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Formulation and in vitro /in vivo Evaluation of Silymarin Solid Dispersion-Based Topical Gel for Wound Healing

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Abstract

Silybum marianum, from which silymarin (SM) is extracted, is a medicinal herb. In the Biopharmaceutics Classification System, it is of the class II type, meaning it is almost completely insoluble in water. It has a number of therapeutic properties, including anti-inflammatory as well as properties that promote wound healing.

The aim of this research is to promote the dissolution and solubility of SM by employing a technique called solid dispersion and then incorporating the formula of solid dispersion into a topical gel that can be used for wound healing.

Solid dispersion is a technique used to enhance solubility and dissolve pharmaceuticals that are not water-soluble. This method is widely used because of its low cost and high efficiency. Because of its capacity to repair skin, the hydrophilic carrier nicotinamide (NA) was selected in this investigation as the carrier. Kneading, solvent evaporation and fusion with a consistent drug-to-carrier ratio were the three separate processes utilized in the preparation of solid dispersion (1:1, 1:3, and 1:5). In addition, the products were examined to determine their physical characteristics and the degree of crystallinity. The selected formula was combined into a hyaluronic acid base gel using the cold method. This gel was then evaluated in vitro for physical qualities and put to an in vivo (animal) examination to determine how it healed wounds.

The study found that the solvent evaporation made SM 25 times more soluble and caused all of it to be released in 20 minutes for a 1:3 ratio.

The additional tests using DSC and XRD demonstrated the amorphous nature of the result. According to FTIR, there was no evidence of interaction between the two.

The gel formula had good qualities, like a pH of 6.6, good spreadability, and drug release within three hours. It also contributed to the rapid healing of wounds.

Keywords: Silymarin, Solid dispersion, Nicotinamide, Wound healing, Topical gel

صياغة وتقييم خارج وداخل الجسم للمتشتت الصلب لمادة سيليمارين كهلام موضعي لعلاج الجروح

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الخلاصه

العشبه الطبيه التي يستخرج منها السيلمارين هي Silybum marianum. و في نظام تصنيف الأدوية الحيوية يعتبر السيلمارين من التصنيف الثاني ، مما يعني أنه غير قابل للذوبان في الماء تقريبًا. له عدد من الخصائص العلاجية ، بما في ذلك الخصائص المضادة للالتهابات وكذلك التي تعزز التئام الجروح.

يهدف هذا البحث إلى تعزيز انحلال وذوبان السيلمارين من خلال استخدام تقنية تسمى المنتشر الصلب ثم دمج صيغة المنتشر الصلب في هلام موضعي يمكن استخدامه في التئام الجروح.

المنتشر الصلب هو تقنية تستخدم لتحسين الذوبان وإذابة المستحضرات الصيدلانية غير القابلة للذوبان في الماء. تستخدم هذه الطريقة على نطاق واسع بسبب تكلفتها المنخفضة وكفاءتها العالية.تم اختيار نيكوتين امايد بسبب قدرته في المساعده في اصلاح الجلد على اعتباره الناقل للصلب المنتشر. العجن وتبخر المذيبات والاندماج مع نسبة ثابتة من الدواء إلى الناقل كانت العمليات الثلاثة المنفصلة المستخدمة في تحضير المنتشر الصلب (١: ١ ، ١: ٣ ، ١: ٥). بالإضافة إلى ذلك ، تم فحص المنتجات لتحديد خصائصها الفيزيائية ودرجة التبلور. تم دمج الصيغة المختارة في هلام أساسه حمض الهيالورونيك باستخدام الطريقة الباردة. ثم تم تقييم هذا الجل في المختبر من حيث صفاته الفيزياوية و على الجسم الحي (على الحيوان) لتحديد كيفية شفاء الجروح.

وجدت الدراسة أن تبخر المذيب جعل السيلمارين٢٥ مره اكثر ذوبانيه وتحرر الدواء كاملا خلال ٢٠دقيقه باستخدام نسبة ١:٣. (سليمارين: نكيوتين امايد)

أظهرت الفحوصات الاخرى باستخدام المسعر الحراري التفاضلي وحيود الاشعة السينية الطبيعة غير المتبلورة للناتج . كما اظهر فحص الاشعة تحت الحمراء على عدم وجود تفاعل بين السليمارين والنيكوتين امايد .

كما اظهرت تركيبة الجل صفات جيدة ، مثل درجة الحموضة ٦,٦ ، وقابلية انتشار جيدة ، وتحرر الدواء في غضون ثلاث ساعات. كما أنه يساهم في سرعة التئام الجروح.

الكلمات المفتاحية : السليمارين، المتشتت الصلب، نيكوتين امايد، علاج الجروح، هلام موضعي

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Introduction

Milk thistle extract silymarin (SM) contains silibin, isosilibin, silydianin, silychristin, and other flavonolignans. There are unknown side effects, and herbalists recommend it [1] . SM is antioxidant, anti-inflammatory, anti-cancer, anti-obesity, antiviral, antibacterial, and liver protective [2].

SM suppresses the creation of leukotrienes and prostaglandins and has antioxidant and anti-inflammatory action on wounds and burns. As a result, SM is a promising therapeutic agent for wound healing [3].The reality is that SM does not really dissolve in water, which presents a challenge when used as a medicine[4] .

Solid dispersion (SD) is one common method used to improve drug solubility. The SD formulation's carriers might have therapeutic and biological effects that improve the skin's condition when applied topically and speed up processes like wound healing. One such carrier is nicotinamide (NA) [5].

