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Research Article

TOPICAL DELIVERY OF PUERARIN USING NANOSTRUCTURED LIPID CARRIER GEL: FORMULATION, CHARACTERIZATION, AND *IN VITRO* ASSESSMENT

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ABSTRACT

Objectives: Puerarin is one of the oldest and most significant edible crude herbs in traditional Oriental medicine and is used as a muscle relaxant, antipyretic, anti-inflammatory, and antidysentery, as well as in the treatment of cardiovascular diseases. Puerarin topical gel based on nanostructured lipid carriers (NLCs) was developed to treat inflammation. This research will focus on preparing and evaluating transdermal drug delivery system formulations using appropriate excipients. Furthermore, NLCs will be incorporated into a transdermal gel to achieve specific delivery. Finally, the performance and efficacy of the optimized transdermal gel containing puerarin-loaded NLC will be thoroughly evaluated.

Methods: Propylene glycol served as a co-surfactant, Tween 20 as a surfactant, oleic acid as a liquid lipid, and stearic acid as a solid lipid made up of NLC. Particle size, differential scanning calorimetry, scanning electron microscopy, and Fourier transform infrared spectroscopy were used to characterize the NLCs using solvent emulsification procedures.

Results: The NLC exhibited impressive entrapment efficiency across all formulations, ranging from 45.90% to 87.80%. Formulation F1 emerged as the optimal NLC composition with an average particle size of 61.70 nm and a polydispersity index of 0.345, indicating a homogeneous particle size distribution. The stability of Formulation F1 was further confirmed by its high-magnitude zeta potential of −13.0 mV. Subsequently, Formulation F6 of the puerarin-loaded NLC gel underwent successful gelling and was evaluated for various parameters which demonstrated optimum results, suggesting the suitability of the nanoparticulate dispersion for further development.

Conclusion: The puerarin NLC gel exhibits potential for extended medication availability in skin tissues and may be applied more effectively to treat inflammatory diseases.

Keywords: Puerarin, Nanostructured lipid carrier, Topical gel, Nanoparticle.

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INTRODUCTION

Colloidal lipid structures, called solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), have been suggested for a variety of delivery routes, including parenteral, oral, and topical routes, offering a controlled release profile of multiple substances. SLNs have the potential to be used for delivery in manufactured goods [1-3]. The stratum corneum is the most superficial layer of the epidermis and it is the primary barrier to drug penetration in transdermal absorption. In recent years, more focus has been placed on the application of nano- and submicron-sized lipid-based carrier systems. The major goals of novel drug delivery are either to accomplish the target drug action using carriers that transport the drug to a specific target site, to provide perpetuated drug action at a fixed rate, or by maintaining a relatively constant, sufficient level of drug in the body with less or no toxicity or adverse effects in the impacted tissue or organ [4-6]. SLNs have drawn much interest as topical/transdermal drug delivery systems because they are made of a physiological and biocompatible solid lipid core [7]. They have proven to be more beneficial than polymeric nanoparticles, microemulsions, and liposomes, including better skin-intended potential, effective incorporation of lipophilic drugs, and good local tolerance. However, there are two significant potential problems associated with the limited use of SLN: drug expulsion throughout storage and inadequate drug payload. As an alternative, next-generation lipid nanoparticles, known as NLC, have been developed to overcome the main drawbacks of SLN. The lipid matrix of NLC is not as perfect as that of SLN, but it still provides sufficient room for the drug molecules to be accommodated and reduces or eliminates drug expulsion throughout storage [8-13]. Each nanocarrier has a unique method of promoting percutaneous absorption. These nanocarriers can alter the physicochemical characteristics of the entrapped drug, which in turn alters the skin penetration profile. These carriers may increase the bioavailability of the drug by extending the duration of skin residence or promoting skin penetration. The permeability of the drug from the lipid-based nanocarrier is explained by the high specific surface area of the nanoparticles, which promotes their strong adherence to the skin surface and provides an occlusive effect. Because the occlusive effect widens inter-corneocyte gaps and reduces coenocyte packing, it can eventually result in increased skin hydration and facilitate drug deposition into viable skin [14-16]. Pure solid lipids constitute SLN, whereas NLCs contain a certain amount of extra liquid lipids that cause flaws in the crystal lattice. One of the following methods yields nanoparticles: solvent emulsification, solvent diffusion, reverse micelle-double emulsion, homogenization followed by ultrasonication, solvent injection, cold homogenization, high-pressure homogenization, microemulsion template, and a recently developed membrane contractor method [17-19]. Puerarin is one of the oldest and most significant edible crude herbs in traditional oriental medicine and is used as a muscle relaxant, antipyretic, anti-inflammatory, and antidysentery, as well as in the treatment of cardiovascular diseases. It is widely distributed throughout the temperate regions of Far East Asia, including Korea, Japan, China, and India. Puerarin and daidzein, in particular, are two isoflavonoids found in large amounts in the genus Pueraria. The biological effects of isoflavonoids are diverse; they possess anti-inflammatory, antithrombotic, antihypertensive, antiarrhythmic, spasmolytic, and cancer-preventive properties [20].

The use of NLCs is a viable method for transdermal administration. However, because of their low viscosity, NLC dispersions are unsuitable for topical application. Therefore, it would be beneficial to convert

NLCs into gels for dermal application [21-23]. The goal of this project was to create and characterize NLCs for transdermal distribution of puerarin. In this study, we examined the effects of varying surfactant concentrations and lipid ratios on the size, encapsulation effectiveness, and *in vitro* release of NLCs. Puerarin-loaded NLC gels are currently unavailable on the market.

MATERIALS AND METHODS

Materials

Puerarin was provided by Yarrow Chem Products (Mumbai, India). Stearic acid, oleic acid, Glycerol Monostearate, Propylene Glycol, Tween 20, Tween 40, Tween 60, Tween 80, Sodium Dihydrogen Phosphate, Dialysis Membrane 12 KDa Himedia (Shree Chemicals, Ratnagiri), 2,2-dipenyl-1-picrulhydrazyl, and other chemicals were procured from Loba Chemicals. Double-distilled water was used for all the experiments.

