Formulation and Characterization of Curcumin 12-Hydroxystearic Acid in Triacetin Organogel for Topical Administration

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Abstract:

Background: Curcumin (CUR) and its derivatives have shown a wide variety of biological activities, such as anti-oxidant, anti-inflammatory, anti-tumor, antimicrobial and antiparasitic effects as well as for the treatment of skin diseases. Due to its physico-chemical limitations such as low aqueous solubility and low bioavailability, we developed curcumin organogel as a topical delivery system to overcome those limitations. The12-hydroxystearic acid (12-HSA) is well known as a low-molecular-weight organogelators (LMOGs) capable of gelling an organic liquid phase. Different concentrations of (12-HSA) in triacetin with 50 mg CUR were gelled and applied for various examinations: tabletop rheology, oil binding capacity, pH measurement, spreadability, *in vitro* drug release, antibacterial activity and oscillatory rheology studies. The results revealed that the organogels transition temperatures from solid to liquid were greater than the normal body temperature, this helped the organogels keep their shape; they had good spreadability,and the organogels pH levels were within the safe range for the skin . *In vitro* release data showed that 4% 12HSA+5%CUR +TA (4TA) gave us 100% release after 6 hours. The selected 4TA illustrated good viscoelastic properties in the amplitude sweep test and a frequency-independent as seen in the frequency sweep test. CUR has good antibacterial action against *Staphylococcus aureus; Streptococcus pyrogen*, *Proteus mirabilis*, and *Escherichia coli*, which prevail at the site of wound injury as this pointed out that 4TA organogel can be used for topical wound healing.

Keywords: Antibacterial, Curcumin, 12-hydroxystearic acid, Organogels, rheology, topical.

صياغة وتوصيف حمض الكركمين 12-هيدروكسيستيريك في الجل العضوي ترياسيتين للاستخدام الموضعي

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الخلاصة:

أظهر الكركمين ومشتقاته مجموعة متنوعة من الأنشطة البيولوجية، مثل مضادات الأكسدة ومضادات الالتهابات ومضادات الأورام والتأثيرات المضادة للميكروبات والطفيليات وكذلك لعلاج الأمراض الجلدية، وبسبب القيود الفيزيو-كيميائية مثل الذوبان المائي المنخفض والتوافر البيولوجي المنخفض، قمنا بتطوير الكركمين العضوي كنظام توصيل موضعي للتغلب على هذه القيود. حمض 12-هيدروكسيستيريك معروف جيدًا بانه عامل عضوي منخفض الوزن الجزيئي قادر على تبلور طور سائل عضوي، تم تصنيع تركيزات مختلفة من 12-هايدروكسيستيرك اسد مع 50ملغ من الكركمين وزيت ال (ترياستين)

وتم التقديم لفحوصات مختلفة: ريولوجيا الطاولة، سعة ربط الزيت، قياس الأس الهيدروجيني، قابلية الانتشار، إطلاق الدواء في المختبر، النشاط المضاد للبكتيريا ودراسات الريولوجيا التذبذبية. أوضحت النتائج أن درجات حرارة انتقال الجل العضوية من الحالة الصلبة إلى السائلة كانت أكبر من درجة حرارة الجسم الطبيعية. كان لديهم قابلية انتشار جيدة؛ وكانت مستويات الأس الهيدروجيني للعضويات ضمن النطاق الآمن للبشرة. ساعد هذا الجل العضوي في الحفاظ على شكلها، وأظهرت بيانات الإطلاق في المختبر أن (4TA)أعطتنا إطلاقًا بنسبة 100 ٪ بعد 6 ساعات. أوضحت (4TA)المحددة خصائص لزوجة مرنة جيدة في اختبار اكتساح السعة ومستقلة عن التردد كما هو موضح في اختبار اكتساح التردد.الكركمين له تأثير مضاد للجراثيم جيد ضد Staphylococcus aureus و Streptococcus pyrogen و Proteus mirabilis و Escherichia coli، والتي تسود في موقع إصابة الجرح ، حيث يشير هذا إلى أنه يمكن استخدام (4TA)العضوي في التئام الجروح الموضعي.

الكلمات المفتاحية: مضاد للجراثيم، الكركمين، (12- حمض هيدروكسيستريك)، عضوي، ريولوجيا، موضعي.

