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Research Article

Leciplex produced on a pilot scale: production and in-vitro characterization.

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Abstract

The present study reported leciplex (LPX) preparation using a simple mixing technique for 10 mL to 200 mL volumes. A D-optimal design was employed to study the effect of different variables on the characteristics of LPX and compare their impact where the batch volume (X_1), mixing speed (X_2), and mixing time (X_3) were set as the independent variables, whereas their impact was studied for, particle size (PS; Y_1), polydispersity index (PDI; Y_2), zeta potential (ZP; Y_3). Furthermore, the LPX batch prepared via optimized parameters was characterized for morphology by transmission electronic microscopy (TEM) and stability study. The PS (Y_1) of different LPX batches were significantly affected by mixing speed (X_2), while the PDI (Y_2) and ZP (Y_3) were influenced considerably by both mixing speed (X_2) and mixing time (X_3). The batch volume (X_1) did not affect the characteristics of the prepared batches. The morphology of LPX was spherical and well dispersed. Furthermore, the optimized LPX batch was stable for 90 days of storage. Finally, it was shown that this fabrication method was appropriate for preparing LPX with good reproducibility and stability.

Introduction

The pharmaceutical industry faces difficulties manufacturing nanoparticle drug delivery systems, frequently preventing them from moving from the bench to the bedside. This is because most traditional preparation techniques cannot be easily scaled up to production levels. In fact, less than 50 liposomal products have been introduced to the market, despite their recognized benefits for drug delivery [1]. The precise and intricate process of manufacturing liposomes involves several steps, all of which have a crucial impact on the final size, stability, and functionality of the finished product [2].

Numerous methods for producing liposomes have been reported, including mechanical ones (film methods, homogenization, sonication, microfluidization, extrusion), ones that rely on substituting aqueous media for organic solvents (ethanol injection, reverse-phase evaporation),

and ones that are based on detergent removal. Many of these techniques, though, are not appropriate for large-scale production [3]. Furthermore, developing and adopting new liposomal systems may be delayed because liposome preparation techniques used in the laboratory setting are difficult to translate to large-scale production [4].

Production of large-scale parenteral liposomes on a large scale is most commonly achieved by ethanol injection followed by extrusion [5]. The reproducibility of liposome particle size and polydispersity index to alternative small-scale manufacturing processes is the cause. On the other

hand, this approach requires about nine unit operations. Further, the complexity increases as every unit operation requires in-process quality control, making it a long and labour-intensive process [6].

Table 1: D-Optimal design for optimization of different batches of LPX.

Factors (independent variables) for LPX design	Levels	
	Low	High
X_1: Batch volume (mL)	100	200
X_2: Mixing speed (rpm)	500	1000
X_3: Mixing time (min)	10	20
Responses (dependent variables)		
Y_1: PS (nm)		
Y_2: PDI		
Y_3: ZP (mV)		

Therefore, there is a need to develop an economical and reproducible manufacturing process capable of controlling liposomes' critical quality attributes. These attributes generally include particle size (typically <100 nm), high drug loading and surface charge [7].

One of the recent lipid-based nanocarriers is leciplex (LPX). LPX is a self-assembled phospholipid-based cationic nanocarrier; the main components of the LPX system are a phospholipid, a cationic surfactant (SAA), and a biocompatible surfactant as Transcutol HP. LPX offers several benefits compared to other nanocarrier systems, including ease of preparation (it only requires one fabrication step), the absence of organic solvent during formulation, and ease of scale-up [7]. Therefore, different batches of LPX were prepared in a single fabrication step using a simple mixing technique for volumes from 10 mL to 200 mL. A D-optimal design was employed to explore the effect of different variables on the characteristics of LPX where the batch volume (X_1), mixing speed (X_2), and mixing time (X_3) were set as the independent variables, whereas their effect was studied for, particle size (PS; Y_1), polydispersity index (PDI; Y_2), zeta potential (ZP; Y_3). Furthermore, the optimized LPX batch was characterized for morphology by transmission electronic microscopy (TEM) and stability study.

