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Formulation and In-Vitro Evaluation of Naringin Loaded Gel for Anti-Inflamatory Activity

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ARTICLE INFO | **ABSTRACT** | **ORIGINAL RESEARCH ARTICLE**

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Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease that involves a sustained inflammatory response of the joints and the eventual destruction of the connective tissues. Naringin, a naturally occurring flavonoid, has strong anti-inflammatory and antioxidant properties, but is hampered in its therapeutic use by poor solubility and low bioavailability. Hence the present study aimed at the formulation and evaluation of naringin loaded nanoemulsion gel for improving the anti-inflammatory efficacy and transdermal drug delivery for RA.

The aqueous titration method was used to formulate the nanoemulsion with oleic acid as the oil phase, Tween 80 as the surfactant and ethanol as the co-surfactant. To define the nanoemulsion region and the nanoemulsion formulation composition, a pseudo-ternary phase diagram was drawn. The optimized nanoemulsion was incorporated in a Carbopol 934 gel base to get the nanoemulsion gel. Characterized: Particle size, 203.7 nm; Polydispersity index (PDI), 0.277 – 0.390; zeta potential, −27.2 ± 1.3 mV (SK-2); surface morphology is Clear, transparent, smooth and homogeneous nanoemulsion droplets, without aggregation or phase separation; physical stability is No phase separation, turbidity, precipitation or color change after centrifugation and freeze – thaw cycles. Permeation studies of the drug diffusion through the skin was done in vitro using Franz diffusion cell.

The anti-inflammatory activity of the formulation was assessed by protein denaturation assay. The nanoemulsion gel exhibited good protein denaturation inhibitory activity, showing dependence on concentration, which demonstrated that the nanoemulsion gel had a good anti-inflammatory activity and prevented protein degradation. Furthermore, the formulation showed improved permeation and physiological stability as compared to the conventional formulation.

Overall, the developed naringin loaded nanoemulsion gel exhibited enhanced solubility, enhanced transdermal permeation and better anti-inflammatory activity which indicate that it can be used as a potential topical therapeutic system for the management of rheumatoid arthritis.

INTRODUCTION

Overview of Rheumatoid Arthritis

Rheumatoid Arthritis Rheumatoid arthritis (RA) is a chronic, progressive, and systemic inflammatory disorder primarily characterized by persistent inflammation of the synovial joints, leading to gradual joint damage, deformity, functional impairment, and various systemic complications. Ahmad N, et Al 2018 and Alamanos, et al 2005[1-2] Rheumatoid Arthritis Rheumatoid arthritis (RA) is regarded as a multifactorial disorder caused by a combination of genetic susceptibility, environmental influences, hormonal imbalance, and immune system dysregulation. Anand David AV, et al 2021[3] RA primarily affects the joints, but it can also involve several extra-articular organs such as the lungs, heart, blood vessels, eyes, and skin. The persistent inflammatory condition associated with RA further increases the risk of complications including cardiovascular disorders, osteoporosis, anemia, and depression.

The main goals of RA management are to keep the inflammation under control, prevent further joint damage, keep the patient moving and enhance the quality of life of the patient. Prompt diagnosis and treatment are important in preventing permanent joint and structural damage. RA's unpredictable course and intricate pathophysiology has made it a significant target for pharmaceutical research, particularly in the advanced drug delivery system. The delivery of drugs utilizing novel techniques such as nanoparticles, nanoemulsions, and transdermal delivery systems is being investigated. There is a great deal of research focusing on novel techniques, including nanoparticles, nanoemulsions, and transdermal delivery systems, for improved drug bioavailability, reduced systemic side effects, and increased therapeutic efficiency. Ronald, et al. & Ashiq K, et al 2023[4-5]

Epidemiology: RA is a progressive immune disease that affects people all over the world. The prevalence of RA in the world population is less than 1% and varies in relation to genetic background, environmental exposures, socioeconomic status and access to healthcare. Beckett, et al 2002 and Berman AY, et al 2023[6-7]

Global Prevalence of RA: The prevalence of RA is different in various ethnic and continental groups. Higher prevalence may be reported in developed countries because of higher-quality healthcare facilities and early detection methods. New figures, however, indicate that the incidence of the disease is on the rise in developing countries because of greater awareness and diagnosis.

KEY OBSERVATIONS

Global prevalence ranges between 0.5–1%

Indigenous populations in some regions show higher susceptibility due to genetic predisposition. Bhardwaj, et al 2008[8]

Table 1.1: Global Prevalence of Rheumatoid Arthritis

Region	Prevalence (%)	Remarks
North America	0.8–1.2	High awareness and diagnosis rate
Europe	0.5–1.0	Strong genetic association
Asia	0.3–0.7	Increasing trend due to lifestyle changes
Africa	0.2–0.5	Underdiagnosis possible
India	00-0.7	Increasing prevalence in urban areas
Worldwide	0.5–1.0	Average global prevalence

Immunological Factors: In rheumatoid arthritis (RA), different types of immune cells (T lymphocytes, B lymphocytes, macrophages, dendritic cells) get abnormally activated, and produce autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). These autoantibodies are capable of forming immune complexes which are deposited within the synovial membrane and activate the complement system, which start and augment inflammation. Choy, et al 2001[9]

Hormonal Factors: In rare instances, hormones may be responsible for the development and progression of rheumatoid arthritis (RA) – women are almost 3 times more likely to be affected than men. Cytokines and hormones like estrogen and

progesterone help control immune function and production; their cycles can upset immune system balance. Changes in hormone levels throughout the menstrual, pregnancy and menopause periods could impact the severity and progression of RA symptoms. During menopause, the decrease in estrogen is accompanied by increased inflammation, which can lead to worse disease in middle-aged women. Cross, Marita, et al 2010[10]

Current Treatment of Rheumatoid Arthritis: Modern therapy uses conventional medications such as NSAIDs, corticosteroids, anti-rheumatic medications and new biologic medications for current treatment of rheumatoid arthritis. Early treatment is crucial to avoid permanent joint damage and handicap. Aulton, et al 2018[11]

NSAIDs: NSAIDs used as first-line therapy for relief with RA. These medications help to decrease pain, swelling and stiffness by blocking the production of prostaglandins, which are chemicals that cause them. According to Aulton, et al 2018[12] NSAIDs are effective in reducing inflammation rapidly but do not stop the disease from getting worse or damage to the joints.

