RESEARCH ARTICLE

Synergistic Herbal–Synthetic Nanoemulgel of Colchicine and Nigella sativa for Enhanced Skin Permeation and Sustained In Vitro Drug Release in Gout Therapy

Iram Jahan[1]*, Garima Garg[1], Gaurav[2]

[1]*Department of Pharmaceutics, IIMT College of Medical Sciences, IIMT University, O-Pocket, Ganganagar, Meerut, 250001, Uttar Pradesh, India*
[2]*Department of Pharmacognosy, Jamia Hamdard Laboratories (India), Meerut Road, Ghaziabad-201003, Uttar Pradesh, India*

Received: 24th July, 2025; Revised: 15th Aug, 2025; Accepted: 27th Sep, 2025; Available Online: 30th Nov, 2025

ABSTRACT

Aims: The objective of this study was to formulate and characterise a synergistic nanoemulgel that integrates colchicine and Nigella sativa oil to enhance transdermal distribution and sustain the release of medicine for gout therapy.

Background: Colchicine, a conventional therapy for gout, suffers from low oral bioavailability and gastrointestinal side effects. Nigella sativa oil, with bio-enhancing and anti-inflammatory properties, offers potential to improve drug permeation and therapeutic efficacy when integrated into nanoemulgel systems.

Methods: We used a Box–Behnken experimental design to identify optimal nanoemulsion formulations using PEG 200, Tween 20, and Nigella sativa oil. Nanoemulgels were synthesised by combining the optimised nanoemulsion with Carbopol 940 gel. Franz diffusion cells were used to evaluate the formulations for droplet size, polydispersity index (PDI), pH, viscosity, spreadability, drug content, and in vitro release. FTIR spectroscopy and transmission electron microscopy (TEM) were used to examine the structure.

Results: The optimised nanoemulgel exhibited a droplet size of 62.23 ± 1.68 nm, a polydispersity index (PDI) of 0.40 ± 0.05, a pH of 6.6 ± 0.34, and a drug content of 99.12 ± 1.12%. In vitro studies of drug release showed that colchicine was released consistently throughout six hours at a rate of 89.4 ± 4.0%, whereas the colchicine solution exhibited rapid release. Nigella sativa oil facilitated enhanced absorption and potential synergistic effects.

Conclusion: The synergistic herbal–synthetic nanoemulgel combining colchicine and Nigella sativa oil presents a promising strategy for enhanced transdermal delivery and sustained drug release in gout management.

Keywords: Nanoemulgel, colchicine, Nigella sativa, gout, transdermal delivery, sustained release, synergistic therapy

How to cite this article: Jahan I, Garg G, Gaurav G. Synergistic herbal–synthetic nanoemulgel of colchicine and Nigella sativa for enhanced skin permeation and sustained in vitro drug release in gout therapy. Int J Drug Deliv Technol. 2025;15(4):1475-1487, DOI: 10.25258/ijddt.15.4.4

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Gout is a kind of inflammatory arthritis characterised by abrupt, intense pain, swelling, and erythema in the joints, often affecting the big toe. It occurs when there is an excess of uric acid in the bloodstream, leading to the formation of urate crystals in the joints. These crystals elicit an immunological response by prompting neutrophils to locate them and release chemicals that induce significant pain and inflammation. Excessive amounts of uric acid result in crystallisation, leading to deposits in the joints and other tissues[1,2]. The development of gout is driven by the accretion of monosodium urate (MSU) crystals in synovial fluid and various tissues, which subsequently triggers an inflammatory response. The immune system recognises these crystals as foreign things, which then triggers the activation of immune cells, including neutrophils. The presence of neutrophils in the joint area is a distinctive hallmark of gout inflammation[3,4]. During gout episodes, the production of pro-inflammatory cytokines and enzymes exacerbates the inflammatory response, resulting in intense pain and swelling. Colchicine is an essential medicine for the treatment of gout. It works by interfering with the neutrophils' capacity to get to the location where crystals are deposited, therefore decreasing the inflammatory reaction. Colchicine is most efficacious when used promptly at the first indication of a gout flare-up, facilitating the rapid alleviation of symptoms. Colchicine is not only used for the action of acute gout attacks, but it is also utilised in smaller amounts for the long-term prevention of recurring gout attacks[5]. This helps to decrease the frequency and intensity of flare-ups. Colchicine is largely acknowledged as the most often prescribed medication for the treatment of acute gout. The typical methods of administering colchicine are by oral or intramuscular routes. Nevertheless, the conventional method of administering colchicine orally may cause gastrointestinal adverse effects such as diarrhoea, bloating, and vomiting[6]. Additionally, the hepatic first-pass

*Author for Correspondence: Iram Jahan

metabolism necessitates the practice of large dosages of colchicine. Intramuscular administration decreases patient acceptability and compliance. Hence, researchers are investigating other methods of administering colchicine. Transdermal delivery is a preferable method of medication administration compared to oral and intramuscular injection since it allows for topical and non-invasive drug delivery via the skin.

Colchicine is a medicine commonly used to treat gout, known for its low permeability across biological membranes. This low permeability can limit its absorption and bioavailability, meaning that individual a portion of the directed dose effectively influences the bloodstream and target tissues. N. sativa, also known as black seed or black cumin, has been studied for its potential to enhance drug permeability and absorption. Research suggests that certain components of Nigella sativa, such as thymoquinone, may influence the function of drug transporters and modulate membrane permeability. This could potentially improve the absorption and bioavailability of drugs with low permeability, like colchicine.

MATERIALS AND METHODS

Materials

Drug sample of Colchicine was conventional as gift sample by ZEE Laboratories Ltd., Delhi 110034, India, Nigella sativa oil Rex Remedies limited (Delhi, India), Methanol Sisco research laboratories, Ltd. (new Mumbai, india), Carbopol-934, Oleic acid, PEG 200, PEG 400, Span 20, 80 Central drug House Ltd. (New Delhi, India), Propylene Glycol, Butanol-1 Numex chemical products (India).

Characterization of Colchicine and Nigella sativa

Fig 1: Shimadzu FTIR-8400S spectrophotometer used for infrared spectral analysis

Figure 2. Black cumin (oil and seed)

Table 1: Observations for Colchicine sample [56,57]

S. No.	Parameters	Inferences	
		Colchicine	Nigella sativa oil
1	Nature	Amorphous powder	Liquid
2	Colour	pale yellow	dark brown
3	Odor	Odorless	characteristic
4	Taste	bitter	slightly bitter
	Solubility of Colchicine		
5	Aqueous solubility (mg/ml)	14mg/ml	
6	Partition coefficient (log P o/w)	1.03	

Physicochemical characterization included organoleptic evaluation, melting point determination (capillary method), and FTIR spectroscopy. TLC confirmed the presence of thymoquinone in Nigella sativa oil.

