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ORIGINAL ARTICLE

Formulation and evaluation of Gel containing Adapalene Loaded Nanostructured Lipid carrier

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ABSTRACT
The current research was used to investigate the development and evaluation of Nanostructured lipid carrier loaded Adapalene- based gel to enhance solubility and efficacy as well as to prolong drug release for an extended period of time *after topical drug administration. The nanostructured lipid carriers of adapalene were formulated by ultrasonication method. Glyceryl monostearate was act as a liquid phase while distilled water was utilized as an aqueous phase. Tween 80 was used as a surfactant. Oleic acid was used as an oil phase and for gelling agent carbapol was used. The parameters such as grittiness, viscosity, and pH of the prepared gel were studied. In vitro drug release study was performed by Franz's diffusion cell. Adapalene gel pH was determined to be 6.8, a value of pH within which transdermal preparations can be used effectively. Gel viscosity was found to be 800 cps when concentration of polymer was constant. Optimized F1 batch were evaluated for drug release upto 6 h (85.72±3.84%), % entrapment efficiency (87±0.05%). The stability study was implemented for optimized batch at 25±2oC temperature and 60±5 %RH for 90 days. Optimization study was successfully conducted using 32 factorial designs. From this study it concludes that NLC transdermal drug delivery formulation is promising alternative technique to the parentral and oral formulation.*
Keywords; *Adapalene, Nanostructure Lipid Carrier (NLC), Homogenization, pH Determination, Spreadability*

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INTRODUCTION

Adapalene belongs to family of drugs used to treat acne and frequently prescribed as topical formulation. It can be used in the treatment of keratosis pilaris additionally for skin conditions. It is sold under the trade names Differin and Adaferin by Galderma. Limited Absorption through the skin; only trace amounts (<0.25 ng/mL) of initial substance have been found in patients plasma having acne ensuring chronic topical application of adapalene [1]. Topical drug delivery systems are localized drug delivery systems for targeted delivery of active agents via skin to treat the cutaneous disorder. These systems are generally used for local skin infections. Drug delivery through the dermal route is becoming growingly popular due to its benefits and inexpensiveness. The dermal route has been a recommended route of drug consumption during the last decades. On the other hand conventional topical drug delivery systems limit less retention and minimum bioavailability. This limitation can be minimized by considerable research to develop novel topical drug delivery systems targeting to enhance safety, efficacy and to reduce side effects [2]. A topical drug delivery system is the more convenient route of localized drug delivery anywhere in the body by routes as ophthalmic, rectal, vaginal, and skin. An active agent applied to the skin for their action involves antiseptics, antifungal agents, skin emollients, and protectants. To reduce the risks and disruption of intravenous therapy and the diverse conditions of absorption like change in pH, the appearance of enzymes, gastric emptying time are other applications of topical preparations. The objective of the current research is to investigate the development and evaluation of nanostructured lipid carrier loaded adapalene-based gel to enhance solubility and efficacy as well as to prolong drug release for a lengthy period after topical drug administration of adapalene. To perform pre-formulation studies of drug and excipients, to carry out compatibility studies of drug and excipients, to prepare the nanostructured lipid carrier loaded adapalene based gel, to evaluate the NLC's loaded adapalene based gel for % entrapment efficiency, In-vitro Drug

Release Studies, Viscosity Measurements, pH Determination, Spreadability, Antiacne activity. Nowadays, solid lipid nanoparticles (SLN) and Nanostructured lipid carriers (NLC) have been already scrutinized as carrier systems for different formulations. As compared to parenteral or oral drug administration topical drug delivery is more preferred as it reduces drug metabolism by first- pass effect. [3,4,5]