Examples: Ibuprofen, Diclofenac, Naproxen, Indomethacin

- **Advantages**
  - ✓ Rapid pain relief
  - ✓ Reduces inflammation
- **Limitations / Side effects**
  - ✓ Gastric irritation
  - ✓ Kidney damage

Naringin as Bioactive Compound: Naringin is a natural flavonoid glycoside widely found in citrus fruits and is considered an important bioactive compound with significant therapeutic potential in the treatment of rheumatoid arthritis. Naringin has attracted considerable attention in pharmaceutical research due to its ability to inhibit inflammatory cytokines, reduce oxidative stress, and modulate immune responses involved in RA pathogenesis. Klareskog,et al 2006[13]

Source of Naringin: Naringin is primarily obtained from citrus fruits, especially grapefruit and orange. It is responsible for the slightly bitter taste of grapefruit juice. Kotta, et al 2016[14]

Naringin is mainly present in the peel, pulp, and seeds of citrus fruits. It belongs to the flavonoid category of phytochemicals and is mostly found in edible fruits and medicinal plants. Kumar S, et al 2018[15]

- **Major natural sources**
  - ✓ Citrus paradisi,
  - ✓ Citrus sinensis,
  - ✓ Citrus limon,

Chemical structure: Naringin is a flavanone glycoside composed of naringenin (aglycone) attached to a disaccharide (neohesperidose) group.

- ✓ Molecular formula = $C_{27}H_{32}O_{14}$ and Molecular weight =580.5 g/mol.

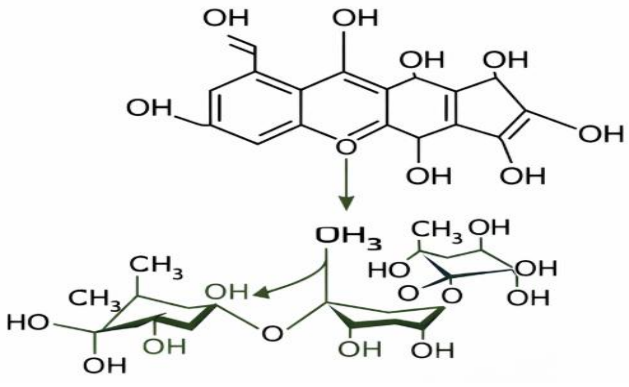

Fig. 1: Chemical structure of Naringin

NANOGEL DRUG DELIVERY SYSTEM

Nanogel drug delivery system is an advanced nanotechnology-based carrier composed of cross-linked hydrophilic polymer networks capable of absorbing large amount of water and retaining their three-dimensional structure. Myasoedova, et al 2010[16] Nanogels are nanosized hydrogel particles generally ranging

from 20–200 nm, which combine advantages of nanoparticles and hydrogels, such as high drug loading capacity, controlled drug release, biocompatibility, and improved stability. Nanogels are capable of encapsulating both hydrophilic and lipophilic drugs and protecting them from enzymatic degradation. Nagar M, et al 2023[17]

ADVANTAGES OF NANOGEL:

High Drug Loading Capacity: Nanogels possess a three-dimensional polymer network structure capable of entrapping large amount of drug molecules within the matrix.

Controlled and Sustained Drug Release: Nanogels release drug slowly over all period through diffusion, swelling, or degradation mechanisms.

High Biocompatibility and Biodegradability: Nanogels are prepared using biocompatible polymers such as chitosan, alginate, gelatin, PEG, and polyvinyl alcohol, which are safe for biological systems.

Reduced Toxicity and Side Effects: Targeted drug delivery using nanogels reduces exposure of healthy tissues to drug molecules. Controlled release reduces sudden increase in drug concentration, minimizing side effects associated with conventional dosage forms. Reduced toxicity makes nanogels suitable for long-term therapy.

Enhanced Skin Permeation: Nanogels possess small particle size and flexible structure, allowing easy penetration through skin layers and biological membranes.

Protection of Drug from Degradation: Nanogels protect drug molecules from enzymatic degradation and chemical instability by entrapping them within polymer matrix.

Suitable for Hydrophilic and Lipophilic Drugs: Nanogels are capable of encapsulating of aqueous-soluble and fat-soluble drugs due to their amphiphilic polymer network structure.

Improved Patient Compliance: Controlled drug release reduces frequency of dose and Noninvasive routes such as topical or transdermal delivery improve patient acceptance and adherence to therapy.

MATERIAL AND METHODS

The materials used in the present study included Naringin, which was procured from Yarrow Chem Products. Other chemicals and excipients such as Tween 20, Tween 80, Ethanol, Distilled Water, Methanol, Oleic acid, Isopropyl Myristate (IPM), Sodium hydroxide, Sodium chloride, Acetone, Disodium hydrogen phosphate, Potassium di-hydrogen phosphate, Olive oil, PEG 400, PEG 200, Acetonitrile, Iodine, Span 20, and Span 80 were obtained from R.V. Northland Institute. All chemicals and reagents used in the study were of analytical grade and were utilized without further purification.

PRE-FORMULATIONS STUDY

Pre-formulation studies were performed to evaluate the physicochemical properties of Naringin and its compatibility with selected excipients. These studies included selection of suitable oil, surfactant, and cosurfactant, determination of drug solubility in various components, and assessment of drug–excipient compatibility to ensure stability and suitability for nano emulsion formulation.

