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Disulfiram Loaded Transdermal Nanoinvasomal Gel Using Carvacrol as Penetration Enhancer

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Abstract

Disulfiram (DSF), an FDA-approved pharmaceutical for the management of alcoholism, has demonstrated its efficacy against several kinds of cancer. DSF has limited solubility, fast metabolism, short duration of action, and instability in physiological environments, mostly caused by rapid degradation in the acidic gastric environment. Therefore, a transdermal gel containing disulfiram, which was loaded into nanoinvasomes, was developed to improve the stability of DSF and enable its effective distribution to tumour tissues. The optimal nanoinvasomal dispersion was developed in the laboratory using a 4:1 ratio of soya phosphatidylcholine to disulfiram, and 1 % carvacrol as a penetration enhancer. Furthermore, three nanoinvasomal gel formulas (IV-g1, IV-g2, and IV-g3) have been developed using hyaluronic acid as the gelling agent at concentrations of 2 %, 2.5 %, and 3 %, respectively. These formulations are subjected to further *in vitro* evaluation to assess their physical appearance, homogeneity, viscosity, spreadability, and *in vitro* drug release. Results indicate that the formula (IV-g3) exhibited better homogeneity, consistency, spreadability, and stability and showed a non-Newtonian flow and a drug content of (99.6 ± 0.4) %. Compared to the DSF suspension (16 %), the IV-g3 showed sustained release with a much greater *in vitro* permeation of DSF (90.03%) over 24 h. The *ex-vivo* skin permeation of rat abdomen skin for IV-g3 was significantly higher (14.72 $cm^{٢}$/ h $\times 10^{-3}$) than that of DSF-suspension (3.44 $cm^{٢}$ / h $\times 10^{-3}$) during an entire day. The enhancement ratio of IV-g3, which was measured at 4.27, surpassed that of the DSF suspension. The study successfully formulated a new transdermal gel by utilizing invasomal nanocarriers. This gel successfully promoted the delivery of DSF through the skin. The transdermal delivery approach exhibited improved stability of DSF and better skin permeability of DSF.

Keywords: Nanotechnology, Transdermal delivery, Invasome, Disulfiram, Hyaluronic acid.

Introduction

Nanotechnology has provided an innovative and superior alternative for treating diseases or for drug carriers. In recent decades, the use of nanomaterials in pharmaceutical therapy has evolved drastically in various medical applications, including vaccination, diagnostic imaging methods and sustained administration of medications or cancer therapy [(1)]. Nanomaterials are materials at the nanoscale that can enhance the stability and permeability of pharmaceuticals, enable controlled drug release, or reduce the toxicity of drugs. The benefits of nanomaterials encompass enhanced colloidal stability, superior dispersibility, and heightened surface reactivity. The transdermal distribution of therapeutic molecules has recently garnered considerable scientific interest as a direct and effective method for treating diseases, attributable to the skin's expansive surface area and convenient accessibility [(2)].

Transdermal delivery systems provide a variety of benefits when compared to alternative approaches. They are non-intrusive and needle-free. In addition, these systems facilitate controlled drug delivery, boosting the quantity of medication that enters the circulatory system while avoiding hepatic metabolism [(3)]. The transdermal drug delivery system is growing as a promising field for technological developments and patenting of technology that administers antineoplastic drugs. Uncovering innovative and imaginative cancer treatments poses a significant global obstacle. Nano-carriers provide an environment for encapsulating and delivering pharmaceutical compounds [(4)]. They can extend the duration of action of drugs and promote their accumulation in tumor tissue as a result of their sizes, surface characteristics, and ability to enhance penetration and retention [(5)]. Nevertheless, the targeting mechanism protects healthy tissue from the harmful effects of drugs and assists in reducing the adverse consequences of cancer treatment [(6)].

Nanoparticles within the size range of 40–200 nm exhibit extended circulation duration, enhanced tissue accumulation, and reduced renal clearance. Conversely, nanoparticles of a size exceeding 200 nm are eliminated more quickly, by lysosomal exocytosis, and hepatobiliary elimination, which involves the liver sinusoids, hepatocytes, bile ducts, intestines, and faecal excretion, whereas the kidneys filter out nanoparticles of less than 10 nm. The size of nanoparticles has a significant impact on the release of medicines. The elevated surface-to-volume ratio of nanoparticles promotes an increased rate of breakdown and accelerated release of drugs [7,8,9].

Invasomes are lipid vesicles composed of phospholipids, ethanol, and either a single terpene molecule or a combination of terpenes like citral, limonene, carvacrol, carvone, and thymol. Ethanol enhances the fluidity of lipids in the vesicle structure, resulting in a softer and less rigid structure compared to normal liposomes. This, in turn, improves its capacity to penetrate the skin [10]. Carvacrol, a phenolic compound, has been found in the volatile oils of *Thymus vulgaris*, *Carum copticum*, *Origanum*, and *Oregano*. Carvacrol is a natural member of monoterpene phenol; carvacrol is the most effective chemical penetration enhancer with a *log p* of 3.28. It has been used as a cosmetic ingredient, and a safe food additive also well known that carvacrol possesses strong antioxidant properties and plays a role as a formulation preservative [11,12].

The thiocarbamate drug disulfiram (DSF; Antabuse®) received authorization from the US Food and Drug Administration (FDA) in 1951 to treat alcoholism. It has been utilized for over 60 years in the treatment of alcoholism. DSF, a drug with low toxicity and manageable side effects, can also be employed for the treatment of complex disorders [13]. The excessive and undesired metabolism of DSF in the liver results in inefficient transportation to tumour tissues, which is a crucial factor contributing to the failure of using DSF as an orally effective antineoplastic agent [14].

