A Unique Strategies for Psoriasis Management: Utilizing Nanoemulsion Based Insitu-Gel Formulation for Topical Administration of Mometasone Furoate

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Abstract:

Mometasone furoate (MF) exhibits low oral bioavailability and can lead to significant adverse effects, rendering oral administration impractical. In order to enhance its solubility and permeability, a nanoemulsion-based in-situ gel was developed for psoriasis treatment. Clove oil, Tween-80 (surfactant), and PEG 400 (co-surfactant) were chosen based on their solubility profiles for MF. A pseudo-tertiary phase diagram was constructed to determine the optimal nanoemulsion formulation. The MF nanoemulsion (MF-NE) was prepared using a drop-by-drop aqueous titration method. The optimized MF-NE demonstrated and thermodynamic stability and was characterized for globule size, zeta potential, refractive index, and viscosity. The final nanoemulsion formulation comprised 2% w/w oil and 20% w/w S_{mix} (surfactant and co-surfactant mixture in 3:1 ratio). This nanoemulsion was incorporated into various concentrations of the gel poloxamer 407. The formulated nanoemulsion-based in-situ gels were evaluated for gelation temperature, pH, drug content, viscosity, in-vitro drug release profiles, and stability. The selected formulation exhibited an enhanced drug release (82.19±1.170%) compared to the marketed gel (53.00±0.818%) preparation. The antioxidant activity of the MF nanoemulsion based in-situ gel was evaluated using the DPPH method. The optimized formulation exhibited non-irritating to the skin in the HET-CAM test. These findings suggest that the nanoemulsion based Insitu gel represents a promising approach to improve the bioavailability and topical delivery of mometasone furoate.

Keywords: Mometasone Furoate, Nanoemulsion, Poloxamer 407, Pseudo-Ternary Phase Diagram, Topical Delivery

1. Introduction

Psoriasis is a complex autoimmune disorder characterized by chronic inflammation of the skin, affecting a significant portion of the global population. The symptoms of psoriasis encompass a spectrum of manifestations including erythema, skin thickening, and scaling, attributed to a combination of epidermal cell proliferation and growth and development of blood vessels [1].

Despite ongoing research, the precise molecular mechanisms driving psoriasis pathogenesis remain elusive. However, it is widely acknowledged that cytokines and growth factors play pivotal roles in accelerating the proliferation of skin cells, culminating in the formation of thick, red patches across various body regions. Proinflammatory cytokines such as interleukin, interferon and TNF-α are key players implicated in the development of psoriatic lesions [2,4]. Psoriasis remains an enigmatic condition with its exact etiology still shrouded in mystery. A myriad of factors, including infections, stress, hormonal imbalances, inflammatory skin disorders, and certain medications like ACE inhibitors, NSAIDs, and lithium, are believed to contribute to its progression. Additionally, smoking has been considered as a potential trigger for this disease [4].

Despite the array of therapeutic options available, ranging from systemic medications such as methotrexate, cyclosporine, or acitretin, to biologics like adalimumab or etanercept, and various topical treatments including corticosteroids, vitamin D_3 derivatives, retinoids, coal tar, or anthralin, along with phototherapy, no one offer a definitive treatment [5,6].

This shortfall is largely attributed to several limitations inherent in existing treatments, such as lack of specificity in targeting, poor drug permeability, low retention rates, development of resistance, and adverse side effects. Addressing these limitations remains a paramount challenge in the quest for more efficacious and tolerable

therapeutic modalities for managing psoriasis [7,9]. Nanocarriers represent a major advancement in addressing the limitations of traditional formulations by allowing for reduced dosing frequency and lower doses, which helps minimize side effects and improve therapeutic outcomes. As a result, they have gained significant attention for their ability to deliver safer and more effective treatments for psoriasis [10].

A wide array of nanocarriers is currently available, encompassing nanoparticles, ethosomes, liposomes, niosome, SLN, micelles, NLCs, nanocapsules, nanofibers, and nanoemulsion in situ gels [11]. Among these, nanoemulsion in situ gels stand out given unique advantages. These formulations combine the benefits of nanoemulsions, such as enhanced skin penetration and controlled drug release, with the convenience of in situ gelation, which allows for prolonged retention at the site of application. Compared with other topical formulation, the nanoemulsion in situ gels have several offer several benefits, including controlled release, fewer skin irritation, drug protection, and targeted drug delivery. [1,12].

This hybrid system offers a promising approach to improving drug bioavailability and therapeutic outcomes in psoriasis treatment, potentially revolutionizing the management of this chronic skin disorder [13,14].

Earlier studies have emphasized the development of nanoemulsion based gel formulations like NLCs of acitretin [15] (Sawant KK et al., 2010), Raza K. et al.,2013 developed and evaluated biocompatible lipid based nanocarriers for tretinoin such as liposomes, ethosomes, SLNs and NLCs [16]. Tomar Sonia et al., 2015 formulated topical gel containing incorporating azithromycin and prednisolone vesicles for the treatment of psoriasis [17]. Madan J.R et al., 2014 prepared SLNs of mometasone furoate [18]. Nanoemulsion-based gel loaded with Clobetasol propionate and calcipotriol [19], Curcumin, resveratrol, and thymoquinone [20], Rajitha Panonnummal et al.,2019 established methotrexate chaulmoogra oil based NEs through emulsification method [21]. Rashid Sheikh Abdur et al., 2021 developed a methotrexate-loaded nanoemulsion using olive oil for psoriasis management [22]. Narang Raj Kumar et al.,2020 formulated a nanoemulgel of clobetasol propionate by homogenization, characterized [23].

Mometasone furoate, a potent glucocorticoid, BCS class II drug has garnered significant attention in the medical community due to its versatile therapeutic applications. It exhibits high anti-inflammatory and immunosuppressive properties, making it a cornerstone in the dealing of various dermatological disease such as eczema, psoriasis, and allergic dermatitis. Topical corticosteroids have a tendency to reduce the thickness of skin which turns favourable in case of psoriasis [24,25,26].

Moreover, its efficacy in managing allergic rhinitis and asthma further underscores its clinical importance. Additionally, studies have clarified its mode of action at the molecular level, shedding light on its intricate interactions with glucocorticoid receptors and downstream signaling pathways. Furthermore, investigations into its safety profile have consistently demonstrated its favorable risk-benefit ratio when used judiciously under medical supervision [27,28].

Nanoemulsions (NEs) are clear, stable formulations with droplet sizes between 20 and 200 nm, making them highly suitable for topical drug delivery. Due to the poor solubility and limited permeability of certain drugs, nanoemulsions are often chosen for their ability to enhance drug absorption. The small droplet size and lipid-based composition improve the solubility of poorly soluble drugs, increase skin penetration, and support better drug loading and retention in the skin [29].

