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Formulation, Characterization And Release Studies Of Colchicine Chitosan Eutectogel Through A QBD Assisted Approach

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Abstract
Objective: *The present study focuses on the development and evaluation of a colchicine-loaded eutectogel as a novel transdermal drug delivery system for the effective management of gout. Transdermal colchicine chitosan eutectogels are preferred over oral formulations because they bypass gastrointestinal irritation and first-pass metabolism, improving bioavailability.*
Methods: *Colchicine Chitosan eutectogel was prepared by dissolving Chitosan in an acidic medium such as using acetic acid along with dissolving Chitosan into it. Characterization was done using Fourier Transform Infra Red Spectroscopy (FTIR) and other preformulation studies. In vitro drug release study was also performed using dialysis membrane and release profile was monitored by kinetic studies. Validation studies were carried out by using High Performance Liquid Chromatography (HPLC) technique.*
Results: *The pH values of all the formulations D14 (C1)-D14 (C4) were found to be in the range of 6.14-6.34. The Viscosity of the gel at different formulation D14(C1)-D14(C4) was found to be in the range from 3223-14206 cP. Considering the determination coefficients, Higuchi model was found (R^2=0.981) to fit the release data best. It could be concluded from the results that the drug was released Deep Eutectic Solvent-Based Matrix (DESM) loaded gel by a prolonged mechanism. In the control gel, 85.47% of the drug was released within 4 hours. In contrast, the DESM-loaded gel formulations (C2–C4) exhibited drug release percentages of 87.29%, 79.34%, and 73.81%, respectively, in phosphate buffer (pH 7.4) over a period of 24 hours. Among these, formulation C2 showed the highest drug release of 87.29% within 24 hours, indicating a sustained and prolonged release profile.*
Conclusion: *The formulated eutectogel exhibited favourable physicochemical characteristics, and a sustained drug release profile, contributing to improved therapeutic outcomes. The findings suggest that eutectogel-based systems hold significant potential for localized and controlled delivery of anti-gout agents. Future research should focus on clinical evaluation, exploration of combination therapies with other anti-inflammatory or phytoconstituents, and large-scale production to facilitate the successful translation of this novel transdermal system into clinical practice.*
Keywords: *Eutectogel, transdermal drug delivery system, gout, drug release, inflammation.*

INTRODUCTION

Transdermal Drug Delivery (TDD) is an advanced and non-invasive method of administering therapeutic agents through the skin to achieve systemic effects [1]. It has emerged as an efficient alternative to traditional routes of drug administration, such as oral ingestion and injections, offering numerous advantages including improved patient compliance, controlled drug release, and reduced side effects [2]. The skin, being the largest organ of the human body, provides an extensive surface area for drug absorption; however, its complex structure and protective function pose significant challenges for drug permeation [3]. The concept of transdermal drug delivery dates back centuries, with ancient civilizations utilizing herbal and medicinal plasters to treat various ailments [4]. However, modern advancements in pharmaceutical technology have led to the development of sophisticated transdermal systems, including patches, gels, and microneedles, designed to enhance drug permeation and efficacy[5]. The primary barrier to transdermal drug delivery is the stratum corneum, the outermost layer of the skin, which acts as a formidable shield against external substances [6]. Overcoming this barrier requires innovative formulation strategies and permeation enhancers to facilitate the transport of drugs across the skin and into the systemic circulation [7].

One of the key advantages of transdermal drug delivery is the avoidance of first-pass metabolism, which is a common limitation of oral drug administration [8]. When drugs are taken orally, they must pass

through the liver before reaching systemic circulation, leading to significant drug degradation and reduced bioavailability [9]. In contrast, transdermal delivery bypasses this metabolic pathway, ensuring a higher proportion of the drug reaches the bloodstream in its active form [10]. This not only enhances therapeutic effectiveness but also allows for lower drug dosages, minimizing the risk of adverse effects [11]. Furthermore, transdermal drug delivery provides a controlled and sustained release of medication, reducing the need for frequent dosing and improving patient adherence to treatment regimens [12]. This is particularly beneficial for chronic conditions such as pain management, hormone replacement therapy, and cardiovascular diseases, where maintaining steady drug levels is crucial for effective therapy [13, 14]. Cholchicine, despite being a **BCS Class III drug** (high solubility, low permeability), its **low molecular weight** (~399 Da) and moderate **log P (~1.017)** suggest it has the potential for transdermal permeation with appropriate enhancers like chitosan or eutectic systems. Highlighting colchicine's **gastrointestinal toxicity**, which is more severe than that of many NSAIDs, strengthens the rationale for bypassing the oral route. These properties suggest it can cross the skin barrier with the help of suitable **permeation enhancers** like chitosan or eutectic solvents. Transdermal delivery bypasses the **first-pass metabolism** and significantly reduces the **gastrointestinal toxicity** associated with oral colchicine. Therefore, despite its limited permeability, colchicine can be effectively delivered through the skin using advanced formulation strategies.

Despite its advantages, transdermal drug delivery is not without challenges. The physicochemical properties of drugs, such as molecular weight, solubility, and lipophilicity, play a critical role in determining their ability to penetrate the skin [15]. Only a limited number of drugs possess the ideal characteristics for passive transdermal absorption, necessitating the use of enhancers such as chemical agents, iontophoresis, and ultrasound to improve permeability [16]. Moreover, interindividual variations in skin characteristics, including thickness, hydration levels, and presence of skin conditions, can affect drug absorption rates and efficacy [17]. Recent advancements in transdermal drug delivery have led to the development of novel technologies aimed at overcoming these challenges [18]. Microneedles, for instance, create microchannels in the skin to facilitate drug penetration without causing significant discomfort [19]. Similarly, lipid-based vesicular systems, such as liposomes and ethosomes, enhance drug solubility and transport across the skin [20]. Additionally, emerging techniques like electroporation and microemulsions continue to expand the potential of transdermal drug delivery, making it a promising avenue for future pharmaceutical innovations [21].

MATERIALS AND METHODS

Materials

Colchicine with 98% purity was purchased from Quad life Science Pvt. Ltd., Punjab. Methanol, Chloroform, Ethanol was purchased from Fisher Scientific India Pvt. Ltd., Maharashtra. Potassium Dihydrogen orthophosphate, Sodium hydroxide was procured from Thomas Baker Pvt. Ltd., Mumbai. Thio-Urea, Citric acid, Urea, Benzoic acid, Succinic acid was obtained as a gift sample from Thermo Fisher Scientific India Pvt. Ltd., Bengaluru. Choline Chloride, Choline Bitartrate was purchased from Tokyo Chemical Industry Pvt. Ltd., Chennai. Malonic acid was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai and n-octanol was purchased from SD Fine-chem. Ltd, Mumbai.

Methods

Pharmaceutical Evaluation Techniques

Pre-formulation studies

A crucial step in the entire development process is pre-formulation. It is the examination of the drug's chemical and physical characteristics before the compounding procedure. These investigations concentrate on the drug's physicochemical characteristics that may have an impact on how well it works and how an effective dosage form is developed. A comprehensive comprehension of these characteristics could eventually justify the necessity for molecular alteration or offer a justification for formulation design. In the most basic scenario, these pre-formulation studies might only verify that the compound's development is not significantly hampered. For the creation of a stable, safe, and effective dosage form, these investigations are an essential protocol. Several analytical methods, including IR spectroscopy, UV spectroscopy, melting point, and others, were used to identify the drug sample that was acquired.

Organoleptic Characteristics

Organoleptic evaluation means the study of drugs using organs of senses. It refers to the methods of analysis like colour, odour, taste etc.

Melting point

The temperature at which a solid and a liquid are in equilibrium at total pressure is known as the solid's melting point. The drug's melting point is ascertained using a melting point instrument. A small quantity of the drug was put into a capillary tube with thin walls that was 75 mm long, 1 mm in diameter, and closed at one end. A thermometer was positioned to assess the temperature in the melting point apparatus, and the capillary containing the sample was suspended to heat it gradually and uniformly. The drug's melting point is determined by measuring the temperature range over which the sample is seen to melt.

Solubility Studies

Solubility is the term used to describe the spontaneous interaction of two or more compounds to create a homogenous molecular dispersion.

A specific quantity of the drug was placed in well-cleaned test tubes with 10ml of each of the following solvents (methanol, ethanol, chloroform, water, and phosphate buffer saline pH 7.4) for a quantitative solubility analysis. The test tubes were then securely sealed. For twenty-four hours at 25 ± 0.5°C, these test tubes were shaken on a water bath shaker. Each sample was centrifuged at 15,000 rpm for 15 minutes after a shaking period of 24 hours, and the supernatant was removed. The supernatant was then filtered, the filtrates were appropriately diluted, and the results were measured using spectrophotometry.

Partition Coefficient of Drug

A measure of a drug's lipophilicity or hydrophilicity and an indicator of its capacity to pass across cell membranes is the partition coefficient (oil/water). The ratio of unionised drug dispersed between the aqueous and organic phases at equilibrium is its definition. The drug's lipophilic/hydrophilic characteristics can be described using the partition coefficient. Drugs are categorized as lipophilic if their P values are significantly larger than 1, and hydrophilic if their P values are significantly less than 1. An oil phase of n-octanol and water is frequently used to calculate the partition coefficient. In the case n-octanol and water:

$$P_{o/w} = C_{n-octanol}/C_{water}$$

As a result, the partition coefficient (Po/w), which is typically expressed as its logarithm to base 10 (log P), is the quotient of two drug concentrations in n-octanol ($C_{n\text{-}octanol}$) and water (C_{water}), respectively.

Shake flask method

The shake flask method was used to carry out the investigation on partition coefficient determination. A specific quantity of the medication (colchicine) was dissolved in 5ml of each of the two solvents (n-octanol: Water) in a 1:1 ratio, and the mixture was left for 24 hours. The two layers were separated and centrifuged for 15 minutes at 15,000 rpm after a 24-hour period. After the proper dilution, the absorbance was measured in a UV spectrophotometer at the corresponding λ max.

HPLC Method Development

Selection of Detection Wavelength

The correct choice of detection wavelength determines the sensitivity of the HPLC process that uses UV detection. The wavelength that provides the best reaction for the drugs to be identified is the ideal one. A diluent containing 100µg/ml of colchicine was created for the current investigation. After that, this solution was scanned in the 200–400 nm UV range, and a spectra was captured.

Selection of Mobile Phase and Optimization of Chromatographic condition

- **Chromatographic conditions**
- **Stationary Phase:** C_{18}, 250 × 4.6 mm, 5µm particle size, OTL/CLM/019
- **Elution mode:** Low pressure gradient mode (35:65 v/v)
- **Mobile phase:** Solvent A was Acetonitrile; Solvent B was 0.1 % O-phosphoric acid.
- **Detector:** UV
- **Absorption maxima:** 245 nm
- **Column Temperature:** 30 °C
- **Flow rate:** 1 ml/min.
- **Injection volume:**20 µl
- **Diluent:** Mobile phase
- **Run time:** 6 minutes.

Standard Stock Solution Preparation

- **Blank:** After passing through 0.22 µ Millipore membrane filters, the diluent was added to the HPLC apparatus.

- **Standard solution preparation:** A solution concentration of 100µg/ml was obtained by dissolving 10 mg of the pure substance in 100 ml of volumetric flask with diluent. After diluting 3 ml of the resultant stock solution with diluents to 10 ml, the concentration was determined to be 30 µg/ml (100%). was put into an HPLC system after being filtered with 0.22 µ Millipore membrane filters.

HPLC Chromatogram of standard

The chromatogram with the best drug retention time was obtained from the HPLC analysis of the standard.

Validation of HPLC method as per ICH guidelines

Linearity and Range

The ability of an analytical method to produce test findings that are exactly proportionate to the analyte concentration in a sample within a specified range is known as linearity. The distance between the highest and lowest analyte levels that have been shown to be determined with an appropriate degree of precision, accuracy, and linearity is known as the analytical method's range. Colchicine's linearity ranges from 25% to 150% of the desired concentration of 30 µg/ml. Colchicine's chosen linearity range was 7.5–45 µg/ml. After passing through a 0.22 µ filter, each dilution was injected.

Procedure

Preparation of linearity stock solution: After dissolving 10 mg of the pure chemical in 100 ml of volumetric flask with diluents (as previously described), the concentration was determined to be 100µg/ml. Filtered through 0.22 µ Millipore membrane filters, 3 ml of the resultant stock solution was diluted with diluents to 10 ml, and the concentration was determined to be 30 µg/ml (100%) before being injected into an HPLC system.

25 % linearity solution (7.5µg/ml): Mix thoroughly after diluting 0.75 ml of the stock solution with the mobile phase in a 10 mL volumetric flask.

50% linearity solution (15µg/ml): In a 10 mL volumetric flask, dilute 1.5 ml of the stock solution with the mobile phase, then thoroughly mix.

75% linearity solution (22.5µg/ml): Mix thoroughly after diluting 2.25 ml of the stock solution with the mobile phase in a 10 mL volumetric flask.

100% linearity solution (30µg/ml): Mix well after diluting 3 ml of the stock solution with the mobile phase in a 10 mL volumetric flask.

125% linearity solution (37.5µg/ml): Mix well after diluting 3.75 ml of the stock solution with the mobile phase in a 10 mL volumetric flask.

150% linearity solution (45µg/ml): In a 10 mL volumetric flask, dilute 4.5 ml of the stock solution with the mobile phase and thoroughly mix.

Precision

Repeatability was assessed with a lower concentration and injected six times, and the percentage RSD was computed. The intra-day and inter-day variation for drug determination was conducted using a concentration in the same day (replicates) and six consecutive days (single injection each day).

- Repeatability
- Inter-day and Intraday

LOD and LOQ

In accordance with ICH recommendations, the developed method's LOD and LOQ were examined. There are various methods for figuring out the LOD and LOQ, depending on whether the operation is instrumental or non-instrumental. Among them here employed method was,

$LOD = 3.3\sigma/S$ and

$LOQ = 10\sigma/S$

Where, σ = the standard deviation of intercept

S = mean of slope in calibration curve.

Accuracy (Percentage Recovery)

The degree of agreement between the value that is recognised as either a conventional true value or an approved reference value is a measure of an analytical procedure's accuracy.

In triplicate, make a working sample solution that is 50% to 150% (50, 100%, and 150%) of the specification limit by adding a placebo that is equal to the test sample minus the API.

Procedure

50% solution: The concentration was 100µg/ml after 10 mg of the pure chemical was weighed and dissolved in 100 ml of diluents (as previously described) in a 100 ml volumetric flask. After diluting 3 ml of the resultant stock solution with diluents to 10 ml, the concentration was determined to be 30 µg/ml. A standard solution containing 15 µg/ml was then added. After that, the solution was labelled, filtered using a 0.22µ nylon syringe filter, and then injected into an HPLC system.

100% solution: The concentration was 100µg/ml after 10 mg of the pure chemical was weighed and dissolved in 100 ml of diluents (as previously described) in a 100 ml volumetric flask. After diluting 3 ml of the resultant stock solution with diluents to 10 ml, the concentration was determined to be 30 µg/ml, and a 30 µg/ml standard solution was added. After that, the solution was labelled, filtered using a 0.22µ nylon syringe filter, and then injected into an HPLC system.

150% solution: The concentration was 100µg/ml after 10 mg of the pure chemical was weighed and dissolved in 100 ml of diluents (as previously described) in a 100 ml volumetric flask. After diluting 3 ml of the resultant stock solution with diluents to 10 ml, the concentration was determined to be 30 µg/ml, and the standard solution was spiked to 45 µg/ml. After that, the solution was labelled, filtered using a 0.22µ nylon syringe filter, and then injected into an HPLC system.

Robustness

Analysing the lower concentration sample while purposefully varying the technique settings allowed for the study of resilience. The percentage RSD was used to quantify the shift in medication reactions. By altering the flow rate and wavelenth±5, the method's robustness was examined.

Procedure

Preparation of standard solution and test solution: After dissolving 10 mg of the pure chemical in 100 ml of volumetric flask with diluents (as previously described), the concentration was determined to be 100µg/ml. After diluting 3 ml of the resultant stock solution with diluents to 10 ml, the concentration was determined to be 30 µg/ml (100%). was put into an HPLC system after being filtered with 0.22 µ Millipore membrane filters.

FTIR of Colchicine

The FT-IR (Fourier Transform Infrared) spectra of a substance or drug can reveal the groups that are present. For structure investigation, FT-IR spectroscopy was employed. For the purpose of identifying any potential drug interactions with excipients, an FT-IR spectrum of a Colchicine was recorded. Take 1-2 mg of Colchicine and placed in FT-IR plate. Scanned the region between 4000 and 400 cm^{-1} of the infrared spectrum was observed.

Drug-excipients Compatibility Study by FTIR

FTIR revealed the drug's compatibility with the excipients. Any chemical or physical interactions between the medication and excipients were found using FTIR. The drug and several excipients were thoroughly combined in a 1:1 ratio. FTIR was used to scan the samples in the 400–4000 cm^{-1} range. To check for incompatibilities and physical changes, the spectra of the pure drug and the drug with excipients were compared.

Powder X-ray diffraction (PXRD)

On a powder x-ray diffractometer (Ultima-4, Rigaku Company, Japan), XRD patterns were captured with a K-beta filter, CuKα radiation, 40 kV of voltage, and 30 mA of current. Over the range of 10.00 to 60.00° diffraction angle (2θ), scanning was used. Physical Mixture of Colchicine, Choline Chloride, and Malonic Acid (1:1:1) Inclusion Complex and Colchicine PXRD patterns were recorded.

Differential scanning calorimetry (DSC)

Using a differential scanning calorimetry (DSC) thermal analyser, the thermal behaviour of colchicine, choline chloride, malonic acid, and their physical mixture (1:1:1) were investigated. As the carrier gas, argon was heated at a rate of 10°C/min and flowed at a rate of 35 cc/min for the DSC analysis. 3mg was the sample size, and observations were made between thirty and three hundred degrees Celsius.

Preparation of Deep Eutectic Solvent Mixture of Colchicine

Choline chloride or choline bitartrate and certain carboxylic acids were combined in constant or varying molar ratios to prepared a Deep Eutectic Solvent Mixture (DESM). The mixtures were sealed in vials and heated in an oven at 75°C until homogenous solutions were formed. Only those liquids that were still liquid were evaluated as room-temperature solvents for model poorly soluble medicines after these samples had been kept at room temperature. Colchicine's BCS class IIIrd that means high solubility but low permeability led to its selection as a model drug. A specific amount of drug was introduced to a blank DESM solvent, and the mixture was vigorously vortexed until the surplus solid was left undissolved in

order to assess the drug's solubility in DESMs. To make sure equilibrium was reached, the resultant solution containing surplus medication was allowed standing for hours.

Influence of various acids with choline chloride

Choline chloride and several carboxylic acids (Thio-urea, Citric acid, Urea, Oxalic acid, Benzoic acid, Malonic acid, Cinnamic acid, and Succinic acid) were combined in same or constant molar ratios to create a Deep Eutectic Solvent Mixture (DESM). The mixtures were sealed in vials and cooked in an oven at 75°C until homogenous solutions were formed. Following that, all DESM were kept or cooled at ambient temperature (24°C) Table 1.

Table 1: Composition of Carboxylic Acid for DESM with Choline Chloride

Sr. No	Formulation Code	Acid used	Molar Ratio (Choline chloride: Carboxylic acid)
1	F1	Thio-Urea	1:1
2	F2	Citric acid	1:1
3	F3	Urea	1:1
4	F4	Oxalic acid	1:1
5	F5	Benzoic acid	1:1
6	F6	Malonic acid	1:1
7	F7	Cinnamic Acid	1:1
8	F8	Succinic acid	1:1

Influence of the various carboxylic acids with Choline Bitartrate

Choline bitartrate and several carboxylic acids (Thio urea, Citric acid, Urea, Oxalic acid, Benzoic acid, Malonic acid, Cinnamic acid, and Succinic acid) were combined in same or constant molar ratios to create a Deep Eutectic Solvent Mixture (DESM). After being sealed in vials, the mixtures were heated to 75°C in an oven to formed homogenous solutions. Following that, all DESM were kept or cooled at room temperature, or 24°C Table 2.

Table 2: Composition of carboxylic acid for DESM with Choline Bitartrate

Sr. No.	Formulation Code	Acid used	Molar Ratio (Choline Bitartrate: Carboxylic acid)
1	F9	Thio-Urea	1:1
2	F10	Citric acid	1:1
3	F11	Urea	1:1
4	F12	Oxalic acid	1:1
5	F13	Benzoic acid	1:1
6	F14	Malonic acid	1:1
7	F15	Cinnamic acid	1:1
8	F16	Succinic acid	1:1

Effect of different molar ratio of selected acid with Choline chloride

Choline chloride and specific carboxylic acids, such as malonic acid, were combined in varying molar ratios to create a Deep Eutectic Solvent Mixture (DESM). After being sealed in vials, the mixtures were heated to 75°C in an oven to formed homogenous solutions. Following that, all DESM were kept or cooled at room temperature, or 24°C Table 3.

