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RESEARCH ARTICLE

Development and Evaluation of Nanostructured Lipid Carriers for Transdermal Delivery of Ketoprofen

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SUMMARY

Ketoprofen is a nonsteroidal anti-inflammatory drug that displays significant gastrointestinal side effects when administered via the oral route and has a low skin permeation profile. The objective of the present work is to utilise nanostructured lipid carriers (NLCs) as a carrier system for the transdermal delivery of ketoprofen. NLCs were prepared via hot homogenisation technique using beeswax, carnauba wax, glyceryl monostearate (solid lipids), linseed oil (liquid lipid) and poloxamer188 (surfactant) and optimized using custom design via JMP. The responses evaluated were drug entrapment efficiency, particle size and drug release profile. The experimental design was evaluated for model fit with the assistance of variance analysis. The optimum formulations were characterized for particle size, zeta potential, scanning electron microscopy (SEM), differential scanning colorimetry, Fourier transform infrared spectrum (FTIR) and also drug content, entrapment efficiency, in-vitro drug release, and ex-vivo drug release profile was studied. The drug loading efficiency between the formulation ranges between 34±0.03-95.06±0.01%. The drug release from the formulations over a 24 h study was found to be 80%±0.09 to 95%±0.06. The maximum desirability was found to be 0.91. The optimum formulation showed a mean particle size of 425.8nm and a zeta potential of -45mV. SEM results revealed slightly agglomerated particles with uneven surfaces. The ex-vivo skin permeation of NLC optimized patch formulation exhibited a higher flux and permeability coefficient than the pure drug patch formulation, and marketed gel (2.5%w/w) FTIR spectra assured the chemical and physical compatibility. Transdermal delivery of ketoprofen via NLCs would be a promising approach for improving skin permeation.

Key Words: *Ketoprofen, permeation, NLC, skin, oils, optimization, ex-vivo, flux*

Ketoprofen'in Transdermal Uygulanması İçin Nanoyapılı Lipid Taşıyıcıların Geliştirilmesi ve Değerlendirilmesi

ÖZ

Ketoprofen, oral yoldan uygulandığında önemli gastrointestinal yan etkiler gösteren ve düşük deri permeasyon profiline sahip, nonsteroidal antiinflamatuar bir ilaçtır. Mevcut çalışmanın amacı, ketoprofenin transdermal iletimi için taşıyıcı sistem olarak nanoyapılı lipid taşıyıcıları (NLT) kullanmaktır. NLT'ler, balmumu, karnauba mumu, gliserl monostearat (katı lipidler), keten tohumu yağı (sıvı lipid) ve poloksamer188 (yüzey aktif madde) kullanılarak sıcak homojenizasyon tekniği ile hazırlanmıştır ve JMP aracılığıyla özel tasarım kullanılarak optimize edilmiştir. İlaç tutma etkinliği, parçacık boyutu ve ilaç salım profili yanıtları değerlendirilmiştir. Deney tasarımı, varyans analizi yardımıyla model uygunluğu için değerlendirilmiştir. Partikül boyutu, zeta potansiyeli, taramalı elektron mikroskobu (SEM), diferansiyel taramalı kalorimetre, Fourier dönüşümlü kızılötesi (FTIR) için optimum formülasyonlar karakterize edilmiş ve ayrıca ilaç içeriği, tuzak etkinliği, in vitro ilaç salınımı, ex-vivo ilaç salım profili incelenmiştir. Formülasyonlar arasında ilaç yükleme etkinliği 34±0.03-95.06±0.01% aralığındadır. 24 saatlik bir çalışma boyunca formülasyonlardan ilaç salınımının %80±0.09 ila %95±0.06 olduğu bulunmuştur. Maksimum arzu edilirlik 0.91 olarak bulunmuştur. Optimum formülasyon, 425.8 nm'lik bir ortalama parçacık boyutu ve -45mV'lik bir zeta potansiyeli göstermiştir. SEM sonuçları, düzgün olmayan yüzeylere sahip hafif aglomere partiküller ortaya çıkarmıştır. NLC ile optimize edilmiş yama formülasyonunun ex-vivo deri geçirgenliği, saf ilaç yama formülasyonuna kıyasla daha yüksek bir akış ve geçirgenlik katsayısı göstermiştir ve ticari jel (%2,5 a/a) FTIR spektrumları, kimyasal ve fiziksel uyumluluğu desteklemektedir. Ketoprofenin NLC'ler yoluyla transdermal olarak verilmesi, deri geçirgenliğini iyileştirmek için umut verici bir yaklaşım olacaktır.

Anahtar Kelimeler: *Ketoprofen, permeasyon, NLT, Deri, yağlar, optimizasyon, ex-vivo, akım*

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INTRODUCTION

The main aim of novel drug delivery is to provide sustained drug action at a fixed rate or by keeping a relatively constant, adequate level of drug in the body with less or no toxicity or side effects in the affected tissue or organ or to achieve the target drug action employing carriers to deliver the drug to a specific target site (Tamjidi et al., 2013).

Nanotechnology's use is extensive in drug delivery, especially for the transdermal delivery of therapeutic agents. Nanocarriers have been a successful tool for delivering the drug to the target site due to their optimized physicochemical properties. Many nanocarriers are available in the form of dendrimers, polymers, magnetic nanoparticles, silicon, carbon materials, lipid-based systems, etc. (Ghasemiyeh et al., 2018).

Lipid-based systems are more promising over other carriers due to lipids' physiologically safe and biodegradable nature. The lipid-based system has been extended into liposomes, solid lipid nanoparticles (SLN), and nanostructure lipid carriers (NLC). These nanostructures can hold hydrophilic and hydrophobic molecules and exhibit very low toxicity. In addition, they extend the drug action by its sustained drug release property, prolonging the half-life. The carrier surface can be remoulded by a suitable chemical approach, which can overcome sensing by the immune system that helps enhance the drug's permeation across physiological barriers (Jain et al., 2016).

The purpose of developing this first-generation lipid nanocarrier system SLN is to achieve drug delivery by several routes of administration in the medical management of physiological complications. NLCs were introduced by Muller, in which drug incorporated matrix was developed by substituting a fraction of solid lipids with liquid lipids. Due to their biocompatibility and superior formulation properties, NLCs have contemplated potentiality over SLNs. The NLC is a colloidal system class containing biocompatible and regulatory permissible lipids supplied as a liquid and solid carrier material, improving the inserted drug's chemical stability (Chaudhary et al., 2013). The advantages of NLC over liposomes are its high drug loading, low cost of raw materials, and good stability. In addition, they can produce smaller particulates decrease drug leakage problems during storage, amplify the bioavailability, and enable the modulation of the medication, which results in controlled and extended-release properties of the product (Zhou et al., 2018).

Ketoprofen is a propionic acid-based non-steroidal anti-inflammatory medication used to reduce the inflammation, stiffness, and pain involved with osteoarthritis and rheumatoid arthritis. Ketoprofen suppresses the synthesis of prostaglandin and thromboxane precursors by inhibiting the activity of cyclo-oxygenase I and II enzymes. It is absorbed rapidly through the gastrointestinal tract.; peak plasma can occur about 0.5 to 2 hours after a dose. When ketoprofen is given with food, the total bioavailability is altered, and the absorption rate is slowed. The elimination half-life in plasma is about 1-5 to 4 hours. Also, like most nonsteroidal anti-inflammatory drugs, ketoprofen presents gastrointestinal adverse side effects. (Reynolds et al., 1993; Manikkath et al., 2017).

Moreover, the short elimination half-life demands frequent dosing. As a result, it generates an effective transdermal formulation of the therapeutic agent that could assist in the elimination of the drug's oral administration-related side effects while simultaneously enhancing therapeutic efficacy and safety. The challenge in conventional transdermal therapy is that only a tiny amount of ketoprofen is absorbed following topical application. It is applied as 2.5% gel 2 to 4 times daily for seven days for local pain relief.

Therefore, in the present study, NLC has been chosen as a carrier system for increasing transdermal delivery of ketoprofen, and the custom design approach was used for optimization.

MATERIALS AND METHODS

Ketoprofen was an endowment from BEC Chemicals Pvt. Ltd, Mumbai, India. In addition to other chemicals, the lipids were purchased from Sigma Aldrich Mumbai.

