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REVIEW ARTICLE

# Gentamicin as Oral Drug Delivery Formulation

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## ABSTRACT

Oral gentamicin (GM) therapy has been challenged by formulating GM in oral formulation. Need for an oral replacement to parenteral delivery has led to renewed attentiveness in excipients like intestinal permeation enhancers which improve oral drug bioavailability. Delivery of a drug by oral route is predominantly restricted by pre-systemic degradation and poor penetration across the gut wall. The major challenge in the oral drug delivery is the development of novel dosage forms to endorse absorption of poorly permeable drugs across the intestinal epithelium. Fifty years ago research on oral absorption enhancers that increases gut permeability was first commenced yet clinical success yet to be achieved. Development has been troubled by lack of adequate reproducibility interest as well as perceived safety concerns. We reviewed some selected permeation enhancement techniques that are advantageous for increasing permeability of poorly permeable drugs like gentamicin (GM).

## KEYWORDS

Gentamicin, Permeability, Oral Formulation, Bioavailability

## INTRODUCTION

Gentamicin is a broad-spectrum bactericidal aminoglycoside antibiotic, produced by fermentation of *Micromonospora purpura* or *M. echinospora*. Gentamicin was introduced in 1958 and showed better effectiveness than earlier aminoglycosides because it was less susceptible to bacterial resistance. It is effective against wide variety of serious bacterial infections caused by susceptible gram-negative and some gram-positive aerobic bacteria.[1, 2] In addition, it's also effective against hard to kill pseudomonas species. Gentamicin is highly water soluble and ishows poor oral absorption and poor protein binding. It is distributed well in body fluids, but poorly in many tissues; thus it is only effective at treating aerobic bacteria.

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It is also absorbed well from denuded skin and the peritoneum, pleural cavity, and joints. The drug is eliminated renally unchanged[1-3]. Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. Like all aminoglycosides, when gentamicin is given orally, it is not systemically active. This is because it is not absorbed to any appreciable extent from the small intestine. It is administered intravenously, intramuscularly or topically to treat infections. It appears to be completely eliminated unchanged in the urine.[4] Due to its high solubility and high polarity, it does not cross cell membranes efficiently, which is an important drawback for the therapy of intracellular infections such as brucellosis, due to the low antibiotic levels achievable inside infected cells. Several reports indicate that gentamicin is more active *in vitro* against clinical isolates of *Brucella* than streptomycin.

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In combined doxycycline–aminoglycoside regimens, gentamicin appears to be more cost-effective and less toxic given the duration of the administration (14–21 days for streptomycin compared with 7 days for gentamicin), with no increases in clinical relapse or treatment failure. These properties make gentamicin an attractive candidate for the treatment of brucellosis provided that the antibiotic can be delivered intracellularly. Therefore, an optimum strategy to treat brucellosis should target a highly active drug to the intracellular compartment and prolong the release of that antibiotic, thereby reducing the number of doses to be administered and minimizing drug side effects[5]. Gentamicin is also used for the treatment of osteomyelitis, an inflammatory bone disease. Osteomyelitis is the microbial infection of the bone medullary cavity, cortex and periosteum that is known to occur during post-operative sepsis after an orthopaedic procedure. However, for the treatment of osteomyelitis, a prolonged systemic antibiotic treatment such as the use of gentamicin, either oral or parenteral, for a period of 4–6 weeks, is known to cause systemic toxicity and patient discomfort.[6]

**Clinical Use**

Gentamicin is used to treat serious gram negative infections. It is indicated for peritoneal dialysis associated peritonitis, Gentamicin is also indicated as an adjuvant to a penicillin or vancomycin for treatment of enterococcal, streptococcal, *Staphylococcus aureus* endocarditis, or in prevention of endocarditis. Gentamicin is supplied in many different forms. Injection solutions are supplied in 2mg/ml, 10mg/ml, and 40mg/ml concentrations. Ophthalmic ointments and solutions are supplied in 3mg/g concentrations. Topical creams and ointments are supplied in 0.1% strengths. When Gentamicin is given for gram-negative infections with susceptibility to pseudomonas, dosing is usually 3-6mg/kg/day IV and IM every 8 hours. Once daily dosing is 4-7mg/kg/day IV. Ophthalmic ointment is twice to three times a day while the solution is given as 1 to 2 drops every 4 hours. For peritoneal dialysis-associated peritonitis, 6 mg/kg is given via intraperitoneal. Dosage adjustments are made based on renal function, cystic fibrosis, and obesity. Gentamicin should reach therapeutic peak levels at around 4-12 mcg/ml and trough levels at 0.5-2 mcg/ml. Peak levels are important because Gentamicin's efficacy is based on high concentrations.

This drug is contradicted in patients with hypersensitivity to aminoglycosides. Patients with renal, vestibular and auditory impairment should use extreme caution when taking this drug. Serious adverse effects include neuromuscular blockage firing, ototoxicity, nephrotoxicity, and respiratory tract paralysis. Gentamicin and other aminoglycosides have a high affinity for tissues in the kidney and 8th cranial nerve. Since aminoglycosides have a very long half-life, the concentration of drug tends to build up in these areas of the body and cause an even greater toxicity. The drug is classified as pregnancy category D and should not be given to women who are pregnant. However, the drug is classified as safe for when mothers are breast feeding. There are several drug interactions. They include: Alcuronium, atracurium, cidofovir, cisatracurium, decamethonium, doxacurium, ethacrynic acid, furosemide, indomethacin, metocurine, mivacurium, polygeline, rapacuronium, tubocurarine, vancomycin, and vecuronium.[7]

