcium being hydrophobic in nature it is insoluble in water and have less bioavailability. To enhance the solubility of the drug it is incorporated into lipospheres. So, as per the result obtained it is proved that liposphere improves solubility of lipophilic drug (BCS-II).

vi) SCANNING ELECTRON MICROSCOPY



3.10 IMAGE OF LIPOSPHERE (MELT-METHOD): Smoother surface with larger pores.



3.11 IMAGE OF LIPOSPHERE (SOLVENT-EVAPORATION METHOD): Rough surface with smaller pores.

- **SCANNING ELECTRON MICROSCOPY**

Microspheres obtained by melt method has smoother surface and larger pores when compared to microspheres prepared by solvent evaporation technique. While the solvent evaporation technique has ended up with rough surface particles with smaller pores. Though the pores are small the increased surface area due to roughness might have been resulted in enhanced drug release from solvent evaporation particles.

b) Evaluation parameters of drug loaded liposphere Formulation variables

i) EFFECT OF CORE: COAT RATIO

The lipid core (stearic acid) is varied by keeping the coat (soya lecithin) and the drug constant. The result showed an increase in particle size and hence an increase in drug entrapment efficiency with increase in lipid content. This might be due to presence of high amount of lipid for encapsulation of the drug. Decrease in drug content with

increase in lipid concentration might be due to dilution of the resulting drug lipid mixture. Reduced cumulative %drug release may be attributed to the increased size of the resultant lipospheres and increased in diffusion pathlength for drug.[19]

Table 3.6 EFFECT OF CORE: COAT RATIO.

FORMULATION	ENTRAPPMENT EFFECIENCY (%)	DRUG CONTENT (%)	PARTICLE SIZE (um)
F-10	74.4 ± 0.577	85.6 ± 0.923	55.6 ± 0.432
F-11	75.4 ± 0.622	72.8 ± 0.654	65.6 ± 0.863
F-12	80.6 ± 0.783	61.4 ± 1.233	75.8 ± 0.567

Table 3.7 IN-VITRO DRUG RELEASE DATA.

TIME (hr)	%CDR	%CDR	%CDR
	F-10 (Mean ±SD) (n=3)	F-11(Mean ±SD) (n=3)	F-12 (Mean ±SD) (n=3)
1	10.89677 ± 0.19026	9.311524 ± 0.26286	8.447146 ± 0.16546
2	23.33453 ± 0.19263	21.88599 ± 0.39429	18.86014 ± 0.16710
3	27.95751 ± 0.33363	26.28717 ± 0.13143	22.62802 ± 0.32766
4	33.73625 ± 0.19262	30.84011 ± 0.13143	26.3959 ± 0.32766
5	47.21996 ± 0.19262	35.6207 ± 0.26286	34.36851 ± 0.41185
6	59.18772 ± 0.38524	45.36323 ± 0.10543	55.8405 ± 0.41040
7	77.89733 ± 0.33363	65.42577 ± 0.39429	79.16913 ± 1.59717
8	93.88516 ± 0.38524	91.68107 ± 0.52572	94.6775 ± 0.92748