The poorly water-soluble drug; can be transformed to nanoparticles using high-pressure homogenization. Considering the molecular weight of the two fractions in the coupled molecule, i.e., of the drug itself and the lipid part, a drug loading of approximately 30–50% is achievable (e.g., as reported for diminazene of about 33% formulated as diminazene diacetate–acid unite with palmitic acid/stearic acid [6]. Nanostructured lipid carriers (NLC) are produced using a mixture of solid lipids and liquid lipids (oils). The overall solid content of NLC could be grown up to 95%. SLN is formulated from solid lipids only. Therefore, after preparation, at least a part of the particles crystallizes in a higher energy modification (α or β`). During storage, this mitigation may be altered to the minimum energy, maximum ordered β modification. Due to this modification's high degree of order, the number of faults in the crystal lattice is small, which leads to drug expulsion. NLC has been developed to reduce the drawbacks related to SLN. They are the second generation of lipid nanoparticles. Compared to SLN, NLC show a maximum loading capacity for active compounds by creating a minimum ordered solid lipid matrix, i.e., by mixing a liquid lipid with the solid lipid, a higher particle drug loading can be executed. Therefore, the NLC has an increased drug loading efficacy in comparison to SLN.

MATERIAL AND METHODS

Materials:

Adapalene was purchased from Intas, Ahmedabad, India. Glyceryl Monostearate, Oleic Acid, Tween 80, Carbopol, Ethanol Was Purchased from Research Lab Fine Chem Industries. The chemicals were employed as obtained without any purification. All remaining reagents employed in this analysis were of analytical grade.

Formulation of NLC Based Gels

Procedure for preparation of NLC's by high shear homogenization followed by ultrasonication method.

In this method lipids (glyceryl monostearate and oleic acid: 2:1 ratio) were melted at 80O C above its melting point. Then Adapalene was added to the molten lipids. Surfactant solution was prepared by dissolving tween 80 in distilled water and heated at 60oC. The dispersion was kept at 80o c and 60 o c respectively, until it appeared optically clear. The lipid phase and a aqueous phase were prepared separately. Hot surfactant solution was then added to lipid phase dropwise and was stirred for 10 minutes on magnetic stirrer. Then dispersion was further mixed using high shear homogenizer at 8000 rpm for 30 minutes followed by ultrasonication for 15 minutes by using a probe sonicator. Then the NLC's so formulated were allowed to cool at room temperature which were further used for characterization. The NLC dispersions were gelled using different polymers like Carbopol934, and HPMC which act as a gelling agent. Depending on the compatibility with Nanoparticulate dispersion, feel aesthetic appeal and ease of spreadability, Carbopol 934 was selected as the gelling agent. Carbopol934 (0.8%) was dispersed using an overhead stirrer at the speed of 800 rpm (Remi, Mumbai, India) until homogeneous dispersion. Different concentrations of Carbopol934 ranging from 0.25 to 1.5 % were used for gelling. The carbopol934 dispersion was neutralized using 0.05%(w/w) triethanolamine. The concentration giving the optimum viscosity was chosen for further studies.[8].

3^2 Full Factorial Design

A 3^2 full factorial design was constructed where the excipients were selected as factors. The levels of these factors were selected based on initial studies and observations. All the other formulation characteristics and processing variables were kept uniform throughout the study period. Polynomial models containing interaction and quadratic terms were formed for the total response variables using the multiple linear regression analysis (MLRA) approach.

The polynomial equations can be used to conclude after considering the magnitude coefficient and the 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.

The statistical validity of the polynomials was confirmed based on analysis of variance (ANOVA) provision in the Design-Expert software. The level of significance was considered at $p<0.05$. The best-fitting mathematical model was adopted depending on the comparison of several statistical parameters, which includes the coefficient of variation (CV), the multiple correlation coefficient(R2), the adjusted multiple correlation coefficient (adjusted R2), and the predicted residual sum of squares (PRESS), provided by the software. Press indicates how well the model fits the data, and for the chosen model, it should be small

relative to the other models under consideration. The 3-D response surface graphs and the 2-D contour plots were also produced by the Design-Expert software (version 12).These plots are very helpful to see the interaction effects of the factors on responses.

