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PHYSICOCHEMICAL ANALYSIS, QUANTITATIVE ESTIMATION, FORMULATION DEVELOPMENT AND EVALUATION OF EMULGEL CONTAINING GINGER EXTRACT USING SIMPLE LATEX DESIGN APPROACH

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Abstract
In the current investigation, a nanoemulgel formulation of Ginger extract was developed and assessed utilizing the simple latex design (SLD) technique. The results of the quantitative phytochemical screening revealed that the extract has optimal amounts of several phytoconstituents, which may contribute to its potential pharmacological effects. The compound GME, when present at doses of 200 and 400 mg/mL, exhibited significant stabilizing effects on HRBC membranes. Specifically, the protection percentages observed were 22.34% and 29.78%, respectively. The comprehensive pre-formulation investigations, including Fourier Transform Infrared (FTIR) and Differential Scanning Calorimetry (DSC) examinations, yielded essential findings about the physical and molecular properties of ginger extract. The investigation of the compatibility between excipients and the subsequent development of nanoemulsion and nanoemulgel have provided evidence of the potential to establish a reliable delivery method for ginger extract. The improved formulation, denoted as F5, demonstrated advantageous attributes including a mean particle size of 228.6 nm, a high zeta potential, and sustained release capabilities. The thorough assessment, including pH, viscosity, drug content, and stability evaluations, combined creates a strong basis for the development of a stable and efficacious ginger extract delivery system, which has promise for possible applications in therapeutic formulations. The optimized formulation will undergo *in vivo* anti-inflammatory studies.

Keywords: Nanoemulgel; ginger extract; Simple Latex Design; evaluation; formulation; development

INTRODUCTION

Ginger, derived from the rhizome of the *Zingiber officinale* plant, has been widely recognized for its culinary and medicinal properties. Ginger extract contains bioactive compounds, such as gingerol, shogaol, and zingerone, which contribute to its pharmacological activities. Ginger extract is associated with anti-inflammatory, antioxidant, gastrointestinal effects, antiemetic, antimicrobial, cardioprotective, antidiabetic, analgesic, and neuroprotective effects. It's important to note that while ginger extract shows promise in various pharmacological activities, further research, including

clinical trials, is necessary to establish its efficacy and safety for specific medical applications(Fajrin et al., 2020; Hsiang et al., 2015; Kausar et al., 2021; Mao et al., 2019).

Emulgel, a term derived from "emulsion" and "gel," refers to a topical drug delivery system that combines the properties of both emulsions and gels. It has gained significance in the field of drug delivery for several reasons i.e. enhanced drug penetration, improved stability, tunable rheological properties, sustained drug release, versatility in formulation, improved bioavailability, ease of application, targeted drug delivery, and cosmetic appeal. Emulgels play a crucial role in drug delivery by providing a versatile and effective platform for the topical administration of drugs. Their ability to combine the advantages of emulsions and gels makes them valuable for a wide range of pharmaceutical and cosmetic applications(Basera, 2015). In present study, we have developed and evaluated nanoemulgel formulation of Ginger extract using simple latex design (SLD) approach.

EXPERIMENTAL SECTION

Materials

Ginger methanolic extract (GME) was purchased and procured from Herb Sky Bio-tech Co. Ltd. China. Liquid paraffin oil was purchased from Moychem Pvt. Ltd., Methanol, Ethanol, Chloroform, Acetone, Potassium dihydrogen phosphate (KH_2PO_4), Sodium chloride, HPMC K4, PEG-200 and Tween80 were obtained from from Loba Chem Pvt. Ltd. Mumbai. All material were use were of analytical grade. Double distilled water was used as aqueous solvent. Remaining all the required chemicals were purchased and procured from Lab Trading Laboratory, Aurangabad, Maharashtra, India.

Organoleptic and Physicochemical Analysis of Extract

The extract was subjected for different physicochemical analysis such color, odor, taste, pH of 1% and 10% solution, test for foreign contents, loss of drying (LOD), different ash values, extractive values, heavy metal estimation, and pesticide residues as per the procedure given in literature(Kamal et al., 2016; Khandelwal K. R., 2005; P K Mukherjee, 2002; Singh et al., 2015).

Preliminary Phytochemical Screening of Extract

The crude drug sample was put through a series of qualitative tests in order to conduct preliminary phytochemical screening on it. These tests were designed to identify the presence of several classes of phytoconstituents. The different qualitative tests such as test for carbohydrates, test of reducing sugars, test for monosaccharides, test for proteins, test for amino acids, test for fats and oil, test for steroids, test for cardiac glycosides, test for anthraquinone glycosides, test for saponin glycoside, test for cyanogenetic glycoside, test for flavonoids, test for alkaloids, test for tannins and phenolic compounds, and test for tannins and phenolic compounds were performed(Chaudhari et al., 2020; Khandelwal K. R., 2005; P K Mukherjee, 2002).

Microbial Content Determination

Solid samples, such as powders, were subjected to disintegration by adding 1g of the sample to 9 mL of sterile distilled water. In contrast, liquid formulations were dissolved or suspended by adding 1 mL of the formulation to 9 mL of sterile distilled water. The experiment included doing serial dilutions and evaluating the viability of the samples using the pour plate technique. The plates were subjected to incubation at a temperature of 37°C for a duration of 24 hours. The plate was positioned onto a colony counter apparatus, and the quantity of colony forming units (CFUs) was recorded. The average microbial content was determined by taking the mean of duplicate measurements. The media used in this study included Nutrient agar, Cetrimide Nutrient agar, Salt Nutrient agar, and MacConkey agar. To identify fungal development in the samples, Sabouraud dextrose agar was used by pouring it onto the plate and allowing it to solidify. Subsequently, a 1ml portion of each sample was evenly distributed over the surface of the agar. The plates were then

placed in an incubator set at a temperature of 27°C for a duration of 72 hours, during which bacterial and fungal counts were conducted(Al-Busaid et al., 2020; Indrayanto, 2018).

Tests for Specific Microorganisms

The utilization of tests designed to detect specific microorganisms in plant extracts is of utmost importance in guaranteeing the safety, purity, and adherence to regulatory standards of the products. The aforementioned factors play a crucial role in safeguarding the well-being of consumers, upholding the quality and reliability of products, and promoting the conscientious utilization of plant-based resources across diverse sectors. Test for *Escherichia coli*, test for *Salmonella spp*., test for *Shigella spp*., test for *Pseudomonas aeruginosa*, and test for *Staphylococcus aureus* contamination were performed as per the reported procedure in the literature(Al-Busaid et al., 2020; Indrayanto, 2018; Parvatikar & Madagi, 2018; Patil et al., 2020, 2021).

Quantitative Phytochemical Analysis

The utilization of quantitative phytochemical analysis is of great significance in diverse disciplines such as pharmacology, herbal medicine, food science, and agriculture. This analytical approach offers precise data regarding the concentration of bioactive compounds present in plant extracts. Consequently, researchers and manufacturers are able to gain insights into the effectiveness and excellence of their products. Here, the plant extract was subjected for different quantitative phytochemical screening such as total carbohydrate content, total protein content determination, determination of total saponin content, determination of total steroid content, determination of total alkaloid content, determination of total content of flavonoids, determination of total tannin content, determination of total phenolic content, and determination of gylcosides were performed(Kalusalingam et al., 2018; Nigam & Arnold, 2018; Tiwari et al., 2017). The name of phytoconstituents, method used and standards are tabulated in Table 1.

Table 1. The name of phytoconstituents, method used and standards

Sr. No	Phytoconstituent	Methods	Standard
1	Carbohydrate	Phenol sulphuric acid method	Glucose
2	Protein	Barford method	Albumin
3	Saponin	Simple solubility method	---
4	Steroids	Liberman-Burchard reaction method	Diosgenin
5	Alkaloids	Bromocresol Green reagent	Atropin
6	Flavonoids	Aluminum Chloride colorimetric method	Quercetin
7	Tannins	Foilin Denis reagent	Tannic acid
8	Total Phenolic	Folin Ciocalteu method	Gallic acid

***In vitro* Anti-inflammatory activity**

HRBC method was used for the estimation of anti-inflammatory activity *in vitro*. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extracts (TME, GME, and TEE at 200 and 400 mg/mL), reference sample (Diclofenac sodium (5mg/mL)) and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%(Saleem et al., 2011).

Percentage protection= 100- (OD sample/ OD control) × 100

Instrumental Analysis of Extract and Extract-Excipients Compatibility Studies

FTIR Analysis

FTIR analysis was done on FTIR spectrophotometer (Ver. 7.03 Shimadzu, Japan) with KBr disc. In the FTIR infrared spectroscopy the spectrum was recorded in the wavelength region of 4000-400 cm-1. 10 mg of drug was mixed with KBr and triturated then it was placed in holder and pressed to form a pellet. Then it was placed under IR beam and a spectrum was obtained on computer.

Differential Scanning Calorimetry (DSC)

Thermogram for Ginger extract was obtained using DSC (Mettler DSC 1 star system, Mettler-Toledo, Switzerland). The drug was sealed in perforated aluminum pan and heated at constant rate of 10°C/min over the temperature ranges of 30-350°C at 20ml/min nitrogen purging.

Screening of Oil, Surfactant and Co-surfactant (Solubility Study)

On the basis of solubility of ginger extract, oils, surfactants and co-surfactants were screened out. The solubility of ginger extract was determined in various oils (oleic acid, liquid paraffin oil, olive oil) Surfactants (tween80, span20, tween 20) and co-surfactant (polyethylene glycol (PEG-200, ethanol, propylene glycol). The ginger extract was taken in excess in centrifuge tubes and 5ml of each of oil, surfactant and co-surfactant was added. The mixtures were shaken in vortex mixer for 15min. After 24 hrs samples were centrifuged for 15min at 3000rpm. The supernatant was then filtered through Whattman filter and diluted with methanol and analyzed in UV-visible spectrophotometer at 281 nm, all samples repeated thrice.