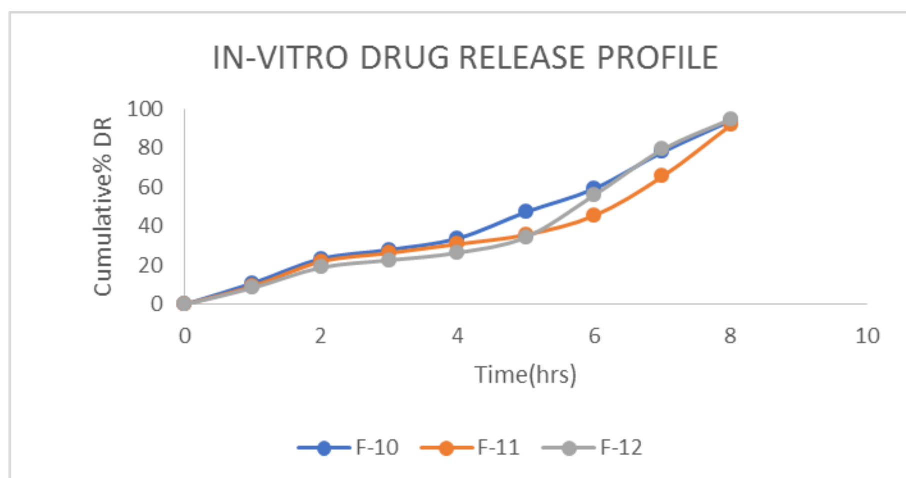


Fig 3.12 In – vitro drug release profile of effect of core: coat ratio.

ii) EFFECT OF AMOUNT OF DRUG

At constant lipid level when amount of drug increases the concentration of mixture increases until saturation. This results in increase in particle size, drug content, entrapment efficiency and cumulative % drug release

Table 3.8 EFFECT OF AMOUNT OF DRUG.

FORMULATION	ENTRAPPMENT EFFECIENCY (%) (Mean ± SD) (n=3)	DRUG CONTENT (%) (Mean ± SD) (n=3)	PARTICLE SIZE (um) (Mean ± SD) (n=3)
F-13	70.23 ± 0.523	73.2 ± 0.657	48.2 ± 0.223
F-10	74.4 ± 0.577	85.6 ± 0.923	55.6 ± 0.432
F-14	84.6 ± 0.568	88.3 ± 0.536	93.6 ± 0.332

Table 3.9 IN-VITRO DRUG RELEASE DATA OF EFFECT OF AMOUNT OF DRUG.

TIME (hr)	%CDR	%CDR	%CDR
	F-13 (Mean ±SD) (n=3)	F-10(Mean ±SD) (n=3)	F-14 (Mean ±SD) (n=3)
1	13.7108 ± 0.19157	10.89677 ± 0.19026	13.48363 ± 0.39966
2	29.33731 ± 0.20123	23.33453 ± 0.19263	28.18482 ± 0.71612
3	32.78561± 0.21345	27.95751 ± 0.33363	36.72835 ± 0.63524
4	35.27604 ± 0.23543	33.73625 ± 0.19262	48.7128 ± 0.55772
5	50.81827 ± 0.38314	47.21996 ± 0.19262	56.971 ± 0.55772
6	66.93521 ± 0.39123	59.18772 ± 0.38524	63.27052 ± 0.63524
7	72.10765 ± 0.38314	77.89733 ± 0.33363	82.27497 ± 0.71612
8	85.90082 ± 0.39123	93.88516 ± 0.38524	96.56801 ± 0.71612

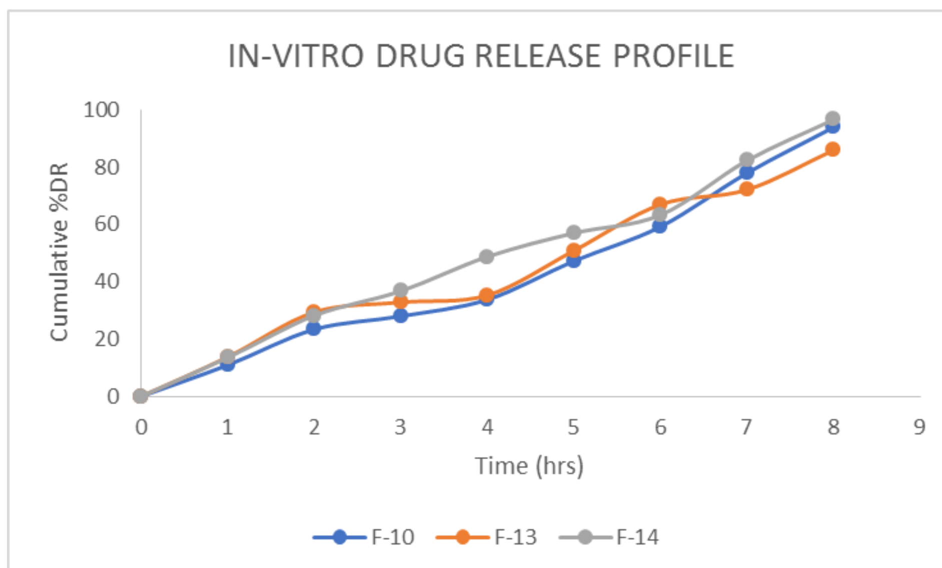


Fig.3.13 IN-Vitro drug release data of effect of amount of drug.

