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Development Of New Enhanced Formulation And Evaluation Of Polymeric Lovastatin Nanoparticles

Kumar Raja Jayavarapu [1]*, Vandana Kona[2], M. Siva Rama Chandu[3] and Nageswara Rao R[4]

[1]* HOD, Department of Pharmaceutical Analysis, Mother Teresa Pharmacy College, Sathupally, Khammam Dist, 507303, India.

[2 & 3] Department of Pharmaceutics, Mother Teresa Pharmacy College, Sathupally, Khammam Dist, 507303, India.

[4] Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, Nandyal, Andhra Pradesh, 518501, India.

ABSTRACT

The main aim of our study is the incorporation of polymeric nanoparticles and solid lipid nanoparticles containing Lovastatin into transdermal patches. In our study, Lovastatin was selected because it possesses the ideal characteristics that a drug should have for formulating into a transdermal drug delivery system that includes high lipid solubility, low molecular mass, effective in low plasma concentration as well as a high degree of first pass metabolism. The aim of our study mainly supports for the prevention of first pass metabolism and achieve the controlled release.

By using various phosphate buffer solutions, solubility studies were conducted and based upon their results phosphate buffer of pH 6.8with 1% Tween 20 was selected as a medium for *invitro* studies. By using FT-IR, compatibility studies were performed between the drug Lovastatin and excipients used in the formulation. In the nano particulated formulation there was no interaction between the drug and the excipients was shown by the compatibility studies. Solvent evaporation method was employed for the preparation of PLNs by using Chitosan, PLA, and PCL as polymers. Micro emulsion technique was employed for the preparation of SLNs by using Stearic acid, cholesterol and Glyceryl mono stearate as lipids. The prepared PLNs (SP1 to SP12) and SLNs (SL1 to SL6) formulations were evaluated in case of various parameters like morphology, particle size, zeta potential, PDI and *invitro* drug release and the results of the above parameters were observed to be in the desired range.

With different concentrations of HPMC, the formulations SP4 and SL6 were selected and incorporated into transdermal patch. Evaluation for the various parameters was done for the prepared patches (TPN1toTPN3and SLNP1toSLNP3) and the results were recorded individually. Based on the results obtained TPN1 and SLNP2 were selected for formulating the transdermal patches by incorporating different permeation enhancers. Evaluation was done for the transdermal formulations (PLP1 to PLP12 and SLP1 toSLP12) and the results were recorded individually. PLP12 (Span 80 as permeation enhancer) and SLP9 (DMSO as permeation enhancer) were observed to be the best formulation and

were selected and subjected to *in vivo* analysis separately and were compared with and without permeation enhancers patches. These final patches showed a significant decrease in the serum cholesterol, LDL, VLDL & triglycerides levels and have increased the HDL and total protein levels significantly.

Key Words: Lovastatin, triglycerides, zeta potential, serum cholesterol.

INTRODUCTION

Transdermal Drug Delivery System

Transdermal drug delivery system (TDDS) can be defined as the dosage forms intended to deliver a drug across a patient's skin in a therapeutically effective quantity. In general, these are also called as "Patches". A special membrane can be utilized by transdermal patches in order to control the rate at which the liquid drug contained in the reservoir within the patch pass through the skin and into the blood stream.

In the year 1970, transdermal patches were first developed. They were first approved by FDA in the year 1979 for the treatment of motion sickness (Scopolamine).After that Nitroglycerin patches were approved in the year 1981 and at present there exists a wide range of patches with drugs like Testosterone, Oxybutinin, Fentanyl, Clonidine, Lidocaine, Nitroglycerin, Nicotine, Estrogen and Scopolamine etc. Usually, the effectiveness of the patches lasts from 1-7 days based on the drug and its dosage. At present, combination patches were also available for hormone replacement and contraception.

