5. SUMMARY

Delivery of antigen using polymeric particles offers many opportunities in fine-tuning the immune response to a particular pathogen to achieve protective immunity. Biodegradable polymeric microparticles are nowadays well recognized as potent immuno-adjuvants. To date, several candidate protein vaccines have been entrapped in the polymeric particles and have shown to enhance the antibody responses against the antigens. So far there are no reports available on the immune response from biodegradable polymeric particles entrapping polysaccharide antigens. Many polysaccharide-based vaccines are available for human use and have consistently shown an efficacy of over 60% in adults. Protection offered by polysaccharide vaccines is relatively short-term, possibly due to the fact that polysaccharide antigens are T-cell independent antigens. Thus, these vaccines need multiple boosting in every 3–5 years. Polymer particle-based vaccine delivery systems provide a viable alternative to multi-dose immunization schedule for many infectious diseases where neutralizing antibody titers provide protective immunity and memory response. Considering this, the current study aims to develop delivery systems entrapping capsular polysaccharide antigens in polymeric particles to enhance its immunogenicity. Vi polysaccharide and pneumococcal capsular polysaccharides (PCP-1) were used as model antigens. Polylactide (PLA) polymers were used for formulation of particles entrapping polysaccharide antigens. Current study also explored the possibility of co-entrapping a carrier protein and a T independent antigen in the same polymeric particle and to evaluate its immunogenicity in experimental animal models. Recombinant PspA and PsaA were used as the carrier proteins with PCP-1 in the co-entrapped formulations. Scaling up of conventional double emulsion-solvent evaporation method for sterile manufacturing and loss of antigen stability during particle preparation are the main problems associated with PLGA/PLA based delivery systems. To address these limitations efforts were made in this work to optimize the spray drying process for formulation polymer particles entrapping antigens. Detailed investigations were also carried out on the interaction of antigen entrapped polymer particles with antigen presenting cells.
The following conclusions were made from the present work.

* **19)** Polymer particle entrapping polysaccharide antigens were formulated using double emulsion solvent evaporation method. Hydrophobic polymers like PLA resulted in higher entrapment efficiency and uniform size distribution.

**20)** Polymer particle co-entrapping T-dependent antigens (polysaccharide antigens) and T-independent antigens in the same polymeric matrix were formulated. Co-entrapment did not interfere with the entrapment efficiency of the polysaccharide antigen.

**21)** Immunization studies using polysaccharide antigens entrapped in polymeric particles resulted in stronger anti-polysaccharide antibody responses. It promoted isotype switching as well as anti-polysaccharide memory antibody responses. This effect was reproducible for two totally different polysaccharide antigens, PCP-1 and Vi polysaccharides.

**22)** Polysaccharide antigens entrapped in nanoparticles elicited stronger antibody responses than microparticles and soluble polysaccharide immunizations. Nanoparticle formulations were effective in inducing anti-polysaccharide memory IgG responses. This effect was observed for both the polysaccharide antigens used in the study.

**23)** Co-entrapped formulations did not improve the anti-polysaccharide IgG responses. The same effect was observed irrespective of the type of polysaccharide antigen used, carrier protein and size of the formulations.

**24)** The polysaccharide component (Vi antigen) interfered with the anti-TT (protein component) antibody responses. Since Vi antigen interfered with anti-TT immune response, a strong anti-TT immune response was not observed. Thus, CD4\(^+\) T-cell help expected from the protein component did not work to improve the anti-polysaccharide antibody responses.

**25)** Immunization studies using polymer particles with higher surface density of polysaccharide antigen improved the anti-polysaccharide antibody responses.

**26)** Challenge experiments with *Salmonella typhi* in mice immunized with Vi antigen entrapped polymer particle formulations elicited stronger recall
antibody response upon challenge, as well as induced stronger anti-Vi IgG1 and IgG2a responses.

27) Immunization with pneumococcal capsular polysaccharide entrapped in nanoparticles protected the mice better when challenged with live pathogenic *Streptococcus pneumoniae* than soluble immunizations. Polymer particle based immunizations resulted in protective antibody response. It also induced memory antibody response which helped to clear the pathogen infected after three months of primary immunization.

28) Phagocytic uptake studies using fluorescent labelled polymer particles revealed that nanoparticles were phagocytosed more efficiently than microparticles. Phagocytic uptake of both nanoparticles as well as microparticles induced cytoskeletal changes in the APCs.

29) Phagocytosis of polymer particles induced remodelling of lysosome clusters, localizing lysosomes preferentially to compartments engulfing particles. This preferential enrichment of lysosomes to compartments engulfing particles was important because this promote the processing and presentation of antigens continuously released from the polymeric particles.

30) Phagocytic studies using Vi polysaccharides entrapped particles proved the anti-phagocytic activities of Vi antigen. Vi interfered with the phagocytosis of particles affecting the processing and presentation of tetanus toxoid. This resulted in reduced anti-TT responses.

31) Vi polysaccharides also inhibited the lysosome remodelling effects of polymeric particles. Phagocytosis of plain polymeric particles induced preferential enrichment of lysosomal clusters to polymer particle engulfed compartments. This effect was abrogated when Vi antigen was present on the particle surface. This suggested the role of Vi antigen in processing and presentation of co-entrapped protein antigens.

32) Optimization of spray drying process parameters, established the pivotal role of fluid feed rate and atomization pressure in regulating particle size distribution of polymeric particles. Process parameters especially feed rate, atomization pressure and inlet temperature had maximum effects on the yield of particle during spray drying.
33) Type of emulsion used, percentage of emulsion stabilizer used in EAP and the percentage total dissolved solids played a major role in regulating size distribution, release profile and entrapment efficiency of spray dried particles.

34) Spray dried particles showed better powder flow properties than particles prepared using conventional double emulsion solvent evaporation methods. This suggested that spray drying method is suitable for large scale manufacture of particles with desired size distribution and flow properties.

35) Immunization studies using spray dried microparticles entrapping PspA elicited higher antibody responses than soluble PspA. This suggested that preparation of antigen entrapped polymer particles using spray drying retains the immunogenicity of antigens.

36) Spray drying W/O/W emulsion directly with alum in external aqueous phase was the most effective formulation strategy to prepare particles entrapping alum and antigen.

37) Particles entrapping alum and antigen demonstrated narrow size distribution without aggregation, good re-dispersibility and good encapsulation efficiency.

38) The surface adsorption of alum was confirmed using elemental mapping and EDX analysis. Alum surface adsorbed on microparticles showed amorphous nature on X-ray diffraction studies where as alum in lyophilized formulations showed crystalline nature.

39) Microparticles co-entrapping alum and PspA prepared using spray drying, on immunization elicited stronger anti-PspA antibody responses than alum adsorbed PspA.

40) Spray dried microparticles entrapping PspA showed good aerodynamic properties. Both non-porous hollow as well as porous particles showed acceptable MMAD and percentage fine particle dose.

41) *In vitro* lung deposition studies suggested that, microparticles prepared using spray drying were suitable for pulmonary delivery of antigen. They are capable of non invasive delivery of entrapped antigen to the lungs.
42) Aerosol immunization of PspA entrapped polymeric particles elicited anti-PspA antibodies suggesting the non-invasive delivery potential of polymer particles.

43) Spray drying is a very useful one-step process for formulation of antigen entrapped polymer particles. Particles with different characteristics can be prepared by varying the formulation parameters during spray drying.