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FORMULATE AND EVALUATE GEL CONTAINING NANOSTRUCTURED LIPIDS OF *PONGAMIA PINNATA*

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ABSTRACT

The objective of present study was to design development and evaluatenanostructure lipid carriers based gel of *Pongamia pinnata* extract for topical application. Nanostructured lipid carriers (NLC) established topical gel of *Pongamia pinnata* extract was formulated for treatment of wound healing activity, NLC were fabricated by melt dispersion ultra-sonication method. NLC containing mixtures of Glyceryl monostearate as solid lipid and Karanj Oil as liquid- lipid and the Tween 80 as surfactant. The particle size of NLC was found between 99.98 and 155.65 nm with PDI varied from 0.256 to 0.349 and zeta potential found between -21.65 to -26.68. The NLC dispersions were gelled by utilizing gelling agent carbopol which have compatibility with nano-particulate dispersion.. From stability study it revealed that formulation is stable and used for topical application.

Keywords: Nanostructured lipid carriers, *Pongamia pinnata* oil, GMS, Tween 80.

INTRODUCTION

Medicinal plants have been utilized as a chief source of the remedy for human illnesses since period age-old. The world's 1.42 billion people are dependent on traditional medications for the management of the various sicknesses. Medicinal herbs are moving from marginal to main stream use with superior number of people looking for remedies and health methods free from side effects caused by synthetic chemicals (Goo Yoon 2013). Herbal treatment has become a piece of global importance for both the medicinal and an economical. In India medications based on herbal source have been utilize as basis of treatment and the cure for numerous diseases (Fardintamjidi, Mohammad Shahedi 2013). Moreover Indian traditional medicine comprises many prescriptions for the therapeutic determinations such as curing of the wounds, inflammation, the skin infections, leprosy, diarrhea, scabies and the venereal diseases upon traditional remedies for the numerous skin diseases. (Subramanian Selvamuthukumar). The use of the medicinal plants as a raw material in production of the novel drugs is over growing because of their potentials in fighting problem of the drug opposition in micro-organisms. Research on the medicinal plants is one of important areas of the research internationally (Magdalene Radtke). Today transdermal drug delivery system (TDDS) is one of most proficient modes of drug application. The TDDS delivers means to sustain drug release and then decrease the intensity of action and then diminish side effects related with its oral treatment (S. Webe 2013). In current years, the substantial efforts have been devoted to utilize potentials of the Nano-technology in a drug delivery subsequently it offers right means of site-specific and the time-controlled delivery of minor or bulky molecular weight drugs and the other bioactive agents. Since establishment of the 1990s lipid nano-particles were getting rising interest from pharmaceutical technology investigation groups worldwide. Nowadays SLN and the NLC have been already inspected as a carrier system for many of the applications. Topical treatment of the skin infections is very attractive, since systemic load of API and the adverse effects are decreased as compared to the parenteral or an oral drug administration (*Patricia Severino* 2012/750891)

1. Colloidal drug carriers
2. Liposomes
3. Ethosomes
4. Transferosome
5. Niosome
6. Microemulsions and nanoemulsion
7. Nano capsules and polymeric nanoparticles
8. Lipid nanoparticles
9. Solid lipid nanoparticles
10. Nanostructured lipid carriers (Sven H. Gohla 2000)

These all declared are drug carriers systems.

NLC's are formed using the mixture of solid - lipids and liquid - lipids (oils). NLC are also composed of the solid hole covered by an emulsifying agent used during production procedure.

NLC mainly classified in different types which is as follows:

1. **The NLC Type I** (or the imperfect crystal model):

Figure 1: Theoretical models for the structure of NLC Black squares stand for drug molecules

Method: The NLC's were formulated by a Melt dispersion ultra-sonication technique.

Pongamia pinnata seeds were grounded and then this will added to the soxhlet apparatus.

Then the extraction were performed adding hexane in to the power *Pongamia pinnata*

Karanj oil to melted GMS which comprised of the lipid phase, kept in a heating water bath at 80 °C

Then to the molten lipid phase, weighed amount of extract was added.

The aqueous phase comprised of surfactant dissolved in water.

Both the phases were maintained at a temperature above the Melting point of the lipid (80°C)

At this temperature, the melted hot lipid phase was then dispersed in the hot surfactant phase, obtaining a pre-emulsion under magnetic stirring (Remi Instruments Ltd., Mumbai, India) at 800 rpm at 61°C for 30 mins

Then this warm pre-emulsion was introduced into a probe sonicator for 20 minutes.

The NLC's so formulated were allowed to cool at room temperature

This model is achieved when blending solid lipids with sufficient amounts of liquid lipids.

2. **The NLC Type II**, (or the amorphous model): is formed when fraternization of special lipids (e.g., hydroxyl octacosanyl hydroxyl stearate, isopropyl myristate) that do not recrystallize after the homogenization and a cooling of Nano-emulsions.

3. **The NLC Type III** (or multiple models): it is composed of the very small oil nano compartments formed inside solid - lipid matrix of a nanoparticle by phase separation process (Ramtekek. H 2012).

