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"DEVELOPMENT CHARACTERIZATION AND *IN-VITRO* EVALUATION OF POROUS NANOPARTICLES CONTAINING STATIN DRUG"

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ABSTRACT

In the present study an attempt was made to prepare and evaluate nanoparticles containing pitavastatin by sonication method. The prepared formulations were characterized by scanning electron microscopy, Fourier transforms infrared spectroscopy, differential scanning calorimetry, X-ray diffraction, nanoparticle prepared were subjected for Particle size, entrapment efficiency, *in-vitro* drug release. The prepared Nanoparticles are spherical and smooth in surface. The size of nanoparticles 978.85 nm and drug entrapment efficiency was in the range of 80.26% to 95.39 %. The DSC analysis and X-ray diffraction studies indicated that the drug was uniformly dispersed in an amorphous state in the formulation. The i*n-vitro* drug release studies indicated nanoparticles containing pitvastatin prepared by probe sonication methods shows good release rate but formulation Pt9 shows retard drug release from nanoparticles because combination of PMMA & HPC helps to release drug slowly for 24 hours. Results indicated that prepared formulation were intact up to 8 hours. Drug released mechanism follows the Non-Fickian transport. The stability test of optimized formulations are carried out according to ICH guideline, which shown that the formulations were stable in temperature condition. By the use of biocompatible and cost effective polymers like PMMA and HPC we can formulate Nano particulate drug delivery of Pitavastatin in controlled manner with good entrapment efficiency.

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INTRODUCTION

Most of the drugs that are available in the market today are administered through an oral route, which is a convenient and cost effective route of administration. Thus, oral bioavailability is one of the key considerations for discovery and development of a new chemical entity (NCE).[1] The prefix "nano'' comes from the ancient Greek through the Latin word nanus meaning very small. Nanotechnology is a known field of research since last century, since "nanotechnology was presented by Nobel laureate *Richard P. Feynman* during 1959. There have been made various revolutionary developments in the field of nanotechnology.[2] Nanotechnology defined as a tiny science. Design characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale. This phenomenon is known as Nanotechnology. By this technique, we can achieve better therapeutic action, better bioavailability and better patient compliance, which include Nanoparticles systems, Liposomes, Nanoemulsions, Nanosuspension and Ligand mediated Nano systems.[3]

Nanoparticles: (nano = 10^9 extremely small; and particles = small piece of matter),

Nanoparticles are tiny materials having size ranges from 1 to 100nm or theses are particulate dispersions or solid particles of drug carriers that may not be biodegradable, with have a one dimension less than 100nm at least.[4] The nanoparticles differ from various dimensions, shapes and sizes apart from their material. Depending upon the method of preparation nanoparticle, nano spheres or nano capsules can be obtained. They can be classified into different classes based on their properties, and its shapes or sizes.[3] These systems can be used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules and this systems helps to deliver the drug at a controlled and sustained rate to the site of action. The nanoparticles show enhanced properties such as high reactivity, strength, surfaced area, sensitivity and stability etc. because of their small size.[4-6] The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concepts. It was realized that the nanoparticles loaded bio actives could not only deliver drugs to specific organs within the body but delivery rate in addition could be controlled as being by standers, burst, controlled, pulsatile or modulated.[7] Cardiovascular disease (CVD) is the leading cause of mortality worldwide, and hyperlipidemia is the most important risk factor for development of CVD. Statins are the first-line drugs for patients with elevated plasma cholesterol, especially low-density lipoprotein cholesterol (LDL-C).[8] Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs).CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide by the year 2020.[9] Statins inhibit HMG CoA Reductase, the rate limiting step of cholesterol biosynthesis. These are used in the treatment of the cardiovascular disease and hyperlipidemia. But it has high first pass metabolism and less bioavailability from 18% -20%, as well as the less half-life 1-3 hours.[8] The statins shows the some untoward effect in 10mg-50mg dose i.e. severe headache memory loss and confusion. Based on these properties to avoid repeated dosing of statins and enhance the bioavailability of statins, Nanoparticles will helps to enhance the bioavailability of pitvastatin also to overcome the adverse effects of drug we selected as targeted porous nanoparticle drug delivery systems for treatment of Hyperlipidemia. Hence, the present work is to Development, Characterization and *In-vitro* Evaluation of Porous Nanoparticles Containing Statin drug by Probe Sonication using synthetic as well as natural polymers.