**Medicinal Chemistry**

Gentamicin acts by binding irreversibly to the 30S subunit of the bacterial ribosome. It is bactericidal because it acts by two different mechanisms. The first mechanism produces a prolonged effect which interferes with proper amino acid polymerization and elongation. The second effect causes a more rapid effect by initiating the misreading of amino acid codons by tRNA. This effect impaires proofreading and causes the incorporation of incorrect amino acids into the protein, thus creating nonsense proteins. These nonsense proteins are thought to be incorporated into the bacterial cell wall causing disruption of its normal function. This allows the first mechanism of action to have enhanced penetration of the cell wall and an

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overall greater effect first mechanism of action to have enhanced penetration of the cell wall and an overall greater effect. The 3 aminosugars linked together by glycosidic linkages are common for all aminoglycosides. Modification in this structure may affect activity or bacterial resistence.[8]

-Altering the first ring (furthest to right) will affect the spectrum of the drug. Methylation of the amines on this ring (which is shown) will decrease bacterial resistance while retaining normal activity.

-Many alterations on the second ring (in the middle) can be made without affecting the activity of the drug.

-Altering the third ring (furthest to the left) involves the amine. It can be changed to a hydroxyl, but its removal abolishes activity.[8]

**Resistance**

Bacterial resistance to Gentamicin is minimal because it is chemically modified by the addition of methyl groups on its hydroxyl and amine groups. However, resistant strains are beginning to emerge. Resistance usually takes place when bacteria use inactivating enzymes that cause the N-acetylation of amine groups, phosphorylation, and adenylation of hydroxyl groups. Since a few of Gentamicin's amine groups are methylated, it is less likely to be inactivated. Resistance may also take place with ribosomal mutations within the bacteria to decrease drug binding. In addition, bacteria may show decreased cell wall permeability to Gentamicin to limit the amount of drug that makes it into the cell.[9]

All drugs are classified according to the biopharmaceutical classification BCS into four categories on the basis of solubility and permeability to rationalize science of drug delivery and simplify complications in the drug registration of newly evolving diverse compounds for regulatory authorities. Among the different classes of BCS the per oral delivery of class 3 and 4 drugs is partially or completely decreased due to their poor intestinal permeability. Due to their in auspicious physicochemical and chemical properties which are difficult to excipient may be added externally to enhance permeation transiently. During the past few decades, noteworthy medical advances have been made in the field of drug delivery with the improvement of new dosage forms and techniques. For the drugs which are not absorbed by oral route other routes of drug delivery such as injection, transdermal, pulmonary or other routes are employed. However, oral route among different probable routes is most preferable because it offers significant advantages of therapeutic effectiveness and patient compliance.

Delivering a drug by oral route is also preferred for its convenience. Tablets and capsules can be prepared in large quantity at low price. Therefore in lead optimization step of drug discovery, oral bioavailability of a drug is important. It depends on various factors the most common being intestinal permeability, solubility during gastrointestinal transit, liberation from dosage form, liability to efflux and metabolism. The importance of solubility and permeability is especially reflected in the adoption of Amidon's Biopharmaceutics Classification System (BCS) by the FDA in 2000, devised as a scientific basis to grant biowaivers for in vivo bioavailability and bioequivalence studies. Various experimental systems are used for permeability enhancement. Although the improved understanding of permeability enhancement has become possible because of use of intact animal models, the inherent complexity of models has hindered definitive experiments to determine biochemical mechanisms. Through the use of in vitro models and techniques, the identification of key components of the barrier functions of epithelia has led to a more clear understanding of permeability enhancement. In this article we reviewed some basic permeability enhancement techniques which are useful for enhancing the permeability of poorly permeable drugs which are included in the class III and IV of the Biopharmaceutics Classification System (BCS) which was adopted by FDA in 2000 as a scientific basis for granting biowaivers for *in*

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*vivo* bioavailability and bioequivalence studies.[10]

Oral gentamicin drug delivery is the most desirable and the preferred method of administrating therapeutic agents. In addition, the oral medication is generally considered as the first choice for investigation in the discovery and development of new pharmaceutical formulations due to convenience in administration, patient compliance and cost effective manufacturing process. The overall process of oral delivery is frequently impaired by several physiological and pharmaceutical challenges that are associated with the inherent physicochemical nature of the drugs and/or the variability in GI condition such as pH, presence of food, transit times, as well as enzymatic activity in the GI tract.[11]

**Potential Absorption Barriers**

Review has been done comprehensively to determine the barriers for the intestinal permeability of drugs. The location of these barriers may be in the unstirred water layer, the mucous layer, the apical and basal cell membrane and cell contents, the tight junctions and the wall of lymph and capillaries.[12]

***Mucous***

A mucous layer consisting of water glycoproteins (mucins), electrolytes, proteins and nucleic acids covers the epithelial cells of the entire intestine. The layer is bound to the apical surface by the glycocalyx, a 500 nm thick glycoprotein structure which is covalently linked to lipids and proteins of the brush border membrane. The unstirred water layer is composed partially of the mucous layer, and it is supposed that the minimal thickness of the unstirred water layer, 100-50µm corresponds with the mucous layer. The mucous layer maintains the pH of the epithelial surface at 6 by acting as a buffer, thus creating an acidic microclimate.

***Apical Cell Membrane***

The shape of the apical cell membrane is like a 1µm thick brush border, and it consists of a 10nm thick double layer of polar lipid molecules containing a hydrophobic and a lipophilic part. The main constituents of lipid are phosphatidylcholine, phosphotidyl ethanolamine, sphingomyline (zwitterionic), phosphatidyl serine, phosphatidylinositol, phosphatidic acid (anionic), cholesterol and lipids.