Introduction

Curcumin (CUR) has demonstrated several properties to show: anti-oxidant, anti-inflammatory, antitumour, antibacterial, and antiparasitic in addition to treating skin disorders [1-6]. CUR is an oil-soluble pigment that is essentially insoluble in water at acidic and neutral pH levels but soluble in alkali. Water-soluble CUR preparations are made by incorporating it into various surfactant micellar systems (e.g., sodium dodecyl sulfate, cetyl pyridinium bromide, gelatine, polysaccharides, polyethylen glycol and cyclodextrins). CUR treatment is----- inadequate because of its physicochemical properties, such as low aqueous solubility and bioavailability, low gastro intestinal dissolution rate, rapid metabolism and rapid systemic elimination. These effects may be overcome by topical administration of curcumin [7]. Gels mainly composed of gelator and solvent that gelled to constitute the three dimensions gels by external stimuli like temperature or pH [8]. The hydrogel or the organogel can be determined by the polarity of the solvent or the continuous phase. In addition, gelators, either low molecular weight gelators such as sorbitan monostearate (Span 60), sorbitan monopalmitate (Span 40), and glyceryl fatty acid esters, or large molecule polymeric organic gelators constitute the solid portion of the gel network [9-12]. Triacetin, also known as glyceryl Triacetate used in cosmetic compositions between 0.8% and 4.0% concentrations as a plasticizer, and solvent. It serves as a medium for the transmission of flavors and fragrances. Numerous studies investigated CUR for topical application to demonstrate CUR-loaded nanosponge in gel to treat psoriasis [13] . Additionally, nano lipid carriers loaded with CUR for enhanced skin delivery while in a separate study, CUR nanocrystals were formulated in the gel to increase CUR solubility [14, 15]. Vigato *et al*., developed CUR for topical administration in polymeric organogel with poloxamer [16]. This study focused on organogels containing a low molecular weight gelator, 12-hydroxy stearic acid (12HSA) and oil triacetin (TA) as the external phase or the solvent of the organogel component and investigate CUR organogel for local antibacterial topical [11]. This study aimed to formulate an organogel to improve the CUR permeation through the topical route, to overcome poor oral bioavailability, rapid metabolism

and short half-life that limit CUR action. Organogels have recently seen as surge interest from the scientific community as potential ingredients in various consumer goods. The reason for this could be due to their intrinsic stability and the simple production methods. Organogel’s smooth application makes them a popular option for cosmetics and transdermal delivery systems [17].

Material and Methods

Materials

Curcumin and 12Hydroxystearic acid (12HSA) were purchased from BiDePharma Technology / China and Hangzhou Hyper Chemicals/ China, respectively. Triacetin (TA) was obtained from Central Drug House (Mumbai). Tween 80 (T80) was bought from Sigma-Aldrich. Formalin was obtained from Sigma-Aldrich, Germany. Ethanol 99%, Phosphate buffer are from alpha chemika, India.

Methods

Preparation of organogel

The blank organogel was produced by weighing 12HSA in the containers then bringing the total mass to 1 g by adding TA in the proportions of 0.5, 1, 2, 3, 4, 5, and 6 wt% of 12HSA respectively, as shown in table (1).

After incubating vials in a water bath at 85°C for ten minutes until a clear solution was obtained, vials were brought out of the water bath and cooled at room temperature. If there were no flow of the organogels during vial inversion, the outcome would be a solid organogel. To produce 5 wt% curcumin gel, we combined 50 mg of CUR with 12HSA and TA up to 1gm , according to Jamali *et al.*, who prepared CUR ointment for topical application [18].

Table 1: The compositions of the CUR organogels.

Formulation name	CUR (mg)	12HSA% (w/w)	TA% Upto (w/w)
0.5TA	50	0.5	100
1TA	50	1	100
2TA	50	2	100
3TA	50	3	100
4TA	50	4	100
5TA	50	5	100
6TA	50	6	100

Saturated solubility of CUR

Two glass bottles and stoppers filled with 4 millilitres of triacetin and phosphate buffer pH 7.4 + 10% w/v Tween80(T80) that already excess CUR was contained (150 mg). The vials were incubated in the water bath at 37°C and 50 rpm for 48 hours. After that, the mixture was spun for 30 minutes, and the supernatant was filtered through a syringe filter with a pore size of 0.45 micrometres [19] . The filtrate of CUR in (triacetin) was diluted with ethanol so that it could be measured by a UV-visible spectrophotometer to find its absorbance as the max absorbance was using the calibration curve equation while filtrate of CUR in phosphate buffer pH 7.4 diluted with the same buffer.

Tabletop rheology (Transitions temperature)

The vials of organogel were heated to 85°C in a water bath. The temperature then dropped 2°C every 15 minutes until it became 32°C. At the end of every 15 minutes, the vilas were tipped to see if the organogel formulation was liquid or solid. During this phase, the solidifying points of all the organogels were found. Then, the vials moved on to the next stage, where the temperature was raised by 2°C every 15 minutes up to 85°C to find the melting points [20].