Hyaluronic acid is an essential compound that plays a vital role in the mechanical and structural aspects of the wound healing process. It is evenly spread throughout the connective tissue, including the subcutaneous tissue and cartilage tissue, and significantly impacts the skin's hydration and flexibility. As people age, their skin experiences a decrease in both the amount and quality of moisture, resulting in dryness and the formation of wrinkles [15]. This study aims to enhance DSF transdermal delivery by enhancing skin permeation, using animal skin models to evaluate the actual permeation of DSF through the skin barrier by designing DSF-loaded nanoinvasomes gel formulations, and subsequently performing evaluation and safety studies and selecting an appropriate concentration of hyaluronic acid as a gelling agent with acceptable properties for topical skin application and optimum physical stability.

Materials and Methods

Materials

Disulfiram and Hyaluronic acid were purchased from Hyper Chem for Chemicals, China. Absolute ethanol, methanol, KH_2PO_4, and NaOH were purchased from Chem-Lab, Belgium. Carvacrol was purchased from Bide Pharmaceutical Ltd. (China). Soybean phosphatidylcholine (SPC90), which has a purity of > 90% was purchased from Henan Guange Biotechnology Co., Ltd, China. The other chemicals and solvents used in this study were of analytical grade.

Preparation of Nano Invasomal Gel

The optimum of invasomal vesicle dispersion prepared by the thin film hydration method that has Particle size:(119.2 ± 2.2), polydispersity index: (0.18 ± 0.05), Entrapment efficiency %: (95.3 ± 0.8), and Zeta potential:(-33.6 ± 1.6) mV. Consisted of a 4:1 ratio of SPC 90 to DSF, 40% ethanol, and 1% carvacrol (under publishing). This formulation was chosen to create three different nano invasomal gel formulations (IV-g1, IV-g2, and IV-g3) using varying concentrations (2, 2.5, and 3) % w/w of hyaluronic acid as the gelling agent. The components specified in Table 1 of the DSF-loaded hyaluronic acid gel formulation were combined and agitated using a magnetic stirrer at a speed of 300 rpm overnight at room temperature until a partially solid gel with a uniform distribution of the DSF- nanoinvasomal dispersion was achieved [16,17].

Table 1. The components of different DSF-loaded nanoinvasome gel formulations

Code**	Disulfiram (mg)	SPC90*** mg	Carvacrol (w/v) %	Hyaluronic acid (w/w) %
IV-g1	25	100	1	2
IV-g2	25	100	1	2.5
IV-g3	25	100	1	3

***Absolute ethanol 40% (v/v) and phosphate buffer (pH 7.4) q.s. ad. to make (5 ml), **: Formula code, ***: Soybean phosphatidylcholine**

Characterization of the Prepared Nano Invasomal Gel

Appearance and pH determination

The prepared nanoinvasomal gel was also tested for homogeneity, also tested for their appearance and the presence of any aggregates, fibres, color, and presence of suspended particles in all formulations were visually inspected against a black-and-white background. Furthermore, the pH of all the formulations was determined using a digital pH meter made by Hanna Instruments. The pH meter probe was submerged in each formulation, and conducted in triplicate, and the average value was taken as the pH of the invasomal gel formulation [18].

Invasomal Gels: Viscosity Determination

The measurements were conducted using the LMDV-60 viscometer. The invasomal gel was placed in a container with a wide opening to allow the viscometer's spindle to be submerged without making contact with the bottom of the container. The invasomal gel samples were allowed to sediment at a consistent temperature for a duration exceeding 30 minutes. The viscosity was determined by gradually increasing the rotational speed from 3 to 60 revolutions per minute (rpm). This is accomplished by using a spindle, specifically spindle number 4. The observations were recorded at a temperature of 25°C [19].

Spreadability Determination of Nano invasomal gels

The spreadability of the novel transdermal gel was determined by compressing 0.5 g of invasomal gel samples between glass plates of known weight. Then, glass plates were sequentially positioned over the sample at 5-minute intervals. The spreading area was determined following the addition of each glass plate, and the findings were presented in terms of spreading area as a function of the applied mass (weight of the glass plate). The slides were carefully affixed to a stand without causing any disruption [20].

Drug Content Determination

About 0.05 grams of nano invasomal gel formulations (IV-g1, IV-g2, and IV-g3) were precisely measured and placed into a 50 mL volumetric flask. Subsequently, ethanol was introduced into the flask until it reached a volume of 50 ml. The resulting mixture was stirred vigorously for 30 minutes. The mixture that emerged was subjected to centrifuging at a speed of 3000 revolutions per minute for 20 minutes. The liquid fraction, commonly referred to as the supernatant, was collected and filtered using a membrane with a pore size of 0.45 mm. The dilute sample was analyzed using a UV spectrophotometer at a wavelength of 217 nm, which corresponds to the maximum absorption (λ max) for DSF [21].

In vitro drug release of invasome-loaded disulfiram gels

A 1-gram sample of the gel, which contained 5 milligrams of disulfiram, was tested using the diffusion technique of dispersing disulfiram into a dialysis bag. DSF was extracted from prepared gels using USP equipment II, 250 mL phosphate buffer solution with a pH of 7.4 was employed as the release medium. The release medium was maintained at a temperature of 37 ± 0.5°C throughout the whole 24-hour experiment. At certain time intervals of 1, 2, 4, 8, 12, and 24 hours, 3 mL samples were taken and the same volume of fresh dissolving medium was added. The samples were then analyzed using a spectrophotometer at a wavelength of 217 nm of DSF. The release test was done three times, and the data obtained from *in vitro* release were recorded as mean ± SD [22].

