CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

One approach to develop a controlled release system of Japanese Encephalitis antigen was encapsulation antigen within polymeric microparticles. DL-PLA, PLGA 85:15, PLGA 75:25 and PLGA 50:50 were used as polymers to prepare microparticles. The encapsulation process was water-in-oil-in-water (w/o/w) emulsion solvent evaporation technique in order to improve the loading of water soluble peptide into PLGA microparticles. The w/o/w technique has since become one of the principal methods for encapsulating proteins and peptides (Ogawa, 1988).

All batches successfully formed microparticles under the condition employed and yielded smooth-surfaced, spherical particles. The formation of microparticles had been attributed to glassy structure with relatively lower crystallinity of polymers. Hence, they had a fairly rigid structure which gave them significant mechanical strength to be formulated (Jain, 1998). However, the effect of copolymer ratio and polymer molecular weight on particle shape and surface morphology were not obviously seen. This agreed with the study by Lacasse (1998). In addition the study of Jeffery (1993) showed the surface of particles prepared at low amount of protein appeared to be smooth and there was no evidence of collapsed particles. Whereas, the surface of particles prepared with high amount of protein appeared pitted and some of the particles had collapsed. This was owing to at high amount of protein, the quantity of polymer presented to completely cover protein was insufficient. However, the effect of amount of antigen added on particle surface morphology was not observed in this present study. This might be due to low amount of antigen added in preparations. The increasing amount of antigen in primary w/o emulsion led to a slight increase in mean particle size

which attributed to increase in antigen entrapment. This result agreed with a previous study from Jeffery (1993).

The emulsion stabilizer used in primary emulsion affected microparticle size. The emulsion stabilizer molecules diffused to emulsion droplet/aqueous phase interface resulting in a presence of stabilizer at surface of emulsion droplet formed during preparation process. This would provide an improvement in protection of droplets against coalescence, resulting in formation of smaller emulsion droplets in which solvent evaporated from the system. These droplets hardened to form microparticles. Therefore, size of microparticles was dependent upon size and stability of emulsion droplets formed during agitation (Nihant, 1994). Previous studies (Jeffery, 1991 and 1993) indicated that a significant reduction in particle size was achieved as concentration of PVA stabilizer increased. However, increasing concentration of polymer dissolved in a fixed volume of DCM resulted in an increase in particle size. The higher polymer concentration of dissolved polymer also increased viscosity of organic phase, which might have reduced efficiency of stirring of solutions. The relationship of microparticle size produced from different molecular weight of polymer and copolymer composition could not clearly conclude because the polymers used in this study had the difference in molecular weight and copolymer composition. However, the increase in polymer molecular weight resulting in increase viscosity of polymer solution which might have reduced efficiency of shearing of solution to break emulsion droplets. Therefore, large size of globules were formed. Additionally, copolymer composition influenced on particle size. The microparticles produced from PLGA 50:50 had smaller size than PLGA 75:25, PLGA 85:15 and DL-PLA, respectively. The more hydrophobicity of polymers, the larger size of microparticles were formed. This was attributed to the more lactide unit of polymer gave higher degree of hydrophobicity of the polymer resulting in high solubility in DCM. During

microencapsulation process, DCM was evaporated resulting in rapid solidification of more hydrophobic polymers and hence the large microparticles were formed. Contrastly, Mehta et al. (1996) suggested that copolymer composition had influence on particle size. Microparticles prepared with PLGA 75:25 had much smaller size as compared to those made with PLGA 50:50. This was owing to high solubility of PLGA 75:25 in DCM. Hence, during microparticle preparation process, a greater fraction of solvent must be removed in order to precipitate and formed microparticles. A polymer with higher solubility also permitted shrinkage of droplets before solidification and required longer solidification time, resulting in much smaller microparticles.

