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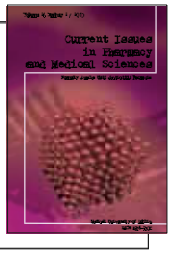

Methods of nanoliposomes preparation

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ABSTRACT

During the last decade, nanoliposomes have attracted great interest of many researchers. This was caused by unusual properties of these molecules, such us their nanosize, biocompatibility, and biodegradability. Due to these properties, they can be widely applied in many fields, especially as carriers of active substances in cosmetics, food technology, agriculture, and nanotherapy. The application of nanoliposomes as drug nanocarriers in medicine allows for more effective treatment of many diseases (e.g. cancers, atherosclerosis, ocular diseases) [1,6,7]. The advantage of these nanocarriers used in medicine is cell-specific targeting, which is indispensable to attain drug concentration, requisite for optimum therapeutic efficacy in the target site, with accompanying side effects limited to the minimum. Furthermore, the efficacy of treatment with these carriers comes from enhancing bioavailability, improving controlled release, and enabling precision targeting of the entrapped compounds, due to a larger increased surface area [7]. The aim of this paper is to present the current knowledge on the conventional methods of preparation of nanoliposomes, including microfluidization, extrusion, sonification technique, with particular emphasis on the advantages and disadvantages of each of these methods.

Keywords: nanoliposomes, liposomes, nanotherapy, nanocariers

INTRODUCTION

Nanoliposomes are bilayer lipid vesicles, whose diameter ranges from ten nanometers to several micrometers. The major ingredients of nanoliposomes are lipid and/or phospholipid molecules. Furthermore, these vesicles may contain sterols in their structure. The most commonly used sterol during the production is cholesterol, which leads to increasing the stability of the vesicles by modulating the fluidity of the lipid bilayer [10]. When the phospholipids are placed in aqueous environment, they form aggregated complexes, where the hydrophobic region avoids contact with water, and the hydrophilic group are directed towards aqueous phase [13]. Delivering sufficient energy to phospholipids, can lead their arrangement in the form of organized, closed bilayer vesicles, such as nanoliposomes [10]. This process can be used to incorporate drugs, vitamins, nutrients to the structure of nanoliposomes. In this article commonly used methods of nanoliposomes preparation are disscused.

Selection of a suitable method of nanoliposomes preparation is not arbitrary and depends on the following parameters: 1) physicochemical properties of the medium in which the lipid vesicle are scattered; 2) shelf-life, optimum size, and polydispersity of the vesicles; 3) potential toxicity, effective concentration substances which are entrapped in nanoliposomes [13].

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MICROFLUIDIZATION

Nanoliposomes are prepared through microfluidization method by using a microfluidizer, which is shown in (Fig.1). The nanoliposomes production method is performed without toxic solvents, simultaneously using high pressure [13]. In this device, the basic principle of operation is based on dividing a pressure stream into two parts, passing each part through a fine orifice, and directing the flows at each other inside the chamber of the microfluidizer [4,10]. Inside the interaction chamber, cavitation, along with shear and impact, reduces the size of particles [10]. The described method has several advantages, the most significant of which are as follows: possibility of obtaining very high efficiencies capture (above 75%), molecules can be produced a large scale in a continuous and repeatable manner. The dissolvent substances which are encapsulated are not prone to sonification, detergents or organic solvents [4]. The negative side of this method involves the use of manufacturing process with very high shearing forces, which in effect contribute to damaging

the structure of the material to be encapsulated [13]. Other relevant drawbacks of the microfluidization method are contamination, material loss, and the relative difficulty with scaling up [13]. The carried out stages are indicated in Tab. 1.

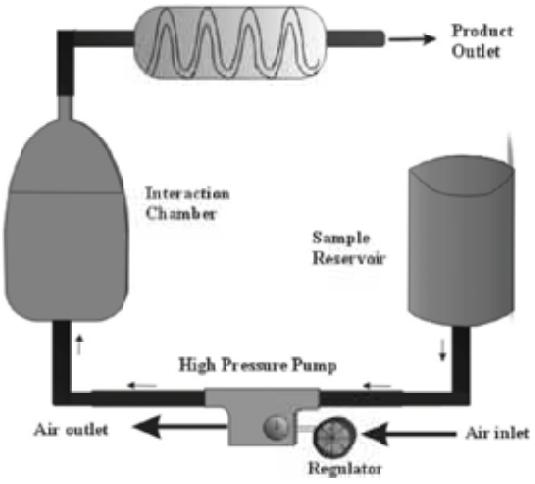

Fig. 1. Microfluidizer apparatus [10]

Table 1. Microfluidization process stages [4,10]

1	Selection of a suitable suspension medium and components of the nanoliposomes- it depends on the intended application.
2	Placement of nanoliposomes ingredients in the suspension medium, followed by the precise mixing of the sample by stirring.
3	Placing the obtained in the previously step suspension in a reservoir, and setting appropriate process parameters such as flow rate air intake, and operating pressure, the size of interaction chamber, and the number of passes.
4	After setting process parameters, the air valve is opened, which in turn leads to liquid dispersion which flows through a filter into the interaction chamber.
5	The dispersion is split in two streams in interaction chamber and they interact at extremely high velocities in dimensionally defined microchannels.
6	Afterwards, the obtained suspension can be recycled through the equipment (several passes occur during nanoliposomes production, usually 3 or 4 before receiving final product). Before the next passing, the suspension must be cooled, on account of temperature increasing in interaction chamber.
7	In the final step, nanoliposome suspension leaves the device at temperature above transition phase in inert atmosphere such as nitrogen or argon for 1 h, which ultimately leads to annealing and stabilizing of sample.