Selection of Optimum Nano Invasomal Gel Formula

The best nano invasomal gel formula was chosen based on a higher % of drug release after 24 h, spreadability, homogeneity, drug content, and viscosity.

Drug and excipient compatibility study by FTIR

An FTIR spectrophotometer was used to evaluate the compatibility of the DSF with other additions in the chosen formula. An examination was conducted on the pure DSF, a physical mixture of hyaluronic acid, carvacrol, and DSF in equal proportions of 1:1:1:1. The analysis also included the nano invasomal gel formula (IV-g3). The samples were compressed using KBr pellets, whereas, for the invasomal gel (IV-g3), the sample was applied to a KBr cell by pouring numerous droplets and covering it with another cell to remove any trapped air. All samples were analyzed using FTIR spectroscopy (Shimadzu, Japan) in the 4000 - 400 cm^{-1} [23].

Field emission-scanning electron microscope

The morphology of the DSF-invasomal dispersion formulation was examined using a field emission scanning electron microscope (Inspect F50, FEI company, Netherlands). Sample preparation is a crucial stage in the visualization of samples using a Field Emission Scanning Electron Microscope. The sample preparation involves the careful collection of nano invasomal dispersion to prevent contamination or damage, the fixation of the sample to preserve the structural integrity of the formula, and the removal of water from the nano invasome through dehydration. Critical point drying is a method employed to eliminate the solvent from the formula while maintaining the morphology of the nano invasome. The formula is then mounted on a stub using conductive adhesive carbon tape. The invasome dispersion formula is coated with a conductive material, specifically platinum. Finally, the nano-invasome formula is ready for imaging

using the field emission scanning electron microscope [24].

Histopathological study of rat skin after transdermal application of the selected nanoinvasomal gel

After 72 hours of transdermal application, six female Wistar rats' sections at the site of the treatment were executed to evaluate any change in the state of the skin was authorized by the Research Ethics Committee for Experimental Investigations, College of Pharmacy, Baghdad University, Iraq, under the protocol number RECAUBCP482023M. Skin samples were collected for histological examination to detect any evidence of irritation. The specimens were stored individually in a solution of 10% formalin in PBS with a pH of 7.4. Afterwards, the specimens were subjected to dehydration using ethanol and subsequently encased in paraffin. The specimens were vertically sectioned into cross sections of 5 µm in thickness and then stained with hematoxylin and eosin. Subsequently, these samples were scrutinized using an optical microscope (Optika® microscopes, Italy), and juxtaposed with skin specimens from the healthy control group. They were meticulously screened for the presence of inflammatory cells or any indications of irritation. In summary, the dorsal hairs of the rats were shaved 24 hours ago. Subsequently, one gram of the IV-g3 substance was uniformly distributed across a shaved region with an area of 4 square centimetres [25,26].

Ex vivo permeation studies

The *ex vivo* permeation test for invasomal gel was conducted using the abdominal skin of mature female Wistar rats, approximately 200 ± 30 g, obtained from the animal house of the College of Pharmacy/University of Baghdad. The experiment uses a glass Franz cell with a receptor compartment that was able to accommodate up to 16 mL and had a diffusion area of 1.1 cm^2. The rat skin was positioned as a barrier between the donor and receptor compartments, with the stratum corneum side facing the donor chamber. The sides of the Franz cell were firmly sealed with Teflon tape [27]. The receiver compartment contained 16 mL of phosphate buffer solution (PB) with a pH of 7.4. The solution in the receiver compartment was stirred using a magnetic stirrer at 100 revolutions per minute, maintaining a temperature of 37 ± 0.5°C. A quantity of one gram of the chosen nano invasomal gel IV-g3 and the DSF suspension was applied (each one separately) onto the skin surface in the donor compartment. The media was systematically removed at different time intervals (0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours), and fresh media was regularly added to ensure that the sink remained in optimal condition. The absorbance of each sample was measured at the wavelength of 217 nm, corresponding to DSF maximum absorbance [28,29]. Three separate measurements were used to calculate the results, and their standard deviations were shown. The lag time was determined from the X intercept. The following equation was used to compute the penetrated flux and apparent permeability coefficient (Papp) [30,31].

$$Jss = (D/dt)/A$$

(Equation 1)

$$Papp = Jss/c_o$$

(Equation 2)

Where: (D /dt) is the rate of DSF permeated (μg/h); A is the surface area of the used membrane (cm^2); C_0 is the initial concentration of DSF.

Stability Study

The invasomal gel was stored at temperatures of 4°C and 25°C for one month, the samples were assessed for their physical characteristics, pH levels, and drug concentration [32].

Statistical analysis

The study's results were subjected to rigorous statistical and scientific analysis using GraphPad Prism version 10.2.0.392. Several statistical approaches, such as paired t-tests and one-way ANOVA (analysis of variance), were used for analyzing and interpreting the results. To qualify for the results to be considered statistically significant, the p-values must be less than 5% ($p < 0.05$) [33].

Results and Discussion

Physical Appearance

The DSF-nanoinvasomal gel formulations (IV-g1, IV-g2, and IV-g3) were identified by their off-white color, uniformity, lack of precipitation, good consistency, and absence of phase separation [34].

pH Determination

The pH values of the drug delivery systems were measured, as transdermal formulations must fall within the pH range of 3 to 9. Deviating from this range could have detrimental effects on the skin's integrity and permeability. Consequently, all the formulations achieved pH values within the permitted range and can be considered safe for use on the skin surface. The pH values of the nano invasomal gel formulae were: IV-g1 = 6.3 ± 0.2, IV-g2 = 6.6 ± 0.15, and IV-g3 = 6.5 ± 0.12, as shown in Table 2. These values indicate that the gels have little chance of causing skin irritation [35].

