International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 06-17 www.ijprajournal.com ISSN: 2249-7781

Physical characterization and Development of Atorvastatin Microspheres for sustained release action with stability assessment.

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Submitted: 01-05-2023 Accepted: 08-05-2023

ABSTRACT: The medicine is evenly placed in a polymer matrix within a micron-sized shell called a microsphere, which serves as the delivery mechanism. Microspheres are naturally biodegradable powders made of proteins or synthetic polymers that typically flow freely and have a particle size of less than 200 nm. After oral treatment, atorvastatin is quickly absorbed, reaching its peak plasma concentrations in one to two hours. Its principal site of action, the liver, extensively processes atorvastatin during first-pass metabolism. About 14 percent of atorvastatin is absolutely bioavailable. In this study the microspheres for atorvastatin was developed and characterized for solubility, FTIR, DSC, partition coefficient, Scanning electron microscopy, entrapment efficiency, drug release as well as stability studies. The formulation remained developed and improved on such parameters where logP was found to be 6.1 and shows a good DSC endotherm peak. The morphological evaluation shows the particle size of 1119 nm with spherical shape. Also, the droplet size was of adequate range and with good polydispersity index. The sustained release profile of the formulation is demonstrated by the in-vitro medicine release features, that also asserts the formation of advanced drug delivery systems. The stability studies also supported the work. Developde microspheres could be working as new drug delivery system as well as can be utilized commercially for statins.

KEYWORDS: Atorvastatin, Microspheres, Sustained release, Drug Delivery System, Release characteristics.

I. INTRODUCTION

Novel drug delivery methods seek to maintain a consistent supply of drugs in the circulation for an extended period of time with predictable and reproducible kinetics [1]. This idea has the advantages of minimising medication-related adverse effects since therapeutic blood levels are regulated rather than fluctuating, improving patient compliance because dosing is done less often, and decreasing the overall amount of medicine supplied. [2, 3]

The medicine is evenly disseminated in a polymer matrix within the micron-sized microspheres, which serve as the delivery mechanism [4]. These are the drugs that have polymer coatings on them. While microspheres are also employed for medication targeting, microcapsules and microspheres prolong drug release [5]. Microspheres are frequently naturally biodegradable [6, 7], free-flowing powders with a particle size of less than 200 nm that are comprised of proteins or synthetic polymers [8, 9]. This is a crucial strategy for providing a prolonged, regulated delivery of a medicinal material to the target location [10, 11]. Pharmaceutical benefits of microspheres are: Taste masking (eg. in case of fish oils, sulfa drugs) [12], Protects drugs from GI-environment [10], enhance solubility of poorly soluble drag by reducing particle size [12], Sustained or controlled drug delivery [13], Targeted release of encapsulated material [14], It can encapsulate live cell [15], Convert liquid to free flowing solids [16], Decreases volatilization of certain drugs [17, 18], Separate drug from unsuited workings (eg. excipients, buffers as well as extra medicines) [19], Safe handling of toxic substances by encapsulation [11, 20]. Natural polymer microspheres, such as those consisting of proteins and carbohydrates [21], are produced via single emulsion, double emulsion [22], phase separation [23] and coacervation, spray drying and congealing, solvent evaporation/solvent extraction, and solvent extraction techniques [21, 24].

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Volume 8, Issue 3 May-June 2023, pp: 06-17 www.ijprajournal.com ISSN: 2249-7781

Oral atorvastatin (3R,5R) 5-(propan-2-yl)-1H-pyrrol1-yl-7-[2-(4-fluorophenyl)-3-phenyl-4-phenyl carbamoyl)-3-phenyl-4-phenyl carbamoyl] 3,5-dihydroxyheptanoic acid is a crystalline powder having a MP of 159.2–160.7°C, a molecular weight of 557.639 g/mol, and properties that make it soluble in methanol, ethanol, distilled water, as well as phosphate buffer at pH 7.4 [25, 26].

