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DEVELOPMENT OF NANOSTRUCTRAL LIPID CARRIER LOADED WITH TEZAROTEIN FOR EFFECTIVE ACNE TREATMENT

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ABSTRACT

Sixteen formulations of nanostructured lipid carriers of Tazarotene prepared by using OVAT (One variable at Time) optimization technique. Variables along with amount of lipid and attention of surfactant had been optimized additionally technique variables as stirring speed and stirring time have been optimized. Particle size and Entrapment efficiency of drug loaded Nanostructured lipid carriers were carried out and the entrapment efficiency of formulations F1 to F16 was found to be 256.65±0.25, 245.65±0.32, 285.65±0.15, 268.98±0.25, 245.65±0.23, 215.65±0.45, 236.65±0.32, 214.47±0.18, 205.65±0.25, 210.74±0.65, 198.85±0.14, 210.58±0.27, 225.68±0.33, 218.78±0.17, 178.85±0.21, and 220.14±0.36 respectively. The Entrapment efficiency of formulation F1 to F16 were found between 63.32±0.54 to 82.23±0.14 respectively. The maximum entrapment efficiency was found in formulation F15 (82.23±0.14). The Drug content of formulation F15 was also found high in formulation F15 select as optimized formulation. The prepared gel at least rpm of 10 exhibited a viscosity of 2898.35±13.45 to 3325.48±10.25cps that indicates that the formulation has the desired viscosity required for semisolid formulation for proper packaging. When the regression coefficient values of were compared, it was observed that 'r2' values of Zero Order was maximum i.e. 0.900 hence indicating drug release from formulations was found to follow Zero Order.

Key Words: Tazarotene, Nanostructured lipid carriers, *Gel, Formulation, Evaluation*

INTRODUCTION

In recent years, it has become evident that the development of novel drugs isinsufficient for guaranteeing progress in drug therapy. A promising approach to overcoming this problem is the development of feasible drug delivery system. During the past decades, some strategies have been developed such as nano-sized drug carrier system [2], which is a great approach in drug delivery with the promising features of protection of drug from degradation and cleavage, controlled release and the delivery of drug molecules to the target sites [3]. Lipid nanoparticles made with a solid matrix is derived with the help of pharmaceutical nanotechnology which gains a huge impact on the pharmaceutical field. Generally a solid lipid nanoparticle is composed of physiological lipids disposed in an aqueous surfactant solution. It has certain benefits like improvement in solubility, bioavailability and also improvement in drug therapy [5]. There are some drawbacks such as loading insufficiency due to formation of perfect crystalline structure, drug expulsion and also high water content in the preparation [6-7].

Different types OF NLCS

There are three types of NLCs such as

(i) TYPE 1: Amorphous structured NLCs(Non crystalline NLCs)-These type of NLCs are developed by preventing the crystallization of the mixing solid and the liquid lipids due to which there is a formation of a amorphous structured lipid matrix which create high amount of space within the lipid matrix in which high amount of drug can be incorporated and reduce the problem associated with SLN preparation [12].

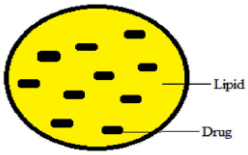


Figure 1.1: Type 1 NCL

(ii) TYPE 2: Imperfect structured NLCs- In these solid lipids and liquid lipids(oils) are blended. During the production process, the liquid lipid particles (nanoemulsions) are cooled from the molten state to room temperature to crystallize and form solid particles. At high oil concentrations a miscibility gap of the two lipids occurs during the cooling phase which leads to phase separation that means precipitation of tiny oily nano compartments [13].

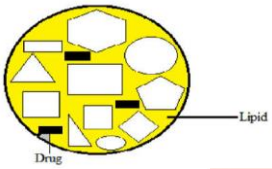


Figure 1.2: Type 2 NCL

(iii) TYPE 3: Multiple structured NLCs- These types of NLCs are made up of oil,
fat, water and stabilizer. Large amount of liquid lipids are used in multiple structured NLCs as compared to other lipids structured formulations. Large amount of liquid lipids are blended with the solid lipids due to which there is a formation of small liquid lipids packets supported by the solid lipid matrix and desired amount of drug can be introduced into the formulation [14-15].

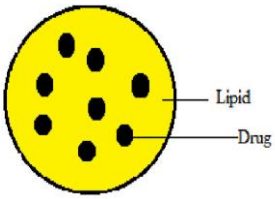


Figure 1.3: Type 3 NCL

Advantages of NLCS

1) NLCs are easy to scale up and inexpensive as compared to polymeric/surfactant based carrier [16].
2) NLCs transport both lipophilic and hydrophilic drug at the same time [17].
3) NLCs are easier to validate and get easy approval from regulatory bodies [18-19].