Table 2. Physical properties of DSF gels (mean ± SD, n = 3)

Code	pH	Drug content%	Spreadability(cm)
IV-g1	6.3 ± 0.2	98.2 ± 0.15	8 ± 0.1
IV-g2	6.6 ± 0.15	99.1 ± 0.25	7.5 ± 0.05
IV-g3	6.5 ± 0.12	99.6 ± 0.4	7.1 ± 0.15

Viscosity determination of the optimized invasomal gel formulation

Different shear rates were used to investigate the viscosity and understand the viscosity properties of the nano invasomal gel. The formulations displayed a decrease in viscosity as the shear rate increased from 6 rpm to 60 rpm, indicating a pseudo-plastic behavior [36]. The gel exhibits shear-thinning behavior, as evidenced by a decrease in viscosity with an increase in shear rate, and returns to its original state when the stress is removed. This behavior is called thixotropic behavior, a desirable property in pharmaceutics that impacts the manufacturing process and dose administration [37]. This characteristic enables effortless extraction of the substance from the container and even distribution of the formulation on the skin. Furthermore, the viscosity increases as polymer concentration rises from 2% to 3% at the same rpm due to more intermolecular entanglements with high polymer concentrations. Formula IV-g3, including 3% hyaluronic acid, exhibited much greater viscosity than the other formulas, as shown in Table 3 [38,39].

Table 3. Viscosity in centipoise of nanoinvasomal gel formulations measured at 25 ˚C in various shear stress values (mean ± SD, n = 3)

Speed (rpm)	IV-g1	IV-g2	IV-g3
6	30412 ± 4.5	28309 ± 4.5	58505 ± 4.6
12	23980 ± 7.6	24862 ± 5.1	48780 ± 4
30	18149 ± 16.1	18444 ± 5.5	30254 ± 5.5
60	10589 ± 8	10592 ± 4	10005 ± 4.6

Spreadability Determination of Nano invasomal gels

The spreadability of gel is a key factor affecting the uniformity of the formulation's application into the skin. The efficacy of a gel is influenced by its spreadability, as a higher spreadability suggests a quicker and more efficient spreading process, leading to better adherence by patients. The gel compositions exhibited excellent spreadability and required minimal shear force. The result of the spreadability of all developed invasomal gel formulations was in the range of (7.1 - 8) cm^2. The spreadability falls as the hyaluronic acid concentration rises. The formulation data presented in Table 2 demonstrate that hyaluronic acid exhibits favorable gel spreadability with minimal shear force [40].

Drug content

The gel preparations exhibited drug content ranging from 98% to 99%, which falls within the acceptable range for achieving optimal therapeutic activity [41]. The outcomes of all these evaluation requirements are displayed in Table 2.

In vitro drug release of nanoinvasome-loaded disulfiram gels

Figure 1 shows the *in vitro* release analysis of various DSF nanoinvasome gel formulas (IV-g1, IV-g2, and IV-g3) which contain (2, 2.5, and 3) % hyaluronic acid respectively in PB pH 7.4. The nano invasome formulae, created using nanoinvasomal technology, demonstrated a considerably larger *in vitro* release. The inclusion of 2 % SPC90, 40 % ethanol, and 1 % carvacrol in these formulae enhanced the solubility of DSF due to its high solubility in these solvents. Furthermore, the nanoinvasomal gel was formulated with elastic vesicles of nanoscale dimensions, hence enhancing solubility through an increased surface area. These results suggested that DSF was sustainedly released, as the % of hyaluronic acid increased due to the gel membranes' surface hydrophilicity improved after the addition of hyaluronic acid in different portions, IV-g3 showed a higher release of DSF after 24 h than IV-g1 and IV-g2 with a significant ($p < 0.05$) in comparison with pure DSF [42-44].

