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FORMULATION DEVELOPMENT AND EVALUATION OF ATORVASTATIN CALCIUM LOADED SOLID LIPID NANOPARTICLES

Upadhyay N[*a], Mohan G[a] , Sharma S[a], Shukla T P[a], Mathur A[a], Manmohan K
[a]NIMS Institute of Pharmacy, NIMS University, Shobha Nagar, Jaipur-303121, India

***Corresponding Author: niranjanpharma1187@gmail.com**

Abstract

The aim of the present study is to develop and evaluate Atorvastatin calcium loaded solid lipid nanoparticles (SLNs). Atorvastatin calcium loaded SLN were prepared by modified solvent injection method and characterized for shape, surface morphology, particle size, and drug entrapment. These SLNs were spherical in shape with smooth surface and possesses particles of size range 307.98 to 706.3 nm. Fourier transformed infrared spectroscopic analysis were performed to study the presence of interaction between the drug and excipients and the state of. The drug release from SLNs formulation was studied which shows the sustained release of drug. On the basis of evaluation of various parameters like particle size, % entrapment effiency, surface charge and in vitro release kinetics 3 batches are considered as an optimized i.e IOP3, IOP7 and IOP9 respectively. They exhibit least particle size i.e. 377.1, 307.98 and 383.4 nm, % entrapment effiency 81.25, 90.88 and 79.35 and zeta potential -34.28, -39.51 and – 36.69. These batches are further preceded for various studied like scanning electron microscopy, IR Spectroscopy.

Keywords: Solid lipid nanoparticles, solvent injection method, Atorvastatin calcium

INTRODUCTION.

The use of lipid particles in pharmaceutical technology has been reported for several years. The first approach of using lipid micro particles was described by eldem et al reporting the production by high-speed stirring of a melted lipid phase in a hot surfactant solution obtaining an emulsion. Solid micro particles are formed when this emulsion is cooled to room temperature, and

the lipid recrystallizes. The obtained products were called "lipid nanopellets", and they have been developed for oral administration [1]. Lipospheres were described by domb applying a sonication process to overcome the drawbacks associated to the traditional colloidal systems, such as emulsions, liposomes, and polymeric nanoparticles, solid lipid nanoparticles (SLNs) have been developed for similar purposes. SLNs are biocompatible and biodegradable and have been used for controlled drug delivery and specific targeting. These colloidal carriers consist of a lipid matrix that should be solid at both room and body temperatures, having a mean particle size between 50 nm and 1000nm [2].

Solid lipid nanoparticles (SLNs) introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles. The importance of nanotechnology in drug delivery is in the concept and ability to manipulate molecules and supramolecular structure for producing devices with programmed functions [3]. Many types of nanoparticulate drug delivery systems are under various stages of development as drug delivery system including polymeric nanoparticles, liposomes, nanoemulsion, lipid drug complexes, polymer drug conjugate, polymer microsphere micelles, ceramic particles and ligand targeted prodrug [4]. The main limitations of polymeric nanoparticles are cytotoxicity of polymers and lack of suitable large scale production. Liposomes and nanoemulsions are suffered from drawbacks like chemical degradation of entrapped drug molecules [5].

To overcome these problem lipid nanoparticles were developed by two different research groups of muller and gasco in 1990. These are novel delivery systems with absorption increasing effects such as occlusion, absorption enhancements, controlled release of food nutrients and active ingredients and excipients used in these are generally recognized as safe status for oral and topical administration, which decrease the danger of acute and chronic toxicity [6].

MATERIALS AND METHODS.

Atorvastatin calcium was purchased from sigma Aldrich, Glyceryl was purchased from HiMedia Laboratories Pvt.Ltd, Mumbai, India and Poloxamer 407 was purchased from Alcon Laboratories Pvt. Ltd, Bangalore, India.

Drug Excipients compatibility Study. [7]

1. Cartension Method.

In this method pure drug lipid and surfactant which is used in formulation development were mixed in specified ratio and allow to stand for 30 days. After 30 days mixed sample of drug + lipid and drug + surfactant was checked for color, caking, liquefaction and % Drug content (recovery).