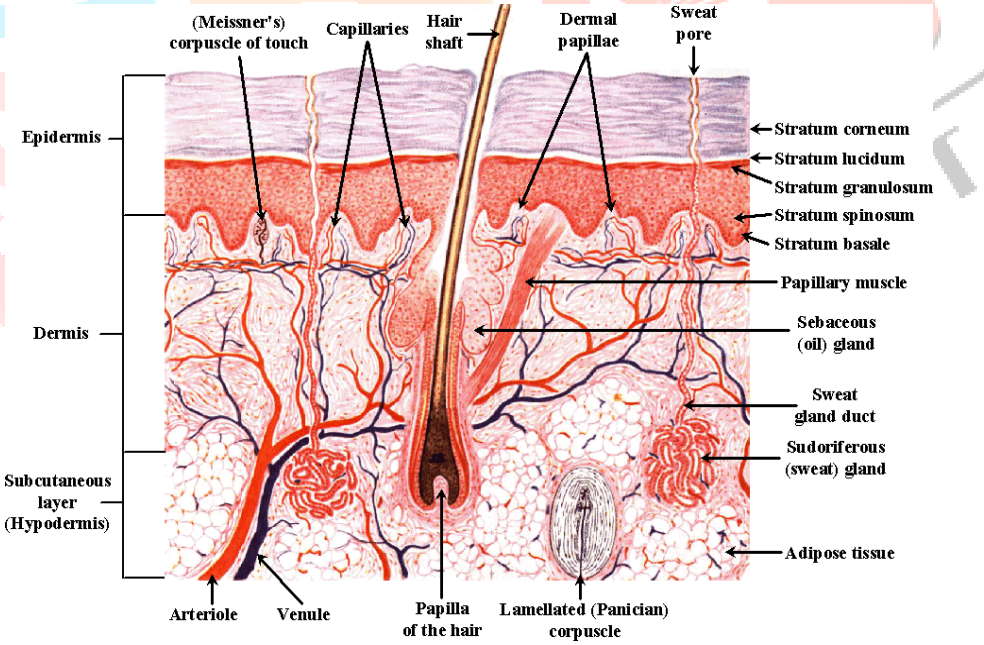


Figure 1: Structure of human skin

Nano Particles

Nanoparticles are smaller than 1000nm. Nanoparticles in the range of 40-500 nm are acceptable for the purpose of drug delivery depending on the route of administration. For the slow degradation of a drug, at a specific site nano particles are used as drug carriers, which targets drug into the skin. The targeting can be active or passive. Efficacy significantly depends upon the method by which a drug is delivered. Some drugs shows maximum pharmacological response with in an optimum concentration range and concentration above or below the optimum

concentration range could cause toxic reaction and no therapeutic effects respectively.

Nanoparticles can be categorized into nano spheres and nano capsules. Solid core structures are called as nano spheres, hollow core structures are called as nano capsules. Nanoparticles can be composed of polymers, polysaccharides, proteins and lipids.

PERMEATION ENHANCERS

For improving the transdermal drug delivery permeation enhancers can be used. In order to reduce the barrier resistance, permeation enhancers penetrates into the skin reversibly. Permeation enhancers are of three types.

Drug vehicle based

This method is based on drug selection, particles & vesicles, chemical potential of drug, pro drugs and eutectic system. The development of structure activity relationships for enhancers and the interaction of enhancers with the stratum corneum will aid in the development of enhancers with minimal toxicity and optimal characteristics.

Physical penetration enhancers

For penetration enhancement, there are various physical and electrical methods that include iontophoresis, electroporation, phonophoresis and photo mechanical waves.

Chemical Permeation Enhancers

The most extensively used technique is the chemical penetration enhancers which enhance a drug's penetration across the skin which results in increased systematic availability. This includes terpenes, dimethyl sulfoxide, urea, azone, pyrolidine, fattyacids, oxazolidinones, mono olein and phospholipids.

Lovastatin reduces cholesterol levels by reversible and competitive suppression of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, an enzyme essential for cholesterol production. It demonstrates low oral bioavailability (G5%) due to fast metabolism in the gastrointestinal tract and liver. Cytochrome P4503A4 catalyzes the conversion of the lactone form of lovastatin into hydroxy acid and its metabolites. To circumvent hepatic first-pass metabolism and improve bioavailability, the intestinal lymphatic transport of drugs may be utilized. The transport of medications through the intestinal lymphatics via the thoracic duct to the systemic circulation at the confluence of the jugular and left sub clavian veins circumvents presystemic hepatic metabolism, hence increasing bioavailability. Highly lipophilic substances, such as long-chain triglycerides, enter systemic circulation through the lymphatic system. Lovastatin, with a water solubility of 0.4×10^{-3} mg/mL, is regarded as a suitable substrate for intestinal lymphatic transport due to its elevated log P value (4.3) and substantial solubility in oils (38 and 42 mg/mL in carbitol and propylene glycol monocaprylate, respectively). Nano particle-based drug delivery methods improve the bioavailability of lipophilic drugs like halofantrine and ontazolast through lymphatic transport of biosynthesized chylomicrons linked to the medications. An alternative method for lymphatic transport of nano- and microparticles involves specific absorption by M cells in Peyer's patches. Polymeric nanoparticles enveloped in hydrophobic polymers are readily absorbed by lymphatic cells within the body [3]. Figure 2 represents the structure of lovastatin.