The most common semisolid formulations for topical medicine administration include gels, creams, and ointments. Topical gel formulations are great for administering medicine since they are easily removed from the skin and have fewer side effects. When compared to traditional topical treatments like creams and ointments, gel formulations provide greater application qualities and durability[6]. One of the pharmacological issues for the topical gel is the solubility of the drug in its carrier, which is typically pure water[7].

The purpose of this research was to determine whether or not SM solubility might be enhanced by combining it with NA, a hydrophilic carrier, in the form of a topical gel and testing the effectiveness of the topical gel for wound healing.

Materials and Methods

Materials

Silymarin, Hyperchem supplied the drug (purity: 98%) (China), nicotinamide (Thomas baker chemicals (India)), Hyaluronic acid, Polyethylene glycol 400 (PEG 400), Methylparaben (sigma Aldrich, Germany)

All of the remaining materials and solvents were used in this paper of analytical grade.

Methodology

SM solid dispersion preparation via kneading (Kn)

As can be seen in Table (1), three distinct formulations (F1, F2, and F3) were made using this approach. In a glass mortar, SM and NA were blended in weight proportions of 1:1, 1:3, and 1:5, then moistened with water-methanol (1:1). This process took 30 minutes. In an oven set at 50 degrees Celsius, the paste was dried for a whole day after being made. When completely dry, the powder was sieved through a No. 60 mesh and placed in a desiccator for further analysis [8].

SM solid dispersion preparation via Solvent evaporation (SE)

Weighed amounts of SM and NA (1:1, 1:3, and 1:5) On a magnetic stirrer, they were dissolved in a sufficient volume of methanol until a solution that was completely clear was observed.; the mixture was dried at 50°C for twenty-eight hours, product then was passed processed through sieve No. 60 and placed in a desiccator pending further analysis [9].

SM solid dispersion preparation via Fusion (FS)

As shown in Table (1), this method was used to make three different formulas (F7–F9). NA was heated to 128 C° until it melted. A weighed amount of SM was then added, and the mixture was stirred with a glass rod until it was evenly spread out. The mixture was then cooled until it turned into a solid mass. A porcelain mortar and pestle were used to break up the product. The finely ground powder was put through a 60-mesh sieve to get particles of the same size. Then, it was put in a desiccator until more investigation could be done[10].

Preparation of the physical mixture

According to the chosen formula, physical mixture (PM) was created by carefully and in the proper proportions combining SM and NA.

Table 1. SD Formulas of SM Composition

Code of formula	Drug: carrier ratio	Method
F1	1:1	Kn
F2	1:3	Kn
F3	1:5	Kn
F4	1:1	SE
F5	1:3	SE
F6	1:5	SE
F7	1:1	FS
F8	1:3	FS
F9	1:5	FS

Preparation of gel

As shown in Table 2, a 2% hyaluronic acid gel base is made by dissolving 2 g of hyaluronic acid (HA) in 70 milliliters of cold, distilled water and allowing it to stand in the fridge overnight. A solid dispersion equal to 1 g of SM was dissolved in PEG 400 and added to the gel base. Methylparaben was then added as a preservative, after adjusting with cold distilled water as necessary, the mixture was stirred with a magnetic stirrer until a homogenous gel formed [11,12].

Table 2. Composition of Gel Formula

Ingredients	Quantity
SD of SM	equivalent to 1 g. SM
Hyaluronic acid	2 g.
PEG 400	22.56 g.
Methylparaben	0.1mg
Water up to	q.s

Evaluation of SD

Percent yield

The yield percentage was determined to assess the efficacy of the used technique. It is determined analytically using the following equation[13].

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoretical wt(drug+carrier)}} \times 100$$

Drug Content

A quantity of the produced SD equal to 10 milligrams of SM was accurately weighed, and then stirred for 10 minutes while in 20 ml of methanol. The solution was then filtered and diluted appropriately with the same solvent. A UV spectrophotometer with a wavelength of 288 was used to determine the medication concentration, and the experiment was repeated three times[14] .

$$\text{drug content} = \frac{\text{Practically obtained mass}}{\text{Theoretical mass}} \times 100$$

Saturation solubility studies

The shake–flask technique was used to conduct saturation solubility investigations using distilled water as the solvent. At 25 °C, an excess quantity of SM pure powder, created SD formulae, was added to 10 mL of water with nonstop shaking for a full two days. Afterward, the samples were screened through a filter. By the use of Millipore filter paper (0.45 m). Distilled water was utilized to dilute the filtrate and examined at 287 nm to quantify the amount of SM that had dissolved[15].

In vitro study of drug dissolution

Utilizing USP type II (paddle type) dissolving test device, the drug release of several formulations of SM- SD was investigated in vitro. As a dissolving medium, preheated to 37 degrees Celsius, 900 ml of phosphate buffered saline (pH 7.4) and 50 mg of drug samples were thrown over its surface. At regular intervals (10,20,30,40, up to 60min), 5ml aliquots were taken and replaced with new dissolving media. At 287 nm, the materials were examined spectrophotometrically. This test was performed three times [16] .Using the following equation, a similarity factor was computed and used for statistical analysis of the dissolution profiles.

$$f2 = 50 \cdot \log\left\{100 \cdot \left[1 + \frac{1}{n}\sum_{t=1}^{n}(Rt - Tt)^2\right]^{-0.5}\right\}$$

(Rt) is the reference dissolution value at time t , (Tt) is the test dissolution value, and (n) is the number of dissolution time points. If *f*2 is greater than or equal to 50 the dissolution profile is deemed similar. If *f*2 is less than 50, the dissolution profiles are not considered similar [17].

The influence of various drug-to-carrier ratios and preparation techniques on the dissolving behavior of SM from a solid dispersion was investigated.