Table 3: Composition of DESM with Different molar ratio of Malonic acid in DESM

Sr. No	Formulation Code	Molar Ratio (Choline chloride: Malonic acid)
1	F6(A1)	1:1

2	F6(A2)	1:2
3	F6(A3)	1:3
4	F6(A4)	1:4
5	F6(A5)	1:5

Effect of Different molar ratio of Choline chloride with Selected acid in DESM

A Deep Eutectic Solvent Mixture (DESM) was prepared by mixing specific carboxylic acids, such as malonic acid, with varying molar ratios of choline chloride. After being sealed in vials, the mixtures were heated to 75°C in an oven to formed homogenous solutions. Following that, all DESM were kept or cooled at room temperature, or 24°C Table 4.

Table 4: Composition of DESM with Different ratio of Choline chloride

Sr. No	Formulation Code	Molar Ratio (Choline chloride: Malonic acid)
1	F6(B1)	2:1
2	F6(B2)	3:1
3	F6(B3)	4:1
4	F6(B4)	5:1

Addition of Drug in Selected DESM

They were combined at various molar ratios to create a Selected Deep Eutectic Solvent Mixture (DESM). After being sealed in vials, the mixtures were heated to 75°C in an oven to prepared homogenous solutions. The drug was then added to each DESM, and it was allowed to dissolve by sonication. Every DESM was kept or cooled at room temperature, which is 24°C Table 5.

Table 5: Composition of DESM with addition of Drug

Sr. No	Formulation Code	Molar Ratio (Choline chloride: Malonic acid)	Drug %w/v
1	F6(A1)	1:01	0.5
2	F6(A2)	1:02	0.5
3	F6(B1)	2:01	0.5

Evaluation of Deep Eutectic Solvent Mixture of Colchicine

Visual appearance

The freshly prepared DESM were visually inspected, and the physical properties of the prepared formulae (such as colour and homogeneity) were assessed.

pH of the Solution

A pH meter was used to determine the pH of each prepared DES. A pH meter was used to measure the pH after 1 mL of each eutectic system was put into a beaker and diluted with 20 mL of distilled water. Every measurement was made three times, and the results are given as the mean ± standard deviation.

Drug Equilibrium Solubility

The prepared DESM with drug was dissolved in deionized water was used to evaluate the drug's equilibrium solubility. Place 1ml of the sample in a 15ml culture tube and dilute it with 10ml of deionized water. To achieve equilibrium, the vials were vortexed for five minutes and then placed in a shaker bath for twenty-four hours. 1ml of the sample was removed from each vial, centrifuged for 10 minutes at 15,000 rpm, and then the supernatant was removed and analysed with a UV spectrophotometer to ensure that any undissolved drug settled.

Drug Content

DESM suspensions (total mount) were centrifuged at 15,000 rpm for 15 min. After centrifugation, take of supernatant was diluted with the addition of 9 ml water. After dissolving water, the drug-containing formulations were filtered via a 0.22 µm syringe filter unit, and the drug concentration was then measured spectrophotometrically using a UV-Vis spectrophotometer. A UV spectrophotometer was used to measure Colchicine's absorption.

$$\%Drug\ Content = \frac{Unentrapped\ drug \times 100}{Total\ amount\ of\ drug}$$

Optimization of Colchicine DESM using central composite design (CCD)

Experimental design

Colchicine DESM was optimized utilizing the design expert program. Using a central composite design (CCD) in accordance with the standard procedure the amounts of malonic acid (B) and choline chloride (A) were chosen as the independent factors based on a trial study, and each was examined at two levels. Throughout the trial, all other formulation and processing variables remained constant. The 13 experimental runs that were examined, their factor combinations, and the conversion of the coded levels to the experimental units used in the study are all compiled in Table 6.

The response variables were Colchicine's aqueous solubility (Y1) and its percentage drug content (Y2) Table 7. Table 8 and 9 show the experimental design of various batches of DESM and the responses that were achieved. A batch of the optimized formulation was chosen for additional research.

Table 6: Summary of Formulation Variables and their levels in CCD

Formulation variables	Unit	(-) Level	(+) Level
A= Choline chloride	Molar	1	2
B= Malonic acid	Molar	1	2

Table 7: Summary of Response Variables

Response variables	Unit
Y1= Aqueous solubility	mg/ml
Y2= % Drug Content	%

Table 8: Formulation trials of Colchicine loaded DESM as per experimental design.

Run	Factor 1	Factor 2
	A: Amount of Choline chloride (molar)	B: Amount of Malonic acid (molar)
1	1.5	1.5
2	1.5	1.5
3	1.5	1.5
4	1	1
5	1.5	2.21
6	1	2
7	1.5	1.5
8	1.5	1.5
9	0.79	1.5
10	2	1
11	1.5	0.79
12	2	2
13	2.21	1.5

Table 9: Concentration of studied parameters from experimental batches

Experimental trial no.	Drug %w/v	Choline chloride (mg)	Malonic acid (mg)
D1	0.5	627	468
D2	0.5	627	468
D3	0.5	627	468
D4	0.5	556	416

D5	0.5	418	460
D6	0.5	417	624
D7	0.5	627	468
D8	0.5	627	468
D9	0.5	440	624
D10	0.5	837	312
D11	0.5	836	328
D12	0.5	558	416
D13	0.5	616	312

Optimized formulation

Number	Amount of choline chloride	Amount of malonic acid	Aqueous solubility	Percentage drug content	Desirability
D14	1	1.01	4.738	84.361	0.994

D14 was selected based on the overall desirability function value (0.963), which integrated multiple response criteria including drug content, viscosity, and in vitro release.While D9 also showed favorable individual responses, D14 demonstrated a superior balance across all key attributes, particularly in terms of drug release (98.83%), spreadability, and stability, which were prioritized in the optimization strategy.

Preparation of Colchicine Eutectogel

Choline chloride and malonic acid were found to be efficacious at a 1:1.01 ratio in the optimized formulation [D14], which was chosen as the optimal formulation for gel conversion. The gel was prepared using Carbopol 934P. The Carbopol 934P was dispersed to a 5ml volume of water and allow to swelled for 24hr. The pH of the Carbopol gel was carefully adjusted to the range of 5.5–6.0 using a neutralizing agent (triethanolamine), ensuring optimal gel consistency and compatibility with skin physiology. After that the optimised formulation of 5ml volume was added to gel phase. Consequently, various gel percentages 0.5%, 1%, 1.5%, and 2% were made in order to achieve the proper gel concentration fully mixing the both phases. To adjust the pH, 1M of NaOH solution was added drop by drop. The gel was assessed based on a number of criteria Table 10.

Table 10: Composition of different Carbopol Eutectogel

Sr. No.	Ingredients	Formulation Code			
		D14(G1)	D14(G2)	D14(G3)	D14(G4)
1	Deep eutectic mixture solution (ml)	5	5	5	5
2	Carbopol 934p(w/v)	0.5	1	1.5	2
3	NaOH solution (ml)	q.s	q.s	q.s	q.s

Preparation of Colchicine chitosan Eutectogel

Choline chloride and malonic acid were shown to be efficient in a 1:1.01 ratio in the optimised formulation [D14], which was chosen as the optimal formulation for converting eutectogel formulation. The gel basis was made with chitosan. Chitosan gel was prepared by dissolving chitosan, a natural polysaccharide, in an acidic solution, typically acetic acid. Chitosan was dissolved in 1% (v/v) aqueous acetic acid, which provided a suitable medium for complete solubilization and maintained the functional properties of chitosan. Gel formulations were prepared with three different chitosan concentrations (1.0%, 1.5%, 2%, 2.5% w/v). Briefly, chitosan was dissolved in acetic acid solution (0.5%, v/v) under a magnetic stirrer at 250 rpm for 4 h. For the DESM formulations, then added to the chitosan solution. Formulations were kept at room temperature overnight in beaker and closed the beaker for the removal of air bubbles Table 11.

Table 11: Composition of different chitosan Eutectogel

Sr. No.	Ingredients	Formulation Code

		D14(C1)	D14(C2)	D14(C3)	D14(C4)
1	Deep eutectic mixture solution (ml)	5	5	5	5
2	Chitosan (%w/v)	1	1.5	2	2.5

Characterization of Eutectogel

Physical appearance

The freshly produced gels were examined visually, and the physical characteristics of the prepared formulations (such as colour and homogeneity) were carefully examined.

pH

The pH meter was calibrated using standard buffer solution such as pH 4, 7 and 9. About 0.1 g of the gel was weighed and dissolved in 10.0 ml of distilled water and its pH was measured. pH was measured in triplicate and average values were calculated.

Viscosity

Rheological investigations were conducted using a viscometer. A L-4 spindle spinning at 5 rpm was used to measure the dial reading after the sample (20 g) had been in a beaker for five minutes to acclimatise. At the speed, the corresponding dial reading from the viscometer was recorded. For every spindle speed drop, the dial reading that matched it was noted. Three attempts were made at the measurements.

Spreadability

Standard-sized glass slides measuring 6.0 cm in length were taken. One side of the glass slide had the 1g eutectogel on it, and another slide was used to sandwich it. Wipe the glass slides' outside to get rid of the adhesive gel. Slides are placed in a platform so that just the top slide can be removed without any problems using the weight (20 g) attached to it. The amount of time it took for the upper slide to move 6.0 cm was recorded. The following formula was used to compute the spreadability, which was measured in triplicate:

$$Spreadability = \frac{Weight \times Length}{Time}$$

Where, S=Spreadability m=Weight tied to the upper slide (20 g) l=Length of the glass (6.0 cm) t=Time taken in seconds.

Drug content

A precisely weighed amount of gel (1gm) was dissolved in a 100 ml volumetric flask, and the volume was increased to 100 ml with water to administer the pharmacological content of the produced gel. Whatman paper No. 41 was used to filter the content. A UV-VIS spectrophotometer was used to spectrophotometrically assess the drug's filtered content [157].

$$\%\ Drug\ Content = \frac{Free\ drug\ into\ gel \times 100}{Total\ drug\ into\ gel}$$

FTIR Study

The FTIR spectrum of optimized formulation was measured by using FTIR spectroscopy.

In-Vitro Drug Release Study

This work was carried out utilizing a Franz diffusion cell setup and a dialysis membrane. The in vitro drug release studies were performed using a dialysis membrane with a **molecular weight cut-off (MWCO) of 12,000–14,000 Da** (Sigma-Aldrich). A 40% ethanol solution is used to treat the dialysis membrane, and it is let to soak overnight. The donor and acceptor compartments of the diffusion cell were separated by the treated membrane. The treated membrane received about 1 gram of gel, and 7.4 pH phosphate buffer was supplied to the diffusion cell's receptor compartment. The entire assembly was mounted on a magnetic stirrer, and the temperature was kept at 37 ±0.50°C while the solution in the receptor compartment was continually swirled with magnetic beads at 100 rpm. 1 ml samples were taken out at intervals of 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours, and the medication was examined using the UV technique. At each sample removal, an equivalent volume of 7.4 pH buffer was added to the receptor phase.

Drug release kinetic studies

The release profile is described by many mathematical functions that form the basis of model dependent approaches. Following the selection of an appropriate function, the release profiles are assessed based on

the model parameters that were derived. The following is a representation of the results from ex vivo permeation investigations in various data treatment models.

- Zero Order model
- First Order model
- Higuchi's Model
- Korsmeyer-Peppas model

Zero order kinetics

It can be used to explain how various modified release pharmaceutical dosage forms, such as some transdermal systems, matrix tablets containing low-soluble pharmaceuticals in coated forms, osmotic systems, etc., dissolve their drugs. Zero order release can be expressed simply as follows:

Q_0-Q_t= K_{0t}

where K0 is the zero-order release constant, expressed in units of concentration/time, Qt is the amount of drug dissolved in time t, and Q0 is the initial amount of drug in the solution (usually, Q0=0). Data from in vitro drug permeation tests were shown as the cumulative amount of drug released vs time in order to investigate the release kinetics.

First order kinetics

Drug dissolution in pharmaceutical dosage forms, such as those that contain water-soluble medicines in porous matrices, can be described using this term. The following equation can be used to explain the drug's release that followed first order kinetics:

$logC = LogC_0 - K.t/2.303$

where t is the time, k is the first order rate constant, and C_0 is the drug's initial concentration. A straight line with a slope of K/2.303 would result from plotting the data as log cumulative percentage of medicine remaining vs. time.

Higuchi's Model

The drug release from a matrix system was predicted by this model. It was first considered for planar systems before being expanded to various geometrical shapes and porous systems. This model is predicated on the following hypotheses: (i) drug solubility is much lower than the initial drug concentration in the matrix; (ii) drug diffusion occurs only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than the thickness of the system; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always achieved in the release environment.

Higuchi was the first to use the square root of a time-dependent process based on Fickian diffusion to create an equation that describes the release of a medication from an insoluble matrix. The following is the simplified Higuchi equation:

$Q_t = KH(t)^{0.5}$

where KH is the Higuchi model's release rate constant and Qt is the quantity of medication released in time t. The medication was released by diffusion process, as indicated by the straight line those results from plotting the data as cumulative drug released vs square root of time. "KH" is equal to the slope.

Korsmeyer-Peppas Model

Drug release from a polymeric system was explained by a straightforward relationship that Korsmeyer derived. The equation put forth by Korsmeyer et al. can be used to characterise the release rates from controlled release polymeric matrices.

$Q = K.t^n$

where Q is the drug's percentage release at time "t." K is a kinetic constant that takes into account the tablets' geometrical and structural properties, and "n" is the diffusional exponent that indicates the release process.

n = 0.45 for Fickian release, 0.45 to 0.89 for anomalous (non-Fickian) transport, and 0.89 for zero order release. Log cumulative percentage of drug releases was plotted against log time using the Korsmeyer-Peppas model

RESULTS AND DISCUSSION

Preformulation Studies

The aim of Preformulation studies was to investigate the physical and chemical properties of a drug substance Table 12. The selected drug colchicine was subjected for investigation of physical characterization parameters such as:

- Organoleptic properties
- Melting point
- Solubility
- Partition coefficient
- UV-visible spectra and validation
- HPLC and validation
- FT-IR spectra of drug and selected excipient

Organoleptic properties

Table 12: Organoleptic Properties of Colchicine

Sr. No.	Properties	Inferences
1	Colour	Pale yellow powder
2	Odour	Odourless
3	Form	Crystalline
4	Taste	Bitter

Discussion: Organoleptic properties of drug colchicine were found to be as per literature. The organoleptic properties of colchicine are given in table 12.

Melting Point

The melting point of a substance was the temperature at which the solid phase gets converted to liquid phase under the total pressure. The melting point determination implies the purity of drug. Melting point of colchicine was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in table 13.

Table 13: Melting Point of Colchicine

Drug	Reference M.P.	Observed M.P.
Colchicine	142-150 °C	146.7±0.83°C -148.3±0.83°C

Discussion: The melting point of colchicine was found to be in range 146.70.83°C-148.3±0.83°C which was in the range of melting point of the pure drug. Hence drug sample was free from any type of impurities.

Solubility studies

Solubility of drug in various solvents, were carried out in order to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 246 nm Table 14. Fig 1.

Table 14: Solubility studies of Colchicine in different solvents

Sr No.	Solvents	Solubility(mg/ml)	Solubility
1	Phosphate buffer 7.4pH	11.270±0.033	Sparingly soluble
2	Water	71.069±0.261	Soluble
3	Methanol	92.973±0.130	Freely Soluble
4	Ethanol	94.363±0.075	Freely soluble
5	Chloroform	95.928±0.419	Freely soluble

Value is expressed as mean ± SD; n = 3

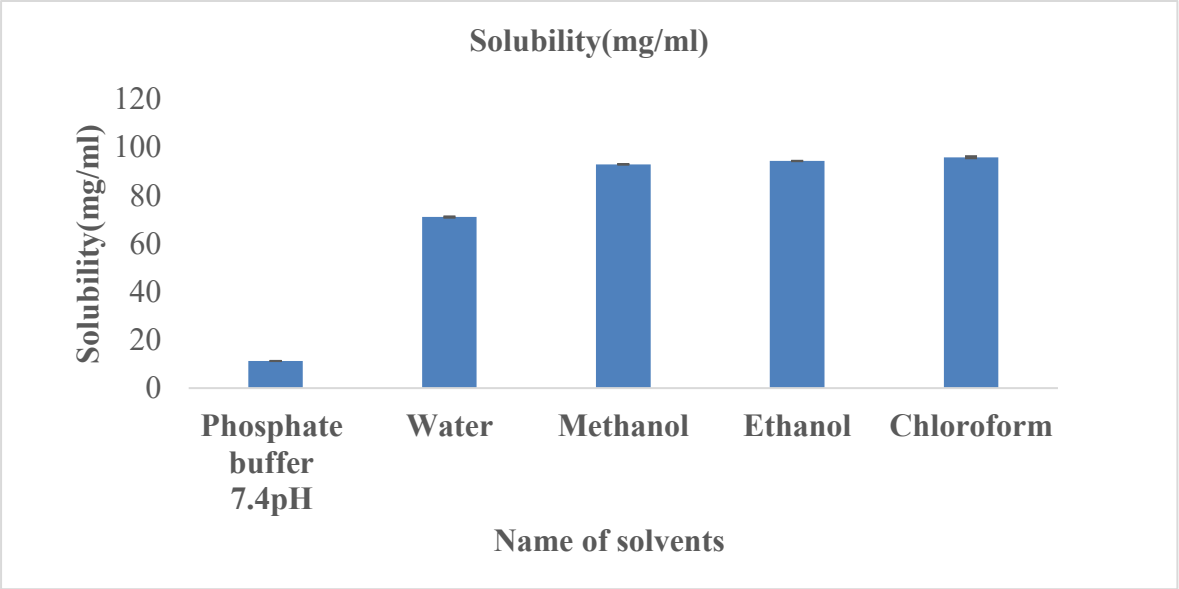

Fig. 1: Solubility study of drug in different solvents

Discussion: From the above data, it was clearly seen that colchicine was freely soluble in chloroform, methanol and ethanol, soluble in water and sparingly soluble in phosphate buffer 7.4pH.

Partition coefficient determination

Partition coefficient of the Colchicine was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This indicated the lipophilicity of drug Table 15.

Table 15: Partition coefficient determination of Colchicine

Partition coefficient of drug	Solvent system	Log P Values	Reference
Colchicine	n-octanol: water	1.017± 0.003	1.0-3.0

Value is expressed as mean ± SD; n = 3

Discussion: The partition coefficient of colchicine in n- Octanol: Water was found to be 1.017± 0.003 this indicates that colchicine was both hydrophilic and lipophilic in nature.

RP-HPLC method development and Validation of Colchicine

RP-HPLC Method

Chromatographic condition

The colchicine was analysed using the HPLC system.