Figure 1: IR Spectrum of Karanj Oil

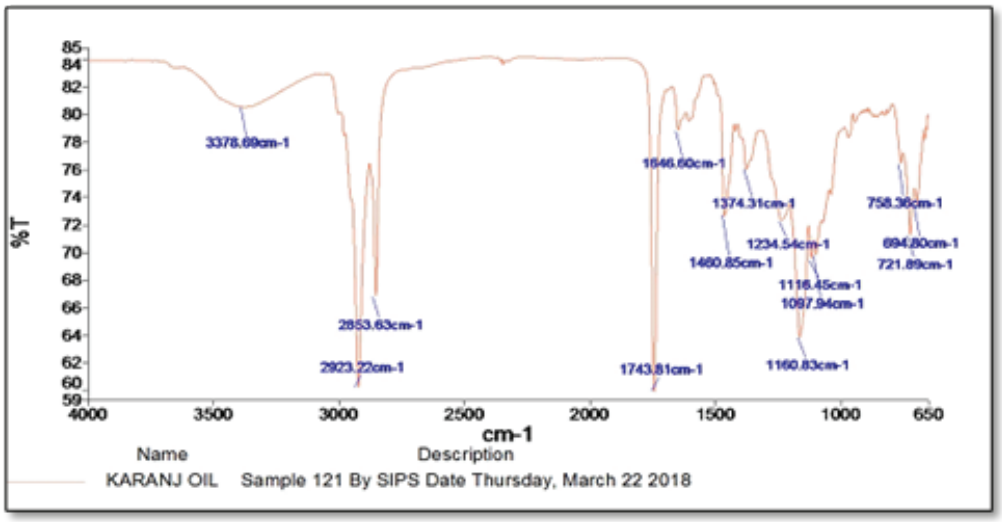


Figure 2: IR spectrum of GMS

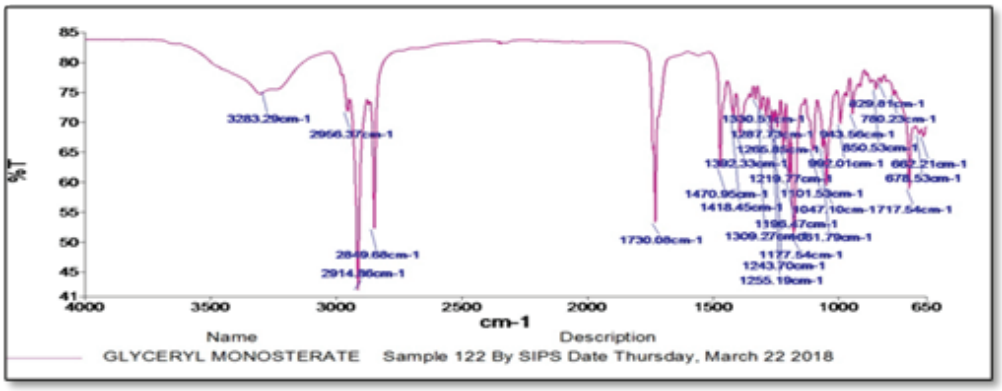


Figure 3: IR spectrum of carbopol

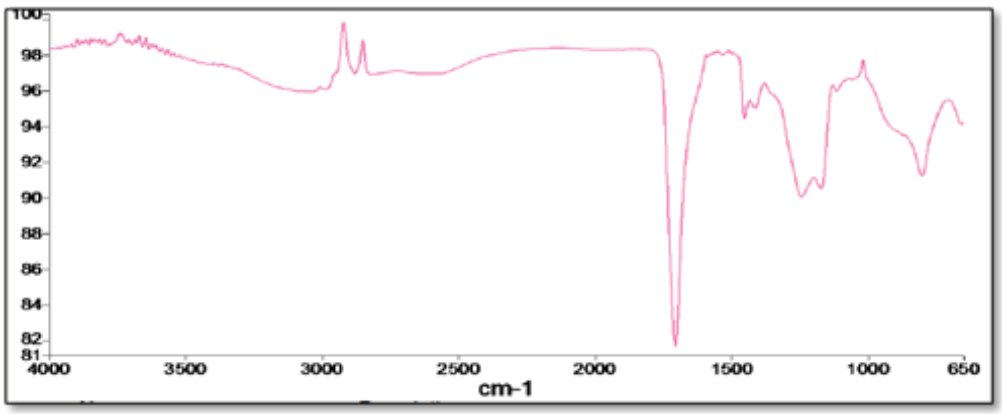
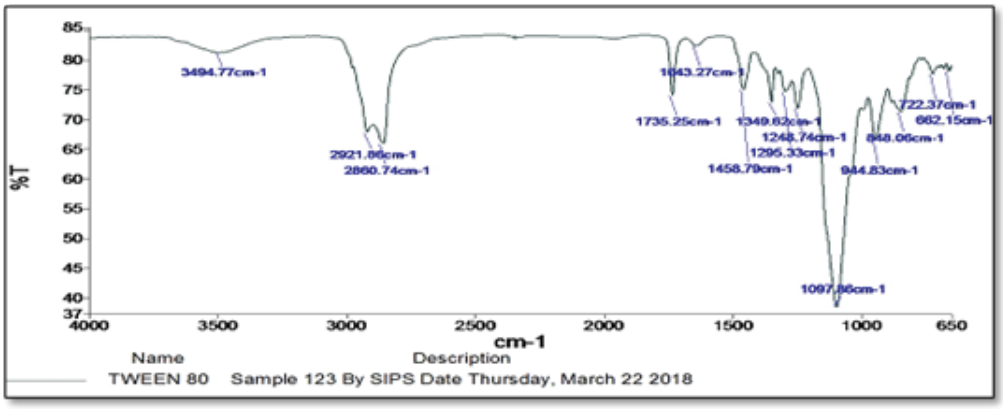


Figure 4: IR spectrum of Tween 80



The drug deliveries through the skin in NLC formulation have various advantages such

As:

1. Increase of skin occlusion
2. Rise of the skin hydration and the elasticity
3. Enrichment of the skin permeation and drug targeting
4. Improve benefit/risk ratio
5. Enhancement of UV blocking activity
6. Improvement of chemical stability of chemically labile compounds

MATERIALS AND METHODS

Materials: *Pongamia pinnata* (Sandip Foundation, Nashik), Glyceryl mono stearate, Tween80, Carbopol (Modern Science apparatus Pvt. Ltd.)

Characterization of Karanj Oil

Determination of the Boiling point

Boiling point was determined to check purity of the oil. Boiling point was determined by capillary tube method by using Thiele's tube containing Liquid paraffin.

The determination of Acid Value

The acid value was calculated by the directly titrating material in alcoholic medium with the aqueous KOH solution. Reagents used are Ethyl alcohol- 95% by volume. The Phenolphthalein indicator- 1gm of phenolphthalein was dissolved in the 100 ml of an ethyl alcohol, the Standard aqueous KOH solution.