2. IR Spectroscopy interpretation method.

The pure drug, atorvastatin calcium and its mixture with the surfactant poloxamer 407 and lipid glyceryl behenate was mixed separately with IR grade KBr and pellets were prepared by applying a pressure of 10 tons in a hydraulic press. The pellets were scanned over a wavelength range of 400cm-1 to 4000cm-1 using an FTIR 8400S model instrument.

Preparation of atorvastatin calcium loaded SLNs.

Solid lipid nanoparticles were prepared by solvent injection technique atorvastatin calcium (80 mg) and specified amount of glyceryl behenate was dissolved in specified quantity of isopropyl alcohol (boiling point 81ºC to 83ºC) with heating at melting temperature of solid lipid. glyceryl behenate is soluble in IPA; however, it requires some heat for ease of solubilization. The resulting solution was rapidly injected into the 10 ml of aqueous phase containing specified amount of poloxamer 407 that was continuously stirred at 400 rpm for 30 min on a magnetic stirrer; 0.1N HCl (4 ml) was added to the dispersion to decrease the pH around 1.5 - 2 to cause the aggregation of SLNs for the ease of separation. Thereafter, the dispersion was centrifuged to 4,000 rpm for 30 min at in REMI cooling centrifuge, and aggregates were resuspended to 10 ml double distilled water containing 4% poloxamer 407 (by weight) as stabilizer with stirring at 1,000 rpm for 10 min.[8]

Experimental design of SLNs.

Table 1. Experimental design of atorvastatin calcium-loaded SLNs.

Formulation code	Amount of drug (mg)	Amount of Glyceryl behenate. (mg)	Amount of Poloxamer 407 (% W/W)	Amount of isopropyl alcohol. (ml)
IOP1	80	240	0.8%	3
IOP2	80	240	0.8%	4
1OP3	80	240	2%	3
IOP4	80	240	2%	4
IOP5	80	400	1%	5
IOP6	80	400	1%	6
IOP7	80	400	2.5%	5
IOP8	80	400	2.5%	6
IOP9	80	560	1.5%	7
IOP10	80	560	1.5%	8
IOP11	80	560	3%	7
IOP12	80	560	3%	8

Evaluation parameter of SLNs.

1. Particle size and Zeta potential. [9]

The particle size of the formulations was determined by laser scattering technique using Malvern Hydro 2000SM (Malvern Instruments, UK) after appropriate dilution with double distilled water. Light scattering was measured at an angle of 90°. The zeta potential was measured using a zeta potential analyzer (Zetasizer Ver. 6.01 Serial Number: MAL1021384 Malvern instrument UK). The samples were diluted with double distilled water prior to zeta potential determination.

2. Entrapment efficiency. [10]

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug in the dispersion medium. The unentrapped drug was determined by adding 0.1 ml of the nanosuspension to 9.9 ml ethanol (95%) in order to dissolve the unentrapped drug; the obtained suspension was centrifuged for 45 min at 6,000 rpm. The supernatant was separated and then filtered through filter paper (0.2-µm). The filtrate was diluted using ethanol and measured spectrophotometrically (Systronic 2203Smart, India.). The entrapment efficiency was calculated using the following equation.

$$\%EE = \frac{W_{inital\ drug} - W_{free\ drug}}{W_{inital\ drug}} \times 100$$

Where "$W_{initialdrug}$" is the mass of initial drug used and the"$W_{free\ drug}$" is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

3. In-vitro drug release. [11]

In-vitro drug release of selected SLNs was performed by dialysis bag diffusion technique. Solid lipid nanosuspension equivalent to 5 mg of atorvastatin calcium was filled in dialysis bag (Dialysis Membrane- 12–14 k Da, pore size 2.4 nm) and immersed in receptor compartment containing 150 ml of phosphate buffer pH 6.8 stirred at 100 rpm at a temperature of 37±0.5°C. Five milliliter of aliquots were withdrawn at regular time intervals (1, 4, 6, 8, 10, 12, 24, 36, 48, hrs) and replenishment of receptor compartment with same volume of fresh dialyzing medium was done and the samples were analyzed for % drug release at 48th hrs.