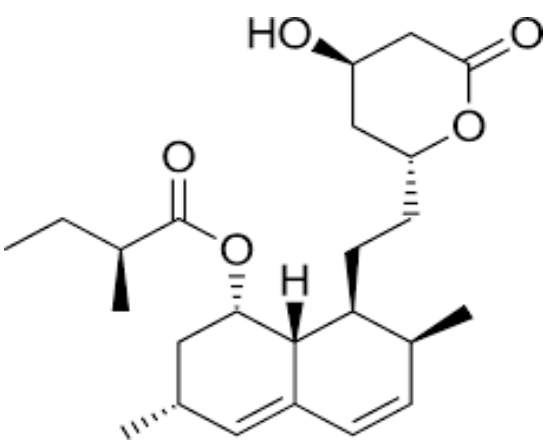


Figure 2. Structure of Lovastatin

In recent years, PNPs have been categorized as biodegradable nanoparticles owing to their excellent entrapment efficiency, controlled release, and reduced toxicity. The nano precipitation technique, also known as the solvent displacement method, is a fundamental, quick, and straight forward procedure. The procedure requires the formation of a precipitate from an existing polymer in an organic solution, followed by the diffusion of an organic solvent into an aqueous medium, with or without a surfactant. It requires two water-soluble solvents that do not cause any issues. In an optimal situation, both the polymer and the drug would dissolve in one solvent while remaining insoluble in the other non-solvent. The amalgamation of the polymer solution with the non-solvent triggers a nano precipitation process, marked by the swift desolvation of the polymer in the aqueous medium. The polymer precipitates instantly upon the total replacement of the organic solvent with the aqueous medium, leading to the swift trapping of the drug. The rapid creation of a colloidal suspension results from the polymer's accumulation at the interface of the organic solvent and water, aided by the rapid diffusion of the organic solvent. This method is most efficacious for hydrophobic substances that are soluble in ethanol or acetone yet demonstrate negligible solubility in water. The objective of this study was to formulate and assess lovastatin polymeric nanoparticles utilizing the modified nano precipitation technique to enhance the solubility and bioavailability of the drug.

Materials and Methods: Materials

Lovastatin was procured from Days healthcare, Hyderabad. Chitosan, sodium tripolyphosphate were obtained from Yarrow chem, Mumbai. Distilled water was used as the aqueous phase. All chemicals and reagents used were of analytical grade.

Method

Pre formulation Studies:

In the rational development of dosage form of a drug, pre formulation testing is the first step. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. To generate information useful to the formulator in developing efficacious, safe and stable dosage form is the overall objective of the pre formulation testing. Hence for identification and compatibility studies, pre formulation studies were carried out on the obtained samples of the drug.

Identification of drug

The sample was examined by IR spectroscopy and was compared with the standard IR spectrum.

Solubility studies of Lovastatin

25mg of Lovastatin was added to each 25ml of distilled water, 0.1N HCl, phosphate buffer solution with pH 4.5, 6.8 & 7.4. The solutions were transferred into 50ml stoppered conical flasks and shaken for 48hours on rotary shaker at 37^0C. To achieve equilibrium, at 4hrs interval 2ml aliquots were withdrawn and filtered. The filtrates were diluted and analyzed for Lovastatin using High performance liquid chromatographic technique.

Standard calibration curve of Lovastatin

To estimate the Lovastatin from the formulations, RP-HPLC method can be used. About 10mg of Lovastatin was weighed and transferred into 10ml volumetric flask containing7ml of phosphate buffer pH 6.8 and Acetonitrile (40:60). 1ml of the above stock solution was transferred into10ml volumetric flask and the volume was made with diluents. From this stock solution the solutions having the concentration range of 1-6µl/ml were prepared. 40:60 ratio of phosphate buffer pH 6.8 and acetonitrile was used as mobile phase with the flow rate of 1ml/min, run time set to 14min
and injection volume being 20µl. Column was calibrated for at least 30minutes with mobile phase prior to the injection of solutions. At 239nm the eluent was measured. By using 0.22µ membrane filter, all the solutions were filtered. Into the HPLC column, the solutions were injected in triplicate by keeping the injection volume constant at 20µl. At 239nm chromatograms were recorded. The calibration curve was diagrammed with mean peak area against concentration and was used to measure the amount of Lovastatin.

Compatibility studies:

Under the experimental conditions, the compatibility of drug and polymers is a significant prerequisite before formulation. The stability and bioavailability of drug scan be altered by the incompatibility between the drugs and excipients which affects the efficacy and safety. Drug-excipients compatibility plays an important role in stable dosage form development. The IR spectrum was recorded in the region of 4000-400 cm^{-1} for the drug, polymer and drug – polymer mixture using KBR pellet method. In the light path, the prepared pellet was placed and the spectrum was recorded.

Figure 3: Photography of FTIR spectrophotometer (BRUKER)

Formulation of lovastatin polymeric nanoparticles and Formulation of polymeric nanoparticles of lovastatin by solvent evaporation method

Lovastatin was dissolved in a mixture of ethanol and 10mM tris buffer, where as polymers like Chitosan, poly lactic acid and poly capralactone was dissolved in a mixture of 0.25% acetic acid and dichloromethane. Lovastatin solution was added drop wise to the 2% span 60 solution and was emulsified under high speed homogenization for15min at 20000rpm.Then added 200ml of water and stirred well in order to complete the precipitation process. To get the free flowing powder of nanoparticles, the nanoparticles suspension was freeze dried at -20°C.

Ingredients (%w/w)	Formulation code					
	SP1	SP2	SP3	SP4	SP5	SP6
Lovastatin	0.2	0.2	0.2	0.2	0.2	0.2
Chitosan	1	2	-	-	-	-
Polylactic acid	-	-	1	2	-	-
Polycapralactone	-	-	-	-	1	2
Span60	2	2	2	2	2	2
Dichloromethane	qs	qs	qs	qs	qs	qs
Ethanol	qs	qs	qs	qs	qs	qs
Acetic acid	qs	qs	qs	qs	qs	qs

Table 1: Formulation of Lovastatin Polymeric Nanoparticles

Preparation of polymeric nano particulated patches of Lovastatin

By dissolving respective quantities of polymer (HPMCK100M) and plasticizer (PEG) in distilled water (50ml), transdermal patches were prepared. In order to remove the air bubbles the mixture was soaked overnight. Into the polymeric solution, 100mg of nanoparticles were incorporated. Glass Petri dishes which were of 25cm^{2}area were taken and the prepared solution was poured into them which were dried at room temperature. The patches were cut in 5cm^2 area after 12 hours and they were packed into aluminum foil.