Further, microparticle size depended on the shearing force to form inner emulsion. In general, higher agitation rate promotes droplet breakage for the turbulent kinetic energy associated with increased droplet breakage. Therefore, higher agitation rate would result in smaller droplets (Kentepozidou, 1995). Therefore, the micro-particles produced with sonicating output of 15 gave a smaller size than those with sonicating output of 9. This result was in agreement to Cohen (1991) and Li (1997) studies.

The antigen content and entrapment efficiency in PLGA microparticles might be improved by increasing the amount of antigen in primary w/o emulsion. Prieto (1994) reported an increase in peptide loading and a slight decrease in encapsulation efficiency as concentration of peptide in internal aqueous phase increased due to a higher percentage of unencapsulated protein. The use of PLGA polymer with different copolymer composition had an effect on antigen entrapment in microparticles. The microparticles produced from PLGA 50:50 encapsulated antigen more than those from PLGA 75:25, PLGA 85:15 and DL-PLA, respectively. This higher lactide-to-glycolide ratio was more hydrophobic resulting in lower entrapment levels for hydrophilic

antigen. It was attributed to the higher penetration of aqueous phase containing antigen into the polymer matrix of hydrophilic polymers than hydrophobic polymers (Jeffery, 1993).

Mehta et al. (1996) suggested that incorporation of peptide into microparticles depended on partition coefficient of peptide between dispersed phase and continuous phase. The copolymer composition had influence on peptide incorporation efficiency. PLGA 75:25 polymer had a greater solubility in dispersed phase and hence required a longer time for solidification. There was a greater diffusion of peptide in non-solidified polymer matrix resulting in peptide partition into continuous phase. In contrast, PLGA 50:50 polymer had a lower solubility in dispersed phase and hence solidified relatively rapidly thus reducing peptide diffusion into continuous phase during emulsification resulting in higher peptide loading. On the other hand, Ogawa (1988) claimed that an increase in internal aqueous phase viscosity by using an increasing concentration of PVA dissolved in internal aqueous phase led to a reduction in partitioning of antigen into external aqueous phase. This also resulted in an increase in antigen entrapment.

Moreover, the antigen entrapment improved by increasing polymer concentration from 1.5 to 5%. The same trends were reported by Yan (1994) and Rafati (1997). The high concentration of polymer in emulsion droplets which tended to restrict migration of antigen from inner aqueous phase to external aqueous phase. However, increasing in shearing force of primary emulsification did not significantly improve antigen loading. Nihant et al. (1994) reported that improvement of protein loading and entrapment efficiency in PLGA microparticles obtained by increasing time of primary emulsification. This improvement was more significant at low polymer concentration. The creation of a fine dispersion of protein containing polymer droplets in primary

emulsion was indicated and an overall improvement in primary emulsion stability. This enhanced the loading of hydrophilic proteins.

In general, microparticles produced by w/o/w emulsion solvent evaporation method exhibit a biphasic profile of protein release, a typical initial burst release and a second phase of release (Rafati, 1990, Hora, 1990, Cohen, 1991 and Yeh, 1995). The initial burst release of antigen is normally considered to be due to the surface-located protein and the release of core-loaded protein which is derived from diffusional release through water-filled networks of pores and channels followed by matrix bioerosion. The second phase of release relies on various parameters such as degradation rate of copolymer matrix, denaturation and aggregation of protein molecules and polymer-protein binding.

In this present study, a very small initial burst effect was observed. This was attributed to low antigen concentration added in preparations resulting in low antigen content in microparticles. Furthermore, antigen was soluble in aqueous phase so that it could be homogeneously encapsulated within microparticles. Therefore, there was a small amount of antigen located or adsorbed on microparticle surface. Additionally, many times of washing in preparation process might reduce adsorbed antigen on microparticle surface. The antigen release from biodegradable polymer matrix might occurred by diffusion through a tortuous, water-filled path in polymer matrix and by polymer matrix bioerosion (Langer 1976 and 1990). However, increasing in polymer concentration further retarded antigen release due to increased viscosity of polymer solution. A highly viscous polymer solution yielded large-sized, compact and dense core microparticles resulting in decrease surface area and porous of microparticles. Therefore, the release of antigen was retarded. This was supported by a previous study of Singh et al. (1995).