Structural components of NLCS: -

Solid lipids Following are some examples of solid lipids

1) Triglyceride (Tristearin, Trilaurin)
2) Monoglyceride (Glycerol monosterate)
3) Fatty acids (Stearic acid, Palmitic acid)
4) Waxes (Cetylpalmitate, Beeswax)

II. Liquid lipids The liquid lipids used are digestible oils obtained from natural sources [25].

(ii) Emulsifier They are used to stabilize the liquid nanoparticle dispersion and also prevent particle agglomeration in the dispersion. Choice of ideal emulsifying agent depends on certain properties like charge, molecular weight and HLB balance [26-27].

Method of preparation : -

I. Solvent based method : - Solvent injection or displacement method and Solvent emulsification evaporation method

II. High pressure homogenization technique: - Hot homogenization technique, Cold homogenization technique and Micro emulsion technique, Melting dispersion method

Applications of NLCS

(i) Oral drug delivery- NLCs have been proved one of the beneficial systems forthe oral administration of poor water-soluble drug having low bioavailability. Lipid nanocarrier protects the drug from the enzymatic attack and also the harsh environment of GIT tract.

(ii) Brain targeting- Targeting of drug to the brain by using the NLCs increases thecerebra spinal fluid concentration and reduces the frequency of dosing and side effects. NLCs of Apomorphine has improved duration of brain targeting and accumulation in brain by intravenous delivery [53-54].

(iii) Tumor targeting- Formation of anticancer drug loaded in nanostructured lipidcarriers can overcome the limitations like low water solubility, high systemic toxicity and insignificant cellular uptake.

(viii) Cosmetic application- Nanostructured lipid carriers are one of the excellentvehicles for cosmetic application due to their excellent characteristics against chemical degradation and enhancement of water content of skin.

(vii) Gene delivery- Gene loaded in NLCs can be used as a non-viral gene transfer vector that offers a promising approach for gene therapy.

Tazarotene is a third-generation retinoid applied topically for the treatment of psoriasis and acne. But its applications are limited due to its poor solubility and bioavailability. Acne vulgaris is the most prevalent disorder in the period before puberty when increased adrenal androgen level causes enlargement of the sebaceous glands and it increased the production of sebum on the face, chest, and back. This disease is caused due to interaction between many causative agents or pathogenic components which lead to formation of the acne and those are seborrhea, follicular hyper keratinization, microbial formation of pilosebaceous unit by *Propionibacteriumacne* and arrival of inflammatory mediators.

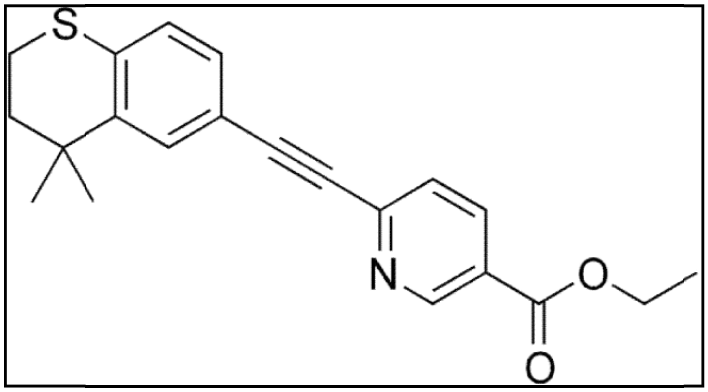


Figure 5.1: Structure of Tazarotene

MATERIAL AND METHOD

Materials which are used in the investigation are listed in Table 1.

Sr. No.	Chemicals	Supplier
1.	Tazarotene	Bioplus Life Sciences Pvt. Ltd. Bangalore
2.	Phosphatidylcholines	Thomas Baker, Mumbai
3.	Disodium Hydrogen Phosphate	S. D. Fine Chem. Ltd., Mumbai
4.	Di potassium Hydrogen Orthophosphate	S. D. Fine Chem. Ltd., Mumbai
5.	Sodium hydroxide	S. D. Fine Chem. Ltd., Mumbai
6.	Methanol	Qualigens Fine Chemicals, Mumbai
7.	Ethanol	Qualigens Fine Chemicals, Mumbai
8.	Chloroform	Qualigens Fine Chemicals, Mumbai
9.	Carbopol 934p	Thomas Baker, Mumbai
10.	Stearyl amine	Thomas Baker, Mumbai
11.	Pluronic F-68	Thomas Baker, Mumbai
12.	Propylene Glycol	S. D. Fine Chem. Ltd., Mumbai

Instruments Used in Investigation

Instruments which are used in the investigation are listed in Table 2.