For the preservation of membrane structures divalent metal ions may be necessary, Ca2+ chalets with negatively charged phospholipids, thus decreasing membrane permeability and lipophilicity. Proteins are entrenched in the lipid bilayer by their hydrophobic segments. For the optimal activities of membrane bound enzymes, fluid state of the membrane is required; cell preserves the membrane transition temperature (Tin), the temperature at which the transition from the stiff gel to the fluid liquid crystalline state occurs, below environmental temperature. A regulating action of cholesterol is employed on membrane structure, increasing fluidity of gel-state membrane and decreasing fluidity of liquid crystalline membrane. Sphingomyline has been proposed to enhance the assembling influence of cholesterol.

The membrane order is also influenced by natural fatty acids, their cis-double bonds distracting phospholipid organization. For this reasons fluidity of fluid state membranes may increase with decreasing cholesterol/ phospholipid molarratio or increasing total lipid/protein ratio and double bond index thus increasing permeability. In rat colonocytes lipid fluidity decreases from proximal to distal transition temperatures amounting to 23-24º C and 26-27º C respectively corresponding with a high enzyme activity in the proximal segment. The transport of molecules across the phospholipid bilayer is commonly correlated with lipid-water coefficient. Subsequently the absorption of strongly hydrophilic substances is restricted by the lipid bilayer e.g. certain antibiotics and peptides. For this reason the transcellular transport of water, ions and polar solutes (e.g. monosaccharides) require other mechanisms e.g. Diffusion through pores and carrier mediated transport.[13]

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### *Basal Cell Membrane*

The basal cell membrane is composed of a 9 nm thick phospholipids' bilayer which contains proteins. The lipid fluidity of the basolateral membrane surpasses apical membrane fluidity probably because of lower content of glycoshingo lipids. Hence the barrier function of the basal membrane is possibly less prominent than that of apical membrane.

### *Tight Junctions*

Tight junctions (zonula occludentes) are regions of close communication between apical ends of epithelial cells. They are constructed of a network of strands, the permeability of tight junction increases with the decreasing strand number, thus determining the 'leakiness' of epithelium. The small intestine contains leaky epithelium, and intestinal permeability decreases in the distal direct ion running parallel with apical cell membrane permeability; the proximal colon is temperately leaky, the distal colon moderately tight. The medium sized solutes (e.g. disaccharides'), ions and water thus establishing route for passive ion permeation. Tight junctions are cation selective & they have been suggested to be impermeable for cations with a molecular weight higher than 350 nm or a diameter exceeding 0.8 nm. Alternatively, it is conceivable that a distribution of pore sizes exists, with a large number of small pores and a few large ones. The structure of tight junction is destabilized by exposure to hypertonic solutions and by Ca2+ depletion. In hamster small intestine sodium coupled solute transports have been suggested to increase junctional permeability towards small peptides, sugars and amino acids.

### *Capillary Wall*

The location of capillary wall is 500 nm underneath the basal membrane. The endothelial cell membrane contains small perforations of 0.4-1 nm radius and the blood capillary wall is fenestrated, fenestrate radius amounting 20-30 nm. On the other hand lymphatic capillaries are provided with an intracellular junction of larger size, permitting passage of particles with a radius up to 300 nm. Particles with a radius smaller than 6 nm are not retained by basement membrane surrounding fenestrated capillaries. Due to the existence of large pores, the intestinal blood and lymph capillaries are not considered to execute an important barrier for drug absorption. However, it is conceivable that strongly hydrophilic drugs will be transported slowly across the capillary wall, compared with hydrophilic compounds, as their absorption site will be limited to the pore area.[14]

## Required Parameters for Effective Oral Drug Delivery

In order to obtain sufficient gentamicin absorption in the gut, novel delivery systems must be developed that are able to overcome the barriers present in the oral route. The main barriers include: the acidic environment in the stomach, basal cell membrane, capillary wall, tight junction the digestive and proteolytic enzymes in the small intestine, the low permeability of the intestinal epithelium to large hydrophilic gentamicin and finally the first pass metabolism of the drug in the liver.[15-18] Protease inhibitors such as Aprotonin, Chymostatin, EDTA and Leupeptin can be used to locally deactivate the proteolytic enzymes of the GI tract. In order to be safe, however, these protease inhibitors have to be linked to high molecular weight hydrophilic matrices to avoid their absorption and possible cell toxicity.[19-21]

Moreover, permeation enhancers have been used to reversibly open the tight junctions of the intestinal epithelium and allow the passive absorption of peptides and proteins by the paracellular pathway. Two main classes of materials including calcium chelators and surfactants are able to increase the permeability of tight junctions and thus improve the absorption of macromolecules and hydrophilic substances. While chelating agents may induce disruption of actin filaments by extracellular Ca+2 depletion, surfactants cause irreversible exfoliation of the intestinal epithelium. However, neither of these substances can be used as permeation enhancers for hydrophilic macromolecules such as peptides or proteins

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due to their interactions with phospholipid bilayer of the intestine and the resulting cell toxicity.[22]

Mucoadhesive polymers such as chitosan and trimethyl chitosan (TMC) have mucoadhesive properties that enable them to attach to the intestinal membrane and interact with the actin filaments of the tight junction to reversibly open them and allow for the passage of hydrophilic peptides across the membrane. These mucoadhesive polymers are shown to be nontoxic and due to their specific interaction with the actin filaments and lack of interference with the phospholipid bilayers of the enterocytes, their use is becoming very common for the induction of the paracellular transport of hydrophilic macromolecules within the opened water filled channels. Their possible use will be discussed in more detail later in this chapter.

