INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
Available online www.irjponline.com

ISSN 2230 – 8407
Review Article

LIPOSOMAL ENCAPSULATION TECHNOLOGY A NOVEL DRUG DELIVERY SYSTEM DESIGNED FOR AYURVEDIC DRUG PREPARATION

M. Hemanth kumar*, V. Spandana
Phytochem laboratories Inc, India

Article Received on: 10/08/11 Revised on: 20/09/11 Approved for publication: 14/10/11

*Email: hemanth@phytolabs.org

ABSTRACT
Liposomal Encapsulation Technology (LET) is the newest delivery method used by medical researchers to transfer drugs that act as healing promoters to the definite body organs. This form of delivery system offers targeted delivery of vital compounds to the body. It has been in existence since the early 70's. Liposomal Encapsulation Technology is a state of the art method of producing sub-microscopic bubbles called liposomes, which encapsulate various substances. These phospholipids or "liposomes" form a barrier around their contents that is resistant to enzymes in the mouth and stomach, digestive juices, alkaline solutions, bile salts, and intestinal flora, found in the human body as well as free radicals. The contents of the liposomes are therefore shielded from degradation and oxidation. This protective phospholipid shield or barrier remains unharmed until the contents of the liposome are delivered right to the target organ, gland, or system where the contents will be utilized. Natural extracts are generally degraded because of oxidation and other chemical reactions before they delivered to the target site. Our research has shown liposomal encapsulated ayurvedic preparations have shown more stability and also more efficiency when compared to traditional preparations. Size of liposomes were measured around 85-200 nm.
KEY WORDS: LET (Liposomal Encapsulation Technology), Natural extracts, liposomes, TBA/water system.

INTRODUCTION

The preparation techniques of liposomes were classified broadly into several categories according to the basic modes of dispersion, e.g., mechanical dispersion, detergent solubilization, and solvent dispersion. Further research to improve the techniques was anticipated to encapsulate sufficient amounts of materials stably and in a reproducible fashion with the possibility of application on an industrial scale, in order to use the liposomes as delivery tools of therapeutic agents. We have explored various new techniques of liposomal preparation. In our extensive research of colloidal formulations for therapeutic agents, we took unique approaches in applying colloidal techniques used for the preparation of microparticulate carriers other than liposomes, e.g., microcapsules or lipid emulsions. Liposomal Encapsulation Technology (LET) is the newest delivery method used by medical researchers to transfer drugs that act as healing promoters to the definite body organs. This form of delivery system offers targeted delivery of vital compounds to the body. It has been in existence since the early 70's. Liposomal Encapsulation Technology is a state of the art method of producing sub-microscopic bubbles called liposomes, which encapsulate various substances. These phospholipids or "liposomes" form a barrier around their contents that is resistant to enzymes in the mouth and stomach, digestive juices, alkaline solutions, bile salts, and intestinal flora, found in the human body as well as free radicals. The contents of the liposomes are therefore shielded from degradation and oxidation. This protective phospholipid shield or barrier remains unharmed until the contents of the liposome are delivered right to the target organ, gland, or system where the contents will be utilized[1]. Natural extracts are generally degraded because of oxidation and other chemical reactions before they delivered to the target site Freeze-drying (also known as lyophilization) has been a standard practice employed to manufacture many pharmaceutical products. The overwhelming majority of these products are lyophilized from simple aqueous solutions. Typically, water is the only solvent that must be removed from the solution via the freeze-drying process. However, there are still many instances where pharmaceutical products are manufactured via a process that requires freeze-drying from organic cosolvent systems.

