International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 3, 2023

Original Article

DIFLUNISAL TRANSETHOSOMES FOR TRANSDERMAL DELIVERY: FORMULATION AND CHARACTERIZATION

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Received: 27 Feb 2023, Revised and Accepted: 06 Apr 2023

ABSTRACT

Objective: The work aimed to obtain an optimum formula of diflunisal transethosome by varying the types and concentrations of edge activators and optimizing the method of preparations.

Methods: Sonication amplitude and sonication time were optimized based on vesicle size and polydispersity index (PDI). Transethosome formulation using different types and concentrations of edge activators would be characterized, including vesicle size, PDI, zeta potential, morphology, entrapment efficiency, and deformability index, which were carried out using the optimum sonication method to formulate the optimum formula.

Results: The result indicates that 30% sonication amplitude for 5 min resulted in the smallest vesicle size with the lowest PDI. Also, F4 containing span 80 as edge activators at a concentration of 0.75% achieved the most favorable outcome, with a spherical shape, vesicle size of 75.32 nm, a PDI of 0.247, a zeta potential of-32.93mV, entrapment efficiency of 75.66% and deformability index of 40.45.

Conclusion: Sonication time of 5 min with an amplitude of 30% is proven to produce optimum diflunisal transethosome, and in comparison to other vesicles, diflunisal transethosome using span 80 was able to have excellent vesicle characteristics, making it extremely promising to be developed as a transdermal delivery system.

Keywords: Transethosome, Diflunisal, Transdermal

DOI: https://dx.doi.org/10.22159/ijap.2023v15i3.47691. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

A salicylic acid derivate known as diflunisal is a class of NSAIDs (nonsteroidal anti-inflammatory drugs) used to treat mild to moderate pain with inflammatory conditions such as osteoarthritis, rheumatoid arthritis, and other pain that is only marketed in an oral dosage form [1]. Diflunisal works by preventing the cyclooxygenase (COX) enzyme from catalyzing the conversion of arachidonic acid into prostaglandin precursors, which results in a reduction in prostaglandin synthesis [2].

Diflunisal is an active pharmaceutical ingredient included in BCS (Biopharmaceutics Classification Systems) class II, meaning it has low solubility and high permeability. This compound is lipophilic with a log P of 4.44 and a molecular weight of 250.2 Da [3], which makes diflunisal an ideal candidate to be formulated into a topical dosage form. Mainly diflunisal is only marketed as a tablet and is reported to have gastrointestinal adverse effects [4].

Topical application with a transdermal delivery system can be used as a diflunisal delivery route. The drug will be applied through the skin to deliver high local drug concentrations at the site of inflammation and therapeutic concentrations in the systemic circulation. In addition, transdermal delivery systems can reduce possible side effects [5]. The main problem faced by transdermal delivery systems is the presence of stratum corneum, which limits drug penetration. Transdermal technologies have primarily been developed to modify the stratum corneum to increase the number of drugs that can permeate. One of the many technologies being developed is lipid-based vesicles [6, 7].

The vesicle system is a carrier used for transdermal delivery systems with several advantages, including biocompatible, biodegradable, enhanced stability, and efficiency of the drugs. The main advantage is that the vesicles can penetrate deeper epidermis layers because the lipid content is comparable to the stratum corneum [8]. Transethosome is one of many formed vesicles. Compared to ethosomes and transfersomes, transethosome have higher entrapment efficiency and enhanced permeability because they contain surfactant (edge activators) and ethanol [9–11]. The usage of surfactants is one of the critical components of the transethosome formula. The surfactants' hydrophilic-lipophilic balance (HLB), alkyl chain length, and concentrations will affect the transethosome vesicle's characteristics [12].

Many factors affect the characterization of vesicles in the process of preparation. To achieve the optimal formula, those factors should be optimized. The sonication process at the preparation stage is one of the factors that can affect vesicle size and impact other characteristics. In this study, sonication time and amplitude would be optimized. This study also aimed to develop optimal diflunisal transethosome formulation by comparing the characteristics of transethosome using different edge activators and their concentrations, namely spans 20 and 80.

