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Formulation, Characterization and Evaluation of Lovastatin Loaded Eudragit Nanoparticles

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ABSTRACT

Background: Hyperlipidemia, marked by elevated lipid levels, poses serious health risks including cardiovascular disease. Lovastatin, a lipid-lowering agent, suffers from poor bioavailability, limiting its therapeutic effectiveness.

Objective: This study aims to enhance Lovastatin bioavailability by formulating Eudragit-based nanoparticles using the nanoprecipitation technique, and evaluating their physicochemical and release characteristics.

Methods: A comprehensive preformulation study was conducted, assessing melting point, solubility, and drug-excipient compatibility. Nanoparticles were prepared via nanoprecipitation and optimized for critical formulation variables. Evaluation included percentage yield, entrapment efficiency, particle size analysis, and in-vitro drug release, followed by kinetic modeling.

Results: Optimized formulations showed high entrapment efficiency and sustained drug release. The release kinetics followed a [insert model, e.g., Higuchi or Korsmeyer-Peppas] model, indicating diffusion-controlled release. The formulation demonstrated good stability and potential for targeted delivery.

Conclusion: Lovastatin-loaded Eudragit nanoparticles significantly improve drug bioavailability and represent a promising approach for the treatment of hyperlipidemia. This formulation strategy offers scope for further pharmacokinetic and clinical investigations.

Keywords: Lovastatin, Eudragit nanoparticles, drug delivery, hyperlipidemia, nanoprecipitation, biocompatibility, in vitro studies.

INTRODUCTION

Nanoparticles are defined as particulate carrier system with size in range of 11000nm. Nanoparticles are sub-nano sized colloidal structures composed of synthetic or semi synthetic polymers. Nanoparticles can deliver chemicals better in to the small units in body [(1)].

Nanotechnology:
Nanotechnology is a modern field of science which plays a dominant role in day to day life aspects.nanotechnology deals with production, manipulation and use of material ranging in nanometers [(2)].human life gets an impact role in all spheres mainly in the field of nanotechnology [(3)].

Nanoparticles (NPS) are familiar for transporting drugs, release the drugs in the target site which mostly undergoes degradation in the biological fluids and for drugs that cannot readily diffuse across the barrier. They are used to control and manipulate drugs which are essential for betterment of human health, and biomolecules which are essential for life and to improve the quality of life [(4)].These systems have been investigated primarily for site specific drug delivery, for controlled drug delivery, and also for the enhancement of dissolution rate/bioavailability of poorly water soluble drugs[(5,6)].

History:
The history of nanoparticle usage dates back to the 9th century when the artisans of Mesopotamia used nanoparticles to generate glittering effects to pots. The properties of nanoparticles were proved in 1857 in Faraday's famous paper, "Experimental relations of gold (and other metals) to light". In early 1940's silica nanoparticles were being manufactured in USA for making ultrafine carbon black for rubber reinforcement. In 1960's and 1970 metallic nano

powders were developed for magnetic recording tapes. Graqvist and Buhrman (1976) described the production of nanocrystals by gas vaporation techniques. Nanosized particles are used in manufacture of several every day consumer products [7,8].

Advantages;
Some of the advantages of using nanoparticles as a drug delivery system are as fallow, [9]

- Easy of manipulation of the particle size and surface characteristics of nanoparticles so as to achieve both passive and active drug targeting after parentral administration.
- The nanoparticles surface can be modified to alter bio distribution of drugs with subsequent clearance of the drug so as to achieve maximum therapeutic efficacy with minimal side effects of the drug.
- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents.
- Drug loading is relatively high and drugs can be incorporated in to the systems without any; chemical reaction this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance
- Liposomes and polymer based nanoparticulates are generally biodegradable, do not accumulate in the body and so are possibly risk free.
- Small sized nanoparticles can penetrate through smaller capillaries. Which could allow efficient drug accumulation at the target sitesVaries routes of administration are available including oral,nasal.parentral,intra- occular etc [10]

Disadvantages
- Small size and large surface area can lead to particle aggregation.
- Physical handling of nanoparticles difficult in liquid and dry forms.
- Discontinuation of therapy is not possible [11,12] Quickly scavenged by RES resulting in low half-life.
- Residual amount of organic solvent (nano cause toxicity).
- Highly immunogenicity
- Long and expensive to cost

Applications of Nanoparticles:
- The main application involved in use of nanoparticles for biomedical applications, such as drug and gene delivery, cancer treatment and diagnostic tools, food etc.
- Nanoparticle involved in drug delivery – the nanoparticle get entrapment of drugs are either enhanced delivery to, or uptake by, target cells and/or a reduction in the toxicity of the free drug to non-target organs[13].
- Nanoparticle systems currently under investigation to be applied in biomedical with the emphasis on cancer therapeutics. There are a variety of nanoparticle systems currently investigated and explored for biomedical applications information and communication technology, power engineering, industrial engineering, environmental engineering, chemical industry, medicine, in pharmaceuticals and cosmetics etc.
- Application of nanoparticles in food i.e., Nanofood is a term used to describe foods that use nanotechnology techniques, tools or manufactured nanomaterials that have been added during cultivation, production, processing or packaging.
- Application of nanoparticles in gene delivery -plays a vital role that can efficiently
- introduce a gene of interest in order to express its encoded protein in a suitable host or host cell.
- Nanoparticles in medicine-Nano medicine aids in early detection and prevention,enhanced diagnosis and follow up of diseases. Invention of nano devices like gold nano particles has made gene equencing less difficult [14].
- Application of nanoparticles in environment [15].
- Application of nanoparticles in cancer treatment [16].
- Application of nanoparticles in drug delivery.
- Application of nanoparticles in energy and electronics.
- Gold nanoparticles and gold nanoshell for immunoassay and cancer detection and treatment
- Silver nanoparticles have been extensively used as electronic product in industry,anti-bacterial agent in the health industry etc. [17].
- Nanoparticles in dietary aspects- Nanotechnology is the method of manipulating matter at the molecular level [18].
- Nanomaterials are used for the fabrication of single processing elements such as filters,