MATERIALS AND METHODS

Pitavastatin was gifted sample from Aarthi Pharmaceutical Mumbai. Hydroxy propyl cellules were purchased from Yarrow chemicals Pvt. Ltd. Poylmethylmethacrylate (PMMA) were purchased from Hi Media laboratories Pvt. Ltd. And solvent chloroform is purchased from SD Fine Chemicals Ltd, and all other chemicals and reagents used were of analytical reagent grade.

Estimation of drug

For the estimation of drug (Pitavastatin), Spectrophotometric method was used by using Phosphate buffer 6.8 pH. Scanning range: 200 to 400nm and Absorbance: 0.2 to 0.8 A. Absorption Maxima of Pitavastatin was found to be: 245nm.

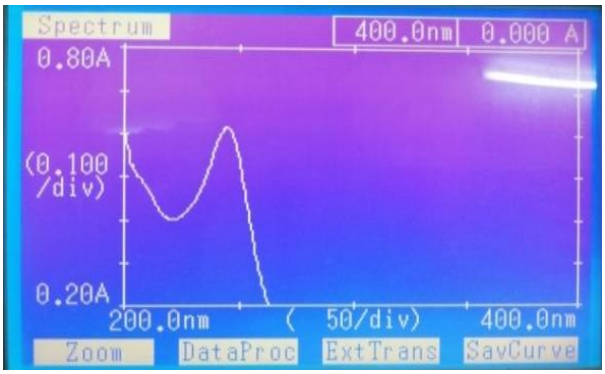


Fig No: - 1 Absorbance Max of Pitavastatin.

Preparation of pitavastatin nanoparticles by sonication method

Dissolve 0.3% of PVA in 100ml of water, then it kept in probe sonicator. Then in another beaker separately dissolve PMMA and HPC polymers in chloroform and add 4mg of pitavastatin drug in PMMA solution and dissolve. After dissolving mixed both the solutions and transfer this solution into the PVA solution in drop wise using syringe while in sonication. Continue the sonication for up to 45-60min to form white precipitation. After completion of sonication remove the solution from the sonication and evaporate the organic layer by using magnetic stirrer. Then the above solution is subjected to centrifugation at 5000rpm up to 30-45min to get nanoparticles and remove the moisture from the preparation by using vacuum drier and collect the dried nanoparticles [10].

Table No: - 01 Formulation table for preparation of nanoparticles by sonication technique.

Ingredients & Formulations	Drug (mg)	PVA (%)	HPC (gm)	PMMA (gm)	Chloroform (ml)
Pt1	4	0.3%	0.5	_	20
Pt2	4	0.3%	1	_	20
Pt3	4	0.3%	1.5	_	20
Pt4	4	0.3%	2	_	20
Pt5	4	0.3%	_	0.5	20
Pt6	4	0.3%	_	1	20
Pt7	4	0.3%	_	1.5	20
Pt8	4	0.3%	_	2	20
Pt9	4	0.3%	0.5	0.5	20/30

CHARACTERIZATION OF NANOPARTICLES

Particle size analyzer:

The particle size and size distribution of nanoparticles can be determined using Malvern Size analyzer with vertically light supplied by an Argon ion laser (cyonics) operated at 40 mW. Experiments were performed at a temperature of 25.5± 0.1^{o} C at a measuring angle of 90^{o} to the incident on beam [11].

Surface charge analyzer:

The zeta potential of nanoparticle was measured in 0.1mW sodium chloride using a Malvern Zeta seizer [3].

