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Formulation and characterization of solid lipid nanoparticles loaded Neem oil for topical treatment of acne

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ABSTRACT

Objective: To investigate the treatment of acne and pimples as well as improves skin elasticity by solid lipid nanoparticles (SLNs) loaded Neem oil. **Method:** Neem oil as a natural agent was incorporated into SLNs prepared by double emulsification method using different concentration of lecithin and Tween 80. The characteristics of SLNs with different concentration of lipid were investigated. **Result:** The average particle size of Neem oil loaded SLNs decreased with increasing concentration of surfactant. SLNs of (221.6 ± 2.0) nm with a Polydispersity index of (0.948 ± 0.040) were obtained at higher concentration of lipid and surfactant. High entrapment efficiency of 82.10% revealed the ability of solid lipid nanoparticles to incorporate a high quantity of Neem oil. Furthermore the stability of SLNs indicated with negligible drug leakage after 3 weeks. **Conclusion:** The result concluded that Neem oil loaded solid lipid nanoparticles with more lecithin content in their colloid exhibit sustained effect which satisfactorily produced the antibacterial action on Acne microbes. Therefore Neem oil loaded SLN was used successfully for prolonged treatment of Acne.

1. Introduction

Solid lipid nanoparticle introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles[1]. Solid lipid nanoparticles are sub-micron colloidal carriers ranging from 50 to 1 000 nm, composed of physiological lipids and dispersed in aqueous surfactant solution. Solid lipid nanoparticles offers unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals. In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed in to solid lipid nanoparticles[2].

Solid lipid nanoparticles are at the fore-front of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research as well as in other allied sciences. They are manufactured from synthetic/natural lipids and ideally suited to optimize drug delivery and reduce toxicity[3].

Colloidal carriers are one of the approaches for the controlled delivery of drugs via dermal route and maintain the controlled release of active substances and targeting to skin layers. In monodispersed systems, solid lipid nanoparticles are the new generation of nanoparticulated active substance carriers and are attracting major attention as novel colloidal drug carriers for topical use[4].

Neem oil is expressed from the seed of the *Azadirachta* indica tree. The tree is part of the Mahogany family *Meliaceae* and it is one of two species in the genus *Azadirachta*, native to India, Srilanka, Malaysia, Bangladesh and Pakistan. In Indian tradition Neem is one of the most important herbal ingredients which not only helps in fighting certain health problems, but also used in the earliest cosmetics and skin care products. Neem oil is used in the treatment of acne and pimples as well as

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improves skin elasticity. The Neem oil normally contains oleic acid (52.8%), stearic acid (21.4%), palmitic acid (12.6%), linoleic acid (2.1%), and lower fatty acids (2.3%)[5].

Acne is caused by bacteria named *Propionibacterium acne (P. acne)*. The *P. acne* bacteria live deep within follicles and pores, away from the surface of the skin. In these follicles, *P. acne* bacteria use sebum, cellular debris and metabolic by-products from the surrounding skin tissue as their primary sources of energy and nutrients. Elevated production of sebum by hyperactive sebaceous glands or blockage of the follicle can cause *P. acne* bacteria to grow and multiply[6].

All components of the Neem oil are strongly anti-bacterial such as Margolone and Mahmoodin which can be used to kill bacteria *P. acne*. And not just that, Neem oil also contains salicylic acid like substances that reduce redness and inflammation. Neem contains active ingredients that are very similar to the ingredients in common medical acne treatment products[7]. In this present research, we investigated solid lipid nanoparticle loaded Neem oil for treatment of Acne.

2. Materials and methods

Soya lecithin was purchased from Yarrow chem products, Mumbai, India. Neem oil purchased from local market, PVA, Tween 80 and Dichloro methane were purchased from Finar chemicals, Ahmedabad India and all other chemicals used were of analytical grade.

2.1. Method of preparation of solid lipid nanoparticle loaded Neem oils

Solid lipid nanoparticle of Neem oil was prepared by w/o/w type double emulsification method[8]. Required quantity of Neem oil was dispersed in aqueous mixture of methanol (75% v/v) and required quantity of Lecithin & Cholesterol was dissolved in dichloromethane. The Neem oil solution was added slowly to lipid mixture and homogenized for 15 min at 15 000 rpm in ultra probe sonicator (Orchid scientific, India) to produced white cloudy primary emulsion. The resultant primary emulsion was poured in to 2% w/v of PVA solution and homogenized for additional 10 min at 15 000 rpm. The resultant w/o/w type emulsion was stored at room temperature. The solvent was evaporated in Rota evaporator at 45 ℃. The stable emulsion was freeze dried at -20 ℃ under reduced pressure to get dried powder of solid lipid nanoparticles.