After oral treatment, atorvastatin is quickly absorbed, reaching its peak plasma concentrations in one to two hours. Its principal site of action, the liver, extensively processes atorvastatin during first-pass metabolism [26]. About 14 percent of atorvastatin is absolutely bioavailable [27]. Having a 0.25 blood-to-plasma concentration ratio, atorvastatin is strongly protein bound (98%) and has a poor red blood cell dispersion [28]. A smaller concentration of atorvastatin, less than 2%, is seen in the urine, by the majority of its elimination occurring via hepatic biliary excretion [29]. Following hepatic and/or extra-hepatic metabolism comes bile elimination [30]. It possesses anticholesteremic properties, HMG-CoA reductase inhibitors, antilipemic properties, and competitively inhibits 3-hydroxy-3-methyl glutaryl-coenzyme A (HMGCOA) reductase, the enzyme that catalyses the conversion of HMG-CoA to mevalonate [31, 32]. This conversion is a first rate-limiting step in the formation of cholesterol [33, 34].

In individuals who are at high risk due to higher blood cholesterol concentration, it acts as a secondary prevention of myocardial infarction, stroke, arterial disease, especially if there are extra atherosclerosis risk factors [33, 35]. In situations of severe medication-resistant dyslidaemia, a bile acid binding resin is added to the therapy with a statin and the prevention of Myocardial infarction and stroke in people with type II diabetes (such as heterozygous familial hypercholesterolaemia) [30, 33].

HMG-CoA reductase, a liver enzyme, that converts HMG-CoA into mevalonic acid, an early precursor to cholesterol, is competitively inhibited by this substance [36, 37]. LDL, very low-density lipoprotein (VLDL), as well as intermediate-density lipoprotein (IDL) were all enhanced by these LDL receptors (IDL) absorption and subsequent elimination, hence restoring lipid homeostasis [32]. Among the pharmacological types, HMG-CoA reductase inhibitors are observedbeing among the mostpositive in lowering blood and LDL cholesterol. Atorvastatin appears to lower triglyceride levels more significantly than other statins do [38]. High-density lipoprotein cholesterol (HDL-C) levels are also increased by around 5 to 15% by atorvastatin. Though, the underlying process is remains ambiguous [33, 39, 40].

It has also been demonstrated that atorvastatin increases nitrous oxide generation. [41] In an in-vitro model, It had been found that smaller the size of the atherosclerotic lesion as well as lower vascular smooth muscle cell proliferation [41, 42]. These results give rise to the possibility that atorvastatin might encourage platelet deaggregation and vasodilatation in dyslipidemic individuals [43, 44].

The study's major goal was to create atorvastatin microspheres as a novel drug delivery method to accelerate release and sustain it for a longer time to improve bioavailability respectively.

II. MATERIALS & METHODS

The atorvastatin was obtained from Anant Pharmaceuticals Pvt. Ltd., while all of the chemicals and reagents were bought from Sigma Aldrich in India and were marketed as being 99 percent pure analytical grade.

SOLUBILITY

Solubility of atorvastatin in water was founded by using Shake flask method. Here an extra amount of drug was shaken with water in conical flask on the shaker, overnight till the equilibrium is achieved. Using a UV-Spectrophotometer set to 242 nm, the supernatant was removed and examined for drug content [45].

PARTITION COEFFICIENT

Shake flask technique was used to get the partition coefficient, where an excess of atorvastatin was shaken between octanol-water systems for overnight till equilibrium is achieved. The two phases were than separated and analyzed using UV- Spectrophotometer at Amax of 242 nm for atorvastatin content [46].

MELTING POINT

A capillary tube was filled with a little amount of the medication. After that, the tube was put into the melting point device [Veego,VMP-D]. The temperature at which the powder melts was observed while the equipment' temperature progressively increased (automatically) [47].

FTIR

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Compatibility study of drug and excipients is mainly used to detect the functional group (present in drug sample and excipients) are compatible or not. FT-IR instrument is used in this analysis and measure the rotation and vibration of molecule influenced by IR radiation at specific wavelength and also detect the structural changes in binding of molecule which helps to detect the type of interaction. Briefly, IR spectrum of sample was taken in the wavenumber 4000-400 cm^{-1} by using KBr solid powder following by fixing $1/8^{th}$ of sample with KBr powder and mix it properly then this powder put into KBr press with 4000 psi pressure then eject the pallet from die and fixed on sample holder of FT-IR instrument. Then, this instrument generating the spectra of sample then comparing this generated spectrum with standard drug to find the interaction [48, 49].

