IJPSR (2021), Volume 12, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 June 2020; received in revised form, 05 December 2020; accepted, 09 December 2020; published 01 June 2021

FORMULATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIER FOR THE MANAGEMENT OF PSORIASIS

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Keywords:

Nanostructure Lipid Carriers, Acetretin, Hot Homogenization method, Surfactant, Skin targeting, Topical delivery

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ABSTRACT: The objective of the research work was to design NLCs gel of Acitretin and perform an *in-vitro* evaluation of different formulations. FTIR and DSC studies revealed that there was no interaction between drug and polymer and with the respective surfactant. A new generation of NLCs consisting of a lipid matrix with a special nanostructure has been developed. These nanostructures improve drug loading and firmly incorporates the drug during storage. The purpose of this study was to prepare Acitretin-loaded NLCs gel effective for topical delivery of Acitretin by using the Hot Homogenization method. Stearic acid as solid lipid, Oleic acid as liquid lipid combination of two types of surfactant: Tween 80 and Sodium lauryl sulphate (SLS) were used to stabilize NLCs dispersion. Physiochemical properties of NLCs gel such as particle size, TEM, entrapment efficiency, and *in-vitro* release studies were investigated. The formulation in which a combination of ionic and non-ionic surfactant was used to demonstrated good entrapment efficiency, sustained *in-vitro* release profile. *In-vitro* release study revealed the biphasic release pattern of NLCs gel system. Results suggested that the combination of two surfactants produces a stable system with good entrapment efficiency and desired particle size. Act NLCs gel formulation with skin targeting may be a promising carrier for topical delivery Acitretin.

INTRODUCTION: Solid lipid nanoparticles (SLN) were developed at the early 1990s as an alternative colloidal lipids system for controlled drug delivery. NLC made of a solid lipid matrix with a specific content of liquid lipid are a new generation of SLN [1]. Nanostructure lipid carriers are the lipid-based colloidal nano-drug delivery system. NLC is advanced solid lipid nanoparticle. NLCs gel has a mean particle size 10-500nm. Nano structuring makes the matrix more flexible and hence shows better drug loading and drug release.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(6).3381-90

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(6).3381-90

NLC gel made of binary mixture of solid lipid and a spatially different liquid lipid as hybrid carrier. A disadvantage associated with SLNs drug expulsion, particle concentration in the aqueous dispersion, and drug loading due to less solubility of the drug in the solid lipid, these disadvantages can be overcome by developing a nanoparticle with a controlled nanostructure, termed as nanostructured lipid carrier (NLC) [2]. They contain extremely low melting points because of the oil and maintain the particle state as solid at body temperature.

Advantages like enhanced drug loading, reduced burst release, better control over the release of drug from the formulation, NLCs is a new generation of lipid nanoparticles which proves to be a good solution for problems like poor drug loading and drug expulsion [3]. NLCs gel can be formulated by using a various technique like high-pressure

homogenization, micro-emulsion method, solvent emulsification evaporation method, solvent displacement method, solvent diffusion method, phase inversion, melt emulsification method, simple sonication method lyophilization, spray drying *etc*. [4]

Acitretin is an oral retinoid utilized in the treatment of severe resistant psoriasis because of the potential for a problem and severe adverse effects. It is commonly used in only very severe cases of psoriasis that have been unresponsive to other treatments [5]. It attaches to nuclear receptors that regulate gene transcription. They produce keratinocyte differentiation and suppress epidermal hyperplasia, leading to the slowing of cell reproduction. Acitretin is readily absorbed and widely distributed after oral administration. A therapeutic effect occurs after two to four weeks or longer. The physical state of Acitretin is yellow to greenish, yellow powder. Acitretin- (trade names soriatane and neotigason) is a second-generation retinoid. Biological half-life is 49 h (range 33 to 96 h) [6].

MATERIALS AND METHODS:

Materials: Acitretin was obtained as a gift sample from Glenmark Pharmaceuticals Ltd., Baddi (H.P.) India. Stearic acid, Oleic acid, Potassium dihydrogen phosphate, and Sodium dihydrogen were purchased from Central Drug House (P) Limited, and Tween 80, was purchased from Qualikem finechem. Limited and other reagents used were of analytical grade. Drug and polymer were evaluated spectometrically for purity, identity [7].

Method for Preparation of Nanostructured Lipid Carrier (NLC):

Preparation of Acitretin-loaded NLCs by Hot Homogenization Method: The drug Acitretin was dissolved in ethanol and mixed with an acetone solution containing a blend of lipid phase stearic acid and oleic acid. The mixture was then added slowly dropwise to a combination of tween 80 and sodium lauryl sulfate were used to stabilize NLC. The mixture was sonicated at 85 ºC for 30 min at 1200 rpm using magnetic stirrer [8]. This primary emulsion was converted to the NLC system using hot homogenizer at 15000 PSI. The emulsion obtained was subsequently cooled down to room temperature with continuous stirring, and the lipid was recrystallized to form a nanostructured lipid carrier (NLC) [9]. The obtained NLC dispersions were lyophilized for further study [10]. The formulations of different ingredients with composition were tabulated in the **Table 1**.

