The aim of the present study was to develop and evaluate porous nanoparticles for the treatment of enterotoxigenic *Escherichia coli* infection using chitosan as polymer. Chitosan is a natural and biodegradable polymer suitable for developing nanoparticles and found to be promising when used as a vaccine adjuvant. Research work has been divided into three sections whose significant findings are summarized below.

**5-Fluorouracil loaded chitosan nanoparticles produced by spray drying technique.**

Chitosan nanoparticles have been developed using spray drying technique. For this part of research study, 5-Fluorouracil has been selected as a model drug. Present part of the research work is aimed to optimize the various operational parameters in order to produce nearly spherical nanoparticles using spray drying technique. Effect of feed rate, inlet temperature, storage time, molecular weight and concentration of chitosan on nanoparticle size, surface morphology has been investigated.

- FTIR and DSC studies confirmed drug–polymer compatibility.
- Gel permeation chromatography studies showed that chitosan is not degraded after spray drying.
- Use of 0.5 ml min$^{-1}$ inlet rate led to formation of discrete spherical particles whereas NP obtained from higher feed inlet rates (3 ml min$^{-1}$ and 6 ml min$^{-1}$) tend to have larger size, rough surface and tend to form aggregates. Hence use of 0.5 ml min$^{-1}$ was selected for further studies.
- NPs obtained with low inlet temperature (120 ºC) were found to be nearly spherical in shape whereas with high inlet temperatures (160 ºC) they were
found to be deformed in shape, so use of low inlet temperature (120 °C) was considered for further studies.

- Study results revealed that NPs prepared at 0 day storage time were found to be nearly spherical with smooth surface, whereas NPs prepared after 3 days storage time showed rough surface with presence of dents. Hence 0 day storage time was found to be promising for further part of research work.

- There is an increase in particle size as molecular weight of chitosan is increased.

- 5-FU3 formulation was able to sustain the drug release for 18 hr and based on *in vitro* data this has been selected for *in vivo* studies. It was found that NP formulation (5-FU3) presented a sustained drug release compared to plain 5-FU, making an increase in AUC curve.

- 5-FU3 formulation was also found to be stable for particle size and drug content over a period of 6 months.
Metoprolol Tartrate loaded porous chitosan nanoparticles produced by spray drying technique.

Controlled release porous nanoparticles of Metoprolol tartrate (MT) were prepared by spray drying using a pore forming agent Ammonium carbonate (AC). The presence of pores in ceramic foams offered the possibility, to use these porous ceramics as carriers for local and controlled delivery of drugs.

- Nanoparticles were successfully prepared by spray drying method. Micromeritic properties for prepared nanoparticles were found to be satisfactory.
- MT was successfully loaded in porous NP by imbibing method.
- Drug loading increased with increase in the amount of pore forming agent.
- FTIR and DSC studies confirmed drug–polymer compatibility.
- SEM figures revealed that the drug loaded nanoparticles were found to be distinct, spherical having a porous surface.
- TEM analysis showed homogeneous drug distribution within the nanoparticles prepared.
- Disappearance of the crystalline peaks of the drug in the prepared nanoparticulate batches compared to the pure drug MT, indicated an amorphous state of the drug in the spray dried formulations as confirmed by X-Ray Powder Diffraction studies.
- Data obtained after GPC analysis showed that no /negligible changes in polymer molecular weight occurred after spray drying of chitosan.
- *In vitro* release studies showed that formulation CH7-CH9 was able to sustain the drug release for more period of time as compared to other batches of formulations prepared.
The best fit model representing the mechanism of drug release from the porous nanoparticles prepared was of zero order.

Formulation CH8 was found to be stable for particle size, zeta potential and *in-vitro* release over a period of 6 months.
F4 loaded porous chitosan nanoparticles produced by spray drying technique.

In the present part of study, F4 fimbriae loaded porous chitosan nanoparticles were developed against ETEC infection using ammonium carbonate (AC) as a pore forming agent. Practicability of F4 fimbriae loaded porous nanoparticles for oral vaccination against ETEC was investigated.

- Porous nanoparticles were successfully prepared by spray drying method using ammonium carbonate as a pore forming agent. Micromeritic properties for prepared batches of nanoparticles were found to be satisfactory.
- F4 was successfully loaded into porous NPs by imbibing method. It was found that as we increase the polymer concentration, an increase in F4 loading took place.
- SEM figures revealed that the drug loaded nanoparticles were found to be distinct, spherical having a porous surface.
- TEM analysis showed the core of nanoparticle matrix and a complete interconnected pore network could easily be viewed within the nanoparticles prepared.
- In vitro villous adhesion assay confirmed that antigen specific receptors is a pre requisite for the initiation of an immune response following oral immunization, by purified F4 fimbriae, and thus the above test proved to be feasible and promising to demonstrate the presence of F4R (F4 receptors).
- It was found that nanoparticles having a low particle size showed profound mucoadhesion as compared to other NP batches with large particle size distribution.
Summary & Conclusion

- It was found that F4 retains its ability in coated NP batches and there is no drastic effect of freeze drying on antigenicity or stability of F4 in NP batches.

- It was clearly shown that due to the presence of interconnected pore network of NPs, F4 gets easily absorbed and distributed throughout the entire NP matrix as confirmed by fluorescence microscope.

- *In vitro* data showed that formulations CH5 and CH6 were able to prolong the release up to 24 hr as compared to other batches of formulations.

- Antibody titre data, clearly revealed that F4 solution and F4 loaded NPs were able to induce an immune response, most prominent in NP group as compared to the solution group.

- Faecal excretion studies clearly proved that enteric coated solid NPs prevent the colonization of small intestine by F4*ETEC* as compared to solution group and therefore can be a promising tool, which will protect the antigen from acidic pH and thus deprive its ability to evoke a premature immune response and will target the antigen to the proper site thus producing a desired immune response.

- CH6 formulation was found to be stable for particle size, zeta potential and serum F4 specific IgG antibody titers responses release over a period of 6 months.

Thus the present research work has been carried out adopting standard procedures to meet the set objectives. The research findings obtained from the studies were found to be satisfactory. It can be concluded present study establish the feasibility of formulating porous chitosan nanoparticles loaded with F4 fimbriae can be effectively used for the treatment of enterotoxigenic *escherchia coli* infection.
Suggestion for Future Work

- Use of other natural polymers to formulate nanoparticles which can be used as vaccine adjuvant,
- Clinical trials,
- Long term stability studies.