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Review

Progress involving new techniques for liposome preparation

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ABSTRACT

The article presents a review of new techniques being used for the preparation of liposomes. A total of 28 publications were examined. In addition to the theories, characteristics and problems associated with traditional methods, the advantages and drawbacks of the latest techniques were reviewed. In the light of developments in many relevant areas, a variety of new techniques are being used for liposome preparation and each of these new technique has particular advantages over conventional preparation methods. However, there are still some problems associated with these new techniques that could hinder their applications and further improvements are needed. Generally speaking, due to the introduction of these latest techniques, liposome preparation is now an improved procedure. These applications promote not only advances in liposome research but also the methods for their production on an industrial scale.

1. Introduction

Liposomes are enclosed vesicles formed by lipid materials, such as phospholipids, dispersed in an aqueous medium. One or more bilayers are formed, which have a similar structure to the cell membrane, separating the inner water phase from the outer [1].

Because of their special structure, liposomes have some excellent advantages when used in drug delivery systems. First of all, the enclosed vesicles can separate the inner phase from the outside one and, thus, improve the stability of the

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encapsulated drug. Secondly, when loaded into liposomes, the poorly water-soluble drug exhibits enhanced bioavailability. In addition, a sustained or controlled drug release profile can be achieved after encapsulation. Also, liposomes possess a good cell affinity with excellent biodegradability. Furthermore, the target effect of liposomal preparations can change the *in vivo* distribution of the loaded drug, improving the therapeutic index of certain drugs [2–5]. Also, many ligands can be modified on the surface of the liposomes and these modifications can alter the characteristics and biological behavior of the final product.

Liposomes, with their special characteristics such as modifiable morphology, particle size, *Zeta* potential, encapsulation efficiency, and drug release profile can enhance the properties of certain drugs. For instance, liposomes with a particle size under 120 nm can produce better tumor accumulation [6]. Moreover, the encapsulation efficiency and stability of liposomes should be as high as possible to produce high drug concentrations as well as preventing drug leakage. Today, a great variety of liposome preparation methods are available and each of them has particular features and special advantages. If suitable methods are selected and corresponding new techniques are used, new liposomal formulations can be prepared easily and efficiently. With the recent advances, more and more novel technologies have been applied to the manufacture the wonderful vesicles in recent years. However, liposome researchers sometimes have a problem when faced with the huge variety of new technologies. In the light of this, we have produced a systematic review of the new technologies to help in the selection of suitable preparation methods to meet different needs.

2. Conventional methods of liposome preparation

Since the pioneering discovery of Bangham, a variety of methods have been reported in the literatures for liposome preparation including thin-film hydration or the Bangham method [7], reversed phase evaporation [8], solvent-injection techniques [9], and detergent dialysis [10]. These are the most commonly used ones. Some techniques have been employed to help reduce the size of vesicles, for instance, sonication [11], high pressure extrusion [12] and microfluidization [13].

Unfortunately, those conventional preparation methods have a number of problems which can be classified into the following four categories: (1) The particle size of liposomes is too large or has a broad distribution so there is a need for post-processing granulation. (2) The organic solvent remaining in the final product is also a serious issue since it not only affects the stability of some protein or polypeptide drugs, but also adversely affects clinical treatment. (3) Since many lipids are sensitive to temperature, the sterilization of liposomal preparations can be a problem. So there is a preference for preparation processes which can be carried out in an ultraclean environment. However, conventional methods do not always fulfill this requirement. (4) In some procedures careful monitoring is needed and this subjective technique might influence reproducibility.

To solve these problems, many novel preparation technologies have been applied for the preparation of liposomes.

3. Novel technologies for liposome preparation

Nowadays, supercritical fluid technology, dual asymmetric centrifugation, membrane contactor technology, cross-flow filtration technology and freeze drying technology have been employed for liposome preparation.

3.1. *Liposome preparation methods based on supercritical fluid technology*

Supercritical fluids are non-condensable fluids, which are very dense at certain temperatures and pressures beyond the critical values. As the line between the liquid and gas phase disappears, supercritical fluids have many particular characteristics compared with conventional fluids. Among these characteristics, solvents with special properties have attracted a great deal of interest from researchers. Remarkably, supercritical carbon dioxide ($scCO_2$) is an excellent organic solvent substitute. In spite of its low cost, it is non-toxic and is not inflammable. In addition, it has a relatively low critical temperature and pressure (31 °C and 73.8 bar) with the dissolution properties analogous to those of nonpolar solvents [14].

3.1.1. *Supercritical anti-solvent (SAS) method*

Regarding classical thin-film dispersion methods, lipids are always dispersed on the inner surface of the glass flask to form a lipophilic thin film. Similarly, the supercritical anti-solvent (SAS) method is being used to achieve a fine and homogenous dispersion of lipid materials. Briefly, in the SAS method, lipids dissolve readily in $scCO_2$ and then precipitate in the form of ultrafine particles. The experimental procedure for SAS is shown in Fig. 1A.

Phospholipid and cholesterol is dissolved in an organic solution and placed in a glass container which, together with a source of CO_2 gas, is connected to pumps linked to a precipitation vessel. Gaseous CO_2 is pumped into a high-pressure precipitation vessel by spraying through capillary tubes and then transformed into a supercritical phase because of the sudden change in temperature and pressure. Subsequently, the lipids are extracted into the supercritical phase as soon as the evaporation of the organic solvent is completely introduced, which lead to supersaturation of the solute in the $scCO_2$ phase and then the lipid materials precipitate. Afterwards, the organic solvent is removed by CO_2 continuously pumped into the vessel to produce fine lipid particles. Finally, liposomes are obtained by directly adding aqueous phase [15].

Data obtained show that SAS liposomes have no significant differences compared with conventional Bangham liposomes in terms of the particle size, encapsulation efficiency and stability [15]. Nonetheless, organic solvent can be removed completely using SAS, which is of great value.