Surface morphology:

Morphology of the particles was examined using Scanning Electron Microscopy (SEM) ZEISS EVO. US SEM. The sample of nanoparticle was loaded on copper sample holder and sputter coated with carbon followed by gold [12].

FT-IR study:

The compatibility of drug and polymer (PMMA, HPC) was analyzed using FT-IR spectrophotophotometer. The FT-IR spectra of combined polymers and drug (Pitavastatin) were compared with standard FT-IR spectra of pure drug. The samples were placed in to sample holder and scanned in the spectral region between 4000 cm^{-1} and 650 cm^{-1}.[13]

Crystallography:

Crystallography is the study of atoms and molecules arrangement in crystal solids. The crystallography of nanoparticles is carried out by a powder X-ray, neutron or electron diffraction to determine the structural arrangement [3].

Determination Production yield:

The production yield of polymeric nanoparticles of drug pitvastatin was determined; as the weight percentage of final product after drying with respect to initial amount of drug, polymer and others material used for the preparation [11].

$$\textbf{Process yield} = \frac{\text{Practical yield}}{\text{Theoritical yield}} \textbf{ X 100}$$

Evaluation parameter of Nanoparticles

***In-Vitro* drug release study**:

The prepared nanoparticles were subjected to *in vitro* dissolution. Dissolution test was carried out using USP XX II type I Baskets method. The stirring rate was 50 rpm, PH-6.8 phosphate buffer was used as dissolution medium and dissolution medium temperature was maintained at 37±1 ^{o}C. Samples of 5ml were withdrawn at regular intervals of time, filtered and replace with 5 ml of fresh dissolution medium, dilutions were made wherever necessary and were analyzed for Pitavastatin at 245 nm by using UV-visible spectrophotometer [14].

Drug entrapment efficiency:

The amount of drug entrapped in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug released}}{\text{Amount of drug initially taken}} \text{ X 100}$$

Estimation of drug content:

A quantity, which was equivalent to 10 mg of drug, was accurately weighed and transferred to 100ml volumetric flask. Then the volume was made up with, PH-6.8 phosphate buffer and shaken or 10 min to ensure complete solubility of the drug. Then the solution was filtered. Same concentration of standard solution was prepared by dissolving 10 mg of standard drug in PH-6.8 phosphate buffer. For both the sample and standard solutions absorbance was measured at 245 nm in UV-Visible spectrophotometer [15].

Short term Stability study of Nanoparticles:

The statin loaded nanoparticle formulation was filled in tightly closed glass vials and subjected to stability testing according to the International Conference on Harmonization (ICH) guidelines for zone III and IV. The packed containers of Nanoparticles were kept under the condition (40±2°C/75±5% RH) in a stability chamber for a period of 45 days. The samples (n=3) were analyzed at 45 days and evaluated for physical appearance, drug content, and drug release studies [16]

RESULTS AND DISSCUTION:-

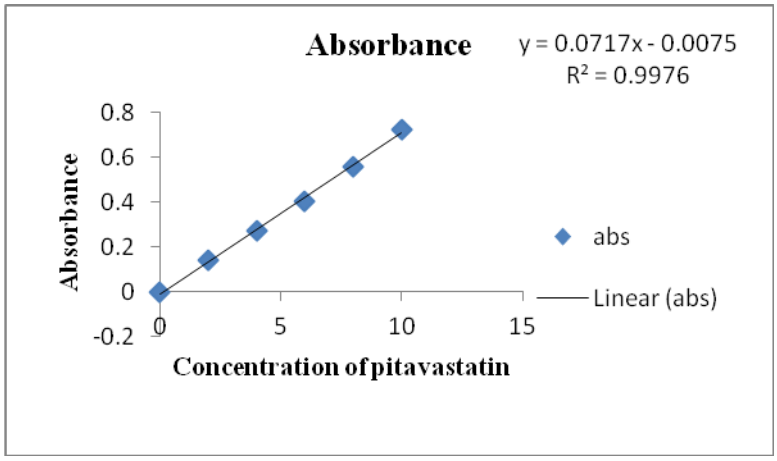
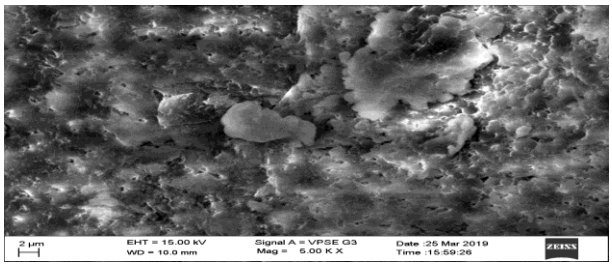