Hyperlipidemia
Hyperlipidemia is a pathological condition characterized by increased concentration of serum triglycerides (TG),Total cholesterol (TC) ,free fatty acids (FFA), and low density lipoproteins cholesterol (LDL-C),along with the exercise accumulation of TG in the liver [19].The increased concentration of blood cholesterol are strongly associated with an elevated risk of the atherosclerosis and cardiovascular disease (CVDs).The WHO predicted that up to 40% of deaths would be related to CVD by 2030affecting approximately 23.6 million people worldwide [20]

Epidemiology

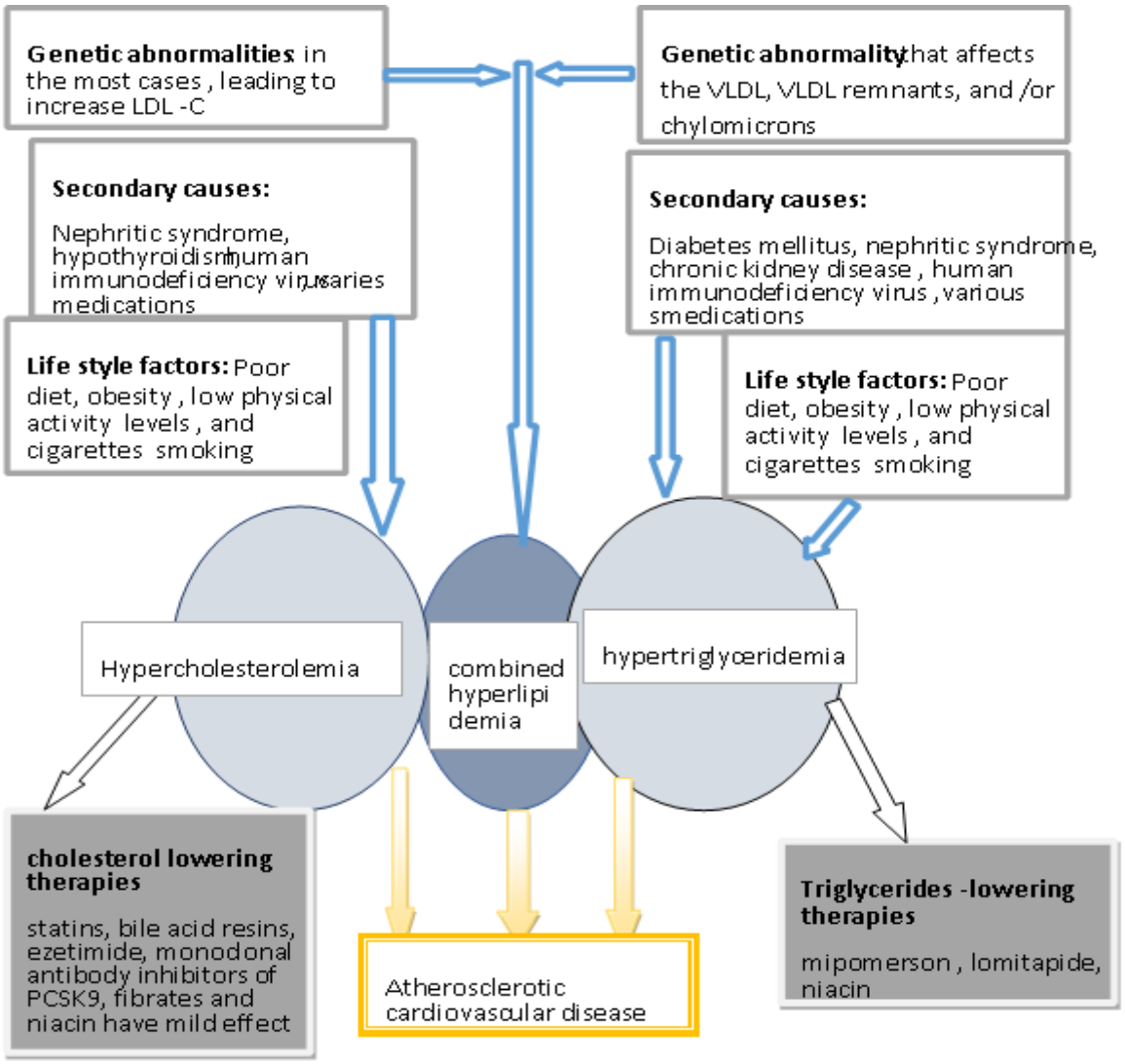
In the United States, more than 100 million, or roughly 53% of adults, have elevated LDL-C levels. Yet, fewer than 50% of patients with high LDL-C receive treatment to reduce their levels, and among those receiving treatment, fewer than 35% achieve adequate control. Further, approximately 31 million American adults have total cholesterol levels that exceed 240 mg/dL, placing them at about twice the risk of ASCVD compared to those with total cholesterol levels that are at goal. [21]

Cholesterol

Cholesterol is a waxy substance found in the blood .With high cholesterol, it can develop fatty deposits in the blood vessels. Eventually, these deposits grow, making it difficult for enough blood to flow through the arteries. Sometimes, those deposits can break suddenly and form a clot that causes a heart attack or stroke.

