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LIPID NANOGEL FOR TRANSDERMAL DELIVERY OF LOVASTATIN : IN VITRO CHARACTERIZATION

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Abstract:

Lipid nanogel was fabricated by incorporating lovastatin loaded solid lipid nanoparticles in carbopol 934P.Lovastatin loaded Solid lipid nanoparticles were prepared by solvent emulsification diffusion technique by using Glyceryl behanate as lipid. The prepared SLNs in the form of nanosuspension were evaluated for Zeta potential, Particle size, Polydispersity index, and percent drug entrapment efficiency. Carbopol 934P was added to the nanosuspension to prepare nanogel. The nanogels were evaluated for physical appearance, pH, viscosity, spreadabilityand*invitro* release profile. Gel formulation was also evaluated for stability.The results indicate that the SLNs have an average particle size of 370 ± 3.67 nm with
0.43 ± 0.12 PDI and -18.8 ± 2.41 MeVzeta potential.The percent drug entrapment was 90.0%. The optimized gel was clear,homogeneous,and have neutral pH near to 7.The viscosity and spreadability of formulated gel were 99054 ± 97.38 centipoise and 18 ± 2.3 mm, respectively. the release from gel was98.6±1.32 and following Higuchi's kinetics. The formulation was stable up to a period of three months.

Keywords: Lovastatin, zeta potential, nanogel, spreadability.

1. Introduction:

Lovastatin is a lactone metabolite isolated from Aspergillus terreus(a fungus) as a prodrug. it is ([(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8 a hexa hydro naphthalen-1-yl] (2S)-2-methyl butanoate) (Figure 1),and reported to have cholesterol-lowering activity(1). It is highly effective andwell-tolerated for patients with moderate hypercholesterolemia (2) for lowering cholesterol level. its high logP value(log P = 4.3)indicates high lipophilicity of the drug. It is reported to have bone formation and chemopreventive activity(3,4).The poor oral bioavailability of <5% due to rapid metabolism in the gut and liver(5), and short half-lives (1-2 h) of its active metabolite,makes lovastatin a potential candidate for sustained release carriers as it takes 48 to 72 h. to achieve steady-state plasma concentrations(6). Hence a formulation with sustained release action and an alternative route like transdermal delivery would be desirable for lovastatin.

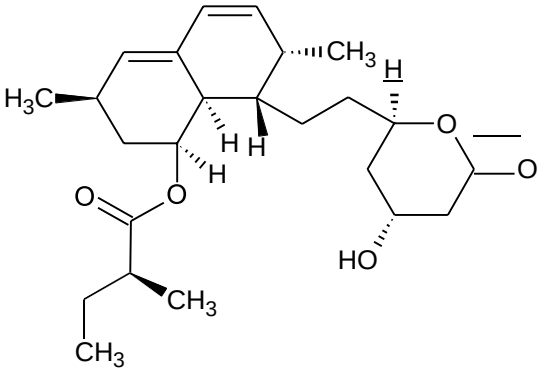

Figure 1: Molecular structure of Lovastatin.

Transdermal delivery is preferred over injectable and oral routes due to the reduction of dosing frequencies, eliminating first-pass metabolism, and is suitable for unconscious or vomiting patients, and can self-administered(7) thus increasing patient compliance. Transdermal delivery route provides both convenient and painless delivery as well as sustained release profile with less side effects.Needle phobia of parenteral delivery can be circumvented by transdermal delivery and the pharmacokinetic profiles of drugs are more uniform so that minimizing the risk of toxic side effects (8).Challenges in transdermal delivery includephysiological barriers in the skin, which limited to small and lipophilic drugs. Large surface area, and availability of various option for transdermal absorption makes the skin a high potential site for application of formulations(9).Lipids are integrated component of human bodies and nanoparticles based on lipids like Solid lipid nanoparticles (SLNs) have been used and evaluated for performance during the last decades for delivery of drugs. SLNshaving uniqueness in encapsulating both hydrophilic and lipophilic drugs(10). They are mainly composed of lipids like glyceryl monostearate, glyceryl behenate, palmitic acid, various fatty acidare solid at room temperature and listed in GRAS(generally recognized as safe)(11).It can improve bioavailability(12),to sustain the release of actives(13).SLNs also provide chemical stability of molecules against oxidation, hydrolysis, and light(14).On application to the skin SLNs form an adhesive layer and present occlusive effect on the skin, which results in reducedtransepidermal water loss(TEWL) from the skin and consequently an increase in skin hydration(15).