Melting point determination

The capillary method and melting point apparatus were used to determine the melting point. A capillary tube was packed with an adequate amount of drug powder, forming a column of approximately 4–6 mm in height. The tube was then placed inside the apparatus alongside a calibrated thermometer. The temperature at which the drug melted was recorded.

Fourier Transform Infrared (FTIR) Spectroscopy

Colchicine and Nigella sativa samples were subjected to FTIR spectra by a Shimadzu spectrophotometer and the potassium bromide (KBr) pellet method (Fig 1), which involved thoroughly mixing 1 mg of each sample with KBr in a 1:1 ratio and then compressing the mixture with a hydraulic press. The spectra were acquired over the wavenumber range of 4000–400 cm^{-1} to identify characteristic absorption bands. The observed spectra were subsequently compared with standard reference spectra for analytical confirmation and interpretation[7,8,9,10].

Thin Layer Chromatography

The seeds and oil of Nigella sativa L. were purchased from a nearby market (Figure 1). One milliliter of methanol was used to dissolve 64 milligrams of seed powder for examination. A mobile phase made of glacial acetic acid

Table 2: Prominent peaks of Colchicine in FT-IR spectra (58)

S. No	Observed Peaks of drug sample (cm-1)	Peaks of pure (cm-1)	Name of group
1	1739.67	1732	C=O
2	2934.49	2935	C-H stretch (Aliphatic)
3	1250.75	1251	C-H (Aromatic)
4	1487.01	1487	C-C stretch in ring
5	1322.11	1323	C-N (Stretch)
6	1283.54	1282	C-O-C
7	3314.44	3246	N-H stretch

and benzene was used in an initial investigation. Two distinct mobile phase systems were used for the comparative analysis: System A, which included benzene and glacial acetic acid in a 1:1 ratio, and System B, which had a combination of carbon tetrachloride, acetone, and glacial acetic acid in a 15.2:3:1 ratio. Commercially available black cumin oil and methanolic extractions of N. sativa seeds were among materials examined. Thin-layer chromatography (TLC) plates that had already been coated had a beginning line drawn with a pencil, and sample solutions were applied as spots along this line[11-14]. The plates were then developed in a chromatography chamber using the respective mobile phase. After development, a finish line was marked with a pencil, and the spots were visualized under a UV lamp to determine the retention factor (Rf) values.

Drug excipient interaction study by FTIR:

The physical incompatibility of Colchicine with a number of excipients, such as the gelling agent (Carbopol 934), surfactant (Tween 20), co-surfactant (PEG 200), and oil phase (Nigella sativa), was evaluated using Fourier Transform Infrared (FTIR) spectroscopy.

Solubility Studies

We assessed the solubility of colchicine in various oils, surfactants, and co-surfactants. We selected Nigella sativa oil, Tween 20, and PEG 200 for formulation development because to their superior solubilisation of colchicine.

Preparation of nanoemulsion

We used a Box–Behnken experimental design to investigate the influence of oil phase concentration, Smix ratio (Tween 20: PEG 200), and water content on the characteristics of the nanoemulsion (NE). To identify suitable nanoemulsion regions, pseudo-ternary phase diagrams were constructed using the aqueous titration technique, also known as the spontaneous emulsification method[15-18]. We combined colchicine with nigella sativa oil in the oil phase. Subsequently, while agitating the mixture with a magnet at 40 °C, we gradually included the aqueous phase until a transparent nanoemulsion was achieved. We examined the phase behaviour by varying the Smix ratios (1:0, 1:1, 2:1, 3:1, 4:1, 1:2, 1:3, and 1:4). For each Smix ratio, we analysed 16 distinct oil-to-Smix combinations (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 1:0.7, 1:0.43, 1:0.25, and 1:0.1).

