2. Literature review

2.1 Oral drug delivery

Oral delivery is the most widely and readily accepted form for easiest administration of wide range of therapeutic drug molecules. It offers numerous advantages over other drug delivery such as non-invasive in nature, cost effective, ease of administration, avoidance of pain and discomfort pertaining to parenteral administration, which ultimately imparts high patient convenience and compliance [1-3]. Oral dosage forms are cheaper to produce as compared to parenteral formulation, due to no need of sterile manufacturing facilities. Oral route is more patient friendly for the pediatric and geriatric patients, especially during the chronic therapy due to lesser clinic visits. Moreover, oral drug delivery has also added advantages for physicians in terms of highly flexible dosing schedules as well as lesser need of trained hospital staff [56-59]. As a result, more than 60% of drug products currently available in the market are administered through oral route [4].

Oral bioavailability of any drug molecule is strongly affected by two parameters: solubility and permeability. Based on these two parameters, all the drug molecules are classified in the Biopharmaceutical Classification System (BCS) by Amidon *et al*. [5]. Regardless of these potential advantages, oral drug delivery faces some of the problems due to undesirable set of physicochemical properties of the drug molecules, which results in poor systemic bioavailability upon oral administration. When drug is administered orally, it must be stable against gastric and enzymatic degradation, throughout the transit through gastrointestinal tract (GIT) before getting absorbed via intestinal epithelial and mucous barrier [57]. The obstacles, which affect the oral bioavailability of drug molecules include, large molecular weight, poor solubility,

ionization at gastrointestinal fluid pH, susceptibility to enzymatic degradation, poor permeation across the intestinal barrier, etc. Moreover, GIT also presents the obstacles through its intrinsic intestinal barrier, which consists of the mucous layer. Penetration through the mucous layer is necessary on order to get absorb through the epithelial cells. Additionally, tight junction between the enterocytes, intestinal metabolism, susceptibility to P-glycoprotein drug efflux pump, etc., also enforces the poor oral bioavailability [3, 9, 10, 60]. As a result, many drugs are currently administered as parenteral formulations.

2.2 Physiology of the gastrointestinal tract

Oral drug delivery can be employed for the local treatment to the GIT as well as for allowing therapeutic molecules to reach systemic circulation. Upon oral administration, therapeutic drug molecules must transit through GIT for being absorbed to the systemic circulation. The GIT is a continuous tube like structure, which starts from the oral cavity and passing further as pharynx, esophagus, stomach, small intestine, large intestine, rectum and finally ends into the anal canal. In which, small intestine is one of the most important organ for regulating the absorption of the any molecule. The absorption potential of the small intestine is mainly due to its macroscopic folds of the inner epithelium, which comprised of the villi and microvilli [58, 61]. The villi are the microscopic finger-like projections, which significantly increase the absorptive surface area in the GIT to around 300-400 m^2 [3, 62]. They act as barrier to tightly regulate the transport of molecules from the intestinal lumen to the lamina propria by their polarized cell monolayer [63]. The epithelial cells are present on the outer side of the basal lamina, which divides the epithelial layer from the lamina propria. Lamina propria contains the network of capillaries, which transport

the molecules across the epithelial barrier to the lymphatic vessels and eventually, to the blood vessels through thoracic duct.

Different types of cells and structures are present on the intestinal epithelium. Villi are covered with enterocytes (absorptive), endocrine cells, Paneth cells (lysozyme and antimicrobial peptide secreting) and goblet cells (mucus secreting), which are interspersed with Follicle Associated Epithelium (FAE). The function of enterocytes is to control the transport of macromolecules and pathogens and to absorb different nutrients from the digested food. Whereas, goblet cells secrete a viscous fluid composed primarily of highly glycosylated proteins (mucins) suspended in a solution of electrolytes called as the mucus. Moreover, intestinal tissue also contains Peyer's patches (PP) in the in the gut-associated lymphoid tissue (GALT). These lymphoid nodules are called as O-MALT (Organized Associated Lymphoid Mucosa). PP are covered with the M cells, which has high transcytosis capacity for antigen sampling to induce immune responses, due to relative less protection offered by mucus layer [1, 64, 65]. They play crucial role in the oral drug delivery application, mainly for peptides and proteins and are able to transport a broad range of materials, including nanoparticles and microparticles. The uptake of particles is occurs mainly through the clathrin mediated absorptive endocytosis and phagocytosis. M cells also reduce the level of membrane hydrolase activity, which in turn influences the particulate uptake. M cells are also found to be located the outside to FAE, but transport and uptake of particulate materials is mainly observed by M cells of FAE [57, 66, 67].

2.3 Nanocarriers in oral drug delivery

A high potential for novel drug delivery system has been attributed to nanocarrier systems. Nanocarrier systems are colloidal systems with particles varying in size from

10 to 1000 nm. Nanocarrier systems, in their various forms, have been attracting ample recognition as a result of extensive investigations towards harnessing their potential and thereby providing a remarkable achievement in the field of medical sciences to combat with life threatening diseases [21]. The nanoscale structures provide endless opportunities by virtue of their nano size as well as their peculiar physicochemical and biological characteristics for delivery of therapeutic agents, i.e., drugs, genes, protein, peptides to diagnostic agents, imaging agents, biosensors etc., precisely and safely to the target site for higher efficacy [68-71]. Therapeutic nanocarrier systems have witnessed to date an ever-increasing interest from academic and industrial arenas in comparison to conventional drug delivery systems due to the multitude of their advantages [72]. They offer various advantages including improvement in solubility rate, controlled and sustained or environmentally responsive drug release, prolongation of therapeutic half-life by surface functionalization, improvement in bioavailability, co-delivery of drugs for generation of synergistic effect as well as suppression of drug resistance, reduction of systemic side effects by site specific delivery [73-75]. Moreover, the *in-vivo* properties such as longer circulating half-life, targeting potential, stimuli responsive drug release profile and diagnostic ability can be easily tailored by means of various modifications which provide better efficacy with enhanced prognosis for the treatment of multiple diseases without any compromising with safety [76, 77].

As a result, multifarious nanocarriers have been engineered for application of therapeutic substance delivery such as polymeric nanoparticles, dendrimers, nanoshells, liposomes, inorganic/metallic nanoparticles (e.g gold, silica), nanogels, micelles, polymeric vesicles, magnetic nanoparticles, bacterial nanoparticles and so on [78]. Amongst all, polymeric nanoparticles, solid lipid nanoparticles and polymer-

lipid hybrid nanoparticles (PLHNs) evolved as the most prominent, ground breaking therapeutic delivery carriers and have continued to manifest dominancy over other drug delivery systems due to their myriad of success as well as opportunity for the treatment of various menacing diseases including cancer [23, 79]. Further, increase in the number of scientific research reports, approved marketed pioneered nanoformulations and numerous nanocarrier based ensuing formulations presently underneath clinical trials as well as entering in the same, are enough to prove their therapeutic potential [80].

2.4 Transport of nanocarriers across the gastrointestinal barrier

Nanocarriers can cross the intestinal barrier by adopting mainly four mechanisms: (i) paracellular pathway (between the adjacent enterocytes), (ii) transcellular pathway (endocytosis through enterocytes), (iii) carrier mediated transport and (iv) receptor mediated transport. Absorption across the intestinal barrier is mainly depends on the physicochemical properties of the drug molecule, such as, molecular weight, lipophilicity, ionization constants, and stability of drug molecule at different pH conditions [81].

2.4.1 Paracellular transport

Paracellular pathway involves the transport of the molecules across the intestinal epithelium by passing through the available intercellular spaces between the adjacent enterocytes. Paracellular transport is mainly passive in nature, which results from the diffusion of molecules and dependent on the concentration gradient across the intestinal barrier. It is mainly controlled by the intercellular tight junctions. The paracellular transport is limited at normal physiological condition due to very small surface area (~ 0.01%) of the intercellular spaces [82]. Moreover, the tight junction

between the adjacent epithelial cells also limits the transport of molecules due to its smaller pore diameter, ranging between the ~3 °A to 10 °A, which ultimately act as rate limiting barrier for the larger molecules and ionic substances. The average diameter of the epithelial tight junction, for the different region of the human intestine is ~7 °A - 9 °A for the jejunum, ~3 °A – 4 °A for ileum and ~8 °A – 9 °A for colon. The molecules having the radial diameter exciding the 15 °A (~3.5 kDa) cannot be transported by the paracellular route. Paracellular transport can be improved by employing the natural as well as synthetic permeability enhancing polymers either in the solution state or in the nanoparticles form. To this end, chitosan and poly (acrylic acids) have been widely employed for the enhancing the paracellular transport through interactions between the protein molecules of the negatively-charged cell membrane and the positive charges of the polymer in the solution state. They also enhance the paracellular transport by forming the complex with the Ca^{2+} of the tight junctions and open the junction reversibly [57, 58, 67, 83-85].

