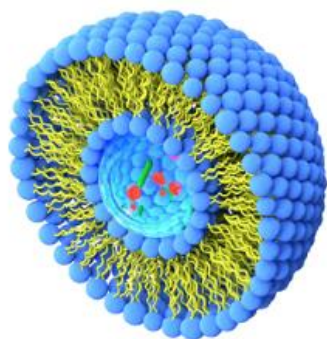


Application Note

Microfluidizer® Technology in Liposome production



INTRODUCTION

Liposomes are spherical lipid vesicles consisting of one or more bi-layered membrane structures. Phospholipids are most often used to form liposomes. Because of their structure - aqueous cores surrounded by lipid bi-layers - liposomes have the unique advantage (compared to other lipid based delivery systems) in that they can also encapsulate and deliver hydrophilic actives including nucleic acids. In addition, liposomes can be formulated to enhance immunogenicity and serve as vaccine adjuvants.

Many methods exist to make liposomes in small quantities, but only a few methods are realistic for production scale manufacturing.

Ultimately the method choice depends on the lipids, time, budget and quality of lipids needed, and the active ingredients to be encapsulated.

Microfluidizer® Technology in Liposome production

CHOICE OF PREPARATION TECHNOLOGIES

Liposome preparation methods fall into either the *top-down* approach, based on the lipid hydration method, or the *bottom-up* approach, which usually involves mixing solvent and non-solvent streams and uses precipitation to create liposome vesicles with the desired size and lamellarity.

Lipid hydration method (Top-down)

The traditional top-down method generates either a thin film or homogeneous mixture of lipid and lipophilic active ingredients and is then hydrated with aqueous solution.

Since phospholipids generally self-assemble into bi-layered structures under hydration and form heterogeneous large unilamellar vesicles (LUVs) and multilayered vesicles (MLVs), this means that various mechanical techniques are needed to perform size reduction or conditioning to the primary size of the vesicles in order to achieve desired size and lamellarity.

Solvent injection method (Bottom-up)

Bottom-up methods, on the other hand, utilize precipitation to directly generate liposome vesicles. The liposome forming ingredients are dissolved in an organic solvent, usually water miscible, and then injected slowly into an aqueous buffer system. Upon injection, small unilamellar vesicles (SUVs) and/or LUVs are formed due to the change of solubility conditions in the phospholipid solution through the replacement of lipid-solubilizing solvent(s) by non-solvent aqueous media.

Devices using this principle commonly use various types of microfluidics chips.

ALTERNATIVE APPROACHES USING MICROFLUIDIZER TECHNOLOGY

Lipid solution method

Microfluidizer processors can also produce liposomes without making the lipid film first. There are a couple of alternative ways to produce liposomes from lipid solutions using Microfluidizer technology:

- All lipid and lipophilic ingredients are dissolved in water miscible solvent such as ethanol then added to the aqueous phase. It is pre-processed with a low shear mixer, and then passed through the Microfluidizer processor^[1]. This is similar to the solvent injection method, but differs in the vesicle formation mechanism since this method creates MLVs during the pre-mix step and then SUVs through the Microfluidizer process. Note that the solvent will still need to be removed after processing.
- Hydrophilic active encapsulations via double emulsion method. An aqueous phase (W1) containing hydrophilic drugs is mixed with the lipid solution (O) (prepared by dissolving all lipid ingredients into water immiscible solvent) to form a water-in-oil (W1/O) emulsion. The W1/O emulsion is subsequently mixed with a second aqueous (W2) to form the W1/O/W2 emulsion. Both emulsions can be produced with the Microfluidizer processor. After removing the solvent, liposome vesicles are obtained inside the final solution. This method can achieve very high encapsulation efficiency. A recent publication reported about 80% successful encapsulation of plasmid DNA inside two different cationic liposome formulations^[2].

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Solvent free method

This novel method works on some liposome formulations and does not require forming either the lipid films or solutions, and is hence completely free of organic solvent.

Liposomes are produced by directly mixing the dry lipid ingredients as well as lipophilic actives with aqueous buffer solutions followed by down-sizing the particles using Microfluidizer processors.