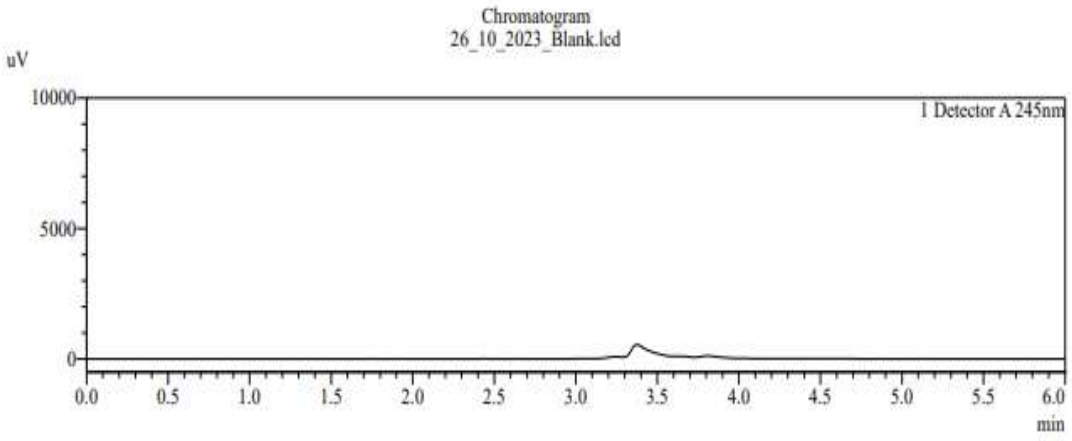

Figure 2. Chromatogram of Blank sample

HPLC Chromatogram of standard

On HPLC analysis of standard, chromatogram was optimized in which Retention time of drugs is shown in Table no.16 and Figure 3 (Chromatogram)

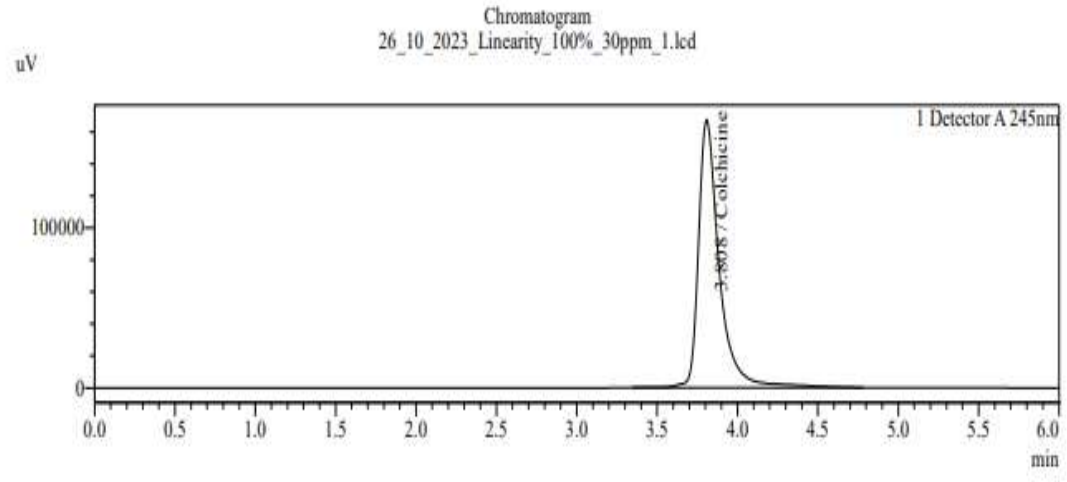

Figure 3. Chromatogram of Standard Colchicine 30µg/ml

Table 16: Data of Standard solution of Colchicine 30µg/ml (100%)

Sr. No.	Compound Name	Retention Time (min)	Area
1	Colchicine	3.808	1499685

Preparation of standard curve of Colchicine by RP-HPLC

Table 17: Calibration curve of Colchicine

Sr. No.	Concentration (µg/ml)	Mean
1	7.5	373499
2	15	759868
3	22.5	1128734
4	30	1517095
5	37.5	1880324
6	45	2317413

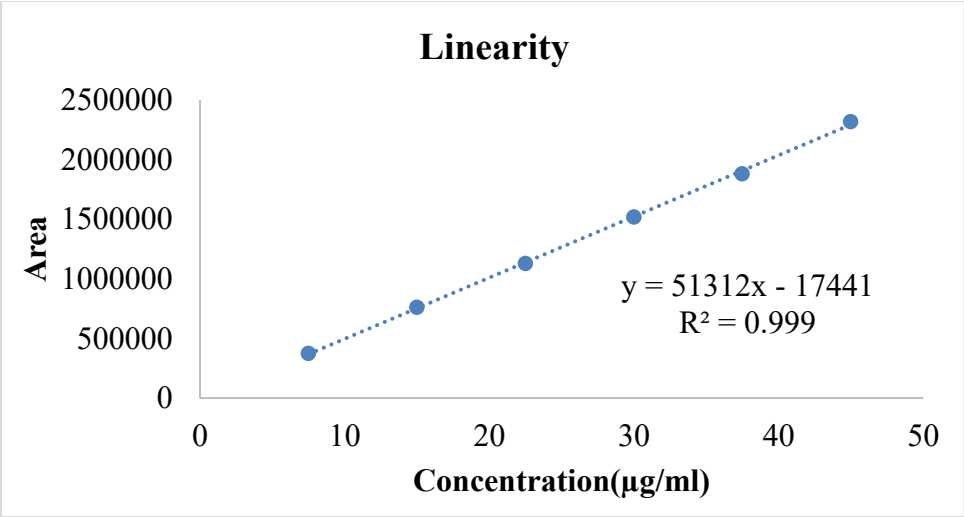

Figure 4: Graph of standard calibration curve of Colchicine by RP-HPLC

Table 18: Result of Statistical parameters for estimation of Colchicine

Statistical parameters	Results

Regression equation: $y = mx + c$	y = 51312x - 17441
Slope (m)	51312
Intercept (C)	17441
Correlation coefficient (r^2)	0.999

DISCUSSION: - The calibration curve for Colchicine was obtained by using the 7.5µg/ml to 45 µg/ml solution. The absorbance was measured at 245nm. The calibration curve as shows in graph indicated the regression equation = 51312x – 17441and R^2 value 0.999 which shows good linearity as shown in Figure 4.

Validation of HPLC method as per ICH guidelines

Linearity

Linearity of Colchicine

A calibration curve was plotted over a concentration range of 7.5µg/ml to 45µg/ml for Colchicine. Accurately measured working stock solution of Colchicine (7.5µg/ml, 15µg/ml, 22.5µg/ml, 30µg/ml, 37.5µg/ml and 45µg/ml) and all the dilutions were filtered through 0.22µ filter and injected. The area of all solution was taken at their respective wavelength. The Linearity was constructed by plotting concentration against area where each reading Table 19.

Table 19: Linearity of Colchicine

Level (%)	Conc.(µg/ml)	Area 1	Area 2	Area 3	Mean	SD	%RSD
25	7.5	365687	394325	360485	373499	18222.43	0.049
50	15	741267	768688	769648	759868	16115.8	0.021
75	22.5	1136066	1089947	1160190	1128734	35690.82	0.032
100	30	1499685	1509484	1542115	1517095	22215.26	0.015
125	37.5	1870068	1889889	1881014	1880324	9928.516	0.005
150	45	2246133	2302249	2403858	2317413	79948.49	0.034

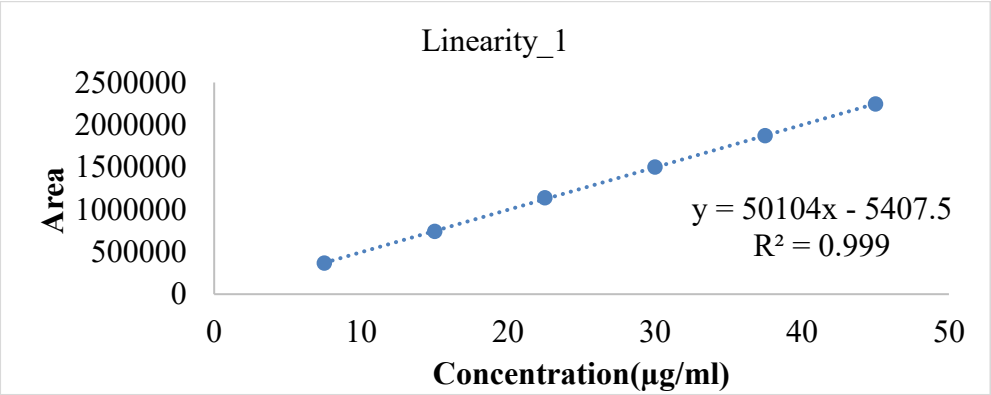

Figure 5: Linearity 1 graph of Colchicine

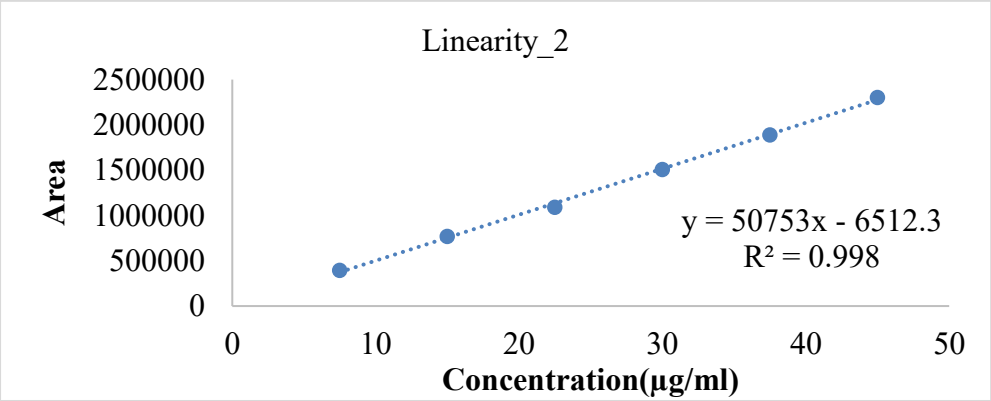

Figure 6: Linearity 2 graph of Colchicine

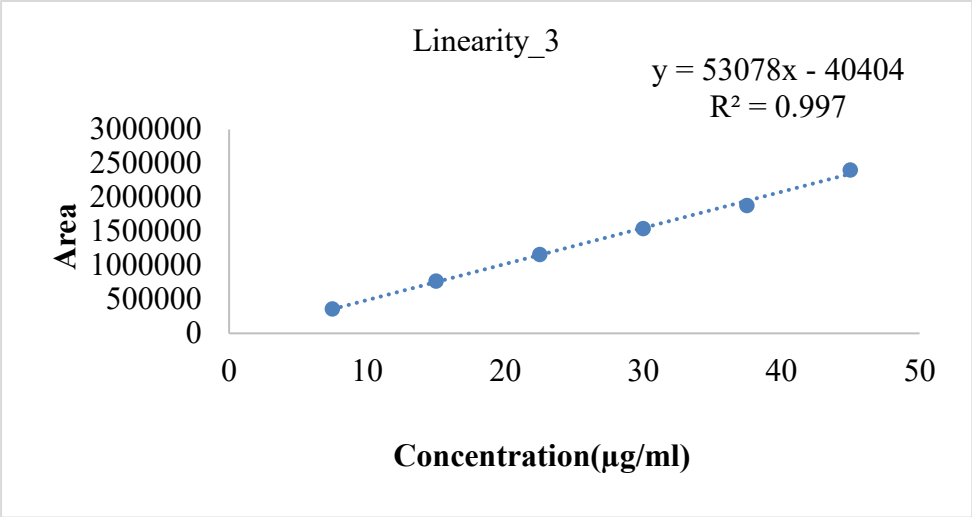

Figure 7: Linearity 3 graph of Colchicine

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the 3 concentration levels 50%, 100% and 150% in pre-analyzed sample. In this method the known concentration of standard drug was added to the assay sample Table 20.

Table 20: Accuracy Study of Colchicine

Level	Concentration Label Colchicine	% Recovery of Colchicine	% RSD
50%	15(µg/ml)	99.367	0.003
100%	30(µg/ml)	99.067	0.012
150%	45(µg/ml)	97.888	0.009

Discussion: The results indicate that the recoveries are well within the acceptance range of 97% – 100%, indicating a good degree of sensitivity of the method towards detection of analytes in sample. Therefore, method was accurate and it can be used for the estimation of drug**.**

Precision

Standard solution of Colchicine was prepared and analyzed as per the proposed method Table 21.

Table 21: Repeatability and Intermediate precision study

Sr.no.	Precision	%Recovery of Colchicine	% RSD
1	Repeatability	100.47	0.012
2	Intraday	101.03	0.006
3	Interday	101.39	0.007

The method was found to be precise due to low values of the %RSD.

LOD and LOQ

Table 22: LOD and LOQ data

Drug	LOD (µg/ml)	LOQ (µg/ml)
Colchicine	1.279	3.877

The Limit of detection and limit of quantification of the method were calculated based on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The results obtained were within the limit.

Robustness

The robustness was studied by analyzing the sample of lower concentration with deliberate variation in the method parameters. The change in the responses of drugs was noted in terms of %RSD. Robustness of the method was studied by change in Column temperature, change in flow rate±0.2 or change in wavelenth±5.

Preparation of standard solution

Weigh 10 mg of pure compound was taken in 100ml volumetric flask and dissolved in 100 ml with diluents (as mentioned above) and concentration found to be 100µg/ml. Take 3ml of this resulting stock solution and dilute upto 10ml with diluents, concentration found to be 30µg/ml (100%) and filtered through 0.22 µ millipore membrane filters and injected in HPLC system Table 23.

Table 23: Robustness data of Colchicine with deliberate change in wavelength

Conc.(µg/ml)	**Wavelength 240nm** (% Recovery)	**Wavelength 250nm** (% Recovery)
30	99.09	96.61
30	99.66	97.53
Mean	99.38	97.07
SD	0.4	0.65
%RSD	0.4	0.67

Table 24: Robustness data of colchicine with deliberate change in flow rate (ml/min)

Conc.(µg/ml)	**Flow rate 0.9ml/min** (% Recovery)	**Flow rate 1.1ml/min** (% Recovery)
30	99.67	97.11
30	99.98	96.00
Mean	99.83	96.56
SD	0.22	0.78
%RSD	0.22	0.81

Discussion: The Percentage RSD should not be more than 2. The %RSD obtained for change of flow rate and wavelength was found to be below 2, which was within the acceptance criteria. Hence the method was robust.

FTIR analysis of pure drug and excipient

FTIR of Colchicine

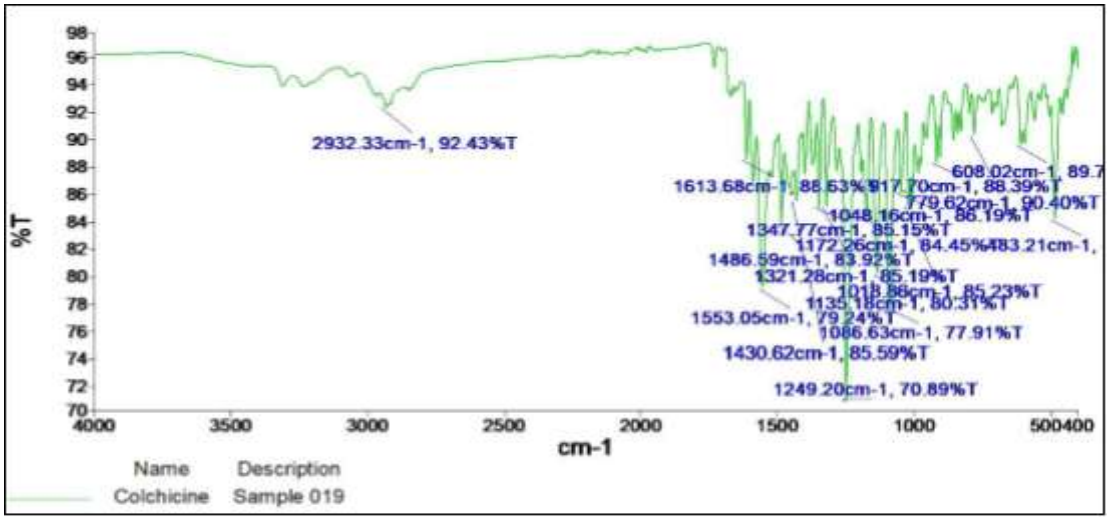

Figure 8: FTIR spectrum of colchicine

Table 25: FTIR interpretation of Colchicine

Characteristics Peaks	**Reported (cm^{-1})**	**Observed (cm-1)**

C-H stretching (alkane)	3000-2840	2932.3
C=C stretching (α, β- unsaturated)	1620-1610	1613.68
C-O vibration	1320	1321.28
C-N stretching (Amine)	1250-1020	1249.2
C-H bending (1,3-disubstituted)	780	779.62

Discussion: The FTIR spectra of Colchicine were shown in the **Figure 8**; **Table 25**. The principal IR absorption peaks of Colchicine at 2932.3cm^{-1} (C-H stretching (alkane)), 1613.68 cm^{-1} (C=C stretching (α, β- unsaturated)), 1486.59cm^{-1} (C-H bending (alkane)), 1249.20cm-1 (C-N stretching (Amine)) and 779.62cm-1 (C-H bending (1,3-disubstituted)) These observed principal peaks. This observation confirmed the purity and authenticity of the Colchicine**.**

FTIR of choline chloride

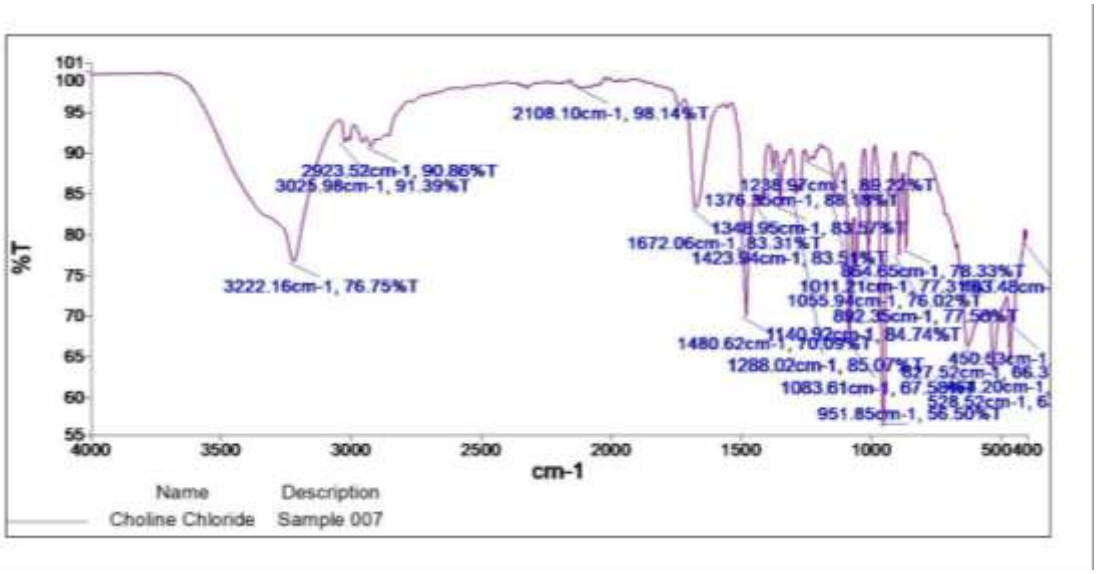

Figure 9: FTIR spectrum of Choline Chloride

Table 26: Interpretation of FTIR spectrum of Choline Chloride

Functional group	**Reported peak (cm^{-1})**	**Observed peak (cm^{-1})**
C–H vibration	3025.82	3025.98
Halogenated organic compounds	1634.16	1672.06
O–H vibration	1441.65	1423.94
C-N vibration	1250-1000	1080.95

Discussion: The FTIR spectra of Choline Chloride were shown in the figure 9 and table 26. The principal IR absorption peaks of Choline Chloride at 3025.98 cm^{-1} (C–H vibration), 1672.06 cm^{-1} (halogenated organic compounds), 1423.94cm^{-1} (O–H vibration) and 1348.95 cm^{-1} (C=O vibration) were all observed in the spectra of Choline Chloride were found to be similar to cited peaks. These observed principal peaks confirmed the purity and authenticity of the Choline Chloride.

FTIR of malonic acid

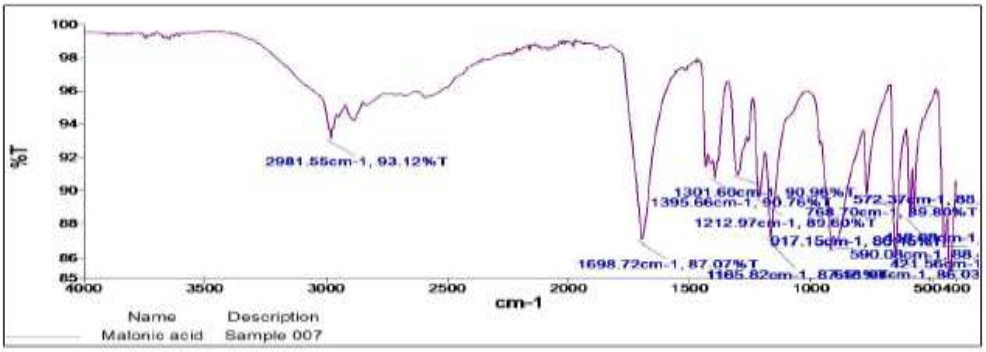

Figure 10: FTIR spectrum of Malonic acid

Table 27: Interpretation of FTIR spectrum of Malonic acid

Functional group	Reported peak (cm^{-1})	Observed peak (cm^{-1})
-OH Stretching	3300	2981.55
C=O Stretching	1690	1698.72
C-C aliphatic stretching	1300	1301.6
C-O	1210	1212.97

Discussion: The FTIR spectra of Malonic acid were shown in the figure 10 and table 27. The principal IR absorption peaks of Malonic acid at 2981.55cm^{-1} (-OH Stretching), 1698.72cm^{-1} (C=O Stretching), 1301.60cm^{-1}(C-C aliphatic stretching) and 1212.97 cm^{-1} (C-O stretching) were all observed in the spectra of Malonic acid were found to be similar to cited peaks. These observed principal peaks confirmed the purity and authenticity of the malonic acid.