Oil binding capacity (OBC)

One gram of organogold was put in a tube and spun at 6000 rpm for 15 minutes. The tubes were turned upside down over a piece of filter paper for 5 minutes to catch the oil that fell from the organogold. Then, the filter paper was weighed to determine how much oil was taken out. This study was done in triplicate to figure out OBC (%), use the formula:

OBC%= (1-massof expressed oil/initial sample mass) *100 ……. Eq (1)

[21].

pH determination

The pH of each organogold was measured by keeping the digital pH metre probe in the organogel and recording the readings until the pH hit equilibrium [16].

Spreadability

The organogel's ability to spread was tested by put 0.5 grams of CUR organogel in a circle on a glass slide with a diameter of 1 centimetre. Then, a second glass slide the same size covered the first slide. After a five-minute break, 500 g of weight was put on the top of the second glass to aid in organogel squeezing and spreading. The increase in the diameter of the organogel spreading was then recorded [22].

In vitro drug release studies

A glass cup of a cross-sectional area of 2 cm^2 and 1 g of organogel inside, wrapped with a cellulose membrane and sealed with a rubber band, a modified Franz cell . The cup was turned upside down to make sure the membrane was level under the surface of 50 mL phosphate buffer with a pH of 7.4 at 50 rpm [23, 24]. Also, 10% w/v T80 was added to the 50 ml phosphate buffer pH 7.4 at 37 °C to ensure the sink state. Shahani *et al*. 2011 found that CUR was more soluble at pH 7.4 with 10% T80 [25]. During the first 24 hours, aliquots were taken out at regular intervals and quickly replaced with a fresh buffer medium. To figure out how much CUR was there, spectrophotometric measurement at 425 nm was done with a UV-visible spectrophotometer [26].

Antibacterial activity

The antibacterial action of CUR was tested against four clinically isolated strains of bacteria: Staphylococcus aureus, Streptococcus pyogen, Proteus mirabilis, and Escherichia coli. The agars were utilized to cultivate the bacteria by dispersing 28 grams of the powder in one litter of deionized water, these agars were made using a Muller Hinton agar plate [27]. The medium was put into sterile plates and left to cool to room temperature before it became rigid. A 0.1 mL bacterial suspension with a constant level of turbidity was spread gently across the surface of the medium with a sterile glass spreader. The wells were made using a cork drill with a diameter of 6 mm, and 50 microliters of organogel were carefully placed in each one. The TA oil was used as a standard as control. The plates were left out for 30 minutes to allow for pre-diffusion, then they were put in an incubator at 37°C for 24 hours [27, 28]. The inhibition zone was measured using a ruler and given in millimetres [29].

Oscillatory rheology studies

The Anton par mcr 302 rheometers with a plate-plate design (PP 25/SN 61895) was used to study the rheology of organogels. Each measurement was done three times at 25°C, and Rheoplus software was used to analyse the results [20]. A scoop of the chosen organogel was put between the two plates for amplitude and frequency sweep tests.

Amplitude sweep

At first, amplitude sweep tests were to start rheology studies. The goal of this test was to find out the storage modulus (G'), the loss modulus (G"), the flow point for each mixture, and the linear viscoelastic area (LVER). So, the range of oscillatory strain

was changed from 0% to 100%, while the rotational frequency stayed the same at 10 rad s^{-1} [30].

Frequency sweep

The second oscillatory test was the frequency sweep. According to the LVER results from the amplitude sweep test for each organogel, the strain applied was between 0.01% and 0.08%. During this test, the frequency changed from 0.1 to 100 rad s^{-1} [31].

Skin Irritation Studies

In this model study, healthy male Wister Albino rats between two and three months old and weighing between 250 and 350 g were used to look into skin irritation. The animal ethics committee at the Iraqi Centre for Cancer Research and Medical Genetics looked over the methods for the skin irritation study and gave their approval. A day before the trial, the hairs on each rat's abdomen were carefully shaved with an electric clipper to not hurt the stratum corneum [32]. Dry cotton was swept over the area to be treated. Researchers looked into skin sensitivity by following the Draize patch test using two groups (gp), each gp with one rat, were divided into:

gp1: Treated with 4TA
gp 2: Treated with 0.8% v/v water solution of formalin, a common irritant.

To figure out how the skin irritation was; a **visual score scale** was used:
"0" meant there was no skin irritation
"1" meant there was minor skin irritation
"2" meant there was clear skin irritation
"3" meant there was moderate skin irritation
"4" meant the skin was scarred[33].

