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International Journal of Pharmaceutics 196 (2000) 151–153

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Bench scale manufacture of multilamellar liposomes using a newly developed multistage pressure filtration device

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Abstract

Liposomes are belonging to the modern kinds of drugs. They are facilitating the secure and well-targeted transport of substances inside the organism, and are gaining increasing importance in the pharmacological treatment of tumors and in gene-therapeutic strategies. Multilamellar liposomes have advantages to one-layer liposomes: the multiplicity of coats increases the effect of a reservoir and makes extremely prolonged releases of drugs possible. This study describes the aseptical manufacturing of different kinds of multilamellar liposomes. It has been shown that it is possible to produce liposome-suspensions with different shares of multilamellar liposomes in the scale of litres under aseptical circumstances using a newly constructed multi-stage-pressure-filtration-device. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Liposomes; Multiple layer vesicles (MLV); Aseptical production

Because of the great variety of liposomes with respect to

- composition,
- size, and
- structure

there are different possibilities for applying these vesicles within the pharmaceutical industry.

Among the different kinds of liposomes the so called multilamellar vesicles (multi layer vesicles; MLV) are of special significance. In addition to the well known advantages of liposomes (e.g. the protected and purpose orientated transport of substances within the organism) these vesicles are able to increase the so-called reservoir effect, i.e. the active substance is released during a long to very long period (Arndt and Fichtner, 1986).

The study described here was aimed at the development of a process allowing the production of sterile and homogenized (with respect to the size of the liposomes) MLV-suspensions in the scale of litres.

The development of the desired generally applicable production process was based on the already known method of liposome production by homogenisation with low pressure extrusion (Ámselem et al., 1990).

Together with the company Schleicher & Schuell, a multi-stage-pressure-filtration-device mainly made of stainless steel was developed:

- The device consists of various filtration stages each having an individual volume of 2000 ml.
- The stages can be used alone as well as in a row.
- The pressure necessary for filtration is transmitted by an inert gas onto the filters. Each

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PII: S0378-5173(99)00410-X

filtration stage can be connected separately with the gas supply by a pressure tube.

- Polycarbonate membranes with a diameter of 142 mm are used as filtermaterial (Ámselem et al., 1993). The size of the filterpores can be chosen arbitrarily.
- In order to meet the demands of the pressure stability and to allow a thorough and easy cleaning of the device, most of its components are made of stainless steel. The sealing rings are made of silicone. All parts of the device can be sterilised in an autoclave.
- In general it is recommended to filtrate a liposome suspension with a temperature above its transition state temperature in order not to destroy the membranes of the liposomes during filtration. But due to the great variety in the composition of liposomes there is a large range of possible transition state temperatures (Talsma and Crommelin, 1992). Consequently, the device had to be equipped with a facility for controlling the temperature of suspensions to be filtrated. To solve this problem, each filtration stage is equipped with a double coat flown through by warm water, which allows to temper the device and, consequently, the liposome-suspension. The tempering works with a circulating pump allowing control of water temperature.
- Because of the stainless steel body of the filtration stages there is a loss of temperature which can vary in dependence on the number of filtration stages connected in a row. Consequently, a temperature control of every filtration stage is vital. In order to provide such control, each filtration stage can be equipped with two sensors for the check of temperature.

The benefits of the device for the production of MLV-suspensions has been demonstrated in several productions of batches containing liposomes consisting of phosphatidylcholine, cholesterol and ascorbylpalmitate.

Table 1
Overview of the volume shares of unilamellar to multilamellar vesicles after pressure filtration[a]

Vesicles	Size of filterpores		
	0.4 μm	0.8 μm	2.0 μm
Unilamellar	51.78	26.08	1.76
Multilamellar	48.22	73.92	98.24

[a] The values are given in volume percentage.

Because it turned out that a final sterilisation of the MLV-suspensions was impossible, in all steps of this batch productions aseptical conditions were assured.

At first the original substances phosphatidylcholine, cholesterol and ascorbylpalmitate were solved in ethanol (Dürr et al., 1994). The ethanolic solution was afterwards sterilefiltrated through a 0.2 μm filter.

All the following steps were carried out under clean room conditions.

The ethanolic solution was vaporized in a flask with a vacuum evaporator.

The so gained lipidfilm was disperged with aqua ad iniectabilia at a temperature of 65°C and afterwards shaken for approximately 24 h.

To show the flexibility of the device regarding the production of MLV, 500 ml of the gained inhomogeneous suspensions of vesicles were filtrated through differently sized polycarbonate membranes.

The particle size distributions of the vesicles in the filtrated liposome-suspensions are shown in Table 1. For the particle size measurements the LS 230 from the Beckmann/Coulter Company was used.

Vesicles with a diameter < 150 nm are called unilamellar. All the vesicles with a diameter > 150 nm are denoted as being multilamellar.

The results mentioned in the listing have been achieved

- one filtration with a filter-size of 2.0 μm and
- with filter-sizes of 0.4 and 0.8 μm after several filtration steps, respectively.

At a storage temperature of 4°C and with light exclusion, the aqueous liposome-suspensions produced with the multi-stage-pressure-filtration-device are chemically stable as well as size stable over a period of at least 6 months.

In summary, it has been shown that it is possible to produce liposome-suspensions with a different amount of multilamellar liposomes in the scale of litres under aseptical circumstances with a newly constructed multi-stage-pressure-filtration-device.

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