TABLE 1: FORMULATION TABLE

Sample	Drug (%w/v)	Ratio of Oleic acid and Stearic acid	Tween 80 (%v/v)	SLS (% w/v)
F 1	10 mg	7:3	1.0	1.0
F 2	10 mg	7:3	1.0	2.0
F 3	10 mg	7:3	1.0	3.0
F 4	10 mg	7:3	2.0	1.0
F 5	10 mg	7:3	2.0	2.0
F6	10 mg	7:3	2.0	3.0
F 7	10 mg	7:3	3.0	1.0
F8	10 mg	7.3	3.0	2.0
F9	10 mg	7.3	3.0	3.0

Characterization of Prepared Nanostructured Lipid Carrier (NLC's):

FT-IR Spectroscopy: An IR spectrum reveals the characteristic peaks of all functional groups present in the drug that helps in the identification of the drug and to determine the interaction of the drug with ingredients [11]. Infrared (IR) spectroscopy was performed using FTIR spectrophotometer (Shimazdu). The spectrum was recorded to 4000 to 600 cm^{-1} [12].

Differential Scanning Calorimetry Analysis (DSC): DSC analysis of Acitretin, stearic acid, physical mixture of stearic acid and oleic acid, and NLC formulations were performed to check the drug- lipid interaction in nanoparticle formulation and crystallinity of drug [13]. All samples (5mg) were heated in aluminum pans using dry nitrogen as effluent gas. The analysis was performed with a heating range of 25-250 ºC and heat at a rate of 10 ºC/min [14].

Preparation of Acitretin Loaded NLCs Gel: Based on the previously mentioned characterizations (particle size, entrapment efficiency, and *in-vitro* release profile of NLC dispersion) NLC formulation

with optimum physicochemical properties were selected [15]. NLC dispersion was incorporated into HPMC gel by continuous stirring. The dispersion was then homogenized by a homogenizer. The gel sonicated at least for 1 hr. under probe sonicator and allowed to stand overnight to remove entrapped air [16].

TABLE 2: FORMULATION CHART OF PLAIN HPMC GEL

Sample Code	HPMC %	Neutralizing agent (TEA) (% v/v)	pH
G 1	1.0	1.5	4.6
G 2	2.0	1.0	6.8
G 3	3.0	0.5	5.1

Evaluation of NLC Gel:

Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates [17].

Spreadability Study of NLC Gel: The spreadability of the gel formulations was determined at 24 h after preparation by measuring the increasing diameter of 1gm gel between two horizontal plates (20 × 20 cm^2) after 1 min. The standardized weight tied on the upper plate was 220gm. The spreadability was calculated by using the following formula.

$$S = M \times L/T$$

Where, S is spreadability, M is weight tied on upper slide, L is the length of the glass slide, **t** is time taken [18].

Visualization of Optical Microscopy: Vesicles Shape can be easily visualized by using optical microscopy.

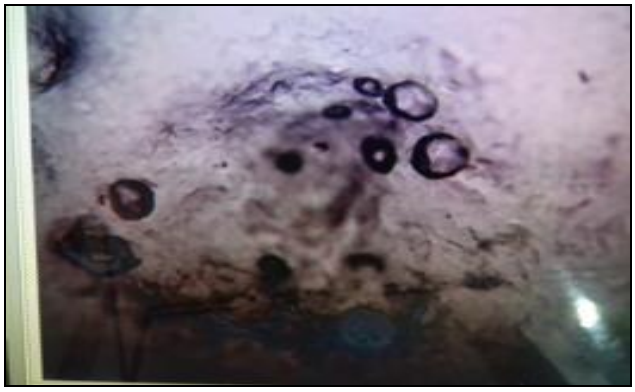

FIG. 1: F9 FORMULATION

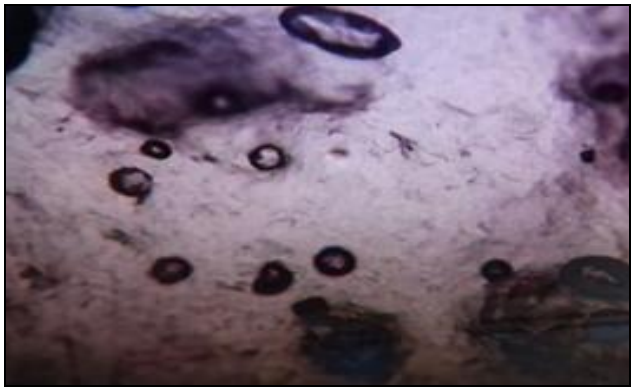

FIG. 2: F3 FORMULATION

Transmission Electron Microscopy (TEM): Morphological characterization of nanostructure lipid carrier was prepared by using transmission electron microscopy. Droplet structure is seen utilizing transmission electron microscopy (TEM). Sample drop is placed in a copper-coated with carbon which has been dried at room temperature, evaluate at a voltage of 120 KVA [19].