Table 1: Formulation of NLC's Based Gel of Adapalene

Batch	Drug (gm)	GMS (gm)	Factor 1 O.A. (gm)	Factor 2 Tween 80 (gm)	Carbopol 934 (gm)	Water (q.s)	Triethanolamine (q.s)
F1	0.1	1	0.2	0.4	0.08	10	q.s
F2	0.1	1	0.2	0.95	0.08	10	q.s
F3	0.1	1	0.2	1.5	0.08	10	q.s
F4	0.1	1	0.5	0.4	0.08	10	q.s
F5	0.1	1	0.5	0.95	0.08	10	q.s
F6	0.1	1	0.5	1.5	0.08	10	q.s
F7	0.1	1	0.8	0.4	0.08	10	q.s
F8	0.1	1	0.8	0.95	0.08	10	q.s
F9	0.1	1	0.8	1.5	0.08	10	q.s

Evaluation of Nanostructured Lipid Carrier (NLC)

Percent entrapment efficiency

Percent entrapment efficiency (% EE) was determined by measuring the concentration of the unentrapped free drug in suspension. In 1ml of NLC dispersion,9 ml ethanol was added. Centrifuge the mixture for 30 min at 500 rpm. The solution was filtered and diluted with ethanol and Adapalene content was determined spectrophotometrically at 312 nm. The percent entrapment efficiency (EE%) and drug loading percentage(DL%) were calculated by using the following Equations.[9]

$$\%EE = \frac{Amount\ of\ drug\ added - Amount\ of\ drug\ in\ supernatant}{Amount\ of\ drug\ added} \times 100$$

In-vitro Drug Release Studies

The drug release from the formulation was determined by using the apparatus known as Franz's diffusion cell. 1 gm of gel was spread uniformly on the surface of the egg membrane (previously soaked in the medium for 24 h) and was fixed to the one end of tube.100ml of acetate of phosphate (pH 5.5) contained in 100 ml beaker. The assembly was placed on the thermostatic hot plate with a magnetic stirrer and maintained at temperature 37°C±2°C. The contents were stirred using the magnetic bar at 100 rpm for a period of 24 h, 5ml of samples were withdrawn at different time intervals and replaced with 5 ml of fresh buffer and after suitable dilution, the sample was analyzed at 312 nm for Adapalene[9].

Viscosity Measurements

The viscosity of NLC'S based gel was determined by using Brookfield rotational viscometer at various rpm. The spindle no.62, was selected based on the viscosity of the gel. The dial reading was taken at rpm and viscosity was measured. Each reading was taken after equilibrium of the sample at the end of 2 minutes. The samples were repeated three times. Based onthe evaluation parameter being out for trial batches, the result of the F1 batch was found to be satisfactory in all attributes and hence selected for further evaluation[10].

pH Determination

The pH of NLC's based gel was measured on a digital pH meter calibrated using pH 4.0 and 7.0 standard buffers before use. NLC's based gel 2.5 gm was weighed accurately and dispersed in 25 ml water. The measurement of pH of formulation was done in triplicate and mean values were calculated.

Spreadability

Spreadability is calculated by apparatus suggested by multimer. A ground glass slide is fixed on this block. A sample of 0.5 gm of NLC's based gel under study is placed on this ground slide. The gel is fixed on the beach formula was pressed between two slides and a 500 gm weight is placed on the top of two slides and left for 5 minutes to expel air and to provide a uniform film of two slides and left for about 5 minutes to expel air and to provide a uniform film of the NLCs based gel between two slides. Excess of the gel is scraped from the edges. The top plate is then subjected to pull the weight. With the help of stirring attaches to the

hook and the time required by the top slide to cover the distance is noted. A shorter interval indicates better spreadability. Spreadability was calculated by using the formula.

$$S = \frac{M \times L}{T}$$

Where, S= spreadability, l-length of a glass slide, m=weight tied to upper slide, t=time taken to separate the slides [10].

Stability Study

The optimized formulation showing optimum gel strength, pH determination and drug release rate were selected for stability studies. The formulation was filled up in glass vials covered with rubber caps and kept in a stable chamber (Remi Instruments Ltd, Mumbai, India) at ambient temperature and humidity for 3 months. Samples were withdrawn at 0,30, 3, 6, 9, and 12 months after the formulation was created. Periodic observations of the physical stability of gel were made for appearances. The formulation was evaluated for gel colour, appearance, pH, and %EE for periodic intervals of one month [9].

