## 1. Introduction

Drug delivery through oral route is the most preferred and readily accepted amongst all other routes, as it offers high compliance and convenience to patients through ease of administration. Moreover, its non-invasive nature, flexible and controlled dosing schedule, cost effectiveness, avoidance of pain and infections associated with inappropriate use of parenteral formulations, make the oral route patients friendly for chronic therapy, especially for pediatric and geriatric patients [1-3]. More than 60% of drug products currently available in the market are administered through oral route [4]. Upon oral administration, the systemic entry of any drug molecule through the gastrointestinal tract (GIT) mainly depends on the two parameters; solubility and permeability. Based on these parameters, Amidon *et al.* have classified all the drug molecules in four classes, named as Biopharmaceutical Classification System (BCS). According to BCS, drugs having high water solubility and low permeability are termed as BCS class-III drugs [5]. BCS class-III (poorly permeable hydrophilic) drugs generally follow the paracellular pathway for permeating through intestinal epithelium. However, the paracellular pathway occupies very smaller surface area as compared to transcellular pathway [6, 7]. Moreover, the poor physicochemical properties, i.e., ionic charge at physiological pH, hydrogen bonding property, substrate to drug efflux pumps like p-glycoprotein, low lipophilicity, high molecular weight and instability due to rapid enzymatic degradation in GIT etc., confine their permeation across GIT, which results in poor oral bioavailability and thereby, restricts their clinical utilization even though they possess high therapeutic potential [7-10].

The rate controlling step during the absorption process of BCS class-III drugs is the GIT permeation. Tight junction between the enterocytes of the intestine restricts the

transport of hydrophilic drugs across GIT. However, the tight junctions are dynamic in nature and can be modulated in order to improve intestinal permeation of the BCS class-III drugs [7, 11]. Several pharmaceutical strategies i.e., prodrug approach, chemical modification by conjugating transporter or receptor recognition molecules, use of permeation enhancer and development of specialized drug delivery system etc., have been employed for enhancing the intestinal absorption of hydrophilic drugs and thereby, oral bioavailability [12-14]. Amongst them, co-delivery with permeation enhancer has drawn considerable attention for enhancing GIT permeability of hydrophilic drugs, by increasing membrane fluidity or reversible opening of the tight junctions between enterocytes [15, 16]. However, the use of permeation enhancer in high dose above the critical enhancement concentration, to achieve significant permeation, results in the irreversible disruption or removal of intestinal barrier and thereby, allows nonspecific absorption of undesirable substances, which leads to severe toxicity [12, 14, 17]. Additionally, the number of adverse reactions i.e., headache, nausea, diarrhoea, emesis, abdominal pain, pharyngitis and dyspepsia etc., reported during the clinical trials, have curtailed their successful translation into an effective oral delivery system [18]. In order to reduce toxicity, chemical modifications as well as prodrug based approach have been utilized, which could enhance drug permeation by means of introduction of hydrophobic moiety [19]. Nevertheless, poor control over the solubility, permeability and hydrolysis rate for maintaining of biological activity poses an obstacle for their further use [14, 20]. All these constraints necessitate the development of novel carrier system, which would enhance the systemic entry of BCS class-III drugs upon oral administration without any toxicity concerns.

In past two decades, nanocarrier systems, in their various forms, have been attracting ample recognition as a result of extensive investigations towards harnessing their potential for combating different life threatening diseases [21]. Different nano-scale vehicles in size range of 10-1000 nm, have already been proven to be an efficient approach for oral delivery of various therapeutic agents [22, 23]. Amongst all, polymeric nanoparticles [24], solid lipid nanoparticles (SLNs) [25] and liposomes [26] have been dedicated tremendous research emphasis for oral bioavailability enhancement of numerous therapeutic molecules, by virtue of their specialized absorption mechanisms across GIT [23, 24]. Upon oral administration, nanocarriers are transported across GIT through paracellular and transcellular transport as well as selective uptake by M-cells in Peyer's Patches (PP), which directly drains them into blood circulation via intra-epithelial lymphoid cells of lymphatic system and thus, increased systemic availability of drug could be anticipated [27, 28]. Despite these rapid progresses, the delivery of BCS class-III drugs using polymeric nanoparticles and SLNs remained obscure owing to their hydrophilic nature, which imparts their faster partition into external aqueous phase during formulation and results in poor encapsulation [29, 30]. In lieu of this, liposomes have demonstrated tremendous potential to encapsulate hydrophilic drugs into the aqueous core with high loading. It has also been reported that liposomal encapsulation imparts lipophilicity through lipid bilayer, which could facilitate the passive transport of hydrophilic drug across the GIT barrier [31, 32]. However, oral delivery potential of liposome is limited by their erratic and unpredictable absorption profile as well as inability to retain their structural integrity at absorption site [23, 33].

