Sys Rev Pharm 2020; 11(6): 794 – 802
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Formulation, Development and Evaluation of Nanostructured Lipid Carrier (NLC) Based Gel for Topical Delivery of Diacerein.

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Article History: *Submitted: 10.04.2020* *Revised: 14.05.2020* *Accepted: 22.06.2020*

ABSTRACT
Diacerein Nanostructured Lipid Carrier (NLC) topical gel was formulated to treat inflammation and the conditions associated with acceleration and prolonged action. Dispersion of nanoparticles was correctly gelled and characterized in pharmacology, pH, proliferation, rheology and in-vitro releases. NLC-based gel safety was assessed with primary skin irritation studies, and pharmacodynamics research confirmed efficacy. Non-steroidal anti-inflammatory drugs (NSAIDs), while known as extreme gastrointestinal and vascular adverse effects without resolving the underlying systemic cartilage injury, are the most widely used agents of osteoarthritis (OA) pain management. Many doctors are elderly. Thus, Diacerein is the preferred drug for strong analgesic benefit in serious OA discomfort. Diacerein is cost-effective and dose-efficient. Gel formulation offers easy application in all quantities at a pain site. The NLC-based gel demonstrated quicker start and extended habits up to 24 hours.
Keywords: Diacerein, Nanostructured lipid carriers (NLC), Topical, Anti-inflammatory, Prolonged action.
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DOI: 10.31838/srp.2020.6.116

INTRODUCTION

Nanostructure lipid carriers (NLCs) are the latest generation of lipid nanoparticles, drawing considerable interest as the latest colloidal drug carriers for topical application. NLC was designed to address SLN limitations. SLN consists of solid lipids, whereas NLC consists of a specially mixed lipid (long-chain) and a liquid (short-chain) mixture, preferably 70:30 with 99.9:0.1. The resulting lipid particle matrix shows a depression in the melting point compared to the original solid lipid but remains strong at body temperature [1]. SLN 's disadvantages are decreased pharmacological load strength, drug expulsion during storage and relatively high dispersion water content (70%-99%)[2, 3]. NLC has a greater drug load potential than SLN for certain active compounds and avoids or minimizes possible expulsion during storage [2]. Liquid lipid solubility is higher than solid lipid for a number of drugs, which improves drug loading [4]. NLC has many features for the topical application route. Carriers consist of low toxicity, moderate cytotoxicity physiological and biodegradable lipids [5]. Small lipid dimensions ensure close contact with the stratum corneum and can increase drug flow through the skin, allowing regulated releases from these carriers due to their definite lipid matrix [5, 6].

Diacerein is an anthraquinone derivative used for osteoarthritis. This is expected to act by inhibiting the active metabolite rhein of interleukin1β. Diacerein. Through decreasing expression of matrix metalloproteinase (MMP)-1 and -3 matrix, and by enhancing the tissue regulator of matrix metalloproteinases to decrease activities of some of the MMPs, the rhein prevents cartilage degradation. Rhine's anti-inflammatory effect reduces interleukin-1beta activity, which plays a significant role in reducing extracellular matrix production, MMP activity and continuous inflammation. Rhein decreases an unexplained cause of pathological osteoblast synthetic activity.

MATERIALS AND METHODS

Diacerien was obtained from Ipca Pharma as a gift sample. Capmul MCM C8 was bought from Abitec, Capmul MCM. Oleic acid was obtained from Loba products, Carbopol 940. Pluronic F68 obtained stearic acid from Gattefosse from Sigma Aldrich, Tween 80 from Fine chem.

Screening of components

One of the crucial factors evaluating the drug's lipid loading capability is the solubility of the drug in molten lipid. Under its potential to emulsify solid-liquid binary lipid (SLB), the NLC preparation surfactant was selected.100 mg SLB is dissolved into 3 mL methylene chloride and applied 5% magnetic surfactant solutions to 10 mL. In subsequent suspensions, the organic component was extracted with 40 TC and saturated with tenfold Milli-Q water. The resulting samples were observed using UV spectroscopy (Labindia) at a limit of 258 nm. Drug solubility in a lipid phase is one of the most critical factors determining the lipid carrier capacity of the drug. Diacerosolubility was assessed in different lipids and surfactants. In screw-capped bottles, the drug was separately applied to liquid lipids and surfactants (5 ml each). Each sample was centrifuged after 24 hours, and 0.5 ml of the transparent supernatant layer was adequately diluted with methanol and spectrophotometrically analyzed at 258 nm.