RESULT AND DISCUSSION

Uv spectroscopic analysis

The calibration curve of adpalene was prepared in phosphate buffer pH 5.5. it showed maximum absorption at 312nm. The straight line obtained in phosphate buffer had regression coefficient of 0.98841. linearity was found in the concentration range from 2-10µg/ml.

Percent entrapment efficiency

Different batches of adapalene loaded nanocarrier gel entrapped the drug in a range of 59.9% to 87%. The highest percentage of drug entrapment was found in batch F1. Because the drug has more solubility in the liquid lipid than the solid lipid, the percentage of encapsulated drug increases as the number of defects in the crystal lattice increases in the presence of liquid lipid in contrast to solid lipid. The high EE values found in this work indicate that the lipid and surfactant compositions used are ideal for the entrapment of formulations in nanostructured lipid carriers.

Table 2: Percent entrapment efficiency of F_1-F_9 Batch

Batch	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
P.E.E.± SD	87±0.05	82.2±0.02	84.1±0.04	75.8±0.03	68.5±0.04	73.8±0.02	83.7±0.03	81.5±0.02	58.9±0.01

SD standard deviation, n=3, P.E.E.: percent entrapment efficiency

In-vitro drug release

In vitro release studies of NLC formulation were performed using the Franz diffusion cell with Cellophane membrane to determine the release of adapalene from the nanostructured lipid carrier. In vitro drug diffusion study was carried out by using Phosphate buffer pH5.5 as diffusion media. The release profiles showed an inflexion point that indicated the NLC had formed in the donor compartment of diffusion cells. Due to its occlusive properties, NLC's based gel showed a higher cumulative amount of permeation than NLC's F1 batch because the nanostructured lipid carrier displayed a higher percentage of drug release in vitro.

Table 3: In vitro release profile of NLCs loaded Adapalene

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	12.61 ±1.22	14.22 ±1.25	18.61 ±1.77	13.24 ±1.44	16.95 ±1.16	23.81 ±2.24	22.41 ±2.27	13.65 ±1.42	15.8 ±1.56
1	27.39 ±1.36	22.34 ±1.66	29.71 ±1.51	23.84 ±2.29	24.97 ±1.37	32.54 ±1.92	32.29 ±1.18	21.71 ±1.79	23.47 ±1.93
2	53.49 ±1.11	32.52 ±1.92	34.52 ±1.98	38.75 ±2.56	37.34 ±1.49	41.36 ±2.61	45.88 ±1.39	32.22 ±1.83	29.08 ±2.22
3	62.05 ±2.52	46.51 ±2.26	48.6 ±2.18	52.36 ±3.42	47.63 ±1.58	50.12 ±2.48	57.79 ±1.86	48.72 ±2.34	34.89 ±3.16
4	72.14 ±2.78	59.39 ±2.50	67.32 ±2.57	60.93 ±3.60	55.69 ±2.66	59.74 ±2.73	63.44 ±1.98	62.26 ±2.56	38.3 ±3.34
5	79.5 ±2.88	71.22 ±3.24	77.85 ±2.68	68.01 ±3.76	60.88 ±2.89	66.06 ±2.82	69.24 ±2.15	70.55 ±2.88	42.2 ±3.76
6	88.93 ±3.12	79.31 ±3.62	84.64 ±2.94	74.47 ±2.14	67.71 ±2.96	72.85 ±3.15	77.02 ±2.32	81.62 ±2.92	45.63 ±3.88

