



Formulation of Lovastatin Solid Lipid Nanoparticles for Transdermal Drug Delivery

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ABSTRACT

Nanoparticles are widely studied drug delivery system because of many benefits like controlled release ability to reach target, smaller particle size, enhancement of therapeutic activity and reduction of toxicity. SLNs (solid lipid nanoparticles) are novel nanoparticulated systems which invited substantial attention as a drug delivery carrier. The design of this study was to develop and evaluate Lovastatin solid lipid nanoparticles, loaded into transdermal patch. Lovastatin, a lipid lowering agent, because of its low bioavailability (5%) and shorter biological $t_{1/2}$ is a suitable drug to formulate into transdermal form. SLNs containing drug and non-toxic lipids (stearic acid, cholesterol and glycerol mono stearate), tween 80 and PEG 400 as surfactant and cosurfactant were prepared using micro emulsion process. FTIR study reports indicated that there was no interaction between Lovastatin and other excipients. SLNs were assessed for their particle size, entrapment efficiency, PDI (poly dispersity index) and *in vitro* studies. Scanning electron microscopy reports shown that the nanoparticles are spherical shape and has size range 132-249 nm. PDI was found out to be in the range of 0.186 – 0.376. Percent entrapment efficiency was between 74.3±0.8 – 93.5±1.8. The SLNs were loaded into a transdermal patch formulated using HPMC in varying concentration. All the prepared patches were assessed for flatness, folding endurance, tensile strength, moisture uptake, moisture content and were determined to be in essential range. The SLNP2 *in vitro* release rate studies shows better release than remaining formulations (SLNP1 & SLNP3) and it was selected as optimized formulation.

KEY WORDS: Lovastatin, lipids, SLNs, micro emulsion, transdermal patch.

1. INTRODUCTION

Lovastatin is [(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] (2S)-2-methylbutanoate falls into the category of statins known as cholesterol-lowering lactones which, in 2011-12, were discovered as being the most widely discovered drug in the universe (Bays, 2001). Lovastatin, a cholesterol lowering agent that is deduced synthetically from *Aspergillus terreus* (a fermentation product) has been determined to minimise both normal and raised LDL-C concentrations. Lovastatin is having plasma half-life of 2 hrs and poor oral bioavailability (<5%) owing to the extensive first pass metabolism. To nullify the first pass effect, the possible methods include transdermal, rectal, buccal, and parenteral routes of administration.

TDDS (transdermal drug delivery system) can be an effective path for the systemic availability of drugs. Transdermal patches are novel drug delivery systems for the skin application to attain a systemic effect. Application of the TDDS system offers many clinical benefits over other routes. It provides a consistent drug release, maintain a steady blood level profile which can reduce systemic side effects, convenient, user complaint, which contribute to improve patient acceptance (Agrawal, 2007). Transdermal delivery can be a potential route for delivery of antihyperlipidemics systemically. As the first pass effect was bypassed, bioavailability can be enhanced. Gastrointestinal irritation that frequently occurs with statin drugs can be bypassed using TDDS. Steady absorption of a drug for a prolonged period eliminates the required for frequent dosing of the drugs which contribute to improved patient compliance (Darwhekar, 2011). Many strategies have been employed to better the transdermal delivery and dermal of drugs, e.g., improving the partitioning between the formulation, increasing the efficient concentration of the drug in the vehicle, the use of chemical penetration enhancers and different physical enhancement methods (Cleary, 2003; Barry, 1983). Furthermore, carrier systems like liposomes, nanoparticles or microparticles have equalled explored (Barry, 2006; Wagner, 2004; Kohli, 2004).

SLNs are biodegradable raw materials which are formed from a matrix of lipids that are physiologically well tolerated (Wissing, 2002). The main advantages of these systems include protection of labile substances from chemical degradation, control of the relinquish of substances referable to the solid state of the lipid matrix, and showing occlusive properties by the formation of films over the skin (Muller, 2000).