The present work aimed to develop lipid nanogel containing solid lipid nanoparticles loaded with lovastatin for transdermal delivery which provide a sustained release profile and may overcome the bioavailability issues of lovastatin.

2. MATERIALS AND METHODS:

2.1. Materials:

Lovastatin was obtained as gift sample from Shreeji Pharma International, Vadodara Gujarat, India, Tween 80, glyceryl behanate, acetone, chloroform, isopropyl alcohol, potassium bromide, sodium chloride were obtained as gift sample from Central Drug House, New Delhi, Carbopol 934P, triethanolamine,propyl paraben,methyl paraben,methanol and ethanol were purchased from Qualigens Fine Chemicals A Division of Glaxo India Limited, Mumbai.

2.2. Fabrication of Lovastatin loaded Solid lipid nanoparticles:

Solid lipid nanoparticles of lovastatin (LSLN) were prepared by solvent emulsification diffusion technique (16).Glyceryl behanate(50 mg) was dissolved in 3 ml solution of chloroform and ethanol (in ratio of 1:1) as internal oil phase.Lovastatin(50 mg) was dispersed in the internal oil phase. Separately,0.8 ml aqueous surfactant solution(1.5%w/v) containing Tween 80 was prepared and taken in homogenizer tube.The above drug dispersion was added drop by drop to aqueous surfactant solution and homogenized at 4000 rpm for 30 min to prepare o/w emulsion. Organic solvent was completely removed by evaporating on a rotary evaporator (Buchi type) at 400 mbar, 45°C, 30 min. Ice cold water was added to this emulsion to make up the volume up to 50 ml with continuous stirring (3000 rpm) for about 3 h. The dispersion was centrifuged for 30 min at 15000 rpm (Remi,India) for the separation of solid lipid materials along with drug followed by redispersing in 50 ml of1.5 % w/v aqueous surfactant (Tween80) solution and sonicated for 5 minto get 50 ml nanosuspension of lovastatin (17).

2.3. Preparation of nanogel loaded with LSNL:

The prepared nanosuspension of lovastatin equivalent to 40 mg was added with 1 to 2% w/v carbopol 934P and kept in cool and dark place for 24 h. Mixture of methyl (0.03% w/w) and propyl parabens (in a ratio 2:1) in propylene glycol was added to above dispersion with continuous magnetic stirring at 100 rpm speed for 2 min(18). The prepared gels were neutralized by addition of
1.0 ml triethanolamine and stirred again to get clear transparent nanogelsloaded withlovastatin(Table 1).

Table 1: Composition of nanogel formulations.

Formulation code	Ingredients			
	Carbopol 934P (%w/v)	**LSLNs nanosuspension (ml)**	**Parabens (methyl and propyl in a ratio of 2:1) (% w/w)**	**Triethanolamine (ml)**
G1	1.0	44.4	0.03	1.0
G2	1.5	44.4	0.03	1.0
G3	2.0	44.4	0.03	1.0

2.4. Characterization of Lovastatin loaded Solid lipid nanoparticles:

Prepared SLNs were characterized for particle size,size distribution,zeta potential and percent entrapment efficiency.

2.4.1. Particle size, size distribution, zeta potential:

Mean particle size was calculated by Photon Correlation Spectroscopy (Nano ZS, Malvern, UK) at room temperature (298°K). For study 1 ml of LSLNs suspension was diluted ten times (10 ml) with distilled water and average particle size, size distribution as polydispersity index (PDI) and zeta potential was measured(19).

2.4.2. Transmission electron microscopy:

Morphological examination of lyophilized drug loaded LSLN was done bytransmission electron microscopy (JEM2100, JEOL, Japan). The LSLNsample was placed on a carbon coated copper grid and dried. The sample staining was done by uranyl acetate. The image of dried sample was recorded using Digital Micrograph® software (Gatan, Inc., USA) (20,21).