MATERIALS AND METHODS

Materials

Diflunisal API was purchased from Apollo Scientific, United Kingdom. Diflunisal analytical standard, ethanol, sodium hydroxide, potassium dihydrogen phosphate, methanol, and dichloromethane were purchased from Merck, Germany. Phosphatidylcholine (phospholipon 90G) was kindly donated by Lipoid, Germany. Span 20 and span 80 were generously gifted from Croda, Indonesia.

Preparation of transethosomes

Transethosome were prepared using the thin-film hydration method. This method was taken from Abd El-Alim *et al.* with a few changes [13]. The formulations are described in table 1. Diflunisal, phospholipon 90G, and edge activators were weighed accurately and dissolved in 20 ml of dichloromethane and methanol (7:3 v/v). Organic solvents were evaporated on a rotary evaporator at 52 °C under vacuum until the thin film was obtained, then streamed with

N2 gas and stored for 24 h at 4 ℃ The thin film was hydrated with ethanol: buffer phosphate (pH 7.4) 3:7 (v/v) for an hour using glass beads. For size reduction, transethosome suspensions were then sonicated.

Table 1: Formulations of diflunisal transethosomes

Formula codes	Concentration (%)				
	Diflunisal	Phospholipon 90G	*Edge Activator*		Ethanol: buffer phosphate (pH 7.4) 3:7 (v/v)
			Span 20	Span 80	
F1	1	2.5	0.5	-	ad 10 ml
F2	1	2.5	0.75	-	ad 10 ml
F3	1	2.5	-	0.5	ad 10 ml
F4	1	2.5	-	0.75	ad 10 ml

Optimization of sonication amplitude

Transethosome were prepared using the procedures outlined in the section *preparation of transethosomes.* The sonication amplitude was 20, 30, and 40%. Then the vesicle size and PDI were measured as described. This optimization is only done on one formula, namely F1.

Optimization of sonication time

Transethosome were prepared using the procedures outlined in the section *preparation of transethosomes***.** The sonication time was 5, 10, and 15 min, respectively. Then vesicle size and PDI were evaluated in all four formulations.

Vesicle size, zeta potential, and polydispersity index (PDI) characterization

Measurements were done using a particle size analyzer (Zetasizer, Malvern) at 25 ℃. Transethosome suspensions were diluted in water for injection (1:10) before being measured. All the formulas were analyzed in triplicate.

Morphology characterization

Morphology of optimum transethosome vesicle was performed by Transmission electron microscopy (TEM). The vesicle was diluted in double-distilled water (1:10), applied to a copper grid (film coated), and measured with an accelerating voltage of 160kV [10].

Entrapment efficiency characterization

The ultracentrifugation method was used to measure entrapment efficiency. The vesicular system was separated to obtain unentrapped diflunisal with 10.000 rpm speed at 4 ℃ for an hour. The supernatant was then diluted in methanol, and the quantity of the unentrapped drug was determined using spectrophotometry UV-Vis at 255 nm. The spectrophotometric conditions were taken from Kaur *et al.* with slight adjustments [14]. The total drug in the vesicular system was also determined by diluting 1 ml of transethosome suspension with methanol. Entrapment efficiency was calculated using the equation below:

$$EE\% = \frac{\text{Total drug} - \text{Amount of the unentrapped drug}}{\text{Total drug}} \times 100\%$$

Deformability index characterization

Measurements were done by extrusion. Transethosome suspensions were passed using a mini extruder over the 50 nm polycarbonate membrane. The amount of the suspension passed the extruder was recorded, and particle size was measured. The following equation is used to determine the deformability index [15]:

$$D = J\left(\frac{rv}{rp}\right)^2$$

D: deformability index

J: penetration rate of the vesicle through a polycarbonate membrane (mg/s. cm^2)

rv: vesicle size after extrusion (nm)

rp: pore size of polycarbonate membrane (nm)

Statistical analysis

One-way ANOVA was used to analyze the various experimental findings using IBM SPSS 24. Data were presented as mean±standard deviation (SD), and all experiments in this study were repeated three times. P-value<0.05 was determined statistically significant.