Pathophysiology of hyperlipidemia

The pathophysiology of hyperlipidemia can be studied under the two-basic classification of hyperlipidemia. The pathophysiology of primary hyperlipidemia involve the idiopathic hyperchylomicronemia in which defect in lipid metabolism leads to hypertriglyceridemia and hyperchylomicronemia caused by a defect in lipoprotein lipase activity or the absence of the surface apoprotein CII31.Moreover, hyperchylomicronemia in cats with autosomal recessive defect in lipoprotein lipase (LPL) activity showed the occurrence of primary hyperlipidemia [22]. In secondary hyperlipidemia, the postprandial absorption of chylomicrons from the gastrointestinal tract occurs 30- 60 min after ingestion of a meal containing fat that may increase serum triglycerides for 3-10 hours .The diabetes mellitus patients have been noted to possess low LPL activity which further caused high synthesis of VLDL cholesterol by the liver ultimately leading to hyperlipidemia. Moreover, hypothyroidisminduced low LPL activity and lipolytic activity has been noted to reduce hepatic degradation Hyperlipidemia: etiology and possible control of cholesterol to bile acids. Furthermore, hyperadrenocorticism increased the synthesis of VLDL by the liver causing both hypercholesterolemia and hypertriglyceridemia [23]



Treatment of Hyperlipidemia
Two methods of treatment are available for the management of hyperlipidemia
1. Therapeutic lifestyle changes
2. Drug therapy

Therapeutic lifestyle changes Diet medications (Low fat diet) ,Regular physical activity ,Smoking cessation ,Weight reduction ,Less cholesterol intake
Drug therapy -HMG-CoA reductase inhibitors (statins) Lovastatin , Simvastatin, Pravastatin, Atorvastatin , Rosuvastatin, Fluvastatin, Pitavastatin

- **Bile acid (Resins) -**Cholestyramine ,Colestipol
- **Fibrates** -Clofibrate , Gemfibrozil
- **Lipolysis and triglyceride synthesis -**Nicotinic acidINTRODUCTION
- **Sterol absorption inhibitor** -Ezetimib[24,25]

Materials
Lovastatin and Eudragit L100 were obtained as gift samples from Hetero Drugs Ltd., Hyderabad. Poloxamer was procured from Sigma-Aldrich, Bangalore. Methanol and acetone (analytical grade) were purchased from SDFine-Chem Ltd., Mumbai. All other chemicals and reagents used in the study were of analytical grade and used as received without further purification.

Method of preparation:
The lovastatin nanoparticles were prepared with the different ratios of drug and eudragit L 100 polymer using the nanoprecipitation technique with slight modification. lovastatin was dissolved in acetone containing concentration from 50-100mg/ml, to add eudragit L100 to form a diffusion phase(volume typically ranging from 0.5-6ml) . This diffusionMaterial and method phase was added into dispersion phase containing 25 ml of 0.5% (w/v) of poloxamer-188 with a constant flow rate (0.5 ml/min). The mixture was stirred for overnight under mechanical stirrer at 500 RPM to evaporate the organic solvent.Then placed in a bath sonicator for 30 minutes. The precipitated mixture was centrifuged (Research Centrifuge R-24) at 10000 rpm for 15min and the sediment was collected, washed with de ionized water and dried to get nanoparticles.

:

Fig : 1 Preparation method of nanoparticle

Preformulation studies
Determination of melting point
The melting point of lovstatin drug was measured by melting point apparatus. lovastatin tend to start melt at 168°Cand end at 170°C, by observing the melting point studies. The lovastatin drug was stable. Solubility of the drug Solubility of lovastatin in different solvent was done, in soluble in water and freely soluble in acetone, ethanol and methanol. 100 mg of drug was dissolved in 10 ml of acetone

Determination of standard calibration curve for lovastatin
The calibration curve of lovastatin was performed with ethanol and it shows regression coefficient value of 0.9996. As per the limits of IP the calibration graph of lovastatin passes regression coefficient and obeys Beers Lambert law.