Compatibility Study

The primary purpose of this work is to determine drug/excipient compatibility and to identify actual and potential associations between medication and excipient as quickly as possible. The drug is in intimate contact with one or more excipients in its solid dosage form, and the latter may affect drug stability. FT-IR technology has been studied in the chemical interaction of the drug, lipid and surfactants. In order to detect any appearance or disappearance of the peaks, the IR spectrum of the concrete mix was compared with the pure medication, lipids and surfactants and peak fitness were determined. The studies were conducted at 55 °

C for 14 days with humidity and moisture in a sealed glass container of individual medicinal products and drugs: Excipient (1:1). Specific IR spectrums were taken before the product, and the medication was put into the glass vials and held for 14 days at 55 ° C. All vials were observed for 14 days for any colour change and liquefaction. Finally, their IR was studied after 14 days. (Table 1)

Table 1: Drug: excipients ratio for compatibility study.

Drug + Excipient	Ratio	Temperature	With moisture	Without moisture
Diacerien	1	55°C	14 days	14 days
Diacerien+ Capmul MCM C8	1:1	55°C	14 days	14 days
Diacerien+ Stearic Acid	1:1	55°C	14 days	14 days
Diacerien+ Oleic Acid	1:1	55°C	14 days	14 days
Diacerien+Pluronic F-68	1:1	55°C	14 days	14 days
Diacerien+ Tween 80	1:1	55°C	14 days	14 days
Diacerien + Carbopol 940	1:1	55°C	14 days	14 days

2.3 Preparation of Nanostructured Lipid Carrier of Diacerein

As liquid lipid, stearic acid as solid lipid and lecithin, NLC prepared between 80 and pluronic f-68. Air was used to solve aqueous process preparation. The lipid method was designed to combine solid lipid and liquid lipid heated at the same temperature and continuous ring (300rpm) at 600 cm. Constant stirring drug was added to lipid phase. The same lipid phase temperature treated the aqueous surfactant layer. The lipid phase was added to the aquatic phase, with continuous riveting at 1500rpm (at the same temperature). The cyclomixer 's micro-emulsion is homogenized to higher rpm and has been kept for 30 minutes over 100 c stable lipid — ultrasonic preparation, with two cycles of 5 minutes each. Hot oil-in - water pre-emulsion was cooled into an ice bath to reinstall lipid and form NLC.

Table 2: Composition of NLC dispersion.

Batch	Solid Lipid	Liquid Lipids (oils)	Ratio of solid to liquid lipid	Surfactants (5%)	Temperature
B1	Stearic Acid	Capmul MCM C 8	6:4	Lecithin	60-65^{0}c
B2	Stearic Acid	Capmul MCM C 8	6:4	Tween 80	60-65^{0}c
B3	Stearic Acid	Capmul MCM C 8	7:3	Tween 80	60-65^{0}c
B4	Stearic Acid	Oleic acid	7:3	Tween 80	55-60^{0}c
B5	Stearic Acid	Capmul MCM C 8	6:4	Pluronic F-68	60-65^{0}c
B6	Stearic Acid	Oleic acid	6:4	Pluronic F-68	55-60^{0}c
B7	Stearic Acid	Capmul MCM C 8	6:4	Tween 80+ Pluronic F-68	60-65^{0}c
B8	Stearic Acid	Oleic acid	6:4	Tween 80+ Pluronic F-68	60-65^{0}c
B9	Stearic Acid	Capmul MCM C 8	6:4	Lecithin+Tween 80 + Pluronic F-68	60-65^{0}c

CHARACTERIZATION OF NLC GEL

Physical appearance

The NLC-based formulations were visually inspected for colour, homogenousness, consistency and pH. Appearance, viscosity, spreadability, extrudability, and formulations were also studied.

Determination of droplet size

Nanostructured Lipid Carrier formula (10 mg) was diluted in a beaker with a constant stirring by a glass rod in 50 ml deionized water. The corresponding NLC was then measured in particle form. The scale of the droplet was calculated by the technique of Dynamic light diffusion (DLS) by means of a zetasizer (Nano ZS, Malvern Instruments, UK). He-Ne laser network, 4.0 mW, 633 nm, temperature 25 ° C.

Zeta Determination Potential

The Zeta potential of the chosen formula was determined by the use of a particle size analyzer (Malvern Zetasizer Nano ZS 90) in laser diffraction analysis. The samples were diluted to distilled water at a ratio of 1:100 (v / v) and mixed with a magnetic agitator for 1 min. Both experiments have been replicated three times.