Determination of the Saponification Value

The oil was then saponified by refluxing it with the additional of an alcoholic KOH solution. The alkali was consumed for

Figure 5: IR spectrum of karanj oil and tween 80

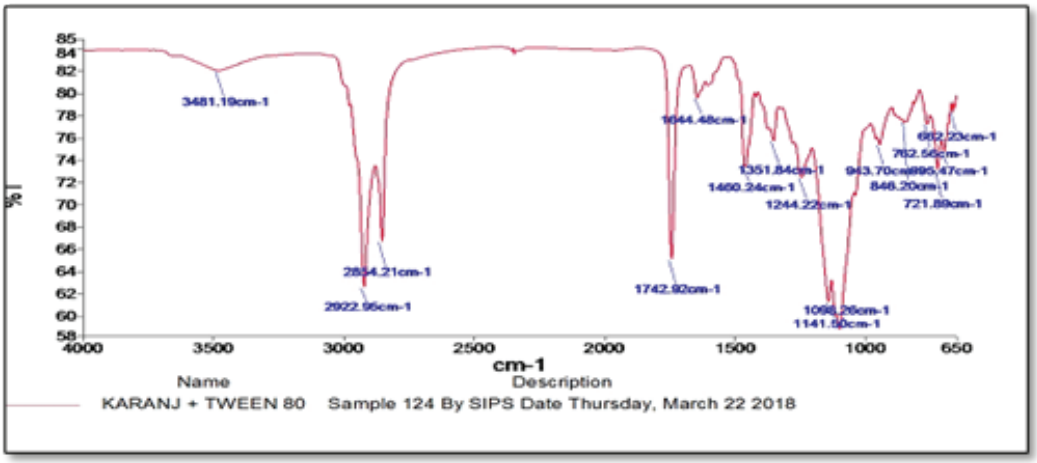


Figure 6: karanj oil and GMS

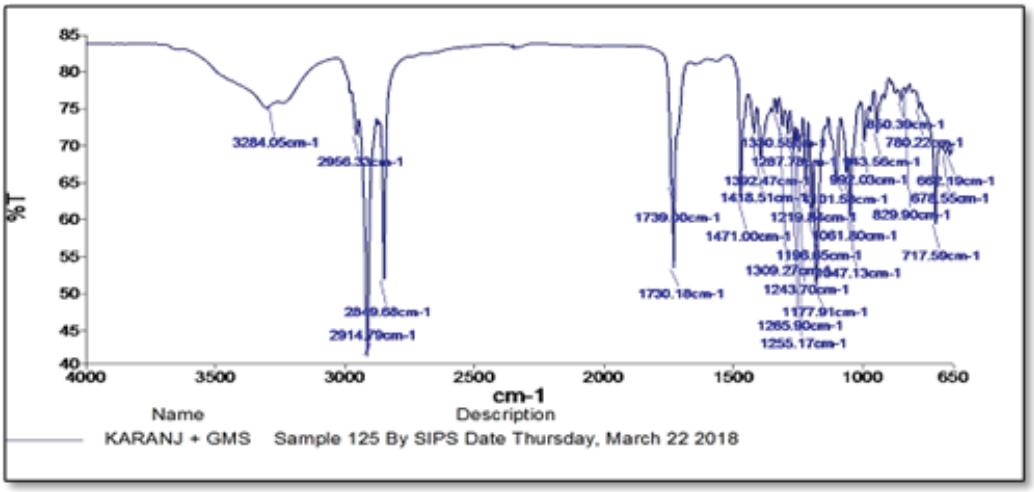


Figure 7: DSC of karanj oil

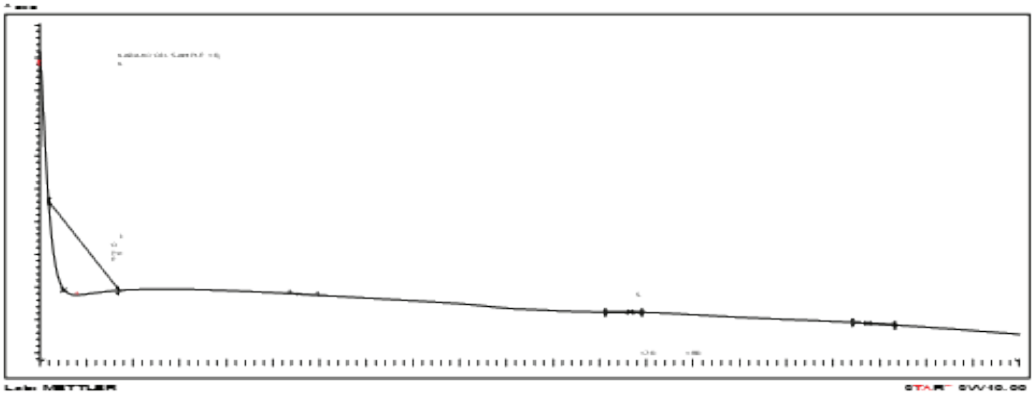
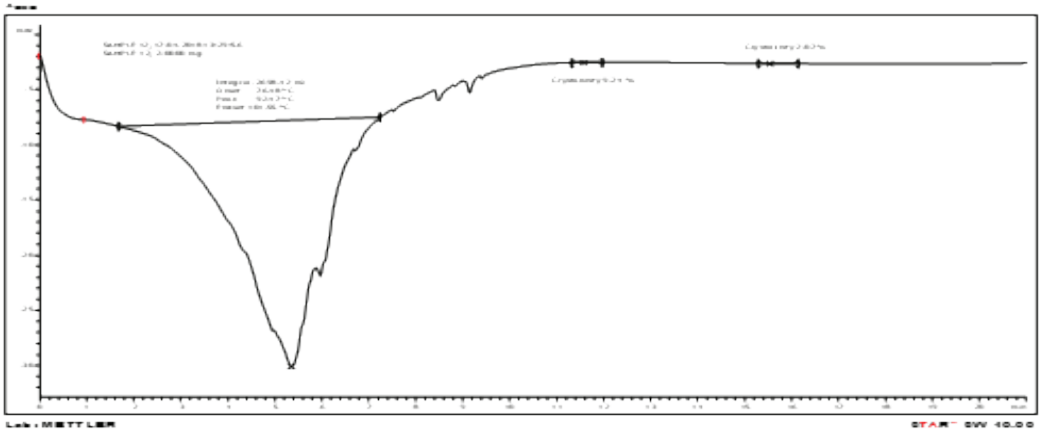


Figure 8: DSC of NLC Formulation.



the Saponification was calculated by titrating with the additional Alkali with standard HCl.