2.4.3. Percent drug entrapment efficiency:

SLNs suspension (10 ml) was taken with a pipette (10 ml, Borosil), and transferred into a centrifuge tube and centrifuged at 10000 rpm for 50 min at room temperature (CENTRIFUGE, REMI), the lipid portion was isolated, and the absorbance of the drug in the supernatant was determined spectroscopically using UV-VIS Spectrophotometer (Shimadzu, Japan) at 238 nm. The concentration of drug was calculated from the calibration curve. The percent drug entrapment efficiency of solid lipid nanoparticle was calculated by the following equation(17):

Percentagedrug entrapment efficiency = $(W_T\text{-}W_S/W_T) \times 100$

Where, W_T is the weight of drug added in the system, W_S is the analytical weight of drug in the supernatant after centrifugation and W_L is the weight of lipid added in the system.

2.5. Characterization of prepared nanogel loaded with LSLNs:

2.5.1. Physical observation:

All the three gel formulations (formulation G1, G2 and G3) were visually observed for clarity and homogeneity(22).Clarity observation of the developed gels were visually inspected against black and white background and graded as turbid, clear, and glassy.The prepared gels were visually observed for homogeneity by keeping the gels in container and tested for presence of any aggregates.

2.5.2. Determination of pH:

pH of all three gels were determined by using by using a calibrated pH meter (Systronics India Limited). The pH meter was calibrated before each use with standard 4, 7 and 9.2 pH buffer solutions,for pH determination 2.0 g of each nanogel was taken and dispersed in 20 ml of distilled water by stirring on magnetic stirrer for 10 min at room temperature and pH was measured.Readings were taken in triplicate(23).

2.5.3. Viscosity study:

The viscosity of nanogel was determined by viscosity of the gels was determined using a viscometer,(Brookfield, DV1MHA) by using small sample adapter having spindle number 1.The gel(100 g) at room temperature(25°C) was subjected to torque ranging from 10 to 100%. Viscosity was calculated in centipoise(cP),viscosity for each formulation was taken in triplicate(24-26).

2.5.4. Spreadability:

Spreadability of nanogel was determined by measuring the spreading diameter of 2.0 gram of sample between two horizontal glass plates (20 cm× 20 cm) after one minute. The standard weight applied to the upper plate was 25 g. Each gelformulation was tested three times(18).

2.5.5. Invitrorelease study:

The in vitro drug release studies of nanogels were carried out using Franz diffusion cell. The dialysis membrane (Mol. Wt. 10,000-12000, Hi Media, Mumbai) after treatment, was mounted in Franz diffusion cell, formulations were placed in donor compartment, and phosphate buffer (pH 7.4 along with 1.5% SLS), as a dissolution medium was placed in the receptor compartment of Franz diffusion cell at 37°C temperature.The assembly was kept on a magnetic stirrer and solution was stirred continuously (100 rpm) using a magnetic bead. The sample (1.0 ml) was withdrawn at predetermined time intervals (0,0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h.) and replaced with equal amount of fresh dissolution media. The samples were analyzed spectrophotometrically (UV-1800, Shimadzu, Japan) at 238 nm(27).The cumulative percentage drug release was calculated. The experiments were performed in triplicate and SD was determined.

2.5.6. Release kinetic study:

In vitro release data of three nanogels were analyzed by applying various kinetic models like zero order ($C = kt$), first order ($LogC = LogC_0 - k^{t/2.303}$), Higuchi's model ($Q = Kt^{1/2}$) and Korsmeyer-Peppas' model ($M_t/M_N = Kt^n$) to check the release pattern and mechanism of drug release(16,28- 30).

2.5.7. Stability study of nanogels:

Stability study of nanogel G2 was carried out at 25°C with 60%RH and 40°C with 75% RH for 90 days, sampling was done at 0, 30, 60 and 90 days for any change in clarity, homogeneity, pH change, viscosity and spreadability (31).

3. RESULTS AND DISCUSSION:

Solid lipid nanoparticles (SLNs) of lovastatin were prepared by solvent emulsification diffusion technique due to its simplicity and suitability in laboratory. Single formulation was prepared with drug to lipid ratio 1:1 and 1.5 percent (w/v) aqueous solution of Tween 80 as surfactant.Particle size of SLNs plays an important role in drug release at target site. Formation of SLNs was confirmed based on the TEM image (Figure 1). Particle size of prepared SLNs were found to be 370 ± 3.67 nm, polydispersity index of SLNs was 0.43 ± 0.12 and the zeta potential was -18.8 ± 2.41 MeV(Table 2).Polydispersity index (PDI) a parameter to predict about physical stability of SLNs should be less than one for better physical stability(32).Zeta potential value of the prepared SLNs depicts the lower chances of agglomeration its high magnitude either positive or negative is necessary for better physical stability(33). Drug entrapment efficiency was found to 90 percent (Table 2).A high drug entrapment could be due to solubility of drug in lipid.