6.3.4EFFECT OF VOLUME OF AQUEOUS PHASE

As the volume of aqueous phase increases at constant stirring speed same amount of energy in the form of sonication is applied to more volume. This in turn decreases viscosity of the liquid which reduces the entrapment efficiency, drug content and particle size. However reduced particle size resulted in an increased cumulative % drug release.[20]

Table 3.10 EFFECT OF VOLUME OF AQUEOUS PHASE.

FORMULATION	ENTRAPPMENT EFFECIENCY (%)	DRUG CONTENT (%)	PARTICLE SIZE (um)
F-15 (25ml)	65.3 ± 0.324	76.4 ± 0.456	66 ± 0.345
F-14 (20ml)	84.6 ± 0.568	88.3 ± 0.536	93.6 ± 0.332
F-16 (10ml)	86.7 ± 0.577	89.5 ± 0.592	95.8 ± 1.245

Table 3.11 IN-VITRO DRUG RELEASE.

TIME (hr)	%CDR (Mean ±SD) (n=3)	%CDR (Mean ±SD) (n=3)	%CDR (Mean ±SD) (n=3)
	F-15	F-14	F-16
1	13.13652 ± 0.36709	13.48363 ± 0.39966	9.646925 ± 0.31336
2	28.10853 ± 0.73419	28.18482 ± 0.71612	21.33072 ± 0.62672
3	32.33013 ± 0.73419	36.72835 ± 0.63524	26.65792 ± 0.62672
4	39.30495 ± 0.73419	48.7128 ± 0.55772	30.88834 ± 0.70394
5	59.70263 ± 1.68224	56.971 ± 0.55772	34.54425 ± 0.20811
6	80.65095 ± 1.14625	63.27052 ± 0.63524	58.69369 ± 0.26728
7	91.29673 ± 1.63140	82.27497 ± 0.71612	81.69412 ± 0.26728
8	97.29263 ± 1.48362	96.56801 ± 0.71612	92.66187 ± 0.62672

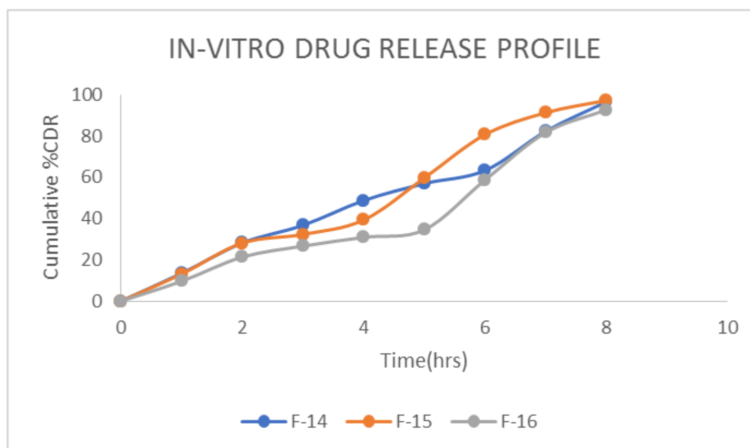


Fig 3.14.

6.3.4 EFFECT OF SONICATION TIME

As the time of sonication increases resulted in breakage of particles which leads to reduction of particle size. As number of lipospheres increases which enhanced hydrophobic space for encapsulation of drug like atorvastatin calcium which itself a hydrophobic in nature. This resulted in increased entrapment efficiency and drug content. Decrease in particle size increases drug release.[21]

Table 3.12 INFLUENCE OF SONICATION TIME.