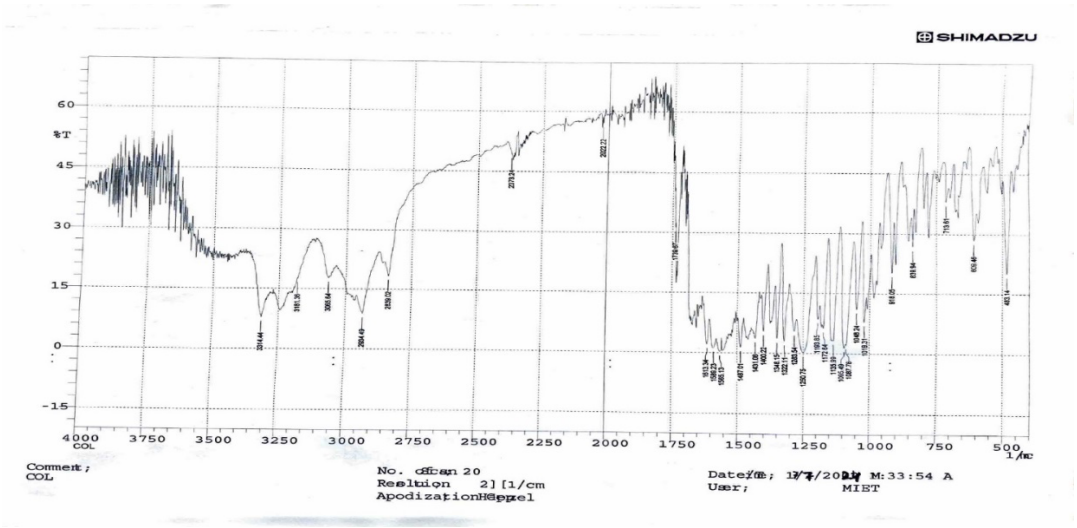

Figure 3: FTIR spectra of Colchicine

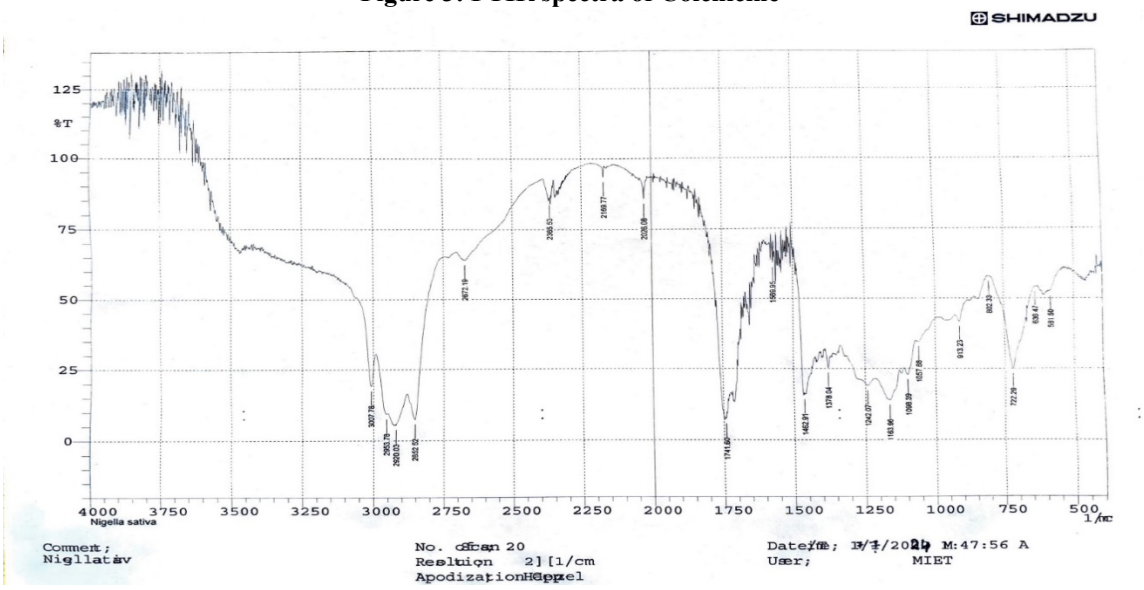

Figure 4: FTIR spectra of Nigella sativa

Table 3: Prominent peaks of Nigella sativa in FT-IR spectra (59)

S.No.	Observed Peaks of drug sample (cm-1)	Peaks of pure (cm-1)(60)	Name of group
1.	3007.78	3006	C–H stretching
2.	1462,91	1402.25	C=C aromatic stretching
3.	2852.52	2853	C–H aliphatic bending
4.	722.29	678.94	R-X- stretching
5.	1057.88	1033.85	-NH_2 (Aliphatic amines) stretching

Each oil–Smix mixture was gently stirred, and the aqueous phase was titrated dropwise with constant agitation to assess nanoemulsion formation. The clarity, transparency, and flowability of the formulations were visually inspected to determine phase transitions[16,19,25-27]. Pseudo-ternary phase diagrams were generated using ProSim software. The three axes represent oil, Smix, and aqueous phase with predetermined mass ratios. The dimensions of the NE region in the diagrams facilitated our selection of the optimal Smix composition. Throughout the titration, the temperature was maintained at 40 °C for five minutes to ensure homogeneous mixing and consistent emulsification[20-24]. These studies provided valuable insights into component compatibility and solubilization efficiency, facilitating the selection of formulation variables that yielded a stable and thermodynamically favorable nanoemulsion system[28]

Evaluation of Nanoemulsion system

Thermal stability studies

We conducted thermodynamic stability studies on the nanoemulsions (NEs) to assess their phase division, clarity, drop size, and drug concentration. These analyses were carried out with minor adjustments to a previously published methodology. These assessments ensured the stability and robustness of the NE formulations under varying conditions[29-33].

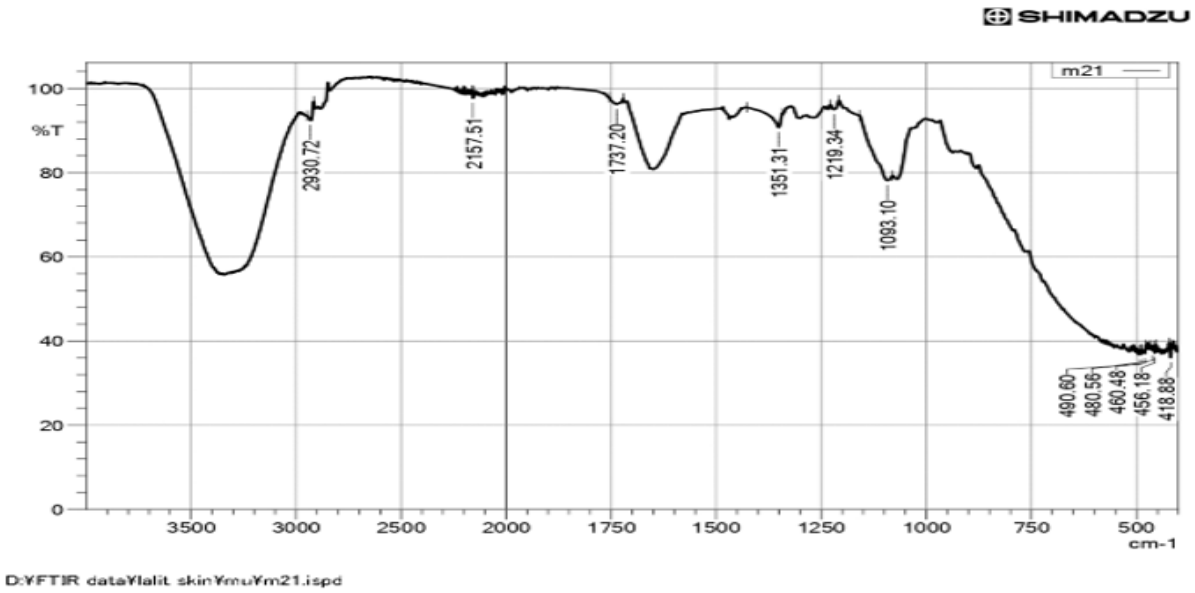

Figure 5: FTIR spectrum of Drug and Nigella sativa with Smix Tween - 20/PEG-200

Figure 6: Thin Layer Chromatography (TLC) Setup for Nigella sativa Extract Analysis and Solvent System Optimization.

Table 4. Rf values of major constituents in Nigella sativa determined with various mobile phase systems[61].

S.No	Sample	Type of mobile phase (v/v)	Rf (thymoquinone)	Rf (dithymoquinone)
1	Methanoic powder solution	Benzene: glacinal acetic acid (1:1)	<0.3	<0.3
2	Oil	Carbon tetrachloride/acetone glacial acetic acid (15.2:3:1)	0.7	0.6

Table 5: Saturation solubility Profile of Colchicine in Different Excipients (Oils, Surfactants and Co-surfactants)

Name of excipients	Drug (mg/ml)
Oil	Colchicine
Nigella sativa oil	52 mg/mL
Nutmeg oil	48 mg / mL
Castor Oil	32 mg/mL
Oleic acid	20 mg / mL
Surfactant	
Cween 20	50 mg / mL
Span 20	47 mg / mL
Span 80	47.23 mg / mL
Cween 80	30 mg / mL
Co-Surfactant	
PEG 200	50.28 mg / mL
Propylene Glycol	46 mg / mL
n-Butanol	30 mg / mL
PEG 400	35 mg / mL

Heating Cooling Cycle

The developed nanoemulsions (NEs) underwent six temperature cycles, each lasting a minimum of 48 hours, ranging from 4°C to 45°C. Subsequent to these cycles, the formulations were evaluated for stability to determine their resistance to phase separation, changes in clarity, and other possible issues.