PREPARATION OF CALIBRATION CURVE

Atorvastatin calibration curve: 10 mg of the medication was weighed and diluted in 10 cc of phosphate buffer 7.4 (containing 20 percent methanol), to produce a 1000 g/ml concentration solution. To create a stock solution with a concentration of 100 g/ml, Using phosphate buffer 7.4, 1 ml of this solution was taken as well as diluted to 10 ml.(20 percent methanol). Different concentrations ranging from 1 to 10 g/ml were generated from this stock solution. By by means of a UV spectrophotometer, At 242nm, the absorbance of these solutions was measured. (ShimazduUV-1800) [50, 51].

FORMULATION AND DEVELOPMENT

The process of solvent evaporation is used to create calcium atorvastatin microspheres. Here, a 1:1 combination of methanol and DCM (dichloromethane) was used to dissolve the necessary amounts of the medication (atorvastatin calcium) and polymer (cudragit S-100).With constant stirring at 800 rpm, the resulting solution was dripped into 100 ml of water containing 0.4 percent PVP. Up until the last drop of organic solvent disappeared, stirring was done.After that, whatman filter paper was used to filter the resultant microspheres. Microspheres were followed by a 24-hour room-temperature drying period. Atorvastatin microspheres are made using the polymer eudragit S-100 at concentrations of 100, 200, 300, and 400 mg, and their size, shape, entrapment effectiveness, and yield percentage are all measured. The microspheres were designed to contain the maximum quantity of medication (i.e 5, 10, 20, & 30mg) [52, 53].

OPTIMIZATION OF PROCESS PARAMETERS

A procedure of solvent evaporation was employed to make microspheres of atorvastatinby altering the concentration of PVA, stirring speed, temperature and internal phase-to-external phase ratio (IP/EP ratio). The microspheres are analyzed for shape, size, entrapment efficiency and % yield [54].

SCANNING ELECTRON MICROSCOPY (SEM)

SEM was employed to review the surface characteristics and shape of microspheres (SEM).Using double-sided adhesive tape, The samples were put immediately just on SEM sample chamber, and the required intensity was used to take photos [55, 56].

PARTICLE SIZE & POLYDISPERSITY INDEX

Malvern Zetasizer Nano ZS was used to perform the dynamic light scattering (DLS) particle size analysis for all the batches (Malvern Instruments, UK). The system was adjusted to 25°C, and the microspheres were dissolved in double-distilled water (DDW) [57].

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

A sample of 5 mg was measured and sealed in an aluminium crucible. The DSC instrument (NETZSCH, DSC-204 F1 PHOENIX) was then placed on the crucible, and the spectra based on temperature transitions were obtained. The DSC spectra of prepared microspheres were taken [58].

ENTRAPMENT EFFICIENCY

To 100ml of phosphate buffer, powder form microspheres were measured out and added (pH 7.4). Centrifuging was completed the resulting mixture at 4 °C and 10,000 rpm. Whatman filter paper was used to filter the supernatant. Then, at 242 nm, it was examined using a UV spectrophotometer. The pellet that had sunk to the bottom was collected and mixed with a millilitre of Triton X-100. After that, it was properly diluted and examined [59, 60].

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IN VITRO DRUG RELEASE

A USP XXIII basket type dissolve test apparatus was used to conduct an in vitro drug release study utilising 900 cc of phosphate buffer pH 7.4 as such dissolving media.. Both the temperature and the stirring speed were kept at 37°C and 1°C, respectively. 5 ml of the samples were removed every hour and replaced with a fresh batch of buffer in an equal amount. Using a UV-Visible spectrophotometer, the amount of atorvastatin was measured at 242 nm [61]

STABILITY STUDIES

Stability testing is done to show how the quality of a pharmacological or or product varies over time when influenced by several environmental factors, such as temperature, humidity, and light [62]. The atorvastatin microspheres formulation was tested for stability using zone III and IV criteria from the International Conference on Harmonization (ICH) and placed in hermetically sealed glass vials. Three months were spent maintaining the packed containers of microspheres at various temperatures, including temperature of room(25+2°C), faster condition (40+2°C/755 percent RH), and oven (50-5°C) [61]. The materials' physical appearance, particle size, and entrapment effectiveness were assessed at intervals of 0, 45, and 90 days, respectively [63].

Visual observations were used to assess the formulation's physical characteristics (colour changes). The microspheres's particle size was evaluated by means of the Malvern Zetasizer.