Histological examination of organogel-treated skin

After euthanized the rats they were undergoing the irritancy test, the skin was cut into pieces and dyed. Then, parts of rat skin were put into blocks of paraffin wax and cut with an electrical microtome into thin slices that were 5 mm long. These skin samples were cut into cross-sections and stained with haematoxylin and eosin (H&E) dyes to investigate under a microscope [34].

Results and discussions

preparation of organogel

Oragnogel mixtures that were solid upon turning vials upside down at room temperature confirmed organogel formation. This helped to determine the minimum gelation concentration (MGC) of 12HSA in TA that was 4% .Then, the CUR was added to three organogels starting from the lower concentration of 12HSA gelled by solvents, as shown in figure (1) and Table (2).

Table 2: The organogels names and their minimum gelation concentrations.

Formula name	12HSA wt%
4TA	4
5TA	5
6TA	6

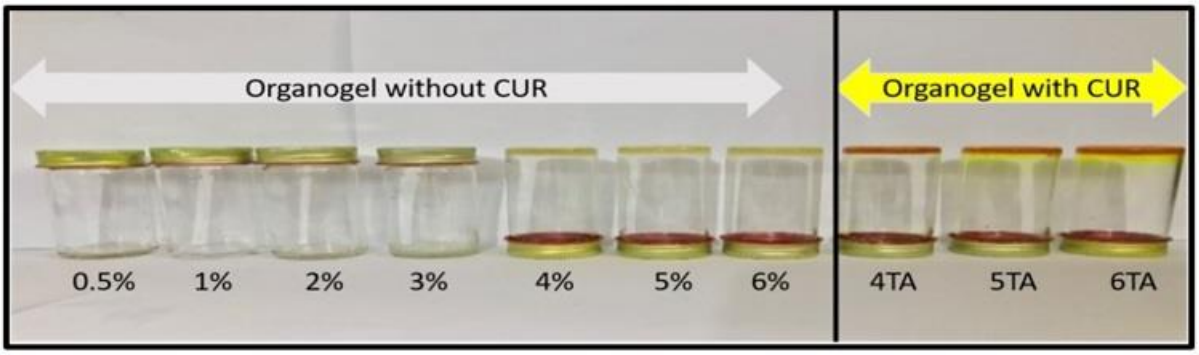

Figure 1 : Organogels of 12HSA in TA as the inverted vials referred to solid organogel at room temperature for organogel without CUR. The last 3 vials represented the CUR organogel, as pointed out in each image. All prepared organogel as wt%

Saturated solubility of CUR

The saturated solubility of CUR was measured to find CUR solubility in oil and buffer, which is essential for *in vitro* releasing CUR from organogels, and ensuring that CUR sinks in the buffer. The solubility was 20.74 and 1.8 mg/ml in TA and phosphate buffer 7.4 + 10 wt% T 80, respectively, as these data were close to previous work [35, 36]. CUR solubility in TA was very high while the CUR solubility in the pH 7.4 phosphate buffer + 10% w/v T80 was lower than TA. Based on the *in vitro* release investigation findings, it was hypothesised that the CUR produced using organogel would be more soluble.

Tabletop rheology (Transition temperature)

Phase changes from gel to sol and sol to gel for thermosensitive organogels were described using tabletop rheology. Table (3) and figure (2) show the transitional temperatures between the gel and sol phases and vice versa. All organogels showed the transition from liquid to organogel higher than body temperatures to confirm the gelation upon application on the skin. The results of this experiment using 12HSA organogel were similar to those of previous study that coupled 12HSA with light mineral oil [37].

Figure 2: Thermal behavior of the organogels (A) Semi-solid state of the organogel at room temperature and (B) sol state of the organogel at 39 °C and above temperature.

Oil binding capacity (OBC)

The binding capability of an organogezl is proportional to the strength of the scaffold [38]. As shown in Table (3), the OBC of organogels in 4TA was significantly lower than in 5TA and 6TA. Additionally, there was a direct correlation between organogel concentration and OBC, which may indicate highly concentrated gelators that effectively entrap the solvents.

pH determination

Since organogel is applied topically, pH was measured and the unsuitable pH could cause skin irritation [16]. The pH values of the CUR organogels are shown in Table (3)

vary between 4.56 and 4.73. These findings were consistent with the previous studies of the CUR pluronic lecithin or-ganogels that indicated no potential for skin irritation as the organogel's pH range (4 -6) was compatible with the skin pH (slightly acidic), reflecting no risk of skin irritation [39].

