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Topical Lipid-Based Nanosphere Delivery System for Enhanced Docetaxel Administration

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Abstract

Lipo-Nanospheres (LPNS) can increase the bioavailability of poorly soluble medicines as an alternative to traditional lipid-bioactive nanocarriers. This novel lipid carrier is characterized by inexpensive components with improving biocompatibility and drug release. The primary goal is to choose the best lipid matrix and transform these poorly soluble molecules into perhaps beneficial transdermal film ; by utilizing the new lipid nano carrier's capabilities of solubilize or disperse the drug in the solid lipid matrix internal core; then incorporated in polyvinyl alcohol transdermal film matrix . The drug used in this study was docetaxel(DCX) , which falls under Class IV of the Biopharmaceutical classification system (BCS) as a dissolution-limited drug. Emulsion melt dispersion was the most effective way to prepare DCX - LPNS. In conclusion, formula 9, which is made up of tristearin, hydrogenated soybean oil and 90G phospholipid, gave the best drug dissolution, which led to the biggest results over 24 hours (98.43±0.829) with a particle size of 156.09±9.564nm. Zeta potential -23.5, drug content 93.56±1.33 and drug loading capacity 6.59±0.04%, are a few advantages of the encapsulation technique ; improve the proper docetaxel incorporation within the lipo-nanosphere, while FESEM provides spherical particles with enhanced mechanical properties. Although FESEM and X-ray diffraction demonstrate that DCX was effectively contained in its amorphous form inside the LPNS. While the histological study determined the safety of the topical DCX-LPNS transdermal film, F12 with 10% PVA transdermal film matrix concentration produced the best sustained release in 24 hours, 89.84% with 90.8±0.19 drug content, transparent, flexible, and non-sticky appearance.

Keywords: Lipo-Nanosphere, docetaxel , Topical.

Introduction

Liposphere in general comprises a single unit of phospholipid layer that encircles a solid lipid core and either clogs the drug or coats it with the drug indeed. The purpose of the emulsifier or stabilizer is to create a layer surrounding the raw material this will improve the lipid nanodrug carrier's dispersion inside the aqueous phase. (1)

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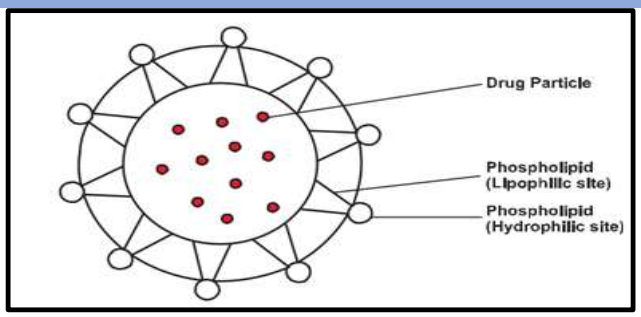


Figure 1.Composition of lipospheres structure. [2]

This solid lipid matrix is an alternative to polymeric-based drug carries; the carrier particles' static contact is facilitated by the lipid matrix's solidification (surface changes); along with low reagent costs, simplicity of fabrication, good stability, and great dispersibility in an aqueous media in the rate at which phospholipid and carrier coating control the release of the trapped substance as shown in Figure. 1[2].The carrier melting point should be higher than 45°C; are more likely to form stable colloidal dispersions [3, 4].

DCX is an anti-neoplastic drug belonging to the second generation of the taxoid family. It is a semi-synthetic equivalent of Paclitaxel; 10-deacetylbaccarin-III. The inactive precursor molecule is obtained from the needles of the rare Pacific yew tree *Taxus baccata* . The chemical formula is $C_{43}H_{53}NO_{14}$, and its molecular weight is 807.89 Da. The chemical has poor water solubility (0.025 µg/ml) and low membrane permeability (1 cm/second X 10^{-6}), as indicated by its log-P and pKa values of 4.1 and 10.97, respectively. The anhydrous DCX has a melting point between (167-169 ºC). Because of its weak solubility and permeability, DCX has a limited capacity to dissolve or permeabilize drugs [5,6]. As shown in Figure 2[5,6].

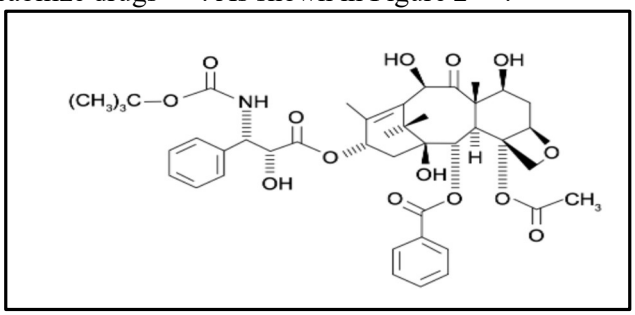


Figure 2. The Structure of Anhydrous docetaxel [5,6].

There hasn't been much success with a single technique for solubility increase when it comes to BCS Class IV, but more complex strategies, such as using lipid-based nano-systems or a chemical prodrug approach, have demonstrated promising outcomes with BCS IV [8]

Anticancer phytomedicines or plant-based pharmaceuticals generally and DCX specifically, are becoming more popular as a natural product a less harmful derivative, and they are an especially appealing option because of their affordability and accessibility. [9] Taxoids' mode of action is distinct from that of all other kinds of plant-based anticancer drugs, such as vinca alkaloids and colchicine, because it involves the hyperstabilization of microtubule DCX is effective in treating patients with metastatic solid tumors that are resistant to paclitaxel. The tubulin heterodimer subunit is the target of the taxoid. [10] DCX is used to treat a variety of cancer types. It was initially approved in the mid-1990s for the treatment of anthracycline-refractory metastatic breast cancer. Studies have shown that patients with metastatic breast cancer treated with single-agent docetaxel or docetaxel-based combination regimens had improved time-to-progression, overall survival, or both. It was only created for intravenous infusion on the market at a dose of 100 mg/m^2 every three weeks (Taxotere®) due to its extreme gastrointestinal toxicity and high hydrophobicity, which causes low bioavailability with first-pass metabolism with P-glycoprotein efflux pumps. [11]Thus, take into consideration DCX as the greatest option for creating novel pharmaceutical forms as antitumor medicines.

However, food-dependent bioavailability can be significantly decreased by preparing the drug in a lipid-based formulation, which can improve lipophilic medicines' solubility and dissolution and speed up the creation of solubilized species, which is the absorption basis. Lipid-based compositions might therefore be utilized to lessen

the drug dose while enhancing its bioavailability [12].