2.4.2 Transcellular transport

Transcellular pathway involves the transport of the molecules across the intestinal barrier through transcytosis mechanism. Transcytosis process involves the endocytosis of the particles through the enterocytes. It starts with the endocytosis process on the apical surface of the intestinal cells, followed by transportation through interstitial fluid and then released at basolateral side. The basolateral membrane of the enterocytes is thinner and hence, more permeable than apical membrane due to the lesser amount of the protein to lipid ratio. The movement of the glucose molecules involves the transcellular transport across the intestinal tissue. Transcellular transport is mainly depends upon the several physicochemical properties such as, molecular size, lipophilicity, hydrogen bonding potential, surface charge, surface hydrophobicity

or the presence of a carrier ligand at the particle surface. It also depends on the physiology of the GIT. Enterocytes and M cells of PP are the main intestinal cells involve the transcellular transport [86]. Amongst both, M cells possess high transcytosis capability and involve in the transport of wide range of molecules and particles from micrometer to nanometer range. They also involve actively in the transport of the protein and peptide molecules. M cells follow any of the mechanism, i.e., clathrin-coated pits and vesicles, fluid phase endocytosis and phagocytosis for the adsorptive endocytosis of the particles or macromolecules. However, the proportion of the particle absorption through transcellular route is low as a result of lower endocytosis via enterocytes. Moreover, this route is limited for the transport of the hydrophobic drug molecules having low molecular weight across the intestinal barrier. The translocation of the particles is mainly occurs through FAE [57, 67, 87-89]. Transcytosis is the main mechanism for the transport of the nanocarriers across the intestinal tissue.

2.4.3 Carrier mediated transport

Carrier mediated transport involves the use of suitable carriers in order to transfer drug molecule or surface modified nanocarriers from the apical membrane to the basal membrane of the enterocytes and ultimately releases in the systemic circulation. This process is mainly involves in the transport of the hydrophilic molecules. It is energy mediated active transport of the specific molecules by specific carriers. Here, receptors of the molecule play an important role in order to get identified by the carrier molecule and to get transport across the intestinal membrane of the GIT. They also transport the molecules against the concentration gradient. Numerous molecules such as small peptides, amino acids and monosaccharides are transported through carrier mediated transport by endocytosis mechanism [57, 67, 90].

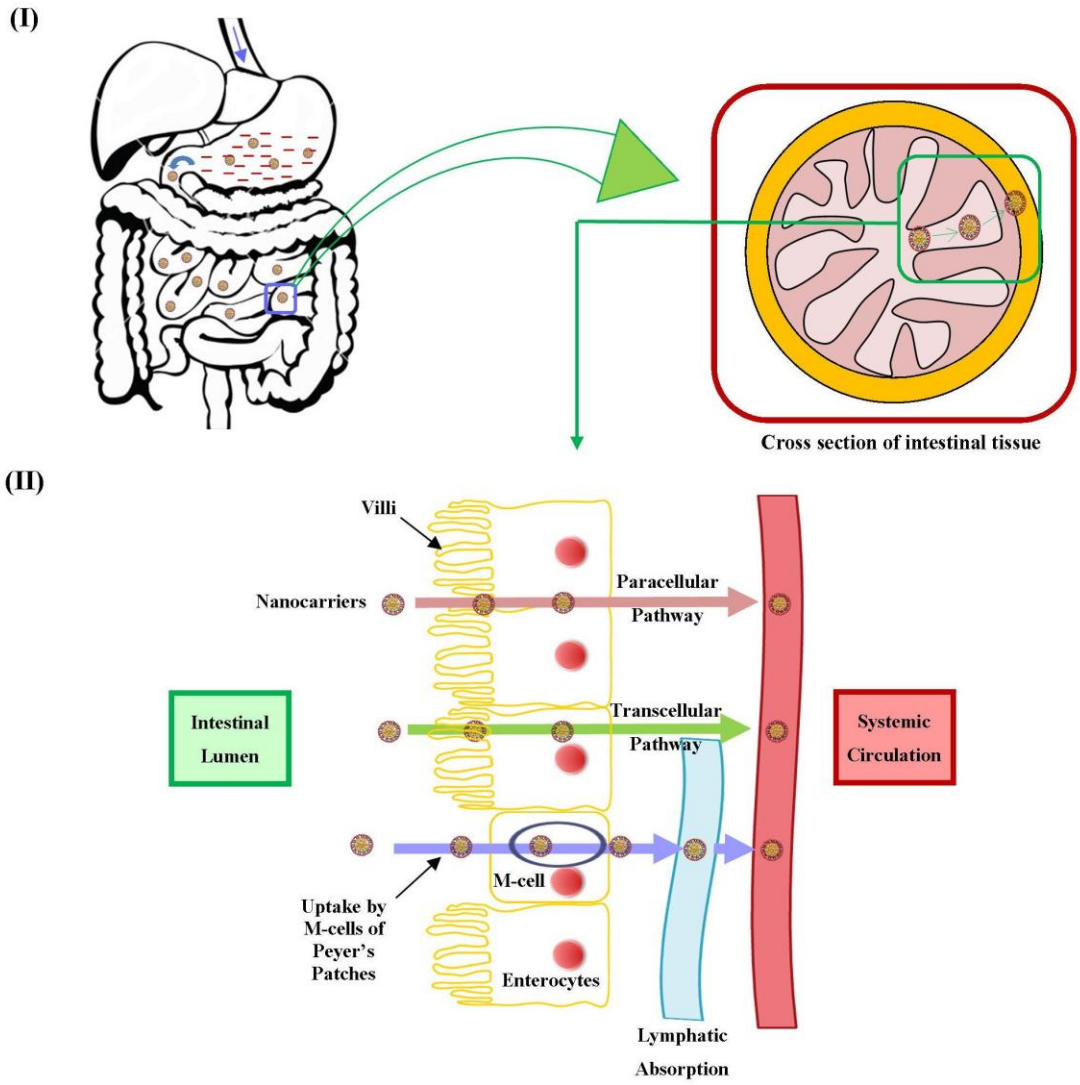


Figure 2.1 (I) Diagrammatic illustration of the gastrointestinal-transit of nanocarriers and subsequent their intestinal permeation. (II) Graphics illustrating the various routes of absorption of nanocarriers across GIT

2.4.4 Receptor mediated transport

Receptor mediated transport involves the recognition of the drug molecule or particles either by acting as receptor specific ligand for cell surface enchored receptors or as a receptor for surface attached ligand in order to transport across the intestinal enterocytes. These transport mechanism generally involves any of the endocytosis process such as phagocytosis, pinocytosis, receptor-mediated endocytosis (clathrin-

mediated), and potocytosis (nonclathrin-mediated). Receptor mediated transport has been widely explored for the targeting ligand enchored nanocarriers as well as oral bioavailability enhancement of protein/peptide and drug molecules. This transport mechanism involves vesicle formation during the endocytosis process and transports the molecules to the blood circulation by following either portal blood vain or intestinal lymphatics. Hydrophilic molecules mainly transported through the portal vein whereas highly hydrophobic molecules transported through the intestinal lymphatics and reaches systemic circulation [57, 67, 91].