4. Selection of optimized formulation.[12]

Optimized formulation will be selected on the basis of least particle size, highest% EE and highest% drug release at 48thhrs, and with maximum desirability factor.

5. Characterization of optimized SLNs formulation.

1. SEM analysis.[13]

Scanning electron microscopy SEM (Carl Zeiss EVO 40, Germany) was conducted to characterize the surface morphology of the SLNs. The samples were mounted on alumina stubs using double adhesive tape, coated with gold in Sputter coater-Polaron SC7640. Then the sample was observed in SEM at an acceleration voltage of 20KV and a magnification of 3.11 KX and 492 X.

2. Fourier transformed infrared (FTIR) analysis. [14]

About 1–2 mg of sample of was mixed with dry potassium bromide and the samples were examined at transmission mode over wave number range of 4000 to 400 cm-1. FTIR studies were carried out on pure glyceryl behenate and atorvastatin calcium as bulk material and SLN loaded with Atorvastatin calcium.

Results and Discussion.

1. Drug Excipients compatibility Study

Characterization of API was performed by FTIR and the result obtained was positive, the IR spectrum shows in **Fig 1** percentage transmission (T %) versus wave number of ATR shows characteristic peaks at 3365, 3255, 2920 and 1615 cm-1, respectively. These peaks are obtained due to presence of O-H, aromatic C-H, methyl C-H and C-O functional group present in the structure of ATR. Peaks between 1553 to 1510 c.m-1 shows the presence of nitrogen bonded with hydrogen. Presence of benzyl group was also predicted by the peak produced at 746 to 505 c.m1.on the basis of interpretation of peaks confirmation of API was done.

Drug compatibility was performed by two methods in which first one was Cartension method in which API and excipients are mixed together and allow to stand for one month and after one month period discoloration, caking, liquefaction and % drug content was determined and the result obtained i.e positive and % drug content was found to be 95.5% for API + glyceryl behenate and 97.9% for API + poloxamer407. IR spectroscopy was another method to compatibilities of API with excipients. The poloxamer in the both ratios (1:1) and (1:2) did not yield any kind of deviation in the finger print region i.e 2000 – 600 c.m-1 as shown in **fig 2** and **Fig 3.** However the little changes in the functional group area between 4000 – 2000 c.m-1. O-H stretch peak is shifted very marginally by 2 c.m-1 which is negligible in fact possibly due to the availability of poloxamer 407 to form H-bounding with the O-H of ATR. C-H stretch also is unaffected but the pronounce effect on C-H stretch of methyl group have been observed

which have been shifted from 2920 – 2970 c.m-1. This seems that a methyl group and hydroxyl methyl group of poloxamer 407 are merging together and forming peak together.. In case of glyceryl behenate it was also not able to alter the position of peak I finger print region i.e 2000- 600 c.m-1 as shown in **fig 4**, **fig 5**. However due to the mixing of ATR with glyceryl behenate, some peaks becomes so weak. So that they are abolished. Otherwise the peak alteration have been observed at C-H stretch, aromatic stretch, methyl C-H stretch have been altered only by 2 cm-1 which is quietly significant. Thus we can conclude that the lipid glyceryl behenate and poloxamer407 do not intercept the ATR and thus is fully compatible with API i.e ATR.

2. Particle size and Zeta potential.

The results had shown that the amounts of glyceryl behenate and poloxamer 407 were critical parameters governing the particle size. The particle size was in the range of 307.98 – 706.3 nm, depending on the lipid loading. And the surface charge of produced SLNs was in the range -39.51 to – 16.01. IOP7 exhibited the least particle size and surface charge among all the tested formulations.

3. Drug entrapment efficiency.

Entrapment efficiency is an important Parameter related with drug delivery system which acts as carriers and their capacity for drug loading. The effects of lipid concentration on drug entrapment efficiency of SLNs were investigated. It is clear that the drug entrapment efficiency of nanoparticles increased from 57.82 to 89.60% and conclusion is that when the concentration of lipid increases in formulation entrapment effiency decreases.

