2. SCOPE AND OBJECTIVES

Bacteraemic infection of *Salmonella enteric serover* (*S*. *Typhi*), responsible for 20 million cases of illness, is the root cause of death in patients with typhoid fever in the resource-poor regions of the world. Treatment of typhoid fever, however, is challenging because of the intracellular nature of the infected bacterium and the lack of availability of the administered drugs at the intracellular compartments. Several vaccines developed so far provide only short term protection against the disease.

Recent studies, however, indicate that controlled delivery of antibiotic loaded nanoparticles (NPs) can overcome these problems because NPs are capable of penetrating cells and release the drugs in to the intracellular compartments (*Clisson et al., 1998*). The use of NPs made from biodegradable polymers to deliver drugs has, therefore, attracted considerable attention of the researchers in recent years. Among them, Chitosan (CS) and Pluronics (PLU) are very promising and have been widely exploited in pharmaceutical industry.

Azithromycin (AZI) is an azalide antibiotic derived from erythromycin, effective against both Gram-positive and Gram-negative bacteria, demonstrating bactericidal activity against Salmonella species. It is also known to be effective for the treatment of multidrug resistant *S*. *typhi* (*Parry, 2004*). AZI therapy with conventional immediate release formulations, however, is associated with several side effects such as nausea, vomiting, abdominal cramping, headache, and dizziness. The incidence of these
adverse effects is due to high peak plasma concentrations of AZI (Christopher&Barradel, 1996). One approach for eliminating the high peak plasma concentration is to develop a nano based drug delivery system to release the drug slowly to the systemic circulation.

In recent years, several studies have, therefore, been carried out on nano-based drug delivery systems of AZI, in order to target and improve the delivery of AZI to the infected cells. AZI nanoparticles in the form of nanosuspension have been studied to overcome the poor bioavailability of AZI (Zhang et al., 2007).

CS is widely used for preparing nanoparticle drug delivery systems because of its non-toxic nature, mucoadhesiveness and biodegradability. Pluronics exhibits sol-gel transition behavior in response to temperature in aqueous medium. In aqueous solutions of 20 to 30% w/w PF127 is liquid at 4- 5°C but gel upon warming to body temperature. When injected in to the body cavity it should, therefore, form in situ gel and hence act as a sustained release depot. The delivery of AZI by means of CS and Pluronics (both PF127 and PF68), however, has not been explored and no studies have been reported so far on the formulation of a simple and easy method for preparing CS-Pluronic based AZI nanocomposite so as to improve the antibacterial activity of AZI.

Docetaxel (DTX) has poor water solubility. A mixture of tween 80 and ethanol (50:50, v/v) is, therefore, used in DTX formulations currently available in the market. Both tween 80 and ethanol, however, are responsible
for hypersensitivities (respiratory distress, hypotension, angioedema, generalised urticaria and rashes) that occur after DTX administration and make premedication of the patients with corticosteroids (Dexamethasone) and antihistamines, a necessity (Weiss et al., 1990; Bernstein, 2000). Despite premedications with corticosteroids and histamine antagonists, reactions like flushing and rash are observed in approximately 40% of patients. Nearly 3% of patients experience potentially life-threatening reactions (Loos et al., 2003).

Further, the oral bioavailability of DTX is only 8.0% due to Pgp efflux in intestine. The pharmacokinetic behavior of DTX is also influenced by the solvent tween 80 present in its formulations. Degradation of tween 80 in plasma is very rapid and binding of the drug with plasma has been observed to be concentration dependent. The nonlinear pharmacokinetics of DTX and taxane, in general, has been attributed to its less metabolic elimination after intravenous administration (three times in a week with every 3h/day) due to the accumulation of the drug in the systemic circulation by micellar entrapment of CrEL in plasma. The pharmacodynamics of the solubilized drug is, therefore, altered. In addition, tween 80 has its own antitumor activity and the complexity of the pharmacological actions exerted by this along with DTX poses a challenging problem.

Efforts have, therefore, been made in the past to enhance the solubility, bioavailability and therapeutic efficiency of DTX by formulating polymersomes, polymeric nanoparticles and liposomes (Afrouz et al., 2009;
Zhihong et al., 2011). However, no investigations have been carried out on a nanocomposite based drug delivery system for DTX using, chitosan (CS), conjugated linoleic acid (CLA) and Pluronic F127 (PF127) in order to enhance its therapeutic efficacy.

In this context, it may be pointed out that CLA has been shown to sensitize tumor cells to chemotherapy. As already mentioned, CS has been widely used for preparing nanoparticle drug delivery systems because of its nontoxic nature, mucoadhesiveness and biodegradability. CS, however, is hydrophilic in nature. CS can be coupled to CLA to obtain CS-CLA to make it strongly amphiphilic in nature. The amphiphilic character of CS-CLA can thus have better biocompatibility and may show good interaction with proteins, enzymes and lipophilic compounds (Chen et al., 2005). CS-CLA, however, can render the highly hydrophobic DTX less soluble. The synthetic biopolymer, PF127, with its low toxicity, unique properties such as amphiphilic nature, bulky structure and large surface area can be used as an excellent solubilizer for the highly hydrophobic drug, DTX. In addition, as already mentioned PF127 exhibits sol-gel transition behavior in response to temperature in aqueous medium. In aqueous solutions of 20 to 30% w/w PF127 is liquid at 4- 5°C but gel upon warming to body temperature. When injected in to the body cavity it should, therefore, form in situ gel and hence act as a sustained release depot.
The objectives of the present investigation were, therefore, to design, develop and evaluate nano-based drug delivery systems for enhanced therapeutic efficacy of the selected drugs, namely AZI and DTX. In particular, the investigation aims to,

- Develop and evaluate an *in situ* gel forming CS and PLU based AZI nanocomposite so as to improve its antibacterial activity and
- Develop and evaluate an *in situ* gel forming nanocomposite delivery system for DTX using CS-CLA and PF127 in order to overcome the problems associated with this drug.

In other words, to carry out investigations to enhance the intracellular drug concentration of the selected drugs, namely AZI and DTX so as to increase their therapeutic efficacy.

**Proposed plan of work**

It was proposed to carry out the work in the following stages;

**Stage I: Development and *in vitro* drug release studies of *in situ* gel forming AZI loaded nanocomposite.**

- Development of calibration curve for AZI
- Formulation of AZI loaded CS-PLU nanocomposite
- FTIR and DSC studies
- Particle size distribution and SEM analysis
- Determination of the process yield of AZI nanocomposite
- Determination of drug entrapment efficiency
- *In vitro* drug release studies
Scope and Objectives

Stage II: Development and *in vitro* drug release studies of *in situ* gel forming DTX loaded nanocomposite

- Analytical method development for DTX using HPLC
- Synthesis of CS-CLA
- Solubility of DTX in PF127 solution
- Formulation of DTX nanocomposite
- Characterization of the DTX nanocomposite
- DSC studies
- Particle size, surface charge and surface morphology studies
- Determination of drug load and encapsulation efficiency
- *In vitro* drug release studies

Stage III: *In vitro* evaluation of the developed AZI loaded nanocomposite and *in vitro* and *in vivo* evaluation of the developed DTX nanocomposite.

- *In vitro* blood compatibility analysis of AZI nanocomposite
- *In vitro* antibacterial studies of AZI nanocomposite
- *In vitro* cytotoxicity studies of DTX nanocomposite
- *In vitro* cellular internalization studies by TEM.
- *In vivo* cytotoxic study of the DTX nanocomposite