TABLE 3: ENTRAPMENT EFFICIENCY OF ACITRETIN NLC GEL

F. CODE	Lipids ratio	Tween 80(ml)	SLS(g)	E.E %
F1	7:3	1	1	61
F2	7:3	1	2	45
F3	7:3	1	3	75.2
F4	7:3	2	1	54
F5	7:3	2	2	74.3
F6	7:3	2	3	68.6
F7	7:3	3	1	62
F8	7:3	3	2	42
F9	7:3	3	3	84.1

Drug Entrapment Efficiency and Drug Loading Determination:

Determination of Entrapment Efficiency: A volume of 2.0 ml of drug-loaded sample was centrifuged at 2500 rpm for 90 min to separate the lipids and aqueous phase. The supernatant solution was separated and analyzed by using UV spectro-photometer at 352 nm using phosphate buffer pH 7.4 as blank. The drug entrapment efficiency of NLC was calculated as

$$\%\text{Entrapment Efficiency (EE)} = \frac{(\text{Wa} - \text{Ws})}{\text{Wa}} \text{x100}$$

$$\text{Drug loading(DL)} = \frac{Wa - Ws}{Wa - Ws + W1} x100$$

Where, EE is entrapment efficiency, DL is Drug loading, Wa stands for the mass of Acitretin added to the formulation, Ws is the analyzed weight of the drug in the supernatant, and Wl is the weight of lipid added.

***In-vitro* Release Study of Nanostructured Lipid Carrier (NLC) of Gel:** *In-vitro* drug release studies were performed by using a modified Franz diffusion cell to evaluate the Acitretin release profile from each formulation the synthetic cellophane membrane was mounted on the Franz diffusion cell. The receptor medium was approximately 45 ml and composed of phosphate buffer, pH 7.4, and stirred by the magnetic bar at 700 rpm to avoid various concentrations within the acceptor medium and to minimize stagnant layers. NLC gel dispersion (equivalent to 1mg of drug) was prepared in the donor compartment. During the experiments, the solution in the receptor side was maintained at 37±0.5 ºC. After a certain time interval, 3ml of the sample medium was withdrawn from receiver compartment through the side tube, and the same volumes of freshly prepared receptor medium were added. The samples were analyzed by UV-Visible spectrophotometer at 253nm [20].

RESULTS AND DISCUSSION:

Melting Point Determination (Capillary Method): The melting point of the drug was determined using the capillary method by the melting point apparatus. The drug was filled in the capillary and sealing the capillary from one end. The sample was situated in the thermometer apparatus, and when the drug started melted, its temperature was recorded and the melting point was 227 ºC.

FTIR spectra of Acitretin: The IR spectra of a pure drug Acitretin drug, lipids, physical mixture, and drug-loaded NLC dispersion were carried out in order to find out the incompatibility study. Pellet for the spectra were prepared using KBr press by spreading the drug sample between the discs. The disc was then placed in the FTIR, and the result was obtained, as shown in the **Fig. 3** and interpretation is shown in **Table 4**.

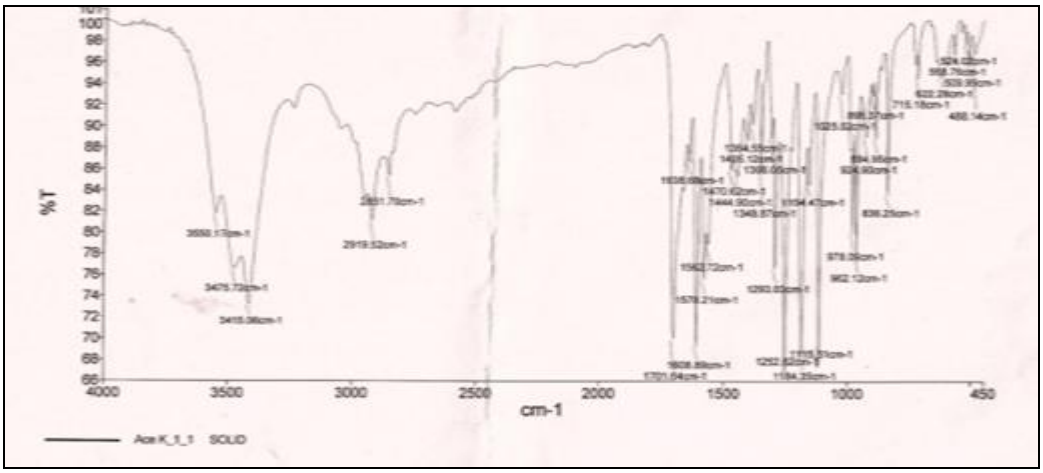

FIG. 3: FTIR SPECTRA OF ACITRETIN

TABLE 4: INTERPRETATION OF IR SPECTRA OF ACITRETIN

Functional groups	Peak value (cm^{-1})	Vibrations
Phenol group	3475.72	Phenolic (OH)
Alkyl Aldehyde	1700, 1608. 89	C=O and C=C vibration
Aromatic-O	1280.73	Ar-O stretching vibration