Centrifugation

The nanoemulsion (NE) formulations were centrifuged at 3,500 rpm for 30 minutes. We selected only those preparations that exhibited no phase separation throughout the freeze-thaw stability testing process.

Freeze-Thaw cycle

Formulations of nanoemulsions (NE) that held up well in centrifugation tests were frozen and thawed at temperatures ranging from -21°C to +25°C. Three freezing cycles were used in this procedure, with a minimum of 48 hours between each temperature storage step.

Characterization of the developed NE

Globule size and PDI analysis

The sample formulation was diluted with 2 milliliters of distilled water in a 50 µL aliquot. With a Zetasizer (Nano-ZS90), the globule size and polydispersity index (PDI) were then examined. Three measurements of the mean droplet size were made to guarantee precision and repeatability[34-38].

Transmission electron microscopy (TEM)

We employed transmission electron microscopy (TEM, CM 200) to look at the morphology of the oil droplets that were dispersed. A drop of the nanoemulsion (NE), which had been diluted with water at a ratio of 1:1000, was put on a copper grid that was covered with carbon. The sample was stained with 2% (w/v) phosphotungstic acid and then looked at after it had dried for a minute[39-42].

Refractive Index and Viscosity

We used Abbe's refractometer to determine the refractive index of the NE formulation. A Brookfield viscometer (MCR101) was used to assess the viscosity of the NE formulations[43-45].

pH and Transmittance

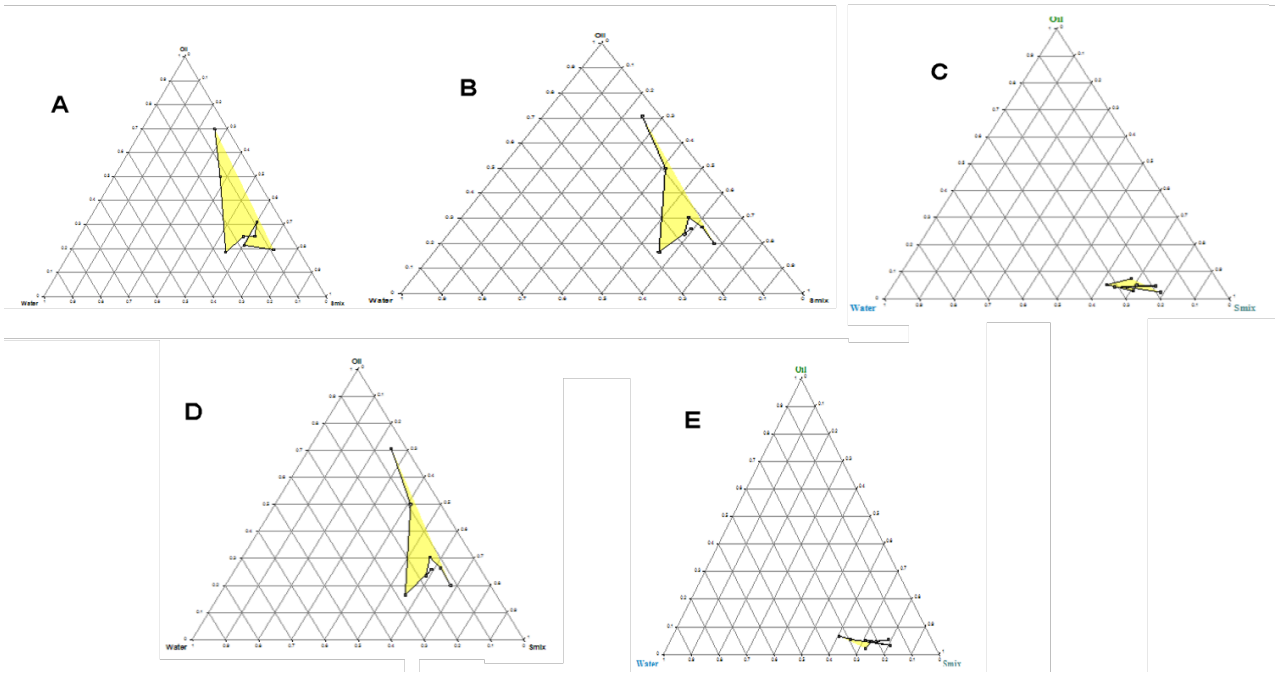

Figure 7: Pseudo-ternary phase diagrams showing nanoemulsion regions at various Smix ratios for the oil, surfactant, and co-surfactant system.

Table 6: Composition of nanoemulsion formulations prepared with different Smix ratios (Tween 20/PEG-200), showing oil, Smix, water, colchicine content, and total weight

Smix ratios (Tween 20/ PEG-200)	Formulation code	Percentage of components				
		Oil (mg)	Smix (Surfactant + Cosurfactant) (mg)	Water (mg)	colchicine (2%) (mg)	Total weight* (mg)
Formulation A	A1	8	30 (15+15)	80	2	120
Smix=ratio 1:1	A2	13	20 (10+10)	85	2	120
	A3	7	26 (13+13)	85	2	120
	A4	9	34 (17+17)	75	2	120
	A5	5	38 (19+19)	75	2	120
	A6	10	42 (21+21)	66	2	120
	A7	5	40 (20+20)	73	2	120
	A8	6	36 (18+18)	76	2	120
	A9	7	24 (12+12)	87	2	120
Formulation B	B1	7	36 (12+24)	75	2	120
Smix=ratio 1:2	B2	3	25 (8.3+16.7)	90	2	120
	B3	6	24 (8+16)	88	2	120
	B4	7	33 (11+22)	78	2	120
	B5	8	30 (10+20)	80	2	120
	B6	8	25 (8.3+16.7)	85	2	120
	B7	4	30 (10+20)	84	2	120
	B8	9	33 (11+22)	76	2	120
	B9	8	24 (8+16)	86	2	120
Formulation C	C1	8	25 (16.7+8.3)	85	2	120
Smix=ratio 2:1	C2	9	30 (20+10)	79	2	120
	C3	7	31 (20.66+10.33)	80	2	120
	C4	13	20 (13.3+6.7)	85	2	120
	C5	6	24 (16+8)	88	2	120
	C6	3	25 (16.7+8.3)	90	2	120
	C7	8	31 (20.66+10.3)	79	2	120
	C8	6	25 (16.7+8.3)	87	2	120
	C9	7	30 (20+10)	81	2	120

The pH of the formulation was measured at 25°C using a calibrated pH meter (Mahtab et al., 2016). We introduced 1 mL of the formulation into a cuvette and using a UV-Vis spectrophotometer to get three measurements at 630 nm to determine the transmittance of light[46-48].

