CHAPTER I

INTRODUCTION

Japanese encephalitis (JE) virus, a mosquito-born flaviviral infection, is the leading recognized cause of children encephalitis in the world, especially in Asia. Approximately 50,000 reported cases and 15,000 deaths are observed annually. Although the disease is transmitted mostly in Asia, the infection in other region are estimated at 5 cases per 1 million population. JE is now the key source of childhood viral neurological infection. By many standards, JE is a major public health problem that potentially can be controlled by proven effective vaccines (Misra and Kalita, 2010; Halstead and Tsai, 2004; Yang et al., 2004).

Inactivated JE vaccine derived from mouse brain or primary hamster kidney cells are currently in use. However, these vaccines require three inoculations which cause the low patient compliance and the injection have to be administered by trained personal. Thus, delivery of vaccine through mucosal route is very practical, non-invasive and alternatively efficacious for vaccine administration in order to achieve more patient compliance with the comfortable way of administration as this route requires neither sterile needles nor trained personnel. Appropriately formulated mucosal vaccines can stimulate all arms of the immune system and could be exploited for protection against pathogens that infect the host through the mucosal surfaces as well as those acquired through other routes (Magistris, 2006; Halsted and Tsai, 2004).

However, the mucosal immune response to most soluble antigens administered by mucosal route has been normally low and required large and frequently administered doses of antigen (McGhee et al., 1992) as a consequence of a mucosal barrier such as nasal clearance and gastric degradation. Hence, nasal delivery could be a selection as compare to oral delivery on an account of the low crucial enzymatic barrier. In order to overcome these barriers from many mucosal routes, there is a great need of adjuvant as a delivery vehicle and also as an immunostimulant to protect antigens from natural host defense mechanisms and effectively enhance the mucosal immune responses (Halstead and Tsai, 2004; Solomon et al., 2003).

Adjuvants are classified into two groups which are particulate and non-particulate adjuvants (O' Hagan, 1998). Many findings have studied so far on the applications and characteristics of nano/microparticles as particulate adjuvant (Singh and O'Hagan, 1998; Singh and O'Hagan, 2003) particularly those made from poly(D,L-lactide-co-glycolide) (Mundargi et al., 2008; Mainardes and Evangelista, 2004; Lee et al., 1999). Biodegradable nano/microparticles of poly(D,L-lactide-co-glycolide) (PLGA) and PLGA-based polymers are widely explored as carriers for controlled delivery of macromolecular therapeutics such as proteins, peptides, vaccines, genes, antigens and protection of these entrapped antigens from mucosal degradation. Furthermore, these devices facilitated to overcome the mucosal barrier and are mainly produced by emulsion or double-emulsion technique followed by solvent evaporation or spray drying. Particle size and surface morphology are key factors which influence the characteristics of PLGA particles as mucosal adjuvant (Tahara et al., 2008; Mundargi et al., 2008; Basarkar et al., 2008; Gutierro et al., 2002) which could be controlled and prepared by different formulation variables. However, there are some drawbacks of PLGA particles as mucosal delivery vehicles, considering mainly on the negatively surfaced charge which edges the adhesion of particles on mucosal surface (Nafee et al., 2007; Basarkar et al., 2007). Therefore, the surface modification of PLGA particles with positively charged substances such as chitosan and Aluminium hydroxide ($AlOH_3$) that hold the mucoadhesive and immunostimulative characteristics would be an noticeable implication. Chitosan is considered as a biodegradable polycationic polymer of low toxicity with mucoadhesive property that has been studied by many groups (Yang et al., 2009; Guo and Gemeinhart, 2008 and Mi et al., 2002) while $AlOH_3$ is an adjuvant of positive charge that enhances the high and long lasting antibody titer of vaccine by its depot property (Kanchan et al., 2009; Katare and Panda, 2006; Gupta et al., 1995). These two substances are potential candidates for surface modification of PLGA particles in order to augment the transport of particles.

Nasal-associated lymphoid tissue (NALT), a mucosal inductive site for the upper respiratory tract, is important for the development of both mucosal and systemic immunity to intranasally introduced particles and antigens (Casteleyn et al., 2010). The preferred sizes of particles taken up by mucosal associated lymphoid

tissue (M-cells), including NALT are considerable different from the optimal sizes of particles taken up by nasal cell (Xiang et al., 2006; Tacken, Torensma and Figdor; 2006; Gutierro et al., 2002). Moreover, the particles need to conquer the nasal clearance and deposited at the mucosal site in order to be taken up and stimulate the immune response (Kamath and Park, 1997).

Thus, this study was aimed to evaluate the achievability of PLGA particles of different sizes with the modification by chitosan and $Al(OH)_3$ on particulate surface to deliver JE vaccine via nasal route. The effect of formulation variables on particles size and surface properties, the in vitro transported mechanism and in vivo study of appropriate doses of vaccine, sizes and surface charges of vaccine entrapped PLGA particles were evaluated.

More specifically, the objectives of this study are :

1. To study the effect of formulation parameters on particle size and particle size distribution of PLGA in order to prepare PLGA particles of suitable sizes for nasal delivery.

2. To develop PLGA particles with suitable sizes for nasal delivery by mucoadhesive substances, chitosan and $Al(OH)_3$, in an attempt to obtain modified PLGA particles as effective nasal vaccine carriers and evaluate their physicochemical properties.

3. To investigate tissue uptake, tissue adhesion, tissue permeability and cytotoxicity of PLGA particles and modified PLGA particles using porcine nasal mucosa as model tissue.

4. To evaluate an appropriate dose of JE vaccine for intranasal administration and the effect of particle size along with surface charge of PLGA particles and modified PLGA particles containing JE vaccine on enhancing the intranasal immune response in experimental animal.