Stable Ethosome-like Catanionic Vesicles for Transdermal Hydrophilic Drug Delivery with Predictable Encapsulation Efficiency

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Abstract: Lipid-like pseudo-double-chained catanionic surfactants have emerged as the attractive materials to prepare potential vesicular carriers in drug and gene delivery applications. In particular, the semi-spontaneous process has been developed to fabricate ethosome-like catanionic vesicles for the transdermal drug delivery. In this work, Arbutin (a water-soluble drug) encapsulation efficiency of ethosome-like catanionic vesicles fabricated from decyltrimethylammonium-tetradecylsulfate (DeTMA-TS, CH₃(CH₂)₉N(CH₃)₃-CH₃(CH₂)₁₃SO₄) and decyltrimethylammonium-dodecylsulfate (DeTMA-DS, CH₃(CH₂)₉N(CH₃)₃-CH₃(CH₂)₁₁SO₄) with various amounts of ethanol and cholesterol in tris buffer solution was experimentally determined. A simple unilamellar vesicle (ULV) model, resulting in the theoretical encapsulation efficiency within ±10% error for most vesicle compositions, was also developed. Such agreement indirectly confirmed the formation of unilamellar vesicles by the preparation method. Stable ethosome-like catanionic vesicles by using catanionic surfactants with the aid of suitable amounts of ethanol and cholesterol, which led to polydispersity index (PDI) values of vesicle size distribution less than 0.3, were successfully prepared and their hydrophilic drug encapsulation efficiencies can be accurately predicted. Furthermore, the linear correlations of the trap volume ratio with both vesicle size and concentration of the extra added CHOL also provided important guidelines for controlling the drug loading of ethosome-like catanionic vesicles. The accomplishments reached for the novel vesicles are useful for developing their transdermal drug delivery applications.

Key words: catanionic surfactant, semi-spontaneous process, ethosome-like catanionic vesicle, hydrophilic drug, encapsulation efficiency, unilamellar vesicle (ULV) model

1 Introduction

The novel catanionic surfactants, also known as ion-pair amphiphiles (IPAs), can be formed from the equimolar mixture of two single-chained oppositely charged surfactants¹⁻⁶. More recently, Manna and Panda⁷ discussed catanionic surfactants as surrogates of phospholipids in some aspects such as the formation of vesicles and its application in drug or DNA delivery. In a recent review article, Tomašić and Mihej⁸ also presented some fundamental facts and recent developments on numerous solid catanionic surfactant systems of synthetic origin.

Due to their abundant sources, low cost, high chemical stability, and designable molecular structures, lipid-like catanionic surfactants have emerged as attractive materials to prepare potential vesicular carriers in drug and gene delivery. Several studies have therefore investigated the cytotoxicity of catanionic vesicles and explored the potentials of catanionic vesicles as delivery carriers⁷⁻¹². Readers are referred to a recent review by Dhawan and Nagarsenker¹³ for the fabrication, stability, and bio-compatibility of catanionic systems in nanotherapeutic applications.

Using catanionic surfactant as the starting material, catanionic vesicles can be prepared from the thin film formations of catanionic surfactants followed by mechanical dispersion¹⁴,¹⁵. This preparation process is similar to that in the conventional liposome productions. Meanwhile, a simple semi-spontaneous process has been developed to fabricate catanionic vesicles using a relatively high content of ethanol¹⁶⁻¹⁸. It should be noted that ethosomes which are permeation-enhancing lipid vesicles containing high...
concentration of ethanol were also developed and investigated in the literature\textsuperscript{19–24}. Ethosome-like catanionic vesicles, that is, catanionic vesicles fabricated with ethanol in the aqueous phase, are therefore expected to be the feasible replacements of ethosomes for transdermal drug delivery.

In the semi-spontaneous process, the formability of catanionic vesicles with the aids of short-chained alcohols (such as ethanol) in water has been proposed and discussed by Yu \textit{et al.}\textsuperscript{25}. Note that the dielectric constant of the aqueous solution decreases with increasing alcohol content. The lowered solvent polarity may reduce the electrostatic repulsion between the hydrophilic polar head groups of catanionic surfactants, resulting in a positive solvophobic effect. In contrast, the lowered solvent polarity may deteriorate the hydrophobic attraction among the nonpolar hydrocarbon chains of catanionic surfactants, leading to a negative solvophobic effect. These competing effects hence result in an optimal alcohol concentration with the maximum solvophobic effect that favors the vesicle formation. Further increasing alcohol concentration, however, can finally lead to complete resolving of the catanionic vesicular system.