Sr. No.	Instruments	Supplier
1.	UV -Visible Spectrophotometer	Labindia 3000+
2.	Fourier Transform Infra Red Spectroscopy	Brucker, Germany
3.	Mechanical Stirrer	Bionics Scientifics, Delhi
4.	Optical Microscope	Lyzer, Ambala
5.	Micro Centrifuge	REMI laboratory, Mumbai
6.	Franz Diffusion Cell	Electro Lab, Mumbai
7.	pH Meter	Accumax India, New Delhi
8.	Electronic Balance	Contech Instruments Ltd. , Mumbai
9.	Melting Point Apparatus	Contech Instruments Ltd. , Mumbai
10.	Hot Air Oven	Oracle Equipments, New Delhi
11.	Vortex Apparatus	Ambros Lab Equipments, Ambala
12.	Brook Field Viscometer	Precision Electro Instrumentation India Private Limited, Thane
13.	Differential Scanning Calorimeter	Perkin-Elmer India Pvt. Ltd., Thane
14.	Rotary Vaccum Evaporator	Microtech Scientific Instruments, New Delhi
15.	IR Moisture Balance	Scope Enterprises, New Delhi
16.	Zeta Sizer	Malvern Instruments, UK
17.	Sonicator	Athena Technology, Thane

Preformulationstudy

Preformulation studies include studies of:

1. The physiochemical properties of drug, and an assessment of their relevance to the final formulation.
2. The chemical and physical stability of drug.

Chemical /physical compatibility of the active with potential excipients.

3 Characterization of Tazarotene

S. No.	Sensory characters	Result
1.	Colour	Light yellow powder
2.	Odor	Odorless
3.	Taste	Tasteless

Table 6.4: Solubility of Tazarotene

Solvent used	Results of Solubility
Distilled Water	Insoluble
0.1 N Hydrochloric acid	Soluble
0.1 N NaOH	Soluble
Ethanol	Freely soluble
Methanol	Freely soluble
Chloroform	Soluble
Phosphate buffer pH 7.2	Soluble

Melting point:

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil [84]. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

Results: Melting point of the Tazarotene was found to be 96ºC

Identification Test using FTIR Spectroscopy This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound. The region from 0.8 µ to 2.5 µ is called Near Infra-red and that from 15 µ to 200 µ is called Far infra-red region.

Identification of Tazarotene was done by FTIR Spectroscopy with respect to marker compound. Tazarotene was obtained as Light yellow crystalline powder. It was identified from the result of IR spectrum as per specification[85].

Sample of pure Tazarotene

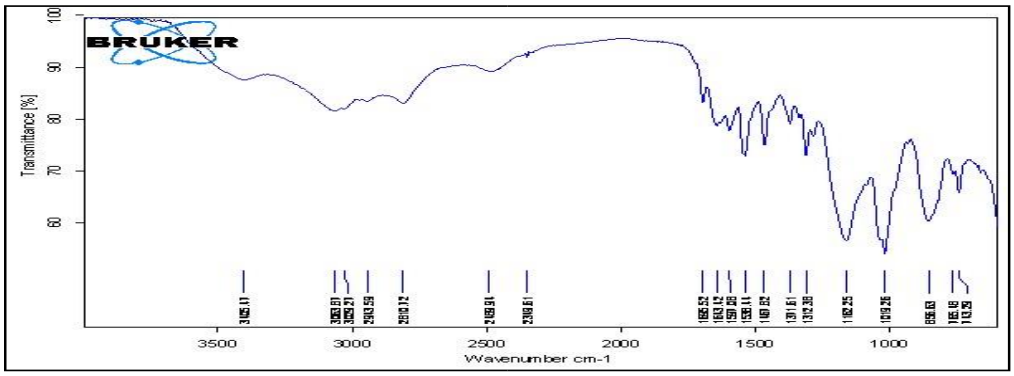


Figure : FT-IR Spectrum of Pure Drug (Tazarotene)

E) Determination of λ_{max} of Tazarotene:

The λ_{max} of Tazarotene was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer. Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.2 pH phosphate buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.2 pH phosphate buffer solution prepare suitable dilution to make it to a concentration range of 10-50 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of Tazarotene versus wave length was shown in figure