Ingredients	Formulation code		
	TPN1	TPN2	TPN3
Nanoparticles (mg)	100	100	100
Amount of HPMCK100M (mg)	500	1000	1500
PEG400 (10%W/W of polymer) (mg)	50	100	150

Table 2: Formulation Table

Preparation of best polymeric nano particulated patch with permeation enhancers

Permeation enhancers were incorporated into the transdermal patches preparation using the best formulation. By dissolving respective quantities of polymer (HPMCK100M) and plasticizer (PEG) in distilled water (50ml), transdermal patches were prepared. In order to avoid the air bubbles, the mixture was soaked overnight. Into the polymeric solution, 100mg of nanoparticles were incorporated. Eucalyptus oil, peppermint oil, DMSO and Span 80 were incorporated as permeation enhancers indifferent ratios. Into the glass Petri dishes (which were of 25cm^2 area) the prepared solution was poured and dried at room temperature. The patches were cut in 5cm^2 area after12hrs and they were packed into aluminum foil.

Ingredients	Formulation code											
	PLP 1	PLP 2	PLP 3	PLP 4	PLP 5	PLP 6	PLP 7	PLP 8	PLP 9	PLP 10	PLP 11	PLP 12
Lovastatin Polymeric Nanoparticles (mg)	100	100	100	100	100	100	100	100	100	100	100	100
HPMC K100M(mg)	500	500	500	500	500	500	500	500	500	500	500	500
PEG400 (mg)	50	50	50	50	50	50	50	50	50	50	50	50
Eucalyptus oil (%)	3	5	10	-	-	-	-	-	-	-	-	-
Peppermint oil (%)	-	-	-	3	5	10	-	-	-	-	-	-
DMSO (%)	-	-	-	-	-	-	3	5	10	-	-	-
Span80 (%)	-	-	-	-	-	-	-	-	-	3	5	10

Table 3: Preparation of Lovastatin polymeric nano particulated Patch with Permeation Enhancers

Formulation of Lovastatin solid lipid nanoparticles and preparation of lovastatin solid lipid nano particles using micro emulsion process at a temperature higher than the lipid melting point, preparation of solid lipid nanoparticles using micro emulsion method was performed. For preparing the solid lipid nanoparticles, stearic acid, cholesterol and glyceryl mono stearate were used.Tween80 and PEG400 were used as surfactant and co surfactant respectively. Deionized water was used as dispersion medium. Different ratios of lipid, surfactant and co-surfactant were weighed. In a water bath, they were mixed at a temperature10^{o}C higher than the lipid melting point. To the same temperature deionized water was heated and added drop wise to the lipid melt under mild stirring. The liquid preparation was agitated at 1000 rpm for 10 sec after each addition and checked for transparency. After stirring, if turbidity persists, the samples were sonicated for 5minutes at a temperature higher than the lipid melting point. When all the ingredients were mixed in suitable ratios for the formulation of micro emulsion, a thermo dynamically static and translucency system was formed. Under mild mechanical stirring, the obtained micro emulsion was then disseminated in cold aqueous medium (5-10^{o}C). The ratio of micro emulsion to aqueous medium was observed to be 1:20.

Sl. No	Ingredients (%w/w)	SL1	SL2	SL3	SL4	SL5	SL6
1	**Lovastatin**	0.2	0.2	0.2	0.2	0.2	0.2
2	**Stearic acid**	1	2	-	-	-	-
3	**Cholesterol**	-	-	1	2	-	-
4	**Glyceryl mono stearate**	-	-	-	-	1	2
5	**Tween 80**	1.5	1.5	1.5	1.5	1.5	1.5
6	**PEG400**	1.5	1.5	1.5	1.5	1.5	1.5
7	**De-ionized Water**	qs	qs	qs	qs	qs	qs

Table 4: Formulation of Solid Lipid Nanoparticles

Preparation of solid lipid nano particulated patch of Lovastatin

Transdermal patches containing solid lipid nano particles of Lovastatin was prepared as described in the table 4 by dissolving respective quantities of polymer (HPMCK100M) and plasticizer (PEG) in distilled water (50ml). In order to remove the air bubbles the mixture was soaked overnight. Into the polymeric solution, 100mg of nano particles were incorporated. Glass Petri dishes of 25 cm^2 area were used and the prepared solution was poured into them which were dried at room temperature. The patches were cut in 5 cm^2 areaafter12 hours and packed into aluminum foil.

Evaluation of Nanoparticles

Morphology of nanoparticles

By using scanning electron microscope (SEM), morphology of nano particles was characterized. It is widely applied to surface micro structure imaging by using a focused beam of high energy electron. In a cover glass, nanoparticles containing Lovastatin was taken and transferred on a specimens tub. Platinum coated dried samples were prepared using sputter coater with a thickness of 100^{o}A. Scanning was done to examine the size and shape after coating.

Particle size distribution

The size of the nanoparticles was analyzed by Malvern zetasizer. In the sample holder the formulation was placed and the particle size was measured. By using distilled water the samples were diluted and measured at 25^{o}C temperature. The auto correlation function of intensity of light scattered from nano particles helps to calculate the diameter. The particles measured were in triplicate.