2.5 Polymeric nanoparticles

Polymeric nanoparticles are colloidal carrier system in the size range from 10 - 1000 nm, generally made from biodegradable or non-biodegradable polymers. They consist of drug carriers in which the active ingredient is dissolved, dispersed, entrapped, encapsulated, adsorbed or chemically attached [21]. Based on the structure and preparation method, they are mainly classified in the two types: nanospheres and nanocapsules. Nanospheres are the polymeric matrix type system whereas nanocapsules are polymeric vesicular system having core-shell type structure [78, 92-94]. Wide spectrum of polymeric nanoparticles, made from biodegradable polymers has been explored for the delivery of therapeutic substances. Some obvious characteristics such as biocompatibility, biodegradability, non-thrombogenic, non-immunogenic, non-inflammatory, stability in biological milieu, non-toxicity and controlled release capability make them highly attractive for delivery of therapeutic agent [68, 95-97]. To make them highly biocompatible, in most cases biodegradable polymers or co-polymeric blocks are used, which also helps in avoiding the phagocytic uptake and thereby, prolongs the systemic circulation. In addition, the rigid polymer matrix provides high structural integrity to polymeric nanoparticles,

which render themselves more stable in biological fluids and during storage in comparison to vesicular systems i.e., liposomes and micelles [98]. They also protect the drug molecule from the harsh gastric environment, enzymatic degradation and drug efflux pumps, which makes the highly attractive drug carrier system for the oral delivery [58].

Polymeric nanoparticles have been found as a better option for delivery of both small and large therapeutic molecules with high drug loading capability. They offer advantages such as higher tissue penetrating ability due to smaller particle size, easy and wide choice of fabrication methods as well as polymers, readily functionalization potential for active targeted delivery, easily tunable and controllable drug loading as well as release behaviour etc., all of which making them highly prominent therapeutic delivery vehicle [99, 100]. Various natural (e.g. gelatin, albumin, sodium alginate and chitosan) polymers and synthetic polymers (Polylactides (PLA), Polyglycolides (PGA), Poly(lactide co-glycolides) (PLGA), Poly-ε-caprolactone (PCL), Poly(acrylic acid)) have been used for the preparation of the polymeric nanoparticles [21]. Amongst all biodegradable polymers PLGA and PCL are widely used for the oral drug delivery application in the form of polymeric nanoparticle. It has been proven to be safe due to its biodegradability and biocompatibility characteristics. They are easily clears off from the body through metabolic pathways after hydrolysis into their monomers. They are mainly prepared by the well reported synthetic methods including nanoprecipitation method, salting out, solvent evaporation method, emulsification method, direct polymerization method, etc. [21, 101-103].

Polymeric nanoparticles also confer flexibility by modulating the physicochemical properties of drug molecules, i.e., size, surface charge, lipophilicity and thereby, control the release of therapeutic agent. Moreover, surface functionalization with

peptides, targeting ligands, antibodies also allows targeted delivery of drug through close interactions with the tissue components. A wide range of drugs can be incorporated in the polymeric nanoparticles, including hydrophilic and hydrophobic small drugs, vaccines, nucleic acids (i.e., DNA or siRNA) and biological macromolecules. The smaller size of the polymeric nanoparticles imparts high surface area, which helps by increasing the contact area with the epithelial tissue and thus, allow to absorb through non-specific endocytosis as well as receptor mediated endocytosis. Furthermore, the simplicity and low cost of the preparation processes make them a highly attractive drug delivery system [58].

In this regard, polymeric nanoparticulate (PNs) based carrier system has been dedicated tremendous research emphasis for oral permeability enhancement of poorly permeable hydrophilic drug molecules, on account of adoption of the special absorption pathways [24]. The selection of the particular fabrication method is primarily depends on the physicochemical properties of the drug molecule (i.e., solubility) and molecular stability [104]. Amongst all, a majority of the methods are developed to encapsulate hydrophobic compounds inside polymeric nanoparticles. Contrary, encapsulation of hydrophilic drugs, inside the matrix of polymeric nanoparticles is really difficult because of rapid partitioning behaviour of drug into external aqueous phase during the fabrication. For better encapsulation of a hydrophilic molecule, the double emulsification solvent evaporation method ($W_1/O/W_2$) is commonly employed [24, 29, 39].

Polymeric nanoparticles have been widely employed for the oral delivery of poorly permeable hydrophilic therapeutic molecules. Liu *et al*. prepared hydrophilic drug; daunorubicin encapsulated PLGA nanoparticles by modified double emulsification solvent evaporation method for enhancing anti-tumor activity by sustaining the site

specific delivery [51]. They have employed partially water soluble organic solvent during the particle formation for the enhancing the encapsulation of hydrophilic drug, which resulted in greater than 80% encapsulation with high payload (6.5% w/w). The effect of different variables, such as ratio of organic solvent, type of surfactant, types of polymer and molecular weight was systematically investigated. Eventually, daunorubicin loaded PLGA nanoparticles exhibited higher cellular uptake and cytotoxicity on the HL-60 cells along with sustained release, confirming the potential of PLGA nanoparticles for the efficient delivery of hydrophilic drugs.

In another instance, Abdelghany *et al*. have developed PLGA nanoparticles by encapsulating aminoglycoside antibiotic, gentamicin for the treatment of *Pseudomonas* infections [105]. They have improved the encapsulation of hydrophilic antibiotic by modulating the pH towards the higher side. PLGA nanoparticles controlled the release of antibiotic up to 16 days and thereby, exhibited significantly improved antimicrobial effect along with corresponding reductions of the surrogate inflammatory indicators interleukin-6 and myeloperoxidase, against the both planktonic and biofilm cultures of *P. aeruginosa* PA01 as well as in a 96-hour peritoneal murine infection model, during *in-vitro* studies.

Similarly, Joshi *et al*. have imperatively tried to develop poorly permeable hydrophilic drug, gemcitabine HCl loaded PLGA nanoparticles for improving the oral bioavailability through enhanced intestinal uptake [106]. They have employed multiple solvent emulsification method for the preparation of PLGA nanoparticles with high entrapment up to 56.48 ± 3.63%. The improvement in the intestinal permeability was confirmed by uptake on the *in-vitro* Caco-2 cells, *ex-vivo* study using intestinal tissue and *in-vivo* absorption by confocal microscopy. Further, improvement in the oral bioavailability was confirmed by *in-vivo* pharmacokinetic

study in rats, which showed 21.47 fold improvements in the bioavailability. Eventually, efficacy of the developed formulation was demonstrated by *in-vitro* cytotoxicity study on K562 leukemia cell lines. Hence, the oral delivery of the gemcitabine HCl loaded nanoparticles can be employed for altering the existing systemic therapy with reduced side effects and enhanced patient compliance. Likewise, Zakeri-Milani *et al*. engineered vancomycin encapsulated PLGA nanoparticles with an objective to improve intestinal permeability for the successful oral administration [24]. The double emulsification solvent evaporation method was employed for the fabrication of nanoparticles. The *ex-vivo* intestinal permeation study by employing single-pass intestinal perfusion technique (SPIP) exhibited the significant improvement in the permeability of vancomycin at different concentration by forming the PLGA nanoparticles.

Recently, Tariq *et al*. developed hydrophilic anthracycline antineoplastic agent, epirubicin loaded PLGA nanoparticles for oral bioavailability enhancement [107]. The optimized nanoparticles showed drug content of 39.12±2.13 μg/mg. The improvement in the oral permeation was demonstrated by *in-vitro* Caco-2 cell line study and *ex-vivo* rat ileum permeation study, which exhibited 2.76 folds and 4.49 folds higher permeation for nanoparticles compared to pure drug. Further, higher permeation for intestinal tissue compared to Caco-2 cell line gave a confirmation regarding the involvement of PP in absorption enhancement. Oral bioavailability study also showed 3.9 fold improvements in the oral bioavailability of epirubicin by forming the PLGA nanoparticles. Further, efficacy of the developed formulation was tested on the human breast adenocarcinoma cell lines (MCF-7) by cytotoxicity studies and exhibited the superiority of epirubicin loaded PLGA nanoparticles over free

epirubicin solution. Henceforth, they could be promising approach for replacing the pre-existing intravenous pharmacotherapy.