The methodology featured in formulations mimicking two commercialized liposomal drug products were produced to achieve vesicle size within clinically appropriate range as well as high drug loading^[3].

WHY MICROFLUIDICS?

High quality liposomes - reliable and uniform shear is generated by the fixed-geometry Interaction Chamber™ and constant pressure pumping system of a Microfluidizer processor effectively producing uniform small unilamellar - vesicles (SUVs). In general, Microfluidizer technology is able to reduce the liposome size down to as small as ~40 –50 nm.

High Concentration - Microfluidics produces liposome solutions with therapeutic level, high concentrations directly, without the need for further downstream processes to concentrate the product. The lipid concentration ranges between 5mM and 25mM^[1]. Microfluidizer technology can produce liposomes with lipid concentrations well within or above the ready-to-be-administered liposomal drug, which is usually one or two orders of magnitude higher than can be achieved from the solvent injection method.

Very fast size reduction of liposome vesicles leads to short process times, especially compared to slow processes such as extrusion or microfluidic chip techniques. A lab scale Microfluidizer processor produces between 1 to 3 liters every 10 minutes. Production units produce hundreds of liters per hour.

Scalability must be considered when moving from preclinical to clinical or even production scales. Most alternative methods are not scalable. For many decades Microfluidizer technology has been shown to be inherently scalable, in quantities up to thousands of liters, producing therapeutic concentrations at any scale.

Solvent independent - solvent is not generally required during the Microfluidizer size reduction process, which means no additional solvent removal process is needed. However if your formulation process requires solvent, that is no problem for the Microfluidizer processor as they are compatible with all commonly used solvents.

cGMP ready systems with CIP and SIP capabilities. Microfluidizer processors are designed for compliance with cGMP and are capable of CIP/SIP operations.

Flexibility - liposomes of different sizes and formulations can be made with the same Microfluidizer processor by varying operational parameters. The size of liposomes can be predicted.

Sterile Filtration -Studies completed with Pall Life Sciences confirm that liposomes produced using a Microfluidizer processor can be filtered successfully through a 220nm filter^[4].

Microfluidizer® Technology in Liposome production

CHOOSING THE RIGHT METHODOLOGY

The pros and cons of the different techniques are summarized in the table below:

METHOD		PROS	CONS
LIPID HYDRATION FOLLOWED BY MECHANICAL SIZE REDUCTION	Microfluidizer Processor	<ul style="list-style-type: none"> • Ease of use • Very efficient & fast size reduction • Smaller & more uniform particle size (vs HPVH, Ultrasonication) • Solvent not necessary • Easier temperature control • Guaranteed scalability 	<ul style="list-style-type: none"> • Use of high shear & certain degrees of temperature rise may affect encapsulated biologics
	Extrusion	<ul style="list-style-type: none"> • Small vesicles & narrow size distribution • Mild pressure & less shear • No temperature changes 	<ul style="list-style-type: none"> • Tedious & unreliable • Only scalable to certain limits • Loss of product - leaking & membrane clogging • Can't completely eliminate pre-existing MLVs • Drug loaded into membrane may impair membrane flexibility reducing ability to extrude
	High Pressure Valve Homogenizing	<ul style="list-style-type: none"> • Next best alternative to Microfluidizers • Only other alternative for larger volumes 	<ul style="list-style-type: none"> • Lower shear & pressure variation lead to inhomogeneous large particles, thus usually requires more passes • High maintenance e.g. wear of valves • Indirectly scalable • Cooling & cleaning issues
	Ultrasonication	<ul style="list-style-type: none"> • Very high cavitational forces near the probe tips 	<ul style="list-style-type: none"> • Localized overheating & poor temperature controllability • Non-uniform shear forces • Localized shear leads to destruction of bilayers • Very low encapsulation efficiency • Lack of scalability • Metal contamination from probe tips
SOLVENT INJECTION	Microfluidic Chip (Lab on a chip)	<ul style="list-style-type: none"> • Low shear • No temperature rise • Narrow distribution • Controllable particle size • Scale up with parallel chips to moderate volume 	<ul style="list-style-type: none"> • Use of organic solvent • Produces very dilute liposomal solutions • Diffusion governed process is inherently slow (must maintain laminar flow) • Additional dilution required to stabilize particles • Extra steps to remove solvent/concentrate product (difficult to remove ethanol) • Solvent/chip compatibility issues (for chip technology) • Heating control is challenging • Industrial scale difficult (need to precisely control flow rates & ratios) • Cost of ownership (one-time use chips)
	Cross-flow Nozzle Injection		