It is noteworthy that lamellarity of vesicles prepared by the semi-spontaneous process is different from those fabricated by the classic mechanical dispersion method. This inevitably can lead to different drug encapsulation efficiency of the vesicles. As illustrated in Fig. 1, the classic mechanical dispersion generally results in multilamellar vesicles (MLVs); in contrast, the semi-spontaneous process generates unilamellar vesicles (ULVs) based on the self-assembly of amphiphiles under the aforementioned solvophobic effect. The important issue of lamellarity and hydrophilic drug encapsulation of the as-fabricated vesicles, however, does not seem to have been tackled in the literature.

The objective of this work is to validate the unilamellar structure of ethosome-like catanionic vesicles prepared by

\textbf{Fig. 1}  \hspace{2cm} (a) Formation of multilamellar vesicle (MLV) by the classic mechanical dispersion method through the preparation of thin films.  \hspace{2.5cm} (b) Formation of unilamellar vesicle (ULV) by the semi-spontaneous process with the aid of ethanol cosolvent.
the semi-spontaneous process and enable accurate predictions of hydrophilic drug encapsulation efficiency through a simple ULV model and the measurements of vesicle size and size distribution by using a popular commercial instrument. Hydrophilic drug Arbutin encapsulation efficiencies of ethosome-like catanionic vesicles fabricated from 5 and 10 mM decyltrimethylammonium-tetradecylsulfate (DeTMA-TS) and decyltrimethylammonium-dodecylsulfate (DeTMA-DS) catanionic surfactants with various amounts of ethanol and cholesterol (CHOL) in 15 mM tris buffer solution were measured. By taking CHOL concentration and experimentally determined vesicle diameter into considerations within the ULV model, the theoretical and experimental results of hydrophilic drug encapsulation efficiency were compared to indirectly validate the unilamellarity of the as-fabricated ethosome-like catanionic vesicles. The agreement between the experimental results of stable ethosome-like catanionic vesicles and the ULV model predictions therefore enabled the accurate evaluation of the hydrophilic drug encapsulation efficiency, providing important guidelines for the hydrophilic drug loading controls of ethosome-like catanionic vesicles.

2 Materials and Methods

2.1 Materials

Cationic surfactant decyltrimethylammonium bromide (DeTMB, 98% pure) was purchased from Acros, China. Anionic surfactants sodium tetradeccylsulfate (STS, 95% pure) and sodium dodecylsulfate (SDS, 99% pure) were purchased from Sigma, Japan. The solvents methanol (99.9% pure) and ethanol (99.9% pure) were purchased from J. T. Baker, USA and Malaysia, respectively. Hydrophilic drug Arbutin (98% pure), buffer saline Trizma base (Tri[hydroxymethyl] aminomethane, 98% pure) and CHOL (99% pure) were purchased from Sigma (Slovakia), Acros (China), and Sigma (USA), respectively. The chemicals were used as received without further purification. All experiments were conducted using ultra-pure water with a resistivity of 18.2 MΩ·cm generated from Milli-Q plus purification system, Millipore, USA.

2.2 Synthesis of catanionic surfactants

Synthesis and analyses of lipid-like pseudo-double-chained DeTMA-TS and DeTMA-DS catanionic surfactants followed the same protocol by Lee et al.\(^\text{25}\). Catanionic surfactants were prepared from the equal molar mixture of single-chained cationic and anionic surfactants by the precipitation method. Catanionic surfactant as precipitate will come out when cationic and anionic surfactants in aqueous solutions of sufficiently high concentrations are allowed to react with each other during gentle mixing. After standing for 1 h, the precipitate was separated from the solution by repeated centrifuging and washing. It was then dried for 36 h under a vacuum and ground into fine powder for further studies. The identification techniques employed for the catanionic surfactant included elementary analysis, mass spectrum, and \(^1\)H and \(^{13}\)C NMR spectra\(^\text{25}\). As-prepared DeTMA-TS and DeTMA-DS were subsequently used as the materials for fabricating ethosome-like catanionic vesicles.

