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Mupirocin Niosomal Gel with Bee Honey & Curcumin as Nano-Drug Delivery in Wound Healing Applications

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Abstract

Wound healing research is still aiming toward complete regeneration and restoration of the skin's function and structure with the least amount of scarring. Controlled and targeted medication distribution to wounds is more convenient than systemic administration, which allows for larger drug concentrations to be delivered to the targeted site over time. The nano wound healing gel demonstrated a dependable administration strategy, excellent local tolerability, and superior drug delivery methods, which can promote faster healing. Recently, niosome formulations have been developed to reduce toxicity while increasing accumulation at the target site. Curcuma longa (CU) and honey are effective at inhibiting the growth of wound-associated pathogens and hastening the healing process. The wound healing potential activity of mupirocin-loaded niosomal gel formulated with honey and curcumin, as well as their blends, by ether injection method and investigated for further studies. FTIR and DSC study reveals the compatibility of the drug and other excipients. In the case of post-approval study the parameters evaluated are entrapment efficiency, drug content, pH, viscosity, spreadability, SEM, in-vitro drug release study, release kinetic study, stability study, and in-vivo wound healing study followed by histopathological study. This study aimed to create an excision wound model in albino rats and compare it to a commercially available ointment (Mupicip by Cipla). The blended formulation (Formulation F7) was administered twice daily, and the wound healing effect was determined by the highest percentage of wound contraction, epithelisation period, and histoarchitecture studies. The obtained results concluded that formulation F7 is considered as best formulation and has shown a higher percentage of wound contraction 99.08%. The histological study also confirms that formulation F7 promotes faster wound healing.

Keywords: Wound healing, Mupirocin, Curcumin, Honey, Niosomal ointment Nano-drug delivery.

Introduction

The wound is the disruption of cellular and anatomic continuity of living tissue, which is the main cause of physical illness. Wound healing is the dynamic process of survival of

a patient by attempting regeneration or restoration of broken tissue and its function (1, 2). Every year, millions of people around the world suffer from acute and chronic wounds and their treatment burden due to its impaired and delayed healing process (3). Different kinds of ulcers including diabetic, vascular, arterial, and pressure ulcers (4-7) are the main factors resulting in complications and intensive treatment in wound healing. Wound healing takes place with three complex interrelated stages, namely inflammation, granulation, and remodeling (8). The mechanism of healing is started due to the secretion of clotting factor fibrin by platelets at the targeted site (9) Growth factors cytokines as a key signal arrives with platelets a source of fibrin clot, helps to treat inflammatory cells in wound healing. The fibroblast is responsible for collagen deposition which helps to provide strength, integrity, and structure to the injured tissue during wound healing and also helps to restore tissue anatomic structure and function (10).

Nanomedicine emerged nanosized particles help in the diagnosis and treat wound healing (11, 12) An optimal drug action application of nanoscience in the pharmaceutical industry is very promising and has grown rapidly. Nnao carrier could be a good transporter for drug molecules to achieve action in the targeted site. Non-ionic surfactant vesicles like niosomes (13, 14) can be used as carriers to transport amphiphilic and lipophilic drugs (15, 16). Nisomes are more stable as compared to similar types of vesicles due to the presence of better surfactants and phospholipids available in them.

A variety of drugs are available for wound healing management like analgesics, nonsteroidal anti-inflammatory, and antibiotics but most of them are responsible for unwanted side effects. In recent years, herbal drugs explicate their potential in wound healing management and revived interest in alternative treatment against synthetic drugs (17). The main object of this research study is to introduce a natural antibiotic mupirocin with the combination of herbal-based products such as curcumin and honey in the form of niosomal gel to control side effects. The significance of this study is to provide quick wound healing of the skin with optimal functional and aesthetic results (18). Mupirocin reversibly inhibits isoleucyl-transfer RNA, which helps in constraining bacterial protein and RNA synthesis. Mupirocin is highly effective against many bacteria (19, 20) and pathogens mostly found in primary and secondary skin infections. Similarly, curcumin and honey have shown anti-inflammatory, antimicrobial, and wound healing properties (21-23) and treat against staphylococcus aureus Pseudomonas aeruginosa and Escherichia coli, the most common causative agent of wound infections (24,25). Curcumin is also used in burn wound infection, sepsis in surgical wounds (26), infections such as bacteremia, septicemia, and wound infection in hospitalized patients (27) Honey free from toxic contaminants, gone through gamma irradiation sterilization technique is safe for medical application (28). Reepithelialization, angiogenesis, and stimulation of skin and immune cells (29) are one of the main wound-healing abilities of honey. The overall aim of this research study is to develop a Niosome, a nanocarrier to improve wound healing treatment by enhancing stability, reducing toxicity, and controlling the release profile of active drugs.

Materials and Methods

Materials

Mupirocin and carbopol are obtained from Divya associates, Vijayawada, India. Miresi is an online site to get organic honey without any preservatives. Curcumin (Curcuma longa) was obtained from Sigma-Aldrich, India. Tween 80, Cholesterol, and glycerin are obtained from SD fine chemicals India. All other excipients were of analytical research grade and contain the highest purity.

Curcumin Preparation Process

Curcumin preparation was carried out with a wide-mouth vial. The calculated amount

of cholesterol and surfactant tween 80 have been taken. The mixture was dissolved in diethyl ether and methanol (8:2) solution. Sealed the vial and heat it with a water bath at 60 °C for 10 min until the cholesterol gets dissolved. The required quantity of Curcumin has to be added to the lipid mixture to obtain a creamy yellow gel (30).