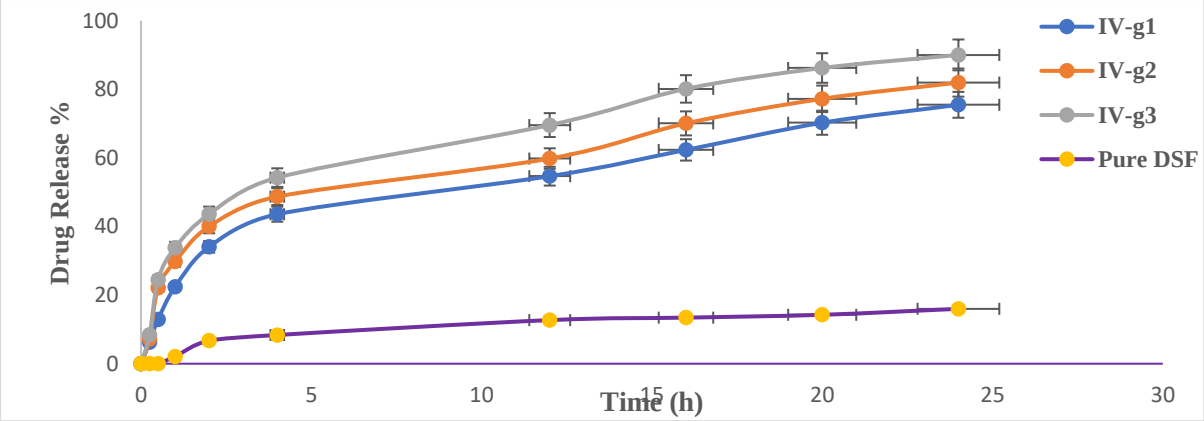


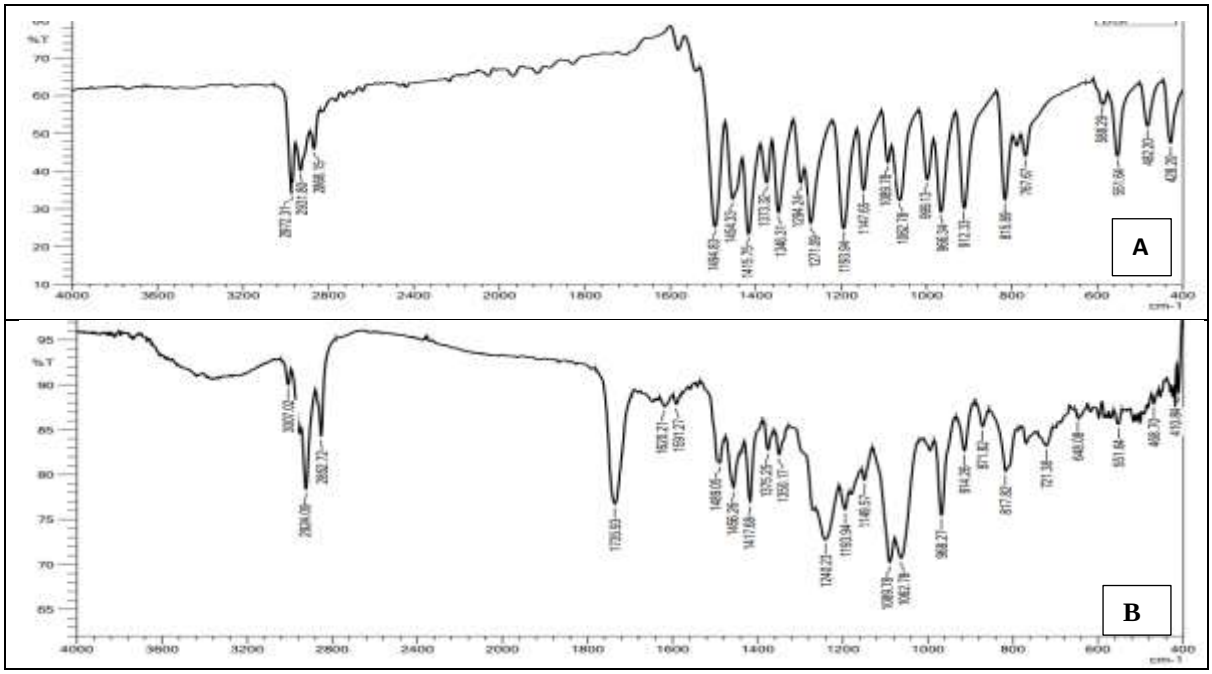
Figure 1. An *in vitro* drug release of nano invasome gel with different formulae (mean ±SD, n=3).

Selection of optimum nano invasomal gel formula

The selection of IV-g3 as the optimal nanoinvasomal gel formula was based on several factors, including its greatest release % after 24 hr., appropriate viscosity, spreadability, good homogeneity, proper pH, and high drug concentration (99.6 ± 0.4%). This formula underwent additional analysis.

Fourier transform infrared spectroscopy (FTIR)

The pure DSF powder's FTIR spectra in Figure 2A showed a C-H stretching vibration at 2972 cm^{-1}, bands at 1494 cm^{-1} and 1271 cm^{-1} corresponding to N-C=S and C=S bond stretching, accordingly, CH_2 and CH_3 deformations from 1346 cm^{-1} to 1454 cm^{-1}, and C-C skeletal vibrations between 1147.6 cm^{-1} and 1193 cm^{-1} that corresponded to the reported spectrum, suggesting that the drug used exhibits a high degree of purity. The physical mixture of the selected nanoinvasomal gel in Figure 2B exhibited similar characteristic peaks as DSF, with reduced intensity. In the selected nanoinvasome gel IV-g3, as shown in Figure 2C. Notably, no new peaks were formed, indicating the compatibility and uniformity of DSF with the excipients in the chosen nanoinvasomal gel formulation. The spectrum confirms the good interaction of DSF with the phospholipids of the nano-invasome chemical structure. However, the fingerprint area of the IV-g3 clearly shows all the characteristic peaks related to DSF, suggesting that its chemical structure remained unchanged during the manufacture of the IV-g3 and maintained its original chemical composition [45,46].