When compared to other colloidal delivery systems, NEs offer key advantages such as the ability to dissolve both hydrophilic and lipophilic drugs, simple preparation, prolonged stability, and improved topical drug delivery. However, their relatively low viscosity can result in reduced drug retention on the skin, which can be a limitation in some applications [30,31].

Previous studies have reported the successful formulation of nanoemulsions based Insitu gel for the ocular delivery of Moxifloxacin Hydrochloride [32], Latanoprost [33], acyclovir [34] and topical delivery of raloxifene hydrochloride [35], curcumin [36], paclitaxel [37], acetazolamide [38], intranasal delivery of temozolomide [39]. Cook MT et al., 2023 reviewed traditional techniques as well as advanced methodology of gelling system for dermal delivery [40]. So, to avoid this problem, develop thermolabile in situ gel formulations for skin tissue, phase transition occurs as temperature raise spontaneously [41].

The most common temperature sensitive polymer that is poloxamer 407, which form micelles and gelation as the temperature increases [34,35,41]. Due to desired released characteristics, compatible with other ingredients, nonirritating, prolonged residence at site, reduction in dose, and minimized unwanted effect make poloxamer 407 a suitable gelling agent [42,43,44].

This paper aims to provide a complete investigation of the potential of mometasone furoate-loaded nanoemulsion in situ gels as an innovative therapeutic strategy for psoriasis. By exploring the formulation's design, characterization, and therapeutic efficacy, we seek to advance the understanding and application of nanotechnology in dermatological treatments, paving the way for improved clinical outcomes in psoriasis management.

2. Materials and methods:

2.1. Materials

Mometasone furoate was graciously provided by Allkind Healthcare Unit III, Baddi (Himachal Pradesh). Clove oil and Rose oil were obtained from CDH (P) Ltd., Gujarat. Tween80 and olive oil were sourced from SD Fine Chemicals Ltd., Mumbai. PEG-400 was obtained from Qualigens Pharma Pvt Ltd., while methanol and glycerol were acquired from Rankem, Maharashtra. All remaining chemicals were of analytical grade.

2.2 Methods

2.2.1 Selection of oil, surfactant, and co-surfactant

A solubility study of MF (a model hydrophobic drug) was conducted using many oils, surfactants, and co-surfactants. An excess quantity of MF was added to 1 mL of each excipient in 2 mL appendrop tubes [32]. Then, mixtures were rotated on a rotary shaker at 200 rpm for 24 hours at 25°C and subsequently, the samples were left to stand for 2 hours. The supernatant was then carefully separated and diluted with methanol, and the solubility of MF in the different excipients was measured using UV spectrophotometry at 248 nm [45]. The absorbance values indicated the solubilizing capacity of the respective oil, surfactant, and co-surfactant for MF.

2.2.2 Construction of phase diagrams

To determine the optimal combination of excipients for preparing nanoemulsions, pseudo-ternary phase diagrams were developed using sigma plot version 10.0 software. These diagrams help to identify the nanoemulsion region by mapping the proportional relationships between the oil phase, surfactant, and co-surfactant. Generally, four ternary phase diagrams were developed for several surfactant and cosurfactant ratio. The S_{mix} was formulated by blending the tween 80 and polyethylene glycol 400 in various ratios: 1:1, 2:1, 3:1, and 5:1. For each phase diagram, the concentrations of the oil phase (clove oil) and S_{mix} were varied to identify the maximum water uptake by the nanoemulsion, ensuring it remained transparent throughout the process. The selected ratios of oil to S_{mix} for constructing the phase diagrams were 1:9 to 9:1. These ratios were chosen to comprehensively explore the range within which the nanoemulsion maintains its transparency, indicating the formation of a stable nanoemulsion system [46,47].

2.2.3 Formulation of MF based nanoemulsion

Using pseudo-ternary phase diagrams, the optimal weight ratios of surfactant, co-surfactant, and oil were established for the preparation of nanoemulsions. Initially, a nanoemulsion was prepared using 0.1% w/w MF as the selected drug, clove oil, tween 80, and PEG 400. The NEs was prepared using the drop-by-drop method, where the oil phase (clove oil) was gradually added to a pre-mixed aqueous phase containing tween 80 and PEG 400. This was done under continuous stirring until a transparent mixture was achieved. Afterwards, a specific quantity of water was slowly incorporated into the mixture. The preparation was equilibrated at ambient temperature for a minimum one hour until clear and uniform NEs were obtained [21,46].

2.2.4 Evaluation of nanoemulsion containing MF

To analyze the physicochemical properties of the formulations, several methods were employed. Initially, the formulations were visually assessed to ensure clarity and absence of any phase separation or precipitation. The globule size, PDI and zeta potential were then assessed instantly afterwards formulation using a Malvern Zetasizer NanoZS (Malvern, United Kingdom). The pH values were measured at room temperature with a Sartorius pH meter (Sartorius, Switzerland). The viscosity of the samples was measured using a Brookfield DVII viscometer (Brookfield Engineering Laboratories Inc., USA) at room temperature. The morphological behavior of optimized MF-NE was determined by TEM analysis (JOEL JEM-1400, Japan). The transparency and clarity of the nanoemulsion were evaluated by measuring the transmittance by UV spectrophotometer at wavelength 650 nm, using purified water as the blank. Visual assessments were performed by diluting the nanoemulsion with purified water and gently stirring it with a magnetic stirrer. Finally, refractive index of the nanoemulsions was measured at 25ºC by an Abbe-type refractometer [47,48,49,50].

2.2.5 Preparation of NEs based insitu Gel

To prepare NE based insitu gel with a targeted gelling temperature, various weight ratios of poloxamer 407 were meticulously weighed and dissolved in the aqueous phase of the chosen formulation. This process was conducted by stirring the mixture at 4°C overnight. Subsequently, the aqueous phase was incrementally incorporated into the pre-prepared oily phase, as detailed in the nanoemulsion method. The resulting mixture was continuously stirred at 4°C for a minimum of one hour to ensure uniformity. The formulations were then stored in a refrigerator until further analysis was conducted [37]. Formulation of nanoemulsion based Insitu gel is given in Table 1.

Table 1. Formulation table for nanoemulsion Insitu gel.

Ingredients	NBG1 (%w/w)	NBG2 (%w/w)	NBG3 (%w/w)

MF	0.1	0.1	0.1
Clove oil	2	2	2
Tween 80	15	15	15
PEG 400	5	5	5
Poloxamer 407 (Pluronic F127)	17	21	23
Distilled water	q.s.	q.s.	q.s.