4. In-vitro drug release of ATR loaded SLNs in phosphate buffer pH 6.8.

In order to develop a prolonged release system, it becomes necessary to understand the release mechanism and kinetics. The in-vitro drug release of formulations is shown in (**Fig 6).** The % drug release of ATR SLNs was plotted as a function of time. The release percentage of formulations (IOP1- IOP12) varied from 63.65% to 93.75% depending upon differences in the glyceryl behenate /poloxamer 407 ratios. The formulation IOP7 formulated using low concentration of glyceryl behenate and high concentration of poloxamer 407 due to which displayed % drug release (93.75%) after 48hrs is achieved.

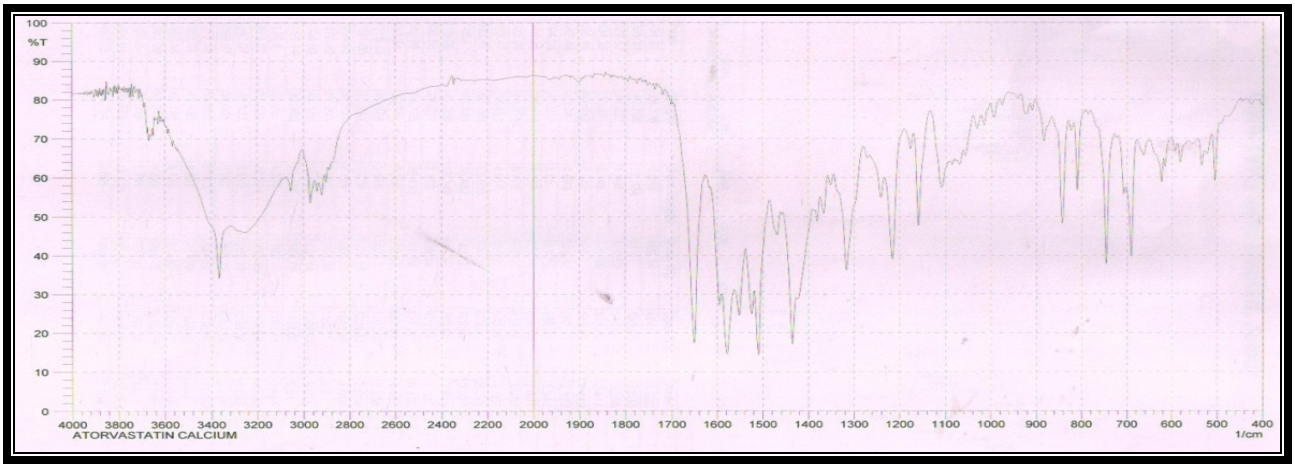

Fig 1. IR Spectra of atorvastatin calcium.

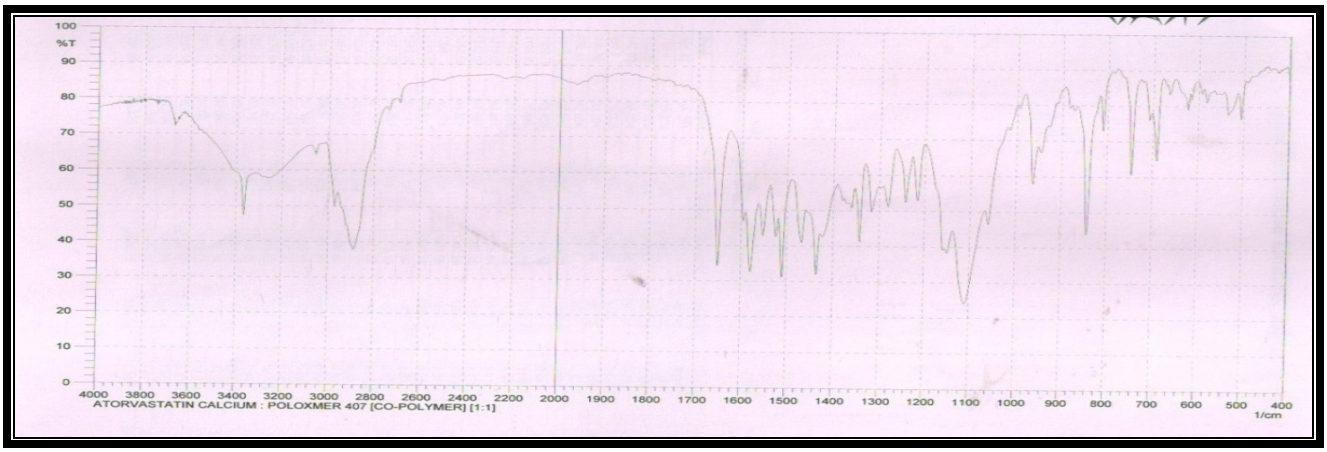

Fig 2.IR Spectra of ATR + Poloxamer 407.(1:1) Ratio.