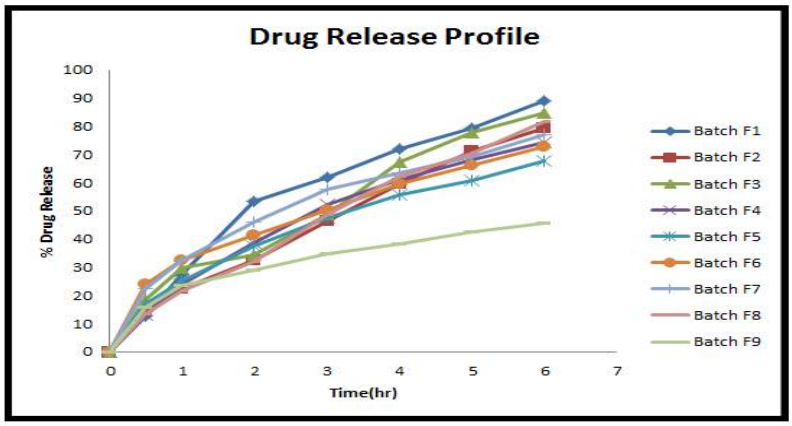

Fig 1: Data showing drug release of NLC dispersion vs time of nine batches [mean±SD, n=3]

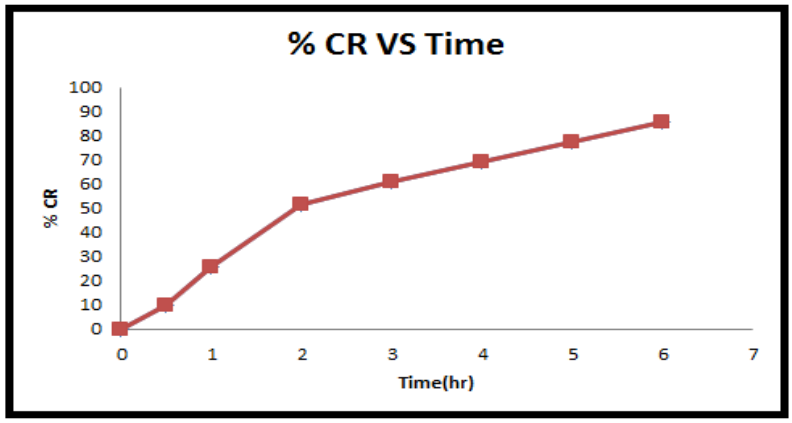

Fig 2: data showing cumulative drug release vrs time of NLC gel (Data of F1 batch)

Rheological behaviour of NLC's based gel

The viscosity of NLC's was determined by using Brookfield rotational viscometer at various rpm. The viscosity of NLC's was determined by using Brookfield rotational viscometer at rpm using spindle no.62. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times. It was revealed that the optimized formulation of F1-F9 exhibits pseudoplastic flow behavior. The characteristic concavity of the program toward the shear rate axis indicates that the developed formulation exhibited pseudoplastic. This pseudo-plasticity results from a colloidal network structure that aligns in the direction of shear, thereby decreasing the viscosity as the shear rate increases.

Table 4: Viscosity value of NLC's based gel formulation

Rpm	Viscosity(cps)								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
10	2100	3240	2580	9180	10560	1800	7860	36470	6600
20	1660	3120	1740	6180	8190	1020	6210	17670	3780
30	1350	2270	1260	4600	6500	900	5600	15800	2840
50	980	1990	920	4400	3230	720	5200	9190	2090
60	800	1386	870	4000	3100	660	4900	7318	1800

pH measurement

The pH of each formulation was determined by using a pH meter. The pH meter was first calibrated using solutions of pH 4.0 and pH 7.0. The pH of all the formulations was found to be in the range of 6.0-7.0, which is around the skin pH.

Table 5: Data showing pH measurement of Formulation

Batch Code	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
pH	6.8	6.2	6.6	6.4	6.0	7.0	6.5	6.9	6.3

Spreadability

The Carbopol used in this batch was 0.8%(0.8 gm) as Carbopol is a polyacrylic acid derivative. It is a form that creates good gel conditions and gives a smooth texture. The same composition was used for the formulation of F1-F9 batches ad gave the same texture for nine batches(F1-F9).It showed that it will give a proper response when applied to the skin. The spreadability of batch F_1 was found to be 2.77 cm. The obtained value indicated a good spreadability of the obtained gel preparations.