Preparation of niosome by ether injection method

Weight amount of polymer and mupirocin taken at different ratios in a 250 ml beaker. Honey is added slowly into the drug and polymeric solution with continuous stirring to obtain a homogeneous mixture. Prepared curcumin gel loaded into a 14-gauge needle and added to mupirocin. The temperature maintained during this mixing is 55-60 °C. Solvents are gradually evaporated from the mixture to obtain a stable vesicle (31). This method also helps to control the size of the formulated niosome, based on the selection of needle and other factors. Formulation codes for different niosomal formulations based on the diverse ratio between polymer, surfactant, and cholesterol are mentioned in Table 1.

Evaluation of Niosomal Gel

Physicochemical evaluation of formulated niosomal gel

All formulated niosomal gels were subjected to visual inspection to determine the homogeneity, color, fluidity, and consistency. Niosomal gel was also viewed under an optical microscope at 50X magnification to further determine the crystallization, agglomeration, and grittiness.

The entrapment efficiency of drug

Niosomal gel from each formulation (1 g) was collected individually and placed in a 100 ml volumetric flask which contain 20 ml buffer solution (pH 7.4) and sonicated for 30 min at room temperature. The mupirocin-loaded niosome were separated from the unentrapped drug with the help of centrifugation at 12000 rpm for 20 min. The supernatant with suitable dilution was taken for UV analysis at 226 nm. The % of encapsulated drugs was determined by using the formula given below.

$$\% \text{ Entrapment efficiency} = \frac{(\text{Amount of drug entrapped})}{(\text{Amount of drug added})} \times 100$$

Table 1: Formulation of niosomal gel loaded with 2% mupirocin (Net Weight 15 g)

Formulation Code	Drug: polymer ratio	Honey	Curcumin	TWEEN80: Cholesterol ratio	Glycerine	Methylparaben	Distilled water
F1	2:1	-	5	4:8	5	0.22	Q.S
F2	2:0	-	-	5:4	5	0.22	Q.S
F3	2:2	2	2	5:3	5	0.22	Q.S
F4	2:3	5	1	6:8	5	0.22	Q.S
F5	2:2	7	4	6:4	5	0.22	Q.S
F6	2:3	3	2	3:5	5	0.22	Q.S
F7	2:2	5	4	5:8	5	0.22	Q.S
F8	2:2	4	2.5	5:8	5	0.22	Q.S

Drug content

Niosomal gel 1 gm was collected individually from each formulation and dissolved with buffer sample pH 7.4 and made up the volume up to 50 ml. 1 ml of the above solution with suitable dilution was taken for determination of absorbance by UV-Visible spectrophotometer at 226 nm.

pH determination of formulated niosome gel

Calibration should be done for the pH meter before its use. 1 gm of formulated niosomal gel was dispersed in 100 ml water and subjected to calibrated digital pH meter to determine the pH of the sample. To avoid unwanted complications each formulation pH was measured in triplicate.

Determination of viscosity

The selected spindle has been attached with a viscometer to determine the viscosity of the sample. The spindle as a rotating element in a fluid measures torque and indicates the viscosity of the fluid. Various formulated niosomal gels were subjected to a Brookfield viscometer to determine the viscosity. The respective reading was noted and represented in cps. The samples are done in triplicate to avoid the error.

Spreadability

An excess sample was taken from each formulation to determine the spreadability of the niosomal gel. Niosomal gel from various sample ware placed in between two glass slides and compressed by 100 gm weight for 5 min to obtain uniform thickness. The weight (125 gm) was applied to the upper glass slide to separate both slides. The time required to separate the slides was individually noted to determine the spreadability.

SEM study

The morphology of the niosomal gel was performed by scanning electron microscope (SEM, Model no. JEOL Model JSM - 6390LV). 1 gm of the sample was added with 100 ml of buffer solution and mixed for 30 min using a magnetic stirrer. Take the supernatant sample to determine the morphology study. It also gives additional information about the shape and size of the niosome.

In-Vitro release studies

Franz diffusion cell with receptor compartment volume 20 ml used to carry out *in vitro* drug release study. Cellulose dialysis membranes (Sigma-Aldrich, Mumbai, India) were soaked in buffer media (pH 7.4) for 24 h at room temperature before experimenting. From various freshly prepared formulations of niosomal gel 3 mg of the sample was loaded in the donor compartment. In the receptor compartment, the buffer media was continuously stirred at 600 rpm and the temperature was maintained at 37±0.5 ^{o}C. After a predetermined time interval, 5 ml samples were collected from the receptor compartment and replaced with the same amount of fresh buffer media. Collected samples were analyzed under a UV Visible spectrophotometer at 226 nm with a suitable dilution. A comparison study of drug release kinetic has been made between formulated niosomal gel concerning commercially available Mupicip manufactured by Cipla. All the experiments were conducted in triplicates and the average were calculated to minimize the error percentage.

Drug release mechanism studies

The drug release mechanism study of mupirocin-loaded niosomal gel was fitted to various release models obtained from *in vitro* dissolution profile. The various kinetic models are zero order, first order, Higuchi model, and Korsmeyer-Peppas model (32) The high correlation coefficient value indicates the best fitting of the release kinetics of any of the models.

Stability studies

The best formulation F7 was packed in an aluminum collapsible tube of 15 gm and considered for the stability test. The stability test was carried out as per ICH guidelines at 25

°C with 60% RH and 40 °C with 75% RH for 3 months. At suitable time intervals, the sample is evaluated for physical appearance, pH, viscosity, spreadability, and drug release profile.

In vivo study

Healthy Wistar albino rats age 8-10 weeks, weight: 200-250g were used to perform the efficacy of formulated niosomal gel (Containing 2% mupirocin) with marketed mupirocin gel on excision wound model.