TDDS formulations were preferable over the tablet (conventional) or capsule preparations as it has several advantages like it controlled release pattern thus minimizing the dosing frequency (Barry, 2001; Mukherjee, 2005). The main aim of this study was to investigate the influence of SLNs on the penetration and permeation of the lipophilic model drug Lovastatin into skin using stearic Acid, cholesterol and glyceryl monostearate as lipids.

2. MATERIALS AND METHODS

Materials: Lovastatin (Days healthcare), Cholesterol (Moly Chem., Mumbai), stearic acid, Glyceryl monostearate, tween 80, PEG 400, (Fine Chemicals), HPMCK 100M (Yarrow). All other chemicals and reagents were of analytical grade.

Compatibility Studies: It is an important requirement to study the drug and excipients under experimental conditions before preformulation. Drug and excipients incompatibility can alter the bioavailability and stability of drugs, thereby, dissembling its safety and efficacy. In the development of a stationary dosage form, the study of drug- excipients compatibility is a crucial process. Early stage of drug-excipients compatibility studies helps the selection of excipients that increases the chances of acquiring a stable dosage form.

The FT-IR spectra were by using BRUKER spectrophotometer and recorded the spectrum in the region of 4000-400 cm⁻¹. The samples (drug, polymer and drug polymer mixture) mixed with 200-400 mg of potassium bromide (KBr). The samples placed between the discs in a hydraulic press and compressed by applying 5 tons pressure upto 5 minutes. The prepared disc was placed in the path of light and the spectrum was recorded.

Method of preparation of Lovastatin SLN using microemulsion process: Preparation of SLN (Gasco, 1993) using microemulsion method was performed at a temperature higher than the lipid melting point. Stearic acid, Cholesterol and Glyceryl monostearate as the solid lipids were used for preparing SLN. Tween 80 and PEG 400 was used as a surfactant and cosurfactant. The dispersion medium used is deionised water. Various ratios of lipid, surfactant and cosurfactant were weighed and mixed at a temperature 10oC higher the lipid melting point in a water bath. Deionised water was heated to the similar temperature as a lipid phase and added drop wise under mild stirring to the lipid melt. After each addition the liquid preparation was agitated at 1000 rpm for 10 Sec and checked for clarity. If turbidity persists after stirring, the samples were sonicated for 5 minutes at a temperature higher the lipid melting point. A thermodynamically static and translucency system was formed when all ingredients were mixed in suitable ratios for the formation of micro emulsion. The obtained micro emulsion was then disseminated in cold aqueous medium (5-10oC) under mild mechanical stirring. The ratio of micro emulsion to aqueous medium was 1:20.

Table.1. Formulation Table

Ingredients	SL1	SL2	SL3	SL4	SL5	SL6
Lovastatin	0.2	0.2	0.2	0.2	0.2	0.2
Stearic acid	1	2	-	-	-	-
Cholesterol	-	-	1	2	-	-
Glyceryl mono stearate	-	-	-	-	1	2
Tween 80	1.5	1.5	1.5	1.5	1.5	1.5
PEG 400	1.5	1.5	1.5	1.5	1.5	1.5
Deionised Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of nanoparticles:

Morphology of Nanoparticles: Morphology of nanoparticles was characterized by scanning electron microscope (SEM) (Essa, 2010). SEM is one of the most limited instruments widely applied to surface microstructure imaging. SEM is a character of electron microscopy that images the sample surface of a solid specimen by using a focused beam of high-energy electrons. Nanoparticles containing Lovastatin was placed on a cover glass and shifted to a specimen stub. Dried samples were taken and coated with a platinum alloy to a thickness of 100° A. After completing the coating, shape and size was examined by scanning.

Particle size distribution: The nanoparticles size was analyzed by employing a Zetasizer, Ver. 6.20 (Malvern Instrument Ltd). The formulation was targeted in the sample holder and the particle size was measured (Elbary, 2008).

Poly dispersibility index (PDI): Poly dispersibility index (PDI): Polydispersity index (Nidhin, 2008) is defined as the particle size distribution of nanoparticles obtained from photon correlation spectroscopic analysis. The PDI was calculated for dispersion homogeneity ranging from 0 to 1. The value close to 0 indicated a homogeneous dispersion and greater than 0.3, high heterogeneity.