Drug: excipient compatibility study by FTIR

A Fourier transform infrared spectrum (FTIR) was used to identify any interaction between ketoprofen and excipients. The sample was analysed by the potassium bromide pellet method using an IR spectrophotometer (Thermo- Nicolet 6700) in the spectral region between 4000-400cm^{-1}(Patwekar et al., 2017).

Preparation of blank formulations

Blank formulations were prepared using liquid lipids linseed oil, solid lipids beeswax, carnauba wax, and glyceryl monostearate alone and in combination with two surfactants, namely tween 80 and poloxamer188. A hot homogenization technique where melted lipids were amalgamated with aqueous surfactant solution under mechanical stirring at 500 rpm to produce coarse oil/water emulsion. This coarse emulsion was further homogenized (Polytron homogenizer) to get a fine emulsion. The resultant emulsion was cooled to room temperature to obtain NLCs. The physical properties of the formulations were observed. Based on physical properties, drug-loaded NLCs were prepared by adding the drug to a melted lipid combination (bee wax, carnauba wax, and glyceryl monostearate), which was further added hot aqueous solution of poloxamer 188, and the same procedure mentioned above was followed (Patwekar et al., 2017).

Utilization of experimental custom design

In pharmaceutical development, quality by design/QbD has improved the resulting formulation integrity by lowering the number of required testing methods, evaluated using various mathematical models. A custom design approach using JMP software version 11 was used to optimize both formulation and processing parameters on the quality attributes. The factors such as phase volume ratio (%), solid lipid: liquid lipid, drug: lipid, stirring speed (rpm), and surfactant (%) were studied on the responses of drug entrapment efficiency (%), particle size(nm) and drug release (%) as given in the (Table 1) and (Table 2). The design generated 12 experimental trials, as given in (Table 3). After experimental trials, responses were collated (Ashwini et al., 2020; Subramanyam et al., 2020).

Table 1. Factors chosen in experimental design

Factors	Levels of factors		
	Low	Medium	High
Phase volume ratio (%)	20		50
Solid lipid: liquid lipid (%)	17		50
Drug: lipid (%)	17		50
Stirring speed (rpm)	10000	12000	15000
Surfactant (%)	0.3		2

Table 2. Responses of experimental design

Responses	Minimum	Maximum
Drug entrapment efficiency (%)	80	90
Particle size(nm)	100	900
Drug release (%)	80	90

Table 3. Experimental runs

Formulation code	Solid lipid: lipid (%)	Drug: Lipid (%)	Phase volume ratio (%)	Surfactant concentration (%)	Stirring speed(rpm)
T1	50	50	50	2	12500
T2	50	50	50	0.3	12500
T3	33.5	33.5	35	1.15	10000
T4	50	50	20	2	10000
T5	17	17	50	2	150000
T6	17	17	20	2	150000
T7	50	50	20	0.3	150000
T8	17	17	50	0.3	10000
T9	33.5	33.5	35	1.15	10000
T10	17	17	20	2	12500
T11	50	50	50	0.3	150000
T12	17	17	20	0.3	12500

Evaluation of NLCs

Drug content and drug entrapment efficiency

For drug content, NLC equivalent to 10 mg of ketoprofen (based on the theoretical drug content) was weighed, transferred into 50 ml of the volumetric flask, and dissolved in (30ml methanol: 20ml ethanol) (stock-I). The 1ml of stock-I preparation was diluted to 10 ml using methanol. The absorbance of 254 nm was measured by UV spectrophotometry. The blank formulation was treated the same way the sample was used as blank (Manikkath et al., 2017). NLC equivalent to 10mg of ketoprofen was weighed and placed in Eppendorf tubes, in 10 ml of methanol, under vigorous vortexing. Centrifugation of the solution was carried out at 5000-10000rpm for 50 min. The resulting supernatant was further diluted with methanol. The absorbance was then measured at 254 nm using methanol as a blank with a UV spectrophotometer (Ramkanth et al., 2018). The entrapment efficiency was calculated by Formula (1) given below (Manikkath et al., 2017).

$$EE = \frac{W - Ws}{W} \times 100, \text{ formula (1),}$$

Where, W = Total drug content, Ws = Free drug content,

Particle size analysis (Horiba SZ-100 particle size analyser):

In nanoparticles, the particle size should not be greater than 1000 nm. The principle involved dynamic light scattering technique <1nm to >1μm. Horiba SZ-100 nanoparticle dynamic light scattering system was used to ascertain the mean particle size, particle size distribution, and zeta potential. All the samples were diluted with double distilled water, and every sample was checked in triplicate at a scattering angle of 90° at 25.2 °C (Fan et al.,2013).

In-vitro release studies for NLC:

Franz diffusion cell assembly was used to perform the *in vitro* studies. About 5mg drug equivalent weight of NLC was put on a cellophane membrane between the donor compartment and receptor compartment of diffusion cell assembly. The donor compartment was moistened by 1ml phosphate buffer of pH 5.5. The receptor compartment is filled with 50 ml of phosphate buffer of pH7.4. Continuous stirring of the receptor compartment at 100-250 rpm employing a magnetic stirrer at a temperature of 35°C. The drug release rate from NLC was carried out by withdrawing 1 ml of receptor fluid every one-hour time interval. The sink condition was maintained by adding an equal volume of phosphate buffer saline (PBS). The spectrophotometric method analyzed the drug concentration after suitable dilution at a λ max of 260 nm (Nair et al., 2015).

Evaluation of experimental study and characterisation of optimum formulations

The responses from the experimental study were substituted into the design and assessed for model fit. The design space was identified from the model, and the desirability function was identified. The surface response curves were obtained. Optimal formulations were prepared and evaluated, and the optimum formula was selected to prepare patches.

Zeta potential determination

Zeta potential is the main characteristic needed for nanoparticles in surface charge estimation. It is a vital factor in the NLC's physical stability. SZ-100 HORIBA Scientific performed the zeta potential measurements. The samples were then diluted with double distilled water, and particles were assessed in triplicate at 90° by electrophoretic light scattering at a temperature of 25°C (Fan et al.,2013).

Scanning Electron microscopy (SEM):

Scanning Electron microscopy model Tescan Vega3 was used to examine the surface morphology of the optimized formulation. NLC dispersion was sputtered with gold and spread on a sample holder. Under argon purging, the figures were acquired at a voltage of 30 kV (Nair et al., 2015).

Differential scanning colorimetry

Differential scanning colorimetry (DSC) analysis was carried out to confirm the thermal behaviour of

pure drugs in the presence of lipids. Approximately 5mg of the sample and drug equivalent formulations were weighed and crimped into non-hermetically sealed aluminum pans. These samples were heated from 0°C to 350°C at 10°C/min. During the measurement, nitrogen was constantly purged at a rate of 40ml/min, and DSC thermograms were documented in Shimadzu DSC-60, Japan (Souto et al., 2005).

Preparation of NLC loaded patch

The transdermal patch used the aluminium-backed adhesive membrane method; A 1.25 % w/v solution (minimum concentration as a film-forming agent) of hydroxypropyl methylcellulose-K100 was dispersed in water, and 1% w/w of polyethylene glycol was added as a plasticizer, followed by the addition of 0.1%w/v of methylparaben. The solution was continuously stirred for 15 min at 1000 pm. Optimized drug-loaded NLC formulations of (0.812g) containing 100mg of the drug were incorporated into the above solution and mixed till a uniform dispersion was obtained. The dispersion was poured over a sheet of an aluminium backing layer covering the area of 67.8cm^2 and dried. A patch formulation of ketoprofen (100mg) was prepared by the same procedure mentioned above for comparison (Patel et al.,2009).

Evaluation of drug loaded patches

Drug content

NLC incorporated patch area of 7 cm^2 containing an equivalent amount of ketoprofen (10mg) was cut, transferred into 50ml of volumetric flask diluted with a mixture of 30ml of methanol and 20 ml warm solution of ethanol (stock-I). 1ml of the stock-I solution was diluted to 10 ml using methanol. UV spectrophotometry measured the absorbance was then measured at 254nm by UV spectrophotometry against the same blank. Note: The pure patch preparation drug content was studied by the same procedure mentioned above (Patel et al.,2009).

Thickness, weight uniformity and folding endurance of patches

Thickness was measured using a screw gauge checking at five different points. 15 -Weight uniformity was measured from five randomly cut (1cmx1cm) patches, and the average weight was calculated (Patel et al., 2009).

A patch strip was cut equally and repeatedly folded at the same spot for folding endurance till it broke (Patel et al., 2009).