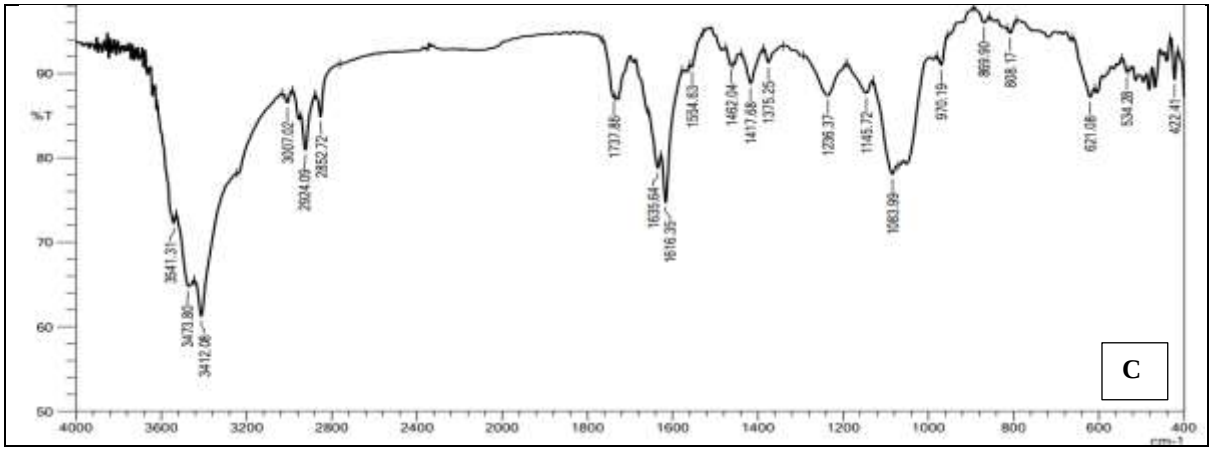


Figure 2. FTIR spectrum of pure disulfiram (A) physical mixture (B), and optimal nano-invasomal gel (C)

Field emission-scanning electron microscope

The Field emission-scanning electron microscope images of invasomal dispersion showed nearly spherical, uniform, and single-layered nano-vesicles. Figure 3 illustrates the active medication contained within invasomal dispersion, appearing as scattered white particles when viewed at high magnification throughout the network. Invasome with a spherical shape was created by mixing 40% ethanol, 2% Soybean phosphatidylcholine, 1% carvacrol, and a concentrated amount of encapsulated drug [47].

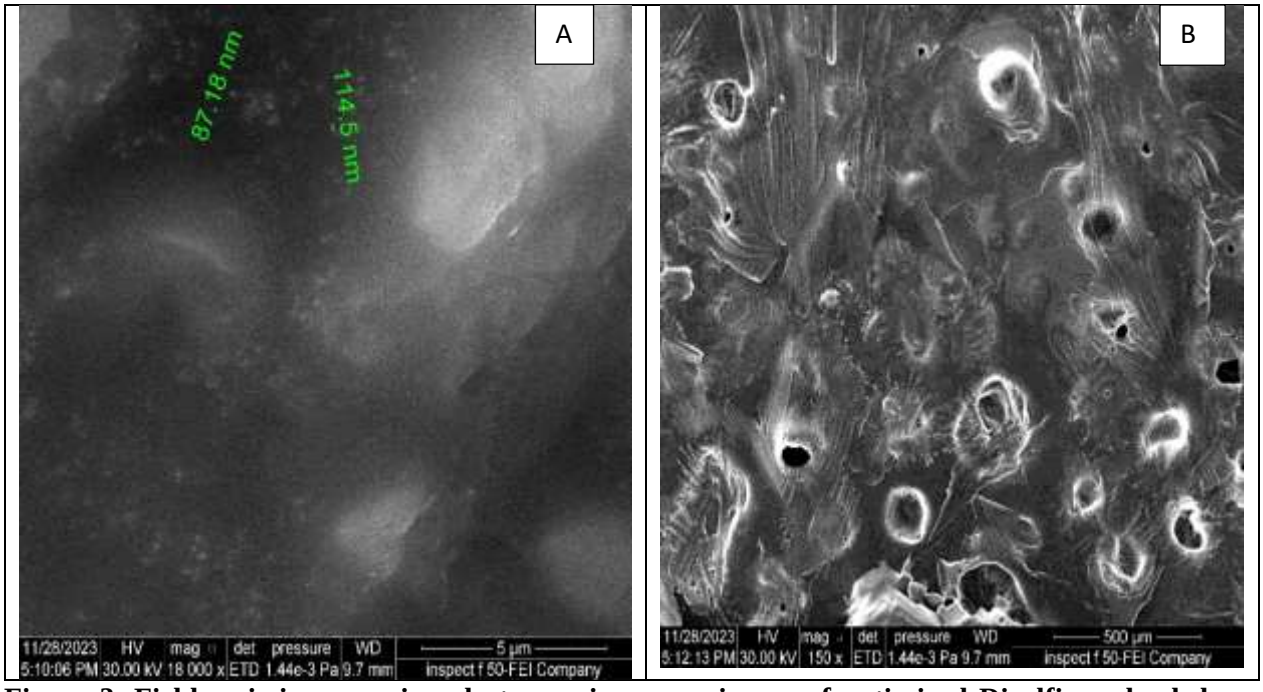


Figure 3. Field emission-scanning electron microscope image of optimized Disulfiram-loaded invasomes formulation at 5 µm (A) and 500 µm(B)

Histopathological study of rat skin after transdermal application of the selected invasomal formulation

Figure 4. shows light photomicrographs taken from the cross-section of control and treated rat skin following 72-hour administration of invasomes loaded with DSF gel (IV-g3). No infiltration of inflammatory cells was detected in the sections of different skin layers indicating the safety of the formulation and the absence of any irritation potential. This indicates the suitability of the aforementioned vesicular formulations for transdermal delivery [48].