Selection of the best formula

the formula that was considered as the optimum formula has been chosen in accordance to its solubility and dissolution tests.

Evaluation of the selected formula

FT-IR Spectroscopy:

FTIR was used to collect the (FTIR) spectra (Shimadzu 8300 Japan). Bromide of potassium was used to compress samples of untreated SM, NA, chosen formula, and PM. Within a scanning range of 4000-0 cm-1, spectra were recorded[18,19].

Powder X- ray diffraction studies (PXRD)

X- ray diffraction studies were used to evaluate the crystalline state of pure SM, NA, a particular SD formula and the PM of the same SD formula. using a PXRD diffractometer (XRD-6000, Shimadzu, Japan) The PXRD was carried out under the subsequent circumstances: target metal Cu, filter K, voltage 40kV, and current 30mA. The samples were scanned at 1.5406 wavelength across a range of 2 including 10-60° [19].

Differential Scanning Calorimetry (DSC)

5mg samples of SM, NA, the chosen formula, and its PM were heated at scanning range of 10 degrees Celsius per minute in an aluminum pan in a nitrogen environment in the 50-300 C temperature range using DSC-60 plus (Shimadzu, Japan). A comparable empty pan was used as a comparison[20].

Evaluation of Gel

Visual appearance

The formula was visually inspected against white and black paper in order to detect any incompatibility resulting from an interaction between SM and excipients or among excipients themselves. A change in shade or precipitation was seen as an indication of incompatibility[21].

Spreadability assessment

The gel formula's spreadability was evaluated by spreading 0.5 grams of the gel in a circle with a 2 cm diameter on a plate of glass and then using a second glass plate on top. For 5 minutes, a 0.5 kg weight was applied to the top glass plate. After dispersing the gel, the diameter of the circle was measured (cm)[22].

Determination of viscosity

The produced gel formula's rheological characteristics were examined using a Myr digital rheometer (Model VR 3000, Spain). Initially, the viscometer is calibrated after being surface-balanced. The material viscosity was tested in triplicate at room temperature. For gel, samples were sheared using spindle R6 at 10, 12, 20, 30, 50, 60, 100, 200 rpm.

Utilizing plots of shear stress (rpm) vs viscosity (cP), the interpretation of rheological behavior was determined[23].

pH determination

By putting the tip of an electrode on the gel's surface and taking a reading with a pH meter two minutes later, the pH was reported[22][23].

Determination of drug content

After dissolving 10 mg of SM in 20 ml of methanol by weight while stirring for an hour, the gel containing the SD was filtered, the filtrate was diluted enough and the absorbance at 288 nm was measured spectrophotometrically against a blank.

In vitro Dissolution

SM was released in vitro from the gel formula using the dissolving apparatus-II (paddle type). A gel containing 50 mg of SM (5 gm) was distributed uniformly on a petri plate and then dissolved in 900 ml of dissolving fluid (phosphate buffer saline, pH 7.4) at 37.0°C. The distance between the paddle and the disk was about 2 cm, and it made 100 rotations each minute. At 30, 60, 90, 120, 150, and 180 minutes, 10 ml samples were taken and replaced with fresh volumes of buffer to maintain the sink condition by keeping constant volume of the buffer [24].

In vivo Evaluation of wound healing activity

Animal

A total of twenty-four Wister albino rats (both sexes) weighing between one hundred and two hundred grams (3 months old) were used in the experiment. These rats were sourced from the Animal House Unit at the University of Baghdad's College of Pharmacy. About 2 weeks before the experiment, The animals were individually caged and allowed free access to water and a standard rodent chow diet (18% Protein, 5% fat, Envigo Teklad Global Rat Food Pellets 2018), food was provided in a standard stainless-steel hopper. The animals were maintained at room temperature (23 – 25°C), 30–40% humidity, and lights on from 7 AM to 5 PM. The rats were maintained in polypropylene cages with husk as the bedding material.

The human care of the animals was according to international guidelines for the care and use of laboratory animals, All of these efforts were made to reduce the number of rats and their suffering, Such as constant checking of animal house air conditioning with standard free access to tap water/ad libitum and cleaning the animal house and cages [25].)

Wound induction and studied groups

The rat's back was shaved with an electrical clipper after it was sterilized with povidone-iodine and anesthetized. Using a sterile scalpel, cuts of approximately 2 centimeters in length were made on the shaved skin of the back, just below the shoulder blades (26). Any cut made here would be out of the animal's reach and safe from self-licking. The count of days began on the day of the injury.

The animals were divided into four groups of six; the first group (negative control) received normal saline solution (as a placebo vehicle) topically on skin wounds once daily; the second group (Positive control) received MEBO topically on skin wounds once daily; the third group received HA base gel; and the fourth group received SM gel.

The wounds were checked every other day and their length was measured.

Statistical analysis

The results of the experiments were analyzed using analysis of variance (ANOVA) for repeated measures, and the outcome was reported as the mean of triplicate samples standard deviation (std). Using SPSS version 29, we determined that a significance level of ($p \leq 0.05$) was statistically significant.

Results and Discussion

Evaluation of SD

The percentage yield of prepared SD

The PY% of the prepared SD for all of the prepared formulas ranged from 90.5% to 101.5% (Table 3), indicating that efficient methods were used to prepare them.

Drug content of SD

All of the prepared SD formulations contained an acceptable amount of the drug, ranging from 90.18 to 101 percent. This demonstrated that the drug was evenly distributed throughout the SD. Table 3 displays the amount of each drug present in the various SM SDs.

