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Formulation and Characterization of Flurbiprofen-Loaded Terpesomes

Zainab M. Al-Qaysi[*,1] and Khalid K. Al-Kinani[2]

[1]Ministry of Health, Diyala Health Directorate, Diyala, Iraq.

[2]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

***Corresponding author**

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Abstract

Flurbiprofen is a non-steroidal anti-inflammatory drug (NSAID) prescribed for the alleviation of inflammation and pain related to rheumatoid arthritis and osteoarthritis. However, like all other NSAIDs, it is associated with gastrointestinal discomfort. Also, multiple dosing is required due to its short half-life which results in decreasing patient compliance and adherence when the drug is given orally. Delivering flurbiprofen systemically through other routes is quite an appealing approach to overcome its distressing adverse effects and reduce its dosing frequency. Flurbiprofen was formulated to be carried in a terpene-enriched ultra-deformable liposomal system called "terpesome" utilizing the thin film hydration technique. Limonene, alpha pinene, and cineole are three types of terpenes of different degrees of lipophilicity that were used in the construction of the terpesome along with soybean lecithin and sodium deoxycholate as an edge activator. A 23. 31 full factorial design was used in designing 23 formulas, and the optimized formula was chosen based on the desirability index of minimum vesicle size and PDI, and maximum entrapment efficiency. The optimized formula (comprised of 50 mg of flurbiprofen, 263 mg of soybean lecithin, 100 microliter of cineole, and 0% SDC) was shown to have a vesicular size of 195 nm, PDI of 0.3, and entrapment efficiency of 62%. Entrapment efficiency was higher in cineole containing terpesomes compared to limonene and alpha pinene counterparts most probably due to the hydrophilic nature (log P = 2.8) of cineole, which has low affinity to the phospholipid content in the vesicle causing repulsion between the vesicle content creating an ample space to efficiently house the flurbiprofen within. The in vitro release study of the optimized formula using cellulose dialysis membrane showed that an 88.74% of the drug was released after seven hours in comparison with the flurbiprofen suspension which showed a complete release after 5 hours confirming the role of the terpesomal vesicles in delaying drug release. In conclusion, terpesomes were prepared successfully with optimum properties to be ready for incorporation into an advanced and suitable drug delivery systems for a desirable enhancement of patient compliance and adherence.

Keywords: Cineole, Flurbiprofen, Lecithin, Terpenes, Terpesomes

Introduction

Flurbiprofen is a potent non–steroidal anti-inflammatory analgesic that is used for acute and chronic rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis treatment. It is a Phenyl alkanoic acid derivative drug acting by inhibition of cyclooxygenase enzyme that is in turn inhibits prostaglandin synthesis which is the principal marker appeared in inflammation, pain, swelling, and fever [(1), (2).] Flurbiprofen is available in markets as 50, 100, or 200 mg tablets or capsules [(3)]. It exhibits a short elimination half-life of about 3.9 h which requires the drug to be administered frequently 2 to 3 times daily [(4)]. To avoid the GIT disturbing side effects besides the fact that FP is commonly used for a prolonged period to maintain a proper therapeutic activity requires delivering FP using other feasible routes like a transdermal delivery system [(5)].Nanovesicles are colloidal carrier systems composed of phospholipids or surfactants that encapsulate an aqueous phase, resulting in the formation of one or more lipid bilayers. These vesicles encapsulate both hydrophilic and lipophilic medications within the aqueous compartment or lipid bilayer, so mitigating drug toxicity by directing the drugs to the specific site of action, which consequently reduces their concentration in other areas of the body [(7).] Moreover, drug trapping within vesicles prolongs the drug's duration in systemic circulation[(8)]. Liposomes and niosomes, the first generation of nanovesicles, were initially discovered but exhibited inadequate physical stability, resulting in drug leakage, aggregation, and fusion, hence constraining their utility and efficacy [(9)]. To address this issue, several recent vesicular systems have utilized terpenes [(10)].

Terpenes are natural chemicals that demonstrate significant improvement of skin penetration [(11)]. They have been employed in the creation of an ultra-deformable vesicular system known as "Terpesomes". Terpesomes comprise

phospholipid(s), ethanol, and one or more terpenes. The phospholipid component forms the bilayer matrix of the nanovesicle, while the embedded ethanol and terpenes enhance the fluidity of the vesicular wall [12, 13]. Numerous pharmaceuticals have been effectively administered via the aforementioned nanovesicle, including zolmitriptan through the transdermal route [12], the antifungal fenticonazole via vaginal and ocular applications [14, 15], and moxifloxacin ophthalmically [16]. Flurbiprofen possesses a molecular weight of 244.26 Daltons, and its log P (octanol/water, pH 7.4) is 3.80. It is primarily processed in the liver, rendering it an appropriate option for alternative delivery methods, including the transdermal route [17]. In this study, we utilize the possibility of delivering flurbiprofen using the Nano vesicular approach through the formulation of Flurbiprofen -loaded terpesome. Three types of terpenes (limonene, cineole and alpha pinene) with different degree of lipophilicity were used to construct a proper deformable vesicle for safe and effective drug delivery.

Materials and Methods

Materials

Flurbiprofen (Energy chemicals , China), Soybean phosphatidylcholine(Shenyang Tianfeng Biological and Pharmaceutical Company , China), Limonene , Alpha pinene, Cineole(Hangzhou® Hyper Chemicals Limited, Zhejiang, China) , Amicon® Ultra Centrifugal tube 10kDa(millipore , Ireland) , Dialysis membrane MWCO 8000-12000(MYM Biological technology , China), Sodium hydroxide , Dihydro potassium phosphate, Methanol ,Ethanol ,Chloroform analytical grade (Alpha® chemika, India), Additional analytical instruments were provided by the College of Pharmacy/ University of Baghdad for the conduction of this study.

Methods

Characterization of drug substance (Flurbiprofen)

Saturated solubility Flurbiprofen saturated solubility was determined in water, phosphate buffer saline (PBS) PH 7.4 at 25 and 37 °C using a shaking water bath for 3 days. The solution was filtered through syringe filter 0.22 micrometer diluted properly with PBS and assessed spectrophotometerically at 246 [18].