Table 6: Data showing spreadability of all batches

Batch Code	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Spreadability (gm.cm/sec)	2.77± 0.05	2.18± 0.04	2.05± 0.04	0.6± 0.02	0.3125± 0.01	0.57± 0.01	0.56± 0.001	0.39± 0.2	0.77± 0.02

Stability Studies

Optimized formulation (F_1 batch) was filled into aluminum tubes as the final dosage form which was subjected to stability studies to determine its physical stability. The stability study was carried out for optimized formulation (F1 batch) at 25±2°C temperature and 60±5% RH for 90 days. From the stability study of the optimized batch, it was found that the NLC based gel remained stable even after exposure to high temperature and moisture conditions, indicating that Adapalene remained chemically stable in NLCs based gel.

Table 7: Data Showing Stability Studies

Parameters	0 day	30 days	60 days	90 days
Colour	Semitransparent White	Semitransparent White	Semitransparent White	Semitransparent White
Visual Appearance	Smooth	Smooth	Smooth	Smooth
pH Determination	6.8	6.6	6.7	6.5
%EE	85.50±0.4	85.41±0.04	85.22±0.06	85.09±0.06

ATR of adapalene

The interaction of the drug with other excipients was studied by infrared spectroscopy. The IR spectra of pure drug adapalene with polymer were recorded by ATR-IR over the range of 4000cm^{-1} to 600 cm^{-1}. The spectra of formulated gel and pure drug were also recorded.The IR spectrum of Adapalene is shown in figure 3.

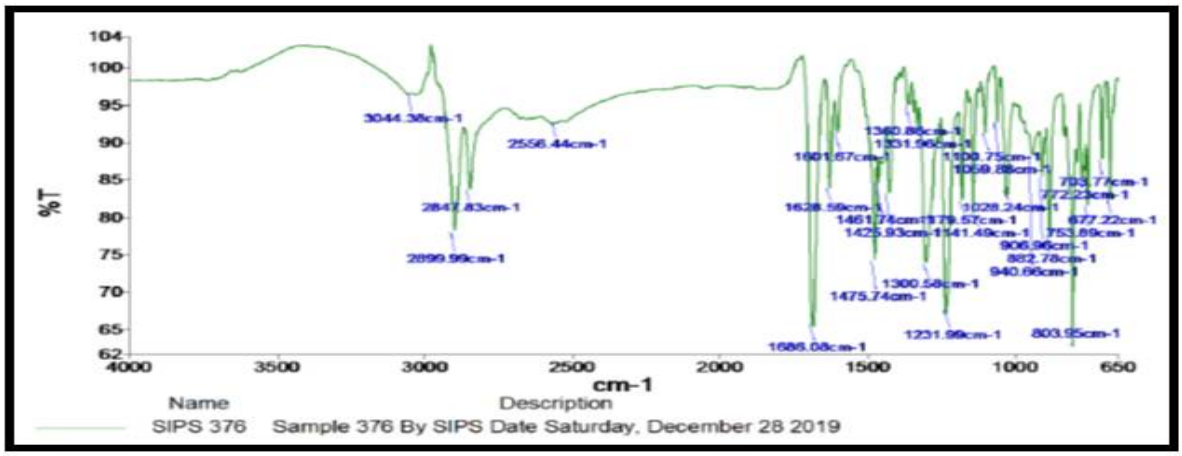

Fig3 :ATR-IR scan of Adapalene

Release Kinetic Methods

Based on In vitro release profile, batch F1 was considered as an optimized batch.Thus, the F1 batch was selected for the study of drug release kinetics.