Poly dispersibility index (PDI)

PDI is the parameter for the determination of particle size distribution of the nano particles. It is a dimensionless number obtained by extrapolation from the autocorrelation function and ranges from 0.01- 0.7 for mono dispersed particles. Poly dispersity index values >0.7, will be having for the samples with very broad size distribution.

Zeta potential

By measuring the zeta potential by laser dropper anemometry using Zetasizer, the charge of the nano particles was determined. By using distilled water, the nanoparticles were diluted. In the electrophoretic cell the samples were kept where the potential of 150mv was established.

***Invitro* release studies**

By using Franz diffusion cell, *in vitro* release of nano particles was determined. The cell has 20ml receptor volume and the area of diffusion was $5cm^2$. The cell was placed in between stirrer and the water bath and maintained at a temperature of 32 ± 0.5^0C. The cellophane membrane which was soaked earlier in the receptor medium was fixed between the receptor and donar chamber of diffusion cell. An aliquot of prepared formulations i.e. 100mg nanoparticles was added to the donar chamber of the cell which was closed with film of paraffin. The receptor medium consisting of 6.8 pH buffer and 1% Tween 20 was stirred with magnetic stirrer. From the receptor compartment 1ml of the sample was withdrawn at the following time intervals that include 1, 2, 4, 6, 8, 10, 12 and 24hours and fresh receptor medium was replaced with equal volume of fresh medium. For a period of 30minutes at cool temperature, the samples with drawn were centrifuged at 20,000rpm. By using HPLC technique, the drug content of the supernatant was estimated.

Release kinetic studies

Various kinetic models were used to determine the drug release and following plots were made:

1. Zero order kinetic model: Cumulative % drug release vs. time.
2. First order kinetic model: Log cumulative of % drug remaining vs. time.
3. Higuchi model: Cumulative % drug release vs. square root of time.
4. Korsmeyer model: Log cumulative % drug release vs. log time.

Evaluation Parameters for PLN and SLN Transdermal Patches

Weight variation

In the cast film, the polymer film was cut at three different places with surface area of $5cm^2$. Each film strip was weighed and the variation of the average weight was calculated.

Thickness of transdermal patches

By using the digital verniar calipers, thickness of nano particulated transdermal patches was measured and the values were recorded in triplicate.

Drug content in nano particulated transdermal patches

$5cm^2$ patch selected were weighed and dissolved in dichloromethane (100ml). The solution was filtered using membrane filter and the samples were analyzed for Lovastatin using HPLC.

Folding endurance

The folding endurance of the prepared patches was measured and expressed as how many times the patch is folded at the particular place till it breaks.

Flatness

From the prepared medication film, longitudinal strips were cut and the length of each strip is measured. Due to the non-uniformity in flatness, the variation in the length was measured. Flatness was calculated by measuring the constriction of the strip where 100% flatness equals to zero percent constriction.

Where,

$$Constriction\ (\%) = \frac{S1 - S2}{S1} \times 100$$

S1- initial strip length

S2- final strip length

Tensile strength

By using weight pulley method tensile strength was determined. The tensile strength can be defined as the weight necessary for breaking the patch.

Moisture content

The patches prepared were individually weighed and dried in a dessicator containing calcium chloride for 24hrs at room temperature. Until they show a constant weight, the films were weighed again and again at a specific interval. The percent moisture content was calculated by using the following formula.

$$\%\ Moisture\ content = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

Moisture uptake

The films which are previously weighed were exposed to 84% relative humidity by using potassium chloride saturated solution in desiccators until a constant weight is achieved. Percentage moisture uptake was calculated by using the following formula.

$$\%\ Moisture\ uptake = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

***Invitro* drug release studies**

For *invitro* release of Lovastatin from the formulated patch, USP dissolution apparatus type II was used with the dissolution medium of 900ml composed of 6.8pH phosphate buffer and 1% tween 20. With an internal diameter of $5cm^2$, a circular patch was used for the study and to skin the patch at the bottom of the dissolution apparatus, a stainless steel ring was employed. All the dissolution studies were performed at a temperature of 37^0C $\pm 0.5^oC$ at 100 rpm. At regular intervals the samples were withdrawn and at each time equivalent amount of fresh medium was added to maintain the constant volume. By using HPLC technique, their concentrations were analyzed. Data obtained from *in vitro* drug release studies were fitted in various kinetic models to study the release kinetics.

Stability studies

The stability studies were implemented for selected formulations at accelerated conditions of $40^0 \pm 2^0C$, 75±5% RH conditions stored in stability chamber for a period of 6months. The patches were examined for their physical appearance, drug content, weight variation, folding endurance, moisture uptake and *in vitro* drug release. Results were analyzed statistically.

RESULTS AND DISCUSSIONS

Standard calibration curve of Lovastatin

From the calibration curve of the Lovastatin, the concentration of Lovastatin in formulations was calculated and results were represented in fig.4.The calibration curve was plotted with mean peak area against concentration and was used to measure the amount of Lovastatin.

