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Measurement of the second virial coefficient of DPPC- and DPPG-liposomes by isothermal titration calorimetry

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Abstract

In this study, we employed a thermodynamic method to obtain information on the second virial coefficient of the interactions between liposomes, and we also determined the stabilities of dipalmitoyl-L-alpha-phosphatidylglycerol (DPPG)- and dipalmitoyl-L-alpha-phosphatidylcholine (DPPC)-liposomes. In this study, an interaction model based on a hard-sphere interaction potential assumption was employed, and we used high-sensitivity isothermal titration nanocalorimetry (ITC) to measure the dilution heat of liposomal suspensions. Information on the second virial coefficient of interactions between liposomes can be derived from the number density of the liposomal suspension. Additionally, we qualitatively demonstrated the repulsive forces of DPPG- and DPPC-liposomes systems. In this work, both the b_2 and b_2/B_0 levels of DPPG-liposome were greater and had better physical stability than those of DPPC-liposome.

Keywords: Second virial coefficient; Interaction potential; Liposome; ITC

1. Introduction

When the physical stability of liposomes is discussed, the phenomena of aggregation and fusion are two important factors. Generally speaking, liposome aggregation does not necessarily induce fusion and must occur prior to it if the fusion phenomenon is to take place. Therefore, interactive forces between liposomes play a significant role in the behavior of liposome aggregations.

With colloid stability, the DLVO theory treats the aqueous phase as a continuum and takes into account only the attractive van der Waals forces and the repulsive electrostatic forces, but it cannot explain the relative stabilities of several non-charged liposomal systems. In addition to repulsive hydration and undulation forces, it has been shown that liposomes can also be stabilized by steric stabilization on surface adsorbed or bound polymers (Needham *et al.*, 1992). Therefore, the real potential or overall interacting force between liposomes can be written as

$$F = -F_{\mathrm{vdw}} + F_{\mathrm{est}} + F_{\mathrm{hyd}} + F_{\mathrm{st}} + F_{\mathrm{und}}. \quad (1)$$

Unfortunately, over the past two decades, the measurement of hydration, undulation, and steric forces has been based on the inter-bilayers system, not on the inter-liposome system. Furthermore, the overall forces between liposomes cannot be directly measured using inter-liposome systems.

The overall interacting force between vesicles, as indicated by the second virial coefficient, has been demonstrated (Bruns, 1996), and it is determined in an experimental system by measuring the osmotic pressure (Brain and Abraham, 1995; Brunettl *et al.*, 1983) or the diffusion coefficient as a function of the volume fraction (Koper *et al.*, 1995; Kurnaz and Maher, 1995, 1997). In our previous study, we proposed a new method of obtaining information on the second virial coefficient of interactions between microemulsion droplet systems (Chen *et al.*, 2000) and liposomal suspension systems (Liu *et al.*, 2000) by microcalorimetry. In this study, we also employed a thermodynamic method to obtain information on the second

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Nomenclature

b_2	second virial coefficient of the fitted polynomial of the dilution heat of a liposomal suspension solution
B_0	virial coefficient
E	internal energy (mJ)
k_B	Boltzmann's constant (J/K)
N	number of liposomes
Q	dilution heat of a liposomal suspension solution (mJ)
R_{HS}	radius of a liposome (nm)
T	absolute temperature (K)

Greek symbols

ε	depth of the square-well potential function (or energy barrier)
ρ	number density of a liposome suspension solution (number/mL)
σ	width of the square-well potential function

virial coefficient of interactions between DPPG- and DPPC-liposomes, respectively. Furthermore, we discuss liposomal stability from the viewpoint of the second virial coefficient of the interaction.

2. Materials and methods

2.1. Liposome system

Dipalmitoyl-L-alpha-phosphatidylcholine (DPPC) and dipalmitoyl-L-alpha-phosphatidylglycerol (DPPG) were purchased from Sigma (U.S.A.). Small unilamellar vesicles (SUVs) or liposomes were prepared by extrusion (Szoka *et al.*, 1980) through unipore poly-carbonate filters (Bio-Rad) with an average diameter of about 120 nm. In this experimental system, there are two types of liposomes: DPPC- and DPPG-liposomes. DPPC-liposomes contain 37.5 mg of DPPC in 10 mL of water. DPPG-liposomes contain 37.8 mg of DPPG in 10 mL of water. Estimation of the liposome number is based on the average liposomal diameter which is about 120 nm and consists of about 7×10^5 lipid molecules. Finally, all samples were diluted with water to the desired vesicle number concentration of about 10^{13} liposomes/mL. The liposomal suspension was prepared without the addition of buffer and salt.