Saponification value of the formulation = 56.1(B-S) N W

Where,

B= is the volume in ml of the standard HCl required for blank.

S=is a volume in ml of the standard HCl required for sample.

N=the normality of standard solution.

W= weight in gram of an oil taken.

Determination of Iodine value

The material was treated in carbon tetrachloride medium with an extra of the iodine monochloride solution in a glacial acetic acid. The excess of iodine mono chloride was treated with the KI and then liberated iodine predicted by the titration with the sodium thiosulphate. Reagents used were Potassium dichromate, the concentrated HCl, a KI solution, the starch solution, the standard thiosulphate solution.

Iodine value = 12.69(V2-V1) W

Where,

V1=is the volume of thiosulphate spent for a test.

V2= is volume of thiosulphate consumed for the blank test.

W= is weight in the grams of an oil taken.

Determination of the Unsaponification Value

The material is completely saponifiable with an

Figure 9: Particle Size Analysis of Batch F5

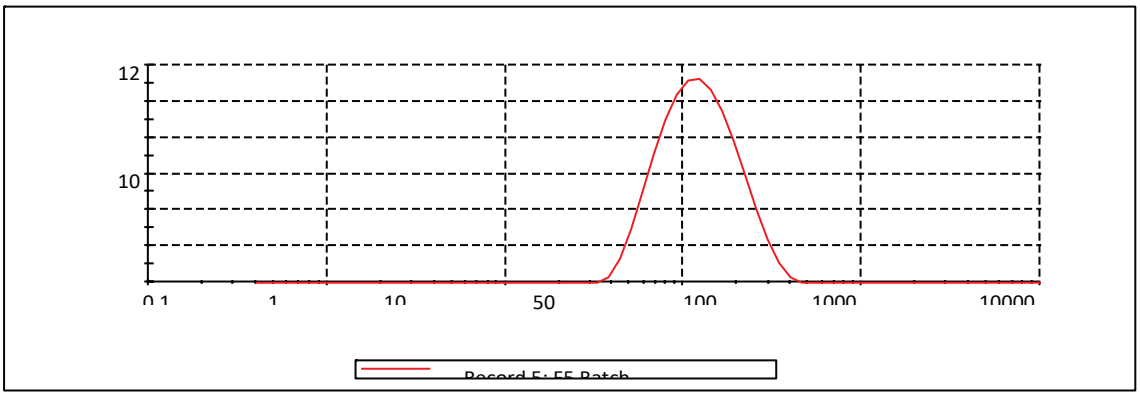


Figure 10: Zeta Potential of Batch F5

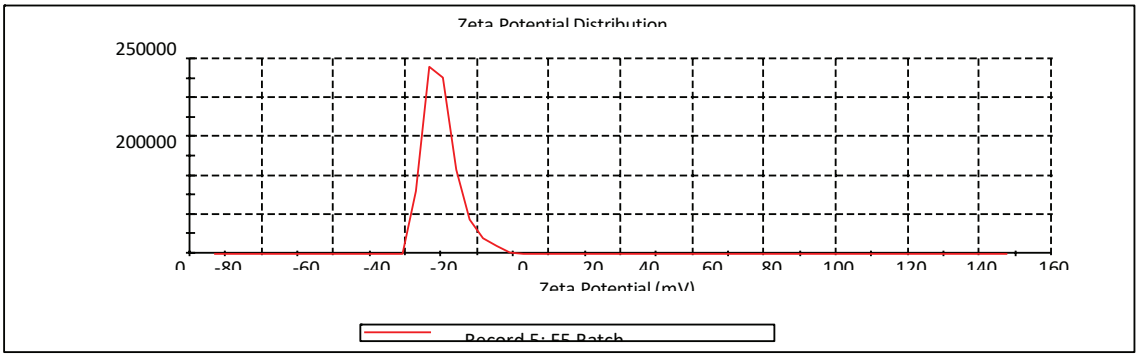
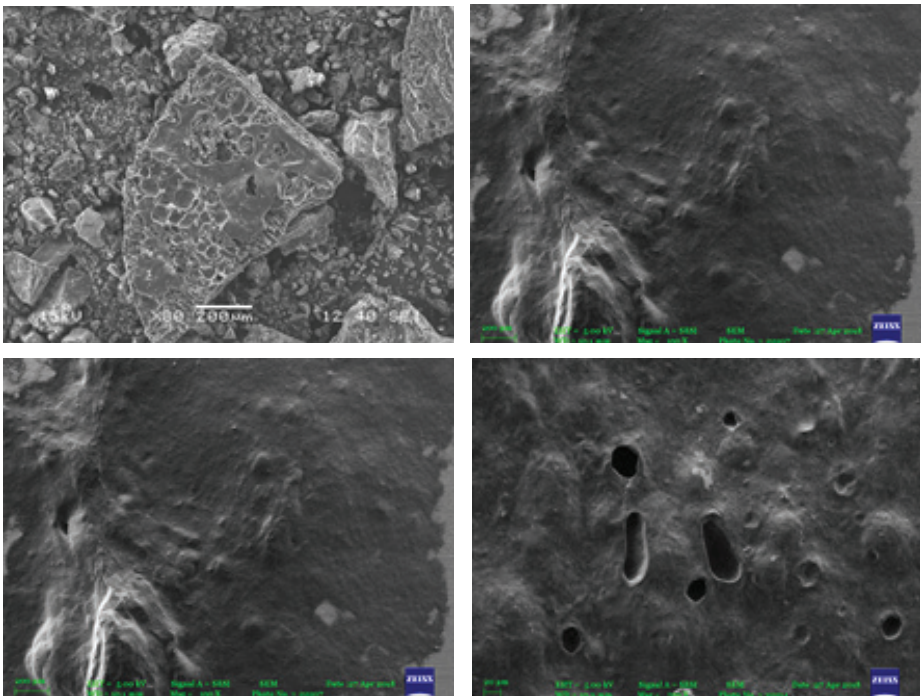