To assess the efficiency of trapping, storage microspheres (2 mg) were precisely weighed, crushed, and resuspended in 50 ml of phosphate buffered saline (pH 7.4). The resultant combination was then filtered, adequately diluted, agitated on a mechanical shaker, and its drug concentration was determined spectrophotometrically at 242 nm [64, 65].

III. RESULTS & DISCUSSION

SOLUBILITY

Any excipient's solubility behavior can be used to gauge how well it will work with various formulations. Drug's 0.12 mg/ml water solubility was established and evaluated for further study.

PARTITION COEFFICIENT

A log P value of 6.1 was discovered.

MELTING POINT

By using the capillary tube technique, it was discovered that the melting point of calcium atorvastatin was 163 °C.

IR

The Infrared Spectra of Atorvastatin calcium (Fig. 1) was measured using KBr disc method.

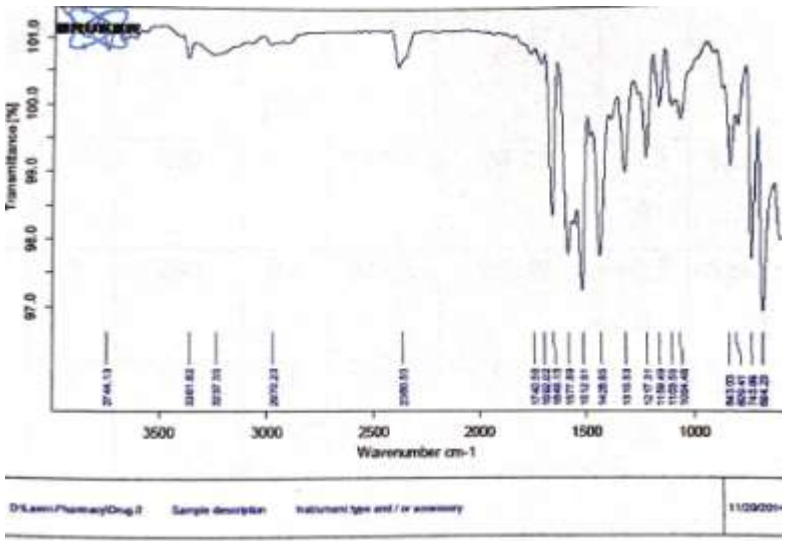

Fig. 1: IR Spectra of Atorvastatin Calcium

CALIBRATION CURVE PREPARATION

The atorvastatin calibration curve was displayed in phosphate buffer at pH 7.4. (20 percent). As seen in Fig. 2, the graph was drawn with concentration (X axis) and absorbance (Y axis) as the two variables. The value of r was discovered to be 0.998.

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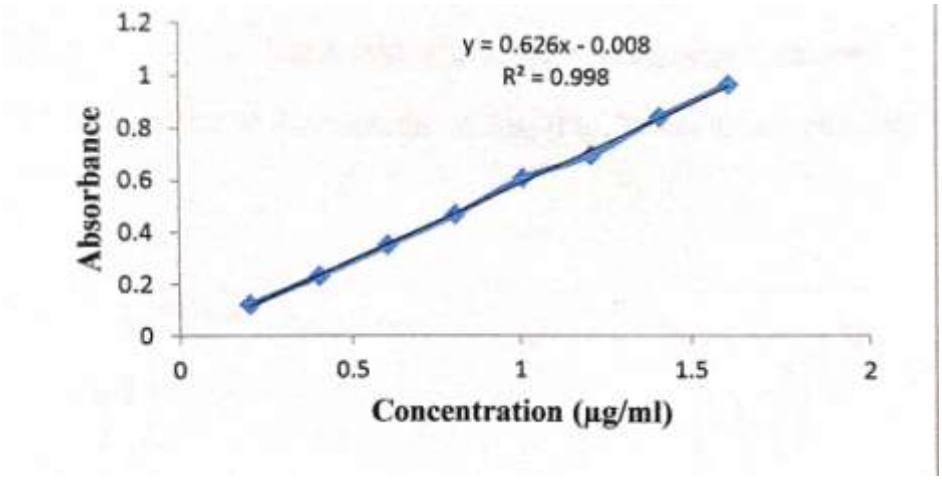

Fig 2: Standard Curve for Atorvastatin Calcium

FORMULATION OPTIMIZATIONS

The process of solvent evaporation was discovered to be the most effective way to encapsulate atorvastatin into eudragit S-100 microspheres., due to its feasibility and high encapsulation efficiency. Multiple studies demonstrated that cudragitS-100 can successfully be used as carrier in drug carrying devices such as microspheres.