Microparticulate and nanoparticulate drug delivery systems have attracted an immense attention as novel carriers for the delivery of lipophilic and hydrophilic substances as well as vaccines. There is a strong belief that nanoparticles of appropriate size may pass the mucosal membranes intactly and deliver their drug load into the systemic circulation. In the case of hydrophilic drugs, nanoparticles should be able to protect such drugs from degradation in the intestinal fluids and improve their penetration and permeation across the intestinal mucosal epithelium. Suitable nanoparticles have mucoadhesive properties which are due to their particle size and the particle's surface charge. However, more and more research shows that only a small fraction of nanoparticles is able to act as a carrier for hydrophilic drug molecules across the enterocytes and to deliver their drug load at the serosal site, which in most cases was not sufficient for a therapeutic effect. It was further shown that a substantial part of the nanoparticles may be internalized into the intestinal epithelial cells.[23-26] Exception is the transport of antigen containing micro and nanoparticles across so-called M-cells with specified particle uptake mechanism capable to induce a sufficient high immunogenic response. More details about the use of micro and nanoparticulate systems will be given below. The following sections will highlight the possible use of mucoadhesive polymers with special emphasis on chitosan and its increasing family of derivatives as well as promising delivery systems for peroral gentamicin delivery.

## Permeability Enhancement Techniques

### *Bile Salts*

Bile, which contains glycine and taurine conjugates of cholic acid and chenideoxycholic acid, emulsifies dietary fat and accelerates lipolysis and transport of lipid products through the unstirred water layer of the intestinal mucosa by micellar solubilisation. The bile salts which escape from active reabsorption in the ileum are metabolized to secondary bile salts deoxycholic acid & lithocholic acid by the bacterial flora. The diminishing order of hydrophilicity is as follows taurine conjugates > glycine conjugates > free bile salts. Polarity increases with the number of hydroxyl groups. Bile salts are capable to bind calcium, their binding properties decreasing with increasing hydrophilicity. No unambiguous data is available on the mechanism of absorption enhancement by bile salts. It may be carried out by effects on the mucous layer and on paracellular and transcellular absorption routes. They have been reported to affect the intestinal glycocalyx structure and to diminish gastric and intestinal mucous. A transcellular absorption enhancing effect is suggested by the phospholipid disordering action of unconjugated and conjugated bile salts. Colonic tight junction structure appears to be influenced by comparatively low bile salt concentrations (5mM and lower) in rabbits and rats. This paracellular absorption promoting effect is suggested to be intermediated by binding of Ca2+. Although bile salts have been validated to enhance drug uptake to a significant extent, applicability of these compounds as safe absorption promoter in man faces many complications, because mucosal damage seems to be correlated with their uptake. On the other

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hand, 2 year therapy with oral chenodeoxycholic acid (350-750 mg/day) for dissolution of gallstones was concomitant with mild side effects (increase of serum level of amino transferase andcholesterol, diarrhoea). This observation designates that long term therapy with bile salts containing formulations may be promising in man. However, the suggested co-carcinogenic & co-mutagenic effects of secondary bile salts discourage the development of bile salts containing pharmaceutical formulations.

***Nano Emulsions***

The nano emulsions can be outstanding vehicles for oral delivery of poorly permeable and/or highly lipophilic drugs since they can be manufactured from excipients that have solubilizing or even permeation enhancing properties. Oral nano emulsions which have a droplet size of less than 150 nm, are almost from the o/w type. Similar to conventional emulsions, they promote enhanced gastrointestinal absorption and reduce inter & intraindividual unevenness for a variety of drugs. Additionally due to their very large interfacial area, they exhibit excellent drug release properties. Moreover nano emulsions may offer a certain degree of protection against degradation or may progress difficult organoleptic properties of the actives. Some nanoemulsions tend to self emulsify in aqueous media, which makes them remarkable for oral formulations. The self-emulsifying formulations can be administered as water free preconcentrates which in situ form nanoemulsions in the gastrointestinal tract fluids.

Pluronics® are class of non-ionic surfactants which are very well known for their very low toxicity. The Pluronics®, also known as poloxamers are triblock copolymers of poly (oxy ethylene)–poly (oxy propylene)–poly (oxy ethylene) [(EO) x (PO) y (EO) x]. They are predominantly used as solubilizes, wetting agent for microemulions and as micro container for drugs after micellization. It has been demonstrated that Pluronics® may influence the carrier mediated transport of drugs depending on their structural composition. This effect might be advantageous for the treatment of drug resistant tumours as well as to enhance the oral bioavailability of actives. Nano emulsion system based on different Pluronics® have been found, that can be used to stabilize lipophilic such that EXE can be suggested to be behaving like class I or class III when complexes to cyclodextrins. This phenomenon requires further in vivo bioavailability studies to be better elucidated. Thus this is an encouraging approach to improve the poor bioavailability of EXE which is commonly used for long term oral administration in post-menopausal breast cancer chemotherapy.