Additionally, peptide molecules have also been delivered by polymeric nanoparticles for oral delivery. Yin *et al*. have developed lectin-conjugated PLGA nanoparticles for the oral delivery of thymopentin [108]. Thymopentin encapsulated PLGA nanoparticles were prepared by double emulsion-solvent evaporation technique using lectin conjugated PLGA for imparting the mucoadhesivity to the nanoparticles. Further, improved bioadhesion and thereby, efficacy was confirmed by pharmacodynamic study using FAC scan flow cytometry, which exhibited significant enhancement of the efficacy as a result of improved permeation of the nanoparticles across the GIT.

Girotra *et al*. developed zolmitriptan loaded PLGA nanoparticles for the effective treatment of migraine [109]. Hydrophilic zolmitriptan was encapsulated by modified double emulsification solvent evaporation method using quality by design approach. *In-vivo* study in rat demonstrated enhanced brain uptake of the nanoparticles up to 14.13 fold compared to free drug. Further, *in-vivo* pharmacodynamic study confirmed the efficacy of the developed system for the treatment of the migraine by delivering the higher concentration of zolmitriptan to the brain.

Li *et al*. prepared 5- Fluorouracil loaded PLGA nanoparticles for the effective oral delivery of anticancer hydrophilic molecule, 5-fluorouracil [110]. Due to the high water solubility of 5-fluorouracil in basic water, the double emulsification solvent evaporation method was employed for higher drug encapsulation. *In-vivo* pharmacokinetic study upon oral administration exhibited significant improvement in the oral bioavailability of 5-fluorouracil by forming the nanoparticles. Further,

improved mean residence time of 5-fluorouracil loaded PLGA nanoparticles in the systemic circulation confirmed the potential of nanoparticles for sustaining the release of hydrophilic 5-fluorouracil. Hence, by forming the nanoparticles the gastric side effects of 5-fluorouracil could be reduced even though oral administration.

2.6 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) have captivated plenty of attention owing to their remarkable effectiveness, versatility and safety in terms of biocompatibility compared to the conventional nanocarriers, such as oil-in-water emulsions, liposomes, microparticles and polymeric nanoparticles [111]. SLNs are comprised of surfactant stabilized, spherical lipid matrix having particle size in nanometer range, in which therapeutic drug molecule are dispersed or entrapped for delivery to the human body. They have the potential to carry lipophilic or hydrophilic drugs and diagnostic agents for controlled and targeted delivery. SLNs capitalize the potential of two prominent therapeutic carrier systems: polymeric nanoparticles and lipidic emulsion, at the same time ameliorating the commonly persisting drawbacks associated with polymeric carriers i.e., temporal and *in-vivo* stability [49]. They are mainly composed of physiologically accepted biocompatible fatty acids and lipids, similar to emulsion. Additionally also imparts stability to drug molecule against chemical degradation in biological media by encapsulating inside solid matrix and thereby, provide the flexibilities in the modulation of the drug release profiles [112, 113].

SLNs are made from solid, biocompatible lipids with the aid of commonly used preparation methods such as high speed homogenization, solvent evaporation, nanoprecipitation, etc. They can be easily prepared at large scale by high-pressure homogenization method. SLNs are prepared from lipids which are solid at room

temperature as well as at body temperature. Different solid lipids have been exploited to prepare SLNs, such as, tripalmitin/Dynasan® 116, trimyristin/Dynasan® 114,tristearin/Dynasan® 118, cetyl alcohol, cetyl palmitate, Compritol® 888 ATO, Glyceryl monostearate, Precirol® ATO5, stearic acid, Imwitor® 900. SLNs emerged as worthful tool for delivery of various drugs as a result of their high physical and chemical stability, biocompatibility, lower toxicity, cheaper excipients, controlled release and easy tailoring potential for targeted delivery, commercial viability and lower cost [111, 114, 115]. Moreover, high encapsulation potential and their ability to cross natural biological barrier in the human body, keep them at the forefront in the rapidly developing field of nanotechnology. It has been reported that lipid core of SLNs stimulates chylomicron formation and facilitates lymphatic uptake, which can easily bypass hepatic first-pass drug metabolism and delivers drug in systemic circulation [116]. Additionally, surface modification also modulates the physicochemical properties of SLNs and thereby, improves their biodistribution and targets to the specific disease cells. SLNs can be administered by various routes including, oral, parenteral and topical/transdermal. Upon oral administration, SLNs improves bioavailability of different drugs by improving the intestinal permeability as well as by protecting from the gastric environment [113]. All these attributes make SLNs excellent carriers for oral drug delivery. Similar to other nanocarrier systems, SLNs get absorb into systemic circulation by paracellular and transcellular pathway through the GIT.

Numerous hydrophilic molecules have been delivered in the form of SLNs. Bhandari and Kaur have developed isoniazid encapsulated SLNs for improving relative bioavailability, pharmacokinetic profile and biodistribution upon oral administration [117]. Isoniazid was encapsulated inside the SLNs by employing modified hot micro-

emulsification technique, which shoed high entrapment efficiency of 69%. Further, *in-vivo* pharmacokinetic study upon single dose oral administration showed significant improvement of the oral bioavailability (~6 folds) as a result of avoidance of first pass metabolism. Moreover, prolongation of drug release due to bypass of reticulo-endothelial system as well as slower drug release from SLNs also reduced the hepatotoxicity and neurotoxicity and subsequent reduction in dose was achieved.

Zidovudine based SLNs have also been prepared with an objective to entrap hydrophilic drug inside the matrix of SLNs [29]. SLNs were prepared by using stearic acid with the help of double emulsification evaporation method. Effect of different formulation and process variables was assessed by employing 3^2 factorial design. The maximum EE for zidovudine was found to be around 27% with particle size of 621 nm. Final outcome of the study exhibited that SLNs prepared using fatty acid have higher capability to entrap hydrophilic drugs as compared to triglycerides. Likewise, Shi *et al*. have developed poorly permeable hydrophilic drug, zanamivir loaded SLNs for enhancing the intestinal permeation [118]. Zanamivir-loaded SLNs were prepared by employing double emulsion solvent evaporation method and different process variables were optimized for higher encapsulation. The cellular transport potential of zanamivir loaded SLNs was evaluated on Caco-2 cell model. Moreover, zanamivir loaded SLNs also showed sustained release potential, indicating that the SLNs have ability to control the release of hydrophilic drug.

In another instance, gentamicin was encapsulated inside the matrix of SLNs for developing the controlled release delivery system for the treatment of *Staphylococcus aureus* infections [119]. The ultrasonic-high homogenization technique was used to encapsulate gentamicin. The positively charged SLNs showed high drug loading efficiency up to 40% and exhibited controlled release up to 96 hr without and burst

release. Ghadiri *et al*. developed paromomycin encapsulated SLNs for enhancing its efficacy as an anti-leishmanial agent [120]. They encapsulated paromomycin by employing two different methods; microemulsion and solvent diffusion method. The effect of different formulation and process variables was assessed in order to obtain SLNs with higher entrapment efficiency and lower particle size. Similarly, Shah *et al*. have encapsulated water soluble antibiotic, ciprofloxacin HCl inside the SLNs [121]. SLNs were prepared by solvent diffusion method. The relationship between the formulation and process variables was established by studying the influence of different variables. Further, *in-vitro* drug release study confirmed the sustained release potential of SLNs, hence, SLNs can be used as efficient delivery system with improved encapsulation and controlled release for hydrophilic drug molecules.

Shelat *et al*. encapsulated hydrophilic anticancer agent, doxorubicin in the SLNs for enhancing the oral bioavailability and thereby, anticancer efficacy [122]. Fatty acid coacervation method was adopted for the preparation of doxorubicin encapsulated SLNs. The SLNs showed encapsulation efficiency nearly about 70 % and exhibited sustained release potential. *In-vivo* pharmacokinetics study in rats revealed that encapsulation of doxorubicin inside SLNs significantly increases oral bioavailability of doxorubicin up to 3.6 folds, which could be further exploited for establishing the safety and efficacy in the treatment of cancer. Similarly, Ren *et al*. tried to encapsulate highly hydrophilic, ganciclovir inside the borneol –functionalized SLNs for improving the brain accumulation [123]. However, their potential to deliver orally is need to be exploited in future.