Spreadability

This study proved the topical distribution capacity of the skin preparation, which aids in the formulation's medicinal efficacy [40]. All of the CUR organogels spreadability test results are shown in

Table (3). Spread circles range in size from 2 cm for 6TA organogel to 2.7cm for 4TA organogel. Our data showed that the easiest-to-spread organogels were those with the lowest 12HSA concentration.

Table 3: Characterization parameter of CUR organogel.

Organogels	(Tsol-gel) ºC	(Tgel-sol) ºC	OBC %	PH	Spreadability in cm
4TA	32.4	39.4	21.9±0.30	4.65	2.7
5TA	31.3	39.6	47.7±0.32	4.73	2.3
6TA	30.5	40	62.1±0.94	4.56	2

In vitro CUR release studies

In vitro release studies were performed utilising CUR organogels for 24 hours in phosphate buffer pH 7.4 to assess the depot property of organogels. As this can be observed in Figure (3), a control consisting of 50 mg CUR mixed with TA were run in the release experiments. The oils are well-known for their ability to slow the release of lipophilic medicines; hence, this control was established **[41]**.

The results showed that the concentration of organogel was inversely related to the amount of CUR that was released. The results showed that the lowest concentration of 12HSA organogels in TA resulted in the maximum CUR release. Similar results were found in a separate investigation with 12HSA/sesame oil organogel, where it was shown that the organogel strength directly affected the amount of released cinnarizine [31]. As shown in Figure (3), the 4TA organogel showed rapid CUR release, and within 6 hours, the release medium contained 100 % CUR. TA control release pattern was close to the 4TA. On the other hand, the organogels of 5TA and 6TA released CUR at about 82% and 79 %, respectively.

From the above findings, a relationship between OBC investigation and CUR release profiles as the lowest amounts of OBC 21.9% in 4TA that released 100 wt% CUR within 6 hours may facilitate CUR migration away from the organogel's scaffold. It may be concluded that, in comparison to the control, CUR organogels slowed the release of CUR by varied

percentages, highlighting the significance of organogel in keeping the CUR within its network [37]. From spreadability tests, the smaller concentrations of CUR organogels the 4TA was the most spreadable. Also, *in vitro* release study illustrated the organogels containing the least amount of 12HSA released the most CUR as in 4TA. Both of these findings bolstered the study's primary objective and contributed to greater skin absorption of CUR. Therefore, 4TA organogel processing occurred for future research.

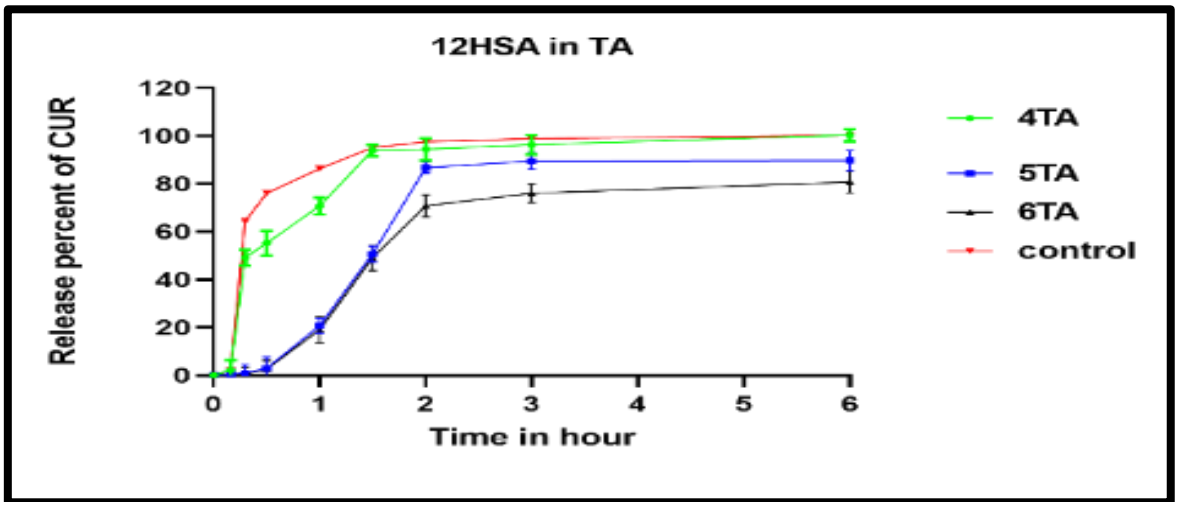

Figure 1 : In vitro CUR release in pH 7.4 sodium phosphate buffer solution (containing 10% w/v Tween80) at 37°C for 12HSA in 4TA, 5TA, 6TA and a control (50 mg of CUR in TA equivalent amount of prepared organogel) as each release curve is an average of triplicate ± standard deviation.