Experimental design

To study the impact of soybean lecithin, sodium deoxycholate (SDC), terpene type, and concentration, a $2^3.3^1$ full factorial design utilizing Design-Expert software® (version 13.0.5.0, Stat Ease Inc., Minneapolis, MN, USA) was used to design 23 formulas as shown in Tables 1 and 2. Vesicle size, PDI, and entrapment efficiency were set as an experimental response. The full factorial design generates experimental points by including all conceivable combinations of the levels of the variables in every independent trial or replication of the experiments [19]. Factorial designs enable the assessment of several interaction components with favorable statistical power and provide the chance to identify interactions among intervention components [20].

Table 1. Full factorial design used for flurbiprofen loaded terpesomes optimization

Factor (Independent Variable)	**Type**	**Level**		
		-1		**1**
A: Soybean lecithin amount (mg)	Numerical	250		500
B: SDC amount (mg)	Numerical	0		10
C: Terpene amount (%)	Numerical	1%		2%
D: Terpene type	Categorical	cineole	Alpha pinene	limonene
Response (Dependent Variable)	**Desirability Constraints**			
Vesicle size (nm)	195 nm			
PDI	0.3			
Entrapment efficiency (EE%)	70%			

Terpesomes preparation method

Flurbiprofen terpesomal formulations were developed by thin film hydration approach (Table 2). Soybean lecithin, terpene, SDC, and Flurbiprofen were thoroughly dissolved in a 12 ml solution with a 2:1 ratio of methanol and chloroform. Using the (Buchi R-110 Rota vapor, Switzerland) rotary evaporator, the solvent combination was evaporated under decreased pressure at 60 °C, 90 rpm for 15 minutes, resulting in the formation of a thin dry layer. The desiccated lipid film was moistened with 10 ml of a 30% ethanolic phosphate buffer solution (PBS) with a pH of 7.4. It was then subjected to rotation in a water bath at 60 °C under normal room pressure for 1 hour to guarantee thorough treatment of the film. Following a 2-hour period of rest at room temperature, the resulting dispersion of terpesomes underwent probe sonication using a probe sonicator (Qsonica, USA) at an 80-watt amplitude for 1 minute (2 seconds on/off). The dispersion was then stored overnight at 4 °C for maturation [12, 20].

Table 2. Prepared flurbiprofen-loaded terpesomes formulations

Formula Code	Soybean lecithin (mg)	SDC(mg)	Terpene amount (µL)	Terpene Type
F1	250	0	100	Cineole
F2	500	0	100	Cineole
F3	500	10	100	Cineole
F4	250	0	200	Cineole
F5	500	0	200	Cineole
F6	250	10	200	Cineole
F7	500	10	200	Cineole
F8	250	0	100	Alpha pinene
F9	500	0	100	Alpha pinene
F10	250	10	100	Alpha pinene
F11	500	10	100	Alpha pinene
F12	250	0	200	Alpha pinene
F13	500	0	200	Alpha pinene
F14	250	10	200	Alpha pinene
F15	500	10	200	Alpha pinene
F16	250	0	100	Limonene
F17	500	0	100	Limonene
F18	250	10	100	Limonene
F19	500	10	100	Limonene
F20	250	0	200	Limonene
F21	500	0	200	Limonene
F22	250	10	200	Limonene
F23	500	10	200	Limonene

In vitro characterization of the prepared terpesomal dispersion

Vesicle size and polydispersity index

The prepared vesicles were evaluated for their size and PDI through photon correlation spectroscopy via the Malvern Zeta sizer (Malvern, UK). A volume of 1 ml of the terpesomal dispersion loaded with Flurbiprofen was diluted with deionized water and then tested using a quartz cuvette employing dynamic light scattering at a temperature of 25 ± 1 °C and a scattered back angle of 175 °C. All measurements were conducted in triplicate [10].

Entrapment efficiency

The entrapment efficiency of the terpesomal dispersion was evaluated using the indirect approach. A quantification of free Flurbiprofen was determined by an ultrafiltration method. A volume of 2 ml amount of terpesomal dispersion loaded with Flurbiprofen was pipetted into an Amicon® Ultra Centrifugal tube with a molecular cutoff size (MWCO) of 10 kDa.

The tube was then centrifuged at room temperature for 30 minutes at 5000 rpm. The concentration of the free and the entrapped Flurbiprofen were measured using spectrophotometry at a wavelength of 246 nm, after appropriate dilution with ethanol. The EE% was determined using the following equation:

$$\mathbf{EE\%} = \mathbf{WT - WF / WT} \times \mathbf{100}$$

Where WT represents the weight of the total drug originally utilized, and WF represents the weight of the free drug computed in the supernatant layer following ultrafiltration of the terpesomal dispersion. The measurements were performed in triplicate, and the values were the mean ± SD [22].

Optimization of Flurbiprofen loaded terpesomes using $2^3.3^1$ full factorial design

By employing Design-Expert software, four independent factors were studied. Soybean lecithin amount, terpene amount, terpene type, and the addition of SDC. The responses were vehicle size, PDI, and EE%. The desirability index of vesicle size was set at 195 nm, PDI was set at 0.3, and EE% was set at 70% (Table 1). For the optimal Flurbiprofen-loaded terpesome selection, statistical analysis of data was conducted using one-way ANOVA at a significance level of $P<0.05$.

Zeta potential

A volume of 1 ml of the optimized flurbiprofen loaded terpesomal dispersion was diluted and examined for evaluating the zeta potential in triplicates using Malvern Zetasizer instrument (Malvern, UK) [21], [23].

Transmission electron microscope

An analysis of the optimized Flurbiprofen terpesomal formula was conducted using transmission electron microscopy (TEM). Following appropriate dilution and centrifugation, one drop of the optimized terpesomal dispersion was

applied onto a carbon coated copper grid. The grid was then stained with uranyl acetate (2% w/v) for 5 minutes, allowed to dry, and then inspected at 80 kV using a Joel JEM 2100 transmission electron microscope (Tokyo, Japan) [24].

