OPTIMIZATION OF HIGH PRESSURE HOMOGENIZATION IN THE PRODUCTION OF LIPOSOMAL DISPERSIONS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: Liposomes are spherical, biodegradable, and biocompatible vesicular systems. These vesicles are built from phospholipid double layers (membranes) surrounding the inner water phase. Liposomes are highly desirable as drug carriers because they can incorporate hydrophilic, hydrophobic and amphipathic drug substances (drugs). The physicochemical properties of liposomes such as size, charge, surface properties and encapsulation efficiency can highly influence their in vivo stability and kinetics.
The aim of our study was to prepare liposomal dispersions and to determine the influence of cycles of high pressure homogenization on some parameters, such as vesicle size and polydispersity index (PDI). Higher homogenization pressures and repeated recirculation led to further reduction in vesicle diameter and heterogeneity.
For preparing liposomal dispersions Phosal IP 40 and Phosal 75 SA were used (Lipoid, Germany). Liposomal dispersions were prepared according to the thin film hydration method. By sampling after each cycle, an estimate was made of how many cycles are needed for the dispersion to have satisfactory parameters (size and PDI).
The size and PDI analysis of the liposomes were carried out by using Zetasizer (Nano series) ZS 90, Malvern Instruments. . High pressure homogenization was carried out in 10 cycles and based on the obtained liposome size values and PDI, was determined how many cycles are needed in the process of homogenization. With each cycle, the size of the liposomes decreased and PDI value was reduced. It has been observed that after 5 cycles of homogenization there is no significant decrease in the size of the liposomes and PDI.
Therefore, in the further production of liposomes with active substances with these raw materials, is recommended to use only 5 cycles of homogenization

KEYWORDS: High pressure homogenization, phospholipids, liposomes, diameter and PDI

INTRODUCTION

The use of liposomes has been quite common lately due to their tendency to enhance the bioavailability and stability of drugs and cosmeticals. Liposomes are spherical, biodegradable, and biocompatible vesicular systems [1]. These vesicles are built from phospholipid double layers (membranes) surrounding the inner water phase. Due to the number of concentrically deposited bilayers, liposomes may be unilamellar, oligolamellar (several phospholipid bilayers) or multilamellar (many phospholipid bilayers). Their diameter ranges from 20 nm to 10 and more μm and they are forming spontaneously, by hydrating the phospholipids in an aqueous medium.

Liposomes are highly desirable as drug carriers because they can incorporate hydrophilic, hydrophobic and amphipathic drug substances (drugs), and they are also physiologically acceptable due to their similarity with biological membranes and biodegradability [2, 3]. Moreover, they are biodegradable, nontoxic, non-immunogenic and biocompatible compounds which can provide several advantages as carriers for the encapsulated molecules such as enhancing their pharmacokinetic and bio-distribution, decreasing their toxicity and providing target selectivity for them [4].

The physicochemical properties of liposomes such as size, surface properties and encapsulation efficiency can highly influence their in vivo stability and kinetics [5,6,7]. In addition, these properties can be modified simply by adding new ingredients to the lipid mixture before liposome preparation and/or by variation of preparation methods.

Various methods have been used for the preparations of liposomes which include mechanical methods like film and ultrasonic method, methods involving replacement of organic solvent, methods involving fusion of prepared vesicles or transformation of size by freeze thaw extrusion (FTE) and the dehydration-rehydration (DR) method [8].

Liposomes can therefore be manufactured with different size, ranging from several nanometers to micrometers [9]. The range of liposome preparation methods has recently been extended by a number of techniques which are based on the use of homogenizers. High pressure homogenizers are efficient multipurpose tools in pharmaceutical industry. Many stud-

ies have proven their convenience for the preparation of small and uniform vesicles. Higher homogenization pressures and repeated recirculation led to further reduction in vesicle diameter and heterogeneity.

According to Bernoulli´s law, static pressure within a fluid decreases at high velocity of flow. If the local pressure is falling below the steam pressure, bubbles filled with steam or gas arise and grow until re-elevation of the pressure causes their implosion. The surrounding water and lipid bilayers are accelerated toward the middle of the buble, followed by shock waves. This effects high local stress and the main results are reduction in the number of bilayers and decreasing diameters of the vesicles [10].

The reduction in size, broadness of the size-distribution, and lamellarity of „handshaken liposomes" during high-pressure homogenization depends on lipid composition, homogenization pressure and number of passages.