2.7 Polymer-lipid hybrid nanoparticles

With the advancement in drug delivery research, strides having been taken to capitalize the positive characteristic features of the two most prevalent nanocarrier systems; liposomes and polymeric nanoparticles at the same time, mitigating some of their intrinsic limitations by combining lipids and polymers to make hybrid supramolecular assembly for expanding their therapeutic horizon. Therefore, an advanced, integrated delivery vehicle known as Polymer lipid hybrid nanoparticles (PLHNs) based tunable architecture has been impressively proposed to achieve the need of pharmacotherapy [23, 36, 50].

PLHNs are colloidal supramolecular assemblies which evolve rapidly as robust delivery architecture and hold great promise as a delivery vehicle [50, 124, 125]. They comprise mainly of two functional building blocks that include polymeric core and single or multiple layer of lipid surrounding on the same. PLHNs are comprised of at least two functional components: the polymer and the lipid, in which various therapeutic substances, targeting ligands, diagnostic and imaging agents can be dissolved, entrapped, adsorbed, encapsulated or covalently attached in to the core-matrix or shell. Each building block has different functional attributes such as lipid containing outer corona confers high biocompatibility through biomimetic characteristic to polymeric core whereas polymeric core imparts high structural integrity in which therapeutic moieties reside with high payload [35, 50]. The lipidic shell also serves as template for decorating the targeting ligands to fulfil the purpose of targeting as well as allows easy surface manipulation for prolonging systemic circulation lifetime through steric stabilization. Moreover, PLHNs enhance the incorporation of hydrophilic drug by virtue of lipid monolayer shield at interface of core and shell which act as a molecular barrier that curtails fast diffusion of

hydrophilic drug into external aqueous media during high speed fabrication step and hence, potentially increases drug loading. Likewise, the sustained release profile of PLHNs attributed to its core-shell type of structure; in particular owing to lipid layer which restricts inward water diffusion thereby slows down the degradation rate of polymer [36]. The lipid shell envelops polymeric core through Van-der Waals forces, hydrophobic interactions, electrostatic interactions or other non-covalent forces. These special attributes of PLHNs are largely accounted for their widespread use in the field of drug delivery applications.

The various materials are used for the formation of core of the PLHNs including polymers such as PLGA, PCL, dextran, or albumin; inorganic materials such as silica, magnetic iron oxide; organic materials such as, polysaccharides, polystyrene, polyelectrolyte capsule, polymer microgel. The various zwitterionic, cationic, anionic, and neutral phospholipids such as soya lecithin, 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), 1,2-dio-leoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) are used to form outer corona. Currently, two different techniques based on bottom-up approach, are used for the preparation of PLHNs. One of this is two step synthesis method and other one is one step synthesis method. In first method, separately prepared polymeric core and lipid vesicles, by two independent protocols are allowed to co-incubate to form PLHNs of desired characteristics. In contrast to two step method, one step synthesis process mainly involves the formation of PLHNs under controlled self-assembling of the elementary molecular constituents, by the virtue of commonly employed methods for engineering of polymeric nanoparticles [126-128].

Apart from parenteral delivery routes, various attempts have also been made by the researchers for increasing patient compliance with reduced cost of therapy, by delivering PLHNs through oral route. Benival and Devarajan have demonstrated the possibility of formation of doxorubicin HCl encapsulated PLHNs, named as "Lipomer", for oral bioavailability enhancement [28]. They used polyglyceryl-6-distearate and poly(methyl vinyl ether-co-maleic anhydride) (Gantrez® AN 119) for achieving high encapsulation (>90 %) with high payload (38.11 ± 0.37% w/w) of doxorubicin inside the lipomer. Doxorubicin encapsulated lipomer prepared by modified nanoprecipitation method, which also showed sustained release. *In-vivo* pharmacokinetic study exhibited 3.84 fold enhancements in the oral bioavailability by forming the lipomer. Further, lipomer also exhibited safety in the rats by significant reducing the cardiotoxicity and nephrotoxicity, which further, indicates the potential of lipomer as a safe drug delivery system.

Ling *et al*. developed novel, self-assembled lecithin-PLGA based PLHNs for improving oral bioavailability of vincristine sulfate by P-gp inhibition [129]. They have employed dextran sulfate as encapsulation enhancer counter-ion to form electrostatic complex with the vincristine, which resulted in the enhanced encapsulation around 93.6% after self-assembling nanoprecipitation method. PLHNs also showed extended release up to 96 hr by avoiding the burst effect. *In-vivo* pharmacokinetic study in rats after oral administration of vincristine encapsulated PLHNs exhibited 3.3 fold improvement in the oral bioavailability. Further, efficacy of the developed system against breast cancer was assessed by cellular accumulation and fluorescence microscopy on the P-glycoprotein over-expressing MCF-7 cells, which showed 12.4 fold higher uptake in the cancer cells as compared to pure drug solution, confirming the potential of the developed system for the oral delivery with efficacy.

Jain *et al*. designed PLHNs for oral delivery of amphotericin-B by using gelatin and lecithin [27]. PLHNs were prepared by a two-step desolvation method, which exhibited encapsulation of 50.61 ± 2.20%. PLHNs also exhibited sustained drug release profile. The improvement in the intestinal permeation was studied on the Caco-2 cell line, which revealed 5.89 fold higher intestinal permeation. Further, *in-vivo* pharmacokinetic studies in rat exhibited a 4.69-fold increase in the oral bioavailability of amphotericin-B upon incorporation inside PLHNs. Additionally, PLHNs also showed significantly lower hemolytic toxicity and nephrotoxicity as compared to marketed Fungisome as revealed in the histopathology and biochemical analysis, confirming the safety of the developed oral drug delivery system. Likewise, Asthana *et al*. have also developed cationic, amphotericin-B encapsulated PLHNs for the Th-1 biased immunomodulation and synergistic antileishmanial activity [130]. However, their oral delivery potential is need to be exploited.

Fang *et al*. encapsulated highly water soluble anticancer agent, salidroside inside the PLHNs for effective anticancer therapy by improving the oral absorption [131]. PLHNs were successfully developed by double emulsification solvent evaporation method. The effect of different formulation variable was systematically investigated to obtain optimized PLHNs with high entrapment efficiency. PLHNs showed sustained release profile without any burst effect. Moreover, anticancer activity was assessed on the 4T1 and PANC-1 cancer cells, which exhibited significantly higher antitumor activity than free drug solution. However, further *in-vivo* pharmacokinetic study is warranted for establishing their oral delivery potential.

Additionally, protein and peptide molecules have also been delivered orally by forming the PLHNs. To this end, Ma *et al*. have encapsulated protein molecule (bovine serum albumin) inside the PLHNs by double emulsification solvent

evaporation technique for oral delivery [132]. Developed PLHNs showed higher encapsulation of 90.82 % with high pay load of 24.73 %. Further, intestinal permeation study showed significant higher transcytosis compared to free protein molecule, which confirmed the enhancement of the intestinal permeation. Henceforth, the developed PLHNs could be a promising delivery platform for the oral administration of proteins and peptide molecules with higher bioavailability.

2.8 Core-shell polymeric nanoparticles

The advent of core-shell polymeric nanoparticles has opened the new avenue in the field of drug delivery [133]. Core-shell polymeric nanoparticles are comprised of the two different polymeric materials, in which one material forms core and other material envelops over the core. They showed numerous superior advantages over the simple polymeric nanoparticles due to their improved biological performance. They showed tremendous physical, biological and chemical properties such as, lower toxicity, higher biocompatibility, lower immunogenicity, increased dispersibility, higher conjugation potential with other biological substances, higher physical, chemical and thermal stability, etc [134, 135]. Both, hydrophobic as well as hydrophilic drugs can be easily delivered by core-shell polymeric nanoparticles. They are also used for the improving the encapsulation potential of the different drug molecules, specifically hydrophilic drugs. They also used to prevent the activation of the host immune system against the by using the biomimetic material, such as amino acids, which makes them biocompatible and less toxic.