Since most proteins and peptides have high molecular weight and are water soluble, not soluble in PLGA polymers, so the release from polymer matrix by partition dependent diffusion is minimal (Hutchinson, 1990). Consequently, the degradation of PLGA might be a crucial factor in determining protein release from polymer matrix. GPC studies showed in vitro degradation of PLGA in PBS pH 7.4 resulted in a progressive decrease in polymer molecular weight versus time. The degradation profiles appeared biphasic with an initial lag time, where only minor changes in molecular weight were measured and a terminal phase characterized by an accelerated polymer degradation. The difference in both lag time and terminal degradation was prominent among PLGA 50:50, PLGA 75:25, PLGA 85:15 and DL-PLA. PLGA 50:50 underwent the fastest degradation with a rapid decrease in weight average molecular weight. Contrastly, DL-PLA degradation was the slowest. It indicated that increasing hydrophobicity of polymers resulted in longer initial lag times and lower terminal degradation rate (Rafler, 1994 and Thomasin, 1996). Two possible mechanisms for enhancing degradation rate are firstly the presence of hydrophilic carboxyl end group in polymer structure may increase the rate and quantity of water uptake. This resulted in an increase in degradation rate of polymer matrix due to a reactant, water. Secondly, carboxyl end group may catalyze degradation reaction (Pettit, 1997). However, nonlinear first-order plot of PLGA degradation versus time was observed. This data indicated that hydrolysis of PLGA polymers was not truly complete random scission. This result was in agreement with the study of Shih (1995 a and b). His studies suggested that degradation of PLGA polymers followed a mechanism with ester hydrolysis at one or both of the chain-ends occuring faster than the internal ester bonds. However, as a result of sustained degradation , the polymeric microparticles were considered particularly useful for controlled antigen delivery over a period of several months.

During microparticle preparation, the antigen was exposed to vigorous conditions such as mechanical agitation and contact with DCM. These conditions might result in irreversible denaturation and loss of antigenicity of proteins. The structural integrity study by using SDS-PAGE revealed identical protein bands from antigen-encapsulated in microparticles and pure JE antigen. The band of E protein, antigenicity epitopes, at molecular weight approximately of 50 kDa was still remained. There was no additional band to indicate the presence of molecular weight fragments or aggregates. Therefore, it indicated that the structural integrity of JE antigen was not significantly affected by encapsulation process.

In this stability study, freeze-dried microparticle preparations were kept at 40 $^{\circ}$ C for 1 month. The temperature did not affect the shape and surface structure of microparticles. This was attributed to the stability of PLGA polymers at temperature 40°C (Aguado, 1992). However, antigen content and in vitro release of antigen decreased less than 10% after stability study. This trend was observed from report by Supajaturas et al. (1997). The study suggested that 10% decrease of antigen was within acceptable level. In addition, SDS-PAGE analysis showed similar protein bands in comparison to initial microparticles. The band of E protein was still observed. It concluded that at high temperature antigen–encapsulated in microparticles loss some antigen content, however, they still maintained the antigenicity of E protein. This result agreed with Gowal et al. (1991). They suggested that freeze-dried JE vaccine was stable for up to two weeks at 40 $^{\circ}$ C.

The solvent evaporation method is one technique of microencapsulation in which organic solvent is easily evaporated from an emulsion system so that solid microparticles are formed. However, it was difficult to use this technique to prepare chitosan microparticles because of difficulty to evaporate of acidic water. Many