Preparation of liposomes with high-pressure homogenizers has many advantages. It avoids organic solvents or tensides, it is versatile in regard to choice of lipids and drugs, it achieves homogeneous distribution of binary lipid mixtures without prior dissolution in organic solvents, any concentration of lipids can be processed, vesicle formation is not dependent on salt concentration or pH of the aqueous solution, labile proteins and peptides can be entraped with low risk of loss of biological activity, the process may easily be scaled up, and the physicochemical stability of vesicles is not affected over at least 5 months [10].

The aim of our study was to prepare liposomal dispersions and to determine the influence of cycles of high pressure homogenization on some parameters, such as vesicle size and polydispersity index (PDI).

MATERIAL AND METHODS

MATERIALS

For preparing liposomal dispersions, phospholipids Phosal IP 40 and Phosal 75 SA were used (Lipoid, Germany).

PHOSAL IP 40	Phospholipids: Phosphatidylcholine + Lysophosphatidylcholine [%] n.l.t. 37; Non-polar lipids [%] 35-45; Ethanol [%] n.m.t. 5; Tocopherols [%] n.l.t. 0.05
PHOSAL 75 SA	Phosphatidylcholine [%] 72.0-78.0; Lysophosphatidylcholine [%] n.m.t. 6.0 Ethanol [%] 8.0-10.0

PREPARATION OF LIPOSOMAL DISPERSIONS

Formulation 1 – Phosal IP 40

Phosal IP 40	10 g
Aqua ad injectabilia	90 g

Formulation 2 – Phosal 75 SA

Phosal 75 SA	10 g
Aqua ad injectabilia	90 g

Liposomal dispersions were prepared by dissolving precise amounts of Phosal IP 40 or Phosal 75 SA in Aqua ad injectabilia. Phase mixing and homogenization was performed by Ultra Turrax Ika T25-Digital, 15 minutes at 5000 rpm.

After that, dispersions were homogenized in a high pressure homogenizer (Emulsiflex-C3, Avestin, Canada) at 500 bar in 10 cycles to optimize homogenization .

The samples were taken after each cycle . By sampling after each cycle, an estimate was made of how many cycles are needed for the dispersion to have satisfactory parameters (based on measurements of liposome size and PDI).

MICROSCOPY

The structures of liposomes were evaluated with optical microscope (BA310 Motic optical microscope, Speed Fair Co. Ltd., Hong Kong, China) at a magnification of 100 x. Microphotographs were taken with Optika Pro 3LT camera processed with Optika Vision Pro Software (Optika Microscopes, Ponteranica, Italy).

SIZE ANALYSIS AND PDI

The vesicle size and PDI analysis of the liposomes were carried out by using Zetasizer (Nano series) ZS 90, Malvern Instruments. The width of the size distribution was indicated by the polydispersity index (PDI). Samples were analyzed 24 h after preparation and diluted with Aqua purificata in a ratio of 1: 1000. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Both liposomal dispersions were milky dispersions, and the presence of liposomes was demonstrated by observation under a microscope at a magnification of 100 x (Figure 1).

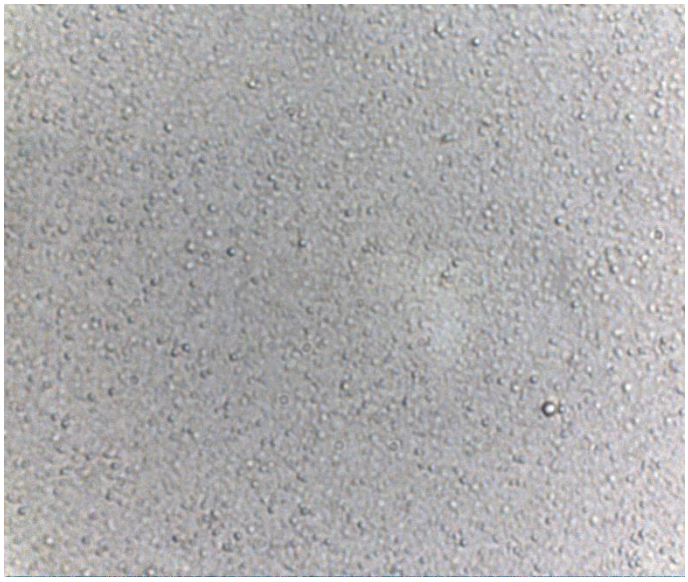

Figure 1. Liposomes under microscope at a magnification of 100 x (small round creatures are actually formed liposomes of different sizes)

Results of the characterization studies (size, polydisperzity index (PDI)) of liposomal dispersions are shown in Tables 1 and 2.

