CHAPTER I

INTRODUCTION

Development of biotechnology has led to the discovery of powerful DNA that can be employed for hereditary disorder improvement, life threatening diseases treatment such as cancer, infectious diseases and vaccination in healthy people to protect them from serious infectious diseases (Alonso, 2004). However, DNA delivery is always restricted by poor stability of DNA in biological environment and limited transport across epithelia causing insufficient bioavailability. Therefore, an appropriate DNA delivery system should be to protect them from biological environments and to facilitate transportation through biological barriers.

Solid lipid nanoparticles (SLN) and chitosan nanoparticles (CSN) have been recently proposed as an alternative carrier for DNA delivery (Olbrich et al., 2001; Agnihotri, Malligarjuna, and Aminabhavi, 2004). This is due to many technological advantages over other existing transfection vectors such as production from generally recognized as safe substances and possibility of steam sterilization and lyophilization (Agnihotri, Malligarjuna, and Aminabhavi, 2004; Tabatt, Kneuer et al., 2004). Especially, the SLN large-scale production with qualified production lines has been reported (Müller, Radke, and Wissing, 2002). The positively charged SLN and CSN would bind to polyanionic DNA via electrostatic force leading to a nanoparticles-DNA complex that will protect DNA from interaction with small molecules in environment (Cui and Mumper, 2001; Olbrich et al., 2001) and will be taken into cell by an endocytosis process (Tabatt, Smeti et al., 2004). However, Calvo et al. (1996) reported that submicron particles could be more taken into cell by endocytosis mechanism than those of microparticles. Therefore, both size and zeta potential value are important particle physicochemical properties, as they determine the physical stability as well as the biopharmaceutical properties of the preparation (Vandervoort and Ludwig, 2006).

In many developed countries, the morbidity and mortality associated with human immunodeficientcy virus (HIV) infection have markedly declined due to the availability and use of highly active antiretroviral therapy (HAART). For some developing countries, the spread of HIV is still aware and accepted as a public health problem. This might be the social programs intended to reduce the spread of HIV have generally proven insufficient and many HIV patients

in poor developing countries could not reach the high cost HAART. However, a long-term usage of HAART is limited by drug resistance development, severe side effects including pharmacokinetic drug interactions. Thus, the new antiretrovirals acting on alternative targets to avoid cross-resistance with old compound and with improved system tolerability profiles are required (Sierra, Kupfer, and Kaiser, 2005). In addition, a safe and cost effective vaccine for the prevention of HIV infection is urgently needed to decrease the epidemic.

The skin has recently been recognized as an attractive route for vaccine delivery because of its immune mechanisms characterization and convenience. Generally, delivery of vaccines through the skin is an invasive procedure, involving needles and syringes to facilitate intradermal, intramuscular and subcutaneous injection. These methods always cause the vaccinated subjects pain and sometimes produce bleeding. In addition, they require trained administrators. In developed countries, immunization without needles or syringes would increase acceptability and would enhance occupational safety for vaccinators and other health providers. This could be particularly critical in the future when it will be necessary to immunize large populations especially in the case of pandemic influenza or bioterror emergency (Levine, 2003).

Although the potential of plasmid DNA (pDNA) vaccine for eliciting antigen specific immune responses by using cationic microparticles, cationic polymers and cationic liposomes as carriers for pDNA delivery has been extensively studied (Locher et al., 2003), there is still a lack of data for *in vitro* and *in vivo* SLN-pDNA and CSN-pDNA delivery especially for transdermal HIV pDNA vaccine delivery. In this study, the effect of SLN and CSN formulation compositions on their physicochemical properties and potential for transdermal delivery of HIV pDNA vaccine were studied. The purposes of this study were:

1. To determine the effect of SLN and CSN formulation compositions on their physicochemical properties.
2. To determine the effect of SLN and CSN formulation compositions on their ability to form complex with HIV pDNA.
3. To determine the toxicity of SLN and CSN in HeLa cell.
4. To determine the potential of SLN and CSN as carriers for *in vitro* HIV pDNA vaccine transfection.
5. To determine the potential of SLN and CSN as carriers for transdermal mice immunization with HIV pDNA.