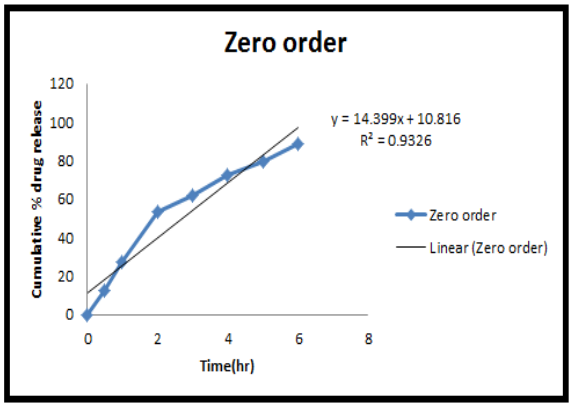

Fig 4: Release kinetic model by zero order

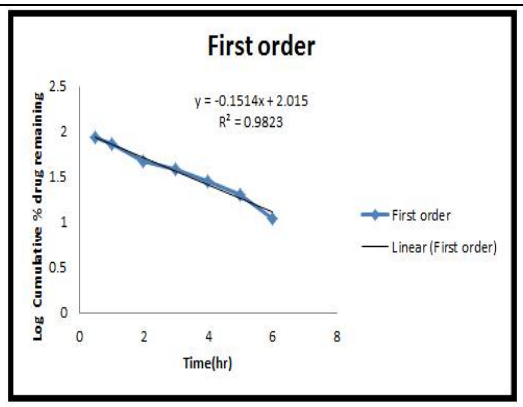

Fig 5: Release kinetic model by First Order

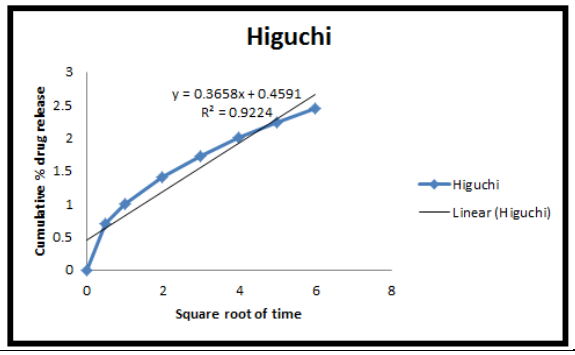

Fig 6: Release kinetic model by Higuchi

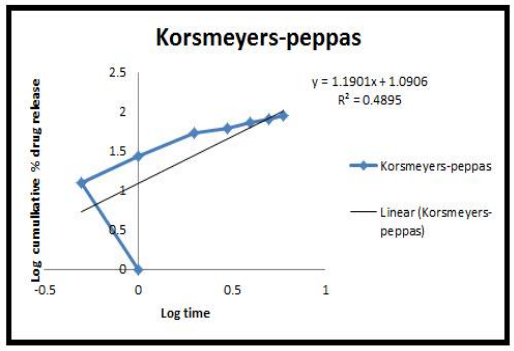

Fig 7: Release kinetic model by Korsmeyers-peppas

The batch F_1 shows the First order model release mechanism and a significant correlation coefficient R^2=0.9823, suggesting that the drug release under physiological conditions occur primarily by diffusion. The mechanism of drug release by diffusion mechanism is further confirmed by plotting zero order, first order, Higuchi, Korsmeyer Peppas plot as log percent cumulative vs log time. Amongst these model's formulation of NLC gel of Adapalene follows First order release mechanism. This indicates that the test product follows matrix diffusion-based release kinetics. The release data from NLC's loaded Adapalene were fitted to the different models. The value of $R^2$was found to be highest for the First order model(R^2=0.9823). This indicates that the test product follows matrix diffusion-based release kinetics.

CONCLUSION

The adapalene loaded nano structured lipid carrier were formulated which aimed for the treatment of acne for delivering the drug in controlled manner. Based on the results of this study, it can be concluded that Excipients and the drug did not interact significantly, indicating that the drug was compatible with the excipients used. As per the optimized Batch (F1) effect of oleic acid (0.2g) and tween 80 (0.4g) on formulation shows optimal gelation. Following in vitro release tests, the f1 batch shows good performance characteristics. Based on above data, it demonstrates that smart nanostructured formulations easily deliver drugs to the target sites with decreased dosage frequency and in a (spatial/temporal) controlled manner to alleviate the side effects experienced with conventional therapies.

ACKNOWLEDGEMENT

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