In vitro release studies: Franz diffusion cell was used to determine the *in vitro* release of nanoparticles, having the receptor volume of 20ml in the cell. The diffusion area was 5cm². The cell placed between the cell stirrer and water bath where the temperature was maintained at 32± 0.5°C. Cellophane membrane having a molecular weight (break up: 6000-8000) soaked previously in the receptor medium which was clamped between the donor and receptor chamber of diffusion cell. Formulated SLNs (100mg) were added to the donor compartment of the Franz diffusion cell which was blocked with a paraffin film. The receptor medium (pH 6.8 buffer + 1% tween 20) was stirred by magnetic bar. From the receptor compartment, 1ml sample was withdrawn at the following time intervals: 1, 2, 4, 6, 8, 10, 12 and 24 h and replaced by 1ml volume of the fresh receptor fluid. The withdrawn samples were centrifuged at 20,000rpm, for 30 minutes, at room temperature. By using the HPLC technique, the drug content in the supernatant liquid was estimated.

kinetics studies: In vitro Release data of various kinetic models were analyzed to describe the kinetics release. The zero order rate Equation. (1) describes the systems where the release rate of drug is independent of its concentration (Hadjiioannou, 1993). The first order Equation. (2) describes the release from the system where the release rate of drug is concentration dependent (Bourne, 2002). Higuchi (Higuchi, 1965) described the release rate of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Equation.(3). Where, k₀ is zero-order rate constant expressed in units of concentration/time and t is the time.

$$Q = K_0t \quad (1)$$

$$\text{Log } C = \text{Log } C_0 - Kt / 2.303 \quad (2)$$

Where, C_0 is the initial concentration of drug and k is first order constant.

$$Q = Kt^{1/2} \quad (3)$$

Where, K is the constant reflecting the design variables of the system.

The following plots were made:

- Zero order kinetic model - Cumulative % drug release vs. time
- First order kinetic model - Log cumulative of % drug remaining vs. time
- Higuchi model - Cumulative % drug release vs. square root of time
- Korsmeyer model - Log cumulative % drug release vs. log time

Preparation of nanoparticulated transdermal patches: Transdermal patches were prepared by dissolving varying concentrations of HPMC K100M (polymer) and PEG (plasticizer) in 50ml of distilled water. The mixture was soaked overnight to get rid of air bubbles. 100mg of Nanoparticles were incorporated into the polymeric solution. The prepared solution was poured into glass petri dishes of 25 cm² area and dried at room temperature (Kulkarni, 2002; Munden, 1967). After 12 h, the patches were cut in 5 cm² area and packed in aluminum foil until used.

Table.2. Formulation of Transdermal Patch

Formulation Code	Quantity of Nanoparticles (mg)	Amount of HPMC K100M (mg)	PEG 400 (10%w/w of polymer) (mg)
SLNP1	100	500	50
SLNP2	100	1000	100
SLNP3	100	1500	150

Evaluation parameters for transdermal patches:

Weight variation: Weight variation the surface area of the polymer film with 5cm² was cut at 3 assorted places in the prepared film. Each film strip weight was considered and average weight was calculated.

Thickness of transdermal patches: Digital vernier calipers was used to evaluate the thickness of nanoparticulated transdermal patches. The values were taken by triplicate.

Drug content in transdermal patch: The patches (n=3) of 5 cm² were weighted and dissolved in 100ml dichloro methane. The solution was filtered through 0.45 µm membrane filter and analyze the samples by HPLC method.

Folding endurance: The folding endurance was measured manually for the prepared patches. It is expressed as the number of times the patch is folded at the same place either to break the patch or to develop visible cracks. This is crucial to verify the power of the patch to resist folding. This also gives an indication of brittleness (Raghuraman, 2002). This was ascertained by folding the patch repeatedly at the same place until the film breaks. The value of folding endurance is calculated by the number of times the patch could be folded at the same place without breaking/cracking (Devi, 2003).