List of instruments

This study employs a range of sophisticated instruments to conduct comprehensive analyses. HORIBA SZ-100 (HORIBA Ltd., Japan) was used for particle size and zeta potential measurements. Spectroscopic analyses were performed using a Shimadzu UV-1800 ultraviolet (UV-Vis) spectrophotometer and Shimadzu 7600 S FT-IR Spectrometer (Shimadzu Corporation, Japan). Thermal properties were investigated using a Hitachi DSC-7020 differential scanning calorimeter (DSC) from Hitachi High-Tech Science. Rheological measurements were performed using an AMETEK Brookfield LDV Prime I Viscometer. For the *in vitro* permeation studies, a molded Franz diffusion cell apparatus was employed. This diverse array of instruments enabled thorough characterization of the materials and formulations under investigation.

Methods

Preparation of Puerarin-loaded NLC (PUE-NLCs)

The PUE-NLCs were prepared using the solvent emulsification method (Table 1). Briefly, as a solid lipid, stearic acid (800 mg) and liquid lipid oleic acid (200 mg) were mixed and heated at 75°C with continuous stirring. Stearic acid and oleic acid form a solid-liquid lipid matrix in nanoparticles (NLCs) because of their high and low melting points, enabling higher drug encapsulation by filling the spaces left by the solid matrix. An aqueous surfactant solution was prepared by dissolving Tween (20, 40, 60, and 80) (500 mg) in water (Q.S. to 50 mL), followed by heating at 75°C. The surfactant solution was added and injected into the hot lipid phase using a syringe (24 gauge) at a stirring rate of 600 rpm to obtain the final mixture (50 mL) [24]. Propylene glycol is used in this preparation as a co-surfactant, which helps dissolve hydrophobic drugs, such as puerarin, in the lipid phase. The puerarin incorporated into the formulation is critical; therefore, puerarin was dissolved in the lipid phase before emulsification. The obtained mixture was magnetically stirred for 40 min at 75°C to strengthen emulsification. NLCs were prepared for the four batches. F1–F3 is control groups in formulation design, component evaluation, and preparation methods without an active drug delivery system. PUE-NLC formulations optimize puerarin dosage based on pre-clinical studies or therapeutic targets.

Table 1: Composition of NLC

Ingredients	F1	F2	F3	F4	Role of ingredients
Puerarin	0.25 g	0.25 g	0.25 g	0.25 g	API
Stearic acid	0.8 g	0.8 g	0.8 g	0.8 g	Solid lipid
Oleic acid	0.2 g	0.2 g	0.2 g	0.2 g	Liquid lipid
Tween 20	0.5 g	-	-	-	Surfactant
Tween 40	-	0.5 g	-	-	Surfactant
Tween 60	-	-	0.5 g	-	Surfactant
Tween 80	-	-	-	0.5 g	Surfactant
Propylene glycol	0.25	0.25	0.25	0.25	Co-surfactant
Water	Q.S. to 50 mL	Q.S. to 50 mL	Q.S. to 50 mL	Q.S. to 50 mL	Vehicle

NLC: Nanostructured lipid carrier, API: Active pharmaceutical ingredient

Preparation of PUE-NLC gel

The compositions of the PUE-NLC gels are tabulated in Table 2. A weighed amount of sodium carboxymethyl cellulose was dispersed in 50 mL of distilled water in a beaker while stirring continuously on a magnetic stirrer. Methyl paraben and propylparaben were dissolved in 5 mL of distilled water and boiled in a water bath. After cooling, it was supplied to the configuration. Pure puerarin was supplied, and the volume was increased to 100 mL with the addition of water. Finally, triethanolamine was added dropwise. After adding triethanolamine gel, the desired consistency was achieved [18]. Collapsible tubes were used to fill the gels. The NLC gels were prepared in six batches. The combinations were stored in dry and cold atmospheres [25,26].

Characterization of NLCs

Particle size and zeta potential

An instrument called HORIBA SZ-100 was used to measure the particle size and zeta potential of PUE-NLCs [27].

Entrapment efficiency

For the separation of the lipid and aqueous phase, 2.0 mL of every drug-loaded sample was centrifuged for 45 min at 12500 rpm. After diluting the supernatant with methanol and filtering it through a 40 µm filter paper, a UV-Vis spectrophotometer was used to measure the amount of medication present at 279 nm (Shimadzu UV-1800) [28]. The trapping effectiveness of the NLC was determined as follows:

$$\text{Entrapment efficiency (\%)} = (Wp - Ws/Wp) \times 100 \quad (1)$$

Where,

Wp: The mass of puerarin added to the formulation

Ws: Analyzed the weight of the drug in the supernatant.

Fourier transform infrared (FTIR) spectroscopic analysis

Analyses of FT-IR spectra are useful in determining the nature of the drug and identifying how it interacts with polymers. The incompatibility investigation was conducted using FT-IR spectral analysis (Shimadzu Model 7600S) for pure puerarin, physical mixture of gel, physical mixture of NLC, formulation of NLC, and formulation of gel. The range of the spectrophotometer was 500 cm^{-1} to 4000 cm^{-1} [29-32].

Table 2: Composition of PUE-loaded NLC gel

Ingredients	F1	F2	F3	F4	F5	F6	Role of ingredients
Puerarin	0.5 g	0.5 g	0.5 g	-	-	-	API
PUE-NLC	-	-	-	0.5 g	0.5 g	0.5 g	
Sodium CMC	1 g	1.5 g	2 g	1 g	1.5 g	2 g	Gelling agent
Methyl Paraben	1 g	1 g	1 g	1 g	1 g	1 g	Preservative
Propyl Paraben	1 g	1 g	1 g	1 g	1 g	1 g	Preservative
Triethanolamine	1	1	1	1	1	1	pH balancer
Water	Q.S. to 100 mL	Q.S. to 100 mL	Q.S. to 100 mL	Q.S. to 100 mL	Q.S. to 100 mL	Q.S. to 100 mL	Vehicle

PUE-NLCs: Puerarin-loaded nanostructured lipid carrier, API: Active pharmaceutical ingredient

DSC
DSC measurements were carried out on Pure Puerarin, a physical mixture of NLC, a physical mixture of gel, formulation of NLC, and formulation of the gel. Sealed aluminum pans with a perforated cover held sample (3 mg) of solid lipids (stearic acid), liquid lipids (oleic acid), drugs (crystalline PUE), surfactants (Tween 20, Tween 40, Tween 60, and Tween 80), and freeze-dried NLC formulations (PUE-NLC). DSC curves were obtained in a nitrogen environment (8 mL/min) at temperatures ranging from 25°C to 250°C at a heating rate of 10°C/min, and a DSC (Hitachi DSC-7020) was used for the investigation [31,33].