Figure 11: SEM image of karanj oil and NLC Formulation



alcoholic KOH solution & then extracted with the petroleum ether. This extract was then evaporated and then the residue was evaluated. An Unsaponifiable material is this residue- the fatty acid present in it. The Reagents which are used an Alcoholic KOH solution, Ethyl alcohol, Phenolphthalein indicator solution.

Unsaponificable matter, percent by weight= 100(A-B)/ W

Where,

W= the weight in grams of an oil taken for test.

A= the weight in grams of residue.

B= the weight in grams of fatty acid.

V= the volume in ml of a standard KOH solution used.

N=is normality of standard KOH solution.

Density and the specific gravity

Density and the specific gravity of Karanj oil was determined by density bottle with capillary tube stopper.

Characterization of nanostructured lipid carrier (NLC)

Fourier-transform infrared (FTIR)

Spectra of karanj oil and pure polymer and the gel were taken to access interaction, if any between drug and the polymer in mixtures. The scanned mixture by using a FTIR spectrophotometer (PERKIN ELMER). The FTIR spectra of the mixtures were related with that of the pure drug and the Polymer to assess the any change in to principal peaks of the spectra of a pure drug and the polymer (Ramnik S 2010).

Differential scanning calorimetric (DSC)

The molecular state of pure drug was calculated by performing DSC analysis. The DSC curve of samples was found by DSC (Mettler Toledo). Each sample was positioned in an aluminum pan and then crimped with an aluminum cover. The heating and the cooling rates were 10°c/mm and all measurements were accomplished over the temperature range of 40-300°C (Ramnik S 2010).

Particle size Photon correlation spectroscopy (PCS)

Is a technique used to conclude mean particle size diameter (mean PCS diameter) and width of a particle size supply expressed as a polydispersity index (PI). The instrument was fortified with laser beam (λ=633 nm). The scattered