Hydrophilic nature of the core-shell polymeric nanoparticles make them easier to dispersed in the biological medium and thus, increases the bio-dispersivity and biocompatibility for the efficient delivery of therapeutic agents compared to

conventional polymeric systems. Additionally, the diversified biological molecules can be easily conjugated on the surface of the core-shell nanoparticles by modulating the polymeric shell material, and thereby, targeted delivery can be achieved. Further, higher surface area with adequate functionalization provides improved pharmacokinetic profile, prolonged blood circulation and improved bioavailability of the drugs at target organ. The shell material also provides protection to the core material against the chemical degradation during the exposure under different biological media, and thus helps in the improving the stability of the encapsulated therapeutic agent. The polymeric barrier over the core material controls the release of bio-actives, which can be modulated easily for spatiotemporal drug release because of ion, temperature, and pH specific degradation of the polymer [136-138]. In this case core-shell polymeric nanoparticles are promising delivery platform for biological applications to that of single polymeric nanoparticles [139, 140, 34].

Different core-shell polymeric nanoparticles have been employed for the oral delivery of therapeutic agents, specifically proteins and peptide molecules. Chitkara and Kumar have prepared bovine serum albumin-PLGA based core-shell polymeric nanoparticles as carrier system for delivery of water-soluble drug, gemcitabine [141]. The core was formed by bovine serum albumin for encapsulating hydrophilic drug whereas, shell was formed by PLGA. Core-shell nanoparticles were prepared by double emulsification solvent evaporation method and effect of different formulation as well as process variables was assessed. Core-shell nanoparticles also showed sustained release profile up to 12 hr due to hydrophobic PLGA barrier. Further, *in-vitro* cytotoxicity study on MG-63 osteosarcoma cells showed significant improvement in the cytotoxic activity due to enhanced cytoplasm co-localization of the core-shell nanoparticles compared to pure drug solution. Results indicated that

core-shell nanoparticles could be a good carrier for delivering the hydrophilic drugs with enhanced efficacy. However, their oral delivery potential is need to be addressed.

Bagre *et al*. developed alginate enveloped chitosan based core-shell polymeric nanoparticles for oral delivery of low molecular weight heparin, enoxaparin [142]. Core-shell nanoparticles were prepared by two step method. In which, chitosan core was prepared by ionic gelation method followed by coated with alginate solution. *In-vitro* permeation study showed significant improvement in the enoxaparin permeation across intestinal barrier. Additionally, *in-vivo* pharmacokinetic study in rat exhibited three fold improvement in the oral bioavailability compared to pure drug. Further, efficacy of the developed system was confirmed by pharmacodynamic study in rat venous thrombosis model, which demonstrated 60% reduction in the thrombus formation. Hence, developed core-shell nanoparticles can be used as potential oral drug delivery system for therapeutic agents.

2.9 Drug specific literature review [143-147]

Drug: Cromolyn sodium

Synonyms: Disodium cromoglycate

CAS number: 16110-51-3

IUPAC Name: Disodium; 5-{3-[(2-carboxy-4-oxo-4H-chromen-5-yl)oxy]-2-hydroxypropoxy} - 4-oxo-4H-chromene-2-carboxylate

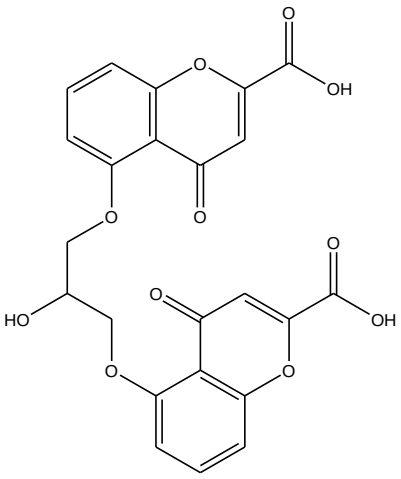
Molecular formula: $C_{23}H_{16}O_{11}Na_2$

Molecular Weight: 512.33 g/mol

State: Solid

Description: White or creamy powder having little odour. Hygroscopic. It is tasteless at first but leaves a slightly bitter after-taste. It is soluble in water and the resulting solution is neutral.

Chemical structure:



2Na+

pKa: 1.1

Storage conditions: Store between 15-30 ºC

Water solubility: 100 mg/mL

Polarity (Log P): -4.80

Melting point: 263-264°C

Categories: Anti-allergic agent

Indication: For the management of patients with bronchial asthma. Also used in the treatment of vernal keratoconjunctivitis, vernal conjunctivitis, and vernal keratitis

Pharmacodynamics: Cromoglicate or cromolyn, a synthetic compound, inhibits antigen-induced bronchospasms and, hence, is used to treat asthma and allergic rhinitis. Cromoglicate is used as an ophthalmic solution to treat conjunctivitis and is taken orally to treat systemic mastocytosis and ulcerative colitis.

Mechanism of action: Cromoglicate inhibits degranulation of mast cells, subsequently preventing the release of histamine and slow-reacting substance of anaphylaxis (SRS-A), mediators of type I allergic reactions. Cromoglicate also may reduce the release of inflammatory leukotrienes. Cromoglicate may act by inhibiting calcium influx.

Pharmacokinetics: In man, oral administration of sodium cromoglycate was followed by a low rate of urinary excretion. The mean urinary excretion of the administered dose over 24 hours was only 0.5%. This indicates that little of the compound is absorbed from the gastrointestinal tract.

Absorption: 1%

Route of elimination: Urine and bile

Half-life: 1.3 hours

Adverse effects: Nausea, vomiting, diarrhoea, abdominal discomfort, headache, insomnia, skin rashes sneezing, cough, unpleasant taste in the mouth, increased wheezing or difficulty in breathing, itching of skin, low blood pressure, shortness of breath, swelling of face, lips, or eyelids, tightness in chest and joint pains have been reported. Hypersensitivity reactions have been reported rarely. Cases of erythema, urticaria or maculopapular rash have been reported and these have cleared within a few days on withdrawal of the drug.

Dosage forms: Aerosol, Capsule, Liquid Solution, Nasal Spray

Toxicology: Sodium cromoglycate has been given to rats at oral doses of 100, 300 and 1000 mg/kg/day for six months. No effects were seen in body weight or food and water consumption which could be related to the administration of the drug. In the haematology, urinalysis, blood biochemistry, serum protein electrophoresis and

ophthalmoscopic studies there were no differences between test and control animals. Organ weights were not affected by treatment. There were no toxic morphological changes in the tissues of any rat in the vehicle control or high dose groups. Detailed examination of bone marrow preparations revealed no effects attributable to daily administration of sodium cromoglycate. A detailed histopathological examination of every segment of the gastrointestinal tract revealed distinct alterations in the gastric mucosa of rats treated at 300 and 1000 mg/kg. The changes were a heightened incidence of increased cornification of the fore-stomach mucosa and basal cell proliferation at the level of the cardia. In addition, a further change noted in the cardia was referred to as mucinous microcyst formation. This latter lesion was not seen in the stomach of any of the high dose recovery animals at the end of the 30-day withdrawal period and appeared, therefore, to be fully reversible.

2.10 Research work done on cromolyn sodium

Ding *et al*. developed noncovalent complex of cromolyn sodium with Sodium N-[8-(2-hydroxy-benzoyl)amino]caprylate (SNAC) for enhancing the oral absorption [40]. The formed complex was evaluated on the Caco-2 cell monolayer for exploring the molecular mechanism of the enhanced absorption. Results suggested that permeation was significantly improved by forming the complex with SNAC. At molecular level, spectroscopic and molecular dynamic simulation studies revealed that the enhanced permeation was attributed to the involvement of intermolecular interaction through 2-hydroxybenzamide moiety of SNAC, which would have weaken the hydrogen bonding between cromolyn and surrounding water molecules and thereby, improved the permeation. Moreover, no any transport marker leakage was detected, indicating the integrity of cell monolayer was remained without any cell damage. Hence, SNAC can be employed for increasing the permeability of cromolyn sodium by oral route.

Alani and Robinson have synthesized amino acid derivative of cromolyn sodium for understanding the oral drug absorption enhancement mechanism [43]. Lipophilicity of the cromolyn sodium remained unchanged after the formation of complex with SNAC. The permeation study on Caco-2 cell line model suggested that the permeability of cromolyn sodium was increased as a result of modulation of membrane fluidity and thereby, opening the tight junction between the cells. The lipophilicity have not any significant impact on the permeation. Hence, from the study they concluded that the increase in membrane fluidity by SNAC plays a crucial role in the permeation enhancement mechanism of cromolyn sodium.