Preparation of the drug-loaded Nanoemulgel

Carbopol 940, the gelling agent, was used in different doses to create nanoemulgel formulations (0.5% to 1.5% w/v). Carbopol 940 was dissolved in distilled water and constantly swirled to create the gel base, which ensured full swelling of the gelling agent. The dispersion was sonicated for fifteen minutes in order to remove air bubbles. To produce a uniform gel dispersion, sodium benzoate, a preservative, and AMP-90, a neutralizing agent, were then added. To create the final DLNE gel, the adjusted DLNE was thereafter slowly added to the gel basis while being gently stirred. The same process was used to create a control gel devoid of NE[49-53].

Physicochemical characterization of Nanoemulgel

Homogeneity

The advanced gels were evaluated for homogeneity through visual review after setting in their respective containers. The gels were examined for their overall advent and the occurrence of any masses or inconsistencies.

Viscosity measurement

To evaluate the viscosity of the set gel, a Brookfield viscometer with spindle number C-50-1 was used. To

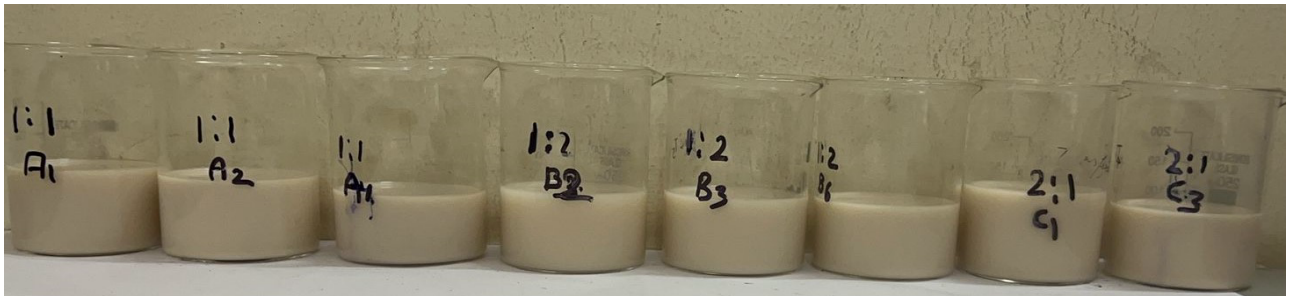

Figure 8: Representative drug-loaded nanoemulsion formulations comprising varying ratios of oil, surfactant, and co-surfactant (1:1, 1:2, and 2:1), selected for further characterization.

Table 7: Physical characterization studies of different nanoemulsion formulations (Mean ± SD, n = 3)

Formulation code	Characterization Parameters				
	Size (nm)	PDI	pH	RI	Viscosity (pas)
A1	62.23±1.68	0.40±0.05	6.6±0.34	1.401±0.006	120.9±4.06
A2	85.3±1.67	0.98±0.04	6.7±0.29	1.402±0.008	53.71±3.03
A3	97.6±1.42	0.89±0.03	6.8±0.26	1.389±0.005	103.0±3.48
C1	99.9±1.21	0.89±0.05	6.9±0.55	1.401±0.006	42.89±2.51
C3	90.2±2.13	0.50±0.02	6.5±0.16	1.404±0.006	101.9±5.95

guarantee precision and dependability, the capacities were carried out in triplicate.

Drug content

To test the medicine in the gel formulations, 100 mL of methanol was combined with 1 g of the gel that included Nigella sativa and colchicine, and the combination was agitated for 30 minutes. The final combination was then conceded over a membrane filter that had 0.45 µm pores. After the filtrate was suitably diluted, the UV spectrophotometric technique was used for analysis. To be confident of correctness, the analysis was done three times.

pH evaluation

A pH meter was used to test the gel's apparent pH three times at 25 ± 1°C.

Extrudability (Tube Test)

The force essential to extrude a 0.5 cm ribbon of gel from a collapsible tube within 10 seconds, measured in grams, served as a simple and effective method to assess the gel's extrudability. The crimped end of the closed, collapsible tube holding the gel was forcefully squeezed. Upon removing the cap, the gel continued to extrude due to the residual pressure that had been imposed was released. Better extrudability is indicated by a greater amount of gel extruded. To guarantee accuracy, the gel formulation's extrudability was assessed three times.

Spreadability

In order to assess the nanoemulgel's spreadability, 0.5 g of the gel was riding onto a glass plate within a previously designated round with a diameter of 1 cm. Placed on top, extra glass plate weighing 500 g was let to rest for five minutes. They next assessed the diameter increase caused by the gel spreading (54). Reliability was ensured by doing the trials in triplicate and measuring the spread's extent using a linear scale.

Texture profile analysis

A probe that was submerged in the formulation at a certain force and velocity was used to perform texture analysis. The TA.XT2 texture analyzer was cast-off to test the nanoemulgel's tensile strength. A 5 kg load cell, a cylindrical probe with a 25 mm diameter, a 0.05 N trigger force, a 5 mm penetration depth, and a 2 mm/s test speed were used to assess mechanical parameters. Every experiment was passed out at room temperature (20°C ± 1°C) in triplicate (mean ± SD). Texture Expert® software was castoff for both data collection and mathematical study (55). The produced nanoemulgel's cohesion, stiffness, consistency, and viscosity index were also assessed.

In Vitro Drug Release Studies

We used a Franz diffusion device to investigate the in vitro drug release of the nanoemulgel formulations. A cellophane

Figure 9: Selected Drug loaded nanoemulsion

membrane encased the donor chamber of the apparatus. This membrane had precisely the correct quantities of the formulation. The system was regulated at 37 ± 0.5°C and rotated at 50 rpm using 7 mL of phosphate buffer (pH 7.4) in the acceptor compartment. Samples were collected at predetermined intervals (0.5, 1, 2, 3, 4, and 6 hours), and spectrophotometric analysis was conducted at a peak wavelength (λmax) of 350 nm. Each experiment was conducted three times.

RESULTS AND DISCUSSIONS

Characterization of Colchicine and Nigella sativa

Organoleptic properties

Colchicine and Nigella sativa were determined for their nature, odor, colour and taste.

Melting point determination

By using capillary method melting point was determined for Colchicine, it was observed f 143-148°C respectively. The organoleptic properties of Colchicine were quite similar to those described in the Colchicine official monograph. There were no discernible variations between the published values and the experimentally obtained data, which included saturation solubility, melting point, and partition coefficient.

FT-IR Spectroscopy

FTIR spectra shows characteristic peaks of drug sample Colchicine and Nigella sativa mentioned in the Table 2 and 3 and almost matched when compared with standard reference as shown in the Figure 3 and 4.