2.2.6 Assessment of gel transition and viscosity of insitu Gel NEs

The gel transition temperature and viscosity of the MF nanoemulsion based Insitu gel formulation were determined using poloxamer 407 as the thermosensitive gelling agent. A rheological study was conducted to assess the formulation viscosity at different temperature (4ºC, 25ºC and 37 ºC) using a rotational viscometer (Brookfield DV-II). The gelation temperature was determined by tube inversion method. The preparation was subjected to gradual heating from 4ºC to 50ºC in a heating bath, and temperature was noted at which the formulation transformed a liquid to a gel. Gelation was deemed complete when the formulation no longer flowed upon inversion of the tube [32,33].

2.2.7 pH and clarity

The pH of the various formulation was measured by a pH meter. 1 g of each sample was placed in 10 mL of purified water. pH measurements were performed in triplicate for each formulation, and the average values were computed. The clarity of each formulation was analysed by visual examination under bright light with a dark background [8,55].

2.2.8 Content of drug

The drug content was assessed by dissolving approximately 1 g of the preparation in 25 mL of methanol. The solution was sonicated for 30 minutes and then further diluted using solvent and measured by UV spectrophotometry at 248 nm, with methanol serving as the blank [8].

2.2.9 In Vitro drug diffusion

The drug release from the NE based nanoemulsion and the marketed product was evaluated using a Franz diffusion cell. A synthetic cellulose membrane with a 0.45 µm pore size (Merck Millipore) was presoaked in purified water and positioned between the receptor and donor chambers. The receptor chambers were filled with a PBS solution at pH 7.4 and methanol (7:3) at constant temperature 37 ± 1°C. The solution was mixed using a magnetic stirrer at 500 rpm. At specific time, sample was withdrawn followed by addition of fresh PBS. The concentration of mometasone furoate in the release medium was measured by UV spectrophotometer at 248 nm [51].

2.2.10 Antioxidant activity of formulation by DPPH method

The antioxidant activity of the mometasone furoate nanoemulsion insitu gel was evaluated using the DPPH radical scavenging assay. Various nanoemulsion gel concentration (0.1,0.2,0.3,0.4,0.5mg/ml) were prepared by diluting the formulation in methanol. An equal volume of the DPPH solution (0.1 mM in methanol) was mixed with in each concentration of the formulation. The reaction mixtures were kept in room temperature for 30 minutes. Afterward, measured the absorbance at 517 nm by UV-visible spectrophotometry. Meanwhile, the nanoemulsion and the ascorbic acid were also examined using the above method. Ascorbic acid was served as the positive control [52,53]. Percentage DPPH scavenging activity was computed by given formula:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.2.11 Skin irritation test (HET-CAM)

The Hen's egg test-chorioallantoic membrane (HET-CAM) was used to measure the skin irritancy of the developed formulations. Freshly fertilized hen's eggs weighing 50-60 g were divided into six groups and incubated at 37 ± 0.5°C with a RH of 67 ± 5% for 10 days. Every 12 hours eggs were rotated and chosen by using a flashlight to carefully monitor the development of embryo. The chosen eggs were carefully opened with surgical instruments to prevent damaging blood vessels. NE based Insitu gel formulations, 0.9% NaCl (negative control) and 1% NaOH as positive control were applied. Irritation score were assessed by visually monitoring changes in the blood vessels, such as hypervascularity, coagulation, and hemorrhage, over a 300-second period. Observations were made at intervals of 0.5, 2, and 5 minutes. [21,54]. Result was assessed as per irritation assessment table, as outline in Table 2.

Table 2. Irritation assessment table

	Score			Cumulative score	Irritation assessment
Effect/time (min)	0.5	2	5	0-0.9	None
Hyperemia	5	3	1	1.0-4.9	Slight
Hemorrhage	7	5	3	5.0-8.9	Moderate
Clotting/coagulation	9	7	5	9.0-21.0	Severe

2.2.12 Stability Studies

Prepared nanoemulsion were centrifuged for 30 min at 5000 rpm and viewed for creaming, phase separation or cracking. For the temperature stability study, nanoemulsion samples were stored at 4°C and room temperature for three months, with regular visual examinations. A stability study was conducted according to ICH guidelines in a stability chamber. Sample was kept in well-stoppered bottles for three months, and the formulation's stability was monitored at 25 ± 2°C temperature and 60% ± 5% RH. The store samples were assessed for appearance, pH, viscosity and drug content at time interval of initial, 1 and 3 months [32,49,51].

3. Results and discussion:

3.1 Screening of oil, surfactant, and co-surfactant

The selection of oils, surfactants, and co-surfactants was based on the drug solubility. Results shown in Fig 1. The findings indicated that clove oil had the highest solubility at 33.56 ± 2.43 mg/ml compared to the other oils. Among the surfactants, Tween 80 exhibited the highest solubility for the drug at 27.2 ± 2.89 mg/ml. Additionally, PEG 400 showed the greatest solubility among the co-surfactants, with a value of 10.6 ± 2.19 mg/ml. The combination of Tween 80 and PEG 400 resulted in a highly clear nanoemulsion, a formulation that has been utilized in previous studies [36]. Based on the screening, these excipients were chosen for further experimentation.

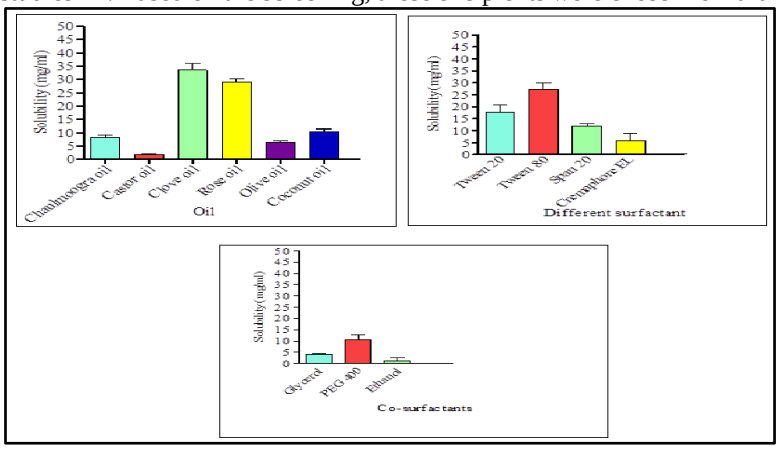

Fig 1: Solubility of MF in different oils, surfactants and co-surfactants

3.2 Phase diagrams studies of formulated NEs

The pseudo-ternary phase diagrams were constructed by the water titration method. As shown in Fig no 2, the nanoemulsion (NE) area was relatively small when using a S_{mix} ratio of 1:1. The decrease in the nanoemulsion area could be due to the limited surfactant quantity, which decreases the number of micelles and, consequently, lowers the solubilization potential. It was observed that increasing the Smix ratio led to a successive increase in the NE area. High concentration of surfactant may cause the skin irritation [20]. Among the four phase diagrams, the S_{mix} ratio of 3:1 demonstrated the largest NE area (Fig. 2B), which led to its selection for the formulation of the nanoemulsion. Tween 80 is a non-ionic surfactant having HLB value of 15.0, enhances the permeation of several antipsoriatic drugs and to create a stable oil/w nanoemulsion with a very low zeta potential, thereby reducing the particle size of the nanoemulsion [30,36].