Table 2: Characterization of Lovastatin solid lipid nanoparticles.

Formulation code	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (MeV)	Drug entrapment efficiency (%)
LSLN	370 ± 3.67	0.43 ± 0.12	-18.8 ± 2.41 MeV	90.0

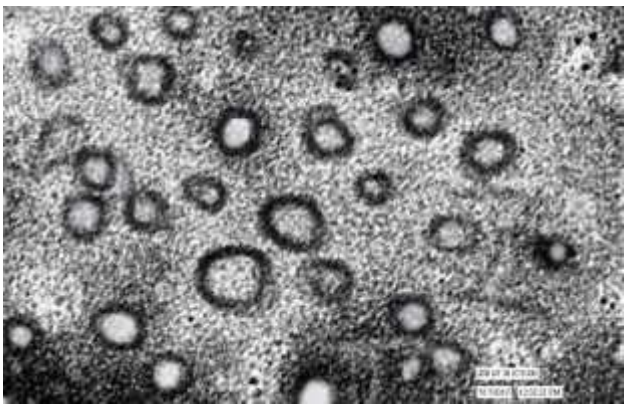

Figure 1: Scanning electron microscopic image of Lovastatin loaded solid lipid nanoparticles.

Prepared nanosuspension were added with three different concentration 1%, 2% and 3% (w/v) of Carbopol 934P as gelling agent, to convert the nano suspension into three gel formulation G1, G2 and G3, respectively. The gels were prepared by mixing gelling agent to nanosuspension with the help of magnetic stirrer at 100 rpm the speed of stirring was kept low to prevent air entrapment in gel during stirring.

The prepared gels were evaluated for various parameters like physical observation, pH, viscosity, spreadability, *invitro* release profile and stability studies.All the three gels were clear and homogeneous. The pH of the nanogelswere 7.8 ± 0.33 for G1, 7.6 ± 0.28 for G2 and 7.1 ± 0.31 for G3 (Table 3).The results showed that all the three gels have almost neutral pH and it is suitable for skin application.

Viscosity of G1 was 90465 ± 37.94 centipoise, G2 99054 ± 97.38 centipoise and for formulation G3 115400 ± 102.1 (Table 3), viscosity seems to be increasing with increase in Carbopol concentration. The spreadability of G1 was 22 ± 4.9 mm, 18 ± 2.3 mm for G2 and for G3 it was found 16 ± 0.98 mm, it reveals the dependency of spreadability on Carbopol 934P concentration. As concentration of Carbopol increases the spreadability decrease it may be due to increase in viscosity of formulation and consequently increase in cohesive strength and decrease in spreadability.

Table 3: Results of characterization of nanogel.

Formulation code	Clarity	Homogeneity	pH	Viscosity (cPs)	Spreadability (mm)
G1	Clear	Homogeneous	7.8 ± 0.33	90465 ± 37.94	22 ± 4.9
G2	Clear	Homogeneous	7.6 ± 0.28	99054 ± 97.38	18 ± 2.3
G3	Clear	Homogeneous	7.1 ± 0.31	115400 ± 102.1	16 ± 0.98

The in vitro release study of three gel formulation was performed for 24 h. Results showing a release of 98.7±3.81% for G1,98.6±1.32% for G2 and for G3 it was 95.8±1.14% after 24 h (Figure 2). Percent release of drug from the gel is not by the concentration of Carbopol up to 1.5% but at

2%Carbopol in G3 it was decreased to 95.8±1.14%, it may be due to slow diffusion of drug on increasing gelling agent concentration.

