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Preparation of liposomes at large scale using the ethanol injection method: Effect of scale-up and injection devices

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ABSTRACT

In the present study, the preparation of liposomes using the ethanol injection technique was reported for volumes from 60 mL to 3 L. Experimental set-up with different scales and injection devices have been tested: (1) a device using a syringe for injection; (2) an experimental set-up using two pumps for injecting both organic and aqueous phases through a Shirasu Porous Glass (SPG) membrane; (3) a pilot plant using a tube dipping into the aqueous phase for injection of the organic phase; (4) a pilot plant using two pumps for injection of both organic and aqueous phases through the SPG membrane. The liposome preparations obtained were characterized for their size, polydispersity index (PDI), zeta potential, and morphology by transmission electronic microscopy (TEM). Two formulations have been tested: drug-free liposome formulation and α-tocopherol-loaded liposome formulation. For both formulations, it was shown that all experimental set-ups were appropriate for the preparation of small vesicles having narrow size distributions, with good reproducibility and stability. The pilot plant using a membrane for injection improves micromixing of the organic phase into the aqueous phase and therefore optimizes the preparation time. Evaporation of ethanol performed directly on the pilot plant has the advantages of being an easy and continuous process.
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Keywords: Liposome; Ethanol injection; Pilot plant; Scaling-up; SPG membrane; Injection device

1. Introduction

Liposomes can incorporate different compound types into their structure due to their inner aqueous core and the amphiphilic characteristics of the lipids which form their outer membrane. Their other characteristics contributing to their industrial success are their ability to decrease drug toxicity, increase drug circulation lifetime, and accumulate at specific sites. Therefore, over the past few years, liposomal drug preparations have been developed from laboratory research to clinical applications, such as Doxil, Myocet, Visudyne (Allen and Cullis, 2013). Not only the pharmaceutical area benefits from liposomes, but also food and cosmetic industries.

Since the pioneering discovery of liposomes by Bangham et al. (1965), many techniques have been reported for liposome preparation including mechanical methods (preparation by film methods, homogenization techniques such as sonication, microfluidization, extrusion), methods based on replacement of organic solvents by aqueous media (ethanol injection method, proliposome–liposome method, reverse-phase evaporation), and methods based on detergent removal (Wagner and Vorauer-Uhl, 2011). However, many of these methods tend to be unsuitable for large scale production. Sonication produces liposomes of varying sizes and is not easily scaled-up. High pressure extrusion by means of a homogenizer or microfluidization (Schneider et al., 1994; Sorgi and Huang, 1996) has been shown to produce reproducible samples

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which are scalable to production size batches. However, the film method required prior to these techniques is difficult to scale-up. Extrusion through membranes of decreasing pore size is also a popular technique for the preparation of liposomes. Polycarbonate membranes are largely used (Wagner and Vorauer-Uhl, 2011), although other membranes have been tested such as SPG membranes (Hwang et al., 2011). Typically, a crude liposome preparation is obtained by film hydration, and then extruded through membranes of decreasing pore size to control liposome size and size distribution. This technique appears also quite difficult to scale-up due to the film hydration method required prior extrusion.

The ethanol injection method is an interesting technique for scaling-up liposomes production. It offers several advantages, e.g., simplicity, fast implementation, and reproducibility as well as the fact that it does not cause lipid degradation or oxidative alterations. The ethanol injection method was first reported in the early 1970s by Batzri and Korn (1973) as one of the first alternatives for the preparation of small unilamellar vesicles (SUVs) without sonication. The method was then extended to other formulations and substances to be incorporated leading to a large range of vesicle sizes (Stainmesse et al., 1992). By the immediate dilution of the ethanol in the aqueous phase, the lipid molecules precipitate and form bilayer planar fragments (Lasic, 1995). Through energy dissipation in the system (by stirring and/or ultrasonication), the fragments of these lipid bilayers tend to decrease the exposure of the hydrophobic parts of their molecules to the aqueous environment, resulting in the curvature of these fragments which take a quasi-spherical structure. In the following years, several studies have investigated the preparation parameters of the ethanol injection technique (lipid concentration and composition, injection velocity, temperature of both phases, stirring rate, etc.) on liposome characteristics (size distribution, zeta potential, drug encapsulation efficiency, etc.) (Kremer et al., 1977; Domazou and Luisi, 2002; Jaafar-Maalej et al., 2010; Justo and Moraes, 2011).