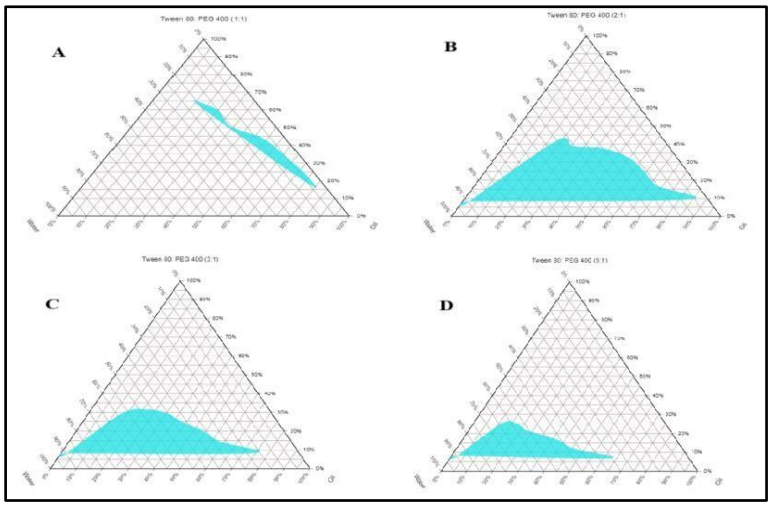

Fig. 2: Partial ternary phase diagrams of system (denoted by shaded) at Smix ratio; A (1:1), B (2:1), C (3:1) and D (5:1) composed of Clove oil, Tween 80 and PEG400 and water (%w/w)

3.3 Physicochemical characterization of MF loaded nanoemulsions

The globule size of optimized batch was observed to be minimum due to the optimal concentration of S_{mix}, as shown in Fig no 3. The reduction in particle size with increasing S_{mix} concentration can be attributed to the enhanced surface area, which facilitates better skin permeation of the drug. Zeta potential is a crucial factor for predicting the physical stability and surface charge of a system. Negative zeta potential values indicate stability due to electrostatic repulsion among particles with the same charge. The optimized formulation had a zeta potential of -13.24 mV, indicating its stability (Table 3). The negative zeta potential in the mometasone furoate nanoemulsions may be due to the presence of negatively charged fatty acid esters in clove oil [56], which was selected for its recognized health benefits, including wound healing, anti-inflammatory, antioxidant, and anticancer properties [36]. The polydispersity index (PDI) ranges from 0 to 1, where 0 indicates a monodispersed system and 1 signifies a polydispersed particle distribution. Optimized batch displayed the lowest particle size and a PDI of 0.216, indicating a homogeneous arrangement of globules. The formulation's conductivity was also measured, revealing a value close to zero, suggesting that the globules possess sufficient surface charge to prevent aggregation. The TEM images (Fig 4) showed that all globules were spherical, with sizes under 100 nm and uniformly distributed, with no signs of aggregation. This is beneficial for enhancing bioavailability, as smaller size offers large surface area that improves cellular uptake and penetration in biological systems. Among all the nanoemulsion batches, some displayed a clear or slightly bluish-white appearance compared to purified water and had a dispersion time of less than 30 minutes, indicating good stability (Fig no 5). Based on these overall evaluation parameters, optimized batch B_4 of nanoemulsion selected for the formulation of topical in situ gel.

Table 3. Physiochemical characterized parameters of optimized MF-NE formulation

Particle size (nm)	PDI	Zeta potential (mv)	Conductivity	pH	Viscosity (cp)	Transmittance (%)	Refractive index
28.43	0.216	-13.24	0.0450	6.20±0.005	1.10±0.020	98.5 ±0.005	1.402 ± 0.007

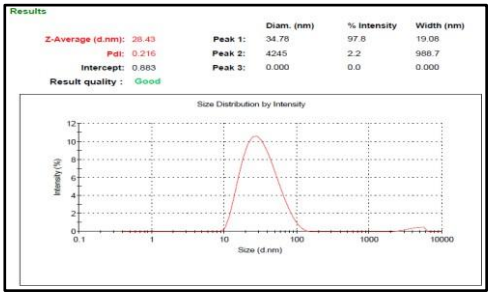

Fig.3: Particle size of optimized NEs

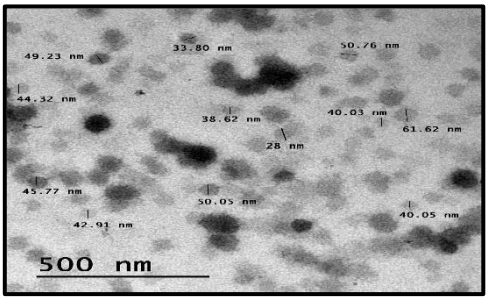

Fig.4: TEM micrograph of MF dispersion.

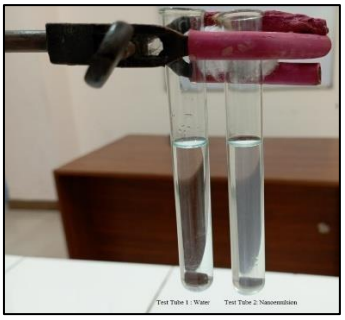

Fig.5: Visual assessment of nanoemulsion with water

3.4 Viscosity and gelation temperature

The sol to gel transition is a key factor in assessing the quality of in situ gels. The viscosity of the mometasone furoate nanoemulsion-based in situ gel demonstrated a temperature-dependent increase. At 4°C, the formulation exhibited low viscosity, facilitating easy administration as a liquid. However, as the temperature increased to 37°C, simulating physiological conditions, the viscosity increased significantly, indicating gel formation. An increase in pluronic concentration resulted in a decrease in gelation temperature. The gelation temperatures are detailed in Table 4 and Fig no 6. The formulation's gelation temperature was observed to be 32 ± 0.40°C, making it suitable for in situ gelation at body temperature following topical or localized application. The gelation properties of Poloxamer 407 enhance the formulation's retention at the application site, allowing for a sustained release of mometasone furoate over time. This thermoreversible gelation improves the drug's bioavailability and ensures localized delivery, preventing premature diffusion. The viscosity profile and gelation temperature are well-suited for topical application, as the formulation remains liquid at room temperature but quickly gels upon contact with body temperature, making it ideal for treating psoriatic lesions.