2.3 Fabrication and characterization of ethosome-like catanionic vesicles

The semi-spontaneous process\(^\text{16,17}\) was used for fabricating the ethosome-like catanionic vesicles with the aid of ethanol. First, the desired amount of catanionic surfactant was dissolved in ethanol. For catanionic vesicle systems containing CHOL, the CHOL additive was also dissolved in ethanol with catanionic surfactant at the specified concentrations. Thereafter, solutions were prepared with 10 and 5 mM catanionic surfactant, 10-30 vol% ethanol, various amounts of CHOL up to 10 and 5 mM, 30 mM Arbutin, and 15 mM tris buffer at pH 7.4. Finally, the catanionic vesicles were generated by passing the solution through a homogenizer at 11,000 rpm for three minutes in a sealed container custom-designed for this procedure.

The dynamic light scattering (DLS) using a He-Ne laser (\(\lambda = 633\) nm, \(\theta = 173^\circ\)) coupled with a computerized particle size analyzer (Nano-ZS, Malvern, UK) was used to determine the size of ethosome-like catanionic vesicles. The scattering intensities at successive time intervals were compared via the correlator, a digital signal processing board, to derive the variation rate of the intensity. The monomodal method (a cumulant analysis) was applied to determine the Z-average vesicle size\(^\text{27}\). The measurement also provided the derived count rate (kcps) and polydispersity index (PDI)\(^\text{27}\).

The ethosome-like catanionic vesicle images were obtained using a transmission electron microscope (TEM, model H-7500, Hitachi, Japan) through the negative staining technique. A few drops of ethosome-like catanionic vesicle solutions were deposited onto carbon-coated Cu grid and dried to prepare the TEM samples. The sample was then stained with one drop of 1 wt% uranyl acetate in water-ethanol (1:1) solution.

2.4 Determination of encapsulation efficiency (E.E. %)

Before purification, 60 µL of the as-fabricated ethosome-like catanionic vesicle dispersion (with initial loading of Arbutin, \(M_r = 1.8\) µ moles) was diluted 100 times to 6 mL with the aqueous solution of ethanol and buffer to the indicated concentrations. The diluted solution was then centrifuged at 4000 g for 8 min using the Ultracel YM-50 centrifugal device (50 kDa MWCO, Millipore). The filtrate was collected and the retentate was re-diluted to 6 mL and then centrifuged for 8 min. Three dilution-centrifugation cycles were conducted to separate all free drug molecules.
from the drug-encapsulated vesicles. Actually the capability of rapid separation between vesicle and free drug by the centrifugal filtration method is beneficial to cope with the problem caused by possible release of drug from vesicles during separation period. To assess the total amount of Arbutin encapsulated in vesicles ($M_e$), 100 μL retentate was withdrawn and mixed with 900 μL methanol to destruct vesicles and release the encapsulated Arbutin. The assessment of encapsulated Arbutin was conducted by a high performance liquid chromatography (HPLC) system with a 25 cm × 4.6 mm column (Ascentis™ C18, 5 mm, Supelco super CO-150, USA), and a L-2200 autosampler (Hitachi, Japan). The mobile phase consisted of an 80% methanol aqueous solution. The flow rate was maintained at 0.5 mL/min throughout the assay by a flow rate controller (model 7100, Hitachi, Japan). Arbutin was detected with a UV detector (model Waters 486 Tunable Absorbance Detector, Germany) at 280 nm. The amount of Arbutin was quantified by integrating the peak areas using the Peak Detector, Germany.

**3 Unilamellar Vesicle (ULV) Encapsulation Model**

By assuming that all the liposomes were unilamellar and packed with lipid molecules of finite volume therein, Yamuchi et al.\(^{28}\) proposed a simple model to evaluate the trap volume of liposomes as a function of vesicle size. Later, Xu et al.\(^{29}\) developed a mathematical model constructed from the surface area of lipid to predict the hydrophilic drug encapsulation inside unilamellar liposomes. In this work, a similar ULV model based on the one proposed by Xu et al.\(^{29}\) was developed to calculate the trap volume percentage, which corresponds to the hydrophilic drug encapsulation efficiency, of the ethosome-like catanionic vesicles.