Antibacterial activity

In the past 50 years, studies on the pharmacological effects of CUR have revealed, its ability to inhibit the growth of bacteria[42]. *Staphylococcus aureus, Streptococcus pyogen, Proteus mirabilis*, and *Escherichia coli* are chosen for this test as skin disorders can be exacerbated by these microorganisms **[43]**. Therefore, it was essential to show that the 4TA could successfully treat a wide variety of skin bacterial strains. Controls consisting of TA and containing 5 wt% CUR were also included on each plate, and their results are displayed in Figure (4) . There was clear zone inhibition by the 4TA against the bacterial strains. We found that the inhibition zones were often greater in the control group. The high solubility of CUR in the oil, as demonstrated by the saturated solubility finding, CUR might be the main reason of showing good antibacterial activity . CUR helped fight bacteria by disrupting their cell membrane. As showen in figure (5), 4TA's zone of inhibition was greatest against Streptococcus pyogen and weakest against *Proteus mirabilis*. Our findings suggest that CUR may be useful in treating skin and chronic wound infections **[44]**.

Figure 4: Antibacterial activity of CUR as each cell represents the agar plates for selected organogel and the 4 strains of bacteria since the C and G mean the control TA and the selected organogel 4TA, respectively.

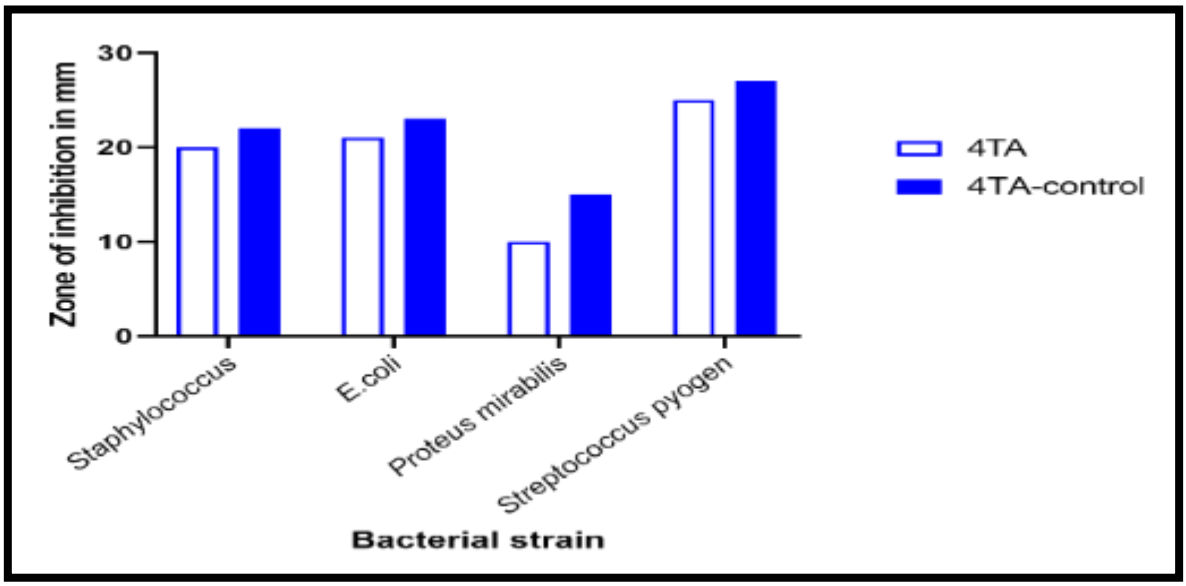

Figure 5: Antibacterial activity of CUR showing the zone of inhibition versus bacterial strain as the bars represent the organogels that showed activity against bacteria.

Oscillatory rheology studies

Amplitude sweep

In this study, the selected organogel (4TA) viscoelasticity was investigated with the amplitude sweep test to determine its G', G", LVER, and flow point,The solid phase, denoted by G', reveals the elastic nature of organogels. In contrast, the liquid phase, marked by G"[21]. A strain gradient from zero to one hundred percent was applied to the scooped organogel . This was until a strain value was achieved beyond which the gel could no longer function as 3-dimensional scaffolds, as the LVER indicates the beginning of gel weakening. The gel eventually breaks down under the strain point at the intersection of G' and G", as this is known as the flow point. The G', and G" curves were similar for the chosen organogel, with G' values being greater by an order of magnitude in LVER than G" values. Organogel formation is established

by this characteristic as suggested by Yan *et al* [45]. In addition, the extent of G' values ranged around 10^6, likewise the prepared organogels of 12HSA in canola oil[46].Finally, the G' and G" values, LVER, and flow points indicated that 4TA organogel was sufficiently robust. Figure (6) and Table (4) display these G', G", LVER and flow point.