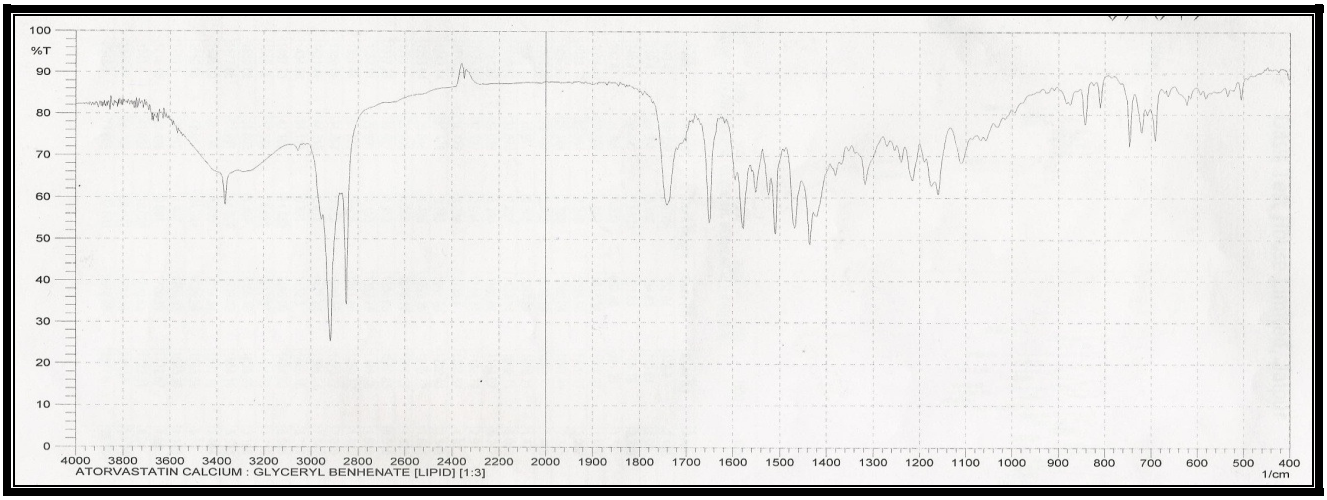

Fig 3.IR Spectra of ATR + Poloxamer 407.(1:2) Ratio.