Preparation of Ginger extract loaded Nanoemulsion

On the basis of their visual observation like transparency and viscosity, 14 formulations were selected out as per factorial design (Table 1) for preparing ginger extract loaded nanoemulsion. The required amount of ginger extract was dissolved in the calculated quantity of oil phase for the said volume of nanoemulsion. The calculated quantity of Smix (surfactant and co-surfactant) were added and mixed thoroughly in beaker using magnetic stirrer at room temperature. Then double distilled water was added drop wise drop till a clear and transparent liquid was obtained. The prepared nanoemulsion was stored in tightly closed suitable container at ambient temperature.

Preparation of ginger extract loaded nanoemulsion in gel (nano-emulgel)

For the preparation of nanoemulgel, HPMC K4 was used as gelling agents in ratio 1.0% (gels made with specified concentration range in the water). The mixed mass was stirred for the time, gels were added slowly into nanoemulsion, mixed and kept aside to settle the air entrapment and assessed next day for visual property. The gelling agent was selected for further preparation of nanoemulgel. The gel made with selected polymer concentration of at various concentrations (1.0%).

Factorial Design

Construction of and Formula optimization using SLD

TW80:TP (Smix) were blended in various proportions, and a mixture of LPO with Smix(s) was created, resulting in LPO:Smix (TW80:TP) ratios as outlined in Table 2 and 3. The optimization of Gin-NEs was carried out using Design-Expert (Stat-Ease Inc., USA), where the variables included concentrations of water (X1), LPO (X2), and Smix (X3), and the corresponding response measured was particle size (Y1) and viscosity (Y2). The statistical relationships between independent variables and 3D Response surface plot were also generated. The formulation layout for the factorial design batches are shown in Table 2 and 3.

Table 2. Levels of variables for optimization

Batch Code	X1: Water (%)	X2: Paraffin Oil (%)	X3: Smix (%)
F1	0	0	1
F2	1	0	0
F3	0.5	0.5	0
F4	0.5	0	0.5
F5	0.16	0.66	0.16
F6	0.5	0.5	0
F7	0.33	0.33	0.33
F8	0	0	1
F9	0.66	0.16	0.16
F10	1	0	0
F11	0.16	0.16	0.66
F12	0	0.5	0.5
F13	0	1	0
F14	0	1	0

Table 3. Simple Latex Design (SLD)

Batch Code	X1: Water (%)	X2: Paraffin Oil (%)	X3: S_{mix} (%)	Particle Size (nm)	Viscosity (cps)
F1	62	2	34	411	1431
F2	64	2	32	461	1530
F3	66	2	32	339	1675
F4	63	2	33	367	1721
F5	**62.21**	**2.64**	**32.16**	228	**1681**
F6	63	2	32	258	1525
F7	62.36	1.32	32.64	380	1635
F8	62	2	34	446	1751
F9	63.56	0.64	32.16	335	1865
F10	64	2	32	410	1745
F11	62.21	0.64	33.56	460	1625
F12	62	2	33	309	1809
F13	62	4	32	331	1715
F14	62	4	32	313	1678

Characterization of Nanoemulsion

Physical Characterization

The prepared nanoemulsion formulations were visually inspected for their colour, transparency, homogeneity and consistency.

Droplet Size and Size Distribution

Droplet size was determined by photon correlation spectroscopy (PCS) that analyses the fluctuations in light scattering due to Brownian motion of the droplets using a Zetasizer (1000 HS, Malvern Instruments, Italy). 0.1ml nanoemulsion was dispersed in 50ml of water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25°C a 90° angle.

Zeta potential analysis

Zeta potential of a droplet is the overall charge that the particle acquires in a particular medium. Knowledge of the zeta potential of nanoemulsion helps to assess the stability of the formulation during storage.

Measurement of pH

The pH values of the nanoemulsion were measured at 25ºC using digital pH meter. 10% w/w dispersion (1gm of nanoemulsion was dispersed in 10 ml of distilled water. At first the reading of pH- meter was adjust with a known pH solution (pH 4 and pH 7). Then the prepared formulations were subjected for pH analysis.

Measurement of Viscosity

The viscosities of nanoemulsion formulation were measured at 250C using Brookfield viscometer (Brookfield DV-E Viscometer) using spindle no 6 at 10, 20.30 and 60 rpm.

Surface Morphological Study

Surface morphology of the nanoemulsion was performed by using SEM/TEM. A nanoemulsion was placed on Formvars coated copper grids and allowed to equilibrate. Excess liquid was removed with a filter paper and dried at room temperature for about half an hour. The dried grid containing the nanoemulsion was visualized using SEM/TEM.

Drug Content Determination

The drug content of ginger extract in nanoemulsion gel was determined by UV-Spectrophotometer. 1.0 g of formulation was accurately weighed, dissolved in 100 ml of methanol: phosphate buffer (2:8). It was filtered and diluted if required. Absorbance was determined using UV spectrophotometer at 281nm.

Spreadability

1g emulgel preparation was placed above ground slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. A weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the two slides. Measured quantity of weight (35g) was placed in the pan attached to the pulley with the help of hook. Time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula.

$S = m \text{ X } t/l \text{ x } 100$

Where; S is spreadability, m is weight placed on upper slide, l is length of upper slide, and t is the time taken.

***In-vitro* Release Study of Ginger Extract Loaded Nanoemulgel**

The *in-vitro* permeation studies were carried out using Franz diffusion cell, which is a reliable method for prediction of drug transport across the skin. These studies were conducted employing dialysis membrane. The receptor compartment of the diffusion cell was filled with 25 ml of phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 100 rpm and the temperature was maintained at 37±0.5 °C throughout the experiments.

Accelerated Stress Stability Study

Stability studies were done as per ICH guidelines for 3 months. The optimized micro emulgel was kept in an amber color glass bottle then placed in an accelerated stability chamber at 40±5 °C temperature and 70±5% RH. After three month, gel was tested for pH, Viscosity, and drug content.

RESULT AND DISCUSSION

Organoleptic and Physicochemical Analysis of Extract

Ginger Methanolic extract (GME) was subjected for organoleptic properties analysis. The organoleptic properties and physicochemical analysis of the obtained extract are tabulated in Table 4.

Table 4. The organoleptic properties and physicochemical analysis of extracts

Parameters	**GME**
Color	Yellowish brown
Odor	Odorless
Taste	Pungent
Texture	Free flowing powder
pH	
1% Solution	5.4
10% Solution	4.1
Foreign content	0%
LOD	4.51%
Ash values	
Total Ash value	18.22%
Acid insoluble ash value	3.17%
Water soluble ash value	3.67%
Sulphated ash value	0.99%
Extractive values	
Alcohol-soluble extractive	0.90%
Water-soluble extractive	1.08%
Heavy metals estimation (present)	Absent
Pesticide residues	Absent

Organoleptic evaluation include the assessment of sensory attributes such as taste, odour, appearance, and mouthfeel. Organoleptic testing is a necessary procedure that products must undergo in order to ascertain their compliance with the predetermined standards set by the firm and the expectations of the client. The evaluation of the sensory attributes of a product is of utmost importance in determining its potential for successful marketing and shelf life. Examining the sensory encounter of a product remains crucial, notwithstanding study findings that establish its safety and fulfilment of nutritious claims. All sensory modalities, including taste, smell, touch, sight, and hearing, are engaged in the process. The extract satisfied the standards for satisfactory sensory quality.

The present study used physicochemical analysis to examine the chemical characteristics of the test compounds. These substances are acknowledged as significant structural components that have a role in penetration, irritation, or sensitization. The physicochemical examination of plant pharmaceuticals is necessary to determine whether medications have been tampered with or polluted. The determination of the overall ash content of various pharmaceutical substances is of utmost importance in order to ascertain their effectiveness and purity. The phrase "foreign organic matter" is used to denote any substance that has any of the aforementioned constituents. In addition to the components explicitly mentioned in the definition and description, or the components for which the limit is specified in the particular monograph, it is possible for additional components derived from the organ or organs used in the production of the drug to be present.

The presence of inorganic residues in herbal remedies is often indicated by their ash content. Silica, carbonate, and phosphate are typical instances of inorganic waste products. These markers are

essential indicators of both the effectiveness and credibility of herbal medicines. Given the circumstances, it is essential to conduct routine testing and analysis of heavy metals, particularly within an industrial context. The association between heavy metal poisoning and various adverse effects on the brain and central nervous system, as well as diminished energy levels, alterations in blood composition, and harm to essential organs such as the lungs, kidneys, and liver, has been established.

There is a correlation between the presence of pesticide residues in food and the occurrence of several health conditions, including cancer, liver and nervous system disorders, as well as visual impairment. The potential long-term consequences include a potential decrease in sperm count and fertility, an elevation in cholesterol levels, an increased susceptibility to newborn mortality, and a range of metabolic and genetic diseases. All of the characteristics fell within permissible limits and were deemed favourable for consideration as a medication candidate within the pharmaceutical field.

Microbial Content Determination

The results of microbial content determinations are exemplified in Table 5.