Molecular structures of ionic moieties of DeTMA-TS, DeTMA-DS and cholesterol utilized in this work are illustrated in Fig. 2. Considering a solution of volume $V$ with the concentration of $C_{IPA}$ for IPA and $C_{CHOL}$ for CHOL, the formed ULVs, as also shown schematically in Fig. 2, have the average diameter $b$ and the bilayer thickness $\delta$. If all IPA molecules are self-assembled into vesicle structures and all cholesterol molecules are incorporated evenly in vesicular bilayers according to the molecular composition, the amount of amphiphile, including both IPA and CHOL, per vesicle $N_v$ can be approximated as:

$$N_v = \frac{A_v}{\langle A \rangle} = \frac{4\pi \left( (b/2)^2 + (b/2 - \delta)^2 \right)}{\langle A \rangle}.$$  \hspace{1cm} (2)

$\langle A \rangle$ and $A_v$ denote the average molecular area of the amphiphile and the total surface area of the vesicular bilayer, respectively. Here, $\langle A \rangle$ is approximated via the ideal mixing of IPA and CHOL where the effect of ethanol on $\langle A \rangle$ is not accounted:

$$\langle A \rangle = \frac{C_{IPA}}{C_{IPA} + C_{CHOL}} A_{IPA} + \frac{C_{CHOL}}{C_{IPA} + C_{CHOL}} A_{CHOL}. $$  \hspace{1cm} (3)

The number of vesicles in the solution $n_v$ can then be evaluated as:

$$n_v = \frac{N_{tot}}{N_v} = \frac{(C_{IPA} + C_{CHOL})V \langle A \rangle}{4\pi \left( (b/2)^2 + (b/2 - \delta)^2 \right)} = \frac{V(C_{IPA}A_{IPA} + C_{CHOL}A_{CHOL})}{4\pi \left( (b/2)^2 + (b/2 - \delta)^2 \right)}.$$  \hspace{1cm} (4)

$N_{tot}$ is the total amount of amphiphile, including both IPA and CHOL, in the solution. Assuming the encapsulating

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Fig. 2  Molecular structures of ionic moieties of IPAs and cholesterol, and schematic representation of the structure of a unilamellar catanionic vesicle composed of IPA, ethanol, and cholesterol.
Table 1  Estimation of molecular lengths and surface areas.

| Molecular length (nm) | Molecular surface area (nm²) |
|-----------------------|----------------------------|
| DeTMA⁺               | 1.54³⁰                     | 0.27³¹             |
| TS⁻                  | 2.05³⁰                     | 0.26³²             |
| DS⁻                  | 1.82³⁰                     | 0.26³²             |
| CHOL                 | 1.80³⁰                     | 0.38³²             |

substance has equal concentration inside and outside the vesicle, the encapsulation efficiency (E.E., %) can thus be expressed as the trap volume percentage, i.e. the ratio between the total trap volume inside vesicles and the volume of the solution:

$$\text{Trapped volume (\%) } = \frac{4}{3} \pi \left(\frac{b}{2} - \delta \right)^3 n_v$$

$$\text{Trapped } \frac{V}{3 \left( \left(\frac{b}{2}\right)^2 + \left(\frac{b}{2} - \delta\right)^2 \right)} \text{CHOL}}$$

(5)

In this work, the bilayer thickness \( \delta \) is taken as twice the length of the longer ionic moiety of IPs, that is \( \delta = 2 \text{ IP}^- \) for DeTMA-TS and \( \delta = 2 \text{ IP}^- \) for DeTMA-DS. The detailed values of molecular lengths and surface areas used in this work are summarized in Table 1. With an experimentally determined vesicle diameter \( b \), therefore, Eq. 5 predicts the hydrophilic drug encapsulation efficiency of ethosome-like catanionic vesicles under the conditions of experiment.

4 Results and Discussion

Figure 4 shows for example the size distributions of as-fabricated Arbutin-encapsulated ethosome-like catanionic vesicles with 10 mM DeTMA-TS, 20 vol% ethanol and various amounts of CHOL. TEM micrographs of empty ethosome-like catanionic vesicles containing 20 vol% ethanol without CHOL and with 10 mM CHOL are shown in Figs. 5(a) and 5(b), respectively. The vesicle sizes in Figs. 5(a) and 5(b) are within the range of 20-140 nm with the average vesicle size in Fig. 5(a) being smaller than that in Fig. 5(b), which supports the results of the DLS analysis. The results of both DLS analysis and TEM micrography confirmed the formation of vesicles in the presence of ethanol and CHOL under the fabrication condition.