literatures had also formulated chitosan microparticles by emulsion-phase separation technique in which glutaraldehyde was added as a cross-linking agent to solidify dispersed chitosan phase (Lin, 1992, Hassan, 1992 and Thanoo, 1992). However, crosslinking agent, glutaraldehyde, is toxic. It can cause irritation to mucosal membrane and may affect structural integrity of protein (Felt, 1998). In order to avoid harsh condition of glutaraldehyde, the emulsification technique via ionotropic gelation was employed to prepare chitosan microparticles (Lim, 1997). Gradually addition of counterion, tripolyphosphate (TPP) solution, into w/o emulsion resulting in gradually increase in volume ratio of dispersed : continuous phase and ionic gelation of chitosan droplets occurred due to interaction between negative groups of TPP and positively charged amino groups of chitosan (Calvo, 1997). However, weak chitosan micro-particles showing a high degree of aggregation were obtained. SEM micrographs indicated that most chitosan microparticles were nonporous and spherical-shaped with rough-surfaced that tended to form aggregates. Increasing chitosan concentration to 3% of MMW and 2 % and 3 % of HMW resulted in very high viscosity of polymer solution. As a consequence, the discrete microparticles were not formed and led to the formation of agglomerates. This was attributed to high viscosity of high polymer concentration and high molecular weight polymers resulting in reduce distance among each particle. The microparticles were closed together and tended to aggregate via Van der Waals forces such as induce dipole-induce dipole.

The increasing in polymer concentration might increase in average particle size (D(v,0.5). Microparticles produced from higher concentration of chitosan solution gave larger size than those produced from lower concentration. This was due to higher aqueous-phase viscosity hindered small globule formation during emulsification and resulting in large-sized globules. This result agreed with the studies of Hasson (1991),

Wang (1996) and Saha (1998). In contrast, Genta (1998) suggested that concentration of polymer did not influence particle size characteristics.

Furthermore, the effect of polymer molecular weight on microparticle size was observed. SEM analysis confirmed that polymer molecular weight had effect on size and appearance of microparticles. The increase in polymer molecular weight resulted in increase in particle size. This was due to high viscosity of high molecular weight polymer. This was in agreement with Wang (1996). His study suggested that chitosan types exerted a strong influence of particle size distribution.

As well as polymer molecular weight, amount of antigen added in preparations showed slight influence on particle size and size distribution. The increment in amount of antigen added in preparations gave large-sized particles because of the increment in antigen-to-polymer ratio resulting in improve antigen entrapment.

In addition, Saha (1998) suggested that increasing amount of Span 80 in primary emulsion greatly decreased mean particle size and size distribution. Wang (1996) suggested that mixed surfactant system of hydrophobic surfactant, Span, and hydrophilic surfactant, Tween showed good sphericity and relatively narrow size distribution.

Antigen content was influenced by amount of antigen added in preparations and chitosan molecular weight. The increment in amount of antigen added in formulations resulted in increase in antigen content. The relationship between amount of antigen added in primary emulsion and percent entrapment efficiency could not clearly conclude. Furthermore, polymer molecular weight had influence on antigen content and entrapment efficiency. The highest antigen content was obtained from microparticles

prepared from LMW CS as resulted by Genta (1998). Increasing polymer molecular weight resulted in decrease in antigen content and percent entrapment efficiency due to high aqueous-phase viscosity of high molecular weight polymer. Additionally, polymer concentration and aqueous-to-oil phase ratio had greatly influence on antigen content. This result contrasted to the previous study of Wang et al. (1996). Their research indicated that increasing polymer concentration from 1 to 3% w/v produced an approximately 4% elevation of drug loading and a 20% increment of drug entrapping efficiency.

In vitro antigen release from chitosan microparticles produced from different molecular weight was investigated. A very slightly initial burst effect was observed. This could be attributed to dissolution/diffusion of antigen from superficial region of chitosan microparticles. However, antigen is water soluble so that it can encapsulate within droplets during emulsification process more than adsorbed on surface region resulting in a small amount of initial burst release from microparticles. However, Calvo et al. (1997) suggested that the high percentage (> 40%) of protein containing in chitosan microparticles, the release profile become biphasic. This could be due to the presence of an important amount of proteins on microparticle surface. In this study, the antigen content in microparticles was extremely low so that the initial burst effect was not clearly observed.

