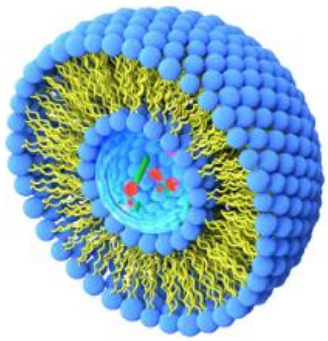
Microfluidics™

Application Note

Microfluidizer® Technology for solvent-free production of liposomes



INTRODUCTION

Liposomes are versatile nanoparticles that are commonly used for delivering both Active Pharmaceutical Ingredients (API) and vaccines.

Traditional methods of creating liposomes involves using solvents which have to be removed, causing additional steps in the processing and extra health & safety considerations.

A new process has been developed that can produce liposomes without the use of solvents. It has been described in a published scientific paper: *Scalable solvent-free production of liposomes*[1] The paper lays out a path for producing liposomes in a greener way which is capable of being scaled for mass-production scale, whilst maintaining high drug encapsulation levels.

This paper summarizes the key points from the scientific paper.

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THE PROBLEM

One of the major issues associated with conventional liposome production is the use of organic solvents in most commonly used liposome preparation methods.

Traditional top-down methods, based on lipid hydration, require a homogenous lipid mixture to be prepared first. This is usually done by dissolving all lipid ingredients in solvents. These solvents must then be removed prior to any subsequent processing steps.

Bottom-up methods require injecting or mixing a lipid in a solvent solution with an aqueous phase during the formation of vesicles.

Regardless of method, the use of organic solvents is not ideal. This is due to potential safety and health concerns since most solvents are flammable or explosive, and can be carcinogenic, and neurotoxic. Furthermore, they can lead to wider health risks through potential environmental contamination if not handled or disposed of properly.

The maximum acceptable amounts for solvents in pharmaceuticals are defined by the *International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q3C guidelines*[2]. According to these guidelines, solvent commonly preferred by liposome manufactures such as chloroform and methanol are class 2 solvents, which should be limited due to their inherent toxicity.

Even solvents considered to have low toxic potential, such as ethanol, which is widely used in the bottom-up methods, can not exceed 5000 ppm or 0.5%. This obviously adds additional burdens on manufacturing processes to remove these residual solvents and achieve concentrations below these limits.

THE SOLUTION

Researchers at the University of Strathclyde have developed a more sustainable and easy-to-adopt manufacturing method that does not require the use of organic solvents.

This novel solvent-free process is achieved using Microfluidizer® technology. In this process liposomes were manufactured by adding powdered lipids to aqueous buffers without organic solvents and then processed using a Microfluidics M110P benchtop unit in order to achieve uniform and efficient vesicle size-reduction. Thanks to the unique Interaction Chamber™ technology within each machine, the whole process can be easily scaled up for mass-production.

The solvent-free process was demonstrated on two commonly made liposomal formulations: *doxorubicin-loaded PEGylated liposome* and *amphotericin B-loaded liposome*.

In both formulations, the target particle size was around 100-110 nm with low polydispersity index (PdI<0.2) and high encapsulation efficiency (>90%).

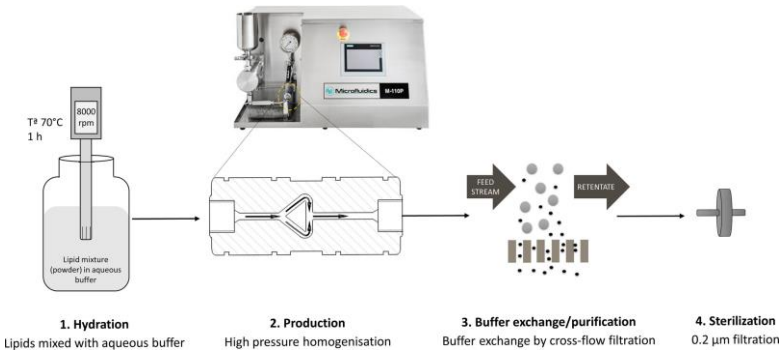

Figure 1 –Schematic representation of liposome manufacture, buffer exchange/purification, drug loading and sterilization.

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THE RESULTS

Results shown in Table 1 below indicate the target physicochemical characteristics that were successfully achieved for both liposomes. These match to products that are already clinically approved.

The morphology showed in Figure 2 confirms the uniformity of the small unilamellar liposome vesicles along with the drug crystals encapsulated for doxorubicin-loaded liposome.

The researchers also demonstrated that the liposome particle size, PDI and drug loading remain unchanged throughout the manufacturing steps. This included tangential flow filtration for buffer exchange and sterile filtration through 0.22 µm filters.

Finally, the drug-release testing of the doxorubicin-loaded liposomes using the USP-4 dissolution apparatus proved the desired slower release profile - 60% doxorubicin release after 6h vs. 100% release after 2h for free drug, for the PEGylated formulation.

REFERENCES

References taken from:

[1] S. Khadke, et al., J. Pharm. Pharmacol., 2020, 72

[2] ICH Harmonised Guideline Q3C(R6)

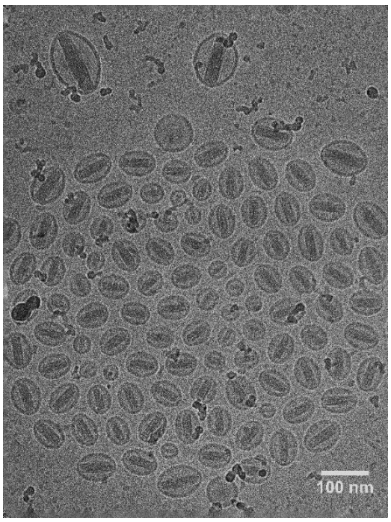

Figure 2 –CryoTEM image of doxorubicin-loaded liposome vesicles produced by a Microfluidizer® processor using the solvent-free approach

	Doxorubicin-loaded liposome	Amphotericin B-loaded liposome
Particle Size (nm)	95	106
PDI	0.19	0.2
Zeta Potential (mV)	-1.5	-40
Drug Loading (%)	95	98

Table 1 – Physicochemical characterizations of doxorubicin-loaded and amphotericin B-loaded liposomes prepared via the solvent-free method