Scanning electron microscopy (SEM)
Through the use of SEM, the surface morphology of the generated NLC formulation was verified. High-resolution SEM can be used to identify surface corrosion, defects, impurities, and fractures. SEM was used to analyze a drop of the diluted NLC preparation that was placed on a coverslip and secured to a brass stub using double-sided adhesive tape. After that, it was dried and covered with a small amount of gold to make it electrically conductive. The sample was then placed under a microscope so that images could be taken [34,35].

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay
The antioxidant assay, known as the DPPH free radical method, uses the transfer of electrons to create a violet solution in ethanol. When an antioxidant molecule is present, this free radical, which is stable at ambient temperature, is reduced, producing a colorless ethanol solution. The DPPH test offers a quick and simple method for the spectrophotometric evaluation of antioxidants; therefore, evaluating multiple items at once may be beneficial [36-38].

0.3 mM ethanolic solution of DPPH was prepared by dissolution of DPPH in ethanol. A final concentration of 10, 20, 40, 60, 80, and 100 µg/mL in ethanol was achieved by diluting the standard solution of puerarin-loaded NLC in ethanol (1 mg/mL). Standard ascorbic acid solution: Ascorbic acid in ethanol (1 mg/mL) sample stock solution was diluted to final concentrations of 10, 20, 40, 60, 80, and 100 µg/mL in ethanol.

Procedure: Each sample extract was combined with two milliliters (mL) of DPPH solution (0.1 mM). The mixture was vortexed and left in the dark for half an hour. The absorbance of the mixture was measured at 279 nm relative to a blank using a spectrophotometer. The inhibition percentage (I%), which represents radical scavenging activity, was computed using the following equation:

$$I (\%) = (A \text{ blank} - A \text{ sample}/A \text{ blank}) \times 100 \quad (2)$$

The plot of inhibition (%) against the concentration of the puerarin-loaded NLC was used to calculate the EC50 value, or the expected concentration of a sample required to scavenge 50% of free radicals. Each determination was performed three times, and the average was recorded. The typical antioxidant utilized was ascorbic acid [39].

Characterization of PUE-NLC gel
Physical evaluation
Physical characteristics including color and uniformity were examined manually.

Washability
After applying the product by hand, it was examined under the flowing water.

Viscosity (rheology)
A Brookfield LDV prime I viscometer device was used to calculate the viscosity. At a temperature of 30°C, the gel sample was placed in a beaker, and the dial reading was recorded for 60 s at 12 RPM with spindle number LV 3. The viscosity of the gel was then calculated and recorded [30].

Spreadability
A spread power source was used to measure the spread capacity. The device has two slides: one glides easily over the solid surface, while the other is mounted on a wooden frame. There was an excess of NLC-based gel (2 g) between the two slides. For 5 min, a weight of one kilogram was applied to the slide to create a consistent NLC-based gel layer and remove air spaces between the slides. The edges of the diaphragms were meticulously cleaned using extra gel. The upper diaphragm had an 80 g pull, and the bottom membrane was placed appropriately. It is important to record how long it takes for the top membrane to wrap around 5 cm in seconds. A higher propagation efficiency is indicated by a shorter time [40]. Subsequently, spread ability was determined using the following formula:

$$S = M \times L/T \quad (3)$$

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

Extrudability
The volume of gel that extrudes or emerges from the tube when pressure is applied is known as extrudability. This is contingent on the formulation's consistency and gelling agent concentration. Fewer patients will accept a product that is inadequately extrudable. Collapsible aluminum tubes were used for the test. The aluminum tube was filled with gel and the cap of the tube was removed. With the aid of the clamp, a 1 kg weight was placed on the crimped end of the tube. For each of the three gel concentrations, the amount of gel extruded from the aluminum tube was measured thrice [41].

pH
A digital pH meter was used to measure the pH of NLC-based gels. The electrode was then submerged in a gel formulation based on the NLC, and a steady reading was recorded. Three separate pH measurements of each formulation were performed [40].

Drug content
The gel samples were precisely weighed in a 10 mL volumetric flask in a fixed quantity of 40 mg. The sample was sonicated for approximately 10 min to achieve full drug extraction after appropriate dilution with phosphate-buffered saline (pH 7.4). These stock solutions underwent filtering and additional dilution after volumes were increased to 10 mL [40]. The absorbance of the solutions was measured using a UV-vis spectrophotometer at 279 nm [16].

In vitro drug release study
A Franz diffusion cell with an active dissemination area of 7.1 cm was used for the experiment. The corneal stratum faces the donor compartment when the egg membrane is positioned between the donor and receptor compartments of a Franz diffusion cell. The stratum corneum was used to collect the NLC-based Gels (1%), or 0.5 mg was used to collect the release profiles. Phosphate buffer (25 mL, pH 7.4) was then added to the receptor container. The receptor material was magnetically extracted at 50 rpm and maintained at 37±1°C. Every milliliter of sample was taken at prearranged intervals, filtered through a 0.45 m pore cellulose membrane filter, and UV analysis was performed. Every sample was immediately replaced with freshly diluted buffer solution in the receptor compartment. The cumulative drug quantity for each formulation was plotted against time (t, h) in the receptor chamber [40].

Statistical analysis
All experiments were performed in triplicate unless otherwise specified. The data are presented as mean±standard deviation (SD). Statistical comparisons between different formulation groups were made using one-way analysis of variance (ANOVA) followed by Tukey's

multiple comparison test for *post hoc* analysis. A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preformulation studies

Determination of λmax

The absorption maximum of puerarin was determined to be 279 nm in phosphate buffer (pH 7.4), and this wavelength was chosen for further research (Fig. 1).

Calibration curve of puerarin

The absorbance data for the standard solutions are listed in Table 3. The absorbance values were plotted against their respective concentrations (Fig. 2). When the curve was plotted, concentrate displayed linearity, indicating that it followed Beer's Lambert law. The regression coefficient values were 0.9890 in 0.1N HCl, 0.9907 in phosphate buffer (pH 6.8), and 0.9927 in phosphate buffer (pH 7.4).

where data are expressed as absorbance determined from each concentration (n=1).