IR spectroscopy

A compatibility and chemical interaction analysis of Flurbiprofen, soybean lecithin, cineole, and the selected optimal FBP loaded terpesomal was conducted using FTIR (8400s, Shimadzu, Japan). The samples were treated with KBr before examination within the spectral range of 4000 to 400 cm^{-1} [25].

Lyophilization of the optimized flurbiprofen formula

In the preparation for thermal examination, the terpesomal dispersion was lyophilized using (Christ Alpha 1-2 LD Plus, Germany). The specimen was subjected to overnight freeze-drying under reduced pressure. The primary drying pressure was 0.021 mbar at a temperature of -50 °C, followed by a final drying pressure of 6.1 mbar at a temperature of 20 °C [26].

Differential scanning calorimetry (DSC)

Thermal analysis instrument (DSC-60 Shimadzu, Japan) was used to quantify the thermograms of Flurbiprofen and the lyophilized optimized Flurbiprofen-loaded terpesomes in order to examine the interaction between Flurbiprofen and the other terpesome components. The tests were done by taking the Flurbiprofen and the lyophilized powder and introduced it in a typical aluminum pan. Then the temperature elevated by 10 °C/min in a range of 50 °C up to 300 °C [22].

In-vitro flurbiprofen release study

A diffusion approach using a dialysis membrane was used to determine the release profile of the optimal Flurbiprofen - loaded terpesomal formulation. A volume of 10 ml of the dispersion, which contained 50 mg of Flurbiprofen, was introduced into the cellulose dialysis bag that had been soaked in PBS for 24 hours. This bag was then firmly affixed to a rod secure to a clamp. The rod was then placed in 500 ml of PBS with a pH of 7.4 in a beaker positioned above a hot plate magnetic stirrer. The temperature was set at 37±0.5 °C and the rotation was set at 50 rpm. A volume of 1 ml was extracted at defined time intervals of 0.5, 1, 2, 3, 4,5, 6 and 7 hours and replaced by 1 ml of fresh PBS to maintain the sink condition. The samples were examined using ultra violet spectroscopy at a wavelength of 246 nm. Flurbiprofen suspension was subjected to in-vitro release test for comparison. The experiment was conducted in triplicate [10].

Release Kinetic Modelling

Utilizing the DD solver and Microsoft Excel 2016 software, we fitted the in vitro release data of the Flurbiprofen - loaded terpesomal formulations to various mathematical equations in order to ascertain the kinetics and release mechanisms of Flurbiprofen from the formulations. The kinetic models used were: zero order release kinetic model (cumulative percentage drug release vs. time), first order release kinetic model (Log cumulative percentage drug retained vs. time), Higuchi release kinetic model (cumulative percentage drug release vs. cubic root of time), and finally Korsmeyer-Peppas release kinetic model (Log cumulative percentage drug release vs. Log time), model with the highest correlation coefficient to be selected as the best fitted model [23], [27].

The Thermodynamic Stability studies

The stability of the terpesomal vesicles obtained was evaluated by subjecting the improved formula to an accelerated thermodynamic stability investigation. The first stage involved the cooling and heating cycle. The formula was cooled at 4°C and then exposed to a heating cycle at 40°C in the oven for 48 hours. Following centrifugation at 3500 rpm for 15 minutes, freezing at 4 °C for 24 hours, and thawing at ambient temperature, the formula reverted to its original state after 1 minute. Evaluated parameters included phase separation, turbidity, vesicle size, PDI, Zeta potential, and EE% [28], [29].

Statistical analysis

The experimental findings are presented in three sets, each accompanied by its respective standard deviations. Statistical analysis of the data was performed using one-way ANOVA. A significance level of $p < 0.05$ was used to determine significant statistical differences.

Results and Discussion

Experimental design

Terpesomes are ultra-deformable nano vesicular delivery system, showed a great ability to encapsulate both lipophilic and hydrophilic drugs were prepared by utilizing the thin film hydration. The statistical significance of four independent variables was evaluated using the Design – Expert algorithm. The study examined three numerical variables: soybean lecithin amount, SDC amount, and terpene amount, as well as one categorical variable, terpene type. Twenty-three Flurbiprofen - loaded terpesomes were successfully developed using Flurbiprofen (50 mg), Soybean lecithin (250 or 500 mg), Terpene (alpha pinene, limonene, or cineole; 1% or 2%, w/v), SDC (0% or 0.1%, w/v) and ethanol (30%) as shown in Table 3.

Table 3. The characterization of the prepared flurbiprofen terpesomal formulas

Formula Code	Vesicle size (nm)	PDI	Entrapment efficiency EE%
F1	171.6 ± 18.7	0.3 ± 0.02	58.06 ± 4.8
F2	126.6 ± 4.09	0.3 ± 0.02	83.1 ± 1.9
F3	266.9 ± 51.54	0.59 ± 0.02	69.2 ± 0.9
F4	294.2 ± 38.7	0.24 ± 0.01	49 ± 1.5
F5	280 ± 1.2	0.6 ± 0.01	90.13 ± 5.3
F6	228.06 ± 26.19	0.29 ± 0.018	86.24 ± 2.6
F7	433.4 ± 191.03	0.64 ± 0.07	76.3 ± 1.05
F8	86.9 ± 7.42	0.2 ± 0	70.46 ± 2.15
F9	94.8 ± 4.4	0.32 ± 0	59.9 ± 3.2
F10	106 ± 1.3	0.24 ± 0.01	70.4 ± 2.15
F11	201.4 ± 12.9	0.5 ± 0.01	89.8 ± 3.1
F12	92.2 ± 4.9	0.17 ±0.014	35.48 ± 1.5
F13	125.83 ± 24.4	0.35 ± 0.01	61.11 ± 1.5
F14	107.9 ± 5.3	0.22 ± 0.007	30.1 ± 2.5
F15	132.26 ± 9.05	0.35 ± 0	47.7 ± 6.45
F16	99.8 ± 5.3	0.19 ± 0.007	41.9 ± 2.15
F17	170.3 ± 11.5	0.44 ± 0.02	58.59 ± 4.5
F18	87.2 ± 4.2	0.17 ± 0.015	24.51 ± 1.67
F19	129.2 ± 13	0.27 ± 0.04	47.37 ± 5.9
F20	191.3 ± 10.38	0.29 ± 0.014	26.53 ± 3.6
F21	204.7 ±12.26	0.51 ± 0	48.8 ± 2.4
F22	148.06 ± 38.2	0.24 ± 0.014	35.07 ± 1.5
F23	233.3 ± 16.6	0.43 ± 0.06	38.3 ± 1.5