Fig No: - 02 Calibration carve of Pitavastatin.

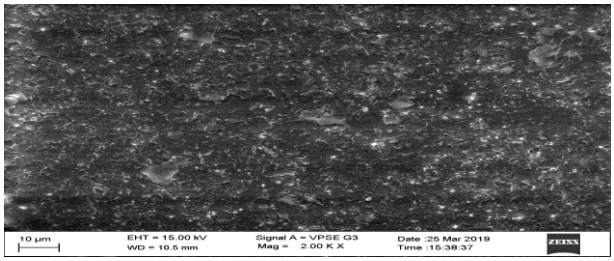
Results the R^2 value of standard graph of drug not more than 0.99 i.e. it obeys beer`s low.

Surface morphology:-

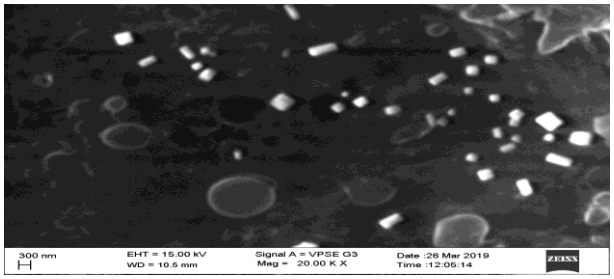
The surface morphology of the prepared nanoparticles was characterized by SEM studies. **Figure no 3** show the SEM images of polymeric nanoparticle containing the drug pitavastatin.



A. (Pt1)



B. (Pt5)



C. (Pt9)

Fig No:- 03 SEM images of formulation (A) Pt1, (B) Pt5 and (C) Pt9.

Table No:-02 Particle size and Drug Entrapment Efficiency of prepared nanoparticle formulations.

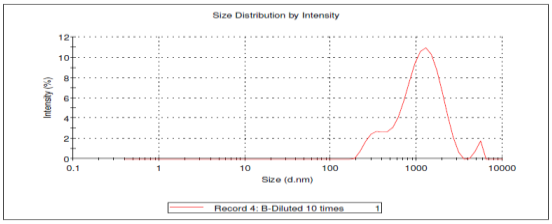
Sl. No	Formulations	Particle size (nm)	Drug EE ($\bar{X}$±SD)*	Production yield %
1	Pt1	987.16	81.23±0.050	86.62
2	Pt2	896.25	85.51±0.036	78.56
3	Pt3	956.12	93.40±0.020	72.92
4	Pt4	905.15	94.63±0.020	80.64
5	Pt5	945.25	80.26±0.036	79.68
6	Pt6	965.25	83.86±0.040	85.67
7	Pt7	957.22	92.69±0.018	82.53
8	Pt8	986.15	88.07±0.025	75.28
9	Pt9	978.85	95.39±0.040	89.20

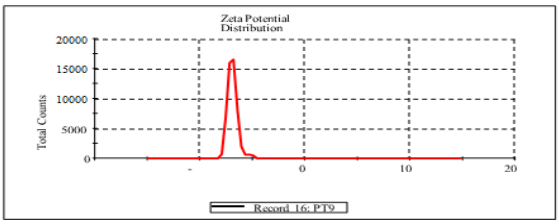
***mean of three readings.