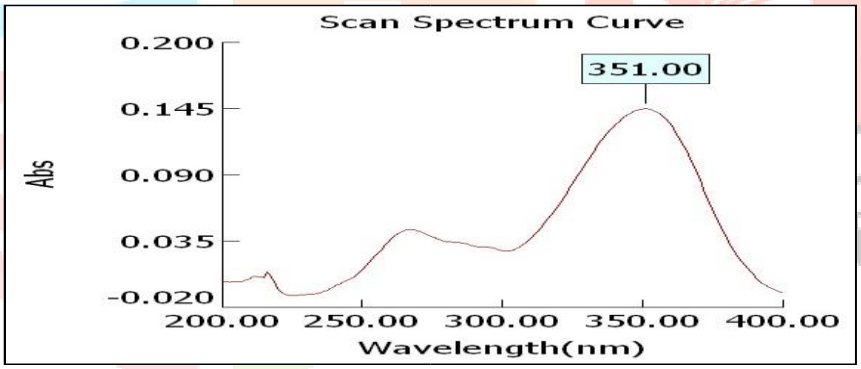


Figure : Wavelength maximaof tazarotene in phosphate buffer pH 7.2

E) Calibration curve of Tazarotene at λ_{max} 351nm

F) Table 5: Calibration curve of Tazarotene

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.138±0.001
2	20	0.355±0.004
3	30	0.567±0.001
4	40	0.785±0.00
5	50	0.981±0.005

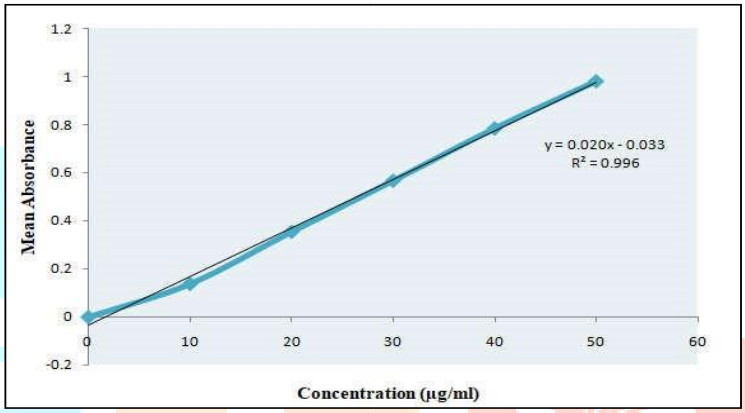


Figure : Calibration curve of tazarotene in phosphate buffer pH 7.2

Preparation of Tazarotene loaded Nanostructured lipid carriers

Nanostructured lipid carriers were prepared by using microemulsion technique[87]and o/w microemulsions were initially prepared. The oil phase, lipophilic surfactant and continuous phase used are glyceryltripalmitate, soy lecithin and pluronic F-68 (hydrophilic surfactant) respectively. The lipid and soy lecithin were melted at 70°C and the drug was added with constant stirring. 10 ml of aqueous surfactant solution containing pluronic F-68 heated at the same temperature was added to the melted lipid with mechanical stirring for 15 min. A clear microemulsion was obtained at a temperature close to the melting point of the lipid used. Stearyl amine was used as a positive charge inducer and added to melted lipid. Nanostructured lipid carriers were obtained by dispersing the warm o/w microemulsion which is added drop wise into ice cold water in a beaker under continuous stirring. After completion of stirring, the Nanostructured lipid carriers dispersion was subjected to ultrasonication for 15 min.

Preparation of Gel Base

Carbopol 934 (1-3% w/v - Nanostructured lipid carriers based gel formulation i.e. G-1 of 1% w/v, G-2 of 2% w/v, G-3 of 3% w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml

and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Nanostructured lipid carriers containing gel in which previously prepared Nanostructured lipid carriers was added. Nanostructured lipid carriers preparation corresponding to 5% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Formulation optimization of gel base

Ingredient (%)	G-1	G-2	G-3
Drug (Invasomes equivalent to 0.1%)	0.1	0.1	0.1
Carbopol 934	1	2	3
Propylene glycol	0.2	0.2	0.2
Water (ml)	100	100	100

Study on the effect of lipid quantity

Components	Formulation COD		
	F1	F2	F3
Lipid	50	100	200
Soy lecithin	1	1	1
Stearyl amine	1	1	1
Pluronic F-68 (1% w/v)	1	1	1
Stirring speed (rpm)	1500	1500	1500
Stirring time (hrs)	3	3	3

Composition of Nanostructured lipid carriers by varying amount of Lipid

Effect of stirring time

Components	Formulation code				
	F4	F5	F6	F7	F8
Lipid	50	50	50	50	50
Soy lecithin	1	1	1	1	1
Stearyl amine	1	1	1	1	1
Pluronic F-68 (1% w/v)	1	1	1	1	1
Stirring speed (rpm)	2000	2000	2000	2000	2000
Stirring time (hrs)	1	2	3	4	5