CONCLUSION

The sample of Colchicine and Nigella **sativa** were physiochemically characterized by melting point and FTIR analysis and was found authentic and pure.

TLC analysis of Nigella sativa essential oil

A UV light was used to examine the chromatogram, and the spots that were found were compared to data from the literature for both the standard and black cumin seed powder. A preliminary investigation utilizing the mobile phase mixture (A) of glacial acetic acid: benzene (1:1) showed insufficient separation, most likely because glacial acetic acid is polar. The study of methanolic black cumin seed powder by thin-layer chromatography (TLC) with mobile phase (B) carbon tetrachloride/acetone/glacial acetic acid (15.2:3:1), on the other hand, showed better separation. The commercial oil's polarity properties, however, made it more suited for separation. The process's effective spot separation was made possible by the use of chloroform. Table 3 displays the retention factor (Rf) values for several commercial oil and powdered black cumin samples under various mobile phases. Clear stain development was guaranteed throughout the development phase when Rf values fell between 0.3 and 0.7, which resulted in optimal separation.

Determination of drug solubility

Colchicine showed maximum solubility in Nigella sativa oil (52 mg/mL), Tween 20 (50 mg/mL), and PEG 200 (50.28 mg/mL). Pseudo-ternary phase diagram analysis revealed prominent nanoemulsion zones at Smix ratios of 1:1, 1:2, and 2:1.

Nanoemulsion Optimization and Characterization

Pseudo-ternary phase diagrams were constructed using Smix mixtures of Tween 20 and PEG 200 in weight ratios of 1:1, 1:2, 1:3, 2:1, and 3:1. The diagrams were plotted with Nigella sativa oil as the oil phase, while Tween 20 acted as the surfactant and PEG 200 served as the co-surfactant. The capacity of each Smix ratio to solubilize the oil phase and affect nanoemulsion production is basically what determines the size of the nanoemulsion zone. Preformulation studies rely heavily on these phase diagrams to help identify possible dispersion systems that are produced at certain ratios of Smix, oil, and the aqueous phase.

Preparation of Nanoemulsion formulation

Design-Expert Software (Version 12, Stat-Ease Inc.) was employed to generate second-order polynomial models and analyze quadratic response surfaces based on a three-factor, three-level Box-Behnken design. This statistical approach enabled systematic optimization of the nanoemulsion system by examining multiple variables and their

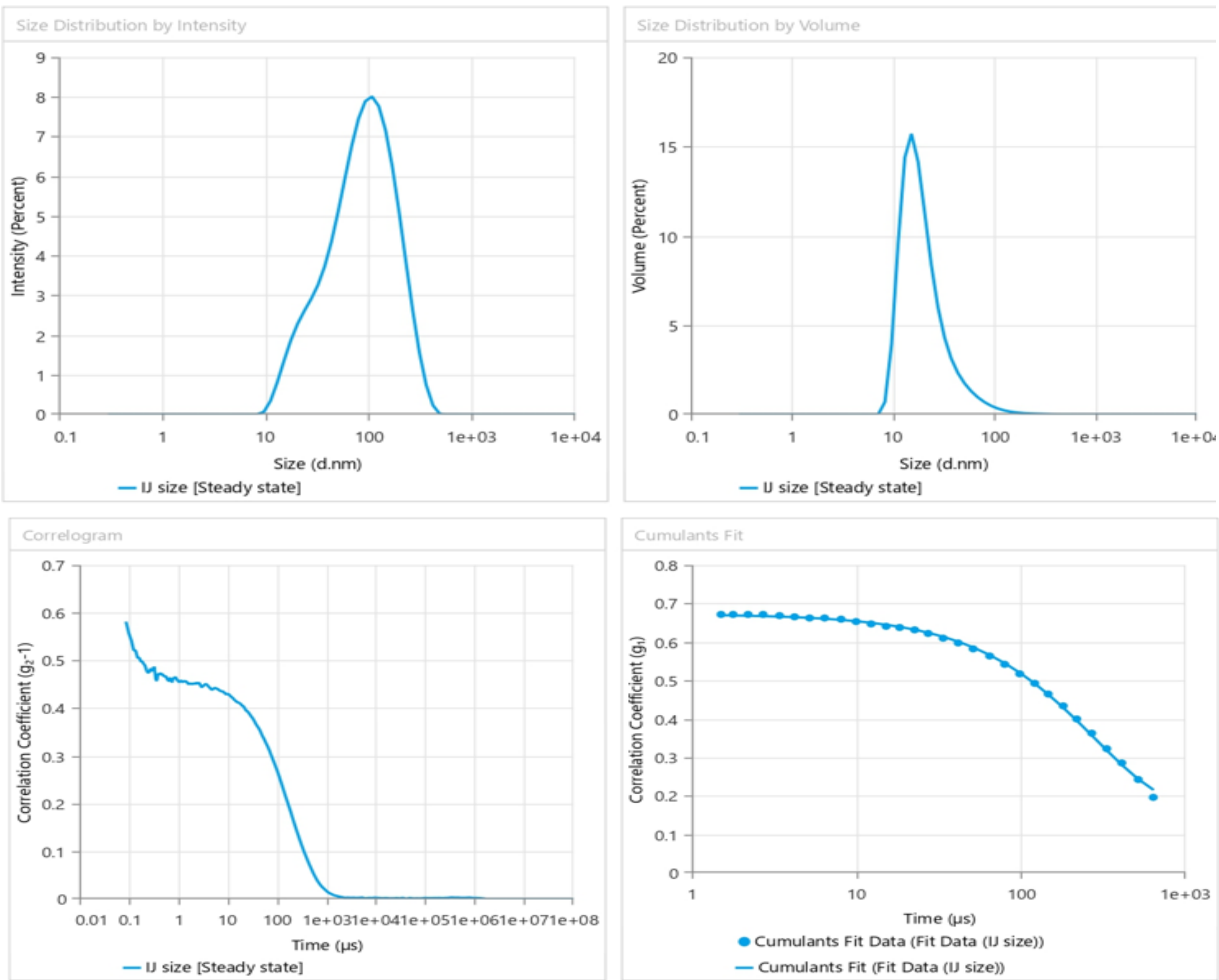

Figure 10: Particle size, distribution profile of the optimized nanoemulsion formulation.