Flatness: One strip is cut from the centre and two from each side of patches. The length of each strip is measured and variation in length is measured by determining percent constriction. Zero percent constriction is equivalent to 100 percent flatness (Arora, 2002)

$$\text{Constriction (\%)} = S_1 - S_2 / S_1 \times 100$$

Where, S_1 - initial length of strip, S_2 - final length of strip

Tensile strength: Tensile strength was checked by weight pulley method (Gannu, 2008). The weight required for breaking the patch was taken as a measure of tensile strength of the patch.

Moisture content: Individually weigh the formulated films and placed in a desiccator which containing the calcium chloride at room temperature for a period of 24hrs. After assigned time interval the films were weighed again and again until they exhibit a constant weight. The percent moisture content was calculated using following formula (Bagyalakshmi, 2007).

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

Moisture uptake: Weighed formulated films were taken and revealed to 84% relative humidity using saturated solution of potassium chloride in desiccator until a constant weight is achieved. % moisture uptake was calculated as given below.

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

drug release studies: Formulated Lovastatin nanoparticulated patch in vitro drug release was executed by using a modified USP type II dissolution apparatus using 900 ml dissolution medium (pH 6.8 buffer + 1% tween20). For the study of *in vitro* release of nanoparticulated patch, a circular patch of an internal diameter 5² cm was used. To sink the patch at the bottom of dissolution apparatus, a stainless steel ring was utilized. All the *in vitro* dissolution studies were conducted at 37±0.5 °C (temperature of the skin) at 100 rpm. At particular time intervals the samples were removed and substituted with an equal volume of fresh dissolution media to maintain sink conditions and their concentrations were examined using HPLC spectroscopy (Jain, 2001).

To analyse the release kinetics, data obtained from in vitro drug release rate studies were accommodated in respective kinetic models: cumulative percent of drug released vs. time (zero order), log cumulative percentage of drug remaining vs. time (first order) and cumulative percent drug released vs. square root of time (Higuchi's model). To determine the drug release mechanism, the release data were confirmed into Korsmeyer and Peppas equation as log cumulative percentage of drug released vs. log time, and the exponent n was computed from the slope of the straight line. If the exponent is 0.5 for slab matrix, then diffusion mechanism is Fickian; if $0.5 < n < 1.0$, mechanism is non-fickian; if n is 1.0, the mechanism is zero order and if $n > 1.0$, then it is super case II transport (Alam, 2009).

3. RESULTS AND DISCUSSION

Compatibility studies: The characteristic peaks for Lovastatin, viz. –OH stretching at 3550, –Ar-H stretching at 3011, aliphatic C-H stretching at 2956 & 2872, C=O stretching at 1698, and C=C aromatic stretching at 1466 cm⁻¹ was also noticed in spectrum of drug with excipients (Fig 1 & 2). There is no appearance or disappearance of any characteristic peaks.

This establishes that the drug and excipients used in the nanoparticle preparation has no interaction.

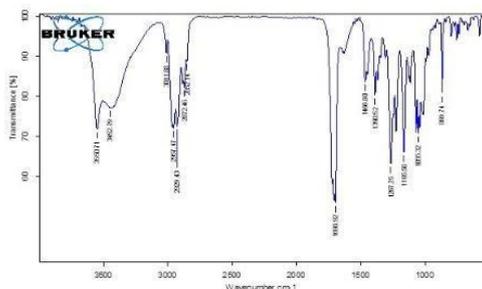


Fig.1. FTIR Spectrum of Lovastatin

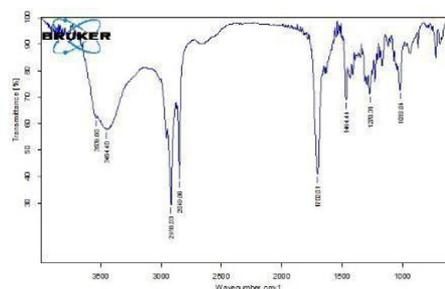


Fig 2. FTIR Spectrum of Lovastatin+Excipients

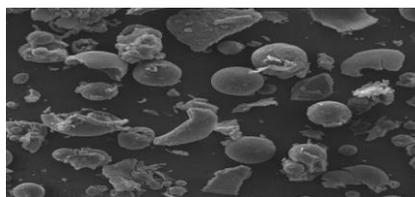


Fig.3. SEM image of Lovastatin solid lipid nanoparticle

Particle size and poly dispersity index: Lovastatin loaded SLNs developed by using cholesterol and stearic acid as the lipid matrix, resulted in bigger particle size compare to SLNs made by employing glyceryl monostearate.