LPNS, a lipid-based system, has the potential to significantly improve the solubility and bioavailability of DCX despite its low solubility. This lipid matrix releases the drug in a controlled manner and has the capacity to produce a reservoir effect, allowing the drug to be released gradually and maintain therapeutic levels in the bloodstream. This release mechanism will also lessen the serious side effects by lowering the blood drug concentration's oscillations and decreasing the need for frequent drug administration improving patient compliance. These ideas for DCX formulation were also observed by *Sumera et al.* when they loaded DCX inside solid lipid nanoparticles; through their published research, they also noted that the benefits of enhanced pharmacokinetic qualities, enhanced efficacies, and reduced side effects have generated a great deal of interest in nanoformulations for DCX delivery. It has been shown that solid lipid nanoparticles are more effective than Taxotere® at delivering DCX, based on prior preclinical research; additionally with efficient DCX delivery for longer circulation times and greater accumulation within cancer cells. [13]

The main aim of this study; By encasing DCX inside a lipid nanocarrier, which can increase cellular absorption by using biodegradable lipids to guarantee safety and efficacy, the study's goal is to better dissolve and stabilize DCX. Combining these strategies with PVA and PEG 600 to prepare transdermal film ; will yield additional benefits, such as prevention of first-pass metabolism, prolonged drug release, decreased P-glycoprotein efflux pumps, fewer direct adverse reactions, and improved patient quality of life ;this is seen as benefits of topical treatments over oral and IV anticancer medication therapy.

Materials and Methods

Materials

Cetyl alcohol was obtained from Alpha Chemika, India; docetaxel was obtained from Hangzhou Hyper Chemicals Ltd., China; phospholipids 80H, 90G, and L-α-Lecithin were provided by Henan Guange Biotechnology Co., Ltd., China. Both Tristearin and hydrogenated soybean oil come from Pharmaffiliates Analytics and Synthetics Ltd., India. Also, India supplies polyvinyl alcohol, Beeswax tween 80, and Phosphate Buffer Saline PH.7.4. The Belgian company CHEM-LAB provides 100% HPLC grade ethanol.

Methods

Formulation and Composition Technique

DCX containing LPNS was created using the emulsion melt dispersion method hybridized with Both homogenization and ultrasonication techniques were employed to create LPNS., also mentioned was initially combined with the lipidic combination or the exterior phase. It dissolved in a hot plate between 70 and 80 °C with a minimum amount of ethanol. The magnetic stirrer was running for fifteen minutes at 950 rpm. Using a hot plate, the exterior hot aqueous phase was heated to 75°C. In addition to a magnetic stirrer set to 950 rpm, add enough dissolved deionized water (DIW) to the mixture for both the surfactant (Tween 80) and the cosurfactant(PVA) and stir for 15 minutes[14]. Subsequently, lipid emulsion was formed by gradually introducing the oil phase dropwise to the aqueous phase while stirring and heating both phases to the same temperature to produce a stable (O/W) emulsion for maintaining the principal lipospheres' uniform dispersion[15]. It was stirred for 15 min. utilizing a mechanical stirrer. Additionally, the main lipospheres are homogenized. It took around 60 minutes to use a homo dispenser to make a homogeneous dispersion after it was quickly cooled to below 20°C by submerging it in an ice bath and continuously whirling to ensure that the DCX loading inside the LPNS was confirmed. It was subjected to the ultrasonic probe (Shimadzu, Japan)for 10 minutes with 50 seconds of work and 10 seconds of rest at 40 watts; then kept in a dry, dark environment [16]. As seen in Table 1.

Table 1. Trail batches for selection of best LPNS.

Substance(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10

DCX	16	16	16	16	16	16	16	16	16	16
Tristearin									25	25
Stearic acid	-	-	25	25	25	25	25	25	-	-
Paraffin wax	25	25	25	25	25	-	-	-	-	-
Cetyl alcohol	25	25	-	-	-	25	25	25	-	-
90 G*	10	-	10	-	-	10	-	-	10	-
80 H*	-	10	-	10	-	-	10	-	-	10
Hydro.soy. oil*	-	-	-	-	-	-	-	-	25	25
Ethanol (ml)	10	10	10	10	10	10	10	10	10	10
L-α-Lecithin	-	-	-	-	10	-	-	10	-	-
PVA*	5	5	5	5	5	5	5	5	5	5
Tween 80(ml)	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9

DI W*	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Hydro. Soy. oil * means hydrogenated soybean oil**, *Phospholipon 90G** represent, soybean Phosphatidylcholine while ***Phospholipon 80H** represent Hydrogenated soybean Lecithin, ***PVA** mean Polyvinyl Alcohol, **DIW*** deionized water **;** The **rate per minute (RPM)** used to prepare each of the abovementioned formulas was 1000 RPM.

Analysis of the Nanoliposphere

Evaluation of particle size, polydispersity index, and zeta potential

Every stable formula was stirred and diluted 100 times with DIW ; at 37 °C using a magnetic stirrer. Using a particle size analyzer (Malvern Zetasizer, UK), the final particle size of each LPNS was analyzed using the dynamic light scattering method, and the PDI was then calculated [17]. Additionally, the zeta potential (zp) was determined. [18].

Fourier transform infrared spectroscopy (FTIR)

The spectrum obtained from Fourier transform infrared spectroscopy (FTIR) was obtained using the pressed-disk approach. A tiny quantity of drug was combined with KBr powder and compressed into a disc. The produced disc was examined using FTIR spectroscopy at 4000-400 wavelengths [19].

Figuring out the drug entrapment efficiency and loading capacity

The filtration/centrifugation method served as an indirect means of determining the loading capacity and the proportion of DCX contained within LPNS. A volumetric flask containing PBS 7.4:ethanol (7:3) was filled with the LPNS Liquid state. After the volume was increased to 10 mL, then added to the upper chamber of an Amicon® Ultra Centrifugal tube with a molecular cut-off size (MWCO) of 10 kDa, the tube was centrifuged for thirty minutes at 4,000 rpm [20].

The medicine that had escaped was soon gathered in the filtrate of the lower chamber then diluted and detected using PBS (pH 7.4): ethanol (7:3) as a blank. It was filtered using a 0.22 µm filter syringe. The drug entrapment efficiency was assessed in triplicate using a UV spectrophotometric technique, with average data being utilized; the equation was used to calculate drug entrapment efficiency and drug loading capacity [21].

$$EE\% = \frac{WT-WF}{WT} * 100 \text{....Eq1}$$

$$DL\% = \frac{WT-WF}{WL} * 100 \text{....Eq2}$$

Where:

WF = weight $free\ drug$ is the weight of the free drug found in the aqueous dispersion ultrafiltration,

WT = weight total $drug$ is the weight of the first drug employed.

WL= is the total weight of the lipid used. The findings are given as averages ± standard deviation[22].