Table 4. Viscosity and gelation temperature of formulation (mean ± SD; *n* = 3).

Formulation	Visual observation at 4°C	pH	Gelation Temperature (°C)	Viscosity (cps)		
				4°C	25°C	37°C
NBG1	Clear	6.93±0.44	32.0±0.40	36.32±0.41	102.21±0.23	625.80±0.47
NBG2	Clear	6.51±0.23	27.5±0.62	48.18±0.90	120.40±0.39	748.23±0.64
NBG3	Clear	6.28±0.18	24.5±0.84	50.16±0.43	117.13±0.40	879.56±0.50

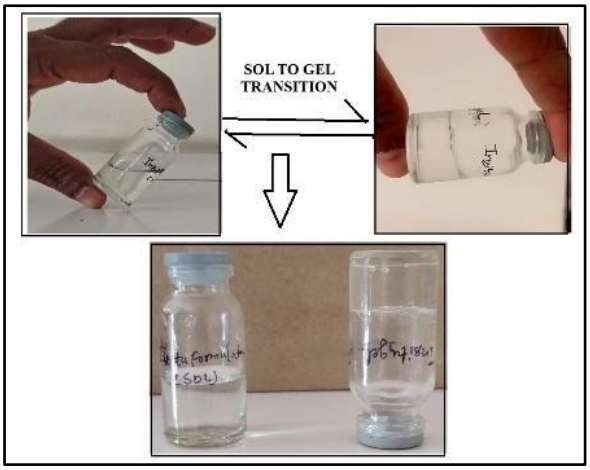

Fig. 6: The sol–gel transition of a thermosensitive in situ gel

3.5 pH and clarity

The pH and clarity results are presented in Table 4. Formulations NBG1, NBG2, and NBG3 exhibited pH values ranging from 6.28 to 6.93, indicating that these gel formulations are close to neutral pH, which is unlikely to cause skin irritation. Specifically, formulation NBG1 has a pH of 6.93 ± 0.44, making it particularly close to neutral and

suggesting it may not irritate the skin. Clarity test results showed that the incorporation of nanoparticles into the in-situ gel formulation did not lead to a significant change in clarity.

3.6 Drug content

The drug content percentage for all formulations varied between 90.49% to 95.34%. It was noted that the highest drug content was achieved with the optimal concentration of the gelling agent, as shown in Table 5.

Table 5: Drug content of formulations

S.No.	Formulation code	% drug content
1	NBG1	95.34±0.264
2	NBG2	94.19±0.182
3	NBG3	90.49±0.318
* Mean ± SD, n=3		

3.7 In Vitro drug release studies

Nanoemulsion-based in situ gel formulated for topical psoriasis treatment displayed a biphasic release profile, which began with a controlled release initially, transitioned to a rapid release phase, and concluded with a sustained release over prolonged period (Fig.7 a). In the initial phase, drug release was slow due to the oil phase encapsulation, which forms a diffusion barrier. The nanoemulsion structure ensures that the drug gradually crosses this barrier, resulting in a controlled release. Following this, the hydrated polymer matrix begins to facilitate the diffusion process, leading to a faster release phase. Eventually, the polymer matrix modulates drug release in a sustained manner, offering prolonged therapeutic benefits for psoriasis. The cumulative drug release for optimized batches, such as NEG1 reached approximately 10-15% in the first hour, progressing to more than 50% by the 6th hour. By the 12th hour, the release rate achieved approximately 82%, indicating effective sustained release potential for prolonged skin application. The slower release in initial hours supports adherence to the skin, enhancing therapeutic efficacy and reducing the need for frequent reapplication. Among various formulations tested, batch NBG1 demonstrated optimal drug release properties with an initial release of 15% at 1 hour, achieving 50% release at 6 hours and reaching a cumulative release of 82% by the 12-hour mark while marketed formulation shows 53% of drug release(Fig no 7 b) it was likely due to the smaller mean size of the internal phase droplets, which were considerably reduced in the nanoemulsion.This formulation was optimized further due to its suitable viscosity, gelation temperature, and clarity, making it a viable candidate for sustained therapeutic action in psoriasis treatment.

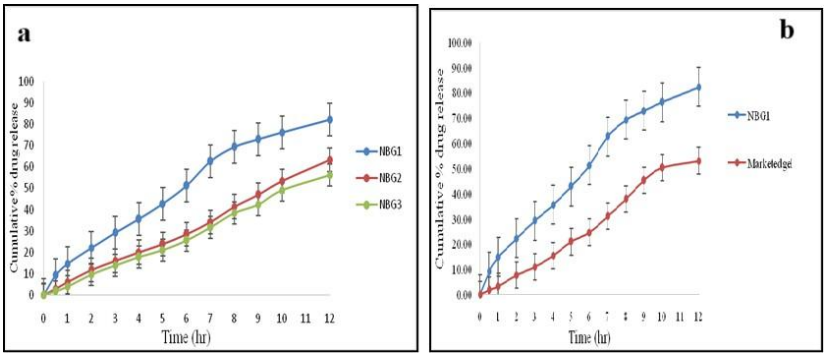

Fig.7: a) Cumulative % drug release of MF nanoemulsion in situ gels and b) marketed formulation

3.8 Antioxidant activity of formulation by DPPH method

As illustrated in Figs no.8 (a) and (b), both the mometasone furoate nanoemulsion and the nanoemulsion based Insitu gel exhibited significant antioxidant activity varying with concentration. The percentage of DPPH radical scavenging increased with higher concentrations of the formulations. The IC_{50} values for each formulation were determined to be 25.74 µg/ml and 22.26 µg/ml, respectively, which are comparable to the standard antioxidant ascorbic acid, with an IC_{50} value of 6.5 µg/ml. This antioxidant activity is possible because of excipients like clove oil, Tween 80, and PEG 400 were used, which are known for their mild radical scavenging properties. The antioxidant effects of the formulation may enhance its therapeutic efficacy in treating psoriasis by mitigating oxidative stress, a key factor in the inflammatory processes associated with the disease. This additional advantage suggests that the formulation could function not only as an anti-inflammatory agent but also as a protective agent against free radical-induced damage in psoriatic lesions.