In addition,other parameters not only antigen content but also polymer molecular weight could influence release of antigen. The faster release from microparticles was observed from microparticles of higher antigen content. This was in agreement with Calvo (1997). Microparticles produced from HMW CS achieved a slower release than those produced from MMW and LMW CS. The release profile of MMW CS and LMW

CS microparticles seemed marginally different. The antigen release from microparticles could be explained by swelling rate of polymer in dissolution medium (Genta, 1998).

In vitro degradation profiles of unloaded chitosan microparticles in PBS pH 7.4 characterized by GPC indicated a progressive decrease in polymer molecular weight along the study period. The molecular weight extremely decreased in an initial period of time followed by a minor change in polymer molecular weight at a terminal phase. This was attributed to the effect of tripolyphosphate in chitosan microparticles. The reaction of TPP could be activated by heat and water resulted in phosphoric acid liberation. The phosphoric acid increased the acidity of the system, therefore hydrolysis degradation was catalyzed. The concentration of phosphoric acid was diluted with time resulting in decrease in degradation rate. However, many literatures indicated that chitosan degraded by enzymatic hydrolysis. Yabuki (1989) expressed that the oligomers of glucosamine were detected and amount of them increased as incubation time proceeded during enzymatic hydrolysis of chitosan. Varum et al. (1989) suggested that enzymatic degradation of chitosan was a first order, random depolymerization. The acetyl groups in chitosan structure were essential for degradation of chitosan. Onishi et al. (1997) supported this result. They observed that polymer molecular weight decreased in a small extent without lysozyme, whereas chitosan microparticles underwent a more extensive degradation by lysozyme. In addition, in vivo degradation was investigated by subcutaneously implant of chitosan microparticles into mice. The result demonstrated that chitosan microparticles could degrade, which suggested biodegradation, gradually over 1 month. This indicated that biodegradation of chitosan microparticles was quite slow and obtained controlled-release properties.

The structural integrity characterized by SDS-PAGE showed that protein bands from antigen-encapsulated in microparticles were similar to those from pure JE antigen.

Moreover, antigenic epitope, E protein, was still maintained at molecular weight approximately of 50 kDa. Several literatures indicated that acidic pH may cause loss of antigenic epitopes. However, Guirakhoo (1992) and Konishi (1993) suggested that PrM in JE virus had also been conferred acid resistance to virus particles. Therefore, pH of chitosan preparations that ranging between 4-5 did not influence on JE antigenicity.

The stability study of chitosan microparticles kept at 40°C for 1 month was investigated. The changes in shape and surface of microparticles were not seen. However, SEM showed the development of microparticulate agglomeration. Further, the increase in average particle size was observed. This was attributed to agglomeration of particles. The decrease in antigen content and in vitro release was range between 4 - 9 % after stability study. Additionally, structural integrity of antigenic epitope still remained. Thus, it concluded that freeze-dried preparations of JE encapsulated in chitosan microparticles were still stable at 40 ° C for 1 month although they lost some properties. The stability study of JE vaccine by Gowal (1991) and Supajaturas (1997) supported this result.

Following a single subcutaneous administration, antigen-encapsulated in PLGA and chitosan microparticles induced antibody titre responses more than fluid antigen. Antigen-loaded microparticles prepared with PLGA 50:50 polymer reached a maximum response and maintained antibody titre level at a period of time. However, the difference of antibody level response provoked by microparticles prepared by different polymer concentration was observed. The microparticles prepared with 5 % PLGA 50:50 polymer reached a maximum antibody level in the same time but could prolong an immune response comparable to those prepared with 1.5 % PLGA 50:50 polymer at the same dose. This might be attributed to the high viscosity polymer solution from high

polymer concentration resulted in compact and dense microparticles with reduced porosity and finally slowed down the release rate. In addition, microparticles produced from high polymer concentration had a large size and wide particle size distribution. For these reasons, antigen release from microparticles prepared with higher polymer concentration were slower and more prolong than those prepared with lower polymer concentration. The effect of dose of administration was investigated. The administration of 150 μg of antigen-loaded PLGA 50:50 microparticles augmented higher titre level and more prolonged effect than administration of 50 μg of the same formulation. Although the administration dose was increased 3 times, the antibody titre level was observed to be elevated by less than 3 times.