This phenomenon could be assigned to the melting point of the lipid. Glyceryl monostearate, having melting point lower than remaining two lipids shown faster lipid crystallisation from the hot homogenized condition resulting in decrease in the size of the particle. Various SLNs formulated with different lipids, the particle size was in the order of Cholesterol > Stearic acid > glyceryl monostearate. A Drug lipid proportion is a critical parameter as the entrapment efficiency increased with increase in lipid phase. However an upper level is crucial to maintain the nanoparticle size in a reasonable range. The PDI of all formulations was found to be in the range of 0.186-0.376. In this study, Tween 80 and PEG 400 were opted as surfactant and cosurfactants which were reported earlier in the literature shall yield finer sized SLN.

Table.3. Physical evaluation of Lovastatin Solid Lipid Nanoparticles

Formulation Code	Particle Size (nm)	PDI	Entrapment efficiency (%)
SL1	181	0.312	75.6±3.2
SL2	192	0.279	81.6±1.9
SL3	249	0.376	74.3±0.8
SL4	243	0.301	77.4±2.4
SL5	132	0.186	91.6±1.6
SL6	139	0.193	93.5±1.8

Entrapment efficiency: The entrapment efficiency is the functional characteristic of polymers, drug and surfactant etc. The entrapment efficiency increases with the increase in the concentration of lipids. This may be due to decrease of surface tension between organic phase and aqueous phase that possibly allows the formation of initially smaller solvent droplets at the site and causes decreased particle size and increase entrapment efficiency. Entrapment efficiency was performed in all six batches (SL1 –SL6). The result obtained for different batches varies from 74.3±0.6 to 93.5±1.8 and are indicated in Table 3. It has been noticed that with enhancing the lipid concentration entrapment efficiency also increases. The maximum entrapment efficiency was found 93.5±1.8 prevailed in batch SL6.

Release studies: From SLNs the Lovastatin in vitro release exhibited a slow initial release at 2 h and followed by a sustained release at a perpetual rate. The initial release, was because of the loosely bounded surface present on the drug could be removed in the initial sink condition. The observed quantity might change with the accumulation and the disaggregation status of the particles. Figure 4 shows the Lovastatin in vitro release profile from nanoparticulate system. The prolonged release of the drug can be assigned to the drug embedment in the solid lipid matrix.

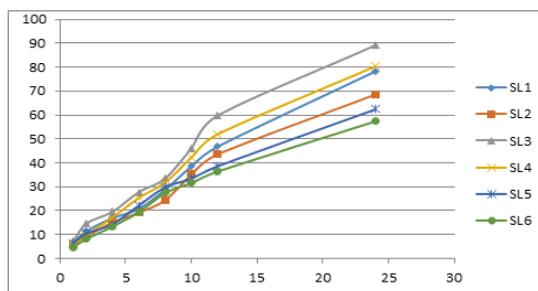


Figure.4. Release profiles of Lovastatin SLNs loaded transdermal patches Table.4. *In-vitro* drug release kinetics from solid lipid nanoparticles formulations