Materials

Soy phosphatidylcholine (SPC) and dimethyldidodecylammonium bromide (DDAB) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Methods

Preparation of leciplex

First, the required amounts of soy phosphatidylcholine (SPC) and dimethyldidodecylammonium bromide (DDAB) in a molar ratio (1: 0.5) were solubilized in 5 % Transcutol HP in a shaker water bath (LWBS-A12, Labtron, Camberley, U.K.) at 70 °C till a clear yellow, homogenous solution was gained. Next, 95% distilled water at the same temperature was added to the lipid mixture with continuous mixing using an overhead Stirrer mixer (Mixer201, Vevor, Germany) in predetermined parameters, as illustrated in Table 1.

Determination of particle size (PS), polydispersity index (PDI), and zeta potential (ZP)

PS, PDI, and ZP were measured for the formulated batches by Zetasizer 2000 (Malvern Instrument Ltd., UK). After proper dilution, the measurement was carried out in triplicate [8].

Optimization of LPX batches

The desirability tool was used to select the optimized manufacturing parameters. Making a batch with the highest ZP values and the lowest PS and PDI values served as the basis for selection.

Morphology Transmission electron microscopy (TEM)

TEM (Joel JEM 1230, Tokyo, Japan) operated at 80 kV was employed to identify the morphology of the optimized LPX batch. After being diluted ten times and applied to the carbon-coated grid, the LPX formula was negatively stained with 1% phosphotungstic acid and dried before visualization [9].

Stability study

The optimized batch of LPX was stored at 4 ± 3 °C for 3 months. The stability was assessed by measuring the physicochemical liposome characteristics of PS, PDI and ZP during storage [10].

Results and discussion

D-optimal design

The independent variables that were studied for the scale-up of LPXs were batch volume (X_1), mixing speed (X_2), and mixing time (X_3). Fourteen batches of LPX were prepared, and particle size (PS; Y_1), polydispersity index (PDI; Y_2), and zeta potential (ZP; Y_3) were measured for each batch, as seen in Table 2. The design output is summarized in Table 3.

Effect of independent variables on the particle size

The PS influence the biodistribution and pharmacokinetics of lipid-based nanocarriers, impacting the efficacy of these carriers [11]. Hence, strict control of the PS is required. The PS of different LPX batches ranged from 108.3±1.4 to 240.6±1.9 nm, as shown in Figures 1 & 2. The PS of LPX batches was not significantly affected by batch

Table 2: Experimental runs and measured response of the D-optimal experimental design of LPX.

Batch No	Batch volume (mL) (X_1)	Mixing speed (rpm) (X_2)	Mixing time (min.) (X_3)	PS (nm) (Y_1)	PDI (Y_2)	ZP (Y_3)
1	200	750	20	142.8±2.1	0.272±0.003	39.6±1.4
2	200	750	10	149.4±1.9	0.205±0.001	36.4±0.6
3	200	1000	10	116.8±1.6	0.214±0.001	41.9±0.8
4	150	500	10	223.1±2.3	0.465±0.001	35.7±0.9
5	100	500	20	157.5±2.0	0.354±0.004	37.5±1.5
6	200	500	10	236.4±3.4	0.470±0.001	28.8±1.7
7	125	750	15	127.7±1.3	0.207±0.001	39.9±0.7
8	200	500	15	142.1±1.1	0.451±0.003	33.0±0.7
9	100	500	10	240.6±1.9	0.480±0.001	40.9±0.8
10	200	750	15	122.6±0.9	0.210±0.001	39.8±0.7
11	200	1000	15	108.3±1.4	0.150±0.001	46.7±0.8
12	100	1000	10	110.6±1.7	0.200±0.005	38.6±0.8
13	100	1000	20	217.6±2.5	0.382±0.002	33.2±1.5
14	100	1000	20	206.3±1.1	0.390±0.004	31.9±1.8