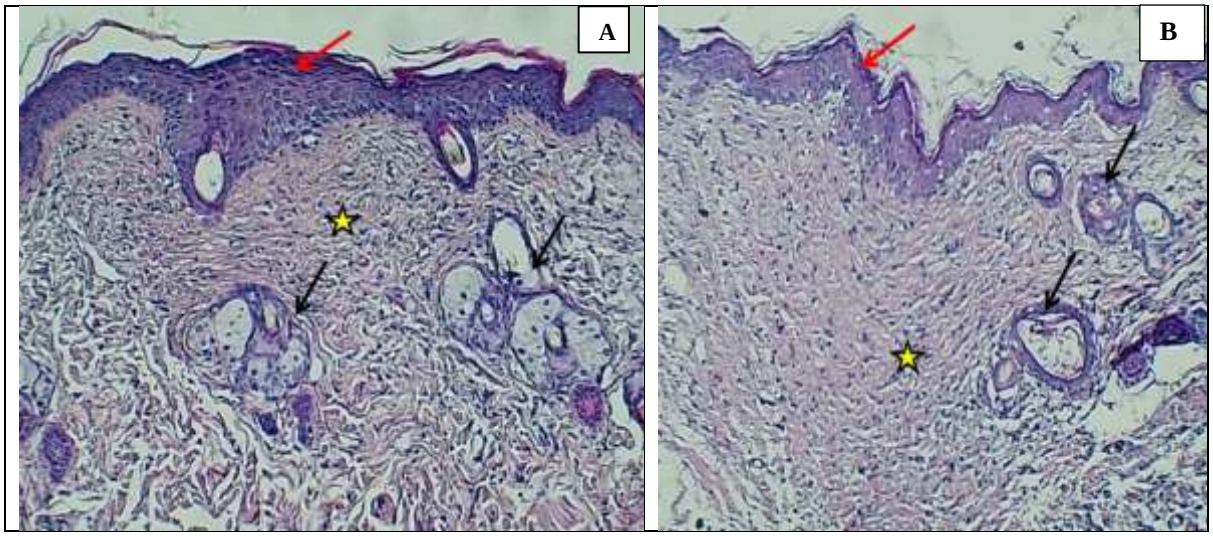


Figure 4. Histopathological examinations (x 100) (H &E) sections of rat skin (A) treated with normal saline (serving as control), (B)treated with nanoinvasomal gel of selected formula

Ex vivo Permeation Study

The drug permeation from the IV-g3 formula is demonstrated in Table 4 and Figure 5. The observed outcomes can be ascribed to the small size of the invasomal vesicle, as well as the presence of ethanol and carvacrol, which function as permeation enhancers. This may cause the invasomal wall to become more elastic and deformable, enabling the nanovesicles to penetrate deeper into the epidermal layer. Furthermore, the flux after the experiment was computed for the sake of comparison. A considerable disparity in flux was observed between the IV-g3 formula (73.638 µg/h/cm^2) and the DSF (17.242 µg/h/cm^2), and the Lag time decreased from 1 ± 0.3 h. for DSF to 0.13 ± 0.2 h [49]. A significant difference ($p<0.05$) in the cumulative amount of the DSF in nano invasomal gel permeated. The presence of the aromatic ring and the phenolic OH group in carvacrol contribute to a greater chemical reactivity than other types of terpenes. Therefore, greater interaction with organic molecules (skin constituents) was expected for the carvacrol [11].

Ethanol in the vesicle interacts with skin lipid molecules in the polar head group region, resulting in reducing the rigidity of the stratum corneum lipids and increasing their fluidity, which may finally lead to an increase in skin permeability. In addition, the ethanol may provide the vesicles with soft and flexible characteristics which enable them to squeeze through the pores in the stratum corneum, which are much smaller than their diameter size [50,30], and also the gel formed by hyaluronic acid adheres well to the skin surface and is an efficient moisturizer, enhancing skin hydration and improving drug permeation [16].

Table 4. Representations of ex-vivo permeation of Disulfiram suspension (DSF-suspension) and optimized invasomal gel formula (IV-g3) through rat skin (Papp = Permeability coefficient, J ss = Steady-state flux).

Ex-vivo permeation parameters	Formulation	
	DSF suspension	IV-g3
J ss (µg/cm^2 /h)	17.242±0.27	73.638±0.4
P_{app} (cm/h ×10^{-3})	3.44	14.72
Lag time (h)	1 ± 0.3	0.13 ± 0.2

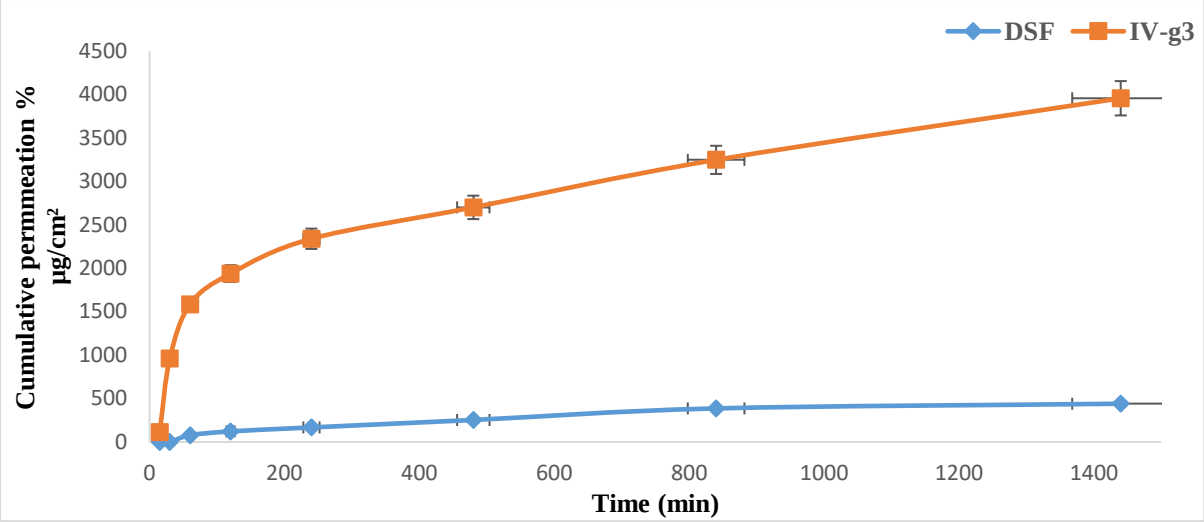


Figure 5. Drug permeation profiles for the optimized nanoinvasomal gel formula (IV-g3), compared with the drug suspension (DSF) as a control, through rate skin membrane for 24 h.