The titre level induced by a single administration of encapsulated microparticles was significantly lower than by given three doses of fluid antigen after week 6 ($p < 0.05$). However, the effect of this parameter on prolonging of antibody titre level did not clearly conclude in the short periods of study. Sah et al (1995) suggested that antigen given in frequent small doses was more effective in stimulating immune response than the same dose administered in one injection.

PLGA 50:50 microparticles exhibited rapid degradation due to increasing number of glycolide unit render of polymer more hydrophilic resulting in a more pronounced water uptake and facilitating hydrolytic cleavage. Therefore, microparticles produced from PLGA 50:50 prolonged antigen release and immune response for a short period. Thomasin et al. (1996) suggested that polymer degradation had been an importance parameter for programming potential response. This was in agreement with O'Hagan (1994) and Coombes (1996). They indicated that antibody levels elicited after administration from OVA-loaded 75:25 PLGA microparticles was significantly greater than that elicited by administration of OVA-loaded 50:50 PLGA microparticles because

75:25 PLGA microparticles exhibited extended degradation time due to increased lactide content of copolymers. As well as polymer degradation, microparticle size is another one factor affecting immune response. The particle size engendered a priming and initial immunogenic response followed by controlled release system. Antibody titre level observed at early stage would be the contribution of small microparticles and the sustained immune response was likely to be associated with the slow antigen release from large microparticles (Men, 1996). Many studies explained these results by small-sized microparticles, approximately less than 10 μm, were first engulfed by APCs especially macrophages, the entrapped antigens processed rapidly within macrophages and migrated into draining lymph nodes. This mechanism led to augment initial immune response. The microparticles that were greater than approximately 10 μm in diameter remained at the site of administration, acted as an antigen depot and provided sustained release of entrapped antigen from microparticles by polymer degradation and finally, might also be partly phagocytosis of fragments of particle with associated antigens, as microparticle degradation and breakdown leading to pulsatile immune stimulation (O ' Hagan, 1993 and Johansen, 2001). There was no inflammation and granulomata formation observed during the study, which was agreed by studies from Visscher (1987) and Eldridge (1991). Moreover, many literatures revealed that PLGA microparticles could elicit cell-mediated immune response (Moore, 1995, Ertl, 1996, Partidos, 1996, and Johansen, 2000).

Antigen-encapsulated in chitosan microparticles could also elicit humoral immune response. They augmented IgG antibody titre level and reached a maximum level at week 6, faster than microparticles prepared with PLGA. This might be attributed to fast release of antigen from chitosan microparticles. Antigen-loaded microparticles produced from chitosan polymer had a smaller size with some rough and uneven surface so that they had large surface area resulting in faster antigen release than

PLGA microparticles. However, chitosan microparticles exhibited lesser prolong effect response than PLGA microparticles. This might be due to fast degradation of chitosan polymer in animal models. In addition, in vitro degradation of chitosan could not completely explained in vivo degradation because of enzymatic degradation of chitosan polymer (Jameela, 1995). Therefore, degradation in vivo was faster than in vitro experiment. However, chitosan microparticles were used as antigen carrier to stimulate and prolonged humoral immune response over a period of time. The ability of chitosan microparticles to stimulate immune response was generated via multiple mechanisms. Calvo (1997) expressed that chitosan microparticles augmented antibody titre response by ability of chitosan to bind and slowly release antigen. Nishimura (1984 and 1986) commented that chitosan induced immune response by stimulated APCs especially macrophages and released of cytokines. Dupuis (1998) revealed that particulate adjuvants such as chitosan microparticles elicited efficient immune response and facilitate antigen uptake and translocation to draining lymph nodes via dendritic cells. Additional, few literatures had been reported effect of chitosan to bind to lipopoly saccharide receptor, CD14 (Otterlei, 1994) and enhance cytotoxic T lymphocyte (CTL) responses (Seferian, 2001).