volumes (p=0.083); the PS of all batches prepared with varied volumes but with the same mixing speed and time was not significantly different, meaning that the LPX could be prepared with varying volumes without altering its PS. On the contrary, increasing the mixing speed significantly reduced the PS of LPX batches (p=0.005), as increasing the mixing speed breaks the vesicles into smaller ones [12]. The mixing time did not significantly affect the PS (p= 0.062); however, upon studying the PS in different LPX batches, it was found that upon increasing the mixing time from 10 to 15 min, the PS was slightly reduced and on further increase, the mixing time to 20 min the PS was increased, indicating a quadratic model. These results could be attributed to vesicle aggregation upon prolonging the mixing time, leading to increased PS.

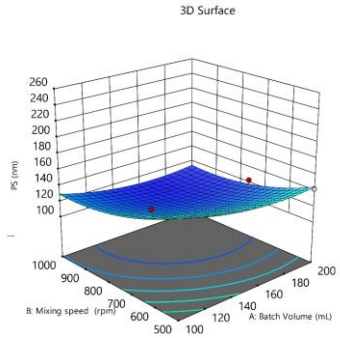

Figure 1: 3D plot for batch volume (X_1) and mixing speed (X_2) effect on PS.

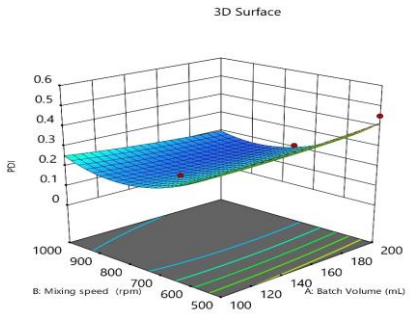

Figure 3: 3D plot for batch volume (X_1) and mixing speed (X_2) effect on PDI.

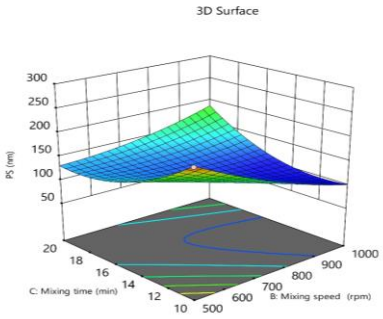

Figure 2: 3D plot for mixing speed (X_2) and mixing time (X_3) effect on PS.

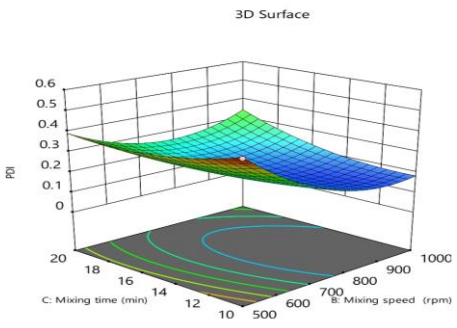

Figure 4: 3D plot for mixing speed (X_2) and mixing time (X_3) effect on PDI.

Effect of independent variables on the polydispersity index

The PDI of LPX batches was not significantly affected by batch volumes (p=0.897), as shown in Figures 3 and 4. The

Table 3: Output data of the D-Optimal design

Source	PS (nm)	PDI	ZP (mV)
p-value	0.0012	0.0258	0.0007
Model	Quadratic	Quadratic	Quadratic
X_1 = A= Batch volume	0.083	0.8978	0.7382
X_2 = B= Mixing speed	0.005	0.0074	0.0009
X_3 = C= Mixing time	0.062	0.3347	0.3723
Adequate precision	17.23	8.278	27.83
R^2	0.9901	0.9517	0.9923
Adjusted R^2	0.9387	0.8429	0.9751
Predicted R^2	0.8486	0.7391	0.8630

PDI of all batches prepared with varied volumes but with the same mixing speed and time was not significantly different; on the contrary, increasing the mixing speed significantly reduced the PDI of LPX batches (p=0.007), as increasing the mixing speed led to more homogenous dispersion [13]. The mixing time did not significantly affect the PDI (p= 0.062); it was found that upon increasing the mixing time from 10 to 15 min, the PDI was slightly reduced, and on further increase, the mixing time to 20 min, the PDI was increased, indicating a quadratic model. These results confirmed the vesicle aggregation upon prolonging the mixing time, leading to increased PS and PDI.