Table 5. The microbial content determinations of extracts

Parameters	**Observation**
Escherichia coli	Absent
Salmonella spp	Absent
Shigella spp	Absent
Pseudomonas aeruginosa	Absent
Staphylococcus aureus	Absent

The establishment of comprehensive standards for the identification and evaluation of microorganisms present in herbal treatments is of utmost importance in mitigating potential health risks to patients. The presence of moulds and coliforms linked with faecal matter suggests the potential for contamination. A study was conducted to evaluate the microbiological contamination of herbal remedies. It was noted that all of the values recorded were found to be within the permissible limits, and no trace of microbial material was detected(Hashimi et al., 2020; Kumatia et al., 2021; Sarraf et al., 2017).

Preliminary Phytochemical Screening of Extract

Phytochemical screening refers to the scientific method of analysing, studying, extracting, and testing with distinct classes of phytoconstituents contained in different areas of the base in order to find new medications. It would then be possible to remove the base's active ingredients for further study. Finding the bioactive components found in medicinal plants is a crucial step towards the discovery and development of novel medications, and the first step in this process is the screening of phytochemicals. The results of preliminary phytochemical screening are tabulated in Table 6. The presence of different phytochemicals in the extract indicates its significant role in different diseases.

Table 6. The results of preliminary phytochemical screening of extract

Chemical Test	**GME**
Test for Carbohyadrate	
Molisch Test	Absent
Test for reducing sugars	
i) Benedict’s test	Absent
ii) Fehling’s test	Present
Test for monosaccharides	

i) Barfoed's test	Absent
Test for Proteins	
i) Biuret test	Absent
ii) Million's test	Absent
iii) Xanthprotein test	Absent
iv) Test for Sulphur containing protein	Absent
v) Precipitation test:	Absent
Test for amino acids	
i) Ninhydrin test	Absent
ii) Tyrosine test	Absent
iii) Tryptophan test	Absent
iv) Cystein test	Absent
Test for Fats and Oil	
i) Solubility test	Absent
ii) Saponification test	Absent
Test for Steroids	
i) Salkowski test	Present
ii) Liebermann – Burchard reaction	Present
iii) Liebermann's reaction	Present
Test for Cardiac Glycosides	
i) Test for deoxysugars (Keller - Killiani test)	Absent
ii) Legal's test (Test for cardenoloids)	Absent
Test for Anthraquinone Glycosides	
i) Borntrager's test	Absent
ii) Modified Borntrager's test	Absent
Test for Saponin Glycoside	
i) Foam test	Present
ii) Test for cyanogenetic Glycoside	Present
Test for Alkaloids	
i) Dragendorff's Test	Present
ii) Mayer's test	Present
iii) Wagner's test	Present
iv) Hager's test	Present
Test for Tannins and Phenolic compounds	
i) Ferric Chloride test	Absent
ii) Lead acetate test	Absent
iii) Dilute Iodine test	Absent
iv) Dilute nitric acid test	Absent
v) Dilute potassium permanganate solution test	Absent
vi) Gelatin test	Absent
vii) Bromin test	Absent
viii) Acetic acid test	Absent
Test for Flavonoids	
i) Shinoda test	Present
ii) Lead acetate test	Present
iii) Ferric choride test	Present
iv) Alkali Test	Present

Quantitative Estimation of Phytoconstituents

Quantitative analysis determines the amount of a phytochemical, as well as its concentration, that is present in a sample of extract. Results obtained through quantitative analysis are tabulated Table 7.

Table 7. Quantitative estimation of different phytoconstituents from extract

Sr. No.	Parameters	Results (%)
1	Carbohydrate	1.09
2	Protein	0.11
3	Saponin	10.37
4	Steroids	11.45
5	Alkaloids	09.99
6	Flavonoids	12.78
7	Tannin	1.67
8	Total Phenolics	1.52

The quantitative estimate was conducted using spectrophotometry, and the absorbance curves were generated by using progressively higher quantities of fractions ranging from 10 mg/mL to 100 mg/mL. The incorporation of a carbohydrate-rich diet is crucial for the preservation of optimal physical well-being. Carbohydrates play a crucial role in sustaining life as they serve as a primary source of glucose for the body. This glucose is then converted into the necessary energy required to facilitate physiological functions and enable engagement in physical activities. Carbohydrates serve as the predominant energy source inside the human body, playing a crucial role in providing fuel to various organs and tissues such as the brain, kidneys, heart muscles, and the central nervous system. Fibre, as a kind of carbohydrate, plays a crucial role in facilitating the process of digestion, while also promoting satiety and regulating blood cholesterol levels(Kurian et al., 2021; Manik Sharma et al., 2017; Sharma et al., 2020). The total carbohydrate content in extract was found to be 1.09 GME. Plant proteins, similar to proteins in general, serve a variety of enzymatic, structural, and functional purposes (photosynthesis, biosynthesis, transport, immunity, etc). In addition to this, they serve as storage media to satisfy the needs of growing seedlings in terms of both development and nutrition(Tsubanova & Trutaieva, 2021). The total protein content in extract was found to be 0.11 in GME. Saponins reduce the levels of lipids in the blood, which in turn lowers the risk of cancer and the glucose response in the blood. Inhibition of dental cavities and platelet aggregation, therapy of hypercalciuria in humans, and antidote for acute lead poisoning are some of the potential applications for a diet rich in saponin(Paseban et al., 2020). The total saponin content in extract was found to be 10.37 in GME.

Plant steroids make up their own distinct class of the chemical substances that may be discovered in both the animal and plant kingdoms. Glucocorticoids are a kind of steroidal substance that is used in the treatment of inflammatory conditions(Ralte et al., 2021). The total steroids content in extract was found to be 11.45 in GME. It can be concluded that this plants might be less effective in inflammatory diseases. Alkaloids serve two purposes in plants: they repel predators and they control the plant's development. Within the realm of medicine, alkaloids are most often used as anaesthetics, cardioprotective agents, and anti-inflammatory pharmaceuticals. Morphine, strychnine, quinine, ephedrine, and nicotine are examples of well-known alkaloids that are often used in therapeutic contexts(Sheikh et al., 2016). The total alkaloid content in extract was found to be 09.99 in GME.

Enhancing one's vascular health and lowering one's disease risk may be accomplished by consuming herbs and drinks that have high levels of flavonoid compounds. It has been shown that the intake of them is connected with an improvement in endothelial function via the activation of protein kinase

B (Akt). This improvement is brought about by vascular endothelial nitric oxide synthase(Do et al., 2019). The total flavonoids content in extract was found to be 12.78 in GME. Tannins may be found in many different parts of plants, including the bark of trees, wood, leaves, buds, stems, fruits, seeds, roots, and galls. Tannins have a protective role in a variety of plant structures, including but not limited to those listed above. Tannins prevent the tree from being infected by bacteria or fungus when they are kept in the bark of the tree. Tannins may also be found in wood(Nayeem et al., 2022). The total tannins content in extract was found to be 1.67 in GME.

Important plant elements called phenolic compounds possess redox characteristics, which are responsible for the antioxidant action of plants. The hydroxyl groups that are present in plant extracts are the ones that are responsible for the facilitation of the scavenging of free radicals(Pulok K. Mukherjee, 2019; Oyedemi et al., 2017; Siddiqui et al., 2017). The total phenolic content in extract was found to be 1.52 in GME. From above screening it was observed that the extract contains optimum concentrations of different phytoconstituents to exert potential pharmacological activities.

In vitro anti-inflammatory activity

Significant stabilization of Human Red Blood Cell (HRBC) membranes is of paramount importance in the field of biomedical research and drug development. The HRBC membrane, which closely mimics the structure and properties of human cell membranes, is frequently used as a model system to investigate interactions between bioactive compounds, pharmaceuticals, and biological membranes. Achieving stable and well-characterized HRBC membranes is vital for studying drug-membrane interactions, understanding cellular transport mechanisms, and assessing the potential toxicity or pharmacological effects of compounds. It also plays a crucial role in the design and evaluation of drug delivery systems, where the interaction between drug carriers and cell membranes can significantly impact the efficiency and safety of drug delivery. Furthermore, understanding the stabilization of HRBC membranes is integral to addressing complex issues related to drug bioavailability, cellular uptake, and toxicity, ultimately aiding in the development of safer and more effective therapeutic agents. GME at different concentrations (200 and 400 mg/mL) showed significant stabilization towards HRBC membranes. The results were tabulated in Table 8.

Table 8. The results of *in vitro* anti-inflammatory activity

Sr. No.	Drug name (mg/mL)	% protection
03	GME-200	22.34
04	GME-400	29.78
07	Diclofenac sodium (5mg/mL)	39.22

Instrumental Analysis of Extract

FTIR Analysis

In Figure 1, the FTIR spectra of ginger extract is given, which show the characteristic band at 3367.20 cm^{-1} (O-H stretching), 2925.64 cm^{-1} (CH_2 methylene stretch), 1639.34 cm^{-1} (C=O stretch) 1459.69 cm-1 (C=C aromatic ring stretch), 1376.02 cm^{-1} (CH_3 methyl bond), 1029.87 cm^{-1} (C-OH stretch CH_2OH) and 611.94 cm^{-1} (Phenolic O-H bond).