Microfluidizer® Technology in Liposome production

CASE STUDY 1 – COMPARING TO THE BOTTOM-UP METHOD

In this case study, Microfluidizer technology is compared with the bottom up method that uses microfluidic chip in preparing liposomes. A high transition temperature liposome formulation was prepared via both methods, each under the optimized conditions.

Results

Liposome characterizations are shown below in Table 2 and Figure 1. Table 2 shows that both methods were able to produce small (< 100 nm) liposomes with very tight distribution (PdIs close to 0.1), but the Microfluidizer processor was able to achieve even smaller vesicle sizes and much smaller variation across the board.

Method	Z-Average (nm)	PdI	Zeta potential (mV)
Bottom up	95.3 ± 3.3	0.12 ± 0.03	-37.1 ± 2.6
Microfluidizer processor	60.9 ± 0.3	0.11 ± 0.01	-43.3 ± 2.3

Table 2 Characterizations of liposome produced by Microfluidizer processor vs. bottom up method.

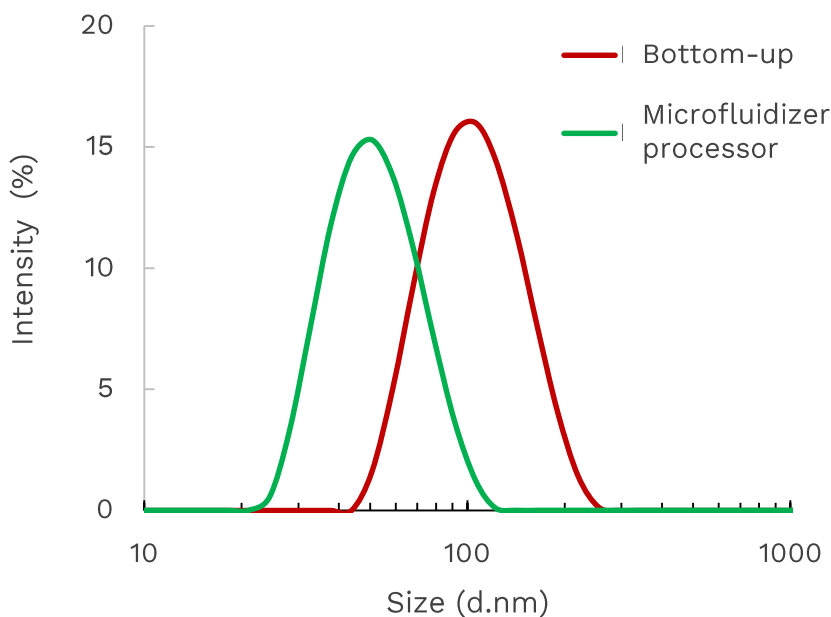


Figure 1 Particle size distributions of liposome produced by Microfluidizer processor vs. bottom up method.

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CASE STUDY 2 – COMPARE TO HIGH PRESSURE HOMOGENIZER (HPH)

In this case study, Microfluidizer technology is compared with traditional high pressure homogenization method in preparing liposomes. A liposome formulation was processed through a Microfluidizer processor and a HPH under identical conditions in terms of pressure and number of passes.

Benefits of fixed geometry & constant pressure

Microfluidizer technology uses a fixed geometry high shear zone and constant pressure pumps. As can be seen from Figure 2, this unique combination allows the Microfluidizer processor to achieve constant process pressure, hence constant shear forces, during the majority of each processing cycle, while HPH generates variable pressures with as much as 50% lower from the targeted pressure. The constant pressure delivered by the Microfluidizer technology ensures that every microliter of product gets the same treatment from 1ml to thousands of liters.