Drug-excipients compatibility studies

FTIR analysis

A compatibility study of the active pharmaceutical ingredient (API) and physical mixtures was carried out using FTIR spectroscopic analysis (Fig. 3). The results of the FTIR spectroscopic analysis were as follows.

API

Puerarin exhibited large bands at 3313.27 cm^{-1} for OH – stretching. –e H stretching was detected at 2923.89 cm^{-1}. The C=C and C-N stretching bands were detected at 1647.36 cm^{-1} and 1146.74 cm^{-1}, respectively. H bending is detected at 857.20 cm^{-1}.

Physical mixture of NLC

The physical mixture exhibited substantial O-H stretching bands at 2923.89 cm^{-1}. C=O stretching was detected at 1748.63 cm^{-1}. N-H bending was detected at 1533.26 cm^{-1}. The O-H bending and CH=CH2 stretching bands were measured at 1410.60 cm^{-1} and 944.20 cm^{-1}, respectively.

Physical mixture of gel

The Physical Mixture exhibited a prominent OH-stretching peak at 2854.01 cm^{-1}. C=O stretching was detected at 1734.37 cm^{-1}. C=C stretching was detected at 1653.07 cm^{-1}. The O-C stretching and CH-CH2 bending were detected at 1115.36 cm^{-1} and 939.93 cm^{-1}, respectively.

Final formulation NLC

The formulation of NLC, including the medications Puerarin, Tween 20, stearic acid, oleic acid, and propylene glycol, exhibited substantial peaks at 2854.01 cm^{-1} for OH- stretching. The C=O stretch was detected at 1740.07 cm^{-1}. The N-H stretching and O-H bending were measured at 1540.39 cm^{-1} and 1339.29 cm^{-1}, respectively. CH-CH2 bending was detected at 947.06 cm^{-1}.

Table 3: Calibration curve of puerarin in different buffer solution

Conc. (mcg/mL)	Abs. (0.1NHCl pH=1.2)	Abs. (pH=6.8 Buffer solution)	Abs. (pH=7.4 Buffer solution)
0	0	0	0
0.2	0.023	0.034	0.012
0.4	0.044	0.053	0.031
0.6	0.061	0.077	0.054
0.8	0.080	0.094	0.063
1	0.098	0.114	0.077

Conc.: Concentrate, Abs.: Absorbance

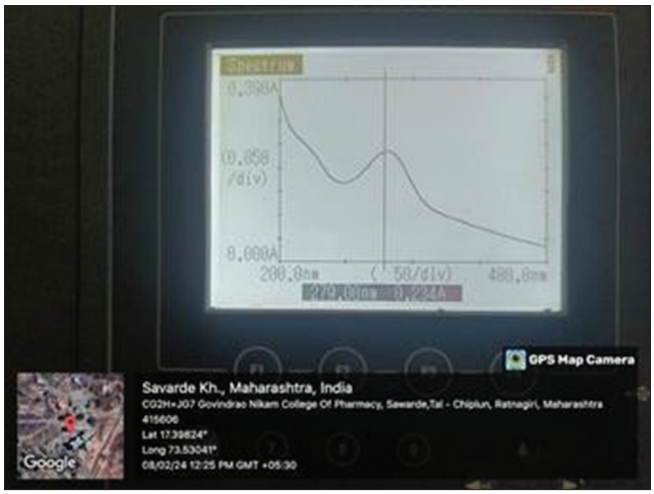

Fig. 1: Ultraviolet spectra of puerarin

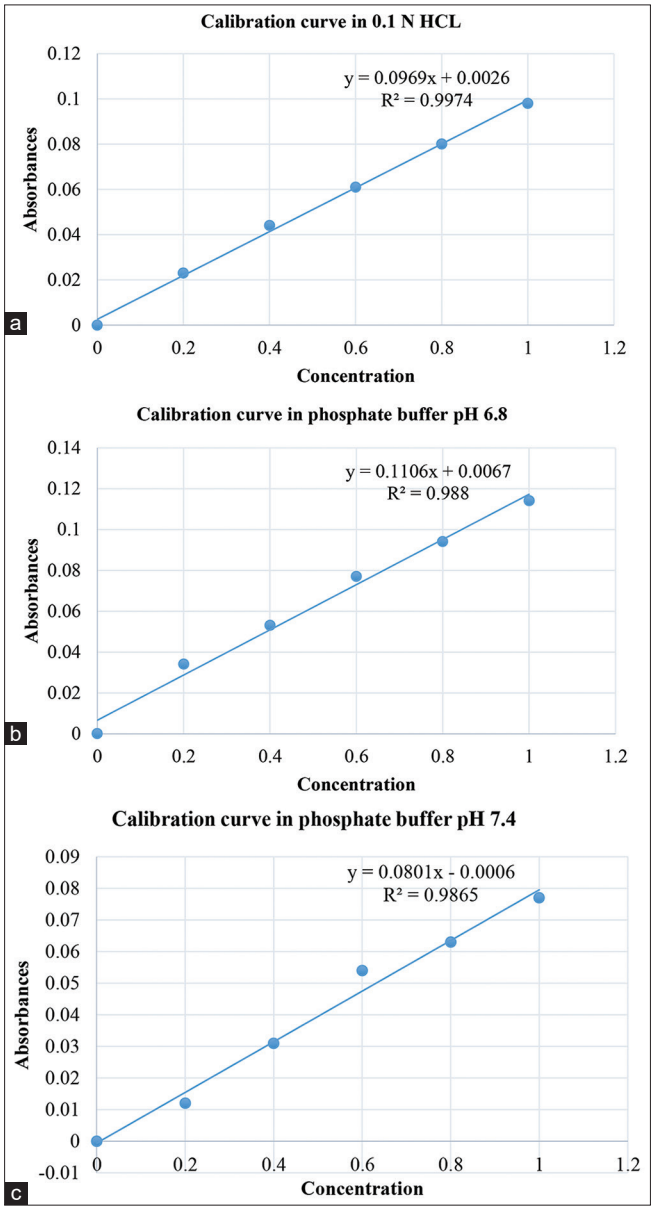

Fig. 2: (a) Calibration curve of puerarin in 0.1N HCl, (b) Phosphate buffer pH 6.8, and (c) Phosphate buffer pH 7.4