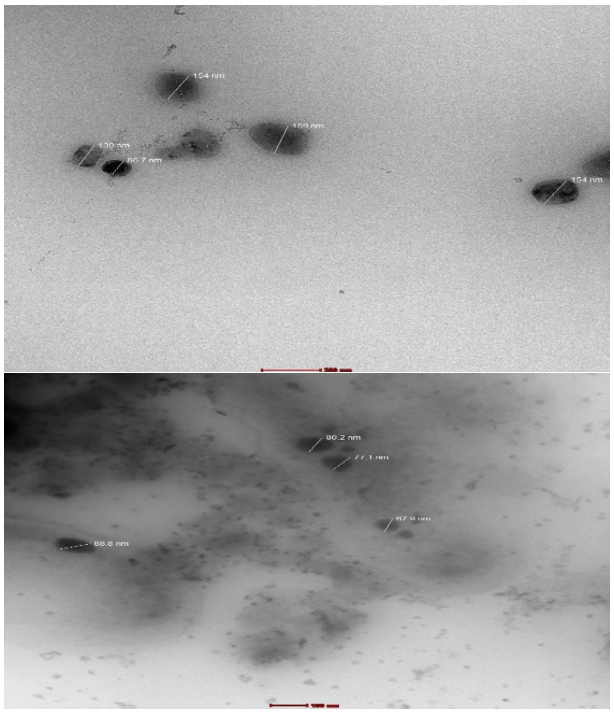

Figure 11: TEM photograph of optimized nanoemulsion

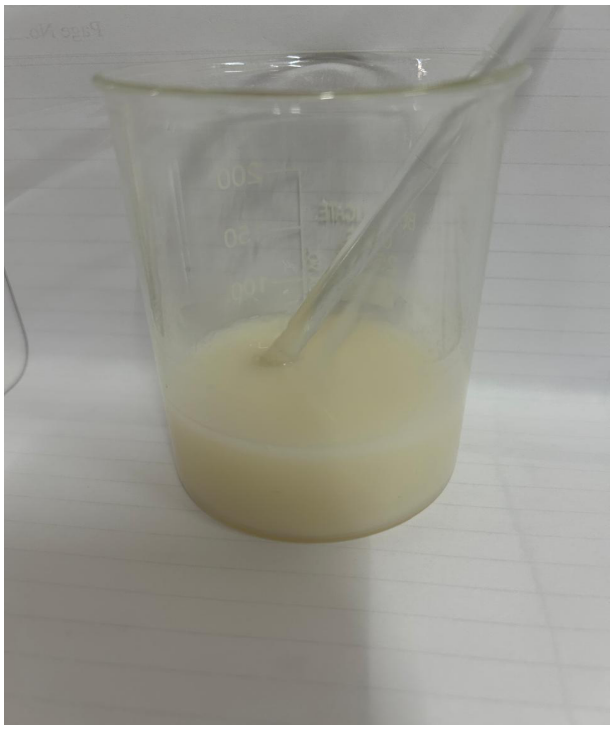

Fig 12: Colchicine and Nigella sativa-Loaded Nanoemulgel

Table 8: Physiochemical characterization of prepared nanoemulgels

Drug loaded nanoemulgel formulation 0.5% carbopol 940 (DLNE gel)	Homo-genity	Mean Viscosity (Pa.s)±S.D. (n=3)	Mean Drug content (%)±S.D. (n=3)	Mean pH±S.D. (n=3)	Mean spreadability (gcm/sec)± S.D. (n=3)	Mean extrudability (gm)±S.D. (n=3)
	Good	157.23 ± 0.67	99.12±1.12	6.55±0.22	14.39 ± 1.66	165.45 ± 2.74
1% carbopol 940	Good	160.2 ± 044	80.26±2.16	6.55±0.75	8.89 ± 0.478	130.11 ± 2.56

interactions. The nanoemulsions were prepared using the aqueous titration method. Enhancing medication penetration and deposition in the dermal layer was the aim of topical drug administration. Taking into account possible skin irritation issues, the ideal ratio of surfactant to co-surfactant was needed to accomplish this. Because of its stability against pH and ionic strength variations and lower toxicity than ionic surfactants, Tween 20 was chosen as a non-ionic surfactant[62]. The co-surfactant PEG-200 is essential for the generation of nanoemulsions (NEs) because it lowers the interfacial tension of the surfactant, which promotes the growth of a more dynamic and flexible interfacial layer. Drug diffusion across phases is made possible by the flexible surfactant layer in this high-energy system, which promotes partitioning and diffusion as a thermodynamically driven process. Furthermore, PEG-200 may increase formulation stability by reducing the necessary surfactant content. Formulations with Smix ratios of 3:1 and 4:1 were disqualified based on the NE area shown in the phase diagram because of the high concentration of surfactant, which might cause skin irritation[63]. From the phase diagram, three Smix ratios have been optimized, 1:1 (A), 1:2 (B), and 2:1 (C) for further studies and results shown in the Table 6.

Thermodynamic stability

Nanoemulsions are created at certain oil, Smix, and water ratios without phase separation; they are kinetically stable but not thermodynamically stable (Ahmed et al., 2018). A few formulations were tested for thermodynamic stability, and the results showed that Ostwald ripening caused instability in several of them (Wennerström and Olsson, 2009). Only stable formulations moved on to drug loading, where the oil phase dissolved Nigella sativa and Colchicine (2 mg/mL each). The most stable formulation, A1, was determined by stability testing and was chosen as the optimized drug-loaded nanoemulsion (DLNE) for further characterisation.

Characterization of the developed NE

Globule size distribution of optimized nanoemulsion

The effects of the Oil:Smix ratio, homogenization speed, and homogenization duration on particle size, PDI, and drug content % were assessed via a total of 27 experimental runs utilizing the Box-Behnken design. Table 7 shows the impacts on various response variables that have been observed.

Transmission electron microscopy (TEM)

Figure 5.22 clearly illustrates that the optimized DLNE exhibited no aggregation, confirming the even and homogeneous supply of spherical particles in the