FTIR of physical mixture

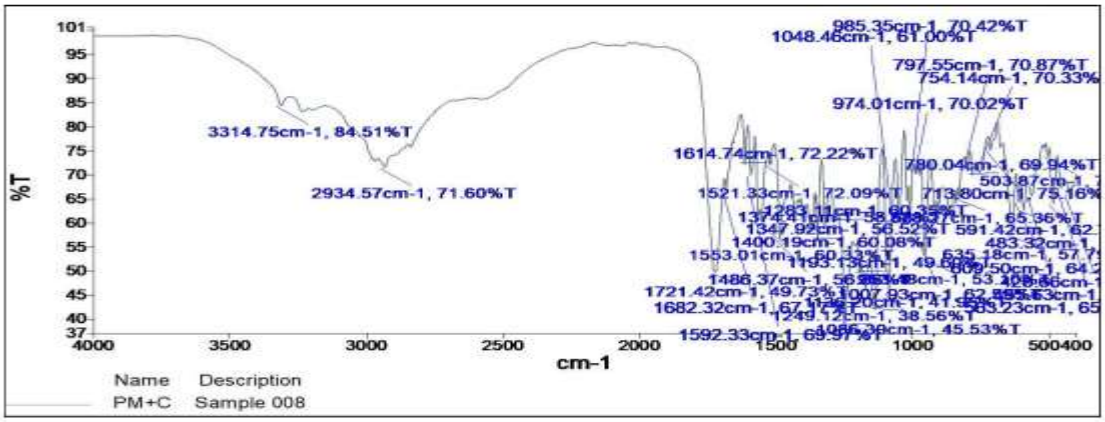

Figure 11: FTIR spectrum of Physical mixture

DSC of colchicine

It is a key analytical technique used to study the thermal behaviour of colchicine. The DSC thermogram of colchicine typically exhibits an endothermic peak corresponding to its melting point. This peak provides insights into the purity and thermal stability of the compound Table 28.

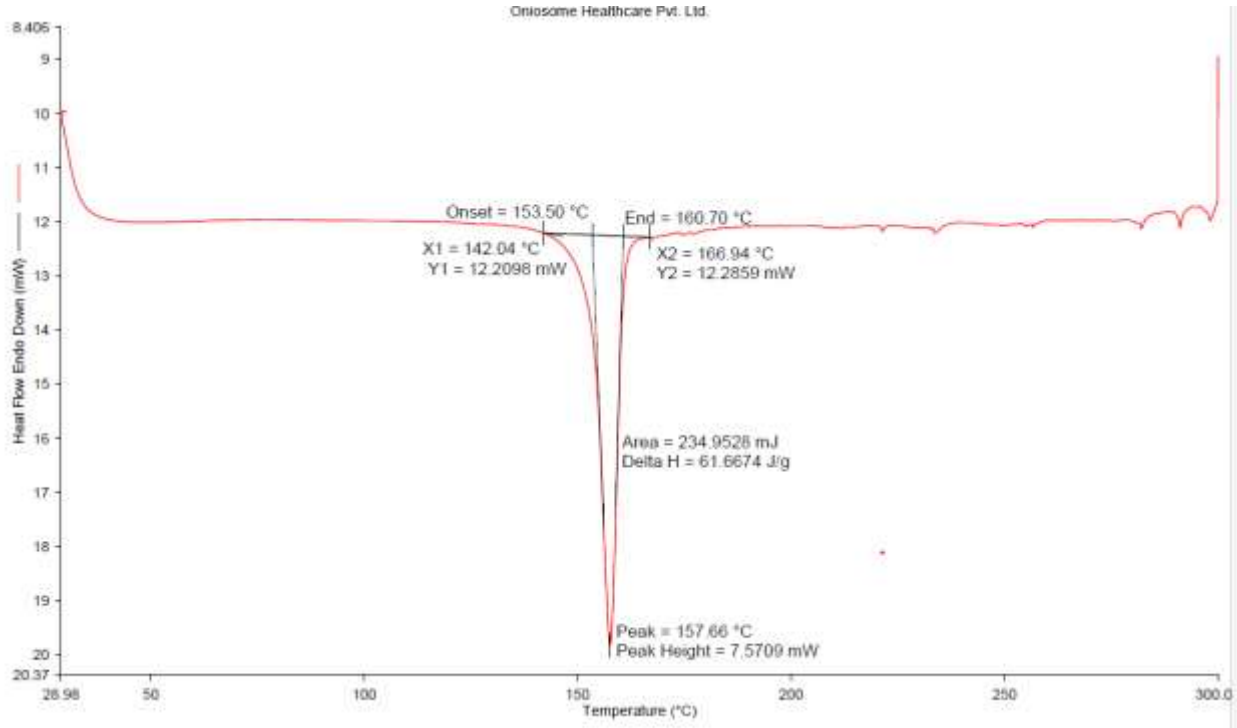

Figure 12: DSC thermogram of colchicine

Table 28: DSC interpretation of Colchicine

Peaks	Reported(°C)	Observed (°C)
Sharp Endothermic Peak represent melting point of Colchicine	Endothermic peak: 155°C	Endothermic peak: 157.66°C

Discussion: The DSC thermogram shows a sharp endothermic peak at 157.66°C indicating the melting point of colchicine purity by a narrow melting range (onset at 153.50°C and end at 160.70°C). These results highlight the purity of drug.

DSC of choline chloride

It is a key analytical technique used to study the thermal behaviour of choline chloride. The DSC thermogram of choline chloride typically exhibits an endothermic peak corresponding to its melting point. This peak provides insights into the purity and thermal stability of the compound Table 29.

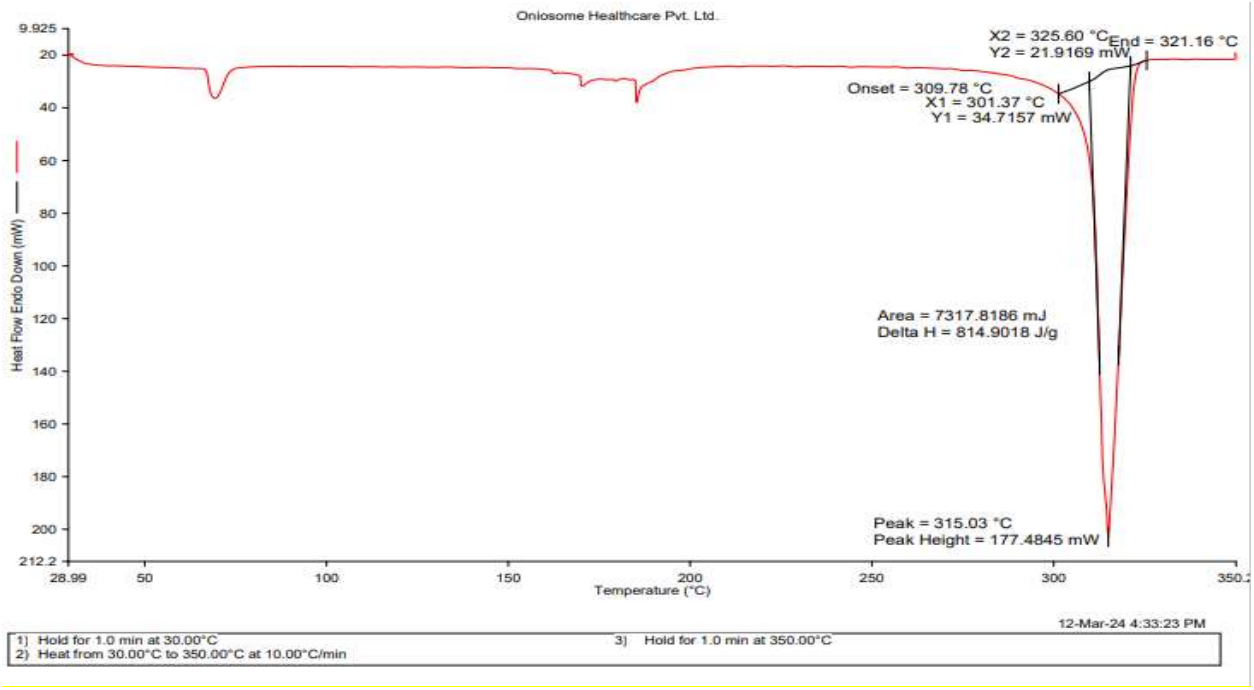

Figure 13: DSC Thermogram of choline chloride

Table 30: DSC interpretation of choline chloride

Peaks	Reported(°C)	Observed (°C)
Sharp Endothermic Peak represent melting point of choline chloride	Endothermic peak: 302°C	Endothermic peak: 315.03°C

Discussion: The DSC thermogram shows a sharp endothermic peak at 157.66°C indicating the melting point of choline chloride purity by a narrow melting range (onset at 153.50°C and end at 160.70°C). These results highlight the purity of drug.

DSC of malonic acid

It is a key analytical technique used to study the thermal behaviour of malonic acid. The DSC thermogram of malonic acid typically exhibits an endothermic peak corresponding to its melting point. This peak provides insights into the purity and thermal stability of the compound Table 31.

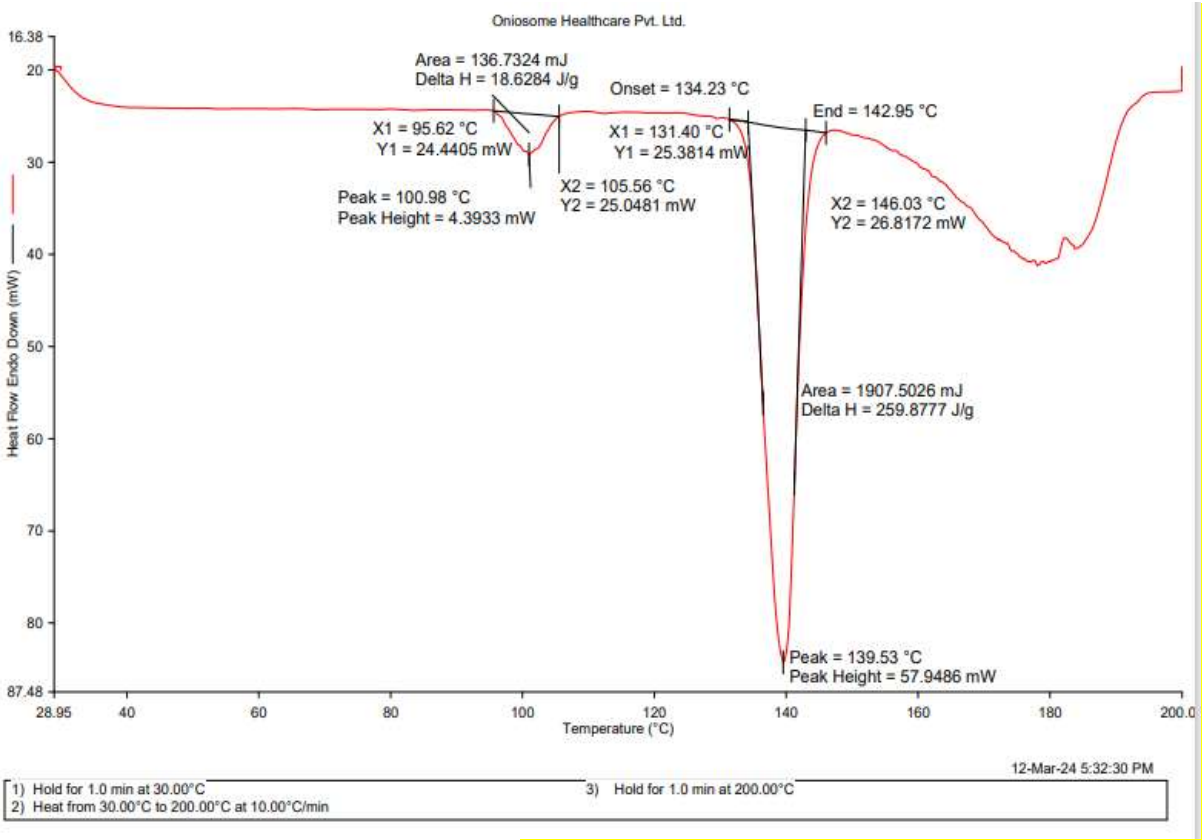

Figure 14: DSC thermogram of malonic acid

Table 32: DSC interpretation of malonic acid

Peaks	Reported(°C)	Observed (°C)
Sharp Endothermic Peak represent melting point of malonic acid	Endothermic peak: 135-175°C	Endothermic peak: 139.53°C

Discussion: The DSC thermogram shows a sharp endothermic peak at 139.53°C °C indicating the melting point of malonic acid purity by a narrow melting range (onset at 134.23°C and end at 142.95°C). These results highlight the purity of drug.

Mechanism of Deep Eutectic Solvent Mixture

The formation mechanism of a deep eutectic mixture (DEM) consisting of choline chloride and malonic acid involves specific molecular interactions that result in a significant reduction in melting point compared to the individual components. Choline chloride, serving as the hydrogen bond acceptor, and malonic acid, acting as the hydrogen bond donor, are carefully selected for their ability to form hydrogen bonds with each other. Upon mixing, hydrogen bonds form between the positively charged nitrogen atom in choline chloride and the negatively charged oxygen atoms of malonic acid, disrupting the crystal lattice structures of both components. This disruption lowers the energy required for molecular movement and leads to a decrease in the melting point of the mixture. Consequently, the DEM exhibits liquid-like behaviour even at ambient or slightly elevated temperatures. The tunable properties of the choline chloride-malonic acid DEM, such as viscosity, polarity, and conductivity, can be adjusted by varying the ratio of the components, allowing for customization to meet specific requirements in various applications.

Influence of various acids with choline chloride

To prepare a Deep Eutectic Solvent Mixture (DESM), choline chloride and different carboxylic acids (Thio urea, Citric acid, Urea, Oxalic acid, Benzoic acid, Malonic acid, Cinnamic acid, Succinic acid) were mixed at different molar ratios. The mixtures were sealed in vials and heated in an oven at 75°C until homogenous solutions were formed. Then all DESM were stored at room temperature [24°C].

Evaluation of Deep Eutectic Solvent Mixture with Choline Chloride

Table 33: Appearance of DSEM with choline chloride at room temperature

Sr. No	Formulation Code	Appearance
1	F1	Crystal form
2	F2	Crystal form
3	F3	Crystal form
4	F4	Crystal solution
5	F5	Crystal form
6	F6	Transparent
7	F7	Crystal form
8	F8	Crystal form

Discussion: DESM using Malonic acid was transparent and clear liquid other than remaining DESM. So, Malonic acid was selected for further process.

Influence of the various carboxylic acids with Choline Bitartrate

To prepare a Deep Eutectic Solvent Mixture (DESM), choline bitartrate and different carboxylic acids (Thio urea, Citric acid, Urea, Oxalic acid, Benzoic acid, Malonic acid, Cinnamic acid, Succinic acid) were mixed at different molar ratios. The mixtures were sealed in vials and heated in an oven at 75° until homogenous solutions were formed. Then all DESM were stored at room temperature [24°C].

Evaluation of Deep Eutectic Solvent Mixture with Choline Bitartrate

Table 34: Appearance of DSEM with choline bitartrate at room temperature

Sr. No	Formulation Code	Appearance
1	F9	Crystal form
2	F10	Crystal form
3	F11	Crystal form
4	F12	Crystal form
5	F13	Crystal form
6	F14	Crystal form
7	F15	Crystal form
8	F16	Crystal form

Discussion: All DESM were in Crystal form at room temperature, so all formulations of DESM using choline bitartrate were rejected.

Due to above observations**,** further process is carried out by using Malonic acid and Choline Chloride.

Effect of different molar ratio of selected acid with choline chloride

To prepare a Deep Eutectic Solvent Mixture (DESM), choline chloride and selected carboxylic acids (i.e Malonic acid) were mixed at different molar ratios. The mixtures were sealed in vials and heated in an oven at 75° until homogenous solutions were formed. Then all DESM were stored at room temperature [24°C].

Evaluation of DESM with Different molar ratio of Carboxylic acid

Table 35: Appearance of DSEM with different molar ratio of carboxylic acid at room temperature [24°C]

Sr. No	Formulation Code	Appearance
1	F6(A1)	Clear solution
2	F6(A2)	Clear solution
3	F6(A3)	Crystal form

4	F6(A4)	Crystal form
5	F6(A5)	Crystal form

Discussion: From the above figures, the F6 (A1) and F6 (A2) and DESM was clear and transparent at room temperature, remaining DESM are crystal formed, so when increase the ratio of carboxylic acid then it is not stable in room temperature**.**

Effect of Different molar ratio of Choline chloride with selected carboxylic acid in DESM

To prepare a Deep Eutectic Solvent Mixture (DESM), of selected carboxylic acids (i.e., Malonic acid) were mixed at different molar ratios of Choline chloride. The mixtures were sealed in vials and heated in an oven at 75° until homogenous solutions were formed. Then all DESM were stored at room temperature [24°C].

Evaluation of DESM with Different molar ratio of Choline Chloride

Appearance

Table 36: Appearance of DSEM with different molar ratio of choline chloride at room temperature [24°C]

Sr. No.	Formulation Code	Appearance
1	F6(B1)	Clear Viscous solution
2	F6(B2)	Crystal form
3	F6(B3)	Crystal form
4	F6(B4)	Crystal form

Discussion: From the above figures, F6(B1) DESM is clear viscous solution, but it is viscous solution at room temperature, remaining DESM are crystal formed, so when increase the ratio of Choline Chloride then it was not stable in room temperature. So, from the above results these shows that the ratio of Choline Chloride and Malonic acid F6(A1), F6(A2) and F6(B1) was suitable for further process.

Addition of Drug in Selected DESM

To prepare a Deep Eutectic Solvent Mixture (DESM), of Selected DESM. The mixtures were sealed in vials and heated in an oven at 75° until homogenous solutions were formed. Then all DESM were stored at room temperature [24°C]. Then added 0.5 w/v% of the drug in each DESM.

Appearance and pH of the Solution without drug

The solutions prepared were subjected to pH estimated and was recorded as shown in table 37.

Table 37: Appearance and pH data of DESM without Drug

Sr. No.	Formulation Code	Appearance	pH
1	F6(A1)	Transparent Solution	5.11±0.02
2	F6(A2)	Transparent Solution	5.13±0.01
3	F6(B1)	Transparent viscous Solution	5.21±0.02

Table 38: Appearance and pH data of DESM with addition of Drug

Sr. No	Formulation Code	Appearance	pH
1	F6(A1)	Light yellow Solution	5.79±0.02
2	F6(A2)	Particles formed	5.73±0.01
3	F6(B1)	Particles formed	5.55±0.04

Discussion: The pH values of formulations without drug and with drug were found to be in the range of 5.11±0.02and 5.79±0.02 respectively as shown in table **39**.

Drug Solubility of DESM [172]

Table 40: Drug Solubility of DESM

Sr. No	Formulation Code	Solubility (mg/ml)

1	F6(A1)	4.764±0.033
2	F6(A2)	4.117±0.015
3	F6(B1)	3.717±0.026

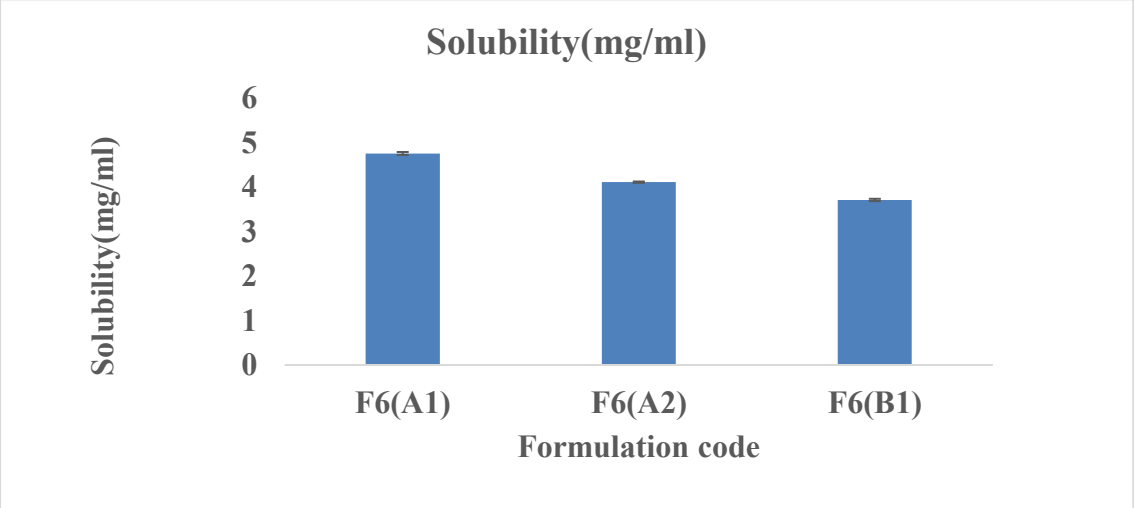

Figure 15: Drug solubility of DESM with addition of drug

Discussion: From the table 40, it was found that maximum solubility of drug in DESM was found to be 4.764±0.033mg/ml in formulation F6(A1) which was subjected to further steps.The solubility of the drug in the **DES-based formulation F6 (A1) was found to be 4.764 mg/mL**, which is notably lower than the solubility observed in **PBS (pH 7.4), i.e., 11.27 mg/mL**. This difference can be attributed to the **nature of the deep eutectic solvent (DES)** composed of **choline chloride and malonic acid**, which provides a polar, hydrogen-bonding environment, but lacks the buffering capacity and ionic strength of PBS. The moderate solubilizing ability of DESMs, while lower than PBS, still offers a **suitable solubility range for topical/transdermal application**, especially considering their role in **enhancing permeability** and **stabilizing drug molecules**.