Table 2 summarizes all physical properties and the experimental encapsulation efficiencies of the as-fabricated Arbutin-encapsulated ethosome-like catanionic vesicles with 10 mM DeTMA-TS, 10-30 vol% ethanol, 15 mM tris buffer, and various amounts of CHOL. It is found that catanionic vesicles could be successfully fabricated with suitable amounts of ethanol and CHOL. Vesicle was unable to
be fabricated with the composition of 10 vol% ethanol and 10 mM CHOL. This is due to the low solubility of relatively high concentrations of catanionic surfactant and CHOL in relatively low concentration of aqueous ethanol solution. Furthermore, double-peak or even triple-peak in vesicle size distribution were found in DLS measurements, resulting in poor description of vesicle size, for the vesicle dispersions fabricated with 25 and 30 vol% ethanol in the absence of CHOL and with 30 vol% ethanol in the presence of 1 mM CHOL. Thus with these compositions, the vesicle formation was not considered to be successful and “NA” was indicated in Table 2. For the other compositions, vesicles exhibited typical single-peak size distribution and the average diameter of stable ethosome-like catanionic vesicles varied from 58 to 125 nm with changing of composition.

The effects of ethanol as co-solvent in water on the formability of ULVs through the semi-spontaneous preparation process utilized in this work has been mentioned previously in the Introduction section. An optimal dielectric constant maximizes the overall solvophobic effect to favor the vesicle formation. At lower or higher concentrations than the optimal ethanol concentration, less formability of vesicles is expected. Figure 6 displays the PDI of ethosome-like catanionic vesicles of various ethanol and CHOL concentrations. In the absence of CHOL, the PDI of vesicles is the smallest at 20 vol% ethanol, suggesting an optimal solvophobic effect at 20 vol% ethanol in this work. Higher or lower ethanol concentration than 20 vol% increases the PDI values. Meanwhile, as a typical bilayer membrane stabilizer, CHOL enhances the stability of the as-fabricated vesicles. Indeed, as also shown in Fig. 6, CHOL was found to effectively lower the PDI values of vesicles and diminish the unfavorable solvophobic effect at ethanol concentrations lower or higher than 20 vol%. With high enough CHOL concentrations, PDI values of ethosome-like catanionic vesicles were reduced to around 0.2 or lower. Although the ethanol-like catanionic vesicles could be successfully fabricated, or the PDI values of the vesicles could be decreased, in the presence of cholesterol, less stable vesicles were formed, if only a small amount of CHOL is added, with a significant extent of aggregation and/or coalescence between vesicles, resulting in a PDI value higher than 0.2. Vesicles with a smaller PDI value are thus generally considered as more stable ones. Vesicles with a smaller PDI value are thus generally considered as more stable ones.

Experimental encapsulation efficiency data statistics of the four measured values were shown in Table 2 by the calculated mean and standard deviation for each available composition. Moreover, the theoretical values of trap volume percentage (theoretical E.E.) calculated by using Eq. 5 with the experimentally determined average vesicle diameter were also included in Table 2. In order to demonstrate the model can successfully predict the encapsulation efficiency, the predicted encapsulation efficiency was compared with the experimental encapsulation efficiency, or the ratio of experimental to theoretical encapsulation efficiency was evaluated, and the results are plotted in Fig. 7.

In parallel with those for DeTMA-TS (10 mM), Table S1-S3 (see Supporting Information) summarize all physical properties and the experimental encapsulation efficiencies of the as-fabricated Arbutin-encapsulated ethosome-like catanionic vesicles with DeTMA-TS (5 mM), DeTMA-DS (10 mM), and DeTMA-DS (5 mM), respectively. Figure S1 (see Supporting Information) shows correspondingly the ethanol and cholesterol effects on the PDI of the vesicle size distribution. Similar results with those of DeTMA-TS (10 mM) were observed. It was also found that at lower or higher concentrations than an optimal ethanol concentration, less formability of vesicles is expected, resulting in higher PDI.
Moreover, CHOL was found to effectively lower the PDI values of vesicles at ethanol concentrations lower or higher than 20 vol%.