Table 4: Amplitude sweep parameters G', G'', LVER, and flow points for the selected organogel as each value is an average and standard deviation of 3 trial. The study was set at a strain from 0% to 100%, angular frequency 10 rad. $s^{-1,}$ and temperature of 25°C.

Organogel	G' (pa)	G" (pa)	LVER (%)	Flow point (%)
4TA	209674 ± 251169	41205.00± 346.80	0.043± 0.005	6.78±0.17

Frequency sweep

This study application was to see if organogels could keep their shape while transported in a three-dimensional scaffold at different speeds. The G' and G" curves for a strong organogel should not intersect. 4TA organogel displayed a higher G' curve that was parallel to the G" curve and did not cross at any frequency (Figure 6). In other words, the organogel was rigid across the whole frequency range. The results were consistent with those found in the 0.88 wt% 12HSA/ dodecane organogel and folic acid organogel in propylene glycol, which showed parallel G' and G" curve [47, 48]. In conclusion, the chosen organogel showed no frequency dependence in the investigated ranges, this outcome might point to that the organogels kept their elasticity alongside different frequency values.

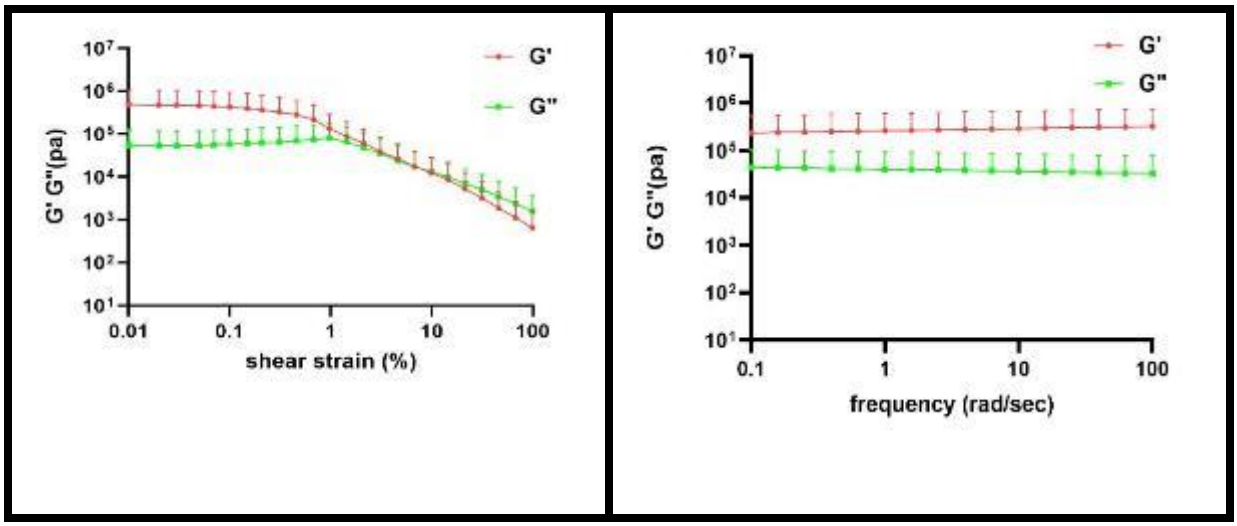

Figure 6: Oscillatory rheology Figures, the left side represents the amplitude sweep, and the right side represents the frequency sweep of the 4TA organogel.

Skin Irritation Studies

This study aims to evaluate the potential for skin irritation from repeated transdermal applications of CUR organogels. Selected organogel 4TA was subjected to a skin irritation test with the findings being compared to those of formalin (the gold standard irritant). Without a score greater than 2, the Draize test as mentioned in section (2.2.10) above indicates no cutaneous irritation as exposed in Table (5) , as there was no evidence of erythema or edoema during the skin irritation study. While formalin caused significant erythema and edoema , Demonstrating that the formulations were nonirritant.

Table 5: Skin irritation test results on Wistar rat dorsal skin for selected organogels.

Group	Edoema	Erythema
Group 1 (4TA)	0	0
Group 2 (Formalin)	4	4

Histological examination

The skin from the irritancy test was studied histologically Epidermis epithelium (keratinized stratified squamous epithelium) appeared normal in histopathological pictures of skin treated with a chosen organogel 4TA. Numerous immature hair follicles and normal sebaceous glands were also visible in the image of 4TA, along with typical dermal fibrous connective tissue made up of fibroblasts and fibrocytes. However, formalin-treated skin showed significant epithelial shedding, a very thin epidermis, deteriorated fibrous tissue, and regressed hair follicles on histological examination. Also, the dermal fibrous tissue was seen to be deteriorated, consisting of fibrocytes and a few regressed hair follicles, all of which were covered by a single layer of epidermal epithelial cells. To conclude, the 4TA histological image revealed no changes on the normal composition of the skin upon application of 4TA. That can be shown in figure (7) below.