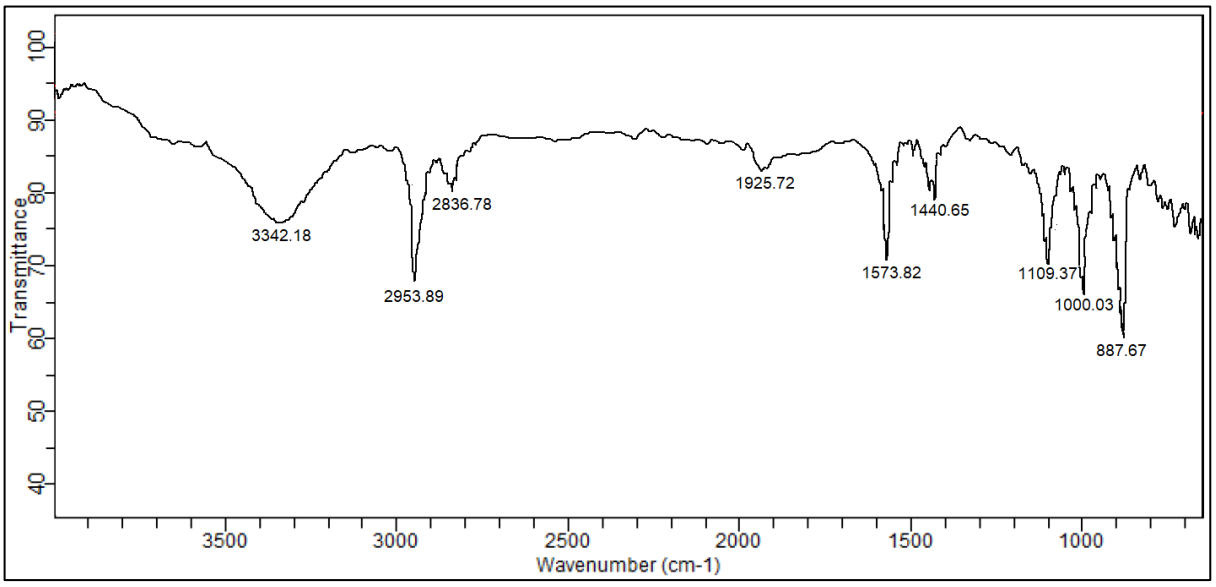

Figure 1. FTIR spectra of Ginger Extract

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) analysis of ginger extract at a scanning rate of 10°C/min revealed a distinct melting endothermic peak at 115.16 ºC, as depicted in Figure 2. This peak indicates the transition from a semi-solid to a liquid state, showing the heat flow associated with this phase change. Furthermore, as the temperature increases, certain components of the plant extract may undergo decomposition, releasing energy in the process. Show small endothermic peak at 237.56 ºC. This decomposition is evident on the DSC curve as an endothermic peak, corresponding to the heat absorbed during this specific thermal event.

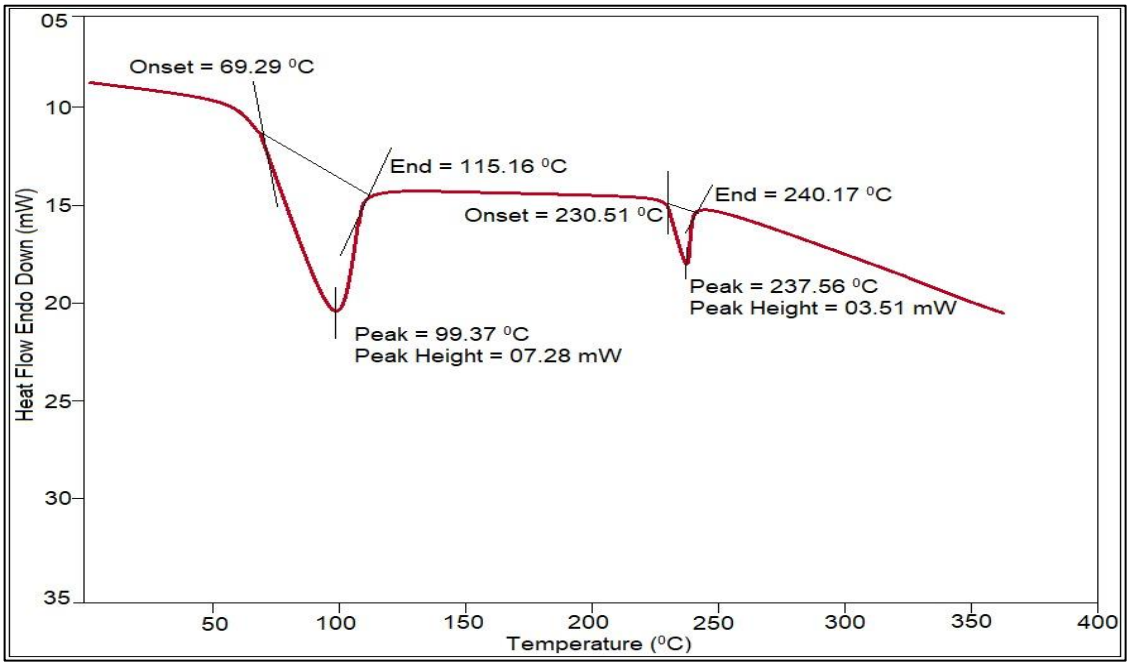

Figure 2. DSC thermogram of Ginger Extract

Extract-Excipients Compatibility Study

FTIR Analysis

In Figure 1, 3-6; the FTIR spectra of ginger extract and physical mixture of all excipients (HPMC K4, Liquid paraffin oil, Tween-80 and PEG-200) is given. The FTIR spectra showed the

characteristic absorption band of ginger extract at 3579.13 cm^{-1} (O-H stretching), 2855.15 cm^{-1} (CH_2 methylene stretch), 1639.34 cm^{-1} (C=O stretch), peak 1461.09 cm^{-1} (C=C aromatic ring stretch), 1377.27 cm^{-1} (CH_3 methyl bond sym), 1047.24cm^{-1} (C-OH stretch (-CH_2OH) and the peak 615.94 cm^{-1} (phenolic O-H bond). All the peaks were found intact and there no significant changes were observed in characteristics peaks of pure extract when compared with pure extract. This indicates the extract is compatible with other excipients.