***Chitosan and Its Derivatives***

Chitosan is a polysaccharide composed of two subunits, Dglucosamine and N-acetyl-D-glucosamine, linked together by s(1- 4) glycosidic bonds. Chitosan, a constituent of crustacean shells and being the second most abundant biopolymer after cellulose is derived from chitin by deacetylation. Chitosan attracts a lot of attention in the pharmaceutical research as a polymeric drug carrier. Chitin and chitosan are copolymers; however, chitin has a limited application because of its poor solubility and reactivity. Chitosan is a fully or partially deacetylated chitin derivative and is consequently soluble in acetic acid and other acidic solvents. This polymer has an apparent pka of about 6.5 and is soluble in acidic solutions with pH values lower than 6.5. Chitosan is a non-toxic, biocompatible polymer that has found a number of applications in drug delivery including that of absorption of hydrophilic macromolecular drugs. Chitosan, when protonated (pH 6.5), is capable to increase the paracellular permeability of polar drugs across mucosal epithelia. Chitosan derivatives have been assessed to overcome chitosan's incomplete solubility and effectiveness as absorption enhancer at neutral pH values such as those found in the intestinal tract. In recent years, significant progress has been made in identifying substances, which may increase the absorption of drugs through the paracellular

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pathways at a wider pH range. The intestinal epithelial cells express apical intercellular attachments, known as tight junctions, connecting the enterocytes with each other. They have some regulation mechanism for the paracellular absorption of hydrophilic compounds like glucose when present in higher amounts and allow the passage of macromolecules through their intercellular space after their opening is triggered externally by specified ionic interactions.

Chitosan with its mucoadhesive and nontoxic properties can act as a significant absorption enhancer by opening the intercellular tight junctions of the epithelia and promoting the paracellular permeation of hydrophilic macromolecules. Nevertheless, chitosan has poor solubility at pH values above 6.5; therefore, water soluble chitosan derivatives, which are soluble in both acidic and basic physiological environments, are good candidates for improving the paracellular permeation of highly polar drugs in the whole GIT. However, just recently a chitosan product has been synthesized by controlled deacetylation process of chitin which results in a soluble chitosan at pH value 7.2.

Different studies were carried out to synthesize and determine the antibacterial activities of quaternary ammonium salt of chitosan. These investigations showed that the antibacterial activities of quaternary ammonium salt of chitosan are much stronger than that of chitosan itself since the cationic charge of the ammonium salt is found to increase the interaction with the negative peptidoglycan residues of the bacterial cell surface and will make the bacterial membrane more permeable.

Trimethyl chitosan (TMC) was initially synthesized and characterized, Further in-vitro and in-vivo studies of the intestinal absorption of gentamicin by quaternized chitosan showed that TMC is able to increase the permeability of gentamicin across the Caco-2 cell monolayer. Moreover, studied the effect of TMC on the intestinal permeation of buserelin. This investigation has shown that TMC, as permeation enhancer, similar to chitosan, is able to open tight junction in a reversible way and increase the permeation of buserelin across the intestinal epithelia both in vitro and in vivo. Moreover, Jonker and coworkers have studied the intestinal paracellular permeation enhancement with TMC of various substitution degrees.[27] their studies clearly demonstrated that their TMCs were able to enhance the intestinal permeation in a neutral pH environment. Furthermore, it was shown that the degree of quaternization of the derivative has a major impact on its permeation enhancing properties across the intestinal epithelia. Due to their unique properties, such as their permeation enhancing effect and enzyme inhibitory capabilities, chitosan and its derivatives also act as antimicrobial agents. These investigations have shown that growth inhibition of chitosan against microorganisms, such as fungi and bacteria depends on the molecular weight of chitosan.

The bioavailability, biodegradability and the extensive studies on chitosan and its numerous derivatives have made them, as multifunctional polymeric permeation enhancers, good candidate polymers for oral drug delivery.

***Self-Micro-Emulsifying Drug Delivery Systems (SMEDDS)***

The GI absorption of poorly permeable drugs i.e. BCS class 4 drugs can be enhanced by using self-microemulsifying drug delivery systems. In this decade a lot of research has been conducted on developing self-micro emulsifying drug delivery systems (SMEDDS). Generally these systems are isotropic mixtures of oils, surfactants and co-solvents /co-surfactants.

Once administered in to the GI system, they are diluted with gastrointestinal fluid and the gastric motility provides the agitation for the formation of a fine oil-in-water (o/w) micro emulsion (SMEDDS). The difference between a SEDDS and SMEDDS is that the former when diluted results in a droplet size between 100 & 300 nm and the later results in a droplet size of less than 50 nm.[28]

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### *Self-Double Emulsifying Drug Delivery System (SDEDDS)*

The self-double emulsifying drug delivery systems (SDEDDS) can be used for enhancing oral bioavailability of drugs with high solubility and low permeability, but their industrial application is inadequate because of low stability. A novel formulation i.e. self-double-emulsifying drug delivery system can be developed which is stable through formulation optimization. SDEDDS can extemporaneously emulsify to water-in-oil-in-water (w/o/w) double emulsion in the diversified aqueous gastrointestinal environment, with drugs encapsulated in the internal water phase of the double emulsions.

Water-in-oil-in-water (w/o/w) double emulsions are complex systems consisting of aqueous droplets dispersed within larger oil droplets, which are they dispersed in an aqueous continuous phase. The internal aqueous droplets encapsulated by the oil membrane can be seen as a storage chamber for hydrophilic drugs. This structure can safeguard the drug dissolved in the internal aqueous phase and have shown great promise for enhancing oral bioavailability of compounds.

Generally w/o/w double emulsions are prepared by improved two step emulsification method. SDEEDS changed the process of second emulsification step, which can self-emulsify to w/o/w double emulsions due to gastrointestinal peristaltic movements *in vivo* instead of simulated emulsification *in vitro*. Similar to SEDDS, SDEDDS can be extemporaneously emulsified in the mixed aqueous gastrointestinal environment. But the formed emulsions are water in- oil-in-water (w/o/w) double emulsions not o/w emulsions, and drugs are encapsulated in the internal water phase of the double emulsions.