Formulation	Entrapment efficiency (%)	Drug content (%)	Particle size(um)
F-17 (10min)	82.4 ± 0.576	72.4 ± 0.425	95.8 ± 1.564
F-14 (15mins)	84.6 ± 0.568	88.3 ± 0.536	93.6 ± 0.332
F-18 (20min)	88.5 ± 0.546	89.6 ± 0.547	78 ± 0.453

Table 3.13 IN-VITRO DRUG RELEASE.

TIME (hr)	%CDR (Mean ±SD) (n=3)	%CDR (Mean ±SD) (n=3)	%CDR (Mean ±SD) (n=3)
0	F-17	F-14	F-18
1	10.3759 ± 0.38737	13.48363 ± 0.39966	10.19575 ± 0.22098
2	22.30131 ± 0.77475	28.18482 ± 0.71612	21.98511 ± 0.78773
3	27.07897 ± 0.87339	36.72835 ± 0.63524	25.3761 ± 0.41408
4	31.53381 ± 0.87339	48.7128 ± 0.55772	28.03673 ± 0.56429
5	34.89108 ± 0.77475	56.971 ± 0.55772	36.17511 ± 0.71775
6	54.47881 ± 0.21582	63.27052 ± 0.63524	46.77901 ± 0.73707
7	87.43107 ± 0.12452	82.27497 ± 0.71612	72.51873 ± 0.86197
8	95.17862 ± 0.93688	96.56801 ± 0.71612	91.51507 ± 0.86197

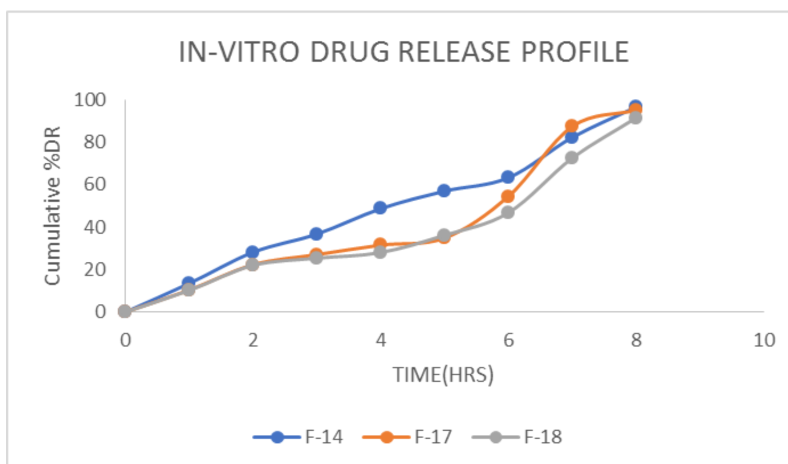


Fig 3.15

6.3.5INFLUENCE OF METHOD OF PREPARATION

Stability of emulsion is more in melt method compare to solvent evaporation method. Since, stability plays an important role in entrapment efficiency it increases in solvent evaporation method. Drug content and particle size decreases in solvent evaporation method because of organic solvents like chloroform. It is toxic in nature it effects particle morphology and atorvastatin calcium dissolve in chloroform easily, it tends to reduce drug content. Particles obtained are smaller in size drug release increases due to large surface area.[22]

Table 3.14 INFLUENCE OF METHOD OF PREPARATION.

TECHNIQUE	FORMULATION	DRUG CONTENT (%)	ENTRAPPMENT EFFECIENCY (%)	PARTICLE SIZE (um)
MELT-DISPERSION	F-14	84.6 ± 0.536	88.3 ± 0.568	93.6 ± 0.332
SOLVENT EVAPORATION	F-14	81.8 ± 0.548	75.6 ± 0.578	35 ± 0.231

Table 3.15 IN-VITRO DRUG RELEASE.