Table 1. Size analysis and PDI of liposomes with Phosal IP 40

Phosal IP40 (1:1000)	Size (nm)	PDI	PDI width
Cycle	385.4 ± 2.629	0.382 ± 0.013	238.1 ± 2.52
Cycle	340.9 ± 5.43	0.302 ± 0.033	187.2 ± 1.20
Cycle	319.1 ± 6.74	0.261 ± 0.010	162.9 ± 1.66
Cycle	306.3 ± 7.85	0.295 ± 0.028	220.6 ± 13.46
Cycle	294.9 ± 0.568	0.258 ± 0.019	149.8 ± 1.84
Cycle	288.1 ± 0.850	0.241 ± 0.006	141.5 ± 1.84
Cycle	283.5 ± 5.122	0.235 ± 0.015	137.4 ± 6.17
Cycle	279.6 ± 5.52	0.229 ± 0.012	133.6 ± 3.46
Cycle	270.9 ± 2.139	0.236 ± 0.014	131.5 ± 4.0
Cycle	257.6 ± 2.066	0.232 ± 0.021	124.0 ± 4.979

Table 2. Size analysis and PDI of liposomes with Phosal 75 SA

Phosal 75 SA (1:1000)	Size (nm)	PDI	PDI width
Cycle	269.8 ± 7.0577	0.395 ± 0.031	169.3 ± 2.25
Cycle	239.1 ± 2.074	0.293 ± 0.027	129.4 ± 7.012
Cycle	220.2 ± 1.102	0.291 ± 0.033	118.7 ± 6.514
Cycle	212.1 ± 5.749	0.283 ± 0.010	112.9 ± 1.626
Cycle	202.8 ± 5.424	0.260 ± 0.005	103.5 ± 3.58
Cycle	198.8 ± 0.723	0.263 ± 0.005	101.9 ± 0.862
Cycle	194.5 ± 1.793	0.249 ± 0.005	96.92 ± 1.269
Cycle	191.1 ± 2.136	0.243 ± 0.007	94.18 ± 1.67
Cycle	190.1 ± 2.042	0.246 ± 0.016	94.24 ± 3.99
cycle	190.7 ± 5.034	0.254 ± 0.012	96.11 ± 4.018

The aim of homogenization under high pressure is to reduce the size of the liposomes and to achieve homogeneity, as well as to increase the stability of liposomal dispersions. High pressure homogenization was carried out in 10 cycles and based on the obtained liposome size and PDI values, was determined how many cycles are needed in the process of homogenization.

The size of liposomes and PDI decreased significantly to 5 cycles, and the following values after fifth cycle were obtained. For Phosal IP 40 the size of liposomes was 294.9 ± 0.568, and PDI was 0.258 ± 0.019. For Phosal 75 SA the size of liposomes was 202.8 ± 5.424, and PDI was 0.260 ± 0.005.

The average size and size distribution of liposomes are important parameters especially when the liposomes are intended for therapeutic use by inhalation or parenteral route [11]. The liposomes with size ≤ 300 nm are able to deliver their contents to some extent into the deeper layers of the skin [12]. So for example, these liposomal dispersions could be used in the preparation of dermal preparations.

Polydispersity index is usually considered as an indicator of particles diameter distribution in a colloidal system. The lower level of this index, the more likely the particle diameter distribution is narrower, so the diameter of particles is more uniform. The value of less than 0.1 for this index shows a homogeneous population and a value greater than 0.3 indicates a high degree of heterogeneity [13]. The values of PDI were lower than 0.3, and the liposomal dispersions can be considered homogeneous.

In our research, with each cycle the size of the liposomes decreased and PDI value was reduced. It has been observed that after 5 cycles of homogenization there is no significant decrease in the size of the liposomes and PDI. Further recirculation can cause vesicle re-growth and therefore, no further homogenization is required after 5 cycles.

The length of homogenization is also very important, and should not be carried out in too many cycles, especially for dispersions with thermolabile active substances. Therefore, we recomend only 5 cycles of high pressure homogenization in preparation of these liposomal dispersions and their use in preparation of dermal products.

CONCLUSION

Based on the results of this research, it can be concluded:

- High pressure homogenization reduced the size of the liposomes and achieved homogeneity.

- The size of the liposomes and PDI values were suitable for further use in the preparation of pharmaceutical and cosmetic products.
- For obtaining liposomal dispersions of satisfactory characteristics, it was sufficient to do 5 cycles of high pressure homogenization, and therefore, in the further production of liposomes with active substances with these raw materials, is recommended to use 5 cycles of homogenization.

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