Animal Preparation

In this current research, all the Wistar albino rats are housed under standard conditions and clinically examined. The animals were acclimatized to the standard laboratory conditions in a cross-ventilated animal house at 25 ± 2 °C, relative humidity 44–56%, and light and dark cycle of 12: 12 hours and fed with a standard diet and water during the study. All the animals were divided into 3 groups having 6 animals each. The animal ethical clearance no IAEC/SCPER/2021-22/13 was approved before performing the study.

Group-I contained control animals (Untreated animals)

Group-II best formulation Niosomal gel (mupirocin, honey, and curcumin loaded gel) was administered

Group-III Application of marketed gel (Mupicip)

Excision wound model preparation

Anesthetic ether (0.05 to 0.1 mg/kg SC) was used to anesthetize the rats (33). Then the rats were subjected to shaving the hair on the back by using a razor. A 15 mm full-thickness wound of 3X3 cm with sterile surgical scalpels was created. The incisions were created in the dorsal lumbar region, 1.5 mm from the midline on the back of rats. Finally, the rats were divided into three separate groups as mentioned earlier. The wounded rats of group I were considered as controlled ones (Left untreated), group II treated with the best formulation F7 (mupirocin, honey, and curcumin loaded gel), and group III with marketed mupirocin gel (Mupicip) applied twice a day until the complete healing of the wound. The wound contraction was measured every 3 days interval to determine the % of wound contraction. The time for wound closure was noted when total healing occurred.

Histopathological studies

The skin specimens were collected in 10% buffered formalin (34) from rats of the 3 different groups after anesthetized. Then, 5-micrometer-thick sections were sliced and stained with hematoxylin and eosin dye for histological examination. The Olympus photomicroscope (400X magnification) was used to evaluate the sliced sections in terms of collagen formation, fibroblast proliferation, keratinization, and epithelisation.

Results and Discussion

FTIR Study

To investigate the compatibility of drugs and polymers, the pharmaceutical industry frequently uses the potent analytical method known as Fourier transform infrared spectroscopy (FTIR). This method examines the interactions between the drug and the polymer as well as any modifications to the polymer's chemical structure that result from the drug's inclusion. As FTIR is a non-destructive and non-invasive method, the chemical makeup of the materials being examined is not changed. FTIR can also pick up electrostatic, hydrogen bonds and van der Waals interactions between the medication and the polymer. These interactions may have an impact on the mechanical and thermal characteristics of the polymer as well as the stability, solubility, and bioavailability of the medication. FTIR spectra of mupirocin, honey and curcumin, and all other excipients and their compositions were considered for compatibility study shown in Figure 1. Obtained spectra of mupirocin confirm that the peak position and intensity of the peaks don't alter due to the addition of honey, curcumin, and other excipients in different for-

mulations. Mupirosin spectra show major peaks 3471.08, 3304.2 cm^{-1} corresponding to OH-stretching 2934.18, 2850.74 cm^{-1} CH-stretching 1723.98, 1658.68 cm^{-1} belongs to C=O stretching 1449.73 cm^{-1} for C-C stretching. Mupirosin with honey spectra appears at 3471.08, 3310.01, cm^{-1} corresponding to OH-stretching 2928.37, 2860.17 cm^{-1} CH-stretching 1721.80, 1658.68 cm^{-1} belongs to C=O stretching 1402.37 cm^{-1} for C-C stretching. Mupirosin with curcumin spectra appears at 3471.88, 3325.24, cm^{-1} corresponding to OH-stretching 2943.61, 2857.27 cm^{-1} CH-stretching 1718.17 cm^{-1} belongs to C=O stretching 1456.26, 1408.37 cm^{-1} for C-C stretching. FTIR spectrum of mupirocin with carbopol shows at 3471.08, 3298.04 cm^{-1} corresponding to OH-stretching 2928.37, 2844.94 cm^{-1} CH-stretching 1718.17, 1652.88 cm^{-1} belongs to C=O stretching1449.23 cm^{-1} for C-C stretching. Mupirocin with Tween 80 shows the peak at 3471.08, 3304.20 cm^{-1} corresponding to OH-stretching 2928.37, 2857.27 cm^{-1} CH-stretching 1723.98, 1652.88 cm^{-1} belongs to C=O stretching1449.23 cm^{-1} for C-C stretching. Similarly, mupirocin with cholesterol composition shows IR frequencies at 3471.08, 3301.30, cm^{-1} corresponding to OH- stretching, 2932, 2859.45 cm^{-1} CH-stretching, 1723.98, 1647.07 cm^{-1} belongs to C=O stretching. The active pharmaceutical ingredient, mupirocin, did not interact chemically with honey, curcumin, or any of the other excipients, according to an FTIR result with a minor change in the FTIR spectrum shown in Figure 2. So, it may be concluded that the active substance is compatible and has retained its functionality.