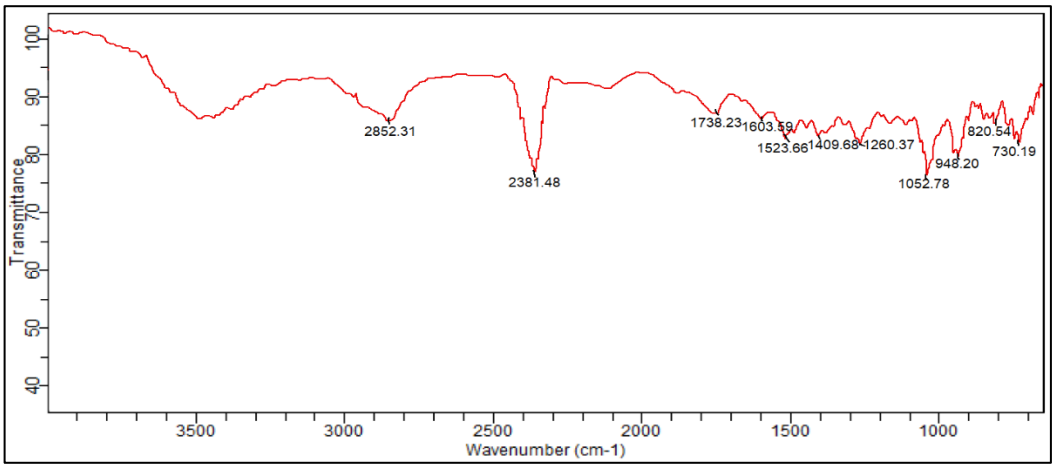

Figure 3. FTIR spectra of HPMC K4

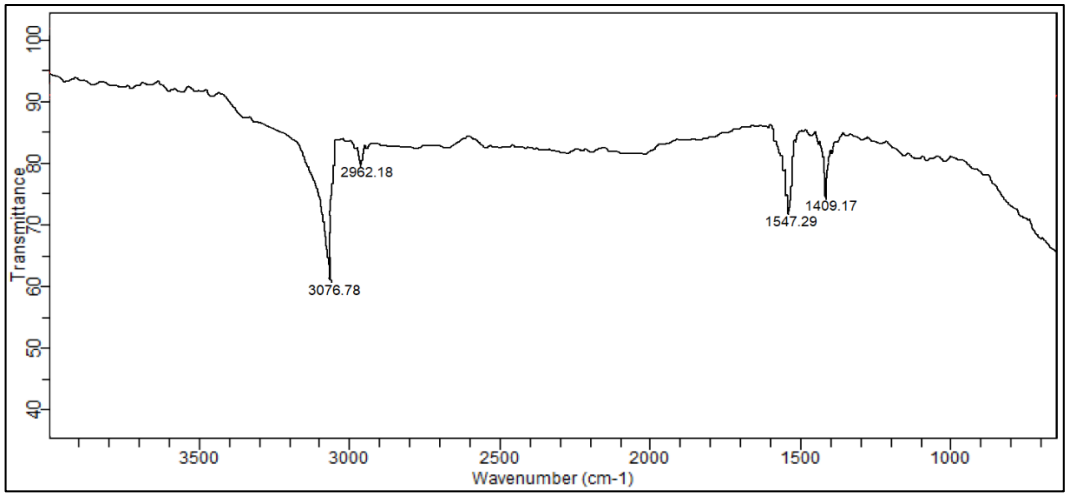

Figure 4. FTIR spectra of Liquid paraffin oil

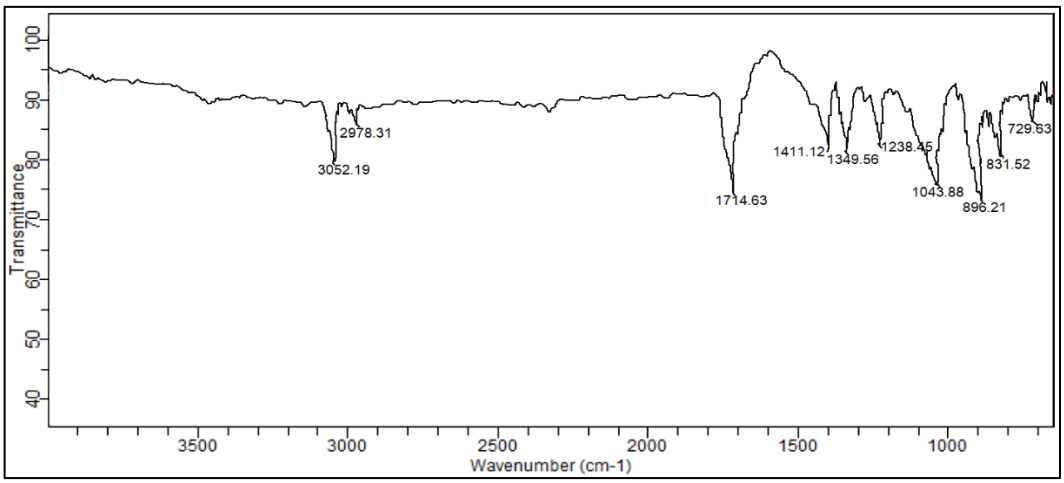

Figure 5. FTIR spectra of PEG-200

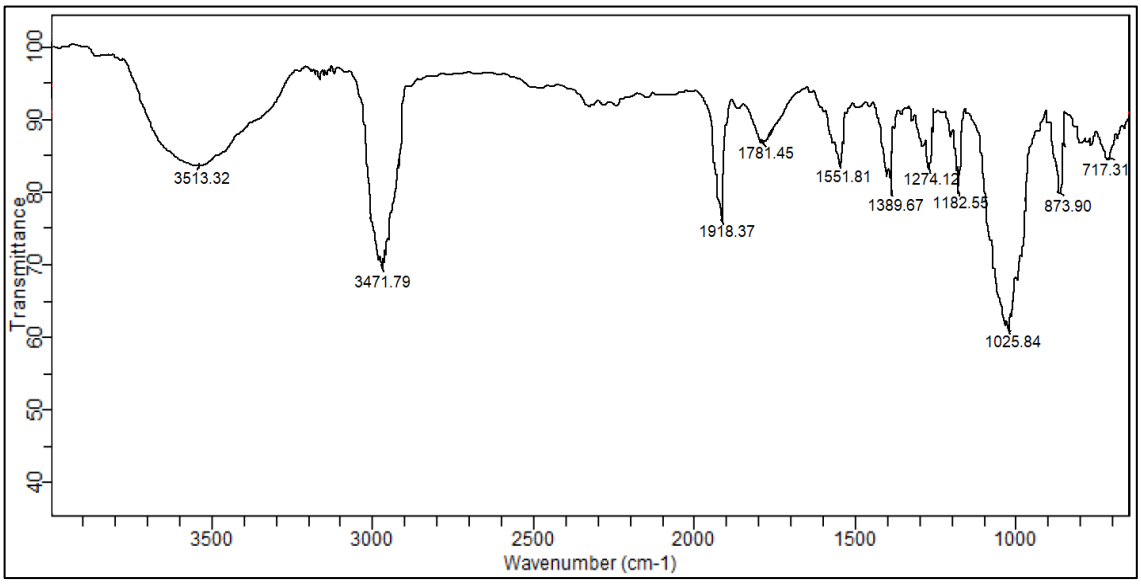

Figure 6. FTIR spectra of Tween 80

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) has been suggested as a quick method for assessing the physical and chemical interactions between components in a formulation. It involves comparing the thermal profiles of pure substances with those of a 1:1 physical mixture to determine suitable excipients for compatibility. In this context, when examining the DSC thermograms, the drug displayed a distinct melting point at 115.16 °C. HPMC K4 and liquid paraffin oil exhibited their respective melting points at 78.18 °C and 62.19 °C in their DSC thermograms, with no observable shifts in these peaks when mixed with venlafaxine HCl. This lack of shift suggests compatibility between the drug and both HPMC K4 and liquid paraffin oil. The comparison of DSC thermograms for ginger extract, individual excipients, and drug-excipient mixtures can be seen in Figures 7-10.

