Micro and nano liposome vesicles containing curcumin for a drug delivery system

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Abstract
Micro and nano liposome vesicles were prepared using a lipid film hydration method and a sonication method. Phospholipid, cholesterol and curcumin were used to form micro and nano liposomes containing curcumin. The size, structure and properties of the liposomes were characterized by using optical microscopy, transmission electron microscopy, and UV–vis and Raman spectroscopy. It was found that the size of the liposomes was dependent on their composition and the preparation method. The hydration method created micro multilamellars, whereas nano unilamellars were formed using the sonication method. By adding cholesterol, the vesicles of the liposome could be stabilized and stored at 4°C for up to 9 months. The liposome vesicles containing curcumin with good biocompatibility and biodegradability could be used for drug delivery applications.

Keywords: microliposome, nanoliposome, curcumin, drug delivery

Classification numbers: 2.04

1. Introduction

In 1965, the first description of tiny close-membraned vesicles of lipids was reported [1]. They were an aqueous volume enclosed by a bilayer lipid membrane, composed of phospholipid and cholesterol, and called a ‘liposome’. There are many types of liposome based on the size and number of lamellae of lipid vesicles. Multilamellar liposome vesicles (MLVs) usually range from 500 to 10,000 nm. Unilamellar liposomes can be defined as small (SUV, 20–100 nm), large (LUV, >100 nm) and very large (giant liposome, >1000 nm) [1–4].

Liposomes have been considered as a ‘magic bullet’ because they can encapsulate hydrophilic and lipophilic drugs. In a spherical structure, the drug molecules can be either encapsulated in aqueous space or insert themselves in the phospholipid bilayer. Liposomes containing drugs have been studied and applied as a delivery system for treatment of various diseases [3–7].

Curcumin, a yellow natural polyphenol, is obtained from Curcuma longa L and also called turmeric. In ancient India, turmeric was thought to have many healthy properties [8]. Curcumin is known to exhibit anticancer, antioxidant, anti-inflammatory, antibacterial and wound-healing properties with low cytotoxicity [9]. However, it is insoluble in water and degrades in certain pH conditions. This causes reduced bioavailability of curcumin in the human body, leading to low intrinsic activity, poor absorption and high rate of elimination [10]. To overcome the problems of poor solubility and low bioavailability, many delivery systems of curcumin have been developed. Curcumin encapsulated in liposome [11], micro-particle-based on albumin [12], chitosan [13], polymer PLGA [14], etc have been reported. Liposomes containing curcumin can be administrated by many routes (oral, intravenous, transdermal, etc) and protect curcumin from degradation.
Table 1. Lipid composition and mean diameter of the liposomes prepared by the hydration method.

| DOPE:CHOL (wt/wt) | 10:1 | 5:1  | 3.33:1 | 2.33:1 |
|-------------------|------|------|--------|--------|
| Mean diameter (μm)| 2.428 ± 0.471 | 2.797 ± 1.050 | 2.944 ± 1.560 | 2.535 ± 0.601 |

Figure 1. Images of the DOPE liposomes (left) and size distribution of the liposomes (right) with different DOPE:CHOL ratios: (a) DOPE:CHOL = 10:1, (b) DOPE:CHOL = 5:1, (c) DOPE:CHOL = 3.33:1 and (d) DOPE:CHOL = 2.33:1.
Various methods of liposome preparation have been reported [15–18]. In this report, liposomes were prepared using the lipid film hydration method and sonication method. The hydration method is used for preparation of MLV liposomes [16, 17] and sonication is used for preparation of SUV liposomes [17–19]. With this synthesis approach, curcumin was contained inside the end-closed liposome. The size and structure of the liposomes were characterized by optical microscopy, transmission electron microscopy (TEM), and UV–vis and Raman spectroscopy.

2. Experimental

2.1. Materials

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol (CHOL) and curcumin (Cur) were purchased from Sigma-Aldrich, US. Chloroform (99.3%–99.5%) was purchased from Prolabo, Germany. De-ionized (DI) water and phosphate buffered saline (1.0 M, pH 7.4, Sigma-Aldrich) were used for the experiments. All chemicals used in the experiments were of pharmaceutical standard and analytical grade.

The reagents (DOPE, CHOL or Cur) were dissolved in chloroform with the ratio of 1 mg per ml solvent. All the solutions were stored at −20°C before use.

2.2. Lipid hydration method

DOPE and CHOL solutions (1 mg ml⁻¹) were mixed in a round-bottom flask with 5 ml chloroform. The weight ratios (wt/wt) of DOPE:CHOL were different (table 1). The flask was attached to a rotary vacuum evaporator (RV06, IKA, Malaysia) at 50°C and 50 rpm. After the evaporation process (2 h), a dry lipid film was formed on the flask wall. The hydration step was carried out by cracking the lipid layer in DI water at 40°C for 1 h and then kept overnight at room temperature.

2.3. Sonication method

In this method, the hydration step was replaced by a sonication method. After a dry lipid was formed, DI water was
added to the flask. The liposome (LIPS) was then prepared by sonication with a bath type sonicator for 10 min. The lipid layer was cracked into the nano-patches by cavitation under an inert atmosphere. This was the main method for downsizing multilamellar (micrometer range) vesicles into nanoscaled unilamellar vesicles [20].