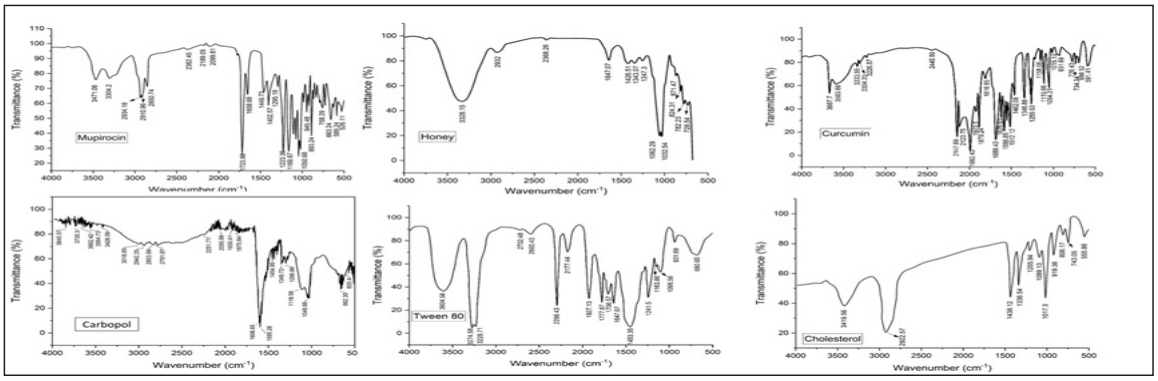

Figure 1: FTIR spectrum of mupirocin, honey, curcumin, and excipients

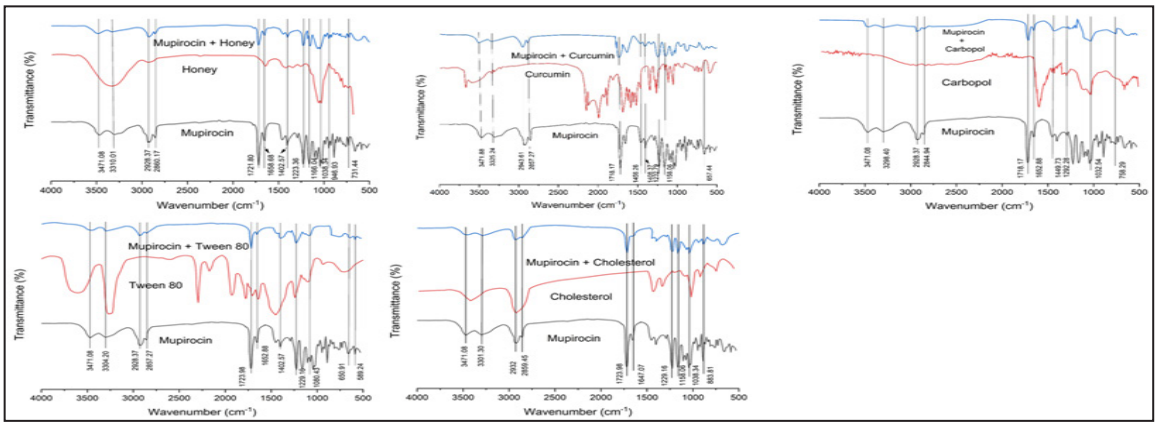

Figure 2: FTIR spectrum of mupirocin mixed with honey, curcumin, and excipients

DSC study

In research on drug-polymer compatibility, differential scanning calorimetry (DSC) is a method that is frequently utilized. DSC can detect any changes in the thermal behavior of the polymer following the addition of the drug since it detects the heat flow connected to thermal transitions in materials, such as melting and solidification. Insights on the compatibility between the medicine and the polymer may be gained by using DSC to identify changes in the glass transition temperature, melting point, and crystallization behavior of the polymer. DSC is more responsive to changes in the polymer's thermal behavior. Changes in these parameters can indicate the formation of new chemical entities resulting from drug-polymer interactions. Mupirocin, honey, curcumin, carbopol, tween 80, and cholesterol endothermic peaks were observed at 77.78°C, 192.44 °C, 172.47 °C, 74.39 °C, 79.15 °C, 149.09 °C respectively shown in figure3. Figure 4 represents the DSC curve for a mupirocin mixture with honey, curcumin, carbopol, and other excipients. Mupirocin with honey represents the spectra at 78.42 °C, mupirocin with curcumin shows spectra at 77.78 °C, mupirocin with carbopol shows spectra at 77.73 °C, mupirocin with tween 80 shows spectra at 77.43 °C and mupirocin with cholesterol shows spectra at 77.75 °C. Obtained results show minor changes in the peak shape and width and intensity, which should be an indication of mupirocin is compatible with other excipients used in different formulations.