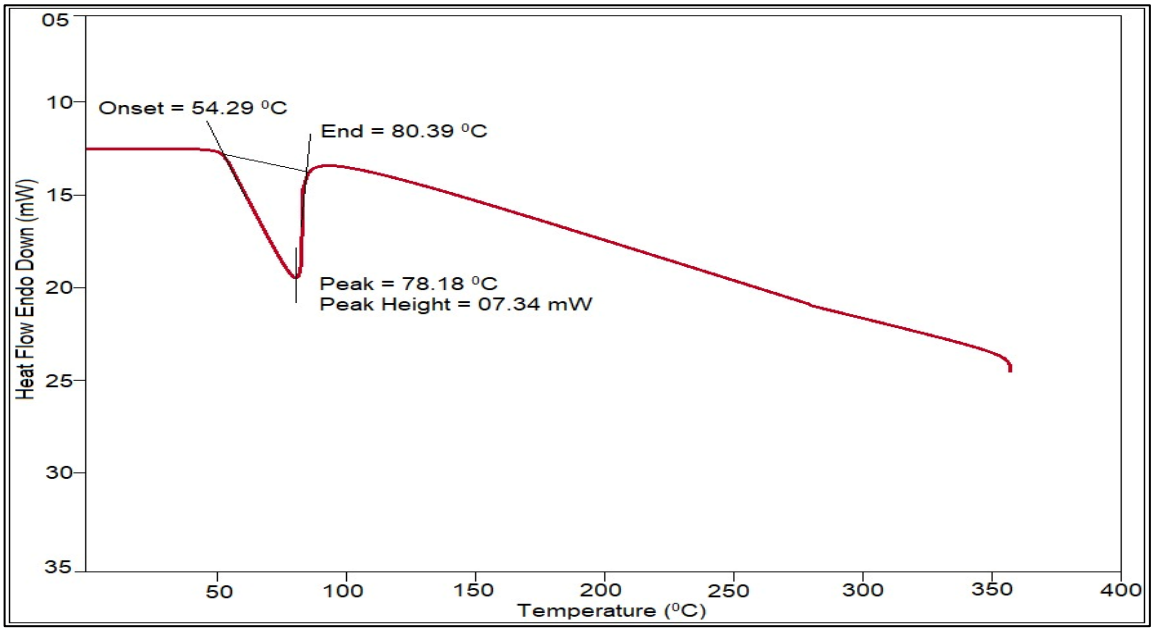

Figure 7. DSC thermogram of HPMC K4

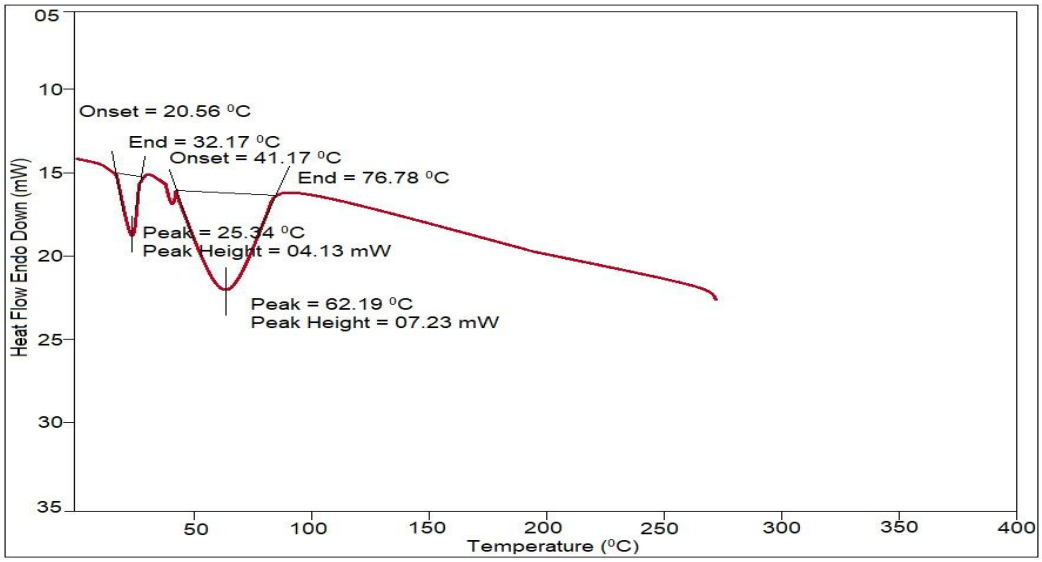

Figure 8. DSC thermogram of Liquid paraffin oil

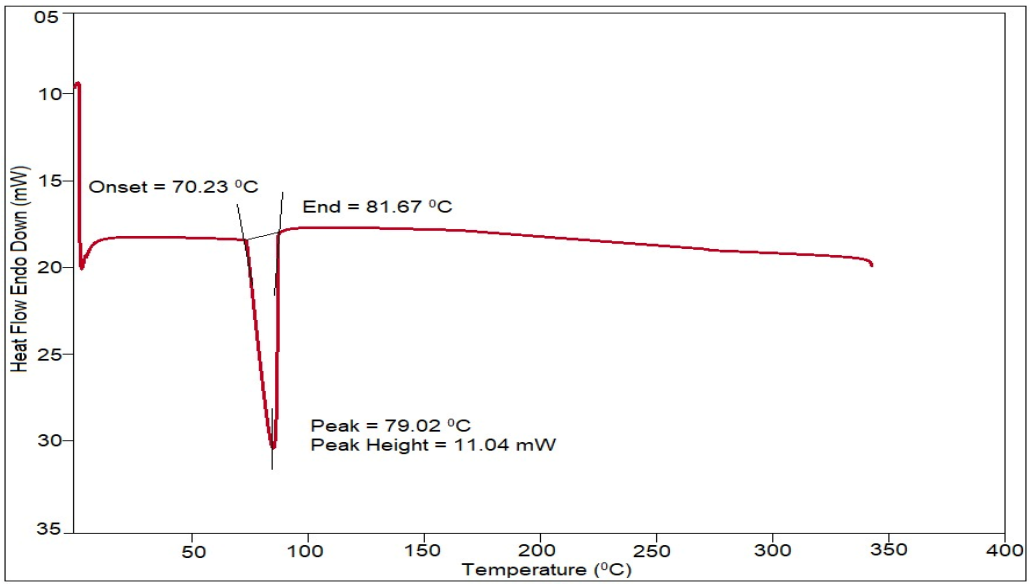

Figure 9. DSC thermogram of PEG-200

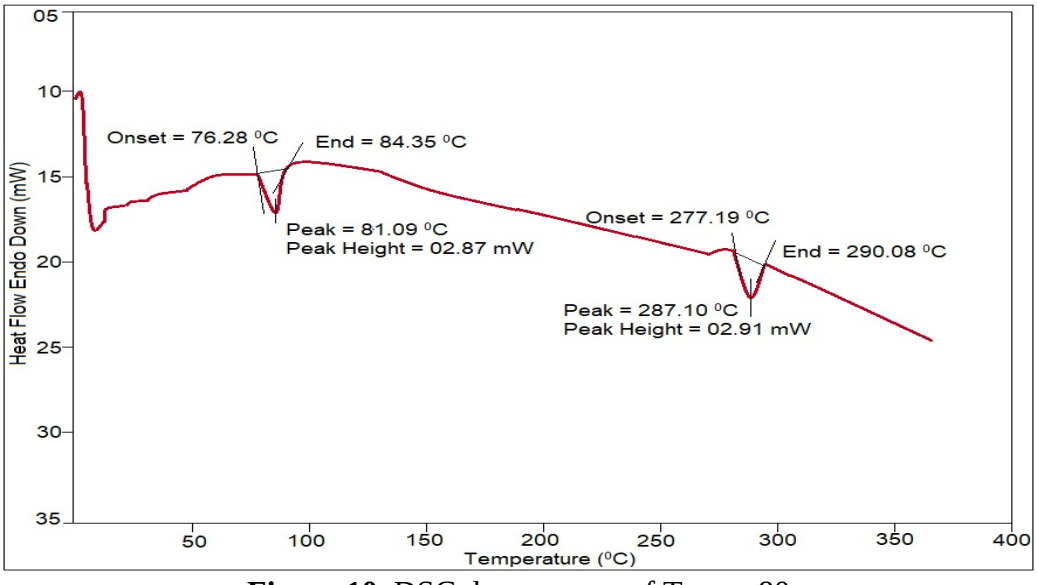

Figure 10. DSC thermogram of Tween 80

Screening of Oil, Surfactant and Co-surfactant (Solubility Study)

Screening of oils, surfactants and co-surfactants are based on their solubility profile for ginger extract as shown in Table 9. The LPO was selected oil, Tween 80 as surfactant and PEG-200 as co-surfactant.

Table 9. Solubility of Ginger extract

Sr. No.	Name of Excipients	Solubility (mg/ml)
1	Liquid paraffin oil	32.12
2	Tween 80	36.02
3	PEG-200	34.58

Formulation and selection of Nanoemulsion and Nanoemulgel

On the basis of their visual observation like transparency and viscosity, 14 formulations were selected out as per factorial design (Table 1) for preparing ginger extract loaded nanoemulsion. For the preparation of nanoemulgel HPMC K4 was used as gelling agents in ratio 1.0% (gels made with specified concentration range in the water).

Formulation Design

The two factors with lower, middle and upper design points in coded and un-coded values are shown in table. The ranges of responses Y1 and Y2 were 258-461 d.nm and 1431-1865 cps respectively. All the responses observed for nine formulations prepared were fitted to main effect model, which was found as the best fitted model for Y1 and Y2, using Design Expert® software. The values of R^2 SD and % CV are given in (Table 10 and 11), along with the regression equation generated for each response. The results of ANNOVA in (Table 10 and 11), for the dependent variables demonstrate that the model was significant for all the response variables. It was observed that independent variables X1, X2 and X3 had a positive effect on the entrapment efficiency and an desired particle size of nano-formulation i.e. nano-emulsion was achieved.

Model Assessment

After putting the data in Design Expert® software for, Fit summary applied to data in that Main Effect Model had been suggested by the software for all the responses. The statistical evaluation was performed by using ANNOVA. Results are shown in (Table 10 and 11). The coefficients with more than one factor term in the regression equation represent interaction terms. It also shows that the relationship between factors and responses is not always linear. When more than one factor are changes simultaneously and used at different levels in a formulation, a factor can produce different degrees of responses.

Table 10. Results of Analysis of Variance for Measured Response (Particle Size)

Parameters	Values
Model	Quadratic Model (Significant)
Model p-value	0.054
Standard Deviation	6.89
Mean	76.67%
CV	09.07%
R^2	0.8021
Adequate Precision	7.7120
Regression Equation	Y1 = 774.63 X1 + 713.37 X2 + 653.37 X3 - 2989.60 X1 X2 – 2647.20 X1 X3 - 2877.87 X2 X3 +5594.40 X1 X2 X3

Table 11. Results of Analysis of Variance for Measured Response (Viscosity)

Parameters	**Values**
Model	Quadratic Model (Significant)
Model p-value	0.054
Standard Deviation	7.68
Mean	75.19%
CV	09.67%
R^2	0.8174
Adequate Precision	7.963
Regression Equation	Y1 = 694.63 X1 + 703.37 X2 + 643.37 X3 - 2979.60 X1 X2 – 2337.20 X1 X3 - 2737.87 X2 X3 +5554.40 X1 X2 X3

Response Surface Plot Analysis

From the 3D response surface plot (Figure 11) and Nanoparticles being nanoparticulated structures, formulation batch amongst all the design batches giving least particle size will be preferred more and selected as an optimized batch. Where F6 Design Batch, with a Smix concentration of about 32.16% and paraffin oil concentration 2.64%, shows the least particle size i.e. 228 nm.

From the 3D response surface plot (Figure 12) and the regression coefficient values of factors, it was concluded the viscosity of nanoemulgel increases with an increase in the concentration of liquid paraffin oil and HPMC K4M. Interaction and nonlinearity was not observed. The results also indicated that the HPMC K4M was given a more significant effect on viscosity as compared to liquid paraffin oil.

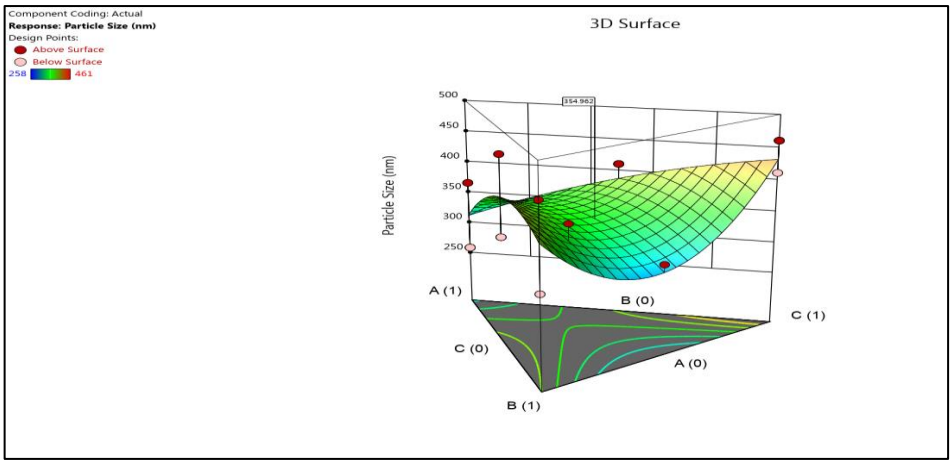

Figure 11. Response surface plots for X1, X2 and X3 on Mean Particle Size (Y1)

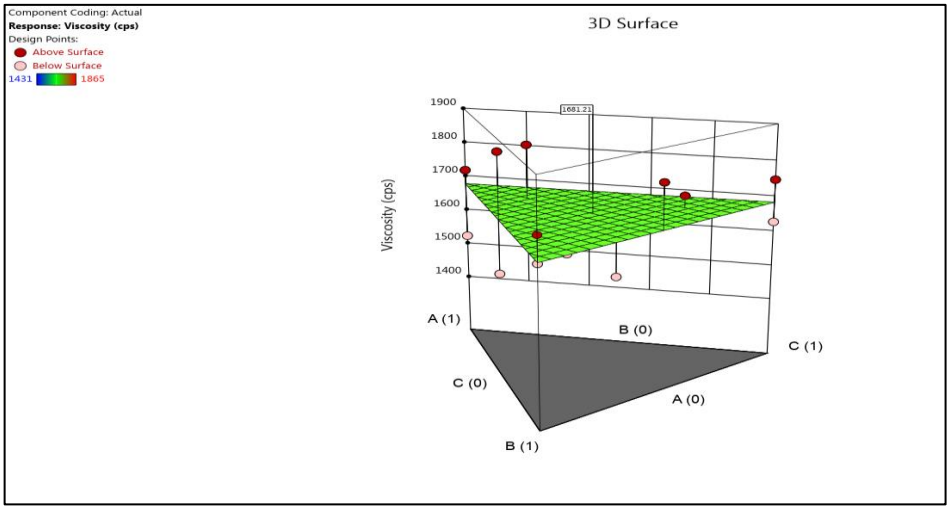

Figure 12. Response surface plots for X1, X2 and X3 on Viscosity (Y2)

Characterization of Nanoemulsion

Physical characterization

All formulations are clear, transparent, and homogenous and no grittiness and no clogs were found and suitable consistency.