Particle size analysis:-

The mean particle size of the nanoparticles was found to be 320.5 nm. **Figure no 4** shows the particle size distribution of pitavastatin loaded polymer nanoparticle

Surface charge:- The zeta potential was found to be -68.0 mV. The result of zeta potential distribution is given in **Figure no 4**.



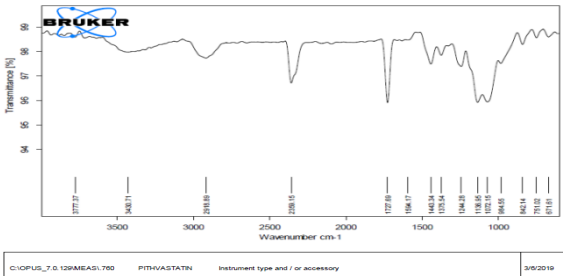


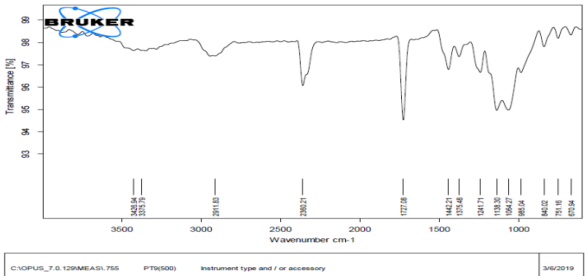
Particle size distribution of prepared formulation Pt9 **Zeta potential of prepared formulation of Pt9**

Fig No:-04 Particle size distribution and Zeta potential of prepared nanoparticle formulation of Pt9.

Drug-polymer interaction study by FT-IR spectrophotometer:

The IR spectra shows peak at 3375 cm^{-1} due to presence of Hydroxyl group (OH). The peak at 2911 cm^{-1} in the IR spectra indicated presence of C-H group. And presence of carboxyl groups the peak at 1727 cm^{-1}. And it shows peak at 670 cm^{-1} due to the presence of C-F group. These similar peaks are obtained in IR spectra of pure drug (Pitavastatin) and Pt9 formulation. It indicates that there is no interaction between drug and polymer used for the preparation of nanoparticles and the results are showed in Figure no 5.



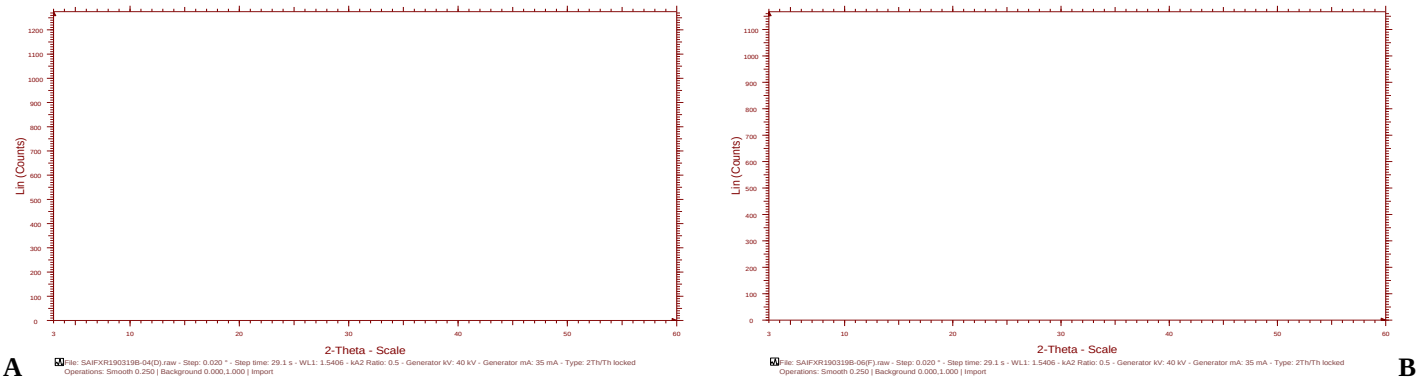


IR spectrum of Pitavastatin **IR spectrum of formulation Pt9**

Fig No:- 05 IR spectrum of Pitavastatin and Prepared formulation Pt9.