Measures of drug loading and trapping efficiency

Indirect measurements of drug loading (percent DL) and trap efficiency (percent EE) with measurement of a drug free concentration in the external aqueous phase have been performed,

$$EE = \frac{(Wa - Ws)}{Wa} x\ 100$$

---- Formula 1

$$DL = \left(\frac{Wa - Ws}{Wa - Ws + Wl}\right) \times 100$$ Formula 2

Where % EE is entrapment efficiency,
% DL is Drug loading, Wastands for the mass of Diacerein added to the formulation,
Ws is the analyzed weight of drug in supernatant and
W$_l$ is weight of lipid added.

pH

The pH of the NLC based gels was determined by a digital pH meter (Hanna Instruments, HI2211). The electrode was then dipped in to NLC based gel formulation and constant reading was noted. The measurements of pH of each formulation were performed in triplicate.

Spreadability of NLC based gel

The spread capacity was measured with the spread power unit. The gadget contains two slides where one slide is set in a wooden frame, and the other slide can ride effortlessly over the fixed surface. There was an excess of NLC-based gel (2 gm) between the two slides. A weight of 1 Kg could be placed on the slide for 5 minutes, thus forming a uniform NLC-based gel film and expelling the air between the slides. The excess gel was carefully removed from the edges of the diaphragms. The bottom diaphragm was adequately mounted, and the top diaphragm had a pull of 80 g. The time (seconds) required by the top diaphragm to cover 5 cm should be noted. A shorter period indicates a better propagation efficiency.
Spread ability was then calculated using the following formula:

$S = M \times L / T$ ----Formula 3

Where, S = Spreadibility
M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide
T = Time (seconds) taken to separate the slide completely.

Extrudability

The NLC based gels were filled into collapsible tubes after formulating them. The extrudability of the formulation has been checked.

Rheological study

The flow activity of the NLC gel formulations was measured at rotational speeds 0.5, 1.0, 2.0, 2.5, 4, 5, 10, 20,50, and 100 rpm using spindle 2 in Brookfield Viscometer (Model RVT, Brookfield Technology Laboratories, Inc., USA). In order to determine the flow behavior of the various formulations, the position of the up and down curves in the rheogram was studied at 25±1 ° C.

Drug content determination

Diacerein NLC-based gel (10 mg) has been dissolved separately into 10 ml of dimethyl acetate, and a 0.1 ml stock solution was correctly measured and converted into 10 ml volumetric flask with 10 ml of methanol and filtering employing Whatman filtering material. UV Spectrophotometer (Shimadzu UV 1800) in αmax analyzed the above solutions. 258 nm. 258. The standard Diacerein calibration curve determined the amount of Diacerein in the formula.

In vitro drug release study

For the experiment, a Franz diffusion cell with an active diffusion area of 7.1 cm2 was used. The egg membrane is placed between the Franz diffusion cell donor and receptor compartments with the corneal stratum facing the donor compartment. Diacerien was taken on stratum corneum, and the release profiles were taken on NLC-based Gels (1 per cent), which were equivalent to 0.5 mg. 25 ml of saline (pH 6.8 phosphate buffer) is filled in the receptor container. The receptor medium was kept at 37 ± 1 daC and magnetically removed at 50 rpm. Samples (every ml) were collected at predetermined times, filtered through a cellulose membrane filter of 0.45 m pores and analyzed by UV. After every sample, the dilution fresh buffer solution was replaced immediately in the receptor chamber. The cumulative amount of drug for the formulations was plotted in the receptor chamber according to time (t, h).

Stability of NLC based gel

The consistency of the NLC-based Diacerien gel was 3 months measured at 45 ° C ± 2 ° C / 75% RH ± 5% RH. The specific measurements included transparency, phase isolation and diacerial examination.

RESULTS AND DISCUSSION

The spreadability measurements from B1 to B5 and findings shown in table 3 were all yellowish viscous clear preparation with homogenous and glossy appearance. The analysis concluded that B2 was selected as a suitable solution based on pH, viscosity, appearance, spredability and extrudability.

Table 3: Physical appearances.

