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Review

Liposomal microencapsulation using the conventional methods and novel supercritical fluid processes

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ABSTRACT

Background: Liposomes are spherical phospholipid vesicles with the capability of versatile **microencapsulation** of hydrophilic and lipophilic compounds. Organic solvent residue has always been a concern in conventional methods of liposome generation.
Scope and approach: Dense gas techniques using **supercritical carbon dioxide** as the **phospholipid-dissolving agent** can provide a green solution for reduction or avoidance of organic solvent use.
Key findings and conclusions: In this review, conventional and dense gas processes of liposomal microencapsulation are evaluated. Comprehensively understanding the current progress of **supercritical fluid** techniques for liposomal microencapsulation will be helpful for development of a non-toxic continuous process of this application in the food and related industries.

1. Introduction

Liposomes are self-assembled spherical vesicles with one or more phospholipid bilayers separating the inner aqueous environment from the outer aqueous medium. It has long been used as a model for biological membrane study due to its similarity to real cell membrane (Chatterjee & Agarwal, 1988; Trier, Henriksen, & Andresen, 2011). The compound of interest was securely encapsulated in the inner aqueous core or within one or more phospholipid bilayers (Arifin & Palmer, 2005; Nii & Ishii, 2005; Xia & Xu, 2005). Based on the size and lamellarity, liposomes can be categorized into four types: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs), and multivesicular vesicles (MVVs). The liposomal size of SUVs varies from 20 to about 100 nm. The sizes of LUVs, MLVs, and MVVs range from a few hundred nanometers to microns. The thickness of one phospholipid bilayer has been reported to be approximately 4–5 nm (Sharma & Sharma, 1997).

Interest in liposomes is greatest in the food, pharmaceutical, and bioprocessing industries, due to the solubility improvement, controlled release, and targeted delivery of active compounds (Decker, Schubert, May, & Fahr, 2013; Fowler et al., 2013; Kiwada, Sato, Yamada, & Kato, 1986; Shieh et al., 1997; Wong et al., 2003). Liposomal microencapsulation has been applied in food industry for the antioxidant, enzyme, and nutraceutical encapsulations (Keller, 2001; Nii & Ishii, 2005; Stone & Smith, 2004). LUV is always preferred due to its higher encapsulation efficiency, simpler process, and better storage stability (Gouin, 2004). By adding cholesterol, surfactants, or carbohydrates into the phospholipid bilayer, the rigidity, fluidity and permeability can be modified for specific purposes (Jo & Kim, 2009; Taylor, Davidson, Bruce, & Weiss, 2005; Yokoyama et al., 2005).

Traditionally, liposomes are prepared by thin-film hydration (TFH, the Bangham method), reverse phase evaporation vesicles (REV), and membrane extrusion. Nowadays, due to the concern of organic solvent toxicity, several new techniques have been attempted to reduce or even completely avoid the use of organic solvents in the liposomal microencapsulation, including microfluidics, rapid expansion of supercritical solutions (RESS), supercritical reverse phase evaporation (SCRPE), and several dense gas processes (Castor, 1996, 2005; Frederiksen, Anton, Hoogevest, Keller, & Leuenberger, 1997; Magnan, Badens, Commenges, & Charbit, 2000; Meure, Knott, Foster, & Dehghani, 2009; Otake, Shimomura, Goto, & Imura, 2006; Utada et al., 2005). The salient features of different liposomal microencapsulation methods are summarized in Table 1. Supercritical carbon dioxide (SC–CO_2) is a

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Table 1
Comparison of conventional and innovative methods of liposomal microencapsulation.

Methods	Organic solvent	Process time	Process type	Liposomal size
Conventional				
TFH	Yes	>1 h	Batch	Variable
REV	Yes	2–3 h	Batch	Variable
Membrane extrusion	Yes	1–2 h	Batch	Variable
Innovative				
Microfluidics	Yes	15–30 min	Semi-continuous	50–100 μm
Dense gas				
RESS-cosolvent	10-20%	15–30 min	Semi-continuous	200–250 nm
Improved RESS	Yes	30 min	Batch	<1 μm
RESS-CCSM	No	30–60 min	Continuous	<20 μm
SCRPE	No	2–3 h	Batch	200 nm
DESAM	Yes	45–60 min	Continuous	100–400 nm
CFN	Yes	20–40 min	Batch	300 nm
CAS	Yes	45–60 min	Continuous	10–100 μm
PGSS	Yes	60 min	Continuous	1–5 μm
DELOS-SUSP	Yes	30–60 min	Batch	100–150 nm

