# CHAPTER I

## INTRODUCTION

Daptomycin is a cyclic lipopeptide antibiotic which consists of a 13-member amino acid cyclic lipopeptide with a decanoyl side-chain (Debono et al., 1988). Daptomycin exhibits bactericidal activity against vancomycin-resistant enterococci and methicillin-resistant staphylococci (Snydmanz et al., 2000; Rybak et al., 2000; Akins and Rybak, 2001).

Daptomycin possesses *in vitro* bactericidal activity with a post-antibiotic effect (PAE) (Bush et al., 1989; Hangerber et al., 1991). However, the first recommended regimen, i.e. 2 mg/kg body weight per day, is terminated because of significantly clinical failures. The clinical failures may have been due to a high degree of daptomycin protein binding, rapid renal clearance, and inadequate distribution to target sites (Bergeron, 1986; Garrison et al., 1990; Lee, Sachdeva and Chambers, 1991; Rybak et al., 1992).

In order to improve therapeutic outcomes of daptomycin, a 4 mg/kg of body weight per day regimen is developed and approved by United States Food and Drug Administration (USFDA) in treatment of complicated skin and soft tissue infections. A 6 mg /kg once-a-day regimen is later approved by the USFDA for the treatment of *Staphylococcus aureus* (*S. aureus*) bacteremia, right-sided endocarditis caused by methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) (Cubist Pharmaceuticals, 2006). However, high dose regimens can result in drug toxicity; for instance, myalgia (Veligandla et al., 2004).

In the treatment of endocarditis, a prototype daptomycin-dextran macromolecular conjugates was proposed to improve biodistribution and to reduce renal clearance of daptomycin (Muangsiri and Kirsch, 2006). The conjugates were successfully prepared with an appropriate molecular size and possess higher affinity for fibrinogen than that of free daptomycin. However, drug release rate from the conjugates was inadequate for clinical applications.

Ionization properties of daptomycin provide an opportunity for electrostatic attachment to cationic carriers, giving rise to another potential drug delivery system. In the pH range of 0-14, daptomycin contains six ionizable groups including an aromatic amine (pKa 0.8), four carboxylic acids (one with a pKa value 3.0 and three with pKa

values of about 5.3) and an aliphatic amine (pKa 10.0). Thus, in the pH range of 3.0 to 9.0, ionic species of daptomycin vary between zwitterionic, monoanionic, dianionic and trianionic forms.

Among non-covalent drug delivery systems, dendrimers are good candidates for non-covalent drug delivery systems since dendrimers have well-defined structures, molecular monodispersity and highly charged surface. Moreover, dendritic formulations exhibit prolonged blood/plasma retention owing to their hydrophilicity, surface characteristics, and molecular size (Bos et al., 2004). These characteristics benefit the design of daptomycin non-covalent drug delivery systems which may overcome problems associated with the free drug molecule and covalent carrier system.

Polyamidoamine (PAMAM) dendrimers are star-shaped polymers with an amine core and polyamide subunits (Frechet and Tomalia, 2001: 587-590). PAMAM dendrimers have tertiary amine groups in the interior or core with pKa ranging from 5.0 to 8.0 and primary amine end groups on the surface with the pKa ranging from 9.0 to 11.0. Both amine functionalities can protonate in the pH range of 3.0 to 10.0 (Cakara, Kleimann and Borkovec, 2003; Maiti et al., 2005). Drug molecules can be entrapped in the interior core as well as electrostatically attached to the amine groups on the surface (Basu, Sandanaraj and Thayumanavan, 2004).

In divergent methods, PAMAM dendrimers are synthesized by stepwise reactions, building the dendrimer up one layer, or "generation," at a time. The core molecule is referred to as "generation 0". Each successive repeating unit along all branches forms the next generation, "generation 1," "generation 2," and so on until the terminating generation. The different numbers of generations determine the size and the amount of surface functionality of the dendrimer (Frechet and Tomalia, 2001: 23-27). In other words, higher generation reflects larger size and more surface functionality for binding.

Several factors are shown to influence formation and characteristics of dendrimeric complexes such as pH of the system and dendrimer generation size (Klajnert, 2003; Leisner and Imae, 2003; Shcharbin, 2005). Objectives of this study were to prepare dendrimeric daptomycin complexes, characterize the complexes and determine the effects of adjustable variables such as pH and dendrimer generation size on binding capacity and affinity. In addition, the results of these investigations were expected to provide insight into the nature of daptomycin interactions with charged macromolecules.