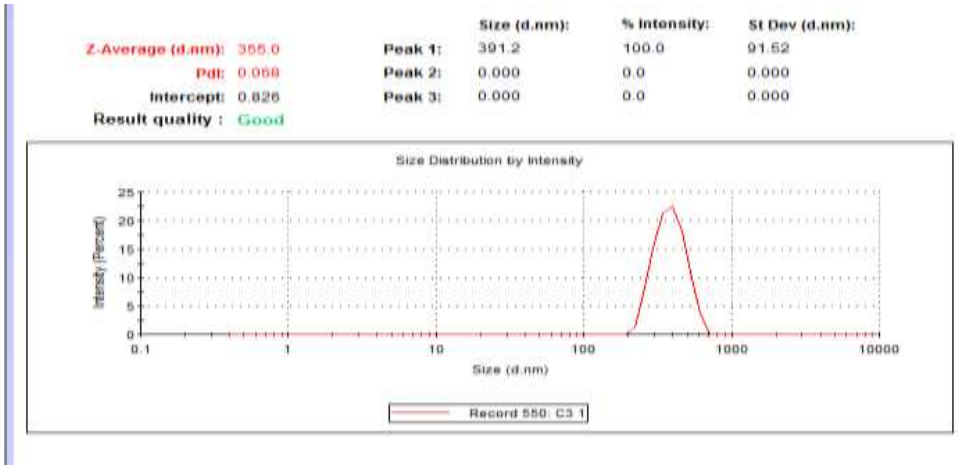
Formulation code	pH	Viscosity (cps)	Appearance	Spreadability	Extrudability
B1	6.6	10250	+	36.45±1.45	+
B2	7.4	12500	+++	35.56±1.45	+++
B3	7.6	12800	++	34.22±1.45	++
B4	7.7	13100	++	33.89±1.45	++
B5	7.8	13400	+	33.45±1.45	+

Where, + average, ++ good, +++ excellent

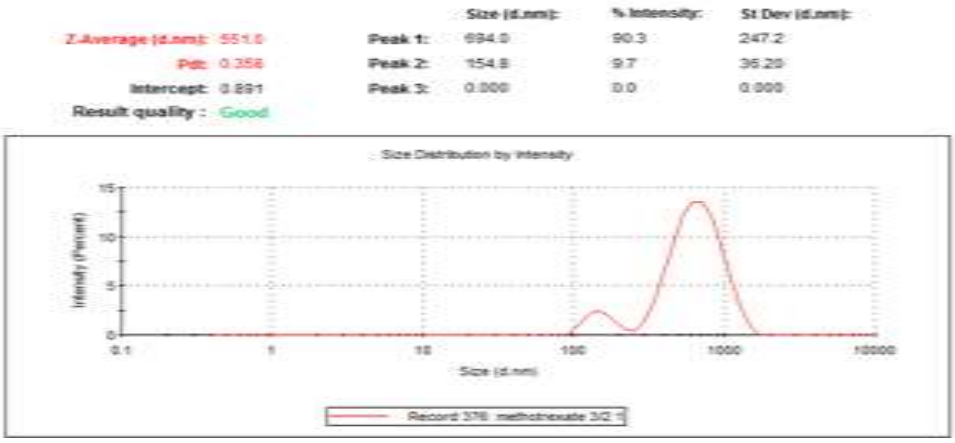
Droplet size and Zeta Potential Determination

ZP governs the degree of repulsion between adjacent or

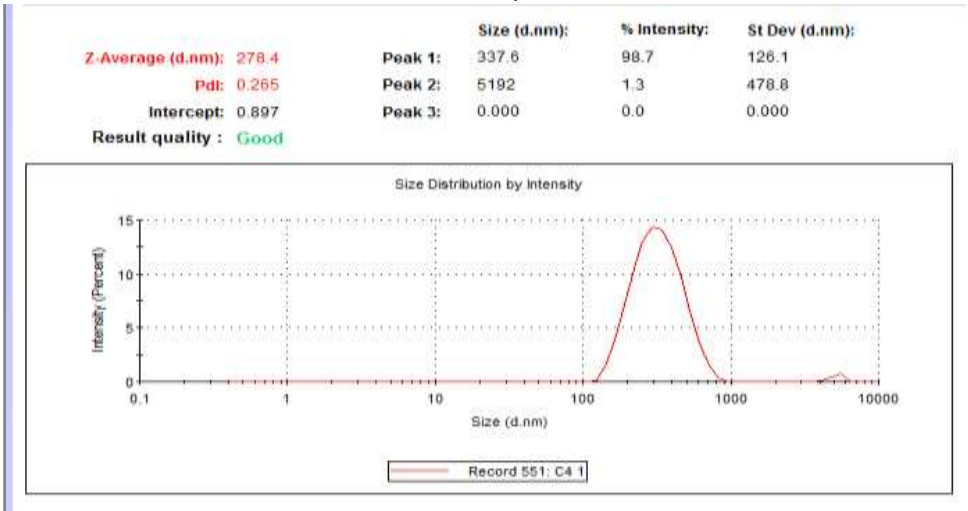
similarly charged and dispersed droplet, it shows the practical application in the stability. ZP values in range of 40 to -40 mV characterize a stable formulation.