Determining the docetaxel Content

One milliliter of the DCX-loaded Lipo-Nanosphere (or 0.16milligrams of DCX) was dissolved in ethanol. The drug was completely extracted at 50°C by adding 10 mL and stirring it for 10 minutes at 750 rpm on a hot plate magnetic stirrer [23] . After diluting the resultant solution with ethanol according to the instructions, it was filtered through a 0.22 µm filter syringe and the concentration of DCX was measured using spectrophotometry. After that, a UV-visible spectrophotometer (Shimadzu, Japan) was used to analyze it spectrophotometrically in ethanol at its λ max (299 nm).[24]

Release within a laboratory

Five milliliters of freshly made DCX Lipo-Nanosphere dispersion were put into a dialysis bag. Using a magnetic stirrer [25]. the dialysis membrane was mixed with; phosphate buffer 7.4: ethanol (70:30); providing 100 ml of releasing medium overall.; at 100 rpm and 37°C, dialysis membrane at a molecular weight threshold

of 3500 Da; The same task was completed by *Yasi Yu et al*. through examining of curcumin-loaded solid lipid nanoparticles; the dispersion was put into a dialysis bag with a molecular weight cutoff of 3500 Da; the buffer solution combined with ethanol was used in the in vitro release media. [26] Sink conditions are typically reached by adding surfactants like tween 20 , sodium lauryl sulphate ; to the dissolving volume, but such a method might not be appropriate for creating a biorelevant dissolution method for a drug that isn't very soluble in water based on BCS Classes for type III and IV drugs . [27] In this instance *Valeria Friuli et al.*, postulated the biphasic dissolution model's foundation; to achieve the sink condition, they suggested utilizing ethanol in the releasing medium because the aqueous layer alone does not saturate, and sink conditions cannot be preserved also the dissolution tests were conducted in two fluids that mimicked the in vivo environment. These settings are the most selective to emphasize the variations in the behavior of the compounds. [28,54]At intervals, 30 min., 1, 2, 3, 4, 5, 6,7, 8, 9, 10, 11, 12,14, 16, 18, 20, 22, and 24 hr. 5 ml of the dissolving liquid were removed. The same volume of the newly created to maintain a steady volume, release medium was added. Utilizing a UV-Vis spectrophotometer and the calibration curve set to 232 nm, the concentration of DCX was determined [29].

Calorimetry using Differential Scanning (DSC)

The crystalline state of a drug is commonly evaluated using this instrument, especially when the drug is in the form of a lyophilized powder of the optimal formula. This is accomplished by using the entire LPNS emulsion formula (with 5% mannitol) that was lyophilized after being frozen for a full day at -80 C- -55 C and 700 bar using a freeze dryer Telstar, model lyoQuest 61665, Spain) for an entire night. Every sample was taken using the sealed aluminum pans. The measurement was carried out under a dry nitrogen gas flow at 100 ml/min, and the temperature was set between 30 and 300 ºC [30].

A total of 8–10 mg of pure DCX, the core and coated LPNS emulsion component, and lyophilized powder from the selected formulation were added to the pan and heated at a rate of 10 ºC/minute; It can also be applied to investigate the drug's and formulation excipients' physical compatibility; the temperature of the sample was monitored for 24 hours using an empty aluminum pan as a reference. [31].

Field emission scanning electron microscopy is referred to as (FESEM)

This technique was created to simultaneously count, size, and sort everything visible in the image. Unconjugated gold particles were added to each sample after it had been incubated to help with focus and act as an internal check for particle sizing. The particles were then fixed, dyed, and processed to prepare them for image analysis on carbon-coated copper grids [32, 33].

X-ray Diffractometry (XRD)

An XRD pattern was established using the X-ray Diffractometer-6000, Shimadzu, Japan. The range in which the data was gathered was 5–50° (2θ). There was a 40 kV voltage, 30 mA current, and an 8 degrees per minute scanning speed [34].

Selecting the optimal formula

The ideal formula was selected based on the zeta potential, droplet size, PDI, and in vitro release studies [35].

Making the LPNS transdermal film prepared

The formula for the chosen DCX lipid nanocarriers was used to load onto biodegradable PVA film based on the findings of earlier analyses. Solvent casting was used to make PVA films filled with either pure DCX or DCX lipid nanocarriers; that will be added to the transdermal film matrix, which is made up of polyvinyl alcohol(PVA); (7%,10%, 12%, and 14% w/v) that has been dissolved in hot DIW by employing a hot plate magnetic stirrer (JOANLAB, China) running at 1000 revolutions per minute; for three hours at 60 degrees Celsius..[36,37] PEG600 (polyethylene glycol 600) with lower molecular weights was more effective when the formula is plasticized, and these films also had superior optical qualities. [38] Ideal concentration for use as a plasticizer at 30% (w/w) [39]; however, to ensure uniformity and the formation of a clear solution, it must be added drop by drop using disposable syringes at a speed of 1000 RPM for 10 minutes 60° C. [40] to guarantee appropriate component mixing and interaction, making it more appropriate for transdermal applications ; after cooling the solution to ambient temperature, any trapped air bubbles were removed by placing it on the sonicator(JOANLAB, China) for 10 minutes. Once the PVA polymer matrix had cooled to room temperature; the DCX-LPNS

and untreated DCX(16mg) were equally distributed throughout the PVA solution for 45 minutes in a(50:50 w/w ratio) at 1000 RPM. [41] The prepared polymeric based on DCX-LPNS; was filled inside a clean glass petri dish with a diameter of(4cm). Turning the funnel over onto the Petri plate; regulated the evaporation rate. They were then dried for 48 hours at room temperature; when they had completely dried, covered in aluminum foil, and kept at room temperature in a desiccator until more assessment research could be conducted. [42] Table 2. lists every component of the transdermal film.

Table 2. Matrix structures for films loaded with LPNS; 50:50 (%w/w).

Formula no.	Loaded-LPNS	Elements of a film matrix(18ml)	
		PVA	PEG600
F11	(18 ml)LPNS that contain 16 mg DCX	7	2.1ml
F12		10	3ml
F13		12	3.6 ml
F14		14	4.2ml

Characterization of transdermal film

The amount of drug in the transdermal film

To confirm their therapeutic efficacy, the drug content of the produced films was examined. Every ready transdermal film (diameter 4 cm, specific area of 12.56 cm^2, area of circle= $\pi d2/4$) was stored separately overnight in a dry dark spot; inside 100 ml of ethanol until the drug was released completely and became fully, soluble. 1ml of the DCX-methanol solutions should be passed through a 0.22 µm membrane filter; and was diluted to 10 ml using ethanol; in the volumetric flask's inside. A UV-visible spectrophotometer (Shimadzu, Japan) set to λmax 299 nm was used to determine the drug concentrations. The drug-free LPNS transdermal film solution was used as the blank; because elevated concentration ethanol destroys certain LPNS-film components when stored for an extended period, influencing the findings of UV-visible readings. The mathematical average of three readings was taken ;(n=3).[43, 44]

Changes in Weight and Thickness

A digital caliper for vernier (KM-DSK-200, Shaanxi, China)was used to measure the thickness of the films, the thickness was measured at five points (four corners and the center), on every film to provide consistent thickness, and the mean was determined .Any variation in mean thickness of more than ± 5% was not included in the study. For weight determination three randomly chosen films were separately weighed to determine the weight variation of each formulation by the electronic balance(G&G, Deutschland); mean and standard deviation values were computed (n=3). [45]