XRD Thermographs:-

The XRD graphs of Pitavastatin has shown 2θ characteristic intense peak between 3 to 27. The formulation Pt9 and Pt9 (placebo) also show the peak in the similar range. This indicates that the drug is amorphously dispersed in the formulation. The data were shown in Figure no 6.



A **B**

Fig No:- 06 XRD Thermograph of A) Pure drug [Pitavastatin], B) Pt9 formulation with Drug.

***In vitro* dissolution studies:**

The results of *in vitro* dissolution studies of prepared nanoparticles are shown in **Figure no 7**. The cumulative percentage of drug dissolution from Pt1 to Pt9 ranges from 84.89 % to 94.44%. But Pt9 shows the less drug release in 'controlled manner' compare to other formulations. Because combination of PMMA and HPC retard the release rate of drug. Hence the formulation shows better retard ether than all other formulation.

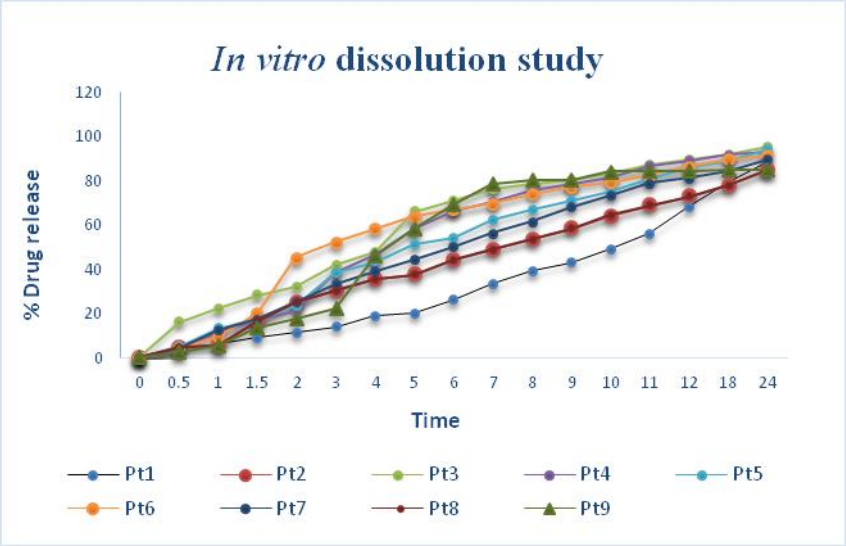


Fig No: - 07 *In vitro* drug release of pitavastatin from Pt1 to Pt9 Nanoparticles in gastric & intestinal fluids.

Release kinetics:

The *in-vitro* release data obtained from all the formulations was subjected to various kinetic release models. Data reveled that release kinetics follows first order drug release kinetics and the 'n' values of First order kinetics shows in the range of 0.042 to 0.088. So it indicates that the release kinetics is Anomalous (Non- Fickian) transport date shown in Table no 3.

Table No 03:- Regression Co-efficient (R^2) values of Pitavastatin Nanoparticles according to Kinetic models.