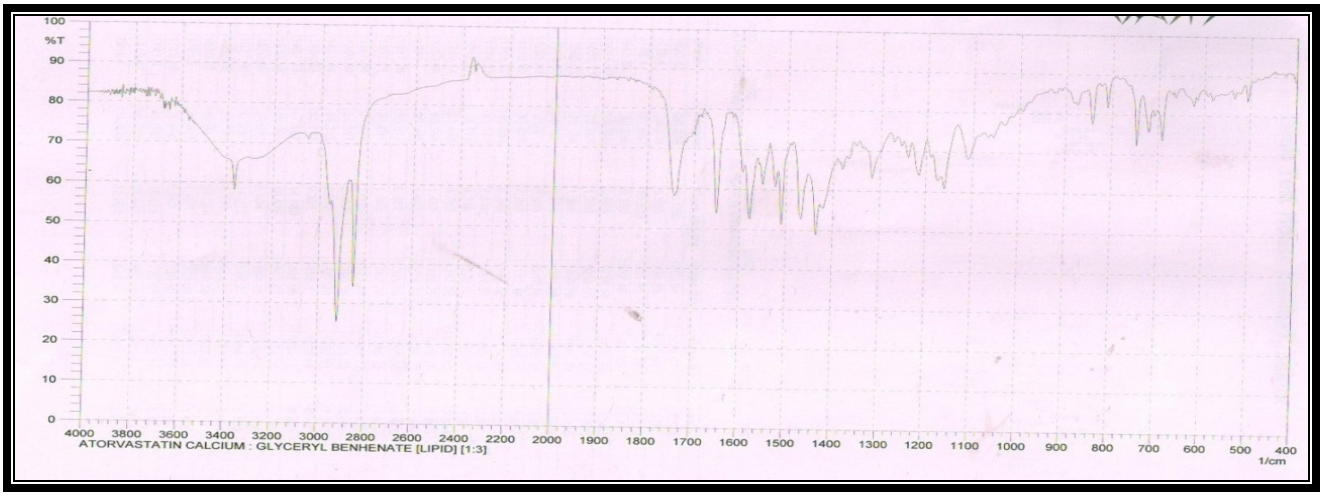

Fig 4.IR Spectra of ATR +glyceryl behenate(1:3) Ratio.

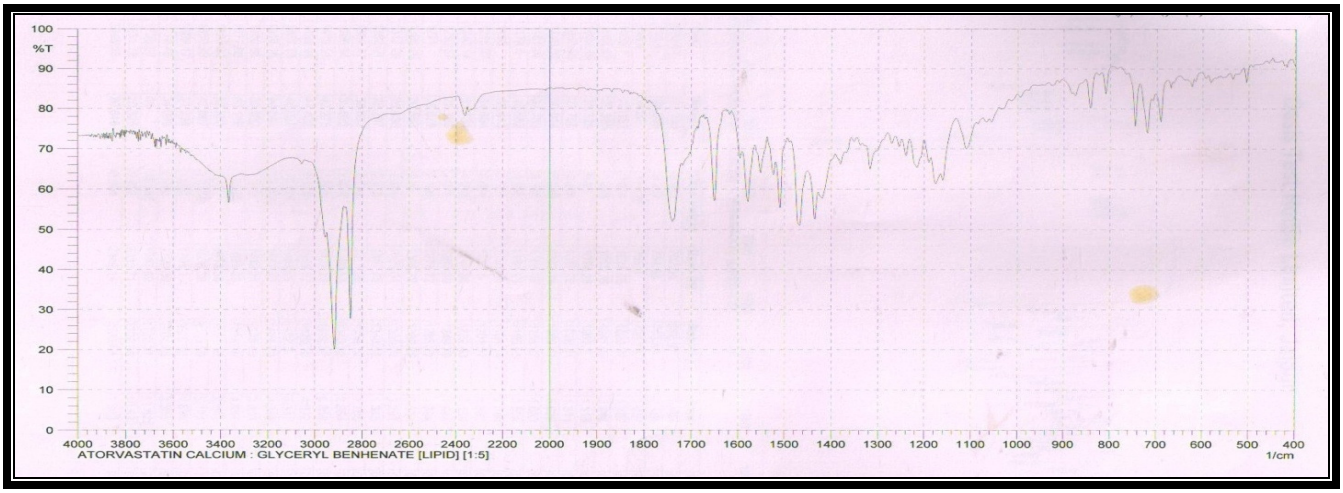

Fig 5.IR Spectra of ATR +glyceryl behenate(1:5) Ratio.

