CHAPTER I

INTRODUCTION

Vaccination against infectious diseases is an attractive alternative to chemotherapy. Although, conventional vaccines use a live attenuated or killed organisms can induce protective immunity, but unacceptable side effect such as mortality and systemic infection may be still induced(1). Then, advance technology trend to be resulted in development of subcellular or antigen vaccines, because these vaccines can elicit specific immune responses, eliminated transfer of undesired pathogens. Unfortunately, these subcellular vaccines are usually not, or only weakly immunity in the absence of an immunological adjuvant(2).

Adjuvants are agents that can induce strong immunity to antigens. They appear to function by creating a depot at site of injection and activating macrophages(3). At present, there are only a few adjuvant using such as aluminium hydroxide (alum), saponin complexed, and Freund's adjuvant, only alum is licensed for safety to use in humans, but it is not always effective adjuvant. The others are toxic, inducing granulomas, acute and chronic inflammations, cytolysis and pyrogenicity(4,5). For these reasons, the new generation of adjuvants are desired and liposomes are discussed as an attractive adjuvant candidates.

Liposomes may be defined simply as phospholipid vesicles consists of phospholipid bilayer enclosing an aqueous compartment. They were brought to the attention as models of cell membranes by Bangham A.D. in 1965(6). Later,

liposomes were discussed in a new role as immunological adjuvant when they were first demonstrated by Allison and Gregoriadis in 1974, using diphteria toxoids (7) as an antigen, and had subsequently been confirmed and expanded in studied of many antigens(8-11).

Liposomes have several suitable properties for an immunological adjuvant (4). The first, since composed of natural lipids, they are biodegradable and nontoxic. The second, they readily disperse from site of injection to the draining lymph nodes, they do not cause granulomas or other local reactions. The third, they are sustained release system, function as an antigen depot. The last importance is their ability to induce cell mediated immunity (CMI). Although liposomes were considerd to be good but there are also several problems in developing liposomal vaccines include the difficulty in producing liposomes on a large scale, the expensive cost of phospholipid as raw materials, and the stability of liposomes during storage.

To overcome these defects various methods of liposomal stabilization were tried(12-27). The two methods that concluded effectively were adjusting cholesterol content of liposomal bilayer (12-17), and coating the surface of liposome vesicles with polymers (18-27).

Pasteurella multocida is a gram negative pathological bacteria, their pathogenesis frequently cause of great economic loss (28). The important disease caused by *P. multocida* were fowl cholera in avian species (29,30) and hemorrhagic septicemia in cattle (31-33). To improve the safety of the fowl cholera vaccine, the immunogenicity of antigens or subcellular extract of *P. multocida* were investigated (34-43). In former studies showed that an antigen extract from *P. multocida* by

KSCN extract were effective antigens and could stimulate protective antibodies (34-37).

In this study, liposomes containing protein extract from *P. multocida* were prepared by double emulsion techniques. The three molar ratios of lecithin to cholesterol (1:1 as cholesterol rich system, 7:2 as cholesterol poor system, and 1:0 as no cholesterol system) were used to study the effect of cholesterol content on physicochemical properties of liposomes. The physicochemical properties such as particle size, particle size distribution, entrapping efficiency, microscopic appearance, electronmicroscopic appearance, DSC thermogram, protein releasing profile at 37 ^{o}C were determined. The appropriate ratio was selected for further study, using polymer stabilized liposomes. For this, the carboxymethylchitosan (CM-Chitosan) in various concentrations (0.02,0.2,0.5% w/v) were used for stabilized liposomes and the comparison of carboxymethylcellulose (CM-Cellulose) in the same concentrations, were studied. The same physicochemical properties of liposomes were again evaluated. Furthermore, the stability of all formulations (at 4 ^{o}C for 3 months) were studied in terms of percent of protein remaining, particle size distribution, and microscopic appearance.

The purposes of this study are

1. To prepare stable polymer coated liposomes containing protein extract from *P. multocida* by double emulsion techniques.
2. To investigate the effects of cholesterol content on the physical properties and stability of liposomes.
3. To investigate the effects of polymer coating on the physical properties and stability of liposomes.