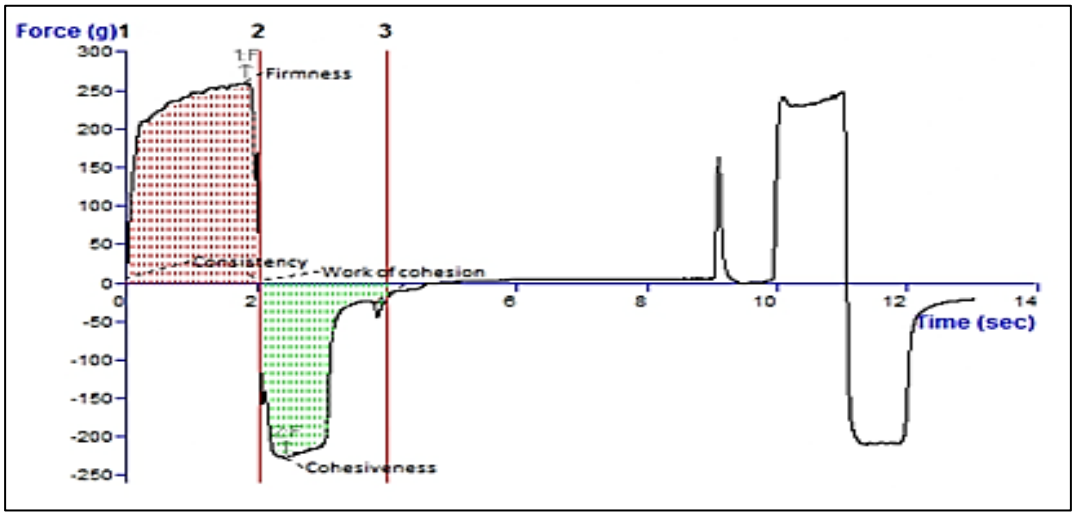

Figure 13: Texture profile analysis graph of optimized DLNE gel

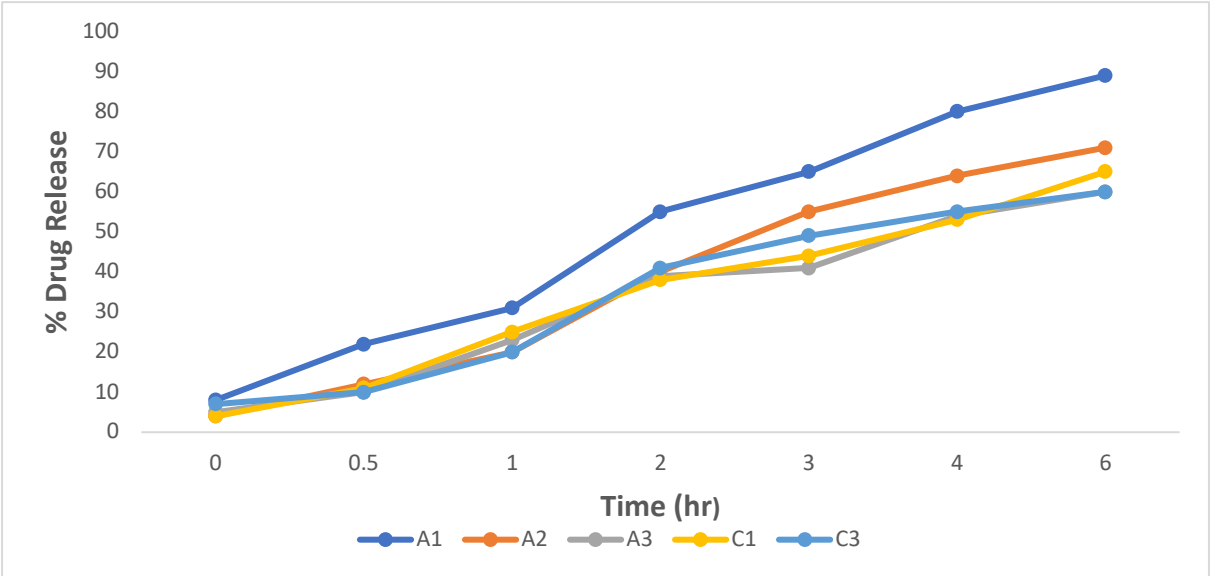

Fig 14: The in vitro release study compared colchicine release from various formulations with that of a colchicine solution in phosphate buffer (pH 7.4) at 37°C.

formulation. These results are reliable with the globule size analysis performed using the Zetasizer (Fig 11).

Physicochemical characterization of drug-loaded nanoemulgel (DLNE gel)

To address the low drug retention of DLNE due to its low viscosity, Carbopol 940, a hydrophilic and non-toxic gelling agent, was incorporated to formulate a nanoemulgel (Mou et al., 2008). Two concentrations (0.5% and 1% w/v) were tested, with 0.5% selected based on key parameters such as appearance, homogeneity, pH (6.55 ± 0.22), spreadability (14.39 ± 1.65 g cm/sec), and drug content (99.12 ± 1.13%). The results for the advanced DLNE gel were as follows: cohesiveness (-37.91±0.031), consistency (117.30±0.021), firmness (78.66±0.66), and viscosity index (-93.84±0.81). The texture analysis, shown in Figure 13, highlighted the formulation's ease of application, confirming the optimized properties of the DLNE gel for effective use.

Drug Release Profile

The in vitro release profile of colchicine from different formulations and its solution across cellophane membranes was analyzed using a Franz diffusion apparatus. The findings are illustrated in Figure 5.24.. Due to its high solubility, approximately 97.8% of colchicine was out from the solution within the first hr. Among the developed formulations, A1 demonstrated the most favorable drug release profile, with 89.4 ± 4.0% of colchicine released after six hours, followed by A2 (71.77 ± 3.5%), A3 (60.67 ± 3.04%), and C1 (65.25 ± 3.04%, 60 ± 2.04%). The significantly lower drug release ($p < 0.05$) from all preparations associated to the colchicine result is attributed to the ability of A1 to form a structured layer within membrane spaces, which regulates drug diffusion while allowing sustained release. Moreover, formulation viscosity plays a crucial role in drug retention and controlled release, with gelling agents contributing to the extended-release pattern observed in A1, making it the most effective formulation for sustained colchicine delivery.

Conclusion

This study successfully developed a synergistic nanoemulgel formulation combining the synthetic drug colchicine and the herbal oil Nigella sativa, demonstrating enhanced physicochemical properties and sustained drug release suitable for transdermal delivery. The optimized nanoemulgel exhibited nanoscale droplet size, good stability, and favorable rheological properties, contributing to effective skin permeation and prolonged colchicine release over six hours. The incorporation of Nigella sativa oil not only improved drug solubility but may also offer additional anti-inflammatory benefits, suggesting a synergistic therapeutic effect. These findings indicate that the colchicine–Nigella sativa nanoemulgel represents a promising strategy for improving topical gout therapy while potentially minimizing systemic side effects. Further in vivo studies are warranted to validate its clinical efficacy and safety.

Acknowledgements

The authors thank the Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, Meerut, for their support and resources provided during this research.

Author Contribution

Conceptualization: Iram Jahan, **Technical Aspects:** Gaurav, Garima Garg, **Data Collection:** Iram Jahan, **Grammatical Correction:** Iram Jahan, Garima Garg

Abbreviations

NE – Nanoemulsion, **NEG** – Nanoemulgel, **FTIR** – Fourier Transform Infrared Spectroscopy, **TEM** – Transmission Electron Microscopy, **PDI** – Polydispersity Index, **Smix** – Surfactant/co-surfactant mixture, **MSU** – Monosodium Urate

TLC – Thin-Layer Chromatography, **PEG** – Polyethylene Glycol, **SD** – Standard Deviation

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