a) Batch 6

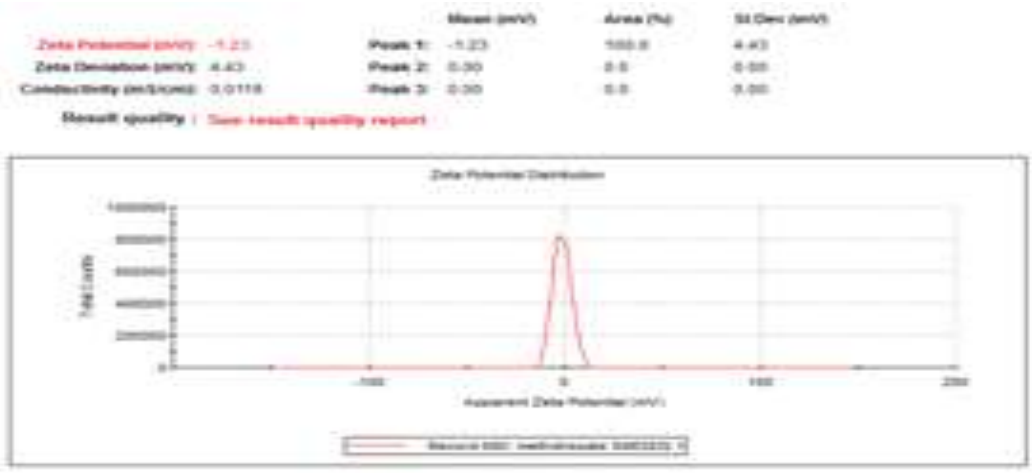


b) Batch 7

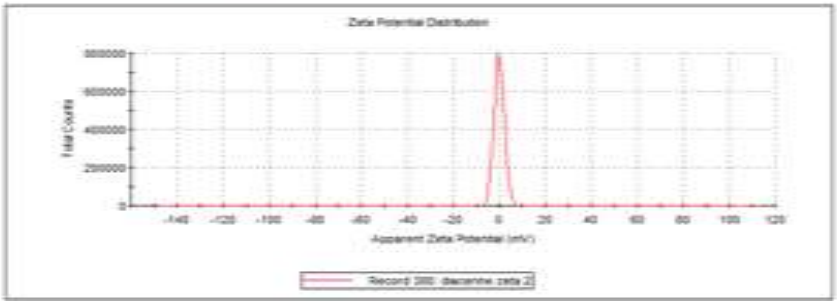


c) Batch B8

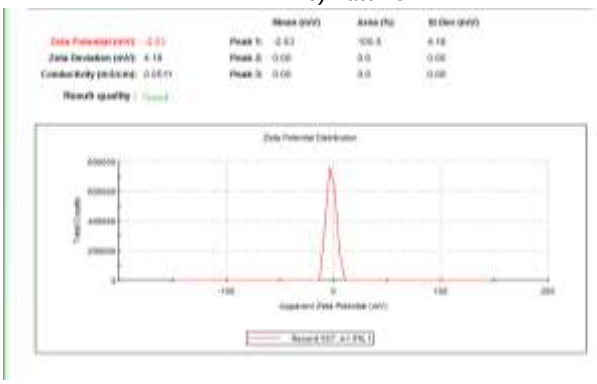
Figure 1: Graph of globule size determination for batches a) Batch 6; b) Batch 7; c) Batch B8.



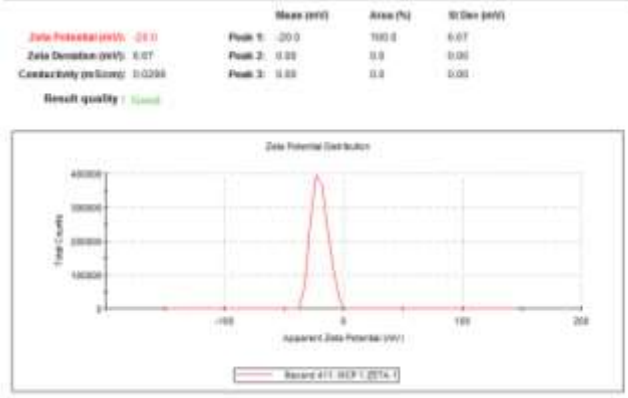
a) Batch 4



b) Batch 5



c) Batch 6



d) Batch 8

Figure 2: Zeta potential of Nanostructured Lipid Carrier for batches a) Batch 4; b) Batch 5; c) Batch 6; d) Batch 8.

Table 4: Droplet size and Zeta Potential Determination.