Composition of Nanostructured lipid carriers by varying Stirring time

Effect of surfactant concentration

Components	Formulation code			
	F13	F14	F15	F16
Lipid	50	50	50	50
Soy lecithin	1	1	1	1
Stearyl amine	1	1	1	1
Pluronic F-68 (1% w/v)	0.5	1	1.5	2
Stirring speed	2000	2000	2000	2000
Stirring time	4	4	4	4

Composition of Nanostructured lipid carriers by varying amount Surfactant

Preparation of drug loaded Nanostructured lipid carriers batches

Components	Formulation code (F16)
Lipid	50
Soy lecithin	1
Stearyl amine	1
Pluronic F-68 (1% w/v)	1.5
Stirring speed	2000
Stirring time	4

Composition of optimized batch

RESULTS AND DISCUSSION

Result for particle size, entrapment efficiency and drug content of drug loaded Nanostructured lipid carriers

Particle size and Entrapment efficiency of drug loaded Nanostructured lipid carriers were carried out and the entrapment efficiency of formulations F1 to F16 was found to be 256.65±0.25, 245.65±0.32, 285.65±0.15, 268.98±0.25, 245.65±0.23, 215.65±0.45, 236.65±0.32, 214.47±0.18, 205.65±0.25, 210.74±0.65, 198.85±0.14, 210.58±0.27, 225.68±0.33, 218.78±0.17, 178.85±0.21, and 220.14±0.36 respectively. The Entrapment efficiency of formulation F1 to F16 were found between 63.32±0.54 to 82.23±0.14 respectively. The maximum entrapment efficiency was found in formulation F15 (82.23±0.14). The Drug content of formulation F15 was also found high in formulation F15 select as optimized formulation.

Result for particle size, entrapment efficiency and drug content of drug loaded nanostructured lipid carriers

Formulation Code	Particle size	Entrapment Efficiency	Drug Content
F1	256.65±0.25	69.98±0.14	96.65±0.25
F2	**245.65±0.32**	**73.32±0.25**	**97.85±0.36**
F3	285.65±0.15	65.74±0.65	96.65±0.15
F4	268.98±0.25	68.78±0.14	95.85±0.25
F5	245.65±0.23	63.32±0.12	96.78±0.14
F6	**215.65±0.45**	**75.85±0.54**	**98.78±0.23**
F7	236.65±0.32	70.23±0.36	97.12±0.47
F8	214.47±0.18	68.98±0.25	98.78±0.32
F9	205.65±0.25	65.74±0.74	97.85±0.25
F10	210.74±0.65	63.32±0.54	96.65±0.65
F11	**198.85±0.14**	**79.98±0.25**	**99.15±0.21**
F12	210.58±0.27	68.78±0.36	98.78±0.14
F13	225.68±0.33	66.32±0.21	97.85±0.74
F14	218.78±0.17	67.74±0.25	98.12±0.36
F15	**178.85±0.21**	**82.23±0.14**	**99.45±0.25**
F16	220.14±0.36	70.14±0.32	99.88±0.22

Table 1: Result for particle size, entrapment efficiency and drug content of drug loaded nanostructured lipid carriers

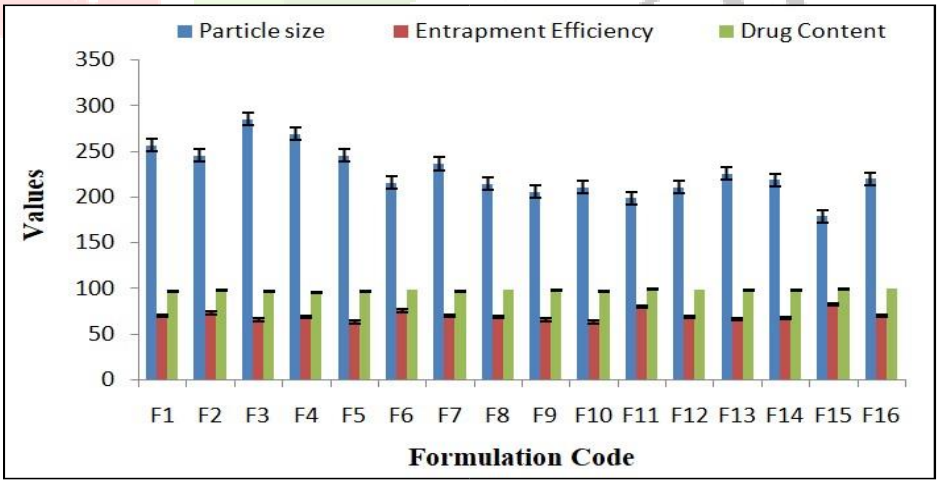


Figure 1: Figure of Particle size, Entrapment efficiency and drug content of drug loaded nanostructured lipid carriers