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		Drug release mechanism
	r ²	Slope	r ²	Slope	r ²	Slope	r ²	Diffusion exponent (n)	
SL1	0.9897	3.1637	0.9667	0.027	0.9586	18.529	0.9858	0.783	Non-Fickian
SL2	0.9801	2.8049	0.9866	0.0212	0.9599	16.518	0.9854	0.796	Non-Fickian
SL3	0.987	3.6417	0.9649	0.0408	0.9639	21.445	0.984	0.786	Non-Fickian
SL4	0.9771	3.3227	0.9876	0.03	0.9788	19.79	0.9959	0.865	Non-Fickian
SL5	0.9795	2.447	0.998	0.0173	0.9852	14.603	0.9921	0.734	Non-Fickian
SL6	0.9721	2.3117	0.9975	0.0154	0.9889	13.875	0.9955	0.807	Non-Fickian

The kinetics release from nanoparticles was shown in Table 4. All the preparations fit First order model, R² values calculated are in the array of 0.9649 to 0.998. Value of exponent n from Koresmeyer model was in the array of 0.734 to 0.865. This is an indication that the dominant drug transport mechanism appears to be non-Fickian diffusion (n 0.45 < n<0.89).

Evaluation of transdermal patches: Results of various parameters studied of transdermal nanoparticulated patches were mentioned in Table 5 and were observed to be in desired range. Folding endurance test results confirmed that the patches would be stable with general skin folding and would not break when applied. Flatness studies results shows that no preparation had the difference in the strip length earlier and later their cuts, showing 100% flatness in all the formulations. No constriction was observed: this indicates that all the transdermal patches have smooth, flat surface and when the patch was applied to the skin smooth surface can be maintained.

Table.5. Evaluation of various parameters of Transdermal Patch

Parameters	SLNP1	SLNP2	SLNP3
Weight variation (g)	0.372±0.015	0.385±0.022	0.392±0.02
Thickness (mm)	0.199±0.012	0.208±0.009	0.224±0.14
Drug content (%)	92.41±2.523	93.22±1.48	89.72±0.892
Folding endurance	96.21±3.231	102±2.458	109.12±1.589
Flatness	100	100	100
Tensile strength (Kg/mm ²)	3.87±0.022	4.82±0.01	5.04±0.121
Moisture content (%)	2.354±0.432	2.920±0.125	3.213±0.251
Moisture uptake (%)	2.7±0.24	2.9±0.32	3.4±0.072

The tensile strength of the SLNP1 to SLNP3 shows the 3.87 ± 0.022 to 5.04 ± 0.112 shows the excellent viscosity. Moisture content consequences shown that increasing the concentration of hydrophilic polymers the

moisture content was observed to enhanced in all the formulations. In the developed patches the moisture content was low, which could assist the prepared formulations stable and brittleness reduces throughout long-term storage (Ubaidulla, 2007). The low absorption of moisture assists the material from microbial contamination and patch bulkiness. Moisture uptake of the prepared formulations (2.7 ± 0.24 to 3.4 ± 0.72) was low, facilitating the prepared formulations to persist stable for usage and longtime storage. The *in vitro* release profiles of different transdermal patches were mentioned in Fig 5. The cumulative percentage drug release for SLNP1, SLNP2 and SLNP3 was observed to be 52.1 ± 0.6 , 46.5 ± 1.4 and 41.2 ± 1.8 respectively at 48 h. It was found that as the concentration of polymer increases the drug release was observed to be decreased. SLNP2 which has shown better release can be considered as best formulation. Burst releases as well as sustained release, both are of interest to dermal application. To facilitate the penetration of the drug, burst release can be helpful. Sustained release supplied the drug over a prolonged period of time.

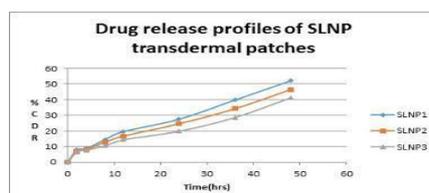


Fig .5. Release profiles of Lovastatin SLN loaded Transdermal Patches Table.6. *In vitro* kinetic studies of SLN Tansdermal patches

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		Drug release mechanism
	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Diffusion exponent (n)	
SLNP1	0.9947	0.9464	0.9863	0.006	0.9685	7.8995	0.9736	0.6122	Non-Fickian
SLNP2	0.9954	0.8348	0.9855	0.005	0.9649	6.9525	0.9783	0.6065	Non-Fickian
SLNP3	0.9858	0.7176	0.9641	0.004	0.9353	5.913	0.9579	0.5700	Non-Fickian