2.2. Liposomal size and electrophoretic mobility measurements

The hydrodynamic diameters of the liposomes were determined by means of a dynamic light scattering (DLS) technique using a photon correlator spectrometer (Malvern Zetasizer 3000) equipped with a He–Ne laser source (wavelength, 633 nm). DLS determinations were made with a reading angle of 90° in all cases. Data analysis was performed using CONTIN software provided by Malvern Instruments (England). Electrophoretic measurements were also performed in the Zetasizer 3000 based on the laser-Doppler microelectrophoresis technique, and the ζ-potential of the liposomes was calculated from their electrophoretic mobility by means of the Smoluchowski equation.

2.3. Isothermal titration nanocalorimetry (ITC)

In this study, we used a highly sensitive nanocalorimetry which employed a Thermal Activity Monitor (Thermometric AB, Sweden) to measure the dilution heat of the liposomal suspension. Briefly, 2 mL of a liposome suspension solution was placed in an ampoule. A series of 150-μL buffer solutions was then titrated into the dispersed liposomes suspension solution using a Hamilton microliter syringe at 30-min intervals after the ampoule and heat sink had reached thermo-equilibrium. The liposome suspension solution was titrated five times per experiment (Fig. 1). All experiments were performed at a temperature of 298 K.

3. Results and discussion

The deviation of a solution from ideal behavior due to two-body interaction in a suitable concentration of solution can be described by vant Hoff's law (Bruns, 1996) Therefore, it is desirable to obtain the value of a second virial coefficient and b_2/B_0 as the interaction potential (ε) index between liposomes.

In this study, we measured the dilution heats of liposomal suspensions (Fig. 1) and combined these results with the virial equation for the non-ideal behavior of liposomal suspensions. The present study proposes the following derivations and discusses the derivation of the second virial coefficient from the dilution heat. In general, dilution heats of liposomal suspensions with the number density of the liposome suspension can be expressed as a function of a second-order polynomial as in Eq. (2):

$$\frac{\mathrm{d}(q/Nk_BT)}{\mathrm{d}\rho} \cong \frac{\mathrm{d}(E/Nk_BT)}{\mathrm{d}\rho} = b_2 + b_3\rho + b_4\rho^2. \quad (2)$$

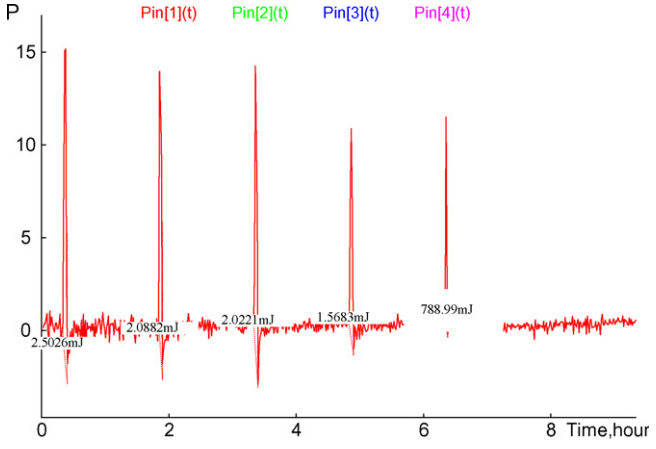

Fig. 1. The dilution heart of a DPPC-liposome suspension.

Table 1
Measurement of the second virial coefficient of DPPC- and DPPG-liposomes by isothermal titration calorimetry

	Zeta potential (mV)	b_2 (nm^3)	Virial coefficient, B_0 (nm^3)	b_2/B_0
DPPC-liposome	−15.4	4.6×10^{11}	3.8×10^6	1.2×10^5
DPPG-liposome	−38.6	8.3×10^{11}	4.4×10^6	1.9×10^5

The values of zeta potential, second-order polynomial, b_2, and the B_0 of virial coefficients of liposomes (values are the mean ± standard deviation, $n = 5$).

For an open liquid system, pressure changes and titration volume are neglected in the system total volume. The observed dilution heat of each titration is comparable to the internal energy change. Furthermore, the internal energy change can be expressed by the virial coefficient and the number density as in Eq. (3):

$$\frac{E}{Nk_BT} = \frac{3}{2} - T\sum_{i=1}^{\infty} \frac{1}{i} \frac{dB_{i+1}}{dT} \rho^i. \quad (3)$$

Comparing Eqs. (2) and (3) reveals that the coefficient of the dilution heat polynomial can be affiliated with the virial coefficients as in Eq. (4):

$$b_2 = -T\frac{dB_2}{dT}. \quad (4)$$

Then, according to statistical mechanics, the relation between the second virial coefficient and the interaction potential energy function $U(r)$ is

$$B_2(T) = -2\pi \int_0^{\infty} \left[\exp\left(-\frac{U(r)}{k_BT}\right) - 1\right] r^2\, dr, \quad (5)$$

if the square-well energy potential function is chosen and plugged into Eq. (5), then Eq. (4) can be rewritten as:

$$b_2 = -T\frac{dB_2}{dT} = -B_0(\lambda^3 - 1)\, e^{\varepsilon/k_BT}\left(\frac{\varepsilon}{k_BT}\right), \quad (6)$$

$$\frac{b_2}{B_0} = (\lambda^3 - 1)\, e^{\varepsilon/k_BT}\left(\frac{\varepsilon}{k_BT}\right), \quad (7)$$