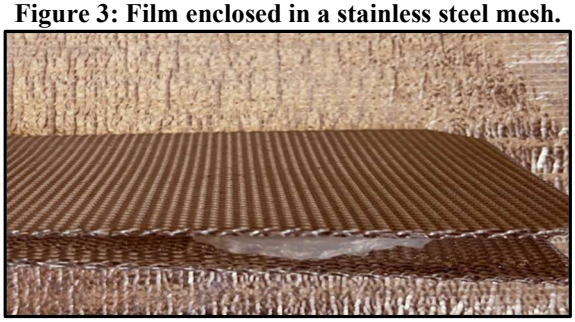
Visual evaluation of the film's composition and quality

The prepared films' stickiness, transparency, and clarity were assessed visually. If it meets the requirements, it was taken for additional assessment. Films that were not created to a high enough standard are discarded. [46]

In vitro dissolution study

Stirring in a beaker containing 100 ml of phosphate buffer slain PH 7.4 with ethanol ; (70:30) ml was used as the dissolving media in these investigations; the transdermal films containing DCX-LPNS were formed by the different film matrix concentrations and the untreated DCX film were examined with a stainless steel mesh was used to secure the film as seen in Figure 3 ; then firmed with stainless steel clips were subsequently inserted into the dissolution media. while the Hot plate magnetic stirrer(JOANLAB, China) at 100 rpm and 37±0.5°C. At intervals of 0, 30 minutes, 1 hr, 2 hr, 3 hrs, 4 hrs till 24 hrs, 5 ml samples were taken out and replaced with 5 ml of brand-new medium solution each time. After filtration through a 0.22 µm filter syringe; the UV-visible spectrophotometer (Shimadzu, Japan) was set to 232 nm where the concentration of DCX was determined. [47,48]

Figure 3: Film enclosed in a stainless steel mesh.



PH of Surface

The surface pH of the transdermal films was measured with a calibrated pH meter (Waterproof Pen, JOANLAB, China); by using standard buffer solutions with pH values of 4 and 7. A(1cm^2) transdermal film segment and (1 mL) of DIW were incubated in a glass tube for two hours at room temperature (25 ± 2.1°C). After the water in the tube was decanted, the pH of the surface was determined using a digital PH meter (Waterproof Pen ,JOANLAB, China). To determine the average pH, the pH electrode was inserted three times into the enlarged portion of the film. [49]

Determining Skin Irritation via Histological Analysis

One day before the skin irritation testing, the electric shaver was used to carefully remove the back hair of the Sprague-Dawley male rats aged 10-12 weeks. Three groups contained(n=3) rats in each group, weighing 200±0.25g, and were taken from the animal house at the University of Baghdad's College of Pharmacy: to conduct studies on skin irritation. The groups included negative control groups that received pH 7.4 phosphate buffer, LPNS-film (drug-free), and the medicated optimal formula containing DCX-LPNS. A specific area of the film 12.56 cm^2; was applied on the shaved skin back of the rats and covered with parafilm then occluded with medicated adhesive tape . 72 hrs. incubation period; After the rats were killed, the applied skin tissue was removed for later histological analysis; this can be seen in Figure 4. [50]

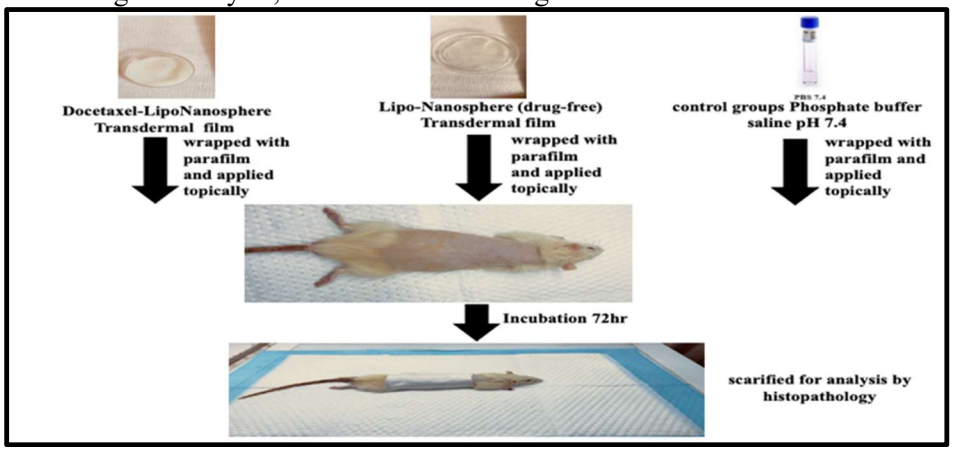


Figure 4 .Transdermal film application and skin preparation for an irritation test.

Details and Statistical Analysis

The mean of three replicate models and the standard deviation represents the experimental results. An analysis of variance (ANOVA) in one direction was carried out to ascertain whether the changes in the applied factors are statistically significant at the level of ($P < 0.05$) and non-significant at the level of ($p > 0.05$) [51].

Results and Discussion

The Optimal Formulation and Composition Technique

The emulsion melt dispersion method was believed to be the best for preparing LPNS since it provided spherical particles with greater mechanical characteristics; the dissolution profiles of LPNS-DXC (F3, F5, F6, F7, F9 and F10) are shown in Figure 5 and 6. concerning pure DCX. Comparable results were observed in *R. Cortesia et al.* [52] ; where they examined several techniques for producing

lipospheres and concluded that the emulsion melt dispersion method works best. Creating nanosized lipospheres through lipid nanoparticulate preparation via homogenization and probe-sonicator makes using conventional surfactants like Tween 80 and an organic solvent miscible with all ingredients easier. Using this idea, *T. BEKERMAN et al.* produced oral cyclosporin nanoparticulate lipospheres. [53] . The best method for producing uniformly sized nanocarriers that boost bioavailability is cavitation technology, which combines high shear homogenization with the assistance of ultrasonic vibrations generated by a probe-sonicator. The energy-efficient approach has the benefit of not requiring specialized workers to operate it. The probe-sonicator and homogenizer investigation is the first by *V. Gurumukhi et al.* to synthesize ritonavir nanostructure lipid carriers with various systematic methods to optimize their bioavailability. [54] The speed of preparation was set at 1000 RPM. That proved smaller particles with limited particle size distribution. Better encapsulation efficiency was found to be 97.23±3.15(F9); the same outcomes were noticed by *S . Babu Natarajan et al.* during the preparation of ofloxacin-loaded liposphere by melt dispersion method [55].

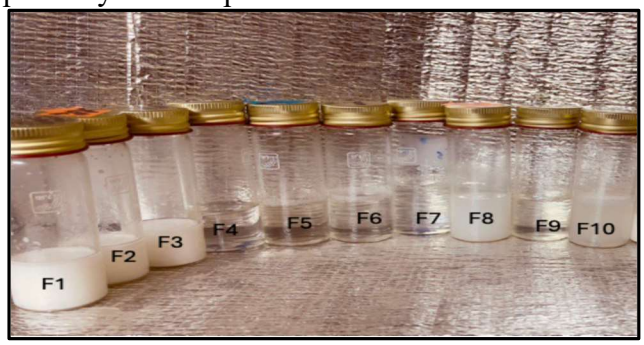


Figure 5. The color and appearance of docetaxel-loaded LPNS for all prepared formulas.