Drug Content of DESM

Table 41: Drug Content of DESM

Sr. No	Formulation Code	% Drug content
1	F6(A1)	84.34±0.398
2	F6(A2)	77.12±0.543
3	F6(B1)	64.78±0.398

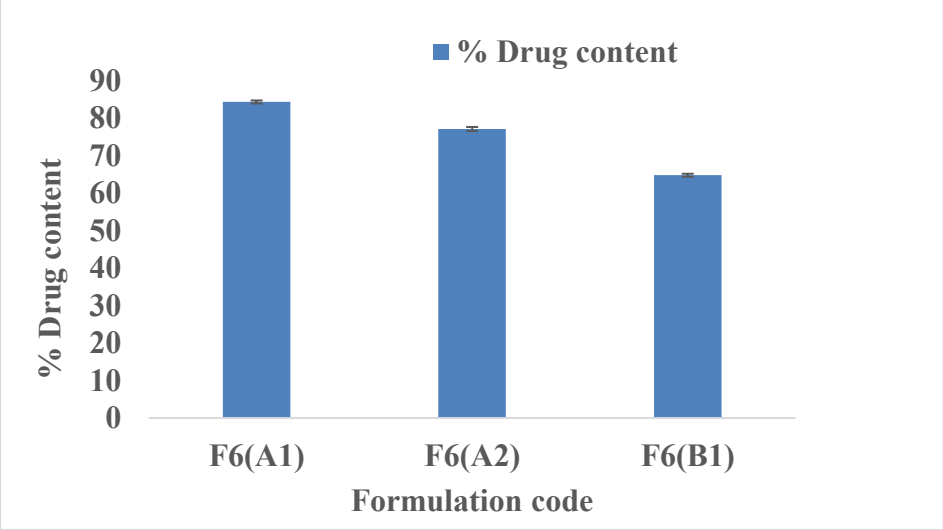

Figure 16: %Drug content of DESM with addition of drug

Discussion: From the table 41, it was found that maximum percentage drug content of Colchicine in DESM was found to be 84.34±0.398in formulation F6 (A1) which was subjected to further steps.

Optimization of Colchicine DESM

Optimization of Colchicine DESM using face central composite design (CCD) Statistical Analysis

Statistical validity of the polynomials was established based on ANOVA provision in the Design expert Software. Three-dimensional (3D) response surface plots and two-dimensional (2-D) contour plots were constructed based on the model polynomial functions using Design Expert software. The plots were useful to see interaction effects on the factors on the responses. Thirteen optimum checkpoints were selected to validate the chosen experimental design and polynomial equations. The formulations corresponding to these checkpoints were prepared and evaluated for various response properties. The resultant experimental data of response properties were quantitatively compared with that of their predicted values and the linear regression plots between observed and predicted values of the response properties Table 42.

Obtained data were subjected to multiple regression analysis. The data were fitted in Eq. (1,2)

$Y_1 = \beta_o - \beta_1 A - \beta_2 B - \beta_1\beta_2 AB - \beta_1 A^2 + \beta_2 B^2$ ..... (1)

$Y_2 = \beta_o - \beta_1 A - \beta_2 B + \beta_1\beta_2 AB - \beta_1 A^2 - \beta_2 B^2$ ..... (2)

Table 42: Responses obtained for studied parameters from experimental batches.

Experimental trial no.	Factor 1 A: Amount of Choline chloride (molar)	Factor 2 B: Amount of Malonic acid (molar)	Response 1 Y1: Aqueous solubility of Colchicine (mg/ml)	Response 2 Y2: % Drug content
D1	1.00	1.00	4.75	84.27
D2	2.00	1.00	3.7	65.68
D3	1.00	2.00	4.15	77.03
D4	2.00	2.00	4.7	82.92
D5	0.79	1.50	4.51	83.94
D6	2.21	1.50	4.2	74.34
D7	1.50	0.79	4.13	71.94
D8	1.50	2.21	4.39	78.07
D9	1.50	1.50	4.59	79.87
D10	1.50	1.50	4.64	82.05
D11	1.50	1.50	4.63	82.05
D12	1.50	1.50	4.51	80.76
D13	1.50	1.50	4.52	80.16

Fitting the model to data

Response data of all formulations were fitted to quadratic model. According to Design Expert software, best-fitted model was quadratic for response Y_1 &Y_2 (Aqueous solubility, % drug content). All the responses were fitted to model to establish full model (FM) polynomial equation:

Coded Equation:

$$Aqueous\ solubility\ of\ Colchicine = +4.578 - 0.117300776 \times A + 0.095961941 \times B + 0.4 \times A \times B - 0.107125 \times A^2 - 0.154625 \times B^2$$

$$\%Drug\ content\ of\ Colchicine = +80.978 - 3.284556275 \times A + 2.377835316 \times B + 6.12 \times A \times B - 0.834 \times A^2 - 2.839 \times B^2$$

Actual Equation:

$$Aqueous\ solubility\ of\ Colchicine = +5.886266504 - 1.349101551 \times Amount\ of\ choline\ chloride - 0.352576118 \times Amount\ of\ malonic\ acid + 1.6 \times Amount\ of\ choline\ chloride \times Amount\ of\ malonic\ acid - 0.4285 \times (amount\ of\ choline\ chloride)^2 - 0.6185 \times (amount\ of\ malonic\ acid)^2$$

$$\%Drug\ content\ of\ Colchicine = +105.7211629 - 33.28111255 \times Amount\ of\ choline\ chloride + 2.103670632 \times Amount\ of\ malonic\ acid + 24.48 \times Amount\ of\ choline\ chloride \times Amount\ of\ malonic\ acid - 3.336 \times (amount\ of\ choline\ chloride)^2 - 11.356 \times (amount\ of\ malonic\ acid)^2$$

Responses

Response 1: Y1 Aqueous solubility

ANOVA for response surface quadratic model

Table 43: Analysis of variance table of aqueous solubility of colchicine [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	1.043579751	5	0.20871595	91.90387239	< 0.0001	Significant
A-Amount of choline chloride	0.110075776	1	0.110075776	48.46965465	0.0002	
B-Amount of malonic acid	0.073669553	1	0.073669553	32.43890635	0.0007	
AB	0.64	1	0.64	281.8111326	< 0.0001	
A^2	0.079831413	1	0.079831413	35.1521577	0.0006	
B^2	0.166322717	1	0.166322717	73.23686463	< 0.0001	
Residual	0.015897172	7	0.002271025			
Lack of Fit	0.001217172	3	0.000405724	0.110551484	0.9495	not significant
Pure Error	0.01468	4	0.00367			
Cor Total	1.059476923	12				

The Model F-value of 91.90 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A++2+-, B++2+- are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 0.01 implies the Lack of Fit is not significant relative to the pure error. There is a 94.95% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good – we want the model to fit.

The "Pred R-Squared" of 0.9702 is in reasonable agreement with the "Adj R-Squared" of 0.9743.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 31.956 indicates an adequate signal. This model can be used to navigate the design space.

Response 2: Y2 % Drug content

Response 2: Y2 % Drug content

ANOVA for response surface quadratic model

Table 44: Analysis of variance table of % Entrapment Efficiency of Colchicine [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	338.9475365	5	67.78950729	101.1936	< 0.0001	significant
A-Amount of choline chloride	86.30647938	1	86.30647938	128.835	< 0.0001	
B-Amount of malonic acid	45.23280632	1	45.23280632	67.52181	< 0.0001	
AB	149.8176	1	149.8176	223.642	< 0.0001	
A^2	4.838650435	1	4.838650435	7.222953	0.0312	

B^2	56.06901565	1	56.06901565	83.69769	< 0.0001	
Residual	4.689294299	7	0.669899186			
Lack of Fit	0.446614299	3	0.148871433	0.140356	0.9307	not significant
Pure Error	4.24268	4	1.06067			
Cor Total	343.6368308	12				

The Model F-value of 101.19 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.
Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A++2+-, B++2+- are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
The "Lack of Fit F-value" of 0.14 implies the Lack of Fit is not significant relative to the pure error. There is a 93.07% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good ~ we want the model to fit.
The "Pred R-Squared" of 0.9715 is in reasonable agreement with the "Adj R-Squared" of 0.9766.
"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 33.827 indicates an adequate signal. This model can be used to navigate the design space.

Response surface (3D)

The effect of independent factors on response, three-dimensional (3D) plots show in Figure 17(a and b) and Fig. 18 (a and b). All of the observed response surfaces formed hillsides with large curvatures confirms that they were typically influenced by the interaction effect of concentrations of dependent factors.

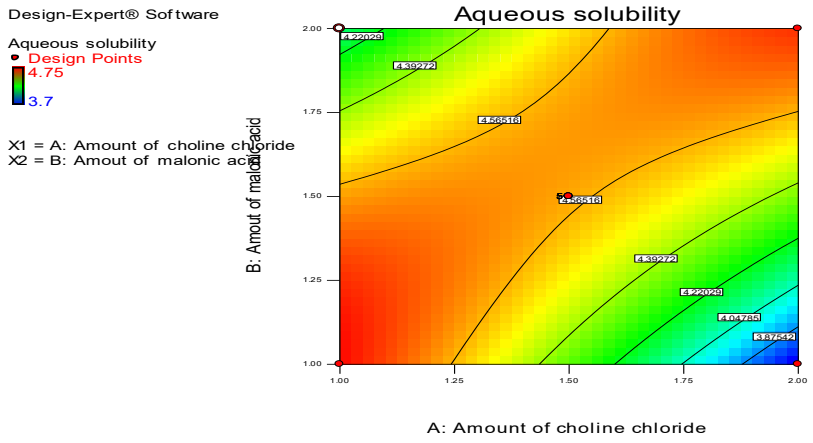

Fig. 17(a): 3D Response surface plot of aqueous solubility of Colchicine

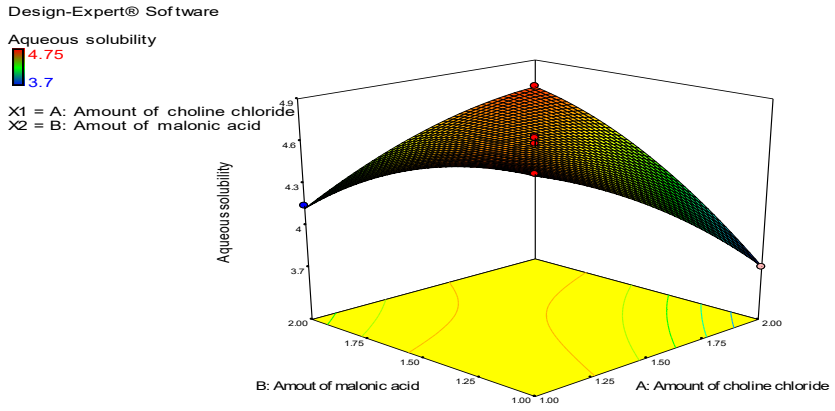

Fig. 17 (b): 3D Response surface plot of aqueous solubility of Colchicine

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Response surface (3D):
% drug content

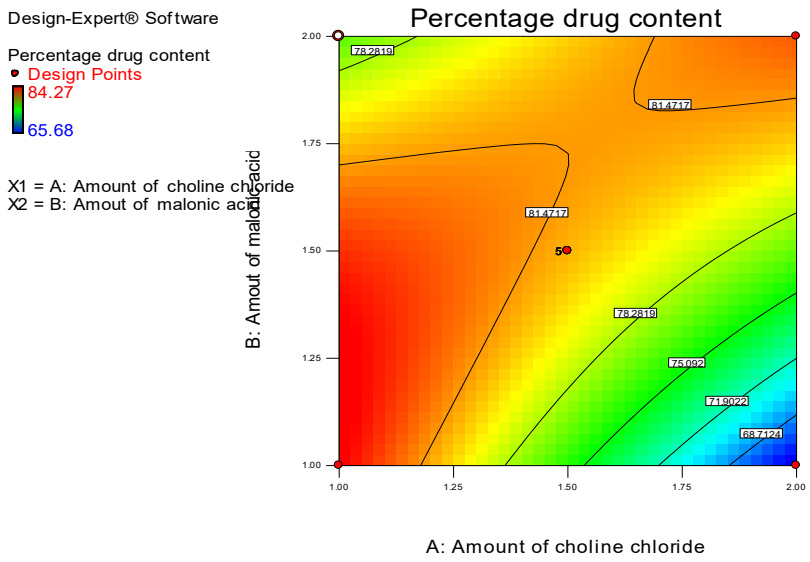

Fig. 18 (a): 3D Response surface plot of % drug content of Colchicine

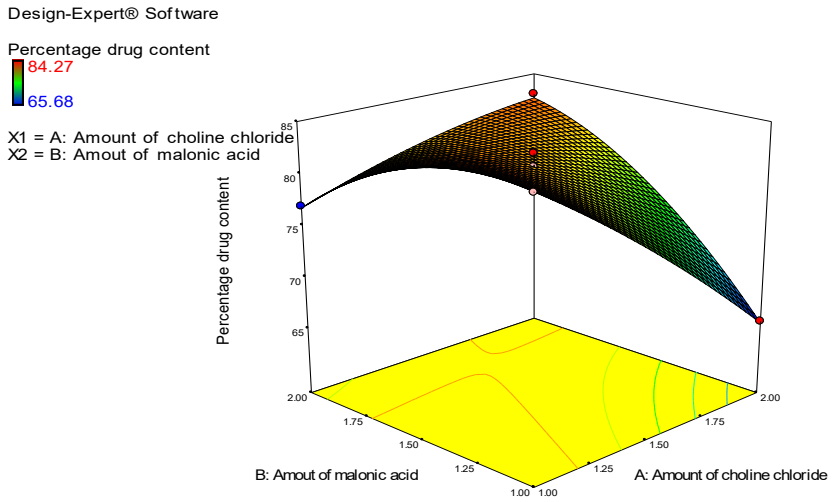

Fig. 18 (b): 3D Response surface plot of % drug content of Colchicine

Normal Plot of Residuals

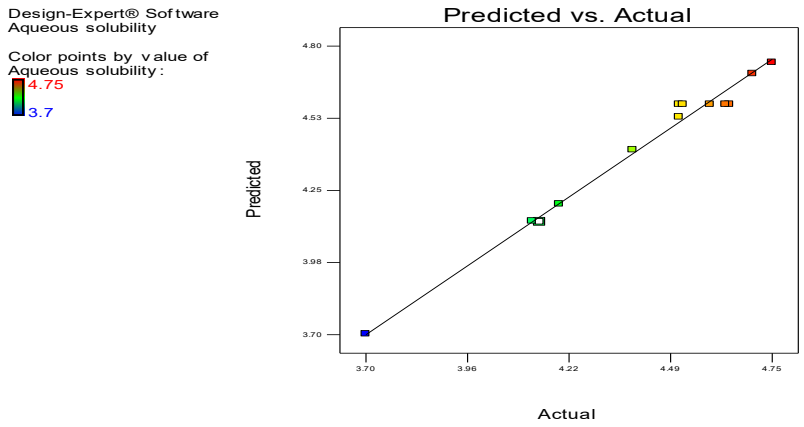

Fig. 19: Predicted vs actual response of aqueous solubility

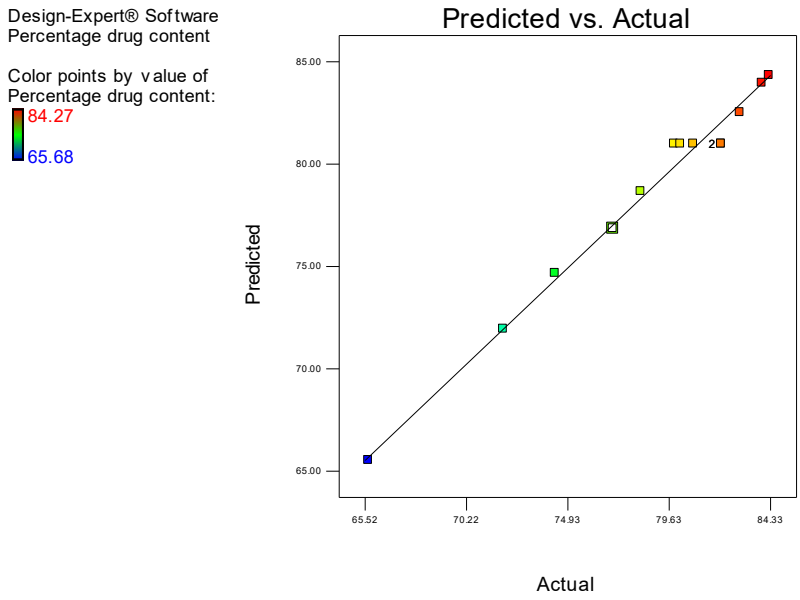

Fig. 20: Predicted vs actual response of % drug content

The normal probability plot indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line. Expect some moderate scatter even with normal data. Look only for definite patterns like an "S-shaped" curve, which indicates that a transformation of the response may provide a better analysis.

The Shapiro-Wilks test for normality is not available on this graph because the test is dependent on the assumption of independence. There is autocorrelation between the residuals, which invalidates the Shapiro-Wilks test. Visual inspection of the graph is sufficient Table 45.

Table 45: Comparison of the optimized formulation, the predicted and the experimental

Serial No.	Predicted response		Observed response		Percentage error	
	Aqueous solubility of Colchicine	% drug content of colchicine	Aqueous solubility of Colchicine	% drug content of colchicine	Aqueous solubility of Colchicine	% drug content of colchicine
1	4.74	84.33	4.75	84.27	0.211	-0.071
2	3.70	65.52	3.7	65.68	0.000	0.244
3	4.13	76.85	4.15	77.03	0.482	0.234
4	4.69	82.52	4.7	82.92	0.213	0.482
5	4.53	83.96	4.51	83.94	-0.443	-0.024
6	4.20	74.66	4.2	74.34	0.000	-0.430
7	4.13	71.94	4.13	71.94	0.000	0.000
8	4.40	78.66	4.39	78.32	-0.228	-0.434
9	4.58	80.98	4.59	79.87	0.218	-1.390
10	4.58	80.98	4.64	82.05	1.293	1.304
11	4.58	80.98	4.63	82.05	1.080	1.304
12	4.58	80.98	4.51	80.76	-1.552	-0.272
13	4.58	80.98	4.52	80.16	-1.327	-1.023

Evaluation of DESM formulation

Visual Appearance: The formulations were examined by visual inspection to verify sample homogeneity (visual appearance), phase separation and presence for aggregates Table 46.

Table 46: Visual Appearance of DESM (D1 to D13)

Formulation code	Visual Appearance	Phase separation	Particles aggregation
D1	Light yellow solution	No phase separation	No particles aggregates
D2	Particles formed	No phase separation	Particles aggregates
D3	Particles formed	No phase separation	Particles aggregates
D4	Light yellow solution	No phase separation	No particles aggregates
D5	Light yellow solution	No phase separation	No particles aggregates
D6	Particles formed	No phase separation	Particles aggregates
D7	Particles formed	No phase separation	Particles aggregates
D8	Particles formed	No phase separation	Particles aggregates
D9	Light yellow solution	No phase separation	No particles aggregates
D10	Light yellow solution	No phase separation	No particles aggregates
D11	Light yellow solution	No phase separation	No particles aggregates
D12	Light yellow solution	No phase separation	No particles aggregates
D13	Light yellow solution	No phase separation	No particles aggregates

Discussion: The data indicates that most formulation (D1, D4, D5, D9, D10, D11, D12 and D13) exhibit a light-yellow solution with no phase separation or particles aggregation that means it was good stability and homogeneity. Remaining formulation such as (D2, D3, D6, D7, and D8) showed particles aggregation but no phase separation. This formulation heaving some incompatibility or stability with each other.

pH of the Solution

For pH measurements, the freshly prepared DESM. After pH was measured. The pH was determined using digital pH meter Table 47.