Sr.No.	Formulation code	Average Particle size (in nm)	Zeta potential
1	B4	1034	-1.23
2	B5	1122	-0.0689
3	B6	355	-2.03
4	B7	551	-1.88
5	B8	278	-20.0

Compatibility by FT-IR Study

IR spectroscopy was used to classify the activity between drug excipients. IR spectroscopy of pure Diacerein and excipients was conducted until the compatibility test was started and the compatibility study was completed. The IR of all samples were taken before the compatibility study and compared with the IR graph.

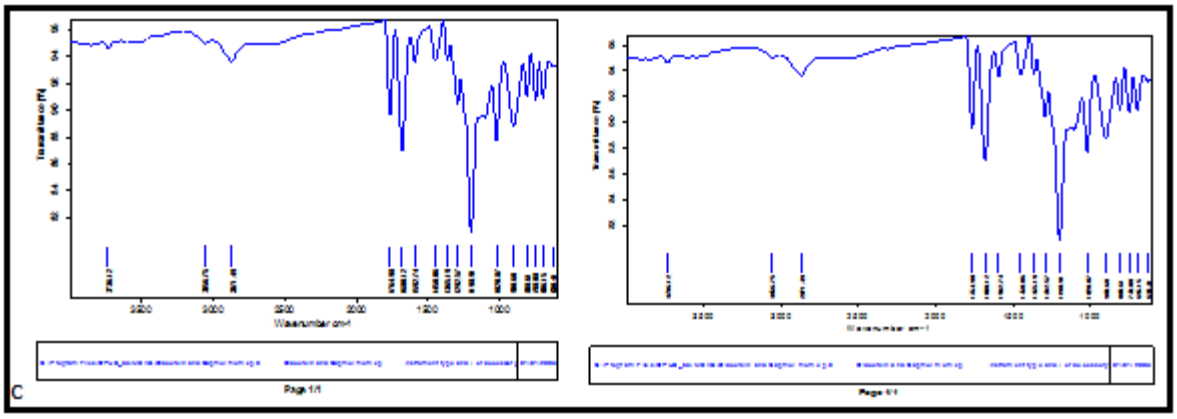


Figure 3: a) IR Spectra of Drug +Capmul MCM C8 (Before); 3b) IR Spectra of Drug +Capmul MCM C8 (After).

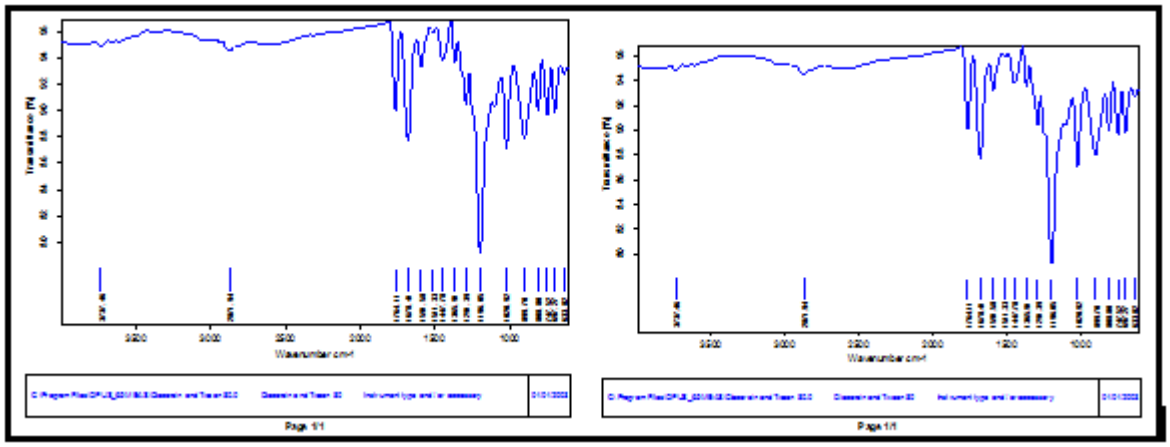


Figure 4: a) IR Spectra of Drug+ stearic acid (Before); 4b) IR Spectra of Drug+ stearic acid (After)