Table 3. Percentage yield and drug content of the prepared SD formulas

Formula code	Yield %	Drug content %
F1	95	95.57
F2	101	101
F3	99	90.18
F4	95.2	99
F5	99.7	99.5
F6	92.9	98
F7	99	101
F8	91	100
F9	90.5	92

Solubility at saturation of pure SM and SM-SD

It was determined that the saturated solubility of SM in water is 0.05 mg/ml. According to USP, this indicates that SM is nearly insoluble in water.

The results of saturation solubility tests on pure SM and SM-SD formulations are presented in Table 4.

Table 4. Saturation solubility of pure SM and prepared SDs

Formula code.	Saturation solubility in mg/ml
SM	0.05±0.01
F1	0.235±0.03
F2	0.806±0.001
F3	0.787±0.003
F4	0.268±0.04
F5	1.25± 0.05
F6	1.07±0.1
F7	0.177±0.03
F8	0.418±0.01
F9	0.603±0.01

In comparison with pure medicine, the solubility of SM was significantly improved by all of the SD formulations except F7. This difference was statistically significant ($p \leq 0.05$) . Because the carrier was hydrophilic, the drug was able to dissolve more readily in water, which contributed to the drug's increased solubility [27].

Furthermore, a substantial improvement ($p<0.05$) was observed by employing a greater drug: carrier ratio within the formula, confirming that the hydrophilic feature had a significant influence on solubility [19].

The process that was used to create the SM-SD had an effect on its solubility; the water solubility of the medication was significantly improved, with SE > Kn > FS being the order in which this occurred. When compared to drug powder, the SD that was formed by the solvent evaporation approach resulted in an approximately 25-fold increase in drug solubility. This is because SD made by evaporating the solvent has a more even distribution of SM in hydrophilic NA than other methods[28].

In vitro dissolution study

The dissolution properties of pure SM were compared with those of four different formulas of SM-SD, each of which was optimized for maximum solubility (Figure 1). The dissolution of the drug from the four SDs formulations (F2, F3, F5, and F6) was faster in comparison with the dissolution of the pure drug in a given time course, with *f*2 values of (9.2, 18.58, 9.5, and 7.5) respectively. This can be attributed to solubility enhancement, which agrees with the Noyes-Whitney equation [29].

Also, the hydrophilic nature of the carrier, the increased wettability of the drug particles, and the smaller size of the drug particles when solid dispersions form as a result of dispersing a drug in another material are all good ways to get around the force between drug molecules and speed up dissolution [9].

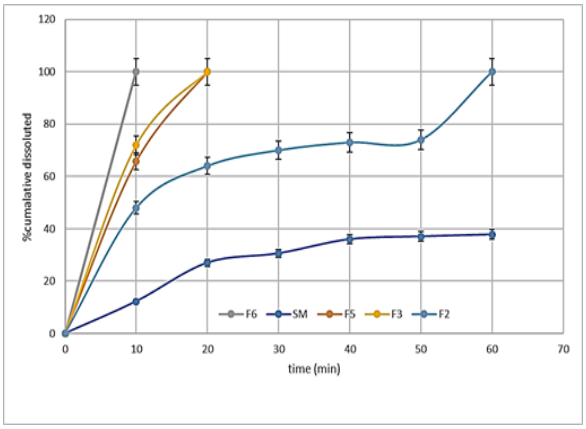

Figure 1. The effect of method and drug carrier: ratio of SD on the *in vitro* dissolution rate of SM at 37°C and a phosphate buffer saline with a pH of 7.4.

Furthermore, the technique of preparation had an influence on the effect of drug: carrier ratio on dissolving. SD formulations (F2, F3) created by Kn demonstrated improved solubility by raising the drug: carrier ratio (*f2* =18.5, 9), but those made by SE (F5, F6) had no influence on dissolution profile since both formulae released more than 85% after 15 minutes[30].

Furthermore Figure 2 showed that enhanced dissolution was obtained by F5 compared to its PM (*f2*= 9.5), while PM showed similar release profile compared to the drug (*f2*= 80.3). These results indicated that the method of preparation rather than the carrier itself had an effect on enhancing the dissolution.

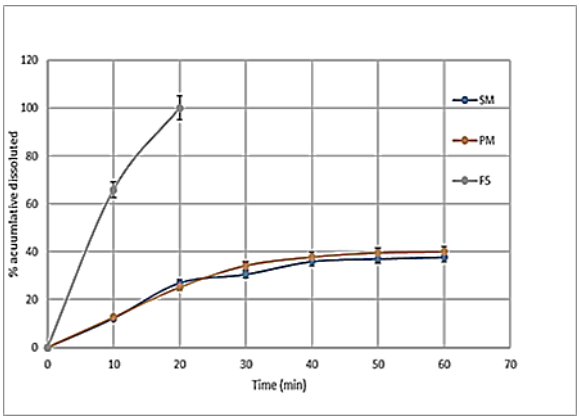

Figure 2. The in vitro release of the F5 compared to that of the pure drug and PM at 37°C and a phosphate buffer saline with a pH of 7.4.

The selection of the best appropriate formula

Because it displayed the greatest improvement in solubility and released more than 85% of the active ingredient within 15 minutes, the F5 formulation, which was produced by SE and composed of SM and NA in a 1:3 ratio, was chosen as the optimal formulation. This formulation was sent in for additional testing to be carried out *in vitro* evaluation of the selected formula

Powder X-Ray Diffraction (PXRD)

Figure 3 depicts the patterns obtained by the SM, NA, F5, and its PM during the PXRD analysis of crystal morphology. SM powder's sharp and intense diffraction peaks revealed its crystalline structure. The locations of the most prominent peaks at **2Θ** of 14 °, 17 °, 19 °, and 24° (Figure 3-A), which confirm the crystalline structure of the drug as demonstrated by the previous results, are shown [31]. The PXRD pattern of NA (Figure 3-B) showed characteristic diffraction peaks at **2Θ** Θ of 15◦, 24.81◦, 26.43◦. indicating that the carrier was also in crystalline state, which were in accordance with the previous results[32].