2.2. Drug polymer compatibility

The samples of pure Neem oil and mixture of Neem oil with lipids were mixed with 100 mg of potassium Bromide (KBr). The samples were compressed to disc by applying pressure of 5 tons for 3 min in a hydraulic press. The prepared pellets were placed in the sample cell and the spectrums were analysed in the region of 4 000-400 cm^{-1}. By comparing the spectrums of pure Neem oil and Neem oil with lipids, the compatibility study was performed[9].

2.3. Surface morphology

Surface morphology of solid lipid nanoparticles loaded Neem oil was observed by Scanning electron microscope (SEM). A small amount of SLN was taken in metal stub. The stub was coated with conductive gold by Hitachi 1010 ion sputter and observed under Hitachi 3000 N Scanning electron microscope (JSM 5610 LV SEM, JEOL, Japan) chamber. The image was scanned at an acceleration voltage of 20 kV with a chamber pressure of 0.8 mmHg[10].

2.4. Particle size analysis

Particle size of nanoparticles was determined using Malvern particle size analyzer (Zetasizer 4000S, Japan). For particle size analysis[11], solid lipid nanoparticles were suspended in double distilled water and one drop was placed on clean slide and the particle size was observed. The average particle size was calculated and significant value ($P<0.05$) was calculated by using Graph pad prism 5.1 software.

2.5. Polydispersity index

Poly dispersity index value was used to characterize the monodispersed and polydispersed nature of nanoparticles. Higher the Polydispersity index values indicate the high level of non uniformity.

2.6. Quantification of drug entrapment efficiency

The entrapment efficiency was calculated by using 100 mg of solid lipid nanoparticles dissolved in 20 mL of dichloromethane and the solution was centrifuged at 12 000 rpm. The supernated fluid was collected and passed through membrane filter. The quantity of drug in the solution was measured by ultra violet spectroscopy at 263 nm[12].

Drug entrapment (%) = Quantity of drug in nanoparticle/ Mass of drug in the formulation×100

2.7. In-vitro release study

In-vitro release of solid lipid nanoparticles loaded Neem oil was carried out by using modified Franz diffusion

apparatus[13]. About 10 mg Neem oil equivalent solid lipid nanoparticles were placed in donor compartment containing phosphate buffer (pH 7.4) at (37±2) ℃. Drug release was assessed by intermittently sampling the receptor medium (5 mL) and fresh phosphate buffer saline solution was replaced. The samples were filtered in membrane filter (0.22 μm) and the amount of drug released was quantified by a U.V. Spectrophotometer at 263 nm.

2.8. Stability studies

For evaluation of stability selected formulation (F4) containing 10:1 ratio of Lecithin & Cholesterol was stored at 5 ℃, 45 ℃ and room temperature for 3 weeks. The entrapment efficiency and particles size measurement were evaluated after storage period of 3 week at 5 ℃ and 45 ℃ and room temperature in the dark condition[14].

3. Results

3.1. Preparation of solid lipid nanoparticles

In the present study, the effect of concentration of lipid mixture (lecithin: cholesterol) and surfactant on preparation of Neem oil loaded solid lipid nanoparticles were evaluated. In these preparations, lecithin and cholesterol were used as lipid and Tween 80 as surfactant. SLNs loaded Neem oil was prepared by double emulsification (w/o/w) using high speed ultra probe sonicator. The aqueous phase of methanolic solution containing Neem oil was poured to organic phase containing lipid at high homogenization speed of 15 000 rpm for 15 min with addition of Tween 80. The Tween 80 solution reduced the interfacial tension between the phases. The uniform size globules were formed as w/o type emulsion. This emulsion was further homogenised with 2% w/v PVA solution as co-surfactant to form double emulsion (w/o/w). The formed emulsion was stabilized and stability was maintained by hardening properties of co-emulsifier. The solid lipid nanoparticles were prepared by using increasing concentration of lipid and surfactant.

Table 1.
Formulation of Neem oil loaded solid lipid nanoparticles.