RESULTS

Preparation and optimization

The first optimization that has been done is the organic solvent evaporation time. Initially, the experiment was carried out to form a thin film was 30 min. However, the organic solvent had not wholly evaporated, so the evaporation was continued for the next 30 min until a thin layer of film was formed and the organic solvent fully evaporated.

Optimization of sonication amplitude

Particle size and particle size distribution of transethosome are crucial parameters, and they are mainly affected by the sonication conditions. Fig. 1 shows the results of particle size and PDI using different sonication amplitudes. It can be seen that there is a significant decrease in the size and PDI compared to without a sonication vesicle. The result also revealed a reduction in particle size and PDI as the amplitude increases up to 30%, but after the amplitude is increased to 40%, there is an increase in both size and PDI. The smallest particle size (122.7±11.46 nm) and PDI (0.241±0.02) is achieved using a sonication amplitude of 30%.

Optimization of sonication time

Sonication time in the preparation method was investigated at 5, 10, and 15 min using 30% amplitude. The particle size and PDI with different sonication times are shown in fig. 2. All sonication times significantly ($p<0.05$) decrease particle size and PDI. The smallest size and PDI for all formulations resulted in using 5 min of sonication time. In contrast, for the sonication time after 5 min, there has been an increase in size and PDI. It is noticeable that sonication time influenced the vesicle's particle size and PDI. The result revealed that increased sonication time linearly decreases the size and PDI until plateau size and PDI are reached. The optimum preparation method was determined: evaporation time for organic solvents was 60 min; the sonication amplitude and time were 30% and 5 min, respectively.

Particle sizes, PDIs, and zeta potentials characterization

Results of particle size, PDI, and zeta potential of transethosome formulations are presented in table 2. The particle size of prepared formulations ranged from 75.32 to 127.17 nm. It was found that F4 formulations using span 80 with higher concentrations exhibited significantly smaller sizes (75.32±1.59) than other formulations ($P<0.05$). According to the data, edge activator concentration increased between 0.5% to 0.75% would decrease the particle size significantly.

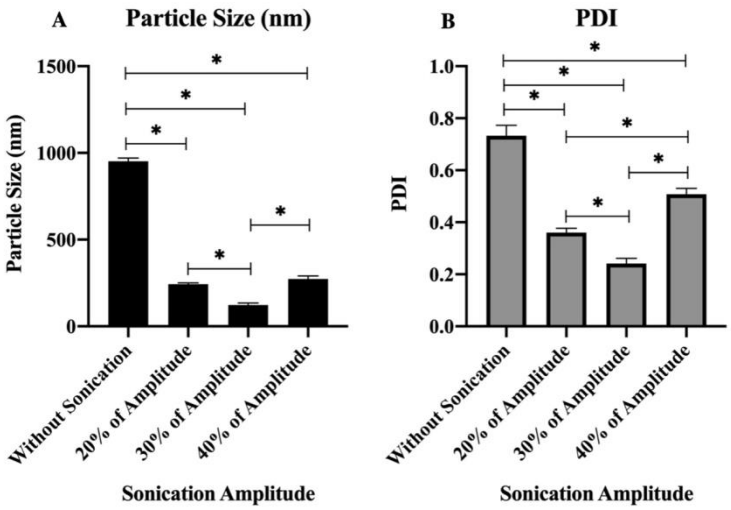

Fig. 1: Particle size (A) and PDI (B) of F1 in different sonication amplitude. Each measurement was performed in triplicate (n=3), and data are presented as mean±SD. Where * = significant difference (p<0.05)