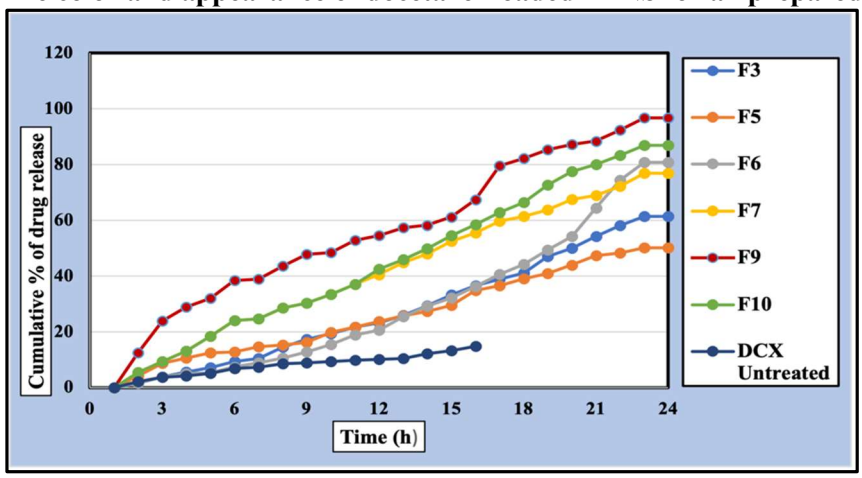


Figure 6. Dissolution profile of LPNS-DXC (F3, F5, F6, F7, F9, F10 and Pure drug)

Docetaxel Concentration, polydispersity, and particle size indexes

Also, as the particle size decreases, the % of EE decreases as seen in F1 and F2, in which respectively 80.74±7.121nm and 73.75±12.55 nm produce EE 44.5±3.53 % and 32±2.82%, and the percent of DCX content was decreased too; these results were along with the results of *S. Pandit et al.* during the preparation of lercanidipine lipospheres, because to the development of tiny lipospheres, which enhance the surface area that is accessible and ultimately cause drug loss in the process this all seen in Table 3 and Figure 7 .[56]

All the ZP values were in the negative signs of F3, F5,F6,F7,F9 and F10. The negative surface charge of the LPNS was caused by the anionic fractions, such as phosphatidylserine and phosphatidylglycerol, in the phospholipids containing 80% phosphatidylcholine (Phospholipon 9G, 80H) [57] .

All formulas that contain phospholipon 90G produce the best EE percent, like F6 , F7 , F9 and F10 gave 92.125±3.005, 88.625±2.298 % 97.23±3.15 and 82.675±3.78 of EE; with the increase of percent of DCX

content; these results indicate the high compatibility between stearic acid, cetyl alcohol, tristearin , hydrogenated soybean oil and phospholipon 90G these results were in line with reports that have already been released *A. TATODE et al.* demonstrated how well phospholipid 90G worked with paclitaxel liposomes [58].Also When phospholipid is present on the coati of spheres prepared correctly, as demonstrated by a negative charge during zeta potential measurement, stable nanoscale spherical particles are produced when the oil phase is added to the aqueous phase. Because they are composed of physiological or physiologically relevant materials, lipophilic and hydrophilic medicines can have up to 95% encapsulation and are well tolerated in biological systems. These outcomes are identical to those that Katta released. *Manogna et.al.* [59] F6 F7 and F9 had higher particle size. Respectively 151.4±0.255 , 184.15±7.10 and 156.09±9.564 which, seen in Table 3, Figure 7 and 8 ; these results further noted by *Kelidari et al.* They found that the greater the particle size, the higher the solid lipid concentration and the impact of mixing solid and liquid lipids. [60]

Table 3. Some Physical Properties of Lipo-Nanosphere.

Formulation code	**Particle size (nm)***	**Polydispersity index***	**%Entrapment efficiency**	**Drug content (%)**	**Loading Capacity (%)**	**Zeta Potential (mV)**
Docetaxel	3416	1.101	-	-	-	-1.987
F1	80.74±7.121	0.252±0.022	44.5±3.53	45.04±0.127	5.89±2.4	
F2	73.75±2.55	0.2592±0.026	32±2.82	39.47±1.33	2.13	-

					3 ± 0 . 9	
F3	143 .65 ±5. 778	0.26 0±0. 41	60.5 ±0.7 07	70. 84 ±1. 86	1 . 4 6 6 ± 0 . 6	- 1 8
F4	194 .18 ±3. 21	0.24 7±0. 036	47.12 5±3. 005	50. 64 ±0. 56	0 . 1 1 ± 0 . 0 4	-
F5	110 .33 ±8. 23	0.34 0±0. 113	52.5 ±3.5 35	65. 24 ±0. 81	0 . 6 6 ± 2 . 5	- 1 6 . 6
F6	151 .4± 0.2 55	0.3 ±0.0 93	92.12 5±3. 005	93. 56 ±0. 188	5 . 9 ± 2 . 5	- 2 8 . 3
F7	184 .15 ±7. 10	0.16 9±0. 019	88.62 5±2. 298	83. 936 ±1. 49	6 . 4 3 3 ± 2	- 2 0 . 6

					. 7	
F8	132 .1± 6.4 71	0.25 8±0. 018	56.25 ±1.7 67	62. 48 ±1. 04	0 . 1 7 ± 0 . 4	-
F9	156 .09 ±9. 564	0.22 0±0. 006	97.23 ±3.1 5	93. 56 ±1. 33	6 . 5 9 ± 0 . 0 4	- 2 3 . 5
F10	95. 796 ±4. 803	0.32 1±0. 012	82.67 5±3. 78	79. 64 ±1. 81	4 . 7 1 3 ± 0 . 9	- 2 0 . 7

*** Average ±Standard Deviation (n=3)**

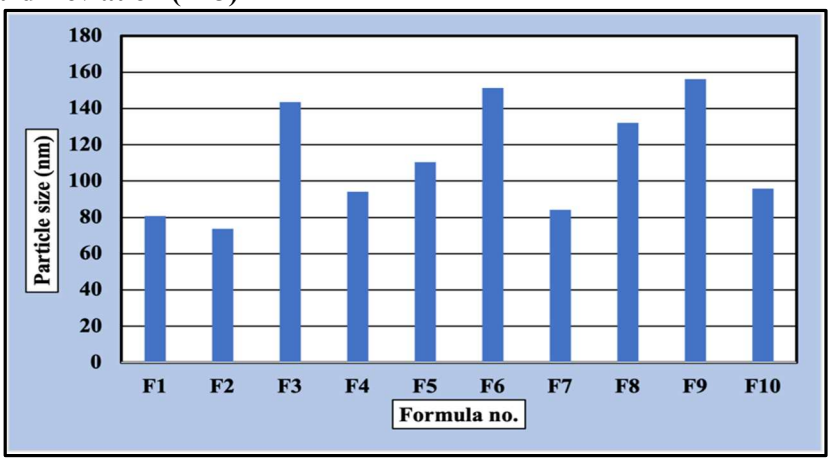


Figure 7. The histogram displays the distribution of particle sizes in the formulas.