S. no	Ingredients	F1	F2	F3	F4
1	Neem oil	1 mL	1 mL	1 mL	1 mL
2	Lecithin	10 mg	20 mg	50 mg	100 mg
3	Cholesterol	10 mg	10 mg	10 mg	10 mg
4	Dichloromethane	10 mL	10 mL	10 mL	10 mL
5.	Tween 80	1%	2%	3%	4%
6.	PVA	2%	2%	2%	2%
7.	Water	q.s	q.s	q.s	q.s

3.2. Drug polymer compatibility

FT-IR spectrum of pure drug and mixture of drug and lipid are shown in Figure 1. Form the spectral study it was observed that there was no significant change in the peaks of pure drug and drug lipid mixture. Hence, no specific interaction was observed between the drug and the lipid used in the formulations.

3.3. Effect of lipid concentration on entrapment efficiency

The Solid lipid nanoparticles were prepared by different proportions of Cholesterol and Lecithin (1:1, 1:2, 1:5 and 1:10). The entrapment efficiency of Solid lipid nanoparticle loaded Neem oil increases with increase in concentration of soya lecithin and Tween 80. The entrapment efficiency was in the range between 67.23%-82.10% as shown in Table 2. The entrapment efficiency (%) was directly proportional to concentration of Lecithin and cholesterol. Normally low entrapment efficiency was observed at low affinity between drug at different solvent (organic and aqueous). The F4 formulation of SLN has showed maximum entrapment (82.1%).

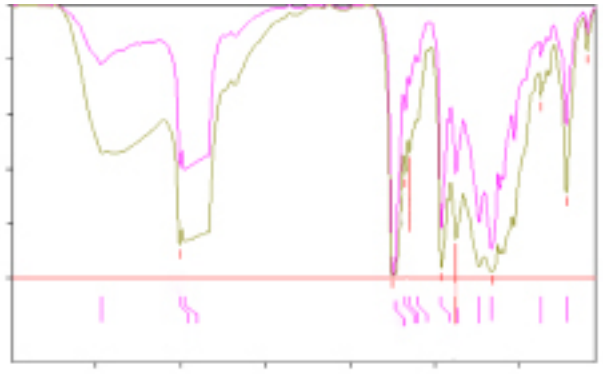

Figure 1. Drug polymer compatibility.

Table 2.
Evaluation of solid lipid nanoparticles.

S.no.	Batch code	Particlesize (nm)	Morphology	Entrapment efficiency (%)	PDI
1	F1	867.5±4.7	Spherical	67.23±8.50	0.461±0.200
2	F2	923.9±2.8	Spherical	71.64±4.80	0.432±0.200
3	F3	746.4±6.2	Spherical	77.98±9.40	0.402±0.800
4	F4	221.6±4.1	Spherical	82.10±4.50	0.948±0.400

PDI: Poly dispersity index (The data were analyzed by two-way analysis of variance with students *t* test and represented as mean ± SD (n=3) using Graph pad prism 2.1 software. Statistical differences were observed at ($P \leq 0.05$).

3.4. Particle size and poly dispersity index

The Solid lipid nanoparticles loaded Neem oil (F1-F4) formulations were observed to be spherical shape with smooth surface. The particle size of all the formulations

(F1-F4) showed between 421.6-867.5 nm as shown in Table 2. The particle size of solid lipid nanoparticles was mainly affected by homogenization speed and saturated lipid concentration. During homogenization of SLN the aggregation was reduced by reducing interfacial tension and stabilised spherical shape particles were formed by addition of tween 80. On increasing the concentration of surfactant, mean particle size of SLNs was reduced. The organic solvent used in these formulations rapidly partitioned into the continuous aqueous medium and the lipid precipitated around the drug. The simultaneous evaporation of the entrapped solvent lead to the formation of spherical shaped solid lipid nanoparticles.

The dispersity index of Solid lipid nanoparticles loaded Neem oil were in range between 0.40-0.46 as shown in Table 2. The PDI was reduced to narrow range due to increasing concentration surfactant.

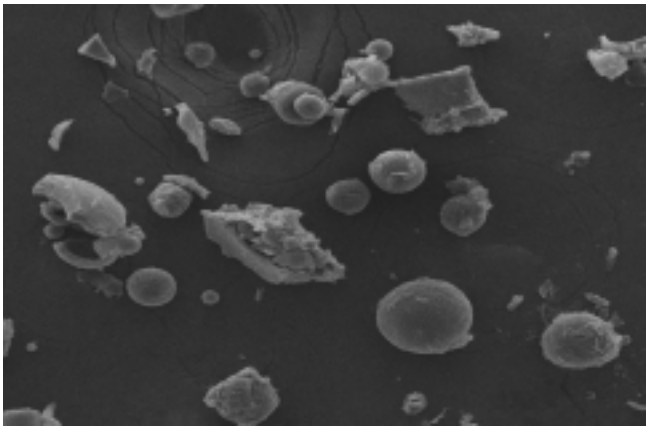

Figure 2. SEM image of SLN loaded Neem oil.