The description of dissolution profile of a model function has been attempted using different kinetics (zero order, first order, Higuchi square root model, Korsmeyer's Peppas model (Table 6). All the formulations (SLNP1- SLNP3) followed first order release kinetics. The correlation coefficients (R^2) were observed to be in the range of 0.9641-0.9863. The data were implemented to Higuchi and the line obtained were comparatively linear ($r^2 = 0.9353- 0.9685$) suggesting that the diffusion might be of drug release. To affirm further drug release mechanism, the data were confirmed to Korsmeyer's Peppas equation. The release exponent 'n' value ($0.5 < n < 1$) of korsmeyer's peppas model indicated that release of the drug from all the patches followed anomalous transport.

4. CONCLUSION

It can be concluded that Lovastatin, poorly water soluble drug converted to SLNs, which are then included in transdermal patch to overcome the problems with oral administration. The Lovastatin SLNs were formulated by micro emulsion process using stearic acid, cholesterol and glyceryl mono stearate as lipids. The physical parameters, entrapment and release studies indicated the formulation SL6 prepared using glyceryl mono sterate was suitable to prepare transdermal patch. The transdermal patches containing SLNs employing HPMC in changing concentrations were referred to various parameters and found that formulation SLNP2 shown the sustained release over a time period of 48 h, which can gain the patient in reducing the dosing frequency. So it is concluded that transdermal patch containing Lovastatin SLNs can represent as a potential drug delivery approach for treating hyperlipidemia. Further, it can be employed for pharmacokinetic and pharmacodynamic studies in suitable animal models.

REFERENCES

1. Agrawal SS, Munjal P, Permeation studies of atenolol and metoprolol tartrate from three different polymer matrices for transdermal delivery, Indian J Pharm Sci., 4, 2007, 535-539.
2. Alam MI, Baboota S, Kohli K, Ali J, Ahuja A, Development and evaluation of transdermal patches of Celecoxib, PDA J Pharm Sci Tech., 5, 2009, 429-437.
3. Arora P, Mukherjee P, Design, development, physicochemical, and *in vitro* and *in vivo* evaluation of transdermal patches containing diclofenac diethyl ammonium salt, J Pharm Sci., 91 (9), 2002, 2076-2089.
4. Bagyalakshmi J, Vamsikrishna RP, Manavalan R, Ravi TK, Manna PK, Formulation development and *in vitro* and *in vivo* evaluation of membrane moderated transdermal systems of Ampicillin sodium in ethanol: pH 4.7 buffer solvent system, AAPS Pharm Sci Tech., 8 (1), 2007, E50-E55.