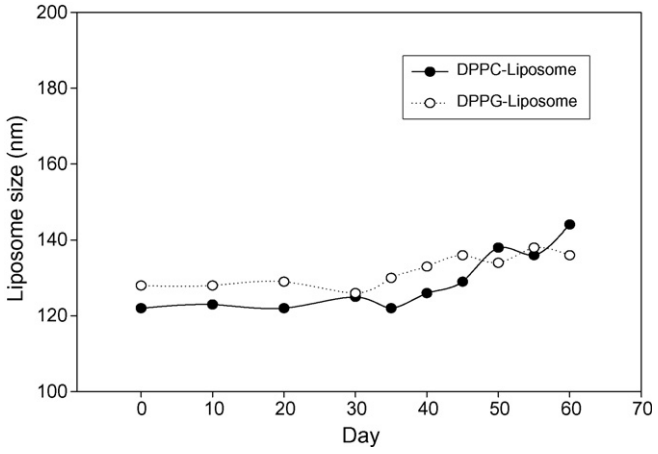

Fig. 2. Sizes of liposomes with times. The number density of the liposome suspension was 10^{13} liposomes/mL (each point value is the average of five experiments).

where $B_0 = (16/3)\pi R_{HS}^3$, $\lambda = (2R_{HS} + \sigma)/2R_{HS}$, and σ and ε denote the width and depth (or energy barrier) of the square-well energy potential function, respectively.

In analyzing the physical meanings of b_2 (nm^3) and b_2/B_0 levels (Table 1), b_2 is desirable for obtaining the value of the second virial coefficient and indicating the effective repulsive force of the liposomes. The value of b_2/B_0 can also be regarded as a liposomal energy barrier for liposomes from the secondary minimum state to the primary minimum state, or as an interaction potential (ε) index between liposomes.

In our study, both DPPC- and DPPG-liposomes had a positive b_2 value, indicating that the overall net interactive force is repulsive between liposomes. This net repulsive force poses a formidable barrier for the aggregation or fusion of liposomes. What is noticeable with the b_2 value is that the effective repulsive force can be effective as far away as 80 times the liposomal radius. This explains how the long-range repulsive force principally decides the physical stability of liposomes. In addition, we speculated that it does not matter if DPPC- or DPPG-liposomes evidently possess the repulsive forces as a result of the ζ-potential of liposomes (Table 1).

In our method (system) of measuring the interaction potential or effective repulsive force between liposomes, we admit the possibility of flaws because the repulsive force was effective as far away as 80 times the liposomal radius in our experiment. In fact, we think that the exact force between liposomes should be smaller than this. This discrepancy had a lot to do with the estimation of liposomal number or density.

Armengol and Estelrich (1995) checked the physical stability of liposomes by measuring their average size by photon correlation spectroscopy. In our study, Fig. 2 displays the respective changes in particle size of DPPC- and DPPG-liposomes. The experimental results suggest that liposomes may be constrained by the electrostatic repulsive forces (long-range repulsive force) which temporarily pause on the second minimum state (or reversible aggregation) for up to 40 days (Fig. 2). In addition, if we consider the aggregation and fusion behavior from the viewpoint of the interaction potential, a more-repulsive interaction potential (higher levels of σ and ε or a greater energy barrier) would result in the liposome system not easily aggregating or fusing (Fig. 2).

4. Conclusion

In this study, we use a thermodynamic model to prove a feasible method for obtaining the second virial coefficient of the interaction potential between liposomes, and this method has potential as a tool to determine the behavior of non-ideal

collisions between liposomes. Furthermore, in terms of the physical meaning of DPPG- and DPPC-liposomes, both the b_2 level and b_2/B_0 level of the former were higher. In other words, DPPG-liposomes have better physical stability.

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利用恆溫熱微卡計量測 DPPC 微脂粒以及 DPPG 微脂粒之第二維里係數

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摘　要

本實驗主要利用高靈敏度恆溫熱微卡計以量測 DPPC 微脂粒以及 DPPG 微脂粒之第二維里係數，同時利用第二維里係數說明微脂粒物理穩定度。實驗系統是利用恆溫熱微卡計量測微脂粒懸浮液之稀釋熱，再配合數學模式之演譯計算出微脂粒懸浮液系統之第二維里係數 b_2 說明微脂粒粒子間交互作用，另外，我們更進一走利用能量位能井方程式說明微脂粒聚集融合之能量障壁參數 b_2/B_0。最後，我們也利用此模式證實，無論是 b_2 或 b_2/B_0 值以及物理穩定度，DPPG 微脂粒都明顯較 DPPC 微脂粒高。