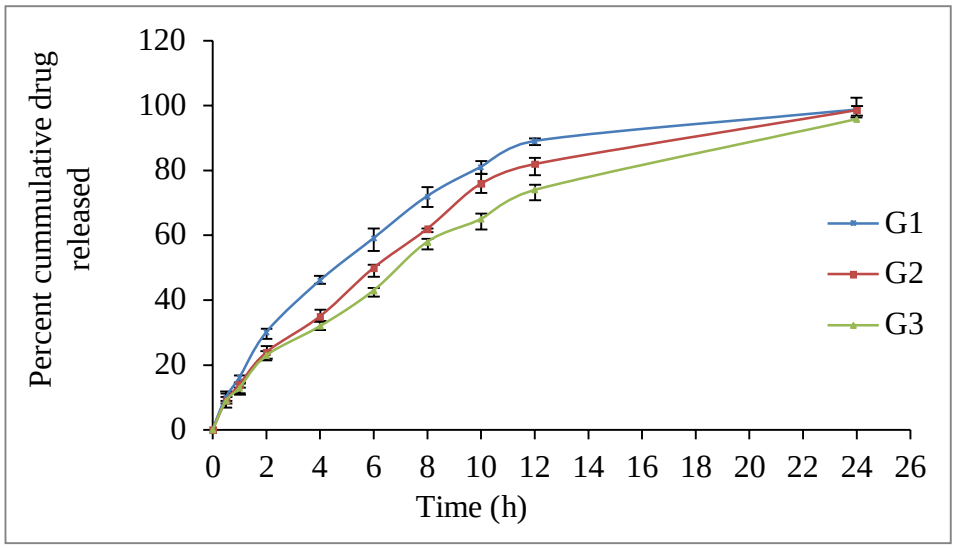

Figure 2: Results of *in-vitro* release study of formulations.

From the above data of viscosity,spreadability and *in vitro* release,formulation G2 was selected for further study like release kinetic study and stability study.Release kinetic study was performed to know the order and mechanism of in vitro release from the formulation G2. Maximum value of correlation coefficient r^2 was found to be 0.917 for Higuchi's kinetics (Table 4), followed by 0.889 for and zero order least correlation was 0.843 for first order suggested that the release kinetics followed mixed order kinetics so for mechanism of drug release KorsmeyerPeppas's model was applied and the value of r^2 was 0.871 and release exponent n was found to be 0.403 reveals that release followed Fickian diffusion.Stability study data reveals that the gel G2 was stable up to 90 days as there is no significant changes occurs in clarity, homogeneity, pH, viscosity and spreadability (Table 5).

Table 4: Results of release kinetic study of formulation G2.

Zero order	First order	Higuchi's model	Korsmeyer-Peppas'model	
r^2	r^2	r^2	r^2	n
0.889	0.843	0.917	0.871	0.403

Table 5: Results of stability study of formulation G2.

Storage condition	Days of sampling	Parameters evaluated				
		Clarity	Homogeneity	pH	Viscosity (cP)	Spreadability (mm)
25±2°C/60±5%RH	0	Clear	Homogeneous	7.6±0.28	99054±97.38	18.00±2.3
	30	Clear	Homogeneous	7.5±0.42	99347±67.34	18.00±3.1

	60	Clear	Homogeneous	7.7±0.74	99421±73.89	18.40±1.8
	90	Clear	Homogeneous	7.8±0.41	99329±87.65	18.10±4.3
	0	Clear	Homogeneous	7.6±0.28	99054±97.38	18.00±2.3
	30	Clear	Homogeneous	7.5±0.34	98675±74.81	18.43±3.8
40±2°C/75±5%RH	60	Clear	Homogeneous	7.4±0.41	98749±37.32	18.57±7.1
	90	Clear	Homogeneous	7.6±0.38	98893±89.46	18.80±2.6

4. CONCLUSIONS:

The present study explores an alternate carrier nanogel which contains solid lipid nanoparticles loaded with lovastatin an antihyperlipidemic drug. The prepared SLNs were not optimized for particle size and PDI since the particle size is not an issue for this study and the chance aggregation is less as the SLNs were loaded in Gel. The prepared SLNs entrapped desirable amount of lovastatin. The nanosuspension incorporated in Gel and provide sustained release of drug following Higuchi's model that is mixed order kinetics. Formulation G2 was evaluated for stability and it was found to be stable up to 90 days. Base on the study results, it can be concluded that the formulated lipids nanogel loaded with Lovastatin SLNs may provide better an alternative to available dosage form and may resolve the issue of bioavailability after further studies on its bioavailability.

Disclosure:

The authors report no conflicts of interest in this work.

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