Stability studies

A stability test was performed on the DSF invasomal gel for one month, as shown in Table 5, with storage temperatures set at 4°C and 25°C. After one month of refrigeration, the gel exhibited a highly satisfactory look. However, when maintained At ambient temperature, the gel experienced a 3% reduction in active DSF content. Therefore, it can be inferred that the invasomal gel exhibits stability and maintains a satisfactory shelf life when stored at a temperature of 4°C [51].

Table 5. Stability study of the selected nano invasomal gel Formula (IV-g3) at various temperatures (4°C, and 25 °C) after 1 month

Parameter	Initial results	4 °C ± SD	25 °C ± SD
Physical appearance	off-white color, uniform	No change	No change
pH	6.5 ± 0.12	6.1 ± 0.12	6.7 ± 0.12
Drug content %	99.6 ± 0.4	98.12 ± 0.2	96.22 ± 0.31

Conclusion

The study successfully developed a transdermal gel using nano-invasomal technology and higher patient compliance. Incorporating natural lipid (soya phosphatidylcholine), carvacrol, and hyaluronic acid as a gelling agent in a 3%. This gel demonstrated sustained release of disulfiram for 24 hours, surpassing the disulfiram suspension in terms of permeation through abdominal rat skin, spreadability, and *in vitro* release.

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Conflicts of Interest

The authors stated no conflict of interest in the manuscript.

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Ethics Statements

The College of Pharmacy/University of Baghdad's ethical committee thoroughly examined and authorized all animal procedures, adhering to established standards for the care and utilization of laboratory animals. The animal study adhered to the ethical criteria for animal research established by the WOAH (Formerly Office International des Epizooties) foundation. The animal study complied with the ethical standards outlined in the criteria established by the National Committee for Research Ethics in Science and Technology (NENT), Norway. The ethical approval code is RECAUBCP482023M.

Author Contribution

The author's responsibilities are described as follows: Preparation, collecting, and analyzing data: Worood Hameed. Designing, reviewing, and supervising the project: Hanan Jalal Kassab. All authors reviewed the results and approved the final version of the manuscript.

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هلام الحويصلات النانوية النافذة المحملة بالدايسلفيرام باستخدام زيت الاوريكانو كمحسن الاختراق عبر الجلد

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الخلاصة

دايسلفيرام (DSF)، الذي يعتبر دواء معتمد من قبل إدارة الغذاء والدواء الأمريكية لعلاج الإدمان على الكحول، قد أظهر فعاليته ضد عدة أنواع من السرطان. يعاني الدايسلفرام من قلة الذوبان، وسرعة التمثيل الغذائي، وفترة فعالية قصيرة، وعدم استقرار في البيئات الفيزيولوجية، والتي تسببها في الغالب تحلل سريع في البيئة المعديّة الحمضية. لذلك، تم تطوير هلام عبر الجلد يحتوي على الدايسلفيرام، والذي تم تحميله في الحويصلات النانويه النافذة، لتحسين استقرار الدايسفرام وتمكين توزيعه بشكل فعال في الانسجة الورمية. تم تطوير تشتت الحويصلات النافذة مثلى في المختبر باستخدام نسبة 1:٤ من فوسفاتيديل كولين المستخلص من فول الصويا إلى دايسلفيرام، و ١بالمئة مستخلص زيت الاوريكانو كمحسن لاختراق الجلد. علاوة على ذلك، تم تطوير ثلاث صيغ لجل الإنفاسوم (IV-g1 ، IV-g2، و IV-g3) باستخدام حامض الهيالورونيك كعامل للتثبيت بتركيزات (٢ ، ٢,٥ ، و ٣) بالمئة. خضعت هذه التركيبات لتقييم إضافي في المختبر لتقييم المظهر الفيزيائي، والتجانس، واللزوجة، وقابلية الانتشار، وإطلاق الدواء في المختبر. أظهرت النتائج أن الصيغة (IV-g3) أظهرت تحسنًا في التجانس، والاتساق، وقابلية الانتشار، والاستقرار، وأظهرت تدفقًا غيرخاضع لقانون نيوتن ومحتوى دواء (٩٩,٦ ± ٠,٤ بالمئة). بالمقارنة مع معلق الدايسلفرام (بالمئة 16) أظهرت IV-g3 الصيغة إطلاقًا أكبر بكثير للدايسلفرام (90.03) بالمئة . كان اختراق الجلد خارج الجسم لهلام IV-g3 في جلد بطن الجرذان أعلى بشكل ملحوظ (١٤,٧٢ سم ٢ / ساعة × ١٠-٣) مقارنة بمعلق دايسلفرام (٣,٤٤ سم٢ / ساعة × ١٠-٣) خلال يوم كامل. أظهرت نسبة التحسين لـ IV-g3، التي بلغت ٤,٢٧، تفوقها على معلق دايسلفرام . كذلك نجح هلام الحويصلات النانوية النافذة في تعزيز توصيل الدايسلفرام من خلال الجلد. أظهرت الطريقة المحسنة للتوصيل عبر الجلد استقرارًا محسنًا للدايسلفرام ونفاذية أفضل للجلد للدايسلفيرام. .

الكلمات المفتاحية: نانوتكنولوجي ، طريقة الاعطاء عن طريق الجلد ، الحويصلات النافذة ، الدايسلفيرام، هايلورونك اسد