PHYSICAL PARAMETER

Naringin was characterized for its basic physical properties including colour, odour, melting point and loss on drying. The melting point of Naringin was determined using the capillary method. These parameters help in confirming the identity, purity, and suitability of the drug for formulation development. Aulton, et al 2018[18]

SOLUBILITY DETERMINATION

The solubility of Naringin was evaluated in various solvents, oils, and surfactant systems to select suitable components for nanoemulsion formulation. Approximately 20 mg of Naringin was added separately to each medium and sonicated for 1 hour to facilitate dissolution. The solubility of the drug was analyzed using UV spectrophotometric analysis and visual observation. Based on the solubility results, suitable oil, surfactant, and co-surfactant systems were selected for further formulation development. Torchilin, et al 2006 and J Drug Deliv Sci Technol, et al 2019[19-20]

ANALYTICAL METHODS

Partition co-efficient

To estimate Naringin's partition coefficient, an excess quantity of Naringin was dissolved in a 10 ml combination of n-octanol and water (1:1) in a separating funnel. To achieve equilibrium, the mixture was agitated constantly

for 30 minutes before being left to stand overnight. After achieving equilibrium, the two layers were separated and centrifuged at 10,000 rpm for 15 minutes. A UV-Visible spectrophotometer with an appropriate wavelength was used to measure the concentration of Naringin in both phases. Tripoli, Enrico, et al 2021[21]

Ultra Visible Spectroscopy

Accurately weighed 10 mg of drug was dissolved in methanol to prepare a 100 µg/ml stock solution. Further dilutions were prepared using methanol, and the solutions were scanned in the wavelength range of 200–400 nm using a UV–Visible spectrophotometer to determine the λ-max of drug. The λ-max value was used for further analytical studies and drug quantification. John R, et al 1998([22]

Fig.2: U. V. Spectroscopy

HPLC analysis

HPLC analysis of Naringin was performed using a C18 column with acetonitrile: water as the mobile phase at a flow rate of 1 ml/min. Detection was carried out at 285 nm with an injection volume of 20 µl. The retention time of Naringin was found to be 4–6 minutes. A calibration curve was prepared using standard solutions of Naringin. Vinogradov, et al 2002[23]

Fourier Transmission Infra-Red (FTIR)

FTIR study was find out to any possible chemical interruption between drug and polymer. The FTIR spectra of pure drug and drug-loaded formulation were recorded using an FTIR spectrophotometer (Spectrum 100, Shimadzu, UK). The analysis helps in identification of functional groups and confirms the compatibility of drug with excipients used in the formulation. Wang Y, et al 2002[24]

Fig.3: Fourier Transmission Infra-Red

DIFFERENTIAL SCANNING CALORIMETRY

Differential Scanning Calorimetry study of drug was performed using a differential scanning calorimeter (Perkin Elmer DSC-7) calibrated with indium. Samples were analyzed in triplicate under nitrogen atmosphere at a heating rate of 10°C/min, gas flow rate of 20 ml/min, and temperature range of −30°C to 200°C using a sample size of approximately 0.5 mg. The DSC thermogram was used to determine the melting point of Naringin and to confirm the purity and compatibility of the drug in the formulation. Aaron M, et al 2011[25]

FORMULATION AND DEVELOPMENT

Construction and development of Pseudo-ternary-phase diagram by water titration method

Oleic acid was selected as the oil phase, Tween 80 as the surfactant, and ethanol as the co-surfactant based on solubility studies, while distilled water was used as the aqueous phase. Pseudo-ternary phase diagrams were constructed by the water titration method to identify the nanoemulsion region. Different S-mix ratios were prepared and mixed with oil in varying proportions to obtain a wide range of compositions. Distilled water was added dropwise with continuous stirring, and the formulations were visually evaluated for clarity, flowability, and phase separation. Systems showing transparent appearance, good flow properties, and no phase separation were considered stable nanoemulsions. Based on the phase diagram analysis, optimized formulation compositions were selected considering drug solubility, stability, and minimum surfactant concentration for preparation of Naringin-loaded nanoemulsion formulations. Yadav VR, et al 2016 and Vrinda R, et al 2023 [26-27]

Preparation of Drug Loaded Nanoemulsion

Accurately weighed 50 mg of Naringin was dissolved in the selected oil phase for preparation of drug-loaded nanoemulsion. The required quantity of surfactant and cosurfactant was added to the oil phase and mixed thoroughly. Distilled water was then added dropwise with continuous stirring until a clear and transparent nanoemulsion was obtained. The prepared nanoemulsion formulations were transferred into airtight containers and stored at room temperature for further evaluation.

Evaluation of Drug Loaded Nanoemulsion formulation

Following parameters are commonly evaluated for optimization of good nanoemulsion formulations.

Preparation of Naringin-loaded Nanoemulgel

The optimized naringin-loaded nanoemulsion is incorporated into a suitable gel base to obtain nanoemulgel formulation for topical and transdermal drug delivery. Accurately weighed quantity of Carbopol 940 is dispersed in distilled water with continuous stirring to obtain uniform gel base. The optimized nanoemulsion containing naringin is slowly added into hydrated gel base under continuous stirring to obtain homogeneous dispersion. Zhang L, et al 2021 and Jing, et al 2021[28-29]

Evaluation Parameters for Optimization of Naringin-loaded Nanoemulgel

The prepared nanoemulgel formulations were evaluated for various physicochemical and performance parameters. The pH of the formulation was determined using a calibrated digital pH meter. Viscosity was measured using a Brookfield viscometer at specified rpm with a suitable spindle to assess the consistency and applicability of the formulation. Spreadability was evaluated by placing a small quantity of formulation between two glass slides and measuring the diameter of spread after applying a fixed weight for a specified time. Homogeneity was assessed visually for smooth appearance and absence of lumps or aggregates. In-vitro drug release studies were carried out using a Franz diffusion cell fitted with a dialysis membrane, with phosphate buffer pH 6.8 maintained at 37 ± 0.5°C as the receptor medium under continuous stirring. The drug release profile was determined by withdrawing of samples at regular time intervals and analyzed using UV spectrophotometer.. Ex-vivo skin permeation studies were also performed using excised animal skin mounted on a Franz diffusion cell to evaluate transdermal permeation efficiency, where cumulative drug permeation and flux were calculated from the obtained results.