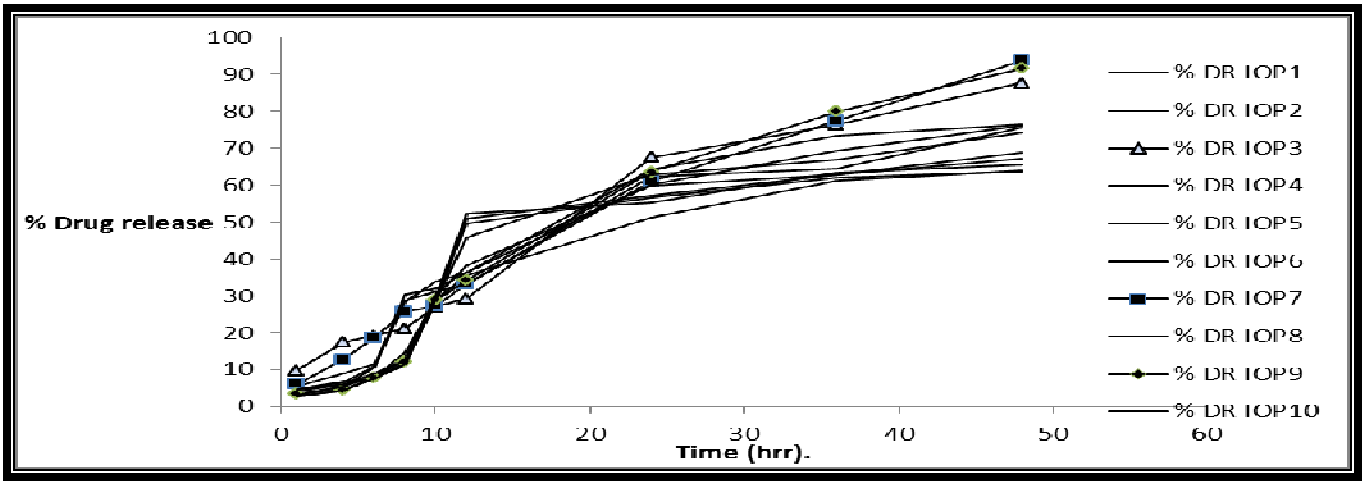

Figure 6. In-vitro drug release profile graph of formulations (IOP1 – IOP12).

5. Selection of optimized formulation.

On the basis of evaluation of various parameters like particle size, % entrapment effiency, surface charge and in vitro release kinetics3 batches are considered as an optimized i.e IOP3, IOP7 and IOP9 respectively. They exhibit least particle size i.e 377.1, 307.98 and 383.4 nm, % entrapment effiency 81.25, 90.88 and 79.35 and zeta potential -34.28, -39.51 and – 36.69. These batches are further preceded for various studied like scanning electron microscopy, IR Spectroscopy and in vivo pharmacokinetic plasma sampling. **Fig- 7.**

6. Characterization of optimized SLNs formulation.

1. Scanning electron microscopy.

Fig 8 shows the shape of the SLNs entrapping ATR; the particles investigated reveal round and homogeneous shading; the figure confirmed that the prepared SLNs were less than 500 nm in size. SEM shows that the drug was on the surface forming ATR layer around the particles; such layer was not present in the unloaded SLNs. This is also in agreement with other investigations, which postulate a drug-enriched shell around a core, lamellar lattice structure of the lipid core and drug-enriched shell makes the ATR SLNs to release prolonged from incorporated core