Mixture of methanol and dichloromethane (1:1) was used as organic phase, as the eudragit gets dissolved in this and this an easily gets evaporated in subsequent steps.

Following that, the formulations underwent PVA concentration optimization, as indicated in Table 1.Microspheres were prepared using different concentrations of PVAand analyzed for size, shape, % yield and entrapment efficiency.

Table 1: PVA concentration optimization

Formulation Code	**Drug (mg)**	**Eudragit S-100 (mg)**	**PV A concn(%)**	**Particle size (nm)**	**Entrapment efficiency (%)**	**Yield (%)**	**Shape**
A 1	5	200	0.2	717.6	34.07	44.19	Spherical
A2	**5**	**200**	**0.4**	**586.5**	**63.49**	**60.5**	**Spherical**
A3	5	200	0.6	566.5	52.64	20.5	Irregular

The 0.4 percent PVA-containing microspheres had a high yield, spherical form, and 63.49 percent entrapment efficiency. It also demonstrates that the relationship between microsphere particle size and PVA concentration was inverse. According to earlier studies, microsphere particle size decreases as PVA content in the continuous medium increases. Raising the PVA percentage causes additional PVA molecules to cover the surface of the droplets, preventing the droplet from coalescing, which causes the creation of tiny emulsion droplets. As a result, 0.4 percent was decided upon as the optimal PVA concentration.

As indicated in Table 2, several batches of atorvastatin microspheres were made by adjusting the high-speed homogenizer's stirring speed (600-1000 rpm).

Table2: Choosing a stirring speed

Formulation Code	Drug (mg)	Eudragit S-100 (mg)	Stirring Speed (rpm)	Particle size (nm)	Entrapment tEfficiency (%)	Shape
B 1	5	200	600	872.6	54.17	Spherical
B 2	**5**	**200**	**800**	**851.3**	**60.5**	**Sphericall**
B3	5	200	1000	653.7	48.37	Irregular

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The highest trapping efficiency was discovered (60,50) in case of B2 formulation and all the microparticles were found to be spherical in shape.

As indicated in Table 3, batches CI to C3 were created by setting the stirring speed to 800 rpm in order to maximise the temperature of the aqueous solution.

Table 3: Selection of the aqueous phase's temperature

Formulation Code	Drug (mg)	Eudragit s-100 (mg)	Temperature of aqueous solution (oc)	Particle size (nm)	Entrapment Efficiency(%)	Shape
C1	5	200	15	754.6	38.22	Irregular
C2	**5**	**200**	**25 (RT)**	**675.9**	**64.15**	**Spherical**
C3	5	200	40	343.7	45.64	Irregular

It was evident from Table 3 that the entrapment efficiency decreased with increasing external aqueous phase temperature as well as decreasing it. For following rounds, the watery PVA solution's temperature was maintained constant at 25°C.

To achieve the best drug polymer (D/P) ratio, batches D1 to D4 were created at 25°C, as indicated in Table 4.

Table4: Choosing the appropriate drug to polymer (D/P) ratio

Formulation Code	Drug (mg)	D/P Ratio	D/P Ratio	Entrapment Efficiency(%)	Particle size (nm)	Yield (%)	Yield (%)
D1	5	100	1:20	49.44	360.8	28.58	Irregular
D2	**5**	**200**	**1:40**	**64.37**	**356.7**	**31**	**Spherical**
D3	5	300	0.083333333	48.64	310.85	40.66	Spherical
D4	5	400	0.097222222	43.37	200.95	72.5	Spherical

As the polymer concentration rises, the particle size decreases.. All the microparticles in formulation D2 remained spherical in shape, and it was discovered that this formulation had a greater entrapment effectiveness. As a result, the drug polymer ratio was set at 1:40.

Batches El to E3 were prepared under optimized conditions (as discussed above) to choose internal external phase (IP/EP) ratio as revealed in Table 5.

Table5: Choosing a drug's IP/EP ratio

Formulation Code	Drug (mg)	Eudragit S-100 (mg)	IP/EP Ratio	Particle size (nm)	Entrapment efficiency (%)	Yield (%)	Shape
E1	5	200	1:10	852.75	62.38	60.5	Spherical
E2	5	200	1:30	226.2	64.12	37.65	Spherical
E3	**5**	**200**	**1:50**	**318.7**	**74.37**	**31**	**Spherical**

Batches F1 to F4 were prepared by keeping PVA concentration at 0.4%, stirring speed 800rpm, temperature of aqueous PVA solution at 25°C as well as D/P ratio 1:40 to improve the amount of drug loaded in final formulation, shown in Table 6.