Table 47: pH of DESM (D1 to D13)

Formulation code	pH
D1	5.70±0.021
D2	5.86±0.030
D3	5.65±0.010
D4	5.89±0.061
D5	5.72±0.026
D6	5.86±0.015
D7	5.58±0.026
D8	5.35±0.026
D9	5.40±0.036
D10	5.74±0.038
D11	5.61±0.020
D12	5.35±0.025
D13	5.24±0.032

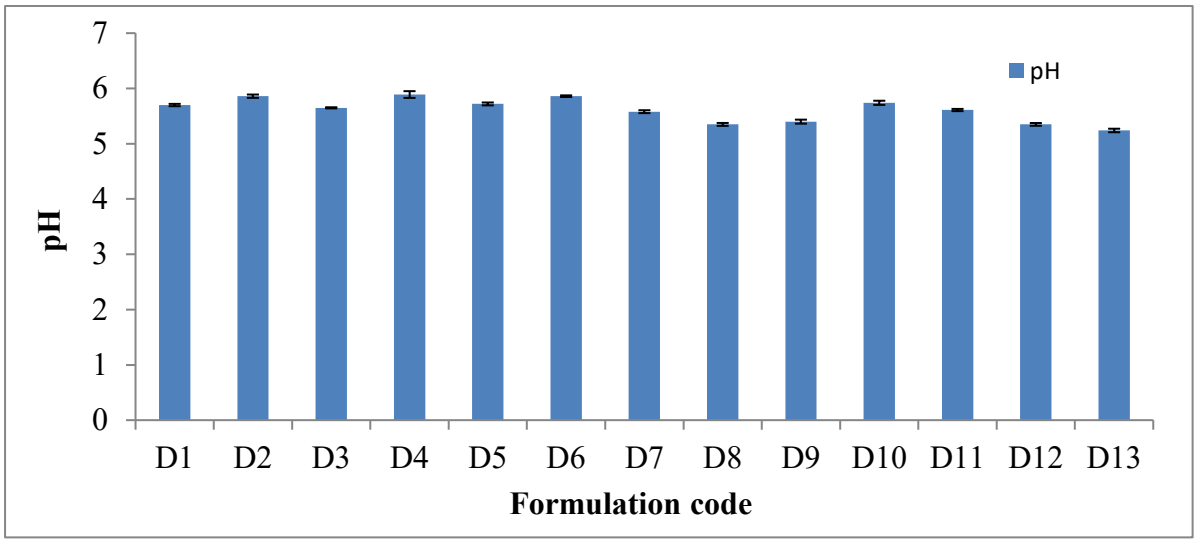

Figure 21: pH of DESM (D1 to D13)

Drug Solubility

Table 48: Solubility of DESM (D1 to D13)

Formulation code	Solubility(mg/ml)
D1	4.75±0.05
D2	3.70±0.05
D3	4.15±0.05
D4	4.70±0.03
D5	4.51±0.04
D6	4.20±0.02
D7	4.13±0.09
D8	4.39±0.03
D9	4.59±0.10
D10	4.64±0.07
D11	4.63±0.03
D12	4.51±0.05
D13	4.52±0.05

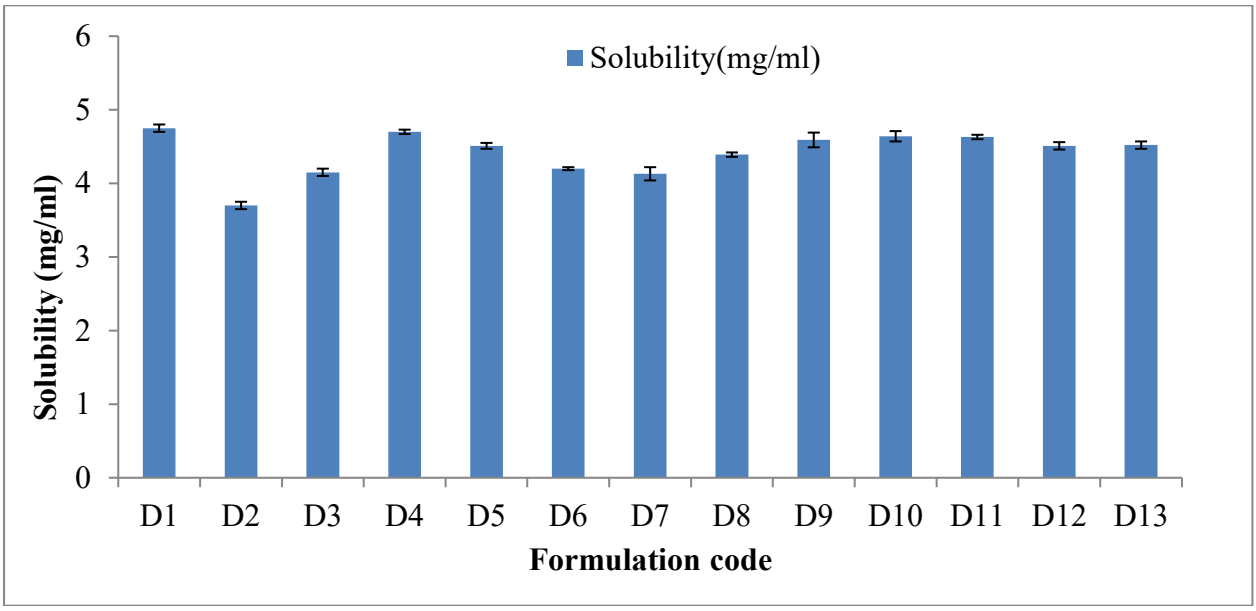

Figure 22: Solubility of DESM (D1 to D13)

Drug Content

Table 49: % Drug content of DESM (D1 to D13)

Formulation code	% drug content
D1	84.27±0.00
D2	65.68±0.970
D3	77.03±0.395
D4	82.92±0.393
D5	83.94±0.828
D6	74.34±0.257
D7	71.94±0.903
D8	78.07±0.891
D9	79.87±0.395
D10	82.05±0.536
D11	82.05±0.394
D12	80.76±0.298
D13	80.16±0.258

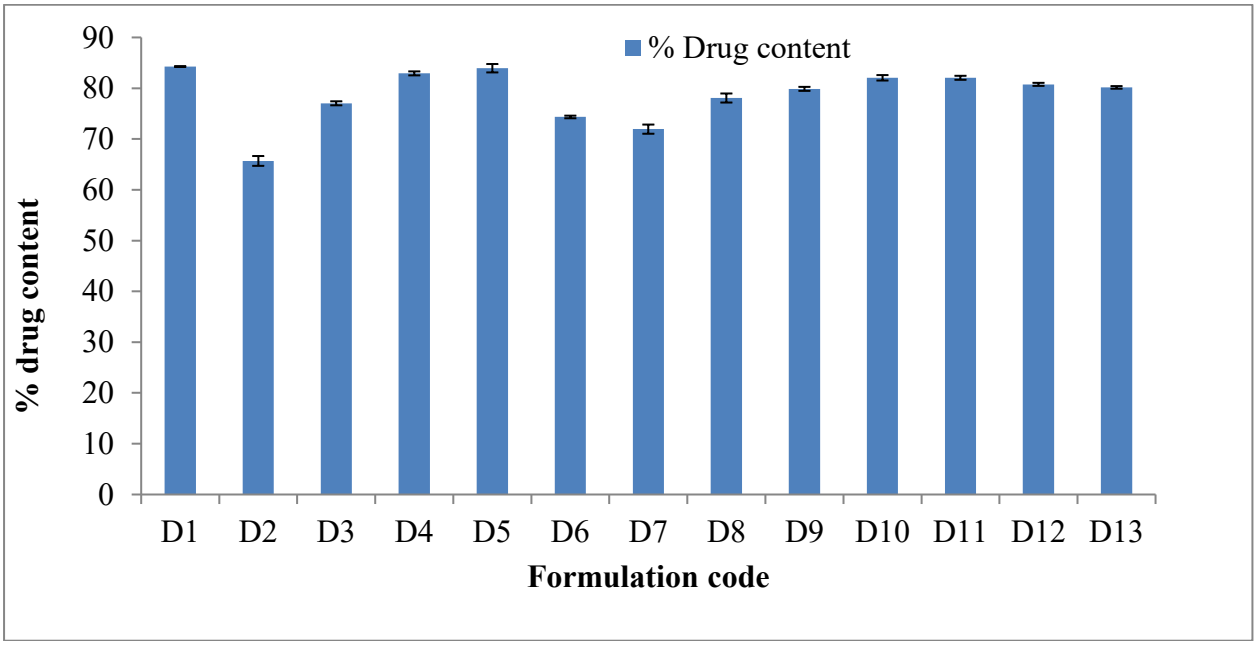

Figure 23: % drug content of DESM (D1 to D13)

Numerical Optimization Solution

Numerical optimization will search the design space, using the models you created in the analysis, to find factor settings that meet the goals you define. First you must have decent models (garbage in equals garbage out). Then you define the goals for each response. Finally, the software will generate a list of potential factor settings that provide responses that meet the criteria you defined Table 50.

Table 50: Numerical Optimization Solution

Numerical Optimization Solution	Factor 1 A: Amount of Choline chloride (molar)	Factor 2 B: Amount of Malonic acid (molar)	Response 1 Y1: Aqueous solubility of Colchicine	Response 2 Y2: Percentage Drug content of Colchicine
D14	1	1.01	4.738	84.361

Check point Analysis

The optimized DESM formulation (D14) presented the experimentally observed values of Aqueous solubility of Colchicine, & % Drug content of Colchicine was 4.725mg/ml and 84.429%. These

experimental values of Aqueous solubility of Colchicine, & % Drug content of Colchicine by the optimized DESM solutions (Table 7.49) were found in agreement with the predicted value of 4.738mg/ml and % Drug content 84.361%) respectively generated by design expert software, suggesting that the optimized formulation was rational and reliable Table 51.

Table 51: Evaluation of design parameters for Optimized Numerical Solutions

S.No.	Formulation Code	Factor 1 A: Amount of Choline chloride(molar)	Factor 2 A: Amount of Malonic acid (molar)	Predicted response		Observed response	
				Aqueous solubility of colchicine	% Drug content	Aqueous solubility of colchicine	% Drug content
1	D14	1	1.01	4.738	84.361	4.725±0.042	84.429±0.542

Evaluation of Optimized DESMVisual Appearance: The formulations were examined by visual inspection to verify sample homogeneity, phase separation and presence for aggregates.

Table 52: Visual Appearance of DESM (D14)

Formulation code	Visual Appearance	Phase separation	Particles aggregation
D14	Light yellow solution	No phase separation	No particles aggregates

Discussion: From the **Table 52**, it was found that the prepared DESM were examined visually and found to be homogenous appearance.

pH of the Solution

For pH measurements, the freshly prepared DESM. The DESM was diluted with water and the pH was measured. The pH was determined using digital pH meter Table 53.

Table 53: pH of DESM (D14)

Formulation code	pH
D14	5.84±0.020

Drug Solubility

Table 54: Solubility of DESM (D14)

Formulation code	Solubility(mg/ml)
D14	4.725±0.042

Discussion: From the **Table 54**, it was found that Aqueous solubility (mg/ml) of colchicine of DESM (D14) was found to be 4.725±0.042. Eutectic (choline chloride:malonic acid ≈1:1.01) was selected after QbD screening to improve colchicine solubility and enable gelation in chitosan (D14(C1–C4)). The observed solubility uplift aligns with extensive reports that choline-chloride–organic acid DESs markedly improve the solubility of poorly soluble drugs via hydrogen-bonding and micro-polarity tuning. Reviews and primary studies show ChCl-based DESs (including **malonic acid** systems) raising equilibrium solubility for diverse APIs and functioning as topical vehicles, with malonic-acid pairs frequently among the better performers. In our system, the DESM facilitated homogeneous gels with consistent drug content and no crystallization, consistent with DES-enabled molecular dispersion reported for other drugs [22-24].

%Drug Content

Table 55: % Drug content of DESM (D14)

Formulation code	%Drug content
D14	84.429±0.542

Discussion: From the **Table 55**, it was found that Percentage drug content of Colchicine of DESM (D14) was found to be 84.429±0.542.

Incorporation of DEMS into eutectogel:

The eutectogel gel of optimized D14 formulation was prepared by dispersing the formulation successfully in 0.5%, 1%, 1.5% and 2% Carbopol 934P and then subjected for characterization.

Evaluation of eutectogel of Colchicine

Appearance and pH of eutectogel of Colchicine

The pH and appearance of eutectogel of colchicine was shown in table 56.

Table 56: pH data of eutectogel of Colchicine

Sr.no.	Formulation Code	Appearance of gel	pH
1	D14(G1)	Gel Not Formed	6.42±0.03
2	D14(G2)	opaque uniform Gel Formed	6.46±0.02
3	D14(G3)	opaque sticky Gel Formed	6.41±0.05
4	D14(G4)	opaque sticky Gel Formed	6.39±0.06

Value is expressed as mean ± SD; n = 3

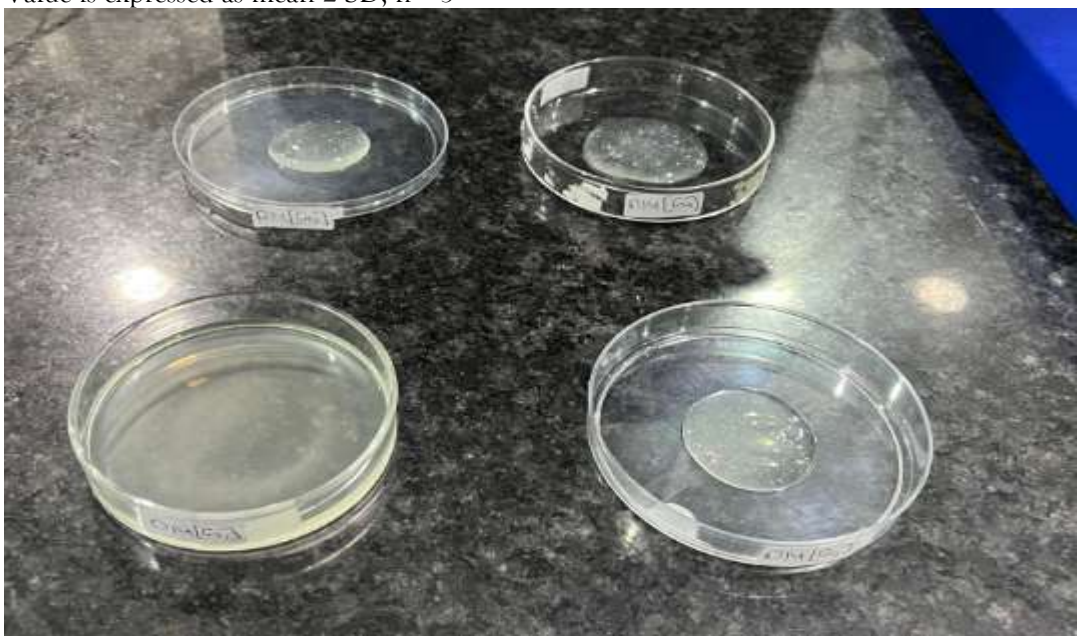

Figure 24: figures of formulation eutectogel D14(G1)- D14(G4)

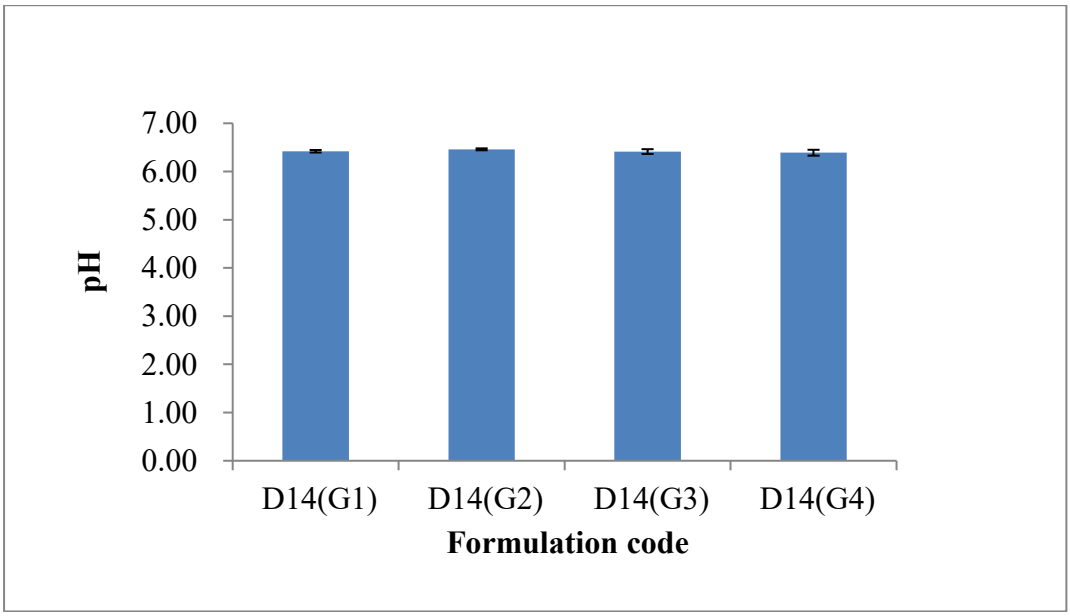

Figure 25: pH of the eutectogel of Colchicine

Discussion: The pH values of all the formulations D14(G1)-D14(G4) were found to be in the range of 6.39±0.06 and 6.46±0.02 as shown in table 56 and figure 25.

Viscosity of eutectogelof colchicine

The viscosity of eutectogelof colchicine is shown in table 57.

Table 57: Viscosity of eutectogel of colchicine

Sr. No.	Formulation code	Viscosity(cPs)
1	D14(G1)	5104±3.06
2	D14(G2)	12320±4.16
3	D14(G3)	14616±2.08
4	D14(G4)	15522±0.58

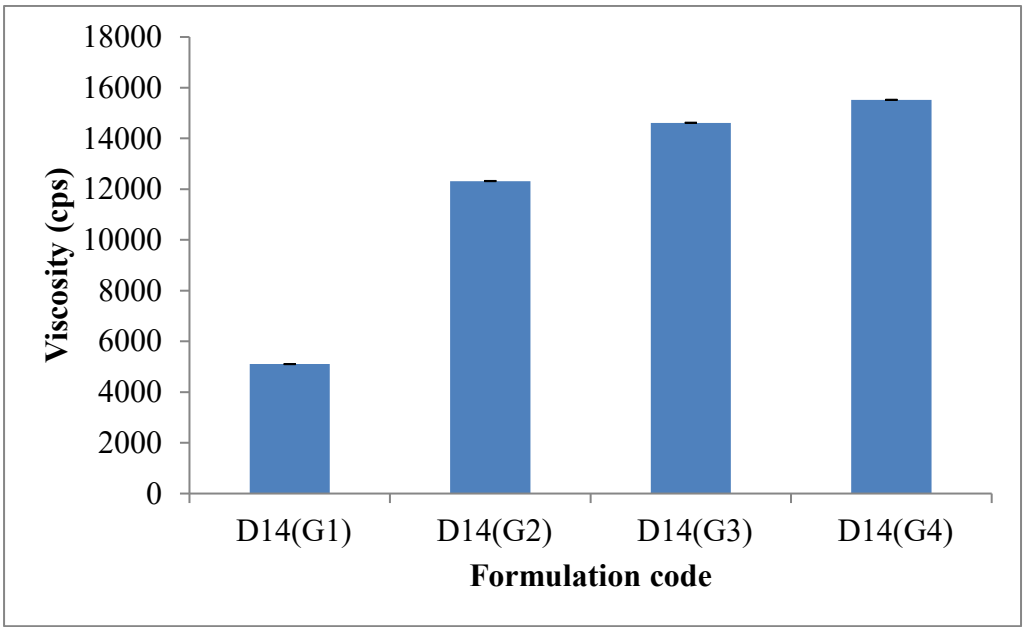

Figure 26: Viscosity of eutectogel of Colchicine

Discussion: The Viscosity of the gel at different formulation D14(G1)-D14(G4) was found to be in the range from 5104±3.06 to 15522±0.58. The chitosan-based eutectogels exhibited lower viscosity values compared to the Carbopol-based formulations. This difference in viscosity directly influences the spreadability, bioadhesion, and consequently, drug retention time on the skin. The higher viscosity of Carbopol gels likely promotes closer contact with the skin surface and enhanced mucoadhesion, which in turn contributes to superior drug release performance. Rheologically, the eutectogels exhibited shear-thinning behavior with viscosities in a transdermal-appropriate range (≈21–33 Pa·s at 10 s^{-1}), which aids spreadability while maintaining residence—behavior typical of chitosan hydrogels and reported for DES-containing semisolids [25, 26].

Spreadability of eutectogel of Colchicine

The Spreadability of eutectogelof colchicine is shown in table 58.