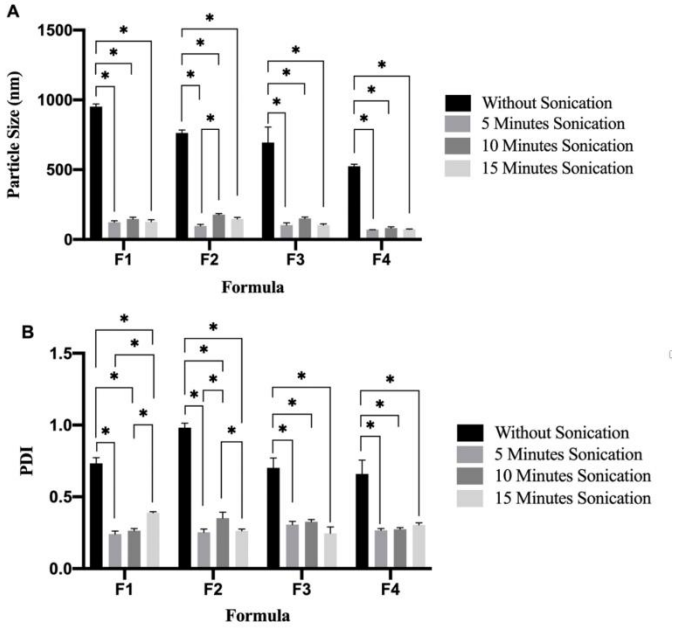

Fig. 2: Particle size (A) and PDI (B) of transethosome in different sonication times. Each measurement was performed in triplicate (n=3), and data are presented as mean±SD. Where* = significant difference (p<0.05)

Table 2: Results of transethosome characterization

Formula codes	Characterization		
	Particle size (nm)	PDI	Zeta potential (mV)
F1	127.17±5.44[b]	0.235±0.01[a]	-32.02±0.35[ab]
F2	99.45±1.26[a]	0.285±0.01[b]	-31.60±0.26[a]
F3	108.63±5.48[a]	0.309±0.01[c]	-32.97±0.42[b]
F4	75.32±1.59[c]	0.247±0.01[a]	-32.93±0.28[b]

Each value shown is the mean±SD (n = 3). While different letters (a,b,c) indicate a significant difference (p<0.05), the same letters indicate a non-significant difference.

PDI measures the width of the unimodal size distribution. The prepared vesicles' PDIs were generally small (<0.5), as seen in table 2, indicating homogeneous dispersion with good homogeneity and narrow size distribution in all formulations. The formula F1 achieves the smallest PDI, but there is no significant difference between F1 and F4 (P>0.05). One more essential characteristic of nanovesicles that might impact their characteristics is their zeta potential. All the formulas show negative zeta potential with values greater than-30mV, indicating less chance for particle agglomeration. The highest zeta potential was produced by formula F3 (-32.97), although there is no significant difference compared to formula F1 and F4.

Morphology vesicles

Fig. 3 shows the morphological results of the selected formula, namely F4. Vesicle was observed at 145.000x magnification. Transethosome vesicle shown to be spherical in shape with no signs of aggregation.

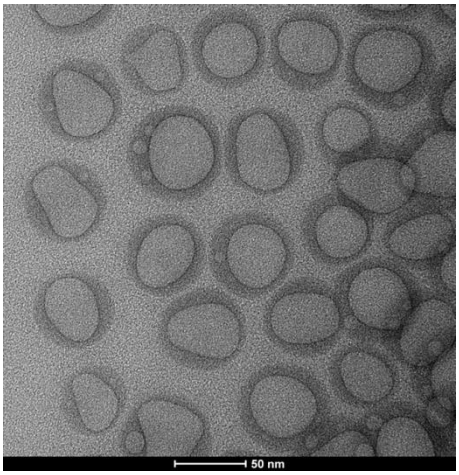

Fig. 3: Transethosome morphology at 145.000x magnification

Entrapment efficiency (% EE)

The entrapment efficiency of transethosome diflunisal was determined by an indirect method. As shown in table 3, the resulting entrapment efficiency ranges from 52.62% to 78.05%, with the highest entrapment efficiency obtained from the formula using span 20 with high concentration (F2). When the surfactant concentration was increased, the entrapment efficiency also increased. In comparing F2 and F4 formulations, F2 revealed a non-significantly higher (P>0.05) entrapment efficiency (76.99±0.89%) than F4 (75.66±0.73).