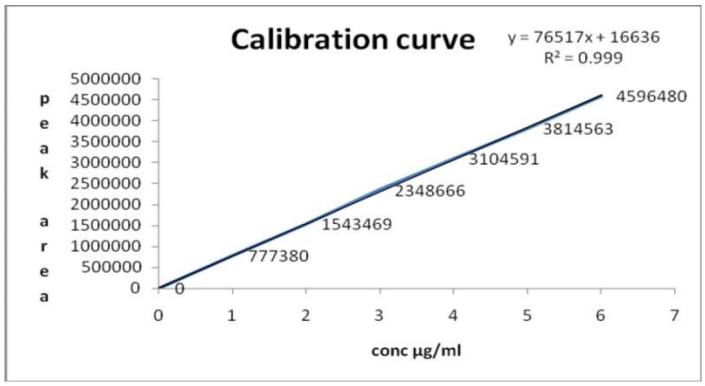


Figure 4: Calibration curve of Lovastatin

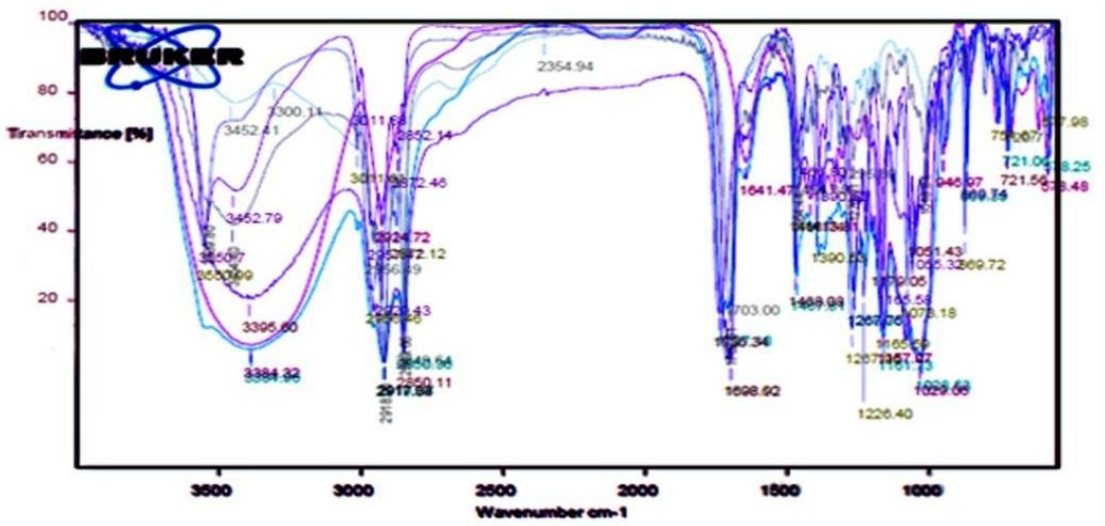
Compatibility studies

Figures from 5 represents the FTIR spectra of Lovastatin & chitosan, Lovastatin & poly lactic acid, Lovastatin & poly caprolactone, Lovastatin & cholesterol, Lovastatin & Stearic acid and Lovastatin & Glyceryl mono stearate. In the spectrum of the drug with excipients, the characteristic peaks for Lovastatin, viz.-OH stretching at 3452cm^{-1},-Ar-H stretching at 3011 cm^{-1}, C-H stretching in CH_3 at 2929cm^{-1},C=O stretching at 1699cm^{-1} and C=C aromatic stretching at1466cm^{-1} and C-O-C stretching at1268cm^{-1} was observed. In the case of Lovastatin and Chitosan mixture significant peaks are –OH stretching at 3384cm^{-1}, C-H stretching in CH_3 at 2919cm^{-1}, C=O stretching at 1727 cm^{-1} and C-O-C stretching at 1268 cm^{-1}. In the case of Lovastatin and poly lactic acid mixture significant peaks are-OH stretching at3395 cm^{-1},C-H stretching in CH_3 at 2917 cm^{-1},C=O stretching at1736 cm^{-1}and C-O-C stretching at1179cm^{-1}. In the case of Lovastatin and poly capralactone mixture significant peaks are-OH stretching at 3550cm^{-1}, C-H stretching in CH_3 at 2956cm^{-1}, C=O stretching at 1698cm^{-1} and C-O-C stretching
at 1165cm^{-1}. In the mixture of Lovastatin and cholesterol, the significant peaks are –OH stretching at 3384cm^{-1}, C-H stretching in CH_3 at 2924cm^{-1}, C=O stretching at 1641cm^{-1} and C-O-C stretching at 1157cm^{-1}. In the mixture of Lovastatin and Stearic acid, the significant peaks are -OH stretching at3454 cm^{-1},C-H stretching in CH_3 at 2918 cm^{-1}, C=O stretching at 1702 cm^{-1} and C-O-C stretching at 1270cm^{-1}. In the mixture of Lovastatin and Glyceryl Monostearate, the significant peaks are –OH stretching at 3452cm^{-1}, C-H stretching in CH_3 at cm^{-1} and C-O-C stretching at 1295 cm^{-1}.No characteristic peaks were appeared or disappeared. In the nanoparticles preparation, there was no interaction between the drug and the excipients used were exhibited 2917cm^{-1}, C=Ostretchingat1703