Table 58: Spreadability of eutectogel of colchicine

Sr. No.	Formulation Code	Spreadability (cm)
1	D14(G1)	7.63±0.021
2	D14(G2)	7.27±0.015
3	D14(G3)	7.03±0.015
4	D14(G4)	6.14±0.042

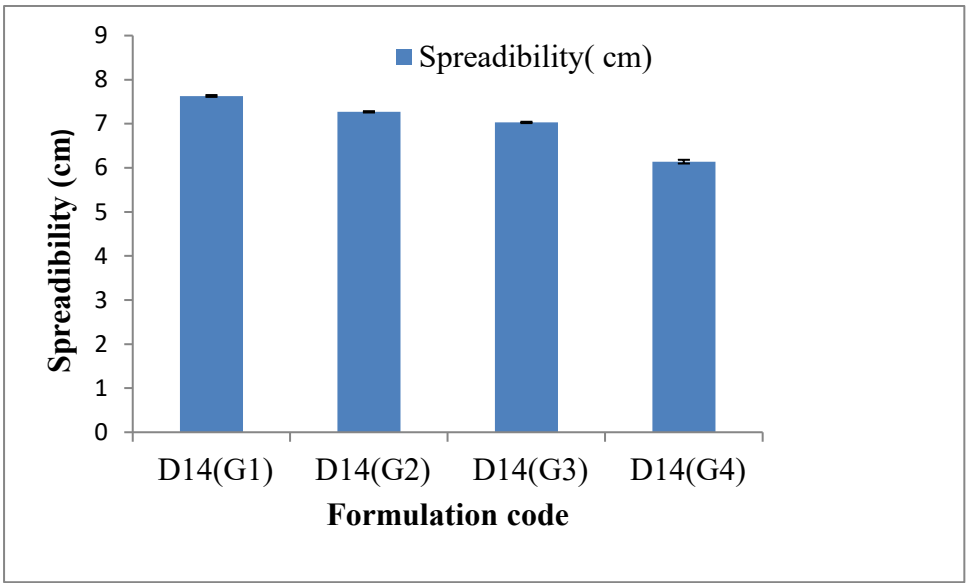

Figure 27: Spreadability of eutectogel of Colchicine

Discussion: Spreadability was an important property of Eutectogel from patient compliance point of view. The diameter was found to be 7.27 cm which is indicative of good Spreadability.

Percentage Drug content of eutectogel of Colchicine

The percentage drug content of eutectogelof colchicine was shown in table 59.

Table 59: % Drug content of eutectogel of colchicine

Sr. no.	Formulation code	% drug content
1	D14(G1)	98.48±0.4
2	D14(G2)	99.61±0.15
3	D14(G3)	97.14±0.3
4	D14(G4)	98.22±0.4

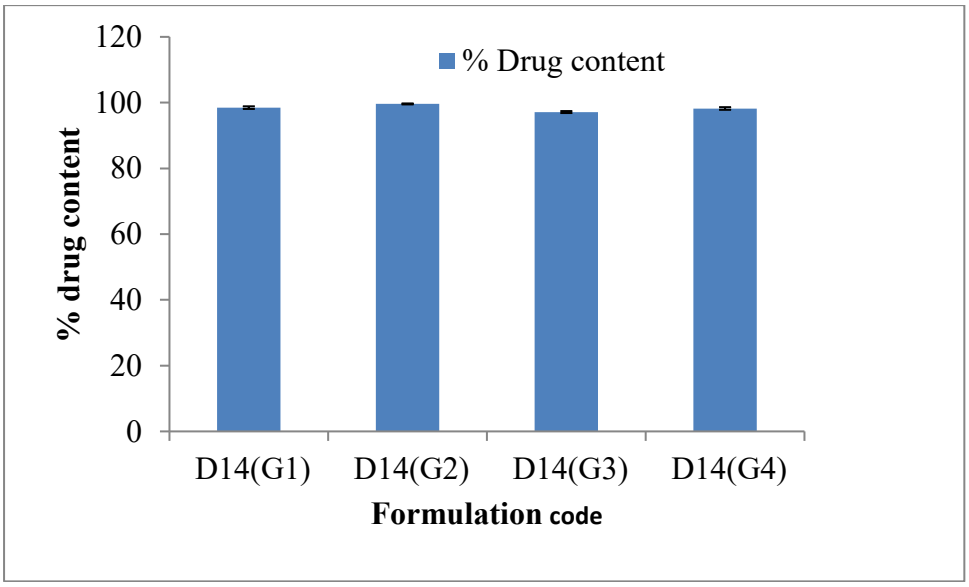

Figure 28: Percentage drug content of the eutectogel of colchicine

Discussion: The drug content of eutectogel colchicine was found to be 97.14±0.3 to 99.61±0.15% respectively. The percentage drug content of all formulations was found to be satisfactory. Hence, the method adopted for gel formulations was found to be suitable. Formulation D14(G2) was selected for

further release study.

In-vitro Drug release study

Table no 60: The in-vitro drug release of pure drug & Formulation D14(G2-G4)

Time (hr.)	% Drug release of control gel	% Drug release of 1% gel formulation G2	% Drug release of 1.5% gel formulation G3	% Drug release of 2% gel formulation G4
0	0	0	0	0
0.25	27.78±0.4	12.92±0.1	6.59±0.05	6.19±0.05
0.5	40.36±0.78	15.29±0.05	9.71±0.07	8.91±0.07
1	55.89±0.65	18.48±0.09	11.8±0.05	11.03±0.12
2	68.99±0.45	24.71±0.02	14.15±0.03	13.81±0.13
3	84.78±0.54	33.86±0.52	17.34±0.08	16.8±0.09
4	86.92±0.26	40.28±0.99	22.84±0.34	22.54±0.09
6	88.89±0.34	49.21±0.26	35.8±0.69	31.17±0.65
8	90.99±0.22	53.72±0.4	44±0.65	40.36±0.52
10	93.89±0.45	70.98±0.75	56.79±0.78	53.98±0.54
12	97.44±0.76	80.87±0.3	66.29±0.79	66.04±0.54
24	98.89±0.89	98.83±0.65	87.29±0.4	84.52±0.65

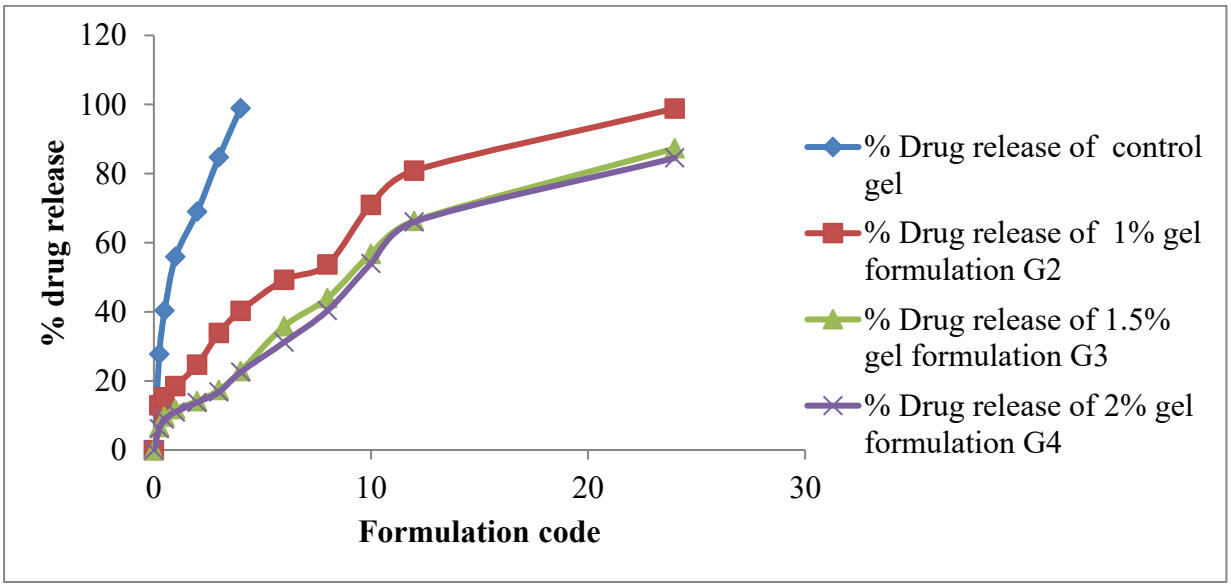

Figure 29: Percent drug release study of & Control eutectogel & Formulation (G2-G4)

Discussion: In vitro drug release for control gel &formulation (G2-G4) was shown in Figure 42. Control gel, 98.92% drug was released within 4hr. On the other hand, the release of drug in eutectogel formulation (G2-G4) showed 98.83%, 87.29%, 84.52% respectively in the Phosphate buffer (pH 7.4) with in 24hr. Drug loaded gel G2 demonstrated maximum drug release up to98.83%, within 24hr followed by prolonged manner. Formulations maintained a mildly acidic pH (≈5.2–6.0), overlapping the physiological stratum-corneum range (≈4.1–5.8), which supports barrier integrity and skin compatibility [27, 28].

Incorporation of DEMS into eutectogel with chitosan

The eutectogel of optimized D14 formulation was prepared by dispersing the formulation successfully in 1%, 1.5% and 2% and 2.5%. Chitosan high medium molecular weight and then subjected for characterization. The term “high medium molecular weight” chitosan refers to chitosan with a molecular weight range of approximately 190–310 kDa, as per the manufacturer's specification. This grade was selected due to its favorable balance of solubility, viscosity, and film-forming capacity, which are critical for gel integrity and controlled drug release.

Evaluation of eutectogel of colchicine

Appearance and pH of eutectogel of colchicine

The pH and appearance of eutectogel of colchicine was shown in table 61.

Table 61: pH data of eutectogel of colchicine

Sr.no.	Formulation Code	Appearance of gel	pH
1	D14(C1)	less Gel property formed	6.27±0.07
2	D14(C2)	opaque Gel Formed	6.34±0.03
3	D14(C3)	opaque sticky Gel Formed	6.35±0.03
4	D14(C4)	opaque sticky Gel Formed	6.14±0.02

Value is expressed as mean ± SD; n = 3

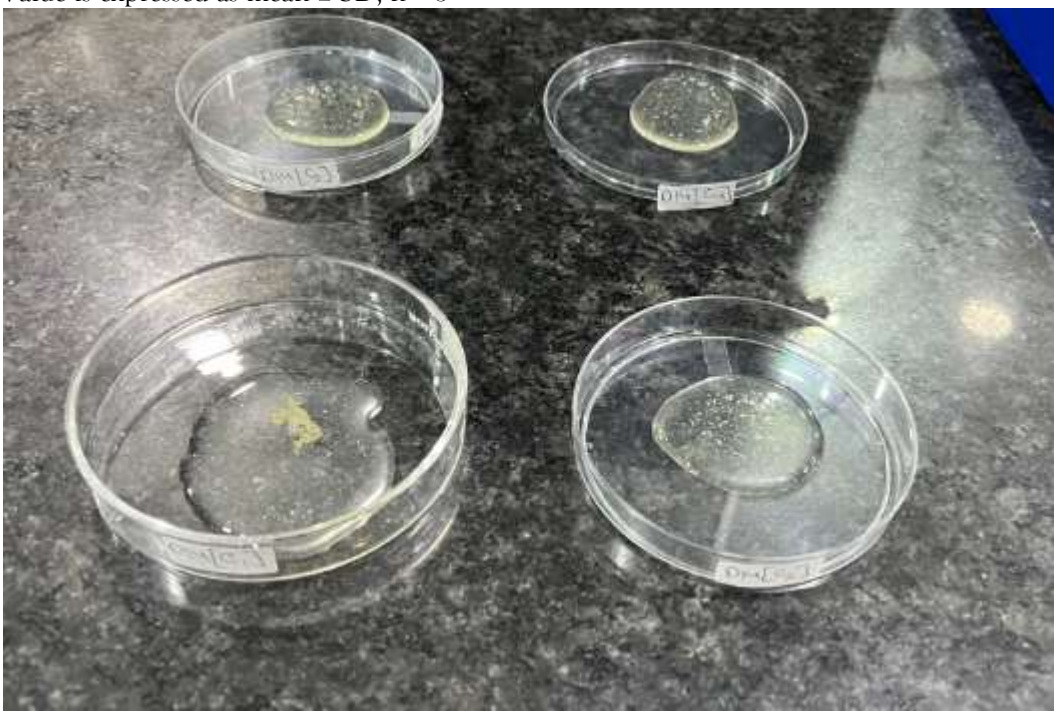

Figure 30: figures of formulation eutectogel D14(C1)- D14(C4)

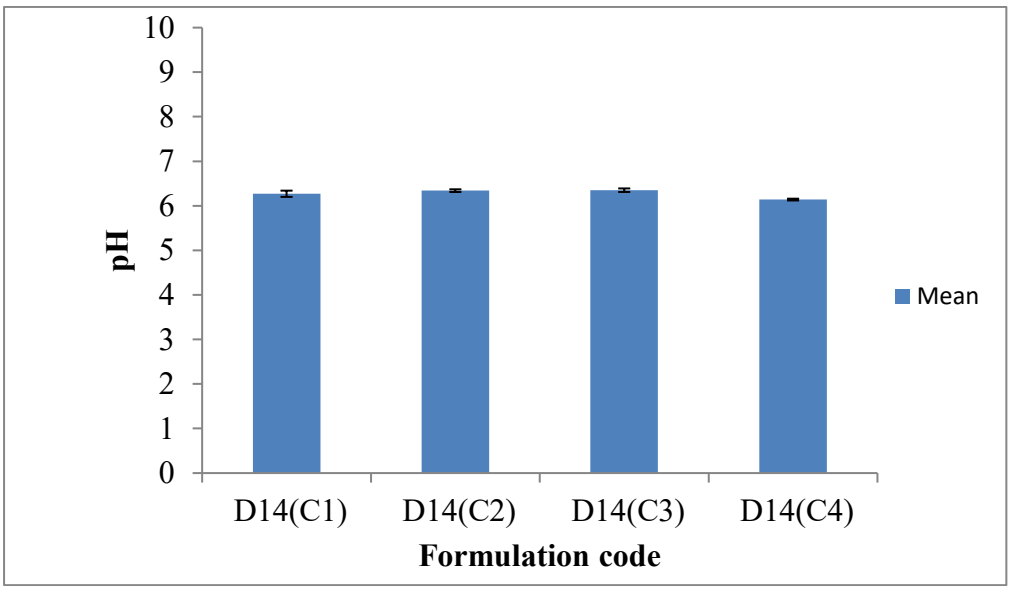

Figure 31: pH of the eutectogel of colchicine

Discussion: The pH values of all the formulations D14(C1)-D14(C4) were found to be in the range of 6.14±0.02and 6.34±0.03as shown in table 73 and figure 31.

Viscosity of eutectogel of Colchicine

The viscosity of eutectogel of Colchicine was shown in table 62.

Table 62: Viscosity of eutectogel of Colchicine

Sr.no.	Formulation Code	viscosity(cps)
1	D14(C1)	3223±2.52
2	D14(C2)	10651±6.08
3	D14(C3)	11232±1.53
4	D14(C4)	14206±5.03

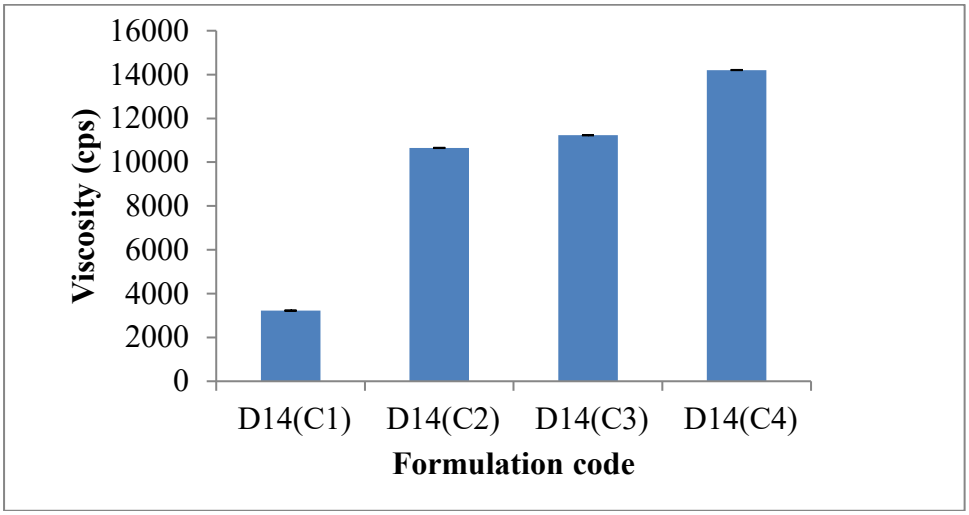

Figure 32: Viscosity of eutectogel of colchicine

Discussion: The Viscosity of the gel at different formulation D14(C1)-D14(C4) was found to be in the range from 3223±2.52to 14206±5.03.

Spreadability of eutectogel of Colchicine

The Spreadability of eutectogel of DESM of colchicine was shown in table 63.

Table 63: Spreadability of eutectogel of colchicine

Sr.no.	Formulation Code	spreadability
1	D14(C1)	4.52±0.010
2	D14(C2)	5.74±0.015
3	D14(C3)	5.34±0.025
4	D14(C4)	5.49±0.067

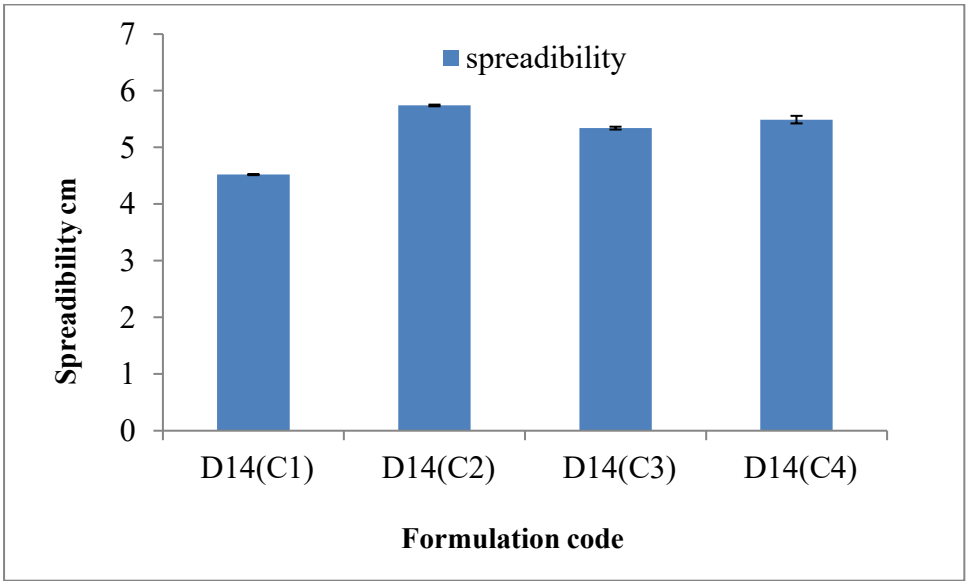

Figure 33: Spreadability of eutectogel of colchicine

Discussion: Spreadability was an important property of eutectogel formulation from patient compliance point of view. The diameter was found to be 5.34 cm which is indicative of spreadability.

Percentage Drug content of eutectogel of Colchicine

The percentage drug content of eutectogelof Colchicine was shown in Table 64.

Table 64: % Drug content of eutectogel of colchicine

Sr.no.	Formulation Code	% drug content
1	D14(C1)	89.20±0.30
2	D14(C2)	90.76±0.40
3	D14(C3)	87.90±0.26
4	D14(C4)	86.51±0.30

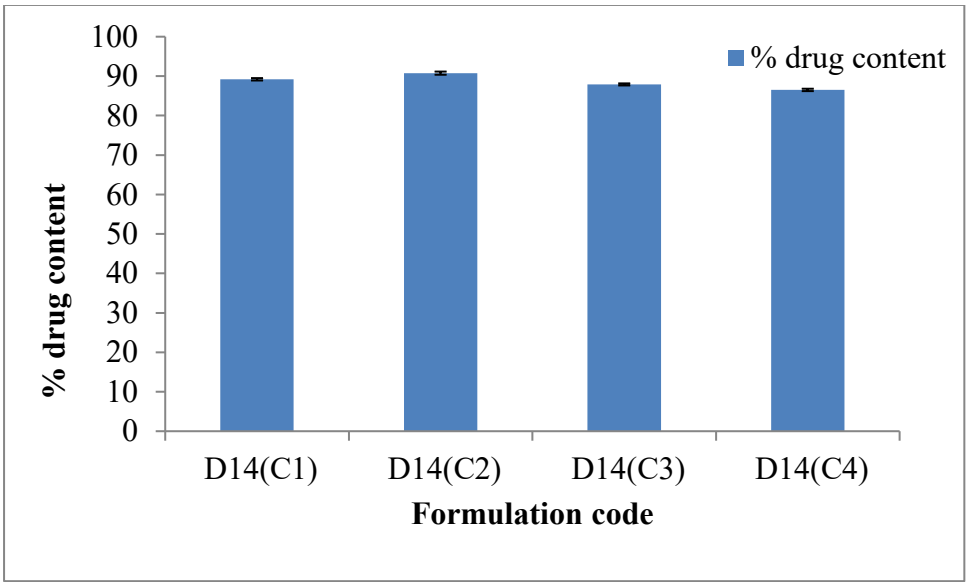

Figure 34: Percentage Drug Content of the eutectogel of colchicine

Discussion: The drug content of eutectogel Colchicine was found to be 86.51±0.30 to 90.76±0.40% respectively. The percentage drug content of all formulations was found to be satisfactory.