The co-solvent system that has been most extensively investigated is the tertiary butyl alcohol (TBA)/water combination. Freeze-drying using TBA/water co-solvent systems was first evaluated by Kasraian and DeLuca in 1995[2,3] . In the subsequent years, the growing interest in using TBA in freeze-drying has led to a series of investigations on TBA/water combination[4-8]. It has been found that freeze-drying using TBA/water co-solvent systems may offer many advantages over simple drying from an aqueous solution, which may include increased drug wetting or solubility, increased sublimation rates and hence decreased drying time, increased predried bulk solution or dried product stability, decreased reconstitution time, and possible enhancement of sterility assurance of the predried bulk solution[1]. It should be noted, though, that there are additional uses for the technique of the freeze-drying from TBA/water co-solvent systems other than the optimization of the freeze-drying process. Observations from our laboratory has shown that freeze-drying of TBA/water co-solvent systems can be used to prepare submicron liposomes of narrow size distribution[9] and oil solution formulations of hydrophilic drugs. Based on our work, it is found that both hydrophilic and hydrophobic species can be simultaneously dissolved in TBA/water cosolvent system with an appropriate volume ratio to form an optically clear Monophase solution. Lyophilization of the resulting Monophase solution will obtain a homogenous codispersion of hydrophilic and hydrophobic species. Further treatment of this kind of solid dispersions will result in the formation of liposomes and oil solution formulations. In this paper, we will mainly discuss two novel technologies developed in our laboratory.

Preparation of Liposomes

The superiority of liposomes as drug carriers has been widely recognized[10].To realize the industrial-scale production of liposomes, a great many preparation methods have been developed[11]. Although some of these methods have been successfully used for the production of liposomes on a large scale, they still suffer from one or more deficiencies including the use of pharmaceutically unacceptable solvents, an additional sizing process, multiple-step drug entrapment procedure, the need for special equipments, and lack of accepted and easy-to-perform technology for sterilization[12,13,14]. A novel procedure is presented here for the preparation of sterile and Pyrogen -free submicron liposomes of narrow size distribution. The procedure is based on the initial formation of an optically clear solution by dissolving liposome-forming lipids and water-soluble carrier materials such as sucrose in TBA/water cosolvent systems, which is then lyophilized to remove solvents after sterilization by filtration through 0.22-mm pores[15,16].

On addition of water, the lyophilized product will spontaneously form homogenous submicron liposomes[17;18].

Lipids and Chemicals

Soybean phosphatidylcholine (SPC, purity of more than 92%), soybean phosphatidylserine (SPS, purity >90%), hydrogenated SPC (HSPC, purity of more than 95%), and soybean phosphatidylglycerol (SPG, purity of more than 95%) vitamin E (95% purity) .All other chemicals were of analytical reagent grade.

Liposome Preparation

SPC and vitamin E was dissolved in TBA and water-soluble carrier materials such as sucrose were dissolved in water. These two solutions were mixed in an appropriate ratio to get a third Monophase solution. After filtration through 0.22-mm pores, the Monophase solution was filled into the 10-mL freeze-drying vials with a fill volume of 2 ml. A laboratory freeze-drier was used. The freeze-drying process was as follows: (i) freezing at -40 C for eight hours; (ii) primary drying at -40 C for 48 hours; (iii) secondary drying at -25C for 10 hours. The chamber pressure was maintained at 20 Pa during the drying process. Lyophilized products were reconstituted by addition of equal volume of water and gentle shaking, which led to the formation of the aqueous suspensions of liposomes.

Size Measurements

The average diameter and size distribution of the liposomes were measured using a submicron particle analyzer.

The preparation of Drug-containing Liposomes

Usually, there are two kinds of approaches for loading drugs into liposomes, namely, passive loading and active loading. If the drug of interest has good solubility in TBA/water and good affinity to PLs due to hydrophobic interaction or electrostatic interaction or both interactions, it is reasonable to encapsulate the drug by passive loading approach, which involves the formation of a dispersion of PLs and drug in carrier materials .If the drug can be loaded in response to some gradients, it is desirable to adopt the active loading method. When active loading is used in combination with our method, a three-component liposome treatment system can be provided, which is composed of dry dispersions for forming empty liposomes, buffer for exterior pH adjustment, and the drug. In fact, sometimes a two component system can also be used, which includes a dispersion of drug, internal buffer such as citric acid and lipids in carrier materials, and a pH adjustment buffer solution.