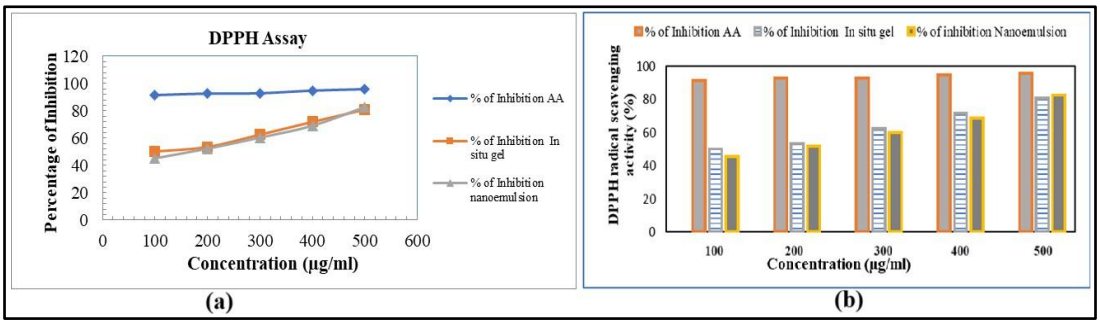

Fig. 8 (a) &(b)The antioxidant activities of MF Formulations.

3.9 Skin tolerance test (HET-CAM)

The HET-CAM assay was performed to evaluate the biocompatibility and irritancy profile of the in-situ gel formulation derived from the nanoemulsion containing mometasone furoate and poloxamer 407. The results indicated that the gel exhibited a low irritancy index, signifying minimal inflammatory response upon application to the chorioallantoic membrane. The mean irritation score was significantly lower than that of the control as shown in Table 6 and Fig.9, demonstrating the formulation's favorable safety profile. These findings underscore the promising biocompatibility of the Poloxamer 407-based in situ gel formulation for potential future research applications.

Table 6: Observations for HET-CAM Test

S.No.	Test substance	Score	Inference
1	0.9% NaCl (negative control)	0	Non irritant
2	Developed formulation (NE based Insitu gel of MF)	0.069	Non irritant
3	1% NaOH (positive control)	9.31	Severe irritant

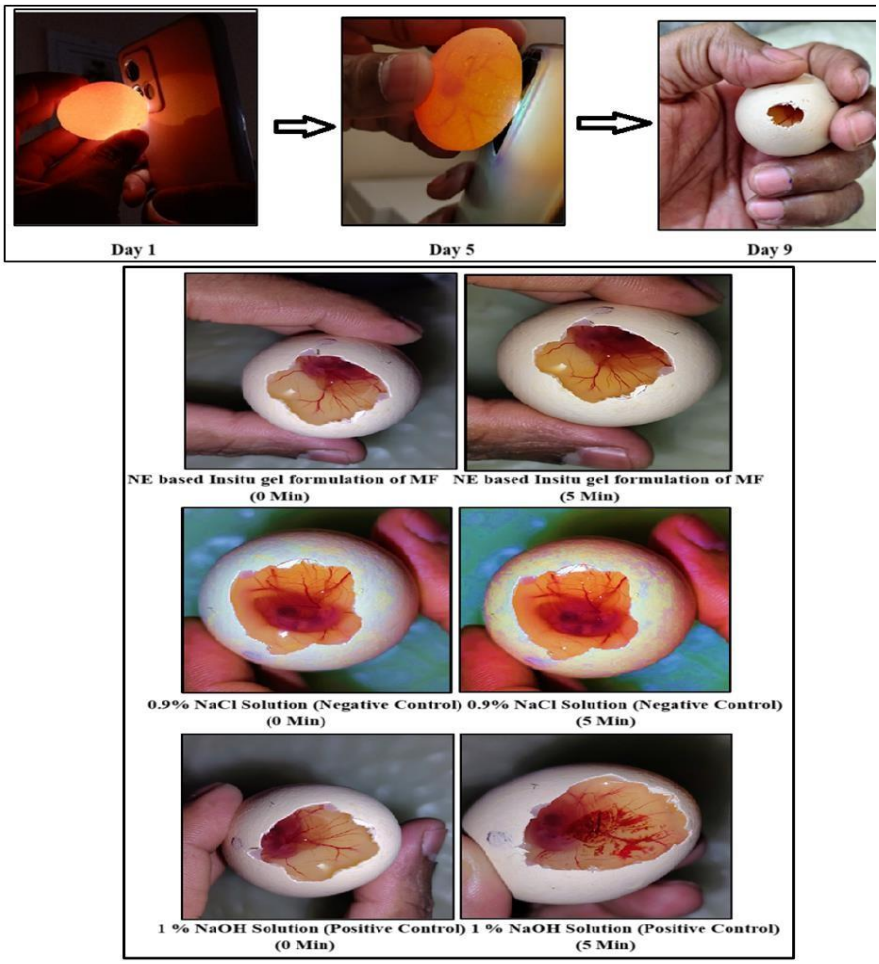

Fig. 9: Image representing the following in CAM at different time intervals

3.10 Stability study

Most nanoemulsion formulations were observed to be thermodynamically stable, exhibiting no phase separation, cracking, or creaming. The stability study of nanoemulsion based Insitu gel revealed a slight increase in the formulation's viscosity, with no significant changes observed in its appearance, pH, or drug content after three months of storage. The results are tabulated in Table 7.

Table 7: Stability study of optimized nanoemulsion Insitu gel

Quality parameters	Initial* (0 days)	25°C ± 2°C/ 65% RH ± 5% RH*	
		30 days	90 days
Appearance	Clear, transparent	Clear, transparent	Clear, transparent
pH	6.93±0.44	6.93±0.26	6.93±0.23
Phase separation	No	No	No
Viscosity (cps)	102.21±0.23	103.43±0.11	105±0.02
% drug content	95.34±0.264	95.33±0.259	95.21±0.153
* Indicates average ± standard deviation, n=3			

4. Conclusion:

The development and evaluation of a thermosensitive insitu gel formulated with mometasone furoate nanoemulsion marks a significant advancement in the management of psoriasis. This formulation takes advantage of the unique properties of thermosensitive gels, allowing it to transition from a liquid to a gel state upon application, which enhances drug retention at the site of action. By encapsulating mometasone furoate in a nanoemulsion system, the formulation improves the drug's solubility and facilitates skin penetration due to the nano-scale size of the emulsion. Furthermore, skin irritation studies indicate that the nanoemulsion-based in situ gel is safe for topical use, with low irritation potential, suggesting it could support long-term therapy. Consequently, this research highlights the potential of integrating nanotechnology with thermoresponsive

polymers to develop effective drug delivery systems for chronic conditions like psoriasis. Future studies should aim to assess the clinical efficacy and safety of this formulation in real world settings to further confirm its viability as a standard treatment option for psoriasis management.

5. References:

1) Kaur, N., Sharma, K., Bedi, N. (2018). Topical nanostructured lipid carrier-based hydrogel of mometasone furoate for the treatment of psoriasis. Pharmaceutical nanotechnology.,6(2):133-43.

2) Salim, N., Ahmad, N., Musa, S.H., Hashim, R., Tadros, T.F., Basri, M. (2016). Nanoemulsion as a topical delivery system of antipsoriatic drugs. RSC advances., 6(8):6234-50.