Moisture content (%):

Each of the prepared patch was weighed separately and placed in desiccators activated silica for 24h, at room temperature till a constant weight was attained (Patel et al., 2009).

Moisture uptake (%):

The weighed patches were placed in a desiccator with potassium chloride saturated solution at room temperature for 24h at relative humidity (RH) of 84%. After 24 h, the patches were weighed again, and formula (2) determined moisture uptake percentage below (Patel et al.,2009).

$$\%moisture\ uptake = \frac{(Final\ weight - Initial\ weight) * 100}{Initial\ weight}$$

Formula (2)

Ex vivo permeation studies

Preparation of skin samples

The research was performed based on approval for use of wistar rats by Institutional animal ethical committee with ethical approval number (KCP/IAEC/ PCEU/37/2019). *Ex-vivo* permeation studies were performed using the abdominal skin of a healthy male albino Wistar rat. The structural similarities of the rat abdominal skin to human skin are owing to its selection for *ex-vivo* studies. In order to ensure the integrity of the skin, hairs from the abdominal region of the rats were shaved using electronic hair remover without scratching or harming the skin surface. After the sacrifice of the rat, full-thickness skin was excised, of which a specific portion of the skin was excised and washed with distilled water before being used in the permeation study (Phatak et al.,2013; Mennini et al., 2016).

Procedure:

Ex vivo studies were performed by modified Franz diffusion cell assembly. Ketoprofen (5mg) equivalent weight of NLC incorporated patch area of 3.5cm^2 and 6.8cm^2 area of patch formulation containing(5mg) of pure drug and 5mg drug equivalent weight of marketed gel of ketoprofen (2.5%) was separately placed on to the skin sample which was inserted between donor compartment and receptor compartment of diffusion cell assembly. The donor compartment is wetted by 1ml phosphate buffer pH 5.5. The receptor compartment is filled with 50 ml of phosphate buffer pH 7.4. The receptor compartment was constantly stirred using a magnetic stirrer at 100 rpm, maintained at 35°C. The drug release rate from NLC was carried out by withdrawing of 1 ml of receptor fluid every one-hour time interval. The sink condition was preserved by adding an equal amount of PBS. The drug concentration was analysed using a spectrophotometric method after suitable dilution against the blank PBS at a λ max of 260 nm. Drug flux (µg/hr/cm^2) at a steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface, and the permeability coefficient

$$Log\ cd = -\frac{log\, cd\,(0) - PSt}{2.303vd} \qquad \text{Equation (1)}$$

Where Cd = Concentration of donor compartment, Cd (0) = Initial Concentration, P= Permeability coefficient, S=Surface area (Mennini et al., 2016).

FTIR Studies

FTIR studies were carried out for NLC formulation and optimal NLC-loaded ketoprofen patch formulation. Samples were assessed by the potassium bromide pellet method in an IR spectrophotometer (Thermo- Nicolet 6700) in the spectral region between 4000-400cm^{-1} (Zhai et al.,2014).

Stability studies

In the stability studies of NLCs, NLC incorporated patch formula was studied at 25 °**±2**° C/60 % RH ±5% and 40°**±2**° C/75 % RH ±5% for three months. The physical nature, drug content, and entrapment efficiency of formulations were checked during and at the terminal stage of the study period (Patwekar et al., 2018).

RESULTS AND DISCUSSION

Preparation and evaluation of NLCs.

Preliminary trials for the preparation of NLC were carried out by preparing placebo formulations. Liquid lipid linseed oil was used with solid lipids carnauba wax, and beeswax individually and in combination. Blank NLCs were prepared in the presence of surfactants, namely poloxamer 188, and at varying concentrations. NLCs obtained using the surfactant poloxamer were found to be free-flowing. Poloxamer has a high hydrophile-lipophile balance value in comparison to tween 80. So, lipid systems get emulsified with higher efficiency, resulting in o/w emulsion contributing to the free-flowing nature of NLCs. Carnauba wax, beeswax, and glyceryl monostearate were used as solid lipid, linseed oil as liquid lipid, and poloxamer as a surfactant in preparing drug-loaded NLCs. The hot homogenization technique was used in preparing NLCs. The formulations were optimized by a custom design approach using JMP 13. The factors selected were solid lipid to liquid lipid ratio, the drug to lipid ratio, surfactant concentration, phase volume ratio, and the stirring speed. The responses chosen were drug entrapment efficiency (%), drug release (%), and particle size.

Drug content and entrapment efficiency

The drug entrapment was between 34±0.03 to95±0.01% shown in (Table 4). The lowest drug content was observed in T1. The low surfactant is insufficient is insufficient to emulsify the large proportion of the lipids used in the T1 formula. In contrast, formula T5 showed a drug content of 69.92±0.1%. Though the concentration of surfactant used in this formula is high, the liquid lipid concentration is high compared to the solid lipid, and subsequent drug leaking in the absence of a low amount of solid lipids from the system; since NLC consists of NLCs comprised of low and high melting lipids, the coexistence of these two is

required to provide increased loading efficiency.

When the oil is mixed with solid lipid, phase separation results in nano-droplets of fat surrounded by a solid matrix, the presence of lipid matrix on the oil surface prevents leakage of drugs from the interior and thus slows down the drug release and higher entrapment efficiency.

Particle size

The particle size of all formulations ranged from 89-950 nm (Table 4). The characteristics and concentrations of surfactant greatly influence the standard and efficacy of nano lipid carriers. The formula T7 and T9 exhibited the largest particle size. There is a large concentration of lipid phase and a lack of poor emulsification in low surfactant concentration. A higher surfactant-to-lipid ratio may advance the emergence of smaller particles and provide higher stabilization of the nano-system due to the scaling down of interfacial tension between the lipid matrix and the hydrophilic phase. The amphiphilic nature helps the surface-active agents be preferentially located in interfacial regions to lower the interfacial pressure between lipid and aqueous phases. Non-ionic emulsifier, especially poloxamer 188, provides another steric stabilization effect that circumvents the aggregation of fine particles in the colloidal system.

Table 4. Results of particle size, drug content and drug entrapment efficiency

Formulation code	Particle size (nm) + SD	Drug content (%)+ SD	Drug entrapment efficiency (%)+ SD
T1	873.6±0.11	55.2±0.09	34±0.03
T2	280.1±0.39	86.8±0.1	72±0.04
T3	597.1±0.07	76.6±0.009	85±0.05
T4	149.7±0.41	99.8±0.03	74.9±0.008
T5	89.3±0.13	75±0.04	69.92±0.1
T6	506.8±0.24	95.2±0.06	93.2±0.6
T7	104.5±0.18	98.2±1.2	95.06±0.01
T8	957.3±0.42	89±0.3	85.2±0.7
T9	942.8±0.1	85±0.7	75.2±0.03
T10	184.8±0.21	85.2±0.2	75.5±0.07
T11	660.2±0.33	88.9±0.006	85±0.02
T12	198.3±0.27	90±0.1	86.2±0.4

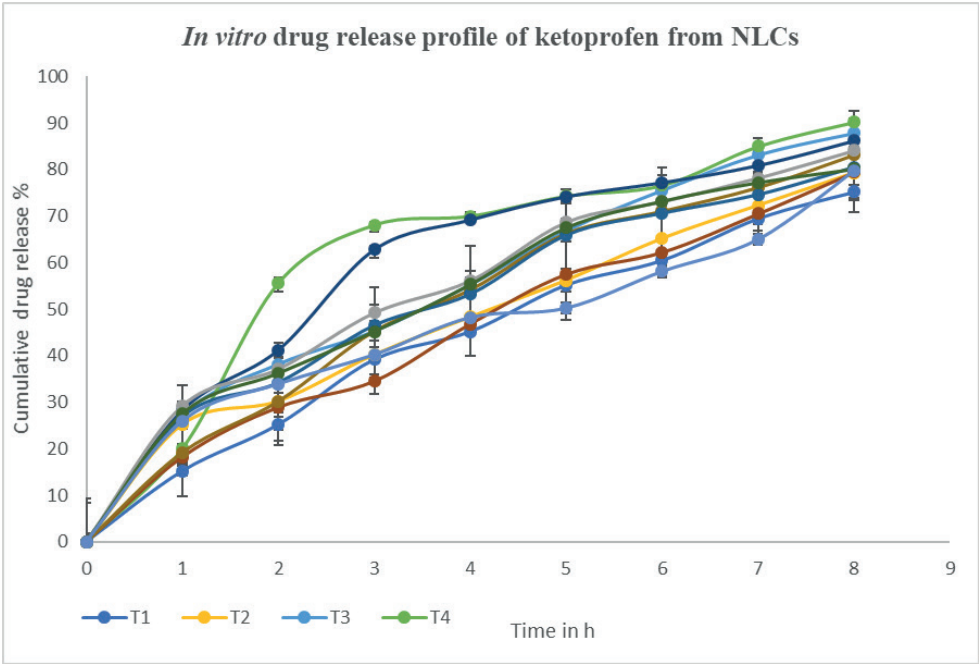

Figure 1. *In vitro* drug release profile of ketoprofen loaded formulations a (T1-T12)

Drug release profile

The formulation drug release over a 24h study was found to be 80%±0.09 to 95%±0.06 shown in (Figure 1). The spatial organization of solid-liquid domains is essential in drug release. The rate at which the drug is released gets affected by the structure of nanoparticles. In typical lipid systems, the uniform distribution of the drug molecules inside the lipid matrix followed by diffusion of these agents through these lipid systems results in drug release. In NLCs, an irregular spatial arrangement of drug molecules, the presence of fluid-natured lipid can cause a rise in the diffusion coefficients of the drugs, thus favouring a faster drug release.