Compatibility Studies between the Drug and Polymer: For the drug mixture compatibility study, the samples were kept at 40 ºC & 75% RH for 4 weeks, and then the sample was evaluated. For the result of the compatibility study that there were no deviations in physical characteristics and optimized formulation. In IR spectra of the physical mixture were compared to IR spectra of drug Acitretin, which shows no interaction between drug and polymer result was obtained as shown in **Fig. 4**.

FTIR Spectra of Mixture:

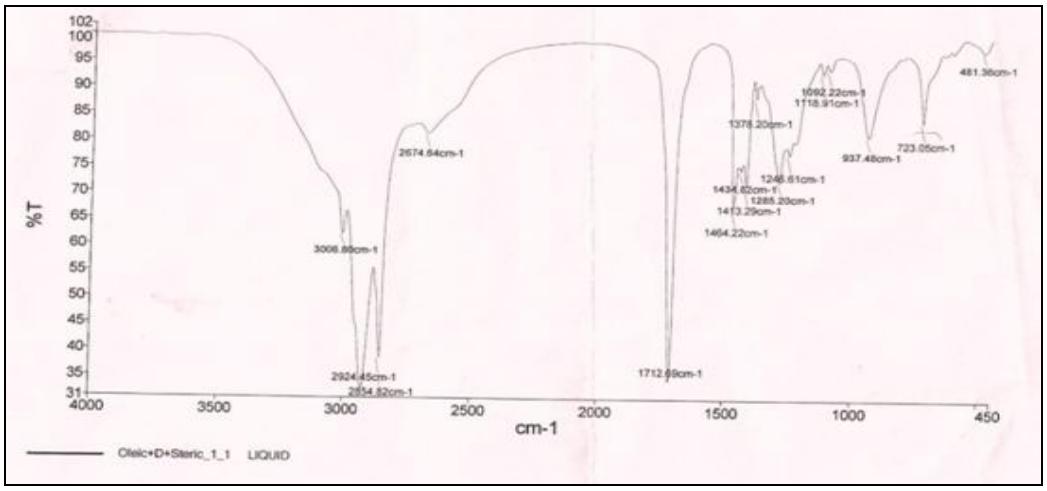

FIG. 4: FTIR SPECTRA MIXTURE OF ACITRETIN, OLEIC ACID AND STEARIC ACID

TABLE 5: INTERPRETATION OF SPECTRA OF MIXTURE [21]

Functional groups	Peak value (cm^{-1})	Vibrations
Phenol group	1464.69	Phenolic (OH)
Alkanes	3006.36	C-H stretch
Alkyl, Aldehyde	1711.59	C=O and C=C vibration
Phenol group	3,595	(OH)

Determination of DSC: DSC provides information about all physical properties of the sample as amorphous and crystalline nature and demonstrates the possible interaction between drug and polymers. The DSC curve of Acitretin showed single endothermic peak at 225 ºC. The DSC profile of optimize formulation shows a low-intensity peak at about 62 ºC and a rise in the curve above 220 ºC. By this, it is confirmed that in a formulation, crystallinity decreases. The thermal behavior of Acetretin and the physical mixture of drug and polymers are shown in **Fig. 5**, and no interaction takes place between drug and polymer [22].