Table6: Selection of Amount of Drug Loaded

Formulation Code	Amount of Drug loaded (mg)	Eudragit S-100 (mg)	Mean Particle Size (nm)	Entrapment Efficiency (%)	Shape
Fl	**5**	**200**	**318.7**	**74.37**	**Spherical**
F2	10	200	449.3	71	Spherical
F3	20	200	528.1	68.32	Spherical
F4	30	200	530.4	67.99	Spherical

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The entrapment efficiency was found to be highest when 5mg of drug was loaded. As the amount of medicine loaded was increased, the size of the microspheres grew. Microspheres were found to be spherical in all the formulations from F1 to F4. So 5mg of drug will be loaded in final formulations.

SEM

The surfaces appearance and form of the microspheres were examined using SEM, as shown in Fig. 3. The microspheres' particle size was established to be around 1119 nm, and their spherical shape was also established.

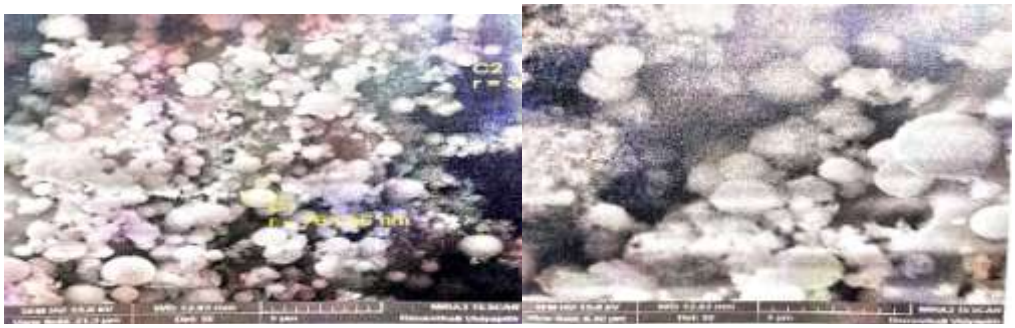

Fig 3: SEM photograph of atorvastatin microspheres

PARTICLE SIZE AND POLYDISPERSITY INDEX

Malvern Zetasizer Nano ZS measured the particle size of microspheres (Malvern Instruments, UK). The system was adjusted to 25°C, and the microspheres were dissolved in double-distilled water (DDW). The improved formulation's average particle size was 75 nm, and the polydispersity index was 0.3.

Differential Scanning Calorimetry (DSC)

The DSC curve for atorvastatin calcium was shown in Fig.4 (a). It exhibits an endothermic peak at 166.3°C, which is also the drug's melting point. Fig. 4 (b) displays the DSC curve for eudragit S-100. The polymer cudragit S-100 shows the peak at 229.8°C. In Fig. 4 (c), it is shown that the peak of only eudragit S-100 is available, since atorvastatin gets completely entrapped in it, because the drug to carrier ratio is very high.