Calibration curve of lovastatin

Table: 1

Concentration(µg/ml)	Absorbance
2	0.108
4	0.197
6	0.291
8	0.385
10	0.484

Drug- excipient compatibility study through FTIR

Fig no .3 .FTIR of lovastatin

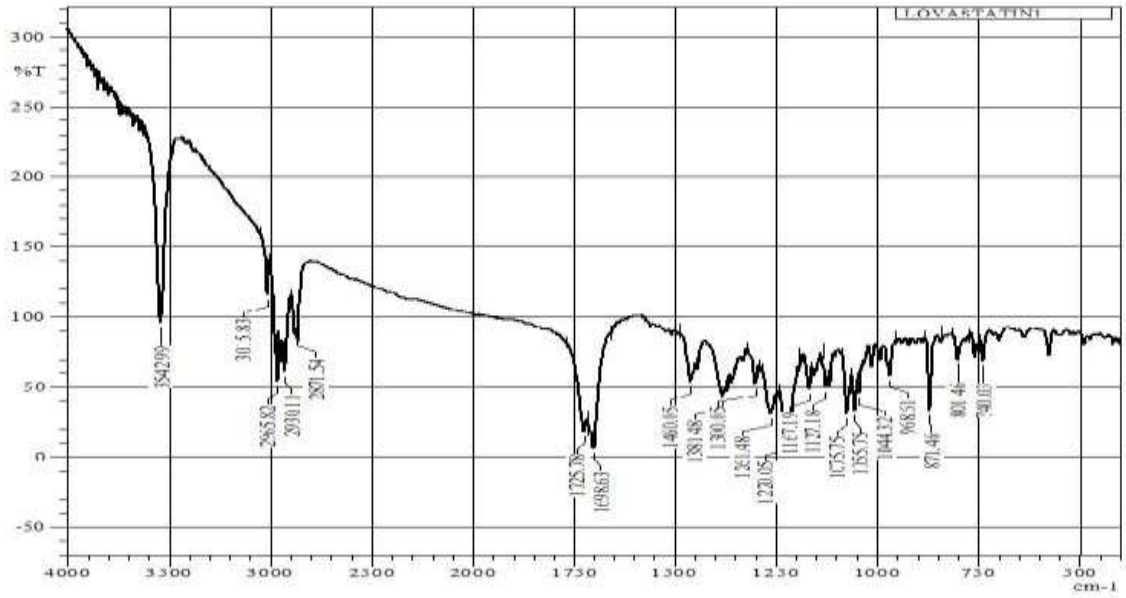


Fig no .4 .FTIR of Eudragit L 100

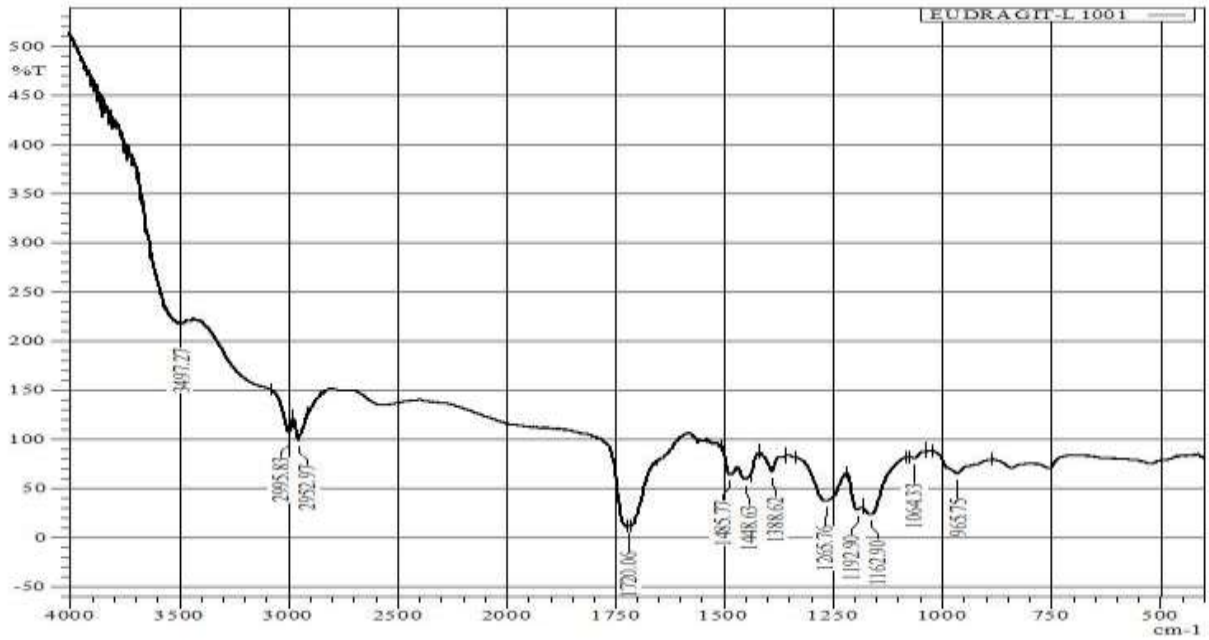


Fig no .5 .FTIR of Polaxamer 188

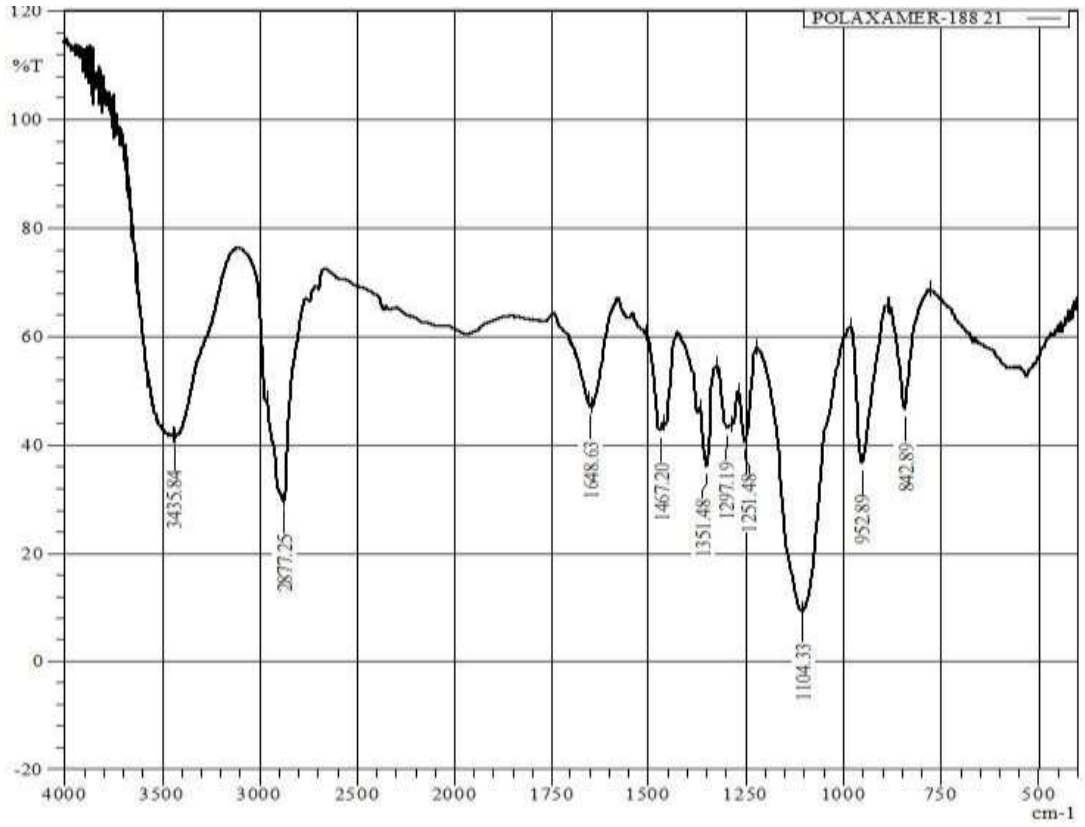


Fig no.6.FTIR of physical mixture

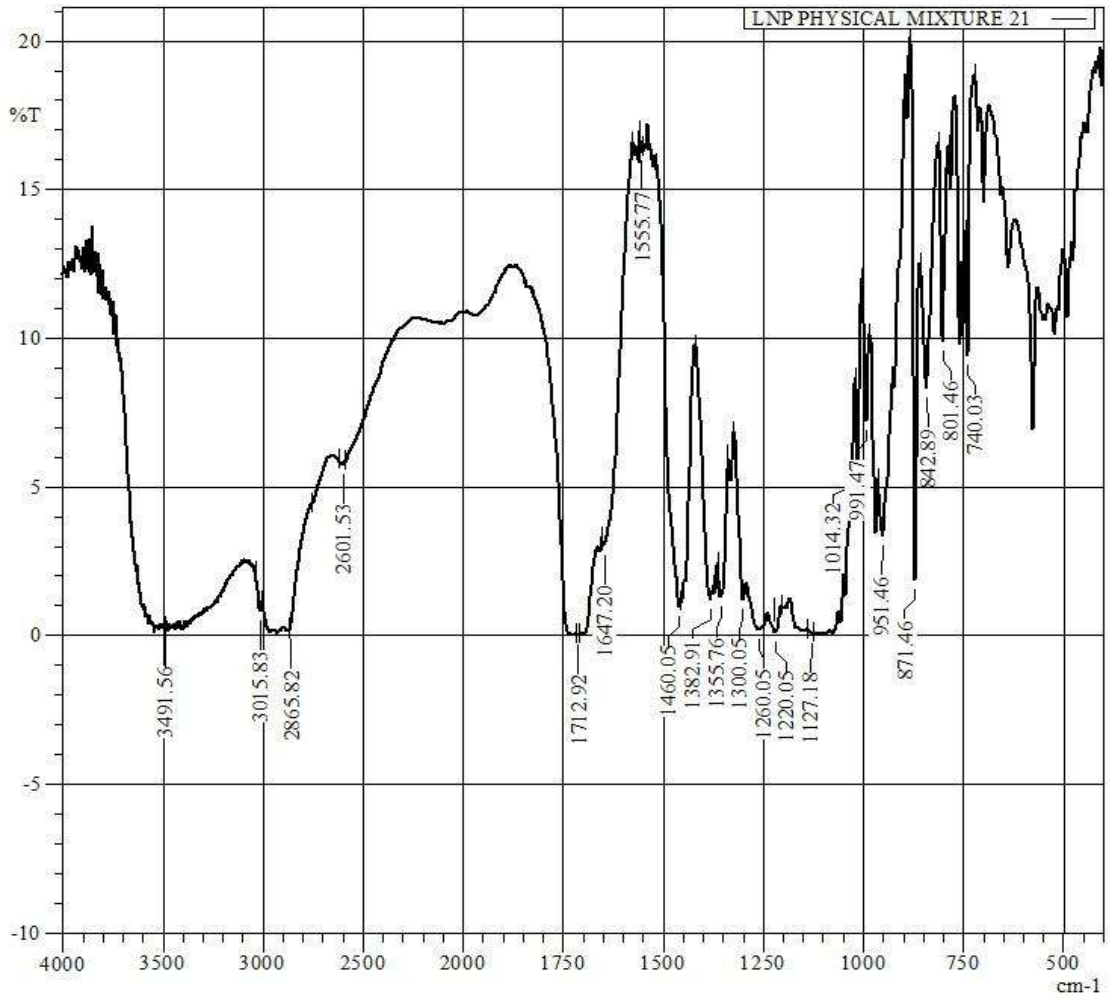


Table:2

S. No	Observed range cm^{-1}				Standard range cm^{-1}	Characteristic group
	lovastatin	Eudragit	Poloxamer	Physical mixture		
1	-	-			<650	Inorganiccompound
3	740.03	965.75	842.89	740.03	1000-650	C-H (out of plane)
4	801.46	-	952.89	842.89 801.46	1000-650	C-H (out of plane)
5	871.46	-	-	894.32 871.46	1000-650	C-H (out of plane)
6	968.61	-	-	991.47 951.46	1000-650	C-H (out of plane)
7	1044.32 1055.75 1075.75 1167.19	1063.33 1162.90 1192.90 1265.76	1104.33 1251.48 1297.19	1260.05 1220.05 1127.18 1014.32	1300-1000	C-O
8	1300.05 1381.48	1388.62 1448.63	-	1382.91 1300.05	1450-1375	C-H (Bending)
9	1698.63	1485.77	1467.20	1460.05	1600-1475	Aromatic ring
10	-	-	1648.63	1647.20	1680-1600	C-C Group
11		1720.06	-	-	1725-1705	C=O Group
12	1725.78	-	-	1712.92	1750-1730	C=O Group
13	2871.54 2930.11 2965.82	2952.97 2995.83	2877.25	2865.82	3400-2400	O-H (stretching)
14	3015.83	3497.27	3435.84	3491.56	3500-3100	N-H (stretching)

Evaluation of Lovastatin Loaded Eudragit Nanoparticle

The variables, factor and responses are fixed by initial trails of experiments, the data what we fixed are feed in design expert software, we get eleven experimental runs from the software. By performing the eleven experiment runs and their evaluation studies, these are the following responses results are observed.