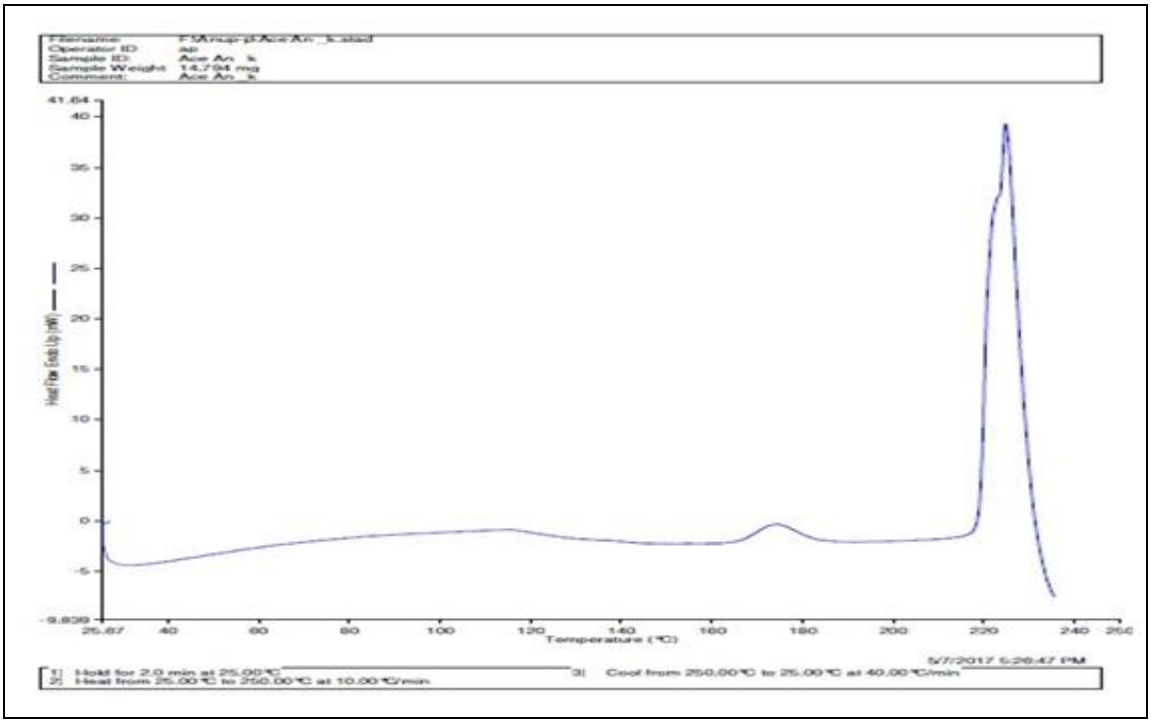

FIG. 5: DSC OF ACITRETIN

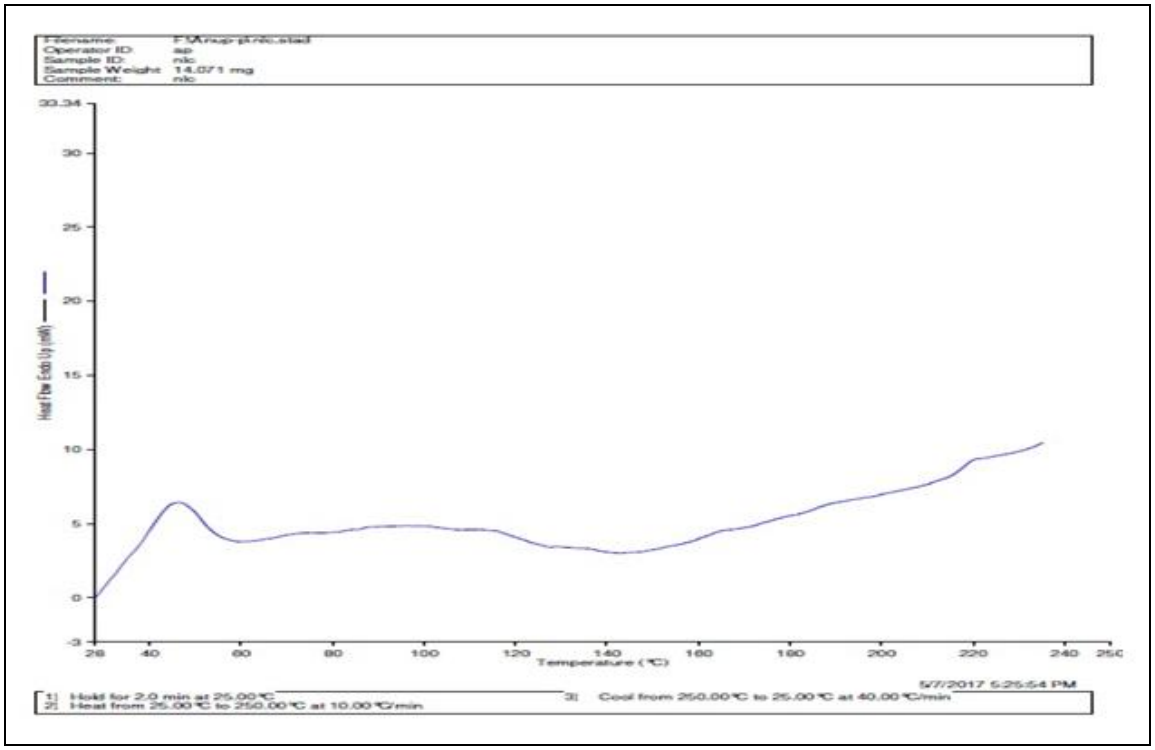

FIG. 6: DSC OF NLC MIXTURE

Evaluation of Plain HPMC Gel: Various evaluation parameters have been studied for evaluation of plain HPMC gel, such as percentage of HPMC, viscosity, and spreadability parameters as given in the **Table 6.**