Figure 5: FTIR spectra of Lovastatin with excipients

Release Studies

The *in vitro* release data of SP1-SP6 formulations was represented in table 3. An initial burst effect of



nanoparticles was because of the some drug particles on the nano particles surface, which was stopped very soon showing the linear behavior thereafter. The drug release from these nanoparticles was governed by some factors like drug- polymer ratio and was indicated by the results. With the polymer concentration increase the drug release rates were decreased in all the formulations. Among all the formulations studied the SP4 shown the slowest release. The release of drug from the formulation was controlled by the high entrapment of the drug in the nano particles. The drug release kinetics of Lovastatin poly mericnano particulated patch were represented in table 4. First order model was fitted by all the formulations and the calculated r^2 values were in the range of 0.916 to 0.986. The exponent 'n' values were between 0.708 to 0.886 from the korsmeyer model indicating the non-fickian diffusion drug transport mechanism.

Formulation code	Time (hrs) Percentage drug release							
	1hr	2hr	4hr	6hr	8hr	10hr	12hr	24hr
SP1	5.50±0.2	11.40±1.4	19.70±0.6	22.90±0.8	27.90±0.2	35.40±1.4	51.70±0.4	64.50±0.6
SP2	4.10±0.6	10.20±0.8	18.40±0.8	21.20±0.2	26.10±0.6	32.30±0.8	47.60±1.2	54.30±0.4
SP3	6.30±0.6	13.40±0.4	21.40±0.2	23.50±1.2	29.70±1.2	35.60±0.2	47.60±0.6	66.40±0.2
SP4	3.20±1.2	7.50±0.2	14.80±1.4	19.20±0.6	23.60±0.4	29.20±1.6	39.80±0.2	52.00±1.2
SP5	6.40±0.8	12.90±0.6	19.90±0.2	23.50±0.2	26.90±1.4	32.70±1.2	50.10±1.2	63.40±0.2
SP6	4.0±1.4	9.50±0.6	16.90±0.6	19.80±0.6	22.60±0.4	30.70±0.8	44.30±0.8	55.60±0.6

*(All the values were calculated as (Mean ± SD, n=3)

***Table 5 Invitro* Release of Lovastatin Polymeric Nanoparticles (SP1-SP6)**

Evaluation of PLN transdermal patches

The evaluation data of TPN1-TPN3 transdermal patches were represented in table 5. When applied, the patches would not break and would maintain their integrity with general skin folding was indicated by folding endurance test. All the formulations had the same strip length before and after their cuts were exhibited by flatness study that indicated100% flatness. All the prepared patches has shown smooth surface and flatness and the constriction was not observed. When the patch was applied to the skin, the smooth surface could be maintained. The TPN1-TPN3 formulations, the tensile strength lies in the range of 3.87±0.013 to 4.02 ±0.111 which indicated excellent viscosity. Moisture content results revealed that with increase in the concentration of hydrophilic polymers, the moisture content was observed to be increased in all the patches. The moisture content of the patches was found to be very less that could help the formulations remain stationary and keep down brittleness during long term storage. From microbial contamination and bulkiness of the patches, the low moisture absorption protects the material. Moisture uptake was observed to be low (2.8 ±0.05 to 3.6 ±0.09) which causes the patches to best able for prolonged storage and usage.

Various parameters	Formulation code		
	TPN1	TPN2	TPN3
Drug content (%)	96.52±1.210	98.53±1.238	96.82±1.569
Weight variation (g)	0.381± 0.023	0.392± 0.021	0.398± 0.031
Thickness(mm)	0.210±0.011	0.215±0.016	0.230±0.013
Flatness	100	100	100
Folding endurance	86.21±4.231	92.11±4.231	98.14±6.231
Flatness	100	100	100
Moisture uptake (%)	2.8±0.05	3.1±0.23	3.6±0.09
Moisture content (%)	2.254±0.534	2.720±0.325	3.103±0.125

*(All the values were calculated as (Mean ± SD, n=3)

Table 6: Evaluation of Various Parameters of Transdermal Patches (TPN1-TPN3)

Time(hrs)	TPN1	TPN2	TPN3
0	0	0	0
2	9.8±1.3	7.1±0.7	6.7±1.1
4	12.8±2.1	9.3±1.1	8.5±1.7
8	12.8±2.1	16.2±1.4	14.2±1.3
12	26.8±0.5	21.5±0.4	19.7±0.2
24	34.0±1.3	29.2±0.6	26.6±0.8
36	41.2±1.2	36.1±1.5	33.3±1.4
48	49.0±1.3	43.7±1.5	39.3±0.7