Compared to conventional thermodynamically unstable emulsions, SDEEDS are stable formulation system. In addition SDEDDS can be filled directly into soft or hard gelatin capsules which are easy for administration and storage.[29]

### *Novel Drug Delivery Systems*

Today, the design and development of novel peroral delivery systems for highly polar drugs are the main goal of many pharmaceutical researchers. The low oral bioavailability of these highly polar drugs like gentamicin due to their low permeation across the intestinal epithelium, the harsh environment of the gastric pH, their rapid degradation by the proteolytic enzymes and their rapid clearance due to the first pass effect are the major drawbacks of developing a successful delivery system, however the delivery of the hydrophilic drug is not difficult to achieve but to enable its absorption in the intestinal tract is the crucial part. Hence, the delivery system has not only to overcome the harsh pH of the stomach and more hydrophillicity and aqueous solubility in the GI tract, but to also increase the permeation of these molecules across the GI epithelium either by opening the tight junctions and increasing the paracellular transport or by increasing the endocytotic passage of the molecules through intracellular transport. In order to achieve this, the delivery system must be able to attach to a specific site in the GI tract long enough for the drug to permeate across the epithelium before the delivery system is being detached by the peristaltic movements of the gut. A number of peroral delivery systems were designed using liposomes, beads, adhesive drug delivery systems, superporous hydrogels, chitosan and its derivatives as well as nanoparticles to protect the drugs from the harsh environment of the GI tract and prolonging the drug's transit time at a specific site of the GI tract for an optimum drug bioavailability.

### *Liposomes*

Long circulating macromolecular carriers such as liposomes can exploit the enhanced permeability and retention effect for the protein drugs. Liposomes are vesicles consisting of one to several, chemically active lipid bilayers. Drug molecules can be encapsulated and solubilized within these bilayers. Different types of phospholipids such as phosphotidyl choline or phosphotidyl inositol may be used in liposomal

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carriers. Liposomes are prepared by sonication, reverse phase evaporation or film formation. Among different types of liposomes, dehydrated-rehydrated vesicles are most commonly used in polar drugs delivery due to the ease of preparation and low amount of stress applied to the polar drugs.

The liposomes can be easily decorated with targeting moieties, e.g., antibodies, hence delivering the choice of drugs to their specific target site. The liposomal composition, encapsulation efficiency, the rate of drug release from lipid bilayers, size and the surface charge are all important factors in successful liposomal drug delivery. Stefanov et al. have used liposomes prepared from phosphotidylcholine (PC) and cholesterol (CH) for oral drug delivery.

Although liposomes with their organized structures have some advantages as drug delivery systems, the extensive leakage of water-soluble drugs entrapped in liposomes during the GIT passage, the low drug entrapment, the heterogeneity of the vesicle size, the poor reproducibility and instability of formulations are some of the disadvantages of using liposome as drug delivery system.[30]

***Microtablets***

Microtablets with diameters of 0.5-3mm containing permeation and/or enzyme inhibitors were designed and investigated for the peroral delivery of hydrophillic protein and peptide drugs. The permeation enhancers must be released rapidly from the dosage form and prior to the release of the drug over a wide area across the epithelium.

In order for the hydrophilic to pass through the epithelium, the site of opening of the paracellular pathway must coincide with the site where the drug is released from the dosage form. Hence, multiple unit dosage forms (MUDFs) were designed to control the release of the drug. The minitablets can be then filled in gelatin capsules and enteric coated to be protected from the acidic condition of the stomach. [31]

***Microspheres***

Spherical microspheres, prepared by complexation between oppositely charged macromolecules such as chitosan and negatively charged molecules such as tripolyphosphate (TPP) or alginates have received a lot of attention as drug delivery vehicles for protein drug delivery purposes. These microspheres can protect the drugs from the hostile environment of the GI tract, improve drug absorption via the paracellular route and control the drug release at a specific site. Luesen et al. and Kotze et al. have applied drug containing chitosan microspheres on Caco-2 cell monolayers and showed a strong increase in the transport of gentamicin, trimetazidin buserelin, insulin and vasopressin derivative. A number of investigations were done by Shu et. al. and Mutara et. al. for controlled release drug delivery. They showed that variables such as drug concentration, type and concentration of chitosan, the pH of TPP solution, volume of the internal and external phases, gelation time as well as drying conditions can all determine the fate of drug release from chitosan beads. Avadi et al. have used enteric coated capsules containing Brilliant Blue chitosan beads as model hydrophilic drug for colon drug delivery. The scintigraphy images have demonstrated that Eudragit S coated capsules containing Brilliant Blue loaded chitosan beads are suitable for colon drug delivery. It can be thus concluded that the non-toxic chitosan microspheres and beads can increase the bioavailability of the peptide and protein drugs by protecting them from degradation, when they are able to mucoadhesively attach to a specific site on the intestinal tract and to increase drug permeation by opening the tight junctions via the paracellular pathway.[32]

***Mucoadhesive Drug Delivery Systems***

Mucoadhesion is the attachment of any type of polymer to the mucus layer via strong interaction between the functional groups of the polymer and those of the mucosa lining of the tissue. The mucoadhesive bonding is attained mostly by physical, chemical and more

importantly through H-bonding. Hence, the presence of hydroxyl, carboxyl and H-bond forming functional groups strongly contributes to the strength of mucoadhesion.