In-vitro characterization of Flurbiprofen-loaded terpesome

Vesicle size, polydispersity index (PDI)

The derived Flurbiprofen -loaded terpesomes exhibited vesicle sizes ranging from 86.9 nm to 433.4 nm and PDI values ranging from 0.17 to 0.64 as reported in Table 3. The vesicle size was strongly predicted by the type of terpene (p = 0.0003) and the quantity of terpene (p = 0.0118), but not by the amount of soybean lecithin and SDC. Concerning the impact of terpene type on vesicle size, there was an inverse relationship between the vesicle size of the terpesomes produced and the lipophilicity of the terpenes. The vesicles derived from cineole exhibited vastly larger vesicle size in comparison to those derived from alpha-pinene and limonene (log P = 2.82, 4, and 4.83, respectively). The observed phenomena can be ascribed to the repulsive contact produced by the lipophilic characteristics of terpesomes and the hydrophilic characteristics of cineole [12]. Besides the fact that cineole molecular weight is higher than limonene and alpha pinene (154.24, 136.24, 136.24 Da, respectively) which contributed to the larger cineole loaded vesicles [30]. Analysis revealed that the dimensions of the vesicles grew proportionally with the rise in terpene content from 1% w/v to 2% w/v, in line with the findings documented by Dragicevic-Curic et al [31]. While increasing the soybean lecithin amount showed no significant increment of vesicle size (p=0.0881) which may be due to the effect of probe sonication. The same went for the SDC addition or exclusion which showed no significant influence (p=0.1426) as shown in Figure 1.PDI is a quantitative metric that quantifies the homogeneity of a sample in relation to the size of its particles. The value of this parameter ranges from 0.0, indicating a sample that is completely uniform, to 1.0, suggesting a sample that is extremely polydisperse with different populations of particle sizes. Generally, values of 0.2 or lower are deemed acceptable in practical applications for nanoparticle materials based on polymers. Pharmaceutical formulations containing lipid-based carriers, such liposomes, are deemed appropriate for drug delivery applications when the PDI value is 0.3 or lower. The observed outcome suggests a uniform dispersion of phospholipid vesicles. The documented PDI values fell within the range of 0.17 to 0.64, as shown in Table 3. PDI values indicate a consistent distribution of vesicle sizes. The statistical investigation showed that the percentage of soybean lecithin (p = 0.0001) and the terpene type (p = 0.0138) had a statistically significant impact on the PDI value. There is a negative correlation between the concentration of soybean lecithin and the uniformity of the dispersion [32]. While the limonene-loaded terpesomes showed the lowest PDI, followed by alpha-pinene and cineole, this may be due to a surfactant effect leading to stabilizing the dispersion as shown in Figure 1.

Flurbiprofen Entrapment efficiency (EE%)

The EE% of the prepared Flurbiprofen terpesomes varied from 24.51 % to 90.13 % as mentioned in Table 3. By generating cohesive layers around the terpesomes, the surfactant characteristics of lecithin may promote drug trapping and limit drug leakage, resulting in a significant increase in Flurbiprofen EE% ($p = 0.006$) with an increase in lecithin concentration [16]. Furthermore, the augmentation of lecithin concentration leads to an elevation in the viscosity of the dispersion, so reducing the external diffusion of the drug [33]. One important role of ethanol in increasing the EE% of Flurbiprofen is its capacity to increase the solubility of Flurbiprofen. ANOVA showed a significant influence of terpene type on the Flurbiprofen EE%. EE% of cineole-containing terpesomes was considerably higher than that of terpesomal vesicles containing alpha-pinene and limonene ($p = 0.003$). Among the terpenes tested, cineole (the most hydrophilic; log P = 2.82) exhibited the highest entrapment, followed by alpha-pinene (log P = 4) and limonene (the most lipophilic among the used terpenes; log P = 4.83) [34]. The hydrophilic character (log p = 2) of cineole, which has a poor affinity for the phospholipid content in the vesicle, leads to repulsion between the vesicle contents. This creates a large area to effectively accommodate the Flurbiprofen within the vesicle [12]. Higher terpene concentration on the contrast reduces the EE%. Dragicevic-Curic et al. proposed that an increase in terpene concentration has a direct correlation with increased fluidity of terpesomes, resulting in a decrease in the EE% [31]. Furthermore, the observed phenomenon could be attributed to the development of pores and the destabilization of the lipid bilayer in terpesomes with higher terpene concentration, as proposed by Albash et al [15]. SDC addition or exclusion contributes to no significant change in the EE% according to ANOVA ($p = 0.7209$) as shown in Figure 1.