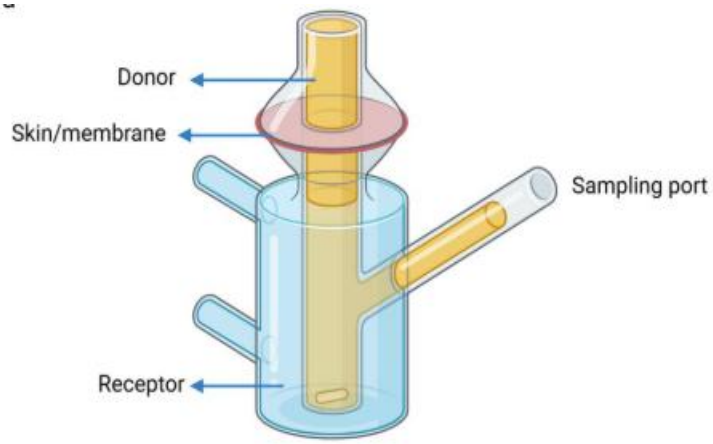

Fig.4: Franz diffusion cell

IN-VITRO ANTI-INFLAMMATORY ACTIVITY (PROTEIN DENATURATION METHOD)

The in-vitro anti-inflammatory activity of the Naringin-loaded nanoemulgel was evaluated using the protein denaturation (albumin denaturation) method, based on the principle that inhibition of protein denaturation reflects anti-inflammatory potential. The reaction mixture was prepared using the nanoemulgel formulation, 1% w/v bovine serum albumin (BSA) solution, and phosphate buffer pH 6.8 to maintain physiological conditions, while Diclofenac sodium was used as the standard drug for comparison. Test, control, and standard solutions were prepared separately and incubated at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes to induce protein denaturation. After cooling to room temperature, the absorbance of the samples was measured using a UV–Visible spectrophotometer, and the percentage inhibition of protein denaturation was calculated to assess the anti-inflammatory activity of the formulation. Zhang, et al 2018[30]

STABILITY STUDY

Stability study of nanoemulgel formulation is carried out according to ICH guidelines to evaluate physical and chemical stability under different storage conditions. Formulations are stored at 25°C ± 2°C / 60% RH, 30°C ± 2°C / 65% RH, and 40°C ± 2°C / 75% RH for specific period of time. Samples are periodically evaluated for changes in pH, viscosity, drug content, homogeneity, and physical appearance. Stability study ensures safety, effectiveness, and shelf-life of nanoemulgel formulation Zhao, et al 2020[31].

RESULT

Physical Parameter

The physical characterization of Naringin revealed that the drug was obtained as a crystalline powder with a pale yellow to light cream colour and was found to be odourless in nature. The melting point of Naringin was determined to be 165 °C, which is in agreement with the reported standard values. The loss on drying was found to be 1.85%, indicating low moisture content and acceptable purity of the drug sample.

Table.3.1: Physical Properties

Parameter	Result
Nature	Crystalline powder
Colour	Pale yellow to light cream
Odour	Odour-less
M.P. (Melting Point)	165 °C
Loss on Drying	1. 85 %

SOLUBILITY DETERMINATION

The pre-formulation solubility studies of Naringin were carried out in various solvents, oils, and surfactant systems to select suitable components for nanoemulsion formulation. The drug was found to be freely soluble in ethanol and methanol, sparingly soluble in acetone and butanol, and insoluble in water, benzene, DMSO, and phosphate buffer solutions, indicating its poor aqueous solubility. Oleic acid was used to represent the oil phase during the screening tests for oils, as maximum solubility was achieved in this medium, and limited solubility in coconut oil, olive oil and IPM. In the surfactant systems screened, Tween 80 exhibited a good solubilizing capacity and was selected as the surfactant, while ethanol was selected as the co-surfactant due to its ability to help to increase the solubility of the drug and formulation stability. Overall the solubility studies demonstrated the feasibility of the selected components for the formulation of Naringin loaded nanoemulsion.

ANALYTICAL METHODS

Partition-co efficient

The distribution of Naringin between octanol and water was found using the shake flask method, where its partition coefficient was determined. The study revealed that the partition coefficient of the drug was 26.90 and the log P value of the drug was 1.43 which falls in the moderate range of lipophilicity. The result obtained was within reported range of literature for log P (0.42-1.82). Considering its balanced lipophilic and hydrophilic nature, Naringin possesses suitable properties for topical and transdermal delivery, showing adequate permeation of skin and release of the drug.

Ultra Visible Spectroscopy

The absorbance of different concentrations was measured using UV-visible spectrophotometer on absorption peak of λ-max 285nm and standard calibration curve of naringin in methanol was constructed. The calibration curve was plotted by taking absorbance of the samples against the concentration, and it was found to be linear between the selected concentration range. The linear regression equation was determined as y = 0.168x + 0.012 with a correlation coefficient (R^2 = 0.998) showing good linearity, accuracy and reliability of the analytical method.

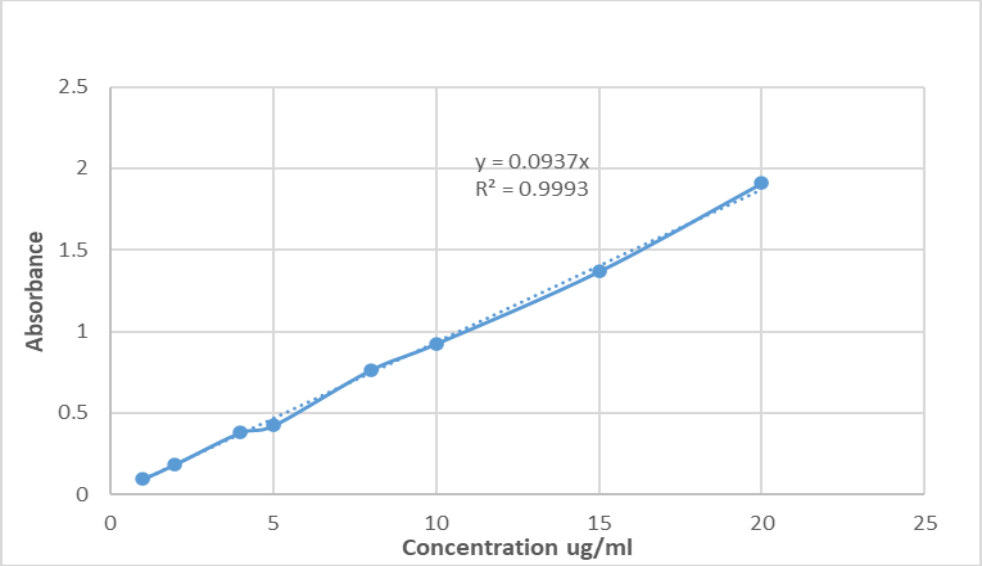

Figure.5: UV Spectroscopy Standard Calibration curve of Naringin

3.2.4 HPLC analysis

y= 163910x + 1716.7

Coefficient of correlation (R^2) = 0.9983

Slope (m) = 163910

Calibration curve of Naringin in mobile phase at 285 nm using HPLC.