The formation process of mucoadhesive bonds include 1) wetting and swelling of the polymers, 2) interpenetration of the mucoadhesion polymer chains and entanglement of the polymer and mucin chains, 3) interfacial interaction of functional groups, 4) formation of weak chemical bonds. The use of mucoadhesive drug delivery systems results in a controlled drug release and attachment at a specific site of the body. Increasing the residence time of the drug delivery systems at the site of absorption in the body may result in prolonging their action. As the GI tract is covered by a mucus layer, the mucoadhesive drug delivery system must be able to attach to a specific site in order to be beneficial. Acrylic acid based polymers have been used extensively for mucoadhesive applications. Their strong bond strength in contact with tissues allows localization of the drug at the site of absorption, increasing residence time at the absorbing tissue and increasing drug bioavailability. Their responsive behaviour to different pH values allows the drug to be released at the desired site of the GI tract.

In order to increase mucus interpenetration, adhesion promoters such as polyethylene glycol (PEG) may be employed, which are not mucoadhesive but contribute to the adhesion process. Moreover, these tethered promoters may be grafted onto polymeric surfaces such that at the one end they are covalently attached to the polymer surface and the other end is free. These grafted chains are able to diffuse into the mucus layer and enhance the mucoadhesiveness of the system. Peppas et al. have done extensive studies on the design and the effect of network morphology of polyethylene glycol (PEG) tethered copolymers as novel mucoadhesive drug delivery systems. They have suggested that the performance of the copolymer is due to the synergistic effect of both polymers: the backbone polymer providing the hydrogen bonds between the hydrogel and mucus layer as well as the adhesive promoter that contributes to the mucoadhesion by increasing the chain interpenetration.[33]

Thiolated polymers are another promising class of mucoadhesive with their capability to form strong covalent bonds through the disulfide binding of the polymers with the mucus gel layer of the mucosa. Thiomers are mucoadhesive polymers with thiol bearing side chains. The disulfide bonds are formed between both the cysteine side of the polymers and glycoproteins of the mucus layer as well as the thiomer itself leading to a strong adherence to the mucus gel layer. At physiological pH values, the oxidation of the thiol groups results in gelling properties due to the formation of inter- and intramolecular disulfide bond. Studies on thiolated poly (acrylic acid) in comparison to the unmodified polymer have shown that the properties of the polymer measured using tensile studies and by using rotating cylinder method were 20 fold increased.[34]

Furthermore, the residence time of the polymer in the small intestine was prolonged by up to 3 fold through immobilization via the thiol groups. Thiolated chitosan was shown to improve mucoadhesion by more than 100- fold. Recently, dosage forms based on thiomers using peptide microparticles were generated via solvent evaporation emulsification method. Because of the formation of the disulfide bonds within the particles they did not disintegrate under physiological conditions for 48hrs and the mucoadhesive properties of the microparticles were improved 3- fold due to immobilization of the thiol groups compared to the control group consisting of the peptide alone. Kast et al. have used thiolated polycarbophil for oral delivery of low molecular weight heparin (LMWH) in rats. In their investigation, they have shown that the absorption of LMWH was significantly increased using the thiolated polymer in comparison to the unmodified polymer. They have reported a 19.9} 9.3% bioavailability in rats compared to the intravenous application.[35]

***Nanoparticles***

Today, a vast number of investigations have been focused on nanoparticles and their role as

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drug delivery vehicles. Nanoparticles were first introduced in the mid-seventies by Birrenbach and Speiser The preparation of nanoparticles was simple, the particles formed were relatively stable and easily freeze-dried; hence, biodegradable polymers were found useful and further developed for drug delivery. Polymeric nanoparticles have the advantages of protecting the hydrophilic, protein and peptide drugs from chemical and enzymatic degradation in the GIT, increasing their stability and absorption across the intestinal epithelium as well as controlling the drug release. A number of techniques such as polymerization, nanoprecipitation, inverse microemulsion can be used to prepare polymeric nanoparticles; however, most of these methods involve the use of organic solvents, heat and vigorous agitation which may be harmful to the polar, peptide and protein drugs. More recently the ionic gelation technique is used as the most favorable method for producing hydrophilic, peptide and protein nanoparticles. The nanoparticles prepared by this method have a suitable size and surface charge, spherical morphology as well as a low polydispersity index indicative of a homogenous size distribution. The lack of using organic solvents, sonication or harsh conditions during preparation reduces the damage to the peptide and proteins and makes this method a favorable one for the preparation of protein loaded nanoparticles. Chitosan nanoparticles with excellent biodegradable and biocompatible characteristics have been used extensively as drug delivery vehicles. However, due to poor solubility of chitosan at pH above 6.0, its quaternized derivatives such as trimethyl chitosan, triethyl chitosan, diethylmethyl chitosan and dimethylethyl chitosan, which are soluble at the intestinal pH, have been used to prepare nanoparticles loaded with insulin. Chitosan nanoparticles loaded with gentamicin were prepared by mixing the positively charged polymer with the negatively charged gentamicin and nanoparticle formation occurred via electrostatic interaction. Studies have shown that gentamicin in nanoparticulate form was more likely to be delivered across the GI tract than in its free soluble form.[36]

The particle size and surface charge are critical factors in nanoparticle absorption. Size is a determining factor for both uptake and biological fate of the particulate systems. Moreover, a size dependent phenomenon exists in the gastrointestinal absorption of the particles. Studies have shown that particles with a size of 100nm were taken up 6- times more than particles with 100μm by the absorptive cells. Hydrophobic particles are absorbed more readily than hydrophilic ones. Thus increasing the hydrophobicity of particles may enhance their permeability through mucus but decreases the translocation through and across the absorptive cells. In the GIT, the particles interact with the mucus before coming into contact with the absorptive cells. Positively charged particles are more prone to uptake as they can associate with the negatively charged functional groups in the mucus.[37]