Table.3. Percentage yield of LNP

Runs	Factor 1 Eudragit L100 (mg)	Factor 2 Poloxamer 188 (mg)	Factor 3lovastatin (mg)	Percentage yield
1.	1000	200	40	73.36**%**
2.	1000	400	40	62.25**%**
3.	1000	600	40	59.14**%**
4.	1000	800	40	78.89**%**
5.	1000	1000	40	80.75**%**
6.	1000	1200	40	86.65**%**
7.	1000	1400	40	64.29**%**
8.	1000	1600	40	88.45**%**

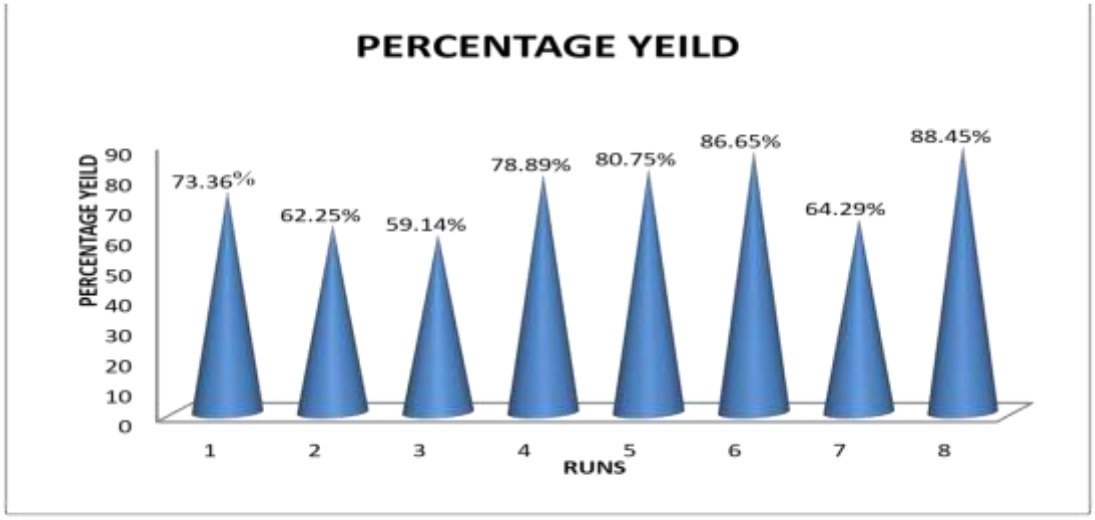


Figure.15. Percentage yield of LNP

Table.4. Drug release and Entrapment of LNP

Runs	Factor 1 Eudragit L100 (mg)	Factor 2 Poloxamer 188 (mg)	Factor 3 Lovastatin (mg)	% EE	%Drug release
1.	1000	200	40	46.56%	80.79%
2.	1000	400	40	39.41%	82.09%
3.	1000	600	40	40.26%	86.36%
4.	1000	800	40	84.08%	98.75%
5.	1000	1000	40	60.87%	92.15%
6.	1000	1200	40	53.12%	89.96%
7.	1000	1400	40	72.65%	91.12%
8.	1000	1600	40	76.29%	94.54%

Figure.8. Entrapment of LNP

Figure.9. % Drug release of F1 –F4 formulation

Figure.10. % Drug release of F5 –F8 formulation In-Vitro Drug release

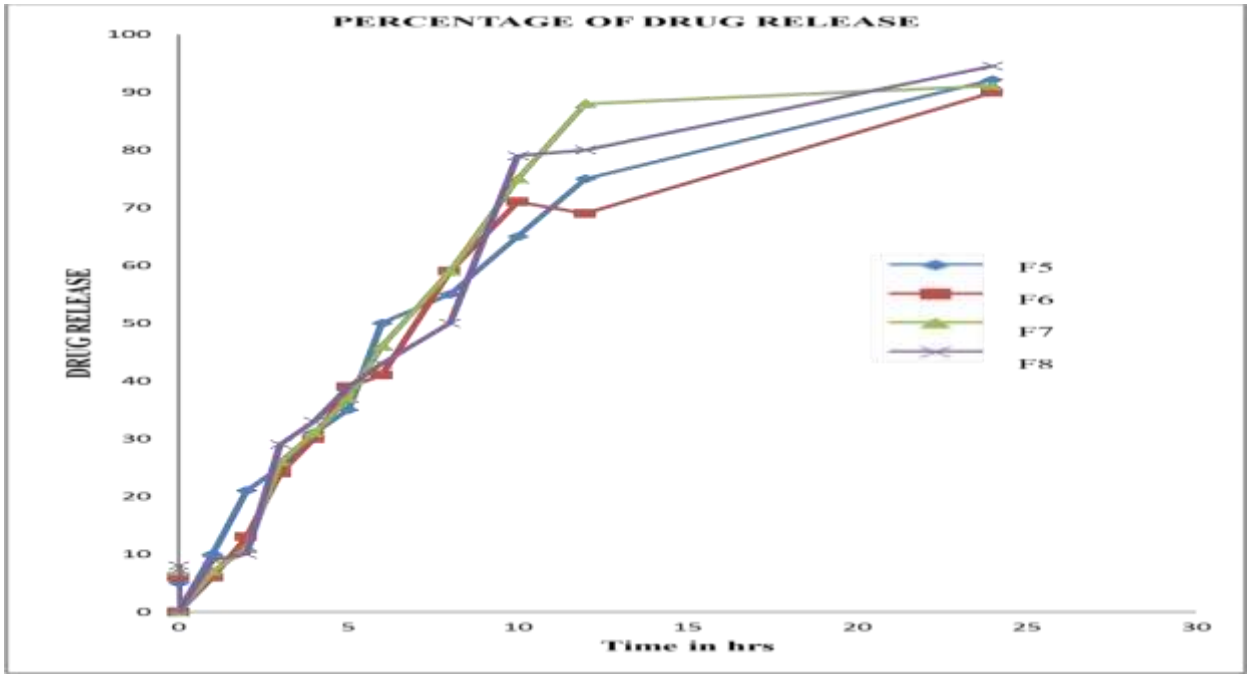


Table 5. Drug release of optimized formula

S.NO	TIME (hr)	% DRUG RELEASE
1	1	4.25%
2	2	14.69%
3	3	23.27%
4	4	26.35%
5	5	34.68%
6	6	48.21%
7	8	57.48%
8	10	63.43%
9	12	85.17%
10	24	98.75%