In order to circumvent these thorny limitations of existing delivery systems, strides having been taken to capitalize the positive attributes of the single nanocarrier system

by designing advanced, core-shell type, promising nanocarriers. They would allow the encapsulation of hydrophilic therapeutic agent inside the core of nanocarrier by forming molecular barrier surrounding the same, during the preparation. The molecular barrier impedes the faster diffusion of hydrophilic drug molecule into an external aqueous phase and hence, potentially increases drug payload. Moreover, core-shell architecture would also be allowed to deliver hydrophilic drug in temporal manner in order to extend the drug release [23, 34-36]. Amongst the array of core-shell type nanocarriers, polymer based and polymer-lipid hybrid based tunable, robust core-shell nanostructures have been impressively proposed for the oral drug delivery.

The drug selected for this work, cromolyn sodium (CS), is a hydrophilic drug, which belongs to BCS class-III. CS was originally explored as a mast cell stabilizer, but recently found that it also possess very broad biologically diversified potential i.e., anti-inflammatory activity, anti-viral activity, anti-cancer activity, etc. [32, 37, 38]. It is currently being used for the pharmacotherapy of various allergic conditions, including allergic rhinitis, food allergy, mastocytosis and bronchial asthma, owing to its membrane stabilizing activity against sensitized mast cells for prevention of their degranulation and thereby, the release of chemical mediators (i.e., histamine and necrosis factor) [32, 39]. CS is currently available in the market for nasal and pulmonary administration, in the form of either solution or powder for allergic indications. However, irritation to the local administration site such as trachea, throat and nasal mucosal epithelia along with dose delivery variation results in poor patient compliance, which preclude the patient to remain adhere with prescribed dosing regimen, even though it is effective [40, 41]. All these limitations pertaining to currently marketed dosage forms, pose an utmost necessity for the development of novel patient friendly, alternative formulation, which would deliver therapeutically

active amount of CS in the systemic circulation without any serious concern. To this end, oral administration of CS would elude all the obstacles and would offer ease of medication with enhanced patient compliance. Additionally, oral delivery of CS would open a door for the treatment of various diseases such as atherosclerosis, coronary artery diseases, pancreatic cancer and viral diseases [23, 32, 39, 42]. Thus, much effort has been devoted for engineering oral formulations of CS that enhances its systemic exposure.

As CS being a hydrophilic molecule, it follows paracellular pathway in order to get absorb from GIT to reach in systemic circulation. However, its unsuitable set of physicochemical properties, i.e., larger molecular weight (512.33 gm/mol) along with high hydrophilicity (100 mg/ml at 20 $^{o}$C) imparted by the two carboxylic groups (pKa = 2.0), hinder its absorption from GIT and render itself orally less bioavailable (< 1%) [43-45]. Further, its hydrophilic nature also prevents its binding with plasma protein and thereby, limits its volume of distribution, which ultimately results in shorter biological half-life (~1.3 hr). Hence, remarkable measures have been taken to improve the intestinal permeation of CS, by developing prodrugs and proliposomal beads, for its successful oral delivery [41, 46, 47]. However, these studies have yielded limited success and further development is still needed for their clinical translation. This indicates that oral delivery of CS still remains challenging and necessitates an exploration of novel delivery strategies with enhanced GIT permeation, for effective oral delivery of CS.

In this perspective, the proposed research work was endeavoured to develop and optimize various nanocarrier systems for oral bioavailability enhancement of poorly permeable, hydrophilic therapeutic molecule, i.e., CS with the hypothesis that if CS is

encapsulated inside nano-carrier systems, there would be an enhancement in the GIT permeation of CS, by virtue of their specialized absorption mechanisms across GIT, i.e., transcellular transport, paracellular transport as well as uptake via M-cells of PP and thus, enhanced oral bioavailability could be anticipated. Moreover, the sustained drug release potential of nanocarrier systems would improve the systemic circulation time of CS, which in turn could reduce dosing frequency and would improve patient compliance.

The present thesis embodies studies on the development, optimization and characterization of four different types of nano-carrier systems such as polymeric nanoparticles, SLNs, core-shell polymer-lipid hybrid nanoparticles (PLHNs) and poly-ε-caprolactone-chitosan based core-shell polymeric nanoparticles (PCCSNs).