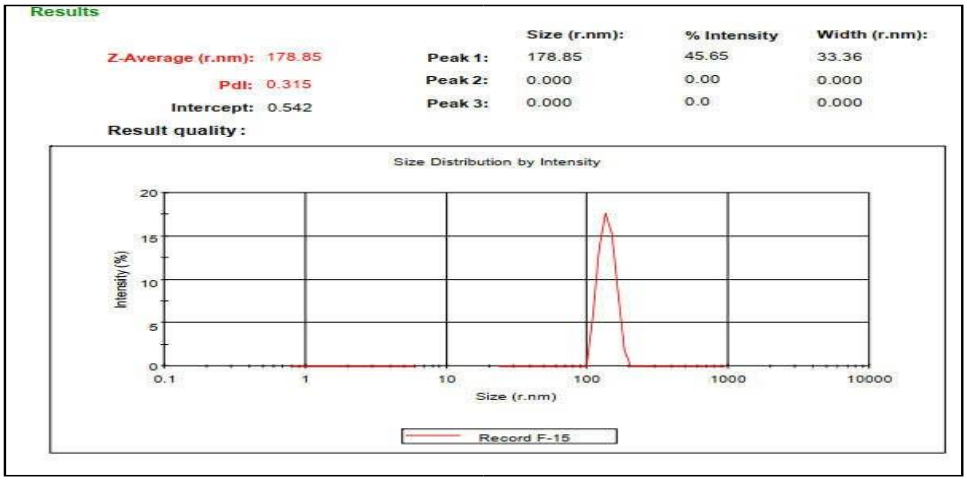


Figure 2: Particle size of Optimized nanostructured lipid carriers

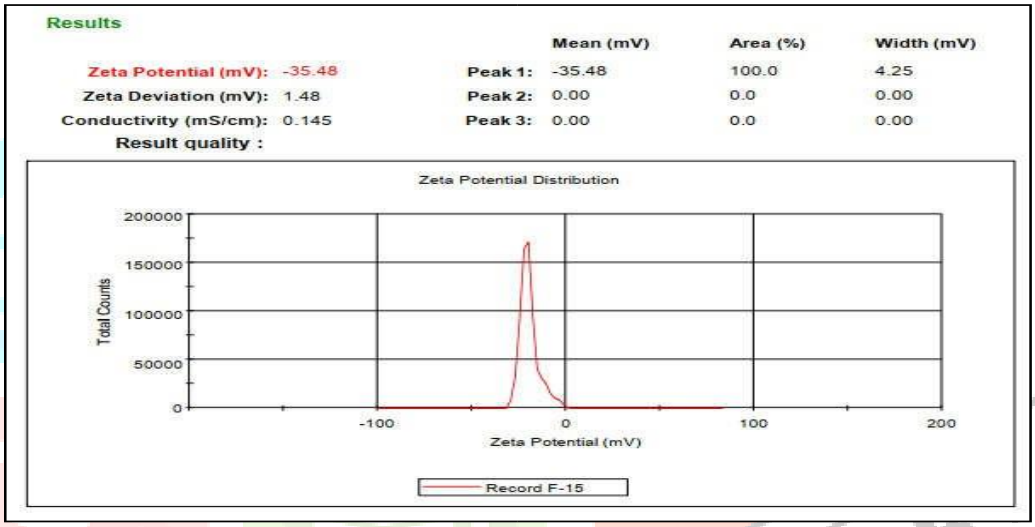


Figure 8.3: Zeta potential of Optimized nanostructured lipid carriers

Results of cumulative drug release of Optimized nanostructured lipid carriers F15

Table 3: Cumulative % drug release

S. No.	Time (hrs)	% Cumulative Drug Release
1	1	10.25
2	2	16.65
3	3	20.25
4	4	26.69
5	5	39.98
6	6	46.65
7	7	59.98
8	8	69.98
9	9	78.85
10	10	86.65
11	12	93.32

Results of characterization of gel based formulation

Characterization of gel based formulation

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
G-1	3325.48±10.25	6.82±0.25	98.28±0.15	178±8	13.25±0.15
G-2	3045.65±9.85	6.70±0.32	99.45±0.35	165±6	12.14±0.17
G-3	2898.35±13.45	6.88±0.32	97.65±0.14	153±5	11.15±0.18