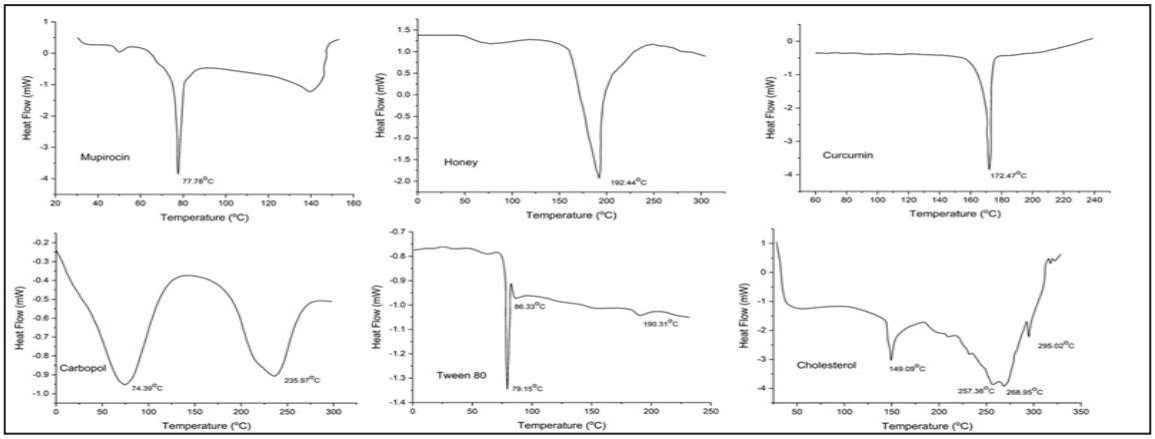

Figure 3: DSC spectrum of mupirocin, honey, curcumin, and excipients

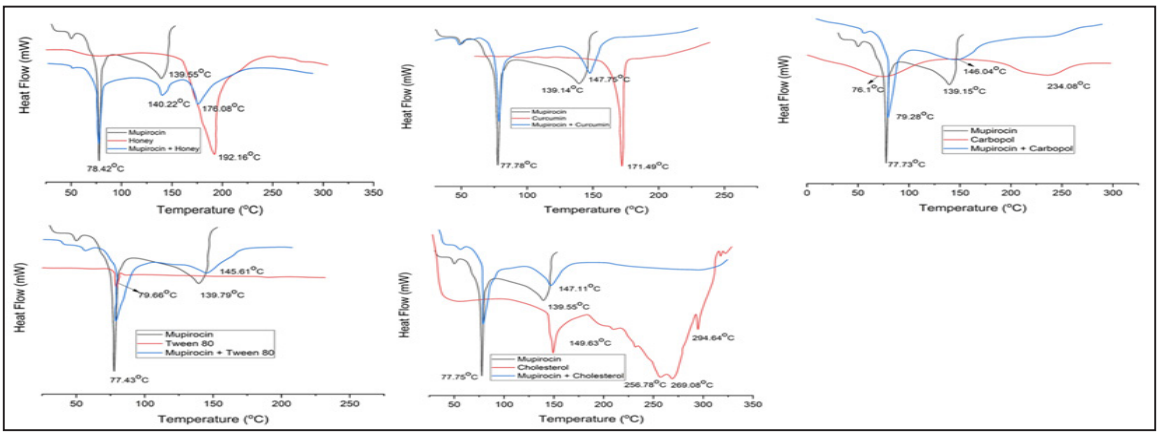

Figure 4: DSC spectrum of mupirocin mixed with honey, curcumin, and excipients

Evaluation study of Mupirocin-loaded niosomal gel

The entrapment efficiency of the drug

Entrapment efficiency (EE) is a measure of the amount of drug mupirocin that is effectively trapped or encapsulated within a drug delivery system, such as formulated niosomal gel. It is an important parameter in drug delivery systems, as it directly affects the drug release rate, bioavailability, and therapeutic efficacy. A high entrapment efficiency is desirable in drug delivery systems, as it indicates that a greater proportion of the drug is being delivered to the target site while minimizing potential side effects from the drug being released in unintended areas. A low entrapment efficiency may result in inadequate drug delivery, lower therapeutic efficacy, and increased toxicity due to higher drug concentrations in non-target areas. Obtained results confirm that 80% to 98% of mupirocin is entrapped in various formulations shown in Table 2. Cholesterol renders the niosomes impenetrable (35) and improves aqueous phase entrapment effectiveness and permeability. With the present formulation of niosome gel (36), nonionic surfactants also significantly improved the drug's ability to be entrapped.

Drug content

Drug content is an important quality control parameter that measures the amount of mupirocin present in a niosomal gel formulation. The drug content of a drug product should be within a specified range. Variations in the drug content of a drug product can occur due to several factors, such as variability in the manufacturing process, changes in the source or quality of the API or excipients, and inadequate mixing or blending of the drug product components. It was observed that a high carbopol content helps to keep more of the medicine in the niosomal gel. The drug concentration was obtained between 84% and 99% shown in Table 2.

Determination of pH

The pH is an important parameter that can affect the stability, solubility, and bioavailability of a drug product. For example, certain drugs may be unstable or insoluble at extreme pH values, and changes in the pH of a drug formulation can affect the rate of drug release and absorption in the body. Therefore, the pH of a drug product is carefully controlled and monitored during the manufacturing process and throughout its shelf life. According to Table 2, all mupirocin-loaded noisome-based gel formulations had a pH between 6.19 and 6.72, which is following the skin pH.

Determination of viscosity

Viscosity is a measure of the resistance of a fluid to flow and is a commonly important parameter in the formulation and processing of various drug products. The viscosity of a semisolid or gel-like drug formulation can affect its spreadability and adhesion to the skin or mucosal surfaces. According to Table 2, the viscosity ranged from 400 to 555 cps.

Spreadability

Spreadability is an important parameter in the development and formulation of topical drug products. The spreadability of a semisolid gel formulation can affect its ease of application, coverage area, and penetration into the skin or mucosal surfaces. The spreadability of a semisolid formulation depends on various factors, such as the viscosity, rheology, surface tension, and particle size distribution of the formulation. Optimization of these factors can ensure optimal spreadability and uniformity of drug distribution on the target surface, which can enhance the efficacy and safety of the drug product. Table 2 shows that the Spreadability of all formulations comprising niosomal gel loaded with mupirocin varied from 4.36 to 5.2 g. cm/s.