Table 5. Statistical evalution of experimental runs

Sl.No	Source	LogWorth	P value
1	Surfactant (%)(0.3,2)	2.032	0.00928
2	Phase volume ratio(%)(20,50)	1.745	0.01799
3	Solid: Liquid lipid(%)(17,50)	1.655	0.02214
4	Drug: Lipid(%)(17,50)	1.445	0.03592
5	Stirring speed(rpm)	1.364	0.04327

Evaluation of experimental design and summary of the response evaluation

In formulation development, method optimization about the amount of the ingredients and process parameter settings plays an essential role in achieving the required characteristics. Custom design is one of the experimental designs which can tackle a wide range of challenges, all within a framework. So, evaluation of critical formulation variables, the custom design approach was adopted, and a statistical evaluation was carried out. (Table 5).

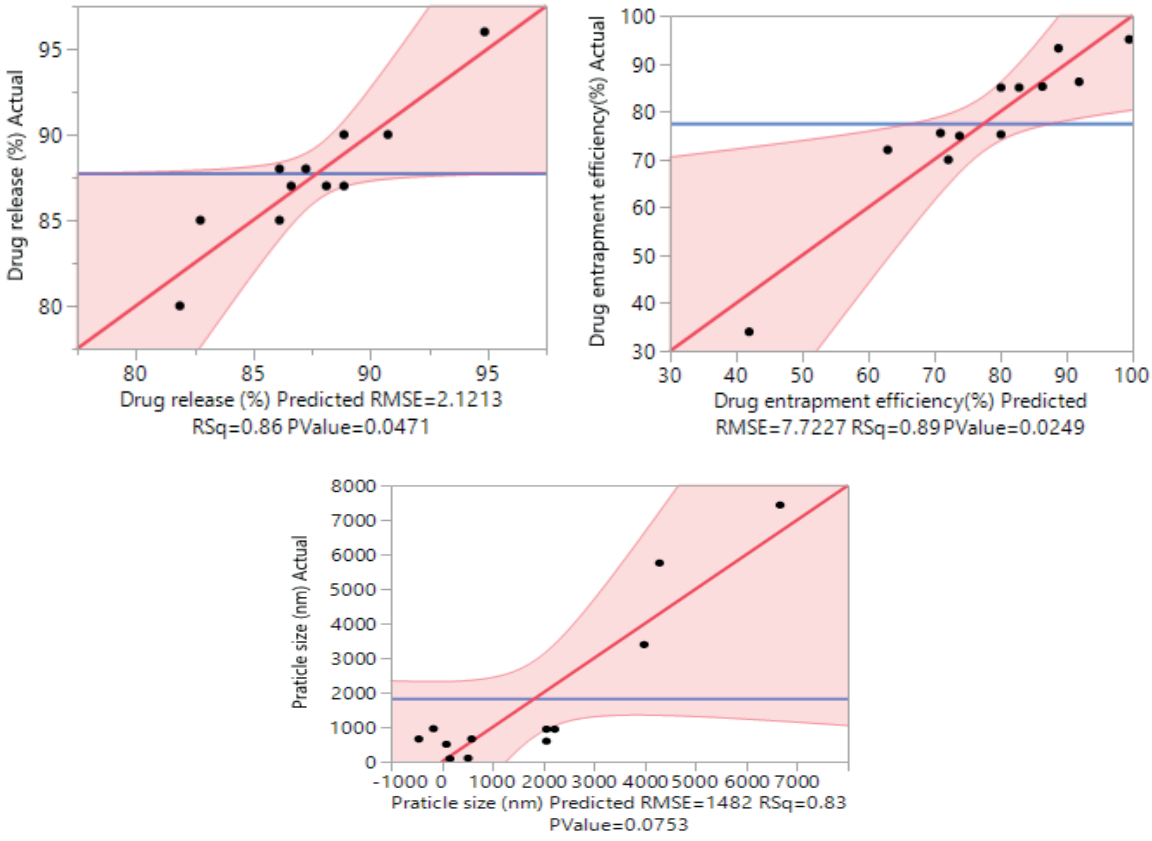

Figure 2. (A) Actual vs Predicted plot of drug release %, (B) drug entrapment efficiency (%) and (C) particle size (nm)

The responses were assessed for their suitability for model fit. P values give the statistical significance of each factor on the selected response. The values ≤ 0.05 indicate that the chosen factors significantly affect overall responses. A highly influencing factor was surfactant concentration, whereas stirring speed did not significantly affect the responses.

The actual vs. predicted plots of the three responses (Figure 2) and the summary of all the factors, level of interactions, and statistical influence is represented in (Table 6,7,8). The desirability function of the model prediction was found to be 0.35 (Figure 3), and the maximum desirability was found to be 0.91. The prediction formula for optimum response is given in equations 2, 3, and 4. Based on the maximum desirability function optimal formula was selected.

Table 6. Response evaluation of entrapment efficiency (%)

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	77.598333	2.229339	34.81	<.0001*
Solid:liquidlipid (%)(17,50)	-5.13375	2.554029	-2.01	0.1006
Drug:lipid (%)(17,50)	-0.50875	2.554029	-0.20	0.8500
Phase volume ratio(%)(20,50)	-8.84375	2.554029	-3.46	0.0180*
surfacant (%)(0.3,2)	-10.49125	2.554029	-4.11	0.0093*
Stirring speed (rpm)[10000]	2.4766667	3.152762	0.79	0.4677
Stirring speed (rpm)[12500]	-10.67333	3.152762	-3.39	0.0196*
Stirring speed (rpm)[150000]	8.1966667	3.152762	2.60	0.0483*

Table 7. Response evaluation of Particle size(nm)

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	77.598333	2.229339	34.81	<.0001*
Solid: lipid (%)(17,50)	-5.13375	2.554029	-2.01	0.1006
Drug: lipid (%)(17,50)	-0.50875	2.554029	-0.20	0.8500
Phasevolume ratio(%)(20,50)	-8.84375	2.554029	-3.46	0.0180*
Surfactant (%)(3,6)	-10.49125	2.554029	-4.11	0.0093*
Stirring speed (rpm)[1000]	2.4766667	3.152762	0.79	0.4677
Stirring speed (rpm)[5000]	-10.67333	3.152762	-3.39	0.0196*
Stirring speed (rpm)[10000]	8.1966667	3.152762	2.60	0.0483*

Table 8. Response evaluation of drug release (%)

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	87.75	0.612372	143.30	<.0001*
Solid:liquid lipid %)(17,50)	0.9375	0.701561	1.34	0.2390
Drug:lipid (%)(17,50)	-1.8125	0.701561	-2.58	0.0492*
Phase volume ratio(%)(20,50)	-1.8125	0.701561	-2.58	0.0492*
Surfactant (%)(0.3,2)	-0.4375	0.701561	-0.62	0.5602
Stirring speed (rpm)[10000]	3	0.866025	3.46	0.0180*
Stirring speed (rpm)[12500]	-2.75	0.866025	-3.18	0.0247*
Stirring speed (rpm)[150000]	-0.25	0.866025	-0.29	0.7844

The desirability approach is a widely used and accepted method for dealing with multiple response processes. It is an objective function that ranges from zero to one. The value depends on how close the upper and lower limits are compared to the optimum. The desirability of the experimental design was found to be 0.35, and the maximum desirability was found to be 0.91. Five factors were chosen for experimental design with low- and high-level values shown in (Table 9).