3.5. In vitro release studies

The *in vitro* release studies of Solid lipid nanoparticles loaded Neem oil at different lecithin concentrations were evaluated by modified Franz diffusion cell. The amounts of drug release of all formulations (F1-F4) were showed in Figure 3. The solid lipid nanoparticles show initial burst release (30 min) of 3.56%-30.05%.

The F4 formulation showed less drug release due to high polymeric matrix. At end of 12 h, limited percentage of drug release was observed around 52.54%. The F4 formulations showed significantly less drug release over other formulations which clearly indicated that they can provide satisfied result for acne treatment over prolonged period of time.

Table 3.
Stability studies of selected formulation.

S.no.	Parameters	Fresh particles	0 ℃	25 ℃	45 ℃
1	Particle size (nm)	221.6	245.7	221.0	239.0
2.	Entrapment efficiency (%)	82.1	82.2	82.0	79.1

Stability studies data were showed after 3rd week.

In order to determine drug release pattern of Solid lipid nanoparticle loaded Neem oils, the release data were substituted to zero, first, higuchi, korsemeyer peppas models. The release constant and regression co-efficient (R^2) was calculated from slopes of appropriate plots. The solid lipid nanoparticles loaded Neem oil were best fitted by zero order release [R^2=0.949 7-0.974 5] followed by higuchi equation [R^2=0.967 2-0.990 3].

The drug release kinetics demonstrates that release was independent of concentration. For explaining the mechanism of drug release from nanoparticles, korsemeyer peppas equation showed good linearity and the release exponent n=0.234 3-0.812 0. This appears to be the coupling of diffusion and erosion mechanism i.e. anomalous diffusion.

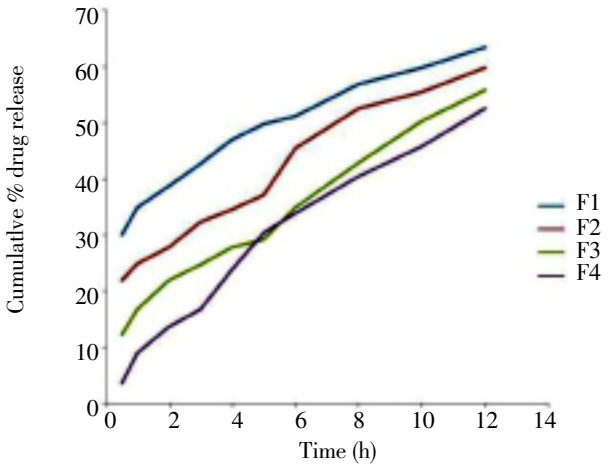

Figure 3. *In vitro* drug release studies.

3.6. Stability studies

During the period of storage, the formulation showed no change in colour, creaming and phase separation. The particle size increased significantly, after storage of 3 weeks at 45 ℃. But there was no significant increase in particle size of SLN stored at 5 ℃ and 25 ℃ after 3 weeks.

The entrapment efficiency was reduced at higher temperature (45 ℃) than other storage conditions. When the particles were aggregated, drug molecule was leaked out from lipid matrix.

The solid lipid nanoparticles loaded Neem oil could successfully deliver the action on acne site with out penetration on to blood stream.

4. Discussion

The Neem oil loaded solid lipid nanoparticles were prepared by double emulsification technique. The Tween 80 solution reduced the interfacial tension between the phases. The uniform size globules were formed as w/o type

emulsion. The formed emulsion was stabilized and stability was maintained by hardening properties of co-emulsifier (PVA). The solid lipid nanoparticles were prepared by using increasing concentration of lipid and surfactant. The FTIR spectrums reveal that drug incompatibles with lipids. The entrapment efficiency (%) was directly proportional to concentration of Lecithin and cholesterol. Normally low entrapment efficiency was observed at low affinity of drug between the solvents (organic and aqueous). The F4 formulation of SLN has showed maximum entrapment (82.1%) due to high affinity of drug with lipid matrix.