**Table 2** Physical properties of DeTMA-TS (10 mM) ethosome-like catanionic vesicles and comparison between experimental and theoretical encapsulation efficiencies.

| DeTMA-TS [10 mM] | CHOL [mM] | Initial diameter (nm) | PDI | Derived count rate (kcps) | Experimental E.E. [%] | Theoretical E.E. [%] | Experiment Theory [%] |
|-------------------|-----------|-----------------------|-----|--------------------------|----------------------|---------------------|----------------------|
| Ethanol [10%]     | 0         | 97.4                  | 0.42| 29874                    | 1.26 ± 0.12          | 2.16                | 58.21                |
|                   | 1         | 124.8                 | 0.30| 45248                    | 2.03 ± 0.27          | 3.10                | 65.51                |
|                   | 3         | 122.1                 | 0.20| 101225                   | 2.94 ± 0.28          | 3.43                | 85.81                |
|                   | 6         | 116.1                 | 0.17| 168942                   | 3.95 ± 0.17          | 3.80                | 103.81               |
|                   | 8         | 109.6                 | 0.16| 189077                   | 4.27 ± 0.44          | 3.92                | 109.06               |
|                   | 10        | N/A                   |     |                          |                      |                     |                      |
| Ethanol [15%]     | 0         | 105.3                 | 0.33| 28076                    | 1.62 ± 0.02          | 2.37                | 67.39                |
|                   | 1         | 77.9                  | 0.23| 40778                    | 1.41 ± 0.18          | 1.77                | 79.85                |
|                   | 3         | 89.5                  | 0.18| 79103                    | 2.38 ± 0.24          | 2.38                | 100.16               |
|                   | 6         | 92.8                  | 0.20| 113312                   | 3.00 ± 0.15          | 2.92                | 102.68               |
|                   | 10        | 106.7                 | 0.21| 115833                   | 4.21 ± 0.33          | 4.14                | 101.59               |
| Ethanol [20%]     | 0         | 57.8 ± 3.2            | 0.22± 0.02| 20511 ± 1240            | 0.59 ± 0.11          | 1.12                | 52.72                |
|                   | 1         | 72.3 ± 3.6            | 0.19± 0.04| 35589 ± 4442            | 1.46 ± 0.18          | 1.61                | 90.68                |
|                   | 3         | 85.5 ± 5.6            | 0.14± 0.01| 73588 ± 8182            | 2.18 ± 0.17          | 2.25                | 100.11               |
|                   | 5         | 100.2 ± 3.5           | 0.16± 0.00| 108653 ± 16171          | 2.84 ± 0.44          | 3.04                | 93.37                |
|                   | 6         | 99.9 ± 2.6            | 0.17± 0.03| 130694 ± 11105          | 3.52 ± 0.44          | 3.19                | 110.32               |
|                   | 10        | 99.1 ± 2.0            | 0.16± 0.01| 150524 ± 19064          | 4.12 ± 0.21          | 3.79                | 108.59               |
| Ethanol [25%]     | 0         | N/A                   | 0.51| N/A                      |                      |                     |                      |
|                   | 1         | 71.4                  | 0.13| 31452                    | 1.59 ± 0.16          | 1.58                | 100.44               |
|                   | 3         | 84.7                  | 0.16| 60484                    | 2.14 ± 0.13          | 2.22                | 96.29                |
|                   | 6         | 97.5                  | 0.14| 100903                   | 2.93 ± 0.31          | 3.10                | 94.51                |
|                   | 10        | 90.3                  | 0.16| 112175                   | 3.74 ± 0.23          | 3.39                | 110.19               |
| Ethanol [30%]     | 0         | N/A                   | 0.70| N/A                      |                      |                     |                      |
|                   | 1         | N/A                   | 0.50| N/A                      |                      |                     |                      |
|                   | 3         | 85.7                  | 0.09| 61592                    | 2.39 ± 0.38          | 2.25                | 106.03               |
|                   | 6         | 99.9                  | 0.11| 97187                    | 3.47 ± 0.30          | 3.19                | 108.75               |
|                   | 8         | 108.7                 | 0.16| 118195                   | 4.10 ± 0.69          | 3.88                | 105.73               |
|                   | 10        | 110.5                 | 0.19| 124605                   | 4.63 ± 0.52          | 4.31                | 107.35               |

Mean ± SD, n = 4

Values. Moreover, CHOL was found to effectively lower the PDI values of vesicles at ethanol concentrations lower or higher than 20 vol%.

**Figure 7** demonstrated a good correlation within ±10% error between the experimental and theoretically predicted encapsulation efficiency. That is, the simple unilamellar vesicle (ULV) model could successfully predict the theoretical encapsulation efficiency within ±10% error for most vesicle compositions. The agreement between the experimental and theoretical E.E.% actually provided indirect evidence of the formation of unilamellar vesicles. For stable ethosome-like catanionic vesicles, fabricated by using catanionic surfactants with the aid of suitable amounts of ethanol and cholesterol, which led to polydispersity index (PDI) values of vesicle size distribution less than 0.3, the hydrophilic drug encapsulation efficiency could be accurately predicted. Less agreement, however, can be observed for the compositions of ethanol concentrations lower or higher than 20 vol% without high enough amounts of CHOL addition, which in general have vesicle PDI values of greater than 0.3 as shown in Tables 2 and S1-S3. With the compositions of ethanol concentrations lower or higher than 20 vol% without high enough amounts of CHOL addition, which in general have vesicle PDI values of greater than 0.3, the vesicles were not stable enough. Thus less agreement was found between the predicted encapsulation...
efficiency and experimental encapsulation efficiency.