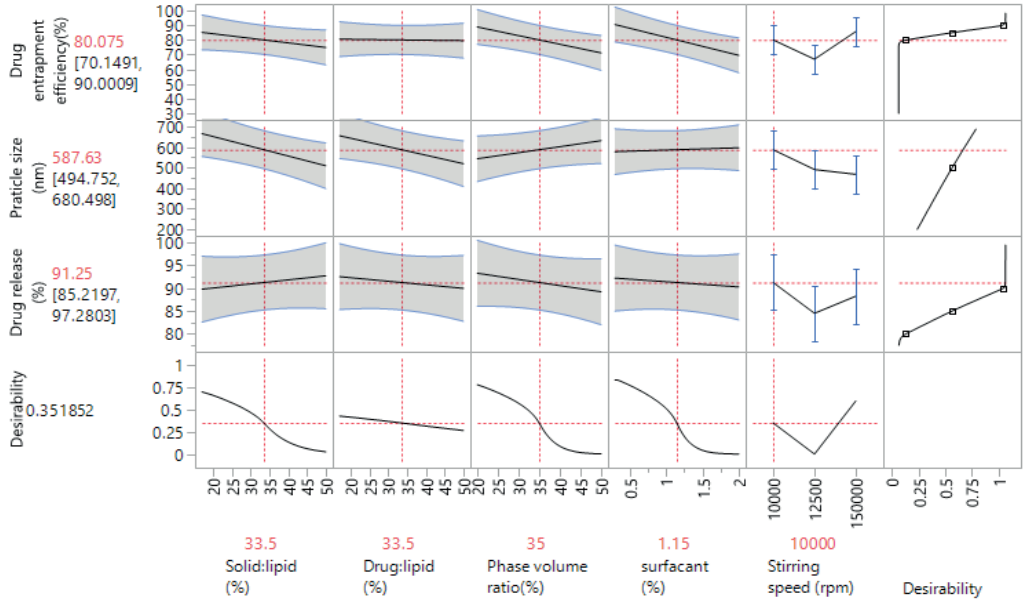

Figure 3. Predication profiler for the experimental runs

Table 9. Prediction formula

	Factors levels	Drug entrapment efficiency (%)	Particle size (nm)	Drug release (%)
Solid: liquid lipid (%)	33.5			
Drug: lipid (%)	33.5			
Phase volume ratio (%)	35			
Surfactant (%)	1.15	80.075	543.06	87.3125
Stirring speed	10000			

Selection of optimal formula and evaluation

Surface response curves and polynormal equations

Surface response curves are a powerful approach for analysing systems and identifying potential trade-offs. Response surface plots such as contour and surface plots are convenient for setting up the required responses and operating conditions. The contour gives the response surface a 2-D view, and the surface plot generally shows a 3-D view. The surface plots indicated first-order effects and no interaction between the responses. Therefore, the curvature effect was found to be minimum. (Figures 4-6).

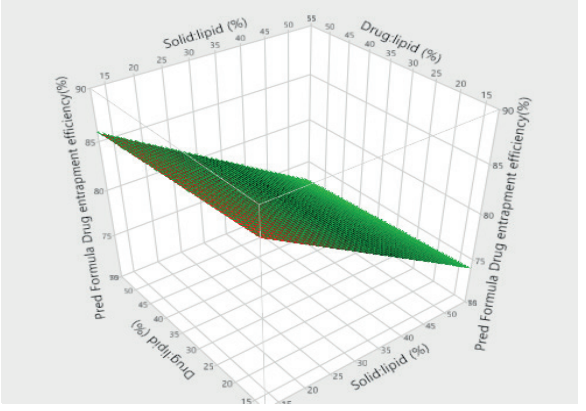

Figure 4. Surface plot of drug entrapment efficiency

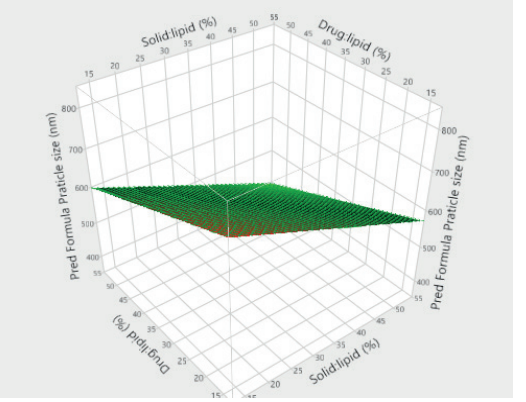

Figure 5. Surface plot of Particle size

80. 07= 77.5983 + -5.13375 * "Solid: liquid lipid (%)" - 33.5) / 16.5) + -0.50875 * ("Drug: lipid (%)") - 33.5) / 16.5) + -8.84375 * (("Phase volume ratio(%)") - 35) / 15) + -10.49125 * ((("surfactant (%)") - 1.15) / 0.85) + ("Stirring speed (rpm)"), "10000", 2.476,"12500", -10.6733,"150000",8.1966- **Equation (2)**

543.06= 515.4583 + -78.203125 * ("Solid: liquid lipid (%)") - 33.5) / 16.5) +-68.053125 * ("Drug: lipid (%)") - 33.5) / 16.5) + 43.996875 * (("Phase volume ratio(%)") - 35) / 15) + 9.52812499999998 * ("surfactant (%)") - 1.15) / 0.85) + ("Stirring speed (rpm)"),"10000", 72.16,"12500", -24.408,"150000", -47.7583- **Equation (3)**

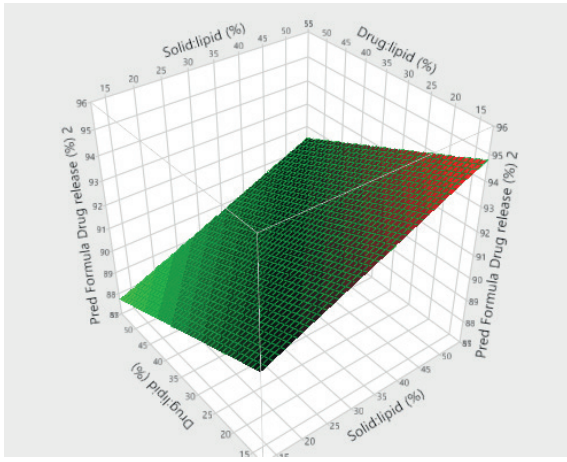

Figure 6: Surface plot of drug release

87.31 = 88 + 1.46875 * ("Solid: liquid lipid (%)") - 33.5) / 16.5) + -1.28125 * ("Drug: lipid (%)") - 33.5) / 16.5) + -2.03125 * ("Phase volume ratio (%)") - 35) / 15) + -0.96875 * ("surfactant (%)") - 1.15) / 0.85) + ("Stirring speed (rpm)"), "10000", 3.25, "12500", -3.5, "150000", 0.25-**Equation (4)**

Evaluation of optimized formula

Particle size distribution and zeta potential

The particle size analysis of optimal formulation showed an average particle size was found to be 425.8nm.

Zeta potential or electrokinetic potential gives an idea about the magnitude of inter electrostatic repulsion in dispersion between the similarly charged adjacent particles**.** The more the zeta potential value, the better the stability of the dispersed system. The zeta potential of the optimal formula was estimated to be -45mV (Figure 7), which indicates the absence of higher-level agglomeration within the system and the stability of NLCs.