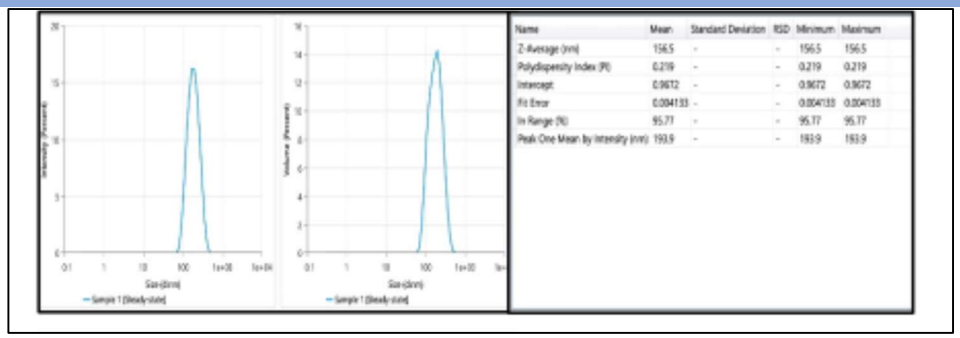


Figure 8. Particle size of the F9 DCX-LPNS optimized formula.

Content and loading of docetaxel.

Besides the high lipophilicity of DCX, which enhances the drug loading inside the nanoliposphere, several Lipophilic medications, especially anticancer medications, are appropriate for efflux transporters, including P-glycoprotein (P-gp). These medications are often sensitive to cytochrome P450 (CYP) enzyme metabolism, leading to notable first-pass clearance .[61] Sometimes, these factors are the main reasons hydrophobic drugs have limited oral bioavailability. Lipid-based nanocarriers would be ideal since they can improve and normalize drug absorption. Triglyceride vegetable oils are the foundation for self-emulsifying drug delivery systems. Since they are widely used in foods worldwide, they are generally recognized as safe. [62] LPNS enhances the percent of EE and DCX loading seen in Table 3; this may be credited to the addition of liquid lipid to solid lipid ; Hydrogenated Soybean oil and tristearin in F9 respectively . This outcome was also observed by *Salerno et al.* through the preparation of nanostructure lipid carriers; they noticed that mixing lipids causes voids in a highly ordered crystal and provides enough room to load a substantial amount of drugs efficiently; this significantly influences their EE%. [63].

Perfect formula

F9 of DCX-loaded LPNS gave the best results as F9 had the optimum zeta potential values (-23.5) mentioned in Table 3 and best drug dissolution over 24 hours (98.43±0.829). Zeta potential, considered the primary parameter for the resistance to aggregation, also displays the dispersed phase's degree of electrostatic repulsion for stability *Algandaby et al*. [64].

Fourier transform infrared spectroscopy (FTIR)

An FTIR technique was employed to ascertain the LPNS chemical makeup; the peaks linked to lipids were found at 2800 cm^{-1}, corresponding to symmetric stretching in CH, and at 2 3400 cm^{-1}, which indicated stretching in O-H and N-H. The highest point was linked to the asymmetric P-O bending of the $PO4^{-3}$ molecule in phospholipids at 528 cm^{-1}. The appearance of peaks at 753 cm^{-1} and 893 cm^{-1}, which correspond to N-H bending and glycosidic C-O-C stretching of Tristearin, respectively, indicated the coating on DCX-loaded Lipo-nanospheres; these peaks were missing in the blank liponanospheres Figure 9, A peak at 1740 $cm^{-1}$was seen in the empty LPNS spectrum. This peak indicates the C=O stretching of the ester bond of the lipid components, which connects the phospholipids' head group to their fatty acid tail. The decline in these peak intensities further validated a good lipid base coating on the DCX. The encapsulation of DCX in blank LPNS was further validated by identifying a peak at 710 cm^{-1}, which represents the fingerprint of the drug's N-H bending of benzamide. [65] Several physical-chemical interactions can occur during the creation of the nanoparticles, including the drug and LPNS components forming hydrogen bonds. This interaction modifies the bond stiffness, which limits the vibration frequency. These results were also recorded by *da Rocha et al.* while preparing DCX solid lipid nanoparticles [66, 67].

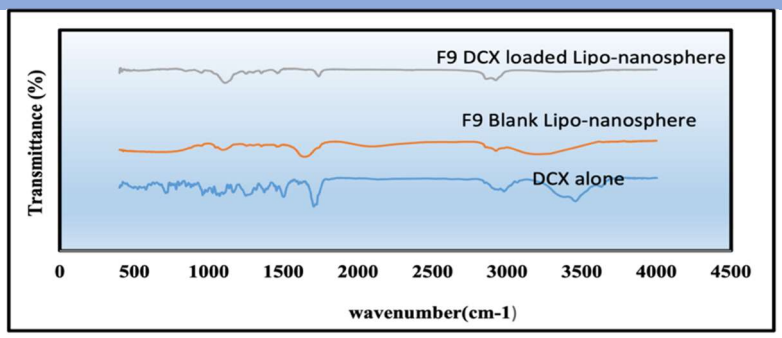


Figure 9. Qualitative FTIR analysis for docetaxel LPNS. A. FTIR of DCX-untreated; B. FTIR of blank F9; C. F9 DCX- LPNS.

Calorimetry using Differential Scanning (DSC)

In Figure 10 A, we can observe a melting peak at 167.25 ◦c, which is close to the melting peak that is recorded by *Lakshmi Prasanna Kolluru et al.* for anhydrous docetaxel at 164.98◦c which indicates the purity of docetaxel [68]. The A comparable collection of peaks was seen in the spectra of the blank and DCX-LPNS. The fact that most of the DCX peaks were absent from the DCX-LPNS indicates that DCX; was completely and successfully confined trapping and/or dispersal inside the hydrophobic core of the LPNS; this was also seen with *Ahmed Al Saqr et al.* during the docetaxel-Loaded Silk Fibroin Nanoparticles [69].