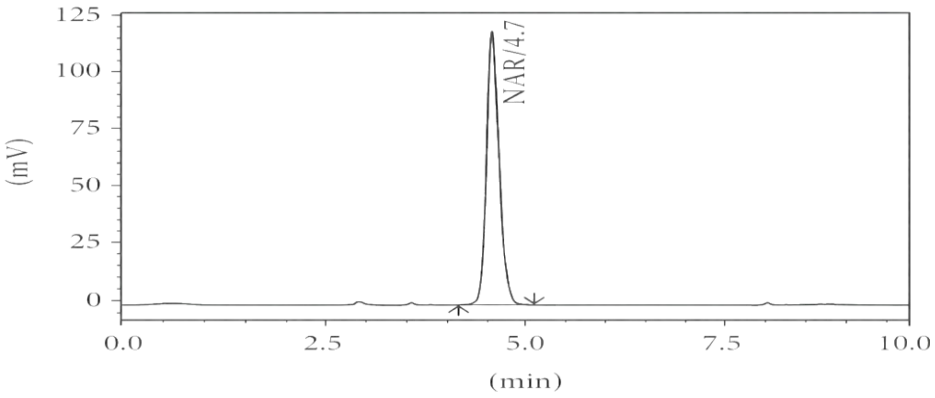

Fig.6: HPLC chromatogram

Fourier Transmission Infra-Red (FTIR)

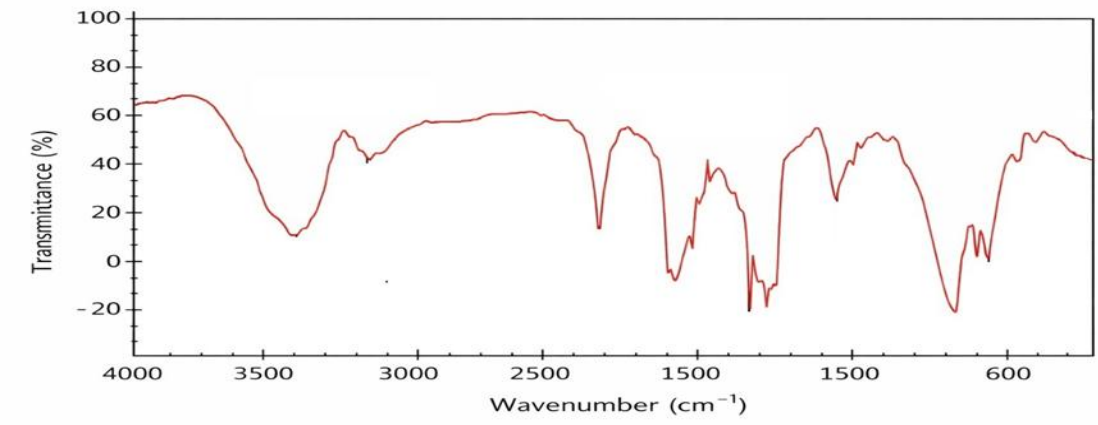

Fig.7: FTIR spectrum of Drug

DIFFERENTIAL SCANNING CALORIMETRY:

The interaction studies substantiated that the selected excipients used in the formulation of the nanoemulsion were compatible with Naringin. The results of UV analysis revealed that the λ-max of the drug remained unchanged and the assay revealed that the amount of drug recovered was between 99.93% – 99.99%, suggesting that it was stable and drug content was uniform. FTIR and DSC analysis revealed that there was no significant drug–excipient interaction, which indicated the suitability of drug and excipients for nanoemulsion formulation.

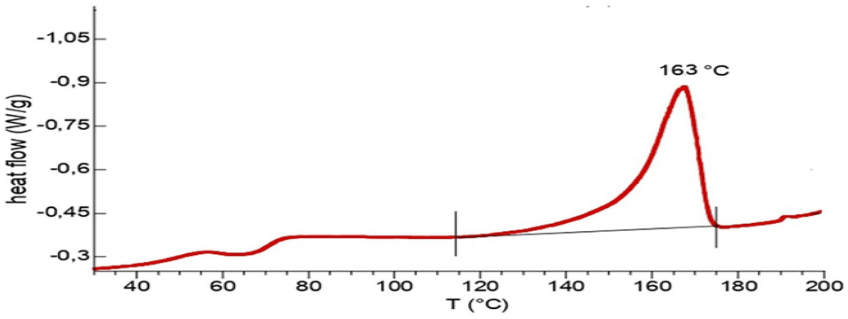

Fig.8: DSC Analysis of drug

FORMULATION AND DEVELOPMENT

Construction and development of Pseudo-ternary-phase diagram by water titration method

For the creation of a naringin-loaded nanoemulsion, oleic acid was chosen as the oil phase, Tween 80 as the surfactant, and ethanol as the co-surfactant based on solubility screening investigations. Different formulations were screened on the basis of physical appearance, including clarity, transparency, and absence of phase separation, for the construction of the Pseudo-ternary Phase Diagram (PTPD). Various S-mix ratios such as 1:0, 1:1, 2:1, 1:2, and 3:1 were prepared and combined with the oil phase in different proportions ranging from 1:9 to 9:1, followed by gradual addition of water to identify the nanoemulsion region. The phase diagrams were constructed using PCB Triangle Software to determine the optimized concentration range for stable nanoemulsion formation. The S-mix ratio exhibiting the largest nanoemulsion region with good transparency and stability was selected for further formulation development.

