

INTRODUCTION

Lipid vesicles have been widely utilized as biomembrane models and as carriers in drug delivery systems. Some anticancer and antifungal liposomal drug delivery systems have already been approved for clinical use.¹ However, the limited stability of lipid vesicles during storage and administration restricts their wider application and development (*in vitro* and *in vivo*). Researchers have attempted to improve the stability of lipid vesicles by several methods: polymerization or chemical modification;^{2,3} polymer-coated lipid vesicles;^{4,5} and lipid vesicles with chemically attached polymers like carboxymethyl chitin.^{6,7} Sterically stabilized lipid vesicles have recently been designed which alter their surfaces through glycolipids⁸ or polymers such as polyethylene glycol (PEG),^{9,10} polyvinyl alcohol, or polyacrylamide.^{11,12} These biologically stabilized lipid vesicles are referred to as stealth lipid vesicles which are heavily hydrated via the attachment of a hydrophilic macromolecule, thus reducing protein adsorption. Kronberg et al.¹³ proved that sterically stabilized lipid vesicles increase the stability of systems that incorporate Tween 80 surfactants into the lipid bilayer in serum compared with corresponding lipid vesicles without Tween 80. However, *in vitro* physical stability after Tween 80 has been added to the liposomes has yet to be carefully investigated. Therefore, the interaction potential between PC lipid vesicles incorporating varying quantities of Tween 80 (or Tween 20) surfactant are examined herein from the perspective of the interactive forces and potentials in order to elucidate the action mechanism and physical stability properties of these lipid vesicles.

Understanding the interaction forces between lipid vesicles is essential for comprehending the aggregation of lipid vesicles as far as their physical stability is concerned. Forces between membranes include long-range electrostatic and Van der Waals forces, and short-range hydration as well as steric stabilization forces. Long-range forces between vesicles can be directly measured by the osmotic method. To measure the interbilayer spacings of multilamellar vesicles (MLVs), the surface balance technique¹⁴ or micro pipette techniques¹⁵ have to be adopted to deter-

mine the small-angle x-ray scattering (SAXS) measurement between bilayer membranes (short-range interactive forces). We believe that incorporating Tween surfactants into the PC bilayer can affect the surface properties of PC liposomes via the interactive forces between lipid vesicles. However, to our knowledge, no study has investigated the interactive net forces between lipid vesicles or the *in vitro* physical stability of aggregation and fusion of Tween surfactant surface-modified lipid vesicles. Therefore, we have developed a microcalorimetry method of obtaining the second virial coefficient of the interaction potential and interactive force between lipid vesicles.^{16,17} Furthermore, we investigate the effect of aggregation and fusion phenomena on the physical stability of Tween-PC lipid vesicles from the perspective of interactive forces and the potential between PC lipid vesicles incorporating various amounts of Tween surfactant.

We previously^{16,17} measured the dilution heat of PC lipid vesicles by isothermal titration calorimetry (ITC), which enables the parameters of the lipid vesicle interaction potential (or the energy barrier) to be qualitatively described from the dilution heat empirical data coupled with a simple statistical thermodynamic model. Therefore, in this study, we examine the interaction potential by measuring the dilution heat of PC lipid vesicles with various amounts of Tween 80 incorporated (or Tween 20) surfactant by ITC. Furthermore, the impact of Tween surfactants on the lipid phase transition temperature was measured via differential scanning calorimetry (DSC) in response to different values of the weight-percent wt% incorporation of Tween surfactants into the PC lipid vesicles. Thus, the aggregation and fusion behaviors of lipid vesicles are explained from both the intervesicle and intravesicle interaction perspectives.

MATERIALS AND METHODS

Materials

Egg PC (99% phosphatidylcholine) or lecithin, DPPC (99% dipalmitoyl-phosphatidylcholine), cholesterol, and α -tocopherol were purchased from Sigma (USA). Tween 20 and Tween 80 surfactants were ob-