The PM (Figure 3-C) showed reduced intensity of SM and NA peaks which mainly due to dilution effect[33] .

F5 shown in Figure 3-D demonstrated the disappearance of the most characteristic peaks of SM, which pointed to the fact that the SM was in amorphous form [14], while NA showed its characteristic peaks but with reduced intensity indicating that it preserves its crystalline nature.Amorphous state of SM within SDs may contribute to the increase in its solubility.

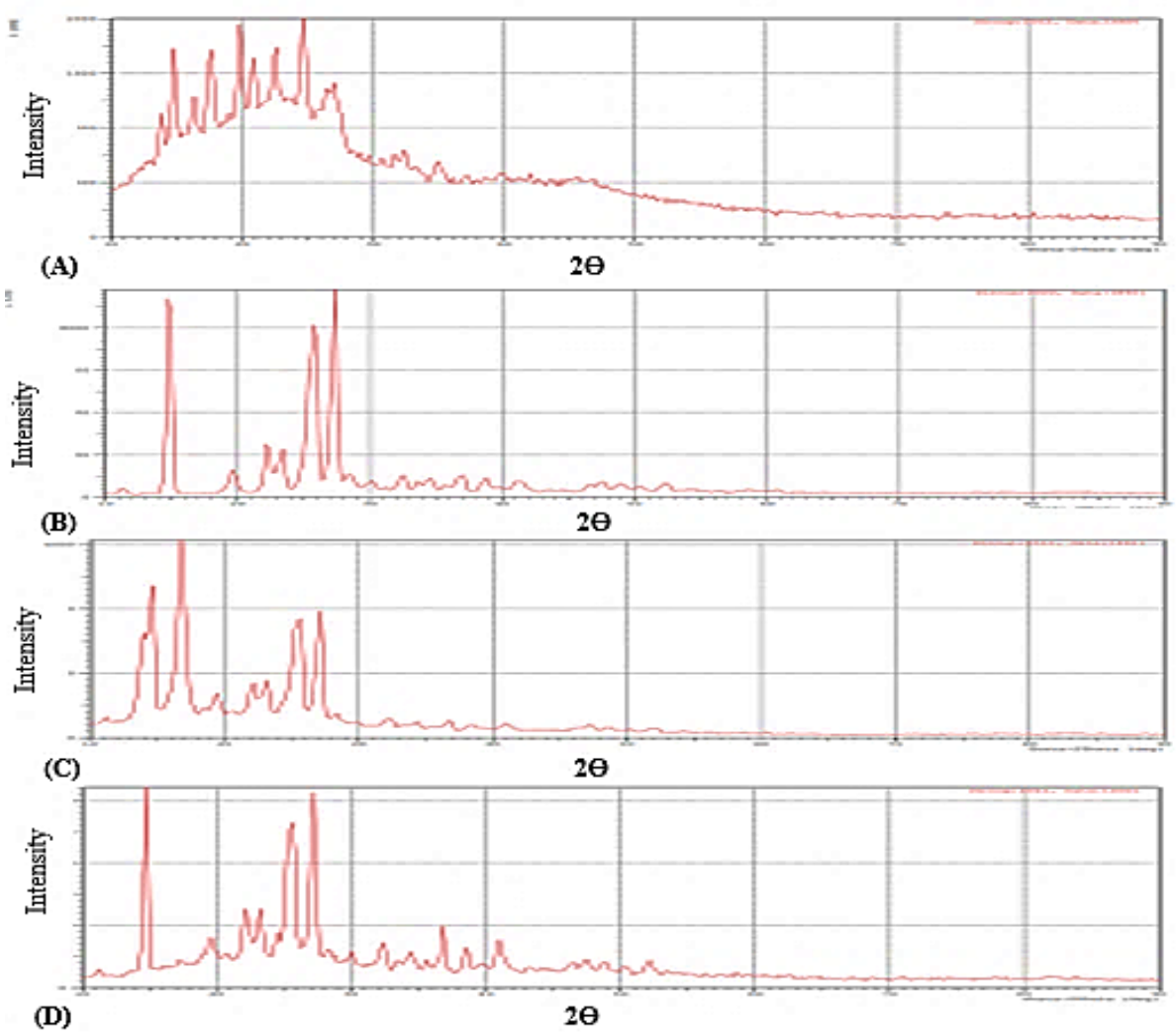

Figure 3. PXRD of (A)SM, (B)NA, (C) PM, (D) F5

Differential scanning calorimetry (DSC)

In Figure 4, the DSC thermograms are displayed. For SM powder, a clear endothermic peak at 179.19 °C, which corresponds to its melting point, was seen [31]. As in earlier studies, the melting endotherm for NA was seen at 130.80 ºC. [32]. The DSC analysis revealed that the physical mixture's NA peak had moved. While the endothermic peak of NA had shifted to a low and was visible in the SD formula with a decrease in melting point, the endothermic peak of SM was not visible in either the physical mixture or the SD formula. These findings suggested that the SM was present in the SM-SD in a different state, that is, SM had changed into an amorphous form, which would account for the increase in solubility and dissolution behavior.