Figure.11. % Drug release of optimized formulation

Characterization of optimized lovastatin loaded eudragit nanoparticle

- **Particle size**

Figure-12. Particle size analysis of LNP

Zeta potential

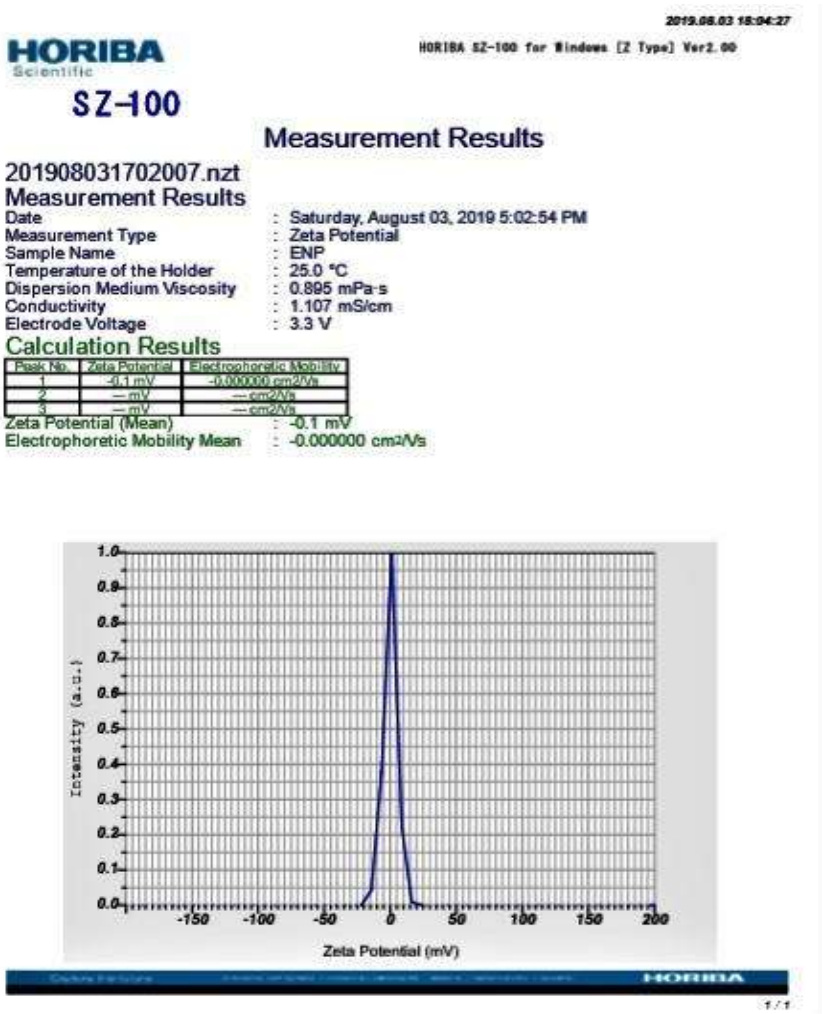


Figure-13. Zeta Potential analysis of LNP

Morphology by SEM

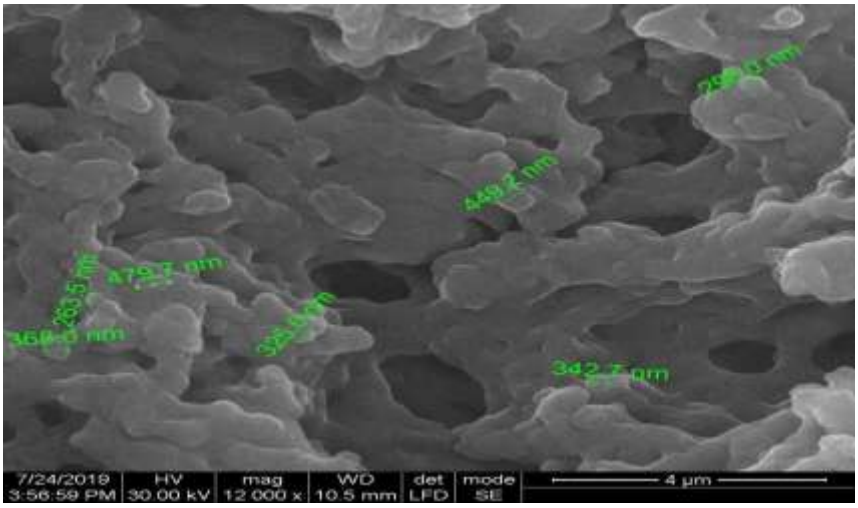


Figure no.14. SEM image of optimized LNP nanoparticle

Table-6. Drug release kinetic model fit

	R	K
Zero order	0.9129	1.3253
T-test	6.324	(Passes)
1st order	0.9615	-0.0188
T-test	9.892	(Passes)
Matrix	0.8539	7.0467
T-test	4.641	(Passes)
Hix.Crow.	0.9486	-0.0055
T-test	8.476	(Passes)