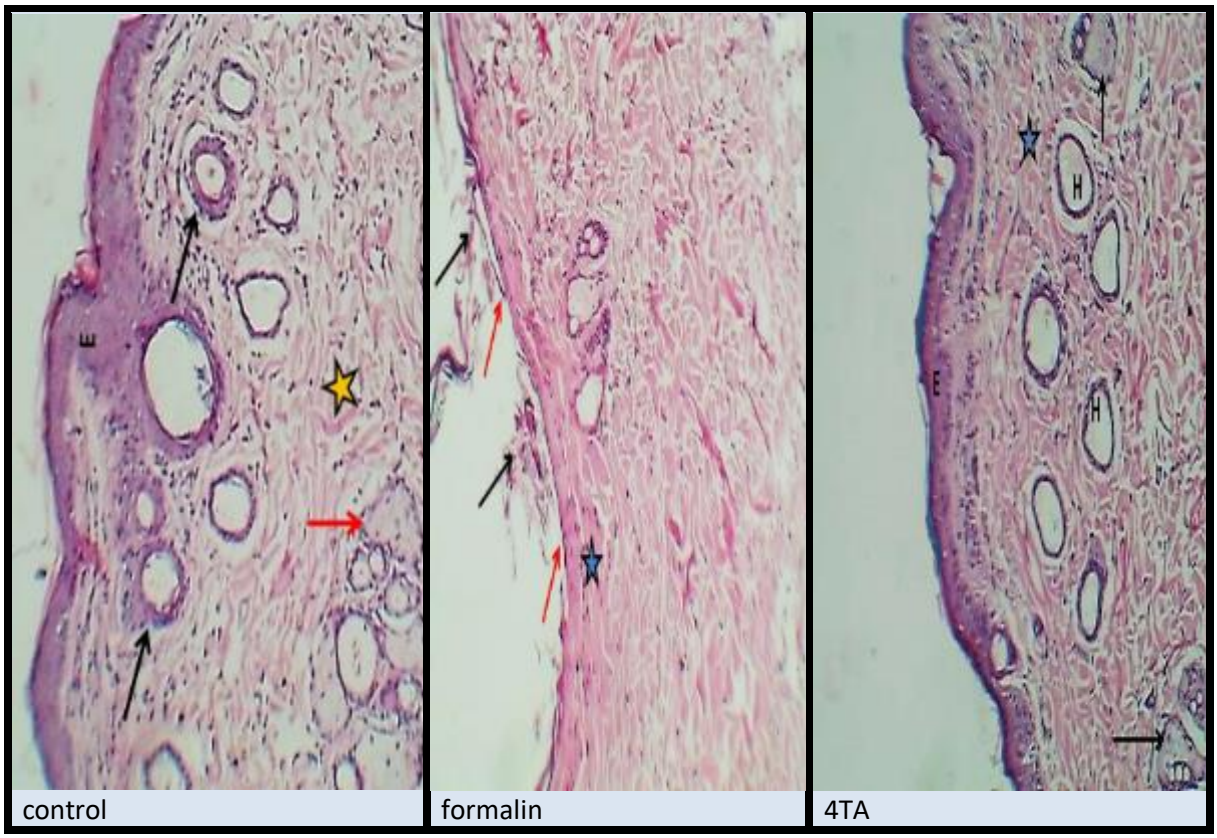

Figure7: Microscopic images histopathology study for CUR organogel, at a magnification of 100x, where epidermis (E), dermis fibrous connective tissue (Asterisk), varying stages of developing hair follicles (Black arrows) & sebaceous glands (Red arrows). Except for formalin, where marked epithelial shedding (Black arrows), very thin epidermis (Red arrows), degenerated fibrous tissue (Asterisk) & regressed hair follicle.

Conclusion

The 4TA organogel was the most spreadable compared with the higher concentrations of 12HSA as well as the 4TA released 100% of CUR faster than other prepared higher 12HSA concentrations. The 4TA was capable of inhibiting the growth of Staphylococcus aureus, Streptococcus pyogen, Proteus mirabilis, and Escherichia coli. The oscillatory findings revealed a strong organogel with no irritancy as revealed by the irritancy test and supported by histological examination.

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