Scanning electronic microscopy:

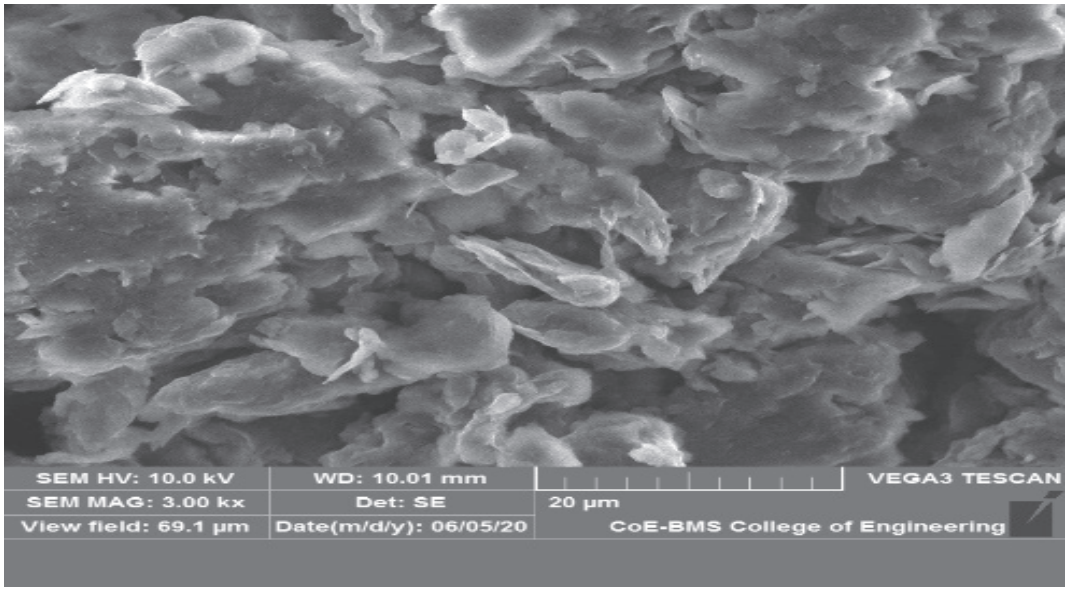

Figure 7: Surface photograph of formulation at different magnification

The surface photograph of the nanoparticles is shown in (Figure 7). The surface picture shows slightly agglomerated particles with uneven surfaces. The effect of high stirring speed disorients the structure of the lipids, which might be one of the contributing factors to the unevenness. At times, the liquid phase containing drug may get ejected during the solidification process, forming an exaggerated version of the liquid phase shell on the solid surface, which might have resulted in such a surface morphology.

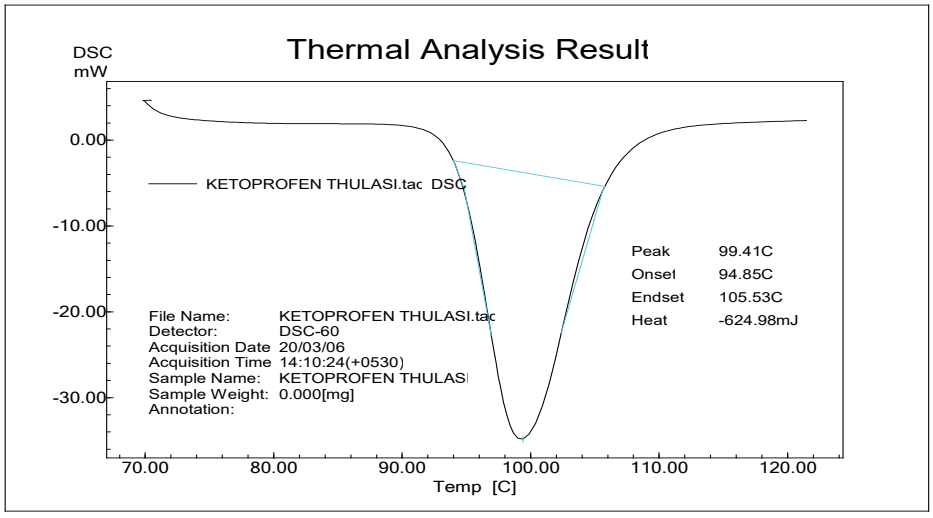

Figure 8. DSC thermogram of a pure drug (Ketoprofen)

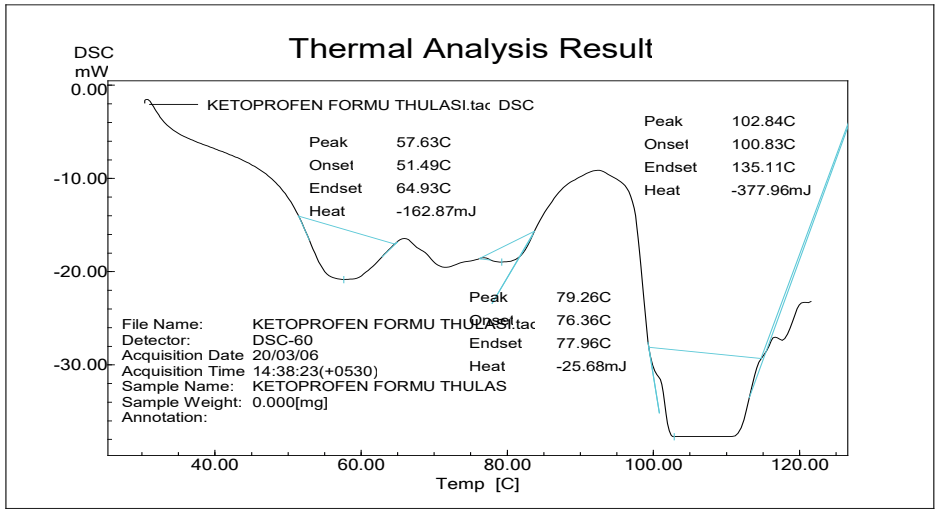

Figure 9. DSC thermogram of NLC formulation

Differential scanning calorimetry

The thermal behaviour of ketoprofen and its NLC were studied using DSC to observe the effect of the lipids on ketoprofen. The DSC thermogram of ketoprofen reveals an endothermic peak at 94.8˚C. In the optimized formulation, the endothermic peak shifted towards lower temperature, i.e., 76.36 ˚C, respectively, shown in (Figure 8) and (Figure 9). This indicates that the lipids (Beeswax, carnauba wax, and linseed oil) and surfactant may have decreased the melting point of NLC of ketoprofen. Thus, this would have resulted in favouring its retention within the matrix of lipids, and on contact with an aqueous medium, it exhibits reversal of effects, thus increasing drug release.

Evaluation of patch formulation

The drug content for the patch formulation was found to be 95.2±0.09, thickness 0.116±0.09, folding endurance 240.2±0.06, weight variation 190±1.2, moisture content 1.3±0. 06. shown in (Table 10).

Table 10. Evaluation of patch

Drug content uniformity (%)±SD	Thickness±SD (mm)	Folding endurance±SD	Weight uniformity (mg)±SD	Moisture content(%)±SD(n=1)
95.2±0.09	0.116±0.09	140.2±0.06	10.5±1.2	1.3±0.06

Preparation of optimum formulation of NLC loaded ketoprofen patch

The optimum formulation from the prediction formula showed 85.4% entrapment efficiency, 425.8nm particle size, and 91.5%± 0.06 of the drug content. The patches were prepared using hydroxypropyl methyl cellulose polymer and polyethylene glycol as a plasticizer.

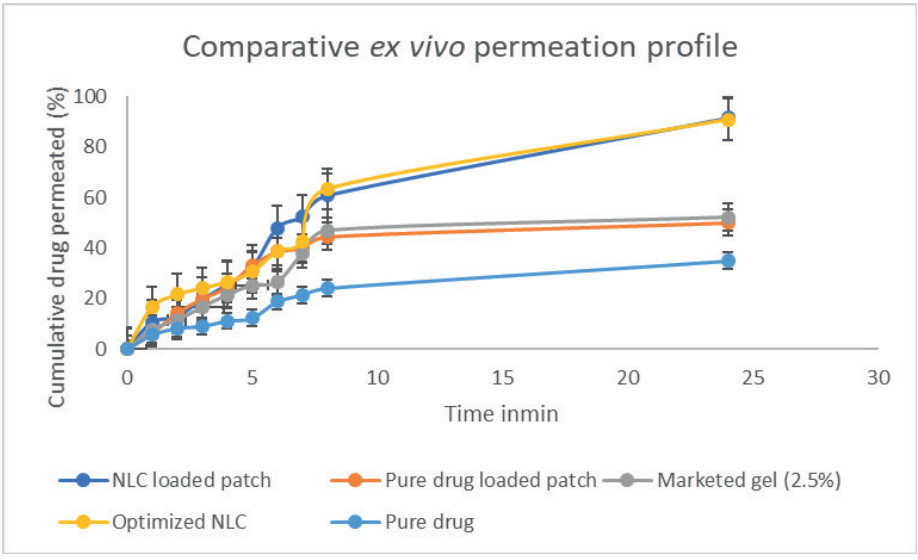

Figure 10. *Ex vivo* permeation profile

Optimized formula from exhibited drug permeation of 90.91±0.98%, whereas pure drug suspension containing the same amount of drug exhibited drug permeation up to 35± 0.14 % in 24 h via the excised rat abdominal skin specimen shown in (Figure 10). NLC's increased skin permeation compared to pure drug patches and marketed formulations is attributed to lipid dynamic behaviour, i.e., the ability of the lipids to get dissolved into the lipid layers of the stratum corneum. Thus, lipid exchange between the stratum corneum and lipid favours ketoprofen's skin delivery. Secondly, as a result of their nano size, these carriers can penetrate the deeper skin layers. Though few particles would endure intact on the surface of the skin, a majority would most likely interact with the stratum corneum, fuse and disrupt the characteristics of the barrier and enhance the drug penetration. Thirdly, the role of poloxamer contributed to the skin permeation of the drug. Surfactants, by virtue of their ability to disrupt the organized stratum corneal layers and their capacity to alter the aqueous fluid content, might have added to the skin permeation.