TIME (hr)	%CDR (Mean ±SD) (n=3)	%CDR (Mean ±SD) (n=3)
0	Melt method	Solvent evaporation method
1	13.48363 ± 0.39966	10.38358 ± 0.17143
2	28.18482 ± 0.71612	25.3958 ± 0.17143
3	36.72835 ± 0.63524	40.33258 ± 0.34286
4	48.7128 ± 0.55772	56.3551 ± 0.34286
5	56.971 ± 0.55772	64.24093 ± 0.35686
6	63.27052 ± 0.63524	72.75534 ± 0.34286
7	82.27497 ± 0.71612	85.09837 ± 1.88574
8	96.56801 ± 0.71612	97.5557 ± 1.00449

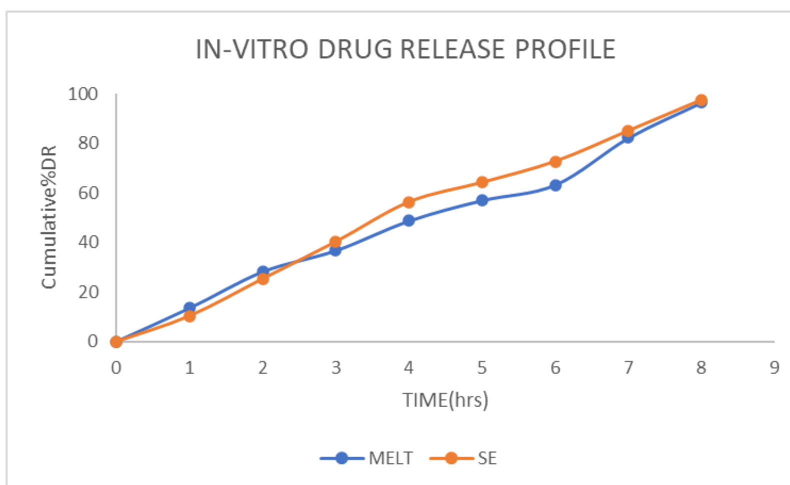


Fig: 3.16.

Table 3.16 Kinetics of drug release of liposphere formulation.

FORMULATION	ZERO	FIRST	HIGUCHI	PEPPAS	KINETICS OF DRUG RELEASE	MECHANISM OF RELEASE
	R^2	R^2	R^2	n value		
F-10	0.964	0.915	0.836	1.32	Zero order	
F-11	0.963	0.873	0.9044	1.18	Zero order	Erosion (Super-case II) transport
F-12	0.904	0.89	0.818	1.28	Zero order	
F-13	0.979	0.926	0.931	1.43	Zero order	
F-14	0.985	0.919	0.958	1.98	Zero order	
F-15	O.943	0.956	0.878	1.57	Zero order	

F-16	0.971	0.936	0.935	1.97	Zero order	
F-17	0.938	0.938	0.879	1.45	Zero order	
F-18	0.951	0.895	0.818	1.28	Zero order	

➢ **6.3.6Kinetic modelling of drug release**

The formulation is followed by the zero-order plot with R2 (0.98) of which show that the rate of drug release was at predictable and constant rate. The n- value of Korsemeyer-peppas equation was found to be 0.97 which showed that mechanism of drug release followed by super case transport-2 mechanism (n>0.85).

5.2.2 Stability studies

Table 3.17 Stability studies data of Lipospheres.

S. No	Evaluation parameters	Short term stability	
		Initial (0 month)	After 3 months
1	Physical appearance	Off white	Off white
2	Particle size	93.6	89.2
3	Drug content	88.3	80.5
4	Entrapment efficiency	84.6	82.8

Table 3.18 IN-VITRO DRUG RELEASE.

TIME (hrs)	%CDR	%CDR
	INITIAL (Mean ± SD) (n=3)	1 MONTH (Mean ± SD) (n=3)
0	0	0
1	13.48363 ± 0.39966	17.03898 ± 0.17463
2	28.18482 ± 0.71612	39.66623 ± 0.34926
3	36.72835 ± 0.63524	53.6596 ± 0.34926
4	48.7128 ± 0.55772	63.6364 ± 0.34926
5	56.971 ± 0.55772	66.43054 ± 0.42361
6	63.27052 ± 0.63524	68.17687 ± 0.54231
7	82.27497 ± 0.71612	82.49681 ± 0.92096
8	96.56801 ± 0.71612	92.21807 ± 0.66756