However, the antibody titre level augmented by antigen-loaded chitosan microparticles was lesser than by antigen-loaded PLGA microparticles given at the same dose. This might be attributed to the less adjuvant effect of chitosan microparticles comparable to PLGA microparticles.

Therefore, antigen encapsulated in microparticles prepared with biodegradable polymers such as chitosan and PLGA could be used for controlled-release delivery system. The microparticle characteristics depended upon several crucial parameters including technique to prepare microparticles, polymer characteristics such as molecular

weight, concentration, copolymer composition and degradation rate. These parameters could affect to sustain antigenn release and prolong immune response. The adjustment of these parameters could be successful in an effective single dose vaccine preparation.

Conclusion

Biodegradable microparticle was one of the greatest ranges of utility in controlled release of any protein and antigen. They could be utilized both in injectable and in oral preparations. PLGA polymers could be successfully used to produce microparticles encapsulating JE antigen by multiple emulsion-solvent evaporation technique. Several formulation parameters principally polymer concentration, copolymer composition, shearing force, and amount of antigen added in primary emulsion extremely influenced microparticle characteristics. The antigen release from PLGA microparticles was mainly controlled by polymer degradation rather than simple diffusion. The hydrolysis of polymer resulted in production of low molecular weight polymer fraction in which reached a critical value, oligomer dissolution and antigen release proceeded. The residual DCM in microparticle was concerned. The higher residue DCM content in microparticles, more than 75 ppm, was not accepted to use in animals and humans. In addition, stability of encapsulated antigens in microparticles during storage was also an important issue. This study concluded that JE antigen encapsulated in freeze-dried microparticle preparations could maintain antigenic epitope after exposure to high temperature, 40°C for 4 weeks.

In vivo study, the antigen containing microparticles exerted an immune stimulating response in term of antibody response. Antibody titre of a single dose administration elevated and maintained over entire time period studies comparable to fluid antigen. Therefore, PLGA microparticles could be flexible delivery systems

capable of encapsulating antigens and allowed development of safe, single-dose vaccines.

As well as PLGA polymers, chitosan, a biodegradable polysaccharide with less antigenic polymer was employed to formulate antigen-encapsulated microparticles. The emulsification and ionotropic gelation technique was used to prepare chitosan microparticles in order to avoid glutaraldehyde toxicity. The microparticles were formed by ionic gelation of chitosan with TPP. The microparticle characteristics such as particle size, antigen content and in vitro antigen release were affected by many formulation parameters including polymer molecular weight, polymer concentration, aqueous-to-oil phase ratio and amount of antigen added in primary emulsion. All microparticle preparations had mean particle size ranging between 6-10 μm that could be subcutaneously administered. The polymer degradation in PBS pH 7.4 was fast in the first four weeks followed by slow degradation along period time studies. However, many literatures revealed that several glycosidases especially lysozyme, an enzyme found in various mammalian tissues, accelerated chitosan degradation. Thus, in vitro degradation was not completely representative to in vivo degradation.

In vivo experiments, JE antigen encapsulated in chitosan microparticles could elicit antibody titre level after a single dose administration for a period of time. Although chitosan microparticles could evoke antibody titre response but titre level induced by chitosan microparticles was less and less prolong effect than microparticles prepared by PLGA at the same dose.

However, both biodegradable polymers, PLGA and chitosan, could be employed as controlled release delivery system of JE antigen for a period of time. The properties of polymers such as molecular weight, polymer composition, viscosity and degradation

rate might affect microparticle characteristics including immune response. In addition, ability of these polymer formulations can be used as vaccine delivery systems in humans in the future because of their high biodegradability, biocompatibility, nontoxicity, no irritant or allergenic and nonantigenicity.