Finally, all suspensions were kept at 4°C for further experiments.

2.4. Preparation of curcumin loaded liposomes

Curcumin loaded liposomes were prepared for both the hydration and sonication methods. Curcumin was mixed with the DOPE and CHOL solutions in the flask with a weight ratio of curcumin/liposome of 1:10 in weight (the weight of liposome is the amount of DOPE and CHOL).

The addition of cholesterol with different concentrations has various effects on the capacity of the liposomes to encapsulate and deliver a drug [7]. For preparation of the curcumin loaded liposomes, a weight ratio of 2.33:1 (the same proportion of cholesterol in the cell membrane) was used.

2.5. Characterization

The size and structure of the liposomes were characterized by an optical microscope (GX41, Olympus, Japan) and TEM (JEM1010, JEOL, Japan). The diameter and distribution of the liposomes were investigated by ImageJ software (Ver 1.50). The properties of the liposomes without curcumin and the liposomes containing curcumin were studied by using a UV–vis spectrometer (CARY 100, Varian, Germany). Raman spectroscopy (LABRam 300, Horiba, France) was used to determine the forms of curcumin.

3. Results and discussion

3.1. Size and structure of the liposomes prepared by the hydration method

Figure 1 shows images of the DOPE liposomes and size distribution of the liposomes with different DOPE:CHOL ratios. The mean diameter and the composition of the phospholipids are shown in table 1. The size of the liposomes was characterized by optical microscopy (figure 2). The results show that the mean diameter of the liposomes does not depend on the lipid composition of the liposomes. The multilamellar vesicles (3–10 lipid bilayers) of the liposomes that were formed by using the hydration method were observed, as in previous studies [1, 16–19].

Without cholesterol, lipid vesicles might be created but then the structures were easily destroyed (as shown in figure 2, top). The cholesterol was added to the formulations of the liposomes to stabilize the phospholipid layers. This is consistent with observations for the role of cholesterol in liposomes [21–23]. The liposomes with cholesterol were then stored for a
longer time (as shown in figure 2, bottom). In this study, the liposomes (DOPE = 10:1) could be stored at 4°C for up to 9 months. During storage, these small liposomes agglomerated together to form larger forms. It was found that the mean diameter of the liposomes increased linearly with time (figure 3).

3.2. Liposome containing curcumin

In this section, curcumin was added in both methods, hydration and sonication, with the weight ratio of 7:3 (DOPE: CHOL). The liposomes containing curcumin (LIPS@CUR) were observed by optical microscopy and TEM. The results showed that the mean size of LIPS@CUR was larger than the mean size of liposomes without curcumin (figure 4). This proved that the presence of curcumin in liposomes increased the size of the vesicles.

Using the sonication method, the size of LIPS@CUR decreased by about four times, from 4.1 μm to 95 nm, and the micro multilamellar liposomes vesicles were changed into nano unilamellar vesicles, which was also observed in the previous studies [17–20].

Figure 5. (a) Chemical structure and (b) Raman spectra of curcumin.

3.3. Raman and UV–vis spectra

The structure of curcumin was investigated by Raman spectroscopy (figure 5). The chemical structure of curcumin is [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], commonly called diferuloylmethane (figure 5(a)). In curcumin, (–OH) and (–OCH3) groups have important implications regarding preferred molecular structures.

The strong peak between 1600 and 1630 cm⁻¹ was attributed to the mixed ν(C=C) and ν(C=O) vibration mode [24–26]. They were observed at 1601 cm⁻¹ (aromatic C=C) and 1626 cm⁻¹ (carbonyl C=O). The peak at 1430 cm⁻¹ was the characteristic peak of phenol C–O [24–26]. While two forms of curcumin structures are defined by the peak at 1249–1250 cm⁻¹, the keto-enol form of curcumin was obtained at 1250 cm⁻¹ in the spectrum. In addition, a methoxy group (R–OCH3) was vibrated at 573 cm⁻¹.

UV–vis spectroscopy was carried out in the wavelength range from 200 to 600 nm (figure 6). Absorption of the liposomes was obtained from 190 to 210 nm, depending on the size of the nanoparticles [27–30]. The micro-MLVs of DOPE liposomes had an absorption peak at 210 nm. On the
other hand, the peak of LIPS@CUR was shifted to 194.74 nm, corresponding to the nanoscale of the vesicles. The peak of curcumin was at 420 nm, which was in good agreement with the literature values [31].

4. Conclusion
In this study micro and nano liposomes were prepared using the lipid hydration and sonication methods. It was found that the mean size of the liposomes depended on their composition and the preparation method. Micro multilamellars were formed using the hydration method while nano unilamellars were prepared using the sonication method. The structure of the curcumin, liposomes and liposomes containing curcumin were investigated by UV–vis and Raman spectroscopy, and optical microscopy. The mean diameter of the LIPS@CUR was 4.1 μm and 95 nm (corresponding to the hydration/sonication method, respectively), which could be stabilized at 4 °C for a long time (up to 9 months in this study). The curcumin loaded liposomes, coupled with biocompatibility and biodegradability, could be feasible for drug delivery purposes.

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