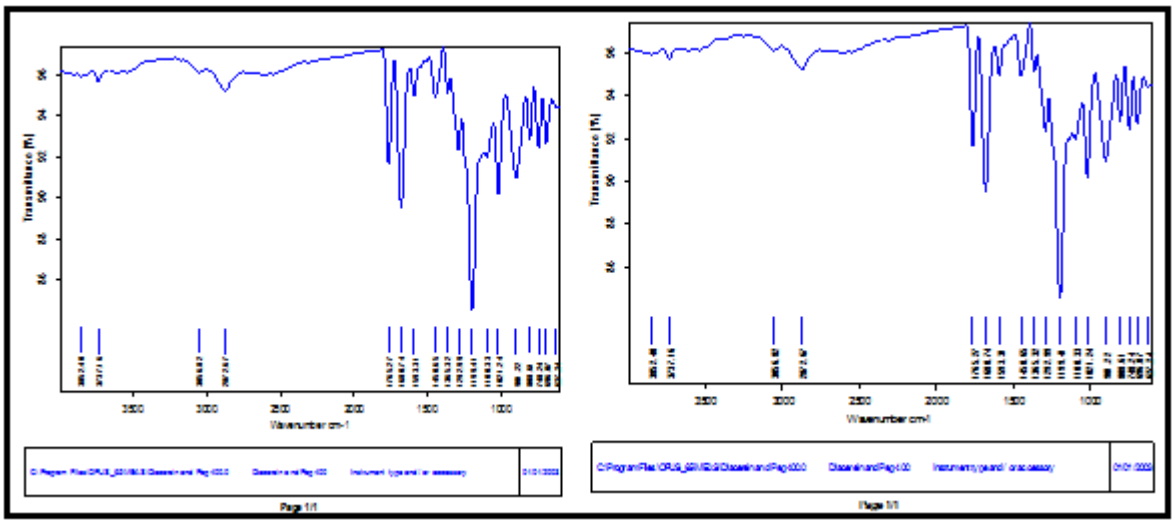


Figure 5: a) IR Spectra of Drug +Tween 80 (Before); 5b) IR Spectra of Drug +Tween 80 (After)

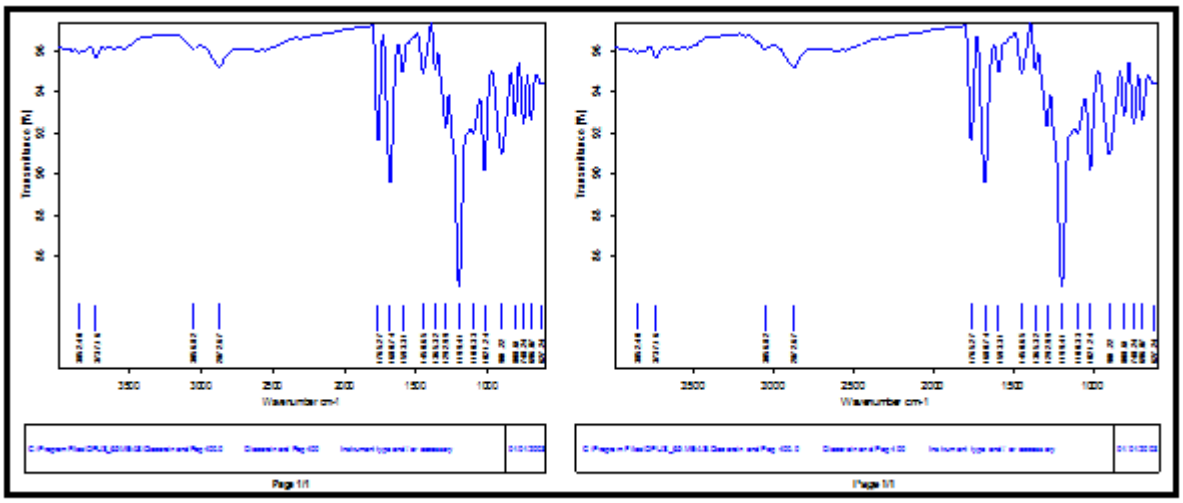


Figure 6: a) IR Spectra of Drug +Pluronic F-68 (Before); 6b) IR Spectra of Drug +Pluronic F-68 (After)

Drug content determination

The drug content of diacerein in all the formulation was found to be in the range 98-99% in Nanostructured Lipid Carrier which indicates complete solubilization of drug in formulation.

Table 5: Drug content Determination.

Formulation	Drug content (%)
B1	97.4
B2	99.20
B3	98.5
B4	98.2
B5	97.97

In vitro Drug Release study

In vitro Drug release of the drug from NLC gel formulation was studied amongst all these five, Formulation B2 showed highest drug release i.e. 97.69%.

Table 6: In-vitro drug release of various NLC gel formulations.