Figure: contour plot for Karanj oil and Tween 80 of particle size

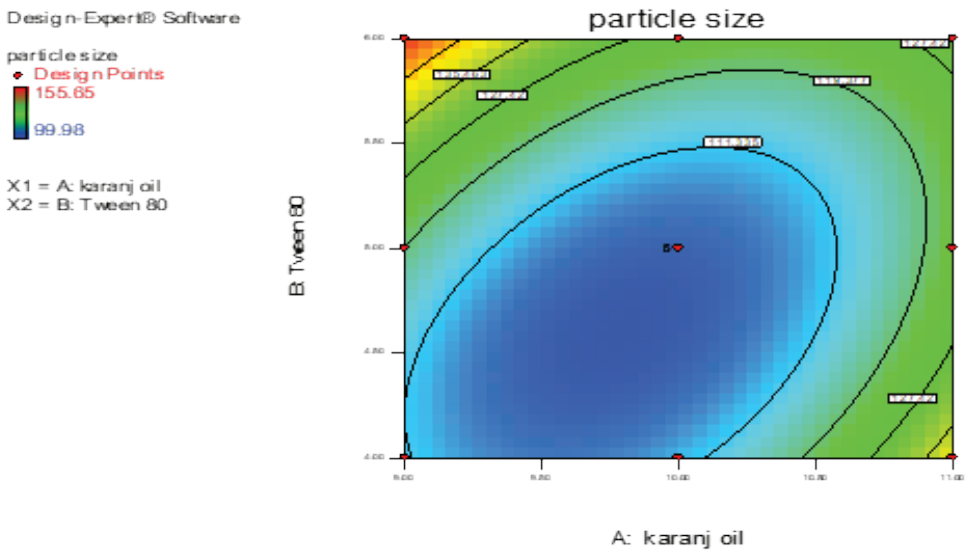


Figure 12: Response surface plot for Karanj oil and Tween 80 Particle Size

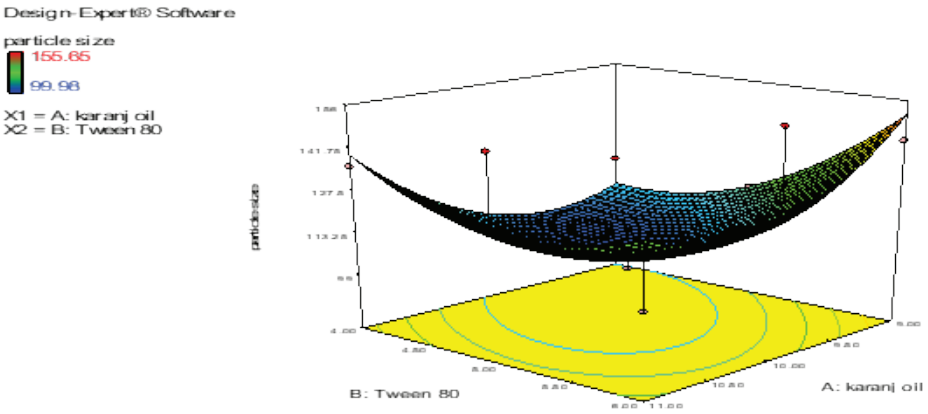


Figure 13: contour plot for Karanj oil and Tween 80 of polydispersity index

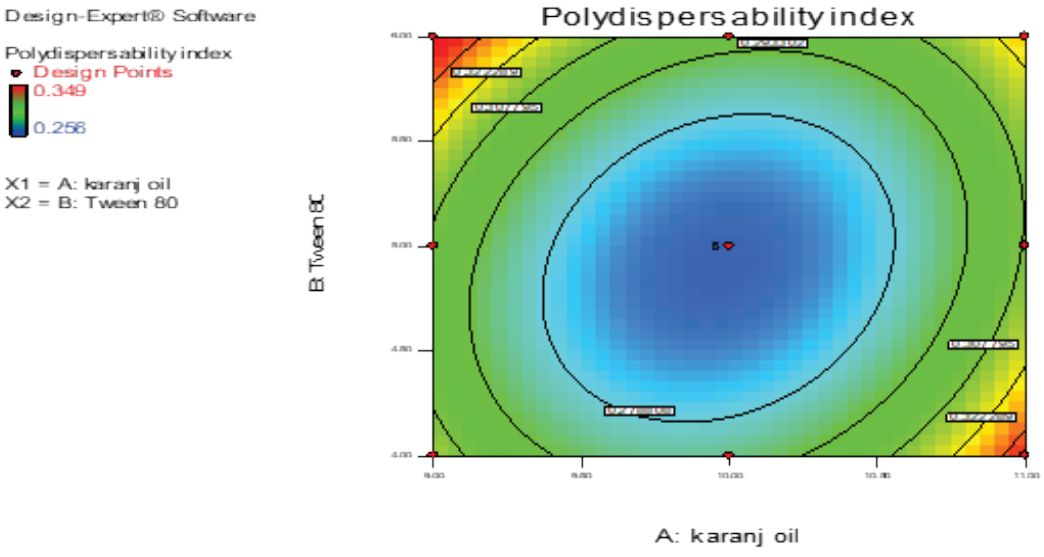


Figure 14: Response surface plot for Karanj oil and Tween 80 polydispersity index