In-vitro Drug release study

Table no. 65: The in-vitro drug release of pure drug & Formulation D14(C2-C4)

Time (hr)	% Drug release of control gel	% Drug release of 1% gel formulation D14(C2)	% Drug release of 1.5% gel formulation D14(C3)	% Drug release of 2% gel formulation D14(C4)
0	0	0	0	0
0.25	9.39±0.26	4.03±0.07	5.04±0.08	4.84±0.08
0.5	19.20±0.4	7.7±0.09	6.44±0.04	6.09±0.07
1	35.59±0.8	10.28±0.07	9.97±0.04	8.95±0.1
2	52.85±0.26	13.44±0.08	13.14±0.09	11.35±0.04
3	66.39±0.69	18.06±0.07	20.33±0.05	18.79±0.05
4	85.47±0.54	21.19±0.05	32.34±0.3	29.66±0.65
6	69.35±0.66	38.63±0.4	42.88±0.54	35.45±0.4
8	87.88±0.78	58.41±0.54	50.48±0.65	40.89±0.79
10	89.36±0.88	68.9±0.4	63.62±0.83	50.48±0.3
12	92.66±0.87	76.36±0.3	70.79±0.26	55.84±0.4
24	96±0.77	87.29±0.4	79.34±0.26	73.81±0.4

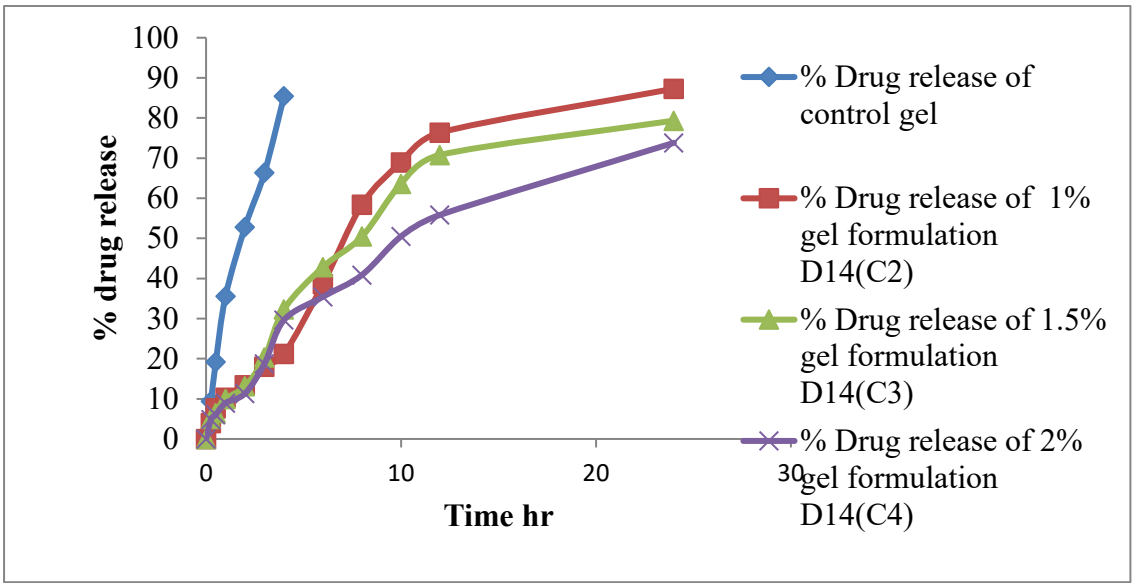

Figure 35: Percent drug release study of & Control gel & Formulation (C2-C4)

Discussion: In vitro drug release for control gel & formulation (C2-C4) was shown in Figure 48. Control gel, 85.47% drug was released within 4hr. On the other hand, the release of drug in DESM loaded gel formulation (C2-C4) showed 87.29%, 79.34%, 73.81% respectively in the Phosphate buffer (pH 7.4) with in 24hr. Drug loaded gel C2 demonstrated maximum drug release up to 87.29%, within 24hr followed by prolonged manner.

Note: Due to above results it shows that in evaluation of gel parameter is best in vitro release and percentage drug content in Carbopol gel then chitosan gel. Because Carbopol gel offers several advantages, particularly in pharmaceutical and cosmetic applications. Firstly, Carbopol has superior viscosity-building properties, allowing for the creation of thicker gels that provide better stability. Its pH sensitivity allows for easy adjustment and optimization of the gel's texture and performance. Additionally, Carbopol gels tend to have a smoother feel and better spreadability on the skin, enhancing user experience. In contrast, chitosan gels can be more sensitive to environmental factors and may have limited formulation flexibility. Overall, Carbopol's versatility and performance make it a preferred choice in many formulations. So, the study of drug release kinetic is followed by Carbopol gel.

Alternatives to improve Chitosan Based Gel Formulation: While eutectic solvents improve solubility and stability of bioactives, additional permeation enhancers can be incorporated to further optimize delivery. For instance, dimethyl sulfoxide (DMSO) acts as a polar aprotic solvent that can disrupt lipid bilayers and transiently open epithelial tight junctions, thereby augmenting chitosan's own ability to enhance paracellular transport. Similarly, terpenes (e.g., limonene, menthol, cineole) modulate membrane fluidity and extract lipids, which can synergize with chitosan's mucoadhesive properties for improved absorption of poorly permeable drugs. Surfactants such as polysorbates or sodium lauryl sulfate can also reduce interfacial tension, solubilize lipophilic molecules, and promote diffusion across mucosal barriers [29-31].

Furthermore, combinatorial strategies are being increasingly investigated, wherein chitosan gels are co-formulated with eutectic solvents and selected permeation enhancers to balance safety, efficacy, and controlled release. These multi-pronged approaches have shown promise in preclinical studies for transdermal, nasal, and buccal delivery systems, suggesting that optimizing chitosan gels requires not only the use of eutectic solvents but also rational incorporation of secondary enhancers tailored to the target tissue and drug molecule.

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FTIR of Final Formulation D14 (G2) eutectogel

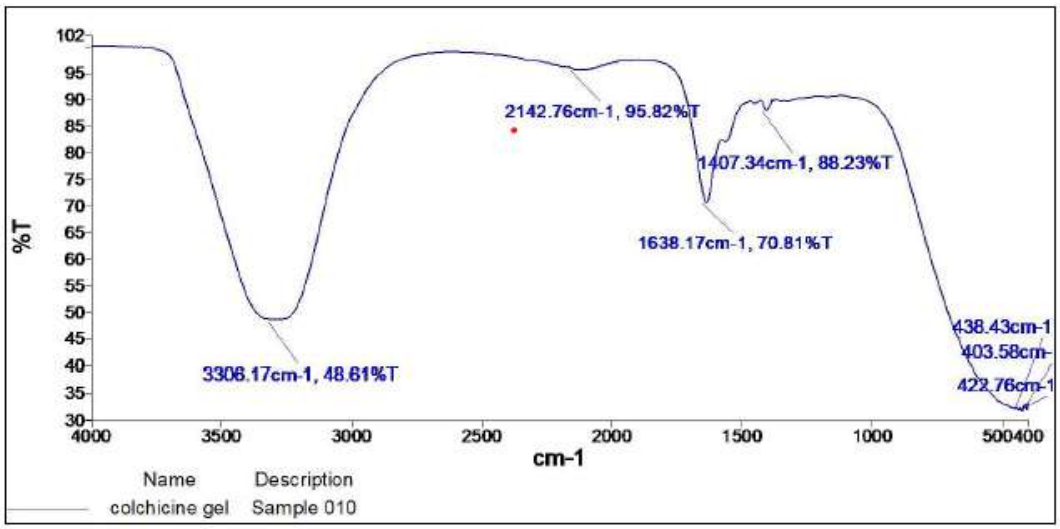

Figure 36: FTIR spectrum of Formulation D14(G2)

Drug release kinetic studies of Carbopol eutectogel

Zero order kinetics

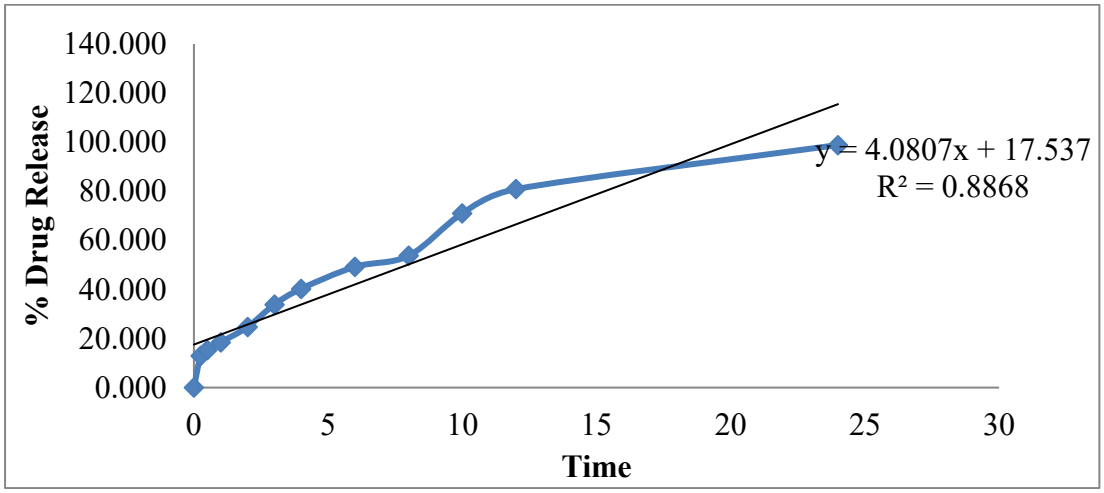

Figure 37: Zero order graph of formulation D14(G2)

First order kinetics

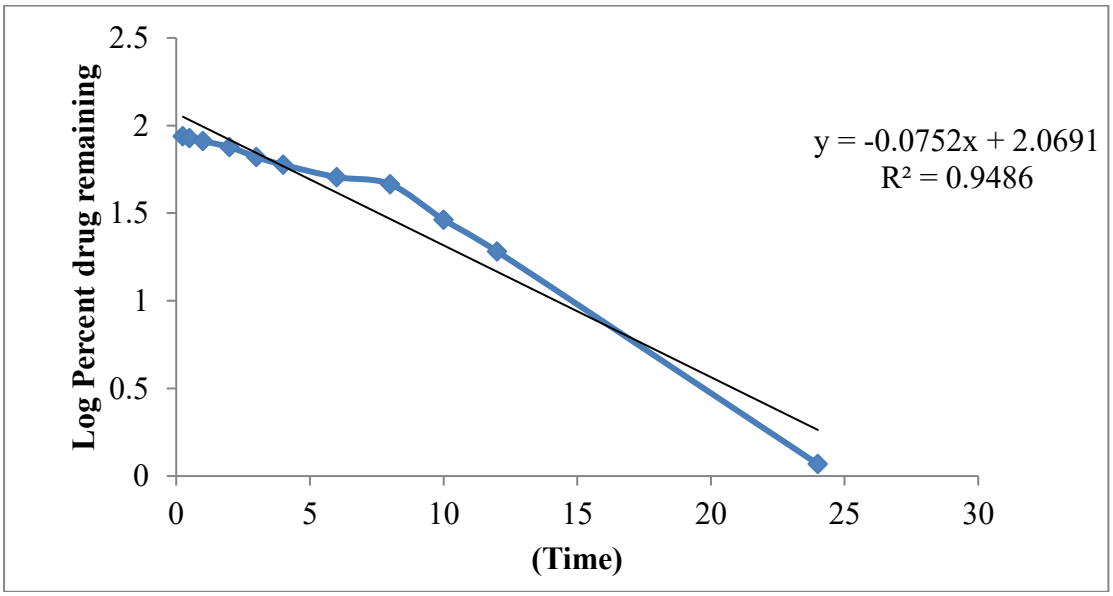

Figure 38: First order graph of formulation D14(G2)

Higuchi's Model

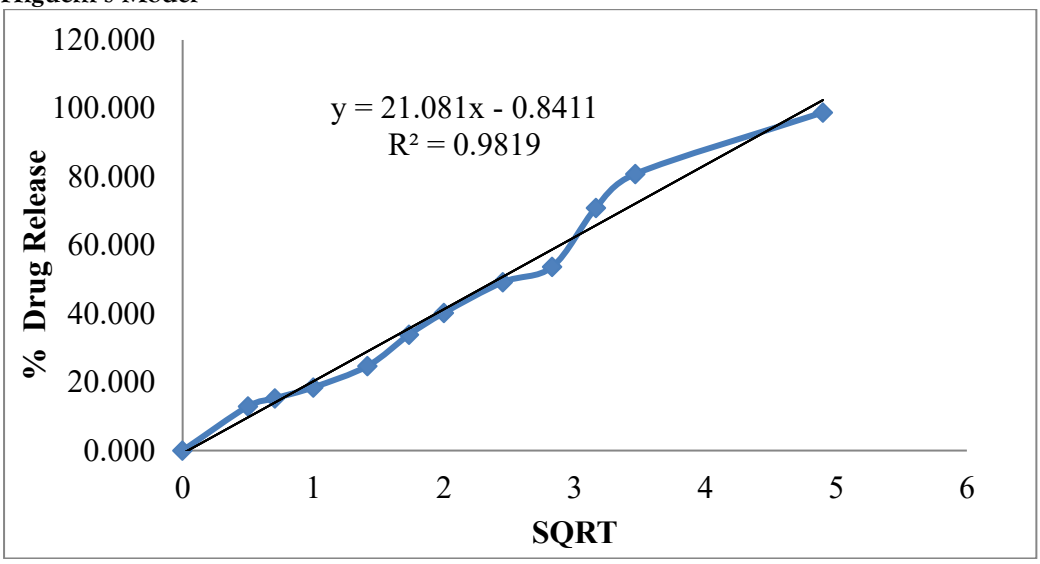

Figure 39: Higuchi order graph of formulation D14(G2)

Korsmeyer-Peppas Model

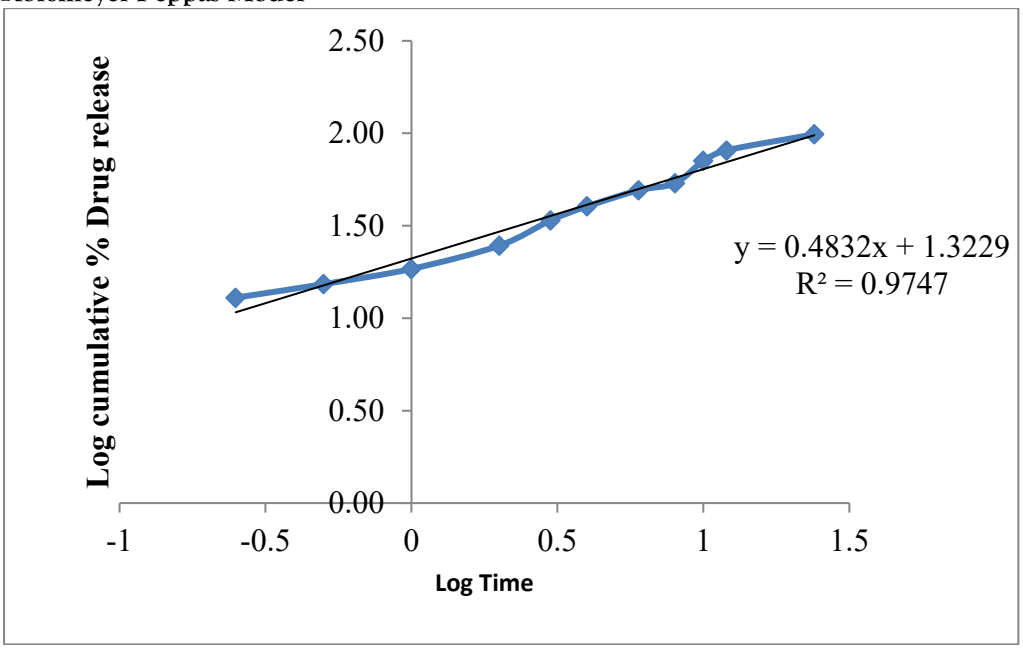

Figure 40: Korsmeyerpeppas order graph of formulation D14(G2)

The **Korsmeyer-Peppas model** was applied to analyze the drug release kinetics, and the **release exponent (n value)** was calculated to determine the underlying mechanism. The obtained **n value was 0.43**, indicating a **Fickian diffusion-controlled release mechanism** for the drug from the eutectogel formulation (for cylindrical geometry).

Table 66: Kinetic equation parameter of formulation D14(G2)

Formulation Code	Zero order		First order		Higuchi		K. Peppas	
	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
G2	4.080	0.886	-0.075	0.948	21.081	0.981	0.483	0.974

Mathematical models are commonly used to predict the release mechanism and compare release profile. For all the optimized formulations, the % drug release vs time (zero order), log percent drug remaining vs time (first order), log per cent drug release vs square root of time (Higuchi plot), and log of log % drug release vs. log time (Korsmeyer and Peppas Exponential Equation) were plotted. In each case, R^2 value was calculated from the graph and reported in **Table 66** and **Figure 38 to Figure 40.** Considering the

determination coefficients, Higuchi model was found (R^2=0.981) to fit the release data best. It could be concluded from the results that the drug was released DESM loaded gel by a prolonged mechanism. Across C1–C4, in-vitro release over 10 h was sustained (≈52–58%), with high Higuchi fits (R^2≈0.96–0.99) and Korsmeyer–Peppas exponents n≈0.41–0.49, indicating diffusion-controlled/Fickian to slightly anomalous transport from a swellable matrix. This matches canonical interpretations (n≈0.43→Fickian; diffusion-governed release), and mirrors many polymeric and nanoengineered systems where Higuchi/Korsmeyer–Peppas dominate [32].

Positioning vs. other colchicine transdermal platforms

Literature on colchicine TDD spans **transethosomal gels**, **drug-in-adhesive patches**, and **microneedles**, which generally drive higher flux or faster release than conventional gels but at the cost of greater formulation complexity or device-dependence. Transethosomal gels significantly increased ex vivo permeation vs. non-ethosomal gels, often delivering more rapid profiles than our eutectogels. Drug-in-adhesive patches tuned with appropriate PSAs have achieved sustained delivery but require specialized adhesives and crystallization control. Dissolvable microneedles yielded rapid release (e.g., ~3.36-fold vs. gel), enabling acute-flare management but requiring a device and puncturing the SC [33-34]. By contrast, the **chitosan–DES eutectogel** here achieves **needle-free**, spreadable administration with **controlled diffusion-led release**, a middle ground between simple gels and device-based systems—valuable where sustained local delivery with favorable skin feel and manufacturing simplicity is desired.

CONCLUSION AND FUTURE PROSPECTIVE

The present research highlights the successful formulation and evaluation of colchicine-loaded eutectogel as a novel transdermal delivery system for the effective management of gout disease. The eutectogel demonstrated excellent physicochemical properties and controlled drug release behaviour, leading to enhanced therapeutic efficacy. The promising results suggest that eutectogel-based drug delivery systems can be an effective strategy for localized and sustained delivery of anti-gout agents.

It was found that using PBS as the release medium does **not accurately replicate the mildly acidic pH and lipid composition of human skin**. This limitation can be overcome by the incorporation of **ex vivo skin permeation or in vivo pharmacokinetic/irritation studies** in future work to better simulate real-world transdermal application. The Quality-by-Design (QbD) approach was applied to identify and optimize **Critical Quality Attributes (CQAs)** relevant to the performance of the eutectogel system. In this study, key CQAs were defined as:

- **Viscosity** (influencing spreadability and retention),
- **Drug content** (ensuring dose uniformity), and
- **In vitro drug release** (reflecting delivery efficiency).

These CQAs were successfully linked to the **Critical Material Attributes (CMAs)** and **Critical Process Parameters (CPPs)** through the CCD model, and their influence was systematically analyzed using response surface methodology.

For future perspectives, further clinical studies are essential to establish the safety, efficacy, and patient acceptability of colchicine eutectogel in human subjects. Additionally, exploring the incorporation of other anti-inflammatory agents or synergistic phytoconstituents within the eutectogel system may further enhance its therapeutic potential. Scaling up the formulation for commercial production, along with detailed toxicological and pharmacokinetic studies, will be the next crucial steps towards the successful development of a marketable transdermal therapy for gout management.

Authors' Contributions

Anil Jindal has written the manuscript.
Rohit Bhatia has conceptualized the manuscript.
Anjana Devi has checked and validated the manuscript.

Conflict of Interest: None
Funding: None
Acknowledgement: Declared None
Ethical Approval: Not applicable

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