Table 2: An evaluation study of mupirocin-loaded niosomal gel

Formulation	Entrapment efficiency (%)	Drug content (%)	pH	Viscosity(cps)	Spreadability (g cm/s)
F1	87.15±0.13	89.25±0.64	6.32±1.36	401.61±3.33	4.79±0.39
F2	93.17±0.72	84.51±0.13	6.31±0.05	400.35±2.94	4.36±0.27
F3	97.73±0.68	96.87±0.19	6.72±0.82	455.51±3.94	4.91±0.86
F4	98.24±0.75	98.82±0.91	6.77±0.36	555.74±5.93	5.2±0.91
F5	97.95±0.83	91.27±053	6.19±0.35	538.32±9.97	4.98±1.74
F6	80.03±0.86	98.13±0.61	6.52±0.17	510.02±11.13	4.63±0.62
F7	94.09±0.57	96.31±0.62	6.41±0.94	535.79±5.68	4.71±0.07
F8	95.61±0.53	97.61±0.75	6.71±0.91	540.84±11.76	4.8±0.74

*Results are exp ressed as of mean ±SD (n=3)

SEM analysis

SEM is a powerful imaging technique that involves scanning a beam of high-energy electrons across a sample surface and detecting the electrons that are scattered or emitted from the surface to generate a high-resolution image. SEM can provide detailed information on the size, shape, and surface characteristics of formulated mupirocin loaded niosome gel. The SEM image of the best formulation F7 was discovered to be a spherical shape and particle sizes ranging from 90 nm to 120 nm. Figure 5 illustrates the SEM image of the best formulation F7, which was found to have a homogeneous distribution of niosome sphere-shaped particles (37).

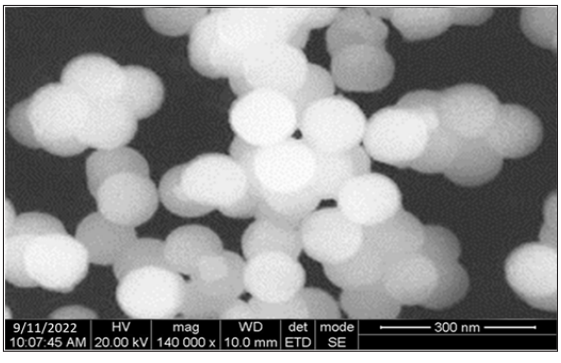

Figure 5: SEM Image of formulation F7 (Mupirocin loaded niosomal gel)

In-Vitro diffusion study

In-vitro diffusion study is a common technique used to assess the rate and extent of drug release from a dosage form. This technique is used to evaluate the release and diffusion of drug molecules from a formulation through a membrane. In this study prepared niosomal gel was placed on one side of a membrane and exposing it to a buffer solution on the other side. The drug molecules then diffuse through the membrane or tissue into the solution, and the concentration of the drug in the solution is measured over time using UV-visible spectroscopy. *An in-vitro* diffusion study was carried out in triplicate using the diffusion medium Phosphate buffer with a pH of 7.4. The percentage of mupirocin drug release at the end of 14 hours varied between 98% and 100% for all niosomal gel formulations shown in Figure 6.

Formulation F1 contains a drug-polymer (mupirocin-carbopol) ratio (2:1) and curcumin without honey shows 98 % drug release at 7h. Formulation F2 contains only drugs without polymer, curcumin, and honey and shows 100 % drug release at 6h. Formulation F2 has shown quick release of mupirocin due to the absence of carbopol in the formulation but the viscosity of honey manages the rate of release till 6h. Formulation F3 contains a drug with an

equal amount of polymer, curcumin, and honey and shows 98% drug release at 8h. Formulation F4 contains a drug-polymer ratio (2:3) high concentration of carbopol and honey and a low concentration of curcumin showing 99% drug release at 9h. Formulation F5 contains an equal amount of drug-polymer ratio (2:2) and a high concentration of honey with a low concentration of curcumin shows 100% drug release at 11h. Honey shows high viscosity and is useful in controlling the release rate of mupirocin at high concentrations. Formulation F6 contains an equal concentration of polymer and honey with a low amount of curcumin showing 99% drug release at 10h. Formulation F7 and F8 contain equal amounts of drug-polymer ratio (2:2) with variation in curcumin and honey showing 100% and 99 % drug release at 14 h and 12 h respectively. Formulation F7 has shown 100% drug release at 14 h, chosen as the best formulation among others.

The retarded release of mupirocin from niosomal gel is due to the concentration and viscosity grade of the polymer used and also due to the addition of a nonionic surfactant in the formulation. Carbopol is a water-soluble polymer that is commonly used as a thickening agent in pharmaceuticals, while honey is a natural product that has been used for medicinal purposes for centuries. Researchers confirm that carbopol and honey were combinedly and used for controlled drug release. The carbopol-honey combination can sustain the release of an active drug with the modulation of ratio. Reports confirm that the carbopol-honey had antibacterial properties, which could be useful in wound healing applications. Mupirocin drug release from niosome gel, which involves the polymer ability to form a gel matrix and its influence on the physicochemical properties of the niosomes. Carbopol can form a gel matrix when it is hydrated, which can entrap the drug and create a network that controls the diffusion and release of drugs. Gelling property of carbopol for topical formulations to preserve the drug molecule for a long time and generate a stable plasma drug concentration. The use of carbopol in niosomal gels for drug delivery is a promising strategy for achieving controlled drug release, and further, enhancing the safety and efficacy of the gel. Available reports confirm that the non-ionic surfactant tween 80 enhances the entrapment efficiency of the active drug in the formulation. Due to the higher concentration of mupirocin drug getting entrapped inside the niosome, it helps Carbopol to sustain the drug and provide better therapeutic efficacy. Overall, the combination of carbopol and honey with non-ionic surfactant tween 80 in niosomal gel, can control the drug release and shows antibacterial properties towards topical wound healing treatment.