Time (Hours)	B1	B2	B3	B4	B5
0	0	0	0	0	0
1	11.57	15.89	13.45	14.32	15.22

2	20.24	29.72	28.47	26.33	25.30
3	27.63	44.27	41.17	37.45	35.80
4	39.79	57.37	42.75	45.2	49.5
5	47.71	77.44	58.92	59.36	51.76
6	51.87	90.65	64.52	66.25	61.63
7	59.80	92.65	82.92	83.75	67.57
8	74.94	97.69	87.05	92.51	71.89

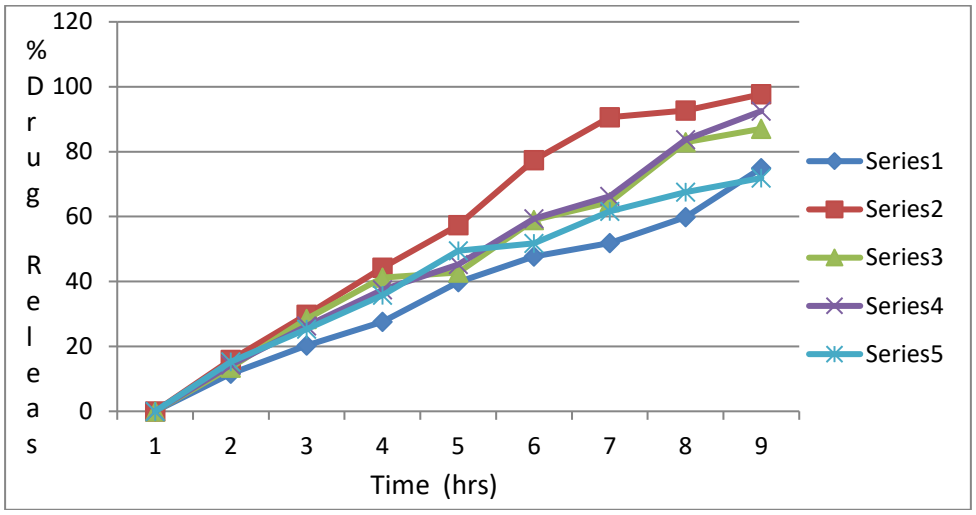


Figure 7: Drug release profile of optimized NLC gel formulation.

Above figure (fig. 7) shows comparison of drug release from five different formulations. In these formulations drug release from B2 is higher as compare to B1, B3, B4 and B5 formulations. The B2 formulation is shows higher drug release at 8 hrs.

STABILITY STUDY

Table 6: Stability parameters studied upto 3 months.

Parameters		Initial	1 Months	3 Months
Drug Content		B2	B2	B2
		99.20%	98.85%	97.56%
Diffusion (%) Medium: 25ml of pH 6.8 phosphate buffer, egg's membrane, 50 rpm.	0hr	0	0	0
	1hr	15.85	15.00	14.50
	2hr	29.00	28.75	27.36
	3hr	43.89	43.68	43.28
	4hr	54.63	54.16	54.02
	5hr	77.29	76.35	76.12
	6hr	89.52	89.14	88.45
	7hr	92.82	92.28	91.96
	8hr	97.52	96.65	96.30
Clarity		Clear	Clear	Clear
Phase separation		No phase separation	No phase separation	No phase separation
Centrifugation test		Stable	Stable	Stable

The NLC gel is filled in aluminum collapsible tubes for stability tests and tubes were held at 45 ° C ± 2 ° C / 75 percent RH ± 5 percent RH in a stability chamber. For physical appearance, diffusion tests, visibility, separation process, centrifugation check, NLC gel was evaluated. After a three month stability test, there is no improvement in NLC gel classification. In Transparency, phase separation and centrifugation test no difference was found. Based on data on equilibrium at 45 ° C ± 2 ° C / 75% of RH ± 5% of RH, it is seen to be stable at 45 ° C ± 2 ° C / 75% of RH ± 5% for 3 months.

CONCLUSION

The most widely used drugs to treat OA pain are non-steroidal anti-inflammatory drugs (NSAIDs). However, they are known to cause serious adverse gastrointestinal and

vascular effects without minimizing structural damage to their substance for a long period in OA medicine. Most patients are older. Diacerein is the medication of choice for sound analgesic effects in serious OA discomfort. At a reduced dosage, Diacerein is cost-effective. Gel preparation makes the pain spot simple to administer in any amount. NLC offers an advantage to nanoparticles that also improve surface and bioavailability.

In the topical treatment of inflammation and associated conditions, Diacerein has formulated topical gel Nanostructured Lipid Carrier (NLC). Specific characterization parameters, including drug quality, pH, spreadability, rheology, and in-vitro release, were identified for nanoparticulate dispersion. Pharmacodynamics study (skin irritation studies) confirmed the safety of the formulated topical gel on NCL. Established NLC-based gel showed quicker start and sustained operation for up to 24 hours.

ACKNOWLEDGMENTS

The authors sincerely thank Dr. Sohan Chitlange, Director, Dr. D. Y. Patil institute of Pharmaceutical Sciences & Research, Pimpri, Pune for valuable guidance and support provided during study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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