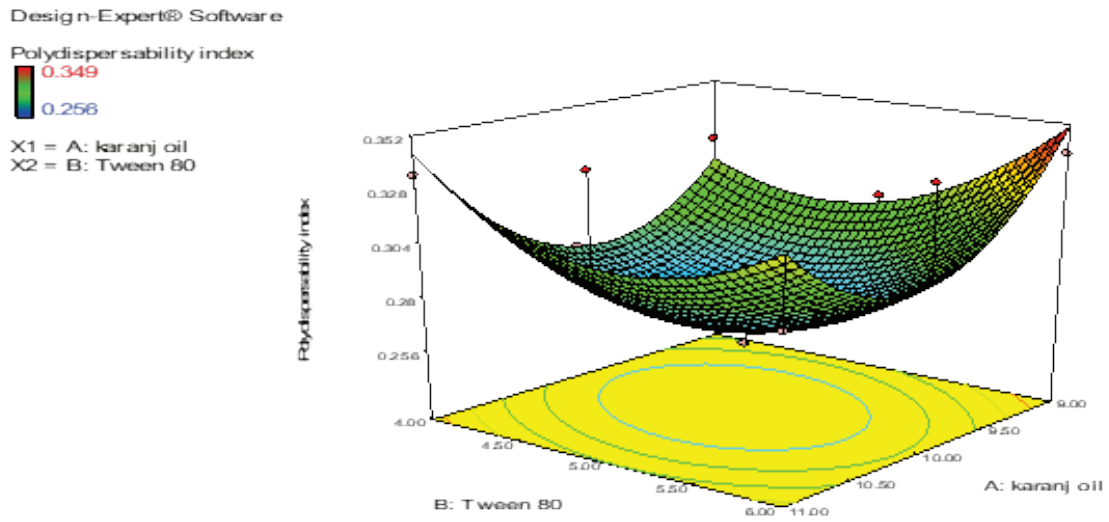


Figure 15: contour plot for Karanj oil and Tween 80 of zeta potential

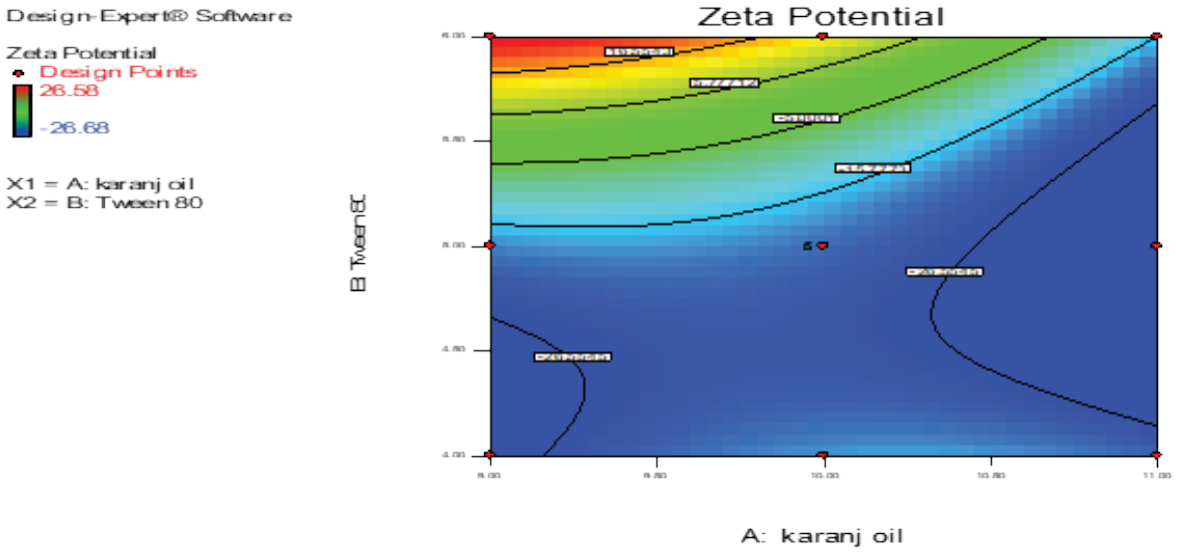


Figure 16: Response surface plot for Karanj oil and Tween 80 Zeta Potential

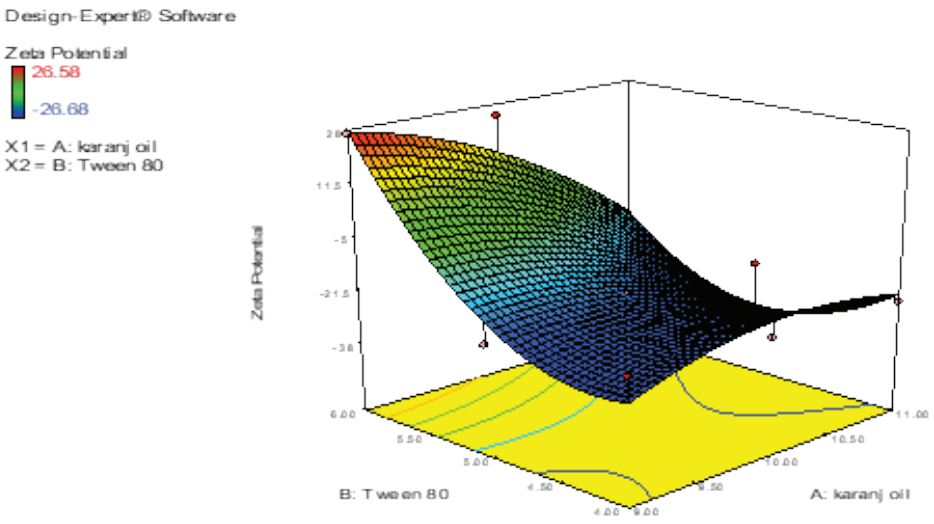


Table 1: formulation batches

Sr. no.	Ingredients	Batches								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Karanj oil (ml)	9	9	9	10	10	10	11	11	11
2	Tween 80 (ml)	4	5	6	4	5	6	4	5	6
3	Glyceryl monostearate (gm)	4.37	4.37	4.37	4.37	4.37	4.37	4.37	4.37	4.37
4	Water (ml)	25.54	25.54	25.54	25.54	25.54	25.54	25.54	25.54	25.54
5	Carbopol(gm)	54.78	54.78	54.78	54.78	54.78	54.78	54.78	54.78	54.78

Table 2: preformulation studies

Parameter	*Pongamia Pinnata*
Boiling point	328-3300c
Acid value	16.83
Saponification value	84.15
Iodine value	84.83
Unsaponification value	2.89
Density	1.204gm/cm3
Specific gravity	0.9011gm/cm3

Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms.

Table 3: Analysis of variance

Factor	Coefficient estimate	df	Standard error	95% cl low	95% cl High	VIF
Intercept	-21.02	1	3.74	-29.87	-12.17	
A-karanj oil	-9.08	1	3.68	-17.78	-0.38	1.00
B- tween 80	15.70	1	3.68	7.00	24.40	1.00
AB	-12.53	1	4.51	-23.19	-1.88	1.00
A2	-7.10	1	5.42	-19.92	5.73	1.17
B2	18.13	1	5.42	5.31	30.96	1.17

Table 4: stability studies

Parameters	0 day	30 days	60 days	90 days
Appearance	Semitransparent Whitish brown	Semitransparent Whitish brown	Semitransparent Whitish brown	Gel strength
(seconds)	55±2.5	54±1.5	55±4.8	55±3.2
Viscosity (cp)	764	785	744	718
Ph Determination	7.2±0.6	7.1±0.8	7.4±0.5	7.3±0.6

light detector is positioned at angle of 173°. This detection angle is known as the backscatter detection and it has the advantages of improved sensitivity and possibility of measuring wide range of sample concentrations. The measuring range of this device is between 0.6 nm to 6 µm. The Samples were diluted with bi-distilled water to an appropriate concentration. The average particle size diameter and PI are given from 30 runs.

Zeta potential

The zeta potential (ZP), imitating an electric charge on a particle surface and indicating physical constancy of colloidal systems. The zeta potential dimensions were performed by utilizing a Horiba zeta sizer Adjusting the conductivity of the distilled water used for diluting the samples avoids the fluctuations of a zeta potential due to dissimilarities in a conductivity. The samples were either diluted with the bi-distilled water adjusted to the conductivity of 50 µs/cm using a 0.9% (w/v) Nail solution or with solution of the surfactant which was used in the formulation (having the same concentration as in the formulation). The pH was adjusted between 6.0 and 7.0. The average of zeta potential is given from 30 runs.

Experimental design of nanostructured lipid carrier (NLC)

Factorial Design

The factorial design is utilized to estimate two or more factors concurrently. The treatments are blends of levels of the factors. Factorial design is used in an experiment of the different factors, or circumstances, the experimental

outcomes are to be elucidated. Factorial designs are the designs of choice for the concurrent determination of an effect of the some factors and their interactions.

Optimization

The optimization of the formulations with respect to one or more characteristics has always been subject of significance and a consideration for those promised in the formulation research. The objective is to produce the mathematical model that will define the responses. Various computations for recent optimization study were completed by using Design Expert® software (Design Expert trial version 8.0.7.1; State-Ease Inc., Minneapolis, MN, USA). The two-factor two-level full factorial design was used for systemic study of mixture of Polymer.

3^2 full factorial design

A 3^2 full factorial design was built where the polymers was selected as factors. The levels of these factors were selected on the basis of initial studies and observations. All the other formulation aspects and the processing variables were retained invariant throughout revision period. The Polynomial models comprising an interaction and the quadratic terms were produced for entire response variables consuming multiple linear regression analysis (MLRA) approach. The polynomial equations can be utilized to draw assumption after considering magnitude coefficient and mathematical sign that the coefficient carries. A high positive or negative value in the equation represent that by making a minor change in the setting of that factor one may obtain a significant change in the dependent variable. 3-D response surface graphs and the 2-D contour plots were also created by the Design Expert® software (8.0.7.1). These plots are very beneficial to see an interaction effect of factors on responses.