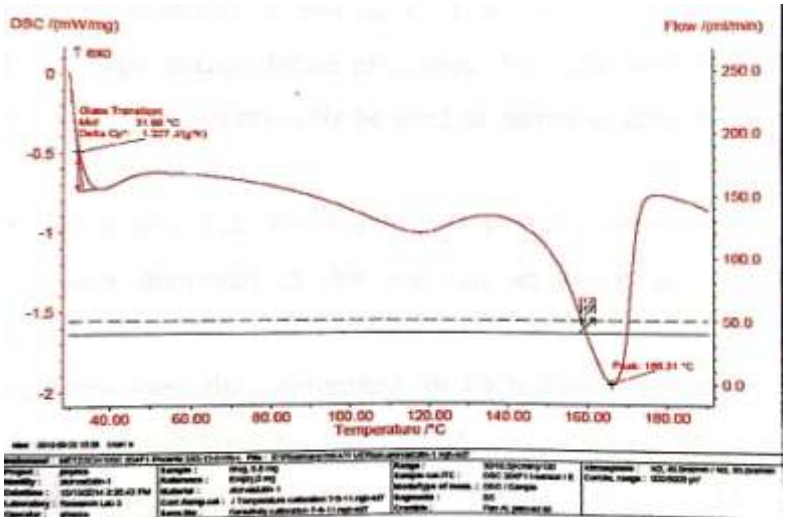

Fig 4 (a): DSC Curve for Atorvastatin Calcium

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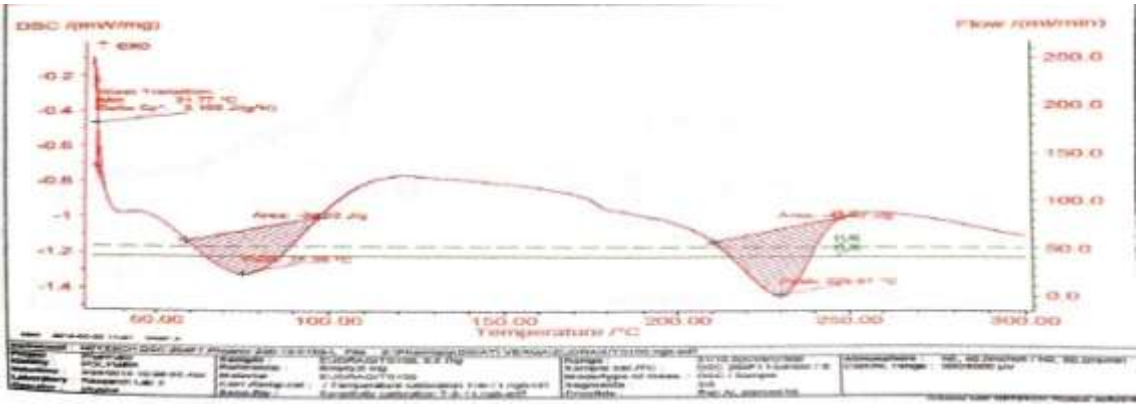

Fig.4 (b): DSC curve for eudragit S-100

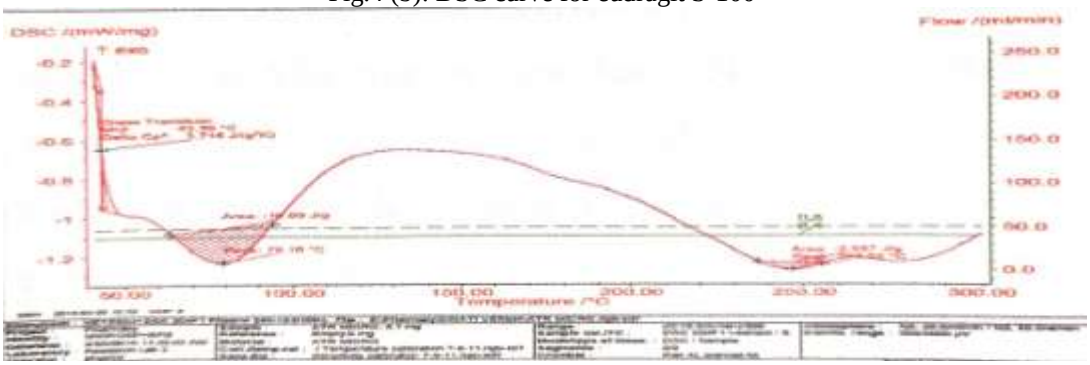

Fig.4 (c): DSC curve for atorvastatin microspheres

ENTRAPMENT EFFICIENCY

By using the centrifugation technique, the entrapment effectiveness of the improved formulation was discovered to be 94.37 percent.

IN-VITRO DRUG RELEASE

Each and every formulation had a regulated release profile. After six hours, about 70% of the medication was released, in case of microspheres with 1:20 drug polymer ratio, whereas it was only 13%, in case of microspheres with 1:80 ratios. Therefore, we draw the conclusion the drug distribution was sustained when the quantity of the polymer rises from 1.20 to 1.80 as shown in Fig.5.