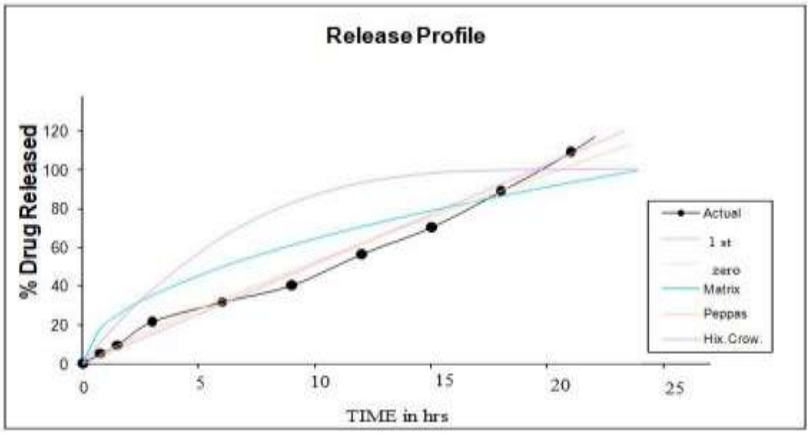


Figure no.15 Drug release kinetic

DISCUSSION

Preformulation Studies
Determination of melting point
The melting point of lovastatin drug was measured by melting point apparatus. lovastatin tend to start melt at **168°C**and end at **170°C**, by observing the melting point studies. The lovastatin drug was stable

Solubility of the drug
Solubility of lovastatin drug was estimate by using tube shake method. Solubility of
lovastatin in different solvent was done, in soluble in water and freely soluble in acetone, ethanol and methanol. 100 mg of drug was dissolved in 10 ml of acetone

Drug- excipient compatibility study through FTIR
The characteristic peak of lovastatin with combination of excipients like Eudragit
L100, poloxamer 188 and drug-mixture show the presence of identification peaks in
mixture and individual samples shows compatibility. The peaks represented the following groups present in lovastatin mixture540.73cm-1 of inorganic compounds,842.89cm-1of CH bond out of the plane, 1460.05cm-1of aromatic rings, 1382.9192cm-1 C-H bending ,1647.20cm-1of C-C group,1712.9292cm-1of C=O group 2865.82cm-1of O-H stretching, 3491.56cm-1of N-H group. The characteristic peaks in Table and figure 11,12, 13, 14 of lovastatin were retained physical mixture, indicating no chemical interaction between the lovastatin and excipients.

Determination of standard calibration curve for lovastatin
The calibration curve of lovastatin was performed with methanol and it shows regression coefficient value of 0.999. As per the limits of IP the calibration graph of lovastatin passes regression coefficient and obeys Beers Lambert law.

Evaluation of Lovastatin Loaded Eudragit Nanoparticles
Entrapment efficiency
The entrapment efficiency of 8 formulations was ranged from 39.41 to 84.08 %, where 4 formulation showed highest entrapment efficiency of 84.08 % and formulation 1 showed lowest entapment efficiency of 39.41 %. The results are shown in table9. Due to the maximum amount of eudragir L 100, Poloxamer 188 and lovastatin are in the formulation 4 so it shows higher entraptment efficiency and in formulation 1 the all the three factors are in minmum concentration so it showed low entrapment efficiency.
increaseing the concentration of lovastatin, eudragit L 100 and Poloxamer188 increasing the entrapment efficiency of the drug was observed.

Percentage Yield
The percenyage yield of 8 formulations was ranged from 59.14 % to 88.85 %, where formulation 8 showed highest percentage yeild of 88.45% and formulation 3 showed lowest percentage yeild of 59.14 %. The results are shown in table 8. The percentage of yeild were increase while increasing the concentrations of the three factors (Eudragit L 100, Poloxamer 188 and lovastatin). In the formulation 8 all three factors are in maximum concentration so it exhibitshigh percentage of yield and the formulation 3 shows low percentage of yeild due to minimum concentration of the the factors.

In-Vitro Drug release study.

The table 9 and figure 17, 18 showing the-drug release study of 8 formulations was
ranged from 80.36% to 98.75 %, where formulation 4 showed highest of 98.75% at the end of 24hour. the brust release of the drug was seen at 1hr with percentage drug release of 4.25% due to release of the drug which is encapsulated on the surface of the particles. After 1hr brust release slow and sustained release of drug was observed due to the slow release of drug from the eudragit polymer membrane by means of errosion or diffusion mechanism.

Drug Release Kinetics
The release constant was calculated from the slop of appropriate plots and the regression coefficient(R 2) was determined.after the comparative evaluation of R 2 values the kinetics of drug release from the optimized lovastatin loaded eudragit nanoparticles was found to follow 1 st order kinetics as the plots of cumulative percentage drug release verses time was linear.

Characterization Of Lovastatin Nanoparticles

Particle size
The Figure 20 particle size has direct effect on the stability, drug release and
bio distribution. The mean particle size of the prepared nanoparticles, were found to be 496.0 nm. The nanoparticles which range from 100-500nm are easily adhereto intestinal epithelium

Zeta potential
The zeta potential of prepared optimized formulation was found to be -0.1mv. It
indicates the nanoparticle have a little anionic charge due to the presence of eudragit that would help in the better intraction of nanoparticle with cationic intestinal lining. Due to lower negative value of zeta potential, results in high attractive force leads to aggregation of nanoparticles .

Morphology by SEM

Scanning electron microscopy is a potential method to study surface characterization of nanoparticles. The optimized formulations are examined under high voltage and the
microphotograph . Particles are formed successfully with smooth topography in spherical or ovate shape with aggregation of nanoparticle. Bright coloured shading was observed due to application of high voltage.

Conclusion

Lovastatin loaded Eudragit nanoparticles were fabricated by using nanoprecipitation
method because of less economic, less laborious and reproducibility technique which
involves only a few steps, in preparation.. All the formulations were prepared randomly, characterised for entrapment efficiency and drug release. The results were analysed and validated. The concentration of polaxamer showed dominant effect on the entrapment efficiency and drug release, where as concentration of Eudragit and lovastatin had small and significant effect on entrapment efficiency and drug release.

Future Recommandation

Lovastatin loaded eudragit nanoparticles were successfully prepared, characterized by
solubility, melting point, particle size, zeta potential and SEM. Evaluated by Percentage yield DR, EE and drug release kinetics. Further the optimized formulation is to be evaluated for in-vitro cell line studies and invivo antihyperlipidemic studies using suitable animal models

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