***Ex vivo* permeation studies of optimal formulation in comparison with ketoprofen loaded patch and marketed gel (2.5%)**

The drug's permeation rate from the NLC patch was 91.5±0.06%, compared to a pure drug, and marketed ketoprofen gel (2.5%) showed a permeation rate of 50.1±0.08% and 52.12±0.06% respectively in 24 h release studies shown in (Table11) and (Figure

11). The NLC formulation showed activity up to 24 h, reaching a maximum at six h, which confirms the prolonged activity of the patch. In contrast, the marketed formulation showed increased activity for the initial eighth. This could be associated with the presence of a significant quantity of alcohol, which is a known skin penetration enhancer. Nevertheless, the marketed formulation exhibited reduced activity at 24 h. In contrast, the pure drug exhibited activity up to 8h. The faster onset of action of the developed NLC patch was confirmed to be comparable to the marketed gel. The sustained action of the NLC patch even at the finish of 24 h could be explained by encapsulation, i.e., when oil is mixed with solid lipid, phase separation takes place, resulting in nanodroplets of oil surrounded by a solid matrix. The lipid matrix on the oil surface prevents drug leakage from the interior and thus slows down the drug release. The *ex vivo* permeation indicated a higher flux and permeability coefficient of NLC patch compared to pure drug incorporated patch and marketed gel of ketoprofen.

Table 11. Comparative steady state flux and permeability coefficient

Time (h)	**Steady state flux Jss (µg/cm²/hr)**		
	NLC loaded patch + SD	**Pure drug loaded patch + SD**	**Marketed gel(2.5%) + SD**
1	0.31±0.02	0.12±0.03	0.22±0.02
2	0.37±0.03	0.17±0.01	0.33±0.03
3	0.56±0.04	0.18±0.06	0.48±0.07
4	0.70±0.05	0.21±0.01	0.62±0.09
5	0.80±0.06	0.25±0.08	0.74±0.01
6	0.94±0.1	0.33±0.9	0.76±0.03
7	1.23±0.04	0.35±0.04	1.05±0.1
8	1.56±0.01	0.39±0.02	1.37±0.01
24	3.05±0.03	0.45±0.05	1.45±0.05

Permeability coefficient Kp ($cmhr^{-1}X10^3$)			
Kp	**NLC loaded patch**	**Pure drug loaded patch**	**Marketed gel (2.5%)**
1	0.75	0.27	0.5