During homogenization of SLN, the aggregation was reduced by reducing interfacial tension and stabilized spherical shape particles were formed by addition of Tween 80. On increasing the concentration of surfactant, mean particle size of SLNs was reduced.

The organic solvent used in these formulations rapidly partitioned into the continuous aqueous medium and the lipid precipitated around the drug. The simultaneous evaporation of the entrapped solvent lead to the formation of spherical shaped solid lipid nanoparticles. From the four formulations, F4 showed significantly less drug release over other formulations which clearly indicated that they can provide satisfied result for acne treatment over prolonged period of time.

The drug release characters were confirmed by following zero order release with higuchi model. The formulation F4 showed korsemeyer peppas values of n=0.234 3-0.812, which reveals that drug release in the mechanism of coupling of diffusion and erosion i.e. anomalous diffusion. The optimized formulation showed stability at room temperature and 5 ℃ but at 45 ℃, particles were aggregated, drug molecule was leaked out from lipid matrix.

It was assumed that the high temperature (45 ℃) increased the kinetic energy of system, which could accelerate the collision of particles which consequently increase the possibility of aggregation.

In this study the potential of SLNs dispersions as carriers for delivery of Neem oil was exploited. Solid lipid Nanoparticles were prepared by the w/o/w type double emulsification method by using bio-acceptable lipids such as Cholesterol and Lecithin and tween 80 as emulsifier. Drug loaded SLNs showed average diameters in the narrow colloidal size range, a good loading capacity and drug release. Results strongly support the potential application of SLNs as drug delivery system in acne treatment.

Conflict of interest statement

The authors report no conflict of interest.

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References

[1] Mehnert W, Mäder K. Solid lipid nanoparticles production, characterization, and applications. *Adv Drug Deliv Rev* 2001; **47**: 165-196.

[2] Rainer H, Muller, Karsten Mader, Sven Gohla. Solid lipid nanoparticles (SLN) for controlled drug delivery. A review of the state of the art. *Eur J Pharm Biopharm* 2002; **50**; 161-177.

[3] Waghmare AS, Grampurohit ND, Gadhave MV, Gaikwad DD, Jadhav SL. Solid lipid nanoparticles: a promising drug delivery system. *IRJP* 2012; **3**: 100-107.

[4] Mukherjee S, Ray S, Thakur RS. A modern formulation approach in drug delivery system. *Ind J Pharm Sci* 2009; **4**: 349-358.

[5] Kaushik N, Vir S. Variations in fatty acid composition of Neem seeds collected from the Rajasthan state of India. *Biochem Soc Trans* 2000; **28**: 6-12.

[6] Makrantonaki, Ruta Ganceviciene, Christos Zouboulis. An update on role of the sebaceous gland in the pathogenesis of acne. *Dermato endocrinol* 2011; **3**: 41-49.

[7] Mishra AK, Singh N, Sharma VP. Use of Neem oil as a mosquito repellent in tribal villages of mandla district, madhya pradesh. *Indian J Malariol* 1995; **2**: 99-103.

[8] Trotta M, Cavalli R, Carlotti ME. Solid lipid microparticles carrying Insulin formed by solvent-in-water emulsion-diffusion technique. *Int J Pharm* 2005; **288**: 281-288.

[9] Liu J, Xiao Y, Allen C. Polymer-drug compatibility: a guide to the development of delivery systems for the anticancer agent, ellipticine. *J Pharm Sci* 2004; **93**: 132-143.

[10] Bouwstra JA, Honeywell-Nguyen PL. Skin structure and mode of action of vesicles. *Adv Drug Deliv Rev* 2002; **54**: S41-S55.

[11] Yener G, Incegul T, Yener N. Importance of using solid lipid microspheres as carriers for UV filters on the example of octylmethoxy cinnamate. *Int J Pharm* 2003; **258**: 203-207.

[12] Anandrao R, Kulkarni, Kmaresh S, soppimath. Solubility study of *Azadirachta indica* A. juss (Neem) seed oil in the presence of co-solvent/Non-ionic surfactant. *J Chem Eng Data* 1999; **44**: 836-838.

[13] Wissing SA, Müller RH. Solid lipid nanoparticles as carrier for sunscreens: *in vitro* release and *in vivo* skin penetration. *J Control Release* 2002; **81**: 225-233.

[14] Lim SJ, Kim CK. Formulation parameters determining the physicochemical transition in triglyceride nanoparticles. *Int J Pharm* 2002; **129**: 159-173.