Table 3: Results of entrapment efficiency

Formula codes	Entrapment efficiency (%)±SD
F1	53.32±0.27[a]
F2	76.99±0.89[b]
F3	51.81±0.09[a]
F4	75.66±0.73[b]

Each value shown is the mean±SD (n = 3). While different letters (a, b) indicate a significant difference (p<0.05), the same letters indicate a non-significant difference.

Deformability index

The most significant advantage of the transethosome delivery system over traditional liposomes is its elasticity. The deformability indexes of all formulas are listed in table 4 and revealed that deformability indexes ranged from 20.24 to 40.45. It was discovered by the data's use of an ANOVA statistical analysis that F4 had a significantly higher deformability index (40.45±0.95) than other formulations and proved that the vesicle could deform and pass through a polycarbonate membrane pore without quantifiable loss seen from the size of the vesicle before and after extrusion.

Table 4: Results of deformability index

Formula codes	Particle size (nm)±SD		Deformability index
	Before extrusion	After extrusion	
F1	127.17±5.44	115.77±1.37	20.24±1.21[a]
F2	99.45±1.26	96.96±1.97	32.07±1.31[b]
F3	105.63±2.26	101.87±1.73	29.49±1.45[b]
F4	75.3±1.59	74.01±0.93	40.45±0.95[c]

Each value shown is the mean±SD (n = 3). While different letters (a,b,c) indicate a significant difference (p<0.05), the same letters indicate a non-significant difference.

DISCUSSION

In this study, we optimized the preparation method of transethosome diflunisal and investigated the characteristics of transethosome diflunisal using different edge activators and concentrations. The thin-film hydration method was chosen to prepare transethosome as it is simple and produces excellent characterization in vesicle size, PDI, zeta potential, and entrapment efficiency [16]. Various factors, like the organic solvent's evaporation time, sonication time, and sonication amplitude, were identified as critical formulation parameters.

Optimization of the method resulted in data that both sonication amplitude and time affect the size and PDI of vesicles. The size and PDI are reduced due to the higher power increasing shear stresses on the solution. Greater homogeneity of the solution is encouraged by the ultrasonic. Vesicle size decreased when the sonication amplitude and time increased until the optimum size was reached, which also applies to PDI. A decrease in PDI was achievable by employing a larger amplitude, which is similar to the trend reported by Silva [17]. It might be due to size reduction by the dispersion energy during sonication, but after a set amount of time and amplitude, the particles begin to agglomerate, increasing in size [18]. As described in the other study, optimum sonication produces smaller vesicle size and higher entrapment efficiency [19].

As mentioned above, the particle size of the vesicle is critical for skin penetration. Vesicle transethosome with a smaller size (<200 nm) allows it to pass through the skin's pores [20]. A total of four formulations of diflunisal transethosomes were prepared to optimize the type and concentration of edge activators. All formulations contain the same concentration of diflunisal (10 mg/ml) and phospholipon 90G (25 mg/ml). Based on these results, the vesicle size decreased with increased surfactant concentrations. As previously reported, increasing the edge activator concentration reduces interface tension which can cause a smaller size of the vesicles and similarly to high ethanol content. High ethanol concentration can cause interpenetration of lipid hydrocarbon chains, causing small vesicle sizes. A similar finding was obtained by El-sonbaty *et al.* during the preparation of luliconazole transethosome [16]. The results in table 2 also indicate that vesicle using span 80 produces smaller vesicle sizes than span 20, which means that vesicle size decrease as the HLB surfactant decrease. The same result studies of niosomes zidovudine using span 20 dan span 80 were reported by Ruckmani [21]. These results may occur due to a reduction in surface-free energy caused by increased hydrophobicity [22].

The polydispersity index (PDI) value, which illustrates a degree of heterogeneity in a dispersed system based on particle sizes, is also determined using DLS. A heterogeneous polydisperse is indicated by a value of 1, while homogeneous dispersion is characterized by a value of 0. A PDI value under 0.5 is considered acceptable [12], where all formulas have a PDI value between 0.2-0.3. The findings showed that optimum sonication could result in transethosome

particle that was more homogenous. Lower PDI values also indicate that the transethosome are stable.