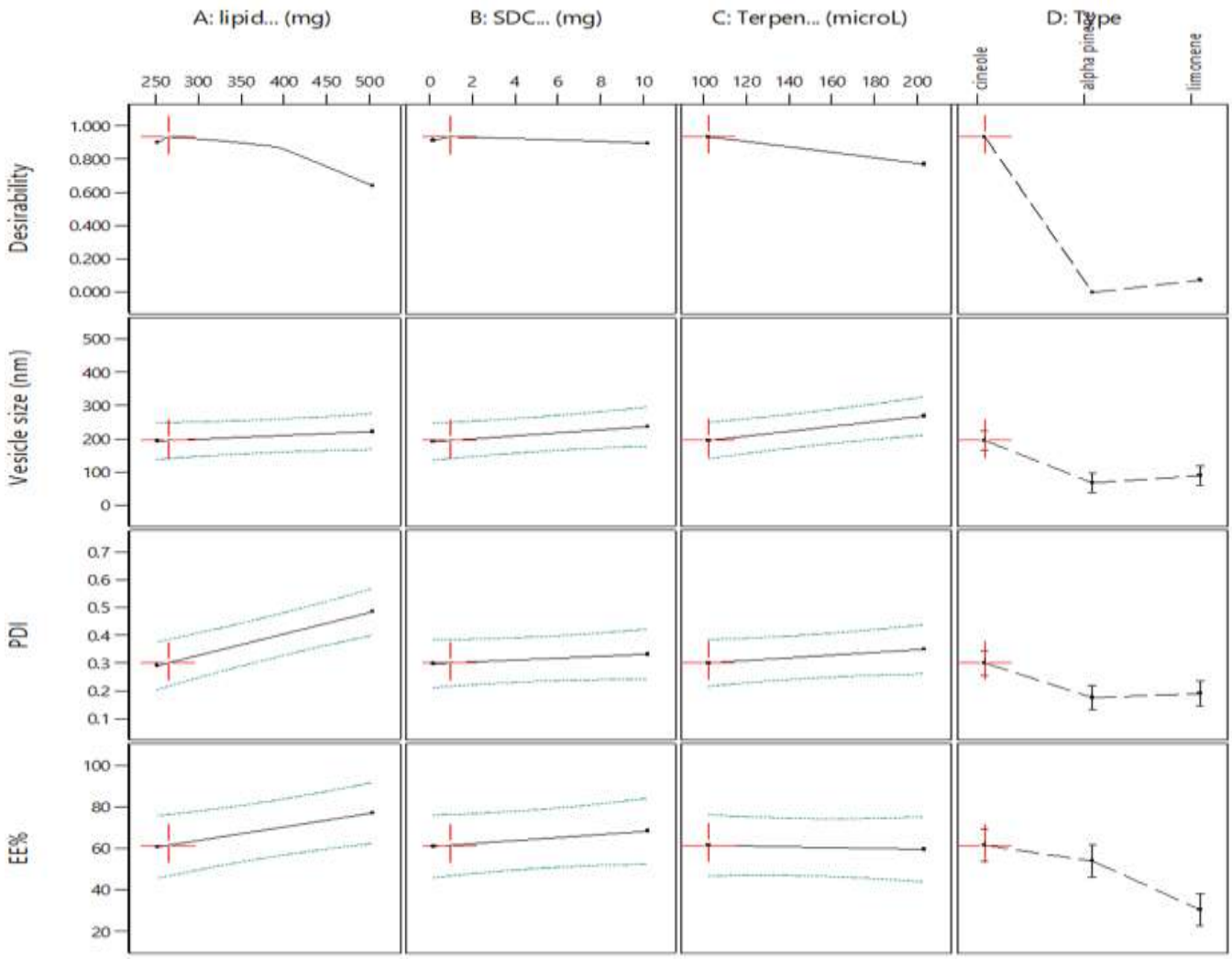


Figure 1. Formulation variables influence on the vesicle size, PDI and EE% of Flurbiprofen-loaded terpesomes

Point prediction optimization

The optimum formula was chosen from the point prediction data generated by the Design Expert software. The optimum formulation consists of 263 mg of soybean lecithin, in which 1% cineole without SDC is employed as the terpene. The predicted values for the responses were vesicle size = 195.49 nm, EE% = 62.9%, and PDI = 0.299. The measured values for the responses were as follows: vesicle size = 193.13 ± 5.2 nm, EE% = 63.23 ± 1.85%, and PDI= 0.32 ± 0.06. The optimized formulation achieved a desirability of 0.945, therefore confirming the validity and robustness of the model as shown in Figure 2.