The first formulation of the present research work was the CS encapsulated polymeric nanoparticles (CS-PNs), which was developed by employing modified double emulsification solvent evaporation method ($W_1/O/W_2$). Polymeric nanoparticles are colloidal submicron size entities ranging from 10-1000 nm in diameter, and are assembled from a wide variety of biodegradable and non-biodegradable polymers [45]. Here, United States Food and Drug Administration (USFDA) approved, biodegradable and biocompatible polymer, poly(D,L-lactide-co-glycolide) (PLGA) was used.

The second formulation was the CS encapsulated SLNs (CS-SLNs), which was also prepared by employing modified double emulsification solvent evaporation method ($W_1/O/W_2$). SLNs are the potential colloidal carriers, comprising of surfactant stabilized solid lipid as a matrix, in which therapeutic drug molecules are entrapped or

encapsulated for delivery to the human body [48, 49]. Here, glyceryl monostearate (GMS) was used as the core forming solid lipid material.

However, CS being a hydrophilic molecule, the encapsulation inside polymeric nanoparticles as well as SLNs was foremost challenge, owing to its lower affinity for hydrophobic carrier, which ultimately resulted in the rapid partitioning into external aqueous phase during the preparation. Hence, the third formulation of CS encapsulated core-shell polymer-lipid hybrid nanoparticles (CS-PLHNs) was prepared. PLHNs are advanced, robust nanocarriers, in which a lipid layer envelopes the polymeric core. They are comprised of positive attributes of both, liposomes as well as polymeric nanoparticles in a single vehicle. The polymeric core confers the structural integrity to the PLHNs, whereas the lipid envelop imparts the biomimetic shield. The enhanced encapsulation yield for hydrophilic drug could be anticipated by covering of lipidic monolayer over the surface of the polymeric core during the preparation. The molecular barrier conferred by lipid, impedes the faster diffusion of hydrophilic drug into an external aqueous phase and hence, potentially increases drug encapsulation [23, 36, 50]. CS-PLHNs were prepared by modified double emulsification solvent evaporation method ($W_1/O/W_2$).

Continuing along the same line, we directed our attempts to develop polymer based core-shell nanocarriers for enhancing the encapsulation of CS as well as improving the intestinal permeation by employing permeation enhancer polymer, i.e., chitosan. Hence, the fourth formulation of CS encapsulated poly-ε-caprolactone-chitosan based core-shell polymeric nanoparticles (CS-PCCSNs) was prepared by employing modified nano-coprecipitation method. Here, poly-ε-caprolactone (PCL) and chitosan were used as polymer.

The process of formulation development and optimization of any nanocarrier system involves the choice and vital understanding of the effect of the several formulation as well as process variables. The choice of suitable formulation and process variables largely depends on the physicochemical property of drug and method used for the preparation [51]. With thorough understanding the effect of different selected variables and controlling these variables in their limits, it is possible to obtain nanocarriers of desired quality traits. The traditional approach for development of any formulation involves changing one variable at a time (OVAT), while keeping others as constant and its effect was studied. But this traditional approach nothing reveals about the interaction of different variables and creates hurdles for optimization of formulation [52]. Moreover, development of formulation involves study of lots of variables which might be or not, affects the performance of product and ultimately increases cost, time and labor. Statistical optimization by using Design of Experiment (DoE) approach provides scope to achieve the target product profile with smallest number of experimental run, manpower, time and cost [53]. The DoE approach has been proven to be most powerful and useful tool based on multivariate analysis for identification of the root cause of variability and alleviate the shortcomings of traditional OVAT approach in a predefined set of experiments [54, 55]. When the number of formulation and process variables is high, then it is necessary to screen a large number of variables. Hence, the most commonly used Plackett–Burman screening design was employed for the preliminary screening of the various formulation and process variables affecting the properties of the different nanocarrier systems (i.e., CS-PNs, CS-SLNs, CS-PLHNs, CS-PCCSNs). The critical formulation and process variables obtained after preliminary screening were applied to response surface methodology (RSM) based Box-Behnken experimental design, for statistical

optimization of the nanocarriers. The optimized different formulations were further characterized for their physiochemical properties, solid state characteristics, morphological properties, *in-vitro* performance, stability study and *in-vivo* behaviour for establishing their oral delivery potential, which have been discussed in detail in this study.