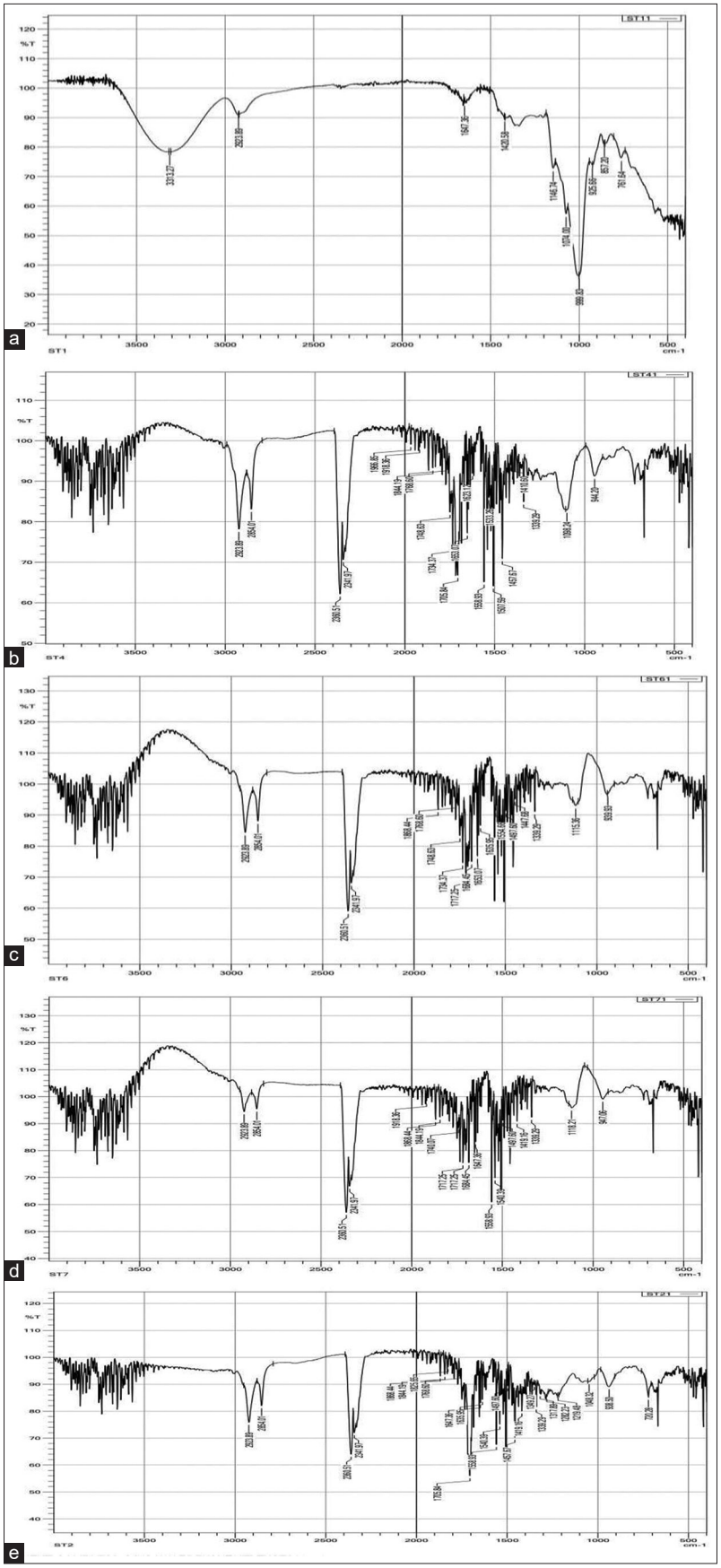

Fig. 3: Fourier transform infrared spectra of (a) puerarin pure drug, (b) physical mixture of nanostructured lipid carrier (NLC), (c) physical mixture of gel, (d) final formulation of NLC, and (e) final formulation of gel