Zeta potential is an essential characteristic of vesicles that can predict the physical stability of the vesicles. Electrostatic repulsion can significantly reduce agglomeration and nanovesicle fusion and increase stability [16, 23]. The zeta potential research demonstrated the vesicular formulations' high stability and showed that they fall within the specified millivolt range. The produced transethosome's zeta potential was influenced by the amount of ethanol in the formulation.

The morphology of transethosome vesicles was visualized under TEM. It could be observed in fig. 3 that the transethosome showed spherical morphology. The binding of phosphatidylcholine heads in the presence of water resulted in the formation of a spherical shape of vesicle to stabilize the system. As a lipophilic drug, diflunisal would be trapped in the lipophilic ends of phosphatidylcholine.

Entrapment efficiency is one of the critical responses in vesicle formulation. We measured the entrapment efficiency with the ultracentrifugation method. With higher concentrations, Span 20 and Span 80 led to higher entrapment. Transethosome with a span 80 resulted in lower entrapment efficiency at both concentrations than a span 20, which was in agreement with pentoxifylline transfersomes studies by Al Shuwaili [24]. It has been commonly reported that lipophilic drugs will result in high entrapment efficiency by low HLB surfactants and long alkyl chains. Span 80 has a lower HLB and longer alkyl chain than span 20. It also has an unsaturated alkyl chain which will increase the permeability of the vesicle membrane so that the entrapment efficiency is reduced [25]. Another reason span 80 results in a lower % EE compared to span 20 is the transition temperature. Span 80 has a lower transition temperature (-12 °C) than span 20 (16 °C). Surfactants with lower transition temperatures could result in irregular structure development and higher vesicle bilayer fluidity, decreasing the drug's entrapment [26]. Other investigations also demonstrate that the type of phosphatidylcholine affects entrapment efficiency, with phospholipon 90G producing a greater entrapment efficiency in the formulation of paclitaxel liposome than another phosphatidylcholine [27].

The sonication process also influences the entrapment efficiency of the vesicle. As reported before, increasing the sonication time would increase entrapment efficiency until an insignificant effect. Longer sonication caused a reduction in entrapment efficiency. Vesicles may be damaged by the cavitation impact of ultrasound, which might then allow drugs to leak and lowered entrapment efficiency [28, 29]. According to other findings, sonication without pauses produced greater entrapment efficiency and particle size than sonication with pauses did [30]. This reinforces the conclusion that an optimal sonication procedure produces high entrapment efficiency.

Deformability is a prominent characteristic of transethosome and represents their permeation ability to pass through pores with a diameter smaller than their size. This study uses 50 nm polycarbonate membranes. The possible synergism between edge activators and ethanol allows the above formula to pass through the polycarbonate membrane and squeeze itself without experiencing damage, as seen in particle size before and after extrusion in table 4. The packing characteristics of the lipid in vesicle bilayers are said to be altered by surfactants and ethanol in transethosome to become weaker, creating more elastic and deformable vesicle that is more effective at delivering drugs to the skin [31].

CONCLUSION

Sonication time and amplitude were successfully optimized. A sonication amplitude of 30% for 5 min resulted in the best particle size and PDI of transethosome. Different types of surfactants, including spans 20 and 80, and their concentration were designed in this work, and transethosome characteristics have been evaluated. In conclusion, the findings of our study demonstrate that adding 0.75% of span 80 in transethosome diflunisal achieved better characteristics of the vesicle represented by smaller size and PDI, zeta potential above-30mV, highest deformability index, and high entrapment efficiency compared to other formulations. The current study has proven that transethosome diflunisal has excellent characteristics to be used as a carrier for transdermal delivery systems.

ACKNOWLEDGMENT

The authors sincerely acknowledge the PUTI research grant 2022 support from the Directorate of Research and Development, University of Indonesia, under contract number NKB-072/UN2. RST/HKP.05.00/2022.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors were responsible for every part of this work and contributed to data analysis, drafting, and revision of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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