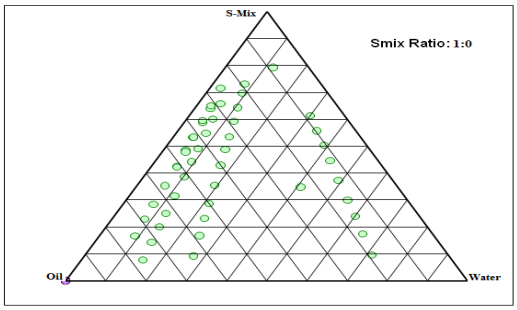

Fig.9: PTPD for the S-mix ratio of 1:0.

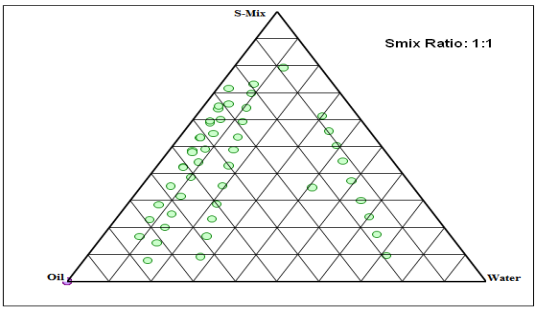

Fig.10: PTPD for the S-mix ratio of 1:1.

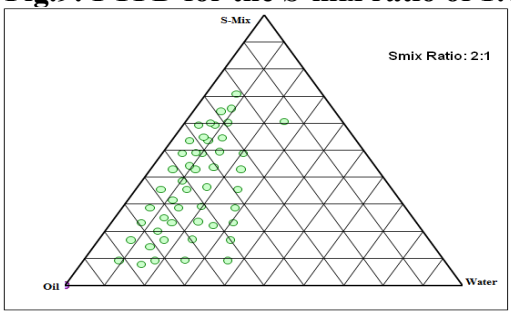

Fig.11: PTPD for the S-mix ratio of 2:1.

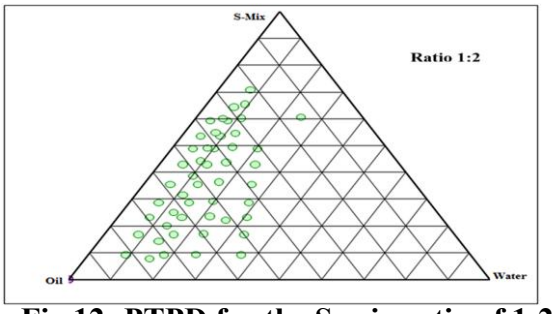

Fig.12: PTPD for the S-mix ratio of 1:2.

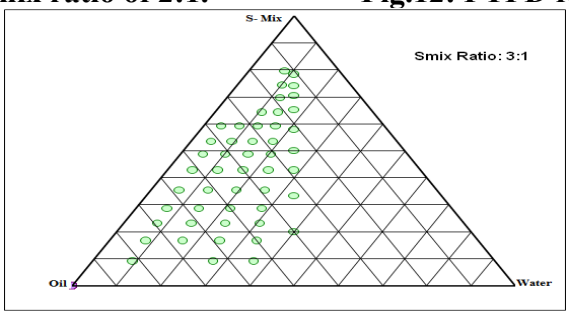

Fig.13: PTPD for the S-mix ratio of 3:1.

PREPARATION OF DRUG-LOADED NANO-EMULSIONS

Naringin-loaded nanoemulsions were prepared by dissolving 20 mg of Naringin in the selected oil phase, followed by the addition of the required amount of surfactant mixture (S-mix). Distilled water was then added dropwise with continuous stirring until clear and transparent nanoemulsions were obtained. Different formulations were prepared using various S-mix ratios such as 1:1, 1:2, and 2:1 with varying concentrations of oil, S-mix, and distilled water. Pseudo-ternary phase diagrams were constructed to evaluate the effect of S-mix ratio on nanoemulsion formation and phase behavior. The phase diagram prepared with Tween 80 alone

(1:0) showed a very small nanoemulsion region, whereas incorporation of ethanol as co-surfactant in 1:1 and 2:1 ratios significantly increased the nanoemulsion area due to better reduction in interfacial tension and improved flexibility of the interfacial film. However, higher co-surfactant concentration (1:2) reduced the nanoemulsion region and promoted gel formation. Therefore, S-mix ratios of 1:0, 1:1, and 2:1 were selected for formulation development as they provided comparatively larger and stable nanoemulsion regions with minimum surfactant concentration. The optimized formulations were further subjected to thermodynamic stability studies, including centrifugation and freeze–thaw cycles, and showed no phase separation, turbidity, color change, or drug precipitation, confirming good physical stability of the nanoemulsion system.

EVALUATION OF DRUG LOADED NANOEMULSION FORMULATION

The in-vitro skin permeation study of Naringin-loaded nanoemulsion formulations was carried out using a Franz diffusion cell to evaluate the effect of different surfactant and co-surfactant ratios on transdermal drug delivery. The cumulative drug permeation increased progressively with time for all formulations, indicating sustained drug release behavior. Among the formulations prepared with S-mix ratio 1:0, NE-2 showed the highest permeation, followed by NE-3 and NE-1. In formulations containing S/CoS ratio 1:1, AK-3 exhibited maximum drug permeation (17100 µg/cm²), demonstrating enhanced skin diffusion due to the combined effect of surfactant and co-surfactant. Formulations prepared with S/CoS ratio 2:1 showed comparatively lower permeation, where SK-2 exhibited the highest permeation among the group. The overall permeation order was found to be AK-3 > NE-2 > SK- Based on the permeation results, NE-2, AK-3, and SK-2 were selected as optimized formulations for further evaluation and nanoemulgel development.The optimized nanoemulsion formulations were further characterized for droplet size, polydispersity index (PDI), and zeta potential. The particle size of the formulations was found within the nanometer range, confirming successful nanoemulsion formation. Among the optimized formulations, AK-3 showed the smallest droplet size (203.7 nm), followed by NE-2 (274.4 nm) and SK-2 (467.5 nm). The PDI values ranged from 0.277 to 0.390, indicating uniform droplet size distribution. The zeta potential values of NE-2, AK-3, and SK-2 were found to be −24.6 ± 1.2 mV, −31.4 ± 1.5 mV, and −27.2 ± 1.3 mV, respectively, suggesting good electrostatic stability and resistance to droplet aggregation. Overall, the results confirmed that the optimized nanoemulsion formulations possessed suitable physicochemical properties for improved transdermal delivery of Naringin.