Accordingly, biodegradable, hydrophobic nanoparticles with sizes between 100-200 nm and positive surface charge may be good candidates for uptake by the epithelial cells. Accordingly, while the use of nanoparticles is highly recommended for gene therapy their use for peptide and hydophillic drug delivery and absorption is debatable also with respect to the minimal drug load they can carry with them in comparison to bigger particles. Even though all of the above mentioned delivery systems gave reasonable results when studied in-vitro and ex-vivo, they were only tested in-vivo using small animals such as mice and rats with intestinal diameters much less than that of humans. For a delivery system to be useful for commercialization, it must have reasonable bio-availabilities in bigger animals such as pigs or dogs with intestinal diameters closer to that of the humans. The small intestinal diameter of smaller animals allows the delivery system to easier come in direct contact with the intestinal wall where in bigger animals and humans reaching the absorbing surface still with full mucoadhesiveness is a big challenge. Moreover, the amount of mucus produced in bigger animals is higher than in the GIT of smaller animals. It was shown for nanoparticles

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developed by Peppas et al. that they lose their mucoadhesive properties in contact with soluble mucins present in the GIT of larger animals before reaching the absorbing surface and are no longer able to open the tight junctions and allow for the paracellular transport of the drugs. Hence, to overcome the above obstacles, delivery systems using superporous hydrogels and gas empowered delivery systems were designed and examined in larger animals such as pigs, rabbits and humans.

***Super Porous Hydrogels (SPH) Based Delivery Systems***

SPH and SPH composites were synthesized by Park et al and adopted by Dorkoosh et al. for intestinal drug delivery. These polymers are able to swell very rapidly up to 200-fold of the original volume upon sucking up the gut fluids and are then able to attach mechanically to the gut wall and bring the dosage form in close proximity of the site of absorption. Consequently, these polymers not only increase the residence time of the dosage form at a specific site in the gut; but also by absorbing the gut fluids they decrease the enzymatic activity. The SPH and SPHC delivery systems were prepared either by inserting the core inside the conveyor system (i.e. core inside, c.i.) or attaching to the surface (i.e. core outside, c.o.).

The core consists of the hydrophilic drug, such as gentamicin. These formulations were then placed in gelatin capsules and enterically coated with Eudragit S100. Hence, the delivery system could safely pass through the acidic environment of the stomach and there after dissolved in the *responsive* pH of the small intestine allowing for the polymer to swell and mechanically attached to the gut wall. The mechanical pressure on the intestinal cells opens the tight junctions allowing for the paracellular transport of the drug across the intestinal membrane.

However, the synthesis and fabrication of the delivery systems is based on SPHs or SPHCs technology, which is difficult and currently32 not commercially feasible on mass production scale. Moreover, their big size (i.e. capsule size 000), is not easily swallowed and may cause variable residence times in the stomach of the patient.[38,39]

***Gas Empowered Drug Delivery System (GEDD)***

The latest approach for a novel drug delivery device is using Gas Empowered Drug Delivery (GEDD) system to deliver hydrophilic drugs such as Gentamicin, peptide and proteins to the intestinal tract and enhance their absorption across the intestinal wall by pushing the active compounds together with the mucoadhesive polymer polyethylene oxide (PEO) and TMC as penetration enhancer to the absorbing membrane of the gut tissue using $CO_2$ gas.

The drug will be then adhered to the mucus layer together with the PEO to prolong the residence time at the mucosal surface and TMC as permeation enhancer will simultaneously trigger the opening of the tight junctions residence time at the mucosal surface and TMC as permeation enhancer will simultaneously trigger the opening of the tight junctions for enhancing drug permeation by the paracellular pathway. The mucoadhesive remnants of the delivery system slide down the mucus membrane and will be shed off at the latest in the large intestine where the dosage form is degraded and expelled. In the GEDD system $CO_2$ acts mainly as the driving force to push the delivery system to the absorbing membrane.

Additionally, it can form a layer around the delivery system protecting it from enzymatic and proteolytic degradation. Furthermore, the $CO_2$ bubbles may act as a permeation enhancer that mechanically opens the tight junctions. In order to protect the drug from the acidic pH of the stomach, the GEDD system was enterically coated with cellulose acetate phthalate (CAP). An increase in drug permeation using this delivery system may be due to the synergistic effect of both the $CO_2$ and TMC in the form of mechanical and chemical enhancement, respectively. The advantage of this delivery system over the superporous hydrogel is its ease of production in large scale.[40,41,42]

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## CONCLUSION

Substantial research exertions have been committed to the development of orally, rectally or nasally applicable formulations of poorly absorbed drugs with absorption improving agent. In recent years a large variety of compounds have been evidently demonstrated to exercise an absorption promoting action. Of these compounds bile salts, cyclodextrins, chitosan, fatty acids, SMEDDS, SDEDDS appears to be contenders of first choice for additional studies, considering their effectiveness and the preliminary data on their safety profiles. Once in the intestinal tract, it must be able to adhere to a specific site long enough to be effective. As the delivery system attaches to the intestinal epithelium it must act as an enhancer to open the tight junctions and enhance the drug permeation across the intestinal wall via the paracellular pathway. Multifunctional polymers such as polyacrylates and chitosans with their multiple derivatives show promising properties as penetration enhancers for the paracellular absorption route of hydrophilic macromolecules by reversibly open the tight junctions; however, their high viscosity and slow dissolution rates make the development of suitable delivery systems using these polymers very difficult. The bioavailability of highly polar drug crossing the intestinal wall must be enough to have a therapeutic effect. Unquestionably, in the near future many more techniques will be discovered to exhibit absorption promoting effects. One such technique is use of animal viruses. As no unambiguous conclusions can be drawn on this issue, additional research is highly desirable insight in the mechanism of permeability enhancement.

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