Droplet Size and Size Distribution

The particle size of the PNs is a fundamental factor because it decides the rate and extent of drug release as well as drug absorption. The smaller particle size offers a larger interfacial surface area for drug absorption and improves the bioavailability. The calculation of polydispersity index takes into account the particle mean size, the refractive index of the solvent, the measurement angle and the variance of the distribution. Low polydispersity index value might be associated with a high homogeneity in the particle population, whereas high polydispersity index values suggest a broad size distribution or even several populations. The optimized formulation batch (F5) showed mean particle size 228.6 nm with PDI 0.282.

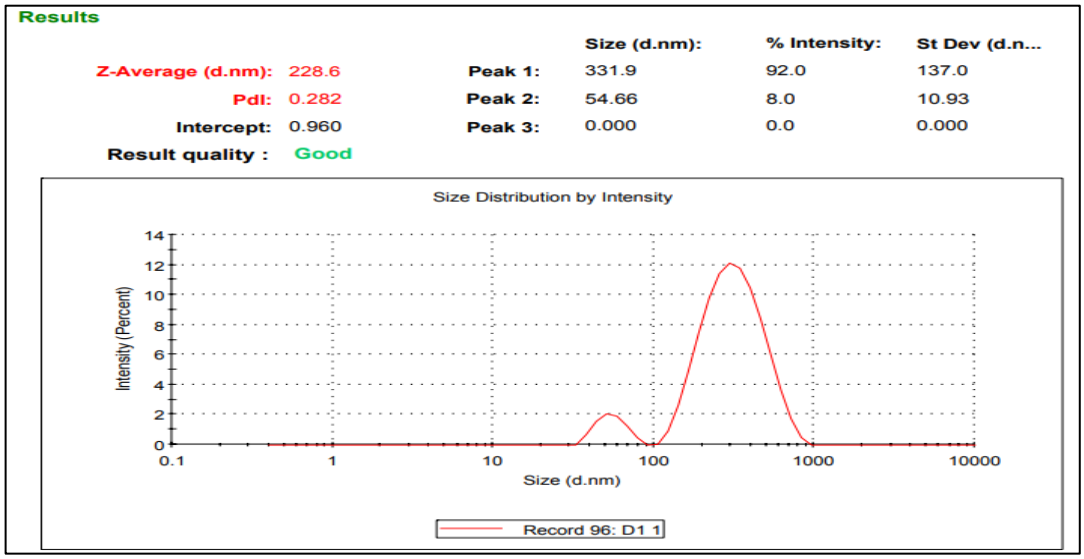

Figure 13. Particle size of F5 formulation

Zeta potential analysis

The zeta potential values of extract loaded nanoemulsion that was found to be -27.3 mV. The high value of zeta potential confirms the stability of nanoemulsion.

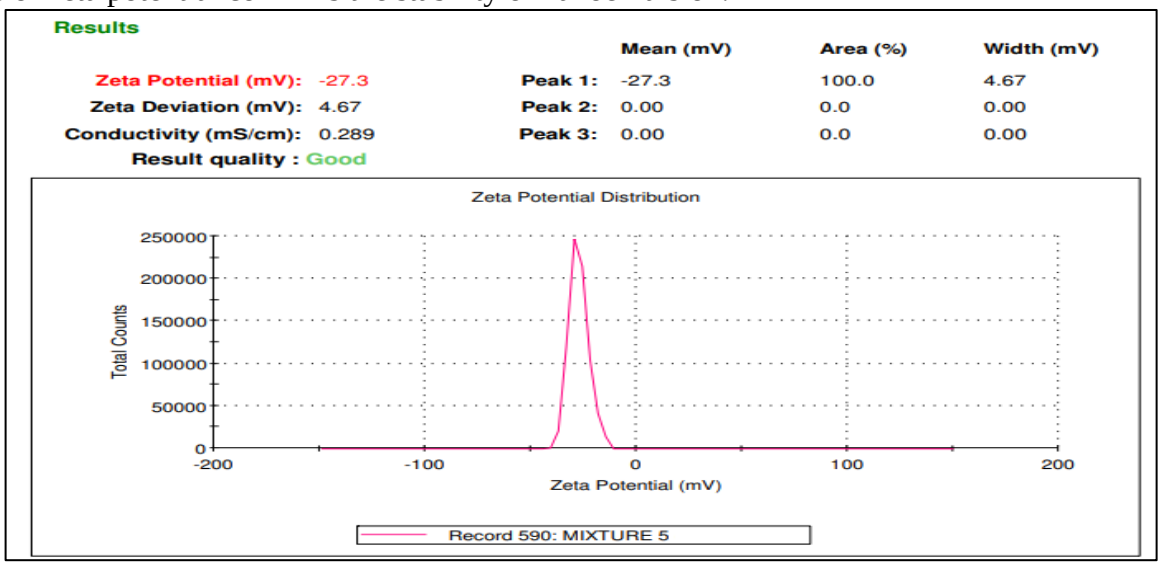

Figure 14. Zeta Potential of F5 formulation

Surface Morphological Study

Surface morphology of the nanoemulsion was evaluated using SEM/TEM from which it can be seen that the droplets have smooth surfaces. Droplets show spherical shape with size 200 nm.

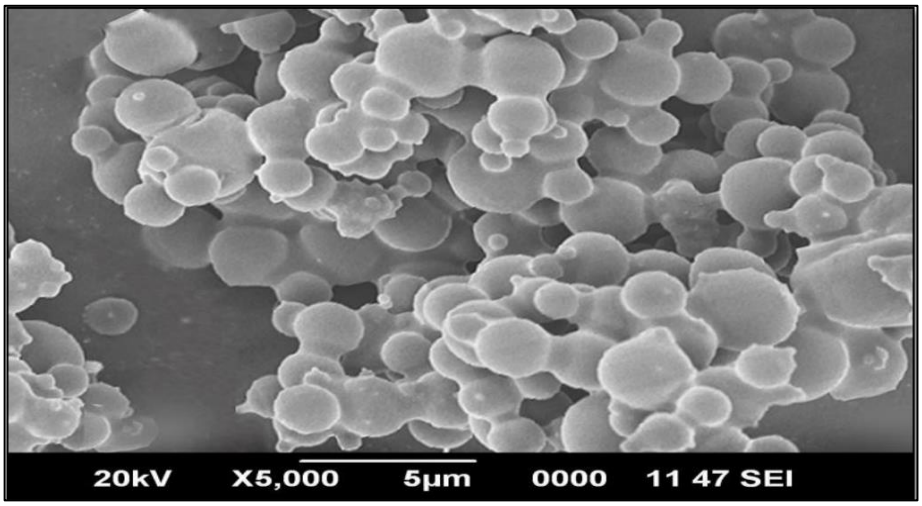

Figure 15. SEM image of F5 formulation

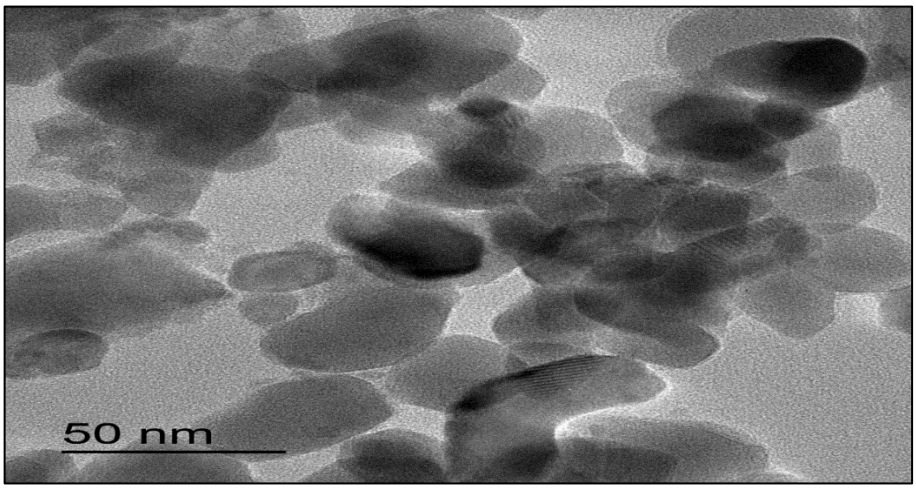

Figure 16. TEM image of F5 formulation

Characterization of Nanoemulsion Gel

Measurement of pH

The pH value for the selected NE and its gel (F1-14) formulation was found to be 6.21-6.96 and 5.21-5.85 as shown in Table 12. The pH of the NE was found to be within the range of pH of skin (5-7) and would not cause any irritation to the skin. Thus, prepared NE formulations are suitable for skin application and the formulated nanoemulgel are also suitable for skin application.