The merits of the encapsulation efficiency predictions through a simple ULV model and the measurements of vesicle size and size distribution by using a popular commercial instrument like Zetasizer are quite obvious. In order that the criterion of successful prediction can be established, the PDI of vesicle size distribution and the accuracy of E.E. prediction at various compositions are categorized as shown in Fig. 8 for DeTMA-TS (10 mM). Figures 8 (a) and 8 (b) show the compositions at which vesicle size distribution exhibited PDI larger than or lower than 0.3 and error of predicted E.E. larger than ±20%, lower than ±20% and larger than ±10%, and lower than ±10%, respectively. Considering the uncertainty and possible error in experimental data, especially at the marginal compositions, this two figures basically coincide with each other. Figure S2 (see Supporting Information) shows correspondingly the similar results for DeTMA-TS (5 mM), DeTMA-DS (10 mM), and DeTMA-DS (5 mM). It is concluded, therefore, precisely predictable hydrophilic drug encapsulation efficiency of ethosome-like catanionic vesicles can be successfully accomplished by this protocol with a proper criterion of PDI less than 0.3.

The validation of the ULV model from Fig. 7 allows us to explore the various factors affecting the overall encapsulating efficiency using Eqs. 2 to 5. Figure 9 shows the correlation between the size of vesicle and the number of DeTMA-TS and CHOL molecules per vesicle and the number of vesicles per 1 mL dispersion. It is clear that, with fixed amount of amphiphiles, more amphiphiles are needed to form larger vesicles and less number of vesicles can be formed in the solution.

For the conditions studied in this work, the calculated trap volume ratio (encapsulation efficiency) of ethosome-like catanionic vesicles by using Eq. 5 shows the positive linear correlation with the vesicle diameter and cholesterol concentration as shown in Fig. 10. Considering a fixed CHOL concentrations, the increased vesicle diameter results in enhanced trap volume ratio as shown in Fig. 10 (a). With the addition of CHOL, which was included in the vesicular bilayer structure, the total amount of vesicular bilayer-forming material would be increased. Thus if a fixed vesicle size was considered, the increased amount of vesicular bilayer-forming material due to CHOL addition would result in an increased number of vesicles within the solution, leading to enhanced trap volume ratio, as shown in Fig. 10 (b). Using Eq. 5, one can generically correlate the
trap volume ratio with the CHOL concentration and the vesicle sizes. The three-dimensional diagram shown in Fig. 10(e) summarizes the combined effects of the added CHOL and the vesicles size on the overall encapsulation efficiency. The linear correlations of the trap volume ratio with both vesicle size and concentration of the extra added CHOL hence provide important guidelines for controlling the drug loading of ethosome-like catanionic vesicles.

5 Conclusions

In this work, ethosome-like catanionic vesicles were fabricated by using catanionic surfactants and various amounts of ethanol and cholesterol in buffer solution by a semi-spontaneous process. Water-soluble drug encapsulation efficiency of as-fabricated ethosome-like catanionic vesicles was estimated theoretically through a simple ULV model and the measurements of vesicle size. A good correlation within ±10% error between the experimental and theoretical encapsulation efficiency for vesicles with PDI values less than 0.3 was found. This provides a validation of unilamellar structure of ethosome-like catanionic vesicles prepared by the preparation method. Furthermore, stable ethosome-like catanionic vesicles with precisely predictable hydrophilic drug encapsulation efficiency can be successfully prepared with the aid of suitable amounts of ethanol and cholesterol, which led to PDI values less than 0.3. The accomplishments reached for the novel vesicles is useful for developing their potential applications in transdermal drug delivery.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess21072

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Fig. 10 Calculated trap volume as functions of (a) vesicle diameter and (b) cholesterol concentration of ethosome-like catanionic vesicles based on the unilamellar vesicle model. (c) Three-dimensional diagram.

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