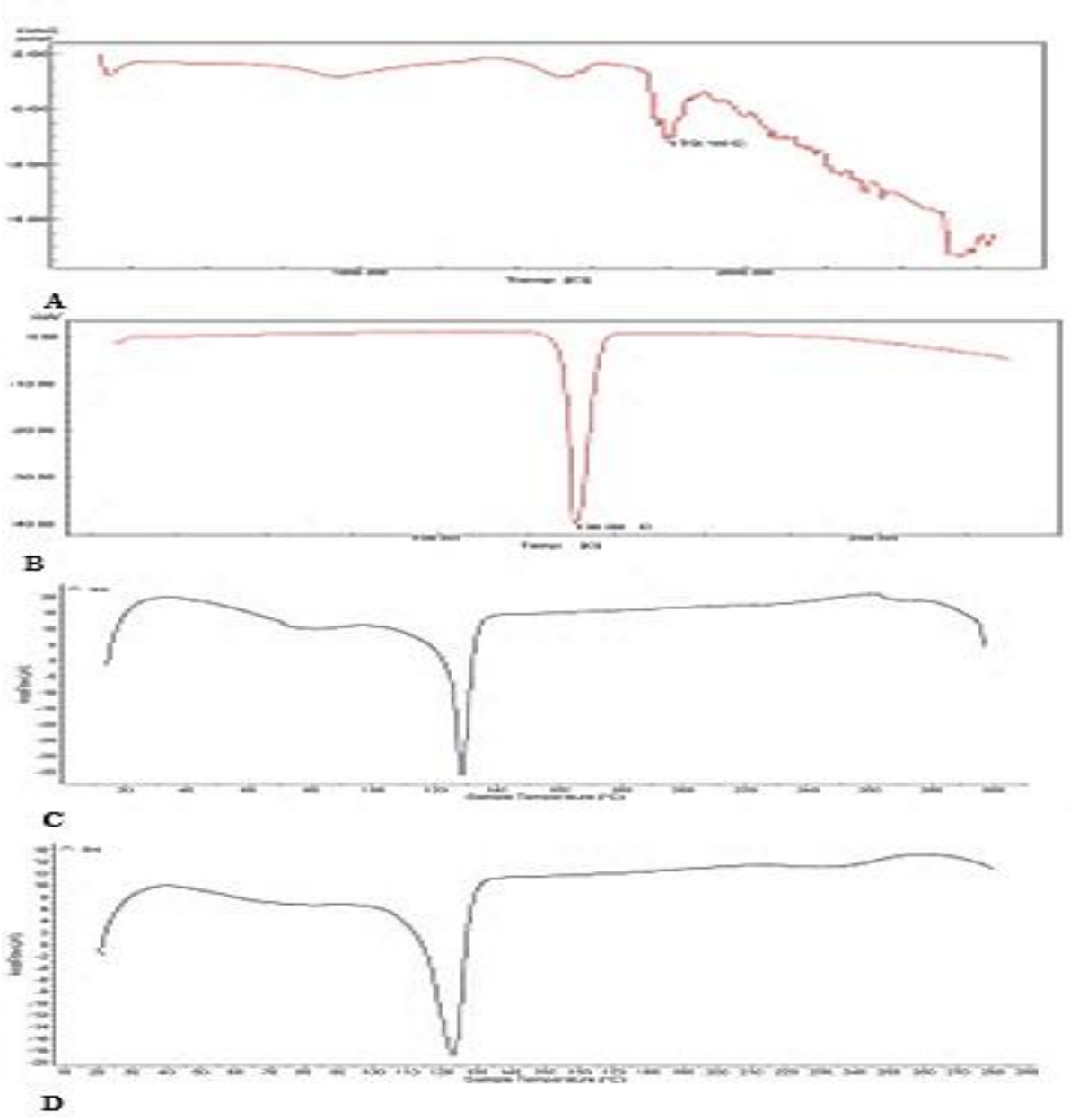

Figure 4. DSC of (A)SM, (B) NA, (C)PM, (D)F5

FTIR analysis

It is well known that vibrational changes in solid materials can detect intermolecular interactions. 3440.39 cm1 (OH stretching vibration), 2947 cm1 (OH bending vibration), 1637.27 cm1 (C=O stretching vibration), 1511–1461 cm1 (skeleton vibration of aromatic C=C ring stretching vibration), 1361 cm1 (C-O-C stretching vibration), 1081–1161 cm1 (in plane = C–H bending vibration), and 6418.18 cm1. These results were consistent with previously published research[34,35].gure 5-B depicts the characteristic symmetric stretching vibrations of the NH2 group in the FTIR spectrum of NA. According to the published values, the C=O stretching vibration appeared at 1618 cm1 and the -CN stretching vibrations appeared at 1422 cm-1 [36]. The spectra of the PMs and the selected formula were comparable. The carrier's failure to modify the SM skeleton indicates the absence of interaction. This indicates that no interactions occurred during the molecular dispersion of the drug in the carrier.