Notes:
TFH: thin film hydration.
REV: reverse phase evaporation vesicles.
RESS-CCSM: rapid expansion of supercritical solution with continuous cargo suction microencapsulation.
SCRPE: supercritical reverse phase evaporation.
DESAM: depressurization of an expanded solution into aqueous media.
CFN: critical fluid nanosome.
CAS: continuous antisolvent process.
PGSS: particles from gas saturated solutions.
DELOS-SUSP: depressurization of an expanded liquid organic solution-suspension.

non-toxic density-adjustable fluid with the solvent behavior similar to hexane (Lopes & Bernardo-Gil, 2005; Shao, Sun, & Ying, 2008). Its moderate critical pressure (7.4 MPa) and low critical temperature (31.1 °C) make SC-CO_2 an ideal candidate for biomaterial processing. Rapid expansion of a supercritical solution with continuous cargo suction microencapsulation (RESS-CCSM) was proposed as a non-toxic and continuous method for the formation of liposomal vesicles using SC-CO_2 as the sole phospholipid-dissolving agent with the continuous cargo suction (Tsai & Rizvi, 2010). The conventional and innovative techniques of liposomal microencapsulation will be described and compared in this review.

2. Conventional methods of liposomal microencapsulation

2.1. Thin film hydration

Thin film hydration (TFH; the Bangham method) was created as a simplified liposomal microencapsulation technique (Bangham, Standish, & Watkins, 1965). Basically, an appropriate quantity of the pure or mixed phospholipids with other lipophilic compounds is dissolved in organic solvents, such as chloroform, methanol, and hexane, followed by organic solvent evaporation in a rotary vacuum evaporator or nitrogen blowing. After the thin lipid film is formed on the bottom of the evaporation flask, a certain quantity of the hydrophilic cargo solution is then added into the flask and mixed with the phospholipid film by vortex shaking or sonication to form liposomes. Liposomes prepared using the Bangham method are usually multilamellar vesicles (MLVs) with relatively low encapsulation efficiency compared to the large unilamellar vesicles (LUVs) made by membrane extrusion (Hope, Bally, Webb, & Cullis, 1985; Trier et al., 2011) and reverse phase evaporation (Yokoyama, Takeda, & Abe, 2002).

Some membrane modifications created a structural change on the liposomal bilayers produced with the Bangham method (Yokoyama et al., 2005). It was found that the liposomes prepared with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/stearylamine (SA) in the molar ratio of 9.5 to 0.5 converted the MLVs into LUVs. The encapsulation efficiency (EE) of the resulting LUVs consisting of the DPPC/SA phospholipid bilayer was ten times higher than that of the MLVs assembled by the DPPC multilayers (Yokoyama et al., 2005).

2.2. Reverse phase evaporation vesicles

Reverse phase evaporation vesicles (REV) was first reported by Szoka and Papahadjopoulos (1978). In the REV process, phospholipids are first dissolved in an organic solvent with a low boiling point, such as chloroform, diethyl ether, or methanol (Szoka & Papahadjopoulos, 1978; Taylor et al., 2005). An aqueous solution containing the active compounds is added into the organic phase to form an emulsion by vortex shaking or low energy sonication. At this step, the emulsion stays in the water-in-oil or reversed micellar system. The hydrophilic domain of the polar lipids attaches to the inner aqueous droplet containing the targeted compound while the fatty acid chains interact with the continuous organic phase. During the evaporation under vacuum, the organic solvent is gradually removed and the continuous phase is converted from the oil phase to the water phase. Eventually, the double emulsion (water in oil in water, W/O/W) is formed as the desired liposome. Although the encapsulation efficiency is relatively high compared to the other methods, a complete removal of the organic solvent is often challenging (Yokoyama et al., 2002).

The calcium cations were encapsulated in liposomes prepared with egg phosphatidyl choline (EPC) by the REV method (Kim, Kim, Jeong, & Kim, 2006). The liposomes were LUVs with the droplet size ranging from 0.25 to 1.8 μm and 63% encapsulation efficiency. These calcium-encapsulated liposomes were then used as meat tenderizer. The effect of the liposomal protection of β-D-glucosidase against the cupric ion was evaluated (Sada, Katoh, Terashima, & Tsukiyama, 1988). The liposomes composed of L-α-dimyristoylphosphatidylcholine (DMPC)/cholesterol/diacetyl phosphate (DCP) in the molar ratio of 7:2:1 were prepared with the REV method. β-D-glucosidase encapsulated in the REV liposome reached 20% of EE and presented 2–3 fold higher enzymatic activity than the unencapsulated glucosidase during 20 days of storage.