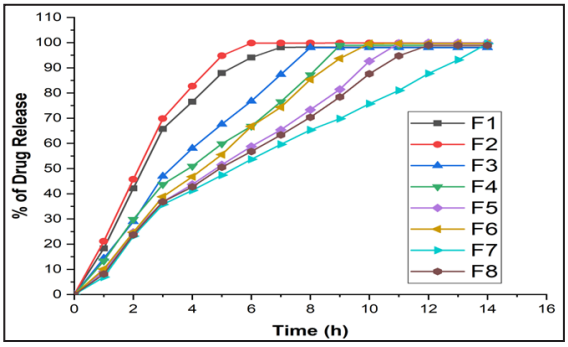

Figure 6: *In-vitro* dissolution of mupirocin-loaded niosomal gel formulations (F1-F8)

Release kinetic study of a mupirocin-loaded niosome-based gel

A release kinetic study is used to determine the release behavior of drugs from drug delivery systems over time. This kinetic study provides important information about the mechanism of the rate of drug release behavior. Obtained drug release data at different time intervals fitting to mathematical models to describe the release kinetics. The mathematical models that can be used to describe the release kinetics of drugs from drug delivery systems, include zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The greatest R^2 value was used as the best-fit criterion for choosing the optimal model. The Korsmeyer-Peppas model is a more complex model that can describe drug re-

lease from a variety of delivery systems, including matrix and reservoir systems, and assumes that the release rate is controlled by both diffusion and erosion. Table 3 displays the results, the drug release followed zero-order kinetics, independent of concentration. The illustration data fit into Peppa's equation, which depicted non-fickian release,

implying diffusion release and a mixture of diffusion and erosion release of the noisome-based gel.

Stability study

A stability study is an important parameter, which involves evaluating the physical, chemical, and microbiological stability of a drug product over time under specific storage conditions. Stability studies reveal that the drug product maintains its quality, efficacy, and safety throughout its shelf life, and establishes appropriate storage and handling conditions.

To establish its self-life, formulation F7 was

Table 3: Release kinetics of mupirocin-loaded niosomal gel formulation (F1-F8)

Formulation	R^2 Values					
	Zero-order plots	First-order plots	Higuchi plots	Korsmeyer-peppas plots		Order of release
				R^2	Diffusional exponent (n)	
F1	0.987	0.961	0.959	0.663	0.983	Diffusion
F2	0.958	0.824	0.956	0.641	0.972	Diffusion
F3	0.972	0.831	0.960	0.726	0.999	Diffusion
F4	0.981	0.943	0.96	0.712	0.983	Diffusion & Erosion
F5	0.989	0.639	0.951	0.791	0.977	Diffusion & Erosion
F6	0.991	0.724	0.947	0.787	0.89	Diffusion & Erosion
F7	0.996	0.992	0.966	0.790	0.894	Diffusion & Erosion
F8	0.985	0.877	0.955	0.798	0.837	Diffusion & Erosion

Table 4: Stability study for mupirocin-loaded niosomal gel formulation F7

Storage condition	Days	Evaluated parameters				
		physical appearance	pH	viscosity	Spreadability	drug release profile (12h)
25°C/60%RH	0	Clear and transparent	6.41	535.79±5.68	4.71±0.07	100%
	30	Clear and transparent	6.49	535.6±5.45	4.69±0.05	98.75%
	60	Clear and transparent	6.5	534.91±5.04	4.68±0.03	98.16%
	90	Clear and transparent	6.5	534.87±5.01	4.68±0.02	97.95%
40°C/75%RH	0	Clear and transparent	6.41	535.79±5.68	4.71±0.07	100%
	30	Clear and transparent	6.46	534.91±5.32	4.70±0.03	97.35%
	60	Clear and transparent	6.51	534.01±5.11	4.69±0.03	96.16%
	90	Clear and transparent	6.52	533.98±4.93	4.69±0.01	95.93%

Results are expressed as of mean ±SD (n=3)

chosen for two different temperatures and related humidity for stability investigation shown in Table 4. The chosen formulation's ultimate purpose is self-life. The results of a 3-month accelerated stability analysis show that there have been no appreciable changes in the physical characteristics (consistency, morphology, and color), pH, viscosity, or spreadability. The drug release profile presented in Table 4 has a slight deviation. The stability of the mupirocin-loaded niosomal gel is confirmed by the obtained findings, which demonstrate no change or a slight deviation in formulation F7.

In vivo wound healing study on an albino rat model

Wound healing is a complex process that involves various cellular and molecular events, including inflammation, angiogenesis, and tissue remodeling. In a rat model study, the niosomal gel loaded with mupirocin, honey, and curcumin (Formulation F7) and marketed available mupicip medicine were applied topically on full-thickness skin wounds considered as group-II and Group-III respectively. Group-I rats are untreated (no application of medicine) and considered a controlled group. Figure 7 depicts the experimental process and the wound healing capability after each treatment. There was no redness, exudation, infection, or suppuration with experimental groups during the monitoring time. All of the wounds had blood scabs on them and were healing. Rats treated with formulation F7 healed wounds more quickly as compared to commercially available mupicip. On the 12th post-operative day, the wound closure area in group II (99.08%) was greater than group III (87.34%), and group-I (80.73%). The niosomal gel showed faster wound closure, increased collagen synthesis, and decreased inflammation compared to the other groups.

Niosomal gel is a novel drug delivery system that can improve the efficacy and bioavailability of drugs by enhancing their penetration into the skin layers and promoting their sustained release. Mupirocin is an antibiotic that inhibits bacterial protein synthesis, thereby preventing bacterial infection and promoting faster wound healing. Honey shows antibacterial, anti-inflammatory, and antioxidant properties. It enhances wound healing by promoting tissue regeneration and reducing inflammation. The niosomal gel loaded with honey retard the release rate of active ingredients into the wound, which can enhance its therapeutic effect and promote faster wound healing. Curcumin is a polyphenol compound with potent anti-inflammatory and antioxidant properties. It helps in reducing inflammation, promoting tissue regeneration, and increasing collagen synthesis. The niosomal gel loaded with curcumin can enhance its bioavailability and provide better wound-healing properties.