From the 1990s, the ethanol injection technique has been implemented at industrial scale for liposomal encapsulation of econazole, an imidazole derivative for the topical treatment of dermatomycosis (Naeff, 1996). Very large batches were reported from 0.5 to 12 kg. The principle of the ethanol injection method was combined to high shear homogenization, which has the disadvantage to bring a supplementary step to the processing method. In the 2000s, several preparations at large scale have been proposed introducing new devices for ethanol injection. For example, Wagner et al. (2002, 2006) and Wagner and Vorauer-Uhl (2011) developed a continuous system which permitted liposome preparation regardless of production scale. The crossflow injection device was made of two tubes welded together forming a cross presenting an injection hole at the connecting point. The organic phase (ethanol, phospholipids and eventually cholesterol and drug) was injected into the aqueous phase through the crossflow injection device. Liposome size was controlled by the local lipid concentration at the injection point depending on process parameters such as injection pressure, lipid concentration and injection rate. Membrane contactors in a tubular (Jaafar-Maalej et al., 2010) or hollow fibre configuration (Laouini et al., 2011) have also been implemented for preparation of liposomes entrapping pharmaceutical or cosmetic agents. In this technique, the ethanol was injected into the aqueous phase through the membrane pores. Akamatsu et al. (2013) also proposed an ethanol injection method through SPG membranes. However, theses authors prepared a lipid thin film prior alcohol injection, which could make the technique difficult to scale-up.

In addition, two different strategies have been proposed for scaling-up: a cross flow recirculation of the aqueous phase across the membrane surface and low frequency oscillation of the membrane surface (~40 Hz) in a direction normal to the flow of the injected organic phase (Laouini et al., 2013b). In a recent study, Zhong et al. (2013) also proposed a scalable method using a microfluidic device for injection. The method consisted of three stages: (1) cross-flow injection using a special Y connector, (2) ultrafiltration for the removal of organic solvents and concentration, and (3) high-pressure extrusion through a polycarbonate membrane to control liposome size. The common point of these injection techniques is a high micromixing efficiency of both phases at the injection point, which is favourable to bilayer planar fragments formation required prior to vesicles preparation.

Final volumes obtained using the ethanol injection method were often lower than 1 L, for example: 10.75 mL (Pons et al., 1993), 30 mL (Pham et al., 2012; Jaafar-Maalej et al., 2010), 600 mL (Laouini et al., 2013b), and up to 800 mL (Justo and Moraes, 2011). Using a crossflow injection device, Wagner et al. (2002) prepared 2.4 L liposomal suspension. In addition, very large volumes were discussed in an economic evaluation, but no experimental data were presented (Justo and Moraes, 2010). The investment and production cost of a liposome production plant was based on assumptions regarding production volume and feedstock to demonstrate the features and economic benefits of the implementation of this process on an industrial scale. The results of the economic analysis suggested that the process was economically feasible for a plant with a daily production capacity of 288 L of liposomal suspension.

In the present study, the preparation of liposomes using the ethanol injection technique was reported for volumes up to 3 L. Experimental set-up with different scales and injection devices have been tested: (1) a device using a syringe for injection ($V = 60$ mL); (2) an experimental set-up using two pumps for injecting both organic and aqueous phases through a SPG membrane ($V = 600$ mL); (3) a pilot plant using a tube dipping into the aqueous phase for injection of the organic phase ($V = 3$ L); (4) a pilot plant using two pumps for injection both organic and aqueous phases through a SPG membrane ($V = 3$ L). Evaporation of ethanol was performed directly on the pilot plant under reduced pressure or using a rotavapor after collecting the preparation in an evaporation flask. Evaporation performed directly on the pilot plant could be scalable; on the contrary, the rotator method could be difficult to be applied to large volumes. In the present study, the rotavapor method was used for comparison to evaporation on the pilot plant.

The liposome preparations obtained were characterized for their size, polydispersity index (PDI), zeta potential, and morphology by transmission electronic microscopy (TEM). Two formulations have been tested: drug-free liposome formulation and α-tocopherol-loaded liposome formulation. The interest of the pilot plant is to translate the preparation at laboratory scale to semi-industrial scale, but also to make the entire process (including the evaporation of ethanol) on the same installation. This avoids eventual contamination related to transfer of the preparation for ethanol evaporation using a rotary evaporator.

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