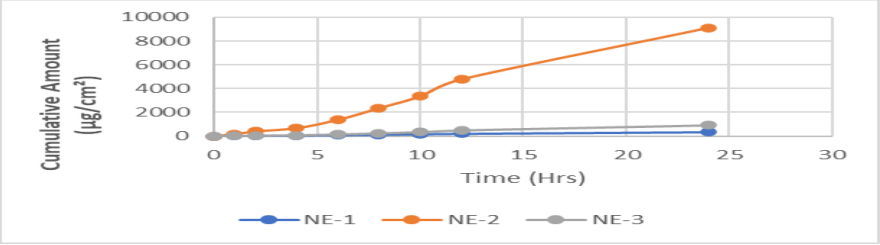

Fig.14: *In-vitro* skin permeation Study of Drug Loaded nano emulsion S-mix ratio 1:0.

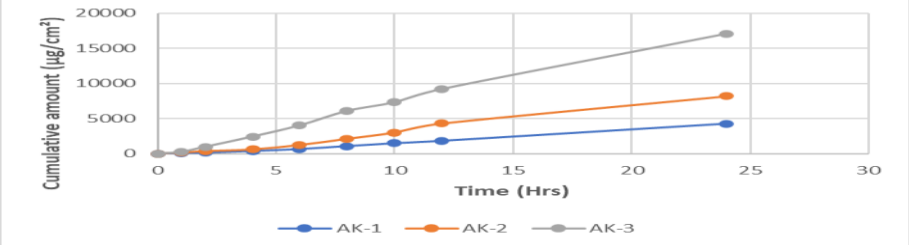

Fig.15: *In-vitro* skin permeation of Drug Loaded nano emulsion of S/CoS ratio 1:1.

PREPARATION OF NARINGIN-LOADED NANOEMULGEL

The optimized nanoemulsion formulation AK-3 was successfully converted to a nanoemulgel (SK-3G) with 1% of Carbopol 934 as gelling agent. The amount of Carbopol 934 required was slowly added to the nanoemulsion with a constant stirring so as to be dispersed evenly. The formulation was then stored overnight for complete hydration and swelling of polymer to get a suitable viscosity and consistency in the formulation as a nanoemulgel. The prepared nanoemulgel was found to be good in terms of homogeneity and no phase separation was observed.Likewise, an easy to prepare gel formulation was made by dispersion of Carbopol 934 (1% w/w) in distilled water with stirring for a long period to prevent the formation of lumps. The dispersion was left to hydrate overnight and then the pH was adjusted to give a clear gel of suitable viscosity by the use of triethanolamine. The simple gel was prepared and the smoothness and uniformity were obtained, which was used as the control formulation.

EVALUATION PARAMETERS FOR OPTIMIZATION OF NARINGIN-LOADED NANOEMULGEL

The prepared nanoemulgel formulation (AK-3G) was tested for its pH, viscosity, spreadability and homogeneity. The pH of the formulation was 6.12 ± 0.08, which is within the acceptable range of skin pH, reflecting the non-irritant and skin-compatible nature of the formulation. The viscosity value (4625 ±55 cPs) indicated the suitable consistency for topical application and good skin surface retention. Spreadability of 6.85 ± 0.21 g·cm/sec showed easy and uniform application; and homogeneity study showed smooth gel with no lumps, thus, it is indicative of uniform distribution of drug throughout the formulation.

In-vitro skin permeation study of optimized nanoemulgel (AK-3G) and neat gel were conducted using Franz diffusion cell for 24 hours. The nanoemulgel formulation showed a sustained release of drug with time and 88% drug release with cumulative permeation of 5866.66µg/cm2 after 24 hrs. The improved permeation was believed to be due to the size of the droplets, which were nano-sized, thereby providing a larger surface area and increasing the permeation of the drug through the membrane. In contrast, the neat gel exhibited lower permeation, as the cumulative drug permeation of the neat gel was 1429.33 µg/cm2 and the cumulative drug release was around 42% at 24 h which represents slower diffusion through the gel matrix. In overall, both the formulations exhibited sustained drug release behavior but nanoemulgel formulation exhibited significantly higher permeation efficiency and better transdermal delivery of Naringin.

The release kinetic studies of optimized nanoemulgel formulation revealed that the drug release showed high linearity (R^2 = 0.9962) and followed predominantly zero-order kinetics, thus showing controlled and constant drug release in the formulation during time. The Higuchi model also gave a good correlation, which proved the diffusion-controlled drug release process from the gel matrix. Moreover, it was found that the Korsmeyer–Peppas model had a high coefficient of correlation with an exponent value (n = 1.12), which is indicative of non-Fickian or anomalous transport which contains both diffusion and polymer relaxation mechanisms. The first order model had the relatively low linearity, reflecting that the release was not predominantly concentration dependent. The low values of the coefficient of variation (CV) values also indicated the reproducibility and consistency of the release data. In conclusion, the optimized nanoemulgel formulation showed controlled, sustained and stable drug release profile which are suitable for effective topical and transdermal delivery of Naringin.