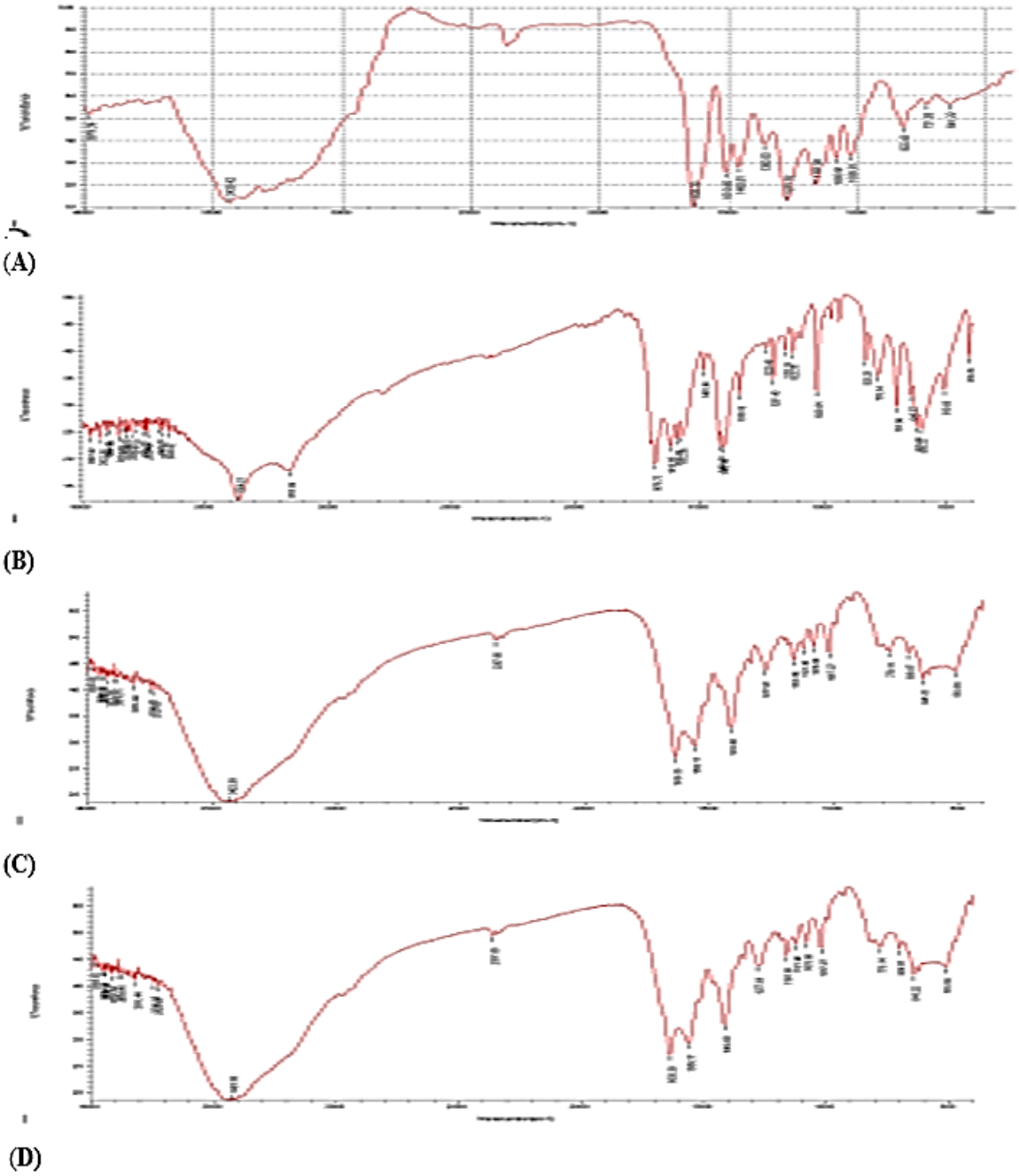

Figure 4. FTIR of (A) SM, (B) NA, (C) PM, (D) F5

Evaluation of gel

Visual appearance

The formulated SM gel was homogenous, yellowish, and opaque in appearance.

pH

Since the pH of the skin is about 5.5, the pH of the prepared gel was found to be 6.6. To avoid irritating the skin, a pH range of 5 to 7 is considered safe[37].

Drug content

The drug content of the gel formula was found to be 99%, which points to a uniform distribution of SD

Viscosity

It was discovered that as the rotation speed (shear rate) increased, the gel viscosity decreased, indicating the pseudoplastic (shear-thinning liquid) flow of the gel preparation. It is a key parameter for describing the gel because it affects how easy it is to spread and how the drug is released[38]. As shown in Figure 5 .

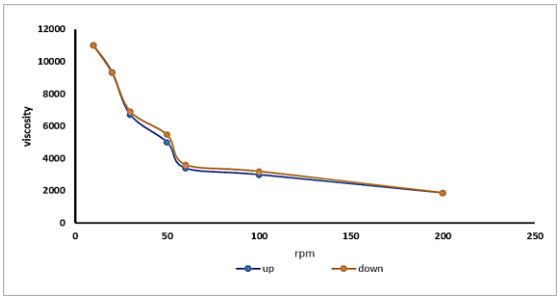

Figure 5. Rheogram of the prepared gel.

Spreadability

The gel spread well (4.76 ± 0.01 cm) and matched previous data [38].

***In vitro* dissolution Study**

Within three hours, the release of SM from the prepared gel formulation reached 100%. The gel release was delayed and extended to three hours to achieve 100% release when compared to the SD release. Because viscosity and drug release properties have an inverse relationship, the retardation of release could be attributed to the gel viscosity[39].

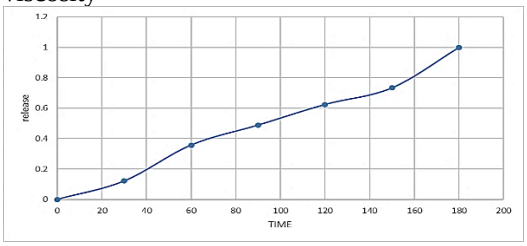

Figure 6. In vitro gel formula release at 37 degrees Celsius and a phosphate buffer saline with a pH of 7.4.