Deshmukh *et al*. developed cromolyn sodium encapsulated proliposomal beads for oral permeability improvement [41]. Phospholipid (distearyl phosphatidylcholine)-cholesterol-surfactant (Tween 80/sodium cholate) mixture was spray coated on the beads containing cromolyn sodium. The developed proliposomal beads were evaluated for permeability study using the Caco-2 cell line and everted rat intestinal sac model, which exhibited significant enhancement in the transport of cromolyn sodium by forming the proliposomal beads compared to pure drug solution and surfactant-free lipid formulations. Further, the cellular damage on the Caco-2 monolayer was also assessed by monitoring the marker molecule, which exhibited that enhanced transport is due to transcellular transport only and not as a result of paracellular transport by modulating the tight junction. Hence, phospholipids-surfactant proliposomal beads can be a good alternative with improved oral delivery of cromolyn sodium.

Sindhumol and Mohanachandran have developed cromolyn sodium loaded ophthalmic inserts using hydroxyl propyl methyl cellulose, methyl cellulose and gelatin as polymers, by employing solvent casting method for increasing contact time,

with improved patient compliance and greater therapeutic efficacy [148]. The ophthalmic inserts exhibited controlled release pattern, which would reduce dosing frequency. Nolan *et al*. formulated nanoporous microparticles of cromolyn sodium by particle engineering through spray drying for pulmonary delivery [149]. *In-vitro* drug deposition study showed significant improvement in the deposition of the developed porous microparticles compared to pure drug. In another instance, pH dependent mucoadhesive in situ gel of cromolyn sodium was developed for improving the bioavailability and sustaining the drug release upon nasal delivery [150].

Nagarsenker and Londhe developed liposomal formulation of cromolyn sodium for inhalation delivery in the prophylactic treatment of asthma [44]. The formed liposomal formulation was found to be stable upon storage at 4-8 ºC. *In-vivo* performance of liposomal cromolyn sodium was evaluated in sensitized guinea pigs after 2 hr of administration of formulations, which exhibited significant inhibition of influx of neutrophils by administering liposomal formulation as compared with pure drug solution at 24 hr, without any significant change in the recovery to normal respiratory pattern.

Gajra *et al*. developed cromolyn encapsulated chitosan nanoparticles for improving the oral permeability and thereby, oral bioavailability [45]. Cromolyn sodium loaded chitosan nanoparticles were prepared by ionic gelation technique using quality by design approach. The optimized formulation showed significant improvement in the intestinal permeability during the *ex-vivo* intestinal permeation study, which further visualized by confocal microscopy.

2.11 Polymer specific review

2.11.1 Poly-(D, L-lactide-co-glycolide) (PLGA)

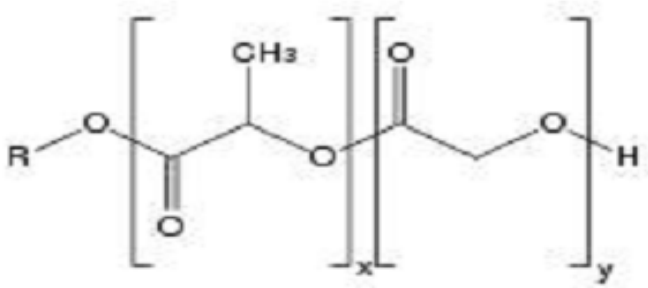
PLGA is one of the most successfully used biodegradable polymers, employed for the development of numerous drug delivery systems. PLGA is a United States Food and Drug Administration (USFDA) approved, co-polymer of poly lactic acid (PLA) and poly glycolic acid (PGA), which is widely used for biomedical applications due to its biodegradability, biocompatibility and excellent mechanical strength. It is enormously used as delivery carrier for drugs, proteins and various macromolecules, including DNA, siRNA and peptides [151].

Polymer composition : 75:25 molar ratio D, L - lactide : glycolide

Molecular weight : 75,000-115000 Da

Molecular formula : -$[(C_6H_8O_4)_x(C_4H_4O_4)_y]$-

Chemical structure:



Where, x = number of units of lactic acid, y = number of units of glycolic acid.

PLA contains an asymmetric α-carbon, which is typically described as the D or L form in classical stereochemical terms. The enantiomeric forms of the polymer PLA can be represented as poly D-lactic acid (PDLA) and poly L-lactic acid (PLLA).

PLGA is generally an acronym for poly D,L-lactic-co-glycolic acid, where D- and L-lactic acid forms are in equal ratio [152].

PLGA is a white to off-white amorphous solid powder. It can be solubilized by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate, acetonitrile. Methyl group of PLA side chain imparts hydrophobicity and hence, more lactide containing PLGA copolymer is highly hydrophobic and degrades slowly. PLGA polymers that are end-capped with esters demonstrate longer degradation half-lives. All PLGAs are amorphous rather than crystalline in nature. PLGA is synthesized by means of random ring-opening co-polymerization of two different monomers, the cyclic dimers (1, 4-dioxane-2, 5-diones) of glycolic acid and lactic acid. Different forms of PLGA can be identified in regard to the monomers' ratio used (e.g., PLGA 75:25 indicates a copolymer has composition of 75% lactic acid and 25% glycolic acid). PLGA can be processed into almost any shape and size, and can encapsulate molecules of virtually any size. Physical properties of PLGA have been shown to be dependent upon multiple factors, including the initial molecular weight, the ratio of lactide to glycolide, the size of the device, exposure to water (surface shape) and storage temperature [152-154].

PLGA has been shown to be extremely safe as a material for macroscopic and microparticle systems. Toxicological studies of PLGA devices suggested that local tissue reactions at the site of application may occur. However, these reactions are generally mild in nature. The degradation products of PLGA are also completely safe as it degrades first into its monomers, lactic and glycolic acid, which further, enter in the tricarboxylic acid cycle (Krebs' cycle), are then metabolized and subsequently, eliminated from the body as carbon dioxide and water [155].

2.11.2 Poly-ε-caprolactone (PCL)

PCL is widely used, USFDA approved polymer for controlled drug delivery and tissue engineering due to its biocompatible and biodegradable nature. PCL is prepared by the ring-opening polymerization of the cyclic monomer ε-caprolactone. Catalyst such as stannous octoate is used to catalyze the polymerization and low molecular weight alcohols can be used to control the molecular weight of the polymer. It is compatible with the wide range of therapeutic agents, which allows the drugs to be encapsulated in the homogenous form across the polymeric matrix and thus, facilitates slower degradation up to several months [156, 157]. PCL has flexible mechanical properties, including Young's modulus, elasticity, tensile strength, elongation at break value, which makes it most suitable for paramedical application, wound dressing, contraceptive and dentistry [158, 159].

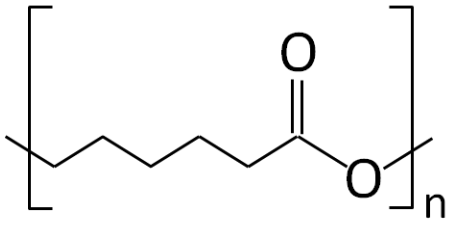
Molecular weight : 75000 – 95000 Da

Melting point : 59 to 64 ºC

Physical nature : Semi-crystalline nature

Molecular formula : $(C_6H_{10}O_2)n$

Chemical structure:



Solubility: Chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone and 2-nitropropane at room temperature. It has a low solubility in

acetone, 2-butanone, ethyl acetate, dimethylformamide and acetonitrile. It is insoluble in alcohol, petroleum ether and diethyl ether at room temperature.

PCL is non-toxic in nature and found to be cyto-compatible with a several body tissues, which makes it an ideal material for tissue engineering. PCL undergoes a two-stage degradation process: first, the non-enzymatic hydrolytic cleavage of ester groups, and second, when the polymer is more highly crystalline along with low molecular weight (less than 3000 Da), the polymer is shown to undergo intracellular degradation as evident by observation of PCL fragments uptake in phagosomes of macrophages, giant cells as well as within fibroblasts. Hence, PCL may be completely resorbed and degraded via an intracellular mechanism once the molecular weight was reduced to 3000 or lesser. It was also noted that in the first stage the degradation rate of PCL is essentially identical to the *in-vitro* hydrolysis at 40 ºC, and obeyed first-order kinetics. It was concluded that the mechanism of PCL degradation could be attributed to random hydrolytic chain scission of the ester linkages, which caused a decrease in molecular weight [158, 160].