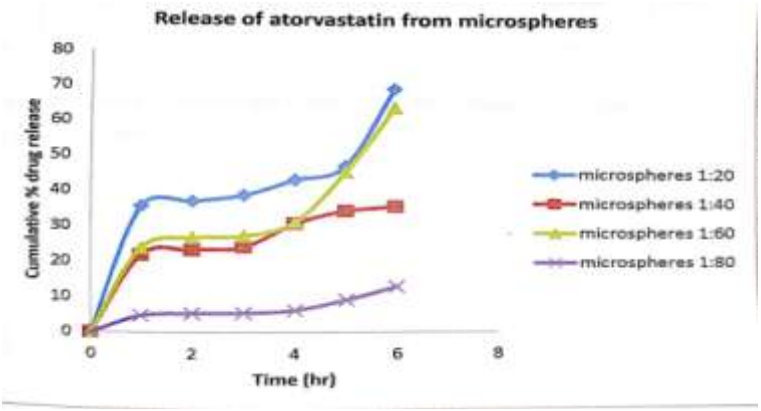

Fig 5: In-vitro drug release from microspheres.

The Higuchi Model is followed by the microspheres; thus the medicine is released from the polymer matrix by diffusion as explained in Table7.

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S.No.	Formulation	Zero Order Release Kinetics. (r2)	First Order Release Kinetics. (r^2)	Hixon Crowell Cube Root Law. (r^2)	Higuchi Model.(r^2)	Korsmeyer Peppas Model.(r^2)
1	1:20 (D:P)	0.9139	0.837	0.9276	**0.9601**	0.6894
2	1:40 (D:P)	0.8554	0.2672	0.8204	**0.8887**	0.1605
3	1:60 (D:P)	0.8011	0.1271	0.1543	**0.8527**	0.2768
4	1;80 (D:P)	0.7769	0.6742	0.8014	**0.8158**	0.603

Table 7: For each formulation developed during the formulation stage, r2 values were calculated for the release profiles that correspond to the zero order, first order, Hixon, Crowell, Higuchi, as well as Korsmeyer Peppas models.

STABILITY STUDIES

At various temperatures (5+3°C, 25+2°C, 40+2°C/75+5 percent RH, and 50+5°C), the stability of atorvastatin microspheres was assessed. Over the course of three months, they were assessed at the scheduled intervals. According to the stability study findings (Table.8), no physical alterations in form or appearance up to three months in all temperature conditions were noted. Particle size did not alter much, however a slight increase in size might be the result of microspheres accumulating from being held under refrigeration (5+3°C).At 25-2°C, 40+2°C, and 50+5°C, it was discovered that the particle size of the microspheres had somewhat decreased, which may have been caused by the microspheres' leftover organic solvent evaporating at higher temperatures. At conditions of refrigeration (5+3°C), room temperature (252°C), accelerated (402°C/755 percent RH), and oven (5015°C), It was discovered that atorvastatin-loaded microspheres had an entrapment effectiveness of 87.34 percent, 91.97 percent, 88.11 percent, and 86.34 percent, respectively, after 3 months of storage. At various storage conditions, it was discovered that there were no notable changes in the formation of the microspheres. According to the findings of the stability investigations, the formulation of atorvastatin microspheres was stable under all circumstances, however it was most stable at room temperature.

Conditions	Time Interval (Days)	Parameters		
		Physical appearance	Particle size (nm)	Entrapment efficiency (%)
Refrigeration (5±3°C)	0	-	1119±4.8	94.37
	45	-	1145±1.2	91.23
	90	-	1179±3.6	87.34
Room temperature (25±2°C)	0	-	1119±4.8	94.37
	45	-	1105±2.8	93.18
	90	-	1103±2.1	91.97
Accelerated (40±20C/75 ±5% RH)	0	-	1119±4.8	94.37
	45	-	1104±2.3	91.13
	90	-	1045±0.4	88.11
Oven (50±50C)	0	-	1119±4.8	94.37
	45	-	1067±3.1	89.19
	90	-	1059±3.6	86.34

Table 8: Stability studies of atorvastatin microspheres

- Indicate no change & + Indicate slight change

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Volume 8, Issue 3 May-June 2023, pp: 06-17 www.ijprajournal.com ISSN: 2249-7781

IV. CONCLUSION

As the solubility of atorvastatin is very low (<1mg), which limits its release from the formulation. Formulation of drug in the form of microspheres can be potentially useful for targeting the drug to the liver. The size of microspheres is less than 1 um, so they are mainly taken by RES organs. So more and more amount of is directly presented to the liver, to block the synthesis of cholesterol. Further this formulation exhibits sustained release profile, which can further be modified in future to provide the prolonged release actions.

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