ANTI- INFLAMMATORY (PROTEIN DENATURATION METHOD)

The in-vitro anti-inflammatory activity of the naringin-loaded nanoemulgel was evaluated using the protein denaturation (albumin denaturation) method, and the results were compared with the standard drug diclofenac sodium. The percentage inhibition of protein denaturation was determined at different concentrations ranging from 20 to 120 µg/mL, and the results are presented in Table 5.29 and Fig. 5.21. Both diclofenac sodium and the naringin

nanoemulgel exhibited concentration-dependent inhibition of protein denaturation. Diclofenac sodium showed percentage inhibition values ranging from 3.21 ± 0.42% at 20 µg/mL to 98.12 ± 1.22% at 120 µg/mL, whereas the naringin nanoemulgel demonstrated inhibition values from 2.12 ± 0.36% to 94.86 ± 1.33% over the same concentration range. The nanoemulgel formulation showed significant anti-inflammatory activity, with inhibition values comparable to the standard drug at higher concentrations. The observed inhibition of protein denaturation indicates the ability of the formulation to stabilize protein structures and prevent inflammatory responses. The anti-inflammatory activity may be attributed to the presence of naringin, a flavonoid well known for its antioxidant and anti-inflammatory properties, including inhibition of inflammatory mediators and prevention of protein denaturation. Furthermore, the nanoemulgel system enhanced the solubility, stability, and penetration of naringin, thereby improving its therapeutic effectiveness and contributing to its pronounced anti-inflammatory activity.

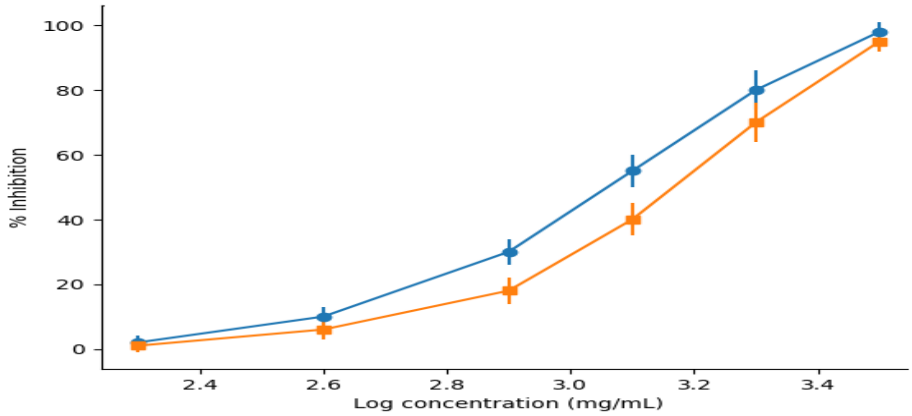

Fig.16: Comparison of % inhibition of protein denaturation of naringin nanoemulgel and diclofenac sodium at different log concentrations (n = 3).

STABILITY STUDY OF NANOEMULGEL FORMULATION

For the stability study of the naringin nanoemulgel formulation, it was stored at 25°C ± 2°C/60% RH, 30°C ± 2°C/65% RH, and 40°C ± 2°C/75% RH for 90 days. Different parameters such as pH, viscosity, drug content, physical appearance and homogeneity were tested at certain time intervals and are presented in the Table 5.30. No phase separation or loss of homogeneity were observed under any of the storage conditions, thus the formulation was physically stable during the study period. The pH value decreased slightly from 6.42 ± 0.05 to 6.39 ± 0.06 at 25°C ± 2°C/60% RH, whereas viscosity and drug content did not vary significantly during the 90 day period with only minor decreases over the period. Same observations were made at 30°C ± 2°C/65% RH, but a small increase in the gel thickness was observed after 90 days. Under accelerated storage conditions (40°C ± 2°C/75% RH), a slightly higher but acceptable loss of pH, viscosity and drug content was noticed with a final drug content of 97.5 ± 1.5% after the completion of the study. Under accelerated conditions minor changes in physical characteristics (slight thickening) were also observed, but the formulation was still homogeneous and stable. The overall results showed the nanoemulgel to be physically and chemically stable under various storage conditions. As no significant changes were observed in evaluated parameters the formulation is stable and found robust that the developed nanoemulgel will retain its quality, efficacy and stability during storage and transportation.

DISCUSSION

Nanoemulgel formulation stability study showed that the formulation prepared was stable under all the storage conditions suggested by International Council for Harmonisation (ICH). The results of the parameters tested such as pH, viscosity, drug content, homogeneity and physical

appearance did not reveal any significant changes, suggesting good physical and chemical stability of the formulation. Moreover, nanoemulgel was stable and free from any signs of phase separation, degradation of the drug, and significant changes in the rheological properties, indicating the stability and reliability of the formulation. Some changes were observed under the accelerated storage conditions (40°C ± 2°C / 75% RH), but the changes were still within the acceptable limits, which indicated that the formulation could withstand the stresses imposed during storage and transportation.

SUMMARY

The present study was aimed at the development of naringin loaded nanoemulgel via transdermal drug delivery system to overcome the poor solubility, low permeability and limited bioavailability of naringin. Naringin, which is a naturally occurring flavonoid glycoside, is richly abundant in citrus fruits and is known to have numerous anti-inflammatory, antioxidant, antimicrobial and therapeutic properties against many chronic diseases. However, due to its low aqueous solubility and lack of permeability across biological barriers, its use is limited as a traditional dosage form.

To overcome the above constraints, the design of nanogel system using nanoemulsion as an efficient carrier for improving solubility, skin permeation and controlled drug release of naringin was worked out. The water titration method was used to prepare the nanoemulsion at room temperature. Nanoemulgel systems have several benefits including the improvement of stability, penetration of the skin, drug release, retention time, lower doses, patient compliance and less systemic adverse effects. Furthermore, transdermal delivery avoids first pass metabolism and thus enhances bioavailability of the drug.

Poor bioavailability of phytoconstituents such as naringin is another problem that restricts their therapeutic use because they lack in solubility in biological media and poor permeation through the skin barrier. The solubilization and thermodynamic activity of lipophilic compounds can be improved by the application of nanoemulsion technology, leading to better diffusion through the Stratum Corneum. Further increasing the viscosity, spreadability and skin surface retention of the formulation, the nanoemulsion is incorporated into a gel system.The following conclusions were drawn from the study:

- The physical and analytical characterization studies confirmed that the naringin sample used in the study was authentic, pure, and complied with standard specifications.
- Drug–excipient compatibility studies demonstrated that naringin was compatible with the selected formulation components including oil, surfactant, and co-s.

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