Effect of independent variables on the zeta potential

The ZP of LPX batches was not significantly affected by batch volumes (p=0.738), as shown in Figures 5 and 6. The ZP of all batches prepared with varied volumes but with the same mixing speed and time was not significantly different; on the contrary, increasing the mixing speed significantly increased the ZP of LPX batches (p=0.0009), as increasing the mixing speed to a reduction in the PS and to an increase in the surface ratio [14]. Increasing the mixing time from 10 to 15 min, the ZP was slightly increased, and on further increasing the mixing time to 20 min, the ZP was decreased, indicating a quadratic model. These results confirmed the vesicle aggregation upon prolonging the mixing time, leading to increased PS and PDI and reduced ZP values.

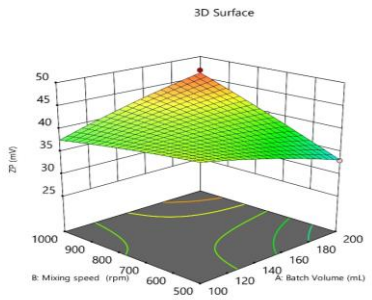

Figure 5: 3D plot for batch volume (X_1) and mixing speed (X_2) effect on ZP.

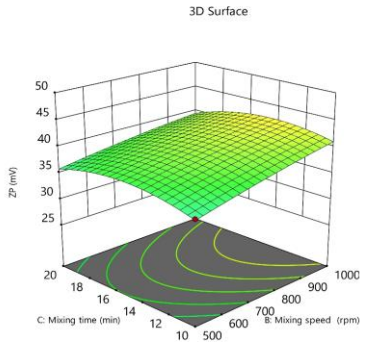

Figure 6: 3D plot for mixing speed (X_2) and mixing time (X_3) effect on ZP.

Morphology

As illustrated in Figure 7, the optimized LPX batch formula's TEM micrographs revealed that the LPX was spherical.

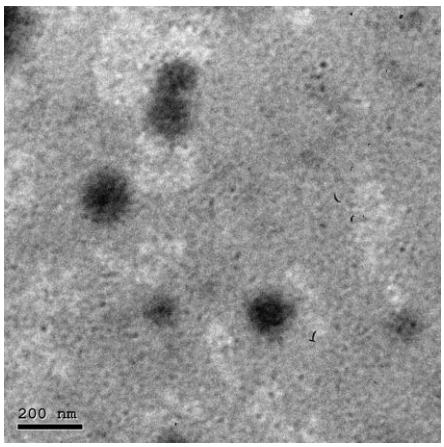

Figure 7: TEM micrograph of the optimized LPX batch.

Stability study

The PS, PDI, and ZP measurements were 111.7±2.8 nm, 0.156±0.003, and 45.6±1.9 mV, respectively. The paired t-test revealed a negligible difference between these findings and the freshly prepared LPX ($p>0.05$). These findings assured the capability of preparing stable LPX batches with a simple mixing technique.

Conclusion

Different LPX batches with varied volumes were prepared using a simple mixing technique. The significant factors impacting the LPX characteristics were mixing speed and time, while the batch volume did not affect any LPX features such as PS, PDI and ZP. These results revealed the capability of scaling up the manufacturing of self-assembled lipid-based nanocarriers via simple mechanical methods. However, further studies are required to study the effect of scaling up on other features, such as drug loading capacity, entrapment efficiency and in vitro drug release.

Disclosure

The authors report no conflict of interest.

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