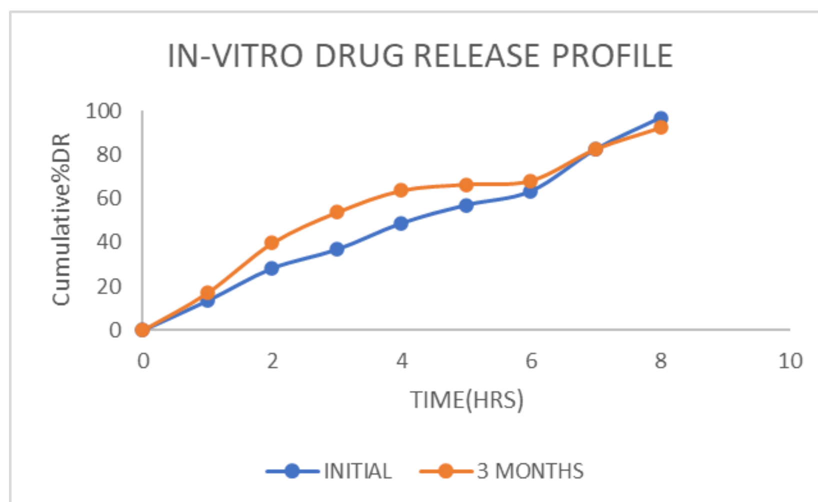


Fig 3.17.

Table 3.19 COMPARISION OF MARKETED PRODUCT AND LIPOSPHERE LOADED TABLET.

EVALUATION TESTS	MARKETED PRODUCT	LIPOSPHERES LOADED TABLET
HARDNESS	4.85	4
THICKNESS	3.83mm	3.12mm
FRIABILITY TEST	0.8%	0.5%
WEIGHT VARIATION	2.7%	3.2%
DISINTEGRATION TIME	25min	40min
DISSOLUTION (%CDR)	97.69%	92.21%

Table 3.20 In-vitro drug release from Liposphere loaded tablet and Marketed tablet.

TIME (mins)	Marketed tablet	Liposphere loaded tablet (Mean ± SD) (n=3)
	% CDR (Mean ± SD) (n=3)	% CDR (Mean ± SD) (n=3)
0	0	0
1	51.09314 ± 0.18718	17.04728 ± 0.73056
2	78.21423 ± 0.16496	36.03315 ± 0.25297
3	83.92186 ± 0.39363	38.66965 ± 0.49481
4	90.41676 ± 0.42345	43.78756 ± 0.36686
5	91.0072 ± 0.56234	51.77459 ± 0.21962
6	93.36898 ± 0.67432	65.07804 ± 0.64892
7	95.33713 ± 0.78935	76.59798 ± 0.81156
8	97.69891 ± 0.39363	92.95977 ± 1.57984