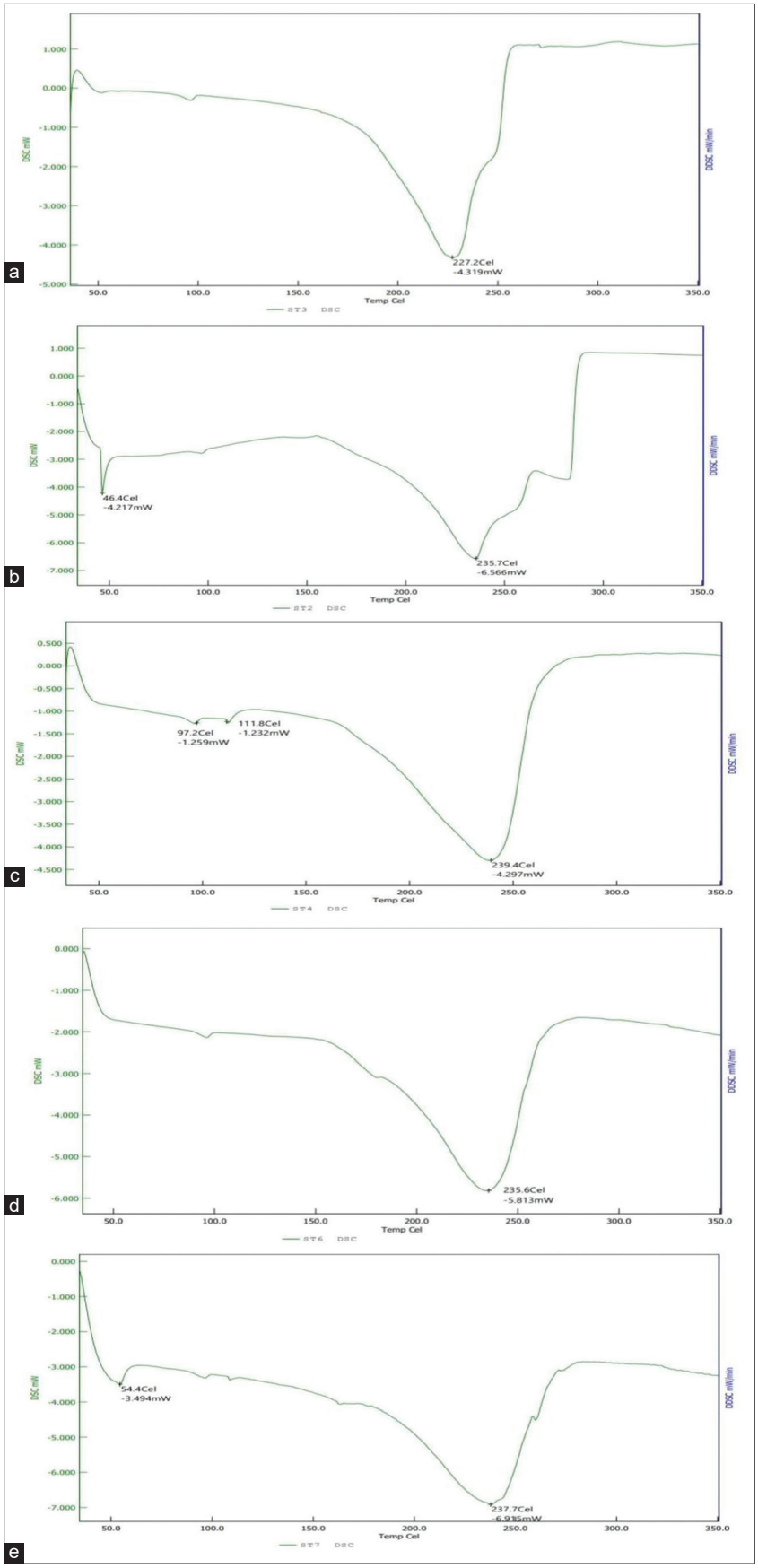

Fig. 4: Differential scanning calorimeter of (a) puerarin pure drug, (b) physical mixture of nanostructured lipid carrier (NLC), (c) physical mixture of gel, (d) final formulation of NLC, and (e) final formulation of gel

Final formulation of gel

The gel formulated with NLC, sodium CMC, methyl paraben, propyl paraben, and triethanolamine exhibited substantial O-H stretching bands at 2923.89 cm^{-1}. The C=O stretching was detected at 1705.84 cm^{-1}. C=C stretching was detected at 1647.36 cm^{-1}. O-C stretching and =CH2 bending were detected at 1219.48 cm^{-1} and 720.28 cm^{-1}, respectively.

DSC

Fig. 4 depicts the thermal properties of (A) the pure drug, (B) the physical mixture of NLC, and (C) the physical mixture of NLC. The DSC thermogram of puerarin revealed a distinct endothermic peak at 227.2°C. The DSC thermogram of the physical mixture of NLC containing Stearic Acid, Oleic Acid, Tween 20, and propylene glycol revealed a characteristic peak at 235.7°C. DSC examination of the pure drug and a physical mixture of NLC demonstrated that there was a negligible change in the melting point of puerarin when mixed with other excipients, showing that the drug and excipients did not interact. The DSC thermogram of a physical mixture of gels comprising sodium CMC, methyl paraben, propyl paraben, and triethanolamine revealed a characteristic peak at 239.4°C. The DSC examination of pure drug and a physical mixture of gel demonstrated that there was a negligible change in puerarin's melting point when mixed with other excipients, showing that the drug and excipients did not interact. The DSC thermograms for the final formulations of NLCs and PUE-NLC Gel exhibit characteristic peaks at 235.6°C and 237.7°C, respectively. The melting point of puerarin changed moderately when mixed with other excipients, suggesting a potential interaction between the medication and excipients in the final formulation.

Formulation studies of NLCs

Determination of solubility of drug in different oils, surfactants, and co-surfactants

The solubility of puerarin in different vehicles was investigated, and the results are presented in Table 4. Puerarin has the highest solubility in the surfactant Tween 20 (253.27 mg/mL). The solubility of puerarin in stearic acid and oleic acid was determined to be 0.163 mg/mL and 0.345 mg/mL, respectively. The solubility of puerarin in propylene glycol was reported to be 54.21 mg/mL. Tween 20 was chosen as the surfactant based on solubility experiments. Standard deviation for the solubility was 42.62.

DPPH assay

An analysis was conducted on the antioxidant activity of the puerarin-loaded NLC-optimized batch. Table 5 and Fig. 5 show the antioxidant activity of the puerarin-loaded NLC and ascorbic acid of the optimized formulation. The standard deviation for the DPPH assay was 27.56% for the antioxidant activity of puerarin and 24.29% for ascorbic acid.

Characterization of PUE-NLCs

Determination of globule size and polydispersibility index

Table 6 and Fig. 6 illustrate the results of the optimized formulation of globule size and polydispersibility. The globule size of the PUE-NLS was found to be between 61.70 nm and 131.00 nm, with a polydispersibility index of 0.345–0.446. The formulation F1 of NLC had the smallest globule size, 61.70 nm, with a polydispersibility index of 0.345. A polydispersibility index of <1 implies that the droplets are distributed uniformly throughout the formulation. The standard deviation for particle size was 25.82, and the polydispersibility index was 0.039.

Determination of zeta potential

The optimized formulation F1 of NLC had a zeta potential of −13.0 mV. The stable formulation was indicated by the negative zeta potential value. The results are shown in Fig. 7.

Morphological studies of NLC using SEM

According to SEM, puerarin was entirely dissolved in the NLC. The conclusion that the particles of the NLC are globular and well separated is supported by the SEM image. However, there was a variation in

Table 4: Solubility study

S. No.	Vehicles	Solubility (mg/mL)±SD
01	Stearic acid	0.163±0.0010
02	Oleic acid	0.345±0.0010
03	Tween 20	253.27±0.27
04	Tween 40	212.06±0.13
05	Tween 60	141.75±0.13
06	Tween 80	95.39±0.07
07	Propylene glycol	54.21±0.09
08	Water	0.03±0.0010

where the values are expressed as mean±SD (n=3). SD: Standard deviation

Table 5: Antioxidant activity (DPPH) assay

Concentration (µg/mL)	% Antioxidant activity of puerarin±SD	% Antioxidant activity of ascorbic acid±SD
10	10.96±0.11	21.83±0.14
20	23.65±0.19	31.83±0.18
40	34.76±0.28	43.16±0.22
60	50.35±0.24	58.82±0.28
80	76.58±0.245	74.49±0.22
100	87.97±0.20	92.15±0.22