TABLE 6: EVALUATION OF PLAIN HPMC GEL

S. no.	Percentage of gel	Viscosity (cgs)	Spreadibility (g × cm/sec)
1	1%	679	2.37
2	2%	2359	4.9
3	3%	10264	9.6

Determination of TEM: The micrograph of the NLC gel illustrated spherical droplets in the nanometer range. Selected nanostructure lipid carrier formulations (1gm) were diluted with 500ml of phosphate buffer pH 7.4. A drop of the emulsion was spread on a copper grid coated film and excess droplet were instantly removed using a filter paper. Then the grid was dried in the air at a certain room temperature before loading in the microscope. The overnight-dried sample was loaded on copper grid in vacuum chamber of TEM and obtained the TEM images. The results showed that the particles were spherical, and no drug crystals of particles visible in the **Fig. 7, 8**. The picture shows some gathering of particles due to the lipid nature of carriers. Some particle shapes are different from spherical due to the drying process of sample treatment.

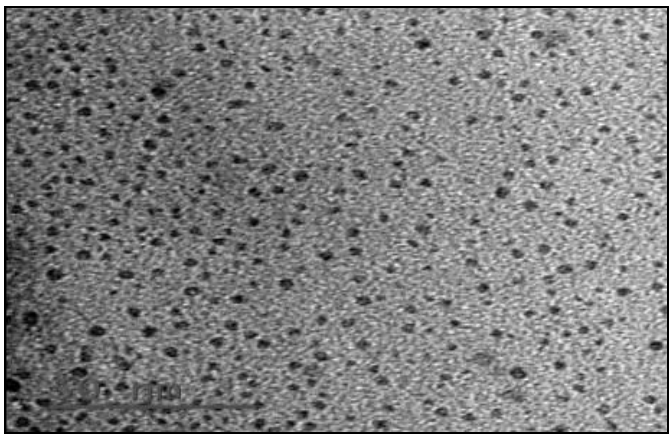

FIG. 7: TEM IMAGE OF NLC GEL AT 11000X OF THE F9G2 FORMULATION

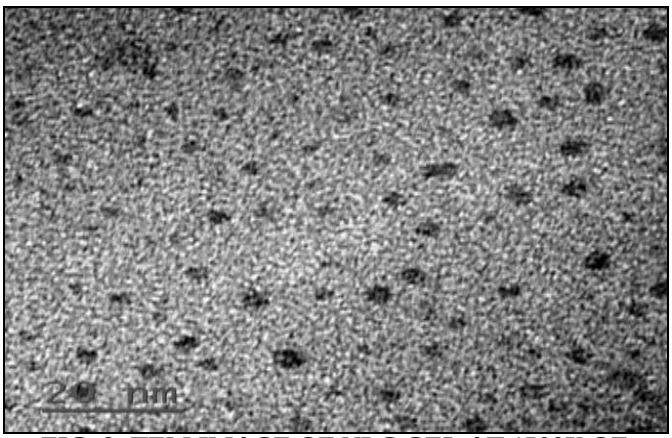

FIG. 8: TEM IMAGE OF NLC GEL AT 1500X OF THE F3G2 FORMULATION

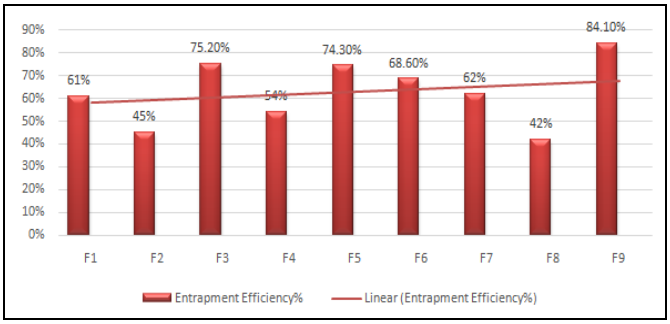

FIG. 9: GRAPHIC PRESENTATION OF %ENTRAPMENT EFFICIENCY

Entrapment Efficiency of Acitretin NLC Gel: Determination of the quantity of drug incorporated in NLC gel is important, since release characteristics.

The quantity of medicament encapsulated per unit weight of the nanoparticles is determined after the release of running medicament and solid lipids from the gel medium. The graphical representation is given below [23].

In-vitro Drug Release:

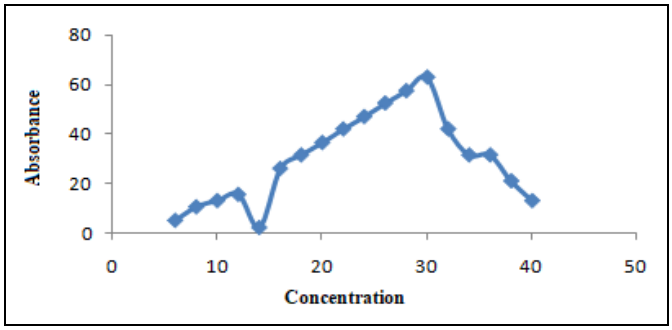

FIG. 10: *IN-VITRO* RELEASE OF FREE DRUG

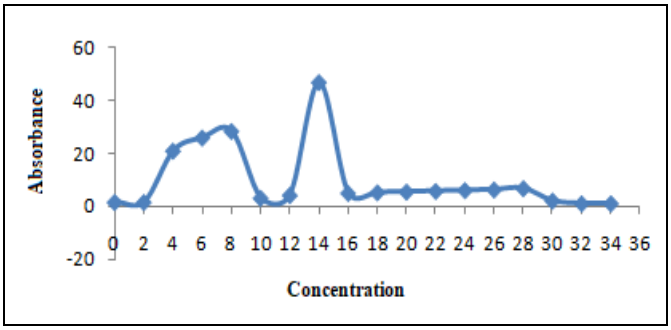

FIG. 11: *IN-VITRO* RELEASE OF DRUG AND GELLING AGENT

In-vitro Release of Optimized two Formulations Acitretin NLC Gel:

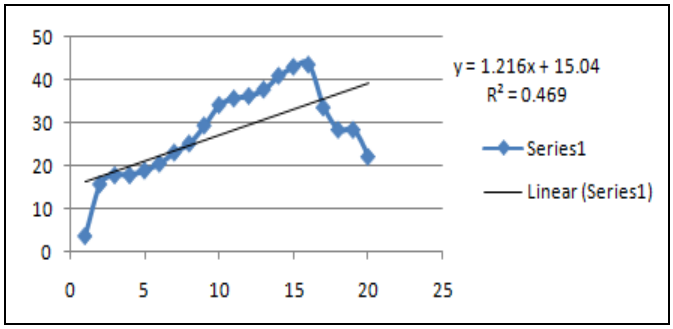

FIG. 12: F3 FORMULATION

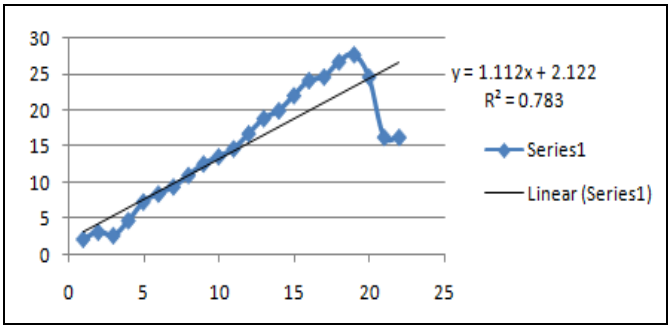

FIG. 13: F9 FORMULATION

TABLE 7: *IN-VITRO* RELEASE PROFILE OF FORMULATION OF F3G2 [24]

Time (min)	Abs. (nm)	Conc.	C(10)	% DR	% Cum
0	0.007	0.36	3.6	1.8	1.8
2	0.030	1.57	15.7	7.85	9.65
4	0.034	1.78	17.8	8.9	18.55
6	0.034	1.78	17.8	8.9	27.45
8	0.036	1.78	18.9	9.45	36.9
10	0.039	1.89	20.5	10.25	47.1
12	0.044	2.05	23.1	11.57	58.6
14	0.048	2.315	25.2	12.6	71.2
16	0.056	2.52	29.4	14.7	85.4
18	0.065	2.94	34.2	17.1	102.5
20	0.068	3.42	35.7	17.85	120.3
22	0.069	3.57	36.3	18.25	138.4
24	0.072	3.63	37.8	18.9	157.3
26	0.074	3.78	38.9	19.45	176.7
28	0.078	3.89	41.0	20.5	197.2

30	0.082	4.10	43.1	21.55	218.7
32	0.083	4.31	43.6	21.8	240.5
34	0.064	4.36	33.6	16.8	257.3
36	0.054	2.84	28.4	14.2	275.5
38	0.054	2.84	28.4	14.2	285.7
40	0.042	2.21	22.1	11.05	296.7

TABLE 8: *IN-VITRO* RELEASE PROFILE FORMULATION OF F9G2

Time (min)	SQRT	Abs. (nm)	Conc.	C(10)	% DR	% Cum
0	0	0.003	0.15	1.5	0.015	0.015
2	1.414	0.008	0.42	4.2	2.1	2.115
4	2.0	0.009	0.47	4.7	2.35	25.61
6	2.449	0.026	1.36	13.6	6.8	32.41
8	2.828	0.028	1.47	14.7	7.35	39.76
10	3.16	0.031	1.63	16.3	8.15	47.91
12	3.46	0.034	1.78	17.8	8.9	56.81
14	3.741	0.036	1.89	18.9	9.45	66.26
16	4.00	0.38	2.0	20.0	10.0	76.26
18	4.242	0.041	2.15	21.5	10.75	87.01
20	4.472	0.043	2.26	22.6	11.3	98.31
21	4.582	0.046	2.42	24.2	12.1	110.41
22	4.690	0.048	2.52	25.2	12.6	123.01
24	4.898	0.052	2.73	27.3	13.65	136.6
26	5.099	0.054	2.84	28.4	14.2	150.86
28	5.291	0.056	2.94	29.4	14.7	165.56
30	5.477	0.058	3.05	30.5	15.25	180.8
32	5.656	0.062	3.26	32.6	16.3	197.1
34	5.830	0.064	3.36	33.6	16.8	213.9