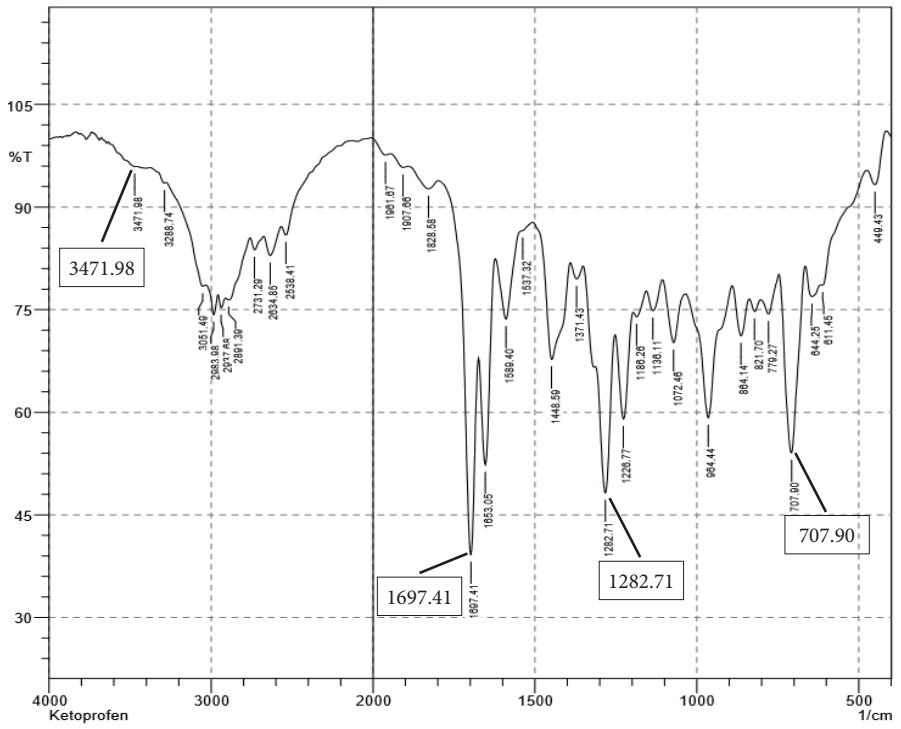

Figure 11: FTIR spectrum of ketoprofen pure drug

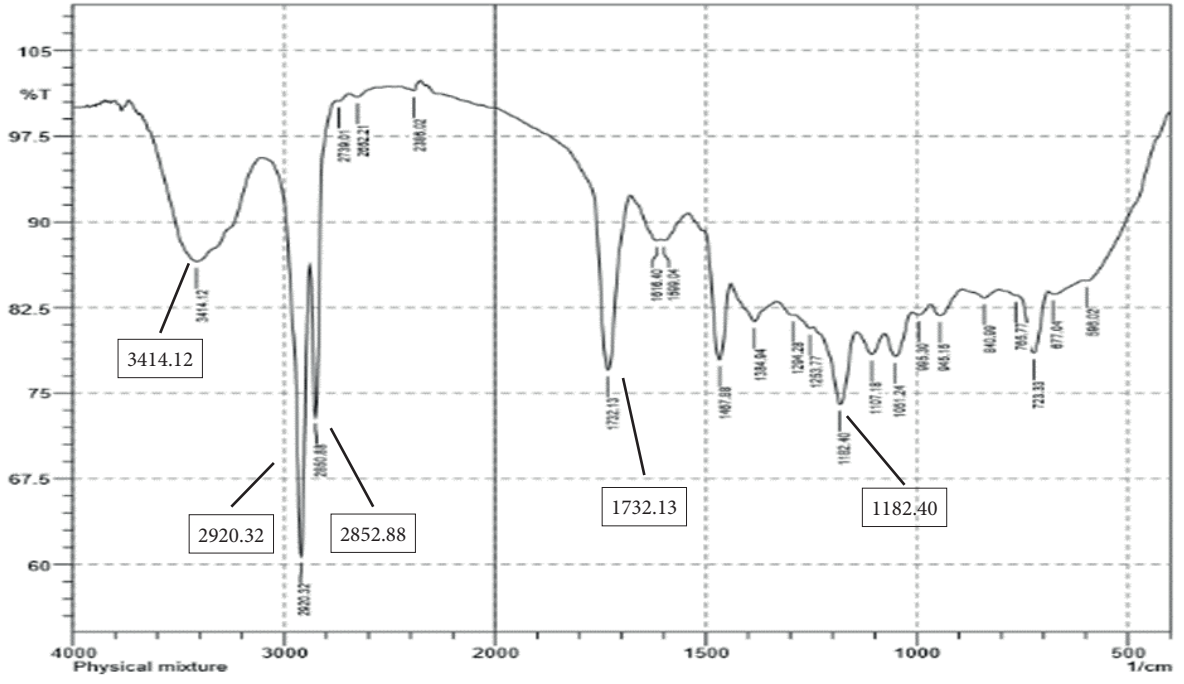

Figure 12: FTIR spectrum of physical mixture

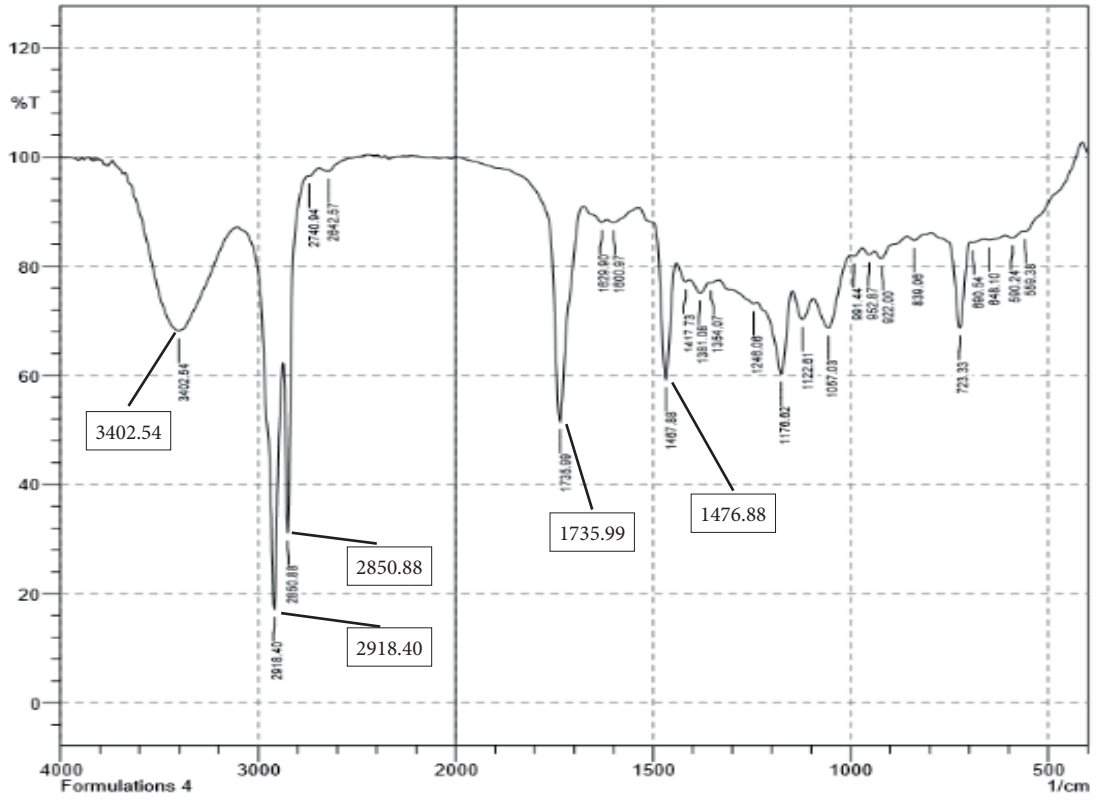

Figure 13: FTIR spectrum of NLC formulation

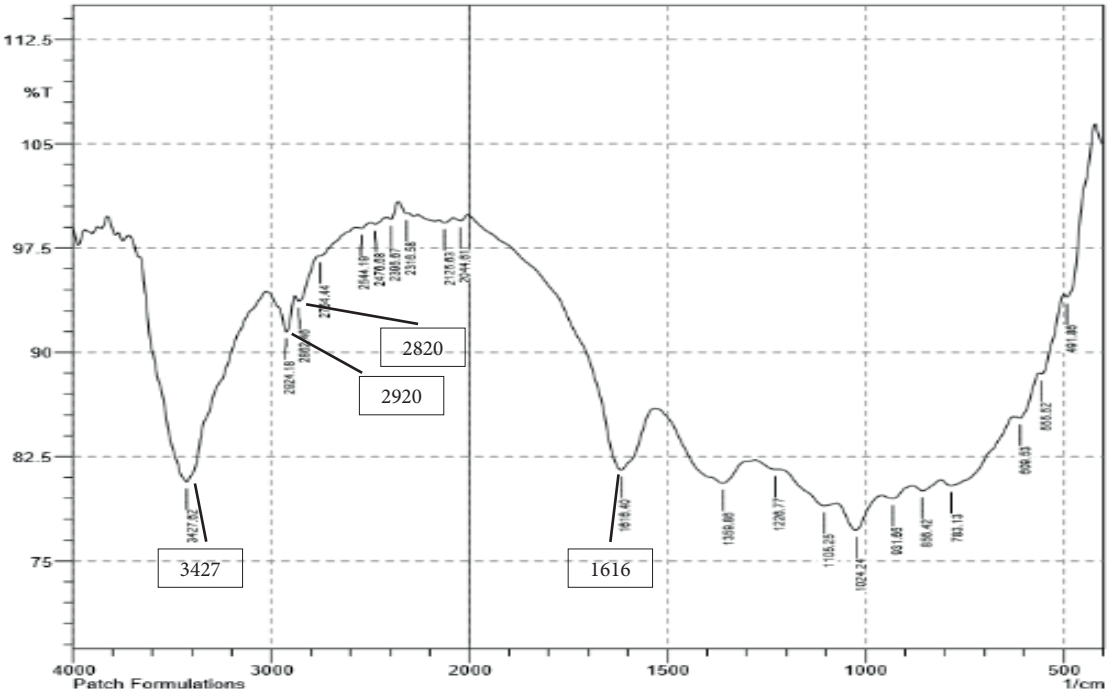

Figure 14: FTIR spectrum of patch formulation

Drug excipients compatibility studies by IR

The compatibility studies by FTIR showed no interaction between the drug and excipients used. The FTIR was performed for drug, drug/excipients and is shown in (Figure 11) and (Figure 12). All the observed ranges were within the stretching range1500-1700 for ketone (C=O),1500-1700 for c carboxylic acid(C=O), and 2000-3000 for aromatic (O-H), 2500-3500 for aromatic (C=C). Both NLC formulation and patch formulation of ketoprofen exhibited the characteristic peaks of ketoprofen as given in (Figure 13) and (Figure 14).

Short term stability studies:

The optimal NLC formula and NLC loaded patch were exposed to short-term stability testing for 90 days at 25°C ± 2°C/ 60% ± 5% RH and 40°C±2°C/75% ±5% RH. The physical appearance result shows no change in the properties at the storage conditions mentioned above (Table 12). It shows that 25°C ± 2°C RH gives better stability conditions than 40°C ± 2°C RH.

Table12. Stability studies

Day	Physical appearance (Optimum NLC formula)		%Drug content ± SD (n=3) (NLC loaded patch)			%Entrapment efficiency ± SD (n=3) (Optimum NLC formula)		
	25±2°C	40±2°C	Initial	25±2°C	40°±2°C	Initial	25±2°C	40°±2°C
0	Free flowing	Free flowing	95.2±0.1	95.2±0.1	95.2±0.1	82.4±0.2	82.4±0.2	82.4±0.2
90	Free flowing	Free flowing	95.01±0.1	94.01±0.8	93.09±0.1	82.2±0.2	82.03±0.1	81.91±0.02

CONCLUSION

NLCs are an effective colloidal drug delivery system for dermal application due to their various beneficial effects on the skin. Considering to be based on non-toxic and non-irritant lipids, they are ideal for inflamed or broken skin. In this present work, ketoprofen-loaded NLC was developed for transdermal application to reduce the systemic adverse effects, escalate the drug's permeation rate and prolong the drug's duration of action.

Ketoprofen was successfully converted to NLCs using natural lipids such as Beeswax, carnauba wax, GMS, and linseed oil as a liquid type of lipid. Poloxamer was an excellent emulsifying agent in this o/w type of system. Hot homogenization was a suitable method to produce NLCs with superior product qualities. The method resulted in free-flowing NLCs with good drug content and entrapment efficiency. The experimental design 'custom design' was suitable for attaining optimum formulations. All the formulations could result in genuinely nanosized particles except one or two higher zeta potentials, an indication of its stability; the zeta potential indicates the excellent stability of the NLCs. The IR studies assure the compatibility of drugs and excipients even after the formulations. The comparative drug release profile assured the improved skin permeation of NLCs to drug-loaded patches and marketed Fastum ® Gel. The nanoparticulate colloidal drug delivery system of ketoprofen using Beeswax, carnauba wax, linseed oil, and poloxamer is anticipated to bestow the clinician with a novel alternative of an economical, reliable, and competent regimen for skin delivery.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Experimentation (TS), Hypothesis, mentor, design, (PS), Framing the manuscript (EJ and MMS).

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