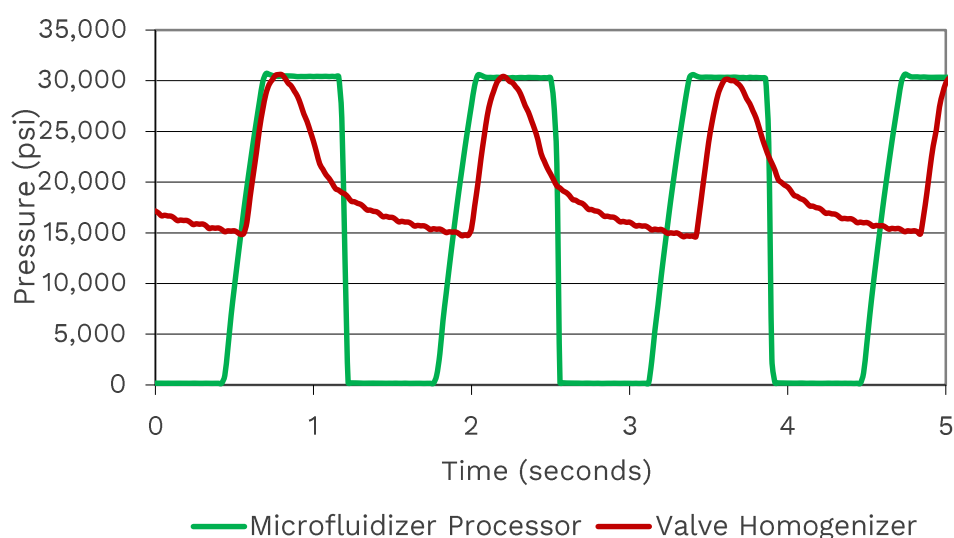


Figure 2 Pressure profiles of Microfluidizer processor vs. homogenizer. Note the homogenizer only peaks at set pressure whereas the Microfluidizer processor processes most of the sample at the set pressure.

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CASE STUDY 2 – COMPARE TO HIGH PRESSURE HOMOGENIZER (HPH) - *continued*

Benefits of fixed geometry & constant pressure - *continued*

The result of the uniform treatment is the small and uniform particle size and distribution showed in Figure 3 and Table 3.

Figure 3 shows the mono-distribution of liposome particles produced by the Microfluidizer processor, whereas liposome produced by HPH under the same conditions has a broad particle size range as indicated by the multi-modal distribution curve.

Table 3 shows the average liposome particle size and proves that Microfluidizer technology is much more efficient in reducing liposome sizes. In this case, Microfluidizer process was able to generate liposomes less than 100 nm after just 2 passes. On the other hand, particle size achieved by HPH even after 3 passes (183 nm) was still much larger than just 1 pass (113 nm) after processing through Microfluidizer processor.

	Microfluidizer processor	Valve Homogenizer
1 Pass	113 nm	268 nm
2 Passes	95 nm	228 nm
5 Passes	72 nm	183 nm

Table 3 Average liposome particle size produced by Microfluidizer processor vs. homogenizer.

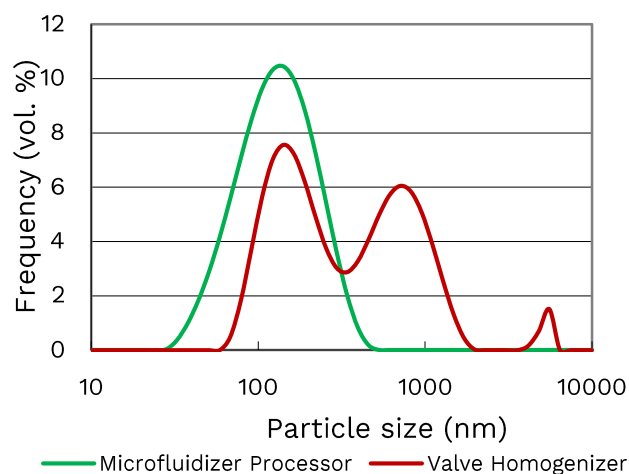


Figure 3 Microfluidizer processor produces small narrow PDI unimodal liposomes compared to the homogenizer method

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