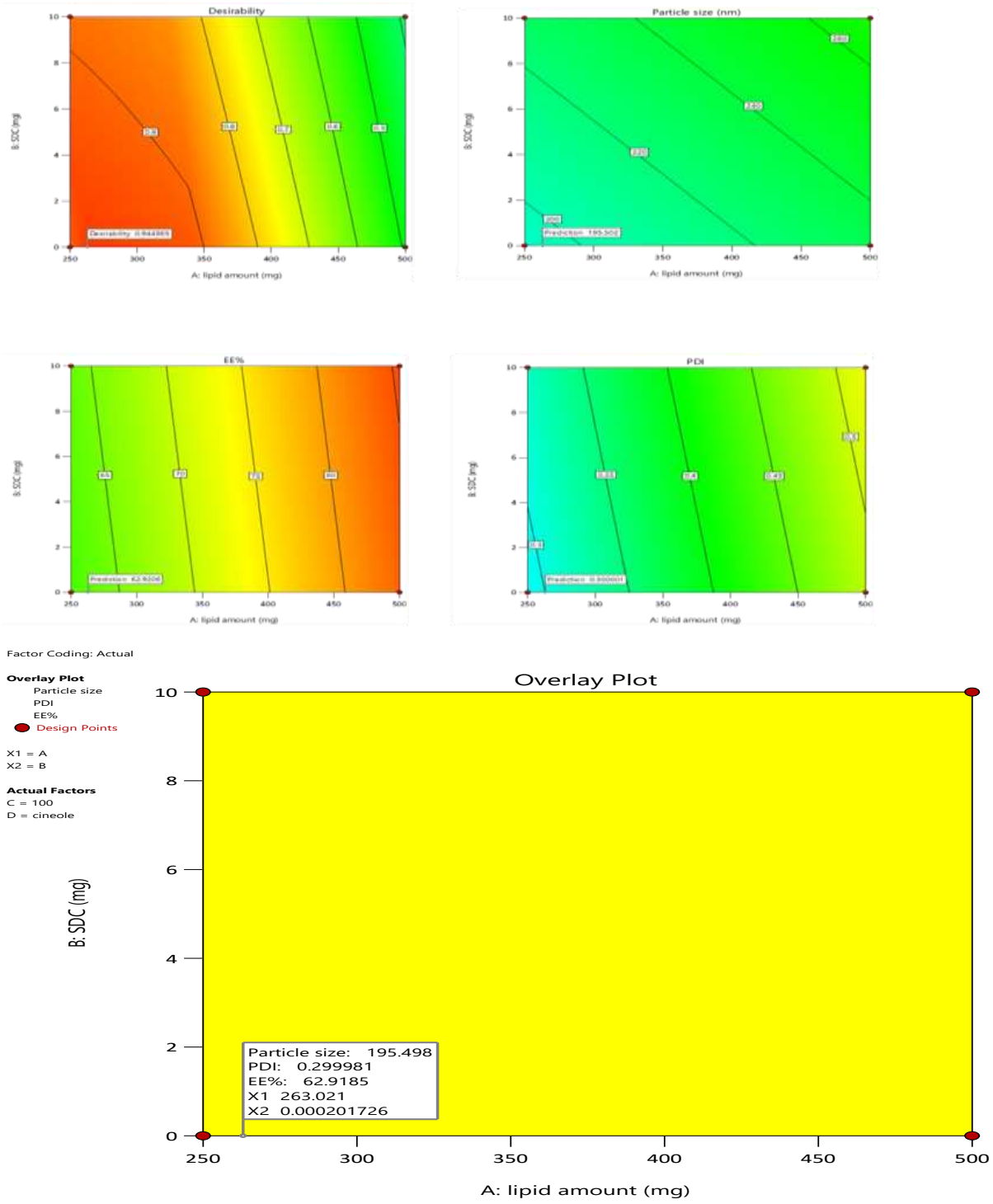


Figure 2. The contour and overlay response surface plots representing the predicted and observed values for the optimized flurbiprofen- loaded terpesomes

Zeta potential

Zeta potential is an important indicator of the repulsion and attraction forces between vesicles, giving an idea about terpesomal dispersion stability The optimum flurbiprofen- loaded terpesomes zeta potential was measured to be -22.26 mV.

Transmission electron microscopy (TEM)

The images taken with TEM (Figure 3) showed the formation of spherical vesicles with clear and definitive margins without any vesicle aggregation. The vesicles measurement in the micrograph was in accordance with the vesicle size measurements provided by the Malvern zeta sizer.

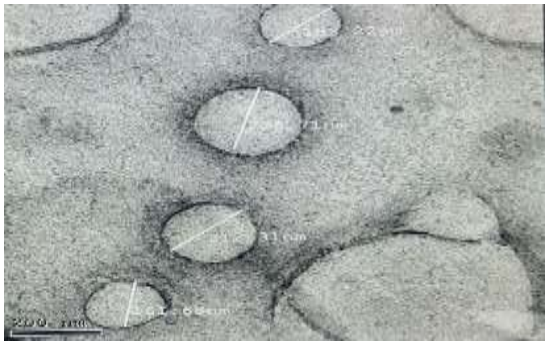
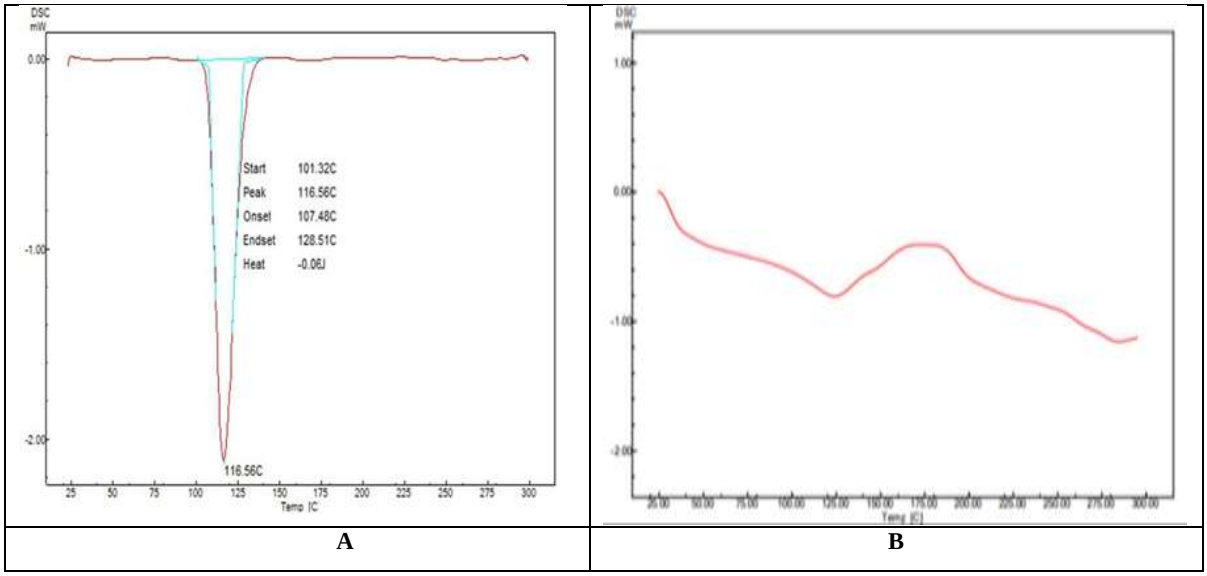


Figure 3. TEM micrograph for the optimized cineole based flurbiprofen terpesomes

Differential scanning calorimetry (DSC)

As illustrated in Figure 4 the Flurbiprofen thermogram exhibited an endothermic peak at 116.56 °C, which aligns with its melting point as measured using the capillary tube method which was at 114 °C both values where in correspondence with Flurbiprofen melting point mentioned in the British pharmacopeia ranged from 114-118 ° C [35]. These results proved the drug purity and crystallinity. The lyophilized Flurbiprofen loaded terpesomes thermogram displayed the no peaks which indicates the drug encapsulation within the vesicle and complete transformation into the amorphous form the same results were observed by Teaima et al with complete disappearance of propranolol endothermic peak due to inclusion within the invasome [36].