Pharmacokinetics Model of F9G2 Formulation: [25]

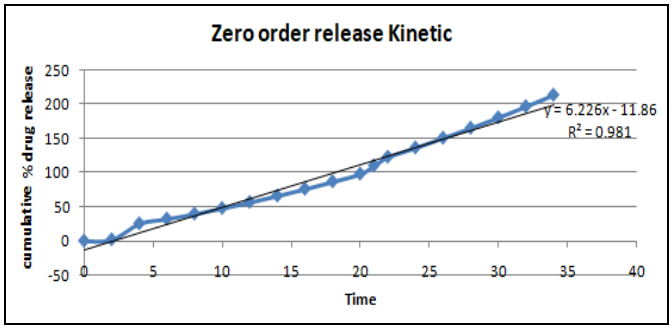

FIG. 14: ZERO ORDER RELEASE KINETICS OF F9G2

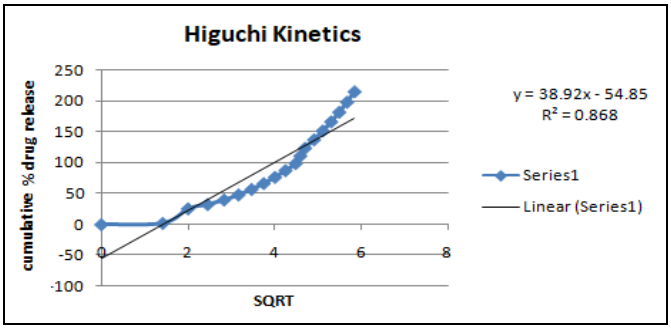

FIG. 15: HIGUCHI KINETICS OF F9G2

CONCLUSION: NLCs dispersion of Acitretin was prepared by hot homogenization method using the different concentration and mixture of surfactants as shown in formulation chart. As given formulation chart revealed that F1, F2, F3, F4, F5, F6, F7, F8 and F9 formulation were prepared with the mixture of oleic acid and stearic acid in ratio of 7:3 and different concentration of surfactant. When the concentration of one surfactant to kept constant and the other is varied in increasing order and vice versa, the result revealed that when the concentration of both surfactants is same and in a maximum amount, the NLC was found to be round shape with good entrapment efficiency as in (1; 1, 2; 2, 3; 3). The entrapment efficiency of NLC formulation ranged from 61 to 84.1%, respectively.

The experimental results showed that the various surfactants had a critical effect on entrapment efficiency. Formulations F2 to F8 showed poor entrapment efficiency that may be due to poor formation of NLCs. The formulation showing good entrapment efficiency (F3, F5, and F9) were selected. The HPMC plain gel was prepared at different concentrations, and evaluation is performed, and it concluded that 2% HPMC plain gel is compatible with the optimized prepared NLCs and were used to prepare NLC gel

formulations. The reason behind the selection of these three formulations was that the ionic and non-ionic surfactant and were used in combination it gives transparent dispersion having good stability. It can be that ionic surfactant (SLS) provide the electrostatic stabilization while non-ionic surfactant (Tween 80) given the stearic stabilization to NLC gel dispersion.

The *in-vitro* release profile of Acitretin NLCs gel formulations was carried out for 48 h by using Franz diffusion cell. The F9G2 formulation showed better drug release than other formulations due to the combination of surfactant in the maximum amount. The *in-vitro* permeation studies of optimized NLCs gel (F9G2) using cellophane membrane barrier was carried out using Franz diffusion cell. The release kinetics of selective F9G2 was evaluated for zero-order and Higuchi's diffusion, as shown in the figure, respectively. The *in-vitro* release study of optimized formulation (F9G2) revealed the biphasic release pattern of NLCs gel system and sustained release for a prolonged period of time.

ACKNOWLEDGEMENT: This research work is supported by the University Institute of Pharmacy, CSJM University Kanpur, Uttar Pradesh, India. We also would like to thank Glenmark Pharmaceuticals Ltd., Baddi (H.P.), India, for a free sample.

CONFLICTS OF INTEREST: There are no conflicts of interest among all the authors with the publication of the manuscript.

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How to cite this article:
Kumari K, Sachan AK, Singh S and Kapoor A: Formulation and evaluation of nanostructured lipid carrier for the management of psoriasis. Int J Pharm Sci & Res 2021; 12(6): 3381-90. doi: 10.13040/IJPSR.0975-8232.12(6).3381-90.