Table 12. Characterization of Nanoemulgel

Sr. No.	Formulation code	pH value	pH value	Viscosity (cps)
1	F1	6.29±0.02	5.35±0.03	1431±2.33
2	F2	6.45±0.03	5.69±0.07	1530±2.28
3	F3	6.55±0.04	5.36±0.04	1675±2.21
4	F4	6.89±0.01	5.67±0.01	1721±2.29
5	F5	6.21±0.03	5.30±0.03	1681±2.31
6	F6	6.39±0.02	5.39±0.05	1525±2.35
7	F7	6.54±0.01	5.49±0.02	1635±2.36

8	F8	6.75±0.04	5.66±0.04	1751±2.39
9	F9	6.85±0.06	5.76±0.07	1865±2.33
10	F10	6.39±0.03	5.21±0.01	1745±2.27
11	F11	6.65±0.02	5.85±0.05	1625±2.26
12	F12	6.84±0.04	5.63±0.03	1809±2.21
13	F13	6.92±0.05	5.49±0.02	1715±2.28
14	F14	6.96±0.03	5.51±0.03	1678±2.31

Measurement of Viscosity

Brookfield viscometer was used to measure the viscosity of nanoemulsion and nanoemulgel (NE gel) at different spindle speeds. Viscosity reveals the rheological properties of all nanoemulsion formulation. All formulation shows shear thinning effect as the shear stress increased the viscosity was decreased. Formulation F5 was found more viscous than other formulations.

Drug Content of Nanoemulgel

Drug content in the nanoemulgel (which is made by adding gelling agent in NE) is supposed to be decreased in some extent because of gelling agent which occupies some volume as it swells in formulations so it was determined by UV spectrometer at 281 nm for the same. The range of percentage drug content of nanoemulsion Gel was 82.77% to 96.91% as shown in Table 13. The percentage drug content of formulations was within a permissible range.

Spreadability

Spreadability determined as % increase in area of gel upon pressing with certain weight. All formulations have shown good Spreadability.

Table 13. Drug content and spreadability of Nanoemulgel

Sr. No.	Formulation code	Drug Content (%)	Spreadability (%)
1	F1	86.19±0.27%	89.18±1.10%
2	F2	87.91±0.29%	87.45±1.14%
3	F3	88.92±0.30%	86.36±1.16%
4	F4	87.38±0.31%	89.91±1.22%
5	F5	96.91±0.32%	95.21±1.25%
6	F6	91.28±0.26%	90.35±1.27%
7	F7	92.38±0.28%	90.89±1.29%
8	F8	93.47±0.32%	91.26±1.28%
9	F9	94.21±0.31%	92.65±1.32%
10	F10	95.38±0.30%	93.78±1.26%
11	F11	91.36±0.28%	89.89±1.24%
12	F12	89.38±0.29%	93.65±1.22%
13	F13	90.65±0.35%	92.45±1.29%
14	F14	91.35±0.39%	91.36±1.32%

***In-vitro* Release Study**

Ginger extract loaded emulgel of all batches were studied *in-vitro* for drug release using Franz diffusion cell. For batches F1-F14, the maximal drug release was found to be about 87.36±2.21%-94.24±3.19% as shown in Table 14. The *in-vitro* release of produced emulgel in phosphate buffer saline (PBS) (PH 7.4) at 37 ºC was studied. Nanoemulgels were dialyzed for 60 min. The quantity of drug released was measured by using a UV-visible spectrophotometer to measure absorbance. A burst drug release was observed in the beginning, which may be due to the smaller particle size that

attributed to the large surface area of the emulgel, apart from it diffusion of the drug from the outer shell of the emulgel may be responsible for initial burst release.

Table 14. *In-vitro* release profile of nanoemulgel

Sr. No.	Time (Min.)	nanoemulgel (F5)
1	0	0
2	60	27.42±1.63
3	120	41.04±2.14
4	180	46.62±1.96
5	240	61.52±2.23
6	300	91.02±2.65
7	360	94.24±2.69

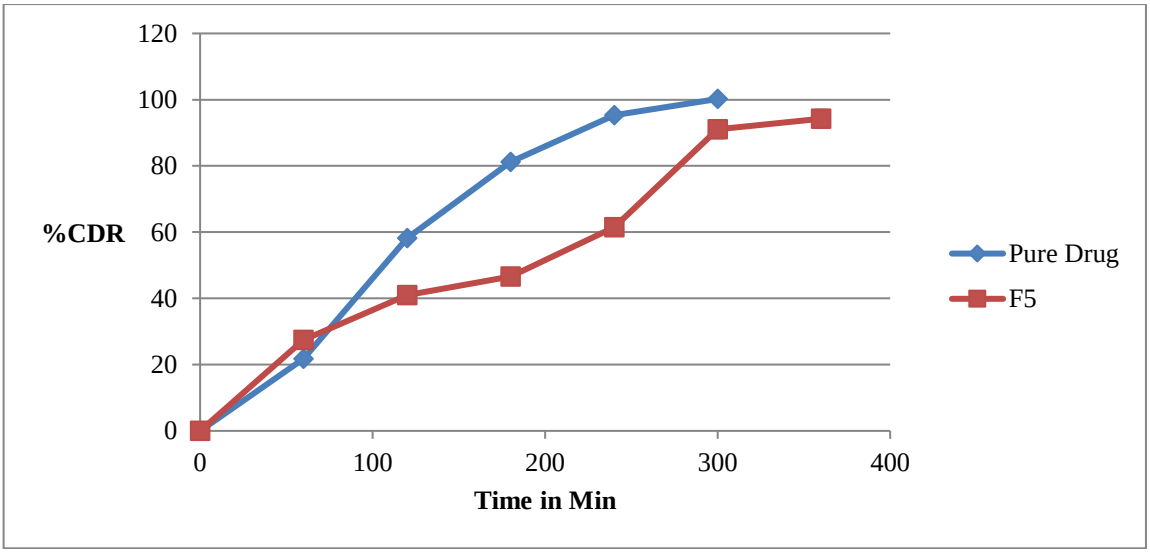

Figure 17. *In-vitro* drug release study of F5

Accelerated Stability Study

The optimized emulgel were subjected to stability studies and the results are given in Table 15. Based on these results it is revealed that, Ginger loaded nanoemulgel (Formulation batch F5) were found to be stable formulation at the given temperature and humidity condition.

Table 15. Stability study of parameters of the optimized formulation (F5)

Parameters	Initial Month	1st Month	2nd Month	3rd Month
pH	5.31 ± 0.03	5.33 ± 0.04	5.29 ± 0.02	5.33 ± 0.03
Viscosity (cps)	1681 ± 2.31	1675 ± 2.28	1698 ± 2.33	1682 ± 2.30
Drug content (%)	96.85 ± 0.32	96.12 ± 0.35	96.78 ± 0.31	96.98 ± 0.39

Discussion

The pre-formulation studies provide valuable information about the physical characteristics of the ginger extract, indicating that it is a yellow-brown resinous amorphous powder with a pungent taste. The FTIR analysis reveals key functional groups present in the extract, such as O-H stretching, CH2 methylene stretch, C=O stretch, C=C aromatic ring stretch, CH3 methyl bond, C-OH stretch, and Phenolic O-H bond. These details are crucial for understanding the molecular structure of the extract. The Differential Scanning Calorimetry (DSC) analysis further elucidates the thermal behavior of the ginger extract, showing a distinct melting endothermic peak at 115.16ºC, signifying the transition from a semi-solid to a liquid state. Additionally, a small endothermic peak at 237.56 ºC indicates the decomposition of certain components.

The compatibility study with excipients, assessed through FTIR and DSC, suggests that the ginger extract is compatible with HPMC K4, liquid paraffin oil, Tween-80, and PEG-200. This is crucial for formulating a stable product. The solubility study aids in selecting suitable components for the formulation, with LPO, Tween 80, and PEG200 chosen as the oil, surfactant, and co-surfactant, respectively. Moving on to the formulation of nanoemulsion and nanoemulgel, a factorial design approach is employed, considering factors like HPMC K4 concentration, paraffin oil concentration, and Smix concentration. The response surface plots and regression equations derived from the ANOVA results provide insights into the effects of these factors on particle size and viscosity.

The physical characterization of the nanoemulsion reveals transparent and homogenous formulations with suitable consistency. The optimized formulation (F5) exhibits a mean particle size of 228.6 nm with good stability, as indicated by the high zeta potential value of -27.3 mV. The nanoemulsion gel is characterized by measuring pH, viscosity, drug content, spreadability, and in-vitro release. The pH values fall within the skin-compatible range, and viscosity measurements indicate a shear-thinning effect. The drug content remains within an acceptable range, and the spreadability of all formulations is satisfactory.

The *in-vitro* release study demonstrates sustained release characteristics, with the optimized formulation (F5) showing a gradual release of ginger extract over time. Finally, the accelerated stability study confirms the stability of the optimized nanoemulgel over three months, as evidenced by consistent pH, viscosity, and drug content values. In conclusion, the comprehensive scientific analysis and characterization of the ginger extract, along with the systematic formulation and evaluation of nanoemulsion and nanoemulgel, provide a solid foundation for the development of a stable and effective ginger extract delivery system with potential applications in various therapeutic and cosmetic formulations.

Conclusion

In present study, we have developed and evaluated nanoemulgel formulation of Ginger extract using simple latex design (SLD) approach. From quantitative phytochemical screening, it was observed that the extract contains optimum concentrations of different phytoconstituents to exert potential pharmacological activities. GME at different concentrations (200 and 400 mg/mL) showed significant stabilization towards HRBC membranes i.e. 22.34 and 29.78% protection, respectively. The thorough pre-formulation studies, including FTIR and DSC analyses, provided crucial insights into the physical and molecular characteristics of ginger extract. The compatibility studies with excipients and the subsequent formulation of nanoemulsion and nanoemulgel demonstrated the feasibility of creating a stable delivery system for ginger extract. The optimized formulation (F5) exhibited favorable characteristics, such as a mean particle size of 228.6 nm, high zeta potential, and sustained release properties. The comprehensive evaluation, including pH, viscosity, drug content, and stability assessments, collectively establishes a robust foundation for the development of a stable and effective ginger extract delivery system, with potential applications in therapeutic formulations. The optimized formulation will be subjected for *in vivo* anti-inflammatory studies.

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