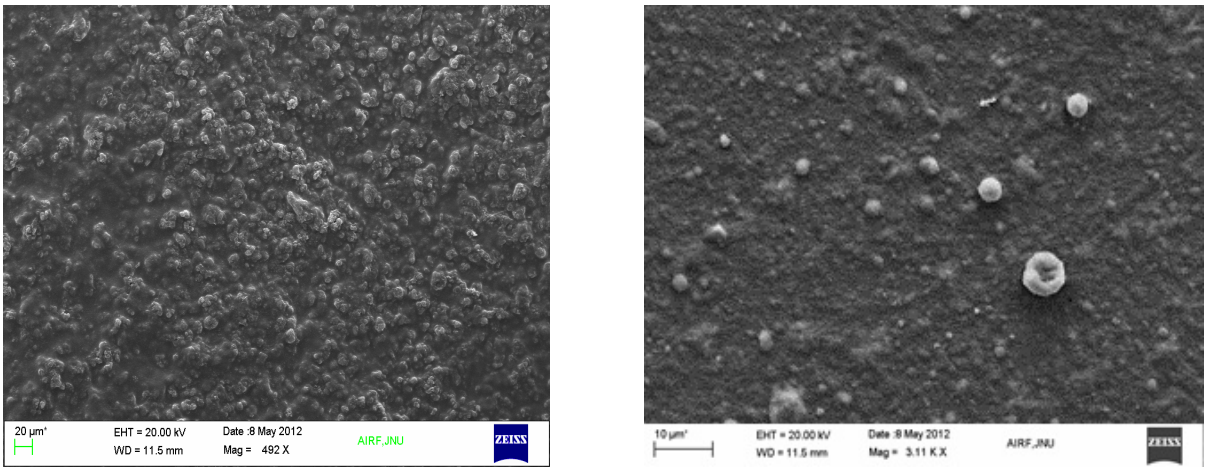

Fig7 . SEM analysis of optimized batch IOP7

2. IR spectroscopic analysis.

The IR spectrum of optimized batch IOP7 tells a different story because of effect of various excipients used in formulation development. It contain 0.08% of API, 40% lipid i.e. glyceryl behenate, 50% IPA and 2.5% poloxamer 407. But the peaks of drug are being stabilized, thus Shows the absorption downfield. Except O-H stretch and C=O stretch. It seems that the shifting of O-H stretch from 3366-3407 cm^{-1} and O-H stretch 1651-1668 cm^{-1} is due to the availability of involvement of H- bond with IPA, water and poloxamer 407. Therefore we can conclude that these excipients are solving the molecule through hydrogen bonding which is favorable for the formulation and we can accept the delayed release of drug from SLNs. The peak position in ATR standard spectra and the shifted peak position in ATR loaded SLNs spectra is described in **Fig 8**

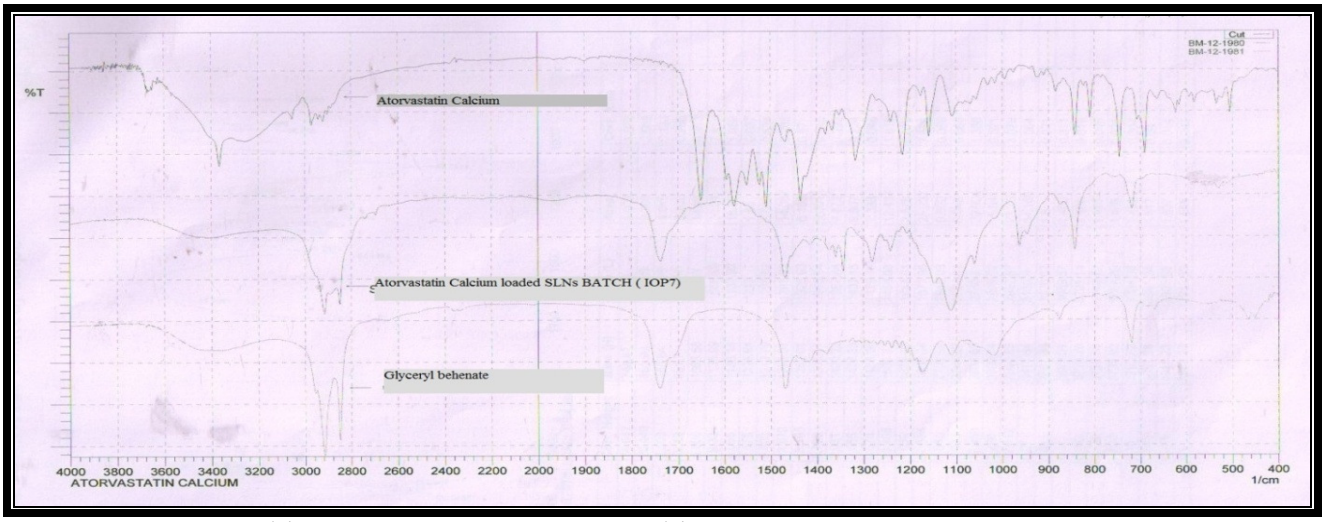

Fig 8. IR Spectra of ATR loaded SLNs batch IOP7, ATR standard and glyceryl benhenate.

CONCLUSION

Solid lipid nanoparticles of ATR were successfully developed with least nanometeric particle size and highest possible entrapment efficiency that could sustain the release of drug for over 48th hrs. In-vitro studies depict the sustained release nature of the formulation, which is potentially desired for the cure of hyperlipidemia. Design enabled to develop an acceptable formulation using minimum raw materials and in minimum time. The release % of ATR loaded SLNs can be increased by modifying the concentration of lipid, surfactant and solvent or by producing SLNs by increasing stirring time or by ultrasonication.

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