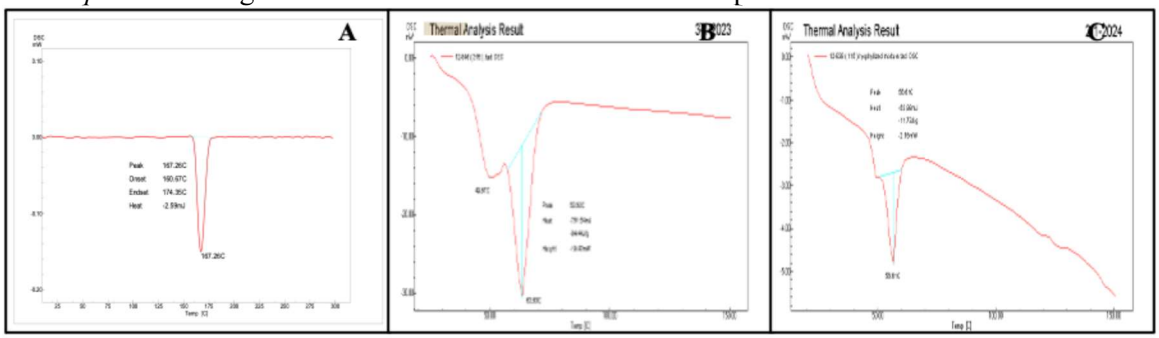


Figure 10 .A. pure DCX , B. physical mixture F9(DCX , Tristearin , Hydrogenated soybean oil ,and 90G phospholipid) and C. F9 lyophilized powder

X-Ray diffraction analysis (XRD)

In 11A's Figure, the in 3θ to-30θ indicates significant diffraction peaks from the unique peak of DCX crystalline structure of DCX was detected by XRD data, exhibiting distinctive diffraction peaks at 7.3, 8.8, 13.7, 17.2, and 20.2 ± 0.2◦ θ as well as at 3θ till 30θ, which causes high crystallinity [70]. A diffractogram of a physical mixture of DCX and tristearin, hydrogenated soybean oil and phospholipid 90G is shown in Figure 11 B.; it indicates a partial deterioration in the crystalline form with fewer but stronger peaks at 2θ = 20.21◦, 23.1, and 26.11◦. The amorphous system of DCX, which is more soluble than the crystalline form, greatly masked the sharp, identifiable peaks in the pure DCX, according to LPNS research. Furthermore, *Khan et al.* described how an amorphous system of nano matrices enhanced the DCX's weak solubility. Olanzapine without the presence of DCX's distinctive peaks in Figure. 11C., the lyophilized powder [71].

Also, in Figure 11 C. When DCX was loaded inside the nanoliposphere lyophilized powder, the crystalline properties of the loaded DCX changed, resulting in a decrease in the distinctive peaks, indicating that DCX was in an amorphous or disordered crystalline phase. These results were the same by *K. Cheng et al.* by preparing biotin-decorated docetaxel-loaded bovine serum albumin nanoparticles [72].

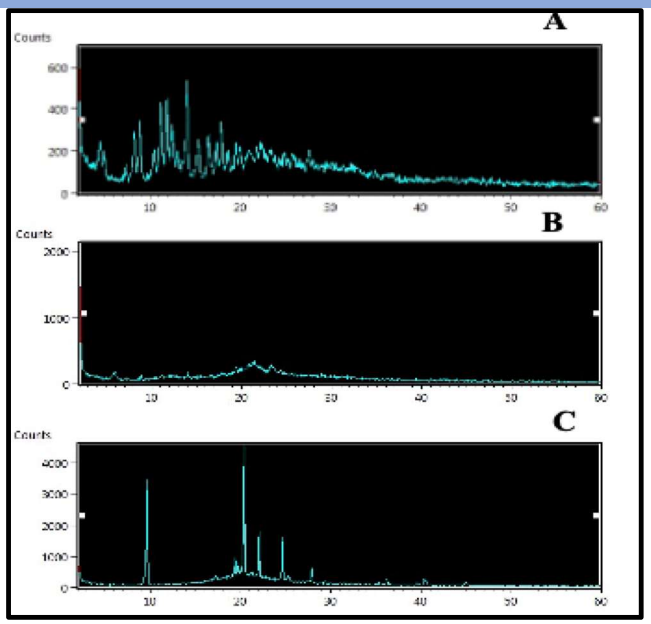


Figure 11. (XRD) A. pure DCX, B. physical mixture F9(DCX, tristearin, hydrogenated soyabean oil, and 90G phospholipid), C. F9 lyophilized powder.

FESEM

The small appropriate quantities of PVA and tween 80 assisted in stabilizing and decreasing interfacial tension by producing stable emulsified oily dispersion and small lipospheres with better nanosized DCX content. These outcomes were the same as seen by *Singh et al.* during the preparation of polar lipid-based lipospheres of peptide drugs [73]; this can be seen in Figure 12

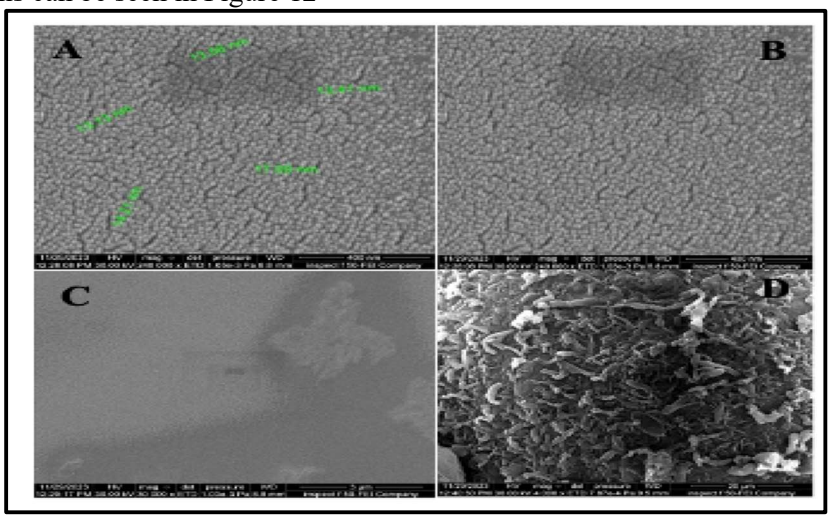


Figure 12. FESEM images of F9 docetaxel LPNS that gave spherical shape particle with different resolutions A.X. 120 000, B. X 240 000 and C. X 30 000 D. docetaxel untreated

Ideal DCX-LPNS film composition

Following the selection of Formula F9, which was the best option, it was mixed with varying concentrations into the PVA matrix. The DCX-loaded LPNS transdermal films ;exhibit the quickest rate of breakdown, releasing roughly 89.84% of the drug from F12 in 24h. Furthermore, DCX-LPNS film dissolved at a rate of 81.9%, 76.92%, and 71.7% for F11, F13, and F114. In the meantime, the untreated DCX offers a 24-hour, roughly 19.2% extended-release rate; This is demonstrated in Figure 13. Additionally based on the data indicated in Table 4 displays the properties all formulas . F12 with 10%(w/v) PVA backbone; was composed of 18ml of PVA

and 18ml of LPNS(w/w) ;has the highest drug content (90.8±0.19) and release profile ; also it is a transparent, flexible, and non-sticky transdermal film; provided the optimal drug characteristics for the transdermal film formulation based on the best medicated Lipo-Nanosphere components formula F9 the same outcomes were observed by *Ankitha Prabhu et al.* ; They used 10% PVA as the patch's basis to create a transdermal patch using curcumin's solid lipid nanoparticle .[74] Even though pure DCX was added to the PVA 10% transdermal film, the DCX release was delayed from 20.14% (16 hours) to 1.33% (20 hours); this could be explained by the fact that DCX's limited mobility within the PVA matrix limited its overall distribution in the release media; *S.M. de Carvalho et al*. also reported similar results when using solid lipid nanoparticles of alpha-tocopherol to create PVA films.[75]Transdermal composite films of chitosan and Tamarind Seed Polysaccharide; have been produced by *Rishabha Malviya et al.* to deliver proteins ;their pH was between 5.7 ± 0.001 and 6.7 ± 0.001, which is lower than the pH range of skin.[76] This result was supported by the outcomes of our experiment ; the surface pH of the films varied from 5.32±0.14 (F13) to 6.7±0.12 (F14).