3) Dinshaw, I.J., Ahmad, N., Salim, N., Leo, B.F. (2021). Nanoemulsions: A review on the conceptualization of treatment for psoriasis using a 'green'surfactant with low-energy emulsification method. Pharmaceutics.,13(7):1024.

4) John, Berth, J. (2009). Medicine., 37(5):235-241.

5) Feletar, M., Foley, P., Brown, M.A. (2008). Developments in psoriasis and psoriatic arthritis. Drug Discovery Today: Disease Mechanisms.,5(1):47-54.

6) Katare, O.P., Raza, K., Singh., Dogra, S. (2010). Novel drug delivery systems in topical treatment of psoriasis: rigors and Vigors. Indian J. Dermatol. Venereol. Leprol., 76:612–621.

7) Preeti, K., Suresh, et al. (2013). Novel topical drug carriers as a tool for treatment of psoriasis: Progress and advances. African Journal of Pharmacy and Pharmacology., 7(5):138-147.

8) Shinde, G., Desai., Shelke, S., Patel, R., Bangale, G., Kulkarni. (2022). Mometasone furoate-loaded aspasomal gel for topical treatment of psoriasis: Formulation, optimization, in vitro and in vivo performance. Journal of Dermatological Treatment.,33(2):885-96.

9) Khurana, B., Arora, D., Narang, R.K. (2018). Topical delivery of nanoemulsion for antipsoriatic drugs. Journal of drug delivery & therapeutics.,8(5-s):1-11.

10) Rahman, M., Akhter, S., Ahmad, J., Ahmad, M.Z., Beg, S., Ahmad, F.J. (2015) Nanomedicine based drug targeting for psoriasis: potentials and emerging trends in nanoscale pharmacotherapy, Expert Opin. Drug Deliv.,12:635–652.

11) Wollina, U., Tirant, M., Vojvodic, A., Lotti, T. (2019). Treatment of psoriasis: novel approaches to topical delivery. Open access Macedonian journal of medical sciences.,97(18):3018.

12) Korting, S.M., Gysler, A. (1992). Topical Glucocorticoids with Improved Benefit/Risk Ratio: do they exist? J Am Acad Dermatol.,27(1): 87-92.

13) Rahman, M., Akhter, S., Ahmad, J., Ahmad, M.Z., Beg, S., Ahmad, F.J. (2015). Nanomedicine based drug targeting for psoriasis: potentials and emerging trends in nanoscale pharmacotherapy, Expert Opin. Drug Deliv.,12:635–652.

14) Goyal, R., Macri, L.K., Kaplan, H.M., Kohn, J. (2016). Nanoparticles and nano fibers for topical drug delivery, J. Controlled Release.,240:77–9.

15) Agrawal, Y., Petkar, K.C., Sawant, K.K. (2010). Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. International journal of pharmaceutics.,401(1-2):93-102.

16) Raza, K., Singh, B., Lohan, S., Sharma, G., Negi, P., Yachha, Y., Katare, O.P. (2013). Nano-lipoidal carriers of tretinoin with enhanced percutaneous absorption, photostability, biocompatibility and anti-psoriatic activity, International Journal of Pharmaceutics, International Journal of Pharmaceutics., 456:65–72.

17) Tomar, S., Singhal. (2015). Formulation and Evaluation of Topical Gel Containing Azithromycin and Prednisolone Vesicles for Treating Psoriasis, International Journal of Pharmaceutical Research & Allied Sciences.,4(4):45-58.

18) Dua, K., Madan, J.R., Khude, P.A. (2014). Development and evaluation of solid lipid nanoparticles of mometasone furoate for topical delivery, International Journal of Pharmaceutical Investigation., 4(2).

19) Kaur, A., Katiyar, S.S., Kushwah, V., Jain, S. (2017). Nanoemulsion loaded gel for topical co-delivery of clobetasol propionate and calcipotriol in psoriasis. Nanomedicine: Nanotechnology, biology and medicine.,13(4):1473-82.

20) Khatoon, Karishma., Asgar, Ali., Fahan, J., Ahmad, Zubair., Hafeez, M., Moshahid, A., Rizvi., Sohail, Akhter., and Sarwar, Beg. (2021). Novel nanoemulsion gel containing triple natural bio-actives combination of curcumin, thymoquinone, and resveratrol improves psoriasis therapy: In vitro and in vivo studies. Drug delivery and translational research 11., 1245-1260.

21) Rajitha., Shammika, P., Aiswarya, S., Gopikrishnan, A., Jayakumar, R., Sabitha, M. (2019). Chaulmoogra oil-based methotrexate loaded topical nanoemulsion for the treatment of psoriasis, Journal of Drug Delivery Science and Technology., 49:463–476.

22) Rashid, S.A., Bashir, S., Ullah, H., Khan, D.H., Shah, P.A., Danish, M.Z., Khan, M.H., Mahmood, S., Sohaib, M., Irfan, M.M., Amin, A. (2021). Development, characterization and optimization of methotrexate-olive oil nano-emulsion for topical application. Pakistan Journal of Pharmaceutical Sciences.,34.

23) Dadwal, A, A., Mishra, N., Rawal, R.K., Narang, R.K. (2020). Development and characterization of clobetasol propionate loaded Squarticles as a lipid nanocarrier for treatment of plaque psoriasis. Journal of microencapsulation.,37(5):341-54.

24) Madgulkar, A.R., Padalkar, R.R., Amale, S.K. (2019). Preformulation studies of intranasal solid lipid nanoparticles of mometasone furoate. Journal of Drug Delivery and Therapeutics.,9(4):526-8.

25) Solanki, A.B., Parikh, J.R., Parikh, R.H., et al. (2010). Evaluation of different compositions of noisome to optimize aceclofenac transdermal delivery. Asia J Pharm Sci.,5(3):87-95.

26) Corazza, M., Virgili, A., Toni, G., et al. (2018). Mometasone furoate in the treatment of vulvar lichen sclerosus: could its formulation influence efficacy, tolerability and adherence to treatment? J Dermatol Treat.,29(3):305-9.

27) Raposo, S., Tavares, R., Gonçalves., Simoes, S., Urbano, M., Ribeiro, H.M. (2015). Mometasone furoate-loaded cold processed oil-in-water emulsions: in vitro and in vivo studies. Drug Delivery.,22(4):562-72.

28) Kaur, N., Sharma, K., Bedi, N. (2018). Topical nanostructured lipid carrier-based hydrogel of mometasone furoate for the treatment of psoriasis. Pharmaceutical nanotechnology.,6(2):133-43.

29) Rajitha., Shammika, P., Aiswarya, S., Gopikrishnan, A., Jayakumar, R., Sabitha, M. (2019). Chaulmoogra oil-based methotrexate loaded topical nanoemulsion for the treatment of psoriasis, Journal of Drug Delivery Science and Technology.,49:463–476.