In vivo wound healing evaluation

The results of studies on how wounds heal in living animals are shown in Table 5. According to the table, all of the groups that received treatment healed their wounds faster than the control group. The SM group was the only one of the other groups that was statistically different from the control group, with a p-value of 0.05. The animals in the SM gel group healed the quickest. The length of their wounds decreased from 17.881 to 0 from the third to the fourteenth day of the study (full wound closure). This faster and better rate of wound healing could be attributed to SM's antioxidative and anti-inflammatory properties, which aid in the treatment of cutaneous wounds[40] [41]. Furthermore, NA's anti-inflammatory actions were effective in supporting the immunological response and hastening recovery; they also aided in boosting fibroblast migration and proliferation, which speeds up wound healing[42]. Furthermore, HA (gel base) is important in wound healing[43]. by regulating the level of inflammation and informing the body to make more blood vessels near the wound. Because HA is antibacterial, it can help reduce the risk of infection when it is put directly on open wounds [44]. Therefore, the combined effect of SM, NA and HA in the prepared gel may explain the shortest healing time.

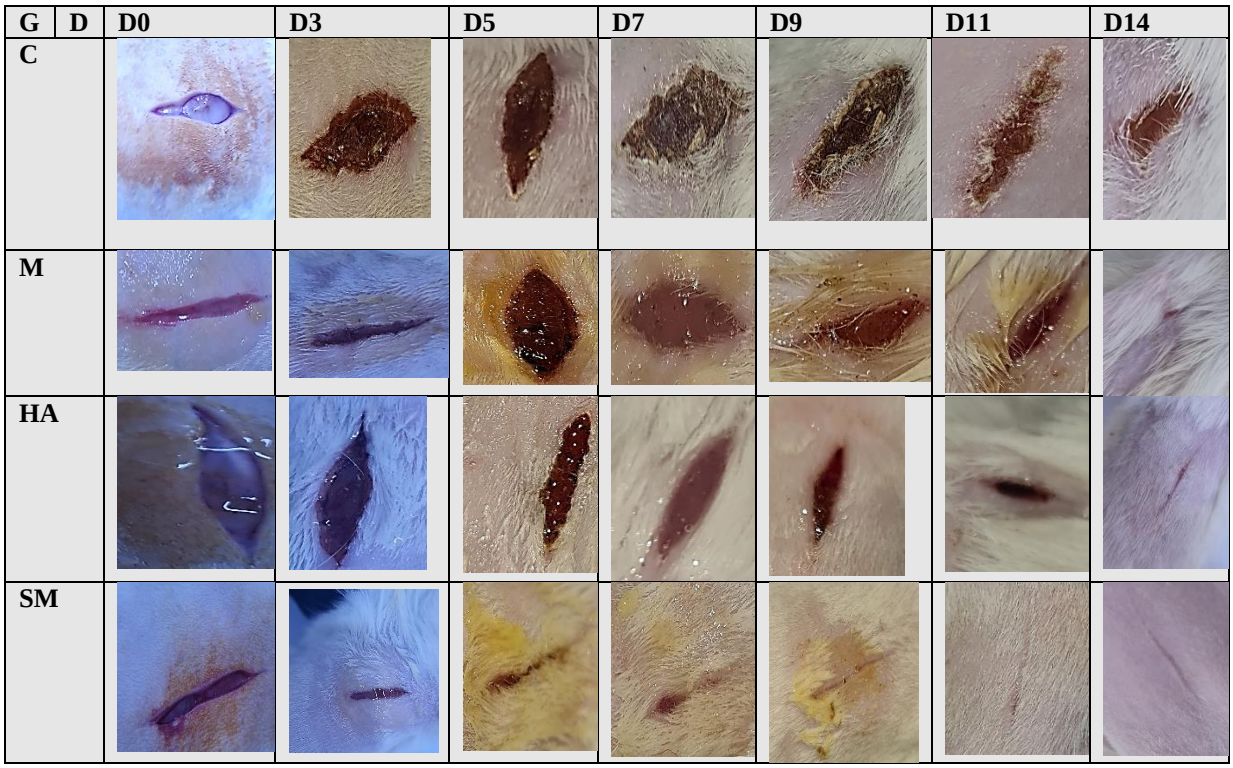

Figure 7. The difference in healing process between the rat groups treated with SM gel in comparison with control (C) , Mebo (M)and HA.

Table 5. Wound length (mm) in control, mebo, base gel and SM gel groups on different days.

groups	Initial	day 3	day 5	day 7	day 9	day 11	day 14
	Length of wound						
control	20	18.93±0.9	17.11±0.76	15.91±1.2	13.7±1.9	8.9±4.9	3.5±3.9
mebo	20	18.83±0.92	17.8±1.7	15.48±1.3	13.63±1.6	8.73±4.6	2±3.1
HA base gel	20	18.86±0.65	16.6±1.7	14.48±0.5	12.43±0.8	9.18±0.9	2.31±2.5
SM gel	20	17.88±1.02	14.71±1.2	12.81±1.2	10.36±0.9	2.36±3.6	0±0

Conclusion

The solubility and dissolution of SM-SD showed a great improvement compared to unprocessed SM. The F5 which was prepared by SE method in 1:3 drug: carrier ratio showed 25-fold increase in solubility and great enhancement in dissolution rate.

Incorporating SM-SD of into hyaluronic acid gel base was successful in formulating a topical gel with acceptable criteria and good wound healing effect.

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Conflicts of Interest

The authors declare that they have no conflicts of interest related to this work.

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Ethics Statements

The research plan was authorized by the local Research Ethics Committee in the University of Baghdad College of Pharmacy (REAFUBCP1552022A

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design Eman B.H.Al-khedairy:; data collection: Bushra Malik Jassim Both authors participate in writing , reviewing and approved the final version of the manuscript

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