Table 4. Physical assessment of films containing docetaxel LPNS

Formula no.	Drug content (%)	Thickness (mm)	Variation in weight(mg)	Surface PH	Appearance	Stickiness
F11	91.12 ±88	0.16± 1.45	196± 1.1	6.15 ±0.01	Transparent, brittle	Sticky
F12	90.8± 0.19	0.18 ± 0.3	200± 0.5	5.32 ±0.14	Transparent, flexible	Non-sticky
F13	88 ±0.1798	0.20 ± 0.3	210± 3.6	5.44 ±0.22	Semitranspar ent, tough	Sticky
F14	80 ±0.053	0.19 ± 0.2	205± 4.4	6.7± 0.12	Semitranspar ent, tough	Non-sticky

*** Average ±Standard Deviation (n=3)**

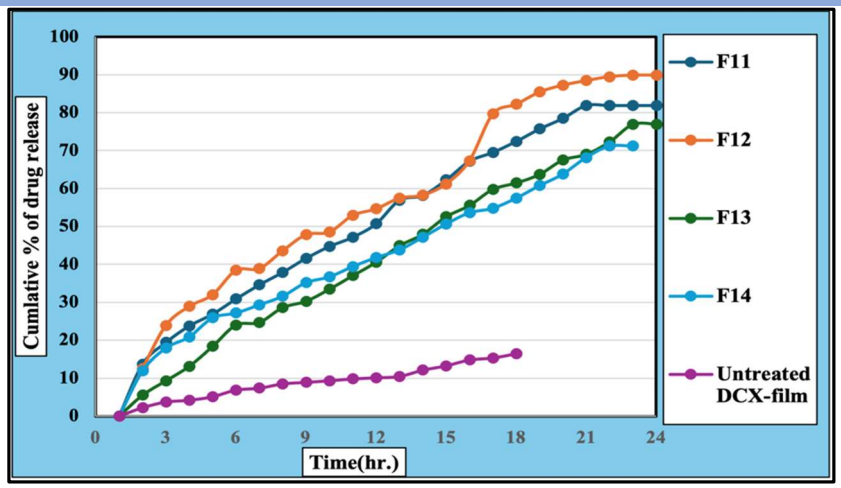


Figure 13. Dissolution profile of LPNS-DXC transdermal film (F11, F12, F13, F14 and Pure drug)

Histological examination and skin irritation

The histological investigation was separated into three groups based on the skin exposure materials; each group was compared to the control group, which consisted of normal dermal cytoarchitecture and epidermal epithelial cells. Groups I, II, and III PBS PH 7.4 , LPNS-film (drug-free), and the medicated optimal formula containing DCX-LPNS stand respectively. In Figure 14; the skin's histological figures were examined , and they showed a normal appearance with normal dermal collagen fibers, normal fibroblasts and fibrocytes, and normal thickness of epidermis epithelial cells. *Lixia Li* et al. observed the same outcomes by preparing a 1,8-cineole nanoemulsion transdermal gel. [77]Also, according to the histological assessment, DCX transdermal film is a safe topical preparation and is in line with the results of the skin irritation test.

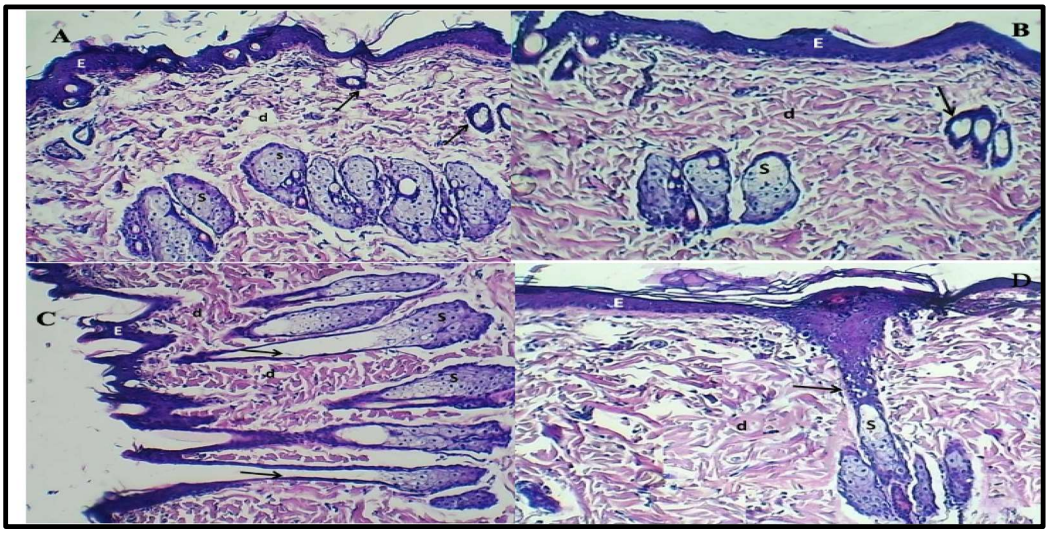


Figure 14. shows the following: A. control Phosphate buffer 7.4 , B. drug-free LPNS transdermal films, C. DCX-LPNS medicated optimum transdermal films; pictures to assess skin histology

Conclusion

Tristearin, DCX, and hydrogenated soybean oil have a high degree of compatibility, which results in the production of an ideal 24-hour sustained-release transdermal film with a high drug content and entrapment efficiency as shown in F12. The encapsulating technique has several advantages including improved physical stability, low ingredient costs, with spherical, nanoscale shapes were formed on a smooth surface using the lipid carrier system, as shown in F9. The duration of the effect of the medicine is a crucial aspect of cancer treatment, and it was made longer with the development of the LPNS transdermal film .While the FTIR, DCS, and XRD measurements showed, DCX transformed into an amorphous form and became trapped inside the LPNS.

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Conflicts of Interest

The authors stated no conflict of interest in the manuscript.

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Ethics Statements

There were no humans or animals used in all experiments.

Author Contribution

The author's responsibilities are described as follows: Preparation, collecting, and analyzing data: Maysam M. Abass. Designing, reviewing, and supervising the project: Shaima Nazar Abd Al Hammed. All authors reviewed the results and approved the final version of the manuscript.

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