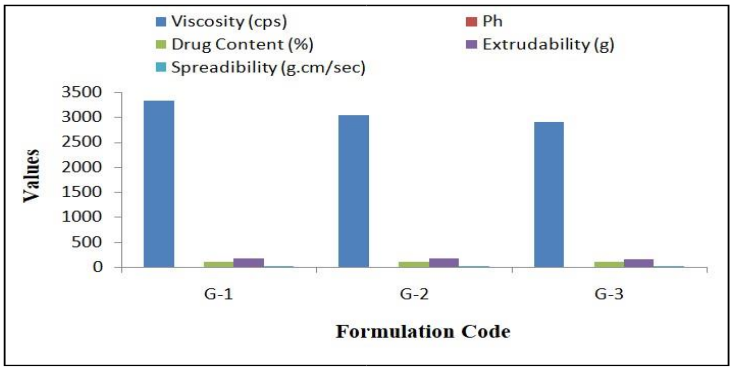


Figure 4:Characterization of gel based formulation

Table 5: *In vitro* drug release study of optimized gel formulation G-2

S. No.	Time (hr)	% Cumulative Drug Release*		
		G-1	G-2	G-3
1	0.5	26.65	20.32	17.78
2	1	37.74	35.65	28.98
3	2	55.65	54.47	33.36
4	4	76.65	68.85	48.85
5	6	92.23	74.45	59.98
6	8	98.85	83.32	66.65
7	10	99.12	95.65	78.85
8	12	99.25	99.45	86.65

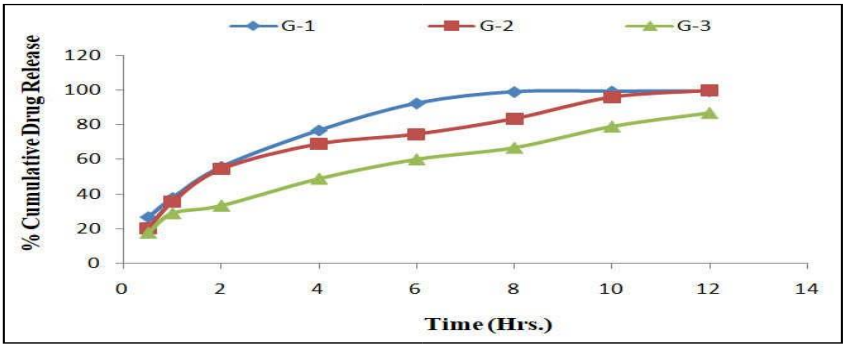


Figure 5:*In vitro* drug release study of optimized gel formulation G-2 Table 8.6: *In vitro* drug release study of optimized gel formulation G-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	20.32
2	1	35.65
3	2	54.47
4	4	68.85
5	6	74.45
6	8	83.32
7	10	95.65
8	12	99.45

Release kinetics of drug encapsulated formulation G-2

Table 8.7: *In-vitro* drug release data for optimized formulation G-2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative *% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.32	1.308	79.68	1.901
1	1	0	35.65	1.552	64.35	1.809
2	1.414	0.301	54.47	1.736	45.53	1.658
4	2	0.602	68.85	1.838	31.15	1.493
6	2.449	0.778	74.45	1.872	25.55	1.407
8	2.828	0.903	83.32	1.921	16.68	1.222
10	3.162	1	95.65	1.981	4.35	0.638
12	3.464	1.079	99.45	1.998	0.55	-0.260