RESULTS AND DISCUSSION

The Formation of Monophase Solution Based on our investigation, the ternary phase diagram of SPC, Vitamin E, TBA, and water mixtures could be divided into at least three main regions, corresponding to a clear solution of SPC, Vitamin E in aqueous TBA, the suspension of stacked hydrated lipid bilayers in excess aqueous TBA, and the suspension of heterogeneous liposomes[9].

For the phospholipids (PLs) that are insoluble in pure TBA (such as SPS and SPG), Monophase solutions can also be obtained by dissolving PLs in the co-solvent systems. However, an additional insoluble region might be found in the corresponding ternary phase diagram. Here, it is important to note the different solubility characteristics of different PLs. For natural PLs with low phase-transition temperatures (Tm), their solubility in co-solvents is almost independent of temperature. Therefore, during the cooling process, the ternary mixtures can maintain the isotropic status. However, it is not the case for PL with a high Tm such as DPPC and HSPC, whose solubility in co-solvents decreases markedly with the decreasing temperature. Accordingly, the appearance of lipid precipitates is inevitable during the cooling process, which might lead to the heterogeneity of final liposome suspensions. In all, the usage of natural PL with a low Tm might be a rational choice provided that our method is used.

The formation of Liposomes

On addition of water, the lyophilized products could spontaneously form liposome suspensions. The size and size distribution of final liposome preparations are strongly dependent on the following factors. Lipid Composition: For the purpose of achieving submicron and monodisperse liposome preparations, only PLs with a low Tm can be employed. Our method is not applicable to the synthetic PLs with high Tm. Furthermore, inclusion of a small fraction of charged PLs (e.g., SPS, Vitamin E) in the formulation could significantly decrease the vesicle size. Reconstruction of the solid dispersion with SPC: SPS: sucrose equal to 9:1:50 (w/w/w) can lead to the formation of liposomes with a volume mean size of 75.7 nm. In contrast, if the formulation did not contain SPS, the liposome size increased to 131.6 nm. Types of Carriers: Water-soluble carriers with different molecular weights can be used in our method with equal ease, but different carriers might exert different influence on the final liposome preparations. When disaccharides such as sucrose and trehalose are used as carriers, the size of final liposomes is usually in the range of 100 to 200 nm, provided that sugar/lipid weight ratio is less than two. However, when water-soluble polymers such as PEG-400 were employed, liposomes in the micron range will be obtained, which could be easily observed in optical microscope. The Carrier/Lipid Ratio: The carrier/lipid ratio might play an important role in the uniformity of final liposome preparations. It is found that the increasing sucrose/lipid ratio resulted in smaller size and narrower size distribution. Accordingly, we may control the uniformity and size of resulting liposome preparation by carefully adjusting the carrier/lipid ratio. The Possible Liposome Formation Mechanism A different liposome preparation method often involves a different liposome formation mechanism; hence, it is desirable to put forward a model to expound why this method results in the formation of submicron and monodisperse liposome preparations.

In the study, the TBA/water volume ratio of initial solutions ranges from 1:1 to 1:2 and the SPC concentration is less than 20 mg/mL. Under this circumstance, we detected no particles in the Monophase solutions using a submicron particle analyzer, whose detection range is from 0.6 to 6000 nm. Therefore, SPC might be dispersed in the cosolvents in the form of single molecule.

When our method is used, the stability problems related to drug retention during shelf time will be resolved because the liposome formation and the drug loading can be performed just prior to clinical use (under the situation that the loading efficiency and capability are acceptable).