2.11.3 Chitosan

Chitosan is the second most abundant natural polymer after cellulose; obtained by alkaline N-deacetylation of chitin. Chitosan is a linear polysaccharide composed of randomly distributed ß-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look ’cottonlike’.

Chitosan is a cationic polyamine with a high charge density at pH < 6.5; and so adheres to negatively charged surfaces and chelates metal ions. It is a linear

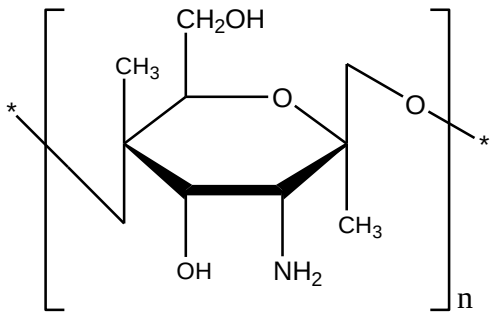
polyelectrolyte with reactive hydroxyl and amino groups allows chitosan to react chemically with anionic systems, which results in alteration of physicochemical characteristics of such combinations. The nitrogen in chitosan is mostly in the form of primary aliphatic amino groups. Almost all functional properties of chitosan depend on the chain length, charge density, and charge distribution. Numerous studies have demonstrated that the salt form, molecular weight, and degree of deacetylation as well as pH at which the chitosan is used all influence how this polymer is utilized in pharmaceutical applications.

Synonyms: 2-Amino-2-deoxy-(1,4)- b - D-glucopyranan; deacetylated chitin; deacetylchitin; b-1,4-poly- D -glucosamine; poly- D-glucosamine; poly-(1,4- b- D -glucopyranosamine)

Chemical Name **:** Poly-b - (1, 4)-2-Amino-2-deoxy- D -glucose [9012-76-4]

Molecular formula : $[C_{16}H_{18}O_8N_2]n$

Chemical structure :



Acidity/alkalinity **:** pH = 4.0-6.0 (1% w/v aqueous solution)

Density **:** 1.35-1.40 g/cm^3

Glass transition temperature: 203 ^oC

Solubility: Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5.

Stability and Storage Conditions: Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. The PhEur 2005 specifies that chitosan should be stored at a temperature of 2-8 ºC.

Chitosan is used in cosmetics and in a number of pharmaceutical formulations. In pharmaceutical formulation used for controlled drug delivery applications, use as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems, and use for gene delivery. Chitosan also used to form gels, films, beads, microspheres, tablets, and coatings for liposomes by using several techniques including spray-drying, coacervation, direct compression, and conventional granulation processes.

Chitosan is incompatible with strong oxidizing agents. The % degree of deacetylation in commercial chitosan is in the range 60-100 %.The degree of deacetylation necessary to obtain a soluble product must be greater than 80 to 85%. It is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. Chitosan is generally regarded as a nontoxic, nonirritant, biocompatible and biodegradable material. It is GRAS listed [161, 162].

2.12 Lipid specific review

2.12.1 Soya lecithin

Soya lecithin is a complex mixture of acetone-insoluble phosphatides that consists chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates as separated from a crude vegetable oil source. The composition of lecithin (and hence also its physical properties) varies

enormously depending upon the source of the lecithin and the degree of purification. Soybean lecithin contains 21% phosphatidylcholine, 22% phosphatidylethanolamine, and 19% phosphatidylinositol, along with other components. Lecithins are diversified in their physical form, from viscous semi-liquids to powders, depending upon the free fatty acid content.

Synonyms: egg lecithin, mixed soybean phosphatides, ovolecithin, phospholipon 100, phosphatidylcholine

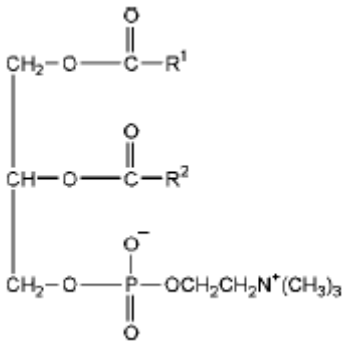
Color **:** Dark yellow or brown colour

Density **:** 0.5 g/cm^3 for powdered lecithin

Isoelectric point **:** 3.5

Saponification value : 196

Chemical structure:



R^1 and R^2 are fatty acids, which may be different or identical

Solubility: Lecithin is soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids.

Stability: When heated, lecithins oxidize, darken, and decompose. Temperatures of 160–180 ºC will cause degradation within 24 hours

Storage condition: Temperatures below 10°C

Lecithins are used in a wide variety of pharmaceutical applications, including in cosmetics and food products. Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents and are included in intramuscular and intravenous injections, parenteral nutrition formulations, and topical products such as creams and ointments. Lecithins are also used in suppository bases. They are also commonly used as a component of enteral and parenteral nutrition formulations. Liposomes in which lecithin is included as a component of the bilayer have been used to encapsulate drug substances; their potential as novel delivery systems has been investigated. This application generally requires purified lecithins combined in specific proportions. Therapeutically, lecithin and derivatives have been used as a pulmonary surfactant in the treatment of neonatal respiratory distress syndrome [163, 164].

2.12.2 Glyceryl monostearate (GMS)

GMS and mono- and diglycerides are used for a variety of esters of long-chain fatty acids, the esters fall into two distinct grades:

40-55 percent monoglycerides: The PhEur 6.0 describes GMS 40-55 as a mixture of monoacylglycerols, mostly monostearoylglycerol, together with quantities of di- and triacylglycerols. It contains 40-55% of monoacylglycerols, 30-45% of diacylglycerols, and 5-15% of triacylglycerols.

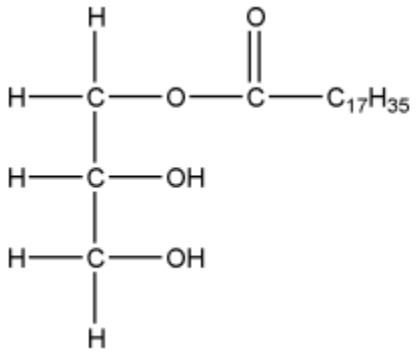
90 percent monoglycerides : The USP32–NF27 describes GMS containing not less than 90% of monoglycerides of saturated fatty acids, chiefly glyceryl monostearate ($C_{21}H_{42}O_4$) and glyceryl monopalmitate ($C_{19}H_{38}O_4$).

Appearance: Glyceryl monostearate is a white to cream-colored, wax-like solid in the form of beads, flakes, or powder.

Chemical name : Octadecanoic acid, monoester with 1,2,3-propanetriol

Empirical formula : $C_{21}H_{42}O_4$

Structural formula:



Molecular weight : 358.6 gm/mol

HLB value : 3.8

Melting point : 55-65 ^oC

Specific gravity : 0.92

Solubility: Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, and fixed oils. Practically insoluble in water, but may be dispersed in water with the aid of a small amount of soap or other surfactant.

The many varieties of GMS are used as nonionic emulsifiers, stabilizers, emollients, and plasticizers in a variety of food, pharmaceutical, and cosmetic applications. It acts as an effective stabilizer, that is, as a mutual solvent for polar and nonpolar compounds that may form water-in-oil or oil-in-water emulsions. These properties also make it useful as a dispersing agent for pigments in oils or solids in fats, or as a

solvent for phospholipids, such as lecithin. GMS has also been used as a matrix ingredient for a biodegradable, implantable, controlled-release dosage form. It is GRAS approved material.

Stability and Storage Conditions: Glyceryl Monostearate shows increase in acid value upon aging owing to the saponification of the ester with trace amounts of water at warm temperature. Antioxidants such as butylated hydroxytoluene and propyl gallate may be added. It should be stored in a tightly closed container, cool, dry place, and protected from light [165, 166].