Formulation	Zero order		First order		Higuchi	Peppas	
	R^2	n	R^2	n	R^2	R^2	n
Pt1	0.951	4.069	0.936	0.059	0.927	0.727	0.581
Pt2	0.838	3.615	0.965	0.036	0.962	0.662	0.680
Pt3	0.693	3.758	0.936	0.059	0.905	0.727	0.681
Pt4	0.710	4.068	0.905	0.055	0.899	0.739	0.685
Pt5	0.790	4.008	0.972	0.088	0.947	0.717	0.608
Pt6	0.648	3.526	0.893	0.046	0.870	0.646	0.593
Pt7	0.800	3.838	0.940	0.044	0.947	0.716	0.609
Pt8	0.838	3.619	0.965	0.036	0.962	0.662	0.680
Pt9	0.609	3.952	0.694	0.042	0.810	0.882	0.736

DSC Thermograms:

The DSC thermograms of pitavastatin and physical mixture of pitavastatin and the polymer showed in Figure No. 08. There is no chemical reaction occurs between the drug and polymer. The Pitavastatin has shown sharp endothermic peak at 136.95^{o}C due to its melting point and drug loaded formulation Pt9 has shown endothermic peak at 54.52^{o}C and the drug free formulation has shown endothermic peak at 53.99^{o}C. This indicates that the drug is amorphous and distributed uniformly through the formulation.

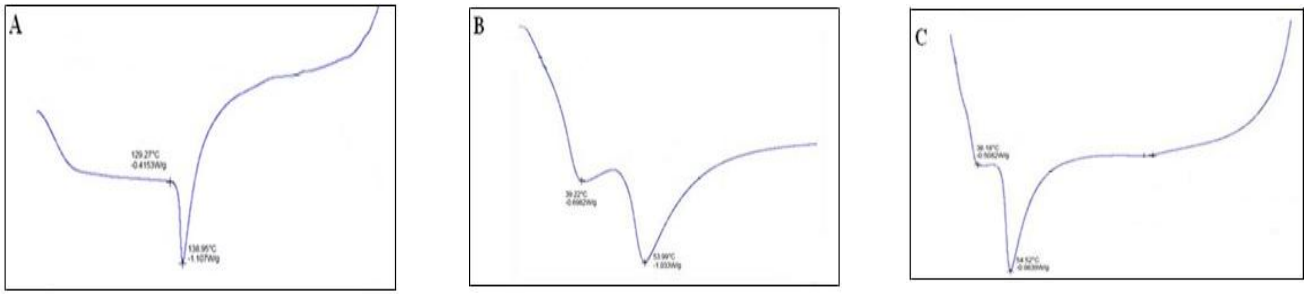


Fig No:-08 DSC Thermograms of A) Plain Pitavastatin, B) Formulation Pt9 Placebo and C) Formulation Pt9.

Stability study:

After storage for 45 days the products were tested for drug content and drug release rate as per the methods described earlier. The results are given in below Table no 4

Table No 04:- Stability studies of formulation Pt 9.

Drug content study of optimized nanoparticle

Formulation	Percentage of drug content ($\bar{X}$±SD)	
	Before stability test	After stability test
Pt 9	95.39±0.040	94.98±0.06

Dissolution study of optimized nanoparticle

Formulation	Percentage of drug release ($\bar{X}$±SD)	
	Before stability test	After stability test
Pt 9	77.15±0.070	77±0.0352

CONCLUSION

Form the results it can be concluded that biocompatible and cost-effective polymer like PMMA can be used to formulate an efficient nanoparticles with good percentage entrapment efficiency and practical yield. The particle size analysis indicated that the particle were in the size range of 896.25 - 987.16 nm, showed good flow properties. The nanoparticles were smooth, as shown by the scanning electron microscopic studies. *In-vitro* drug release showed that release from the nanoparticles gets successfully retarded for over 24h. The formulations were found to be stable in short term stability studies. By considering the results obtained from nanoparticle of pitavastatin prepared by sonication method using PMMA and HPC shows better retard efficiency than individual polymers. So it can be suggested that there is further scope for *in-vivo* and pharmacokinetics study. Hence the nanoparticle technique well formed to be suitable for hyperlipidemia drug.

Present work indicates that using biocompatible and cost effective polymers, efficient nanoparticle form of Pitavastatin with good percentage entrapment efficiency can be formulated. Thus the prepared Nanoparticle could be promising delivery system for Pitavastatin with controlled drug release.

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