REFERENCES

1. Teagarden DL, Baker DS. Practical aspects of lyophilization using nonaqueous co-solvent systems. Eur J Pharm Sci 2002; 15:115.
2. Kasraian K, DeLuca PP. Thermal analysis of the tertiary butyl alcohol-water system and its implications on freeze-drying. Pharm Res 1995; 12:484.
3. Kasraian K, DeLuca PP. The effect of tertiary butyl alcohol on the resistance of the dry product layer during primary drying. Pharm Res 1995; 12:491.
4. Wittaya-Areekul S, Nail SL. Freeze-drying of tert-butyl alcohol/water cosolvent systems: effects of formulation and process variables on residual solvents.J Pharm Sci 1998; 87:491.
5. Oesterle J, Franks F, Auffret T. The influence of tertiary butyl alcohol and volatile salts on the sublimation of ice from frozen sucrose solutions: implications for freeze-drying. Pharm Dev Technol 1998; 3:175.
6. Nuijen B, et al. Pharmaceutical development of a parenteral lyophilized formulation of the novel antitumor agent aplidine. PDA J Pharm Sci Technol 2000; 54:193.
7. Wittaya-Areekul S, et al. Freeze-drying of tert-butanol/water cosolvent systems: a case report on formation of a friable freeze-dried powder of tobramycin sulfate.J Pharm Sci 2002; 91:1147.
8. Van Drooge DJ, Hinrichs WL, Frijlink HW. Incorporation of lipophilic drugs in sugar glasses by lyophilization using a mixture of water and tertiary butyl alcohol as solvent. J Pharm Sci 2004; 93:713.
9. Li C, Deng Y. A novel method for the preparation of liposomes: freeze drying of Monophase solutions. J Pharm Sci 2004; 93:1403.

10. Maurer N, Fenske DB, Cullis PR. Developments in liposomal drug delivery systems. Expert Opin Biol Ther 2001; 1:923.
11. Walde P, Ichikawa S. Enzymes inside lipid vesicles: preparation, reactivity and applications. Biomol Eng 2001; 18:143.
12. Buboltz JT, Feigenson G. A novel strategy for the preparation of liposomes: rapid solvent exchange. Biochim Biophys Acta 1999; 1417:232.
13. Wang W. Lyophilization and development of solid protein pharmaceuticals. Int J Pharmaceuticals 2000; 203:1.
14. Crowe JH, Crowe LM. Preservation of liposomes by freeze-drying. In: Gregoriadis G, ed. Liposome Technology. Boca Raton: CRC Press, Inc., 1993: chap 14.
15. Crowe JH, Crowe LM. Factors affecting the stability of dry liposomes. Biochim Biophys Acta 1988; 939:327.
16. Crowe LM, et al. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim Biophys Acta 1986; 861:131.
17. Evans JR, Fildes FJT, Oliver JE. Process for preparing freeze-dried liposome compositions. US Pat. 4311712, 1982.
18. Drummond DC, et al. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol Rev 1999; 51, 691 .

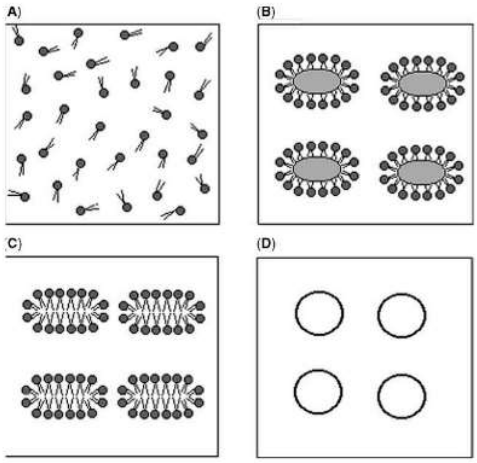

Figure 1 .Schematic representation of the liposome formation process. (A) The homogenous solution of SPC, Vitamin E and sucrose in TBA/water mixture. (B) Solid TBA hydrates surrounded by SPC. (C) Rearrangement of SPC to form PBFs. (D) Liposome formation on addition of water. Note: sucrose is not shown.
Abbreviations: SPC, soybean phosphatidylcholine; TBA, tertiary butyl alcohol; PBFs, phospholipid bilayers fragments.

Source of support: Nil, Conflict of interest: None Declared