5. Barry BW, Dermatological Formulations, Percutaneous absorption, Drugs and the Pharmaceutical Sciences, Marcel Dekker, Inc., New York and Basel, 18, 1983.
6. Barry BW, Novel mechanisms and devices to enable successful transdermal drug delivery, Eur J Pharm Sci., 2, 2001, 101-114.
7. B Ramu, N. Ramakrishna, Meruva Sathish, D. Anoocha (2015). Formulation of telmisartan Hcl Fast Disintegrating Tablets by Sublimation Technique. International Journal of Pharm Tech Research. 8(3), 330-339.
8. Bays HE, Moore PB, Drehobl MA, Effectiveness and tolerability of Lovastatin in patients with primary hypercholesterolemia: pooled analysis of two phase II studies, Clin 377, www.ajptr.com Ther, 8, 2001, 1209-1230.
9. Bourne DW, Pharmacokinetics, In: Modern pharmaceuticals, Ed 4, Banker GS, Rhodes CT, Eds., Marcel Dekker Inc, New York, 2002.
10. Cleary G.W, Transdermal and transdermal-like delivery system opportunities: today and the future, Drug Delivery Technology. 3 (5), 2003, 35-40.
11. Darwhekar G, Jain K, Patidar KV, Formulation and Evaluation of Transdermal Drug Delivery System of Clopidogrel Bisulfate, Asian J Pharm Life Sci., 1 (3), 2011, 269-277.
12. Devi VK, Saisivam S, Maria GR, Deepti PU, Design and evaluation of matrix diffusion controlled transdermal patches of Verapamil hydrochloride, Drug Dev Ind Pharm., 29 (5), 2003, 495-503.
13. B. Ramu, Chandrul KK, Pandiyan PS, BioAnalytical Method Development of Repaglinide Drug Delivery Systems, Journal of Drug Delivery and Therapeutics. 2019;9(6):140-142 <http://dx.doi.org/10.22270/jddt.v9i6.3718>
14. S. Ullas Kumar, B. Ramu, G. Srikanth et al (2016). Formulation and evaluation of sustained release verapamil hydrochloride using natural polymers. Int J Appl Pharm Sci Res. 1(2):76-87. Doi: 10.21477/ijapsr.v1i2.10179
15. Gannu R, Vishnu YV, Kishan V, Rao YM, Development of Carvedilol transdermal patches: evaluation of physicochemical, *ex vivo* and mechanical properties, PDA J Pharm Sci Technol., 62 (6), 2008, 391-401.
16. Gasco M.R, Method for producing solid lipid microspheres having narrow size distribution, United States Patent, USS 188837, 1993.
17. Hadjiioannou TP, Christian GD, Koupparis MA, Quantitative calculations in pharmaceutical practice and research, VCH Publishers, Inc, New York, USA, 1993.
18. Higuchi T, Mechanism of sustain action medications, theoretical analysis of rate of release of solid drug dispersed in solid matrices, Journal of Pharmaceutical Sciences, 52, 1963, 1145-1149.
19. Jain NK, In Advances in controlled and novel drug delivery, Edn. 1, CBS Publishers, New Delhi, 2001, 108.
20. Kohli AK, Alpar HO, Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge, Int J Pharm., 275 (1-2), 2004, 13-17.
21. Kulkarni RV, Mutalik S, Hiremath D, Effect of plasticizers on the permeability and mechanical properties of Eudragit films for transdermal application, Ind J Pharm Sci., 64 (1), 2002, 28-31.
22. Kumar S, Tyagi LK, Chandra A, Chemical penetration enhancers: an approach for better transdermal drug delivery, Int J Pharm Res Dev., 3 (7), 2011, 87-95.
23. Maryadele J.O. Neil, Edu. In, Edn13, Merck Index, NJ, USA, 2001, 868.
24. Mukherjee B, Kanupriya, Mahapatra S, Das S, Patra B, Sorbitan monolaurate 20 as a potential skin permeation enhancer in transdermal patches, J Appl Res., 5, 2005, 96-108.
25. Muller RH, Souto EB, Radtke M, PCT application PCT/EP00/04111, 2000.
26. Munden BJ, George HD, Banker GS, Evaluation of polymeric materials, J Pharm Sci., 53 (4), 1967, 395-401.
27. Nidhin M, Indumathy R, Sreeram KJ, Balachandran U, Synthesis of iron oxide nanoparticles of narrow size distribution on polysaccharide templates, Bull Mater Sci., 31, 2008, 93-96.
28. Raghuraman S, Velrajan R, Ravi B, Jeyabalan D, Benito J, Sankar V, Design and evaluation of propranolol hydrochloride buccal films, Ind J Pharm sci., 64 (1), 2002, 32-36.
29. Ubaidulla U, Reddy MVS, Ruckmani K, Ahmad FJ, Khar RK, AAPS Pharm SciTech., 8 (1), 2007, E13-E20.
30. Wagner H, Kostka KH, Adelhardt W, Schaefer UF, Effects of various vehicles on the penetration of flufenamic acid into human skin, Eur J Pharm Biopharm., 58 (1), 2004, 121-129.
31. Wissing SA, Müller RH, The influence of the crystallinity of lipid nanoparticles on their occlusive properties, Int. J. Pharm., 242 (1-2), 2002, 377-379.