EXTRUSION

Preparation of nanoliposomes by using extrusion method involves the use of fine-pore filters, whose size depend on volume preparation particles [7,8]. The fundamental principle of the method is physically extruding the vesicles under pressure via polycarbonate filters of defined pore sizes [7,8]. Fig.2 presents an extruder, which is used for manufacturing nanoliposomes preparation. The subsequent stages to nanoliposomes preparation by using extrusion methods are indicated in Tab. 2.

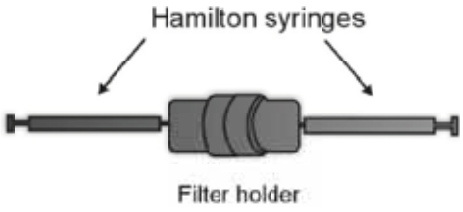

Fig. 2. Extruder [10]

Table 2. Extrusion technique steps [4,10]

1	Preparation of a sample consisting of micrometric liposomes (MLV), which are modified for the next stages of the liposomes.
2	Placement of one or two-stacked polycarbonate filters into the stainless steel filter-holder of the extruder (see Fig.2) It is essential to assemble extruder apparatus fully before inserting in the heating block.
3	Placing the extruder stand/heating block onto a hot place, and then inserting a thermometer into a wall in the heating block. The liposome suspension should be kept above the phase transition temperature of the lipids, for this purpose the hot plate needs to be switched
4	Filling the syringe fully with buffer, and then passing through the extruder; the aim is to reduce the dead volume. In the following step, the buffer is removed.
5	Putting the liposome suspension into one of the syringes (donor syringe) of the extruder; subsequently carefully placing the syringe into one end of the device by applying a gentle twisting. In the next stages, placing the second syringe (receiver syringe) into the other end of extruder, moreover before starting next stages, make sure that receiver syringe plunger is set to zero.
6	Placing the fully assembled device into the extruder stand. Follow inserting the stainless-steel hexagonal nut in such way that any two opposing apexes fall in the vertical plane [4,10]. In order to ensure good thermal contact between syringes and heating block, swing-arm clips should be used .
7	Within about 5-10 minutes temperature of nanoliposomes suspension reaching the temperature of the heating block.
8	The contents of the filled syringe is transferred to the empty syringe by pushing the plunger slightly and slowly. The next step includes gently pushing the plunger of the alternate syringe to transfer the suspension back into the original syringe.
9	In order to obtain nanoliposomes of the desired properties, extrusion process should be repeated. The number of passages through filters is always odd, the minimum number of passes is seven.
10	After the end of extrusion, the extruder should be taken out of the heating block, and then the filled syringe should be removed from the extruder. The obtained nanoliposomes sample should be injected into a clean vial.
11	In a subsequent stage, the extruder components can be cleaned first by rinsing with ethanol, and then with distilled water.
11	The final step includes keeping nanoliposomes at temperature above transition phase under inert atmosphere such as nitrogen or argon for 1 h, which ultimately leads to annealing and stabilizing the sample.

SONICATION TECHNIQUE

Sonification technique is widely used for the manufacture of nanoliposomes. This method involves treating hydrated vesicles for several minutes with a titanium-tipped probe sonificator in a temperature-controlled environment. Successive stages of producing nanoliposomes by using sonification technique are presented in Tab. 3.

Table 3. Sonification technique steps [4,10]

1	Dissolving the appropriate combination of the phospholipid components (with or without cholesterol), in either chloroform or in chloroform--methanol mixture [4,10].
2	The resultant mixture should be filtered to remove minor insoluble components. Additionally, ultrafiltration is used to reduce or eliminate pyrogens.
3	Transfer of the solution to a flask (pear-shaped or a round-bottom), followed by placing it in a rotary evaporator. The aim is to remove the solvents at temperature above phase transition under negative pressure. This procedure is performed until a thin layer of dried lipid components in the flask is obtained.
4	Removal of traces of the organic solvents using a vacuum pump or flushing the flask with an inert gas (e.g. nitrogen or argon).
5	Adding glass beads (e. g with 500 μm diameter) into a flask containing dry lipids and then adding a suitable aqueous phase such as distilled water or buffer.
6	The dried lipids can be dispersed into the hydration fluid by the vortex mixing for 1-5 minutes or hand-shaking the flask. In this stage, micrometric (MLV) type liposomes are obtained.
7	In the next step, flask containing MLV is transferred to the probe (tip) sonicator (see Fig.3.) For probe sonication, place the tip of the sonicator in the MLV flask and sonicate the sample with 20 s ON, 20 s OFF intervals, for a total period of 10-15 minutes.
8	After sonication is carried out properly, we receive the final product-nanoliposomes.

CONCLUSION

Nanoliposomes can be widely applied in many fields, especially as carriers of active substances in cosmetics,

food technology, agriculture, and nanotherapy. Procedures described above make it possible to obtain nanoliposomes with the desired sizes, and properties. Selected type of conventional methods of preparation of these particles, largely depends on properties of substances which are incorporated into this structure. It is worth emphasizing that these methods have numerous advantages, such as that particles can be produced on a large scale in a continuous and repeatable manner, ease and simplicity of carrying out methods, and high efficiency of the process. Another major point regarding methods preparation of nanoliposomes are limitations resulting from the inability of adjusting this method from small to industrial scale. Moreover, high shear forces and potentially toxic solvents used during nanoliposomes production give start to new methods, especially- heating methods.

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