Characterization of the NLC based Gel

Homogeneity

All advanced gels were confirmed for the homogeneity by the visual inspection after gels have been fixed in a container. They were verified for their appearance and a presence of any aggregates.

Grittiness

All preparations were estimated microscopically for existence of any noticeable particulate matter which was perceived under light microscope. Hence obviously the gel formulation satisfies requirement of the freedom from particular matter and from the grittiness as preferred for any topical preparation.

Viscosity measurements

The apparent viscosity values were calculated for the gel formulations using a Brookfield viscometer DV-E using spindle no. 6 at 1 to12 rpm.

PH determination

The pH was calculated for each gel, using pH meter, which was standardized previously each practice with the buffered solutions at pH 4 and 7. The electrode was dipped in NLC's based gels and readings were noted on a pH meter.

Gel strength

Determination this is defined in terms of the time (in seconds) essential by a 35g piston for penetration about 5 cm distances, through a 50g gel formulation. The Test was executed using 'Gel strength apparatus modified at the laboratory (Figure 6.1). NLC based gel of karanj oil (50 g) was located in 100 ml-measuring cylinder. The piston (weight: 35 g) was then positioned on to gel. The gel strength was calculated as a time required moving piston 5 cm down through gel. In cases that took more than 5 minutes to drop an apparatus into gel, additional weights were employed on top of apparatus and gel strength was defined by minimal weights that pushed an apparatus 5 cm down through gel.

Stability Study

From the stability study of the optimized batch, it was found that the NLC based gel remained stable even after exposing to high temperature and moisture conditions, indicating that karanj oil remained chemically stable in NLC's based gel.

RESULT AND DISCUSSION

Preformulation studies

The karanj oil is checked for its boiling point, acid value, saponification value, iodine value, unsaponification value, the density and specific gravity. The outcomes are showed in the table no 1

Fourier transforms infra-red spectroscopy (FT-IR) analysis

The FT-IR studies were performed in order to examine interaction of drug to polymer.FT-IR studies of drug; the polymer, the physical combination of drug & polymer of NLC were done. From the spectra's it can be determined that the principle peak value of drug remains unchanged in nano sponges and there was no interaction between drug and the polymer.

Differential Scanning Calorimetry

DSC measurements were performed on Differential Scanning Calorimeter with thermal analyzer DSC-61000 (Metller Toledo USA). The drug, the polymer, physical mixture of the drug and the polymer (1:1) and optimized formulation were examined by the DSC.

All accurately weighed samples (approximately 2 mg of samples) were located in locked aluminum pan, and then the samples were heated under a nitrogen flow (10_ml/min) at scanning rate of the 100C per min from 25 to 300C. A vacant aluminum pan was utilized as the reference.

Particle size and PDI

The all batches i.e., Batch F1 to F9 showed their particle size in range of 99.98 nm to 155.65 nm. But the optimum particle size was observed in F5 batch i.e., 99.98 nm. The PDI showed their range in 0.256 to 0.349 the optimized batch i.e., Batch F5 shows 0.256.

Zeta potential

The zeta potential for all batches was checked by Horiba zetasizer. The batches F1 to F9 Shows zeta potential in range of -22.34 to -26.68 the zeta potential for batch F5 was establish to be -22.34 mv representing good physical stability.

Scanning electron microscopy (SEM)

Morphology of drug particle was studied by SEM technique. The morphological characteristics of nano sponges are as shown in fig

Evaluations of NLC Based GEL

Homogeneity

All developed gels were tried for homogeneity by a visual examination after gels have been set in the holder. They were verified for their appearance and presence of any aggregates. There were no aggregates in the formulation. Gel appears semitransparent white.

Rheological behavior of NLC's based gel

The apparent viscosity values were calculated for the gel formulations using a Brookfield viscometer DV-E with spindle no. 6 at 1 to 12 rpm. In the gel state all formulations were observed to be exhibiting the pseudo plastic flow. The viscosity of F5 batch shows viscosity up to 765 cp and it was revealed that optimized formulation exhibits pseudo plastic flow behavior.

PH determination

The pH of all the formulations was found to be in a range of 7.4–7.9, which is around the skin ph. This is approximately neutral and may be suited for topical application without any discomfort. Topical gel must have the suitable gel strength so as to be applied effortlessly and can be reserved at the skin without outflow after application. The gel strength for batch F5 was then observed to be 55±2.5.

Grittiness

All preparations were assessed microscopically for the existence of any considerable particulate matter which was observed under the light microscope. Hence clearly gel preparation achieves necessity of the freedom from the particular matter and from the grittiness as anticipated for any topical preparation. The gel was free from a particulate matter and grittiness when was seen under the microscope.

Stability Studies

Optimized formulation (F5 batch) was filled into aluminum tubes as the final dosage form which was exposed to stability studies to conclude its physical stability. From the stability study of an optimized batch it was establish that NLC based gel persisted stable even after exposing to the high temperature and moisture conditions, indicating that Karanj oil stayed chemically steady in NLC's based gel.

DISCUSSION

The current work concentrates on development of the nanostructured lipid carriers (NLC) for topical application. It also shows the advantages of using the NLC topical formulations and studies the factors that affect these advantages. In order to minimize these adverse effects, NLC based gel of *Pongamia pinnata* was given by topical route. An other advantages of topical route including fast onset of action, improved patient compliance, better drug utilization, reduced fluctuation. NLC's of *Pongamia pinnata* were formulated by a melt dispersion ultrasonication method. Pre-formulation studies were achieved by an identification IR determination. The formulations were exposed to various evaluation parameters like particle size Analysis, zeta potential. Particle size of NLC was found in range between 99.98 and 155.65 nm with polydispersity index varied from 0.256 to 0.349. The NLC dispersions was developed by utilizing the gelling agent carbopol which have compatibility with nano particulate dispersion, feel attractive appeal and comfort of spread ability. The NLC based gel of *Pongamia pinnata* was evaluated for viscosity study, gel strength, and stability studies. It is revealed from the stability study that optimized formulation is stable and used for topical application.

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