A **B**

Figure 4. (A) Pure flurbiprofen thermogram (B) Optimized flurbiprofen loaded terpesome thermogram

FTIR

The FTIR spectra of Flurbiprofen, Soybean lecithin, cineole and the optimized Flurbiprofen loaded terpesome were displayed in Figure 5 respectively. The FTIR spectra of the pure drug exhibits distinct absorption peaks for the carbonyl stretching band at 1701 cm^{-1} and the C–F stretching peak at 1217 cm^{-1}. The bands identified at 1620, 1579, 1512, and 1481 cm^{-1} correspond to the stretching modes of biphenyl rings. The bands detected within the range of 3120–3030 cm^{-1} correspond to the stretching vibration of the C–H bond. The FTIR spectra of lecithin exhibited peaks at 2922 and 2850 cm^{-1} of the CH2 stretching vibration, 1734 cm^{-1} corresponding to the symmetrical C=O stretching vibration, and 1246 cm^{-1} corresponding to the PO_4 antisymmetric stretching bands. An FTIR analysis of cineole reveals a prominent absorption at 985 cm^{-1}, which is attributed to the symmetrical

bending out of the CH2 plane. The bands observed at 1080 cm^{-1} and 1215 cm^{-1} correspond to symmetric and asymmetric stretches of the C–O–C group, respectively. An additional distinctive band, associated with the deformation of CH3, may be observed at 1374 cm^{-1}. The optimized terpesome loaded with Flurbiprofen exhibits peaks at 3415, 2926, 2854, 1258, and 1085 cm^{-1}, which are comparable to the lecithin peaks located at 985 cm^{-1} for cineole and 1637, 1458 cm^{-1} for Flurbiprofen which indicates compatibilities between the terpesome components.

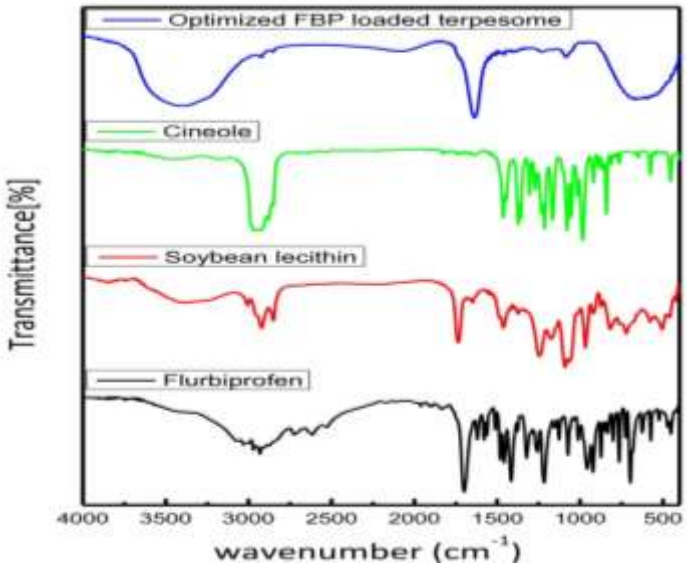
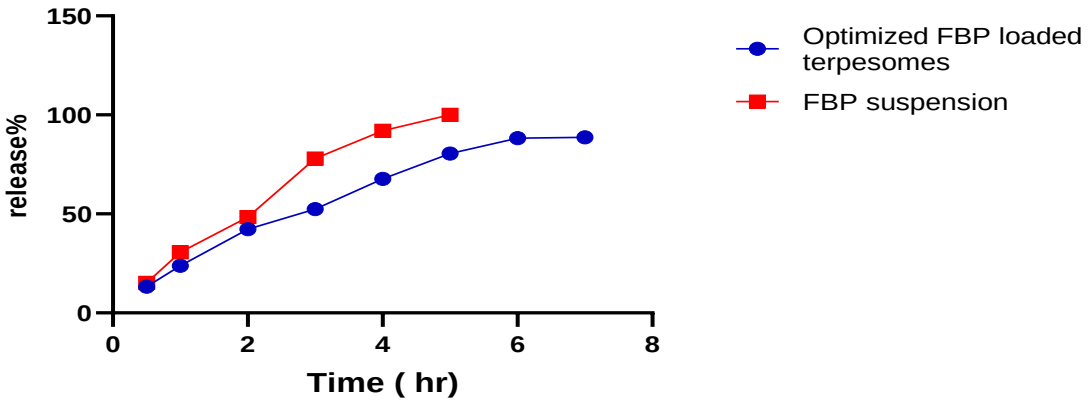


Figure 5. FTIR spectrum for Flurbiprofen, Soybean lecithin, Cineole and the optimized Flurbiprofen loaded terpesome

In vitro release studies

The in vitro drug release profile of the optimized Flurbiprofen loaded terpesomes compared to Flurbiprofen suspension is shown in Figure 6. The flurbiprofen suspension showed complete release (100%) after 5 h. while the optimized Flurbiprofen loaded terpesomal formula released 80.55% after 5 hours followed by 8% increment in the next 2 hours (88.22% and 88.74%). This can be justified as the terpesome like any other colloidal vesicles systems act as a drug reservoir releasing its cargo in a sustained fashion, similar results were obtained by Tawfik et al [12].

Figure 6. In - vitro release profile of the optimized



Flurbiprofen loaded terpesome Vs Flurbiprofen suspension in PBS

Release kinetic modelling

Diverse mathematical models were employed to simulate the release of Flurbiprofen from the optimized formulation. Table 4 presents the values of release constants and regression coefficients. The most precise release model is identified by the highest R2 values. The table indicates that the Korsmeyer-Peppas model has the highest R2 value. Consequently, it was identified as the most appropriate model for elucidating the

process of Flurbiprofen release from the optimized terpesomal vesicles. An exponent value of n, beyond 0.5, signifies that the drug transport mechanism is defined by non-Fickian anomalous transport. This indicates that both diffusion and erosion contribute to the drug release mechanism. Comparable outcomes for the exponent values were demonstrated for the release of candesartan from invasomes [21]. The Flurbiprofen suspension, which is expected to adhere to first-order kinetics, has demonstrated adherence to the Korsmeyer-Peppas model, despite the absence of any vesicular system from which the drug may be eroded, highlighting the limitations of the release kinetics model. Numerous models employ oversimplified settings that fail to adequately reflect the true circumstances of drug release, potentially resulting in inaccuracies in estimating the release profile [37].