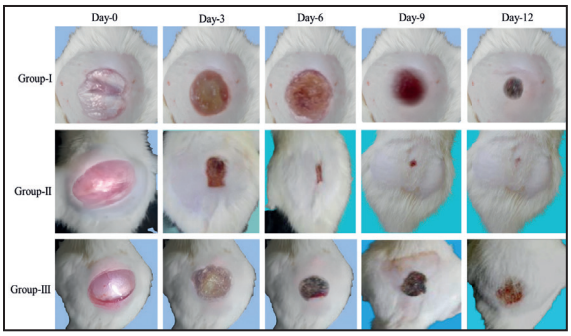

Figure 7: Images represent wound repair on excision wound model days 0-12.

The use of niosomal gel loaded with mupirocin, honey, and curcumin may provide a novel and effective approach to the treatment of skin wounds in animal models. Overall, the niosomal gel (formulation F7) can promote faster wound healing by preventing bacterial infection, reducing inflammation, endorsing tissue regeneration, and increasing collagen synthesis.

Histopathological study

In the case of niosomal gel loaded with mupirocin, honey, and curcumin on a rat model, a histopathological examination can provide insights into the mechanism of action and safety of the treatment. The examination showed a significant increase in the number of fibroblasts,

which are cells responsible for producing collagen, the primary component of the extracellular matrix in wounds. Figure F8 represent the histopathological study of group-I, group II, and group III where (a) Rearrangement of intact epidermis/ epithelial cells; (b) Well-developed blood vessel, more organized collagen fibers, formation of mononuclear cells, neovascularisation, and hair follicle growth; and (c) Hair follicle development. Re-epithelization and remodeling of epithelial cells. (d) Maximum number of fibroblasts and newly-formed blood vessels, angiogenesis (H & E photograph captured with an Olympus photomicroscope at 400X magnification)

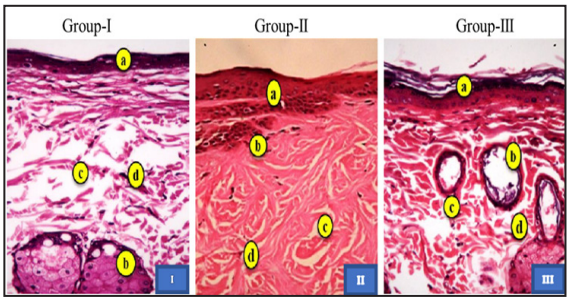

Figure 8: Histopathological photomicrographs of wounded tissue of rats on day 12

Histological examination of the lesion areas of the treated groups confirmed an increase in cellular infiltration, angiogenesis, fibroblast depositions, and collagen depositions. Due to the chemotactic effect of the niosomal gel (Formulation F7) in group II, the mechanisms of topical action on the lesion site may have attracted inflammatory cells. Further mitogenic activity may have increased cellular proliferation and contribute substantially to wound healing. On the 12th day, group II exhibited significantly smaller wound areas than group I &III.

This finding indicates that the niosomal gel may promote tissue regeneration and faster wound healing. Moreover, there was a significant decrease in the number of inflammatory cells, such as neutrophils and macrophages, in the wound site of the rats treated with the niosomal gel. This finding indicates that the niosomal gel may have anti-inflammatory effects, which can promote faster wound healing by reducing inflammation and preventing the formation of chronic wounds. The histopathological examination also showed that the use of the niosomal gel did not cause any significant adverse effects or tissue damage. This finding indicates that the niosomal gel is safe and well-tolerated in the rat model.

Conclusion

Treatment of wound healing problems depends on the severity of the wound and its treatment procedure such as the application of antibiotics to treat infection, surgical debridement to remove dead tissue, wound dressings to promote healing, and lifestyle changes such as improved nutrition and exercise. In some cases, advanced wound care techniques such as nano drug delivery or growth factor therapy may be necessary to promote wound healing. In conclusion, the combination of mupirocin with honey and curcumin in a niosomal gel has the potential to provide a novel approach to wound healing treatment. Niosomes may serve as an effective drug delivery vehicle because it helps in controlling rapid drug release, targeted drug release and improve penetration. The combination has been shown to provide a synergistic effect in promoting wound healing, due to the antibacterial and anti-inflammatory properties of these compounds. Compatibility studies between drug, polymer, and other excipients, confirm by FTIR and DSC studies. Obtained results reveal entrapment efficiency, drug content, pH, viscosity, spreadability, and *in vitro* diffusion studies within the limit of IP. Formulation F7 is considered the best formulation. SEM analysis confirms the spherical shape of particles in the range of 90-120 nm. Kinetic studies depicted non-fickian release, implying diffusion release and a mixture of diffusion and erosion release of the noisome-based gel. The stability study confirms that obtained niosomal gel is stable with minor deviation. The niosomal gel formulation F7 healed wounds more quickly as compared to commercially available mupirocin gel. On the 12th post-operative day, the wound clo-

sure area in group II (99.08%) was greater than group III (87.34%), and group-I (80.73%). The histopathological examination for formulation F7 showed increased fibroblast activity and decreased inflammation, indicating that the niosomal gel can promote faster wound healing without causing significant tissue damage and has shown superior therapeutic options compared to conventional dose forms.

Ethics

All the *In-Vivo* procedures were approved by the Institutional Animal Ethic Committee (IAEC) as per CCSEA (Committee for Control and Supervision of Experiments on Animals), India.

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Conflict Of Interest

The authors declared no conflict of interest.

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