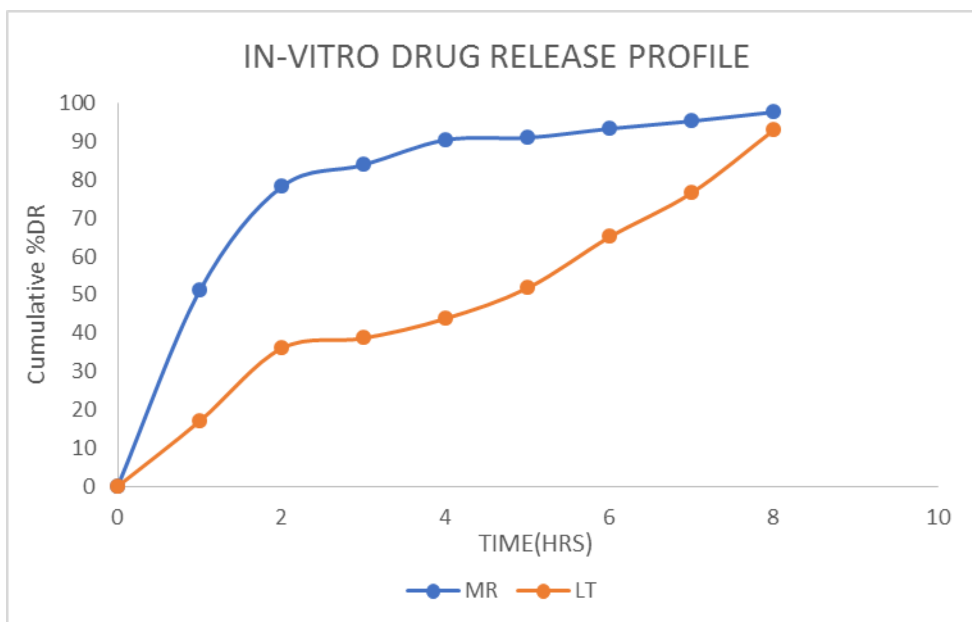


Fig.5.18

6.4Evaluation tests for marketed tablet and liposphere loaded tablet

- Weight variation test: The result obtained were within the acceptable range i,e < 5%.
- Hardness test: The acceptable range of hardness of a tablet is 3 to 5g/cm^2 the result obtained was under this limit.
- Thickness test: The result obtained was under acceptable range < 5%
- Friability test: The friability test limit for most of the tablets should be not more than 1% so, the result obtained was under the limit.
- Disintegration time: The acceptable disintegration time range is 45mins for uncoated tablets the obtained results were under the limit.
- Dissolution test: The drug release from both the product was at T_8 hr but it was found to be more from marketed tablet compare to liposphere loaded tablet.

Table 3.21 Kinetic modelling comparison In-vitro drug release of Liposphere loaded tablet and marketed tablet.

Formulation	Zero order	First order	Higuchi	Peppas plot	Kinetics of drug release	Mechanism of drug release
	R^2	R^2	R^2	n		
Marketed tablet	0.887	0.198	0.744	1.804	Zero-order	Erosion (Super-case II) transport
Liposphere loaded tablet	0.989	0.312	0.563	1.637	Zero-order	

Kinetic modelling of drug release

The formulation is followed by the zero-order kinetics with R^2 of which show that the rate of drug release was at predictable and constant rate. The mechanism of drug release followed by erosion (super-case-2) transport (n>1) which means diffusion is very rapid compared to relaxation.

CONCLUSION

- The aim of the study was to formulate and evaluate the lipospheres of an Anti-hyperlipidemic drug atorvastatin calcium for sustained drug delivery. From this study, the following conclusions were drawn: Pre formulation helps to choose the excipients compactible with the drug in the formulation and to fulfil the requirements of formulation. The standard graph was prepared using methanol and concluded that the standard graph was found to be linear in the range of 5-25ug/ml. FT-IR was performed to study the physical and chemical interaction between the drug and the excipients used. It was observed that there was no interaction between the drug and the excipients. Nine formulation of Atorvastatin calcium using stearic acid as core and soy-lecithin as coat by melt-dispersion method. The optimized formulation was prepared again with solvent-evaporation method to compare the influence of methods of liposphere formulation on its properties.
- Atorvastatin calcium liposphere formulations was evaluated for particle size, %drug entrapment efficiency, % drug content and %drug release of all the formulations and were found to be satisfactory. After considering all the results obtained F-14 was found to be optimised formulation. The mechanism of drug release for optimized formulation was found to be zero order and follows super case-transport II which means diffusion is more rapid compare to relaxation. The best formulation of Atorvastatin calcium lipospheres was formulated into tablet using required amount of lactose, magnesium stearate and talc. The prepared tablets were evaluated by weight variation, hardness, friability, thickness, disintegration, entrapment efficiency. Drug content and %drug release of the formulations was found to be satisfactory.
- The mechanism of the drug release of the tablet was found to be Zero order release and follows super-case-2 transport. The study was concluded that lipospheres loaded tablet showed less atorvastatin calcium release as compared to lipospheres. It was concluded that lipospheres improves the solubility of poorly-soluble drugs like Atorvastatin calcium.

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