Table 4. Release kinetic modelling for optimized Flurbiprofen loaded terpesome and the Flurbiprofen suspension

Formula	Zero order		First order		Higushi model		Korsmeyer–Peppas model		
	K_0	R^2	K1	R^2	K_H	R^2	K_{KP}	n	R^2
Optimized Flurbiprofen Terpesomes	0.149	0.885	0.002	0.886	0.333	0.944	0.259	0.668	0.984
Flurbiprofen suspension	0.224	0.939	0.002	0.940	0.421	0.901	0.308	0.762	0.982

The Thermodynamic stability study

The optimized flurbiprofen-loaded terpesomal dispersion successfully underwent centrifugation, freeze-thaw cycles, and heating-cooling cycles without exhibiting any obvious turbidity or phase separation. The vesicle size, PDI, Zeta potential, and EE% remained consistent throughout all phases of dispersion, indicating enhanced homogeneity and stability of the overall system.

Conclusion

In the current study, flurbiprofen-loaded terpesome with optimum properties for delivering flurbiprofen systemically through the transdermal route was prepared successfully. The terpesomal system was prepared with optimum properties with optimum vesicle size, PDI and zeta potential to cross through skin and maintain a good stability under various stress conditions without significant change in its physical form showing to sings of phase separation along with proper entrapment efficiency of flurbiprofen within the vesicle structure (62%). The in vitro release profile showed a proper release over the course of 7 hours reaching 88.74% and following the Korsmeyer-Peppas model. The optimized terpesomal dispersion is to be included into a proper and suitable future transdermal dosage form to be delivered safely to the patient enhancing their compliance.

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Conflicts of Interest

The authors assert that they own no conflicts of interest associated with this study.

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Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Zainab M. Al-Qaysi, Khalid K. Al-Kinani; data collection: Zainab M. Al-Qaysi; analysis and interpretation of results: Zainab M. Al-Qaysi, Khalid K. Al-Kinani; draft manuscript preparation: Zainab M. Al-Qaysi, Khalid K. Al-Kinani . All authors reviewed the results and approved the final version of the manuscript.

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تحضير و تقييم التربيسومات المحملة بالفلوربايبروفين

زينب محمد القيسي*[1،] و خالد كاظم الكناني[2]

[1]وزارة الصحة العراقية، دائرة صحة ديالى، ديالى، العراق.

[2]فرع الصيدلانيات، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

الخلاصة

فلوربيبروفين هو دواء مضاد للالتهابات غير الستيرويدية يُستخدم لتخفيف الألم والالتهاب المرتبط بالتهاب المفاصل الروماتويدي والتهاب المفاصل العظمي. ومع ذلك، مثل جميع مضادات الالتهاب غير الستيرويدية الأخرى، يرتبط هذا الدواء بمشاكل الجهاز الهضمي. بالإضافة إلى ذلك، يتطلب تناول جرعات متعددة بسبب فترة نصف عمره القصيرة داخل الجسم، مما يؤدي إلى انخفاض التزام المريض واستمراره في تناول الدواء عند إعطائه عن طريق الفم. يعد توصيل الفلوربيبروفين عبر طرق ايصال دوائية اخرى غير الفموية نهجًا جذابًا للتغلب على آثاره الجانبية المزعجة وتقليل تكرار الجرعات. تمت صياغة الفلوربيبروفين ليتم تحميله في نظام ليبوسومي فائق التشكل وغني بالتربينات يُسمى "التيربيسوم" باستخدام تقنية ترطيب الفيلم الرقيق. تم استخدام ثلاثة أنواع من التربينات ذات درجات مختلفة من الليبوفيلية في تركيب التيربيسوم وهي الليمونين، والألفا بينين، والسينول، إلى جانب ليسيثين الصويا ودي أوكسيكولات الصوديوم كعامل مساعد. تم استخدام تصميم تجريبي كامل بعاملين بمستويين ($3^{1}.2^{3}$) لتصميم ٢٣ تركيبة، وتم اختيار الصيغة المثلى بناءً على مؤشر الرغبة الذي يهدف إلى الحد الأدنى لحجم الحويصلة، وانخفاض مؤشر التشتت وأقصى كفاءة احتجاز داخل الحويصلة. تتكون الصيغة المثلى من ٥٠ ملغ من الفلوربيبروفين، و٢٦٣ ملغ من ليسيثين الصويا، و١٠٠ ميكرولتر من السينول، و٠٪ من دي أوكسيكولات الصوديوم. أظهرت هذه الصيغة حجم حويصلة يبلغ ١٩٥ نانومتر، ومؤشر التشتت ٠,٣، وكفاءة احتجاز ٦٢٪. كانت كفاءة الاحتجاز أعلى في التيربيسومات التي تحتوي على السينول مقارنةً بالليمونين والألفا بينين، وذلك بسبب الطبيعة المحبة للماء للسينولمعامل تقسيم = ٢,٨)، الذي لديه توافق منخفض مع محتوى الفسفوليبيد في الحويصلة مما يسبب تنافرًا بين مكونات الحويصلة ويخلق مساحة كافية لاحتجاز الفلوربيبروفين بكفاءة. أظهرت دراسة الإفراج في المختبر للصيغة المثلى باستخدام غشاء السليلوز أن ٨٨٪ من الدواء تم إفرازه بعد ثماني ساعات مقارنة بمعلق الفلوربيبروفين الذي أفرز أكثر من ٩٠٪ بعد أربع ساعات، مما يؤكد دور الحويصلات التيربيسومية في تأخير إفراز الدواء. في الختام، تم تحضير التيربيسومات بنجاح بخصائص مثلى لتكون جاهزة للإدماج في أنظمة توصيل دوائية مناسبة لتعزيز امتثال المريض واستمراره في تناول الدواء.

الكلمات المفتاحية: الفلوربايبروفين , التربينات , التربيسومات ,سينول , لثسين.