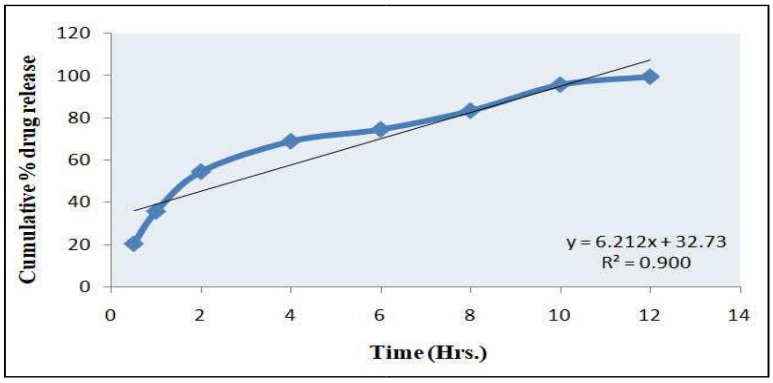


Figure 6: Cumulative % drug released Vs Time

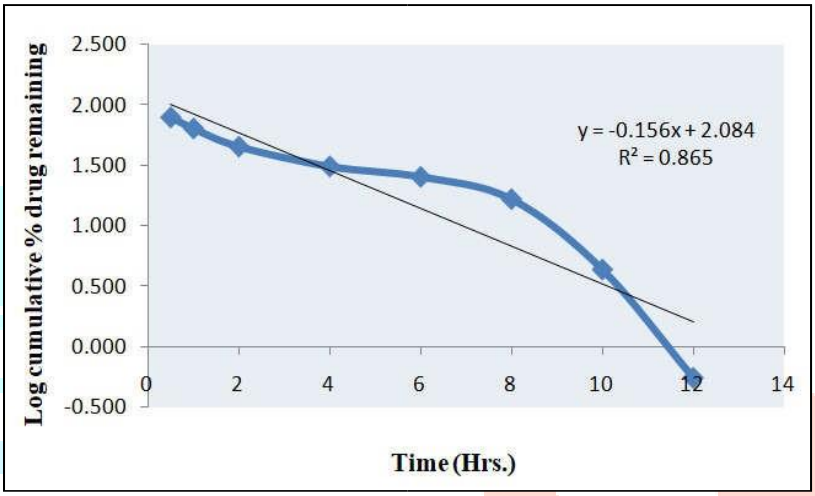


Figure 7: Log cumulative % drug remaining Vs Time

(First Order Kinetics)

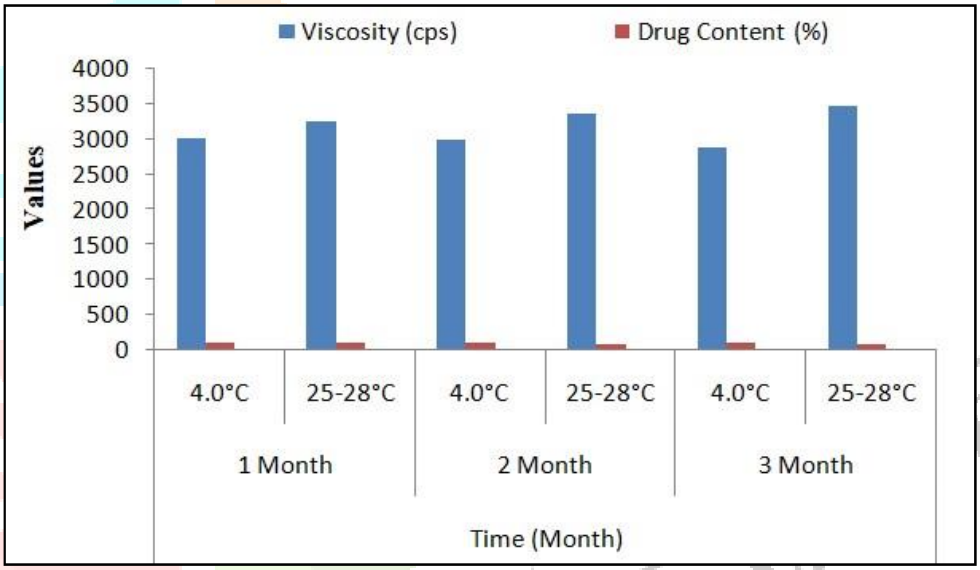
Table 8: Regression analysis data of optimized gel formulation G-2

Batch	Zero Order	First Order
	R^2	R^2
G-2	0.900	0.865

Results of stability

Table 9: Stability of optimized formulation

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0 ± 0. 2°C	25-28 ± 2°C	4.0 ± 0. 2°C	25-28 ± 2°C	4.0 ± 0. 2°C	25-28 ± 2°C
Viscosity (cps)	3022.45	3256.45	2985.65	3365.85	2878.45	3478.74
Drug Content (%)	99.12	98.45	99.05	97.75	99.00	97.12
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid



CONCLUSION

pH of prepared gel was measured by using digital pH meter. The pH of the Gel was found to be in range of 6.70±0.32 to 6.88±0.32 which is good for skin pH. All the formulation of Gel was shown pH nearer to skin required i.e. pH of G1- 6.82±0.25, 6.70±0.32 and G3-6.88±0.32. Spreadability plays considerable role in patient compliance and ensures uniformapplication of Gel to a larger area of the skin. The spreadability of the formulation G-2 was calculated as 12.14±0.17cm/sec. The low value of spreadability coefficient of the Gel was sufficient suggesting easy spreading and no signs of grittiness. The lower value of spreadability indicates the lesser work required to spread the Gel over the skin, which means formulation was easily spreadable by applying small amount of shear.

Drug content of drug incorporated gel for formulation G-1, G-2 and G-3 was found to be98.28±0.15**,** 99.45±0.35 and 97.65±0.14 respectively. The maximum drug content was found in formulation G-2 (99.45±0.35), select as optimized formulation.

When the regression coefficient values of were compared, it was observed that 'r^2' values of Zero Order was maximum i.e. 0.900 hence indicating drug release from formulations was found to follow Zero Order.

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