Where the values are expressed as mean±SD (n=3).
DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, SD: Standard deviation

Table 6: Globule size and polydispersibility index of NLC

Formulation code	Particle size	PDI
F1	61.70 nm	0.345
F2	131.00 nm	0.446
F3	108.10 nm	0.414
F4	115.50 nm	0.365

NLC: Nanostructured lipid carrier, PDI: Polydispersibility index

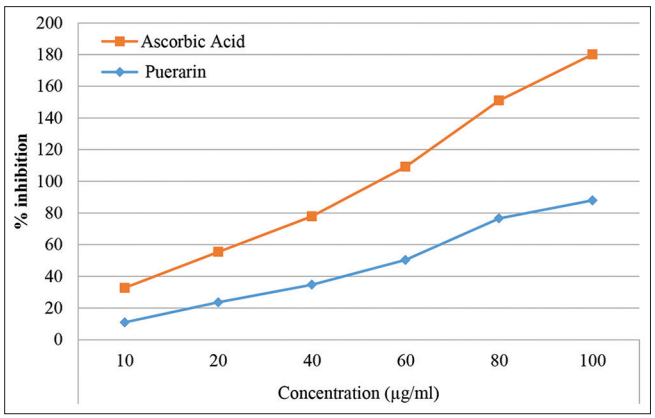

Fig. 5: Antioxidant activity (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay

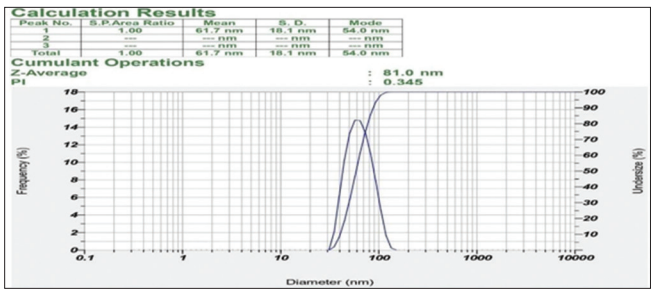

Fig. 6: Globule size and polydispersibility index of formulation F1 of nanostructured lipid carrier

size. The NLC image (Fig. 8) shows that the particles have an irregular surface area and a limited particle size distribution.

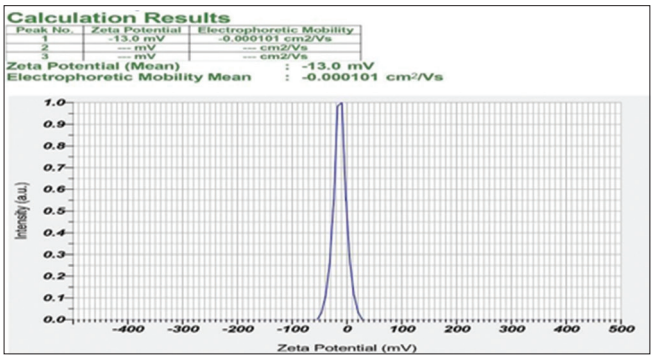

Fig. 7: Zeta potential of formulation F1 of nanostructured lipid carrier

Table 7: Entrapment efficiency of NLC

Formulation code	Entrapment efficiency (%)±SD
F1	87.80±0.17
F2	79.48±0.29
F3	45.90±0.31
F4	65.41±0.09

Where the values are expressed as mean±SD (n=3). NLC: Nanostructured lipid carrier, SD: Standard deviation

Determination of entrapment efficiency

The EE of puerarin (Table 7) in the NLC was between 87% and 80%. It was discovered that the optimized formulation F1 of NLC had an entrapment efficiency value of 87-80%. The standard deviation for entrapment efficiency was 15.87.

Formulation studies of gel formulation

Physical evaluation of gel formulation

The physical properties of the gel, such as appearance, color, odor, consistency, and washability, were assessed for six batches of NLC gel. Table 8 shows the outcomes of the formulation evaluations. Physical evaluation of gel formulations is shown in Fig. 9.

Physico-chemical evaluation of gel formulation

The physicochemical properties of the gel, such as pH, spreadability, extrudability, drug content, viscosity, and *in vitro* release, were investigated for six batches of NLC gels. Table 9 shows the physicochemical properties of these formulations.

In-vitro drug release of gel formulation

An *in vitro* drug diffusion study was performed using a diffusion cell assembly. The drug-loaded NLC gel was evaluated at 279 nm wavelength using a dialysis membrane as a barrier containing phosphate buffer solution (pH 7.4) as a medium. *In vitro* drug-release profiles are shown in Table 10. Fig. 10 shows a graph of the % cumulative drug release.

Table 8: Physical evaluation of NLC gel

Evaluation parameters	F1	F2	F3	F4	F5	F6
Appearance	Clear and transparent	Clear and transparent	Clear and transparent	Clear and transparent	Clear and transparent	Clear and transparent
Color	Pearl white	Pearl white	Pearl white	Pearl white	Pearl white	Pearl white
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
Washability	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable

NLC: Nanostructured lipid carrier

Table 9: Physicochemical evaluation of NLC gel

Evaluation parameters	F1±SD	F2±SD	F3±SD	F4±SD	F5±SD	F6±SD
pH	6.48±0.016	6.30±0.020	6.43±0.016	6.27±0.020	6.36±0.012	6.51±0.012
Spreadability (g*cm/s)	21.18±0.012	23.11±0.008	26.64±0.016	22.64±0.012	25.59±0.012	27.8±0.012
Extrudability (%)	83.45±0.008	80.96±0.008	88.23±0.008	84.94±0.012	86.52±0.008	92.02±0.008
Drug content (%)	95.82±0.008	97.21±0.008	96.46±0.008	98.08±0.012	98.82±0.008	99.13±0.008
Viscosity (cp)	3325.10±0.008	3479.30±0.012	3542.20±0.008	3543.00±0.012	3571.90±0.008	3593.40±0.012

Where the values are expressed as mean±SD (n=3). NLC: Nanostructured lipid carrier, SD: Standard deviation