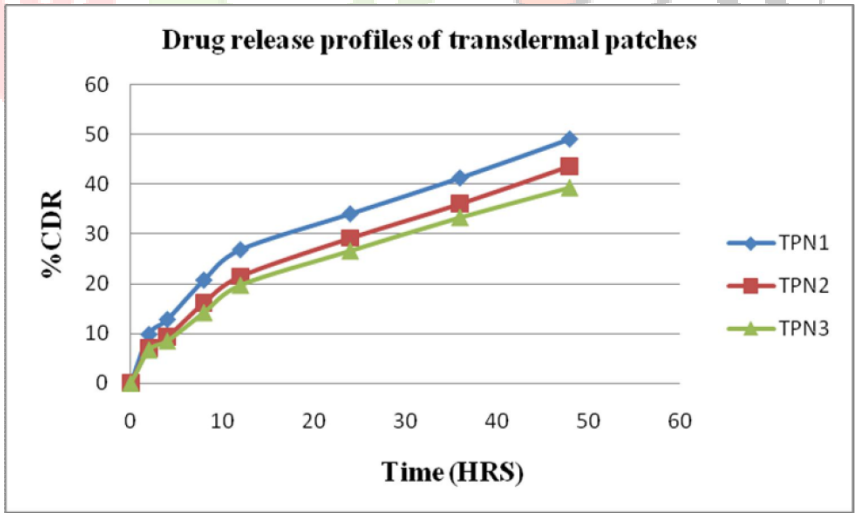


Table 7and Figure 6 represent the *invitro* release data of TPN1-TPN3

The percentage drug release for TPN1-TPN3 were found to be 49.00±1.3, 43.70 ±1.5 and 39.30 ± 0.7 respectively at end of 48 hours. The drug release was observed to be decreased as the concentration of polymer raises. Better release was shown by TPN1andwas considered as the best formulation.

Discussion: General Discussion

At present, Cardio vascular diseases (CVD) are the most prevalent causes of morbidity and mortality world wide. Dyslipidemias are one of the most important contributing factors of CVD and statins are considered to be highly effective cholesterol-lowering agents. In patients with and without CVD, they significantly reduce the cardio vascular morbidity and mortality. Among the statins drug class, Lovastatin was the most popular hypo lipidemic drug. In order to prevent the first pass metabolic process and also to achieve the controlled release, Lovastatin polymeric nanoparticles and Lovastatin solid lipid nanoparticles were incorporated into transdermal patches in the present study.

Pre formulation studies

Fourier Transform Infra red Spectroscopy (FTIR) was used to perform the compatibility studies between the Lovastatin and the excipients used in the formulation and no incompatibility issues were observed. The FTIR spectra for pure drug Lovastatin and other excipients was graphically represented in the figure 5.2 the observed frequencies were found to be 3452 cm^{-1}, 2929 cm^{-1}, 1699 cm^{-1} and 1268 cm^{-1} indicates the presence of O-H, C-H, C=O and C-O-C stretching vibration corresponding to Lovastatin. The characteristic peaks of Lovastatin & chitosan, Lovastatin & poly caprolactone, Lovastatin & cholesterol, Lovastatin & stearicacid and Lovastatin & Glyceryl monosterate were observed to be 3384cm^{-1}, 3395cm^{-1}, 3550cm^{-1}, 3454cm^{-1}, 3452cm^{-1} indicates the presence of O-H stretching, the frequencies of 2919cm^{-1}, 2917cm^{-1}, 2956cm^{-1}, 2924cm^{-1}, 2918cm^{-1}, 2917cm^{-1} presence of C-H stretching, frequencies of 1727cm^{-1}, 1736cm^{-1}, 1698cm^{-1}, 1641cm^{-1}, 1702cm^{-1}, 1703cm^{-1} indicates the presence of C=O stretching and 1268cm^{-1}, 1179cm^{-1}, 1165cm^{-1}, 1157cm^{-1}, 1270cm^{-1}, 1295cm^{-1} the presence of C-O-C stretching respectively. Compatibility studies revealed that there was no interaction between the drug and the excipients in the nanoparticles preparation.

SUMMARY AND CONCLUSION

For the delivery of anti- hyper lipidemic drugs systematically, a potential route of choice being transdermal delivery in which the first pass metabolism can be eliminated and the bioavailability can be enhanced. Lovastatin is a cholesterol lowering agent that has the 2hours plasma half life with poor bioavailability i.e. less than 5% owing to the first pass effect.

By using various phosphate buffer solutions, solubility studies were conducted and based upon their results the medium selected for *invitro* studies was phosphate buffer pH 6.8 and 1% Tween 20 was selected as a medium for *invitro* studies. By using FTIR, compatibility studies were performed between the drug Lovastatin and excipients used in the formulation. No interaction between drug and the excipients was observed in the formulations made with nanoparticles as per compatibility studies. Solvent evaporation method was employed for the preparation of PLNs by using chitosan, PLA, and PCL as polymers. Micro emulsion technique was employed for the preparation of SLNs by using Stearic acid, cholesterol and Glyceryl mono stearate as lipids. The prepared PLNs (SP1toSP12) and SLNs (SL1 to SL6) formulations were evaluated incase of various parameters like morphology, particlesize, zetapotential, PDI and *invitro* drug release and the results of the above parameters were observed to be in the desired range. With different concentrations of HPMC, the formulations SP4 and SL6 were selected and incorporated into transdermal patch. Evaluation for the various parameters was done for the prepared patches (TPN1toTPN3 and SLNP1 to SLNP3) and the results were recorded individually. Based on the results obtained TPN1 and SLNP2 were selected for formulating the transdermal patches incorporating different permeation enhancers. Evaluation was done for the transdermal formulations (PLP1 to PLP12 and SLP1to SLP12) and the results were recorded individually. PLP12 (Span 80 as permeation enhancer) and SLP9 (DMSO as permeation enhancer) were observed to be the best formulation.

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