30) Salim, N., Ahmad, N., Musa, S.H., Hashim, R., Tadros, T.F., Basri, M. (2016). Nanoemulsion as a topical delivery system of antipsoriatic drugs. RSC advances.,6(8):6234-50.

31) Yousef, S.A., Mohammed, Y.H., Namjoshi, S., Grice, J.E., Benson, H.A., Sakran, W., Roberts, M.S. (2019). Mechanistic evaluation of enhanced curcumin delivery through human skin in vitro from optimized nanoemulsion formulations fabricated with different penetration enhancers. Pharmaceutics.,11(12):639.

32) Prajapati., Patel, A.G., Paliwal. (2021). Fabrication of nanoemulsion-based in situ gel using moxifloxacin hydrochloride as model drug for the treatment of conjunctivitis. Food Hydrocolloids for Health.,1:100045.

33) Khattab, A., Marzok, S., Ibrahim, M. (2019). Development of optimized mucoadhesive thermosensitive Pluronic based in situ gel for controlled delivery of Latanoprost: Antiglaucoma efficacy and stability approaches. Journal of Drug Delivery Science and Technology.,53:101134.

34) Mahboobian, M.M., Mohammadi, M., Mansouri, Z. (2020). Development of thermosensitive in situ gel nanoemulsions for ocular delivery of acyclovir. Journal of Drug Delivery Science and Technology.,55:101400.

35) Singh, T.P., Ahmad, F.J., Jain, G.K., Verma, N. (2021). Formulation development and characterization of nanoemulsion-based gel for topical application of raloxifene hydrochloride. Indian J Pharm Educ Res.,55(4):996-1007.

36) Ahmad, N., Ahmad, R., Al-Qudaihi,A, A., Alaseel, S.E., Fita, I.Z., Khalid, M.S., Pottoo, F.H. (2019). Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC advances.,9(35):20192-206.

37) Unal, S., Tekeli, M.C., Dogan, O., Aktas, Y. (2023). Thermosensitive Pluronic® F127-based in situ gel formulation containing nanoparticles for the sustained delivery of paclitaxel. Med Science., 12(1):224-30.

38) Morsi, N., Ibrahim, M., Refai, H., El, Sorogy, H. (2017). Nanoemulsion-based electrolyte triggered in situ gel for ocular delivery of acetazolamide. European journal of pharmaceutical sciences.,104:302-14.

39) Bayanati, M., Khosroshahi, A.G, A.G., Alvandi, M., Mahboobian, M.M. (2021). Fabrication of a thermosensitive in situ gel nanoemulsion for nose to brain delivery of temozolomide. Journal of Nanomaterials.,2021(1):1546798.

40) Kolawole, O.M., Cook, M.T. (2023). In situ gelling drug delivery systems for topical drug delivery. European Journal of Pharmaceutics and Biopharmaceutics.,184:36-49.

41) Rassing, J., McKenna., Bandyopadhyay, S., and Eyring, E. (1984). Ultrasonic and 13C-NMR studies on gel formation in aqueous solutions of the ABA block polymer Pluronic RF 127, Journal of Molecular Liquids.,27(3-4):165–178.

42) Dumortier, G., Grossiord, J.L., Agnely, F., and Chaumeil, J.C. (2006). A review of poloxamer 407 pharmaceutical and pharmacological characteristics, Pharmaceutical Research.,23(12):2709–2728.

43) Mansuri, S., Kesharwani, P., Jain, K., Tekade, R.K., and Jain, S. (2016). Mucoadhesion: a promising approach in drug delivery system. Reactive and Functional Polymers., 100:151– 172.

44) Lusiana, and Müller-Goymann, C.C. (2011). Preparation, characterization, and in vitro permeation study of terbinafine HCl in poloxamer 407-based thermogelling formulation for topical application. AAPS PharmSciTech.,12(2), 9611:496–506, 2011.

45) Kanagathara, N., and Kavitha, K. (2014). Optimization and solubilization study of novel nanoemulsion formulation for 5-fluorouracil by applying pseudoternary phase diagram, Asian J. Pharm. Clin. Res.,7(2):137-139.

46) Mazonde, P., Khamanga, S.M., Walker, R.B. (2020). Design, optimization, manufacture and characterization of Efavirenz-loaded flaxseed oil nanoemulsions. Pharmaceutics.,12(9):797.

47) Baboota, S., Shakeel, F., Ahuja, A., Ali, J., Shafiq, S. (2007). Design development and evaluation of novel nanoemulsion formulation for transdermal potential of celecoxib. Acta Pharm.,57:315–332.

48) Shankar, R., Tiwari, V., Mishra, C., Singh, C., Sharma, D., Jaiswal, S. (2015). Formulation and evaluation of ketoconazole nanoemulsion gel for topical delivery. American Journal of Pharmtech Research.,5(5):446-62.

49) Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F.J., Khar, R.K., Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. European journal of pharmaceutics and biopharmaceutics.,66(2):227-43.

50) Alam, P., Ansari, M.J., Anwer, M.K., Raish, M., Kamal, Y.K., and Shakeel, F. (2017). Wound healing effects of nanoemulsion containing clove essential oil, Artif. Cells, Nanomed., Biotechnol.,45(3):591–597.

51) Alam, M.S., Algahtani, M.S., Ahmad, J., Kohli, K., Shafiq-un-Nabi, S., Warsi, M.H., Ahmad, M.Z. (2020). Formulation design and evaluation of aceclofenac nanogel for topical application. Therapeutic Delivery.,11(12):767-78.

52) Rahman, M.M., Islam, M.B., Biswas, M., Khurshid, Alam, A.H. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC research notes.,8:1-9.

53) Zhao, Z.J., Cui, X.D., Ma, X.L., Wang, Z.H. (2021). Formulation of a Self-Nanoemulsifying Drug Delivery System of Buckwheat Flavonoids and Evaluation of Its Antimicrobial Activity. Journal of Nanoscience and Nanotechnology.,21(5):3050-8.

54) Kapoor, A., Gupta, G.D. (2020). Optimization and characterization of ion activated ocular in-situ gel formulation for bacterial conjunctivitis. Int J App.,12(4):182-91.

55) Perminaite, K., Marksa, M., Stanciauskaite, M., Juknius, T., Grigonis, A. (2021). Ramanauskiene K. Formulation of ocular in situ gels with Lithuanian Royal Jelly and their biopharmaceutical evaluation in vitro. Molecules.,26(12):3552.

56) Ahmad, N., Ahmad, R., Alam, M.A., Ahmad, F.J. (2018). Quantification and brain targeting of eugenol-loaded surface modified nanoparticles through intranasal route in the treatment of cerebral ischemia. Drug research.,68(10):584-95.