Table 10: *In vitro* drug release

Time (h)	% Cumulative drug release (mean±SD)					
	F1	F2	F3	F4	F5	F6
0	0±0	0±0	0±0	0±0	0±0	0±0
1	11.16±0.012	10.28±0.008	17.56±0.0124	11.16±0.0124	12.13±0.0124	19.47±0.0081
2	23.14±0.012	19.10±0.008	22.66±0.008	17.16±0.012	20.43±0.012	29.69±0.0124
3	29.63±0.012	30.15±0.008	27.37±0.008	21.26±0.0081	28.60±0.008	40.90±0.0169
4	36.99±0.016	36.68±0.012	32.00±0.0124	28.57±0.016	39.12±0.0124	47.49±0.008
5	41.44±0.0124	42.34±0.012	38.19±0.0081	33.41±0.0124	48.19±0.008	52.57±0.0081
6	49.07±0.0081	48.90±0.016	46.95±0.0124	45.11±0.008	54.51±0.0124	59.97±0.0124
7	54.47±0.008	55.25±0.0124	54.43±0.0124	52.82±0.008	63.55±0.008	66.50±0.008
8	60.99±0.008	61.99±0.012	64.34±0.012	64.88±0.0124	69.45±0.0081	74.62±0.0081

Where the values are expressed as mean±SD (n=3). SD: Standard deviation

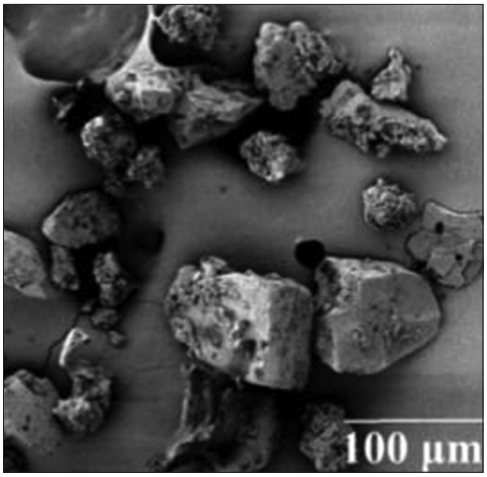

Fig. 8: Scanning electron microscopy of formulation F1 of nanostructured lipid carrier

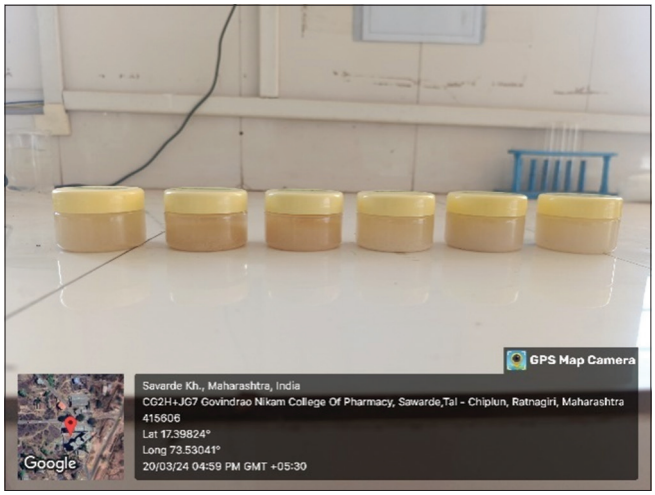

Fig. 9: Physical evaluation of gel formulation

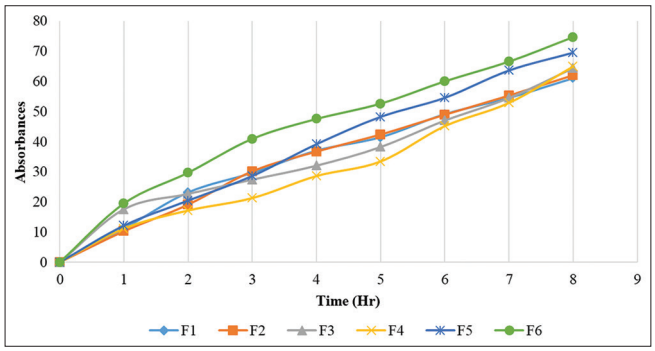

Fig. 10: The graph of % cumulative drug release

CONCLUSION

FTIR and DSC analyses of Puerarin, Tween 20, stearic acid, oleic acid, and their physical combination revealed no significant interaction. Puerarin-loaded NLCs were prepared using the solvent emulsification process with Tween 20 as a surfactant because puerarin is more soluble than the other surfactants. Particle size, zeta potential, DPPH assay, and entrapment efficiency were tested on four batches of NLC formulations 4 batches. NLCs were added to a gel matrix containing sodium CMC as a gelling agent. Preliminary experiments on six batches of NLC-loaded gel formulations of 6 batches assessed appearance, color, consistency, and washability, as well as physicochemical parameters such as pH, spreadability, extrudability, drug content, viscosity, and *in vitro* release in phosphate buffer (pH 7.4). The optimized NLC-loaded gel formulation F6 demonstrated superior stability and controlled-release properties compared to the other batches. These findings suggest that the developed NLC-loaded gel formulation F6 could be a promising topical delivery system for puerarin, offering potential applications for the treatment of various skin conditions. The optimized NLC-loaded gel formulation F6 exhibited excellent skin compatibility and minimal irritation during the dermal safety studies. Further investigations revealed that the NLC-loaded gel significantly increased the bioavailability of puerarin compared to conventional topical formulations. Clinical trials are currently underway to evaluate the long-term efficacy and safety of this novel puerarin delivery system in patients with specific dermatological disorders.

INFORMED CONSENT STATEMENT

Not relevant.

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FUNDING

None.

CONFLICTS OF INTEREST

There are no conflicts of interest among the writers. The funders had no part in the design of the research, in the collection, analysis, or interpretation of data, in the writing of the paper, or in the decision to publish the findings.

AUTHOR CONTRIBUTIONS

Conceptualization, Madan Dadaso Pomaje; Data curation, Shruti Shriram Tikam, and Ashwini Bhauso Patil; Investigation, Methodology, Project administration, Resources, Software, Validation, writing – Original draft, Writing – review & editing, Madan Dadaso Pomaje, Shruti Shriram Tikam, Ashwini Bhauso Patil, Viraj Abhay Kamat.

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